PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(4): 1273–1284
https://doi.org/10.14719/pst.3999

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

A multi-trait targeted genotype selection approach for screening drought tolerance in teosinte–maize hybrids

Jaishreepriyanka Ramaswamy[1], Ravikesavan Rajasekaran[2]*, Iyanar Krishnamoorthy[3], Uma Doraisamy[4] & Senthil Natesan[5]

[1]Department of Genetics and Plant Breeding, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India
[2]Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India
[3]Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India
[4]Department of Bioinformatics, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641003, India
[5]Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641003, India

*Email: chithuragul@gmail.com

ARTICLE HISTORY
Received: 30 May 2024
Accepted: 16 September 2024

Available online
Version 1.0 : 10 November 2024
Version 2.0 : 15 November 2024

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Jaishreepriyanka R, Ravikesavan R, Iyanar K, Uma D, Senthil N. A multi-trait targeted genotype selection approach for screening drought tolerance in teosinte–maize hybrids . Plant Science Today. 2024; 11(4): 1273–1284. https://doi.org/10.14719/pst.3999

Abstract

Drought tolerance is a complex trait, and screening based on multiple characteristics rather than relying solely on direct selection indices would lead to more efficient selection. This study aims to select hybrids under both optimal and water deficit conditions to achieve genetic gains in yield traits while maintaining other secondary traits, such as anthesis-silking interval, leaf chlorophyll content, delayed leaf rolling, leaf senescence, relative water content, and ROS scavenging. This will be accomplished using the multi-trait genotype ideotype distance index. Thirty teosinte-maize hybrids, along with four check varieties, were evaluated for two categories of traits: 16 morphometric traits and 14 physiological and biochemical traits, under well-watered (WW) and water-stressed (WS) conditions. Significant variation among the genotypes was observed for most traits, and the moderate to high heritability of many traits suggests the potential for direct selection to improve those traits. A significant correlation between these traits and yield, as well as intercorrelations among traits, indicates the advantage of indirect selection based on secondary traits. Assuming a selection intensity of 15% and equal weights given to all the traits, five genotypes were selected based on the MGIDI for each class of traits in both environments. Notably, genotypes G19, G20, and G29 were consistently selected across both environments. The selection process resulted in desired positive and negative gains for most of the traits studied, with particularly high positive gains in single plant yield of 35.6% under WW conditions and 69.3% under WS conditions. The strength and weakness plots effectively highlight the advantages and limitations of the selected genotypes in each environment. The multi-trait-based selection approach proved to be an effective tool for identifying genotypes and designing breeding strategies for stress tolerance.

Keywords

Multi-trait index; water stress; drought; genetic gains; teosinte-maize hybrids

Introduction

While domestication and selective breeding have significantly advanced crop improvement over centuries, they have also unintentionally reduced the genetic diversity of many cultivated plants. In recent decades, researchers have increasingly turned to crop wild relatives—undomesticated ancestors or close relatives of crops—for their potential to bridge these genetic gaps (1). This approach is particularly promising for a globally important crop like maize, as wild relatives offer a wealth of novel genetic variations that can be strategically introduced into maize to enhance its resilience and performance.

Teosinte, a group of primitive wild grass species native to Mexico and Central America, is a key example. *Zea mays* subsp. *parviglumis* is considered the progenitor of modern maize (*Zea mays* subsp. *mays*) (2). Other diploid species of these wild grasses include *Zea luxurians*, *Zea mays* subsp. *mexicana*, and *Zea diploperennis*. Despite sharing many genetic similarities with its wild ancestor teosinte, modern maize has a vastly different phenotype due to the loss and gain of various genetic traits during domestication (3). Successful hybridizations between maize and teosinte, with teosinte used as the pollen parents, have been achieved, resulting in the synthesis of fertile hybrids (4, 5).

Notable variations among the hybrids included traits such as tillering, prolificacy (with more ears per plant), the protective stony casing of kernels, and kernel row numbers (6–8). Singh et al. used *Z. mays* ssp. *parviglumis* in hybridization with maize lines and observed significant variations for traits like plant height, flowering time, the number of ears per plant, and grain yield per plot among the recombinant inbred lines. A study on the phenotypic and genomic characterization of maize and *Z. mays* ssp. *parviglumis* reported 25% similarity using Jaccard's similarity coefficients, further confirming the presence of wild alleles that can be applied to diversify the maize genetic background (8). Thus, hybridization with teosinte has the potential to narrow the genetic gap in domesticated maize. Several novel alleles from teosinte could be utilized to develop maize hybrids with improved agronomic characteristics and greater resilience to environmental stresses, including drought.

Maize yields are significantly impacted by drought stress in addition to biotic factors. There is an urgent need to develop drought-tolerance crops due to adverse climatic conditions, such as extended drought periods and irregular monsoon rainfall. In developing countries like India, drought is the leading cause of major crop yield losses, and high temperatures are expected to exacerbate its effects. Drought tolerance is a complex trait influenced by various mechanisms across plant morphology and development stages (9). One study found substantial variation in morphometric and physiological traits related to drought in *Z. mays* ssp *mexicana* genotypes (10). Another experiment evaluated *parviglumis*-derived recombinant maize lines for drought tolerance, noting that the introgression of wild alleles into the maize background led to significant improvements in drought tolerance (11).

Drought stress is the most devastating and severe of all abiotic stresses affecting crops. In maize, drought stress during flowering is particularly critical, as it significantly impacts kernel yield. A delay in silking, caused by a slower rate of silk elongation under drought conditions, is commonly observed (12). Drought stress increases the anthesis-silking interval (ASI) due to reduced silk growth rate, and a lower ASI is typically associated with greater drought tolerance. Severe drought during the reproductive stage reduces yield by negatively affecting traits such as the number of kernel rows, the number of kernels per row, the number of kernels per cob, and overall plant yield (13). Other traits impacted by drought include leaf rolling, stomatal closure, and leaf chlorophyll content (14). Grain yield is the primary trait for direct selections; however, several reliable secondary traits are significantly correlated with grain yield, making them valuable for indirect selection to enhance yield (15, 9). For more efficient selection, a multivariate method that consists of multiple traits, including both grain yield and secondary traits, should be prioritized over selecting genotypes based solely on yield performance. The multi-trait genotype ideotype distance index (MGIDI), a multi-trait-based selection index, can be used to identify genotypes with desirable mean performances across multiple traits, overcoming the limitations of direct selection and other linear indices (16).

An effort was made to utilize the MGIDI index to select suitable hybrids for drought tolerance based on various morphometric, physiological, and biochemical traits.

Materials and Methods

In this study, research was conducted on wide hybridization between maize and teosinte species, using *Zea mays* ssp. *mays* inbreds as female parents. The materials included 10 contemporary maize inbreds and the wild relatives, *Zea mays* ssp. *mexicana*, *Zea mays* ssp. *parviglumis*, and *Zea luxurians.* The list of lines, testers used in the hybridization program, and the hybrid combinations evaluated for drought tolerance are presented in Table 1. Crossings were conducted during the summer and kharif seasons of 2022, and 30 newly generated F_1s hybrids along with the parent lines were separately evaluated for drought tolerance, along with four check varieties, during the summer of 2023 at the Department of Millets, Tamil Nadu Agricultural University, Coimbatore. The 30 hybrids and four checks were grown in a randomized block design with two replications under both well-watered (WW) and water-stressed (WS) conditions. The check varieties included popular commercial hybrids CO (H)M 6, CO (H)M 8, CO (H)M 11, and S6668. CO (H)M 6 and CO (H)M 8 are well-known for their high yields, CO (H)M 11 is a recently released high-yielding drought- tolerant hybrid, and S-6668 is a popular hybrid with high yield and superior photosynthetic efficiency (17, 18). Each genotype was planted in a plot consisting of two rows, each 4 m long, with a spacing of 60 × 25 cm between plants. Water stress in the stress environment was induced by withholding irrigation after 40 DAS, creating stress before anthesis, and extending up to 77 DAS, after which rewatering was performed. All other agricultural practices were followed as per the recommended guidelines. Observations were recorded for morphometric, physiological, and biochemical traits in both well-watered and water-stressed environments.

Table 1. List of experimental materials.

Lines		Testers		Hybrids					
L_1	VL1018299	T_1	*Zea luxurians*	G1	$L_1 \times T_1$	G11	$L_1 \times T_2$	G21	$L_1 \times T_3$
L_2	VL1018300	T_2	*Zea mays* ssp. *parviglumis*	G2	$L_2 \times T_1$	G12	$L_2 \times T_2$	G22	$L_2 \times T_3$
L_3	DMR E 63	T_3	*Zea mays* ssp. *mexicana*	G3	$L_3 \times T_1$	G13	$L_3 \times T_2$	G23	$L_3 \times T_3$
L_4	UMI 1223			G4	$L_4 \times T_1$	G14	$L_4 \times T_2$	G24	$L_4 \times T_3$
L_5	UMI 1200		**Checks**	G5	$L_5 \times T_1$	G15	$L_5 \times T_2$	G25	$L_5 \times T_3$
L_6	UMI 1230	C1	CO (H)M 6	G6	$L_6 \times T_1$	G16	$L_6 \times T_2$	G26	$L_6 \times T_3$
L_7	UMI 1200 B$^+$	C2	CO (H)M 8	G7	$L_7 \times T_1$	G17	$L_7 \times T_2$	G27	$L_7 \times T_3$
L_8	UMI 1230 B$^+$	C3	CO (H)M 11	G8	$L_8 \times T_1$	G18	$L_8 \times T_2$	G28	$L_8 \times T_3$
L_9	UMI 1201	C4	S6668	G9	$L_9 \times T_1$	G19	$L_9 \times T_2$	G29	$L_9 \times T_3$
L_{10}	UMI 1205			G10	$L_{10} \times T_1$	G20	$L_{10} \times T_2$	G30	$L_{10} \times T_3$

Morphometric traits

Drought escape is one of the key mechanisms of drought resistance, with earliness and short antithesis-silking intervals (ASI) being crucial for plants to escape drought stress. A short ASI ensures pollination with viable pollen and adequate seed set (19). A larger leaf area is essential for maintaining photosynthesis, thereby supporting grain filling and yields (20). Additionally, traits such as the number of tillers, ears, kernel rows, and test weight are important yield-contributing factors, vital for improving performance under both well-watered and water-stressed conditions (21).

The following morphometric traits were selected for the investigation:

- In each plot, days to 50% anthesis (DA) and days to 50% silking (DS) were recorded when 50% of the plants began shedding pollen and 50% of plants showed silk emergence, respectively. The anthesis-silking interval (ASI) was calculated as the difference between DA and DS.
- The number of tillers (NOT), number of ears (NOE), number of leaves (NOL), and number of tassel branches (TB) were counted on five randomly selected plants in each plot, and the mean was recorded.
- Similarly, plant height (PH), ear height (EH), leaf length (LL), leaf width (LW), and tassel length (TL) were measured in centimeters on five randomly selected plants per plot, and the mean values were recorded.

Post-harvest observations were taken from the cobs of five randomly selected plants per plot. Cob length (CL) and cob girth (CG) were measured in centimeters on five cobs from each of the five harvested plants, and the mean was calculated for each plot. Kernel row number (KRN), and kernels per row (KPR) were also recorded for each plot. The cobs were then shelled, and the weight of 100 kernels (test weight, TW) was recorded. The mean grain weights from five plants were adjusted to a standard moisture content of 15.5% (22) and used to calculate the single plant yield (SPY).

Physiological traits

The dynamics of leaf characteristics, photosynthesis, and water use efficiency play a crucial role in maintaining grain yield under water stress. While leaf rolling can reduce transpirational water loss, it significantly impacts the production of photosynthates, which ultimately affects grain filling. Delayed leaf rolling, however, can enhance tolerance to water stress (23). The following physiological characteristics were selected as suitable indicators of water stress tolerance:

- The leaf chlorophyll content was measured using a SPAD meter (SPAD 502, Minolta Co., Tokyo, Japan) on the third leaf from the top for 10 randomly selected plants in each plot. The average SPAD readings were taken three times at 8, 19, and 26 days after drought induction (SPAD8, SPAD19, SPAD26) and once at 4 days after rewatering (SPAD4R).
- Leaf rolling scores were recorded plot-wise, using a scale of 1 to 5, with 1 indicating a flat leaf and 5 indicating a fully rolled (onion leaf-like) leaf. These scores were taken at 8, 19, and 26 days after drought induction (LRS8, LRS19, LRS26), and once at 4 days after rewatering (LRS4R).
- Leaf senescence scores (LSS) were assessed plot-wise at 26 days after drought induction using a scale of 1 to 10, where 1 represented almost all leaves green and 10 represented all leaves dead.
- Relative water content (RWC30) was measured at 76 days after sowing (DAS) as a percentage, using the formula:

$$\text{RWC} = \frac{(\text{Fresh weight - Dry weight})}{(\text{Turgid weight - Dry weight})} \times 100 \qquad \text{......(Eqn. 1)}$$

- The average was calculated from five plants per plot.
- The chlorophyll fluorescence (CF) was measured on 75 DAS, with the minimum (F_0) and maximum fluorescence (F_m) recorded. The F_v/F_m ratio was derived using an OS30p+ handheld chlorophyll fluorometer (Opti - Sciences, Inc.) on photosynthetically active leaves from four plants per plot after 20 min of dark adaptation.

Biochemical traits

Plant stress leads to the accumulation of reactive oxidative species (ROS) in cells, which can severely disrupt cellular homeostasis. However, plants possess an innate ability to counteract the effects of these oxidants through enzymatic scavengers such as peroxidase and catalase (24). Osmolytes like proline also help scavenge free radicals and restore cellular homeostasis under water stress (25). In this study, the activity levels of peroxidase, catalase, and proline were measured. Leaves from four plants per plot were collected, immediately frozen in liquid nitrogen, and stored in a -40˚C deep freezer for subsequent laboratory enzyme analyses. Catalase activity (CAT) and peroxidase activity (POX) were determined as μM^{-1} min^{-1} $g^{-1}$of tissue (26) and units' min^{-1} $g^{-1}$of tissue (27), respectively. Proline content (PRO) was determined as µmoles g^{-1} of tissue (28).

Statistical analysis

The data were analyzed using two–way ANOVA with the TNAUSTAT package (29) to assess significant variation among the genotypes. Correlation network plots and MGIDI values were generated using R studio and R version 4.3.3. (30, 31) Pearson's correlation coefficients were calculated, and network plots for the correlations were produced using the corrr package (32). A linear mixed-effects model was created using the *gamem()* function from the metan (33) package, and genetic parameters were estimated. A likelihood ratio test with a two-tailed chi-squared test, using one degree of freedom, was performed to test the significance of random effects in the model.

The *mgidi()* function from the metan package was used to calculate the MGIDI scores. The MGIDI estimates for hybrids in each environment were derived based on the best linear unbiased predictions (BLUPs) for the genotypes. The MGIDI index was computed in four major steps: (i) rescaling traits so that all had a similar range of 0–100, (ii) using factor analysis to reduce data dimensionality, (iii) designing an ideotype based on the desired trait values, and (iv) calculating the distance between each genotype and the planned ideotype (34).

Equal weightage was assigned to all traits, with positive directionality for most traits, except for ASI, TL, and TB from the morphometric traits, and LRS8, LRS19, LRS26, LRS4R, and LSS from the physiological and biochemical traits. A moderate selection intensity of 15% was assumed to balance achieving selection gains while maintaining genetic diversity.

Results and Discussion

Genotype × Environment interaction

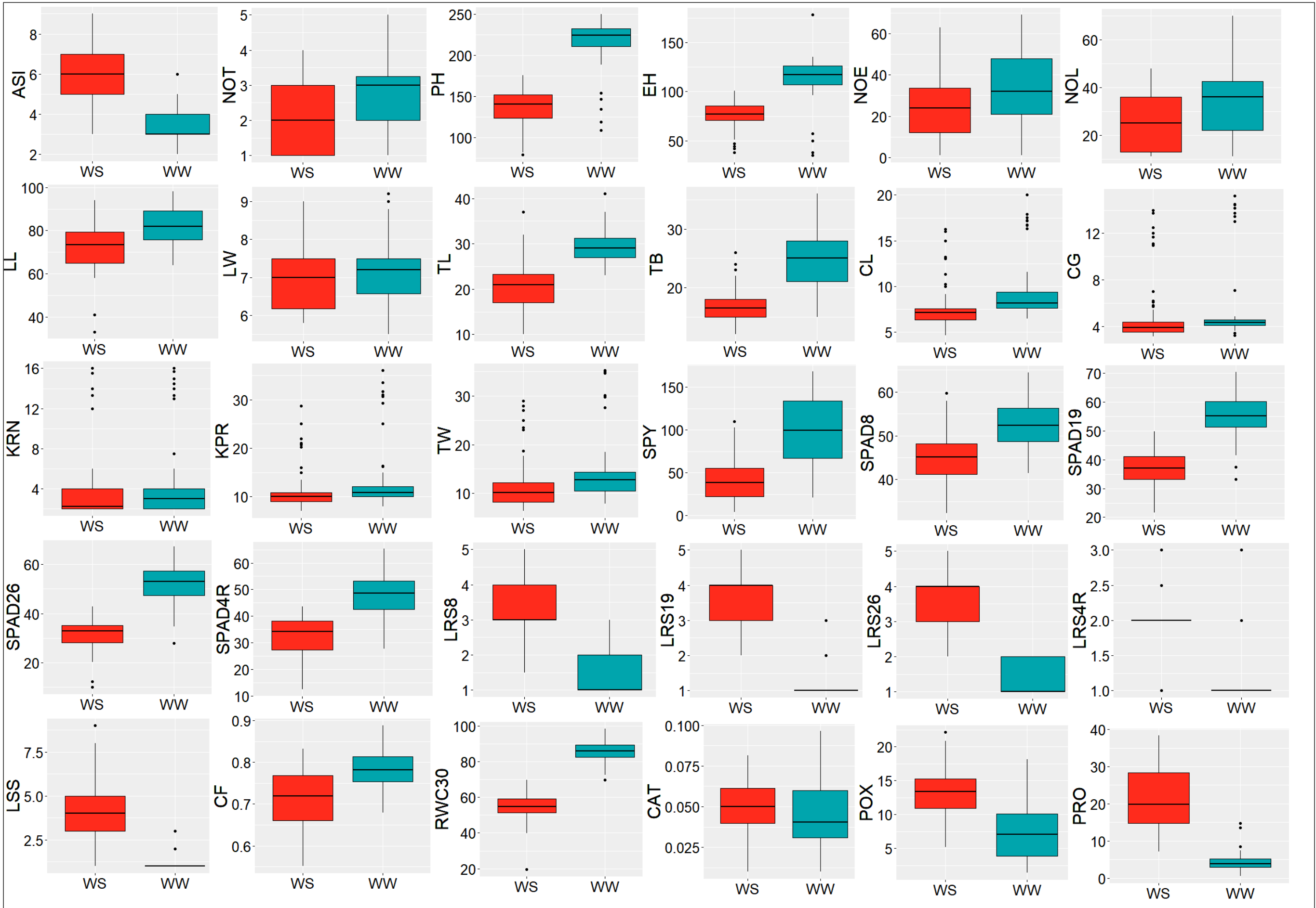
The two-factor analysis of variance (Supplementary Table 1a and b) revealed significant variations among genotypes for all traits studied. The environment also displayed significant variation across all traits. Furthermore, the genotype × environment (G×E) interaction was significant for most traits, except for TW, SPAD8, SPAD19, and CF. According to Kumar et al., significant effects of genotype, environment, and G × E interaction indicate ample variation among genotypes and environments (35). This suggests that the genotypes showed notable variations under water stress conditions, and the studied morphometric, physiological, and biochemical traits have the potential to identify tolerant genotypes.

Fig. 1 presents the mean performances of 34 genotypes for 30 morphometric, physiological, and biochemical traits under well-watered (WW) and water-stressed (WS) conditions. The mean performances of the traits varied across environments, indicating sufficient variation for selection. The lower mean yield in the WS environment can be attributed to poor performances in yield-contributing traits such as the number of tillers, ears, kernel rows, kernels, and test weight. Additionally, poor physiological characteristics, including reduced chlorophyll content, early leaf rolling, high leaf senescence, decreased relative water content, and low antioxidant activity, further contributed to the reduced yield under stress conditions.

The effect of water stress on the studied traits and the relative changes in trait means under WS conditions compared to WW conditions are illustrated in Fig. 2. It was observed that flowering traits such as DA, DS, and ASI, along with leaf rolling scores (LRS8, LRS19, LRS26, and LRS4R), leaf senescence score (LSS), and biochemical traits including CAT, POX, and PRO, exhibited a positive change compared to the WW environment. In contrast, the remaining traits showed a negative change under WW conditions. The relative changes in trait means compared to the well-watered environment were substantial. Leaf width (LW) exhibited the smallest change, decreasing by only 2.00%, whereas proline activity (PRO) demonstrated the highest increase, rising by 398.7% over the WW conditions. The mean SPY showed a reduction of 56.4% under the WS condition.

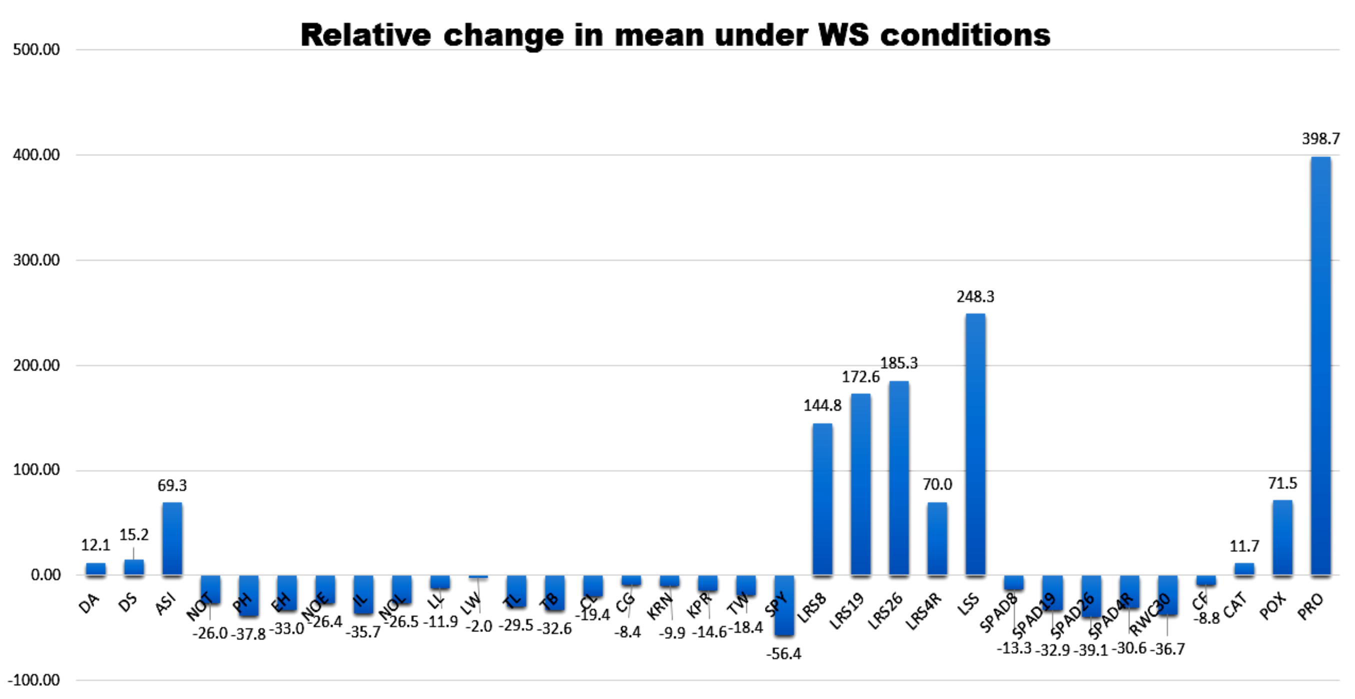
Likelihood ratio test

The likelihood ratio test (LRT) for the mixed-effect model revealed significant differences among genotypes for most traits, except for SPAD8 and chlorophyll fluorescence (CF) under well-watered (WW) conditions and SPAD8 and SPAD19 under water-stressed (WS) conditions. This indicates a high level of genetic variability among the genotypes. Table 2 presents the genotypic variance (GV), phenotypic variance (PV), heritability (h), accuracy (As), and the correlation coefficients with single plant yield (SPY) for the different traits in both environments. The experimental results revealed low (below 30%), moderate (30-60%), and high (above 60%) heritability levels, as per the classifications of heritability (36). Under WW conditions, all traits except tassel length (TL), SPAD8, SPAD19, leaf rolling score at 26 days (LRS26), CF, and relative water content at 30 days (RWC30) exhibited high heritability. Similarly, under WS conditions, most traits, except SPAD8, SPAD19, LRS26, and LRS4R showed high heritability. SPAD8 was observed to have low heritability in both environments. High to moderate heritability indicates that genotypic variance contributes significantly to phenotypic variance, with minimal environmental influence, suggesting successful



ASI – Anthesis silking interval, NOT – No. of tillers, PH – Plant height, EH – Ear height, NOE – No. of ears, NOL – No. of leaves, LL – Leaf length, LW – Leaf width, TL – Tassel length, TB – Tassel branches, CL – Cob length, CG – Cob girth, KRN – Kernel row number, KPR – Kernels per row, TW – Test weight, SPY – Single plant yield, SPAD8, SPAD19, SPAD 26, SPAD4R – SPAD at 8, 19, 26 days after drought induction and 4 days after rewatering respectively, LRS8, LRS19, LRS26, LRS4R – Leaf rolling score at 8, 19, 26 days after drought induction and 4 days after rewatering respectively, LSS – Leaf senescence score, CF – Chlorophyll fluorescence, RWC30 – Relative water content, CAT – Catalase activity, POX – Peroxidase activity, PRO – Proline content.

Fig. 1. Mean performances of the 34 genotypes for the studied traits under WW and WS environments.



ASI – Anthesis silking interval, NOT – No. of tillers, PH – Plant height, EH – Ear height, NOE – No. of ears, NOL – No. of leaves, LL – Leaf length, LW – Leaf width, TL – Tassel length, TB – Tassel branches, CL – Cob length, CG – Cob girth, KRN – Kernel row number, KPR – Kernels per row, TW – Test weight, SPY – Single plant yield, SPAD8, SPAD19, SPAD 26, SPAD4R – SPAD at 8, 19, 26 days after drought induction and 4 days after rewatering respectively, LRS8, LRS19, LRS26, LRS4R – Leaf rolling score at 8, 19, 26 days after drought induction and 4 days after rewatering respectively, LSS – Leaf senescence score, CF – Chlorophyll fluorescence, RWC30 – Relative water content, CAT – Catalase activity, POX – Peroxidase activity, PRO – Proline content.

Fig. 2. Relative change (%) in the mean of traits under water-stressed (WS) conditions.

Table 2. LRT and estimates of genetic parameters of the traits, along with their correlation with SPY under WW and WS environments.

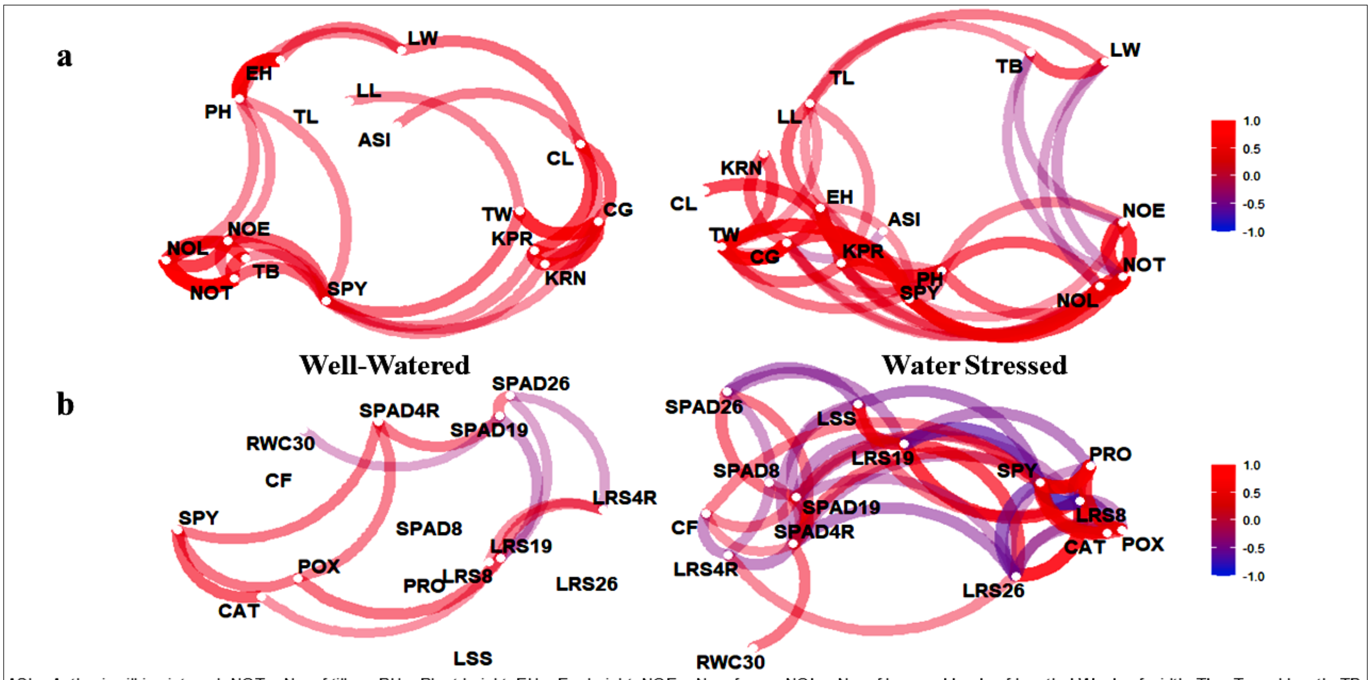
Traits	Well-watered condition (WW)						Water-stressed condition (WS)					
	LRT	GV	PV	h	As	r	LRT	GV	PV	h	As	r
ASI	35.82	1.03	1.27	0.81	0.95	0.11	21.97	1.34	1.91	0.70	0.91	-0.24
NOT	29.46	0.12	0.15	0.80	0.93	0.45	48.47	0.10	0.11	0.90	0.97	0.56
PH	24.67	566.43	780.64	0.73	0.92	0.34	68.48	494.35	528.64	0.94	0.98	0.37
EH	36.13	383.57	470.24	0.82	0.95	0.15	76.82	157.71	166.01	0.95	0.99	0.32
NOE	48.93	3.64	3.89	0.94	0.97	0.47	65.11	2.71	2.82	0.96	0.98	0.31
NOL	26.82	1.38	1.78	0.78	0.92	0.46	36.98	1.06	1.27	0.84	0.95	0.58
LL	56.60	70.05	77.35	0.91	0.97	0.16	49.96	94.08	106.52	0.88	0.97	0.21
LW	72.12	0.63	0.67	0.94	0.98	-0.02	83.14	0.70	0.73	0.96	0.99	-0.22
TL	10.07	6.42	12.52	0.51	0.82	-0.05	42.01	25.32	29.84	0.85	0.96	-0.07
TB	39.28	0.24	0.28	0.84	0.95	0.33	50.73	0.12	0.14	0.89	0.97	-0.15
CL	74.05	9.54	10.09	0.95	0.99	0.05	62.34	5.02	5.45	0.92	0.98	0.26
CG	112.96	10.48	10.66	0.98	1.00	0.33	99.05	7.62	7.81	0.97	0.99	0.54
KRN	80.33	0.54	0.57	0.94	0.99	0.34	93.22	0.52	0.55	0.94	0.99	0.20
KPR	67.70	0.57	0.63	0.91	0.98	0.35	60.12	0.26	0.29	0.90	0.98	0.50
TW	68.59	42.56	45.50	0.94	0.98	0.45	66.57	30.24	32.47	0.93	0.98	0.63
SPY	65.29	1543.12	1662.34	0.93	0.98	1.00	55.86	513.69	568.67	0.89	0.97	1.00
SPAD8	0.29ns	2.66	28.59	0.09	0.41	0.05	0.75ns	4.74	31.59	0.15	0.51	0.18
SPAD19	4.13	15.96	46.54	0.34	0.71	0.19	1.45ns	6.92	33.33	0.21	0.59	0.39
SPAD26	19.48	43.10	64.56	0.67	0.89	0.10	23.25	30.29	42.60	0.71	0.91	0.27
SPAD4R	23.59	48.50	67.86	0.71	0.91	0.41	32.70	40.28	50.80	0.79	0.94	0.42
LRS8	22.94	35.21	50.09	0.70	0.91	0.14	27.49	82.16	119.03	0.69	0.93	-0.76
LRS19	16.53	30.24	48.51	0.62	0.88	0.16	31.61	93.89	120.69	0.78	0.94	-0.62
LRS26	10.96	18.59	34.96	0.53	0.83	-0.04	15.90	70.26	147.54	0.48	0.87	-0.58
LRS4R	9.64	16.23	32.23	0.50	0.82	-0.07	14.16	31.85	54.05	0.59	0.86	-0.14
LSS	26.99	0.003	0.005	0.74	0.92	-0.18	30.27	45.27	58.13	0.78	0.93	-0.48
CF	0.66ns	0.0003	0.002	0.14	0.50	-0.20	18.73	0.003	0.005	0.66	0.89	0.37
RWC30	13.18	20.41	35.57	0.57	0.85	-0.27	60.71	64.67	70.51	0.92	0.98	0.20
CAT	65.29	0.0004	0.0004	0.93	0.98	0.49	82.08	0.0003	0.0003	0.96	0.99	0.57
POX	66.14	14.85	15.96	0.93	0.98	0.41	51.08	9.33	10.52	0.89	0.97	0.54
PRO	86.48	5.90	6.13	0.96	0.99	-0.10	91.69	68.32	70.54	0.97	0.99	0.72

GV - genotypic variance, **PV** - phenotypic variance, **h** - heritability, **As** - accuracy of genotype selection, **r** - correlation with SPY, **ASI** - anthesis silking interval, **NOT** - no. of tillers, **PH** - plant height, **EH** - ear height, **NOE** - no. of ears, **NOL** - no. of leaves, **LL** - leaf length, **LW** - leaf width, **TL** - tassel length, **TB** - tassel branches, **CL** - cob length, **CG** - cob girth, **KRN** - kernel row number, **KPR** - kernels per row, **TW** - test weight, **SPY** - single plant yield, **SPAD8**, **SPAD19**, **SPAD 26**, **SPAD4R** - SPAD at 8, 19, 26 days after drought induction and 4 days after rewatering, respectively, **LRS8**, **LRS19**, **LRS26**, **LRS4R** - leaf rolling score at 8, 19, 26 days after drought induction and 4 days after rewatering, respectively, **LSS** - leaf senescence score, **CF** - chlorophyll fluorescence, **RWC30** - relative water content, **CAT** - catalase activity, **POX** - peroxidase activity, **PRO** - proline content. **ns** - non-significant.

selection for these traits (37). Utilizing highly heritable traits in breeding programs could enhance selection efficiency, particularly under drought stress (20). The accuracy of trait predictions ranged from 0.41 (SPAD8) to 1.00 (cob girth, CG) in the WW environment and from 0.51 (SPAD8) to 0.99 (ear height, EH; number of ears, NOE; leaf width, LW; cob girth, CG; kernel row number, KRN; catalase, CAT; and proline, PRO) in the WS environment. The high accuracy observed for most traits indicates a strong correlation between predicted and actual genotypic values, suggesting reliable predictions for these traits.

Genetic correlation/inter-relationship

Genetic correlations were calculated based on Pearson's model, and network plots were generated separately for morphometric traits, as well as physiological and biochemical traits, under both environments (Fig. 3). Traits with stronger associations clustered together, with red paths indicating positive correlations and blue paths representing negative correlations. The morphometric traits showed positive inter-correlations under well-watered (WW) conditions, whereas under water-stressed (WS) conditions, both stronger positive and some negative



ASI – Anthesis silking interval, NOT – No. of tillers, PH – Plant height, EH – Ear height, NOE – No. of ears, NOL – No. of leaves, LL – Leaf length, LW – Leaf width, TL – Tassel length, TB – Tassel branches, CL – Cob length, CG – Cob girth, KRN – Kernel row number, KPR – Kernels per row, TW – Test weight, SPY – Single plant yield, SPAD8, SPAD19, SPAD 26, SPAD4R – SPAD at 8, 19, 26 days after drought induction and 4 days after rewatering respectively, LRS8, LRS19, LRS26, LRS4R – Leaf rolling score at 8, 19, 26 days after drought induction and 4 days after rewatering respectively, LSS – Leaf senescence score, CF – Chlorophyll fluorescence, RWC30 – Relative water content, CAT – Catalase activity, POX – Peroxidase activity, PRO – Proline content.

Fig. 3. Network plots for genetic correlation a. between the morphometric traits and b. between the physiological and biochemical traits under different environments.

correlations were observed among these traits. Several morphometric traits, including number of ears (NOE, 0.47), number of leaves (NOL, 0.46), number of tillers (NOT, 0.45), test weight (TW, 0.45), kernels per row (KPR, 0.35), plant height (PH, 0.34), kernel row number (KRN, 0.34), tassel branches (TB, 0.33), and cob girth (CG, 0.33), exhibited significant positive correlations with single plant yield (SPY) under WW conditions. Under WS conditions, the traits TW (0.63), NOL (0.58), NOT (0.56), CG (0.54), KPR (0.50), PH (0.37), ear height (EH, 0.32), NOE (0.31), and cob length (CL, 0.26) showed significant positive correlations with SPY (Table 2). Physiological and biochemical traits, such as catalase activity (CAT, 0.49), SPAD4R (0.41), and peroxidase activity (POX, 0.41), also showed significant positive correlations with SPY under WW conditions, while relative water content (RWC 30) had a negative correlation (-0.27). Under WS conditions, proline content (PRO, 0.72), CAT (0.57), POX (0.54), SPAD4R (0.42), SPAD19 (0.39), chlorophyll fluorescence (CF, 0.37), and SPAD26 (0.27) were positively correlated with SPY, while leaf rolling scores (LRS8, -0.76; LRS19, -0.62; LRS26, -0.58) and leaf senescence score (LSS, -0.48) had strong negative correlations with SPY. The inter-correlations among traits, along with the magnitude and direction of these correlations, provide valuable information for the simultaneous improvement of multiple characteristics. Comparing the correlations of traits under different environments offers insights into the effectiveness of indirect selection based on stronger or weaker trait associations across conditions. Traits such as plant height, leaf area, and yield-related factors were found to have strong positive correlations under both environments, making them important targets for selection. Although physiological and biochemical traits were grouped separately, they also influenced morphometric traits and grain yield under stress. For example, high chlorophyll content, photosynthetic efficiency, and larger leaf area under water stress positively impacted yield and related traits. Under WW conditions, no significant associations were observed between SPAD, leaf rolling, or leaf senescence traits and grain yield. However, under WS conditions, SPAD exhibited a strong positive correlation with grain yield, while leaf rolling and leaf senescence had significant negative correlations. Dordas et al. reported similar positive correlations between grain yield, chlorophyll fluorescence, and chlorophyll content in maize inbreds and hybrids under drought stress (38). Effendi et al. also found a higher correlation between yield and relative water content, along with a negative correlation with leaf rolling in maize genotypes under drought stress (23). Antioxidant enzymes and proline play a protective role during stress, showing positive correlations with grain yield under WS conditions. Previous studies have reported similar associations among enzyme activity levels under stress (24). Indirect selection for high chlorophyll content, increased antioxidant activity, and reduced leaf rolling and leaf senescence under WS conditions could enhance grain yield under water-stress environments.

Explanatory factor analysis

The factor loadings for the studied morphometric, physiological, and biochemical traits are presented in Table 3a and b. The 16 morphometric traits were grouped into four principal components, which explained a cumulative variance of 81.10% under well-watered (WW) conditions and 79.7% under water-stressed (WS) conditions. For the 14 physiological and biochemical traits, six principal components explained a cumulative variance of 75.10% under WW conditions, while four principal components accounted for a cumulative variance of 71.40% under WS conditions.

Table 3a. Explanatory factor analysis for morphometric traits under WW and WS environments.

Morphometric traits	Well-watered				Water-stressed			
	FA1	FA2	FA3	FA4	FA1	FA2	FA3	FA4
ASI	0.14	0.12	0.06	0.87	-0.49	-0.45	-0.31	0.41
NOT	-0.53	-0.18	-0.71	0.05	0.24	-0.86	0.23	-0.16
PH	-0.02	-0.9	-0.21	-0.03	-0.27	-0.38	0.05	-0.72
EH	-0.01	-0.9	0.01	0.05	-0.3	-0.09	-0.12	-0.8
NOE	-0.63	-0.33	-0.34	-0.35	0.53	-0.68	0.18	0.01
NOL	-0.53	-0.27	-0.72	0	0.23	-0.87	0.17	-0.2
LL	0.43	-0.33	-0.1	0.63	-0.28	-0.13	-0.6	-0.53
LW	0.69	-0.43	0.27	-0.03	-0.33	0.28	-0.73	-0.08
TL	-0.66	0.13	0.08	-0.33	0.66	-0.25	0.12	0.28
TB	-0.19	-0.13	0.79	-0.02	-0.19	-0.21	0.77	-0.05
CL	0.97	-0.02	0.05	0.05	-0.93	0.18	-0.04	-0.15
CG	0.97	0.05	0.02	0.15	-0.94	0.12	-0.1	-0.19
KRN	0.95	0.06	-0.04	0.18	-0.93	0.24	-0.03	-0.06
KPR	0.97	0.02	-0.03	0.16	-0.94	0.01	-0.06	-0.13
TW	0.96	0.02	-0.03	0.17	-0.91	0.07	-0.12	-0.27
SPY	0.44	-0.24	-0.61	-0.13	-0.59	-0.53	0.02	-0.25
Eigen values	7.38	2.79	1.64	1.16	6.95	3.22	1.52	1.06
Variance %	46.1	17.5	10.3	7.27	43.4	20.1	9.48	6.64
Cum. variance %	46.1	63.6	73.8	81.1	43.4	63.6	73	79.7

ASI - anthesis silking interval, **NOT** - no. of tillers, **PH** - plant height, **EH** - ear height, **NOE** - no. of ears, **NOL** - no. of leaves, **LL** - leaf length, **LW** - leaf width, **TL** - tassel length, **TB** - tassel branches, **CL** - cob length, **CG** - cob girth, **KRN** - kernel row number, **KPR** - kernels per row, **TW** - test weight, **SPY** - single plant yield.

Table 3b. Explanatory factor analysis for physiological and biochemical traits under WW and WS environments.

Physiological &biochemical traits	Well-watered						Water-stressed			
	FA1	FA2	FA3	FA4	FA5	FA6	FA1	FA2	FA3	FA4
SPAD8	-0.14	-0.19	-0.12	-0.26	0.15	0.74	0.18	-0.17	0.74	0.19
SPAD19	-0.24	-0.08	0.14	-0.77	-0.12	-0.1	-0.17	-0.25	0.71	0.35
SPAD26	0.12	0.04	-0.15	-0.79	0.02	0.09	-0.03	0.11	0.24	0.85
SPAD4R	-0.39	-0.52	-0.44	-0.4	-0.06	0.18	-0.41	0.06	0.65	0.25
LRS8	-0.22	-0.01	-0.11	-0.33	0.08	-0.82	-0.86	-0.18	0.13	0.21
LRS19	-0.41	0.38	-0.06	-0.53	0.46	-0.03	-0.68	-0.11	0.24	0.46
LRS26	-0.76	-0.06	-0.09	-0.01	0.2	-0.06	-0.92	-0.05	0.14	0.07
LRS4R	-0.6	0.13	-0.21	-0.48	0.17	-0.09	-0.24	0.57	0.42	0.38
LSS	-0.79	0.14	0.09	-0.02	-0.16	0.02	-0.4	0.01	0.18	0.69
CF	-0.07	-0.06	-0.88	0.05	0.15	-0.04	-0.04	0.02	0.68	0.2
RWC30	-0.1	0.56	-0.28	0.53	-0.15	0.01	-0.32	0.27	0.72	-0.3
CAT	0.05	0	0.08	-0.03	-0.95	-0.04	-0.61	-0.39	-0.14	-0.04
POX	0.12	-0.9	-0.11	0.12	-0.05	0.1	-0.31	-0.83	0	-0.05
PRO	-0.46	-0.02	0.48	0.18	0.22	-0.41	-0.18	-0.82	0.24	0.06
Eigen values	3.31	2.22	1.52	1.29	1.1	1.07	4.87	2.51	1.51	1.12
Variance %	23.7	15.8	10.9	9.2	7.89	7.62	34.8	17.9	10.8	8
Cum. variance %	23.7	39.5	50.4	59.6	67.5	75.1	34.8	52.7	63.4	71.4

SPAD8, **SPAD19**, **SPAD 26**, **SPAD4R** - SPAD at 8, 19, 26 days after drought induction and 4 days after rewatering, respectively, **LRS8**, **LRS19**, **LRS26**, **LRS4R** - leaf rolling score at 8, 19, 26 days after drought induction and 4 days after rewatering, respectively, **LSS** - leaf senescence score, **CF** –-chlorophyll fluorescence, **RWC30** - relative water content, **CAT** - catalase activity, **POX** - peroxidase activity, **PRO** - proline content.

MGIDI index and selection gains

The MGIDI values were calculated, and the genotypes were ranked (Fig. 4) separately based on morphometric traits as well as physiological and biochemical traits for both environments. A selection intensity of 15% was applied, and five genotypes were selected in each environment for each trait category. The selected genotypes and their MGIDI values are presented in Table 4. Based on the morphometric traits, the genotypes G19 (2.44), G20 (2.45), C1 (2.58), C3 (2.77), and G14 (2.81) were chosen under well-watered (WW) conditions, while C4 (3.21), G29 (3.23), G20 (3.61), G27 (3.72), and G22 (3.76) were selected under water-stressed (WS) conditions. The checks, C1 and C3, which are known for high yield and superior agronomic traits, ranked among the top genotypes under WW conditions, whereas check C4, a high yielder, was selected under WS conditions for morphometric traits. Furthermore, the genotypes G29, G20, G27, and G22 were ranked higher than the drought-tolerant check C3 for morphometric traits under WS conditions. For physiological and biochemical traits, genotypes G25 (2.39), G19 (2.53), G11 (2.53), G15 (2.61), and G29 (2.67) were selected under WW conditions, while G27 (1.50), G19 (1.67), G17 (1.88), G30 (2.03), and G29 (2.09) were selected under WS conditions. The selected genotypes ranked higher than the checks in both environments for physiological and biochemical traits. Under WS conditions, G27 and G29 were consistently selected based on both trait categories. Genotypes G19, G20, and G29 were selected in both environments, indicating their robustness. The selected genotypes for each environment and the three common genotypes across both environments are illustrated in Fig. 5. Singamsetti et al. utilized MGIDI selection to choose maize hybrids across optimal, drought, and waterlogging conditions (39).

The selected genotypes for each class of traits and environments resulted in desired selection gains (SG) for the mean performance of most traits (Supplementary Table 2), with the exception of NOT, NOE, NOL, TL, TB, SPAD26, and LRS19 in the well-watered (WW) environment, and LW and TL in the water-stressed (WS) environment. For morphometric traits, desired positive gains were observed for PH, EH, LL, CL, CG, KRN, KPR, TW, and SPY, while a desired negative gain was seen for ASI in both

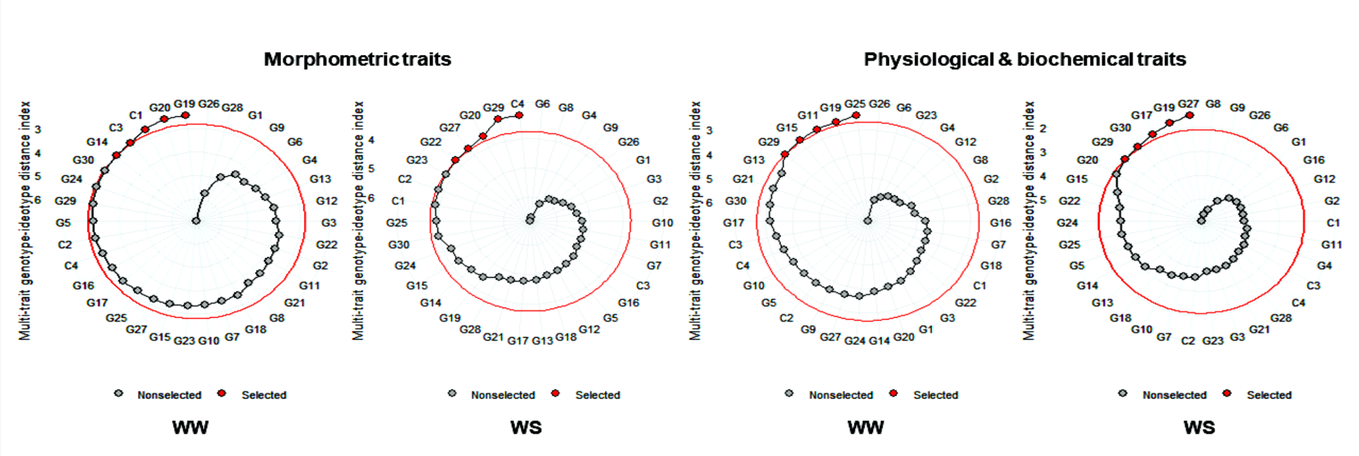


Fig. 4. Ranking of genotypes in ascending order of MGIDI under different environments based on morphometric traits and physiological and biochemical traits.

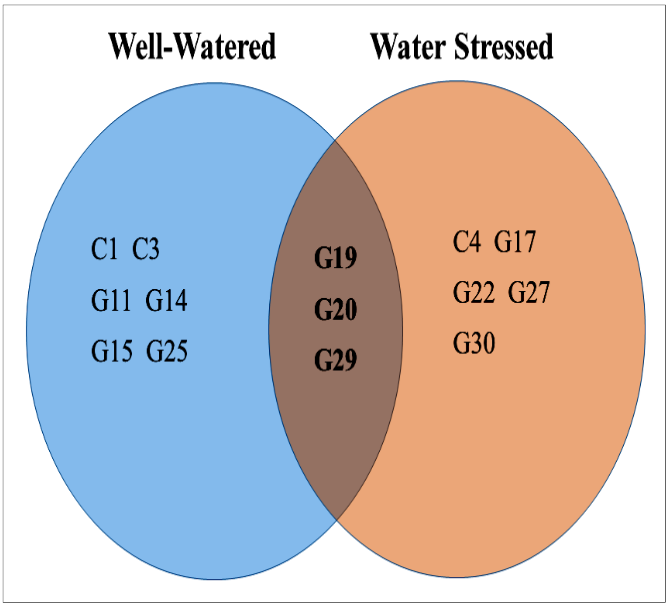


Fig. 5. Venn diagram showing the common hybrids selected across WW and WS environments.

Table 4. MGIDI of selected hybrids in WW and WS environments based on morphometric traits and physiological and biochemical traits.

Morphometric traits				Physio & Biochemical traits			
Well-Watered		Water-Stressed		Well-Watered		Water-Stressed	
Genotype	MGIDI	Genotype	MGIDI	Genotype	MGIDI	Genotype	MGIDI
G19	2.44	C4	3.21	G25	2.39	G27	1.50
G20	2.45	G29	3.23	G19	2.53	G19	1.67
C1	2.58	G20	3.61	G11	2.53	G17	1.88
C3	2.77	G27	3.72	G15	2.61	G30	2.03
G14	2.81	G22	3.76	G29	2.67	G29	2.09

environments. Additionally, LW showed a positive gain only in the WW environment, and NOT, NOE, and NOL had positive gains, while TB exhibited a negative gain only in the WS environment. Traits such as CL, CG, KRN, KPR, and TW were identified as major contributors to SPY in both environments. Ngugi et al. identified short ASI as a key ideotype for selecting drought-tolerant maize genotypes (19). For physiological and biochemical traits, desired positive gains were achieved for SPAD8, SPAD19, SPAD4R, CF, RWC30, CAT, POX, and PRO, while negative gains were noted for LRS8, LRS26, LRS4R, and LSS in both environments. Under WS conditions alone, SPAD26 showed a desired positive gain, and LRS19 exhibited a desired negative gain. Kamphorst et al. used SPAD as an indirect indicator for screening drought tolerance in popcorn genotypes (40). Li et al. utilized CF to screen drought-tolerant barley genotypes (41). Alvi et al. observed increased POX and CAT activity in drought-tolerant maize genotypes (24). Effendi et al. found that maize genotypes with delayed leaf rolling and lower LRS displayed greater drought tolerance and maintained higher RWC (23). Monteoliva et al. concluded that delayed leaf senescence and lower LSS could contribute to water stress tolerance (42). The selection gains achieved for grain yield, as well as key secondary traits, ensure that the genetic improvements in yield and yield-related traits do not result in unintended reductions in other important characteristics. A study that selected elite rice genotypes based on MGIDI reported similar genetic gains, further demonstrating the effectiveness of this selection method (43).

Strength and weakness plot

The strengths and weaknesses of the selected hybrids across both environments are illustrated in Fig. 6. Factors that contribute most to a hybrid's MGIDI score are positioned closer to the center of the radar plot, while the least contributing factors are farther from the center. Therefore, if a genotype performs well for a particular factor, that factor will have a smaller contribution to the MGIDI score for that genotype.

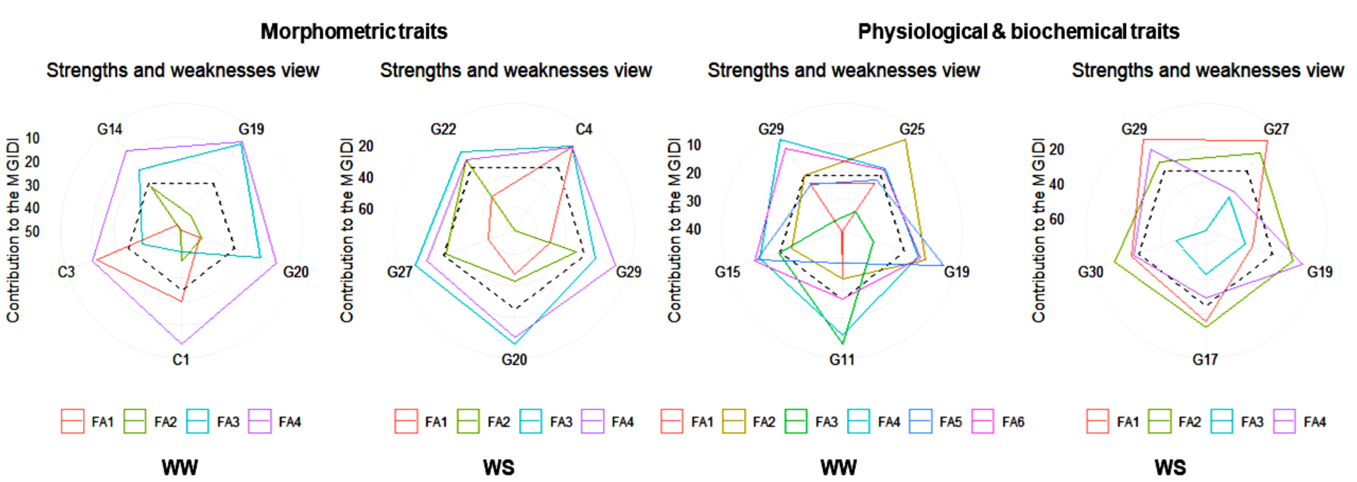


Fig. 6. Strength and weakness view plot as the proportion of each factor to the MGIDI values over WW and WS environments for morphometric traits and physiological and biochemical traits.

The strength and weakness plot for morphological traits under the well-watered (WW) environment reveals that genotypes C1 and C3 exhibited strengths for factor FA1, which is associated with the traits cob length (CL), cob girth (CG), kernel rows number (KRN), kernels per row (KPR), test weight (TW), and leaf width (LW), all showing positive selection gains. Genotype G14 demonstrated strength for factor FA2, related to plant height (PH) and ear height (EH), with positive selection gains. Genotypes G19 and G20 were stronger in factor FA3, which pertains to single plant yield (SPY), while all selected genotypes showed strength related to factor FA4, which includes anthesis-silking interval (ASI) and leaf length (LL). Although checks C1 and C3 are high-yielders, they were noted to perform poorly for factor FA3 due to their lower performance in number of tillers (NOT) and number of ears (NOE). Genotypes G14 and G19, which showed higher performance for factors FA3 and FA4, could be further improved in cob-related traits and plant height to reduce their contribution to the index score. Under the water-stressed (WS) environment, genotype C4 showed strength in factor FA1, associated with ASI, CL, CG, KRN, KPR, TW, and SPY. In contrast, all other selected genotypes, except C4, were stronger in factor FA2, which relates to NOT, NOE, and NOL, also exhibiting positive selection gains. All genotypes showed strength in factor FA3 for leaf length (LL) and tassel branches (TB), as well as in factor FA4 for traits PH and EH. Genotypes G22, G29, and G27 were particularly strong in factors FA2, FA3, and FA4, but they could benefit from improvements in ASI, yield, and related traits under WS conditions.

For physiological and biochemical traits under the well-watered (WW) environment, all genotypes exhibited weaknesses in factor FA1, which includes leaf rolling scores (LRS26, LRS4R) and leaf senescence score (LSS). However, genotypes G25 and G19 demonstrated strengths in factor FA2, associated with SPAD4R, relative water content (RWC30), and peroxidase (POX), all showing positive selection gains. Genotype G11 was stronger in factor FA3, related to chlorophyll fluorescence (CF) and proline (PRO), while genotypes G29 and G11 excelled in factor FA4, related to SPAD19, with positive selection gains. Genotypes G19 and G15 showed strengths in factor FA5 for catalase (CAT), while G29 and G15 were stronger in factor FA6, which includes SPAD8 and LRS8. Notably, genotypes G29 and G19, also selected under water stress (WS) conditions, demonstrated better performance in factors FA2, FA4, FA5, and FA6, indicating their strengths in chlorophyll content, enzyme activity, and relative water content. Under the WS environment, genotypes G29, G27, and G17 showed strengths in factor FA1 for traits LRS8, LRS19, LRS26, and CAT. All five genotypes excelled in factor FA2, which encompasses LRS4R, POX, and PRO. Genotype G29 was the weakest in factor FA3, which includes SPAD8, SPAD19, SPAD4R, CF, and RWC30. Meanwhile, genotypes G19 and G29 demonstrated strengths in factor FA4 for SPAD26 and LSS. Overall, genotypes G29, G17, and G30 were strong across factors FA1, FA2, and FA4, suggesting that improvements in chlorophyll content, photosynthetic efficiency, and relative water content could help reduce the contribution of these traits to the MGIDI.

Similarly, an experiment applied MGIDI-based selection to fodder maize genotypes, highlighting the strengths and weaknesses of the selected inbreds for various fodder component traits (44). Another study utilized radar plots to identify the strengths and weaknesses of maize hybrids selected under different water regimes, including drought and waterlogging (39). The radar plot serves as an easy and effective tool for understanding the shortcomings of selected hybrids and suggests improvements for the weaker characteristics. The results recommend using MGIDI to effectively select ideal hybrids and genotypes, facilitating better decision-making in crop improvement programs.

Conclusion

In conclusion, MGIDI proves to be a valuable multivariate approach for the effective selection of genotypes based on multiple traits. The study identified genotypes G19, G20, and G29 as consistently selected across both environments, highlighting their resilience to stress conditions. The progenies of these selected hybrids could serve as valuable pre-breeding lines for developing drought-tolerant maize. Furthermore, the plots generated from the analyses facilitate the identification of the strengths and weaknesses of the selected genotypes, enabling targeted improvements of the weaker traits in these genotypes.

Acknowledgements

The authors wish to acknowledge the support provided by the Department of Millets, Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore.

Authors' contributions

JR conducted the research experiments and wrote the manuscript. RR and IK designed the study and supervised it. RR, IK, UD, and SN helped in conducting the experiments. IK helped in the statistical analysis and interpretation. RR and IK helped in correcting and revising the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

Supplementary data

Supplementary Table 1a. Two-factor analysis of variance for the morphometric traits.

Supplementary Table 1b. Two-factor analysis of variance for the physiolo gical and biochemical traits

Supplementary Table 2. Selection differential for the mean performance of hybrids under different moisture conditions.

References

1. Volk GM, Khoury C, Greene S, Byrne P. Introduction to crop wild relatives. In: Volk GM, Byrne P. Crop Wild Relatives and their Use in Plant Breeding. Fort Collins, Colorado: Colorado State University; 2020 [cited 2024 May 29].
2. Adhikari S, Joshi A, Kumar A, Singh NK. Diversification of maize (*Zea mays* L.) through teosinte (*Zea mays* subsp. *parviglumis* Iltis & Doebley) allelic. Genetic Resources and Crop Evolution. 2021;68(7):2983-95. https://doi.org/10.1007/s10722-021-01170-z
3. Hossain F, Muthusamy V, Bhat JS, Zunjare RU, Kumar S, Prakash NR, Mehta BK. Maize breeding. In: Fundamentals of Field Crop Breeding; 2022 May 6. pp. 221-58. Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-16-9257-4_4
4. Baltazar BM, de Jesús Sánchez-Gonzalez J, de la Cruz-Larios L, Schoper JB. Pollination between maize and teosinte: an important determinant of gene flow in Mexico. Theoretical and Applied Genetics. 2005;110:519-26. https://doi.org/10.1007/s00122-004-1859-6
5. Liu Z, Cook J, Melia-Hancock S, Guill K, Bottoms C, Garcia A, et al. Expanding maize genetic resources with predomestication alleles: maize–teosinte introgression populations. The Plant Genome.2016;9 (1):plantgenome2015-07. https://doi.org/10.3835/plantgenome2015.07.0053
6. Niazi IA, Rauf S, da Silva JA, Munir H. Comparison of teosinte (*Zea mexicana* L.) and inter-subspecific hybrids (*Zea mays* L. × *Zea mexicana*) for high forage yield under two sowing regimes. Crop and Pasture Science. 2015;66(1):49-61. https://doi.org/10.1071/CP14155
7. Singh NK, Kumar A, Chandra H, Pal K, Verma SS. Enhancement of maize allelic diversity using wild relative teosinte (*Zea mays* ssp. *parviglumis*). Indian Journal of Plant Genetic Resources. 2017;30(3):253-57. http://dx.doi.org/10.5958/0976-1926.2017.00032.8
8. Adhikari S, Joshi A, Singh NK. Phenotypic characterization and microsatellite marker analysis of elite maize inbred and teosinte (*Zea mays* ssp. *parviglumis*) accession. Pantnagar Journal of Research. 2019;17(2):123-28.
9. Dao A, Sanou J, Gracen V, Danquah EY. Selection of drought tolerant maize hybrids using path coefficient analysis and selection index. Pakistan Journal of Biological Sciences: PJBS. 2017;20(3):132-39. https://doi.org/10.3923/pjbs.2017.132.139
10. Bondok AE, Mousa WM, Rady AM, Saad-Allah KM. Phenotypical, physiological and molecular assessment of drought tolerance of five Egyptian teosinte genotypes. Journal of Plant Interactions. 2022;17(1):656-73. https://doi.org/10.1080/17429145.2022.2085335
11. Kumar A, Singh NK, Jeena AS, Jaiswal JP, Verma SS. Evaluation of teosinte derived maize lines for drought tolerance. Indian Journal of Plant Genetic Resources. 2020;33(1):60-67. http://dx.doi.org/10.5958/0976-1926.2020.00009.1
12. Messina CD, Hammer GL, McLean G, Cooper M, van Oosterom EJ, Tardieu F, et al. On the dynamic determinants of reproductive fail-

ure under drought in maize. *In Silico* Plants. 2019;1(1):diz003. https://doi.org/10.1093/insilicoplants/diz003

13. Sah RP, Chakraborty M, Prasad K, Pandit M, Tudu VK, Chakravarty MK, et al. Impact of water deficit stress in maize: Phenology and yield components. Scientific Reports. 2020;10(1):2944. https://doi.org/10.1038/s41598-020-59689-7
14. Farooq M, Wahid A, Kobayashi NS, Fujita DB, Basra SM. Plant drought stress: effects, mechanisms and management. Sustainable Agriculture. 2009;153-88. https://doi.org/10.1051/agro:2008021
15. Al-Naggar AM, Atta MM, Ahmed MA, Younis AS. Direct vs indirect selection for maize (*Zea mays* L.) tolerance to high plant density combined with water stress at flowering. Journal of Applied Life Sciences International.2016;7(4):1-7. https://doi.org/10.9734/JALSI/2016/28582
16. Fellahi ZA, Hannachi, Benmahammed A. Utilization of multi-trait genotype-ideotype distance index (MGIDI) increases expected genetic gains for simultaneous improvement of wheat traits. In: Conference: BGRI 2021 Technical Workshop; 2021 Oct 6-8.2021 [cited 2024 May 29].
17. Maize characteristics of hybrids. [Internet]. TNAU Agritech Portal; 2023 May [cited 2024 May 29.
18. Joshi NE, Chandrashekar CP. Precision nutrient management in maize (*Zea mays* L.) under northern transition zone of Karnataka. Journal of Farm Sciences. 2017;30:343-48.
19. Ngugi K, Collins JO, Muchira S. Combining, earliness, short anthesis to silking interval and yield based selection indices under intermittent water stress to select for drought tolerant maize. Australian Journal of Crop Science. 2013;7(13):2014-20.
20. Blancon J, Buet C, Dubreuil P, Tixier MH, Baret F, Praud S. Maize green leaf area index dynamics: genetic basis of a new secondary trait for grain yield in optimal and drought conditions. Theoretical and Applied Genetics. 2024;137(3):68. https://doi.org/10.1007/s00122-024-04572-6
21. Raut SK, Ghimire SK, Kharel R, Kuwar CB, Sapkota M, Kushwaha UK. Study of yield and yield attributing traits of maize. American Journal of Food Science and Health. 2017;3(6):123-29.
22. Mulvaney MJ, Devkota PJ. Adjusting crop yield to a standard moisture content: SS-AGR-443/AG442, 05/2020. Edis. 2020;2020(3). https://doi.org/10.32473/edis-ag442-2020
23. Effendi R, Priyanto SB, Aqil M, Azrai M. Drought adaptation level of maize genotypes based on leaf rolling, temperature, relative moisture content and grain yield parameters. In: IOP Conference Series: Earth and Environmental Science; IOP Publishing. 2019 [cited 2024 May29].270(1):1-11.,http://dx.doi.org/10.5958/0976-26.2020.00009.1
24. Alvi AK, Ahmad MS, Rafique T, Naseer M, Farhat F, Tasleem H, et al. Screening of maize (*Zea mays* L.) genotypes for drought tolerance using photosynthetic pigments and anti-oxidative enzymes as selection criteria. Pakistan Journal of Botany. 2022;54(1):33-44. https://doi.org/10.30848/PJB2022-1(1)
25. Hasan MM, Ma F, Prodhan ZH, Li F, Shen H, Chen Y, Wang X. Molecular and physio-biochemical characterization of cotton species for assessing drought stress tolerance. International Journal of Molecular Sciences. 2018;19(9):2636. https://doi.org/10.3390/ijms19092636
26. Azevedo RD, Alas RM, Smith RJ, Lea PJ. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiologia Plantarum. 1998;104(2):280-92. https://doi.org/10.1034/j.1399-3054.1998.1040217.x
27. Chance B, Maehly AC. Assay of catalase and peroxidase. In: Methods in Enzymology; Academic press; 1955 [cited 2024 May 29].2:764-75. http://dx.doi.org/10.1016/S0076-6879(55)02300-8
28. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39:205-07. https://doi.org/10.1007/BF00018060
29. Manivannan N. TNAUSTAT – Statistical Package; 2014. R Core Team._R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2024.
30. RStudio Team. RStudio: Integrated development for R (Version 2023.12.1+402 “Ocean Storm”). RStudio, PBC, Boston, MA. 2024.
31. Kuhn M, Jackson S, Cimentada J. Corrr: Correlations in r [Internet]. 2022 [cited 2024 May 29].
32. Olivoto T, Lúcio AD. metan: An R package for multi-environment trial analysis. Methods in Ecology and Evolution. 2020;11(6):783-89. https://doi.org/10.1111/2041-210X.13384
33. Olivoto T, Nardino M. MGIDI: Toward an effective multivariate selection in biological experiments. Bioinformatics. 2021;37(10):1383-89. https://doi.org/10.1093/bioinformatics/btaa981
34. Kumar B, Choudhary M, Kumar P, Kumar S, Sravani D, Vinodhana NK, et al. GGE biplot analysis and selection indices for yield and stability assessment of maize (*Zea mays* L.) genotypes under drought and irrigated conditions. IJGPB [Internet]. 2024;84(02):209-15.
35. Johnson HW, Robinson HF, Comstock RE. Estimates of genetic and environmental variability in soybeans. Agronomy Journal. 1955;47:314-18. https://doi.org/10.2134/agronj1955.00021962004700070009x
36. Magar BT, Acharya S, Gyawali B, Timilsena K, Upadhayaya J, Shrestha J. Genetic variability and trait association in maize (*Zea mays* L.) varieties for growth and yield traits. Heliyon. 2021;7(9). https://doi.org/10.1016/j.heliyon.2021.e07939
37. Dordas CA, Papathanasiou F, Lithourgidis A, Petrevska JK, Papadopoulos I, Pankou C, et al. Evaluation of physiological characteristics as selection criteria for drought tolerance in maize inbred lines and their hybrids. Maydica. 2018;63(2).
38. Singamsetti A, Zaidi PH, Seetharam K, Vinayan MT, Olivoto T, Mahato A, et al. Genetic gains in tropical maize hybrids across moisture regimes with multi-trait-based index selection. Frontiers in Plant Science.2023;14:1147424. https://doi.org/10.3389/fpls.2023.1147424
39. Kamphorst SH, Amaral Júnior AT, Lima VJ, Guimarães LJ, Schmitt KF, Leite JT, et al. Can genetic progress for drought tolerance in popcorn be achieved by indirect selection?. Agronomy.2019;9(12):792. https://doi.org/10.3390/agronomy9120792
40. Li RH, Guo PG, Michael B, Stefania G, Salvatore C. Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. Agricultural Sciences in China. 2006;5(10):751-57. https://doi.org/10.1016/S1671-2927(06)60120-X
41. Monteoliva MI, Guzzo MC, Posada GA. Breeding for drought tolerance by monitoring chlorophyll content. Gene Technol. 2021;10:165.
42. Pallavi M, Prasad BM, Shanthi P, Reddy VL, Kumar AN. Multi trait genotype-ideotype distance index (MGIDI) for early seedling vigour and yield related traits to identify elite lines in rice (*Oryza sativa* L.). Electronic Journal of Plant Breeding. 2024;15(1):120-31. https://doi.org/10.37992/2024.1501.020
43. Palaniyappan S, Ganesan KN, Manivannan N, Ravichandran V, Senthil N. Multi trait genotype-ideotype distance index-A tool for identification of elite parental inbreds for developing heterotic hybrids of fodder maize (*Zea mays* L.). Electronic Journal of Plant Breeding.2023;14(3):841-49. http://dx.doi.org/10.37992/2023.1403.098