Article

Osmoaconditioning of six maize populations subjected to accelerated aging

Jorge Covarrubias Prieto
Manuel Jerónimo Arriaga
Adriana Basilio-Apolinar
J. Gabriel Ramírez-Pimentel
Juan Carlos Raya-Pérez[§]

Technological National of Mexico-Technological Institute of Roque. Carretera Celaya-Juventino Rosas km 8. Roque, Celaya, Guanajuato, Mexico. CP. 38110. (jor_covarru-jrg@hotmail.com; jeross456@gmail.com; adriana.apolinar@hotmail.com; garamirez@itroque.edu.mx; juraya@itroque.edu.mx).

[§]Corresponding author: juraya@itroque.edu.mx.

Abstract

The pre-treatment of the seeds allows to improve their behavior in the field, through an increase in germination, vigor and lengthen the shelf life. In this investigation the effect of the pretreatment with potassium nitrate (3%) plus gibberellins (GA_3) (0.06%) in two periods: 6 and 12 h on the seed of five races of maize, including an experimental hybrid (Roque B16) was quantified. The objective of this research was to undergo osmoaconditioning to these races of maize and then to accelerated aging and quantify their effect on the percentage of germination. The Roque B16 hybrid showed no difference in germination percentage and maintained a good germination percentage after 60 days of storage. The Elotes Occidentales (purple pigment) race exhibits a similar behavior between the control and the treated seed at 7 and 30 days of storage, at 60 days it indicated a significant fall. Elotes Occidentales (red pigment) showed a significant decrease at 7, 30 and 60 days of storage with the 12 h treatment. The Palomero race showed a significant decrease in germination at seven days with the 12 h treatment, Amarillo Roque evidenced a significant decrease at 7 and 60 days of storage with the 12 h osmoaconditioning, the Pozolero race revealed a significant decrease at 7 and 30 days of storage with 12 h and at 60 days with both periods of treatment (6 and 12 h). The Amarillo and hybrid varieties were statistically equal with a higher germination percentage during storage. Purple and Pozolero were very affected by the storage at three months. Rojo was also affected, but to a lesser extent than the previous two.

Keywords: ashes, conductivity, creole race, gibberellins, potassium nitrate.

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Introduction

The seeds reflect their importance in the maintenance of genetic diversity, through them the propagation of the species is made (Amador-Alférez *et al*., 2013). The seed of good quality is the cornerstone for the establishment of a good population in any crop. The trend of the seed industry is increasingly specialized, with companies dedicated exclusively to the production of good quality seeds. Therefore, an important research area has been created in the search for seeds with higher physical, sanitary, physiological and genetic quality (Jahnke *et al*., 2016).

Evans and Turnbull (2004) consider that seeds with good physiological quality are those that have a high percentage of germination and vigor, mainly. In addition, it confers advantages such as the extension of the shelf life, a minimum of waste and production of uniform plants in nurseries, seedbeds and fields. Luna *et al*. (2012) mentions that physiological quality depends on biotic and abiotic factors that can easily alter maturation, damage the crop, drying, storage, distribution and its establishment in the field; the seed is susceptible to being damaged at any time during its production, so its management from maturation to sowing requires a high degree of specialization.

According to Romano *et al*. (2008), one of the quality components that shows signs of deterioration is the vigor of the seeds, followed by a decrease in the germination percentage, in the production of normal seedlings and finally death. The factors that characterize the vigor of the seeds are rapid germination, speed of development after germination and integrity and normality of the seedlings that emerge from a vigorous seed. Given the importance of the vigor of the seeds, research is carried out to understand the genetic nature and effects on the establishment of seedlings in the field, as well as the effects on the yield and longevity of seeds in storage (Sveinsdottir *et al*., 2009).

Various theories have been formulated regarding the biological phenomena that lead to seed deterioration. Stresses the research conducted in the last fifteen years, related to the action of free radicals in the deterioration of mitochondrial membranes, as the first event in the deterioration of seeds. Aging alters the activity of the H+ATPase of tonoplast, peroxidase, protease and DNA polymerase (Svenisdottir *et al*., 2009).

Polyethylene glycol (PEG) has been recommended as an ideal osmotic substance for seeds, because it does not penetrate cell membranes, is not toxic and keeps its osmolarity constant in small quantities, which allows an acceptable aeration of the medium; To avoid the effect of hypoxia in the seed, which causes the accumulation of toxic metabolites (Finch-Savage *et al*., 2004), it has been found that the behavior of seedlings of monocot species is different from that of dicotyledons if they develop under stress osmotic with PEG. In general, monocots are more sensitive to stress at the level of the radical system (Campos-Álvarez *et al*., 2002; Méndez-Natera *et al*., 2008).

Lara *et al*. (2014) found that nitrates stimulate germination by decreasing ABA concentration and nitric oxide production, thus interrupting dormancy, as well as stimulating antioxidant enzymes. Ilbi and Eser (2002) point out that KNO_3 is involved in protein synthesis and carbohydrate

metabolism, and consequently, delay seed aging. In this investigation the effect of osmoaconditioning with potassium nitrate (KNO_3) (3%) and gibberellins (GA3) (0.06%) in two periods: 6 and 12 h, on the seed subjected to accelerated aging in six maize populations was evaluated, including an experimental hybrid.

Materials and methods

The seed was obtained from the experimental field of the Technological Institute of Roque (ITR) of the project of genetic improvement of maize of the spring-summer harvest (S-S) 2015. The field is located at 20° 34' 53'' north latitude, 100° 49' 36.5'' west longitude and altitude of 1 769 m. The genetic materials used were: Elotes Occidentales (purple or red pigmented), Pozolero, Palomero, Roque Amarillo, Roque B16 hybrid. 100 seeds were used per treatment (25 seeds for four repetitions; each batch was submerged for 6 and 12 h at room temperature in 100 mL of a solution of potassium nitrate (3%) plus gibberellic acid (0.06%), the control was not osmoaconditioned.

At the end of the treatment time the solution was removed, placing the seeds on a paper towel to weigh them immediately and let them dry at room temperature for 5 days, at the end of this period the weight was re-taken, so that will recover the initial weight. Accelerated aging was done in a beaker with 250 ml of distilled water, a metal mesh was placed and 220 seeds were placed on it. The beakers were closed with aluminum foil and incubated in the growth chamber at 42 °C for 96 h and a relative humidity of 100%.

After this time, 200 seeds of each genotype were evaluated; Paper dowels were made to perform the standard germination test at 25 °C for 7 days. The test was performed according to Moreno's method (1998) with slight modifications. Electrical conductivity: to measure it 25 seeds were used per repetition; were managed in triplicate of each population and treatment. The seeds were placed in polyethylene cups, 50 mL of distilled water was added, left at a temperature of 20 °C for 24 h, at the end of time the reading was taken directly from the glass with the seed submerged with an Acuapro brand digital conductimeter and the rest of the reading was obtained in the glass with the control water, the results were expressed in mS cm^{-1}.

For the pH measurement, 10 g of maize seeds were taken, they were soaked with 40 ml of distilled water in a beaker and ground in a blender, the homogenate was allowed to stand at room temperature for one hour and then pH was measured with the help of a table potentiometer.

Ash determination

The chemical method with reference 08-01 1995 of the AACC for the quantification of total ashes is also used to determine ashes in maize grains. The percentage of ashes to six maize populations was determined in two treatments and one control, three repetitions were used, was performed by incineration at a temperature of 500 °C. A completely randomized design with two treatments and four repetitions was used. The analysis of variance was carried out with the SAS program (2001) version 8.0. The comparison of means was carried out by means of the Tukey test ($\alpha \leq 0.05$).

Results and discussion

There were no significant differences between the control and the treatments in any of the times for the Roque B16 Hybrid, none of the parts in this seed was sensitive to deterioration, since as Pérez de la Cerda *et al*. (2007), the different parts of the seeds do not deteriorate or age simultaneously and those most sensitive to deterioration should be the focus of attention in quality studies. The endosperm of maize is mainly composed of starch, which is not as sensitive to deterioration as the embryo; this is mainly composed of proteins and oil, while the endosperm represents 80% of the dry weight of the maize seed, 20% refers to the embryo.

It is known that proteins, lipids and DNA are more sensitive to damage. The type of endosperm influences the germination and vigor of the seed, corneal endosperm maize resists deterioration better (Pérez de la Cerda *et al*., 2007). For this B16 genotype, hybrid vigor would explain the resistance to seed deterioration, as indicated by Greaves *et al*. (2015). In the imbibition variable, the 12 h treatment absorbed a greater amount of solution; as the soaking time lengthens the seeds absorb more water (Table 1).

Table 1. Comparison of Roque B16 population averages, for the variable's imbibition, electrical conductivity, pH and percentage of ashes. Roque, Guanajuato. 2016.

HRoqueB16	Imbibition (mg)	Electric conductivity (µs $cm^{-1)}$	pH	Ashes (%)
Control	0 c	5.54 b	6.05 b	1.07 b
6 h	13.79 b	20.52 a	6.20 a	1.29 a
12 h	15.84 a	21.11 a	6.19 a	1.29 a
Tukey	1.45	2.13	0.03	0.13

Sousa Paiva *et al*. (2006) indicated that, during imbibition, the increase in the weight of the *Swietenia macrophylla* seeds was only significant in the first two phases; that is, that of rapid absorption and then not absorb water for a certain time (until the protrusion of the radicle). In electrical conductivity, the Roque B16 seeds soaked for 6 and 12 h exceeded the control (5.54 µs/cm), the electrical conductivity is based on the principle that as the seeds deteriorate, the differential permeability of cell membranes is altered, so that, when placed in an aqueous solution, the less vigorous seed suffers loss of cytoplasmic compounds such as amino acids, ions and sugars of low molecular weight, when the seeds are rehydrated they must have the ability to reorganize the membranes to avoid the loss of solutes (Yu *et al*., 2015).

Roque B16 values were similar to those reported by Hilmig and Mendez-Natera (2011), both unaged and aged seeds, with electrical conductivity values of 20 to 32 µs cm^{-1}.

The results for the Elotes Occidentales race (purple pigmentation) are shown in Table 2; at 7 and 30 days after the treatment, no differences were observed between the control (non-osmoaconditioned seed) and the treatments (osmoaconditioned and subject to accelerated aging), at 60 days the control had a germination percentage higher than the treatments, there was a negative effect of the treatments since the germination was too low and lower than the control, at this stage the germination was less than 85% in control and treatments.

Table 2. Comparison of means by means of the Tukey test in the standard germination variable of the Elotes Occidentales race (Morado and Rojo) at 7, 30 and 60 days of storage, Roque, Guanajuato. 2016.

Treatment	Elotes Occidentales			
	Rojo	Morado		
	60 days	7 days	30 days	60 days
Control	75 a	80 a	80 a	77 a
6 h	14 b	77 a	49 b	30 b
12 h	22 b	45 b	67 ab	45 b
Tukey	19.5	22.2	20.4	25.7

Treatments with equal letters are statistically equal (Tukey, $p< 0.05$).

The endosperm of this seed is floury, which explains the negative effect of accelerated aging on germination and agrees with the results of Pérez de la Cerda *et al*. (2007) who point out that the corneal endosperm confers more resistance to deterioration and with Duran-Hernández *et al*. (2011) who indicate that the seed with greater vigor resists accelerated aging better. Germination and vigor are quality indicators, they are attributes that allow us to assess the establishment of the plant in the field.

The size, shape and type of endosperm, vitreous or mealy are important characteristics in a seed. 35 maize populations evaluated by Guillén-de la Cruz *et al*., (2018) showed a great variation in seed germination ranging from 28-100%. The populations with vitreous endosperm tended to have greater vigor, germination and normal seedlings than those of floury endosperm. Pigmented maize is seeds with a flour-like endosperm (Gómez *et al*., 2017). Gutiérrez-Hernández *et al*. (2011) report that the Oaxaca maize variety has a good behavior, since it produces normal seedlings with seeds subjected to humid heat, which contrasts with the results obtained here.

Maize seed contains more than 80% unsaturated fatty acids, as part of total lipids, which makes it more susceptible to deterioration (McCann *et al*., 2007). Table 2 shows the results of the Elotes Occidentales (red pigmentation) race, which shows that after seven days, the osmoaconditioning at 12 h caused a significant decrease in germination; at 60 days the germination percentage of the two treatments decreased (6 and 12 h), which were lower than the control; in this variety, germination in all cases was less than 85%.

The degradation of oligosaccharides during imbibition could explain the low germination, in addition to stress due to the accelerated aging test, as indicated by Gurusinghe and Bradford (2001). In nature, the seeds must be able to enter cycles of hydration and dehydration and be able to germinate when conditions are conducive (Footitt *et al*., 2015), but the domestication process seems to have suppressed or diminished this property.

There were significant differences between the treatments and the control in the variables imbibition, electrical conductivity and ashes in the population of Elotes Occidentales blue pigmentation (Table 3).

Table 3. Comparison of means of the Elotes Occidentales (blue pigmentation) population, for the variables imbibition, electrical conductivity, pH and percentage of ashes. Roque, Guanajuato. 2016.

Occidental Azul	imbibition (mg)	Electric conductivity (µs cm^{-1})	Ashes (%)
Control	0 c	5.75 c	1.25 b
6 h	18.57 b	37.60 a	1.64[a]
12 h	21.63 a	26.70 b	1.66 a
Tukey	2.44	5.52	0.17

Treatments with equal letters within each variable are statistically equal (Tukey, $p< 0.05$).

Table 4 shows the comparison of means of the Roque Amarillo population, treatments at 6 and 12 h had a reduction in germination; they were inferior to the control. This behavior was similar at seven and 60 days. In the control and in the treatment at 6 h, germination was at least 85%. At 30 days no differences were observed between the treatments and the control. Apparently, the corneal characteristic of the endosperm influenced to obtain a good level of germination in this population, in comparison with the floury endosperm populations that showed low germination (Pérez de la Cerda *et al*., 2007).

Table 4. Comparison of means by means of the Tukey test in the standard germination variable of the Roque Amarillo and Pozolero population at 7, 30 and 60 days of storage, Roque, Guanajuato. 2016.

Treatments	Amarillo	Pozolero White		
	7 days	7 days	30 days	60 days
Control	97 a	47 a	45a	44a
6 h	85 ab	55 a	46 a	14 b
12 h	57bc	21 b	28 b	5 b
Tukey	31.8	17.14	15.3	25.5

Treatments with equal letters within each variable are statistically equal (Tukey, $p< 0.05$).

Gutiérrez-Hernández *et al*., (2011) mention that there are different degrees of tolerance to accelerated aging; seed exposure to dry heat causes fewer normal seedlings, less accumulation of dry matter and higher proportion of dead seeds. Table 4 shows the comparison of means of the White Well, the 12-hour treatment had a negative effect on germination and was lower than the control in the three storage times, the six-hour treatment was statistically the same as the control seven and 30 days, but at 60 days, this treatment showed a negative effect and was statistically inferior to the control.

This population is floury endosperm, which affects germination over time, reflected in a very low germination. Villaroel and Méndez-Natera (2007) point out that the growth conditions affect the resistance of the maize seed to storage conditions, those in cold areas better resist refrigeration, this and the type of endosperm could be part of why these researchers found a favorable effect of accelerated aging on germination, different from the results obtained in this research.

In electrical conductivity, the treatments exceeded the control in Roque Amarillo, the electrical conductivity estimates the integrity of the cell membrane, the losses of cytoplasmic solutes with electrolytic properties are indicative of the rapid deterioration of the seeds (Hilmig and Méndez-Natera, 2011). The treatments of 6 and 12 h did not show differences between them (Table 5), the potassium nitrate did not influence the fluidity to the cell membranes. Ashley *et al*. (2006) indicate that potassium plays an important role in basic cellular functions; as well as in the regulation of osmotic pressure and membrane potential.

Table 5. Comparison of population means Roque Amarillo for the variable's imbibition, electrical conductivity, pH and percentage of ashes. Roque, Guanajuato. 2016.

Roque Amarillo	Imbibition (mg)	Electric conductivity (µs cm^{-1})	pH	Ashes (%)
Control	0 c	1.88 b	6.15 c	1.18 b
6 h	16 b	14.08 a	6.27 b	1.43 a
12 h	21.88 a	14.74 a	6.29 a	1.42 a
Tukey	1.01	1.96	0.01	0.16

Treatments with equal letters within each variable are statistically equal (Tukey, $p< 0.05$).

The pH of the seeds was increased at 6 and 12 o'clock, beating the control (6.15). Hilmig and Méndez-Natera (2011) reported a very narrow pH range, between 4.9 and 5.6 in maize seeds; the seeds that recorded a pH greater than 5.8 were killed; however, the pH obtained in this investigation were higher than those reported by this author, and it differs in that the seeds of this case were not killed.

As a percentage of ashes, the 6 and 12 hour treatments presented values higher than the control by 18%. Sánchez (2004) determined ash contents from 1.4 to 1.54% in five yellow maize genotypes, in the data presented here it is clearly observed that the control showed lower ash content, while in the treatments the percentage of ash increases, the values found here are in accordance with the values reported by the aforementioned author, the application of potassium nitrate improved the ash content in the treated seeds (Table 6).

Table 6. Comparison of population means Elotes Occidentales (red pigmentation) for the variable's imbibition, electrical conductivity, pH and percentage of ashes. Roque, Guanajuato. 2016.

Elotes Occidentales	Imbibition (mg)	Electric conductivity (µs cm^{-1})	pH	Ashes (%)
Control	0 c	4.78 c	6.03 c	1.09 b
6 h	10.82 b	34.44 a	6.24 b	1.42 a
12 h	25.23 a	29.22 b	6.27 a	1.57 a
Tukey	0.95	2.75	0.02	0.22

Treatments with equal letters within each variable are statistically equal (Tukey, $p< 0.05$).

The FAO (1985) points out that the mineral that is most abundant in the seed is phosphorus in the form of potassium and magnesium phytates, being entirely in the embryo, with values of approximately 0.9% in common maize and about 0.92% in opaque maize-2.

In six hours imbibition maize showed superior absorption Pozolero 12 h and the control, in a first phase, the seed absorbs water proportionally to the soaking time, but there comes a point where the absorption decreases, at 12 h soaking, the seeds decrease the absorbed water, which was observed by Méndez-Natera *et al*. (2008) for the maize, caraota (bean) and quinchoncho (pea) crops with rate increases until 8 and 10 h after imbibition, respectively (Table 7).

Table 7. Comparison of population means Pozolero white, for the variables, imbibition, electrical conductivity, pH and percentage of ashes. Roque, Guanajuato. 2016.

Pozolero white	Imbibition (mg)	Electric conductivity (µs cm^{-1})	pH	Ashes (%)
Control	0 c	8.74 b	6.17 b	1.31 b
6 h	23.41 a	38.41 a	6.29 a	1.53 a
12 h	21.43 b	44.88 a	6.31 a	1.65 a
Tukey	0.81	8.04	0.02	0.2

In electrical conductivity, the control (8.74 µs cm^{-1}) showed lower ion output compared to treatments. Nitrate is indirectly involved in protein synthesis and carbohydrate metabolism and in delaying seed aging. Its effect on tomato seeds (*S. lycopersicum*) increased the total protein content and nitrate reductase activity, although it did not affect the germination percentage, uniformity and synchrony. The treatment effect could be observed, positively, in low quality seeds (Lara *et al*., 2014).

Figure 1 shows the means adjusted for minimum squares. It is observed that the Amarillo and hybrid varieties were statistically equal with a higher percentage of germination during storage with little effect of the latter. The Morado and Pozolero varieties were greatly affected by the three-month storage. This is because its endosperm is starchy, the Rojo variety was also affected but to a lesser extent than the previous two; this effect of decrease in germination by storage time was notorious until the third month.

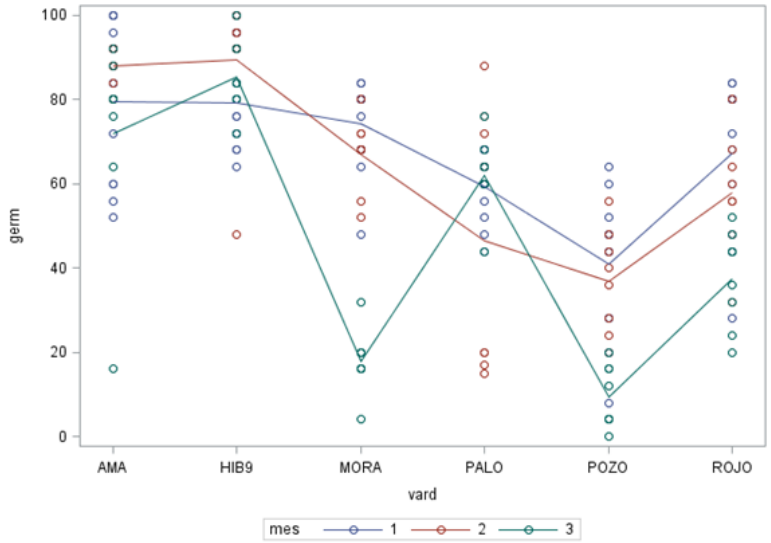

Figure 1. Illustration of the interaction variety x storage time in the germination variable. Germ= germination percentage; var: variety; AMA= yellow; HIB9, hybrid; MORA= Elotes Occidental Morado; PALO= palomero maize; POZO= Pozolero white maize; ROJO= Elotes Occidentales Rojo; trat= treatment 1 (0 h), 2 (6 h) and 3 (12 h).

According to Finch-Savage *et al*. (2004) the accumulation of harmful metabolites, during the soaking test under anaerobic conditions, the percentage of germination decreases, especially in sensitive cultivars. Ventura *et al*. (2012) point out that the beneficial effect of osmoaconditioning can be subsequently lost due to oxidative stress.

In the Palomero the variables imbibition and electrical conductivity in the two treatments were not significantly different ($p \geq 0.05$) but exceeded the control; maize seed usually has an acidic pH, which ranges between 4-5. The 6-hour treatment reached values of 6.16, some authors point out that the pH variance of the maize is a condition for the quality of the seed, since very small alterations usually activate the proteins and enzymes of the internal structure mainly embryo.

The analysis of variance of the Palomero Toluqueño population for the standard germination variable indicated that there were no significant differences between treatments. The percentage of ashes, the two treatments are not significantly different ($p \geq 0.05$), but are higher than the control, the application of potassium nitrate had a significant effect (Table 8).

Table 8. Comparison of means of the Toluqueño palomero population for the variable's protein, imbibition, electrical conductivity, pH and percentage of ashes. Roque, Guanajuato. 2016.

Palomero Toluqueño	Imbibition (mg)	Electric conductivity (µs cm^{-1})	pH	Ashes (%)
Control	0 b	1.84 b	5.95 c	1.11 b
6 h	11.85 a	9.93 a	6.16 a	1.74 a
12 h	12.46 a	11.01 a	6.05 b	1.72 a
Tukey	1.04	1.82	0.01	0.2

Figure 2 shows that the treatment time at 12 h most strongly affected the Palomero, Amarillo and hybrid population were the least affected with the treatments, had more germination than the other populations. Morado and Palomero behaved similarly regarding the treatment. Palomero and Rojo behaved similarly.

There is a clear effect of the treatments on the germination of the maize seed. The treatment at 12 affects in a more pronounced way the germinative capacity of the seed, so other times and other substances could be evaluated to discover any substance that could have a positive effect on the germination and vigor of the seed, which also help withstand aging, as described by other researchers (Finch-Savage *et al*., 2004; Villaroel and Méndez-Natera (2007), although it is difficult to determine the correct imbibition time to then dry the seed without suffering damage (Ventura *et al*., 2012).

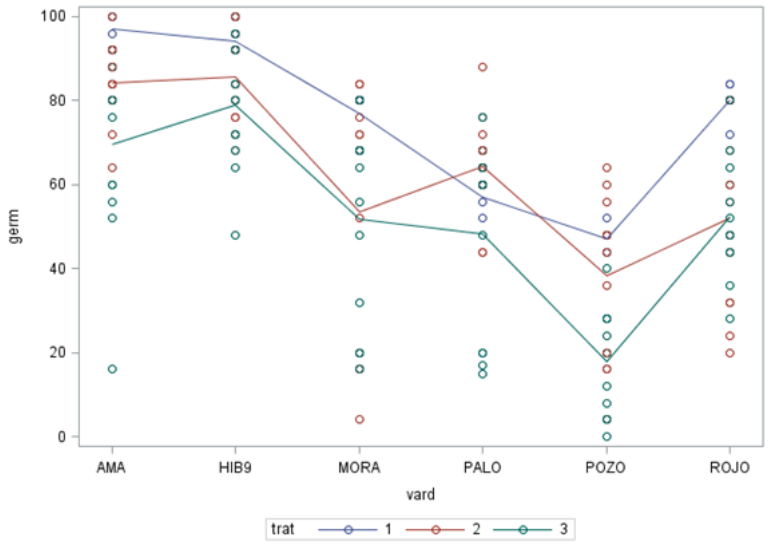

Figure 2. Illustration of the interaction variety x treatment of osmoaconditioning in the germination variable. Germ= germination percentage; var: variety; AMA= yellow; HIB9, hybrid; MORA= Elotes Occidentales Morado; PALO= palomero maize; POZO= Pozolero white maize; ROJO= Elotes Occidentales Rojo; trat= treatment 1 (0 h), 2 (6 h) and 3 (12 h).

Conclusions

Osmoconditioning did not improve seed resistance to accelerated aging damage. There was a negative effect of the treatments on the germination of the seed, although the one of hybrid origin resisted the treatments, followed by the one of corneal endosperm (Roque Amarillo), the Palomero, although it is of hard endosperm it did not show the resistance of the mentioned varieties, but neither is the susceptibility of soft endosperm, Elotes Occidentales and Pozolero. Osmoconditioning at 12 h has a more adverse effect than treatment at 6 h. The results support the proposal that accelerated aging gives a good idea of the physiological quality of the seed.

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