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Osmotic stress tolerance in forage oat varieties (*Avena Sativa* L.) based on osmotic potential trials

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Abstract. Forage oats (*Avena sativa* L.) are globally important for milk and meat production, and, to a lesser extent, for the human diet. In Mexico, oats are a strategic crop, occupying the fourth place in cultivated area, only after maize for grain, bean, and sorghum for grain. Droughts are the main problem for oat production in Mexico. This study evaluated the germination and seedling growth of several oat varieties in response to drought stress simulated by PEG-6000 treatments of different osmotic pressure in order to identify drought-resistant genotypes. The Teporaca genotype was the most outstanding in the three levels of OP compared to its control with 0.0 of Osmotic Potential (OP). The Teporaca genotype showed the largest root length and the lowest diminishment of root length under osmotic stress conditions. This genotype also had the largest shoot length in the three osmotic stress levels. Regarding root fresh weight, Babicora stands out with 98.5% and Teporaca with 43% in the most severe level. Teporaca, Menonita, and Babicora showed the outstanding root dry weights of 346.5%, 327.2%, and 251.2%, respectively. These varieties had higher root dry weight than their own controls in water in the most severe level of OP. In conclusion, the Teporaca, Menonita, and Karma genotypes showed the highest osmotic stress tolerance and could be used as sources of favorable alleles to improve oat drought tolerance.

Key words: *Avena sativa*, polyethylene glycol 6000, osmotic pressure.

INTRODUCTION

Forage oats (*Avena sativa* L.) are the sixth most important cereal in the world, right after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), barley

(*Hordeum vulgare* L.), and sorghum (*Sorghum bicolor (*L.) Moench) (Mariscal-Amaro et al., 2009; Basha, 2020).

Drought is one of the most important problems for worldwide oat production (Farooq et al., 2009; Canales et al., 2021). In Mexico, 670,527 ha were sown with forage oats in 2020. Oats are a strategic crop, occupying the fourth place in terms of cultivated area, only after maize for grain (7,472,356 ha), bean (1,711,962 ha), and sorghum for grain (1,484,126 ha) (SIAP, 2021).

Forage oats are cultivated mainly in rainfed conditions (534,898 ha), representing 79.7% of the total cultivated area in México (SIAP, 2021; Salmerón, 2000; Amado et al., 2000; Osuna-Ceja et al., 2013). In arid and semi-arid regions, drought stress is the main limitation for forage and grain yield, especially for non-irrigated oats (Zhao et al., 2021) and cereals (Batool et al., 2021). To improve drought tolerance, the characteristics of tolerant oat varieties can be genetically selected, such as the leaf water potential and the capacity for osmotic adjustment (González et al., 2008). Moreover, plant precocity, although not necessarily described as a response to osmotic adjustment, is also a physiological characteristic that responds to drought (González et al., 2008).

Under severe water deficit, plants increase their synthesis of abscisic acid (ABA), which reduces the plant cycle and generates a larger concentration of photoassimilates destined for grain production (Maldonado et al., 1997; Coelho et al., 2020). The root is the first organ exposed to the drying soil and the origin of the drought tolerance response (Schachtman & Goodger, 2008). The lack of water in the soil can increase the synthesis of ABA in the roots, which is transported to the bud, causing stomatal closure (Li et al., 2020). In addition, the lack of water can lead to morphological and physiological changes in plants (Canales et al., 2019); in a moderate water deficit, plants tend to reduce the leaf area to minimize water loss and increase root growth (Coelho et al., 2020). To tolerate the osmotic stress of sodic-saline soils, plants also generate proline, which is synthesized in sub-cellular leaf and root compartments (Maldonado et al., 1997; Liu et al., 2020).

Water deficit and nitrogen deficiency (N_2) are the two most important factors limiting physiological activities in crops (Li et al., 2020). In oats, a severe water deficit causes an earlier increase in N_2 absorption, reduces the N_2 daily accumulation rate, and shortens the vegetative cycle (Coelho et al., 2020).

Low molecular hydrophilic substances collectively called osmolytes including mannitol, sugars, and salts modulate osmotic pressure of cell cytoplasm, have been used to control the osmotic pressure, but they have serious disadvantages; for example, they are subject to microbiological decomposition and affect plant metabolism (Kaul, 1966). Hydric stress conditions can be simulated in vitro by using polyethylene glycol (PEG-6000) in controlled environments. The use of PEG-6000 is effective for evaluating genotypes with osmotic stress tolerance at the seedling stage (Michel & Kaufmann, 1973; Basha, 2020). This method has been shown to be efficient in species such as corn, barley and rice (Lu & Neumann, 1998), sorghum (Tsago et al., 2014), wheat (Jatoi et al., 2014), beans (Jimenez-Galindo et al., 2018) and oats (Basha, 2020).

Droughts are currently one of the main problems that prevent crop plants from expressing their genetic potential (Sánchez-Martín et al., 2012). Identifying sources of drought-tolerant germplasms (Sánchez-Martín et al., 2012) and developing cultivars with better drought adaptation is a priority in breeding programs (Canales et al., 2021). Due to the agroclimatic conditions of the Mexican highlands, especially the scarce and irregular distribution of precipitation, it is necessary to identify genotypes of drought-tolerant oats. Therefore, the purpose of this study was to evaluate osmotic stress tolerance in ten varieties of oats selected by the polyethylene glycol PEG-6000 method in order to identify tolerant parents that may improve commercial oat varieties.

MATERIALS AND METHODS

Plant material

Ten genotypes of *Avena sativa* L. with diverse genetic backgrounds were used (Table 1).

Table 1. Agronomic traits of ten genotypes of oats (*A. sativa*) evaluated for osmotic stress tolerance

Genotype	Source	Drought Response	Characteristics	Grain color	100 seeds weight (g)
Babicora	INIFAP	Tolerant	Precocity	Brown	32.9
Bachiniva	INIFAP	Tolerant	Precocity and high yield	Light brown	36.3
Cuauhtemoc	INIFAP	Unknown	High yield	Light creamy brown	33.0
Cusarare	INIFAP	Unknown	High yield	Pearly	36.9
Karma	INIFAP	Tolerant	Tolerant to rust fungi	Light brown	34.4
Menonita	INIFAP	Moderate tolerant	Resistant to rust fungi	Light yellow	34.2
Papigochic	INIFAP	Unknown	Intermediate cycle	Light brown	28.6
Tamo 386 (Tamo)	TEXAS	Unknown	Long cycle	White	32.9
Teporaca	INIFAP	Tolerant	Resistant to rust fungi	Creamy	33.0
Turquesa	INIFAP	Unknown	High yield	Creamy	32.3

INIFAP (National Institute of Forestry, Agriculture and Livestock Research).
Texas Agricultural Experiment Station, Texas A&M University.

Experimental design

This study was conducted under laboratory conditions, germinating seeds at 28 °C. The study design was completely randomized in a factorial arrangement. Factorial combinations were evaluated in 10 genotypes and four levels of osmotic potential (OP) with three repetitions and two experiments. The experimental unit was a Petri-dish with 10 seeds of each genotype. Levels of osmotic pressure were prepared as 0.0, -0.05, -0.15, and -0.30 MPa using PEG-6000, based on the equation given by Michel & Kaufmann (1973). The bioassays were performed in Petri-dishes of 9.5 cm in diameter with filter paper and 8 mL of a solution containing 0.0, 50.0, 100.0, or 150.0 g of PEG-6000. Seeds were considered germinated when the root or shoot had more than 10 mm in length. After seven days, roots and shoots of the seedlings were measured and weighed. Root and shoot tissues were placed on a stove at 35 °C for seven days and then weighed.

Statistical analysis

An ANOVA was performed using the GLM (General lineal model) procedure (PROC GLM) of the SAS (SAS Institute 2016). The sources of variation were the genotype, experiment, and the interaction genotype × experiment. Genotypes, experiment, and the interaction genotype × experiment were considered fixed effects. Individual ANOVAs were performed by stress levels. The Tukey test was used at $p < 0.05$ to compare means.

Principal Component Analyses (PCA) were carried out using SAS software (SAS Institute 2016). All data were previously standardized with mean = 0 and standard deviation = 1. The first component was used for ordering the genotypes because it explained most of the variability across the OP levels (OP1 = 51.9%, OP2 = 55.4%, and OP3 = 49.5% of the variability explained), and it was considered an osmotic stress tolerance index. Furthermore, 100 seeds for each genotype were weighed (Table 1).

RESULTS

Significant differences were found between varieties in almost all analyzed traits and OP, except for the following: root length at -0.05 and -0.30, shoot length at -0.05, root dry weight at -0.05 and -0.15, shoot fresh weight at -0.15, and shoot dry weight at -0.05 and -0.30.

No significant differences were found between experiments and genotype × experiment interaction at -0.05. No significant differences were found between experiments and genotype × experiment interaction, except for root fresh weight at -0.15.

Significant differences were found between experiments for shoot length, root fresh weight and shoot fresh weight at -0.30. Additionally, there were found significant differences in genotype × experiment interaction in germination and root fresh weight at -0.30.

The Teporaca genotype registered the lowest percentages of germination in all PEG concentration levels (Fig. 1), but the Teporaca germinated plants were more tolerant to osmotic stress than the rest of the varieties (Fig. 2). Moreover, the Teporaca genotype had larger shoot length and root fresh weight compared to the rest of the genotypes in all osmotic stress levels. The Babicora genotype also showed high root fresh weight in the third osmotic stress level (Fig. 1). Teporaca, Menonita, and Babicora had the highest root dry weight in the most severe level of osmotic stress evaluated. Regarding shoot fresh weight, Teporaca stands out in all the OP levels, and Babicora and Cuauhtémoc do so in the last OP level (Fig. 1).

Although there were no significant differences among groups in the osmotic potential of -0.15 and -0.30, the germination of the Teporaca genotype was better in -0.05 OP than in its control. The root and shoot length were much longer in the Teporaca genotype in all levels of osmotic stress. Babicora and Teporaca had the highest root fresh weight in the most severe level of osmotic stress. Teporaca and Menonita had the highest root dry weight in the most severe levels of osmotic stress (Fig. 1).

The results of PCA combining all osmotic pressures confirmed the results observed in the univariate analysis (Figs 1, 2 and 3). By osmotic pressure, the first PC1 explains 51.9% at -0.05 MPa, 55.4% at -0.15 MPa and 49.5% at -0.30 MPa. The genotypes respond positively or negatively to the increase in OP levels. According to the first principal component (which was considered as the tolerance index), the most tolerant genotypes at -0.05 MPa were Teporaca and Karma. The most outstanding genotypes at -0.15 MPa were Teporaca, Turquesa, and Karma. The most tolerant genotypes at -0.30MPa were Teporaca, Babicora, Menonita, and Karma. Tamo was the most susceptible genotype at all OP levels (Fig. 3). The PCA showed a positive response of the Babicora genotype: in the first level, the OP was negative; in the second level, it began to increase; and in the third level, it increased significantly. The Menonita genotype responded similarly. Conversely, Karma always responded positively (Fig. 3).

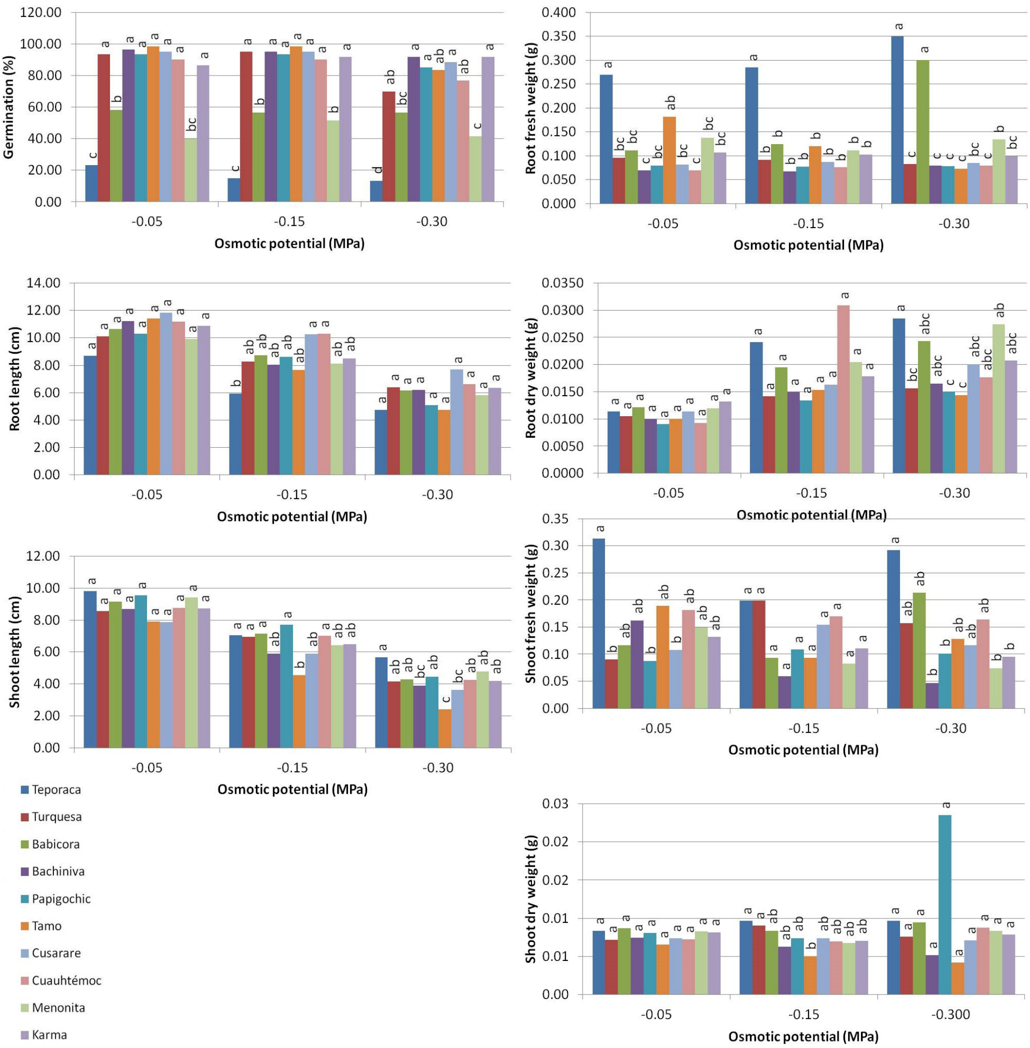

Figure 1. Effect of the different osmotic potentials generated by concentrations of PEG6000 of original data of on different germination traits of the ten oat varieties. The *LSD* for the interaction (genotype × experiment) was calculated with the equation $LSD = Distribution\ T\ (\alpha - DF) * \sqrt{EMS} * \frac{2}{n\ repetitions}$

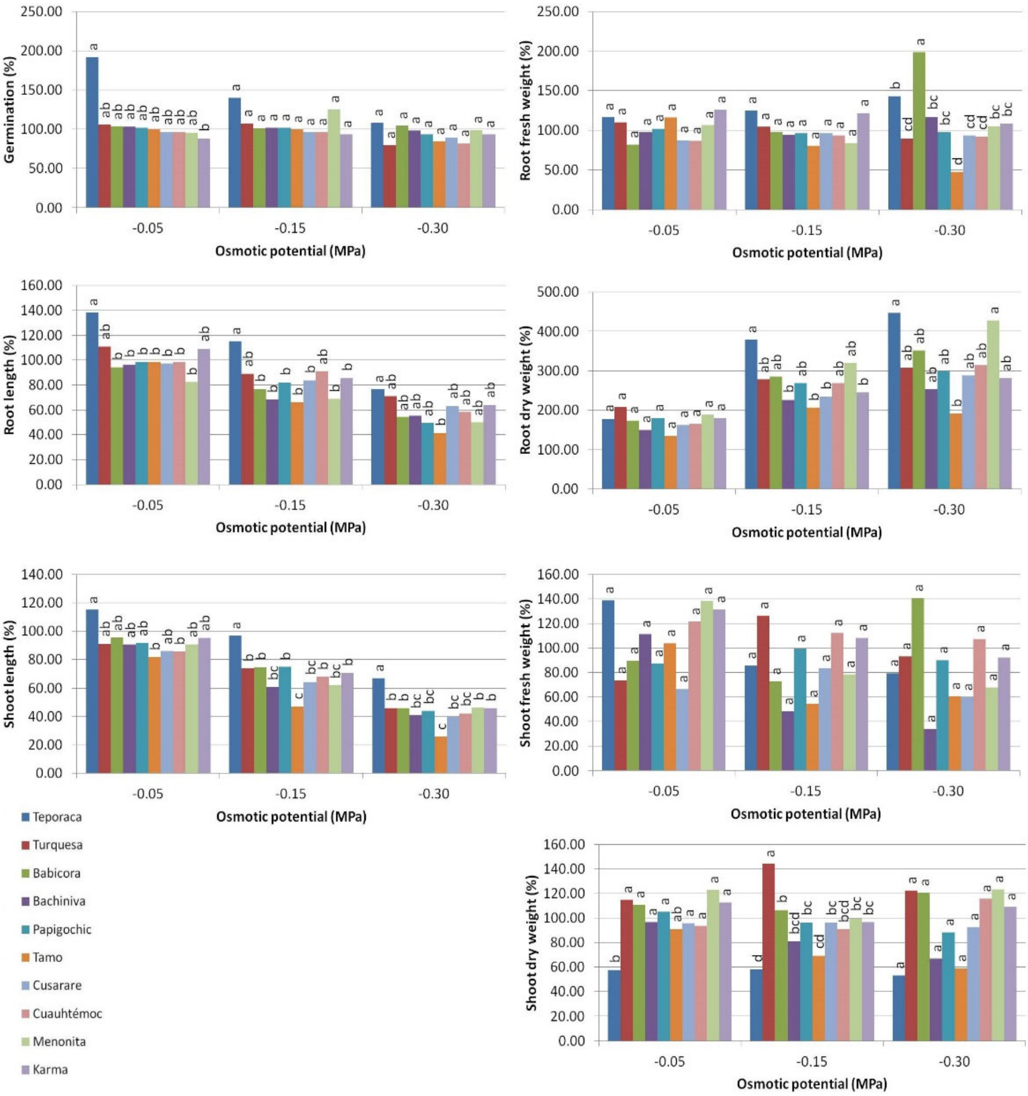

Figure 2. Effects of the osmotic potential on percentage data of ten oats varieties evaluated in vitro under an osmotic potential generated by increasing concentration of PEG6000. The control group is not shown because it is the 100% for each genotype and for seach characteristic. The *LSD* for the interaction (genotype × experiment) was calculated with the equation $LSD = Distribution\ T\ (\alpha - DF) * \sqrt{EMS} * \frac{2}{n\ repetitions}$.

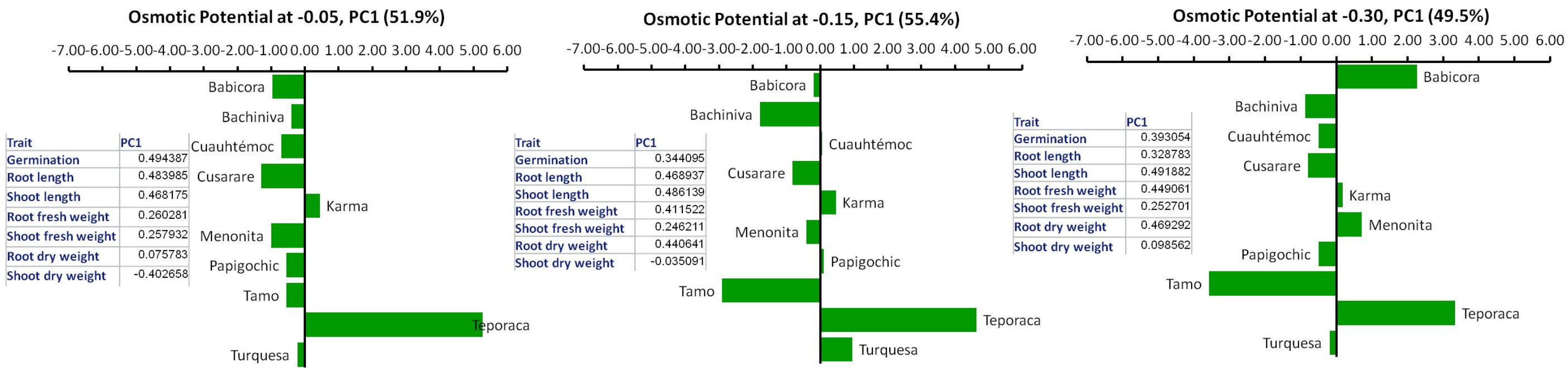

Figure 3. Effect of osmotic potential on ten oat genotypes evaluated in vitro under osmotic potential generated by an increasing concentration of PEG-6000.

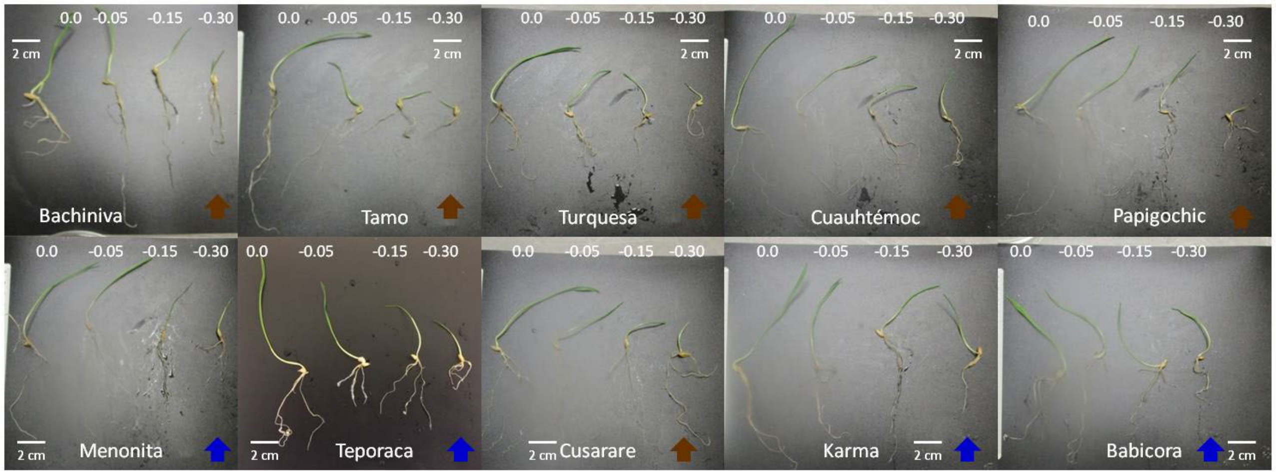

Figure 4. The in vitro effect of osmotic potential on ten oat genotypes evaluated under osmotic potential generated by an increasing concentration of PEG-6000. Blue arrows show longer roots at -0.30MPa, and brown arrows show shorter roots at -0.30 MPa.

The PCA showed outstanding growth and development in the Teporaca genotype in the three osmotic stress levels. In the PCA plot in Fig. 5, Teporaca (genotype no 9) is found in the B rectangle at the top of the Y axis and to the right of all genotypes on the X axis. This can be interpreted as Teporaca being the most tolerant genotype at -0.05. Teporaca is also the most outstanding at -0.15 MPa, as indicated by its appearance in rectangle C (Fig. 5). In rectangle D, Teporaca is also the second most tolerant genotype at -0.30 MPa, only after Babicora. The genotypes with the best response to osmotic stress are Teporaca, Babicora, Menonita, and Karma (Fig. 5). The genotypes with the worst response to osmotic stress are Tamo and Cusarare.

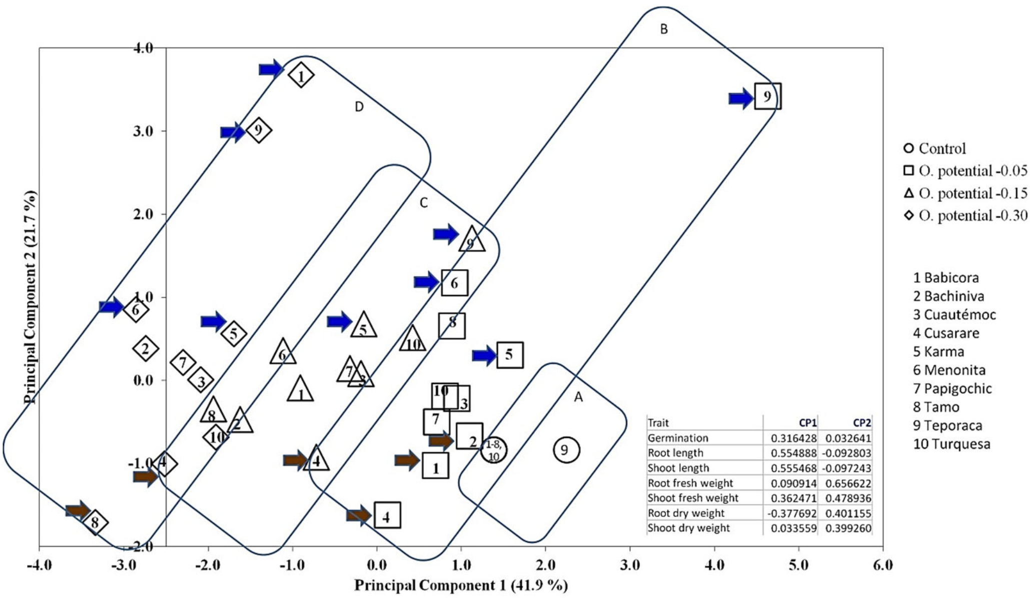

Figure 5. Plot of PC1 vs PC2 for ten oat genotypes and three OP levels. The Blue arrows show genotypes with the best response to OP, and brown arrows show the genotypes with the worst response.

DISCUSSION

The low germination of Teporaca is possibly one of the reasons why farmers did not sow it with the same intensity as other varieties. However, germinated plants from this genotype present a higher tolerance to osmotic stress than the rest. Canales et al. (2021) found that the resistant genotype showed a mild and slow increase in abscisic acid production that allowed maintaining transpiration longer. This response was linked to an increase in root hydraulic conductance by increasing total root length, the length of the thinnest roots, and root conductivity. The study agrees with the present study because the Teporaca genotype, which is the most tolerant, shows the largest roots at all osmotic pressure levels.

Evaluating osmotic stress tolerance in laboratory conditions is backed by Michel & Kaufmann (1973) and Basha, 2020), which measured root length and total root volume. These characteristics indirectly denote the capacity for water exploration and absorption, which help to explain better the behavior of the genotypes under hydric stress (Jimenez-Galindo et al., 2018; Canales et al., 2019). In addition, the present results agree with Gorny (1995) and Górny & Szolkowska (1996), who found enhanced rooting and improved drought tolerance in progenies of spring barley and oats after a selection for longer juvenile roots. Such characteristics are more complicated to measure in field experiments on irrigation drought (Górny & Szolkowska, 1996). Oat drought resistance is related to many phenological, morphological, and physiological factors (Larsson & Górny, 1988); probably the most important factor is genetic variation in the plant root system (Larsson & Górny, 1988). Using root characters to evaluate drought resistance of breeding materials has been suggested repeatedly (Derera et al., 1969; Taylor & Klepper, 1979).

Tolerance to osmotic stress by Teporaca, Menonita, and Babicora is backed by yields obtained in the field (with precipitations lower than 300 mm), which indicate that these three genotypes outperform the Bachiniva genotype (Salmerón, 2000). Osmotic stress tolerance of the Karma genotype in the present study is supported by field experiments performed by Villaseñor et al. (1998) and Salmerón et al. (2010). In addition, Sánchez-Martín et al. (2012) showed the potential of multivariate analysis as a robust approach to target key mechanisms responsible for drought tolerance in oats. Multivariate analysis can help breeders by accelerating genotype selection in large breeding populations.

The present study contrasts with the drought tolerance reported for the Bachiniva genotype by (Salmerón, 2000; Zamora, 2002) since we found a lower osmotic stress tolerance for this genotype. The contradiction is probably due to previous research assumed Bachiniva's drought tolerance only with field yield data. Relating this genotype with high yield, even in years with little and poor distribution of rain and the present experiment was only carried out under laboratory conditions. The reported tolerance of the Bachiniva genotype is possibly justified by its precocity (Salmerón, 2000), and the apparent contradiction between our study and the previous ones is probably due to the lack of testing of Bachiniva in drought tolerance experiments (Salmerón, 2000; Zamora, 2002).

The Tamo genotype produces a high amount of forage and has a long growing cycle (McDaniel, 1987; Aseeva & Melnichuk, 2018). This last characteristic probably makes it very susceptible to drought, supporting what was observed in the present study and others. Although precocity is linked to osmotic adjustment, several studies indicate that it is a physiological response to drought (González et al., 2008).

In the present study, the most outstanding genotype in terms of osmotic stress tolerance is Teporaca, followed by Babicora, Menonita, and Karma. Searching for agronomic traits and their use for oat breeding is very important (Ociepa, 2019). Evaluating seedlings might ease the phenotyping of plants in the previously mentioned populations Sánchez-Martín et al., 2012; (Canales et al., 2019).

CONCLUSIONS

We identified four commercial varieties of oats with enhanced tolerance to OP: Teporaca, the best option, Babicora, Menonita, and Karma. These varieties are a potential source of favorable alleles for osmotic stress tolerance and should be useful for oat improvement. Tamo and Cusarare were the most susceptible varieties to osmotic stress. On the contrary to susceptible genotypes, tolerant genotypes 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. PEG-6000 can be effectively used in genetic improvement studies to select in early stages oat lines with outstanding osmotic stress tolerance. Future research should focus on mapping the genetic regions responsible for osmotic stress tolerance using the PEG method for phenotyping biparental or MAGIC populations.

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