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Protective action of nitric oxide in sesame seeds submitted to water stress[1]

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ABSTRACT - The objective in this work was to investigate the effect of nitric oxide (NO) like protective agent in sesame seeds submitted to different osmotic potentials. The treatments, in total of eight, were water (control), water plus sodium nitroprusside (SNP) and the other treatments with PEG 6000 and PEG 6000 plus SNP: - 0.1 MPa, -0.1MPa +200 µM of SNP, 0.2 MPa, -0.2 MPa +200 µM of SNP, -0.3 MPa and -0.3 MPa, +200 µM of SNP. Were done the following determinations: germination, first count of germination, speed germination index, hypocotyl length, radicle length, dry mass of hypocotyl and radicle. It was quantified the activity of the antioxidative enzymes, superoxide dismutase, catalase, ascorbate peroxidase and total peroxidase. The experimental design was completely randomized with five replications. The water restriction reduced the germination of sesame seeds, however, the presence of nitric oxide (NO) due to the application of SNP, was beneficial, promoting increase in germination, vigor and seedlings. There was an increase of antioxidative enzymes activity in the period of 0 to 24 hours, demonstrating organization of antioxidative system in all long the time. The association of PEG 6000 to SNP, increased the activity of antioxidative enzymes, evidencing an efficient system of elimination of ROS formed during the exposition to water deficit.

Index terms: *Sesamum indicum* L., sodium nitroprusside, vigor, germination, antioxidative system.

Ação protetora do óxido nítrico em sementes de gergelim submetidas ao estresse hídrico

RESUMO - O objetivo neste trabalho foi investigar o efeito do óxido nítrico como agente protetor em sementes de gergelim submetidas a diferentes potenciais osmóticos. Os tratamentos, no total de oito, foram: água (controle), água acrescida de nitroprussiato de sódio (SNP) e os demais tratamentos referentes às concentrações de PEG e PEG acrescido de SNP: 0,1MPa, 0,1MPa +200 µM de SNP, -0,2 MPa, -0,2MPa +200 µM de SNP, -0,3 MPa e -0,3 MPa, +200 µM de SNP. Foram feitas as seguintes determinações: germinação, primeira contagem de germinação, índice de velocidade de germinação, comprimento de hipocótilo, radícula e massa seca de hipocótilo e radícula, além da quantificação da atividade das enzimas superóxido dismutase, catalase, ascorbato peroxidase e peroxidases totais. O delineamento estatístico utilizado foi o inteiramente casualizado com cinco repetições. A restrição hídrica reduz a germinação de sementes de gergelim, entretanto, a presença de óxido nítrico (ON) proporciona aumento na germinação e vigor. Há aumento da atividade das enzimas do sistema antioxidante no período de 0 a 24 horas, demonstrando organização do sistema antioxidante nas sementes de gergelim. A associação do PEG-6000 ao SNP aumenta a atividade das enzimas antioxidantes evidenciando um eficiente sistema de eliminação de ERO durante a exposição ao estresse hídrico.

Termos para indexação: *Sesamum indicum* L., nitroprussiato de sódio, vigor, germinação, sistema antioxidante.

Introduction

In the germination process, the first step in the events sequence that culminate in the resumption of embryo growth is the imbibition. The water absorbed by seeds is directly or indirectly involved in all other stages of the subsequent metabolism.

The seeds imbibition plays a key role in the germination process (Bewley et al., 2013). Between the various environmental factors that can influence this process, the availability of water is one of the most important. The water

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deficit occurrence in plants leads to the reduction of germination speed and the delaying of the seedlings development. Stronger restrictions come to harm the final germination percentage due to the delay or the non-occurrence of the metabolic processes necessary for germination (Rahimi, 2013). Prolonged exposure to water deficit generates several natural reactions in seeds. One of the consequences of this exhibition is the generation of reactive oxygen species (ROS), which leads to the oxidative stress, what means, there is an increase in the lipid peroxidation and a decrease in the activity of oxygen reactive species (ROS) sequestrant enzymes (Yao et al., 2012), affecting the structures and cell metabolism (Wang et al., 2009).

One of the defense mechanisms modulated by plants against oxidative stress is the antioxidant system that acts and constitutes an important primary defense against free radicals generated in seeds under stress conditions, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) (Mittler, 2002).

Studies have shown that chemical compounds such as nitric oxide (NO), act in plants protection exposed to stress factors. Multi-functional molecule that acts in many physiological events, has among other functions, the cytoprotective by the ability to regulate the level and toxicity of ROS. Sodium nitroprusside (SNP) is currently the most used donor of nitrogen oxides that produce NO.

The donor use of NO in plants is being extensively explored in research to be an important signaling molecule present in many phases of plant development and essential element in the responses to biotic and abiotic stresses in plants. Studies show that NO is able to increase wheat seeds germination submitted to salt stress (Zheng et al., 2009), stress by heavy metals such as arsenic (Singh et al., 2013), copper (Hu et al., 2007), lead and cadmium (Kopyra and Gwóźdź, 2003), in addition to increasing the accumulation of dry matter in pumpkin seedlings under salt stress (Fan et al., 2013).

The NO action in the increase of plants tolerance to different types of stress has been strongly correlated with their ability to protect plant cell from oxidative damage. Seeds submitted to water stress and the NO influence as a protective molecule requires further studies.

Thus, the objective in this study was to investigate the NO effect as a protective agent and signaling in sesame seeds submitted to different osmotic potential, through evaluations of physiological and biochemical characteristics and antioxidant enzymes activity.

Material and Methods

Sesame seeds (*Sesamum indicum* L.) were used from the agricultural year 2013, from EMBRAPA Cotton. Initially, the preliminary tests were done to determine the concentrations of polyethylene glycol solution of molar mass 6000 (PEG 6000) and NO donor solution (sodium nitroprusside (SNP) to be studied. The amount of SNP was the one capable of reversing or mitigating the actions of the water stress. After that, the sesame seeds, in five replicates of 50, were put to germinate on a paper towel moistened with 3 mL of the solution, referent to the following treatments in Petri dishes: water (control), water plus sodium nitroprusside (SNP) and the other treatments with PEG 6000 and PEG 6000 plus SNP; - 0.1 MPa, -0.1 MPa +200 µM of SNP, 0.2 MPa, -0.2 MPa +200 µM of SNP, -0.3 MPa and -0.3 MPa, +200 µM of SNP, summing up to a total of eight treatments.

The seeds of each treatment were kept in B.O.D. regulated with alternate temperature of 20-30 °C, with the presence of constant light (Brasil, 2009). The following evaluations were done:

Germination percentage: in the sixth day after sowing, the percentage of normal seedlings was evaluated (Brasil, 2009).

First germination count: consisted of a record of the number of normal seedlings obtained in the third day after sowing, and the values were expressed in percentage (Brasil, 2009).

Germination speed index: daily counts were done of the number of seeds that issued a radicle higher than 1.0 mm and the index were calculated according to Nakagawa (1999).

Length of the hypocotyl and the radicle: the seeds of each treatment, in five replicates of 25, were sown in gerbox, following the methodology described above for the germination test (Pires et al., 2016). A measurement of the length of hypocotyls and the radicle was done in the seedlings classified as normal with a graduated ruler. The results were expressed in cm. seedling^{-1}.

Dry matter of the hypocotyl and the radicle: the seeds used to measure the length of the hypocotyl and the radicle were separated in hypocotyl and radicle, and later dried in an oven for 72 hours at 65 ºC. The results were expressed in mg.seedling^{-1} (Brasil, 2009).

Activity of the main enzymes of the antioxidant system: determined using seeds soaked for 12 and 24 hours in water and in solutions of PEG and PEG increased by SNP. The enzyme extracts were obtained by maceration of 0.2 g of seeds in ice, followed by the addition of 2.0 mL of the following means of homogenizing: potassium phosphate buffer 0.1 M and pH 6.8, ethylenediaminetetraacetic acid (EDTA) 0.1 mm, phenylmethylsulfonyl fluoride (PMSF) 1 mm and polyvinylpolypyrrolidone (PVPP) 1% (p/v). After that, the extract was centrifuged at 15,000 g for 15 minutes at 4 °C and the supernatant was collected, where determinations were done on the activities of ascorbate peroxidase enzyme (APX), peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD).

The activity of APX was determined by the addition of 200 µL of raw enzyme extract at 2.9 mL of reaction medium of ascorbic acid 10 mM and H_2O_2 10 mM in potassium phosphate buffer 100 mM, pH 6.0. A decrease in the absorbance was observed at 290 nm, at 25 ºC, during the first minute of the reaction. The enzyme activity was calculated using the molar extinction coefficient of 2.8 $mM^{-1}.cm^{-1}$ and expressed in µmol $min^{-1}.mg^{-1}$of protein (Sano et al., 2001).

The activity of POX was determined by the addition of 50 µL of raw enzyme extract at 2.97 mL of reaction medium composed of potassium phosphate buffer 100 mM and pH 6.8, pyrogallol 150 mM and hydrogen peroxide 125 mM (Kar and Mishra, 1976). The increase in the absorbance during the two first minutes of reaction at 420 nm at a constant temperature of 25 ºC determined the production of purpurogallin. The enzyme activity was calculated using the molar extinction coefficient of 2.47 mM^{-1}. cm^{-1} and expressed in µmol min-^{1}. mg^{-1} of protein.

To quantify the CAT activity, was added in 30 µL of enzyme extract, 0.98 mL of sodium phosphate buffer 0.05 M, pH 6.8, H_2O_2 0.0125 mM dissolved in buffer. The enzymatic activity was determined by monitoring the absorbance decrease at 240 nm for 2 minutes, in intervals of 15 seconds and calculated based on the extinction factor of 36 mM^{-1}. cm^{-1}.

The activity of SOD was determined by the addition of 30 µL of raw enzyme extract at 2.95 mL of the reaction medium composed of sodium phosphate buffer 100 mM at pH 7.8, methionine 50 mM, p-nitro blue tetrazolium (NBT) 1 mM, EDTA 5 mM and riboflavin 100 mM. The reaction was conducted at 25 ºC in a reaction chamber under lighting of a 15 W fluorescent lamp, kept inside a box internally coated with foil. After five minutes of exposure to the light, the lighting was interrupted and the blue formazan produced by NBT photo reduction was determined by the absorption at 560 nm in a spectrophotometer. A SOD unit was defined as the amount of necessary enzyme to inhibit the NBT photo reduction in 50%. The SOD activity was expressed in U min^{-1}. mg^{-1} protein. To determine the content of proteins, the method used was Bradford (1976) with a standard curve constructed with bovine serum albumin (BSA) as a reference protein.

For all determinations, the statistical design was entirely randomized with five replicates. The data was submitted to a variance analysis (ANAVA) with the help of the statistical program SISVAR® (Ferreira, 2011) and the averages obtained for the treatments were compared by the Tukey test at a 5% significance. The averages obtained in the treatments with and without SNP were compared by the F test at 5% of probability and for the enzyme determinations the Tukey test was also used, at 5% significance. The graphs were plotted with the help of Sigma Plot program.

Results and Discussion

Sesame seeds had 98.4% of germination under optimal conditions (water germination), occurring reduction in germination under water stress with values of 31.6% in lower osmotic potential (-0.3 MPa), 40% under osmotic intermediate potential (-0.2 MPa) and 66.8% in greater osmotic potential (-0.1 MPa) (Figure 1A). It was observed that the different values of water potential studied acted reducing germination. Despite this, the germination reached in the osmotic potential of -0.1 is above 60%, considered minimal for Brazilian marketing sesame seeds (Brasil, 2013). Reducing the percentage of seed germination in water deficit conditions is attributed to lower diffusion of water through tegument.

The SNP application does not affect the seeds germination in water, since there was no stress and the germination conditions were ideal (Figure 1), but allowed to obtain significant seed germination increase in treatments with PEG 6000 in the three concentrations tested (Figure 1A).

By the first germination count (FC) and germination speed index (GSI) results, it turns out that the water deficit in any of the tested potential reduced the germination speed (Figure 1B). There was marked reduction in germination speed with increasing of water stress, i.e., with decreased of osmotic potential of the PEG 6000 solutions. When PEG solutions were used, there was an increase in the germination speed (Figures 1B and 1C) using SNP. The water restriction effects on seed germination have been reported in several studies for different species. Ávila et al. (2007) found an accentuated reduction in canola (*Brassica napus*) seed germination when submitted to most negative osmotic potential. Pereira and Lopes (2011) observed germination, germination speed and performance drastic reduction of jatropha (*Jatropha curcas*) seedlings in the potential of -0.2 MPa and Teixeira et al. (2011) working with crambe (*Crambe abyssinica*) seeds verified significant reduction of germination and vigor in seeds submitted to osmotic potential more negative, without no formation of normal seedlings in lower potential at -0.6 MPa. The seed germination reduction when exposed to water stress is expected, since the solutes presence as PEG decreases the water absorption by the seeds.

Water restriction reduced the sesame seeds germination, however the NO presence due to the SNP application stimulated germination. The mechanism by which the NO stimulates germination has not been fully elucidated, but some studies show the NO effective effect in promoting seed germination. Deng and Song (2012) found the NO efficiency in promote germination when lettuce seeds were submitted to water stress, as well as Sarath et al. (2006) with *Panicium virgatum*. One

hypothesis used by these authors is the NO direct effect on abscisic acid - ABA endogenous content. Sarath et al. (2006) demonstrated that the SNP application partially reverses the ABA inhibitory effects on germination, radicle elongation and the coleoptile emergence in *Panicum virgatum*.

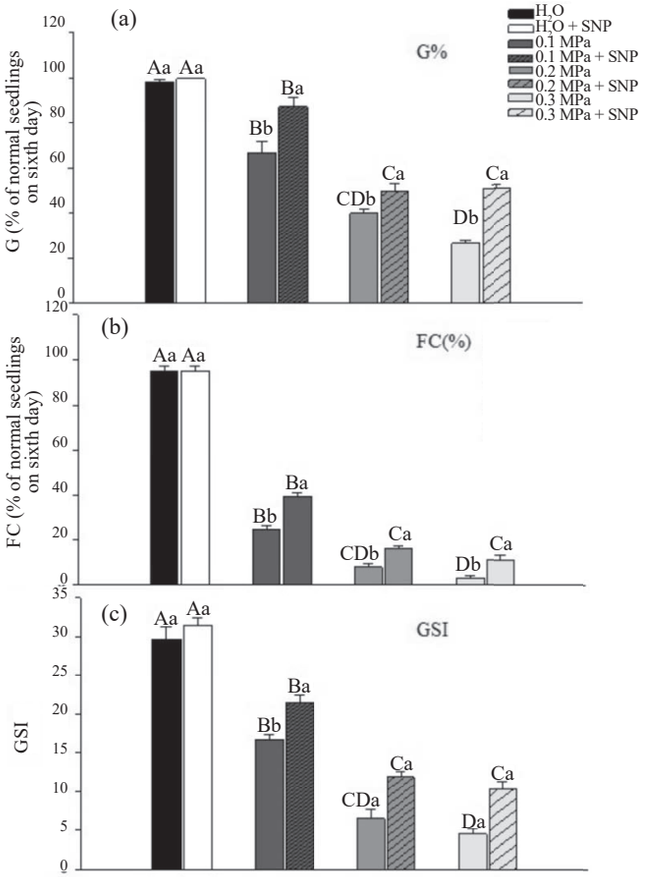

Figure 1. A- Germination (G%), B- first germination count (FC%) and C- germination speed index (GSI) of *Sesamum indicum* seeds submitted to imbibition in water (control), water + SNP, PEG 6000 solutions at -0.1 MPa, -0.2 MPa and -0.3 MPa added or not of SNP. Averages followed by the same upper case does not differ by Tukey test at 5%. Average of each treatment with and without SNP followed by the same lower case does not differ each by F test at 5%. The bars indicate the average standard deviation (n = 5).

Regarding the hypocotyl length (HL) (Figure 2A), it was observed reduction as the osmotic potential became more negative. In the potential of -0.3 MPa were obtained values for the HL 75% lower than those observed in seedlings grown under adequate conditions of water availability. In general, the root system was less compromised by water restriction than the shoots development (Figure 2B). These results can be explained by the fact that seedlings submitted to the most rigorous water stress, in general, tend to invest more biomass and develop larger root system as a survival strategy. The distribution of the root system in depth/length due water insufficiency is regarded as an indicator parameter of tolerance to drought and can confer adaptation in some species (Santos et al., 2015).

It was observed greater dry matter accumulation on seedlings of control treatment which presented dry mass of hypocotyl - DMH of 20.07 mg.seedling^{-1} (Figure 2C) and dry mass of radicle - DMR of 19.54 mg.seedling^{-1} (Figure 2D). When seeds were submitted to osmotic potential of -0.1 MPA there was reduction of 53% and 62% in DMH and DMR, respectively. In the potential of -0.2 MPa, the reduction was 69% and 74%, and in the potential -0.3 MPa the reduction of DMH and DMR in relation to control was 84% and 88%, respectively. This reduction of the seedlings' dry mass in function of water restriction can be explained by the lower speed of the physiological and biochemical processes or by difficulty of hydrolysis and mobilization of seed reserves (Bewley et al., 2013).

The NO application was able to reverse significantly the DMH and DMR reduction caused by water stress, except the DMH in the lower potential -0.3 MPa. Regarding DMH, reversal of reduction in the higher osmotic potential -0.1 MPa was 4.2 percentage points and 4.18 points at the intermediate potential -0.2 MPa. Regarding the DMR, reversal of reduction in the higher osmotic potential was 1.94 percentage points, 2.31 points at the intermediate potential and 3.02 in the lower potential (Figures 2C and 2D).

Adverse situations or biological dysfunctions lead to the formation of reactive oxygen species. Sesame seeds defense mechanism submitted to different concentrations of PEG were evaluated by means of the antioxidant enzymes activity (Tables 1 to 4). In general, it is observed increased activity of enzymes SOD, CAT, and APX with the progressive increase in the imbibition period from 0 to 24 hours. The POX, where treatments in the intervals 0 and 12 hours did not differ. These results indicate an apparent organization of the antioxidant system in sesame seeds over time. However, it was found that the period 0 h and 12 h did not differ statistically the treatments compared to the control for all enzymes, showing that these times are insufficient for organizing the antioxidant apparatus in sesame seeds submitted to stress by water deficit. Already in the 24 hours period, it is possible to observe differences in enzyme activity compared to control in practically all treatments.

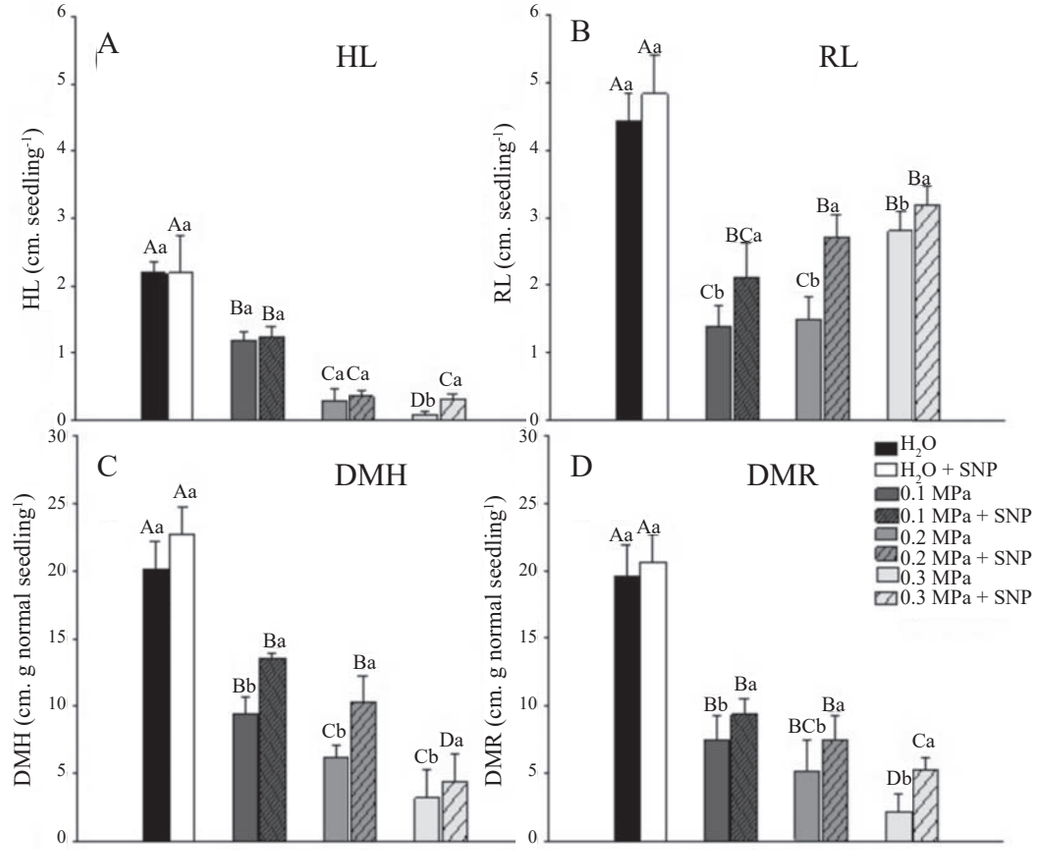

Figure 2. A- Hypocotyl length - HL (cm.normal seedling), B- Radicle lenght – RL (cm.normal seedling), C- dry mass of hypocotyl - DMH (cm.normal seedling), D- dry mass of radicle - DMR (cm.normal seedling) of *Sesamum indicum* seedlings submitted to imbibition with water (control), water + SNP, higher osmotic potential (-0.1 MPa), higher osmotic potential plus SNP (-0.1 MPa + SNP), osmotic intermediate potential (-0.2 MPa) plus SNP (-0,2 MPa + SNP), low osmotic potential (-0.3 MPa) and plus SNP (-0.3 MPa + SNP). Averages followed by the same upper case does not differ by Tukey test at 5%. Average of the treatment followed by the same lower case does not differ by F test at 5%. The bars indicate the average standard deviation (n = 5).

Table 1. Enzyme activity of superoxide dismutase (SOD) *Sesamum indicum* seeds after 0, 12 and 24 hours of imbibition in solutions with different osmotic potentials supplemented or not of SNP.

	SOD (U min^{-1}. mg^{-1}. protein)		
Treatment	0 h	12 h	24 h
H_2O	0.38 ± 0.01 Ba	0.87 ± 0.01 Ba	1.09 ± 0.06 Ae
-0.1 MPa	0.38 ± 0.01 Ca	1.08 ± 0.03 Ba	1.22 ± 0.14 Ae
-0.1 MPa +SNP	0.38 ± 0.01 Ca	1.21 ± 0.14 Ba	2.18 ± 0.32 Ac
-0.2 MPa	0.38 ± 0.01 Ba	0.79 ± 0.03 Ba	1.69 ± 0.52 Ad
-0.2 MPa +SNP	0.38 ± 0.01 Ba	1.37 ± 0.06 Ba	2.68 ± 0.31 Ab
-0.3 MPa	0.38 ± 0.01 Ca	1.27 ± 0.30 Ba	1.75 ± 0.18 Ad
-0.3 MPa +SNP	0.38 ± 0.01 Ca	1.33 ± 0.32 Ba	3.29 ± 0.23 Aa
CV(%)	----------	23.21	19.43

Averages followed by the same lower case in the column does not differ each other by Tukey test at 5%. Average followed by the same upper case in the line does not differ each other by Scott-Knott test at 5% probability. Average ± standard deviation.

It is worth remembering that in 0 h, there was no contact of the seeds with solutions PEG or PEG plus SNP, there was no seeds contamination. Thus, it was calculated the values of each enzyme, assigning fixed value for all treatments in this interval.

Table 2. Enzyme activity of catalase (CAT) *Sesamum indicum* seeds after 0, 12 and 24 hours of imbibition in solutions with different osmotic potentials supplemented or not of SNP.

	CAT (µmol $min^{-1}.mg^{-1}$. protein)		
Treatment	0 h	12 h	24 h
H_2O	15.64 ± 1.24 Ca	29.51 ± 2.13 Ba	32.82 ± 2.56 Af
-0.1 MPa	15.64 ± 1.24 Ca	33.68 ± 1.97 Ba	42.88± 4.23 Ae
-0.1 MPa +SNP	15.64 ± 1.24 Ca	34.62 ± 1.89 Ba	50.31 ± 3.67 Ac
-0.2 MPa	15.64 ± 1.24 Ca	29.58 ± 2.31 Ba	49.53 ± 3.21 Ad
-0.2 MPa +SNP	15.64 ± 1.24 Ca	34.04 ± 3.14 Ba	55.44 ± 4.23 Ab
-0.3 MPa	15.64 ± 1.24 Ca	33.26 ± 3.18 Ba	49.13 ± 3.10 Ad
-0.3 MPa +SNP	15.64 ± 1.24 Ca	34.09 ± 2.33 Ba	63.26 ± 3.67 Aa
CV(%)	----------	18.45	23.21

Averages followed by the same lower case in the column does not differ each other by Tukey test at 5% probability. Average followed by the same upper case in the line does not differ each other by Scott-Knott test at 5% probability. Average ± standard deviation.

Comparing the different treatments in the period 24 hours of imbibition (Tables 1 to 4), generally for all studied enzymes, there was higher enzyme activity in the most negative osmotic potential (-0.3 MPa) with lower activity in the potential 0 MPa (control). It is also observed increased activity of antioxidant enzymes in treatments with SNP application compared to the same treatment without SNP. It is assumed that the SNP application contributes to reducing ROS concentration during the sesame seeds germination process. According to Dong et al. (2014), NO promotes tolerance to water stress due to stimulate the increased activity of antioxidative enzymes, then providing greater protection against oxidative stress during the seed germination process.

In Table 3, there is the APX activity, at the time 0 h it was not observed enzyme activity (data not shown), however, there was catalase enzyme activity (CAT) (Table 2). The fact of the APX does not show activity in dry seeds is also reported by Bailly (2004) that justifies a higher catalase activity to supply APX absence, as both act dismuting superoxide in oxygen and hydrogen peroxide.

Already in the interval of 24 h after start of imbibition, similarly to what happened with SOD (Table 1), the peroxidase activity of ascorbate (APX) and peroxidase (POX) (Tables 3 and 4, respectively) increased the higher osmotic potential. It was also observed increased activity of both enzymes in treatments plus SNP.

It is interesting to observe that for all the analyzed enzymes, isolated treatments of -0.2 and -0.3 MPa did not differ, which may suggest that oxidative stress in both potential was similar. Similar results were found in the germination test, first germination count and germination speed index, where the two water potentials were significantly resembled in those parameters analyzed.

Table 3. Enzyme activity of ascorbate peroxidase (APX) in *Sesamum indicum* seeds after 12 and 24 hours of imbibition in solutions with different osmotic potentials supplemented or not of SNP.

	APX (µmol $min^{-1}.mg^{-1}$. protein)	
Treatment	12 h	24 h
H_2O	0,11 ±0.01 Ba	1.39 ±0.40 Af
-0.1 MPa	0.05 ±0.01 Ba	1.67 ±0.32 Ae
-0.1 MPa +SNP	0.13 ± 0.02 Ba	3.75 ± 0.21 Ac
-0.2 MPa	0.03 ±0.01 Ba	3.28 ± 0.19 Ad
-0.2 MPa +SNP	0.09 ±0.01 Ba	4.91 ± 0.31Ab
-0.3 MPa	0.06 ±0.01 Ba	3.59 ± 0.08Ad
-0.3 MPa +SNP	0.05 ±0.02 Ba	5.72 ± 0.08 Aa
CV(%)	21.93	15.38

Averages followed by the same lower case in the column does not differ each other by Tukey test at 5% probability. Average followed by the same upper case in the line does not differ each other by Scott-Knott test at 5% probability. Average ± standard deviation.

Table 4. Enzyme activity of peroxidase (POX) in *Sesamum indicum* seeds after 0, 12 and 24 hours of imbibition in solutions with different osmotic potentials supplemented or not of SNP.

	POX (μmol $min^{-1}.mg^{-1}$. protein)		
Treatment	0 h	12 h	24 h
H_2O	8.54± 1.03 Aa	12.14 ± 1.78 Aa	18.45 ± 2.34 Af
-0.1 MPa	8.54± 1.03 Ba	13.17 ± 1.23 Ba	31.39 ± 4.32 Ae
-0.1 MPa +SNP	8.54± 1.03 Ba	9.23 ± 1.29 Ba	52.99 ± 3.21 Ac
-0.2 MPa	8.54± 1.03 Ba	10.45 ± 2.01 Ba	44.39 ± 2.28 Ad
-0.2 MPa +SNP	8.54± 1.03 Ba	10.34 ± 1.45 Ba	59.12 ± 3.21 Ab
-0.3 MPa	8.54± 1.03 Ba	11.87 ± 2.65 Ba	48.45 ± 2.89 Ad
-0.3 MPa +SNP	8.54± 1.03 Ba	12.45 ± 3.12 Ba	65.45 ± 4.67 Aa
CV(%)	----------	21.36	19.22

Averages followed by the same lower case in the column does not differ each other by Tukey test at 5% probability. Average followed by the same upper case in the line does not differ each other by Scott-Knott test at 5% probability. Average ± standard deviation.

Conclusions

The increase of PEG concentrations, reduces of germination and seed vigor, as well as initial seedling growth.

The nitric oxide presence due to the sodium nitroprusside application is beneficial, providing increases in germination and vigor.

The SNP increase the antioxidant enzymes activity, indicating an efficient removal system of reactive oxygen species formed during exposure to water deficit and allow increased tolerance of this species to the water restriction and stimulate seed germination.

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