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Effects of drought and heat stresses on physiological and yield traits of maize landraces in Mexico

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Summary

Drought and heat stress are major constraints for crop productivity worldwide. Maize is the most important crop in Mexico contributing to food security. Maize landraces in Mexico exhibit remarkable diversity and most farmers continue to cultivate them. The present study investigated the effects of combined drought and heat stress on the physiological and yield responses of maize landraces in Mexico. Six maize landraces and two improved varieties were evaluated. Two treatments were used: a well-watered control and drought stress, applied at the reproductive stage for 20 days. Maize physiological and yield responses were analyzed by one-way ANOVA. Five drought tolerance indices were calculated and multivariate analyses were used to classify drought-tolerant from sensitive maize genotypes. Drought and heat stress reduced the photosynthetic rate (P_n), stomatal conductance (g_s), transpiration (T_r), and increased water use efficiency (WUE) compared to well-watered plants. Yield under drought conditions was 46% lower than under irrigation conditions and yield losses were caused mainly by a significant reduction in ear and kernel weights. Multivariate analyses identified the maize genotypes XNM, SB, and CHTZ as drought-tolerant. Tolerant varieties help farmers mitigate climate challenges and sustain food supply. Moreover, local maize landraces offer the potential for discovering valuable tolerance traits.

Keywords: climate change, drought tolerance, grain yield, heat tolerance, stress indices, *Zea mays*

Introduction

Maize (*Zea mays* L.) is the most important crop in Mexico, representing over 50% of the caloric intake for the poorest sectors of the population and significantly contributing to food and nutritional security. It occupies the largest planted area in the country, is mostly grown under rain-fed conditions, and is cultivated by many small-scale farmers (Ureta et al., 2020). Maize originated in Mexico and spread to wide areas worldwide. Maize landraces in Mexico show remarkable diversity and the ability to thrive in various climates, from arid to humid conditions and from temperate to tropical environments (Pace et al., 2024). Farmers in Mexico continue to use maize landraces, with roughly 80% of the maize cultivation area dedicated to local varieties (dos Santos et al., 2024). Landraces constitute an important aspect of global crop genetic resources, and their diversity is continually evolving-including in response to climate change (Pace et al., 2024).

Mexico has been identified as particularly vulnerable to the impacts of climate change, especially in agriculture due to increased temperatures and changes in precipitation patterns, resulting in yield losses (Ureta et al., 2020; Camacho-Villa et al., 2021). Global warming and heat waves have become more prevalent and seriously threaten crop productivity (El-Sappah et al., 2022; Djalovic et al., 2024). In the last decades, high levels of drought have been reported in the spring-summer agricultural cycle affecting the area planted with maize (Ibarra et al., 2020; Ureta et al., 2020). Drought is the most important factor limiting maize productivity in regions that depend on rainfall. The combined drought and heat stresses caused disproportionate damage to plant growth and productivity. Maize is highly vulnerable to drought and heat stress during the reproductive stage. If the water deficit occurs at the flowering and grain-filling stages of maize it can disrupt the flowering process and increase anthesis-silking interval, reduce cross-pollination, resulting in poor seed formation, all of which contribute to a decrease in maize yield (Nelimor et al., 2019). High temperatures above 35 °C affect the vegetative and reproductive growth of maize. High-temperature stress caused tassel blasting, reducing pollen production and viability, reducing pollination rate, shortened grain-filling period, and reduced kernel and grain weight (Djalovic et al., 2024). Overall, yield losses due to these altered morpho-physiological traits at the reproductive stage were estimated to be higher than 45% (El-Sappah et al., 2022). Additionally, drought and heat stress cause the deterioration of several metabolic processes in maize plants, including a severe break in photosynthesis, reduced stomatal conductance, and internal leaf CO_2 concentration, resulting in increased yield losses compared to the separate effects of the two stresses (Nelimor et al., 2019; El-Sappah et al., 2022; Rasheed et al., 2023). Chlorophyll fluorescence and leaf gas exchange analysis are frequently employed to assess the maize's response to abiotic stresses (Singh et al., 2022). Despite efforts to enhance drought or heat tolerance in maize through physiological traits, these improvements have not resulted in increased grain yields because grain yield and stress tolerance are complex traits regulated by multiple genes with low heritability and are characterized by genotype-environment interactions (Rasheed et al., 2023).

Despite the frequent existence of combined drought and heat stress episodes under field conditions, most studies have focused on independent drought or heat stress responses in plants. Also, several drought tolerance indices were used to identify drought-tolerant genotypes, based on yield loss as compared with irrigated conditions. The relationship between grain yield and drought tolerance indices might be used to screen good genotypes that are suitable to grow under drought conditions (El-Sabagh et al., 2018). Principal component analysis, biplot, and clustering methods are also employed to compare stress-tolerant and stress-sensitive maize genotypes (Badr et al., 2020; Khatibi et al., 2022).

Effects of drought and heat stress have significant impacts on maize productivity, and food security may be affected (Ureta et al., 2020). Hence, adaptation and mitigation of the adverse impacts of climate are crucial to sustaining food supply (Djalovic et al., 2024). Under

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extreme conditions, drought and heat stresses forced farmers to abandon their farmlands. To manage these stresses, farmers will require maize varieties with increased tolerance to drought and heat stress that minimize the risk of failure. To develop such maize varieties, there is a need for continuous access to a wide range of alleles that are scattered in germplasm resources, particularly in the landraces (Nelimor et al., 2019; Rasheed et al., 2023).

The present study aimed to elucidate the effects of combined drought and heat stress in maize landraces by exploring the physiological response of plants along with grain yield in southern Mexico.

Material and methods

Field experiments and treatments

The field experiment was conducted at the Uxmal experimental site, located at Muna, Yucatan (20° 24’ 36.2” N and 89° 45’ 29.5” W). In this region, the climate is warm-sub humid with summer rains. The sowing date was February 10 to allow drought and heat stress to be imposed during the dry and hot season between February to May 2024. Delayed planting in the dry season allowed high temperatures during the reproductive stage. During the experiment, daily maximum temperatures exceeded 35 °C for 86 days, with an average maximum temperature of 37 °C observed during the flowering period, clearly representing conditions of heat stress or high temperatures. Only 91.2 mm of precipitation was recorded between the sowing and anthesis periods. Site weather conditions during the field experiment were taken from a meteorology station 200 m from the experimental site and data are given in Fig. 1 (CONAGUA, 2024). The soil was classified as Cambisol type with 0.28% total N, and the total content of P, K, Ca, and Mg were 0.036, 1.29, 0.34, and 1.0 g kg^{-1}, respectively.

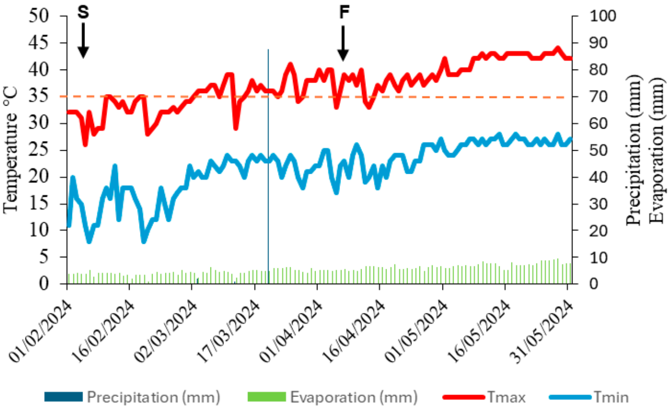

Fig. 1: Maximum and minimum temperatures and precipitation from February to May 2024, at the Uxmal experimental site, located at Muna, Yucatan. The horizontal dashed line indicates heat stress, while the black arrow marks the sowing (S) and flowering (F) periods

Eight treatments consisting of six maize landraces and two improved varieties were evaluated. The landrace populations locally named Nal tel (NTA, NTB and, NTR), Xmejen nal (XNA, XNB and, XNM) were collected from farmers in the state of Yucatan in southeastern Mexico. The improved varieties correspond to Sac Beh (SB) and Chichen Itza (CHTZ) released by INIFAP, Mexico (Aguilar et al., 2010).

Experimental design

The experiment was arranged in a randomized complete block design, with three replicates assisted with a drip irrigation system. Water was supplied every third day at a flow rate of 1 L per hour for 4 hours. The experimental unit comprised four rows 4 m in length, 0.8 m wide, and 0.2 m within plants in each row, containing a total of 80 plants. The final plant density was 62,500 plants ha^{-1}. Weeds were treated with atrazine plus s-metolachlor (4 L ha^{-1}) as pre-emergent treatment and Bentazon (2 L ha^{-1}) as post-emergent. The plants were fertilized with a dosis of 150-70-00 (N-P-K, respectively), applied fractionally; all P and 18% of total N at sowing and the remaining N 35 days later. Additionally, two applications of Spinetoram (100 mL ha^{-1}) and *Poliquel® Multi* (2 L ha^{-1}) were made during crop growth 15 and 45 days after sowing (das).

Two different water regimes were used: a well-watered control and drought stress. Drought stress was imposed by stopping irrigation between 54 to 74 days after sowing to produce drought stress during the reproductive stage. Maize plants were subjected to severe drought stress followed by recovery irrigation. Soil water potential was monitored throughout the experiment using soil tensiometers. Before the recovery irrigation, the soil water potential was 75 ± 5 kPa.

Physiological traits

The physiological response of maize genotypes was measured at two phenological stages: pre-flowering (45 das), and at flowering (68 das), the last one, under well-irrigated and drought stress conditions, using an LI-6400XT infrared gas analyzer (IRGA) (LI-COR, Lincoln, Nebraska). Fifteen measurements were taken between 7:00 h and 10:00 h on the central part of the second mature leaf (considering the flag leaf as the first) across five leaves per treatment, with three readings taken per leaf. IRGA was set with a photon flux density of 2500 µmol m^{-2} s^{-1} and a CO_2 concentration of 400 µmol mol^{-1} and the physiological traits measured were photosynthesis rate (P_n), stomatal conductance (g_s), transpiration (T_r), and water use efficiency (WUE) calculated as P_n/T_r (dos Santos et al., 2024).

Agronomic and yield traits

Days to anthesis (DA), days to silking (DS), anthesis-silking interval (ASI), ear weight (EW), ear diameter (ED), ear length (EL), number of ear rows (NR); number of kernels per row (NKR), 100-kernel weight (100KW), kernel length (KL), kernel width (KW), kernel thickness (KT), and grain yield (GY) were measured. Grain yield was calculated to kg ha^{-1} adjusted to 12% moisture.

Drought tolerance indices

Five drought tolerance indices were calculated using formulas from Tab. 1.

Data analysis

The data obtained from the experiments were analyzed using a combined analysis of variance (ANOVA) with three replicates across two environments (irrigated and drought) for the agronomic response variables.

Then a t-test ($p < 0.05$) was conducted between environments for each maize genotype.

Drought tolerance indices of maize genotypes were used to compare genotypes based on their response to drought. We performed a Principal Component Analysis (PCA) with all drought tolerance indices to synthesize all measured indices into a small set of principal components to create a biplot diagram. Additionally, we used the unweighted pair-group mean average (UPGMA) clustering method based on Gower distance implemented with the software PAST version 4.17 (Hammer et al., 2001).

Tab. 1: Drought tolerance indices used to evaluate maize genotypes in Yucatan, Mexico.

Drought tolerance indices	Code	Equation	References
Mean productivity	MP	$\frac{Yd + Yi}{2}$	ROSIELLE and HAMBLIN, 1981
Geometric mean productivity	GMP	$Yd \times Yi$	FERNANDEZ (1992)
Tolerance	TOL	$Yi - Yd$	ROSIELLE and HAMBLIN, 1981
Stress tolerance index	STI	$\frac{Yi \times Yd}{Y\bar{\iota}^2}$	FERNANDEZ (1992)
Stress susceptibility index	SSI	$\frac{1 - \frac{Yd}{Yi}}{1 - \frac{Y\bar{d}}{Y\bar{\iota}}}$	FERNANDEZ (1992)

Yi –yield under irrigation conditions, Yd - yield under drought conditions, $Y\bar{d}$ – mean yield under drought conditions; $Y\bar{\iota}$ - mean yield under irrigation conditions.

Results

Effects of drought and heat stresses on physiological traits

Maize physiological traits were measured under optimal conditions and heat stress under irrigation (Fig. 2). Under optimal conditions, all maize landraces showed a high photosynthetic rate. The genotypes XNM and CHTZ showed the highest photosynthetic rate with 48 µmol m^{-2} s^{-1}. Heat stress caused reductions in photosynthesis rates in all maize genotypes. XNB showed the greatest reduction in photosynthetic rate, dropping by 48%. NTR only reduced 8% in photosynthetic rate (Fig. 2A). Similarly, heat stress caused reductions in stomatal conductance in all maize genotypes. XNB, NTB and CHTZ showed greater reductions with decreases of 61%, 50%, and 50% in stomatal conductance. NTR and SB both reduced 26% of the stomatal conductance rate (Fig. 2B). Contrarily, heat stress increased the transpiration rates of all maize genotypes. NTR and SB showed the greatest increase with 79% and 75% respectively. NTB and XNB only increased by 4% and 6% in transpiration rate (Fig. 2C). Additionally, heat stress significantly reduced WUE, with a 46% decrease observed across maize genotypes (Fig. 2D).

Furthermore, maize physiological traits were measured under drought conditions and compared with well-watered plants at the flowering stage (Fig. 3). Under well-irrigated conditions, maize genotypes NTR and XNM showed higher photosynthetic rates with 41.2 and 39.2 µmol m^{-2} s^{-1} (Fig. 3A). Drought stress caused reductions in photosynthesis rates in all maize genotypes. NTB, XNA and, NTA showed the greatest reduction in photosynthetic rate, dropping by 69%, 64% and, 60%, respectively. XNB only reduced 2% in photo-

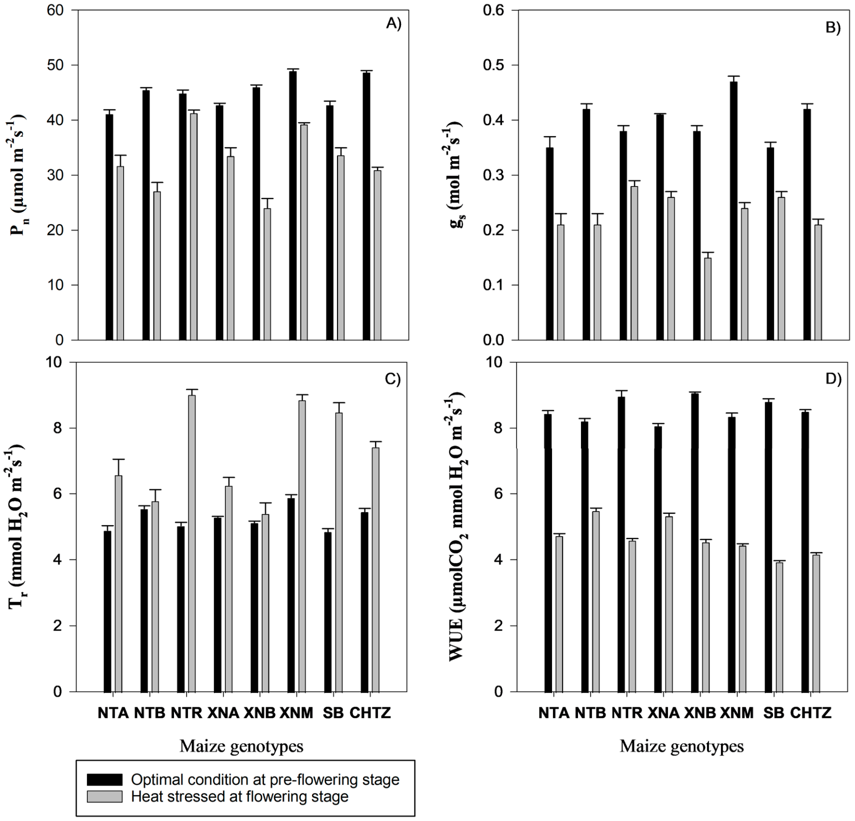

Fig. 2: Photosynthesis (P_n, A), stomatal conductance (g_s, B), transpiration (T_r, C) and water use efficiency (WUE, D), of maize genotypes assessed at pre-flowering and flowering stages under optimal and heat stress conditions in Yucatan, Mexico. Data are means ± SE

synthetic rate (Fig. 3A). Similarly, drought stress caused reductions in stomatal conductance in all maize genotypes. XNA and NTB showed the greatest reduction in stomatal conductance, with decreases of 77% and 76%, respectively. XNB reduced 13% of the stomatal conductance (Fig. 3B). In addition, drought stress significantly decreased the transpiration rates with a 50% decrease observed across maize genotypes (Fig. 3C). Contrarily, drought stress increased the WUE of all maize genotypes. Under drought conditions, WUE was significantly higher on XNA with 7.52 µmol CO_2 mmol H_2O, followed by XNM with 6.9 µmol CO_2 mmol H_2O (Fig. 3D).

Effects of drought and heat stresses on yield traits

The combined analysis of variance (Tab. 2) showed highly significant differences ($p \leq 0.01$) for most traits studied across the sources of variation in genotypes (G) and environments (E). Additionally, the interaction between genotypes and environments (G x E) showed significant differences for the variables DA, DS, EW, NKR, 100KW, and KL. Moreover, a higher coefficient variation in some characteristics indicated variation in stress response between maize genotypes.

Drought conditions significantly impacted most yield traits of maize compared to irrigated plants under high temperatures (Tab. 3). Drought conditions delayed DS while increasing ASI. Additionally, drought reduced ear traits such as EL, ED, EW, NR, and NKR. It also significantly affected kernel weight and size, reducing 100KW, KL, and KW.

Also, maize genotypes showed variability within all traits evaluated. The variables DA and DS ranged from 61 days in genotype SB to 76 days in XNA, and from 66 days in genotype XNM to 81 days in XNA, respectively. Ear traits EL and ED varied from 10.3 cm and 2.9 cm in genotype NTB to 14.8 cm and 4.1 cm in genotypes CHTZ and XNM, respectively. Similarly, EW ranged from 33.5 g in NTB to 110.6 g in XNM. The number of ear rows (NR) ranged from 12 in SB to 14 rows in NTR. The number of kernels per row (NKR) varied from 21.7 kernels in genotype NTR to 35.2 kernels in CHTZ. Kernel trait 100KW ranged from 11.9 g in NTB to 24.2 g in XNM. Kernel traits KL and KW varied from 7.7 mm and 6.7 mm in NTB to 10.0 mm and 8.4 mm in XNM. Finally, KT ranged from 3.3 mm in NTB to 4.1 mm in genotype XNB.

Tab. 2: Statistical values of yield traits of maize genotypes evaluated with irrigation and drought under heat stress in Yucatan, Mexico.

	Source of variation	Genotypes (G)	Environments (E)	Interaction (G × E)	Error CV (%)
DA	580.67***	10.93***	2.99***	0.28	0.78
DS	613.37***	93.79***	4.49***	0.28	0.73
ASI	32.93***	40.69***	1.46***	0.0	0.00
EL	63.42***	83.75***	2.55	1.82	10.77
ED	3.46***	9.79***	0.13	0.10	9.19
EW	18811.93***	33381.55***	475.51*	205.35	21.74
NR	8.30**	10.26*	1.61	2.53	12.31
NKR	578.69***	866.99***	72.16***	20.78	16.40
100KW	341.62***	527.52***	64.53***	18.98	24.95
KL	13.26***	34.59***	1.44***	0.51	8.19
KW	9.44***	6.18***	0.85	0.50	9.39
KT	1.61***	0.0028	0.34	0.19	12.07
GY	13262.11***	21949.75***	227.84	151.20	22.88

Days to anthesis (DA), days to silking (DS), anthesis-silking interval (ASI), ear length (EL), ear diameter (ED), ear weight (EW), number of ear rows (NR); number of kernels per row (NKR), 100-kernel weight (100KW), kernel length (KL), kernel width (KW), kernel thickness (KT), and grain yield per plant (GY). *, **, *** indicate significant effect with $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively.

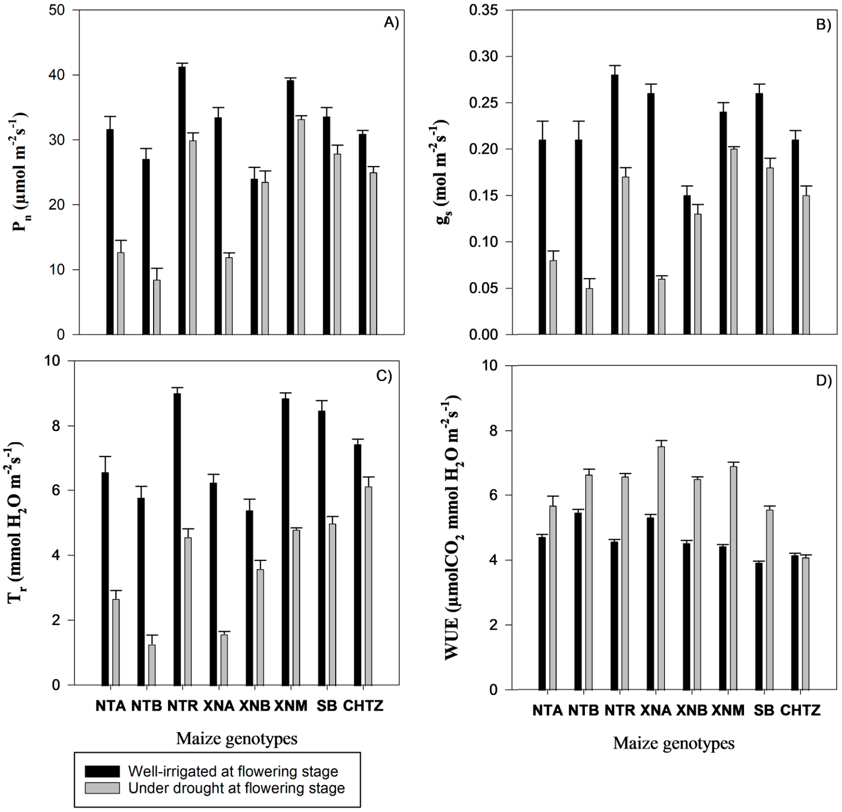

Fig. 3: Photosynthesis (P_n, A), stomatal conductance (g_s, B), transpiration (T_r, C) and water use efficiency (WUE, D), of maize genotypes assessed at flowering stage under irrigation and drought conditions with heat stress in Yucatan, Mexico. Data are means ± SE, n = 15.

Tab. 3: Mean values of yield traits of maize genotypes evaluated with irrigation and drought under heat stress in Yucatan, Mexico.

Genotypes	Environments (E)	DA	DS	ASI	EL	ED	EW	NR	NKR	100KW	KL	KW	KT
NTA	Irrigated	64.5^{ns}	67.5^{b}	3.0^{b}	11.9^{a}	3.4^{a}	56.0^{a}	13.8^{ns}	30.2^{a}	13.4^{ns}	8.7^{a}	6.9^{ns}	3.1^{b}
	Drought	64.6^{ns}	68.6^{a}	4.0^{a}	10.5^{b}	3.1^{b}	30.4^{b}	13.2^{ns}	20.8^{b}	12.7^{ns}	7.6^{b}	6.5^{ns}	3.5^{a}
NTB	Irrigated	68.5^{ns}	73.5^{b}	5.0^{b}	10.6^{ns}	3.1^{a}	42.5^{a}	12.8^{ns}	27.4^{a}	13.1^{a}	8.4^{a}	6.9^{ns}	3.2^{ns}
	Drought	68.6^{ns}	75.6^{a}	7.0^{a}	9.9^{ns}	2.7^{b}	25.3^{b}	12.4^{ns}	22.6^{b}	10.8^{b}	7.1^{b}	6.5^{ns}	3.5^{ns}
NTR	Irrigated	67.5^{b}	73.5^{b}	6.0^{b}	12.0^{a}	3.7^{a}	57.2^{a}	14.8^{ns}	23.0^{ns}	16.3^{a}	9.5^{a}	7.2^{a}	3.7^{ns}
	Drought	68.5^{a}	75.5^{a}	7.0^{a}	9.5^{b}	2.9^{b}	27.2^{b}	13.7^{ns}	20.4^{ns}	11.6^{b}	7.6^{b}	6.3^{b}	3.5^{ns}
XNA	Irrigated	75.5^{b}	79.5^{b}	4.0^{b}	12.9^{a}	3.3^{a}	53.0^{a}	12.7^{ns}	28.0^{a}	17.2^{ns}	8.2^{ns}	7.5^{ns}	4.0^{ns}
	Drought	76.5^{a}	81.5^{a}	5.0^{a}	11.2^{b}	2.9^{b}	30.7^{b}	12.1^{ns}	20.1^{b}	16.0^{ns}	7.9^{ns}	7.7^{ns}	4.0^{ns}
XNB	Irrigated	72.5^{b}	75.5^{b}	3.0^{b}	13.2^{a}	3.9^{a}	82.3^{a}	12.8^{ns}	28.3^{a}	23.3^{a}	8.4^{ns}	7.9^{ns}	4.1^{ns}
	Drought	74.6^{a}	78.6^{a}	4.0^{a}	11.8^{b}	3.3^{b}	43.5^{b}	12.2^{ns}	18.8^{b}	17.1^{b}	8.1^{ns}	7.9^{ns}	4.1^{ns}
XNM	Irrigated	62.5^{ns}	65.5^{ns}	3.0^{ns}	15.0^{a}	4.4^{a}	133.5^{a}	12.4^{ns}	33.3^{ns}	30.1^{a}	10.6^{a}	9.0^{a}	4.0^{a}
	Drought	62.5^{ns}	65.4^{ns}	3.0^{ns}	13.0^{b}	3.9^{b}	87.7^{b}	13.2^{ns}	31.3^{ns}	18.3^{b}	9.4^{b}	7.9^{b}	3.6^{b}
SB	Irrigated	60.5^{ns}	65.5^{b}	5.0^{b}	14.8^{ns}	3.9^{a}	106.3^{a}	12.8^{ns}	34.5^{ns}	22.2^{a}	9.9^{a}	8.2^{ns}	3.7^{ns}
	Drought	60.5^{ns}	66.5^{a}	6.0^{a}	14.5^{ns}	3.5^{b}	74.7^{b}	12.0^{ns}	34.8^{ns}	16.7^{b}	8.9^{b}	7.8^{ns}	3.6^{ns}
CHTZ	Irrigated	64.5^{ns}	68.6^{b}	4.0^{b}	15.5^{a}	3.9^{a}	112.1^{a}	13.2^{ns}	35.9^{ns}	21.0^{a}	9.8^{a}	8.0^{ns}	3.6^{ns}
	Drought	64.5^{ns}	69.5^{a}	5.0^{a}	14.0^{b}	3.7^{b}	91.3^{b}	12.4^{ns}	34.4^{ns}	17.4^{b}	9.1^{b}	7.9^{ns}	3.5^{ns}
ALL	Irrigated	67^{ns}	71.1^{b}	4.1^{b}	13.2^{a}	3.7^{a}	81.7^{a}	13.2^{a}	30.1^{a}	19.5^{a}	9.2^{a}	7.7^{a}	3.7^{ns}
ALL	Drought	67.6^{ns}	72.8^{a}	5.1^{a}	11.8^{b}	3.2^{b}	50.9^{b}	12.7^{b}	25.5^{b}	15.4^{b}	8.2^{b}	7.3^{b}	3.7^{ns}

Days to anthesis (DA), days to silking (DS), anthesis-silking interval (ASI), ear length (EL), ear diameter (ED), ear weight (EW), number of ear rows (NR); number of kernels per row (NKR), 100-kernel weight (100KW), kernel length (KL), kernel width (KW) and kernel thickness (KT); Distinct letters within the same column indicate significant differences between environments (irrigated-drought) for the same genotype at a significance level of $p < 0.05$, in the t-test. ns - indicates non-significant differences, n = 10.

Estimates of drought tolerance indices

The mean yield under irrigation conditions (Yi) for all maize genotypes was 2654 kg ha^{-1}. The maximum yield was reached on maize genotypes CHTZ, SB, and XNM with 5729, 5461, and 4994 kg ha^{-1}, respectively. Under drought conditions, the mean yield (Yd) was 1429 kg ha^{-1}, 46% less than under irrigation conditions. This study's stress intensity index was equal to 0.46 (Tab. 4).

Higher MP, GMP, TOL, and STI index values indicate genotypes with higher yield potential under drought and irrigated conditions. Based on those indices, maize genotypes CHTZ, SB, and XNM tolerance levels were more pronounced whereas XNA, NTR, and NTB were sensitive (Tab. 3). The SSI index favors genotypes with good grain yield under drought conditions. A high value of SSI indicated more sensitivity to drought. Based on the SSI index, maize genotypes CHTZ, NTB, SB, XNM, and XNB had the lowest SSI values indicating more tolerance to drought conditions.

Multivariate analysis of drought tolerance indices

The PCA and UPGMA multivariate analyses consistently and effectively classified drought-tolerant from sensitive maize genotypes based on drought tolerance indices (Fig. 4). The first two components of the PCA analyses explained 97.8% of the total variability between maize genotypes. The first component was strongly correlated with the MP, GMP, TOL, and STI drought tolerance indices. The second component was highly correlated with the SSI index. The PCA biplot diagram illustrated the drought tolerance of maize genotypes based on the tolerance indices (Fig. 4A). The arrangement of maize geno-

Tab. 4: Estimates of drought tolerance attributes for maize genotypes evaluated with irrigation and drought under heat stress in Yucatan, Mexico.

Genotype	Yi kg ha^{-1}	Yd kg ha^{-1}	MP kg ha^{-1}	GMP kg ha^{-1}	TOL kg ha^{-1}	STI	SSI
NTA	1784.9	602.5	1194	1037	1182	0.15	1.4
NTB	866.2	488.1	677	650	378	0.06	0.9
NTR	614.4	241.1	428	385	373	0.02	1.3
XNA	287.8	44.4	166	113	243	0.00	1.8
XNB	1495.0	797.4	1146	1092	698	0.17	1.0
XNM	4993.5	2670.7	3832	3652	2323	1.89	1.0
SB	5461.2	2869.7	4165	3959	2592	2.22	1.0
CHTZ	5728.8	3715.8	4722	4614	2013	3.02	0.8
Mean	2654.0	1428.7	2041.3	1937.8	1225.3	0.94	1.15
CV (%)	87.6	99.5	91.5	93.8	77.9	130.7	28.7

Yi –yield under irrigation conditions, Yd - yield under drought conditions, MP – mean productivity, GMP – geometrical mean productivity, TOL – tolerance, STI – stress tolerance index, SSI – stress susceptibility index.

types reveals the formation of two distinct groups, characterized by drought tolerance and drought-sensitive. Group 1 was formed by the maize genotypes CHTZ, SB, and XNM with high drought tolerance response and high scores in PC1 on the right of the PCA diagram. On the left of the PCA diagram, Group 2 was formed by maize genotypes XNA, NTA, NTR, XNB, and NTB which were drought-sensitive. The UPGMA diagram (Fig. 4B) also split maize genotypes into two distinct groups here called A and B. The group A, which included the drought-tolerant genotypes CHTZ, SB, and XNM, contrasted with Group B, consisting of the drought-sensitive genotypes XNB, NTA, XNA, NTB, and NTR.

Discussion

In 2024 in Yucatan, we experienced a heat wave lasting 34 days, with temperatures exceeding 40 °C that affected the reproductive period of maize growth and significantly impacted our results.

Evaluating drought and heat tolerance under field conditions is probably the most relevant approach in maize breeding programs to identify germplasm tolerance. However, field evaluation of drought and heat tolerance is extremely difficult because drought and heat does not occur at a specific growth stage or with the right intensity and duration (CASTRO NAVA et al., 2014). So, this study was conducted under appropriate conditions for screening maize germplasm for drought and heat tolerance.

Effects of drought and heat stresses on physiological traits

The detrimental effects of drought and heat stresses on maize physiology are well documented (CAIRNS et al., 2013; HUSSAIN et al., 2019; EL-SAPPAH et al., 2022; DJALOVIC et al., 2024). Nevertheless, most studies used elite maize cultivars and less is known on maize landraces. The elite maize cultivars exhibit limited genetic variation, particularly regarding heat stress tolerance. Therefore, exploiting existing natural resources in landraces and wild maize accessions holds promise for identifying potential responsive traits (CAIRNS et al., 2013; DJALOVIC et al., 2024). Additionally, maize landraces are crucial for smallholders, who rely on them for human and animal feed (OLIVEIRA et al., 2020). These farmers play a key role in preserving maize diversity *in situ*, and they must achieve sufficient yields to ensure food security (URETA et al., 2020).

Heat stress reduced the photosynthetic rate and stomatal conductance compared to optimal conditions. Heat stress significantly increased transpiration and water use efficiency levels in all genotypes. Under no water limitations, maize genotypes NTR, XNM, SB, and CHTZ exhibited a positive physiological response to heat stress by increasing transpiration to reduce leaf temperature and maintain a high photosynthetic capacity. HUSSAIN et al., (2019) also observed increased g_s and T_r levels in two maize hybrids under heat stress compared to well-watered control plants. However, in that study, these effects were non-significant, which may be related to the intensity of the heat stress. DJALOVIC et al., (2024) explained that crop response to heat stress may vary substantially with changes in moisture levels in the air and soil. The relative humidity, vapor pressure deficit, solar radiation, and soil moisture are reported to determine the impact of heat stress as it may elevate the level of heat stress severity by enhanced photoinhibition and tissue temperature. As a C4 plant, maize P_n was tolerant of relatively high leaf temperatures, with inhibition not observed until leaf temperature exceeded 37.5 °C (CRAFTS-BRANDNER and SALVUCCI, 2002). CARTER et al. (2016) reported that tropical maize plants grown under irrigated conditions showed no yield reduction under heat stress. In contrast, the yield of rainfed maize was significantly reduced due to heat stress.

On the other hand, heat stress during the reproductive stage is detrimental to reproductive success and seed set, leading to biomass and yield loss (DJALOVIC et al., 2024).

It has been reported that temperatures above 38 °C impair pollen formation, damage pollen tubes, and increase pollen sterility. It also led to a reduction in the number of silks, drying of silks, decreased stigma receptivity, and suppression of the fertilization process. Additionally, higher temperatures slow down silk elongation, accelerate pollen shedding, and extend the anthesis-silking interval (DJALOVIC et al., 2024).

Under combined drought and heat stress at the flowering stage. Drought stress reduced the photosynthetic rate, stomatal conductance, transpiration, and increased water use efficiency compared to well-watered plants. Maize genotypes NTR, XNM, and SB exhibited a positive physiological response to drought stress by maintaining a relatively high photosynthetic capacity and WUE compared to well-watered plants. HAYANO-KANASHIRO et al., (2009) also observed a reduction in the photosynthesis rate and stomatal conductance of three Mexican maize genotypes, which included two drought-tolerant and one susceptible after 10 and 17 days of drought stress. The authors also showed that maize genotypes had great recovery capacity after stress. Similarly, HUSSAIN et al., (2019) reported that the photosynthetic rate, transpiration rate, and stomatal conductance were significantly reduced by combined drought plus heat stresses in two maize hybrids compared with each stress. The authors also noted that T_r was increased by heat stress, while drought or drought plus heat stress decreased it, which was also observed in our study. NELIMOR et al., (2019) explained that under heat stress, plants open their stomata to cool their leaves by transpiration, but when plants

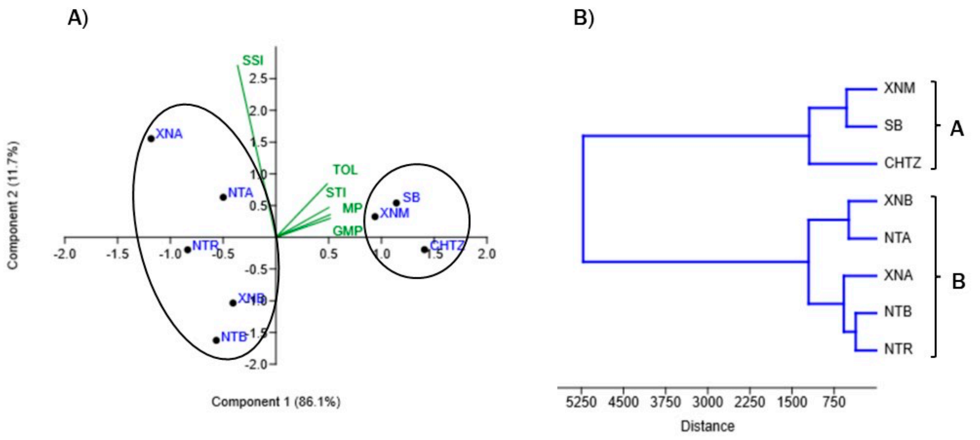

Fig. 4: PCA (A) and UPGMA (B) diagram of maize genotypes based on their response to drought stress indices

have to keep their stomata closed to reduce water loss during combined drought and heat stress conditions, the leaf temperature remains high, resulting in increased yield losses compared to the separate effects of the two stresses.

Effect of drought and heat stress on yield traits

The statistical differences detected in the ANOVA between genotypes were attributed to the genetic variation in the maize landraces evaluated, that belong to different maize races of Mexico. The statistical differences between environments (irrigation-drought) detected by ANOVA are explained by the contrast in the soil moisture conditions to which the genotypes were subjected. Likewise, the variation between the environments directly affected the degree of phenotypic expression, particularly in some morphological variables. The significant interaction between maize genotypes and environments for some traits indicates that maize genotypes have a distinct response when exposed to drought and heat stresses and exhibit variability in their capacity to withstand abiotic stress. Therefore, the selection of tolerant genotypes is considered more effective when evaluated under both stressed and non-stressed conditions (IBARRA et al., 2020). These observations corroborated the results of other authors who suggested the presence of significant genetic variability for tolerance to abiotic stresses in tropical maize landraces (CAIRNS et al., 2013; NELIMOR et al., 2019).

In the present study, drought and heat stress conditions reduced the yield and related attributes of maize genotypes evaluated in Yucatan, Mexico (Tab. 2). The maize landraces XNB, NTA, XNA, NTB, and NTR suffered great yield losses when subjected to drought stress and high temperatures during anthesis and grain filling periods. Yield losses were caused mainly by a significant reduction in ear and kernel weights, with a 38% reduction in ear weight and a 21% decrease in grain weight. Additionally, we observed a 2-day delay in DS and a 1-day increase in ASI across all genotypes.

Several studies have reported the detrimental effects of combined drought and heat stresses on the growth and yield traits of different maize genotypes (HUSSAIN et al., 2019). In Northeast Mexico, with high temperature (≥ 35°C) and water stress (non-irrigated), CASTRO-NAVA et al., (2014) reported a significant reduction of the grain yield (> 47%), caused mainly by a significant reduction of the grain number per ear (> 41%), and the grain individual weight (> 17%) in two maize landraces compared to the control environment. Additionally, there was an increase in ASI (> 2 days), DA (> 8 days), and DS (> 10 days).

The combination of drought and heat stress has a significantly greater detrimental impact on crop growth, development, and productivity than the effects of each stress applied individually (CASTRO-NAVA et al., 2014). Tolerance to combined drought and heat stress is genetically distinct from tolerance to either stress alone and, tolerance to either stress alone did not confer tolerance to combined drought and heat stress (CAIRNS et al., 2013).

Even with an irrigation system in place, high temperatures alone could have a significantly negative impact on maize yield (EL-SAPPAH et al., 2022; DJALOVIC et al., 2024). CASTRO-NAVA et al., (2011) evaluated 28 maize landraces from Tamaulipas, in Northeast Mexico under high temperatures (≥ 35 °C). The excessively high temperatures during the reproductive period and growing season caused a reduction in grain yield by over 34%, primarily due to a decrease of more than 29% in the number of grains per ear. CICCHINO et al., (2010) also observed that heating during the pre-silking period caused a larger delay in the silking date than in the anthesis date, and an increase in male and female sterility. Additionally, heating consistently led to a reduction in both plant and ear growth rates, as well as a decrease in the harvest index.

Estimates of drought tolerance indices

Selection of drought tolerance genotypes based on drought tolerance indices, considering yield under non-stress and drought stress conditions, is an efficient strategy, especially under unpredictable rainfed conditions with various yearly drought scenarios (EL-SABAGH et al., 2018; BONEA, 2020). Several researchers have introduced various drought tolerance indices (FERNANDEZ, 1992). While assessing drought tolerance genotypes using a single index appears contradictory, selecting based on a combination of indices provides an effective strategy for improving drought tolerance (BONEA, 2020). The most effective indices for identifying drought tolerance genotypes are those that correlate with grain yield under both conditions (EL-SABAGH et al., 2018).

In this study, yield under drought conditions was 46% lower than under irrigation conditions, and maize genotypes CHTZ, SB, and XNM were consistently identified as drought tolerant. The stress intensity index ranges from 0 to 1, and a value of 0.46 indicates a moderate stress level. BONEA (2020) screened maize hybrids in Romania based on several drought tolerance indices, including abiotic tolerance index (ATI), stress susceptibility tolerance index (SSPI), stress tolerance index (STI), mean productivity (MP), relative drought index (RDI) and golden mean index (GMI). The authors used the ranking method, based on a combination of indices, to determine the most suitable drought-tolerant hybrid and recommended two hybrids (Felix and P 9903) as drought-tolerant. Also, they reported a 36% grain yield reduction in drought conditions compared to non-stress conditions with a stress intensity index of 0.37, indicating low severe stress. Similarly, KHATIBI et al. (2022) used multiple indices to study the response of maize hybrids to drought stress in Iran. The estimated indices showed that the SC647 and KSC704 hybrids performed well under both conditions and exhibited drought tolerance. IBARRA et al. (2020) evaluated a group of secreting (S2) maize lines in both irrigated and drought environments in Morelos, Mexico. They selected lines with tolerance to water deficit based on drought susceptibility indices (ISS) and the tolerance index (TI). Based on the grain yield under irrigation, a 78% reduction was observed under drought conditions. The ISS and TI indices identified three lines as tolerant. In Zamorano, Honduras, GÓMEZ-CERNA et al., (2021) investigated 30 maize landraces to assess their drought stress tolerance across various environments and seasons. They employed several indices, including geometric mean indices and percentage yield reduction. The authors reported that the landrace accessions Capulín, Olotillo, Indio, Negro, and Tuza Morada demonstrated good grain yield and stability under the applied stress conditions. However, they observed an average yield reduction of 57% under severe stress and 38% under moderate stress compared to the yield under non-stress conditions.

Multivariate analysis of drought tolerance indices

The PCA and UPGMA multivariate analyses were congruent and classified drought-tolerant and drought-sensitive maize genotypes based on drought tolerance indices. BADR et al. (2020) employed PCA analysis to cluster 40 maize accessions based on their drought tolerance indices in their study. The authors identified a cluster comprising five accessions with the highest indices values, which were classified as drought-tolerant. BADR AND BRÜGGEMANN (2020) also used the PCA biplot analysis to identify traits that differentiate maize genotypes for drought tolerance. The soil water content during drought and the relative water content during drought exhibited significant influence on the clustering of accessions, with the authors identifying one genotype as the most tolerant. KHATIBI et al., (2022) also employed PCA biplot analysis, finding that the first and second principal components interpreted 79% of the changes in drought tolerance indices for stress conditions. They called the first principal component the performance potential component and the second principal component the stress sensitivity component. Additionally, cluster analysis using UPGMA effectively distinguished drought-tolerant from drought-sensitive maize genotypes, indicating significant genetic differences between the two groups. Similar clustering patterns of maize genotypes have been previously reported by Nelimor et al., (2019).

Conclusion

Maize genotypes NTR, XNM, and SB exhibited a positive physiological response to drought stress by maintaining a relatively high photosynthetic capacity and WUE compared to well-watered plants. Drought and heat stresses reduced the yield and related attributes of maize genotypes evaluated in Yucatan, and maize genotypes CHTZ, SB, and XNM were consistently identified as drought tolerant based on multiple drought tolerance indices with higher yield potential under drought and irrigated conditions. The PCA and UPGMA multivariate analyses consistently and effectively classified drought-tolerant from sensitive maize genotypes based on drought-tolerance indices.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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