ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

Effect of PEG8000 and NaCl on germination and seedling traits of tropical maize (*Zea mays* L.)

Efecto del PEG8000 y el NaCl en la germinación y características de plántulas de maíz tropical (*Zea mays* L.)

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Abstract. Among the different types of abiotic stresses, water is the most damaging for crops. In the current research, the effect of water stress, induced with Polyethylene Glycol 8000 (PEG8000) and Sodium chloride (NaCl) solutions, was studied in maize genotypes. A randomized block design (RBD) with a factorial arrangement and four replications of 25 seeds each was used. The studied maize genotypes were RC_2C39, RC_2C51 (wild varieties of the State of Tabasco backcrossed with the endogamic line CML247 of the International Center for the Improvement of Maize and Wheat), wild Tamulte, VS536, and Asgrow 7573 hybrid, study osmotic pressures (OP) were 0, -1.0, and -2.0 MPa. The percentage seed germination (G%), and seedling length (SL, in cm), root length (RL, in cm), fresh seedling weight (FSW, in g), and fresh root weight (FRW, in g) were evaluated on seedlings after 12 days of treatment imposition in the five corn genotypes (*Zea mays* L.). The results showed significant differences in the study variables before and after the stresses as the concentrations of PEG8000 and NaCl increased. In general, the RC_2C39, RC_2C51, and VS536 genotypes had a better response to PEG8000 and NaCl as compared to wild Tamulte and Asgrow 7573 hybrid for all measured variables. The OP of -2.0 MPa is suggested to separate drought-tolerant from intolerant corn cultivars in the Mexican tropical areas.

Keywords: Water stress; Germination; Osmotic potential; Drought-resistant maize.

Resumen. Entre los diferentes tipos de estrés abiótico, el estrés hídrico es considerado el más nocivo para los cultivos. En la presente investigación se estudió en un diseño de bloques al azar en arreglo factorial con cuatro repeticiones de 25 semillas cada una, el efecto del estrés hídrico simulado con soluciones de PEG8000 y Cloruro de sodio (NaCl) en los genotipos de maíz RC_2C39, RC_2C51 (criollos del estado de Tabasco retro-cruzados con la línea endogámica CML247 del Centro Internacional de Maíz y Trigo), Criollo Tamulte, VS536 y Asgrow 7573). Las presiones osmóticas (PO) fueron a 0, -1.0 y -2.0 MPa. Se evaluó el porcentaje de germinación (%G), longitud de plántula (LP en cm), longitud de la raíz (LR, en cm), peso fresco de plántula (PFP, en g), y peso fresco de raíz (PFR, en g), a los 12 días después de la siembra en cinco genotipos de maíz (*Zea mays* L.). Los resultados mostraron una respuesta diferencial significativa ante el estrés, en la medida que se incrementó la concentración de PEG8000 y NaCl. Los genotipos RC_2C39, RC_2C51 y VS536, respondieron en general mejor a los dos productos osmóticos que el Criollo Tamulte y Asgrow 7573 en todas las variables medidas. Se sugiere usar la PO de -2.0 MPa para discriminar cultivares de maíz con tolerancia a sequía para el trópico Mexicano.

Palabras clave: Estrés hídrico; Germinación; Potencial osmótico; Maíz resistente a sequía.

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Received 22.X.2015. Accepted 29.IX.2016.

INTRODUCTION

The maize crop (*Zea mays* L.), together with wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), occupy most of the world cultivated area (Bonilla, 2008), and they generate the majority of agricultural labor. During 2014, 177 million hectares were planted with maize in 125 countries (FAOSTAT, 2014), yielding an approximate production of 948 million tons (Maluenda, 2014). This quantity of grain could be considered as exceptional when taking into account that a similar planted area in 2012/2013 led to a total production of 863 million tons (Maluenda, 2014).

In Mexico, the importance of maize is not only determined by its per capita consumption of 330 g/d, but also because the grain is used as raw material in industry, and it is consumed either indirectly or in the way of its byproducts (Anonymous, 2006). According to the *Sistema de Información Agroalimentaria y Pesquera* (Agri-food and Fishing Information System) (SIAP, for its acronym in Spanish, 2015), 7.5 million hectares were planted with maize during the 2014 agricultural cycle, which represent 51% of the cultivated area in Mexico (Naix'ieli-Castillo, 2014). Of this cultivated area 7.07 million were harvested, representing 94.6% of the total maize planted area (FND, 2014). On the basis of these numbers, Perea (2015) reported that Mexico was not only the world's eight maize producer in the 2014/15 cycle, but also the second major importer of such an important crop.

Of the total area cultivated with maize each year in Mexico, 85% is represented by temporary or seasonal land, leading to losses due to drought (CEFP, 2013). In the State of Tabasco, the predominating type of climate is warm and hot Am(f)"(i') g. Rainfall is abundant in summer (on average, between 2500 and 3000 mm of precipitation), and the mean annual temperature is 25 °C (Ruíz-Álvarez et al., 2012). However, maize crops are frequently affected by drought stress, whether at the germination, seedling, flowering, or grain filling stages of developmental morphology, all of which are essential for their survival in the land (Mokhberdoran et al., 2009).

Several studies have been conducted under water stress at the maize early stages. This is because of the importance of this crop for men, and since each year the areas where it is cultivated are prone to suffering drought periods. This emphasizes the importance of finding the adaptation mechanisms to this stress. Osmotic-regulating substances such as polyethylene glycol of 6000 and 8000 molecular weight (PEG6000, PEG8000), and sodium chloride (NaCl) have proved to be effective in generating drought conditions during the germination of seeds of forest species (Cordero & Di Stéfano, 1990); maize (Jia et al., 2001; Mohammdkhani & Heidari 2008; Shahriari et al., 2014); wheat (González et al., 2005; Sayar et al., 2010); rice (Mokhberdoran et al., 2009); pearl millet (Govindaraj et al., 2010), rye (Hamidreza et al., 2013); sorghum (Tsago et al., 2014), bean (Domínguez et al., 2014), and B*rassica juncea* (Toosi et al., 2014).

The purpose of this research was to evaluate the effect of water stress under laboratory conditions on the germination and growth of seedlings of five maize genotypes. This will contribute to identify possible differences among them that could be used as useful criteria in the selection of tolerant and susceptible species to water stress.

MATERIALS AND METHODS

The study was conducted under laboratory conditions at the Academic Division of Biological Sciences (DACBiologicas, for its acronym in Spanish) of the Universidad Juarez Autonoma de Tabasco (UJAT, for its acronym in Spanish), Mexico. The plant material comprised two backcrosses (RC_2C39 x CML247 and RC_2C51 x CML247), wild Tamulte, the synthetic variety VS536, and the Asgrow7573 hybrid. Seeds were disinfected using a commercial 5% chloride solution for 5 minutes. Then, they were rinsed with bi-distilled water to remove the chloride excess. The seeds of each genotype were immediately placed in each solution of PEG8000 or NaCl with osmotic potentials of 0, -1.0, and -2.0 MPa during 48 h. After the pre-conditioning period concluded, 25 seeds of each genotype were placed on blotting paper (Sanitas Mexico), covered with another piece of paper dampened with bi-distilled water to be later wrapped to form rolls that were placed in aluminum trays. The osmotic potentials for PEG8000 were determined using the method described by Michel & Kaufmann (1973). For NaCl, osmotic solutions were prepared on the basis of the Van't Hoff equation (Ben-Gal et al., 2009).

The experiment was carried out using a randomized complete block design (RCD) with a factorial arrangement and four replications of 25 seeds each per treatment. The trays with the experimental material were kept in the laboratory at a day temperature of 23 °C, with a 12 h light-12h darkness cycle.

Treatments were maintained for up to 12 days, time at which the following measurements were made:

Germination Percentage (G%): Defined as the final number of germinated seeds x 100. The data obtained were arcsine transformed $\sqrt{Y + 0.5}$.

Seedling Length (SL, in cm). It was measured from the stem base to the tip of the main leaf in five randomly selected seedlings, both by treatment and repetition.

Root Length (RL, in cm). Measured from the stem to the tip of the main root in five randomly selected seedlings, both by treatment and repetition.

Fresh Seedling Weight (FSW, in g). Total weight of the coleoptile of five randomly selected seedlings, both by treatment and repetition. Weight was determined using an Ohaus digital scale.

Fresh Root Weight (FRW, in g). The roots of five randomly selected seedlings, both by treatment and repetition, were weighted with an Ohaus digital scale.

Analysis of variance and multiple mean comparisons (Duncan P<0.05) were carried out in SAS 9.04 version for Windows (2004).

RESULTS

The analysis of variance (Table 1) was only significant in the Solution x Osmotic Potential (SO x OP) interaction for the fresh seedling weight (FSW) variable. In regards to the Genotypes x Osmotic Potential (G x OP) interaction, there was significance in the seedling length (SL), root length (RL), and fresh seedling weight (FSW) variables, while for the Genotypes x Osmotic solutions (G x SO) interaction, the fresh seedling weight (FSW) and fresh root weight (FRW) variables were significant. The five variables measured showed significant in the source of variation Genotypes (G), Osmotic Solutions (SO) and Osmotic Potentials (OP); there was no significance in the fresh root weight (FRW) and fresh seedling weight (FSW) variables, at other measured variables were observed significance. The coefficients of variation (CV) gave acceptable values, with the exception of fresh root weight (FRW) which was high (28.84%).

In general, and in accordance to the Duncan mean comparison test (P≤0.05) (Table 2), the two backcrosses (RC_2C39 and RC_2C51) and the synthetic variety (VS536), surpassed wild Tamulte and the hybrid Asgrow7573 in terms of the variables measured.

When comparing in terms of osmotic pressures, it can be seen (Fig. 1) that the highest germination percentage was observed with bi-distilled water (0 MPa), and that both wild Tamulte and Asgrow7573 showed lower percentages of germination at this potential. The increase in osmotic potential reduced the germination percentage (Fig. 1) proportionally in all the evaluated genotypes.

Table 1. Analysis of variance for germination percentage (G%), seedling length (SL), root length (RL), fresh seedling weight (FSW), and fresh root weight (FRW).
Tabla 1. Análisis de varianza para porcentaje de germinación (PG), longitud de plántula (LP), longitud de raíz (LR), peso fresco de plántula (PFP), peso fresco de raíz (PFR).

FV	Gl	G%	SL	RL	FSW	FRW
G	4	0.334 **	117.489 **	31.012 **	6.927 **	0.871 **
R/G	15	0.039**	11.133 ns	8.202 ns	0.855 ns	0.262 ns
SO	1	0.089 **	44.287 **	44.165 **	2.945 **	0.310 ns
G x SO	4	0.030 ns	0.820 ns	17.263 ns	1.845 **	0.308 **
OP	2	4.472 **	13.778 ns	535.308 **	0.229 ns	12.643 **
G x OP	8	0.031 ns	17.800 *	20.873 **	1.683 **	0.261 ns
SO x OP	2	0.034 ns	20.432 ns	11.422 ns	2.297 **	0.216 ns
G x SO x OP	8	0.008 ns	4.271 ns	8.997 ns	0.486 ns	0.112 ns
EE	75	0.017	8.106	8.487	0.548	0.167
CV (%)		15.47	16.20	22.08	20.55	28.84

*, ** Significant differences for P<0.05 and 0.01, respectively; ns=non significant, G= Genotypes, R/G=Repetitions nested in Genotypes, OS= Osmotic solutions, PO= Osmotic pressures, EE= Experimental error, CV= Coefficient of variation in percentage.

Table 2. Average values for the variables measured in five tropical maize cultivars (*Zea mays* L.) exposed to osmotic potential.
Tabla 2. Promedios para las variables medidas en cinco cultivares de maíz tropical (*Zea mays* L.) sometidos a tres sustancias osmóticas.

Genotypes	G(%)	SL	RL	FSW	FRW
RC_2C39 x CML247	59.33 a	19.39 a	13.28 ab	3.78 ab	1.32 b
RC_2C51 x CML247	59.83 a	19.39 a	13.25 ab	4.22 a	1.68 a
VS536	61.33 a	18.01 ab	14.32 a	3.60 b	1.48 ab
wild Tamulte	47.67 b	14.03 c	13.80 a	2.74 c	1.17 b
Asgrow7573	40.00 c	17.06 b	11.32 b	3.68 ab	1.42 ab

Duncan's multiple range test (P≤0.05); equal letters indicate statistical similarity.
G(%)=Germination Percentage; SL=Seedling length; RL=Root length; FSW=Fresh seedling weight; FRW=Fresh root weight.

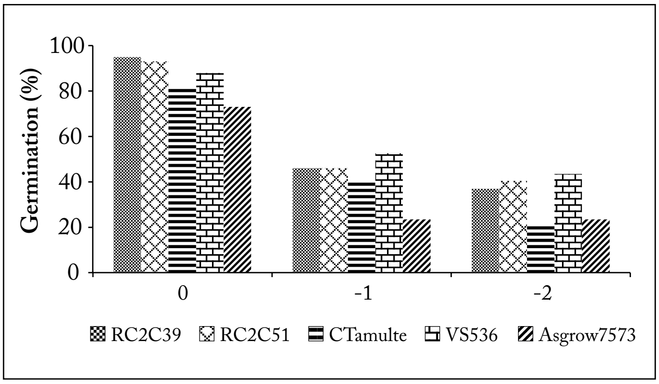

Fig. 1. Response to germination of tropical maize cultivars to different osmotic pressures (MPa).
Fig. 1. Respuesta a la germinación de cultivares tropicales de maíz a diferente presión osmótica (MPa).

Figure 2 shows the response of genotypes to germination percentage in the two solutions evaluated (PEG8000 and NaCl). Note (Fig. 2) that though small, the difference in germinated seeds was greater in the first than in the second osmotic solution. However, when comparing among genotypes, RC_2C51 and VS536 had a higher germination in NaCl.

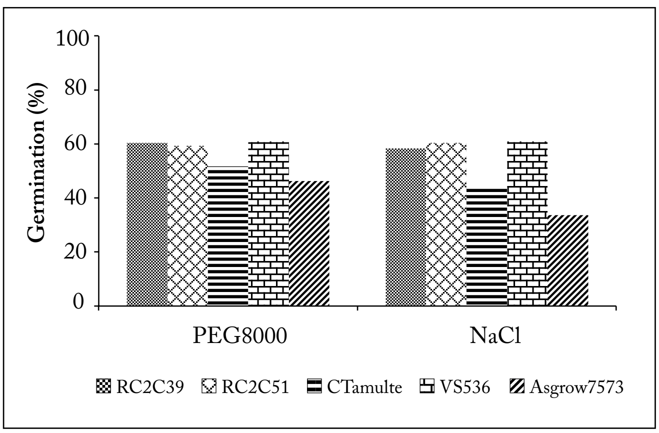

Fig. 2. Response to germination of tropical maize cultivars to two osmotic substances.
Fig. 2. Respuesta a la germinación de cultivares tropicales de maíz a dos substancias osmóticas.

The response of the evaluated genotypes in terms of seedling length was greater for the five genotypes in the PEG8000 condition *versus* the NaCl condition (Fig. 3). In the case of root length, the behavior of the genotypes was the same, that is, greater in PEG8000, with the exception of RC_2C39 that had a slightly higher root length in NaCl than in PEG8000.

All the genotypes showed a similar behavior (Fig. 4) in regards to the seedling fresh weight (SFW) and fresh root weight (FRW) variables. That is, the average of both variables was greater in PEG8000, with the exception of RC_2C39 and VS536 that had a greater Fresh weight of seedling (FWS) and Fresh root weight (FRW) in the NaCl solution.

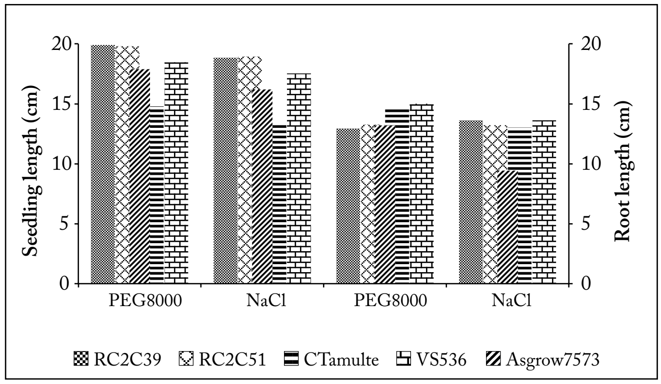

Fig. 3. Effect of two osmotic substances on seedling length (SL) and root length in five tropical maize cultivars.
Fig. 3. Efecto de dos substancias osmóticas en longitud de plántula (LP) y longitud de raíz (LR) en cinco cultivares tropicales de maíz.

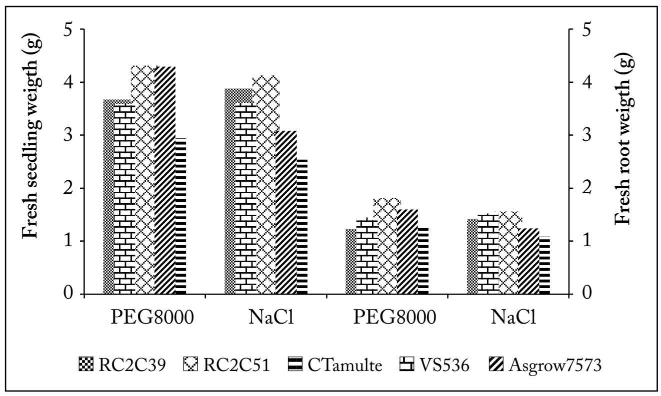

Fig. 4. Effect of two osmotic substances on the fresh seedling weight (FSW) and Fresh root weight (FRW) in five tropical maize cultivars.
Fig. 4. Efecto de dos substancias omóticas en peso fresco de plántula (PFP) y preso fresco de raíz (PFR) en cinco cultivares tropicales de maíz.

DISCUSSION

The differences found for genotypes (G), treatments (OP), and the genotype x treatments (G x OP) interaction agree with those reported by Tsago et al. (2014). These authors found differences *in vitro* in length of coleoptile and root length in six sorghum genotypes tested for drought tolerance using PEG6000. This is also similar to results of Castro-Montes et al. (2009) on seedling length and number of leaves per maize sprouting with the addition of PEG8000. Results similar to those found in this research were reported for husk tomato (*Physalis ixocarpa* Brot.) by Marín et al. (2007) with several osmotic agents, including PEG8000.

The results of this research agree with those reported by Al-Taisan et al. (2010) who, while evaluating osmotic pressures lower than the ones used in this study, found that germination decreased both with PEG8000 and NaCl. At a -1.2

MPa osmotic pressure of PEG8000, these authors reported 39.0% of germination, and 35.0% at -1.5 MPa, while it was even lower when using NaCl. In this research, germination percentages with PEG8000 averaged 55.73% for both levels (-1.0 and -2.0 MPa), and 51.41% for NaCl. These values exceed those reported by Mendez Natera et al. (2011). Biasutti & Galiñanes (2001), who evaluated polyetilenglicol 8000 (-1.5 MPa) in corn found that germination and coleoptile length was more affected by the osmotic potential that in our research. Domínguez et al. (2014) found that germination decreased as the osmotic potential was reduced in all the bean genotypes that they assessed. The decreased germination when osmotic potential is lower (-2.0 MPa) is possibly due to a toxic reaction of both PEG8000 and NaCl. These results are similar to those observed by Rangel-Fajardo (2014) when they exposed maize seeds to different concentrations and inhibition times of PEG8000. Another possible explanation of the decreased germination could be attributed to the fact that high concentrations of PEG8000 and NaCl prevent the absorption of water by seeds, caused by the high osmotic potential developed in the solution, and to a reduction in available oxygen by limiting its solubility and diffusion, because of the high viscosity of PEG8000 (Silmara & Juliano, 2004).

On the other hand, Sayar et al. (2010) found differences by variety in germination percentages. In their report, these authors point out that NaCl had a lower effect in germination than PEG8000 in the two varieties of wheat evaluated in their study. The contrasting behavior of the genotypes that they evaluated in regards to those obtained in the current research with maize (NaCl concentrations had a higher effect on maize varieties), could be due to the use by such authors of osmotic potentials lower than those assessed in this study (the highest osmotic potential these authors used was -0.8 MPa). Also the differences observed in both results could be due to intrinsic responses of each genotype as it was reported by Al-Taisan et al. (2010) in E*phedra alata*, a bushy growth type variety in Arabia, where both PEG8000 and NaCl affected germination at lower osmotic potentials.

The reduction in growth of the seedling and roots at a lower osmotic potential (-2.0 MPa) agrees with results reported by Shahriari et al. (2014) in sweet maize, Domínguez et al. (2014) in bean, González et al. (2005) in the wheat variety INIFAT RM-36, and Radhouane (2007) in pearl millet. In these reports, an increase of osmotic potential in the solution where the plants grew, both the seedlings and roots exhibited a lower growth as well as a decrease in the fresh weight of the stem and root, similarly to our study. In their work, González et al. (2005) pointed out that use of PEG6000 has a potential use in selecting drought-tolerant genotypes at early stages of genetic improvement programs aimed at enhancing resistance to drought.

CONCLUSIONS

The variables measured in tropical maize cultivars subjected to simulated drought using PEG8000 and NaCl were considerably affected. A differential behavior was observed among the evaluated cultivars, with the two backcrosses (RC_2C39 and RC_2C51) and VS536 standing out mainly as the most tolerant in terms of germination and seedling length. The extremely poor behavior of Asgrow7573 was due to the fact that it is a hybrid genotype formed by progenitors that are not suitable for drought conditions.

The three salient genotypes will be evaluated with osmotic potentials of -2.0, -3.0, and -4.0 MPa of PEG8000 to increase tolerance to drought. This is because the referred cultivars possess a certain degree of tolerance to osmotic stress conditions, and because in accordance to the results obtained, PEG8000 was more effective than NaCl.

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