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Priming of *Coffea arabica* seeds improves the germination quality and stimulates antioxidant system enzymes

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Abstract

Physiological conditioning is reported to result in faster and more uniform seedling emergence in the field and induces tolerance to environmental adversities. This study aimed to evaluate the efficiency of physiological conditioning on *Coffea arabica* L. seeds and drying rates through germination tests and enzymatic activity. *C. arabica* seeds from Catuaí Amarelo, with water contents of 43, 35 and 12%, were conditioned with distilled water, cathodic and anodic water, ascorbic acid, and sodium nitroprusside. The efficiency of the treatments was analyzed through tests of germination, emergence, electrical conductivity, and seedling growth. The activities of the enzymes superoxide dismutase, catalase, and ascorbate peroxidase were also measured. No priming agent was found to improve germination on seeds dried up to 12% water content, as little or no differences were found for those at 43%, while either ascorbic acid or sodium nitroprusside effectively improved vigor on 35% water content seeds. Our results indicate that sodium nitroprusside or ascorbic acid can be used as molecules to improve coffee seed vigor.

Keywords: Antioxidant enzymes, Coffee, priming.
Abbreviations: ANOVA_analysis of variance, APX_ascorbate peroxidase, AsA_ascorbic acid, B.O.D._Biochemical Oxygen Demand, CAT_Catalase, CRD_Completely random Design, DTT_Dithiothreitol, EC_Electrical Condictivity, EDTA_Etilenediaminetetraacetic salt dissodic, ESI_Emergence Speed Index, HL_Hypocotyl Length, NBT_Nitroblue Tetrazolium, NO_Nitric Oxide, PMSF_Phenylmethylsulfonyl Fluoride, RL_Root Length, SL_Seedling Length, SNP_Sodium Nitroprusside, SOD_Superoxide Dismutase

Introduction

Physiological conditioning, also called priming, is a technique reported to result in improvements in seed germination and vigor in a wide range of species (Paul and Roychoudhury, 2017). In addition, this technique also results in improved antioxidant system efficiency (Gammoudi et al., 2020; Ali et al., 2020), leading to a better response to abiotic stress. In the literature, many treatments have been reported as efficient for priming, such as cathodic and anodic water, as other signaling molecules.

The cathodic fraction (reduced) of an aqueous electron solution has a direct antioxidant property without needing to apply any other component. In studies regarding cathodic water efficiency as a proacting agent against desiccation effects on the embryonic axis in the recalcitrant species *Strychnos gerrardii* and *Boophane distich* during cryopreservation steps, Berjak et al. (2011) observed that cathodic water is a probable endogenous antioxidant activity stimulation agent, mitigating oxidant damage related to the cryopreservation steps. On coffee seeds, this positive effect was also observed by the cathodic action during seed imbibition.

Nitric oxide (NO) is a molecule that acts as a signalizer in plants, and studies regarding its actions indicate that it participates in plant development, pathogen defense, and abiotic stress responses. In most cases, the stress response is a product of its interactions with phytohormones (Ataíde et al., 2015; Sanz et al., 2015). Molecules acting as NO donors, such as sodium nitroprusside, have been widely reported due to their effectiveness in increasing seed stress tolerance. These effects were reported for diverse species such as *Senna macranthera* (Silva et al., 2015), *Vigna radiata* (Roychoudhury et al., 2021), *Eucalypitus urophylla* (Pereira et al., 2020), *Urochloa ruziziensis* (Oliveira et al., 2022), and cotton seeds (Guaraldo et al., 2023).

Ascorbic acid (AsA) is an essential constituent in plant growing tissues and acts to control cell division and expansion, hormone biosynthesis, reactivation of antioxidant enzymes, and photosynthesis and respiration (Zha et al., 2020). The use of AsA for priming has been cited as effective in other species, such as wheat (Farooq et al., 2013). AsA is a plant regulator and increases seed vigor after its exogenous application (Zha et al., 2020). In chicory seeds,

AsA efficiency in physiological conditioning was reported, with an increase in germination and seedling development as stress tolerance (Sadeghi and Robati, 2015).

Much research has been carried out using the physiological conditioning technique to improve coffee seed germinative potential, evaluating temperature and treatment time, aiming to establish an effective method (Carvalho et al., 2014). However, studies on coffee seed evaluation conditioning using exogenous molecules compared to water with different charges are incipient, such as comparisons with different water contents. Thus, this study was carried out to evaluate the influence of physiological conditioning on stored coffee seed germination by using different conditioning agents.

Results

Seed germination

After harvest, seeds had a 93% germination percentage, and eight months after storage, germination decreased to 80%, and the water content remained at 43%. Regarding priming effects, at first and final germination counts, seeds with 12% water and sodium nitroprusside (SNP) resulted in higher values, as both cathodic and anodic water resulted in lower values. No differences were observed for priming effects on seeds with 35 and 43% water content (Table 1). For cotyledonary leaves at 45 days, the results for seeds with 12% water content were similar to those observed for first germination counting at the same water content. For 35% water content, superior results were found for ascorbic acid (AsA) and sodium nitruprusside.

By analyzing seedling emergence at 45 days (Table 2), there was no significant difference among conditioning agents on seeds with 12% water content. For those with 35% water content, higher values were found for those conditioned with SNP. For seeds with 43% water content, higher values were observed for anodic water, distilled water, and AsA. Regarding water content, 12 and 35% represent the inferior and superior values, respectively. After 60 days, no difference among priming agents was found in emergence for seeds dried at 12% water content, as SNP resulted in higher values for those dried at 35% water content. On seeds at 43%, cathodic water resulted in lower values and no difference among other treatments.

Seed electrical conductivity

As observed in the electrical conductivity test (Table 3), for seeds submitted to drying to 12% water content, independent of the conditioning agent, higher conductivity values were observed, with the lowest observed at 35%. By comparing conditioning solutions within each water content, those with 12%, conditioned with anodic water and water have lower and higher results. For 35%, no significant differences were observed. For 43%, those conditioned on cathodic after having higher values of electrical conductivity.

Seedling growth

No differences were found among priming agents for both seeds at 35 and 43% water content. However, a higher hypocotyl length was found on seedlings dried to 35% than on those dried to 43% (Table 4). No effect on priming agent was found for root length on seeds at 35% water content, while cathodic water resulted in higher values for those at 43% water content (Table 4). By comparing water content, seeds with 43% had higher root length than those at 35%. For the root/hypocotyl length ratio, no effect of the conditioning agent was found for seeds at 35% water content, while for seeds at 43% water content, lower values were found for those treated with cathodic water (Table 4). Once no seedling with expanded cotyledon was found after 45 days on seeds dried to 12% water content, no measurements on those treatments were done.

Sixty days after sowing, once we found seedlings developed from seeds dried at 12% water content, we carried out an analysis of root and aerial part dry mass (Table 5). Independent of the drying ration and conditioning agent, no significant differences were found for either of these traits (Table 5).

Antioxidant system activity

For superoxide dismutase (SOD) enzyme activity (Fig 1A), higher values were observed in 12% water content seeds treated with AsA, and lower values were observed in seeds treated with distilled water. For 35% water content seeds, higher values were observed for SNP and AsA. Seed with 43% water content, in SNP and AsA also have superior results.

By analyzing catalase (CAT) enzyme activity (Fig 1B), in seeds with 35% water content, higher values were found for water, SNP, and AsA. For seeds with 43% water content, lower activity was found for seeds conditioned on distilled water. For seeds with 12% water content, higher values of catalase activity were found for SNP and water, with lower values observed for anodic water.

As observed in Fig. 1C, ascorbate peroxidase (APX) activity for 12% water content seeds conditioned with AsA had lower activity, while SNP had higher activity. In contrast, seeds with 35% water content conditioned in AsA had higher activity, while lower activity was observed for anodic water. For seeds with a 43% water content, higher activity was found for AsA and cathodic water.

Discussion

The benefits of SNP as priming agents, as seen in our research, were also reported for other species, such as *Dalbergia nigra* (Ataíde et al., 2015). By comparing the results with the water content, it was possible to observe that seeds with a 43% water content conditioned with AsA and cathodic water were not significantly different from seeds with a 35% water content conditioned with the same conditioning agents.

Santos et al. (2016) observed that watermelon seeds imbibed on AsA had improvements in seed germination and seedling growth, highlighting the significant effect of the germination stage on initial seedling development. Higher vigor and germination for wheat seeds treated with ascorbic acid was also observed (Farooq et al., 2013).

Kaiser et al. (2016) studied SNP-treated cabbage seeds under salt stress and observed and recommended a concentration of 0.01 mmol/L, for which better results were found. However, it is important to highlight that NO concentrations that promote germination may vary according to the species and liberator used, as in other conditions, as observed here.

After 45 days, we observed that higher values were observed for seeds dried up to 35%, while lower values were found for 12% water content seeds, indicating that physiological conditioning is not effective for low water content seeds. Regarding seedling emergence after conditioning, Song et al. (2009) studied *Suaeda salsa* seeds

Table 1. Germination, normal seedlings, and seedlings with expanded cotyledon after 15, 30 and 45 days from sowing (respectively) from coffee seeds with different water content submitted to physiological conditioning.

Conditioning Agent	Fist Count			Fistal Count			Cotyledonar leaves		
	Water content			Water content			Water content		
	12	35	43	12	35	43	12	35	43
Anodic Water	12 Cc	78 Aa	59 Ab	14 Cc	80 Aa	63 Ab	14 Cc	80 Aa	63 Ab
Ascorbic Acid	29 Bb	74 Aa	68 Aa	33 Bc	84 Aa	75 Ab	33 Bc	84 Aa	75 Ab
Cathodic Water	16 Cb	70 Aa	60 Aa	18 Cc	76 Aa	64 Ab	18 Cc	76 Aa	64 Ab
Water	23 Bc	79 Aa	65 Ab	28 Bc	85 Aa	67 Ab	28 Bc	85 Aa	67 Ab
Sodium nitroprusside	46 Ac	82 Aa	58 Ab	50 Ac	90 Aa	62 Ab	50 Ac	90 Aa	62 Ab
Coefficient of variation (%)	15,04			13,01			14,06		

Averages followed by the same letter, uppercase for columns and lowercase for lines, are not different according to Tukey's test at 5% probability.

and observed that an increase in NO concentration did not stimulate seedling emergence or aerial dry mass for seed

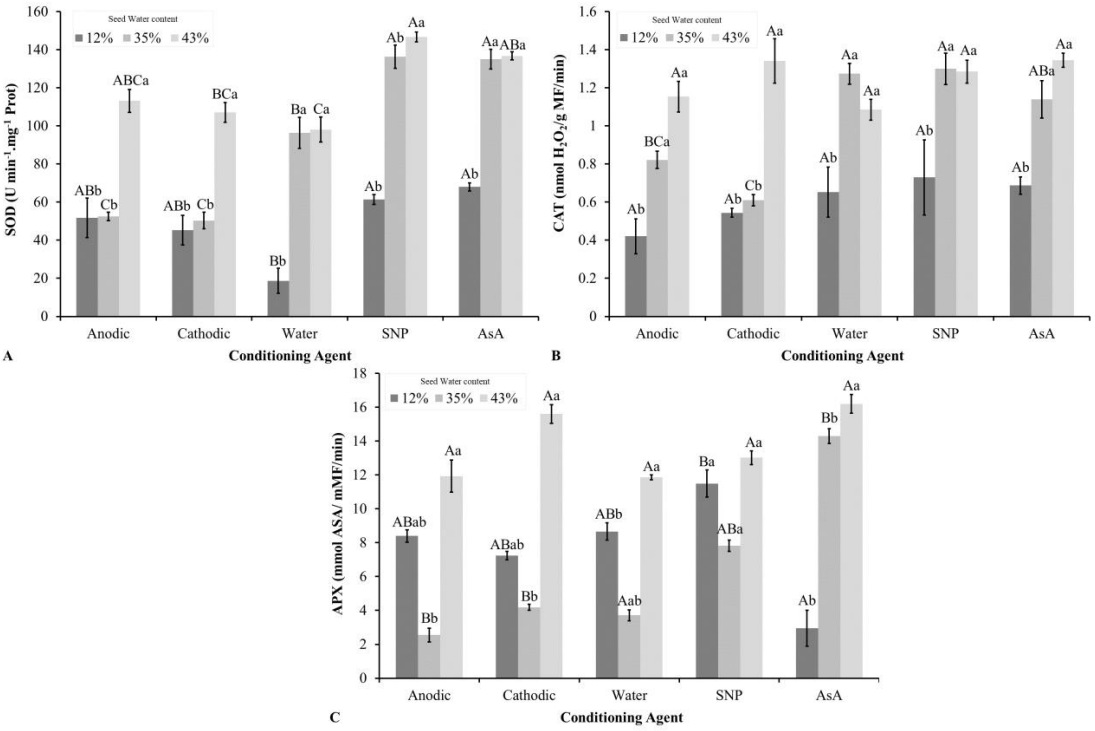

Figure 1. Enzymatic activity of Superoxide Dismutase (A), Catalase (B), and Ascorbate Peroxidase (C) on seeds under effect of conditioning agents and seed water content. Same letters over the bars indicates no differences among treatments according to Tukey's test at 5% probability which uppercases compare conditioning agents in each seed water content and lowercases compares water content in each conditioning agent.

Table 2. Average values for seedling emergency at 45 and 60 days and emergency speed index developed from coffee seeds under effect of different water content and conditioning agents.

Conditioning Agent	45			60			ESI		
	Water content			Water content			Water content		
	12	35	43	12	35	43	12	35	43
Anodic Water	5Ac	59Ba	46Ab	22Ac	82Ba	59Ab	0.45Ab	2.24Ba	1.7Aba
Ascorbic Acid	2Ac	66Ba	45Ab	20Ab	81Ba	69Aa	0.38Ab	2.73Aa	1.7Aba
Cathodic Water	6Ac	60Ba	29Cb	15Ac	74Ca	50Bb	0.34Ab	1.95Ba	1.0Bb
Water	3Ac	64Ba	53Ab	12Ac	80Ba	66Ab	0.17Ab	2.72Aa	2.1Aa
Sodium nitroprusside	6Ac	79Aa	38Bb	9Ac	95Aa	56Ab	0.12Ab	2.61Aa	1.8Aba
Coefficient of variation (%)	20.12			14.34			30.63		

Averages followed by the same letter, uppercase for columns and lowercase for lines, are not different according to Tukey's test at 5% probability.

Table 3. Electrical conductivity on coffee seeds under effect of different water content and conditioning agents.

Conditioning Agent	Water Content		
	12	35	43
Anodic Water	12.4Ca	4.8Ac	10.1Cb
Ascorbic Acid	14.9Ba	5.9Ab	7.6Db
Cathoric Water	13.4BCb	5.8Ac	17.5Aa
Water	17.3Aa	5.2Ab	6.8Db
Sodium nitroprusside	13.7BCa	5.3Ab	13.3Ba
Coefficient of variation (%)	10,47		

Averages followed by the same letter, uppercase for columns and lowercase for lines, are not different according to Tukey's test at 5% probability.

Table 4. Length of hypocotyl, root, and relation from three measures on coffee seedlings, submitted to different drying and conditioning agents.

Conditioning Agent	Hypocotyl length		Root length		Ratio HL/RL	
	Water Content		Water Content		Water content	
	35	43	35	43	35	43
Anodic Water	5.8 Aa	4.9 Ab	4.0 Ab	6.6 Aa	0.7 Ab	1.3 Aa
Ascorbic Acid	6.1 Aa	5.4 Ab	3.5 Ab	6.0 Aa	0.6 Ab	1.1 Aa
Cathodic Water	6.1 Aa	5.1 Ab	2.7 Ab	4.0 Ba	0.4 Ab	0.8 Ba
Water	6.5 Aa	5.1 Ab	3.7 Ab	6.2 Aa	0.6 Ab	1.2 Aa
Sodium nitroprusside	5.9 Aa	5.1 Ab	4.0 Ab	6.3 Aa	0.7 Ab	1.3 Aa
Coefficient of variation (%)	7.72		16.94		16.13	

Averages followed by the same letter, uppercase for columns and lowercase for lines, are not different according to Tukey's test at 5% probability.

Table 5. Dry mass of root and aerial part from coffee seedlings developed from seeds with different water content and conditioning agents.

Conditioning Agent	Root Dry Mass				Aerial part Dry Mass			
	Water content				Water content			
	12	35	43	Average	12	35	43	Average
Anodic Water	0.10Aa	0.21 Aa	0.23 Aa	0.17 A	0.85 Aa	1.68 Aa	1.44 Aa	1.32 A
Ascorbic Acid	0.11 Aa	0.25 Aa	0.23 Aa	0.20 A	0.86 Aa	1.86 Aa	1.38 Aa	1.36 A
Cathodic Water	0.10 Aa	0.21 Aa	0.20 Aa	0.17 A	0.80 Aa	1.79 Aa	1.41 Aa	1.33 A
Water	0.10 Aa	0.21 Aa	0.23 Aa	0.18 A	0.84 Aa	1.89 Aa	1.45 Aa	1.39 A
Sodium nitroprusside	0.12 Aa	0.26 Aa	0.23 Aa	0.20 A	0.88 Aa	1.84 Aa	1.46 Aa	1.40 A
Average	0.11 b	0.23 a	0.22 a		0.85 c	1.82 a	1.43b	
Coefficient of variation (%)	19.84				10.36			

Averages followed by the same letter, uppercase for columns and lowercase for lines, are not different according to Tukey's test at 5% probability.

treatments with and without salt stress. For a water content of 43%, we highlight the final emergency for cathodic water conditioning, which was lower than the others. Seeds imbibed in AsA did not statistically differ regarding water contents of 35 and 43%. For the emergency speed index (ESI), no differences were found for 12% water content seeds. Low ESIs were found for seeds, with 35% conditioned on both cathodic and anodic water and 43% conditioned on cathodic water.

Due to their desiccation sensitivity and low longevity, coffee seeds are classified as intermediate, not surviving more than 12 months at 15°C, and these seeds do not respond well to drying up to 10% water content or less (Hong and Ellis, 1996). Bot germination and seedling emergence were at lower values as seeds were dried into 12% water content. Comparing the results, we found that although seeds with 43% water content had higher root length than those with 35%, there was no difference in dry mass (Table 4), which suggests that there was no difference in dry mass accumulation in roots from 43%.

The higher SOD activity for seeds conditioned on AsA and/or SNP can be associated with the germination of these seeds (Table 1). NO donors stimulate antioxidant enzyme activity, reducing oxidative stresses caused by abiotic conditions. This response may be linked to the action of NO as a signaling molecule (Sanz et al., 2015). In studies of sunflower, it was observed that for low-vigor seeds exposed to thermal stress (35°C), osmoconditioning allows higher SOD activity (Barros et al., 2021).

SOD studies have been commonly used as an additional tool

for analyzing the physiological quality of maize, soybean, scarlet eggplant, pepper, and sesame seeds (Pires et al., 2016; Santos et al., 2016; Saibi and Brini, 2018; Berwal and Ram, 2019; García-Caparrós et al., 2021; Cavasin et al., 2023). Studies on cotton (Guaraldo et al., 2023) and Brachiaria (Oliveira et al., 2022) have highlighted the action of the antioxidant system on seed and seedling stress responses, in addition to the action of priming agents in stimulating this system in the stress response. Importantly, SOD and CAT act together and are integrated into the antioxidant system, neutralizing toxic metabolites to controlled levels (Mittler, 2017). In addition, a reduction in CAT activity may be linked to seed deterioration and results in increasing H_2O_2 concentrations, which may occur under stress conditions (Pires et al., 2016). We may highlight that an increase in this enzyme may also be linked to higher metabolic activity, which may be the case for our results on the 43% content seeds.

The importance and action of ascorbate peroxidase (APX) is the same as that of CAT, preventing H_2O_2 accumulation (Kumar et al., 2015). In plants, ascorbate peroxidase is an important antioxidant acting on preventing and/or reducing the damage from metabolic processes in either ideal or stress conditions (Zhang et al., 2013). The stimuli of priming on the antioxidant system have been reported for sunflower (Oliveira et al., 2022), cotton (Guaraldo et al., 2023), rice (Ahmad et al., 2015), and maize (Hussain et al., 2015).

Despite the number of priming efficiency reports and the efficiency of the agents in this technique (Kaiser et al., 2016; Ribeiro et al., 2019; Oliveira et al., 2022), there is no universal priming treatment, i.e., the report of a method/agent for a species does not necessarily indicate that it will be efficient for any species or cultivar (Iqbal and Ashraf, 2013; Akbari et al., 2020; Chouhan and Mandal, 2021; Nascimento et al., 2021). For our research, we observed that sodium nitroprusside and ascorbic acid were efficient treatments to improve coffee seed vigor, with SNP standing out as the best treatment for this end. However, as seeds are dried up to 12% water content, no differences could be found, with values below those observed for seeds under higher water content.

Material and Methods

Experimental area

This study was conducted at the Federal University of Lavras (UFLA) in the Central Laboratory of Seed Research (LCPS). Coffee seeds of the cultivar Catuaí Amarelo IAC 62 were produced by the group Fazenda Bom Jardin, located in the municipality of Bom Sucesso, Minas Gerais - Brazil.

Aiming to decrease coffee seed vigor to test priming efficiency, seeds were stored at 10°C and a relative air humidity of 50% until experiments were conducted at 8 months after harvest. Both before and after this storage, we carried out germination tests according to (Brasil, 2009), i.e., in paper roll conditioned at 30°C, seed germination was measured for four replicates of 25 seeds each. Additionally, at both time points, the water content was measured by the oven method at 103°C for 24 hours (Brasil, 2009).

Experimental design

This research was conducted on a completely randomized design in a double factorial of 3 (seed water content) x 5 (conditioning agents). For water content, we used seeds after eight months of storage that were not dried (43% water content) and those dried at 35% and 12%. The five priming treatments consisted of solutions of cathodic and anodic water, 100 µM sodium nitroprusside (SNP) (Faraji and Sepehri, 2018) and 50 µM ascorbic acid (AsA) (McCue et al., 2000), with distilled water used as the witness.

Osmotic conditioning was carried out by immersing seeds in distilled water, cathodic water, anodic water, SNP, or AsA. A total of 500 seeds with no silver skin were immersed in 250 mL of each solution. Osmotic conditioning was carried out in a *biochemical oxygen demand* (B.O.D.) chamber at 25°C adapted with an air compressor that maintained solution aeration to avoid seed asphyxiation. Priming was conducted under these conditions at 25°C for 7 days in the dark. The time was chosen according to the findings of Rosa et al. (2010). After conditioning, seeds were washed on running water to remove the residual priming agents before the physiological tests were started.

Seed drying

Drying was carried out by placing seeds in hermetically closed recipients with 60 grams of activated silica gel inside a type B.O.D. (biochemical oxygen demand) at 25°C/dark. The seeds were dried from the initial water content (43% fresh basis) until reaching 35% or 12%. Drying was carried out to observe whether the decrease in seed vigor caused by this process could be reversed with the use of priming on coffee seeds (Ellis et al, 1990).

Cathodic and anodic water preparation

Cathodic water was produced according to the method of Berjak et al. (2011), with the following modifications: solutions with 0.5 mM $CaCl_2$ and 0.5 mM $MgCl_2$ were electrolyzed by using a potential difference of 60 V using an electrophoresis vat. The solution content was split into equal portions, and electrophoresis was carried out for 1 hour at room temperature, producing 500 mL of anodic water (oxidized) with pH 3-4 and 500 mL of cathodic water (reduced) with pH 11-12. The circuit was completed using a saline bridge based on agar and potassium chloride.

Seed germination tests

We carried out germination tests with four replicates of 25 seeds for each treatment on a Germitest paper roll with a volume of distilled water two and half times heavier than the paper weight. Rolls were kept in a germination chamber at 30°C under constant light (Brasil, 2009). Germination was carried out in a Mangelsdorf-type germination chamber, which has an interior water reservoir that maintains the air and consequently the paper rolls and humidity. Every 7 days, water on the reservoir was reposed to maintain the volume, and germination test rolls were (if necessary) moistened with distilled water by aspersion.

Germination (radicle protrusion) was measured 15 days after sowing, and normal seedlings were measured 30 days after sowing. Seedlings with one main and two lateral roots and well-developed stems (more than 3 cm cotyledonary loop) were considered normal. After 45 days, the percentage of seedlings with expanded cotyledonary leaves was measured.

Seed electrical conductivity

Electrical conductivity was conducted by using four replicates of 25 seeds each, placed on plastic bags with 50 mL deionized water and conditioned on BOD at 25°C for 24

h. Readings were carried out after the immersion of the electrode in imbibition water, and the results are expressed in $\mu Scm^{-1}g^{-1}$. The following equation was used for calculations:

Equation 1. Formula used to calculate electrical conductivity. Caption: EC = Electrical conductivity. Weight was expressed in grams (g).

$$ECsample = \frac{(ECsolution - ECwater)}{Weight}$$

Seedling emergence test

A seedling emergence test was executed with a mixture of substrate soil:sand at a proportion of 1:1 by volume. For each treatment, four replicates of 25 seeds were used. Emergency tests were conducted on plastic trays kept in a growing chamber at 30°C, with the substrate moistened at every counting. Every three days, the number of seedlings that emerged from the substrate was counted. When seedlings with cotyledons completely emerged from the substrate, they were considered to have emerged. We calculated the emergency speed index (EVI) by using the equation proposed by (Maguire, 1962). We used for calculations the percentage of seedlings that emerged from sowing to 60 days, when values were stabilized. At the end, for normal seedlings, the aerial part was separated from the root with a scalpel, placed in paper bags and dried in an oven at 60°C for 4 to 5 days until a constant mass was observed, and this value was considered the dry mass for the root and aerial part.

Seedling growth analysis

Seedling development was evaluated through the GroundEye System, version S800, composed of a capitation module with an acrylic tray and a high-resolution camera integrated into software for evaluation. We used ten normal seedlings with expanded cotyledons randomly chosen from each treatment from the emergency tests at 45 days from sowing, which were placed on the tray of the capitation module to obtain the images.

To configure the background color for analysis, we used the model CIELab with a luminosity index of 0 to 100, dimension 'a' from -13.9 to 46.1 and dimension 'b' from -57.1 to -40.6. After calibration of the background color, image analysis was used. From image analysis, we extracted the average values of root length (RL), hypocotyl length (HL), seedling length (SL), and ratio RL/HL.

Antioxidant-system enzyme activity

Catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) were analyzed through spectrophotometry (Biemelt et al., 1998). A sample of 50 seeds was macerated in the presence of polyvinylpyrrolidone and liquid nitrogen and stored in an ultrafreezer at -86°C. For extraction, 0.050 g of material from each treatment was added to 1.5 mL of extraction buffer composed of 1452 µL of 100 mM potassium phosphate (pH 7.0), 15 µL of 100 mM EDTA, 15 µL of 100 mM ascorbic acid, 23 µL of 500 mM DTT, and 12 µL of PMSF. The material was homogenized by vortexing and centrifuged at 14,000 rpm at 4°C for 20 minutes. The supernatant was collected and transferred to a new microtube.

SOD activity was evaluated through extract capacity to inhibit nitro tetrazolium (NBT) blue photoreduction. A volume of 5 µL of extract was added to 195 µL of incubation solution containing 100 µL of 100 mM potassium phosphate (pH 7.8), 40 µL of 70 mM methionine, 3 µL of 10 µM EDTA, 35 µL of distilled water, 15 µL of 1 mM NBT, and 2 µL of 0.2 mM riboflavin. The plate was illuminated in fluorescent light for seven minutes before reading at 560 nm and was used as a bank, with water replacing the sample (Giannopolits and Ries, 1977).

To evaluate CAT activity, 9 µL of extract was added to 100 mM potassium phosphate solution previously incubated in buffer at 30°C. To this mix, 10 µL of 250 mM hydrogen peroxide was added. Activity was analyzed in spectrophotometer equipment by measuring the decrease in absorbance every 15 seconds for three minutes and monitoring the decrease in hydrogen peroxide (Havir and McHale, 1990). For APX, 9 µL of extract was added to 100 mM potassium phosphate + 0.5 mM ascorbic acid solution previously incubated at 30°C. To this mix, 9 µL of 250 mM hydrogen peroxide was added. The decrease in ascorbate absorbance was measured at 290 nm every 15 seconds for three minutes on ELISA equipment (Nakano and Asada, 1981).

Data analysis

The results were analyzed through ANOVA by using SISVAR® software (Ferreira, 2011), and averages were compared by Tukey's test at 5% probability. Tables and graphs were created in Microsoft Office Excel®.

Conclusions

Priming was not effective in improving coffee seed germination if the seeds were dried to 12% water content.

For coffee seeds at 35 and 43% water content, priming with either ascorbic acid or sodium nitroprusside is effective in improving seed vigor.

Superoxide dismutase enzyme activity can be potentially related to coffee seed physiological quality.

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