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Identification of Mexican maize races (*Zea mays* L.) with drought tolerance using osmotic potential experiments for genetic breeding

J.A. Batista-Rascon[1], J.C. Jiménez-Galindo[2,*], N. Ramírez-Cabral[3], G. Castellanos-Pérez[1] and J.J. Figueroa-Gonzalez[3]

[1]Autonomous University of Chihuahua, Agrotechnological Sciences Faculty, V. Carranza y Escorza s/n, Col. Centro, Chihuahua, 31000 Chihuahua, México
[2]Agriculture and Livestock Research, National Institute of Forestry, Av. Hidalgo No 1213, Cuauhtémoc, 31500 Chihuahua, México
[3]National Institute of Forestry, Agriculture and Livestock Research, Km. 24.5 Carretera Zacatecas-Fresnillo Calera de Víctor Rosales, Zacatecas. C.P. 98500, México
[*]Correspondence: cruz2477@yahoo.com.mx

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Abstract. Maize (*Zea mays* L.) is the third most important cereal crop worldwide after wheat and rice per cultivated area with 249,225,876 hectares and the most important crop for number of harvested grain tons with 1,482,997,259 in 2021. Some native Mexican maize races could be a source for drought tolerance to improve commercial cultivars and hybrids. The experiments were conducted using various osmotic pressures (OP) induced by polyethylene glycol (PEG-6000) (0, -0.05, -0.15, -0.30 and -0.49 MPa) simulating an increase of drought stress in ten maize genotypes. The main objectives of this study were the evaluation of germination and seedling growth components in response to drought stress and the identification of sources of drought tolerance in Mexican maize races. Apachito-r showed an increased germination in 110.4%, Cristalino-079 had a decreased germination in 98.7% and Cristalino-279 reduced its germination in a 91.1% compared to the control. Apachito-r outstands in root length at -0.05 OP increasing 200.1% and at -0.49 increasing 129.8%. The values for stem length were decreasing as the OP was increasing and only Apachito-r showed a significant difference at -0.30 MPa decreasing 39.8% respect to its control. Cristalino-279 showed significant difference in the variable root fresh weight and its value outstand at -0.15 increasing 267.2%, at -0.30 increasing 281.6% and at -0.49 MPa increasing 189.3% compared to the control in water. The variable root dry weight had the highest value for Apachito-r at -0.05 MPa increasing in a 189.4%, decreasing at -0.15 in 72% and at -0.30 MPa in a 79.8% and increasing at -0.49 MPa in 112.3%. Also noteworthy are E-zapata-r increasing 190.5% and Cristalino-061 increasing 142.9% at -0.30. E-zapata-r at -0.49 increased 115.1%. Cristalino-279 showed significant difference in the variable stem fresh weight and its value outstand at -0.05, -0.15 and -0.30 MPa increasing 146.7%, 103.7% and 60.2% respectively. Finally, in stem dry weight the tendency was to decrease as OP was increasing, however Cristalino-279 showed differences at -0.30 decreasing in 89.5% and at -0.49 MPa increasing in a 143.5% respect to the control. The most drought tolerant genotypes were Cristalino-279, Apachito-r, Azul and 8-carreras-PP. The most tolerant genotypes showed greater root length, greater root fresh and dry weight, better germination and greater stem length.

Resistant and susceptible genotypes are ideal material to understand the physical and chemical mechanisms related to drought tolerance. Cristalino-279 shows the best level of drought tolerance at all levels of osmotic pressure, this genotype can be used as a source of drought tolerance for the improvement of commercial maize.

Key words: native maize races, drought tolerance, osmotic pressure, polyethylene glycol.

INTRODUCTION

Worldwide, maize (*Zea mays* L.) is the third most important cereal crop, after wheat and rice (Huang et al., 2015) per cultivated area with 249,225,876 hectares harvested in 2021 (FAOSTAT, 2023); and the most important crop in the world, per grain harvested tons, with 1,482,997,259 (Shiferaw et al., 2011; FAOSTAT, 2023). Maize is listed as an essential crop for world food security, for humans and in animal consumption (Campos et al., 2004; Prasanna, 2012). Moreover this crop is a staple grain with economic and social importance (Ureta et al., 2020; Magorokosho & Tongoona, 2003; Iqbal et al., 2021).

Climate change is increasing average temperatures, modifying patterns of precipitation and reduction water availability, this phenomena is likely to increase in frequency and intensity in the coming decades (Hendrix & Glaser, 2007; Lobell & Burke, 2008). Due to climate change, grain yield and quality will decrease, because of the increment of biotic and abiotic stresses, among these, dry and heat stresses are the most remarkable affecting negatively growth and yield traits of maize (Sabagh et al., 2018; Zenda et al., 2018; Islam et al., 2019; Dong et al., 2020; Raj et al., 2020; Liu et al., 2022; SINGH et al., 2022).

Mainly, farmers grow the maize under rain-fed conditions (Hellin et al., 2014). Biradar et al. (2006) mentioned that globally more than 50% of the total cultivated area of maize is under rain-fed conditions.

Some native Mexican maize races have been reported as tolerant to drought, such is the case of Apachito, Cristalino de Chihuahua and Azul (Ruiz Corral et al., 2008; Ruiz Corral et al., 2013). Other genotypes from other regions of the world have been identified such as hybrids with good response under drought conditions as H3, H4, H8, H11, H15, H19, H27 and H29 (Qayyum et al., 2012). Tohono O'odham Z16 maize was reported with high drought tolerance (Shisanya & Hornetz, 1997). Arun-2 the most drought tolerant maize was reported by (Magar et al., 2019). Genotypes BC678 and BC404 were reported as resistant to drought stress (Khayatnezhad & Gholamin, 2012). Ahmad et al. (2015) found two hybrids with good drought tolerance 6525 and 32B33. Naghavi et al. (2013) found three genotypes with highest tolerance to drought KSC-720, KSC-710-GT and KSC-700. ND476 genotype were reported with high level of drought tolerance (Dong et al., 2020). And from the tested genotypes by (Iqbal et al., 2021), genotype 249 and genotype 252 were the best genotypes for maize improvement for tolerance to drought stress.

The loss in maize production is accounted approximately 16% in lowland tropics (Edmeades et al., 2006) and it reached up to 60% in severely drought-affected regions/ seasons (Ribaut et al., 2009). Current trends of climatic change show a likehood of increase water scarcity and reduce maize productivity by 15–30% (Lobell et al., 2014).

Recommended drought tolerance adaptation strategies include farmers' increased use of tolerant maize genotypes (Hellin et al., 2014). Among different plant adaptive strategies to water stress (WS), drought avoidance is one of the most important, can be used for enhancing crop yield under WS conditions (Blum, 2011). This can be achieved in a variety of ways, including adjustment of growth rate and growth pattern of shoot and root (Comas et al., 2013). It is already known that the ability of the plant for water uptake depends on root system, root structure, and access to water in soil which in turn determine the functionality of plant shoot. Thus, extent of drought avoidance or tolerance in plants can be determined by a number of biometric attributes such as leaf number and structure, root length and branching pattern, leaf waxy layer, leaf rolling etc. (Blum, 2011; Comas et al., 2013). Since crop sensitivity at the germination growth stage governs overall success of a crop, it is advocated that biometric attributes at the early growth stages can be used as indicator for crop performance at later growth stage or as a selection criteria for improving crop resistance against drought (Lobell et al., 2008; Reynolds & Tuberosa, 2008; Blum & Blum, 2011; Comas et al., 2013). This argument can be supported by the fact that several germination and seedling growth indices are frequently used as predictors to appraise drought tolerance in crop plants (Comas et al., 2013; Ayalew et al., 2015).

One of the most plausible techniques to simulate uniform drought includes the use of metabolically inactive compound such as Polyethylene glycol which has been widely employed by a number of workers to study the effects of WS in different groups of plants (Waheed, 2014; Ahmad et al., 2015; Islam et al., 2019; Zeng et al., 2019; Badr et al., 2020; Raj et al., 2020; Bukhari et al., 2021; Badr et al., 2020; Suhartono, 2021). Some efforts have been carried out in different crops to find sources of tolerance to drought, such is the case of (Jimenez-Galindo et al., 2018) that found that the tolerant bean genotypes, detected with PEG-6000, T-cafe and R-bufa, showed more root length and root dry weight. Therefore these traits could help us indirectly to select tolerant genotypes in beans (Jiménez Galindo & Acosta Gallegos, 2013). In addition (Ortega-Ortega et al., 2023) found that tolerant genotypes detected with PEG-6000 bioassays of oat to drought have higher germination rate with respect to their control in water, longer roots, and higher root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight. In this case Teporaca genotype was the most tolerant genotype and had the best response in field experiments with irrigation-drought experiments.

The main objective of this research is to assess drought tolerance in a collection of native Mexican maize races to identify the best sources of drought tolerance at early stages.

MATERIALS AND METHODS

Plant material

Ten genotypes with diverse genetic backgrounds into six Mexican maize races (*Zea mays* L.) recollected by INIFAP at Chihuahua State, were used, to study drought tolerance on germination, and root and stem development characters using polyethylene glycol (Table 1). The Apachito, Cristalino de Chihuahua and Azul races, tolerant to drought, were selected (Ruiz Corral et al., 2008; Ruiz Corral et al., 2013). In addition, we studied the races: Gordo, Palomero, E-Zapata-r and 8-Carreras-PP with unknown response to drought tolerance.

Table 1. Agronomic traits of ten genotypes of maize, evaluated for drought tolerance

Genotype	Origin	Drought response	Race	Seed color
Apachito-r	INIFAP	Tolerant[1, 2]	Apachito	Pink
Gordo	INIFAP	Unknown	Gordo	White
Palomero	INIFAP	Unknown	Palomero de Chihuahua	Yellow
E-Zapata-r	INIFAP	Unknown	Unknown	White
Cristalino-282	INIFAP	Tolerant[2]	Cristalino de Chihuahua	Yellow
Azul	INIFAP	Tolerant[2]	Azul	Black
Cristalino-079	INIFAP	Tolerant[2]	Cristalino de Chihuahua	Yellow
Cristalino-279	INIFAP	Tolerant[2]	Cristalino de Chihuahua	White
Cristalino-061	INIFAP	Tolerant[2]	Cristalino de Chihuahua	White
8-carreras-PP	INIFAP	Unknown	8-carreras	White

[1]Apachito race was reported as tolerant to dry conditions by (Ruiz Corral et al., 2008). [2]Apachito, Cristalino de Chihuahua and Azul races were reported as tolerant to dry conditions by (Ruiz Corral et al., 2013).

Experimental design

Two experiments were conducted in Lab where seeds were incubated at 28 °C in a complete randomized design in a factorial arrangement. Factorial combinations of ten maize genotypes and five osmotic pressures levels were evaluated with three repetitions. The experimental plot was one petri dish of 10 cm in diameter. Ten seeds of each accession were used per repetition with a layer of filter paper, to which 8 mL of the previously PEG-6000 prepared dilutions were added. Obtaining a total of 10 genotypes and 5 levels of osmotic pressure of 0.0, -0.05, -0.15, -0.30 and -0.49. To carry out the evaluation, dilutions of polyethylene glycol (PEG-6000) were prepared in distilled water at 0, 50, 100, 150, and 200 g of PEG-6000 per liter of distilled water, to reach an osmotic pressure (OP) of 0.0, -0.05, -0.15, -0.30 and -0.49, respectively, based on the equation provided by (Michel & Kaufmann, 1973). The variables germination, root and stem length, root fresh weight, stem fresh weight, root dry weight and stem dry weight were measured. Seeds were considered germinated when the root or stem was approximately 10 mm in length or more. After seven days, the roots and stems of the seedlings were measured in centimeters and on the same day the fresh root and stem were weighed on a precision balance in grams. The root and stem tissue were cut and placed in a stove at 45 °C for seven days to record the root and stem dry weight in grams. All data were expressed as percent reduction or increase respect to their own control in water with 0.0 OP.

Statistical analysis

An analysis of variance (ANOVA) was made using the GLM (General Linear Model) procedure (PROC GLM) of SAS. Sources of variation were genotype, stress level, and genotype × stress level interaction. Genotypes, osmotic pressure and genotype × osmotic pressure interaction were considered fixed effects. Besides, an individual ANOVA by stress level was also carried out. We used Tukey at $p < 0.05$ for mean comparisons. In addition, Principal Component Analyzes was performed with all traits using the SAS Software for Windows 9.0 statistical package (SAS Institute. Inc. Cary, N.C., USA, 2002). Previously, all data were standardized with mean = 0 and standard deviation = 1. The first component was used to ordering genotypes because it explained most of the variability across Osmotic Pressure levels (OP1 = 44.7%, OP2 = 36.5%, OP3 = 45.0% and OP4 = 40.3% of the explained variability) and was considered as an index of tolerance to drought.

RESULTS AND DISCUSSION

Of the ten maize races under study, three stand out in percentage of germination: Apachito-r which had an increased germination in 110.4%, Cristalino-079 with a decreased germination of 98.7% and Cristalino-279 that reduced its germination in 91.1% compared to control at high osmotic pressure at -0.49 OP. No significant differences were obtained at -0.05, -0.15 and -0.30 MPa between maize races (Figs 1 –7).

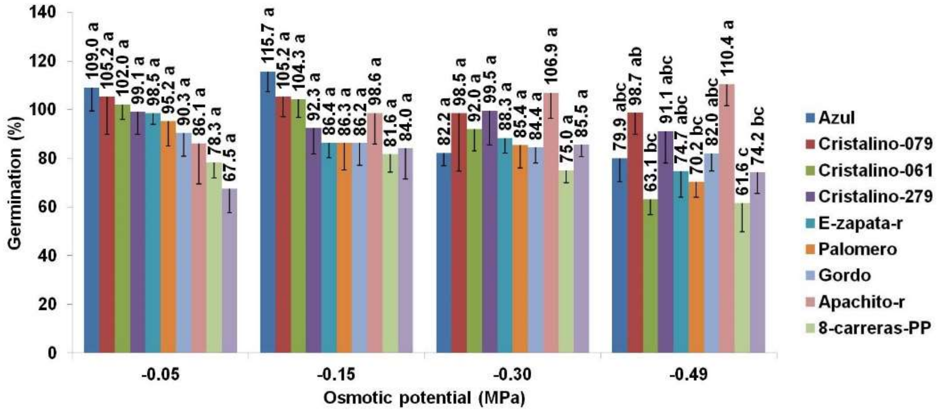


Figure 1. ANOVA for germination (%) effects by the osmotic potential of percentage data of ten maize genotypes evaluated in vitro conditions under different osmotic potentials generated by increasing concentration of PEG-6000. The control group is not shown because it is the 100% for each variety and for each trait. The *LSD* for the interaction (genotype*PEG) was calculated with the formula *LSD* = Distribution T (α-DF) * √EMS * 2 / n repetitions.

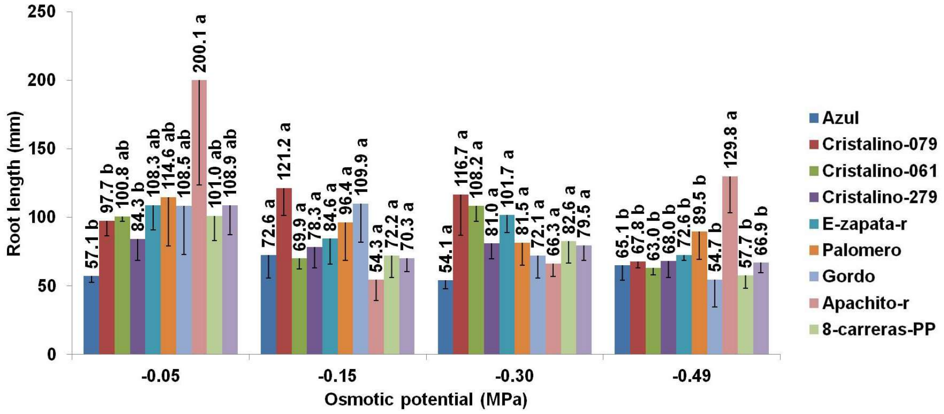


Figure 2. ANOVA for root length effects by the osmotic potential of percentage data of ten maize genotypes evaluated in vitro conditions under different osmotic potentials generated by increasing concentration of PEG-6000. The control group is not shown because it is the 100% for each variety and for each trait. The *LSD* for the interaction (genotype*PEG) was calculated with the formula *LSD* = Distribution T (α-DF) * √EMS * 2 / n repetitions.

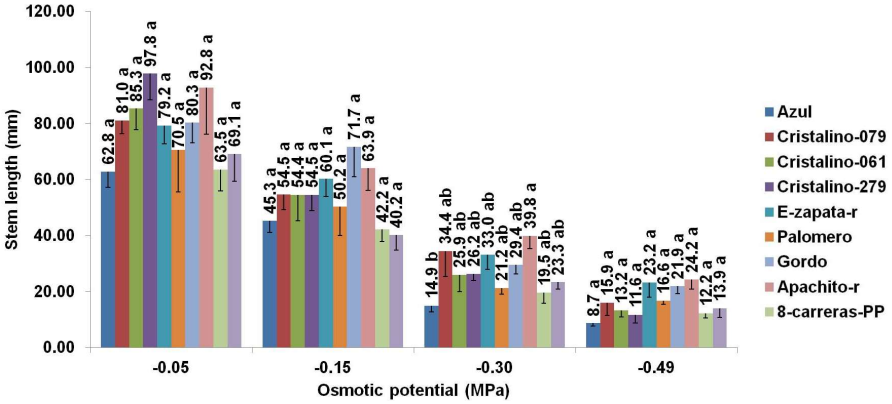


Figure 3. ANOVA for stem length effects by the osmotic potential of percentage data of ten maize genotypes evaluated in vitro conditions under different osmotic potentials generated by increasing concentration of PEG-6000. The control group is not shown because it is the 100% for each variety and for each trait. The *LSD* for the interaction (genotype*PEG) was calculated with the formula *LSD* = Distribution T (α-DF) * √EMS * 2 / n repetitions.

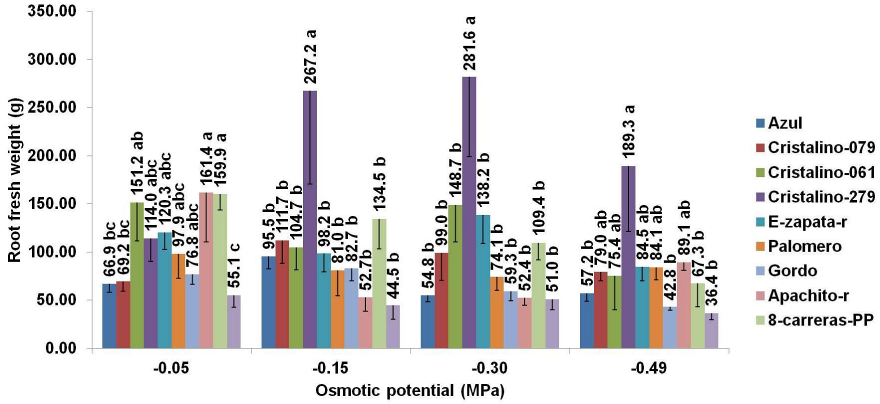


Figure 4. ANOVA for root fresh weight effects by the osmotic potential of percentage data of ten maize genotypes evaluated in vitro conditions under different osmotic potentials generated by increasing concentration of PEG-6000. The control group is not shown because it is the 100% for each variety and for each trait. The *LSD* for the interaction (genotype*PEG) was calculated with the formula *LSD* = Distribution T (α-DF) * √EMS * 2 / n repetitions.

Apachito-r outstands in root length at -0.05 OP increasing 200.1% compared to control. No significant differences were found at -0.15 and -0.30 between maize races; and at -0.49 only Apachito-r was different to the other races, increasing 129.8% respect to its control in water. The values for stem length were decreasing as the OP was increasing and only Apachito-r showed a significant difference at -0.30 MPa decreasing 39.8% respect to its control. Cristalino-279 showed significant difference in the variable root fresh weight and its value outstand at -0.15 increasing 267.2%, at -0.30 increasing 281.6% and at -0.49 MPa increasing 189.3% compared to the control in water. The variable root dry weight had the highest value for Apachito-r at -0.05 MPa increasing in

189.4%, decreasing at -0.15 in 72% and at -0.30 MPa in a 79.8% and increasing at -0.49 MPa in 112.3%. Also noteworthy are E-zapata-r increasing 190.5% and Cristalino-061 increasing 142.9% at -0.30. E-zapata-r at -0.49 increasing 115.1% compared to the control. Cristalino-279 showed significant difference in the variable stem fresh weight and its value outstand at -0.05, -0.15 and -0.30 MPa increasing 146.7%, 103.7% and 60.2% respectively. Finally, in stem dry weight the tendency was to decrease as OP was increasing, however Cristalino-279 showed differences at -0.30 decreasing in 89.5% and at -0.49 MPa increasing in 143.5% (Figs 1–7).

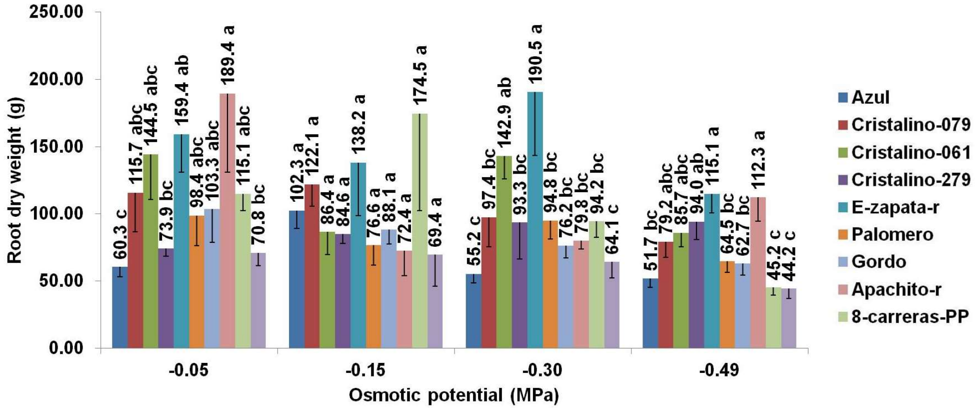


Figure 5. ANOVA for root dry weight effects by the osmotic potential of percentage data of ten maize genotypes evaluated in vitro conditions under different osmotic potentials generated by increasing concentration of PEG-6000. The control group is not shown because it is the 100% for each variety and for each trait. The *LSD* for the interaction (genotype*PEG) was calculated with the formula *LSD* = Distribution T (α-DF) * √EMS * 2 / n repetitions.

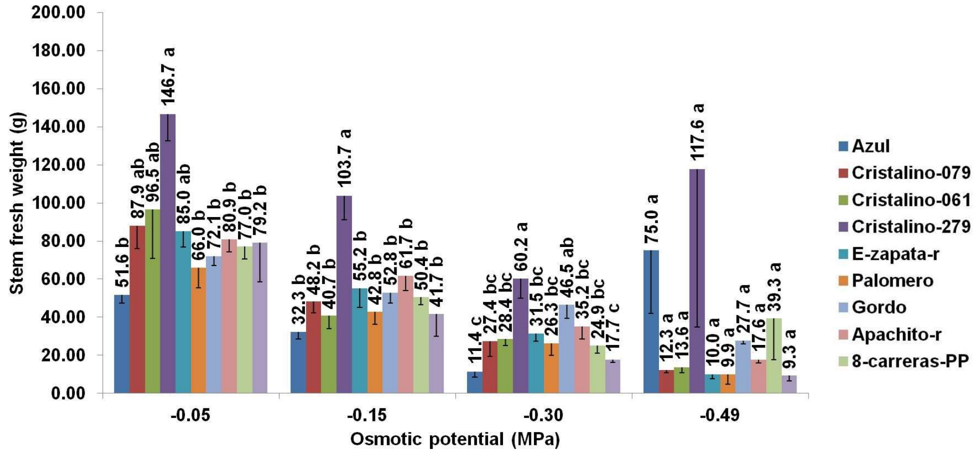


Figure 6. ANOVA for stem fresh weight effects by the osmotic potential of percentage data of ten maize genotypes evaluated in vitro conditions under different osmotic potentials generated by increasing concentration of PEG-6000. The control group is not shown because it is the 100% for each variety and for each trait. The *LSD* for the interaction (genotype*PEG) was calculated with the formula *LSD* = Distribution T (α-DF) * √EMS * 2 / n repetitions.

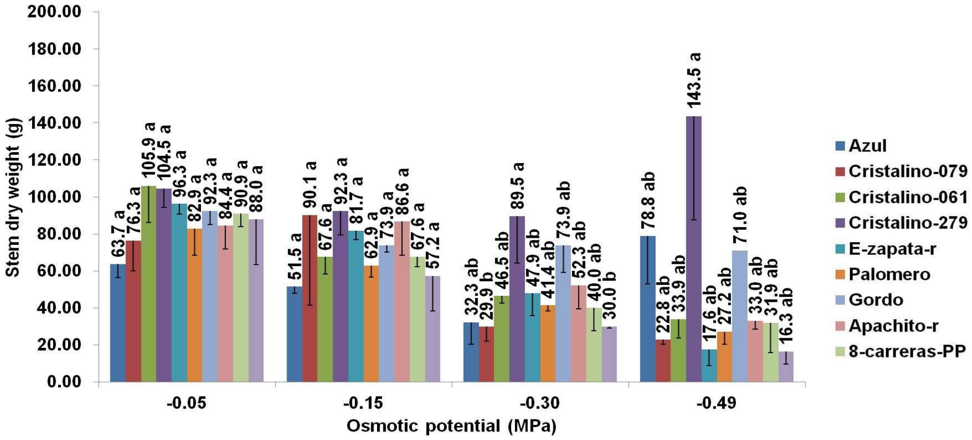


Figure 7. ANOVA for stem dry weight effects by the osmotic potential of percentage data of ten maize genotypes evaluated in vitro conditions under different osmotic potentials generated by increasing concentration of PEG-6000. The control group is not shown because it is the 100% for each variety and for each trait. The *LSD* for the interaction (genotype*PEG) was calculated with the formula *LSD* = Distribution T (α-DF) * √EMS * 2 / n repetitions.

Many studies have been carried out to search sources of drought tolerant maize using PEG-6000 (Bashir et al., 2016; Khayatnezhad et al., 2010; Magar et al., 2019). Sabagh et al. (2018) mention that due to drought stress, yield of maize reduce significantly, especially in Arid and Semi-Arid regions around the world. The use of drought tolerant maize genotypes has potential to stabilize the grain yield of maize (Sabagh et al., 2018). Therefore, developing cultivars tolerant to drought stress is challenging for breeders to face the future climate changing conditions (Sabagh et al., 2018; Iqbal et al., 2021). The main traits that have been used to evaluate drought tolerance with PEG-6000 are: germination, root length, stems length, fresh and dry weight of the root, fresh and dry weight of the stem (Bashir et al., 2016; Jimenez-Galindo et al., 2018; Ortega-Ortega et al., 2023). Other researchers have been measured number of crown roots, number of seminal roots, primary root length, number of lateral roots fresh root weight, dry root weight and these genetic variations can be used to develop high yielding drought tolerant maize genotypes through selection and conventional breeding approaches (Qayyum et al., 2012). Mexico is the primary center of origin and diversity for maize (*Zea mays* L.) (Hellin et al., 2014; SINGH et al., 2022). For this reason, it is extremely important to search for the best sources of drought tolerance in maize races from northern Mexico where the maize genotypes have been mostly exposed to severe droughts, perhaps for thousands of years. However, few studies have been carried out with maize races from Mexico.

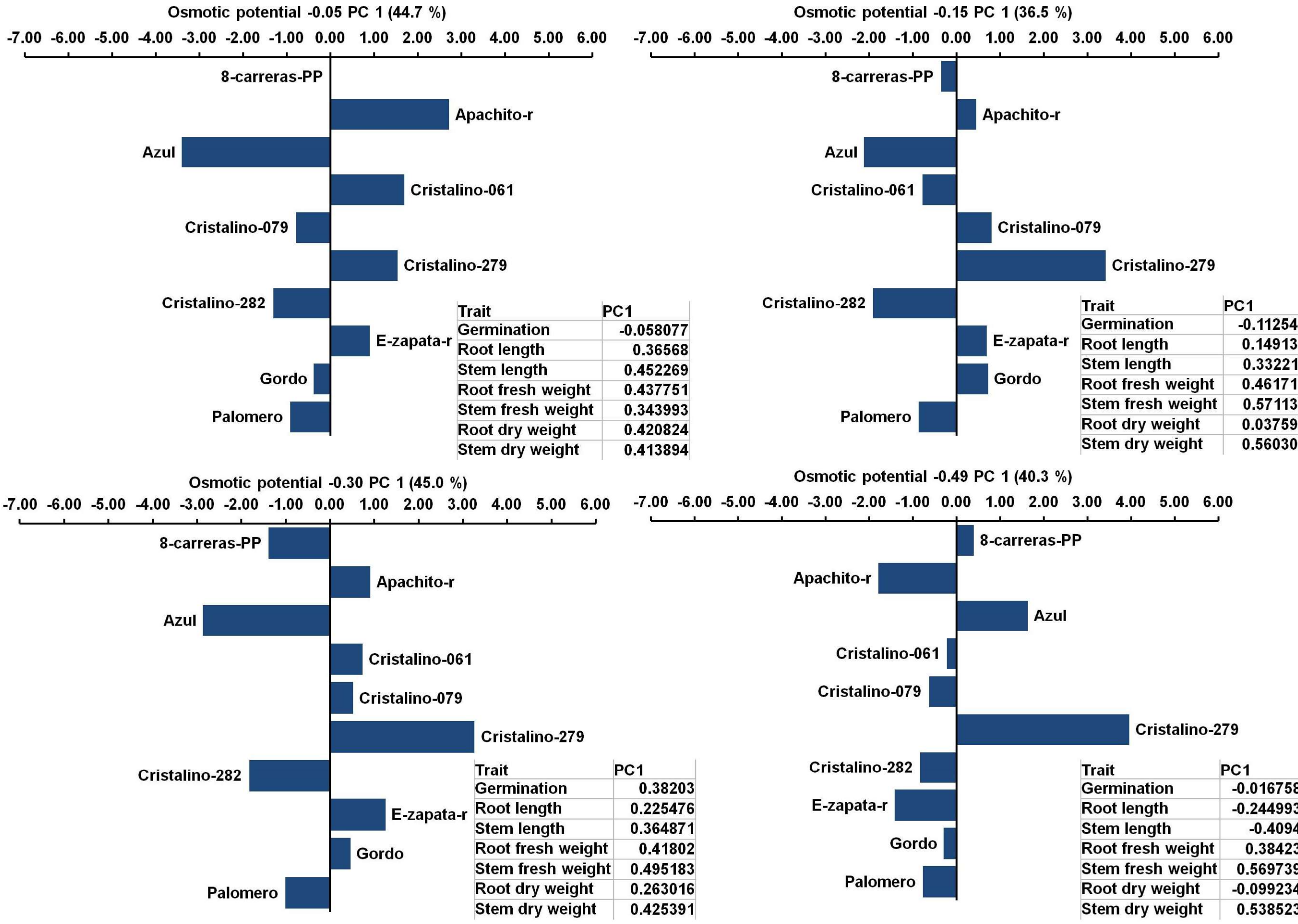
On the other hand, when evaluating root growth, it was also observed that the Apachito-r genotype showed greater length in root growth at -0.05 and -0.49 of OP. Our results are agree with (Zeng et al., 2019) that mention that the growth and development of maize roots are closely related to drought tolerance. While when evaluating the length of its stem, significant differences were only found at -0.30 of OP. These results are consistent, because the Apachito-r genotype has been reported as adaptable to drought stress conditions, but only in some period of its production cycle (Ruiz Corral et al., 2013), although no significant differences were found at all OP concentrations.

When evaluating the total fresh weight of the root, a greater weight was observed at -0.05 of OP, in the races: Cristalino-061, Apachito-r and 8-carreras-PP. In addition, the higher weight found in the Cristalino-279 genotype at -0.15, -0.30 and -0.49 from OP. However, it is necessary to highlight that not all the genotypes of the Cristalino de Chihuahua race presented a good response to drought as mentioned by (Ruiz Corral et al., 2013), highlighting only the Cristalino-279 genotype. Maize selection by farmers and by environmental factors led to the evolution of a large number of distinct Mexican maize races (Perales & Golicher, 2014). This is probably due to the great variability of Cristalinos de Chihuahua that has been preserved in the mountains of the state by the Tarahumaras. This native group, and with the help of the environment, have selected genotypes for different colors and their response to biotic and abiotic factors. This is also consistent because Mexico is the main center of origin for bean and maize (SINGH et al., 2022), as mentioned by (Jiménez-Galindo et al., 2023) where it is mentioned that maize Mexican races, have been exposed for thousands of years to adverse biotic and abiotic factors. We are agree with (Eagles & Lothrop, 1994) that mention that Mexican maize races is of ancient origin and has a distinct morphology, karyotype, and isozyme frequency. Previous research describes that the Cristalino de Chihuahua maize race tolerates varying degrees of WS during its production stages, and it is a race that commonly grows in conditions of low humidity and with high temperatures around 31 °C (Ruiz Corral et al., 2013). Therefore, it can be considered that the higher fresh weight of the root, which was observed in the Cristalino-279 genotype, is because of its greater tolerance to drought stress compared to Apachito-r, Cristalino-061 and 8-Carreras- PP, during root development.

In contrast, when evaluating root dry weight, the maize races with the highest weight were: Apachito-r, E. Zapata-r and Cristalino-061, the three maize genotypes, showed significant differences at -0.05 from OP; E. Zapata-r and Cristalino-061 at -0.30 and Apachito-r and E. Zapata-r at -0.49 of OP. Although it is known that the Cristalino-279 genotype obtained greater root weight when fresh, but not dry, it could be suggested that the root of Cristalino-279 in contrast to the races: Apachito-r, Cristalino-061 and E. Zapata-r, maintains a greater amount of water in its root, possibly because drought stress has induced the expression of proteins such as aquaporins (Chávez Suárez et al., 2014), these proteins regulate and transport water, under conditions of abiotic stress, such as drought. Therefore, it could be suggested that the lower amount of dry weight of the Cristalino-279 genotype is due to the fact that the evaporation rate was higher when subjected to dehydration, since possibly its roots are capable of retaining a greater amount of water, in contrast to the other races that obtained higher root dry weight.

Principal Component Analysis

On the other hand, when performing the principal component analysis, to evaluate the effects of the increasing osmotic potential (PEG-6000) of the maize genotypes in vitro, the principal component (PC1) explains 44.7% at -0.05 MPa, 36.5% at -0.15 MPa, 45.0% at -0.30 MPa and 40.3% at -0.49 MPa of OP. The PC1, when considered as an index of tolerance to drought, it was observed, that the maize races evaluated, with greater tolerance to drought at -0.30 and -0.49 of OP, were Cristalino-279, Azul and 8-carreras-PP, in contrast to Apachito-r, E-Zapata-r and Palomero, which proved to be maize races with greater susceptibility to drought at -0.49 MPa.



Trait	PC1
Germination	-0.058077
Root length	0.36568
Stem length	0.452269
Root fresh weight	0.437751
Stem fresh weight	0.343993
Root dry weight	0.420824
Stem dry weight	0.413894

Trait	PC1
Germination	-0.11254
Root length	0.14913
Stem length	0.33221
Root fresh weight	0.46171
Stem fresh weight	0.57113
Root dry weight	0.03759
Stem dry weight	0.56030

Trait	PC1
Germination	0.38203
Root length	0.225476
Stem length	0.364871
Root fresh weight	0.41802
Stem fresh weight	0.495183
Root dry weight	0.263016
Stem dry weight	0.425391

Trait	PC1
Germination	-0.016758
Root length	-0.244993
Stem length	-0.4094
Root fresh weight	0.38423
Stem fresh weight	0.569739
Root dry weight	-0.099234
Stem dry weight	0.538523

Figure 8. Effect of osmotic potential in ten maize genotypes evaluated *in vitro* under osmotic potential generated by an increasing co PEG-6000.

Apachito-r responds well at -0.05, -0.15 and -0.49 MPa. Therefore, it is observed that the best genotype for tolerance to drought was Cristalino-279, which maintained tolerance at all different levels of OP evaluated. Fig. 8 shows whether the genotypes respond positively or negatively as the OP level increases. Cristalino-279 responds positively (Fig. 8).

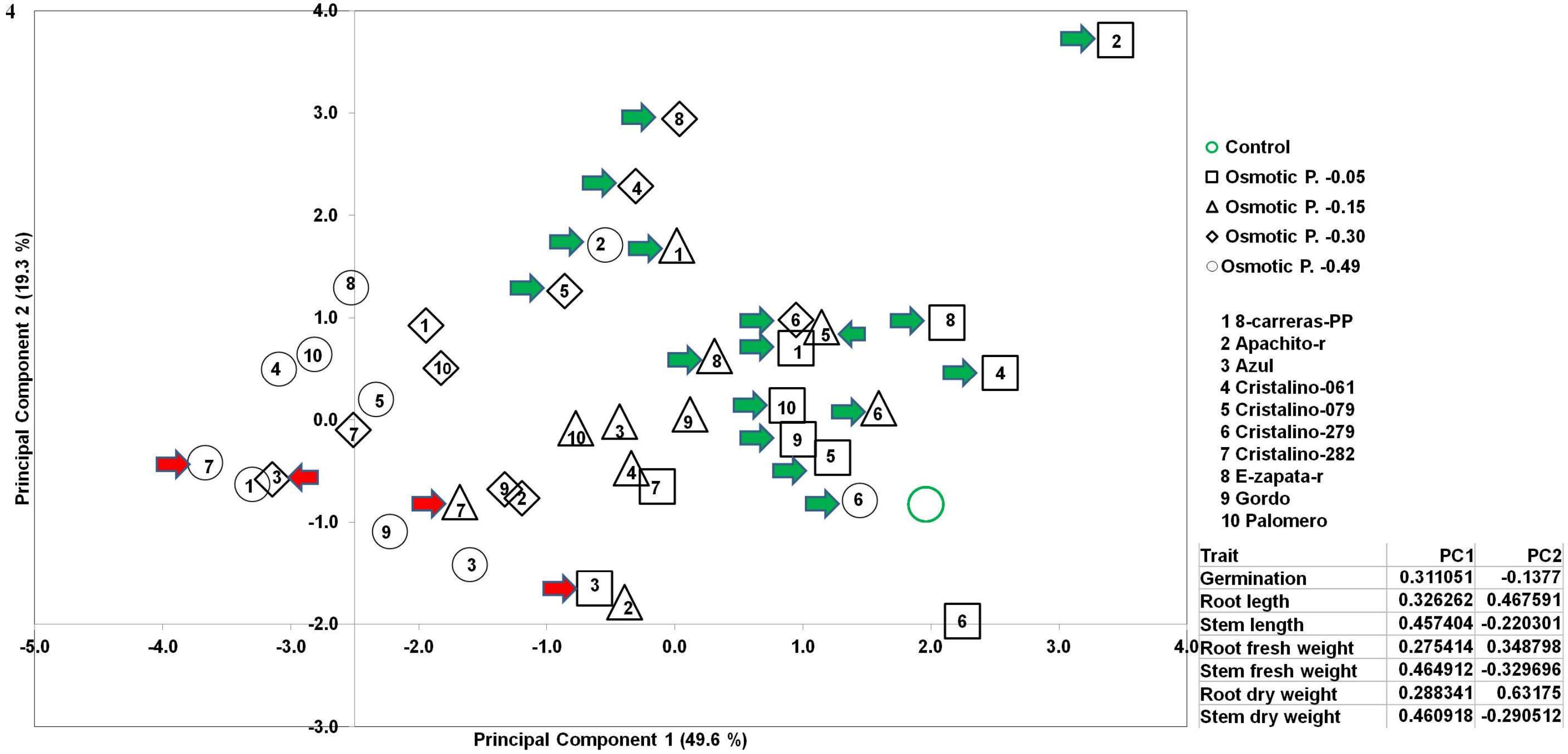
Research has been reported that in maize germination under drought stress, enzymes such as isocitrate lyase (ICL) and malate synthase (MS) are expressed, these belong to the glyoxylate cycle, which is activated under different phonological stages of the plant kingdom, including germination (Ihle & Dure III, 1972; Longo et al., 1975). Furthermore, it has also been shown that both ICL and MS are responsible for inhibiting the action of abscisic acid (ABA), a phytohormone responsible for seed dormancy; in contrast, the germination phytohormone gibberellic acid (GA) is activated (Paek et al., 1998). Therefore, it is probable that Apachito-r, Cristalino-079 and Cristalino-279 genotypes stand out in germination, under drought stress conditions, induced with PEG-6000, due to a higher expression of the enzymes ICL and MS, allowing a greater AG activity in the seeds. The drought tolerance mechanism may be associated with accumulation of osmoprotectants as proline and soluble sugars (Mohammadkhani & Heidari, 2008). The accumulation of soluble sugars is strongly correlated to the acquisition of drought tolerance in plants (Hoekstra et al., 2001).

However, the enzymatic activity of the Mexican maize races mentioned should be studied in the future.

On the other hand, when evaluating the fresh weight of the stem, the best genotype in all the levels induced by drought with PEG-6000, was the Cristalino-279 genotype. Similarly, when evaluating stem dry weight, significant differences were observed at -0.30 and -0.49 of OP, which favor higher, stem dry weight again to the Cristalino-279 genotype. As can be seen, in this evaluated parameter of dry and fresh weight of the stem, Cristalino-279 again obtained greater weight, due to the fact that the Cristalino race is reported as one of those that best adapts to drought stress conditions (Ruiz Corral et al., 2013). Although the other selected races are high-yielding, it is consistently shown that the Cristalino-279 genotype excels in stem weight, both fresh and dry, which could suggest that in addition to retaining a greater amount of water in the stem, could also be a great producer of biomass and therefore, the possibility that it excels in grain yield, although the necessary evaluations are still to be carried out to verify this parameter. Finally, it can be suggested that the races with the highest yield, in different parameters evaluated, were both Apachito-r and Crisitalino-279, both maize races, reported in previous studies (García-Lara & Serna-Saldivar, 2019), also native to the Sierra de Chihuahua. We are agree with (Wen et al., 2012) about the knowledge of genetic diversity within and among maize races is essential for using them in plant breeding.

The maize races with the best response for the variable root length, under drought conditions at -0.49 OP, were the genotypes: Cristalino-279, Azul and 8-carreras-PP. Apachito-r responds well at -0.05, -0.15 and -0.30 (Fig. 9).

Figure 9. Effect of osmotic potential in ten genotypes of maize evaluated *in vitro* under osmotic potential generated by an increasing concentration of PEG-6000. Green arrows show longer roots at -0.49 of OP, and red arrows show shorter roots at -0.49 MPa.



Trait	PC1	PC2
Germination	0.311051	-0.1377
Root legth	0.326262	0.467591
Stem length	0.457404	-0.220301
Root fresh weight	0.275414	0.348798
Stem fresh weight	0.464912	-0.329696
Root dry weight	0.288341	0.63175
Stem dry weight	0.460918	-0.290512

Figure 10. Plot of PC1 vs PC2 for ten maize genotypes and four levels of OP. Green arrows show genotypes with best response to OP, and red arrows show the genotypes with the poor response when OP was increasing.

On the other hand, when performing the principal component analysis at the four OP levels (-0.05, -0.15, -0.30 and -0.49), between PC1 (49.6%) and PC2 (19.3%). It was observed that the genotypes with the best response to the OP at -0.05 were Apachito-r, E-Zapata-r, Cristalino-061, 8-carreras-PP, Palomero, Gordo and Cristalino-079 but when subjected to OP values of -0.15, the best were 8-Carreras-PP, Cristalino-079, Cristalino-279 and E-Zapata-r and, although when increasing the OP to -0.30, the more tolerant were E-zapata-r, Critalino-061, Cristalino-279 and Cristalino-079. Finally, it was shown that when increasing the OP to -0.49, the genotypes with the highest resistance proved to be in order of greater than less tolerance to drought: Cristalino-279 and Apachito-r. In contrast, it was shown that the genotypes most susceptible to drought for OP levels of -0.05, -0.15, -0.30 and -0.49 were: Azul, Cristalino-282, Azul and Cristalino-282, respectively (Fig. 10).

Cristalino-279 shows the best level of drought tolerance at all levels of osmotic pressure, this genotype will help to develop maize drought resistance breeding in Mexico and the world. In addition, to carry out genetic research and improvement in maize we are preparing a MAGIC population, with this races, to study the genomic regions for biotic and abiotic stresses, among them drought tolerance, and in addition grain quality and agronomic traits in a future. Now we have the first recombination with crosses Cacahuacintle × Bofo × Cristalino-079 × Cristalino-279 × Azul × Cristalino-282 × Apachito-r × Apachito-b.

CONCLUSIONS

The most drought tolerant genotypes were Cristalino-279, Apachito-r, Azul and 8-carreras-PP. In contrast to Cristalino-282, the genotype most susceptible to drought. The most tolerant genotypes showed greater root length, greater root fresh and dry weight, better germination and greater stem length. Susceptible genotypes have shorter root length, lower fresh and dry root weight, lower germination and shorter stem length. Resistant and susceptible genotypes are ideal material to understand the physical and chemical mechanisms that are related to drought tolerance. Cristalino-279 shows the best level of drought tolerance at all levels of osmotic pressure and this genotype can be used as a source of drought tolerance for the improvement of commercial maize. In future research we should focus on mapping the genomic regions responsible of drought tolerance using PEG method for phenotyping MAGIC populations from Mexican maize races.

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