Evaluation of maize (*Zea mays* L.) inbred lines for tolerance to Aluminum toxicity using selection indices

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Abstract. Selection of genotypes with better yield performance when exposed to stress (biotic or abiotic) is key objective for every plant breeding program. Aluminum toxicity is one of the significant constraints to maize production in acidic soils in many parts of the world. The aim of this study was to identify desirable genotypes with Al tolerance based on selection indices at seedling stage and identify the most effective selection indices under Al stress conditions. 75 Quality Protein Maize (QPM) genotypes were evaluated in CRD with three replicates in the laboratory using a nutrient solution screening method. Additionally, the same genotypes were also screened in the greenhouse arranged in RCBD with three replicates using soil bioassays. The genotypes were exposed to aluminum (Al) toxicity and non-toxic conditions. Data were collected and seven selection indices namely: Stress susceptibility index (SSI); stress tolerance index (STI); geometric mean productivity (GMP); Tolerance index (TOL); Stress Resistance Index (SRI); Percentage Reduction (PCRD); and Mean Productivity (MP) were calculated. Dry Matter (DM) yield under Al toxicity and non-toxic conditions had highly significant ($p < 0.001$) effects with GMP, STI, SRI and MP, suggesting that these indices have the potential to differentiate genotypes for Al tolerance. Significant and negative correlations were observed between DM and PCRD, TOL, and SSI. Indices that had highly significant and very strong correlations amongst themselves were PCRD with SSI ($r = 1.00$), and GMP with STI ($r = 1.00$). The most desirable selection indices that can be used to select tolerant QPM genotypes included GMP, STI, SRI, PCRD, and TOL as they had strong correlations with dry matter under Al toxicity. The tested genotypes were classified into four groups using the principal component (PCA) analysis known as QPM genotypes with high performance under both environments (Group 1), those with high performance under optimum conditions (Group 2), genotypes with high performance under Al stress conditions (Group 3) and those with low performance under both conditions (Group 4). The top five most tolerant maize inbred lines were CML 486, QSY 2, IBL 9, IBL 5 and IBL 4. These tolerant genotypes could be used as parent material for hybridization programs in developing Al tolerant cultivars.

Key words: aluminum toxicity; selection indices, quality protein maize; tolerant; sensitive.

INTRODUCTION

Maize (*Zea mays* L.) is among the most cultivated crops in the world and is consumed by people and livestock in various forms. Although maize is an important crop,

its production is fraught with numerous biotic and abiotic constraints that result in low yields. One of the major abiotic limitations in maize production is the existence of acidic soils, as a result of low potential of hydrogen (pH), (Kaul, 2016; Tandzi et al., 2018). A soil pH was below 4.5 causes the release of high and toxic Al and Mn concentration levels resulting in root damage and decreased overall plant production (Hue et al., 2021; Zama et al., 2022) while reducing the activity of beneficial microorganisms in the soil. Al toxicity in sub-soils is particularly harmful because it causes shallow rooting, drought susceptibility, and poor use of subsoil nutrients lowering the production of maize crops (Lidon & Barreiro, 2002). The metal is found in soluble forms which affect soil biological systems (Arora et al., 2017). Al concentration is pH dependent, thus when pH is low, aluminum becomes soluble resulting in upsurge of aluminum ions in the soil (Bojórquez-Quintal et al., 2017). A high concentration of Al in the soil influences the physiology of maize plants as they will be battling to minimize effects of the stress. Several researchers have confirmed that even in minute concentrations, Al affects the absorption of water and nutrients from the soil, and eventually results in the decline of crop growth and yield (Matsumoto, 2000; Ma & Furukawa, 2003; Ma, 2007; Zhao & Shen, 2018).

There are several strategies that lessen aluminum toxicity impacts on maize production. Developing tolerant cultivars through selection is one of the most promising control strategies. Plant breeders exploit existing genetic variability for Al toxicity tolerance in maize as a basis for developing tolerant cultivars, which offer a cheap and sustainable control option (Malekzadeh et al., 2015). Several authors have reported advantages of different selection indices for screening stress tolerant genotypes. These include tolerance index (TOL) and mean productivity (MP) (Rosielle & Hamblin, 1981); geometric mean productivity (GMP) (Fernandez, 1992; Kristin et al., 1997), stress resistance index (SRI) (Blum et al., 1988), stress tolerance index (STI) (Fernandez 1992); stress susceptibility index (SSI) (Fisher & Maurer, 1978), and percentage reduction (PCRD) (Choukan et al., 2006), and they can be used to advance the efficiency of selection. These indices provide a chance to evaluate the sensitivity and tolerance of some of the QPM germplasm to Al stress. The selection indices have been developed based on yield performance under optimal and stress conditions to identify the most stress tolerant genotypes (Merga et al., 2019). It is apparent that effectiveness of indices seems to be dependent on genotypes used and environment in which they are evaluated, including the interaction between the two. Previous studies have used selection indices to select genotypes for several stresses, including heat, drought (Anwar et al., 2011; Cairns et al., 2013; Tandzi et al., 2015; Teklay et al., 2020), and soil acidity tolerance (Merga et al., 2019). However, these indices have not been administered in the selection of QPM inbred lines exposed to acidic soil conditions. Against this background, the current evaluation was conducted seeking to identify the most effective selection indices for screening Al tolerance in available QPM genotypes at seedling stage.

Evaluation of dry matter (DM) yield has been reported to be useful and effective in several studies that aimed to estimate grain yield during vegetative growth phases (Valle et al., 2009; Zielewicz et al., 2021). In Valle et al. (2009), evaluation of Al-tolerant and Al-sensitive wheat genotypes exposed to different soil aluminum concentrations revealed that grain yield was significantly and linearly correlated ($R^2 = 0.96$, $p < 0.0001$) with the plant dry matter, thus making DM a good indicator to estimate yield. Similarly,

in a maize study, Ball et al. (2000) reported that grain number per plant was directly proportional to the plant biomass, which further reveals how plant biomass is linked to grain yield. This is because most of the dry matter is mobilized from the pre-anthesis accumulated reservoirs of organs such as stems (Rahimi Jahangirlou et al., 2021). The stem weight is critical for increasing grain weight as the leaves are the source and the kernels are the sink, thus a strong source-sink activity (Zhao et al., 2020). Additionally, wide acceptability of genotypes is mainly on the basis of many desirable traits and not only on its grain yield potential (Bello et al., 2010). Therefore, evaluation was done at seedling stage because seedlings are generally more sensitive to environmental stresses than mature plants (Teskey et al., 2012). Therefore, it was crucial to take advantage of dry matter accumulation while evaluating the QPM genotypes under Al stress. Screening of QPM genotypes was therefore conducted at seedling stage in this study since grain yield can be predicted from plant biomass as assessed at the seedling and vegetative growth stages.

The correlation coefficient shows the average relatedness between two variables and it is one of the simplest ways to ascertain the association between variables (Khatibi et al., 2022). It is known as an effective method to measure the relationship between variables because it is based on the covariance method. Similarly, Principal Component Analysis (PCA) suggests the degree of contribution of traits towards the variation among treatments. It explains which variable or trait is the most discriminating and has greater variability among treatments (Ene et al., 2016). The present study was conducted in different environments with the overall objective to identify Al stress tolerant genotypes among quality protein maize inbred lines during vegetative stage using selection indices, cluster, and principal component analyses. The study's objective sought to determine the most desirable selection indices that can be used to select tolerant QPM genotypes. Selection was done once in the laboratory under nutrient solution, and twice using soil-based screening method in the greenhouse. The nutrient solution and soil bioassay methods are the most commonly used techniques for screening genotypes at seedling stage (Ahlrichs et al., 1990). These methods permit preliminary screening of numerous genotypes and thus reduce the number of potentially useful genotypes to be further evaluated under field conditions.

MATERIALS AND METHODS

Experimental study location

Two experiments were conducted at Fort Hare University in South Africa. One experiment was conducted in the Plant Breeding and Genetics Laboratory, while the other experiment was conducted in a plastic tunnel with uncontrolled conditions as described by Zishiri et al. (2022). Daily temperature was recorded using a thermometer and, in the laboratory, the average temperature was 25 °C while in the plastic tunnel average temperature was 25 °C in the first season and 25 °C in the second season. Light was provided by fluorescent bulbs that emit 400–700 nanometer wave lengths in the laboratory, and in the plastic tunnel, the clear polyethylene plastic that made the tunnel allowed passage of light to the plants.

Germplasm

The 75 maize germplasm used in this study were collected from CIMMYT Zimbabwe (19), CMMMYT Mexico (4 used as tolerant checks), and Quality Seed (Pty) Ltd of South Africa (52). The list of germplasm is presented in Table 1.

Experimental Procedure

Laboratory experiment. The nutrient solution-based procedure used in the laboratory was adopted from Zishiri et al. (2022). Seeds of each genotype were surface sterilized for five minutes while being constantly shaken with a 0.1% sodium hypochlorite solution. After giving the seeds a thorough wash for five more minutes with tap water, they were rinsed three times with distilled water. The seeds were then placed in petri dishes with moistened filter paper, and they were incubated for seven days at 27 °C. Five uniformly germinated seeds of each genotype were placed in plastic trays with Hoagland solution. The trays were then left at room temperature for three days (seventy-two hours) thus allowing seedlings to grow in the nutrient solution. Following that, the seedlings were moved to a stress solution supplemented with 600 μM aluminum sulfate ($Al_2 [SO_4]3$) (Chanda et al., 2015) to discriminate Al tolerant and sensitive maize genotypes, whilst the other Group of seedlings was kept in a non-toxic solution (control) that didn't include any aluminum. Al sulphate is regarded as the greatest stress to plants (Ma & Furukawa, 2003). Data were collected from five uniform seedlings per genotype in a completely randomized design (CRD), with each treatment being reproduced three times (Zishiri et al., 2022). In the laboratory, the experiment was administered for only 72 hours and the nutrient solution was changed frequently to avoid nitrate fluctuations. The composition for the nutrient solution (in milligrams per liter, mg L^{-1}) 0.03 Mo, 14.6 Mg, 48.1 Ca, 0.16 Zn, 42.61 N, 0.03 Mn, 0.32 B, 0.03 Cl, 23.5 K, 0.02 Na, 0.06 Cu, 1.67 Fe (added as $FeSO_4·7H_2O$) (Choudhary & Singh, 2011; Chanda et al., 2015). The nutrient solution pH was then adjusted to 4.0 using 1M HCl solution and left unadjusted thereafter. Each treatment was replicated five times.

Greenhouse experiment. The experimental design and protocol used for the greenhouse-based soil bioassay was the same as described by Zishiri et al. (2022). Uniform soil was collected from the Fort Hare University farm, sieved through a mesh wire and amended with a basal fertilizer hygrofert (183 g kg^{-1} Mg, 153 g kg^{-1} N, 69 g kg^{-1} P, and 14 g kg^{-1} S). The soil was filled in pots measuring 60 cm height and 11cm diameter and watered to field capacity and incubated with $Al_2 [SO4]_3$ 2 days before planting. Three seeds per genotype were sown in each pot and later thinned to one 3 days after crop emergence. The $Al_2 [SO_4]_3$ was applied at a rate of 24 mg kg^{-1} of soil adopted from Holland et al. (2021). Holland et al. (2021) reported that 24 mg kg^{-1} of soil is the critical concentration of exchangeable hydrogen cations in the soil above which toxicity is observed for cereals. A pressure plate apparatus was used to determine the field capacity of the soil (Nyambo et al., 2018). Soil pH measurements were taken bi-weekly and it was always under 4.5, signifying that Al^{3+} in the soil was available to the plant during the experimental period. The experiment was replicated three times in a randomized complete block design (RCBD).

Table 1. List of germplasm used in the study

	Inbred Lines form CIMMYT Zimbabwe
1.	IBL 1 [CLQRCWQ50/CML312SR)-2-2-1-BB-1-B-B]
2.	IBL 2 - [CML202/CML144] F2-1-1-3-B-1-B*6/[GQL5/[GQL5/[MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-BB] F2-4sx]-11-3-1-1-B*4]-B*5-1-B
3.	IBL 5 [CML144/SNSYNF2[N3/TUX-A-90]-102-1-2-2-BSR-B*4]-B-4-3-B*4-1-B
4.	IBL 6 [CML150/CML373]-B-2-2-B*4-4-B-B
5.	IBL 7 [CML159/[CML159/[MSRXPOOL9] CIF2-205-1(OSU23i)-5-3-X-X-1-BB] F2-3sx]-8-1-1-BBB-4-B-B
6.	IBL 8 [CML182/TZMI703]-B-9-1-BB-#-BB-2-B-B
7.	IBL 9 [CML202/CML144] F2-1-1-3-B-1-B*6-2-B
8.	IBL 10 [CML205/CML176]-B-2-1-1-2-B*5-1-B-B
9.	IBL 11 [CML389/CML176]-B-29-2-2-B*4-B
10.	IBL 12 [GQL5/[GQL5/[MSRXPOOL9] CIF2-205-1(OSU23i)-5-3-X-X-1-BB] F2-4sk]-11-3-1-1-B*5-3-B-B
11.	IBL 13 [GQL5/GQL5/CML202] F2-3sk]-11-4-1-3-B*4-B
12.	IBL 14 [TZMI703/CML176]-B-3-2-B*5-4-B-B
13.	IBL 15 CLQRCWQ50-BB-1-2-B-B
14.	1BL 16 CML176-#-B-2-B
15.	IBL 17 CML181-B-1-5-B-B
16.	IBL 18 CML182-BB-B
17.	IBL 20 CML491-B-3-11-B-B
18.	IBL 21 CML492-BB-2-1-B-B
19.	IBL 22 WWO1408-1-1-2-B*4-#-B-B-B

Inbred Lines from Quality Seeds (Pvt) Ltd South Africa

white maize				yellow maize			
20.	QSW 1	37.	QSW 19	48.	QSY 1	65.	QSY 21
21.	QSW 2	38.	QSW 20	49.	QSY 2	66.	QSY 23
22.	QSW 3	39.	QSW 21	50.	QSY 3	67.	QSY 24
23.	QSW 4	40.	QSW 23	51.	QSY 4	68.	QSY 27
24.	QSW 5	41.	QSW 24	52.	QSY 5	69.	QSY 28
25.	QSW 6	42.	QSW 25	53.	QSY 7	70.	QSY 28
26.	QSW 7	43.	QSW 26	54.	QSY 8	71.	29 E
27.	QSW 8	44.	QSW 27	55.	QSY 9		
28.	QSW 9	45.	QSW 28	56.	QSY 10		
29.	QSW 10	46.	QSW 30	57.	QSY 12		
30.	QSW 12	47.	QSW 32	58.	QSY 13		
31.	QSW 13			59.	QSY 14		
32.	QSW 14			60.	QSY 16		
33.	QSW 15			61.	QSY 17		
34.	QSW 16			62.	QSY 18		
35.	QSW 17			63.	QSY 19		
36.	QSW 18			64.	QSY 20		

	Inbred Lines from CIMMYT Mexico
72.	CML 304*
73.	CML 425*
74.	CML 486*
75.	CML 439*

*: resistant checks from Mexico.

Data collection

There were two harvests: one from the laboratory experiment and the other harvest from the greenhouse experiment. In the laboratory experiment, plants were harvested three days after being exposed to Al toxicity. In the greenhouse, plants were harvested four weeks after seeding. In all cases, the plant samples were baked at 65 degrees Celsius until they reached a steady weight. Using a microgram balance (d = 0.1 mg, Sartorius AG Gottingen CP 64, Germany), the total dry matter yield under acidic (Ys) and non-acidic (Yp) soils was determined for each genotype. The following selection indices were calculated using data gathered from the environments with and without aluminum treatment:

$$\text{Stress tolerance index (STI): } (Ys * Yp)/ \bar{Y}p^2 \text{ (Fernandez, 1992)} \qquad (1)$$

$$\text{Stress resistance index (SRI): } \frac{Ys}{Yp} \text{ (Blum et al., 1988)} \qquad (2)$$

$$\text{Percent Reduction (PCRD): } [(Yp - Ys)/Yp] * 100 \text{ (Choukan et al., 2006)} \qquad (3)$$

$$\text{Geometric mean productivity (GMP): } \sqrt{(Ys)(Yp)} \text{ (Fernandez, 1992; Kristin et al., 1997)} \qquad (4)$$

$$\text{Tolerance Index (TOL): } Yp - Ys \text{ (Rosielle \& Hamblin, 1981)} \qquad (5)$$

$$\text{Mean productivity (MP): } \frac{Yp+Ys}{2} \text{ (Rosielle \& Hamblin, 1981)} \qquad (6)$$

$$\text{Stress Susceptibility Index (SSI): } (1 - Ys/Yp)/(1 - \bar{Y}s - \bar{Y}p) \text{ (Fischer \& Maurer, 1978)} \qquad (7)$$

where Yp = biomass of genotype in a non-stress environment; Ys = biomass of genotype in stress environment; $\bar{Y}p$ = mean biomass of genotype in a non-stress environment; $\bar{Y}s$ = mean biomass of genotype in a stress environment.

Statistical analysis

Dry weight data obtained under stress (Ys) and non-stress (Yp) conditions from the two experiments (laboratory and greenhouse experiments) were subjected to analysis of variance (ANOVA) using GenStat 17th edition to obtain the means. Computation of the indices was performed using Excel Microsoft 365. Using Genstat Release 17.1, multivariate analysis techniques such as ranking method, correlation analysis, and principal component analysis (PCA) were used to determine the most acceptable indices for choosing genotypes tolerant to Al toxicity. The ranking method used was recommended by Farshadfar et al. (2012), with few modifications. In this method, a genotype that exhibited the highest values for each of the indices received a ranking score of 1. The average ranking score for all the indices was calculated, thus the discrimination of the QPM genotypes relative to their tolerance to Al toxicity was performed based on the average ranking score across the indices. The PCA was based on the correlation matrix of the selection indices and the Biplot analysis.

RESULTS

Classification of genotypes based on dry matter yield performance under Al toxic and non-toxic conditions

Results of dry matter yield under Al toxicity and non-Al toxicity conditions, and the different selection indices calculated are presented in Table 2. CML 486 exhibited

the highest dry matter (23.3 g) under Al toxicity, while QSW 30 (32.2 g) had the highest dry matter performance under non-Al stress conditions. Based on the yield loss percentage of genotypes under stress and non-stress conditions, stress susceptibility index (SSI) identified CML 486 (0.10), CML 439 (0.33), QSW 32 (0.46), IBL 4 (0.47), and QSW 16 (0.23) as the most tolerant genotypes, while IBL 8 (1.49), QSW 4 (1.49), 28E (1.61), and QSY 21 (1.62) were the most susceptible genotypes.

Selection indices. Stress tolerance index (STI) selected CML 486 (0.94), QSY 2 (0.91), IBL 9 (0.85), IBL 5 (0.85), and IBL 4 (0.79) as the top five most tolerant genotypes, whereas QSY 19 (0.35), QSY 9 (0.33), 29 E (0.30), QSY 12 (0.29), and QSY 21 (0.27) were the most sensitive genotypes. The five genotypes with the highest STI values were 1st, 2nd, 3rd, 25th, and 4th by DM yield under stress conditions (Table 2). According to geometric mean productivity (GMP), genotypes with the highest stress tolerance were CML 486 (23.9), QSY 2 (23.5), IBL 9 (22.7), IBL 5 (21.8), and IBL 4 (21.6), whereas genotypes with the lowest stress tolerance were identified as QSY 19 (14.6), QSY 9 (14.2), 29 E (13.5), QSY 12 (13.2), and QSY 21 (12.7). Based on mean productivity (MP), QSY 2 (23.9), CML 486 (23.9), IBL 5 (23.2), IBL 9 (23.0), and QSW 30 (22.7) were selected as most tolerant, while QSY 20 (16.0), QSY 24 (16.0), QSY 9 (15.7), QSY 21 (15.3), and QSY 12 (14.9). Three of the most tolerant genotypes by MP (CML 486, QSY 2, and IBL 9) and two of the most sensitive genotypes (QSY 12 and QSY 21) were also identified by STI and GMP. Stress susceptibility index (SSI) selected CML 486 (0.10), CML 439 (0.33), QSW 32 (0.46), IBL 4 (0.47), and QSW 16 (0.53) as the highest performing genotypes. Three of the genotypes identified by SSI as the most tolerant (CML 486, CML 439, QSW 32) were also considered most tolerant by tolerance index (TOL). Stress resistance index (SRI) considered genotypes CML 486 (1.63), IBL 4 (1.11), QSW 32 (1.09), CML 439 (1.06), and QSY 23 (1.04) as genotypes exhibiting the highest stress resistance, while genotypes QSY 10 (0.26), QSY 12 (0.22), QSW 4 (0.22), 29 E (0.15), and QSY 21 (0.14) displayed the least stress resistance. According to percentage reduction (PCRD), the most desirable genotypes were CML486 (4.3), CML 439 (14.5), QSW 32 (20.2), IBL 4 (20.8), QSW 16 (23.4), where QSY 12 (62.8), IBL 8 (66.0), QSW 4 (66.1), 29 E (71.2), and QSY 21 (71.6) were considered undesirable as they exhibited the highest percentage dry matter yield under Al toxicity conditions. Similarly, these genotypes were also identified by SSI. Based on mean rank determined by average performance of genotypes across indices, CML 486 was identified as the most tolerant genotype, followed by IBL 4, while QSY 21 was identified as the most sensitive to Al stress, followed by 29 E (Table 3).

Mean rank values of genotypes based on stress tolerance index

Genotype ranking based of selection indices calculated from dry matter yield performance under Al stress and non-stress conditions is presented in Table 3. The mean rank of the 10 highest and lowest performing genotypes is presented in brackets. The most tolerant genotypes were CML 486 (0.94) < QSY 2 (0.91) < IBL 9 (0.85) < IBL 5 (0.79) < IBL 4 (0.77) < QSW 32 (0.77) < IBL 13 (0.76) < IBL 20 (0.74) < QSW 26 (0.74) < QSW 32 (10.73), while the lowest performing genotypes with highest mean rank scores were QSY 21 (0.27), QSY 12 (0.29), 29 E (0.3), QSY 9 (0.33), QSY 19 (0.35), QSW 23 (0.37), QSW 5 (0.37), QSY 13 (0.37), QSY 20 (0.37), and QSW 4 (0.38).

Table 2. Estimates of stress tolerance attributes for the ten highest & lowest-performing genotypes selected under Al stress conditions on dry matter and corresponding values for selection indices

Ten highest performing genotypes

Genotype	Ys	Yp	SSI	STI	TOL	GMP	MP	SRI	PCRD
CML 486	23.3 (1)	24.4 (36)	0.10 (1)	0.94 (1)	1.0 (1)	23.9 (1)	23.9 (2)	1.63 (1)	4.3 (1)
QSY 2	19.5 (2)	28.3 (6)	0.70 (17)	0.91 (2)	8.8 (26)	23.5 (2)	23.9 (1)	0.98 (8)	31.0 (17)
IBL 9	194 (3)	26.6 (18)	0.61 (13)	0.85 (3)	7.2 (16)	22.7 (3)	23.0 (4)	1.03 (6)	27.0 (13)
IBL 4	19.2 (4)	24.3 (38)	0.47 (4)	0.77 (5)	5.1 (5)	21.6 (5)	21.8 (11)	1.11 (2)	20.8 (4)
QSY 23	18.8 (5)	24.8 (33)	0.55 (8)	0.77 (6)	6.0 (12)	21.6 (6)	21.8 (10)	1.04 (5)	24.2 (8)
QSW 32	18.7 (6)	23.5 (49)	0.46 (3)	0.73 (11)	4.8 (3)	21.0 (11)	21.1 (17)	1.09 (3)	20.2 (3)
IBL 13	18.0 (7)	25.6 (26)	0.67 (15)	0.76 (7)	7.6 (18)	21.5 (7)	21.8 (9)	0.93 (10)	29.5 (15)
QSW 16	17.7 (8)	23.1 (54)	0.53 (5)	0.68 (18)	5.4 (8)	20.2 (18)	20.4 (24)	0.99 (7)	23.4 (5)
QSY 14	17.5 (9)	23.6 (48)	0.58 (10)	0.68 (17)	6.0 (13)	20.3 (17)	20.6 (23)	0.95 (9)	25.6 (10)
CML 435	17.0 (10)	24.9 (31)	0.71 (18)	0.70 (14)	7.9 (22)	20.6 (14)	21.0 (18)	0.85 (16)	31.6 (18)
Ten lowest performing genotypes									
QSY 13	10.0 (66)	22.3 (60)	1.24 (56)	0.37 (70)	12.3 (48)	15.0 (70)	16.2 (69)	0.33 (59)	55.0 (56)
QSW 5	9.8 (67)	23.1 (53)	1.30 (60)	0.37 (67)	13.3 (54)	15.1 (67)	16.5 (65)	0.30 (66)	57.7 (60)
QSW 23	9.7 (68)	23.2 (51)	1.31 (61)	0.37 (69)	13.5 (56)	15.0 (69)	16.5 (66)	0.30 (67)	58.1 (61)
QSY 10	9.6 (69)	25.6 (24)	1.41 (70)	0.41 (63)	16.1 (67)	15.7 (63)	17.6 (53)	0.26 (70)	62.6 (70)
QSY 19	9.4 (70)	22.7 (57)	1.32 (63)	0.35 (71)	13.3 (52)	14.6 (71)	16.0 (70)	0.29 (68)	58.4 (63)
QSY 9	9.0 (71)	22.4 (59)	1.35 (66)	0.33 (72)	13.4 (55)	14.2 (72)	15.7 (73)	0.27 (70)	59.7 (66)
QSW 4	8.8 (72)	26.0 (21)	1.49 (73)	0.38 (66)	17.2 (70)	15.1 (66)	17.4 (56)	0.22 (73)	66.1 (73)
QSY 12	8.1 (73)	21.7 (63)	1.4 2(71)	0.29 (74)	13.6 (58)	13.2 (74)	14.9 (75)	0.22 (72)	62.8 (71)
29 E	7.2 (74)	25.1(30)	1.61 (74)	0.30 (73)	17.9 (71)	13.5 (73)	16.2 (68)	0.15 (74)	71.2 (74)
QSY 21	6.8 (75)	23.9 (45)	1.62 (75)	0.27 (75)	17.1 (68)	12.7 (75)	15.3 (74)	0.14 (75)	71.6 (75)

Ys: dry matter in stress environment; Yp: dry matter in the non-stress environment; GMP: Geometric Mean Productivity; SRI: Stress Resistance Index; PCRD: Percent Reduction; STI: Stress Tolerance Index; TOL: Tolerance index; MP: Mean Productivity; SSI: Stress Susceptibility Index.

Table 3. Mean rank values for ten highest & lowest-performing genotypes selected under Al stress conditions based on stress tolerance index

Genotype	Ys	Yp	SSI	STI	TOL	GMP	MP	SRI	PCRD	SI	Rank
CML 486	23.3	24.4	0.1	0.94	1	23.9	23.9	1.63	4.3	0.04	1
QSY 2	19.5	28.3	0.7	0.91	8.8	23.5	23.9	0.98	31	0.31	2
IBL 9	19.4	26.6	0.61	0.85	7.2	22.7	23	1.03	27	0.27	3
IBL 5	15.4	30.9	1.13	0.79	15.5	21.8	23.2	0.56	50	0.5	4
IBL 4	19.2	24.3	0.47	0.77	5.1	21.6	21.8	1.11	20.8	0.21	5
QSY 23	18.8	24.8	0.55	0.77	6	21.6	21.8	1.04	24.2	0.24	6
IBL 13	18	25.6	0.67	0.76	7.6	21.5	21.8	0.93	29.5	0.3	7
IBL 20	16.4	27.3	0.9	0.74	10.9	21.1	21.8	0.72	40	0.4	8
QSW 26	15.9	28.2	0.99	0.74	12.3	21.1	22	0.65	43.7	0.44	9
QSW 32	18.7	23.5	0.46	0.73	4.8	21	21.1	1.09	20.2	0.2	10
QSW 4	8.8	26	1.49	0.38	17.2	15.1	17.4	0.22	66.1	0.66	66
QSY 20	10.6	21.4	1.14	0.37	10.8	15.1	16	0.38	50.6	0.51	67
QSY 13	10	22.3	1.24	0.37	12.3	15	16.2	0.33	55	0.55	68
QSW 5	9.8	23.1	1.3	0.37	13.3	15.1	16.5	0.3	57.7	0.58	69
QSW 23	9.7	23.2	1.31	0.37	13.5	15	16.5	0.3	58.1	0.58	70
QSY 19	9.4	22.7	1.32	0.35	13.3	14.6	16	0.29	58.4	0.58	71
QSY 9	9	22.4	1.35	0.33	13.4	14.2	15.7	0.27	59.7	0.6	72
29 E	7.2	25.1	1.61	0.3	17.9	13.5	16.2	0.15	71.2	0.71	73
QSY 12	8.1	21.7	1.42	0.29	13.6	13.2	14.9	0.22	62.8	0.63	74
QSY 21	6.8	23.9	1.62	0.27	17.1	12.7	15.3	0.14	71.6	0.72	75

Genotype	SSI	STI	TOL	GMP	MP	SRI	PCRD	Mean rank
CML 486	1	1	1	1	2	1	1	5
QSY 2	17	2	26	2	1	8	17	10
IBL 9	13	3	16	3	4	6	13	9
IBL 4	4	5	5	5	11	2	4	8
QSY 23	8	6	12	6	10	5	8	10
QSW 32	3	11	3	11	17	3	3	11
IBL 13	15	7	18	7	9	10	15	13
QSW 16	5	18	8	18	24	7	5	15
QSY 14	10	17	13	17	23	9	10	17
CML 435	18	14	22	14	18	16	18	18
QSY 13	56	70	48	70	69	59	56	61
QSW 5	60	67	54	67	65	66	60	62
QSW 23	61	69	56	69	66	67	61	63
QSY 10	70	63	67	63	53	71	70	62
QSY 19	63	71	52	71	70	68	63	65
QSY 9	66	72	55	72	73	70	66	67
QSW 4	73	66	70	66	56	73	73	64
QSY 12	71	74	58	74	75	72	71	70
29 E	74	73	71	73	68	74	74	69
QSY 21	75	75	68	75	74	75	75	71

Ys: dry matter in stress environment; Yp: dry matter in the non-stress environment; GMP: Geometric Mean Productivity; SRI: Stress Resistance Index; STI: Stress Tolerance Index; PCRD: Percent Reduction; TOL: Tolerance index; SSI: Stress Susceptibility Index, MP: Mean Productivity.

Correlation analysis of dry matter yield and stress selection indices for 75 QPM genotypes exposed to Al toxicity and non-Al toxicity conditions

To determine the most suitable tolerance criteria, the correlation coefficients between dry matter yield performance and quantitative Al tolerance indices under Al toxicity and non-toxic environments were calculated (Table 4). Significant and positive relationships were found between Ys and GMP (0.90), MP (0.76), SRI (0.96), and STI (0.90), and negative and significant associations were observed with PCRD (-0.90), SSI (-0.90), TOL (-0.74). Similarly, Yp displayed significant and positive relationships with GMP (0.46), MP (0.68), PCRD (0.40), SSI (0.40), STI (0.46), and TOL (0.65). No significant correlation coefficient was observed between Yp and Ys (-0.04). Correlation coefficients observed between PCRD with SSI, and GMP with STI were very strong (1.00).

Table 4. Correlation coefficients of dry matter yield and selection indices of 75 QPM genotypes exposed to Al toxicity and non-Al toxicity conditions

	GMP	MP	PCRD	SRI	SSI	STI	TOL	Yp	Ys
GMP	-								
MP	0.96***	-							
PCRD	-0.62***	-0.40***	-						
SRI	0.75***	0.58***	-0.96***	-					
SSI	-0.62***	-0.40***	1.00***	-0.96***	-				
STI	1.00***	0.96***	-0.61***	0.76***	-0.61***				
TOL	-0.37***	-0.12ns	0.95***	-0.86***	0.95***	-0.37**	-		
Yp	0.46***	0.68***	0.40***	-0.20***	0.40***	0.46***	0.65***	-	
Ys	0.90***	0.76***	-0.90***	0.96***	-0.90***	0.90***	-0.74***	0.04ns	-

Ys: yield under stress; Yp: yield under control; TOL: Tolerance index; STI: Stress Tolerance Index; SRI: Stress Resistance Index; SSI: Stress Susceptibility Index; SI: Stress Index; GMP: Geometric Mean Productivity; MP: Mean Productivity; PCRD: Percentage Reduction. Levels of significance are represented by ***; **; and ns for $p < 0.001$, $p < 0.01$; non-significant, respectively.

Principal component analysis of dry matter yield and selection indices of 75 QPM genotypes exposed to Al toxicity and non-Al toxicity conditions

Ten principal components described the dry matter yield variability amongst genotypes. The first principal component (PC) had an Eigen value greater than 1 (7.21) and expounded 92.8% of the variation, while the subsequent PC had an Eigen value of 0.704. The most discerning indices under PC1 were GMP, PCRD, and Ys. In contrast, for PC2, the most discriminating indices were GMP, MP, Yp and Ys. The third component emphasized GMP, PCRD, TOL and Ys, and contributed 0.13% of the total variation. (Table 5).

PCA Biplot

The relationships between the different indices and genotypes with regards to principal components were further illustrated by a PCA biplot (Fig. 1). Smaller angles between the dimension vectors going in the same direction displayed high correlation between the indices relative to genotype discrimination.

Figure 1. Principal component analysis biplot displaying genotypic variation based on selection indices and dry matter yield under Al stress and non-stress conditions.

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For example, acute angles between Ys and SRI, GMP and STI were observed, signifying the presence of a strong positive relationships amongst the indices with yield under the stress environment (Ys). PCA classified genotypes according to their performance under the two contrasting environments into four groups: genotypes with high performance under both environments (Group 1), genotypes with high performance only under optimum conditions (Group 2), genotypes with high performance only under Al stress conditions (Group 3) and genotypes with low performance under both conditions (Group 4). Genotypes with superior performance were plotted further in the direction of the vector line and closer to the vector line. Genotypes such as CML 439, IBL 2, CML 304, QSW 15 and QSY 17 exhibited high PC1 and low PC2, displaying low sensitivity to Al stress and high yield respectively (Fig. 1). On the other hand, genotypes QSW 30, IBL 5, IBL 8, QSY 5, and QSY 27 were high performers under non-stress conditions. Some genotypes exhibited both high PC1 and PC2, and these include CML 486, IBL 4, IBL 9, QSW 32, and QSY 23. These genotypes are likely superior and stable genotypes in the two contrasting environments characterized by Al stress and non-stress. (Fig. 1).

Table 5. The first two principal components and contribution of selection indices towards variability, and dry matter yield under stress and non-stress environments

Selection index	PC1	PC2
GMP	0.869	0.448
MP	0.057	0.473
PCRD	-0.937	0.013
SRI	0.018	0.013
SSI	-0.021	0.000
STI	0.006	0.027
TOL	-0.261	0.290
Yp	-0.073	0.618
Ys	0.187	0.328
Eigenvalue	9.28	0.704
Proportion of total variance (%)	92.8	7.04

GMP: Geometric Mean Productivity; MP: Mean Productivity; PCRD: Percentage Reduction; TOL: Tolerance index; STI: Stress Tolerance Index; SSI: Stress Susceptibility Index; SRI: Stress Resistance Index; SI: Stress index; Ys: yield under stress; Yp: yield under control.

DISCUSSION

Dry matter yield under optimum and Al stress conditions, and selection indices

The selection of genotypes that tolerate Al toxicity is of major importance. Several researchers affirmed that selection indices are often used in studies related to screening of genotypes for stress tolerance (Khan & Mohammad, 2016; Merga et al., 2019). In the current evaluation, an attempt was made to apply selection indices to evaluate QPM genotypes for Al tolerance at the seedling stage. To identify suitable indices, seven different indices were calculated based on dry matter yield under Al toxicity and non-stress conditions. Analysis of variance demonstrated highly significant differences amongst the selection indices. This implied that the indices can be used to effectively to select tolerant genotypes. The significant interaction between genotype and environment implied that the genotypes performed differently under the two environments. The differential performance of genotypes in the two environments could be attributed to the inherent differences between the growing media that were used, that is soil versus a

nutrient solution-based assay. Other physico-chemical parameters of the soil might have contributed the differential responses of genotypes.

Ranking of 75 QPM genotypes based on the different selection indices

The discrimination of QPM genotypes' level of tolerance or susceptibility to Al stress based on only a single criterion might be contradictory and misleading (Steiner et al., 2020). For example, IBL 4 was ranked position 4 by Ys, SSI, and PCRD, position 5 by STI, TOL, GMP, position 2 by SRI and position 11 by MP. Thus, differentiation and separation of genotypes for stress tolerance levels must be carried out considering all selection indices (Karantin et al., 2019). STI was used in this study to identify high yielding genotypes under Al stress environment. It is reported that the larger the STI value for genotype exposed to stress conditions, the higher the yield potential and stress tolerance (Fernandez, 1992). Thus, the genotypes that had high STI values can be considered as the most tolerant genotypes to Al toxicity. Corroborating these findings was Khatibi et al. (2022) who suggested STI as an effective indicator in genotype selection. GMP was similar to STI and MP in discriminating genotypes and the higher values of these indices indicated higher tolerance to Al toxicity. Several authors have affirmed that GMP, MP, and STI are more desirable and suitable indicators for evaluating stress tolerance (Anwar et al., 2011; Gholinezhad et al., 2014; Teklay et al., 2020; Khatibi et al., 2022). TOL is the yield difference between stress and non-stress conditions (Hossain et al., 1990; Merga et al., 2019). Genotypes with high TOL and SSI values were considered sensitive to Al stress and low yielding, and the reverse is true for low TOL and SSI values. The results of this study were in agreement with assertions that the lower the SSI and TOL values, the less sensitive the genotypes are to stress (Teklay et al., 2020), suggesting that smaller values of these indices are preferred. High values of SRI denoted genotypes with relatively high stability but was somewhat biased towards selecting genotypes that yield high under Al stress conditions. Similar results were reported by Merga et al. (2019) on sunflower (*Helianthus anus*, L.). Similar to TOL and SSI, PCRD selected genotypes with lowest yield reductions under Al stress environment. This index seemed to be also biased towards selecting few genotypes with relatively high DM yield potential.

Correlation between dry matter yield and selection indices

An appropriate index must have a correlation that is significant with yield under control and stress conditions (Gholinezhad et al. (2014). The correlation revealed both negative and positive relations between the indices, displaying similarity and dissimilarity among the indices in genotypic ranking. The results clearly illustrated the interactions which corroborate a better understanding of stress responses. Our results demonstrated strong and significant correlations between GMP, STI and MP with dry matter yield under stress and non-stress conditions. Similar results were reported by Gholinezhad et al. (2014) on sunflower (*Helianthus anuus* L.), Khan & Mohammad, (2016) on wheat (*Triticum aestivum* L.), and Merga et al. (2019) on Tef (*Eragrostis tef*). SSI, PCRD, and TOL on the other end displayed the existence of strong inter-relationships with dry matter under Al toxicity and amongst themselves, confirming similarity between these indices for genotype ranking. Agreeing to this result

is Paula et al. (2019) who asserted that indices that indicate high yield potential under stress or non-stress conditions are in high correlation amongst themselves. Combining these indices could be a better predictor of yield under stress conditions and could be recommended for screening maize genotypes for Al tolerance. The same has been reported by Farshadfar et al. (2012), who asserted that the most appropriate selection indices for stress-tolerant genotypes were indices that generally have a relatively strong correlation with dry matter and among themselves. The results of this evaluation were consistent with Borzoo (2015) on barley, Selamawit Abebe et al. (2021) on sweet potato, who all found a significant positive and negative correlation among indices. Conclusively, use of selection indices was very effective in identification of tolerant genotypes in our study. A previous study by Zishiri et al. (2022) also identified CML 486 as tolerant to Al toxicity based on the nutrient solution technique. It is interesting to note that in the current evaluation, the same genotype was also identified as tolerant genotype by six (SSI, STI, TOL, GMP, SRI, PCRD) out of seven indices, demonstrating that these indices were effective and ideal for identifying superior QPM genotypes.

Principal component analysis (PCA)

The principal component (PC) analysis was performed to establish indices that were suitable to select superior genotypes under the two contrasting environments. The results revealed that STI, GMP, MP and SRI had strong positive correlation, while PCRD, SSI and TOL had strong negative correlation with DM yield under Al toxicity (Ys). PC1 and PC2 were selected and regarded worthy of elucidation and interpretation as they contributed 99.9% of the total variation which helped reduce the dimensionality of the data. Similar results were reported by Drikvand et al. (2012), Amare et al. (2019), and (Khatibi et al., 2022) who stated that the first two principal components explained greater than 99% of the total variation. The results of this study suggested that selection of indices which had high PC1-PC2 were suitable for identifying genotypes for both environments. Principal components 1-2 identified suitable indices under both settings, and this suggested that discriminating genotypes exhibiting high PC1 and PC2 was appropriate for both environments. The components established that their selection and correlation between yield potential of the genotypes were perceptively assenting with earlier reports (Thomas et al., 1996; Khan & Kabir, 2015; Yasir et al., 2013).

CONCLUSION

From the current evaluation, the most desirable selection indices that had the ability to select tolerant QPM genotypes were GMP, STI, SRI, PCRD, SSI as they had strong with DM yield under stress conditions. Principal component analysis classified genotypes into four categories according to their performance under Al toxicity and optimum conditions. The top five most tolerant maize inbred lines were CML 486, QSY 2, IBL 9, IBL 5 and IBL 4. These most tolerant inbred s could be used as parent material for hybridization programs in developing Al tolerant cultivars.

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