Drought stress effect induced by polyethylene glycol (PEG) on germination and seedling stage in maize landraces from Yucatan, Mexico.

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Abstract

Drought stress negatively affects germination, vegetative growth, biomass production, and yield traits in maize (*Zea mays* L.). In this study, we investigate the effects of osmotic stress induced by polyethylene glycol (PEG) solutions on germination, early seedling growth traits, and the physiological response of ten maize genotypes in Yucatan, Mexico. Additionally, drought tolerance indices were calculated and used to differentiate drought-tolerant genotypes. The data were examined by two-way analysis of variance and multivariate analysis. The results showed that PEG treatments significantly reduced germination and retarded seedling growth of maize genotypes. The physiological response of maize genotypes was also affected. Under drought stress, maize genotypes showed reduced carbon assimilation, stomatal conductance, and transpiration, but increased water use efficiency. Additionally, variability was observed in drought tolerance traits among different maize genotypes. According to PCA analysis based on variation in drought tolerance indices, three maize genotypes are drought-tolerant, including NTR, NTA, and NTB. These genotypes are suitable for cultivating areas where water availability is limited and for selecting tolerant genotypes to drought in breeding programs.

Abbreviations

ANOVA: Analysis of variance
An: CO_2 assimilation rate
An-DTI: CO2 assimilation rate drought tolerance index
CHTZ: Chichen Itza
Ci: Intercellular CO_2 concentration
DTIs: Drought tolerance indices
DW-DTI: Dry weight drought tolerance index
E: Transpiration
E-DTI: Transpiration drought tolerance index
gs: Stomatal conductance
gs-DTI: Stomatal conductance drought tolerance index
GS: Germination speed
GS-DTI: Germination speed drought tolerance index
%G: Germination percentage
G-DTI: Germination drought tolerance index
H: Hybrid H-562
NTA: Nal tel amarillo
NTB: Nal tel blanco
NTR: Nal tel rojo
PCA: Principal component analysis
PEG: Polyethylene glycol
SB: Sac beh
SNICS: National Seed Inspection and Certification Service
SL-DTI: Seedling length drought tolerance index
UXM: Blanco Uxmal
WUE: Water use efficiency
WUE-DTI: Water use efficiency drought tolerance index
XNA: Xmejen nal amarillo
XNB: Xmejen nal blanco
XNM: Xmejen nal morado

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide, due to its high production volume and diverse utilizations in food, animal feed, and various industrial sectors (Queiroz *et al.*, 2019). In Mexico, maize is a staple crop, central to the diets of both urban and rural consumers, with an average consumption exceeding 100 kilograms per capita annually (Erenstein *et al.*, 2022). It occupies the largest planted area in the country, nevertheless, the country faces an approximate deficit of 8.3 million tons per year, which is satisfied with the imports (Ureta *et al.*, 2020). Modern technology and infrastructure investment are needed to ensure a sustainable future for the country's maize industry and food security (Fonteyne *et al.*, 2023). Drought is the main global challenge of agriculture, and this problem is expected to worsen with climate change (Ureta *et al.*, 2020; Mustamu *et al.*, 2023). Mexico has been identified as vulnerable to the impacts of climate change, particularly in its dry tropical regions where severe droughts pose significant challenges to agricultural production, farm sustainability, and the food security of local communities (Álvarez-Iglesias *et al.*, 2018; Ureta *et al.*, 2020). The loss in maize production is approximately 16% in the lowland tropics (Rasheed *et al.*, 2023). According to Mi *et al.* (2018), drought stress can cause maize seed production losses up to 65%, depending on genotype, plant growth stage, intensity, and duration of drought stress. Drought-tolerant plants are needed in dry regions to prevent maize yield losses (Mustamu *et al.*, 2023). Breeding for drought tolerance is the most convenient approach for managing climatic variability and effectively addressing drought-related challenges (Rasheed *et al.*, 2023). Breeding programs for drought tolerance have focused on elite germplasm. Nevertheless, maize variability for drought tolerance is limited within elite germplasm, and tropical germplasm offers unexplored resources for breeding (McMillen *et al.*, 2022).

Farmers in Mexico continue to use traditional maize landraces, with roughly 80% of the maize cultivation area dedicated to these heritage 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). Maize landraces show remarkable diversity and the ability to thrive in a wide range of climates, ranging from arid to humid conditions and from temperate to tropical environments (Hellin *et al.*, 2014).

In addition, most breeding programs for drought tolerance have focused on yield and adult vegetative phases, while little attention has been devoted to drought tolerance at early stages of development. Maize's initial growth stage is more sensitive to water stress than the later growth stages (Badr *et al.*, 2020). The lack of moisture in the soil directly affects seed germination and seedling growth of a maize plant (Ashraf *et al.*, 2007). Maize plants display a variety of morphological, physiological, and biochemical responses to drought stress (Rasheed *et al.*, 2023). Employing solutions with varying low water potentials to germinate seeds offers a convenient approach to investigating the impact of water stress on different plant groups (Badr *et al.*, 2020). Polyethylene glycol (PEG) is a metabolically inactive compound frequently used to induce drought stress at early germination and seedling growth stages (Álvarez-Iglesias *et al.*, 2017). Moreover, in conditions of water scarcity, maize experiences a decline in both growth and yield attributed to a decrease in photosynthetic capacity. These characteristics of maize make it an excellent model plant to examine the physiological mechanisms of water stress tolerance and to identify some drought-tolerant genotypes (Pace *et al.*, 2024). Physiological attributes such as leaf gas exchange could be used as indicators for the selection of genotypes for drought tolerance (Pace *et al.*, 2024). In addition, drought sensitivity indices based on the response of traits under stress conditions compared to the control have been recently applied to evaluate maize drought tolerance (Badr *et al.*, 2020). Principal component analysis and clustering methods are also increasingly used for comparisons of drought tolerance in maize (Badr *et al.*, 2020).

The present study aimed to investigate the effects of osmotic stress induced by PEG on germination, early seedling growth traits, and the physiological response of maize genotypes in Yucatan, Mexico.

Materials and methods

Maize genotypes

The experiment was conducted in the laboratory of INIFAP, Yucatan, Mexico. Ten maize genotypes from Southeast Mexico were used to study the effect of drought stress on germination and seedling growth traits and the maize physiological response. Seven short-cycle maize landraces with different grain colours were selected, including NTA, NTB, NTR, XNA, XNB, XNM, and UXM. In addition, two improved varieties, SB and CHTZ (Sac beh and Nukuch nah released by INIFAP), and one hybrid, H (H-562 released by INIFAP), adapted to the dry tropics and high yielding, were used.

Experimental treatments

Seeds of each genotype were selected for size homogeneity, then surface sterilised for 5 min in 1% sodium hypochlorite and then rinsed twice in distilled water. The study was performed in the between-paper method, with paper towels rolled and held in transparent plastic bags (ISTA, 2017). Two water stress and one control treatments were evaluated. Water deficit conditions were created by using the PEG-8000 solution by maintaining the osmotic potentials of - 0.75 MPa and - 1.0 MPa, estimated following Burlyn (1983), while distillate water with zero osmotic potential was used as a control. Sixty seeds from each genotype and osmotic potential were used, distributed among three replicates, containing twenty seeds each. The solutions were continuously monitored to maintain paper moisture for each treatment. Seeds that showed a minimum radicle length of 3 mm were counted as germinated. Seed germination was counted daily, and germination percentage was noted up to six days after seed sowing. The germination speed (GS) and total germination percentage (%G) were calculated according to Maguire (1962).

$$GS = \Sigma \frac{ni}{ti} \tag{1}$$

Where ni = number of seeds germinated within the consecutive time intervals and ti = time elapsed between the start of the test and the end of the interval.

The seedlings of each maize genotype were allowed to establish for six days. Then, 10 healthy and uniform maize seedlings of each genotype were transplanted to a new paper towel under the same conditions and osmotic potentials described above. Plants were grown in the laboratory at T_{min} of 20.6 °C, and T_{max} of 28.7 °C with a 16/8 h light-dark period and 75% relative humidity. The moisture of the paper rolls was continuously monitored, and water or PEG solutions were added to control and stress seedlings to keep the paper rolls wet, and the seedlings were left to grow further for 14 days. At the end of the study, the length of roots and shoots, vigour index, and fresh and dry weights traits were calculated.

$$\text{Vigor index} = \text{germination percentage} \times \text{seedling length (root + shoot)} \tag{2}$$

Physiological response

When maize seedlings reached 21 days after sowing, gas exchange and the physiological response of genotypes were measured using an infrared gas analyzer LI-6400XT (*LI-COR*, Lincoln, Nebraska) set with a photon flux density of 2500 μmol m^{-2} s^{-1} and CO_2 concentration of 400 μmol mol^{-1}. Fifteen measurements were taken on the central part of each leaf (five leaves per treatment, with three readings taken per leaf) The physiological traits measured were CO_2 assimilation rate (A_n), intercellular CO_2 concentration (C_i), stomatal conductance (gs), transpiration (E), and water use efficiency (WUE) calculated as A_n/E (dos Santos *et al.*, 2019).

Data analysis

The experiment was set up in a completely randomised design, with three replicates. Data for various morpho-physiological traits were subjected to a two-way analysis of variance (ANOVA) using Infostat 2020 statistical software. The Kolmogorov-Smirnov test was used to test data normality, and the homogeneity test was conducted using Levene's test. The significance level for the multiple comparisons of means was set at $\alpha < 0.05$ according to Tukey's method.

Drought tolerance indices (DTIs) were calculated for germination (G-DTI), germination speed (GS-DTI), seedling length (SL-DTI), dry weight (DW-DTI), as well as for the physiological traits CO_2 assimilation rate (A_n-DTI), stomatal conductance (g_s-DTI), transpiration (E-DTI), and water use efficiency (WUE-DTI) as the ratio of response of traits under stress conditions compared to the control, where the highest DTI value indicates drought tolerance (Badr *et al.*, 2020; Mustamu *et al.*, 2023).

$$DTI = \frac{\text{(mean values under water stress at -1.0 Mpa osmotic potential)}}{\text{(mean values under control 0 Mpa osmotic potential)}} \tag{3}$$

Box and whisker plots were created using SigmaPlot 14.5 software to illustrate the variation of DTIs across different osmotic potentials and genotypes. In addition, the DTIs mean values were used to construct a PCA using the PCA analysis. The PCA was applied to assign the variables to genotypes and to classify which of them were more sensitive or tolerant to drought stress. The PCA utilises orthogonal transformation to convert a set of possibly correlated variables into a set of linearly uncorrelated variables called principal components. This transformation is defined in such a way that the first principal component has the largest possible variance (Badr *et al.*, 2020). PCA is sensitive to the relative scaling of the original variables in the PCA scatter plotting visualisation. The SigmaPlot 14.5 software was used to perform the PCA data analysis and biplot visualisation.

Results and Discussion

Germination, seedling growth, and drought tolerance responses of maize genotypes

The results of the ANOVA showed significant effects ($p \leq 0.05$) for the main effects of maize genotypes and osmotic potential levels, as well as for interaction, for all evaluated traits measured (Table 1). The significant interaction between maize genotypes and osmotic potentials for most of the evaluated traits indicates that maize genotypes have a distinct response when exposed to different drought levels. Among the evaluated maize genotypes, 90% exceeded the standard germination percentage established by the SNICS, which is 85% for maize seeds in Mexico, indicating, in this study, high physiological seed quality (Magdaleno-Hernández *et al.*, 2020). However, maize genotype XNM exhibited

Table 1 - Analysis of variance for the germination and seedling traits of maize genotypes under drought stress

	p-value of traits								
Source of variation	% Germination	Germination speed	Vigor index	Root length	Shoot length	Root fresh weight	Shoot fresh weight	Root dry weight	Shoot dry weight
Genotypes (G)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0161	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Osmotic potential (Ψs)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
G x Ψs	< 0.0001	< 0.0001	0.0129	0.0003	0.0010	0.0001	< 0.0001	< 0.0001	0.0015
CV (%)	22.6	58.9	52.4	30.5	58.5	36.0	74.5	43.7	68.2

CV: coefficient of variation

a lower rate of 72%.

Figure 1 shows that a drought stress level led to a significant reduction in seed germination and seedling traits of maize genotypes. The germination percentage and speed decreased from 95.7% and 15 seeds per day in the control group to 80.2% and 5 seeds per day, respectively, when seeds were exposed to lower osmotic potential (-1.0 Mpa) (Figure 1A and 1B). Also, increasing drought stress levels significantly reduced both seedling length and dry weights of maize seedlings compared to the control group (Figures 1C and 1D). At lower osmotic potential, seedling length was reduced by 60.6% and seedling dry matter by 50% compared to the control group (Figure 2). Moreover, the lower and upper limits of the boxplots indicated variation in stress response between maize genotypes. Maize genotypes

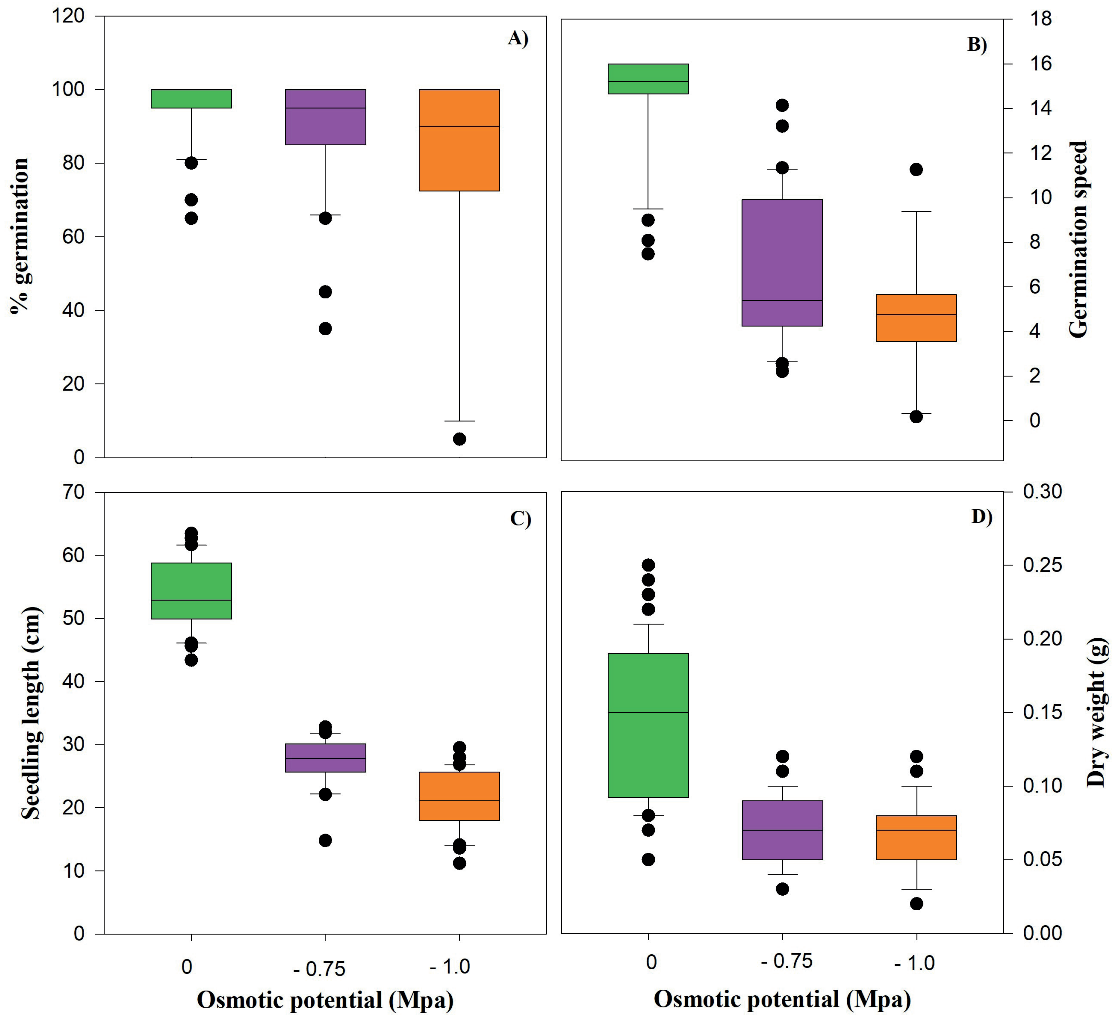

Fig. 1 - Total germination A), Germination speed B), Seedling length C), and Dry weight D) of maize genotypes under drought stress simulated with PEG treatments at different osmotic potentials

Table 2 - Interaction of maize genotypes with osmotic potentials simulated with PEG solutions on germination and seedlings maize traits

Genotype	Ψs (Mpa)	GS (seed day^{-1})	%G	VI	RL (cm)	SL (cm)	RFW (g)	SFW (g)	RDW (g)	SDW (g)
NTA	0	16.0^{a}	100^{ns}	47.4^{a}	23.0^{a}	24.0^{a}	0.46^{a}	0.38^{a}	0.05^{a}	0.03^{a}
	- 0.75	11.7^{b}	100^{ns}	27.3^{b}	16.0^{b}	11.6^{b}	0.32^{b}	0.15^{b}	0.03^{b}	0.01^{b}
	- 1.0	8.3^{c}	100^{ns}	17.2^{c}	11.6^{c}	7.8^{c}	0.24^{c}	0.09^{b}	0.03^{b}	0.01^{b}
NTB	0	16.0^{a}	100^{ns}	46.2^{a}	21.3^{a}	24.7^{a}	0.44^{a}	0.33^{a}	0.04^{a}	0.03^{a}
	- 0.75	11.0^{b}	98.3^{ns}	27.1^{b}	15.3^{ab}	12.0^{b}	0.25^{b}	0.13^{b}	0.02^{b}	0.01^{b}
	- 1.0	9.7^{b}	100^{ns}	16.1^{c}	9.9^{b}	6.3^{c}	0.26^{b}	0.08^{b}	0.02^{b}	0.01^{b}
NTR	0	16.0^{a}	100^{a}	51.0^{a}	22.2^{a}	28.9^{a}	0.51^{a}	0.45^{a}	0.05^{a}	0.04^{a}
	- 0.75	10.0^{b}	98.3^{a}	26.2^{b}	15.4^{b}	11.8^{b}	0.35^{b}	0.22^{b}	0.03^{b}	0.02^{b}
	- 1.0	5.7^{c}	85^{b}	20.4^{b}	14.2^{b}	10.2^{b}	0.33^{b}	0.17^{b}	0.03^{b}	0.02^{b}
XNA	0	14.7^{a}	100^{a}	59.1^{a}	27.9^{a}	28.1^{a}	0.80^{a}	0.70^{a}	0.10^{a}	0.06^{a}
	- 0.75	4.0^{b}	85^{b}	23.0^{b}	16.7^{b}	10.2^{b}	0.54^{b}	0.20^{b}	0.06^{b}	0.02^{b}
	- 1.0	3.3^{b}	80^{b}	16.4^{b}	11.5^{b}	8.7^{b}	0.50^{b}	0.17^{b}	0.06^{b}	0.02^{b}
XNB	0	15.3^{a}	98.3ns	54.3^{a}	25.6^{a}	29.9^{a}	0.69^{a}	0.63^{a}	0.08^{a}	0.05^{a}
	- 0.75	4.3^{b}	95.0^{ns}	27.2^{b}	18.6^{b}	10.1^{b}	0.43^{b}	0.17^{b}	0.05^{b}	0.01^{b}
	- 1.0	5.0^{b}	96.7^{ns}	22.0^{b}	14.6^{c}	8.3^{b}	0.45^{b}	0.16^{b}	0.05^{b}	0.02^{b}
XNM	0	8.0^{a}	71.7^{a}	43.9^{a}	27.1^{a}	32.9^{a}	0.98^{a}	0.85^{a}	0.13^{a}	0.07^{a}
	- 0.75	2.3^{b}	48.3^{a}	13.1^{b}	17.3^{b}	9.6^{b}	0.53^{b}	0.17^{b}	0.07^{b}	0.02^{b}
	- 1.0	0.0^{c}	6.7^{b}	0.9^{c}	8.5^{c}	5.9^{c}	0.56^{b}	0.16^{b}	0.08^{b}	0.02^{b}
UXM	0	14.7^{a}	93.3^{a}	52.0^{a}	26.4^{a}	28.9^{a}	0.92^{a}	0.77^{a}	0.11^{a}	0.07^{a}
	- 0.75	4.7^{b}	81.7^{b}	23.5^{b}	18.7^{b}	10.0^{b}	0.54^{b}	0.17^{b}	0.06^{b}	0.02^{b}
	- 1.0	3.7^{b}	70.0^{c}	12.1^{b}	18.0^{b}	9.0^{b}	0.56^{b}	0.19^{b}	0.07^{b}	0.02^{b}
SB	0	14.3^{a}	100^{a}	53.2^{a}	25.8^{a}	29.4^{a}	0.90^{a}	0.73^{a}	0.12^{a}	0.07^{a}
	- 0.75	4.3^{b}	98.3^{a}	23.5^{b}	17.0^{b}	10.9^{b}	0.59^{b}	0.22^{b}	0.08^{b}	0.02^{b}
	- 1.0	3.7^{b}	93.3^{b}	17.0^{b}	16.8^{b}	7.6^{c}	0.61^{b}	0.19^{b}	0.08^{b}	0.02^{b}
CHTZ	0	15.3^{a}	95^{a}	55.6^{a}	26.4^{a}	29.2^{a}	0.79^{a}	0.68^{a}	0.10^{a}	0.06^{a}
	- 0.75	6.0^{b}	85^{ab}	24.6^{b}	17.8^{b}	13.1^{b}	0.54^{b}	0.26^{b}	0.07^{b}	0.03^{b}
	- 1.0	5.0^{b}	70^{b}	23.4^{b}	14.3^{b}	10.2^{b}	0.49^{b}	0.20^{b}	0.06^{b}	0.02^{b}
H	0	15.7^{a}	98.3^{ns}	48.5^{a}	21.7^{a}	27.3^{a}	0.75^{a}	0.71^{a}	0.09^{a}	0.06^{a}
	- 0.75	6.0^{b}	100^{ns}	28.0^{b}	17.9^{b}	10.0^{b}	0.56^{b}	0.21^{b}	0.07^{b}	0.02^{b}
	- 1.0	4.7^{c}	96.7^{ns}	19.0^{c}	11.4^{c}	8.3^{b}	0.50^{b}	0.18^{b}	0.06^{b}	0.02^{b}

Values are means; Ψs - Osmotic potential simulated with PEG solutions; GS germination speed; %G germination percentage; VI vigor index; RL root length; SL shoot length; RFW root fresh weight; SFW shoot fresh weight; RDW root dry weight; SDW shoot dry weight. Distinct letters within the same column indicate significant differences between osmotic potentials for the same genotype at a significance level of $p < 0.05$, in the Tukey test. ns - indicates non-significant differences.

exhibited variability in their capacity to perceive, react to, and withstand stress across various stress levels.

Under drought stress conditions, maize genotypes showed a significant decrease in germination percentage, germination speed, vigour index, root, and shoot lengths, as well as fresh and dry weights, in comparison to the control group (Table 2). A significant delay in seed germination was observed for all genotypes treated with PEG. Nevertheless, the decreased germination in response to water stress was not uniform in all genotypes. Maize landraces NTA and NTB did not exhibit significant differences between water stress treatments and the control. Similarly, the PEG treatments significantly retarded the seedling growth of all maize genotypes. The mean of the measured traits showed successive reductions in drought stress levels. The mean vigour index was 51.1 for the control (0 Mpa) and 24.4 and 16.5 for PEG treatments (-0.75 and -1.0 Mpa, respectively). Root length measured 24.8 cm for the control and 17.1 and 13.1 cm for the PEG treatments, while shoot length was 28.3 cm for the control and 10.9 and 8.2 cm for the PEG treatments. The root fresh weight was 0.72 g for the control, and 0.46 and 0.45 g for the PEG treatments, while the shoot fresh weight was 0.62 g for the control and 0.19 and 0.16 g for the PEG treatments. Finally, root dry weight was 0.09 g for the control and 0.05 g for both PEG treatments, while shoot dry weight was 0.06 g for the control and 0.02 g for both PEG treatments (Table 2).

PEG solutions delayed germination of all maize genotypes and decreased total germination in some cases compared to the control group. Moreover, the speed of

Fig. 2 - Photographs illustrating the impact of water stress on seedlings of two maize genotypes at different osmotic potentials. A) maize landrace (Nal tel); B) maize hybrid (H-562)

germination revealed differences in the dynamics and progress of the germination process between maize genotypes. Hence, combining total germination with other indices, such as speed of germination, allows efficient comparison of the effects of drought stress on maize genotypes (Álvarez-Iglesias *et al*., 2017). PEG molecules attract and absorb water molecules, preventing the seed from receiving the necessary moisture for germination. Additionally, PEG molecules interfere with signal transmission, which can prevent the seed from receiving the necessary chemical signals for germination (Mustamu *et al*., 2023). Additionally, Khan *et al*. (2019) found that drought stress inhibits germination through the release of reactive oxygen species (ROS) that damage cell structures and metabolic processes.

PEG solutions affected the maize seedling growth of genotypes under study. Severe drought stress induced by PEG solutions resulted in the reduction of 60.6% seedling length and 50% seedling dry matter compared with the control group (Figure 1). Osmotic stress causes low water availability, resulting in decreased cell division and elongation. This is due to a decrease in turgor pressure and cell growth. Therefore, drought stress induced by PEG reduced dry and fresh biomass and root and shoot length (Queiroz *et al*., 2019; Mustamu *et al*., 2023). Nevertheless, roots are less sensitive than shoots to growth inhibition at low osmotic potentials, and thus root length and dry weight are less affected than shoots (Álvarez-Iglesias *et al*., 2017). In several studies, similar findings have been reported regarding seedling growth traits, including Álvarez-Iglesias *et al*. (2017), Queiroz *et al*. (2019), and Badr *et al*. (2020). Under drought stress conditions, developing a root system is considered an adaptive mechanism for optimising soil water uptake, increasing the possibilities of finding water sources (Álvarez-Iglesias *et al*., 2017). Reduction in shoot growth can also be considered an adaptive response to avoid water loss by evapotranspiration.

Many studies on plant responses to drought stress induced by PEG solutions regarding seed germination and seedling growth have been recently reported with similar findings. Indeed, Khodarahmpour (2011) showed that the osmotic potential of -1.2 Mpa reduced the seed germination and shoot length of maize seedlings by 71 and 90%, respectively. Kappes *et al*. (2010) found that the germination of different maize hybrids ranged from 36 to 65% at the osmotic potential of -1.2 Mpa. Rangel-Fajardo *et al*. (2019) found that an osmotic potential of -0.75 Mpa decreased seed germination and seedling length of maize hybrids in Yucatan, Mexico by 87% and 94%, respectively. Mustamu *et al*. (2023) reported that germination speed in local maize from Indonesia decreased by 50% at a 50% PEG concentration.

Drought-tolerant indices of maize genotypes are illustrated in Figure 3. Significant differences ($p < 0.05$) between maize genotypes were identified for the germination tolerant index (Figure 3A), speed germination tolerant index (Figure 3B) and seedling length tolerant index (Figure 3C). In the germination tolerant index (Figure 3A), maize genotypes NTA, NTB, XNB, and the hybrid (H) had the highest values, while the landrace XNM was highly affected by PEG solution and had the lowest value, with a mean of 0.1. Maize landrace NTB was statistically superior to the other maize genotypes and had the highest speed germination tolerant index with a 0.63 mean value (Figure 3B). Likewise, maize landrace NTA ranked second with a 0.52 mean value. The landrace XNM resulted in the lowest speed germination tolerant value (Figure 3B). The seedling length (root + shoot) tolerant index is shown in Figure 3C. The mean seedling length tolerant index for all maize genotypes was 0.4. Maize UXM had the highest and most significant value with 0.5, followed by NTR with 0.47. Both genotypes were less affected by PEG osmotic solutions. The landrace XNM resulted in the lowest tolerance value of 0.25. The dry weight tolerance index was statistically similar between maize genotypes. The mean dry weight tolerant index was 0.48 and ranged from 0.42 in the NTA to 0.55 in the NTR (Figure 3D).

The drought tolerance index (DTI) is a reliable indicator for identifying drought-tolerant or sensitive maize genotypes. Therefore, maize genotypes NTA and NTB, with high germination and speed germination, were considered drought tolerant at the germination stage. At the seedling stage, differences between maize genotypes were less evident. Nevertheless, maize landraces UXM and NTR were less affected by PEG osmotic solutions and are considered moderately drought tolerant at the seedling stage. In similar studies, Queiroz *et al.* (2019) and Mustamu *et al.* (2023) also observed a significant decrease in root and shoot lengths as osmotic potential increased, pointing out maize drought-tolerant genotypes.

The mean dry weight drought tolerant index (DW DTI) was 0.48, ranging from 0.42 in the NTA to 0.55 in the NTR. Mustamu *et al.* (2023) reported that the dry weight DTI ranged between 0.28 and 0.68 across 16 local genotypes from Indonesia, and maize genotypes with DTIs greater than 0.5 were considered drought tolerant. Queiroz *et al.* (2019) documented dry weight DTI values of 0.68, 0.42, and 0.26 at -0.2, -0.4, and -0.8 Mpa, respectively, for a maize hybrid from Brazil.

Physiological and drought tolerance responses of maize genotypes

The results of the ANOVA were significant ($p \leq 0.05$) for the main effects of maize genotypes and osmotic potential levels, as well as for interaction, for all physiological traits measured (Table 3).

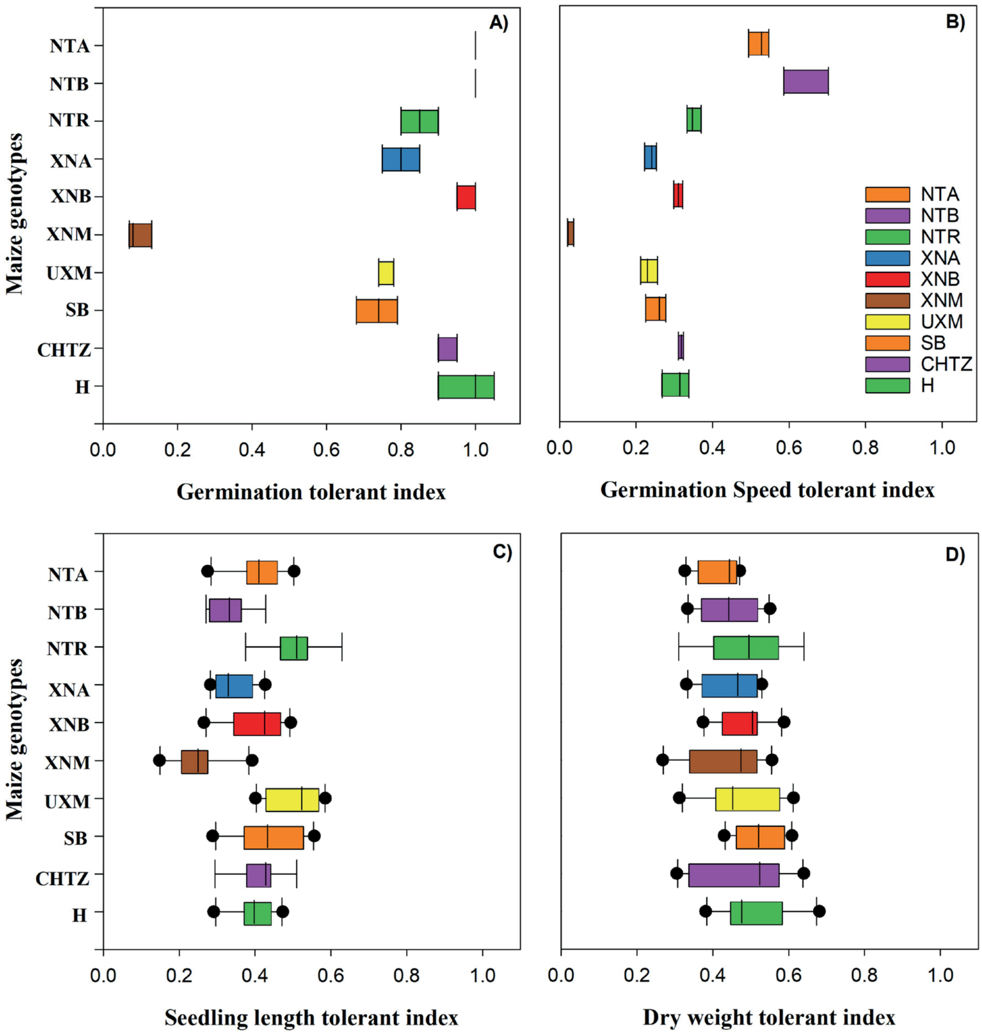

Fig. 3 - Drought tolerance indices for germination and seedling growth responses of maize genotypes. A) Germination tolerant index B) Speed germination tolerant index, C) Seedling length tolerant index, D) Dry weight tolerant index

Figure 4 showed that an increase in drought stress level led to a significant reduction in the carbon assimilation rate (A_n), stomatal conductance (g_s), and transpiration (E) of maize genotypes. The A_n was reduced by 25% and 70% at -0.75 Mpa and -1.0 Mpa osmotic potential, respectively, compared to the control group. Stomatal conductance was reduced by 33% and 67%, and water loss by transpiration was reduced by 53% and 81%, respectively, compared to the control group (Figure 4). On the contrary, water use efficiency (WUE) rises as osmotic potential increases (Figure 4D). WUE increases by 42% and 33% at -0.75 Mpa and -1.0 Mpa osmotic potential, respectively, relative to the control group.

PEG osmotic solutions significantly affected the physiological response of maize genotypes (Table 4). Under drought stress conditions, maize genotypes showed a significant decrease in carbon assimilation rate (A_n), stomatal conductance (g_s), and transpiration (E) in comparison to the control group (Table 4). Nevertheless, the response to drought stress was not uniform in all genotypes. Under control conditions mean A_n was 5.4 μmol m^{-2} s^{-1} within maize genotypes. At -0.75 Mpa of osmotic potential, maize genotypes XNA and SB showed the highest carbon assimilation rate with 8.7 and 7.2 μmol m^{-2} s^{-1}, respectively. At -1.0 Mpa of osmotic potential, maize genotypes NTR and XNB were superior to the other genotypes, exhibiting carbon assimilation rates of 4.7 and 3.7 μmol m^{-2} s^{-1} (Table 4). Mean intracellular carbon was 212.6 μmol m^{-2} s^{-1} between maize genotypes and control conditions. At -0.75 Mpa, the landraces NTR, NTA, and NTB exhibited the highest amounts of intracellular carbon with 330.7, 278.1, and 271.3 μmol m^{-2} s^{-1}, respectively, while at -1.0 Mpa, genotypes NTA, XNA, and NTB accumulated 785.5, 782.9 and 505.6 μmol m^{-2} s^{-1}, respectively. Under control conditions mean gs was 0.06 mol m^{-2} s^{-1} within maize ge-

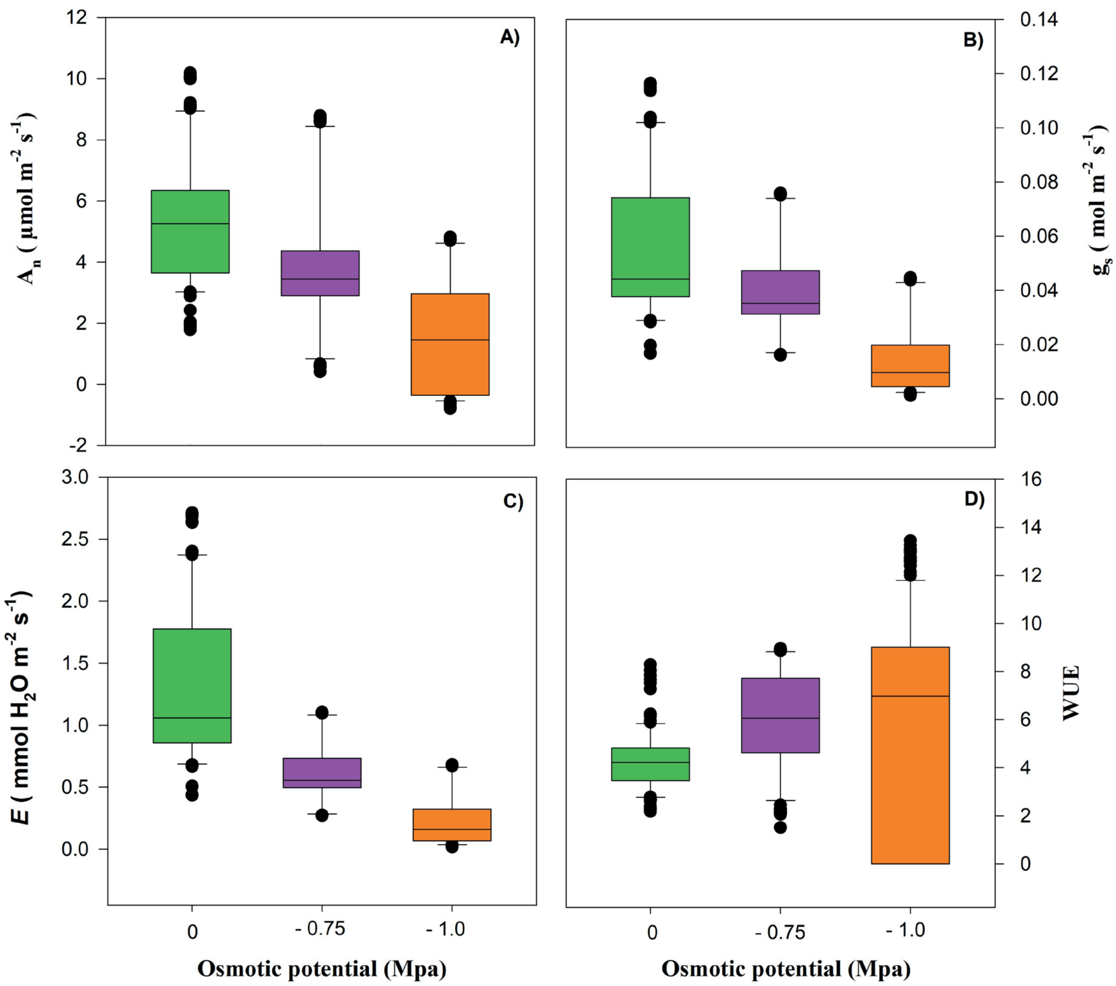

Fig. 4 - Physiological response of maize genotypes under drought stress simulated with PEG treatments at different osmotic potentials. A) CO2 assimilation rate (An); B) stomatal conductance (gs); C) transpiration (E) and D) water use efficiency (WUE)

notypes. At -0.75 Mpa, gs was 0.08 mol m^{-2} s^{-1} in SB and 0.06 mol m^{-2} s^{-1} in XNA. However, at -1.0 Mpa, it reached its maximum at 0.04 mol m^{-2} s^{-1} in the landrace NTR. Mean transpiration under control conditions was 1.4 mmol H_2O m^{-2} s^{-1}. At -0.75 Mpa, maize genotypes SB and XNA exhibited transpiration rates of 1.10 and 0.97 mmol m^{-2} s^{-1}, respectively, whereas at -1.0 Mpa, the highest transpiration rate was observed in the maize genotype NTR, with 0.68 mmol m^{-2} s^{-1}. Water use efficiency was 4.3 under control conditions for all maize genotypes. At -0.75 Mpa, maize genotypes XNA, H, and CHTZ showed the highest WUE with 8.9, 8.4, and 7.7 µmol CO_2 mmol H_2O, respectively. However, at -1.0 Mpa, maize genotypes SB, CHTZ, and H were the most water use efficient with 12.8, 9.3, and 9.1 µmol mmol, respectively.

Monitoring leaf gas interchange can detect drought stress intensity (Álvarez-Iglesias *et al.*, 2018). Ashraf *et al.* (2007) explored the physiological response of seven maize lines from Pakistan subjected to PEG-induced water stress (-0.66 Mpa). They reported that photosynthetic capacity could be used as an efficient selection criterion for screening maize germplasm for water stress tolerance. The central role of photosynthesis in maize response to drought is regulated by stomatal opening, a complex process affected by many factors. Genotypes reduce stomatal conductance to limit water loss, but stomatal closing also limits CO_2 availability. Stomatal closure represents one of the earliest responses to drought, protecting the plant from water loss (transpiration) and increasing its water use efficiency (Álvarez-Iglesias *et al.*, 2017). In our study, under moderate drought stress (-0.75 Mpa), six maize genotypes maintained or increased stomatal conductance (NTA, NTB, NTR, XNA, SB, and H). Among these genotypes, SB and H also increased An and WUE, suggesting that these landraces have a good physiological response

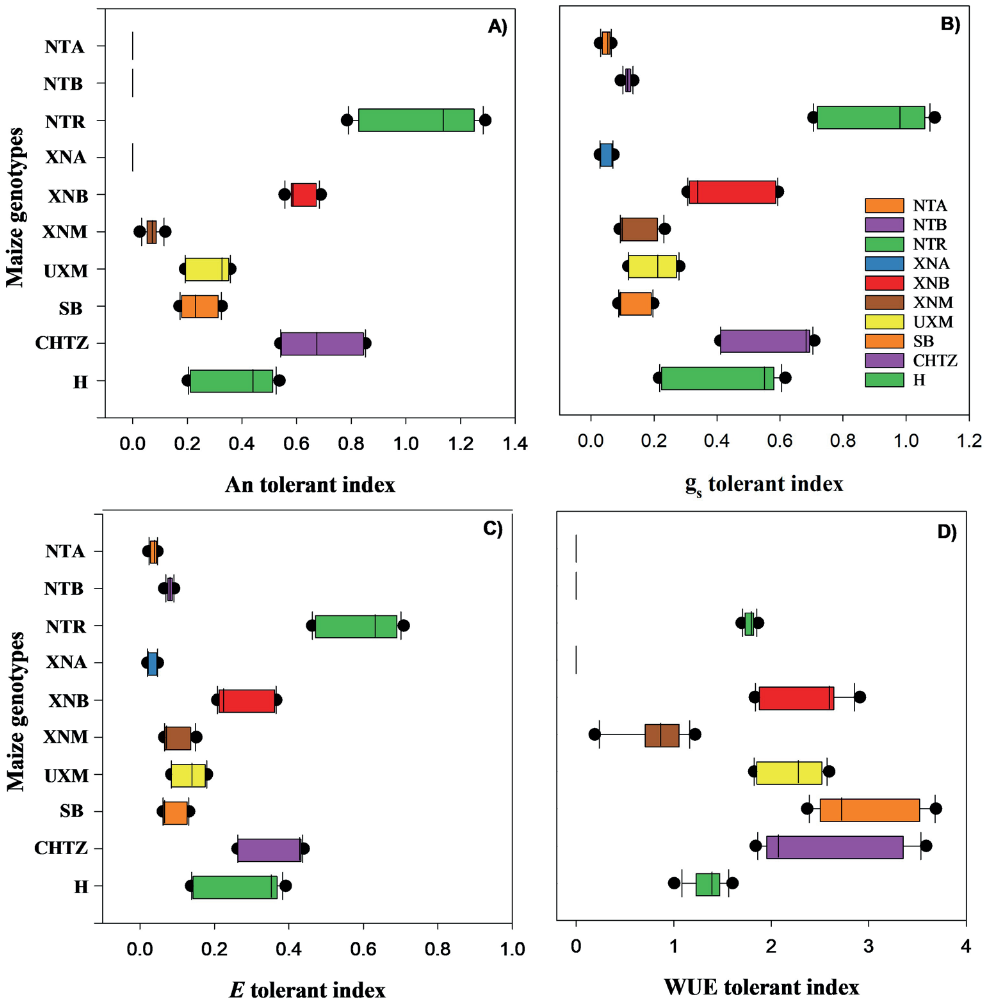

Fig. 5 - Drought tolerance indices for physiological responses of maize genotypes. A) CO2 assimilation rate (An) tolerant index; B) stomatal conductance (gs) tolerant index; C) transpiration (E) tolerant index: and D) water use efficiency (WUE) tolerant index

Table 3 - Analysis of variance for the physiological response of maize genotypes under drought stress

	p-value of traits				
Source of variation	Carbon assimilation (An)	Stomatal conductance (gs)	Carbon intracellular (Ci)	Transpiration (E)	Water use efficiency (WUE)
Genotypes (G)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Osmotic potential (Ψs)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
G x Ψs	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV (%)	70.9	71.5	62.4	84.2	54.9

CV: coefficient of variation

under moderate drought stress. Under severe drought conditions, all maize genotypes closed their stomatal and stomatal conductance was drastically reduced. The better physiological response with greater water use efficiency was on SB, CHTZ, H, UXM, NTR, and XNB maize genotypes. Among these, NTR was the only one with a similar An under drought stress and control conditions, so, NTR was considered drought tolerant.

Physiological drought-tolerant indices of maize genotypes are illustrated in Figure 5. Statistically significant differences ($p < 0.001$) were observed in ANOVA analysis among maize genotypes for A_n, g_s, E, and WUE tolerant indexes. Maize genotypes NTR and CHTZ had the highest and statistically significant values for A_n, g_s, and E tolerant indexes (Figure 5A, 5B, and 5C) while the landraces NTA, NTB, and XNA resulted in lower values for the same indexes. Also, in Figure 5A, the landraces NTA, NTB, and XNA resulted in a zero tolerant index value as a result of a negative carbon assimilation rate (respiration). In the WUE tolerant index (Figure 5D), the mean tolerant index value between maize genotypes was 0.46. The maize genotypes SB, XNB, UXM, and CHTZ had the highest indexes with 2.72, 2.59, 2.28, and 2.07 median values, respectively.

Principal component analysis of drought tolerance indices in maize genotypes

The PCA diagram illustrating the clustering of 10 maize genotypes according to their DTIs values is shown in Figure 6. The first two components of the PCA analyses explain 78.4% of the total variability between maize genotypes and were used to group the genotypes in the PCA diagram. The first eigenvector was strongly correlated with the physiological response and seedling growth DTIs. The second eigenvector was associated with germination and germination speed DTIs. The

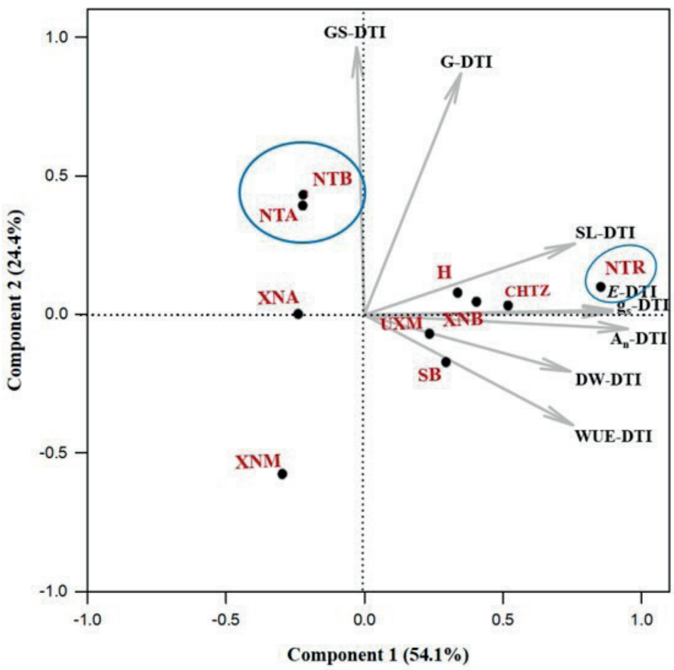

Fig. 6 - PCA diagram illustrating the clustering of 10 maize genotypes according to multiple drought tolerance indices values. Blue circles denote maize genotypes considered drought-tolerant

Table 4 - Interaction of maize genotypes with osmotic potentials simulated with PEG solutions on physiological response traits

Genotype	Ψs (Mpa)	An (μmol m^{-2} s^{-1})	Ci (μmol m^{-2} s^{-1})	gs (mol m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	WUE (μmol CO_2 mmol H_2O)
NTA	0	3.13^{a}	255.3^{b}	0.04^{a}	0.83^{a}	3.80^{a}
	- 0.75	2.42^{b}	278.1^{b}	0.04^{b}	0.53^{b}	4.56^{a}
	- 1.0	- 0.47^{c}	785.8^{a}	0.002^{c}	0.03^{c}	0.0^{b}
NTB	0	3.90^{a}	219.7^{c}	0.04^{b}	0.87^{a}	4.53^{a}
	- 0.75	3.28^{b}	271.3^{b}	0.05^{a}	0.72^{b}	4.54^{a}
	- 1.0	- 0.3^{c}	505.6^{a}	0.002^{c}	0.07^{c}	0.0^{b}
NTR	0	4.60^{a}	231.6^{b}	0.04^{b}	1.16^{a}	3.9^{b}
	- 0.75	0.59^{b}	330.7^{a}	0.05^{a}	0.27^{c}	2.2^{c}
	- 1.0	4.74^{a}	212.6^{c}	0.005^{c}	0.68^{b}	7.0^{a}
XNA	0	7.39^{b}	202.6^{b}	0.07^{a}	1.69^{a}	4.5^{b}
	- 0.75	8.67^{a}	158.8^{c}	0.06^{b}	0.97^{b}	8.9^{a}
	- 1.0	- 0.66^{c}	782.9^{a}	0.003^{c}	0.04^{c}	0.0^{c}
XNB	0	6.10^{a}	260.7^{a}	0.09^{a}	2.09^{a}	3.0^{c}
	- 0.75	3.50^{b}	226.2^{b}	0.04^{b}	0.57^{b}	6.1^{b}
	- 1.0	3.70^{b}	210.3^{c}	0.03^{c}	0.53^{b}	7.0^{a}
XNM	0	7.50^{a}	223.0^{b}	0.08^{a}	2.01^{a}	3.9^{b}
	- 0.75	3.00^{b}	234.5^{b}	0.03^{b}	0.51^{b}	5.8^{a}
	- 1.0	1.13^{c}	304.3^{a}	0.02^{c}	0.33^{c}	3.1^{c}
UXM	0	6.96^{a}	218.1^{b}	0.07^{a}	1.78^{a}	4.0^{c}
	- 0.75	4.39^{b}	236.0^{a}	0.05^{b}	0.73^{b}	6.0^{b}
	- 1.0	1.91^{c}	161.5^{c}	0.01^{c}	0.22^{c}	8.7^{a}
SB	0	6.30^{b}	200.3^{b}	0.06^{b}	1.46^{a}	4.5^{c}
	- 0.75	7.23^{a}	226.9^{a}	0.08^{a}	1.10^{b}	6.6^{b}
	- 1.0	1.45^{c}	46.7^{c}	0.01^{c}	0.11^{c}	12.8^{a}
CHTZ	0	3.68^{a}	214.3^{a}	0.03^{a}	0.91^{a}	4.0^{c}
	- 0.75	2.90^{b}	188.7^{b}	0.02^{b}	0.37^{b}	7.7^{b}
	- 1.0	2.98^{b}	145.2^{c}	0.02^{b}	0.32^{c}	9.3^{a}
H	0	4.09^{a}	101.3^{c}	0.02^{b}	0.62^{a}	6.8^{c}
	- 0.75	4.14^{a}	173.5^{a}	0.03^{a}	0.50^{b}	8.4^{b}
	- 1.0	1.42^{b}	149.6^{b}	0.01^{c}	0.16^{c}	9.1^{a}

Ψs – Osmotic potential simulated with PEG solution. Values are means; An - Net carbon assimilation (negative values at An represent photorespiration); Ci – Carbon intracellular; gs – Stomatal conductance; E – Transpiration; WUE – Water use efficiency. Distinct letters within the same column indicate significant differences between osmotic potentials for the same genotype at a significance level of $p < 0.05$, in the Tukey test.

PCA diagram describes the drought tolerance in response to variables and assigns variables to genotypes. This analysis has been widely proposed in the literature (Badr and Brüggemann 2020; Badr *et al.*, 2020). In the PCA scatter diagram, the arrangement of maize genotypes clearly shows the formation of two distinct groups characterized by high DTI values. Maize genotype NTR maintained a high physiological and seedling growth response under drought stress, reflected by its high scores in PC1, while genotypes NTA and NTB, exhibited a great germination response under drought stress. Therefore, maize genotypes NTR, NTA, and NTB are considered tolerant to drought stress at an early growth stage. Diverse mechanisms of drought adaptation are displayed by these populations depending on the feature measured and the stage of development.

These results agree with those reported in Álvarez-Iglesias *et al.* (2017) for various maize hybrids, which were classified into five clusters representing four functional units in the cluster analysis. Later, Badr *et al.* (2020) employed a PCA scatter plot to show the clustering of 40 maize accessions according to their grand DTI values. The authors identified a cluster comprising five accessions with the highest grand DTIs identified as drought tolerant. In addition, 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.

Conclusions

All PEG treatments significantly reduced germination and retarded seedling growth traits of maize genotypes. Drought stress also led to reduced carbon assimilation rate (A_n), stomatal conductance (g_s), and transpiration (*E*) in maize genotypes. Conversely, water use efficiency (WUE) increased under drought. The DTIs values were very useful in differentiating traits and maize genotypes. PCA analysis based on variation in DTIs grouped the maize genotypes with high DTIs. The results highlighted different responses of maize genotypes at germination, seedling, and physiological traits that, when considered together, allowed an efficient discrimination of maize genotypes. Therefore, maize genotypes NTR, NTA, and NTB were tolerant to drought stress at an early growth stage. These genotypes could be considered as parents for drought-tolerant varieties or direct cultivation. Drought-tolerant maize varieties have potential implications for crop productivity, food security, and sustainable agriculture, especially in the face of climate change. Further studies should be carried out under field conditions to validate these findings.

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