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Osmopriming with selenium: physical and physiological quality of tomato seeds in response to water deficit

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ABSTRACT: Tomato is a crop sensitive to water deficit and the responses of seeds to germination under these conditions involve biochemical, physiological, and molecular processes. The aim of this study was to evaluate the physical, physiological, and biochemical changes in tomato seeds osmoprimed with selenium (Se) and subjected to water deficit during germination. Tomato seeds of the LA 4050 and LA 3475 accessions were osmoprimed with PEG 6000 solution (-1.0 MPa) plus Na_2SeO_4 (12.5 µM) or only with PEG 6000 solution for 24 h or unprimed. The seeds from the different treatments were subjected to water deficit (-0.3 MPa) or to control (0 MPa). Image analyses were performed (X-ray), physiological variables were obtained by the germination test and activities of SOD, CAT and POX enzymes were determined. Osmopriming, especially with PEG, provided the seeds of both accessions with higher densities and gray mean when compared to unprimed ones. Seeds under water deficit showed lower physiological performance and lower enzymatic activity. Se did not induce seed resistance to water deficit. Osmopriming with PEG 6000 at -1.0 MPa is an efficient technique for improving the physiological quality of seeds of tomato accessions susceptible and tolerant to water deficit. Moreover, the antioxidant enzyme CAT proved to be an indicator of oxidative stress tolerance in tomato seedlings under water deficit.

Index terms: abiotic stress, enzymatic activity, image analysis, *Solanum lycopersicum* L., *Solanum pennellii* L.

RESUMO: O tomate é uma cultura sensível ao déficit hídrico e as respostas de sementes à germinação sob essas condições envolvem processos bioquímicos, fisiológicos e moleculares. O objetivo do trabalho foi avaliar as alterações físicas, fisiológicas e bioquímicas em sementes de tomate osmocondicionadas com selênio (Se) e, posteriormente, submetidas ao déficit hídrico durante a germinação. Sementes de tomate das linhagens LA 4050 e LA 3475 foram osmocondicionadas com solução PEG 6000 (-1,0 MPa) mais Na_2SeO_4 (12,5 µM) ou apenas com solução de PEG 6000 por 24 h ou não condicionadas. As sementes provenientes dos diferentes tratamentos foram submetidas ao déficit hídrico (-0,3 MPa) ou ao controle (0 MPa). Foram realizadas análises de imagens (raios X); obtenção de variáveis fisiológicas pelo teste de germinação e atividades das enzimas SOD, CAT e POX. O osmocondicionamento, sobretudo com PEG, proporcionou às sementes de ambas as linhagens maiores densidades e média de cinza quando comparadas às não condicionadas. As sementes sob déficit hídrico apresentaram menor desempenho fisiológico e atividade enzimática. O Se não induziu resistência às sementes ao déficit hídrico. O osmocondicionamento com PEG 6000 a -1,0 MPa é eficiente para melhoria da qualidade fisiológica de sementes dos acessos de tomate suscetíveis e tolerantes ao déficit hídrico. Além disso, a enzima antioxidante CAT se mostrou indicadora da tolerância ao estresse oxidativo em plântulas de tomate sob déficit hídrico.

Termos para indexação: estresse abiótico, atividade enzimática, análise de imagens, *Solanum lycopersicum* L., *Solanum pennellii* L.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a vegetable of great economic importance worldwide, with production of around 186 million tons (FAOSTAT, 2021). It is a crop considered sensitive to water deficit at different stages of development (Shi et al., 2014; Klunklin and Savage, 2017; Alordzinu et al., 2021), which is the main abiotic factor affecting the world's agricultural cultivation (Parkash and Singh, 2020).

In the seed germination process, water promotes the resumption of embryo growth and consequently the protrusion of the radicle. In addition, it is the vehicle responsible for the translocation and transformation of reserves, fundamental for seed germination and seedling growth (Bewley et al., 2013; Marcos-Filho, 2015; Obroucheva et al., 2017). In general, there are few studies aimed at obtaining tomato genotypes tolerant to water deficit in the germination stage, which limits the number of commercial cultivars with satisfactory levels of drought tolerance (Maciel et al., 2017). According to Bernier et al. (2008), this fact is related to the complexity of water deficit tolerance, which is controlled by many genes and is affected by stress intensity.

Rousseaux et al. (2005) highlight the existence of wild tomato species that occur naturally in water-scarce environments and serve as a genetic source of morphological, physiological, and biochemical traits. Among these species, *Solanum pennellii* originates from dry regions, has a complete genome sequence (Bolger et al., 2014), and has 76 segmental introgression lines (ILs), each of which carries only one homozygous introgressive segment (Eshed and Zamir, 1995; Liu and Zamir, 1999; Chitwood et al., 2013). These segments cover the entire genome of the donor *S. pennellii* (LA 716) on the genetic background of *S. lycopersicum* (cultivar M82), sensitive to water deficit (Eshed and Zamir, 1995). When compared to *S. lycopersicum*, *S. pennelli* has several characteristics that make it more efficient in water use and less susceptible to water deficit, such as lower stomatal frequency, greater leaf thickness, smaller leaf tissue volume, lower chlorophyll content and lower photosynthetic activity per leaf area unit (Kebede et al., 1994; Easlon and Richards, 2009). In this context, crosses of ILs of *S. pennellii* with *S. lycopersicum* have been used to define quantitative trait loci (QTL) relevant to stress tolerance (Lippman et al., 2007; Kamenetzky et al., 2010). Pessoa et al. (2022) identified introgression lines (ILs) derived from *S. pennellii* more tolerant to water deficit during the germination process. Among the selected lines, IL 1-4-18 (accession LA 4050) was classified as a candidate for further studies to validate it as tolerant to this characteristic.

In addition to the effect of the genotype, some strategies can be used to increase plant tolerance to environmental stresses. In this context, recent studies have confirmed the role of selenium (Se) in increasing plant resistance to various abiotic stresses (Hasanuzzaman and Fujita, 2011; Nawaz et al., 2013; Zahedi et al., 2020). Therefore, the supply of Se by priming or osmopriming could constitute a strategy aimed at mitigating the effects of water deficit during the germination process of tomato seeds.

The relationship between antioxidant enzyme activity and osmopriming is widely reported in the literature. Balabusta et al. (2016) evaluated the behavior of cucumber seeds at low temperatures and observed the beneficial role of melatonin applied via osmopriming as a promoter of the activity of enzymes, such as superoxide dismutase (SOD). The physiological pretreatment of seeds with Se was used in rapeseed (*Brassica napus*), boosting the antioxidant defense system (Hasanuzzaman and Fujita, 2011). In wheat seeds, pretreatment with Se led to an increase in the total contents of sugar and total free amino acids (Nawaz et al., 2013). On the other hand, Galviz-Fajardo et al. (2020) observed that osmopriming with salicylic acid (SA) in tomato seeds under water deficit did not lead to improvement in their performance. However, these authors observed increased antioxidant enzyme activity. Thus, osmopriming of tomato seeds with Se, as well as its relationship with the increase in antioxidant activity, can bring important answers for the increase in seed tolerance to water deficit.

In view of the above, the aim of this study was to evaluate the physical, physiological and biochemical changes in seeds of two contrasting tomato accessions in terms of tolerance to water deficit osmoprimed with Se and, subsequently, subjected to water deficit during germination.

MATERIAL AND METHODS

Osmopriming

The experiment was carried out on the campus of the *Universidade Federal de Viçosa* (UFV), in Viçosa, Minas Gerais, Brazil. Tomato seeds of the line IL 1-4-18 (accession LA 4050; tolerant to water deficit) and the cultivar M82 (accession LA 3475; sensitive to water deficit) were used. Initially, the seeds were obtained from the Germplasm Bank of UFV. The seedlings were produced and cultivated in a protected environment until fruit production, following all the recommendations for tomato cultivation (Nick et al., 2018). After maturation, the fruits were harvested and the seeds were extracted, washed, and dried (Carvalho and Nakagawa, 2012).

The seeds were subjected to osmopriming (method of controlled hydration with soaking in osmotic solution) through immersion in 500 mL of sodium selenate (Na_2SeO_4) solution. The solution concentration was 12.5 µM (defined after pre-tests with doses of 0.0, 12.5, 25.0, 37.5 and 50.0 µM), or without Se, both in polyethylene glycol solution (PEG 6000) at -1.0 MPa (296 $g.L^{-1}$), for 24 hours. The control treatment consisted of unprimed seeds.

The seeds were kept in the osmopriming solution, in Erlenmeyer® flask (capacity of 1000 mL), at 15 °C, in a B.O.D. chamber. The Erlenmeyer® flasks were sealed and subjected to forced aeration using an air pump and under constant illumination.

After the osmopriming periods, the seeds were washed in running water and dried at room temperature (25 ± 2 °C), until the hygroscopic equilibrium (approximately 12% moisture), which was monitored by successive weighing.

Physical analyses (X-ray test)

The seeds were subjected to analysis of their internal morphology by means of the X-ray technique, using 160 seeds of each treatment, fixed in groups of 20 seeds in an orderly and equidistant way on adhesive paper. This procedure was performed to allow the individual identification of each seed for further analysis.

Radiographic images were generated by a Faxitron device, model MX-20 (Faxitron x-ray Corp. Wheeling, IL, USA). The device was adjusted to a voltage of 23 kV and the seeds were exposed to radiation for 10 seconds, at a focal length of 41.6 cm. The image contrast was calibrated at 970 (width) x 2300 (center). The images were saved in *Tagged Image File Format* (TIFF) and then analyzed.

Image analysis was performed in ImageJ® software, with help of the *IJCropSeed* macro (Medeiros et al., 2020a). The variables obtained in this analysis were: area (mm); perimeter (mm); relative density ($gray.pixel^{-1}$) and gray mean ($gray.mm^{-1}$).

Physiological analyses

After acquisition of radiographic images, the seeds were evaluated for their physiological quality. For the germination test, eight replications of 25 seeds were arranged in *gerbox* boxes. The seeds were sown on two sheets of *Germitest* paper, moistened with 2.5 times the weight of dry paper with PEG 6000 solution (-0.3 MPa) (water deficit) or distilled water (0 MPa) (control). The boxes were packed in transparent plastic bags and placed in BOD germination chamber, under constant temperature of 25 °C and 8 h photoperiod (Brasil, 2009).

Daily counts were performed and the following parameters were calculated: percentage of root protrusion (radicle greater than 2 mm) (RP); normal seedlings obtained at 14 days after sowing (NS); first germination count obtained on the fifth day after sowing (FC) (Brasil, 2009), germination speed index (GSI) (Maguire, 1962); germination synchrony (Sync) (Primack, 1980); and fresh matter of seedlings (FM) ($mg.seedling^{-1}$).

Biochemical analyses

For the analysis of the activity of antioxidant enzymes, the seeds were placed to germinate, as described for the germination test. After 14 days, the seedlings were frozen in liquid nitrogen and stored at -20 °C. The activity of the enzymes superoxide dismutase (SOD) (Beauchamp and Fridovich, 1971), catalase (CAT) (Anderson et al., 1995) and peroxidase (POX) (Kar and Mishra, 1976) were determined by obtaining the crude extracts through maceration of 0.2 g of plant material in liquid nitrogen, followed by the addition of 2 mL of extracting medium, potassium phosphate buffer (0.1 M, pH 6.8), containing ethylenediaminetetraacetic acid (EDTA) (0.1 mM), phenylmethylsulphonyl fluoride (PMSF) (1.0 mM) and polyvinylpolypyrrolidone (PVPP) 1% (w/v). The homogenate was centrifuged at 19,000 *g* for 15 min., at 4 °C.

Protein content was determined using BSA as standard (Bradford, 1976). 1 mL of the Bradford reagent was added to 50 µL of the enzymatic extract, and the mixture was shaken. After 20 minutes, the sample absorbance was read in a spectrophotometer at 595 nm. The data were used to calculate the activities of antioxidant enzymes.

Experimental design and statistical analysis

The experiment was carried out in a 3x2 factorial scheme, corresponding to three osmopriming treatments (with Se, without Se and unprimed seeds) and two levels of water deficit during germination (with or without water deficit). The design used was completely randomized, with four replications. The data were subjected to analysis of variance. The means of the osmopriming treatments were compared by the Tukey test ($p < 0.05$). The means obtained for treatments with water deficit during germination were compared by the F test ($p < 0.05$). The two accessions were evaluated individually. In addition, principal component analysis (PCA) was performed. All analyses were performed in R statistical software (R Core Team).

RESULTS AND DISCUSSION

Through the results of the physical attributes by the X-ray test, it was possible to observe that the osmopriming (especially with PEG) provided the seeds of both accessions with higher relative density and higher gray mean when compared to unprimed seeds (Table 1).

Table 1. Physical attributes obtained by X-ray test of tomato seeds of the accessions LA 4050 and LA 3475 subjected to different osmopriming treatments.

			LA 4050	
Treatment	AREA (mm)	PER (mm)	REL. DENS. (gray.pixel^{-1})	GRAY M. (gray.mm^{-1})
PEG	3.99 B	7.77 B	72.31 A	75.57 A
PEG + Se	4.27 A	8.03 A	67.19 B	70.17 B
Unprimed	4.22 AB	8.02 A	64.16 C	66.25 C
Mean	4.16	7.94	67.8	70.6
CV (%)	4.61	2.36	3.3	3.19
			LA 3475	
Treatment	AREA (mm)	PER (mm)	REL. DENS. (gray.pixel^{-1})	GRAY M. (gray.mm^{-1})
PEG	4.71 B	8.48 B	71.16 A	74.16 A
PEG + Se	5.02 A	8.74 A	67.82 B	70.98 B
Unprimed	5.05 A	8.79 A	66.14 B	68.41 B
Mean	4.93	8.67	68.3	71.1
CV (%)	3.14	1.71	2.78	2.84

Area; perimeter (PER); relative density (REL. DENS.); and gray mean (GRAY M.). Means followed by the same uppercase letter in the column, comparing the effect of osmopriming, do not differ from each other by the Tukey test ($p < 0.05$). CV: coefficient of variation.

In the literature, there are several reports on the relationship between tissue density and the gray mean obtained by the X-ray test with the physiological quality of seeds (Abud et al., 2018). In general, higher tissue density is related to greater integrity and development of the embryo, in addition to a greater accumulation of reserves (Cheng et al., 2015). This information is confirmed in seeds of several species, such as tomato (Borges et al., 2019), pepper (Medeiros et al., 2020b.), soybean (Pinheiro et al., 2021), jatropha (Medeiros et al., 2020c), *Anadenenthera peregrina* (Pinheiro et al., 2022) and others.

Unlike tissue density, some physical attributes of seeds have a greater relationship with genotype, such as area and perimeter. However, the mean area of the seeds of the accession LA 4050 did not differ when comparing PEG + Se treatments with those of unprimed seeds and unprimed seeds with PEG. For the variables area and perimeter and considering the seeds osmoprimed with PEG, it was possible to observe that the seeds of both accessions were smaller (with smaller area and perimeter) (Table 1). These results may be related to the drying process after osmopriming, with these seeds reaching lower moisture content compared to seeds osmoprimed with PEG + Se and unprimed seeds.

It is possible to visualize that, regardless of the evaluated line, seeds that were osmoprimed, both with PEG and with PEG + Se, showed greater cellular differentiation in the embryo than unprimed seeds (Figure 1).

In osmoprimed seeds, it was possible to observe spaces between the embryo and the endosperm, which can be associated with greater embryo development due to the consumption of part of the endosperm during the imbibition promoted by the osmopriming (Liu and Zamir, 1999). During this process, the seeds have their metabolism reactivated and metabolic events of the germination process occur, with beginning of the translocation of reserves (Farooq et al., 2019). Thus, after treatment, these seeds germinate faster and with greater uniformity (Figure 1).

The analysis of seed images through the X-ray test is an important tool for various objectives and species. The X-ray test has assisted in the evaluation of the internal morphology of seeds and its direct relationship with physiological performance, in a fast and nondestructive manner (Rahman and Cho, 2016; Abud et al., 2018; Medeiros et al., 2020b; Pinheiro et al., 2021).

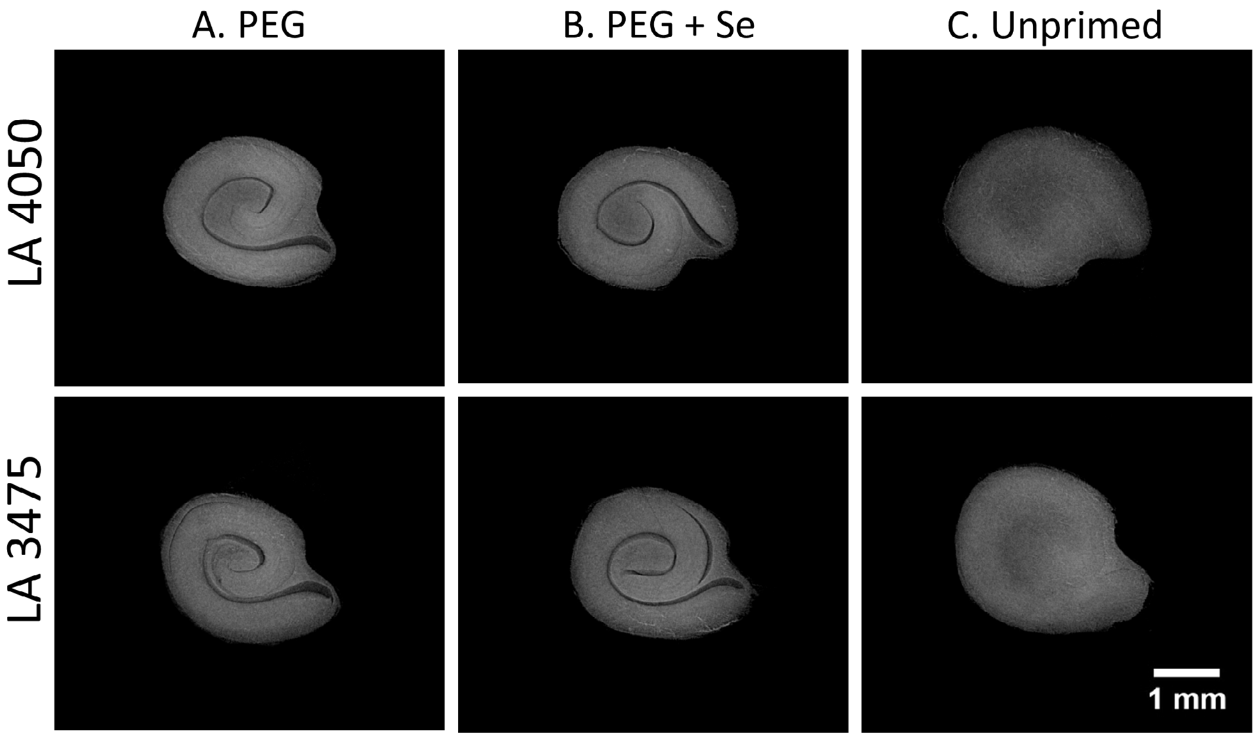

Figure 1. Radiographic images of seeds of two tomato accessions: LA 4050 (tolerant to water deficit) and LA 3475 (sensitive to water deficit), osmoprimed with PEG (A), osmoprimed with PEG + Se (B) and unprimed (C).

In general, the water deficit affected the performance of seeds of the two accessions analyzed, causing reduction in the percentage of seeds with root protrusion (RP), normal seedlings (NS) and normal seedlings in the first count (FC) of the germination test, among others (Tables 2 and 3).

For seeds of the accession LA 4050, which has greater tolerance to water deficit, osmopriming with PEG and PEG + Se contributed to obtaining higher values of RP, NS and GSI, under water deficit. In addition, germination synchrony (Sync) was higher in seeds osmoprimed with PEG, regardless of stress imposition (Table 2). As observed for this accession, Sadeghi and Robati (2015) investigated different priming methods in chicory (*Cichorium intybus* L.) seeds and observed increments in germination percentage and initial growth of seedlings under water deficit. For LA 3475, osmopriming of the seeds, in general, did not alter their performance, which was lower under water deficit condition (Table 3), except only for FC, GSI and Sync, for which seeds osmoprimed with PEG and PEG + Se showed higher values. Finally, no differences were observed in the fresh matter (FM) of seedlings of the accession LA 4050, regardless of osmopriming and water deficit treatment (Table 2). For the accession LA 3475, a lower fresh matter of seedlings was observed under the water deficit condition (Table 3).

Therefore, water deficit reduced germination, initial growth and uniformity of seedlings of the two tomato accessions. Similar results were found by Florido et al. (2018) in seeds of contrasting tomato accessions in terms of tolerance to water deficit, as these authors observed that under low osmotic potentials, the materials had a reduction in germination. In general, water deficit during seed soaking causes reductions in tissue hydration speed and oxygen diffusion, delay in the onset of enzymatic activity, reduction of meristematic growth, problems in cell elongation, wall synthesis and radicle emission (Marcos-Filho, 2015; Obroucheva et al., 2017). In addition, different responses to water deficit depend on the species and/or cultivar used, as well as the time and duration of the condition.

From the results obtained, it was clear that the osmopriming favored the physiological performance of tomato seeds, especially those of the accession LA 4050, which is more tolerant to water deficit. This technique exposes

Table 2. Physiological attributes of tomato seeds of the accession LA 4050 (tolerant to water deficit) subjected to different osmopriming treatments and water deficit during the germination process.

Treatment	RP (%)			NS (%)			FC (%)		
	Deficit	Control	Mean	Deficit	Control	Mean	Deficit	Control	Mean
PEG	86 Ba	96 Aa	91	82 Ba	93 Aa	88	49	94	72 A
PEG + Se	91 Aa	98 Aa	94	86 Ba	97 Aa	91	43	93	68 A
Unprimed	69 Bb	95 Aa	82	56 Bb	98 Aa	77	29	96	63 A
Mean	82	96		75	96		40 b	94 a	
CV (%)	6.61			7.19			16.27		
Treatment	GSI			Sync			FM (mg.seedling^{-1})		
	Deficit	Control	Mean	Deficit	Control	Mean	Deficit	Control	Mean
PEG	3.97 Ba	7.10 Aa	5.53	0.246	0.552	0.399 A	13.6	33.1	23.3 A
PEG + Se	4.18 Ba	7.07 Aa	5.62	0.171	0.403	0.287 B	11.7	27.4	19.5 A
Unprimed	2.41 Bb	7.17 Aa	4.79	0.149	0.495	0.323 AB	75.7	36.9	56.3 A
Mean	3.52	7.11		0.189 b	0.483 a		33.7 a	32.4 a	
CV (%)	7.8			21.65			132.29		

Root protrusion (RP); normal seedlings (NS); first germination count (FC); germination speed index (GSI); germination synchrony (Sync); and fresh matter of seedlings (FM). Means followed by the same lowercase letters, comparing the effect of osmopriming treatments, do not differ by the F test ($p < 0.05$). Means followed by the same uppercase letters, comparing the effect of water deficit, do not differ from each other by the Tukey test ($p < 0.05$). CV: coefficient of variation.

Table 3. Physiological attributes of tomato seeds of the accession LA 3475 (sensitive to water deficit) subjected to different osmopriming treatments and water deficit during the germination process.

Treatment	RP (%)			NS (%)			FC (%)		
	Deficit	Control	Mean	Deficit	Control	Mean	Deficit	Control	Mean
PEG	86	96	91 A	75	93	84 A	38.5	90	64 A
PEG + Se	83	97	90 A	71	93	82 A	36	89.5	63 A
Unprimed	85	92	88 A	73	79	76 A	23.5	59.5	42 B
Mean	84 b	95 a		73 b	88 a		33 b	80 a	
CV (%)	5.08			8.74			20.71		

Treatment	GSI			Sync			FM (mg.seedling^{-1})		
	Deficit	Control	Mean	Deficit	Control	Mean	Deficit	Control	Mean
PEG	3.56 Ba	6.711 Aa	5.13	0.206	0.403	0.304 A	10.9	34.2	22.6 A
PEG + Se	3.49 Ba	6.45 Aa	4.97	0.216	0.314	0.265 AB	9.3	33.1	21.2 A
Unprimed	3.13 Ba	4.25 Ab	3.69	0.175	0.268	0.222 B	15.5	33.1	24.3 A
Mean	3.39	5.8		0.199 b	0.328 a		11.9 b	33.5 a	
CV (%)	13.24			22.69			24.58		

Root protrusion (RP); normal seedlings (NS); first germination count (FC); germination speed index (GSI); germination synchrony (Sync); and fresh matter of seedlings (FM). Means followed by the same lowercase letters, comparing the effect of osmopriming treatments, do not differ by the F test ($p < 0.05$). Means followed by the same uppercase letters, comparing the effect of water deficit, do not differ from each other by the Tukey test ($p < 0.05$). CV: coefficient of variation.

seeds to low water potentials, which allows partial soaking, activating germination events such as DNA and RNA repair and synthesis, respiratory activity, reserve mobilization and enzymatic synthesis, but without the occurrence of radicle protrusion. Thus, osmopriming promotes greater uniformity and synchronism in germination, as well as in the emergence and development of seedlings (Chen and Arora, 2013; Farooq et al., 2019). Borges et al. (2019) found that the X-ray technique for tomato seeds at different maturation stages was efficient for studying physiological performance. These authors observed that seeds with embryos classified as normal showed higher germination and those with larger internal free area showed lower germination. Therefore, these results corroborate those observed in the present study (Tables 1 and 2; Figure 1).

Osmopriming works as an exposure of the seeds to a pre-germination stress, leaving them with a "stress memory". Therefore, when seeds are exposed to a stress situation after being osmoprimed, germination and initial establishment of seedlings occur faster compared to those that have not undergone treatment (Bruce et al., 2007). Similarly to the results observed in this study, it has been reported that osmoprimed tomato seeds have shown faster imbibition, in addition to an increase in the extensibility of the radicle cell walls and weakening of the endosperm, resulting in a reduction of the phase prior to radicle emergence (Haigh and Barlow, 1987).

Indeed, the accessions responded differently to the treatments. For the accession LA 3475 (sensitive to water deficit), although osmopriming did not result in higher germination, unprimed seeds showed longer time needed for germination (FC and GSI) and lower germination synchrony. For seeds of the accession LA 4050 (tolerant to water deficit), the osmopriming treatments (PEG and PEG + Se) were beneficial when compared to unprimed seeds, under water deficit condition. In this case, osmoprimed seeds without Se showed improvement in physiological performance. Thus, it can be emphasized that the genotype can result in distinct responses to Se supply and osmopriming.

Unlike what was observed in this study, in wheat seeds, osmopriming with Se at different concentrations led to different responses among cultivars under water deficit, but resulted in improvement of physiological parameters of seedlings (Nawaz et al., 2013). The response of seeds to osmopriming with Se depend on the level of stress to which they were exposed, species and/or cultivar, duration, concentration and time (Khaliq et al., 2015; Nawaz et al., 2013). Thus, studies related to the role of this technique using Se should be further explored for tomato seeds.

In seedlings of the accession LA 4050 grown from unprimed seeds, for the activity of SOD and POX there was significant interaction between the osmopriming and water deficit factors (Figure 2A and B). For the CAT enzyme, this interaction was not significant, and the factors were presented separately. There was an increase in the activity of this enzyme under water deficit conditions (Figure 2C and D). Under water deficit, the activity of antioxidant enzymes did not differ between osmopriming treatments. However, in the control, an increase in enzymatic activity was observed in seedlings grown from osmoprimed seeds. Osmopriming with PEG reduced CAT activity, but the values did not differ from those obtained for seeds osmoprimed with PEG + Se (Figure 2D).

In seedlings of the accession LA 3475, there was interaction between the osmopriming and water deficit factors for the activity of SOD, POX and CAT enzymes (Figure 3). Under water deficit, the activities of SOD and POX did not differ between osmopriming treatments. In the control, osmopriming with PEG promoted an increase in the activity of the enzymes compared to the other osmopriming treatments. In unprimed seeds, there was no difference between SOD and POX activity under deficit or without water deficit (Figure 3A and 3B), which was also observed in seeds

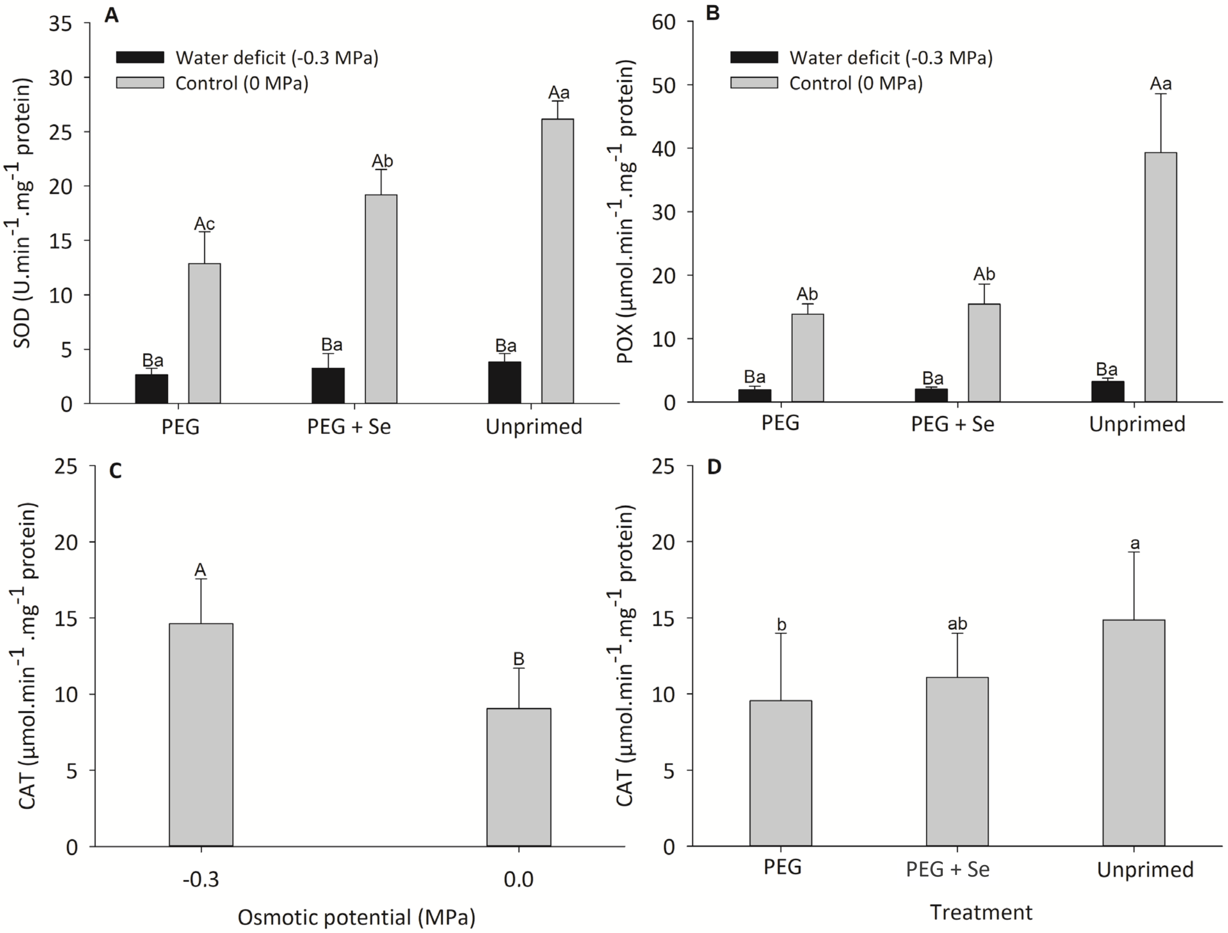

Figure 2. Activity of the enzymes superoxide dismutase (SOD) (A), peroxidase (POX) (B) and catalase (CAT) (C and D) in tomato seedlings of the accession LA 4050 grown from seeds osmoprimed with PEG or Se and subjected to water deficit during germination. Means followed by the same lowercase letters, comparing the effect of osmopriming, do not differ from each other by the Tukey test ($p < 0.05$). Means followed by the same uppercase letters, comparing the effect of water deficit, do not differ from each other by the F test ($p < 0.05$).

osmoprimed with PEG + Se regarding the activity of POX (Figure 3B). Under water deficit, there was lower CAT activity in seeds osmoprimed with PEG. In the control, seedlings grown from unprimed seeds had higher activity of this enzyme compared to those grown from osmoprimed seeds (PEG and PEG + Se). No difference was observed in CAT activity in seedlings under water deficit or without deficit, grown from seeds osmoprimed with PEG or PEG + Se.

Considering the accession LA 3475 under water deficit, lower CAT activity was observed in seedlings grown from seeds osmoprimed with PEG when compared to those osmoprimed with PEG + Se and unprimed (Figure 3C). In the control treatment, seedlings grown from unprimed seeds showed higher CAT activity. In seedlings of the accession LA 4050, CAT activity was increased under water deficit (Figure 2C and D). In addition, unprimed seeds produced seedlings with higher activity of this enzyme when compared to those osmoprimed with PEG. Differently from what was observed in this study, especially for both accessions, the osmopriming with salicylic acid (SA) in tomato seeds subjected to different levels of water deficit resulted in an increase in SOD activity, but the responses of CAT remained virtually unchanged (Galviz-Fajardo et al., 2020). CAT in general plays a more important role among peroxidases in eliminating excess H_2O_2, as already reported in tomato seeds (Badek et al., 2016).

Responses to water deficit in seeds involve several biochemical, physiological and molecular processes (Samota et al., 2017). As observed, osmopriming with Se did not result in greater activity of enzymes of the antioxidant defense system. In seedlings under water deficit, there was lower activity of SOD and POX enzymes. In the control treatment

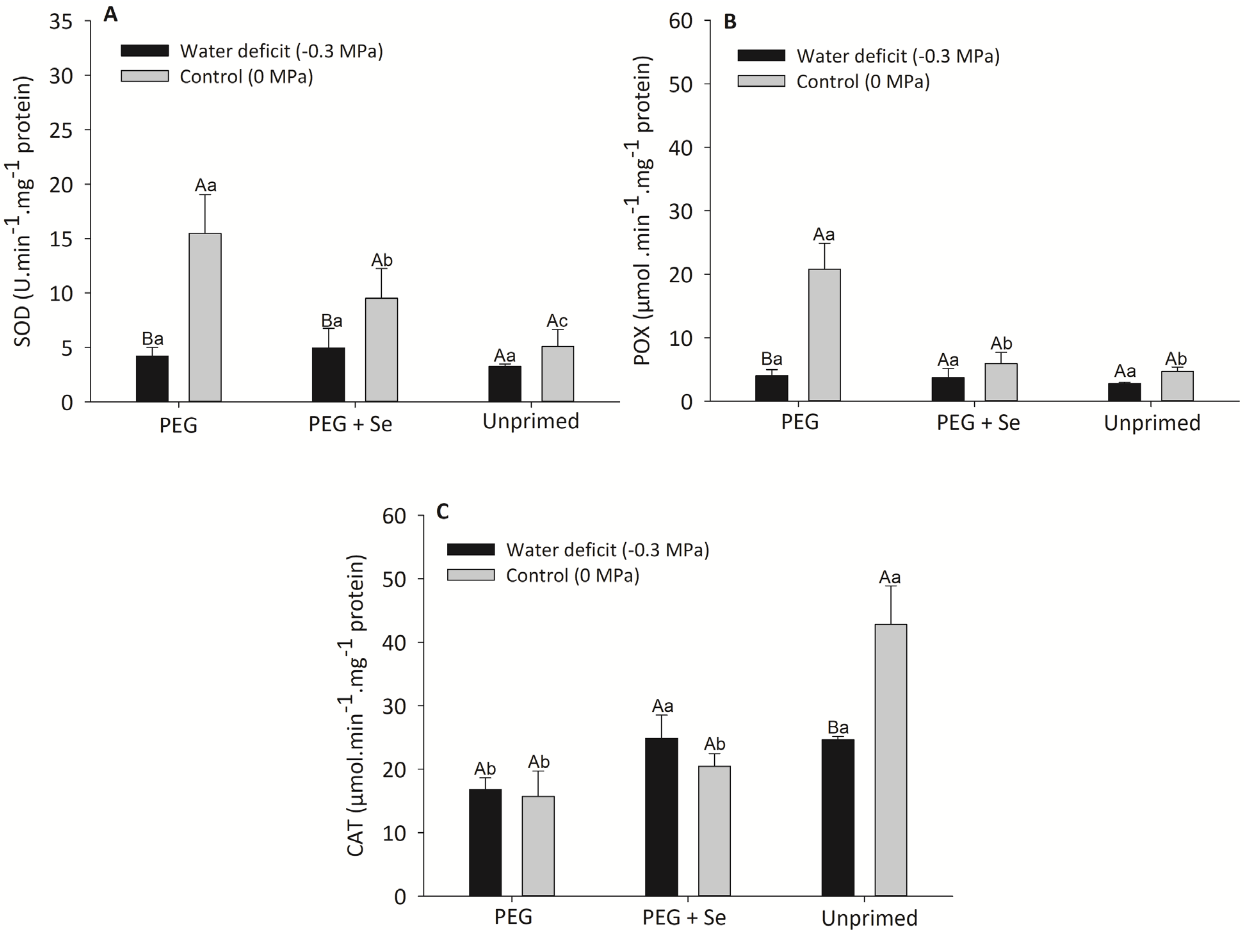

Figure 3. Activity of the enzymes superoxide dismutase (SOD) (A), peroxidase (POX) (B) and catalase (CAT) (C) in tomato seedlings of the accession LA 3475 grown from seeds osmoprimed with PEG or Se and subjected to water deficit during germination. Means followed by the same uppercase letters, comparing the effect of water deficit, do not differ from each other by the F test ($p < 0.05$).

(without water deficit), seedlings of the accession LA 3475 grown from seeds osmoprimed with PEG and seedlings of the accession LA 4050 grown from unprimed seeds showed higher activity of the enzymes SOD and POX compared to the other treatments. The role of Se in the antioxidant system (including in osmoprimed seeds) of plants under stress is to regulate the overproduction of ROS, especially superoxide ion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) (Feng et al., 2013). However, at high concentrations, Se is a pro-oxidant, causing reduction in plant growth and increase in peroxidation, besides leading to overproduction of ROS (Hawrylak-Nowak, 2013).

SOD is the first enzyme in the line of defense against ROS in plants under abiotic stresses, catalyzing the dismutation of $O_2^{\bullet -}$ into O_2 and H_2O_2. Despite being more toxic, $O_2^{\bullet -}$ has low stability, being quickly converted to H_2O_2. H_2O_2 is neutralized by CAT, POX and other peroxidases, resulting in water as a final product (Mittler, 2017). Hasanuzzaman and Fujita (2011) reported that osmopriming with Se improved the tolerance of rapeseed to water deficit by increasing the activities of enzymatic and non-enzymatic antioxidant systems.

Khaliq et al. (2015) found contrasting results to those of the present study in rice seeds osmoprimed with Se, which led to an increase in antioxidant activity and increase in seedling emergence and growth. Nawaz et al. (2013) performed foliar spraying with Se in maize plants under water deficit and observed that exogenous Se caused an increase in the activity of the antioxidant enzymes SOD, CAT, POX and APX. In addition, the observed results show the difficulty in adjusting the osmopriming time and Se concentration to optimize the responses of seeds/seedlings, since high concentrations of Se can cause toxicity in plants, depending on the species and the environment (Wiesner-Reinhold et al., 2017). Tomato is a non-Se-accumulator species, and quantities greater than 25 µg of Se per gram of dry weight of roots and leaves, in general, are toxic to the species (White, 2016). For tomato, Se supplied at low concentrations (5 to 10 µM) through the roots led to an increase in the synthesis of phenolic compounds in leaves and reduced the levels of some chemical elements in the roots. At higher concentrations (25 to 50 µM), there was an increase of around 3 to 5 times in the enzyme glutathione reductase (GSH) in the leaves (Schiavon et al., 2012). However, the form in which Se was supplied may influence the results, and previous studies that performed osmopriming with Se in tomato seeds were not found in the literature.

Rady et al. (2020) observed that the application of Se in tomato plants through foliar spraying and in the soil (0, 20, or 40 mM) promoted regulatory role of Se in plants under low water availability due to its positive influence on enzymes and non-enzymatic components of the antioxidant defense system. Thus, enzymatic activity occurs in a joint and integrated way in order to maintain the balance of ROS at the intracellular level (Mittler, 2017). In this context, Bailly et al. (2008) conceptualize the “oxidative window for germination”, which basically defines the critical levels of the ROS threshold regarding their dual role, such as cell signaling and their toxic effects. Thus, very low or very high ROS levels are harmful, while maintaining ROS levels within this range is essential (Mittler, 2017).

In summary, there was variation in the responses of the two genotypes evaluated regarding the activity of enzymes of the antioxidant defense system (Figures 2 and 3). For LA 4050, higher enzymatic activity was observed in unprimed seeds, while CAT showed higher activity under water deficit. According to Steinhauser et al. (2011), the introgression of part of the genome of *S. pennelli* in the cultivar M82 (LA 3475) led to an increase in the enzymatic activity evaluated in the pericarp of ripe fruits in the accessions obtained. In this context, the results observed in this study show that the higher activity of CAT in seedlings under stress conditions contributes to the greater tolerance of this accession to water deficit; in addition, there was greater tolerance of this genotype to water deficit (Pessoa et al., 2022). According to Mhamdi et al. (2012), CAT is the main enzyme responsible for neutralizing H_2O_2 in peroxisomes, which reinforces the importance of its activity under stressful situations. In the accession LA 3475, higher activity of SOD and POX was observed in osmoprimed seeds. However, CAT activity was higher in unprimed seeds. Under water deficit, the activity of antioxidant enzymes was lower compared to the control. Kurek et al. (2019) mention that the reduced accumulation of CAT transcripts may result in oxidative stress due to greater accumulation of ROS, which may have occurred for the most susceptible line.

Through principal component analysis (PCA), it was observed that for both accessions, components 1 (PC1) and 2 (PC2) explained more than 90% of the variability of the data (Figure 4). The physiological quality vectors (green color) were mostly concentrated in the positive scores of component 1 (PC1+), close to the vectors referring to antioxidant

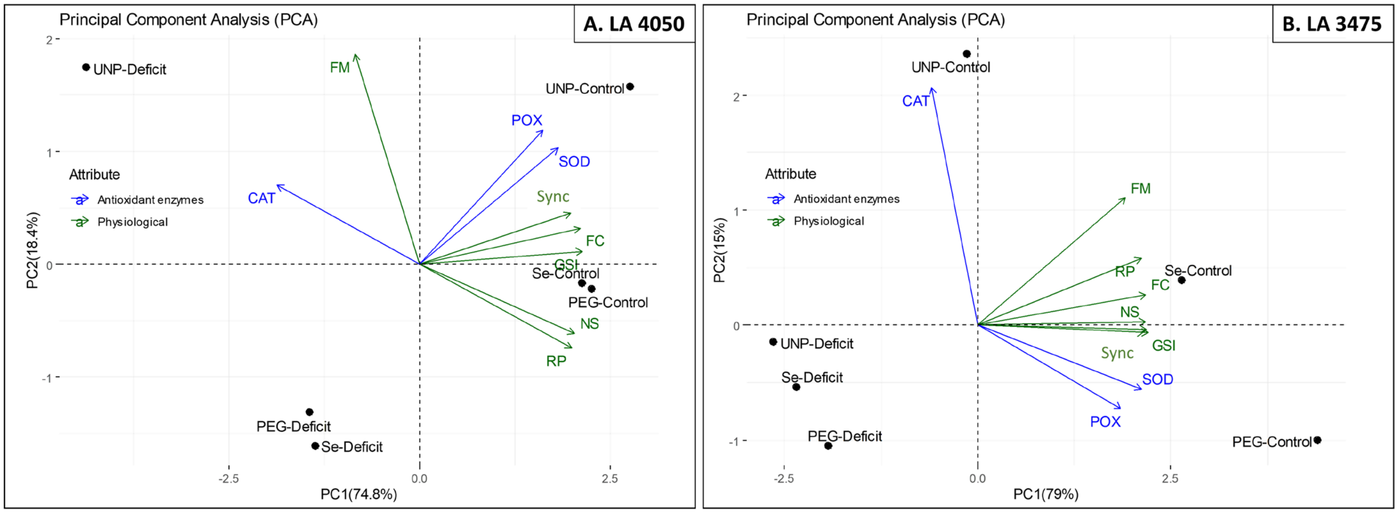

Figure 4. Biplot of principal component analysis (PCA) obtained by the linear combination of physiological variables and antioxidant enzymes of seeds and seedlings of the tomato accessions LA 4050 (A) and LA 3475 (B). The seeds were subjected to different conditions of osmopriming (PEG, PEG + Se and unprimed seeds) and water deficit during germination.

enzymes (blue color) SOD and POX and the control treatments (without water deficit). On the other hand, the treatments with water deficit (Unprimed-Deficit, PEG-Deficit and Se-Deficit) were concentrated in the negative scores of component 1 (PC1-), close to the vector of CAT (Figures 4A and B).

The PCA helped to summarize and understand the data obtained in the present study. In summary, the water deficit reduced germination, root protrusion and germination speed, increasing the mean germination time and reducing the uniformity of germination of tomato seeds, regardless of treatment. In addition, the osmopriming of seeds with Se, in general, did not improve their performance. Furthermore, the analysis of vectors in PCA helped to observe that the enzymes SOD and POX were not indicators of oxidative stress caused by water deficit for tomato seeds. On the other hand, CAT was a more evident indicator of oxidative stress in seeds subjected to water deficit, especially in LA 4050 (tolerant to water deficit). Osmopriming with PEG proved to be a promising alternative for the seeds of the two accessions and can be used to reduce germination time and increase the uniformity of seedlings under water deficit (Figures 4A and B).

CONCLUSIONS

Osmopriming with Se (12 µM for 24 h) is not an efficient method to improve physiological quality and activation of antioxidant enzymes in tomato seeds susceptible and tolerant to water deficit.

Osmopriming with PEG (-1.0 MPa for 24 h) has the potential to increase the tolerance of tomato seeds to water deficit during the germination process.

The antioxidant enzyme CAT is an indicator of oxidative stress tolerance in tomato seedlings under water deficit.

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