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Osmolyte accumulation and antioxidant metabolism during germination of vigorous maize seeds subjected to water deficit

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ABSTRACT. The objective of this work was to evaluate the alterations of antioxidant enzyme reserves and antioxidant enzymes during germination under water deficit in maize hybrids and to associate with seed vigor, determining the mechanisms related to tolerance for this stress. Two three-way maize hybrids were characterized by their vigor at different levels of water deficit induced by polyethylene glycol 6000. Next, the seeds were hydrated at different osmotic potentials (0.0, -0.3, and -0.9 MPa) and removed at different times to assess the levels of the total soluble protein, total soluble sugars, proline, starch, and antioxidant enzymes, such as superoxide dismutase, catalase and ascorbate peroxidase. The analysis of variance, Tukey test at 5% and principal component analysis (PCA) were used. The vigorous hybrid (HT1) was more efficient than the low vigor hybrid seeds (HT2) in mobilizing the total soluble protein during the initial stages of germination and the total soluble sugars before and after root protrusion under water deficit in addition to increasing the catalase activity at the different osmotic potentials that were assessed.

Keywords: *Zea mays* L.; vigor; antioxidant enzymes; reserve compounds.

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Introduction

Vigorous seeds are capable of adapting to a wide range of adverse environmental conditions for survival (Marcos-Filho, 2015), including drought, and thereby ensuring the establishment of an adequate seedling stand in the field. In this respect, seedling germination and their emergence under a water deficit have been shown to be dependent on the physiological quality of the seeds (Ávila, Braccini, & Scapim, 2007).

The germination process begins with water being imbibed by the seed (Brito et al., 2015). Water is vital to cell metabolism during germination and is responsible for several internal processes within the seed, including enzyme activation and the solubilization and transport of reserve compounds, primarily in the hydrolytic digestion of the stored reserves (Marcos-Filho, 2005). The accumulated reserves are used after seed hydration, particularly in stage two of the germination, triggering intense metabolic activity, such as respiration and changes in the protein, lipid and carbohydrate content (Han, Yin, He, & Yang, 2013).

Most stored compounds are used at the end of the stage two/three germination to sustain seedling growth until it becomes autotrophic; however, energy is expended on the growth and development of the radicle and plumule before germination is complete (Bewley, 2001). The seed hydration declines under a water deficit, altering and limiting the mobilization of the stored reserves to the growing tissue, which delays the germination process.

A defense mechanism in response to the stress is the accumulation of organic osmolytes, such as proline and soluble sugars in the cytoplasm during drought (Li, Li, Zhang, Liu, & Guan, 2013). Proline is one of the main organic osmolytes and favors seedling adaptation to water stress (Agostini, Machado-Neto, & Custódio, 2013). Drought also leads to the accumulation of reactive oxygen species (ROS); however, such enzymes as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) minimize the oxidative damage under these conditions (Amirjani & Mahdiyeh, 2013; Anjum et al., 2017).

To determine the biochemical components and the differences in their contents that lead to drought tolerance in seeds, studies on the germination and the initial growth of seedlings may help to identify which mechanisms explain the differences associated with seed vigor.

In this respect, the aim of this study was to evaluate the alterations of antioxidant enzyme reserves and antioxidant enzymes during germination under water deficit in maize hybrids and to associate with seed vigor, determining the mechanisms related to tolerance for this stress.

Material and methods

Plant material

Two conventional triple-crossed maize hybrids (HT1 and HT2) were used. Both hybrids were produced in the 2015/2016 growing season and provided by a local company in the municipality of Coxilha in the state of Rio Grande do Sul. Both were previously characterized in terms of vigor according to Prazeres and Coelho (2017). The HT1 hybrid showed higher germination (96%), higher vigor by accelerated aging (89%) and a low electrical conductivity (10.81 μS cm^{-1} g^{-1}). The hybrid HT2 had a germination of 84%, low vigor by accelerated aging (22%) and a high electrical conductivity (19.56 μS cm^{-1} g^{-1}). In addition, root protrusion occurred in less time in the high vigor hybrid (HT1) for all of the osmotic potentials compared to its low vigor counterpart (HT2).

The HT1 hybrid was harvested in bulk, with an axial flow with 15% water content in the seeds, and the HT2 hybrid was harvested manually with 32% water content. Both hybrids were artificially dried at the company in a forced circulation oven with temperatures between 38 and 45ºC until reaching a humidity of 11%. The seed samples were received immediately after harvest and processed in a research laboratory for homogenization and to reduce the working sample (900 g), in line with the Rules for Seed Analysis (*Ministério da Agricultura, Pecuária e Abastecimento* [MAPA], *Secretaria de Defesa Agropecuária* [SDA], 2009). The work sample was divided into replicates, also by means of the homogenizer, and after this procedure, the sample remained in a dry chamber with 50% humidity and a temperature of 10 ± 2ºC until use in the experiment.

Assessment of germination and vigor under water deficit

Germination was evaluated according to the Rules for Seed Analysis (Brazil, 2009). The seeds were placed at equal distances apart on germitest paper, which was rolled up and stored at 25ºC. The normal seedlings were counted after seven days. The vigor was determined by simulating a water deficit using two levels (-0.3 and -0.9 MPa) of polyethylene glycol 6000 (PEG 6000) according to the osmotic potentials found in Villela, Doni Filho, and Sequeira (1991). The seeds were maintained at 25ºC in rolls of germitest paper, and the normal seedlings were evaluated after seven days. The time required for 50% of the seeds to germinate (T50) was assessed during germination and water deficit simulation and counted from the moment the radicle reached 2 mm, as measured with a pachymeter.

Seed hydration and water deficit simulation

Three conditions were used for seed hydration: the control (0.0 MPa), using distilled water, and two simulated levels (-0.3 and -0.9 MPa) of water deficit using polyethylene glycol 6000 at 25ºC, with rolls of germitest paper being used as the substrates. The hydration times in water were 0, 12, 24, 36, 48, and 72h; the hydration times at an osmotic potential of -0.3 MPa were 0, 24, 36, 48, 72, 96, and 120h; and the hydration times at an osmotic potential of -0.9 MPa were 0, 24, 48, 72, 96, 144, 168, and 192h. For each time period, approximately 20 g of seeds were collected for biochemical analyses.

The seeds containing PEG 6000 were washed with distilled water. Next, the endosperm was removed from all the seeds to quantify the composition of the biochemical and the enzymatic compounds at the different hydration times. The hydrolysis rate and the mobilization of the reserve compounds during germination were determined based on the difference between initial levels and the levels at root protrusion for each hybrid.

Extraction and quantification of biochemical compounds

The total soluble protein was extracted using 0.5 grams of maize endosperm, which was ground with liquid nitrogen and added to 2 mL of potassium phosphate buffer at 100 mM (pH 7.5), 3 mM dithiothreitol and 4% (p/v) polyvinylpolypyrrolidone (Azevedo, Alas, Smith, & Lea, 1998). The extract was centrifuged at 2270 g for 30 min at 4ºC. The supernatant (20 μL) was collected to read the samples at 595 nm in a

spectrophotometer according to the method described by Bradford (1976) using bovine serum albumin (BSA) as the protein standard. The remaining supernatant was stored at -20ºC and used to determine the enzymatic activity of the SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), and APX (CE 1.11.1.11).

The proline content was measured using the method described by Bates, Waldren, and Teare (1973) with 0.2 g of ground endosperm, homogenized in 10 mL of 3% sulfosalicylic acid and centrifuged at 2,270 *g* for 20 min. at 15ºC. Next, 2 mL of the diluted supernatant (200 µL of the sample in 1.800 µL distilled water), 2 mL of acidic ninhydrin, and 2 mL of glacial acetic acid were added to a glass test tube for 1 hour at 100°C and cooled in an ice bath for 10 min. Then toluene was added to the reaction mixture, and the test tube was shaken for 15 seconds using a vortex mixer and allowed to rest for 10 minutes. The chromophore was aspirated from the top (pink) and the absorbance was read at 520 nm using toluene as a blank. The proline content was determined using a standard curve and calculated based on the fresh weight as follows: µmoles of proline g^{-1} fresh weight = [(µg proline mL^{-1} x mL toluene)/115.5 (molar weight) µg $µmole^{-1}$] / [(g sample)/5].

The total soluble sugar content of the seed endosperm was calculated in line with the methodology described by Clegg (1956). The endosperm (0.25 g) was dried at 65ºC for 48 hours, ground and homogenized with 25 mL of 80% ethanol at 60°C for 15 minutes and later centrifuged for 7 minutes at 10,000 *g*. The supernatant was separated, and the precipitate was homogenized with 30 mL of 80% ethanol. The supernatants were mixed, and a 200 µL aliquot was removed for quantification in a spectrophotometer. The aliquot was added to 2 mL of an anthrone reagent. The samples were placed in a water bath for 3 minutes and cooled, and the absorbance was measured in a spectrophotometer at a wavelength of 620 nm.

The starch content was measured using the precipitate derived from total soluble sugar extraction. We added 20 mL of 0.2 N sulfuric acid to the test tube, which was subsequently sealed, shaken and placed in a water bath for 2 hours at 100°C. A 10 µL aliquot was removed from the extract and diluted in 990 µL of distilled water, and 400 µL of the diluted solution was placed in a glass test tube and added to 600 µL of distilled water and 3 mL of an anthrone reagent. Next, the samples were placed in a boiling water bath for 3 minutes, cooled in an ice bath for 5 minutes and the absorbance was measured in a spectrophotometer at 620 nm. The result was multiplied by a factor of 0.9 to convert glucose into starch according to the methodology proposed by McCready, Guggolz, Siliviera, and Owens (1950).

Determination of antioxidant enzyme activity

Superoxide dismutase (SOD) activity (EC 1.15.1.1) was analyzed using the method reported by Giannopolitis and Ries (1977). The reaction consisted of combining 30 µL of the extract, 150 µL of 1.3 µM riboflavin, 225 µL of nitrotetrazolium blue (NBT) and 2.5 mL of a mixture composed of 26.7 mL of 50 mM sodium phosphate buffer (pH 7.8), 450 µL of 0.1 mM ethylenediamine tetraacetic acid (EDTA) and 11.7 mL of 13 mM methionine. The test tubes were placed in a chamber containing fluorescent lamps for 5 minutes for reaction (purple) purposes. The reaction rate was determined by measuring the absorbance at 560 nm in a spectrophotometer.

The catalase (CAT) activity (EC 1.11.1.6) was analyzed according to Aebi (1984). The reaction consisted of adding 10 µL of the extract to 2 mL of a mixture containing 16 mL of 50 mM potassium phosphate buffer (pH 7.5) and 20 mL of 30% hydrogen peroxide (H_2O_2). The decomposition of the H_2O_2 was analyzed directly by measuring the decreasing absorbance at 240 nm over 120 seconds.

The ascorbate peroxidase (APX) activity (CE 1.11.1.11) was evaluated using the methodology described by Nakano and Asada (1981). The reaction mixture was composed of 20 µL of the total soluble protein extract, 80 mM potassium phosphate buffer (pH 7.0), 5 mM ascorbate, 1 mM EDTA and 1 mM of 30% hydrogen peroxide (H_2O_2). The entire reaction was performed at 30ºC, and the buffer, ascorbate stock solutions, and EDTA remained in a water bath during the test. The absorbance reduction at 290 nm was monitored in a spectrophotometer from 0 to 60 seconds.

Statistical analysis

A randomized block design was used, with a factorial scheme consisting of two hybrids and three osmotic conditions, four repetitions for the physiological assessments and three repetitions for the remaining analyses. Analysis of variance (ANOVA), Tukey's test at 5% probability and principal component analysis (PCA) were applied, using R software (R Core Team, 2016).

Results and discussion

The results obtained demonstrated that the percentage of normal seedlings from the maize seeds exposed to a water deficit declined significantly with an increase in the osmotic potential of the substrate, as shown in Table 1. The hybrid HT1 exhibited a higher germination percentage (0.0 MPa) of normal seedlings under the water deficit than HT2 for all of the osmotic potentials studied. There was a drastic decline in the percentage of normal seedlings for HT2 at -0.9 MPa, likely as a result of the damage to different physiological processes as a function of the lower water availability in the substrate.

Table 1. Mean percentages of normal seedlings for germination (0.0 MPa) and vigor under water deficit conditions (-0.3 and -0.9 MPa) in hybrids HT1 and HT2.

Hybrids	0.0 MPa	-0.3 MPa	-0.9 MPa
HT1	96 aA	94 aA	64 aB
HT2	84 bA	68 bB	30 bC
Means	90	81	47
%CV	4.02	5.12	12.39

*Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically according to Tukey's test at 5% probability.

Similar trends have been reported by other researchers, who found a significant decline in the germination capacity of maize seeds as a function of reduced osmotic potential caused by polyethylene glycol 6000, helping to identify drought-resistant maize genotypes (Khayatnezhad, Gholamin, Jamaati-e-Somarin, & Zabihi-e-Mahmoodabad, 2010; Vaz-de-Melo et al., 2012; Abreu et al., 2014; Partheeban, Chandrasekhar, Jeyakumar, Ravikesavan, & Gnanam, 2017), as observed in the present study.In hydration with water, hybrid HT1 achieved protrusion in 36 hours and HT2 achieved protrusion in 48 hours; at -0.3 MPa, HT1 obtained protrusion in 48 hours and HT2 in 96 hours; and at -0.9 MPa, protrusion occurred in 72 and 168 hours for HT1 and HT2, respectively. According to Prazeres and Coelho (2017), root protrusion was achieved in less time in the high vigor hybrid (HT1) than its low vigor counterpart (HT2) for all of the osmotic potentials. Root protrusion was used as a reference point for the mobilization of the stored reserves.

There was a significant difference between the hybrids in the levels of the total soluble protein, total soluble sugars, proline and starch during germination at the osmotic potentials studied (Figures 1 and 2). The mobilization of the total soluble protein (TSP) until root protrusion in water (0.0 MPa) was greater for HT1 (36.43%) than HT2 (31.51%), and the aforementioned levels were higher for HT1 in the initial germinations stages (Figure 1a). According to Soriano et al. (2011), the early use of nitrogen from the soluble protein is essential in meeting the demand for amino acids in the early phases of germination.

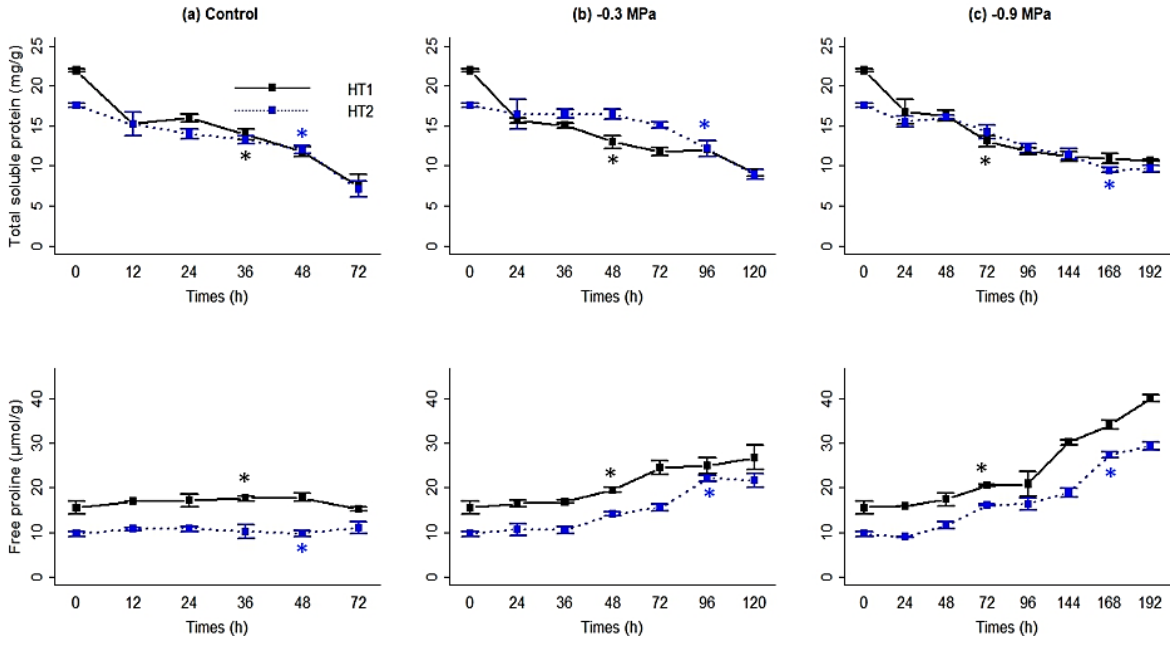

Figure 1. Changes in the total soluble protein and free proline levels in the endosperm of maize seeds during germination in water (control) and negative osmotic potentials (-0.3 and -0.9 MPa). *Indicates root protrusion. The error bar indicates the mean ± standard deviation.

Due to the negative osmotic potential (-0.3 MPa), the mobilization of the TSP reserves until root protrusion was 40.8% (48h) for the hybrid HT1 and 30.81% for HT2 (96h). The greater use of the TSP in the HT1 may serve a dual purpose in cell expansion and as osmoprotection, maintaining drought tolerance and enabling rapid root protrusion (48h) compared to HT2, even under stress conditions. By contrast, at -0.9 MPa, the TSP mobilization was 40.35% and 46.07% for the HT1 and HT2, respectively. Despite the high TSP mobilization observed for the low vigor hybrid, its performance was not superior, and the seeds were exposed to PEG 6000 for an extended period of time, confirmed by the considerable delay in root protrusion (168h).

The changes observed by Li et al. (2017) in a study of sweet corn seeds also demonstrated that the total soluble protein content declined linearly for the water treatment during germination, whereas the mobilization occurred slowly for negative osmotic potentials (-0.3, -0.6, -0.9, and 1.2 MPa). The results obtained in the present study indicated lower TSP mobilization in the low vigor hybrid for seed hydration in water (0.0 MPa) and at -0.3 MPa, leading to delayed root protrusion when compared to the hybrid HT1.

There was a difference between the hybrids in terms of the free proline (PRO) content during the seed hydration for all the osmotic potentials assessed (Figure 1). For the hydration in water, the levels remained stable over time with means of 16.71 µmol g^{-1} for HT1 and 10.33 µmol g^{-1} for HT2 (Figure 1a). From the start of the hydration until the root protrusion (36h), the PRO levels increased 13.31% for the hybrid HT1 but only 0.5% for HT2 (48h). The seeds that remained under a water deficit showed a significant increase in the PRO levels during the exposure time (Figure 1b and c).

The proline content rose in the seeds at the negative osmotic potentials (-0.3 and -0.9 MPa), increasing by 25.68 and 128.81% for HT1 and HT2, respectively, at -0.3 MPa. In PEG 6000 at -0.9 MPa, the PRO level varied from 15.53 µmol g^{-1} to 20.60 µmol g^{-1} (72h) for HT1 and 9.67 µmol g^{-1} to 27.4 µmol g^{-1} for HT2 over 168h, which corresponds to an increase of 32.65 and 183.3%, respectively, for HT1 and HT2. Although the PRO increases were lower in the hybrid HT1 than HT2, osmoprotection was observed in the seeds exposed to PEG 6000, evident in the rapid root protrusion and improved physiological performance observed under a water deficit.

Queiroz and Cazetta (2016) studied whether maize seeds are capable of developing osmoprotective mechanisms when germinating under low osmotic potentials (-0.3, -0.6, -0.9, and -1.2 MPa).The authors observed that the proline content in the embryonic axis of the maize seeds germinating under a water deficit is directly proportional to the stress intensity and does not correlate with the levels found in the endosperm. In the present study, the hybrid HT2 (low vigor) showed a greater increase in the PRO than HT1 when exposed to PEG 6000 but did not achieve drought tolerance, resulting in delayed root protrusion, which was not associated with the physiological quality of the seeds.

The hybrids differed in relation to starch hydrolysis and the mobilization of the total soluble sugars as a function of time and the osmotic potentials, as analyzed in Figure 2.

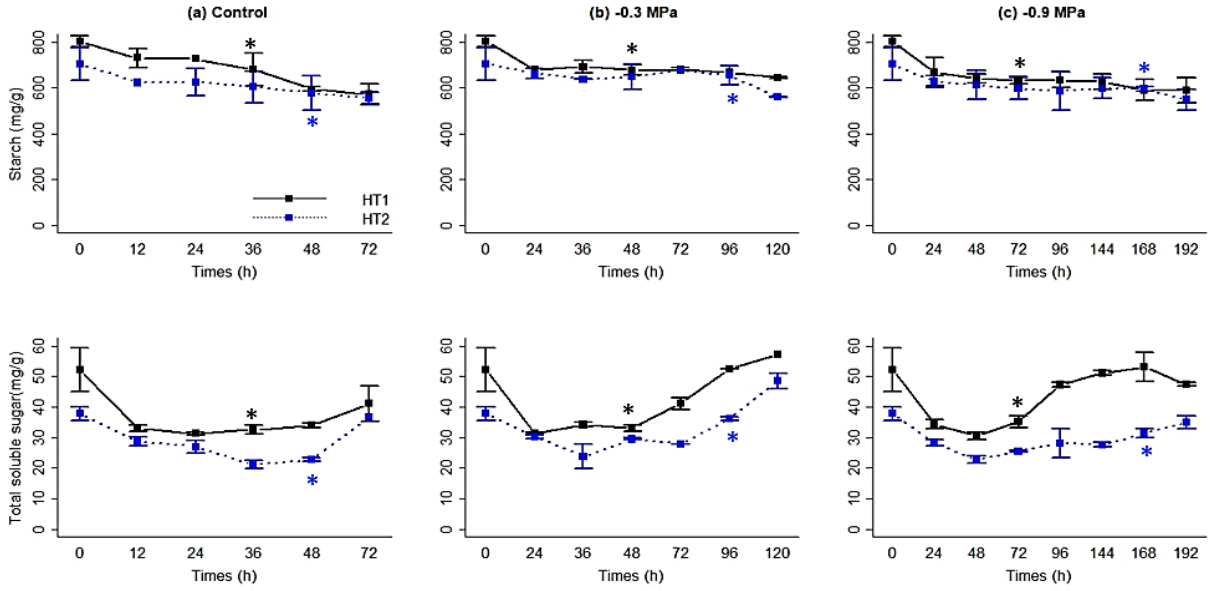

Figure 2. Changes in starch and the total soluble sugar levels in the endosperm of maize seeds during germination in water (control) and negative osmotic potentials (-0.3 and -0.9 MPa). *Indicates root protrusion. The error bar indicates the mean ± standard deviation.

The initial starch content was higher in HT1 compared to HT2 with 801.2 mg g^{-1} and 703.3 mg g^{-1}, respectively (Figure 2). The starch hydrolysis until root protrusion for HT1 was 15.03% during germination in water, 15.52% at -0.3 MPa and 21.07% at -0.9 MPa. The starch content remained stable for HT2 during the treatment in water and at -0.9 MPa, declining at -0.3 MPa from 96h onwards (root protrusion) by 6.95%. The slight decline in the starch content for HT2 confirms the hybrid's slow metabolism, due to its lower vigor in relation to HT1.

The starch in the storage organs of seeds (endosperm) is degraded and converted into soluble forms that can be easily transported to the growth regions to produce energy (Bewley, 2001). These data demonstrate the superior starch hydrolysis of the high vigor hybrid in relation to its low vigor counterpart under the conditions tested. However, high starch hydrolysis was required at the start of the seed hydration, since the seed uses the total soluble sugar that has already been degraded to meet the initial energy needs for the embryo growth.

The mobilization of the total soluble sugars (TSS) differed between the hybrids over time for the different osmotic potentials studied (Figure 2). The early TSS mobilization was observed in the seeds hydrated in water and for the PEG 6000 treatment. According to Pritchard, Charlton, Baker, and Graham, (2002), this rapid decline in the TSS before root protrusion may occur as the soluble sugars are used as a form of energy prior to the degradation of the stored reserves.

The TSS mobilization before root protrusion was 37.53 and 39.73% for HT2 and HT1 seeds hydrated in water, respectively. A 36.6% decline in the TSS was observed for the hybrid HT1 at an osmotic potential of -0.3 MPa, demonstrating its rapid metabolism in relation to HT2 with only 4.76%. At -0.9 MPa, the mobilization before root protrusion reached 32.48% for HT1 and 16.93% for HT2. After root protrusion, a significant increase in the TSS content was observed in both hybrids at negative osmotic potentials, albeit more accentuated for HT1 at -0.9 MPa. The rise in total soluble sugars after the root protrusion indicates that this compound may be associated with protecting or maintaining the structure of the cell components, acting as an osmoprotection for seeds under stress (Seki, Umezawa, Urano, & Shinozaki, 2007).

Li et al. (2017) also observed a rise in the TSS levels after the root protrusion in sweet corn seeds under negative osmotic potential (-0.3 and -0.9 MPa), beginning at 48 hours and peaking at 72 hours. In the present study, the TSS content increased linearly for HT1 and HT2 at -0.3 MPa after the root protrusion. However, a larger increase was observed for HT1 after 72 hours as a function of the more severe water deficit (-0.9 MPa), peaking at 168 hours with a TSS level of 53 mg g^{-1}, whereas the hybrid HT2 only reached 31.4 mg g^{-1} over the same time period.

The total soluble sugars are highly sensitive to environmental stresses, and sucrose and hexose play dual roles in gene regulation, evident in the positive regulation of genes related to stress (Rosa et al., 2009). In this case, the results indicate the difference between the hybrids can be explained by the TSS mobilization before and after the root protrusion, suggesting that the compound may be related to drought tolerance in the high vigor seeds, such as the hybrid HT1.

Disaccharides (sucrose, trehalose), the raffinose family of oligosaccharides, and fructan are three major water soluble sugars involved in stress responses whose varying levels influence the production of the reactive oxygen species in the chloroplasts, mitochondria and peroxisomes of plant cells (Keunen, Peshev, Vangronsveld, Ende, & Cuypers, 2013).

Significant variations were observed in the superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities for seed hydration in water and at negative osmotic potentials (Figure 3).

The SOD activity increased significantly for all the osmotic concentrations assessed (Figure 3). A gradual increase was observed for both hybrids when the seeds were exposed to PEG 6000 at -0.3 MPa. The high SOD activity proportionally increased the hydrogen peroxide content (H_2O_2) by dismutation of the superoxide radical, leading to greater activity of the antioxidant enzymes, such as catalase and ascorbate peroxidase (Silva, Dias, Sekita, & Finger, 2018). In this case, the result suggested the presence of the SOD activity but no difference between the hybrids with respect to the physiological quality of the seeds.

A difference was observed between the hybrids for the catalase (CAT) activity during germination (Figure 3). For the hydration in water, a significant increase in the CAT activity was observed for both hybrids immediately after root protrusion, reaching 18.70 µmol $min.^{-1}$ mg^{-1} protein for HT1 and 13.97 µmol $min.^{-1}$ mg^{-1} protein for HT2, with equal levels observed at 72 hours. Under a water deficit (-0.3 MPa), the CAT activity increased prior to root protrusion for both hybrids (Figure 3b) but was significantly higher for HT1

after 24 hours of hydration. A difference was also observed past 144 hours (-0.9 MPa) with a peak in activity for HT1 that declined sharply after 168 hours of hydration.

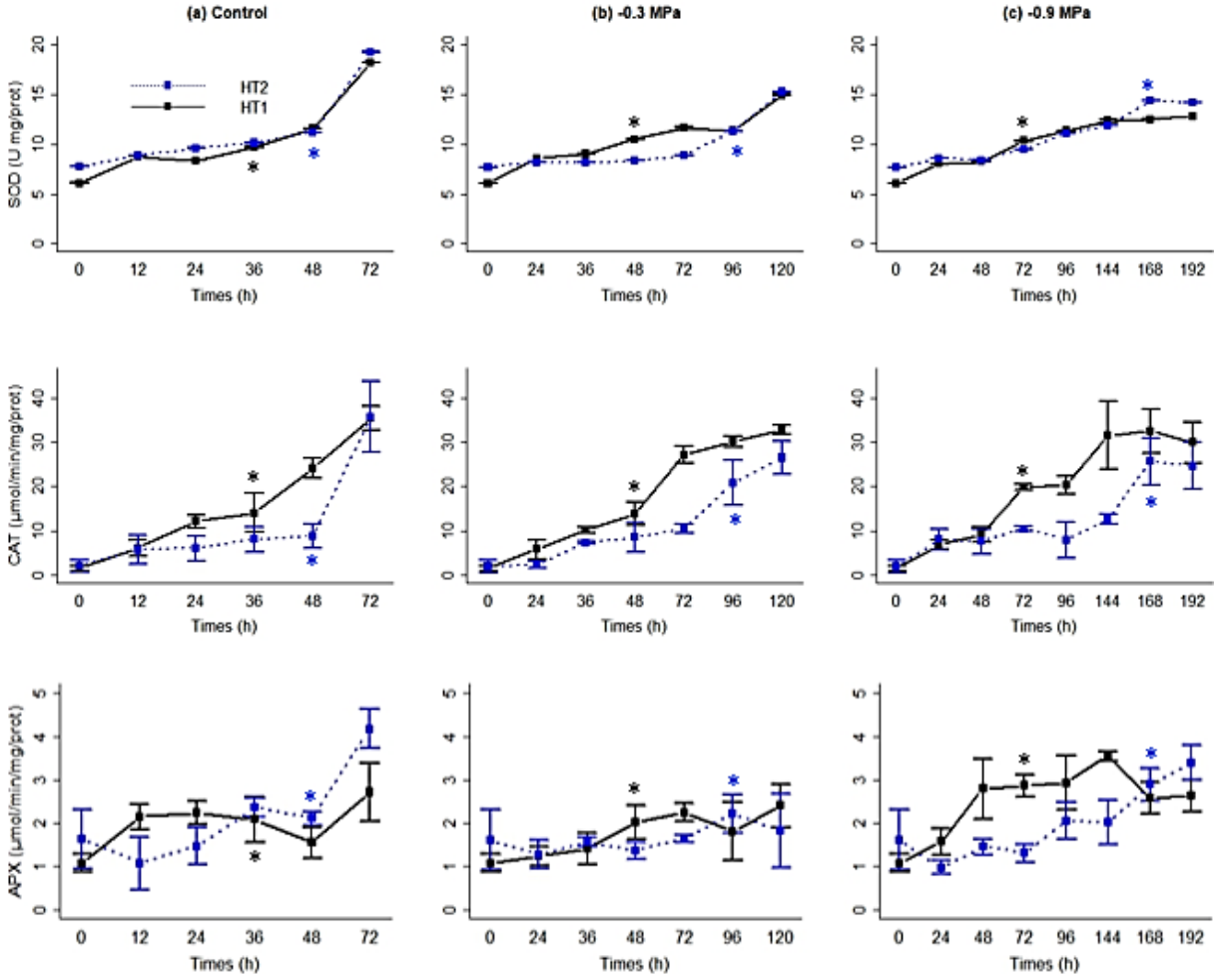

Figure 3. Changes in the activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in the endosperm of maize seeds during germination in water (control) and negative osmotic potentials (-0.3 and -0.9 MPa). *Indicates root protrusion. The error bar indicates the mean ± standard deviation.

Similar results were also found by Abreu et al. (2014) using electrophoresis, with greater CAT expression in the maize breeding lines exhibiting improved physiological quality, which is considered promising in terms of drought tolerance. In our study, the increase in CAT differed between the hybrids under certain conditions, particularly during germination under a water deficit (-0.3 MPa) and before root protrusion at -0.9 MPa. This enzyme may have resulted in a greater drought tolerance (vigor) for HT1, which showed earlier root protrusion in comparison to HT2.

The ascorbate peroxidase (APX) activity differed between the hybrids during seed hydration in water and at an osmotic potential of -0.9 MPa (Figure 3). In water, the APX increased after root protrusion for both hybrids, while at -0.3 MPa the hybrids remained stable throughout the assessment period. The ascorbate peroxidase activity was greater for HT1 at -0.9 MPa before and after root protrusion, with lower levels observed for HT2 and similar activity after 168 hours for both hybrids. In this case, the APX activity was similar for both hybrids in most of the water hydration scenarios and under negative osmotic potentials, with no difference being observed between the hybrids in terms of their physiological quality.

The principal components analysis (PCA) was performed to better describe the results indicating different biochemical and enzymatic behavior between the hybrids. Based on the findings obtained, the total variation (Figure 4) corresponded to 75.6%, with the first PCA (PCA1) responsible for 54.7% of the data variation and the second PCA (PCA2) accounting for 20.9%. When more than 70% of the total variance is preserved, the scatter plot provides a good picture of the data structure (Varmuza & Filzmoser, 2008). A difference was observed between the hybrids in relation to the biochemical components and the antioxidant enzymes (Figure 4).

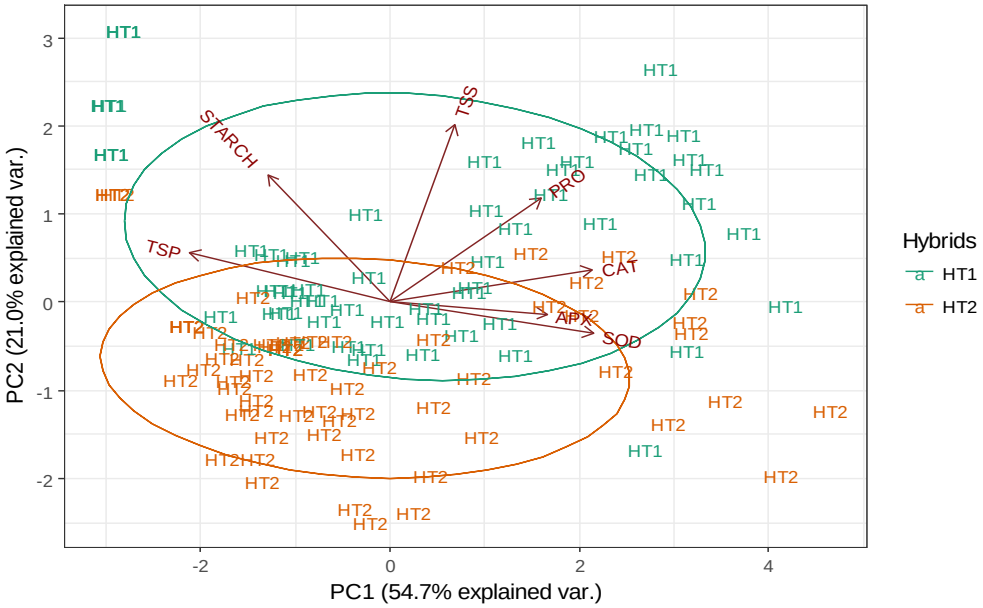

Figure 4. Principal component analysis (PCA) of biochemical components and antioxidant enzymes as a function of hybrids HT1 and HT2. TSP: Total soluble protein; TSS: Total soluble sugars; PRO: Free proline; CAT: Catalase; APX: ascorbate peroxidase; and SOD: superoxide dismutase.

The principal component analysis (PCA) indicated that the hybrids were allocated into different components. In the component PC1+/PC2+, the genotypes were grouped as a function of the total soluble sugars, proline, and catalase; in PC1+/PC2- according to the ascorbate peroxidase and superoxide dismutase; and in PC1-/PC2+ by the starch and total soluble protein, with no variables remaining in PC1-/PC2-. This grouping made it possible to determine which variables were most associated with the different hybrids. The PCA indicated a greater association between the HT1 and catalase, proline, TSS, starch and TSP, while hybrid HT2 was associated with the variables ascorbate peroxidase and superoxide dismutase. The results demonstrate that the high vigor hybrid used a larger number of mechanisms to tolerate a water deficit.

The eigen values were calculated (Figure 5) to complement the PCA.

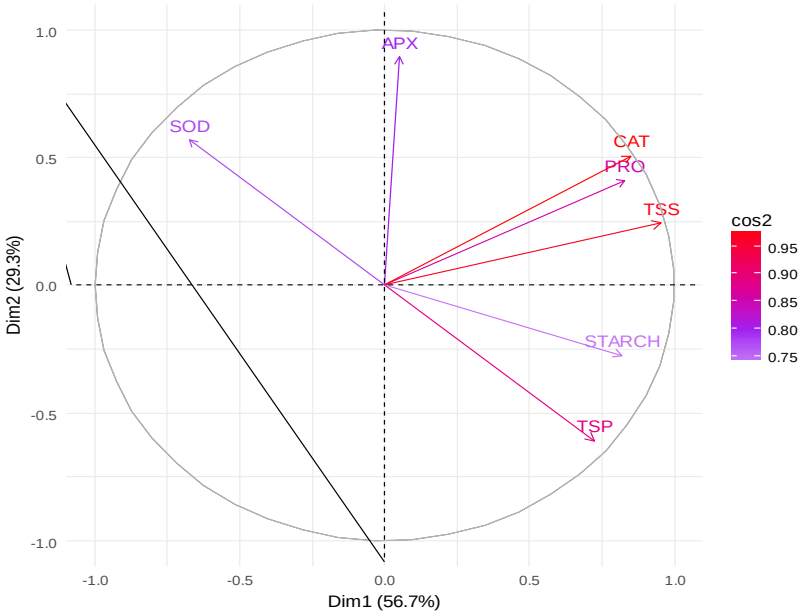

Figure 5. Eigen values identifying the variables that most contributed to data variability in PC1 and PC2. TSP: Total soluble protein; TSS: Total soluble sugars; PRO: Free proline; CAT: Catalase; APX: ascorbate peroxidase; and SOD: superoxide dismutase.

Based on the eigen values obtained, the variables that most significantly contributed to the data variability in the PCA model were the catalase, TSS, and TSP, while the superoxide dismutase, ascorbate peroxidase, starch, and proline contributed least significantly.

Vigor is related to the hydrolysis and mobilization of the reserves and the antioxidant enzyme activity over certain time periods during germination and at different osmotic potentials, indicating better drought tolerance in vigorous seeds compared to their low vigor counterparts. The variables chosen can be used as decisive biochemical markers in genotype selection as a function of the physiological quality.

Conclusion

The difference in the vigor between the hybrids can be explained by certain components of the hydrolysis and mobilization of the reserves and antioxidant enzymes under control conditions and a water deficit. The vigorous hybrid (HT1) was more efficient than the low vigor hybrid seeds (HT2) in mobilizing the TSPs during the initial stages of germination and the TSS before and after root protrusion under water deficit, as well as increasing the catalase activity at the different osmotic potentials assessed.

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