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Differential effect of osmopriming on three maize populations (*Zea mays*)

Efecto diferencial del osmoacondicionamiento sobre tres poblaciones de maíz (*Zea mays*)

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Technological Innovation: seed pre-treatment could help deal with the effects of stresses such as drought.

Industrial application area: areas prone to experiencing water stress, cold, anoxia during planting and seedling emergence.

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Resumen

México posee una gran diversidad de maíz (*Zea mays*), clasificados en más de 50 razas. Esta diversidad se preserva fundamentalmente en áreas de cultivo bajo régimen de lluvia donde los insumos se utilizan moderadamente o no se utilizan. La caracterización de estas poblaciones ha incluido a la calidad nutrimental y su efecto en la industria tortillera. El osmoacondicionamiento de la semilla antes de la siembra consiste en un proceso de imbibición en agua, solución salina u otros compuestos, como el polietilenglicol (PEG). Este tratamiento incrementa la germinación, el vigor y el establecimiento de la plántula en campo. En esta investigación se caracterizó la respuesta de tres poblaciones de maíz al osmoacondicionamiento. La población Tuxpeño osmoacondicionada mostró un incremento significativo en la germinación, en longitud de plúmula y raíz; en la población Celaya no se observaron diferencias estadísticas respecto al testigo. El PEG 6 y 12 hr incrementó el peso fresco de plántula de Tuxpeño y el KNO₃-AG₃ a 6 y 9 hr del Celaya; el PEG 12 hr incremento peso seco de plántula de Tuxpeño; la biomasa seca de raíz de la población Cónico Norteño, se incrementó con nitrato a 9 y 12 hr; la biomasa fresca de este órgano se incrementó en Cónico Norteño con PEG 9 hr y KNO₃-AG₃ a 9 y 12 hr; el KNO₃-AG₃ tuvo el mismo efecto en la

poblacion Celaya a los tres tiempos de incubación (6, 9 y 12 hr). Para las poblaciones de Celaya y Tuxpeño se podría recomendar el osmoacondicionamiento cuando la semilla pudiera ser sometida a estrés como hipoxia. En el Cónico Norteño no se estimuló la germinación. En las tres poblaciones hubo un incremento en longitud de plúmula, raíz primaria y secundaria. El osmoacondicionamiento se recomienda para condiciones de escasez de agua o hipoxia durante la siembra.

Palabras clave: germinación, giberelinas, imbibición, vigor.

Abstract

Mexico has a great diversity of maize populations (*Zea mays*), classified in more than 50 races. This diversity is essentially preserved in dryland cultivation areas where inputs are used moderately or not used. Populations characterization has included nutritional quality and tortilla. Seed osmopriming before planting consists of a water imbibition procese, saline solution or other compounds, such as polyethylene glycol (PEG). These treatments increase germination, vigor and seedling establishment in field. In this research, three maize populations response to osmopriming was evaluated. Tuxpeño population osmopriming showed a significant increase in germination, plumule and root length; Celaya population showed no statistical differences with control. PEG 6 h and 12 hr treatments increased Tuxpeño seedling fresh weight; KNO₃-AG₃ at 6 hr and 9 hr tratments increased Celaya seedling fresh weight; PEG 12 hr treatment increased Tuxpeño seedling dry weight; root dry weigth of Conico Norteño population was increased with KNO₃-AG₃ at 9 hr and 12 hr; fresh weigth of this organ was increased in Conical Norteño with PEG 9 hr and KNO₃-AG₃ at 9 hr and 12 hr; nitrate- had the same effect in Celaya at three incubation times (6, 9 and 12 hr). Celaya and Tuxpeño populations osmopriming could be recommended when seed could be subjected to stress such as hypoxia. Conico Norteño germination was not stimulated. There was an increase in plumule, primary and secondary root length for all three populations. Osmopriming is recommended for water stress or hypoxia during planting.

Keywords: germination, gibberellins, imbibition, vigor.

INTRODUCTION

Mexico maize diversity is classified in more than 50 races (1). This diversity is mainly preserved in dry farming areas where inputs are moderately used or not used. Populations characterization has included nutritional and “tortilla” quality. Seed osmopriming consists of water soaking, salts and other compounds, such as polyethylene glycol, before planting. These treatments increase germination, vigor and field establishment (2). Seeds should not germinate during imbibition, but once they are planted they will germinate evenly. Seed

high quality is essential for its germination and proper establishment in the field. Pre-planting treatments that help improve seed performance in the field have recently been investigated with greater emphasis. Most studies aim to identify osmoaconditioning substances suitable for each crop, know the optimal concentrations of each substance, determine the temperature and the optimal time of imbibition. In this research, three maize populations response to osmopriming was evaluated. Hypothesis was that maize populations would respond similarly to

osmopriming. Process in which seeds absorb water or aqueous solutions of potassium nitrate, gibberellins, polyethylene glycol, sodium silicate, polyamines and others, is known as osmopriming (2).

It is desirable to find the convenient aeration and drying method and simplify the process to implement it on a commercial scale. Studies on seeds of species of commercial interest have shown that pregerminative treatments increase their viability and vigor (3), reinforce the emergence of seeds with low vigor by increasing antioxidant activity. In treated sorghum seed (*Sorghum bicolor*) the permeability of the membrane is decreased and the levels of reducing and proline, sugars, soluble proteins and free amino acids are increased, and three main families of transcription factors are activated. Protein synthesis genes are activated, but also others related to the degradation of peptides and proteins (2). Silicon treatment, prior to the planting corn, relieves the symptoms of drought stress in two populations, one sensitive and the other stress tolerant. The 6 mM silicon treatment increased the length and weight of the aerial and root part and increased the levels of photosynthetic pigments. It also increased antioxidant enzyme activities (4). The treatment of the maize seed before sowing with ascorbic acid increased the weight of the aerial part and the root of the seedling when sown in conditions of acid soil and aluminum stress. In field trials, an aluminum-tolerant genotype increased its productivity after pretreatment of the seed with ascorbic acid. This treatment has the advantage that it is given dry, without imbibing the seed (5).

Climate change alters precipitation patterns and agricultural cycles; The use of techniques such as osmopriming can alleviate these negative effects (6). When osmoconditioning Elotes Occidental, Bofo Celaya and Palomero pigmented corn seed, it was found that this

treatment has a positive effect on the length of the seedling and root, which could help the establishment of seedling in the field, in addition to the seed germinating faster (7,8). The objective of this research was to study the effects of osmoconditioning on three maize populations (Celaya, Northern Conical and Tuxpeño).

MATERIALS AND METHODS

In this research, three populations belonging to three maize populations were used: Cónico Norteño, Tuxpeño and Celaya. For osmotic solutions preparation, a concentration of 300 g/L of polyethylene glycol 1000 (SIGMA®) and 30 g/L of potassium nitrate (Golden Bell®) plus 0.1 g/L of gibberellic acid (Rali Agropecuaria®) were used, obtaining a solution of PEG and another of potassium nitrate added with gibberellic acid. Before subjecting seeds to the corresponding treatments, a sample was taken for each treatment consisting of 100 seeds, which was splitted into four replications of 25 seeds; each replication was placed into a glass bottle, adding 25 mL of PEG osmotic solution. The same procedure was followed for the treatment with $KNO_3 + AG_3$, placing 4 replicatons of 25 seeds and adding 25 mL of this solution to each container. Treatments were: PEG (Polyethylene glycol 6, 9 and 12 hours; KNO_3 plus AG_3 at 6, 9 and 12 hours); once time was elapsed, osmotic solution was eliminated and seeds weight was recorded; the samples without osmopriming (0 hr) of each population were used as control. Once the seeds were weighed, they were dried out at room temperature during 6 days to recover its original weight.

Standard germination, vigour (plumule length), soak test and titratable acidity were determined for each treatment, according to ISTA (1976, 1981) under a completely randomized design using four replications of 25 seeds for each treatment. Normal seedlings

count was carried out on the fourth and seventh day. Plumule length, primary root length and secondary root length, were recorded (cm) on seventh day. Germination percentage was determined with the total of normal seedlings obtained on the fourth and seventh day.

Thousand seeds weight. To carry out this test, directions of ISTA (9) were used. Eight replications of 100 seeds of each population for each treatment were taken; counting was done manually and they were weighed on a Setra SI 410S® balance, thus obtaining sample total weight and extrapolating to thousand seeds weight.

Germination test: 100 osmoprimed seeds (PEG or $\text{KNO}_3 + \text{AG}_3$) and control group were taken; each sample was splitted into four replications 25 seeds each, placed on paper towels moistened with sterile water, forming five rows of 5 seeds separated by uniform spaces; radicle tip was placed pointing towards the bottom of the towel and the embryo towards top. Two additional pre-moistened paper towels were placed to cover seeds and rolled up to be placed in the germination chamber (Control System Labs®) at 25°C with 80% relative humidity in open-topped polyethylene bags. Every third day they were moistened with sterile water. Two counts were made, first, on the fourth day and the second on the seventh day.

Plumule length test; it was performed according to ISTA (9). Ten normal seedlings were random selected from each treatment; both plumule and root from each normal seedling were weighed separately; they were placed in the MEMMERT® drying oven during two days at constant temperature (60°C); at the end of the drying period samples were weighed.

Soak test.

Four replicates 25 seeds each maize population were placed in a 300 mL plastic container; sterile water was added so that all seeds were covered with water, with a margin of 2 cm between the surface of the water and the seed; container was hermetically closed. Sample was kept at temperature of 25 °C ± 1 °C during 24 hr; water was eliminated and seeds weight was determined after imbibition period. Finally, standard germination test was performed as was described earlier.

The acidity in the sample, expressed as malic acid, was calculated using the following formula:

$$\text{acidity} \frac{g}{L} (\text{malic acid} = V \times N \times 67 / v).$$

Where: V = volume (mL) of 0.1 N sodium hydroxide solution spent on the titration of the sample, N: normality of the solution of sodium hydroxide, v: volume (mL) of the sample, 67: chemical equivalent of malic acid (8, 10). ANDEVA was performed with the SAS 9.0 program; means comparison was performed according to Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

INDIVIDUAL SEED WEIGHT AND PROTEIN CONTENT

The weight of one thousand seeds of the Celaya genotype was 273.3 g, the Tuxpeño, 262.5 g and Cónico Norteño, 256.7 g; when evaluated two races of maize (11), the Tabloncillo had a weight of 310.7-364.1 g and the Chalqueño presented values that ranged from 367-509 g, for Cónico Norteño (12) give a value of 233 g Celaya, 305 g Tuxpeño and Norteño 372 g; the protein content of the first one was 8.8%, Celaya 8.9% and Tuxpeño 10.5%. In pericarp thickness, the Northern Conic is the one with the thinnest. Weight of a thousand seeds is the most convenient and independent factor of the variety.

VIGOR TEST

The analysis of variance showed that there were differences between osmopriming treatments between the three populations in the vigor test. In the comparison of means (Table 1) these differences are shown; the least affected was Celaya; in all three populations differences were obtained with respect to control; Conical Norteño, was the one that had the least response to treatment. Conical Norteño and Tuxpeño had a good response to KNO₃-AG₃ treatment at 12 h, while the Celaya population had a very low response.

GERMINATION

The beneficial effect of osmopriming is manifested in a rapid and uniform germination after the removal of the PEG and its drying at room temperature. The genetic background of the populations studied is probably influencing the response to osmopriming, since they had a different response to treatment. It would be desirable to determine if with other incubation times the germination of the Celaya population might not be affected. Some researchers have expressed the need to determine the effects of osmopriming for each species and seed lot,

because although in general positive effects are observed, in some cases they could be negative (13). There were no positive differences in osmopriming for plumule length (Table 1) between the treatments and the control in Tuxpeño and Celaya. For the conditions that were used in this experimentation, no clear positive effects were observed for this variable; as mentioned above, possibly other imbibition times or the use of other substances could have a positive effect on this variable.

Ahmmad *et al.*, (14) found differential effects among the substances used to seed osmocondition. The positive effects of osmopriming on the seventh day for the variable primary root length were only observed for Tuxpeño population (Table 1), where KNO₃-AG₃ stands out at 9 and 12 h and PEG at 6 and 12 hr; in Celaya some treatments were equal to control. Osmopriming effect turns out to be positive in some populations, but not all. This is consistent with the recommendations regarding the need to determine the effect of the treatment for each lot or population. It has been reported that the beneficial effect of osmopriming in the initial times could be lost if the treatment time is extended (15).

Table 1. Means comparisons for germination trait (vigor) in three maize populations: Cónico Norteño, Tuxpeño y Celaya. Roque, Gto., 2015.

Treatment	Germination (%)			Plumule (cm) 7d		Prim Root (cm) 7d	
	Cónico N	Tuxpeño	Celaya	Tuxpeño	Celaya	Tuxpeño	Celaya
Control	81 ab	84 bc	98 a	6.38 ab	8.50 a	8.21 cd	12.75 ab
PEG 6	70 b	91 ab	92 a	6.30 ab	7.88 ab	9.41 abc	10.08 c
PEG 9	71 b	94 a	96 a	3.69 b	7.18 ab	8.96 bcd	13.62 a
PEG 12	78 ab	92 ab	96 a	6.18 ab	6.38 ab	11.17 ab	13.00 ab
KNO ₃ + AG ₃ 6	79 ab	79 c	91 a	6.15 ab	5.15 b	6.92 d	13.86 a
KNO ₃ + AG ₃ 9	80 ab	90 ab	81 b	6.52 ab	5.60 ab	11.48 a	14.23 a
KNO ₃ + AG ₃ 12	84 a	85 abc	71 c	6.95 ab	6.40 ab	10.05 abc	10.81 bc
Tukey	12.72	9.62	8.24	2.83	2.97	2.43	2.30

^a Treatments with the same letter within each variable are statistically equal (Tukey $p < 0.05$).

N: norteño; d: days; plumule: plumule length; PEG: polyethylene glycol; AG₃: Gibberellic acid; prim root: primary root.

Conico Norteño had the lowest germination percentage, including the control; however, despite the seed being subjected to the stress

of imbibition, a germination percentage similar to the control was maintained (Table 2). The constitution of the seed itself could

explain the different effects to the treatments. Cold damage during germination can be overcome by osmopriming of the seed; something similar to what was observed in this work, since a certain germination percentage is maintained (16). The effect of osmo conditioning was positive when imbibition was also applied to seeds (Table 2); this effect was notorious in Conico Norteño and Tuxpeño, but the effects were uneven. The 12 hr PEG treatment was positive in both populations, while 9 hr

$\text{KNO}_3\text{-AG}_3$ was only positive in Conico Norteño; $\text{KNO}_3\text{-AG}_3$ 12 hr seems to have a negative effect after 9 hours of seed incubation. On the fourth day, the positive effects of osmopriming on plum length are not clearly shown (Table 2); more time is required for its manifestation. Although the results were not uniform, osmopriming is generally recommended for difficult conditions, especially water shortages (13,17).

Table 2. Means comparison of germination in imbibition test (soak test) in three maize populations: Cónico Norteño, Tuxpeño y Celaya. Roque, Gto., 2015.

Treatment	germination %			Plumule 4 d cm		
	Cónico N	Tuxpeño	Celaya	Cónico N	Tuxpeño	Celaya
Control	62 ab	83 a	80 ab	4.57b	3.13b	7.88a
PEG 6	59 ab	76 ab	85 ab	3.87b	4.33ab	5.65b
PEG 9	65 ab	88 a	71 b	3.47b	3.73b	8.54a
PEG 12	65 ab	86 a	75 ab	4.85ab	6.02a	8.20a
$\text{KNO}_3 + \text{AG}_3$ 6	57 b	87 a	76 ab	3.62b	3.38b	8.32a
$\text{KNO}_3 + \text{AG}_3$ 9	71 a	78 ab	80 ab	6.82a	3.50b	7.45a
$\text{KNO}_3 + \text{AG}_3$ 12	71 a	64 b	88 a	3.72b	2.50b	5.53b
Tukey	12	18.13	16.04	1.82	1.92	1.72

^a Treatments with the same letter within each variable are statistically equal (Tukey $p < 0.05$).

PEG: polyethylene glycol; AG_3 : Gibberellic acid; d: days; plumule: plumule length; N: Norteño

On the seventh day it was observed that some treatments (PEG at 9 and 12hr, $\text{KNO}_3\text{-AG}_3$ at 6 and 12hr) were superior to the control in the three populations (Table 3), which shows that osmopriming if it is effective to increase plumule length, that is, it has an effect on seedling vigor, as reported by other authors (3,13). This effect was not as noticeable on the fourth day of the evaluation, so it is necessary to evaluate also on the seventh day

as established by the test. The PEG 9 hr was the treatment that had the greatest effect for this variable, since in the Conico Norteño it almost doubled the length of the control formula, very similar to the effect of nitrate-12 hr; nitrate- treatment had more variable effects than PEG. The effect of osmopriming may vary depending on whether only water or any substance is used, as found by other authors (16,18).

Table 3. Means comparison for length of plumule trait (imbibition, “soak test”) in three maize populations: Cónico Norteño, Tuxpeño y Celaya. Roque, Gto., 2015.

Treatment	Plúmule length day 7 (cm)		
	Cónico N	Tuxpeño	Celaya
Control	6.48 d	7.34 d	8.34 b
PEG 6 hr	7.73 cd	11.34 b	13.69 a
PEG 9 hr	11.47 a	12.55 ab	13.15 a
PEG 12 hr	9.43 abcd	10.98 b	6.64 b
KNO ₃ + AG ₃ 6	10.66 abc	10.63 bc	13.15 a
KNO ₃ + AG ₃ 9	8.08 bcd	8.37 cd	9.31 b
KNO ₃ + AG ₃ 12	11.30 b	14.20 a	14.46 a
Tukey	1.82	1.92	1.72

^aTreatments with the same letter within each variable are statistically equal (Tukey p<0.05).

N: norteño; PEG: polyethylene glycol, AG₃: Gibberellic acid.

In the imbibition test, some treatments had positive effects superior to the control in the three populations evaluated (Table 4). The three-time PEG had positive effects on the Tuxpeño; within these, the PEG 9 hr was consistent in its effects on the three populations. The positive effects of KNO₃-AG₃ were most noticeable in Tuxpeño and Celaya. In the Northern Conical population, treatments PEG 9 and KNO₃-AG₃ 9 hr were those that showed superior effects to the control. Despite possible hypoxia damage due to the imbibition test, some treatments had a positive effect; Finch-Savage et al., (13) found that at 20 °C osmopriming has little effect on germination after having subjected

the seeds to imbibition. Finch-Savage et al., (13) point out that the effects of osmopriming can be neutral, positive or negative, as observed in these population.

In secondary root length in the Northern Conical population, the best treatments were PEG 9 hr and KNO₃-AG₃ 9 hr; in Tuxpeño all the treatments surpassed the control. In the Celaya breed, a positive effect was observed especially on KNO₃-AG₃ at three times and PEG 9 and 6 hr (Table 4). In general, a positive effect of the treatment on the seeds is observed, which has led other researchers to propose osmopriming as a way to improve the performance of the seed in the field (2, 17).

Table 4. Means comparisons for primary and secondary root length traits in “soak test” in three maize populations: Cónico Norteño, Celaya y Tuxpeño. Roque, Gto., 2015.

Treatment	Primary root length (cm)			Secondary root length 7 days (cm)		
	Cónico N	Tuxpeño	Celaya	Cónico N	Tuxpeño	Celaya
Control	5.78 c	8.47 c	9.85 bc	7.10 c	6.18 b	7.34 de
PEG 6	6.63 c	13.58 a	9.62 bc	8.27 bc	9.66 a	9.74 cd
PEG 9	12.09 a	13.08 a	10.88 abc	9.43 ab	9.71 a	12.09 abc
PEG 12	9.12 b	13.07 a	8.97 c	7.35 bc	8.91 a	6.38 e
KNO ₃ + AG ₃ 6	6.04 c	10.86 abc	12.53 ab	4.76 d	9.21 a	13.60 ab
KNO ₃ + AG ₃ 9	10.24 ab	9.33 bc	10.58 abc	11.52 a	10.42 a	11.48 bc
KNO ₃ + AG ₃ 12	8.88 b	12.15 ab	13.64 a	9.04 bc	8.99 a	14.21 a
Tukey	2.16	3.22	3.23	2.12	2.54	2.66

^aTreatments with the same letter within each variable are statistically equal (Tukey p<0.05).

PEG: polyethylene glicol; AG₃: Gibberellic acid, N: norteño

The fresh and dry weight of the plumule are closely related in terms of the effect of osmopriming (Table 5) in Conico Norteño and Tuxpeño populations; means comparison is very similar for both variables. In Tuxpeño and Celaya some treatments were superior to control. The PEG 12 hr was the one that showed superior consistency to control in

Tuxpeño and Celaya and was similar to control in Cónico Norteño; In this population some of the treatments were equal to control (PEG 12 hr and KNO₃-AG₃ 9 hr). Whaid et al., (17) found that pretreatment of corn seed with hydrogen peroxide (H₂O₂) increases seedling length and dry weight; osmopriming improves germination and vigor.

Table 5. Comparison of means test for the variable fresh and dry weight (g) of plumule (seventh day) in three maize populations: Cónico Norteño, Celaya, Tuxpeño. Roque, Gto., 2015.

Treatment	Cónico N		Tuxpeño		Celaya
	FWP	DWP	FWP	DWP	FWP
Control	2.45 ab	0.24 ab	2.23 bc	0.20 bc	3.80 c
PEG 6	2.33 b	0.23 ab	3.15 ab	0.26 ab	3.75 c
PEG 9	2.14 b	0.19 b	1.82 c	0.17 c	4.08 abc
PEG 12	2.65 ab	0.23 ab	4.04 a	0.34 a	4.88 abc
KNO ₃ + AG ₃ 6	1.87 b	0.18 b	2.05 c	0.19 bc	5.12 ab
KNO ₃ + AG ₃ 9	3.36 a	0.30 a	2.83 bc	0.24 bc	5.37 a
KNO ₃ + AG ₃ 12	1.82 b	0.17 b	1.91 c	0.17 c	4.83 abc
Tukey	0.93	0.08	0.13	0.08	1.23

^a Treatments with the same letter within each variable are statistically equal (Tukey p<0.05).

PEG: polyethylene glycol; AG₃: Gibberellic acid; FWP: fresh weight plumule; DWP: dry weight plumule.

The KNO₃-AG₃ 9 and 12 hr showed consistency in its positive effect on the fresh and dry root weight in the three populations (Table 6). In Tuxpeño some treatments were similar to the control; in Northern Conical KNO₃-AG₃ 9 and 12 hr they were superior to the control and it is in the only population where osmopriming responded positively in

both variables. In the Celaya population, treatments with KNO₃-AG₃ were superior to the control for fresh root weight. Zhang et al., (2) found that osmopriming improves the viability of the root and Dell'Amico et al., (19) point it out as a way to induce radical development.

Table 6. Comparison of means test for the variable fresh and dry weight of radicle and plumule (seventh day) in three maize populations: Cónico Norteño, Celaya y Tuxpeño. Roque, Gto., 2015.

Treatment	Cónico N		Tuxpeño		Celaya	
	FWR	DWR	FWR	DWR	FWR	DWR
Testigo	0.27 c	0.05 c	1.74 a	0.15 ab	0.68 b	0.18 ab
PEG 6	0.21 c	0.07 bc	0.97 d	0.11 b	0.64 b	0.14 b
PEG 9	0.41 b	0.07 bc	1.34 bc	0.14 ab	0.89 ab	0.16 ab
PEG 12	0.23 c	0.06 c	1.64 ab	0.16 a	0.71 b	0.18 ab
KNO ₃ + AG ₃ 6	0.19 c	0.06 c	1.30 bcd	0.17 a	1.22 a	0.20 a
KNO ₃ + AG ₃ 9	0.45 ab	0.10 a	1.56 abc	0.16 a	1.27 a	0.19 a
KNO ₃ + AG ₃ 12	0.57 a	0.09 ab	1.21 cd	0.17 a	1.30 a	0.18 ab
Tukey	0.13	0.02	0.37	0.05	0.47	0.04

^a Treatments with the same letter within each variable are statistically equal (Tukey p<0.05).

PEG: polyethylene glycol; AG₃: Gibberellic acid; FWR: fresh weight radicle; DWR: dry weight radicle

For the titratable acidity variable, all treatments were superior to control in the Celaya population; in Tuxpeño all treatments were equal to control; in Conico Norteño, only the 9-hour KNO₃-AG₃ treatment was similar to the control (Table 7). Zhang et al., (1) found that the pool of soluble proteins and free amino acids are higher in osmoconditioned seeds; these molecules certainly contribute to maintaining or balancing the pH; Ventura et al., (14) mention

that during rehydration amphiphilic molecules are redistributed in the seed, something that also occurs with water (18). The decrease in titratable acidity in the treated seeds could be due to the use of citric acid molecules, malic acid for new molecules synthesis or as a metabolic substrate (9). In the seed the high concentrations of some metabolites could lead to a eutectic phase, which is modified on the hydration of the seed (20).

Table 7. Comparison of means for the variable titratable Acidity in maize populations: Cónico Norteño, Tuxpeño y Celaya. Roque, Gto., 2015.

Treatment	titratable Acidity g/Kg		
	Cónico N	Tuxpeño	Celaya
Control	0.42 ab	0.32 ab	0.23 b
PEG 6	0.28 d	0.30 ab	0.28 ab
PEG 9	0.37 bc	0.30 ab	0.28 ab
PEG 12	0.37 bc	0.32 ab	0.28 ab
KNO ₃ + AG ₃ 6	0.35 bcd	0.31ab	0.29 a
KNO ₃ + AG ₃ 9	0.47 a	0.28 b	0.27 ab
KNO ₃ + AG ₃ 12	0.32 cd	0.35 a	0.28 ab
Tukey	0.01	0.07	0.05

^a Treatments with the same letter within each variable are statistically equal (Tukey p<0.05).

^a Tratamientos con la misma letra dentro de cada variable son estadísticamente iguales (Tukey p<0.05).

PEG: polyethylene glycol; AG₃: Gibberellic acid

So that, there were no positive differences in soak test for plumule length among treatments and control in Tuxpeño and Celaya populations. Osmopriming effects on the seventh day for primary root length trait were only observed for Tuxpeño population. Osmopriming effect was positive when soak test was also applied to seeds.

On the seventh day it was observed that some treatments (PEG at 9 and 12hr, nitrate at 6 and 12hr) were superior to control in the three populations (Table 3), which show that osmopriming was effective to increase plumule length, that is, it was an effect on seedling vigor. In soak test, some treatments had positive effects and significant to control in the three populations evaluated (Table 4).

The three-time PEG treatments had positive effects on Tuxpeño population; within these, PEG 9 hr was consistent in its effects on the three populations. Positive effects of nitrate were significant in Tuxpeño and Celaya populations. In Conico Norteño population, treatments PEG 9 and nitrate 9 hr were significant different to control. Secondary root length in Conico Norteño population, the best treatments were PEG 9 hr and KNO₃ 9 hr; all treatments in Tuxpeño population were significant different to control. Plumule fresh and dry weight are closely related in terms of the effect of osmopriming (Table 5) in Conico Norteño and Tuxpeño populations. KNO₃ 9 and 12 hr treatments showed consistency in its positive effect on fresh and dry root weight in the three populations. For titratable acidity

trait, all treatments were superior to control in the Celaya population.

CONCLUSIONS

Tuxpeño population, in the germination variable, responded favorably to treatments; no differences were observed in the Celaya and in Conico Norteño population a negative effect was observed. Stress due to excess water (soak test, hypoxia) significantly decreased the germination of Conico Norteño and to a lesser extent that of Tuxpeño and Celaya. Regarding the length of the plumula, a positive effect of osmopriming was observed when soak test was applied and this was more conspicuous on the seventh day, in the three populations. In radicle length, on the seventh day greater length was obtained in the Celaya and the Tuxpeño populations. When they underwent soak test, the three populations tended to show greater of primary root and secondary root length on the seventh day. In fresh and dry seedling weight some treatments had a positive effect on all three populations; in dry root weight there was a positive effect on Celaya and Tuxpeño. In general, a positive effect on fresh and dry weight of plumula was observed. For the Celaya and Tuxpeño populations, osmoconditioning could be recommended when planning to use the seed under stress conditions, such as hypoxia. For the Conico Norteño it does not seem advisable to stimulate germination, but if the length of the plumule and the root, as happened with the other two populations, so treatment for water scarcity conditions could be recommended. Most researchers recommend osmopriming for aged seeds or when it is expected that they will undergo some kind of stress.

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