Research article

Effects of water deficit on radicle apex elongation and solute accumulation in *Zea mays* L

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ABSTRACT

In this study, we examined the effects of water deficit on the elongation of radicles of maize seedlings and on the accumulation of solutes in the radicle apices of two maize varieties: VS-22 (tolerant) and AMCCG-2 (susceptible). Sections of radicle corresponding to the first 2 mm of the primary roots were marked with black ink, and the seedlings were allowed to grow for 24, 48, and 72 h in polyvinyl chloride (PVC) tubes filled with vermiculite at three different water potentials (Ψ_w, −0.03, −1.0, and −1.5 MPa). The radicle elongation, sugar accumulation, and proline accumulation were determined after each of the growth periods specified above. The Ψ_w of the substrate affected the dynamics of primary root elongation in both varieties. In particular, the lowest Ψ_w (−1.5 MPa) inhibited root development by 72% and 90% for the VS-22 and AMCCG-2 varieties, respectively. The osmotic potential (Ψ_o) was reduced substantially in both varieties to maintain root turgor; however, VS-22 had a higher root turgor (0.67 MPa) than AMCCG-2 (0.2 MPa). These results suggest that both varieties possess a capacity for osmotic adjustment. Sugar began to accumulate within the first 24 h of radicle apex growth. The sugar concentration was higher in VS-22 root apices compared to AMCCG-2, and the amount of sugar accumulation increased with a decrease in Ψ_w. Significant amounts of trehalose accumulated in VS-22 and AMCCG-2 (29.8 µmol/g fresh weight [FW] and 5.24 µmol/g FW, respectively). Starch accumulation in the root apices of these two maize varieties also differed significantly, with a lower level in VS-22. In both varieties, the proline concentration also increased as a consequence of the water deficit. At 72 h, the proline concentration in VS-22 (16.2 µmol/g FW) was almost 3 times greater than that in AMCCG-2 (5.19 µmol/g FW). Trehalose also showed a 3-fold increase in the tolerant variety. Accumulation of these solutes in the root growth zone may indicate an osmotic adjustment (OA) to maintain turgor pressure.

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1. Introduction

Water deficit affects the growth of crops and can reduce their productivity by up to 70% (Boyer, 1982; Edmeades et al., 1993). However, when plants perceive a water deficit, they may activate adaptation mechanisms at the physiological, metabolic and genetic levels (Chaves et al., 2003). The main adaptations include the maintenance of cell turgor, changes in the fluidity and composition of cell membranes, reductions in photosynthetic activity, changes in carbon metabolism, and solute accumulation (Chaves and Oliveira, 2004). Drought tolerance depends on the magnitude of the changes in the above-mentioned adaptations (Baud et al., 2004; Massonnet et al., 2007).

For maize, varieties that tolerate moderate drought stress have been selected, and this tolerance is based on the capacity to make physiological and metabolic changes (Cramer et al., 2011; Skirycz and Inzé, 2010). The root is the primary sensory organ of water

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deficits in the soil, and in maize seedlings, the root apex continues to grow even in substrates with water potentials (Ψ_W) as low as −1.6 Mpa (Sharp and Davies, 1979). This growth characteristic is an adaptation to water-limiting conditions, and it is crucial for the formation of the aerial portion of the plant (Hsiao and Xu, 2000; Sharp and Davies, 1979; Westgate and Boyer, 1985; Yamaguchi and Sharp, 2010). Several authors have concluded that, in maize plants, the root architecture, length and water extraction are closely related; however, other studies have shown poor relationships between water uptake and root length and density compared with other crops, such as chickpea and lentil (Valdez et al., 2013). When exposed to water deficit conditions, the elongation zone of the maize radicle has the capacity for osmotic adjustment, and an increase of 45% in the proline concentration has been observed (Sharp et al., 1990; Voetberg and Sharp, 1991).

Trehalose is a new and little-studied player in drought tolerance in plants. Trehalose is the most widespread disaccharide in nature, occurring in bacteria, fungi, insects and plants. Typically, trehalose is synthesized in a two-reaction process, in which trehalose 6-phosphate (T6P) is first synthesized from glucose 6-P and UDP-glucose by the enzyme trehalose phosphate synthase (TPS) and then dephosphorylated by trehalose 6-P phosphatase (TPP). Trehalose is degraded by the enzyme trehalase, yielding two molecules of glucose (Avonce et al., 2006; Zentella et al., 2009). The intermediary T6P regulates starch synthesis and is used by chloroplasts of prokaryotic origin to report the carbon metabolic status of the cytosol in plants (Kolbe at al., 2005).

Although physiological studies related to the radicle elongation of maize plants under water stress have been performed, it is very important to continue to identify and obtain maize germplasm that possesses key physiological and biochemical characteristics that allow it to adapt to adverse drought conditions. Materials that are considered to be drought tolerant are expected to exhibit greater water use and accumulation efficiencies, which will allow them to continue growing under conditions of water limitation. Therefore, the objective of the present study was to determine the effects of a decrease in substrate moisture content on both the longitudinal growth of the radicle zone and the pattern of compatible drought-related solutes in two contrasting maize varieties, VS-22 and AMCCG-2.

2. Materials and methods

2.1. Vegetative material

Two varieties of maize were evaluated: VS-22, a variety selected under environmental conditions in which water was the main constraint, and AMCCG-2, a variety selected for grain quality in industrial processes (nixtamalization) and presumed to lack tolerance to water deficits. Both varieties were developed at the Colegio de Postgraduados, Texcoco, State of Mexico, and recommended for the high-elevation valleys of central Mexico (Puebla, Tlaxcala, Veracruz, Mexico and Hidalgo). They are both characterized by an intermediate life cycle length (180 d), a high harvest index, and tolerance to a number of viral diseases (Aquiles Carballo, 2013, personal communication).

2.2. Treatments and measurements

Vermiculite (grade 1) was used as the substrate. After washing it with water and drying in an oven at 115 °C for 72 h, 100 g of vermiculite was weighed into plastic bags. To each bag, 10, 12.5, or 100 mL of distilled water was added. Then, the bags were sealed and incubated for 48 h to homogenize the moisture content. The Ψ_W was determined for each of the substrates by incubating

samples for 4 h in a psychrometric chamber (Wescor C-52 Inc., Logan, Utah, USA), which was then connected to a dew point microvoltmeter (Wescor HR-33T Inc., Logan, Utah, USA). The values of Ψ_W were −1.5 MPa (severe water deficit), −1.0 MPa (moderate water deficit) and −0.03 MPa (well-watered) for the 10-, 12.5- and 100-mL additions of distilled water, respectively.

Seeds of uniform size and shape were selected and disinfected with 10% commercial sodium hypochlorite (Clorox®) for 15 min. The seeds were germinated in trays with a bed of cotton at a slope of 35° for 48 h at a temperature of 25 ± 1 °C in the dark (Ellis et al., 1985) to avoid lignification and to ensure that the sugars to be quantified were not the product of photosynthesis (Aroca and Ruiz, 2012). The germination period was sufficient for the radicles to achieve a length of 35—40 mm. At that time, the radicle of each seedling was marked with black ink at 2.0 mm from the apex to analyze the effect of water deficit on this elongation zone. Subsequently, the seedlings were transplanted into polyvinyl chloride (PVC) tubes that were 40 mm in internal diameter and 100 mm in length and that contained the vermiculite substrates. The seedlings were subjected to different water regimes (Ψ_W): well-watered (0.03 MPa), moderate water deficit (−1.0 MPa), and severe water deficit (−1.5 MPa). Each tube was sealed at the ends with black polyethylene and was fitted with a rubber band to maintain constant vermiculite Ψ_W. The seedlings were then allowed to continue to grow for 24, 48 or 72 h in the dark at a temperature of 25 ± 1 °C.

After completing the radicle growth period, the PVC tube system was opened, and the radicles were collected. The displacement of the previously marked 2.0-mm segment was then quantified with a ruler. The root growth segments were collected and stored at −36 °C for future analyses. A completely randomized experimental design was used, with a factorial arrangement of treatments in which the factors (and levels) were the varieties (VS-22 and AMCCG-2), the different Ψ_W values (−0.03, −1.0 and −1.5 MPa), and the growth periods (24, 48, and 72 h).

2.2.1. Statistical analysis

The data were analyzed using analysis of variance (ANOVA). The experimental units consisted of groups of 60 seedlings with 5 repetitions of 2 independent experiments. Significant differences between the treatment means were separated with Tukey's honestly significant difference (HSD) test at a P value of <0.05. All analyses were performed using the SAS statistical package (SAS, 2009).

2.3. Water (Ψ_W), osmotic (Ψ_O) and turgor (Ψ_T) potentials of the radicle

Small sections from the apices of the radicles below the initial 2-mm mark were cut, placed in a psychrometric chamber (Wescor C-52 Inc., Logan, Utah, USA), and maintained there for 3 h. After the elapsed time, the Ψ_W was determined. The osmotic potential (Ψ_O) was determined on the same tissue samples after they were frozen in liquid nitrogen and maintained again for 3 h in a psychrometric chamber. The turgor potential (Ψ_T) was calculated as the difference between Ψ_W and Ψ_O. The osmotic adjustment (OA) was estimated as the difference between the values of Ψ_O in the radicles of the seedlings under water deficit and under well-watered conditions. To estimate the contribution of an individual solute to the OA, the differences in the concentrations of individual solutes between the water deficit and the well-watered treatments was expressed on a molar basis relative to the amount of water present in the radicle apex samples and was then calculated as the Ψ_O of extra individual solutes using the following equation: (Ψ_O extra individual solutes $= (C*R*T)$), where C is the concentration of the solution expressed in Mol L-1, R is the universal gas constant and T is the

absolute temperature in degrees Kelvin (-273 °C). The formula used to calculate the contribution of extra solutes to OA was: (%) $= 100 \times ((\Psi_{\text{O extra individual solutes}}/\text{calculated OA})$ (Nio et al., 2011).

2.4. Solute quantification

2.4.1. Quantification of soluble sugars and starch

For the determination of the solute concentrations in the radicles, sections from the same marked zones of root apical development were analyzed. From the roots (50 mg, FW), sugars were extracted with 5—6 successive 10-min incubations in 80% ethanol at 70 °C and were quantified according to the method described by Scholes et al. (1996). The extracts were evaporated at 50 °C, dissolved in 1 mL of distilled water, placed in Eppendorf tubes, and stored at -38 °C. The measurements of glucose, fructose, and sucrose were conducted enzymatically by the sequential addition of hexokinase, phosphoglucose isomerase, and invertase. The absorbance values were obtained using a spectrophotometer and microplate reader (Multiskan Ascent, Labsystem, Thermo). The calculations were performed using standard calibration curves, which were previously prepared for each of the sugars and expressed in μmol/g FW. The remaining root tip tissue was homogenized in 1 mL of distilled water; it was autoclaved for 30 min and then incubated with 100-μL aliquots of a buffered solution of hydrolytic enzymes (100 μL of 500 mM MES-KOH, pH 4.5, 50 μL of 4 mg mL^{-1} amyloglucosidase, and 50 μL of 90 mg mL^{-1} α-amylase). The starch content was quantified by measuring the glucose produced by the enzymatic hydrolysis of starch.

2.4.2. Trehalose

Trehalose quantification was performed according to the protocol described by Avonce et al. (2006). A 200-mg FW portion of radicle that had been frozen in liquid nitrogen was weighed and pulverized with a mortar and pestle. The samples were then resuspended in 1 mL of high-performance liquid chromatography (HPLC)-grade water and transferred to 1.5-mL Eppendorf tubes. Next, the samples were incubated at 95 °C in a dry bath and centrifuged at 14,000 rpm for 5 min. Subsequently, the samples were deionized in a 1-mL column prepared with cationic and anionic resins and filtered through 0.22-μm Millipore filters. The samples were injected into an HPLC (Waters) with a 600 controller, a 2414 refractive index detector, and a 717 autosampler (automatic injector). A 25 cm × 4.6 mm Supelcosil column (LC-NH2) was used. An acetonitrile-water mixture was used (75:25) for the mobile phase at a flow rate of 1 mL min^{-1}.

2.5. Proline

The proline content was evaluated using the colorimetric method (Bates et al., 1973), which is based on the reaction of ninhydrin with proline. Maize radicles (50 mg FW) were ground and mixed with a solution of ninhydrin acid, 3% sulfosalicylic acid, and glacial acetic acid at a ratio of 1:1:1 (v/v/v). The samples were incubated in a water bath at 100 °C for 1 h. Then, the reaction was stopped with ice, and the chromophore was extracted by adding 4 mL of toluene. The concentration of proline in the tissue was calculated using the calibration curve created for this amino acid.

3. Results

3.1. Radicle elongation

The maximum root tip elongation was observed after 72 h under well-watered conditions in both maize materials. Radicle growth inhibition was gradual as substrate water deficit increased.

Moderate Ψ_W (-1.0 MPa) reduced radicle growth by approximately 56% compared to the well-watered plants in both maize varieties; however, after 72 h, the lowest Ψ_W (-1.5 MPa) reduced radicle growth by approximately 72% and 90% in VS-22 and AMCCG-2, respectively (Fig. 1). It is important to note than even at the lowest Ψ_W, radicle growth was not completely inhibited, and VS-22 showed the least negative effect. There were significant differences in root elongation (P $\leq$ 0.05) both between varieties and as a function of the substrate Ψ_W. In addition, the section of elongated root was slim and did not exhibit secondary root growth when subjected to moderate and severe water deficits.

3.2. Water (Ψ_W), osmotic (Ψ_O) and turgor (Ψ_T) potentials of the radicle

The results presented in Fig. 2A show that the Ψ_W values of the radicle in both varieties after 72 h of growth were reduced as the water deficit in the substrate increased, reaching values of -0.3 and -2.5 MPa for VS-22 and AMCCG-2, respectively. The Ψ_O was also reduced, as the water deficit was more severe in both varieties (Fig. 2B). The variety VS-22 had the lowest Ψ_O, observed when the radicle was grown at substrate Ψ_W values of -1.0 and -1.5 MPa. This reduction in the Ψ_O maintained turgor pressure in the radicle in both materials. The variety VS-22 had the highest Ψ_T, with values of 1.0 and 0.78 MPa (Fig. 2C) when grown under moderate and severe drought conditions, respectively.

3.3. Solute accumulation

Under well-watered conditions, glucose, fructose, and sucrose were detected in the radicle apices of the VS-22 and AMCCG-2 maize varieties (Fig. 3). After 24, 48, and 72 h of growth, the accumulations of these solutes substantially increased when the radicle was subjected to a moderate or severe water deficit. The accumulations of these three sugars differed significantly (P $\leq$ 0.05) among growth times. These increases were greatest at the lowest substrate Ψ_W (-1.5 MPa) after 72 h of root growth. Under these conditions, the VS-22 variety had higher accumulations of glucose (1.9-fold), fructose (2.9-fold) and sucrose (4.1-fold) relative to the AMCCG-2 variety.

The total soluble sugars accumulated in the radicle apices subjected to moderate (-1.0 MPa) and severe (-1.5 MPa) water deficit for 72 h gradually increased to 40% and 32% of the total measured solutes accumulated in VS-22 and AMCCG-2, respectively (Fig. 4). The concentrations of total soluble sugars (Glucose, fructose and sucrose) in VS-22 under moderate and severe water deficit accounted for a 13.5% and 28% OA, respectively, versus AMCCG-2, with 0% and 11.9%, respectively (Table 1).

Another important osmolyte, trehalose, also accumulated at low values of Ψ_W. This disaccharide was not observed when the Ψ_W of the substrate was -0.03 MPa in either maize variety (Fig. 5). Trehalose appeared in the radicle apex when the Ψ_W of the substrate was decreased to moderate water deficit. In particular, the VS-22 variety exhibited a greater root apical accumulation of trehalose (8 μmol/g FW) than AMCCG-2 during the first 24 h of growth, and this level increased substantially until 72 h, reaching 29.8 μmol/g FW. The AMCCG-2 variety did not accumulate trehalose until 48 h of moderate water deficit, reaching 5.24 μmol/g FW. After 72 h under the lowest Ψ_W, a 3-fold increase in the trehalose level was detected in the VS-22 variety compared to AMCCG-2.

Starch was present in the root apices of the VS-22 and AMCCG-2 varieties after 24 h of growth at a substrate Ψ_W of -0.03 MPa (Fig. 6). When the exposure time was increased to 72 h, the length of the apex elongation zone increased and the starch content decreased. For both maize varieties, the same tendency for
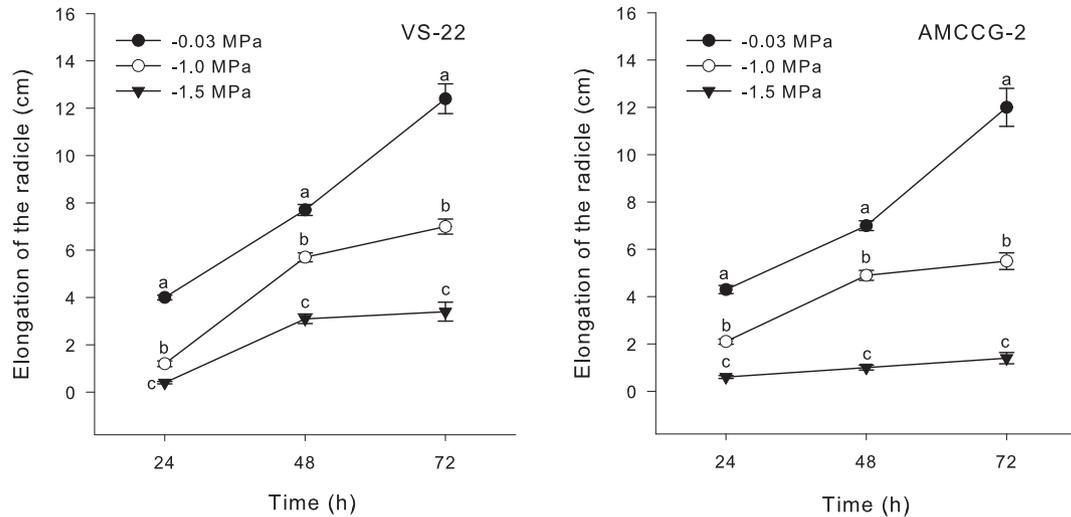
Fig. 1. Elongation (cm) of radicle apices of two maize varieties (VS-22 and AMCCG-2) grown under three substrate water potentials (Ψ_W; −0.03, −1.0 and −1.5 MPa) for three time periods (24, 48, and 72 h). The values represent the means ± SE ($n = 10$). Different letters indicate significant differences between treatments at each time measured (Tukey $P < 0.05$).

decreased starch was observed, though more drastically, under severe water deficit for 72 h. After 48 h of root growth at moderate water deficit, a 70% increase in starch was detected for VS-22; however, by 72 h, the starch content decreased significantly, possibly because of the more severe water deficit. In the susceptible variety, the starch content decreased by only 30%.

Proline was observed in the radicle apices of both varieties of maize after all three root growth times (24, 48 and 72 h) at a substrate Ψ_W of −0.03 MPa (Table 2). When the substrate Ψ_W was decreased to severe water deficit, there was notable proline accumulation. This accumulation was the most evident in the VS-22 variety after 72 h; the proline content increased 5-fold compared with the value observed for well-watered seedlings. In contrast, the corresponding increase in proline for AMCCG-2 was approximately 4-fold greater. The proline accumulation in VS-22 was 3-fold higher than that in AMCCG-2 at 72 h. The proline concentration differed significantly between the two varieties among the Ψ_W values and growth development times tested.

The contribution of solutes to the osmotic adjustment is outlined in Table 1. For the tolerant variety (VS-22) after 72 h of growth

under moderate water deficit, sucrose and trehalose appeared to be the most important solute contributors. When the water deficit increased, glucose and proline were the most important solutes in the OA. In the susceptible variety (AMCCG-2) under moderate water deficit, none of the solutes contributed to the OA. The increment of the water deficit increased the participation of sucrose; in fact, this solute was the only one for which a notable increase was observed.

4. Discussion

The elongation of the radical apex of maize continued for 72 h, even under severe water deficit conditions. The decrease in the substrate Ψ_W caused a differential decrease in the longitudinal growth of the radicle apex between the two maize varieties. In VS-22, radicle apex elongation was reduced by 72% compared with the well-watered conditions, while in AMCCG-2, the elongation was reduced by 90%. Nonetheless, radicle growth was maintained in both varieties. It was observed that VS-22 grown under drought conditions for 72 h presented a higher level of solutes in the radical
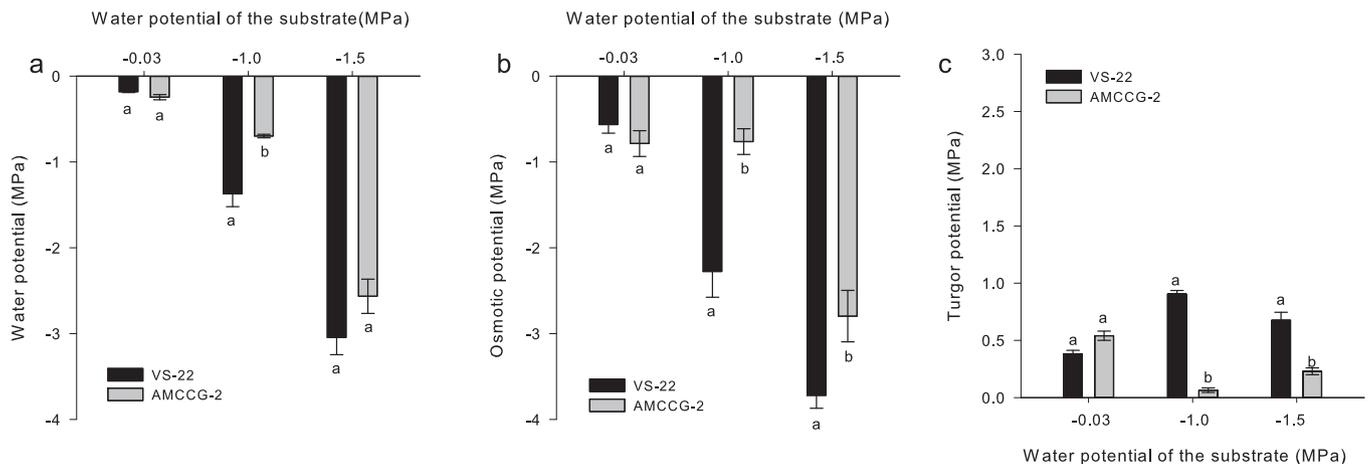
Fig. 2. Water potential (A), osmotic potential, (B) and turgor potential (C) of radicle apices of two maize varieties (VS-22 and AMCCG-2) grown under three substrate water potentials (Ψ_W; −0.03, −1.0 and −1.5 MPa) for 72 h. The values represent the means ± SE ($n = 10$). Different letters indicate significant differences between treatments at each time measured (Tukey $P < 0.05$).
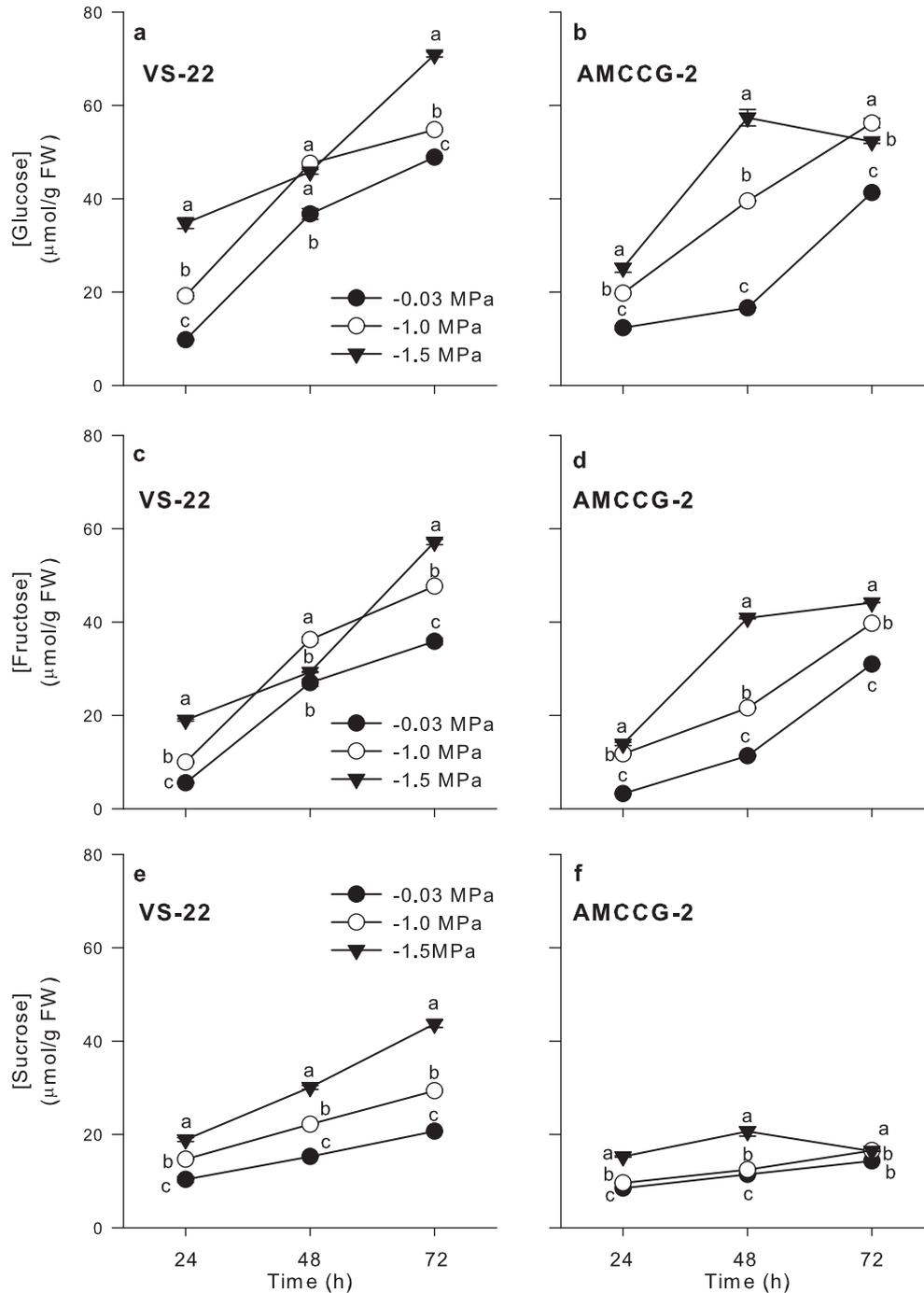
Fig. 3. Accumulations of glucose (A and B), fructose (C and D) and sucrose (E and F) (μmol/g FW) in radicle apices of two maize varieties (VS-22 and AMCCG-2) grown under three substrate water potentials (Ψ_W; −0.03, −1.0, and −1.5 MPa) for three time periods (24, 48 and 72 h). The values represent the means ± SE (n = 10). Different letters indicate significant differences between treatments at each time measured (Tukey P < 0.05).

apex compared to AMCCG-2, which may suggest that the accumulation of compatible solutes maintained the radicular apex growth (Sharp and Davies, 1979). It was previously reported that drought-tolerant rice, wheat and sugar cane varieties showed better root development compared to susceptible varieties. However, in other species, positive correlations between root size and tolerance to this abiotic stress were not found, which can be explained by the variations in the anatomical, morphological and hydraulic characteristics of plant species (Aroca and Ruiz, 2012). In addition, severe water deficit affected the cellular functioning of

the radicle and was associated with a mean Ψ_W of −3.0 MPa in the radicle tissue. Under these conditions, longitudinal growth progressed in a restrained manner, possibly because the zones of cell elongation and differentiation are located near the apex (Baluska et al., 2001). These results indicate that a continuous gradient of moisture is established in the root tissue (Saab et al., 1990; Sharp and Davies, 1979).

Pritchard et al. (1993) observed that the walls of the transition zone of the elongation region and the radicle apex of maize are very sensitive to acidification, which stimulates cellular elongation;
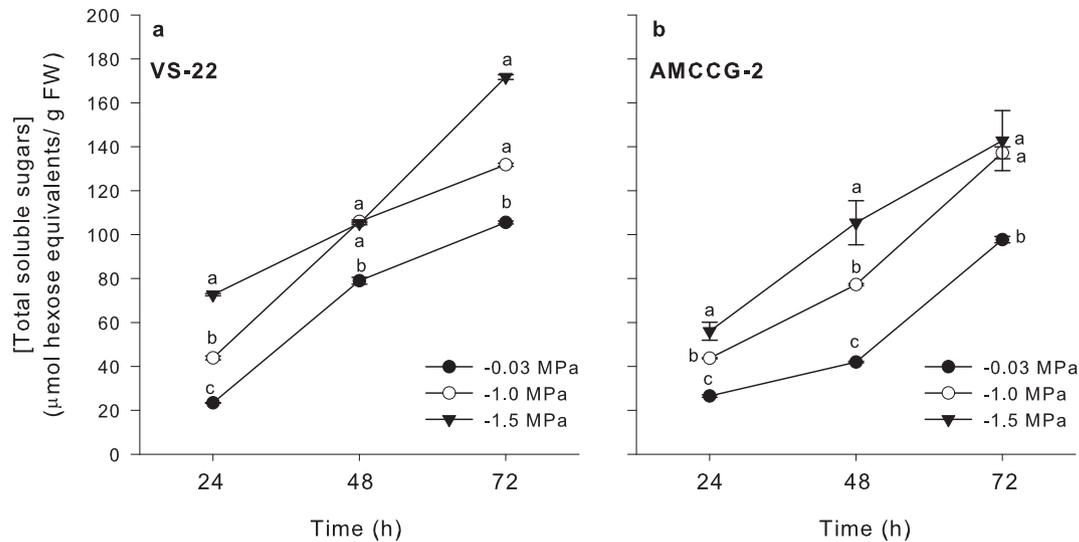
Fig. 4. Total soluble sugars (μmol/g FW) accumulated in radicle apices of the maize varieties VS-22 (A) and AMCCG-2 (B) grown at three substrate water potentials (Ψ$_W$; −0.03, −1.0 and −1.5 MPa) for three time periods (24, 48, and 72 h). The values represent the means ± SE ($n = 10$). Different letters indicate significant differences between treatments at each time measured (Tukey P < 0.05).

Table 1
Solute contributions to osmotic adjustment (%) in the radicle apices of VS-22 and AMCCG-2 maize varieties under two different water regimes (moderate and severe water deficit) after 72 h of growth.

| Varieties	Contribution to OA%						
72 h	MPa	Glucose	Fructose	Sucrose	Trehalose	Proline	Total
VS-22							
Moderate water deficit severe water deficit	−1.0	4	3.5	6	6.0	3.9	23.4
	−1.5	8	7	13	10	19	57
AMCCG-2							
Moderate water deficit severe water deficit	−1.0	0	0	0	0.5	0	0.5
	−1.5	3	1.9	7	5	6	22.9

therefore, cells can maintain growth even under severe water deficit. The increase in length and the maintenance of root development in maize VS-22 under moderate and severe water deficit conditions are indicative of an adaptive mechanism for drought tolerance, preventing the adverse effects caused by these water deficits.

The continued development of the root under water-limited
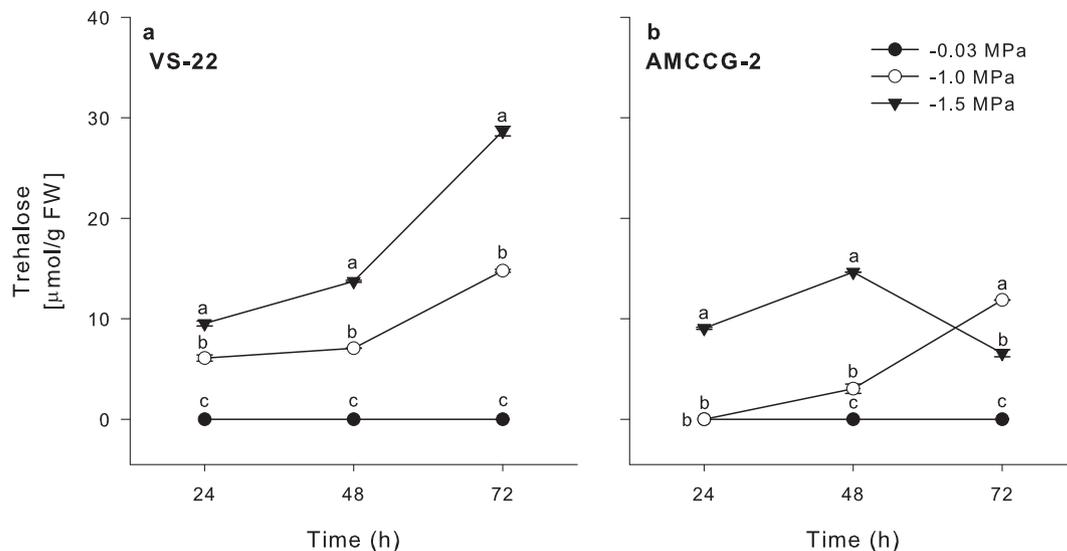
Fig. 5. Trehalose accumulation (μmol/g FW) in radicle apices of two maize varieties (VS-22 and AMCCG-2) grown at three substrate water potentials (Ψ$_W$; −0.03, −1.0 and −1.5 MPa) for three time periods (24, 48, and 72 h). The values represent the means ± SE ($n = 10$). Different letters indicate significant differences between treatments at each time measured (Tukey P < 0.05).
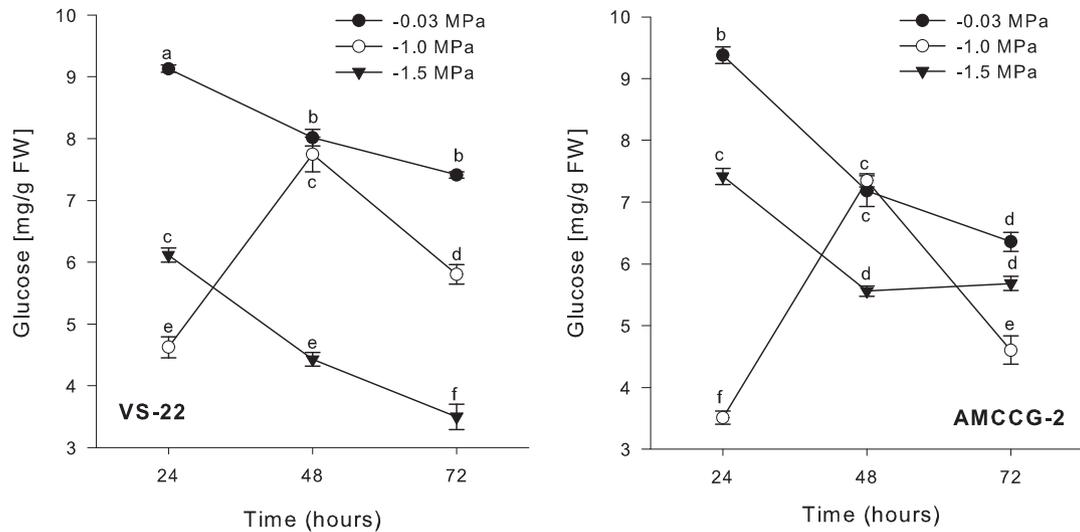
Fig. 6. Starch concentrations (mg g^{-1} FW) in radicle apices of two maize varieties (VS-22 and AMCCG-2) grown under three substrate water potentials (Ψ_W; −0.03, −1.0 and −1.5 MPa) for three time periods (24, 48, and 72 h). The values represent the means ± SE ($n = 10$). Different letters indicate significant differences between treatments at each time measured (Tukey P < 0.05).

conditions is considered a determining characteristic that guarantees plant establishment and survival. The root provides effective anchorage to the substrate and penetration into deeper layers of the soil, where water can be located, absorbed, and transported to the stem to maintain the growth of the aerial portion of the plant (Baluska et al., 2010; Chaves et al., 2003; Chaves and Oliveira, 2004).

The Ψ_W values in the radicles of the two maize varieties growing under severe water deficit conditions for 72 h decreased to −3.0 and −2.4 MPa for VS-22 and AMCCG-2, respectively. The Ψ_O was also reduced, and the value was lower in VS-22 (−3.7 MPa) compared with AMCCG-2 (−2.7 MPa). These results were similar to those of Serraj and Sinclair (2002), who reported a reduction in Ψ_O that was reflected by an increase in solute accumulation in the root tissue. In this study, the monosaccharides glucose and fructose, the disaccharides sucrose and trehalose, and starch and proline all accumulated in the radicles of both VS-22 and AMCCG-2. The detection of these sugars, especially glucose, fructose, sucrose and trehalose, during the very early stages of the development of maize seedlings subjected to water deficit has rarely been documented, and the studies performed to date have evaluated total sugars, total reducers, abscisic acid (ABA), and proline, among others (Chaves and Oliveira, 2004). At this stage of plant development, these solutes are not the product of photosynthesis. Instead, they are derived from storage in the endosperm, and their abundances are

correlated with a simultaneous reduction in Ψ_O, as observed in maize. In higher plants, the presence and accumulation of some of the solutes mentioned above have been reported under conditions of water deficit (Chaves et al., 2003; Chaves and Oliveira, 2004), but evidence for their presence during the very early developmental stages of the root, as shown in this study, has not been previously documented.

The magnitudes of the decreases in the Ψ_W and Ψ_O of the root maintained the turgor in both maize varieties, with a higher value of Ψ_T for VS-22 (0.67 MPa) than AMCCG-2 (0.16 MPa). This result suggests that both varieties performed OAs. Notably, the VS-22 variety (which was selected from a water-limited environment) exhibited a better performance in terms of turgor maintenance, which may suggest that VS-22 is better adapted to water-limited conditions compared to AMCCG-2.

The results clearly demonstrate a difference between the two varieties, not only in their sensitivity to the substrate Ψ_W but also in their capacity to accumulate solutes. The VS-22 variety, which is considered drought tolerant, had a greater accumulation of solutes relative to AMCCG-2. In the radicle apex, VS-22 accumulated 1.9-fold greater glucose, 2.9-fold greater fructose, and 4.1-fold greater sucrose than AMCCG-2. The concentrations of trehalose and sucrose increased while the concentration of starch decreased in the VS-22 variety after 72 h of severe osmotic stress. In contrast, in AMCCG-2, the concentrations of trehalose and sucrose increased, whereas the starch concentration remained high. This finding indicates that, in drought tolerant plants under osmotic stress, the starch that accumulates in amyloplasts can be used to make sucrose and trehalose for OA. It is known that T6P regulates starch synthesis through the activation of ADP-glucose-pyrophosphorylase (Kolbe et al., 2005). An increase in sugars has been observed in maize seedlings under water deficit as an active response related to OA (Nio et al., 2011). This response has also been observed for maize roots by Nayer and Reza (2008), who noted that the roots of maize 701 accumulated almost double the amount of sugars when the Ψ_W was −1.76 MPa compared to non-water deficit conditions. The authors inferred that this accumulation was attributed to a decrease in starch concentration from glucose splitting.

Conversely, in numerous plant species, such as *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays* L, and *Solanum tuberosum*, during periods of water stress, starch is degraded, and the final products

Table 2
Proline accumulation (μmol/g FW) in the radicle apices of the maize varieties VS-22 and AMCCG-2 grown under three different water regimes (well-watered, moderate and severe water deficit) and for three time periods (24, 48, and 72 h). Different letters indicate significant differences (Tukey 0.05). Each value represents the mean ± SE ($n = 10$).

Variety	Ψ_W (MPa)	Hours of growth		
		24	48	72
VS-22	−0.03	0.09 ± 0.12 c	1.38 ± 0.11 c	2.31 ± 0.32 c
	−1.0	1.7 ± 0.16 b	3.67 ± 0.26 b	4.40 ± 0.27 b
	−1.5	6.17 ± 0.80 a	7.30 ± 0.36 a	16.2 ± 1.3 a
AMCCG-2	−0.03	0.76 ± 0.09 b	1.04 ± 0.1 c	1.32 ± 0.3 b
	−1.0	1.30 ± 0.14 a	3.7 ± 0.40 b	4.27 ± 0.37 a
	−1.5	0.54 ± 0.03 c	6.1 ± 0.42 a	5.19 ± 0.90 a

The values represent the means ± SE (n = 10). Different letters indicate significant differences among treatments at each time measured (Tukey P < 0.05).

are used for sucrose synthesis (Smith et al., 2005). In addition, sucrose can function as a molecular signal of stress (Koch et al., 1996). These results support the idea that a water deficit induces an efficient translocation of carbon towards the root apex in VS-22, which could be considered an adaptive strategy that imparts drought tolerance.

Trehalose was also detected when the radicle developed under moderate and severe water regimes. The content of this disaccharide significantly increased as a function of treatment time, and the values recorded for VS-22 were up to 3.2-fold greater than those recorded for AMCCG-2. Notably, to date, the detection of this solute in the very early stages of root development and under the experimental conditions of this study has not yet been reported. Therefore, the function of trehalose at this stage is not well defined, although it is likely that it contributes to radicle growth or that it acts as an osmoprotectant that contributes to the maintenance of elongation (Kolbe et al., 2005; Quistian-Martínez et al., 2011).

Trehalose is known to accumulate in large amounts in organisms that are highly resistant to desiccation, such as certain resurrection plants, tardigrades, and yeasts (Zhang et al., 2009). The main evidence for the presence of trehalose in plants comes from genome sequencing projects for species such as *A. thaliana*, *Oryza sativa*, and *Z. mays*, among others (Paul et al., 2008; Zhou et al., 2014). Trehalose appears to be associated with the adaptation of plants to abiotic stress and functions as an osmoprotectant. The intermediate trehalose 6-phosphate appears to be involved in stress signaling, the metabolic regulation of carbon, and photosynthesis (Paul et al., 2008; Iturriaga et al., 2009). There is evidence that rice improved for drought tolerance also accumulates more trehalose in comparison with non-tolerant varieties. This report and our data suggest that the capacity to accumulate trehalose and to use it to cope with drought is one of the consequences of plant breeding for drought tolerance (Quistian-Martínez et al., 2011). Transgenic *A. thaliana* and *Oryza sativa* that accumulate greater amounts of trehalose have been produced. This increased trehalose accumulation which makes the plants more tolerant to different types of abiotic stress, such as drought, salinity, cold, and thermal shock (Kolbe et al., 2005).

It has been previously reported that varieties of rice bred for drought tolerance are able to accumulate more trehalose under drought in comparison with non-tolerant varieties (Quistian-Martínez et al., 2011). In this work, similar results are reported for maize; in addition, it is demonstrated that this sugar also plays an important role in the OA in the radicle, an important organ that senses the water content status in the soil. As in rice, in maize, a greater capacity for accumulating trehalose seems to be one of the traits associated with selection that improves drought tolerance in plants.

In contrast to the accumulations of the above-described osmolytes, although the presence of starch was detected, the content did not increase notably. Furthermore, the starch concentration decreased by 76% in VS-22 and by 61.5% in AMCCG-2 after 72 h of radicle growth under severe water deficit conditions compared to the previous growth periods. The reduction was more drastic in VS-22 (76%). The VS-22 variety may have decreased its starch content in response to the adverse environment, which is an adaptive response to the degradation of endosperm starch, as proposed by Smith et al. (2005). Alternatively, the increment in the concentrations of total soluble sugars could result from degradation processes (e.g., enhanced starch degradation), de novo synthesis (e.g., increased formation of hexose), or reduced consumption of photosynthate as growth is impeded (Arndt et al., 2001).

The proline accumulation in the tolerant VS-22 variety was 3-fold higher than that of the susceptible AMCCG-2 variety after 72 h. Proline accumulation in plants under water deficit has been documented as an adjustment that plants make to reduce their Ψ_O and maintain turgor (Nayer and Reza, 2008). Therefore, proline accumulation is associated with the prevention of membrane damage (Hare et al., 1999; Farooq et al., 2009).

Proline accumulation in the primary radicles of maize seedlings is involved in OA and the maintenance of root development at very low Ψ_W (Baluska et al., 2010; Serraj and Sinclair, 2002), which may explain why, even at a Ψ_W of −1.5 MPa, root elongation continued for the VS-22 maize variety for at least 72 h.

The mechanisms of water stress tolerance may be associated with the accumulation of solutes such as sugars and proline. The accumulation of soluble sugars is correlated with drought tolerance (Verslues and Sharp, 1999); proline itself can also be of principal importance in stress adaptation (Nayer and Reza, 2008) and can serve as a selection marker for tolerant cultivars, even in very young seedlings (72 h).

The different organic osmolytes accumulated in response to water deficit in plants are often involved as antioxidants and osmoprotectants, regulating plant responses such as increased reactive oxygen species (ROS) production, which may cause oxidative damage in plants. In addition, these compatible solutes, as natural products of normal metabolism, play important roles in cell signaling, as they act as secondary messengers in triggering subsequent defense reactions in plants (Farooq et al., 2009).

The growth of radicles under stress conditions and the increase in solutes such as glucose, fructose, sucrose, trehalose, and proline have demonstrated that drought tolerance is based on the capacity of each maize plant variety for adjustments in solute concentrations. These studies can serve as the basis for the development of biochemical markers for the selection and genetic improvement of maize.

Acknowledgments

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