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ARTICLE

Chemical treatment and mobilization of reserves of soybean seeds under water deficit

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ABSTRACT: Seed treatments with chemical phytosanitary products can compromise mobilization of seed reserves for the seedlings under water deficit conditions. The aim of this study was to evaluate the physiological quality, the initial seedling development, and the mobilization of reserves in soybean seeds treated with phytosanitary products under water deficit. The trial was set up in a completely randomized design in a 4 × 4 factorial arrangement: chemical treatments (control, Thiamethoxam, Fludioxonil + Metalaxyl-M, and Fipronil + Pyraclostrobin + Tiophanate-methyl) × osmotic potentials (0, -0.1, -0.2, and -0.3 MPa). We analyzed the following variables: germination; hypocotyl length, root length, and total seedling length; seedling length vigor index; cotyledon dry matter weight; seedling dry matter yield; seed reserve reduction; relative dry matter yield; seed reserve reduction rate; and conversion efficiency of seed reserves into seedling dry matter. Seed quality and seedling development decline under water deficit conditions. The treatment with Fipronil + Pyraclostrobin + Thiophanate-methyl results in lower phytotoxicity. The estimated cotyledon dry matter (CDM) weight is an indicator of phytotoxicity and water deficit. Mobilization of reserves is compromised in seeds treated with insecticides, reducing the capacity of conversion of reserves into dry matter. There is an inversely proportional relationship between the reduction in conversion and the efficiency of conversion of reserves of treated soybean seeds under water deficit.

Index terms: *Glycine max* (L) Merrill, osmotic potential, seed quality, seed treatment, translocation of reserves.

RESUMO: O tratamento químico de sementes com produtos fitossanitários sob condições de déficit hídrico, podem comprometer a mobilização das reservas das sementes para as plântulas. Objetivou-se avaliar a qualidade fisiológica, o desenvolvimento inicial de plântulas e a mobilização de reservas em sementes de soja tratadas com produtos fitossanitários sob déficit hídrico. O ensaio foi instalado em delineamento inteiramente casualizado em esquema fatorial 4 x 4: tratamentos químicos (Controle; Thiametoxam; Fludioxonil + Metalaxil-M e Fipronil + Piraclostrobina + Tiofanato-metílico) x potenciais osmóticos (0; -0,1; -0,2 e -0,3 MPa). Foram analisadas as variáveis: germinação, comprimento do hipocótilo, da raiz e total das plântulas, índice de vigor do crescimento, peso de matéria seca de cotilédones, rendimento de massa seca de plântulas, redução de reservas, rendimento relativo de matéria seca, taxa de redução de reservas e eficiência de conversão das reservas de sementes em matéria seca de plântulas. Há redução da qualidade de sementes e desenvolvimento das plântulas em condições de déficit hídrico. No tratamento com Fipronil + Piraclostrobina + Tiofanato-metílico a fitotoxidez é menor. O peso de matéria seca estimada de cotilédones (CDM) é um indicativo para fitotoxidez e déficit hídrico. A mobilização das reservas é comprometida em sementes tratadas com inseticidas diminuindo a capacidade de convertê-las em massa seca. Há uma relação inversamente proporcional entre a redução e a eficiência de conversão das reservas em sementes de soja tratadas sob déficit hídrico.

Termos para indexação: *Glycine max* (L) Merrill, potencial osmótico, qualidade de sementes, tratamento de sementes, translocação de reservas.

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INTRODUCTION

In economic terms, soybean (*Glycine max* (L) Merrill) is increasingly consolidated as one of the main commodities of the world and is one of the main products of Brazilian agriculture (Bianchi et al., 2022). Soybean production is prominent among the economic activities of agribusiness and is stimulated by the increase in global population, along with the multiple uses of the crop: for human consumption, animal consumption, and biodiesel production (Wu et al., 2018; França-Silva et al., 2023).

Biotic and abiotic stresses can negatively affect soybean yield (Brzezinski et al., 2015). Biotic stresses, such as pests and diseases, can lead to reduction in germination percentage and hurt initial establishment of plants, resulting in yield losses (Silva et al., 2019). Abiotic stresses, such as lack of water, high and low temperatures, and restricted photoperiod, bring about significant changes in the soybean cycle (Rodrigues et al., 2001).

Seed treatment with chemical phytosanitary products aims at reducing the impacts caused by biotic stresses (Soares et al., 2019) and consists of application of chemical or biological compounds on seeds (ABRASEM, 2015). The application of chemical products, above all fungicides and insecticides, in different situations and with different objectives has helped to select the best products, combinations, doses, and soybean genotypes regarding seed quality (Brzezinski et al., 2015; Ferreira et al., 2016; Santos et al., 2018; Carvalho et al., 2020; Medeiros et al., 2023). Nevertheless, it is important to emphasize that some chemical groups can cause phytotoxic effects in diverse circumstances (Carvalho et al., 2020), and information is limited on how these products respond in situations of water deficit and how they can cause reduction in seed physiological quality (Lacerda et al., 2021).

Water deficit is considered the great challenge of current agriculture since, in many situations, sowing occurs under inadequate soil moisture conditions, aiming at sowing a second crop in Brazil (Munns and Tester, 2008; Ferrari et al., 2015). Water plays a fundamental role in germination, triggering chemical reactions and metabolic processes for digestion of reserves and mobilization of these reserves for the embryo (Corte et al., 2006; Taiz et al., 2017). Low availability of water in imbibition affects the germination process and, therefore, simulation of water deficit in the germination test is appropriate for representing water available to the seed (Barbero et al., 2011). That way, it is also possible to observe the responses of germination and of mobilization of reserves under water deficit conditions, simulated for example, by a polyethylene glycol 6000 (PEG 6000) solution (Villela et al., 1991; Pereira et al., 2020).

Mobilization of reserves is considered the second step of germination, where the substances acquired during seed formation are directed to the development of structural components of the seedling (Corte et al., 2006). First, the embryo initiates the germination process with its own reserves, but maintaining this process will depend on the flow of soluble components from its reserves to the regions in development (Henning et al., 2010). For that reason, the properties related to the dynamics of the seed reserves are considered potential characteristics for evaluation of seed lots that show greater tolerance to stress conditions (Dantas et al., 2017).

Although chemical treatment with phytosanitary products is a considerably consolidated practice for soybean seeds, it is important to obtain information regarding its effect on seed physiological quality, on seedling development, and on mobilization of reserves under water deficit conditions.

Under these considerations, the aim of this study was to evaluate the physiological quality, the initial seedling development, and the mobilization of reserves in soybean seeds treated with phytosanitary products under water deficit conditions.

MATERIAL AND METHODS

The experiment was conducted in the Seed Laboratory of the *Universidade Federal de Uberlândia* (UFU), in the state of Minas Gerais, Brazil. Seeds of the soybean cultivar Brasmax Desafio RR - 8473 RSF were used. First, the seeds were homogenized in an 18-channel divider. After that, the seeds were initially characterized so as to evaluate physical and physiological quality using the following tests: 1000-seed weight (g), moisture content (%), and germination (%) according to the Rules for Seed Testing (*Regras para Análises de Sementes – RAS*) (Brasil, 2009); strong normal seedlings (Krzyzanowski et al., 2020); and electrical conductivity ($\mu S.cm^{-1}.g^{-1}$) (Vieira and Marcos-Filho, 2020).

After initial characterization, the trial involving chemical treatment of seeds under water deficit was conducted in a completely randomized design (CRD), with the statistical treatments distributed in a 4 × 4 factorial arrangement consisting of four chemical treatments of seeds [control (water), Thiamethoxam, Fludioxonil + Metalaxyl-M, and Fipronil + Pyraclostrobin + Thiophanate-methyl] and four osmotic potentials (0, -0.1, -0.2, and -0.3 MPa), with four replications. To obtain the osmotic potentials, aqueous solutions were used, composed of deionized water and polyethylene glycol (PEG 6000) prepared according to the specifications proposed by Villela et al. (1991).

Seed chemical treatment was performed manually in plastic bags through constant and vigorous shaking until complete coverage of the seeds by the mixture. The volume of the mixture was standardized at 600 $mL.100\ kg^{-1}$ of seeds. The dose used to prepare the mixture followed the recommendations contained in the instructions for each product, along with distilled water in the amount equivalent to that lacking to complete the specified volume (Table 1). No polymer or drying agent was used.

After the treatment, the seeds remained three days under laboratory conditions for the phytosanitary products to be fixed to the seeds. After that, the tests began to be set up to evaluate the physiological quality of the seeds under water stress conditions. The following variables were analyzed:

Germination (%): 200 seeds from each chemical treatment were used, divided into four replications of 50 seeds and distributed on sheets of germination paper moistened with the polyethylene glycol (PEG 6000) solutions in an amount equivalent to 2.5 times the weight of the unmoistened paper, without later addition of the solution. The PEG 6000 solutions were prepared with the following osmotic potentials: 0 (distilled water), -0.1, -0.2, and -0.3 MPa. Then the sheets of germination paper were organized in roll form and placed in transparent plastic bags to prevent loss of water by evaporation and to ensure the desired osmotic potential. They were then placed to germinate in a Biological Oxygen Demand (B.O.D.) type chamber regulated to 25 °C under a 12-h photoperiod. On the eighth day, germinated seeds were counted, and the criterion used was that of normal seedlings (Brasil, 2009), with the results expressed in percentage.

Seedling length (cm): 20 soybean seeds treated with the respective phytosanitary products were placed in moistened germination paper, with the same osmotic potentials: 0 (distilled water), 0.1, 0.2, and -0.3 MPa. The seeds were arranged in two rows traced in a longitudinal direction containing 10 seeds and uniformly spaced to allow free development of the seedlings. Each treatment was composed of four replications. The rolls were placed in plastic bags and then placed in a Biological Oxygen Demand (B.O.D.) type chamber under the same conditions as in the germination test. On the fifth day, hypocotyl length, primary root length, and total normal seedling length were measured, with the aid of a millimeter ruler.

Table 1. Active ingredients, commercial products, classification, and application rates for soybean seed treatments.

Active ingredient (a.i.)	Trade name	Classification¹	Dose of the commercial product²	Amount of water³
Thiamethoxam	Cruiser 350 FS	I	300	300
Fludioxonil + Metalaxyl-M	Maxim XL	F + F	100	500
Fipronil + Pyraclostrobin + Thiophanate-methyl	Standak Top	I + F + F	200	400
Control	-	-	-	600

Classification¹: I: Insecticide; F: Fungicide; Dose of the commercial product²: $mL.100\ Kg^{-1}$ of seed; Amount of water³: $mL.100\ Kg^{-1}$ of seed; Total volume: 600 $mL.100\ Kg^{-1}$ of seed.

Seedling length vigor index (SLVi): this was determined for each treatment using the equation proposed by Abdul-Baki and Anderson (1973):

$$SLVi = seedling\ length\ (cm)\ \times germination\ (\%)$$

In regard to mobilization of reserves, four replications of 50 seeds were weighed to estimate the total seed reserves. Moisture content of the seeds was then determined by placing them in a laboratory oven regulated to 105 °C for a period of 24 hours, obtaining constant weight. They were then removed and once more weighed, and then discarded. After that, four replications of ten seeds were placed to germinate under the same conditions as the germination test. The seeds were distributed longitudinally at the upper third of the germination paper, with their micropyles turned in the direction of the paper base (Pereira et al., 2015). On the eighth day, the radicle, the hypocotyl, and the cotyledons were separated and dried in a forced air circulation oven at 65 °C for 72 hours. After that, the vegetative material was weighed for evaluation of the dry matter of the radicle, hypocotyl, and cotyledons; and the values obtained were later estimated for that equivalent to 50 seedlings (Pereira et al., 2015).

Mobilization of reserves was calculated based on the values of dry matter that were estimated, using the equations proposed by Pereira et al. (2015):

- Seed reserve reduction: $SRR\ (g) = SDM - CDM$
- Relative dry matter yield: $RDMY\ (g) = \frac{SDDM}{10}$
- Seed reserve reduction rate: $SRRR\ (\%) = \left(\frac{SRR}{SDM}\right) \times 100$
- Conversion efficiency of seed reserves into seedling dry matter: $CESR = \frac{SDDM}{SRR} \times 100$

Where:

SRR: seed reserve reduction for 50 seeds; SDM: seed dry matter estimated for 50 seeds; CDM: weight of dry matter estimated for 50 pairs of cotyledons; RDMY: relative dry matter yield; SDDM: dry matter yield estimated for 50 seedlings; SRRR: seed reserve reduction rate; CESR = conversion efficiency of seed reserves into seedling dry matter (%).

Analysis of variance by the F test was used on the data, and the means were clustered by the Scott-Knott test at 5% probability. Statistical analyses were carried out with the SISVAR statistical software (Ferreira, 2011). In addition, multivariate principal component analysis (PCA) was carried out, as well as Pearson correlation, for all the traits evaluated. The R statistical software was used in all the analyses (R Core Team, 2022).

RESULTS AND DISCUSSION

In relation to initial physiological quality, the soybean seeds met the minimum standard for seed commercialization (80%) established by legislation (Brasil, 2013) (Table 2). High seed quality is extremely important for reliability and consistency of results. The seeds have a 1000-seed weight (1000SW) of 195 grams, 11% moisture content, 94% germination, high vigor of strong normal seedlings (86%), and electrical conductivity of 66 $\mu S.cm^{-1}.g^{-1}$.

At the 0 MPa potential, the chemical treatments did not have an effect on germination. Germination of the soybean seeds was negatively affected by the reduction in osmotic potential, regardless of the seed treatment (Figure 1).

Table 2. Physiological characterization of seed of the soybean cultivar Brasmax Desafio RR - 8473 RSF by first germination count (FGC), final germination count (G), percentage of strong normal seedlings (SNS), and electrical conductivity (EC).

1000-seed weight (1000SW) (g)	Moisture content (%)	FGC (%)	G (%)	SNS (%)	EC ($\mu S.cm^{-1}.g^{-1}$)
195	11.0	94	94	86	66

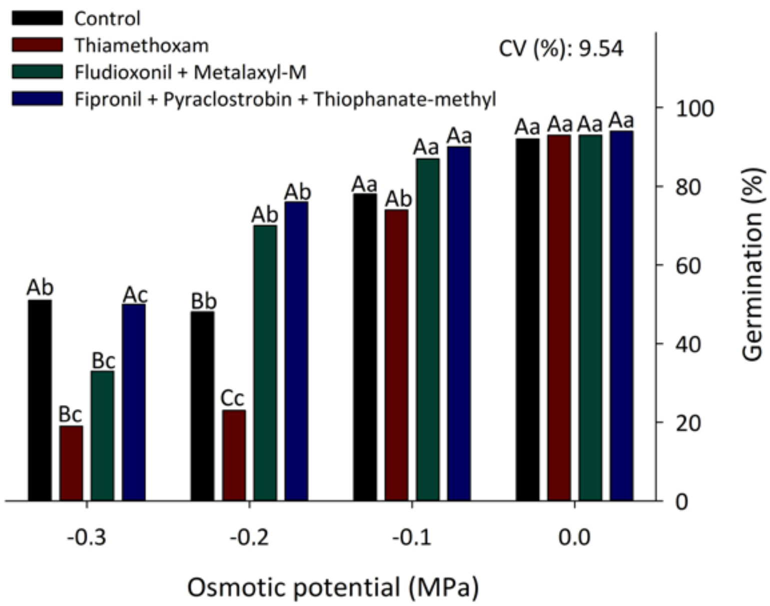

Figure 1. Germination (%) of treated soybean seeds, cultivar Brasmax Desafio RR - 8473 RSF, under water deficit at different osmotic potentials. Bars with the same uppercase letter among the seed treatments and the same lowercase letter among the osmotic potentials do not differ statistically from each other by the Scott-Knott test at 5% probability.

These results are in agreement with those obtained by other authors studying the response of soybean seeds under water stress (Pereira et al. 2015; Soares et al. 2015). Water deficit also compromises seed germination in other crops, such as common bean (Paiva et al., 2018), peanut (Steiner et al., 2019), and crambe (Silva et al., 2019).

Reduction in seed germination under low osmotic potentials is due to reduction in the availability of water necessary for activation and maintenance of seed metabolism (Bewley et al., 2013). Another factor that can also explain this reduction in germination is the high molecular weight of the polyethylene glycol (PEG 6000), which is not absorbed, due to its high viscosity, compromising the availability of oxygen to the seeds during the germination process (Braccini et al., 1996).

In treatment of seeds with the insecticide Thiamethoxam, there was lower germination percentage at the potential of -0.2 MPa compared to the other potentials (Figure 1). In addition, in the seed treatment with Thiamethoxam, as the osmotic potential decreased, germination decreased as of the potential of -0.1 MPa. These results suggest that this insecticide molecule may have caused toxicity to the soybean seeds under conditions of negative osmotic potentials. Beneficial physiological effects through seed treatment with Thiamethoxam were reported by Castro and Pereira (2008) and Rocha et al. (2020). These authors observed that the treatment of soybean seeds with insecticide molecules affects germination, and that insecticide molecules have greater toxicity than fungicide molecules.

In seed treatment with Fludioxonil + Metalaxyl-M and Fipronil + Pyraclostrobin + Thiophanate-methyl, there was less reduction in germination at -0.2 MPa (Figure 1). Seed treatment with Fipronil + Pyraclostrobin + Thiophanate-methyl proves to be less toxic under water stress conditions in soybean seeds, not differing from the control treatment at the potential of -0.3 MPa. Balardin et al. (2011) reported that the treatment with Fipronil + Pyraclostrobin + Thiophanate-methyl increased germination and seedling emergence of soybean under water deficit, showing that the combination of insecticide/fungicide is vital for enhancing the benefits brought about by seed treatment. Brzezinski et al. (2015) observed that chemical treatments tested containing fungicides and insecticides in combination (such as abamectin + thiamethoxam + fludioxonil + mefenoxam + tiabendazol) favor crop establishment; however, they do not change the yield performance of soybean.

There was a statistical difference for hypocotyl length, primary root length, total seedling length, and seedling length vigor index when the seeds were under different osmotic potentials (Figure 2).

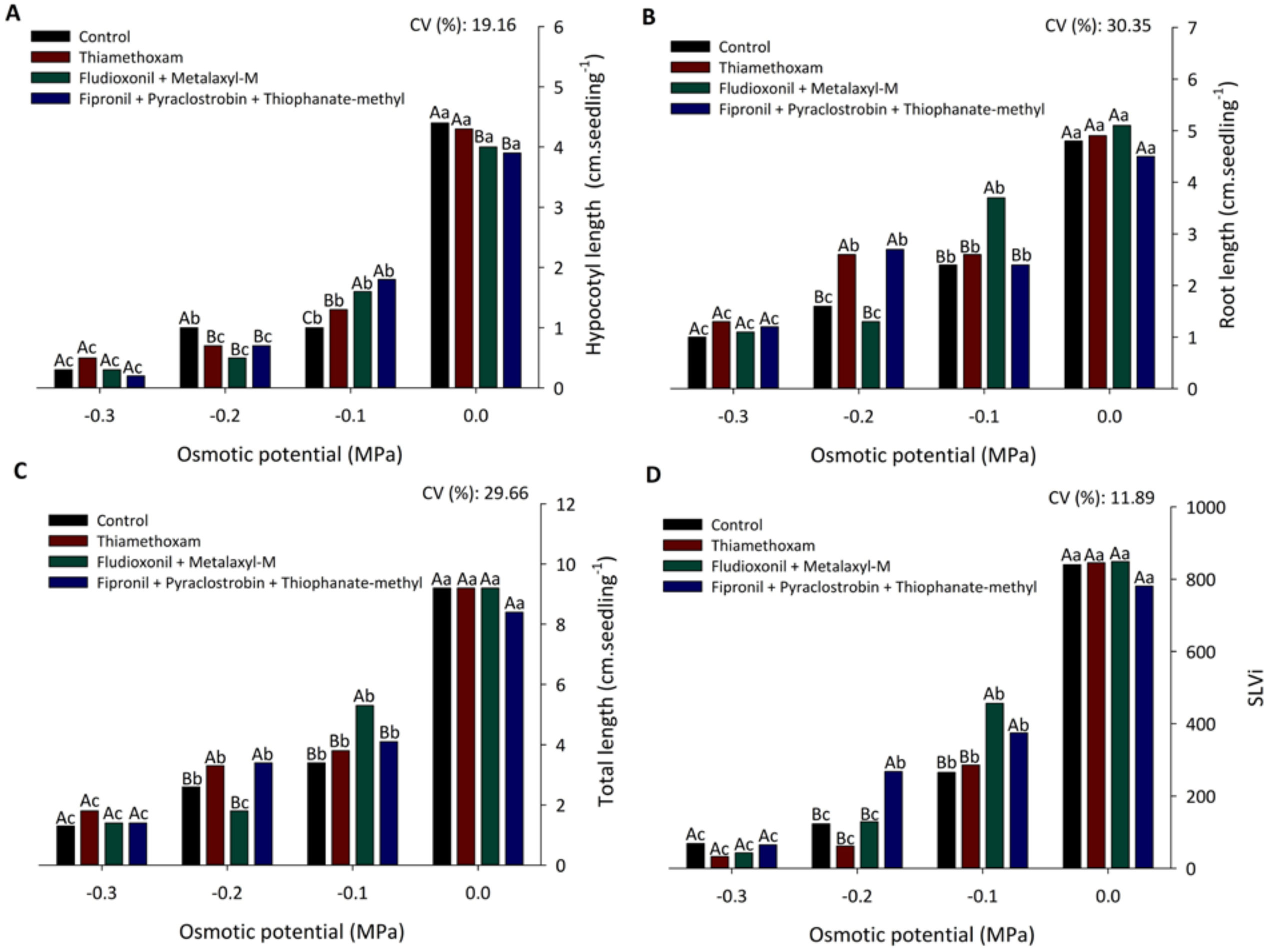

Figure 2. Hypocotyl length (A), root length (B), and total length (C) of seedlings and seedling length vigor index (SLVi) (D) of soybean, cultivar Brasmax Desafio RR - 8473 RSF, coming from seeds under water deficit at different osmotic potentials. Mean values followed by the same uppercase letter among the seed treatments and by the same lowercase letter among the osmotic potentials do not differ statistically from each other by the Scott-Knott test at 5% probability.

At the potential of 0 MPa, the seedlings showed better performance (hypocotyl length, primary root length, and total seedling length) in relation to the other potentials (Figures 2A, 2B, and 2C). As the osmotic potential decreased, seedling performance or performance of their parts declined, which reinforces that even under moderate water deficit conditions, there is reduction in seedling development (Bewley et al., 2013).

Taiz and Zeiger (2013) reported that reduction in seedling growth is the first factor that can be measured under negative osmotic potential conditions. As reported, seedling growth is hindered under water stress conditions (Ionov et al., 2013). According to Medeiros et al. (2015), reduction in seedling length occurs due to changes in cell turgidity caused by reduction in protein synthesis under water stress conditions. Upon reducing turgor pressure, lower water availability suppresses cell expansion and growth, affecting metabolism (Bewley et al., 2013).

The seedlings originating from seeds treated with Fludioxonil + Metalaxyl-M and Fipronil + Pyraclostrobin + Thiophanate-methyl had shorter hypocotyl length at the potential of 0 MPa (Figure 2). Nevertheless, the root length and total seedling length was greater when the seeds were treated with Fludioxonil + Metalaxyl-M (-0.1 MPa), Thiamethoxam, and Fipronil + Pyraclostrobin + Thiophanate-methyl (-0.2 MPa) under water deficit. At the potential of -0.3 MPa, the treatments did not differ statistically. Balardin et al. (2011) reported that in the presence of water stress, seed treatment with Fipronil + Thiophanate-methyl + Pyraclostrobin brought about a significant increase in plant stature, root length, root volume, cotyledon dry matter, root dry matter, and leaf area. Carvalho et al. (2020) report phytotoxicity caused by soybean seed treatment is clearly shown by the root length trait. In that respect, differences can be observed in the root length of seedlings coming from seeds treated with different products, above all under water deficit (-0.1, -0.2, and -0.3 MPa) (Figure 2B).

The seedling length vigor index significantly declined through exposure to water stress for all the seed treatments (Figure 2D). These results show the negative effect that water shortage leads to in soybean seedling length, as observed in the shorter length of the hypocotyl and of the primary root (Figures 2A and 2B). Similar results were obtained by Steiner et al. (2019) when they investigated the effects of salinity and of water deficit on the quality of peanut seeds of different sizes and on the initial performance of seedlings.

Regardless of the chemical treatment used, there was a decrease in the seedling length vigor index (SLVi) (Figure 2D). Obviously, the best results of this index were obtained at the osmotic potential of 0 MPa. However, it is noteworthy that the active ingredients Fludioxonil + Metalaxyl-M and Fipronil + Pyraclostrobin + Thiophanate-methyl maintained a higher vigor index and greater seedling length than the other treatments did. A better result was also found at the potential of 0.2 MPa for Fipronil + Pyraclostrobin + Thiophanate-methyl. At the potential of -0.3 MPa, all the indices were statistically equal for all the products used. We reiterate that better results appear in the use of combinations (insecticide + fungicide or insecticide + fungicide + nematicide) in seed treatment.

In relation to mobilization of reserves, there was significant interaction between the soybean seed treatment and the water stress potentials. The results of cotyledon dry matter (CDM) obtained after germination show greater reallocation of reserves at the potential of 0 MPa than at the other potentials, regardless of the seed treatment used (Figure 3A). At this potential (0 MPa), the untreated seeds and the seeds treated with Thiamethoxam had greater reallocation of reserves. At the osmotic potential of -0.2 MPa, the treatment with Thiamethoxam has a greater amount of the reserves in the cotyledons, resulting from water stress and possibly the effect of phytotoxicity. At the osmotic potential of -0.3 MPa, the seed treatment with Fludioxonil + Metalaxyl-M translocated more reserves to the seedlings; however, there was greater accumulation of reserves compared to the accumulations at the other potentials (0 and -0.2 MPa).

Seedling dry matter yield (SDDM) was greater at the potential of 0 MPa, a potential at which seed quality was not compromised. At the osmotic potentials of -0.2 MPa and -0.3 MPa, there was lower relative yield for the seeds treated with Thiamethoxam (Figure 3B). In the seeds of the control and Fludioxonil + Metalaxyl-M treatments, there was greater dry matter yield at the most negative osmotic potential. Pereira et al. (2015) found that there is correlation between CDM and SDDM because lower dry matter weights in the cotyledons were positively reflected in the dry matter yield for seedling establishment.

Seed reserve reduction (SRR) declined with the reduction in osmotic potential (Figure 3C). At the potential of 0 MPa, there was greater reserve reduction compared to the other seed treatments. At the potentials of -0.1 MPa and -0.2 MPa, the control treatment also differs statistically from the others. This may have occurred as a result of phytotoxicity at negative osmotic potentials. The treatment with Thiamethoxam exhibited lower performance than the other treatments at the osmotic potential of -0.3 MPa, similar to what can also be observed in Figure 3D for relative dry matter yield (RDMY). With more severe water deficit (-0.3 MPa), seeds treated with Fludioxonil + Metalaxyl-M have greater seed reserve reduction (SRR) (Figure 3C), as well as greater relative dry matter yield (RDMY) (Figure 3D). Seed reserve reduction (SRR) is positively correlated with seed dry matter (SDM), because seeds with more dry matter have more reserves to be mobilized (Pereira et al., 2015). Soltani et al. (2006) also reported this and furthermore found that this is connected with the initial quality of the seeds and with efficiency in converting mobilized reserves.

Pereira et al. (2015) and Oliveira et al. (2020) reported that the relative dry matter yield is directly related to the availability of reserves. Thus, seeds with greater initial size and weight could have a greater quantity of reserves for seedling development (Pádua et al., 2010; Pereira et al., 2013) and, moreover, exhibit greater efficiency in conversion of energy released by respiration.

Nevertheless, it should be emphasized that in the same stress situation, vigorous seeds have a higher respiratory

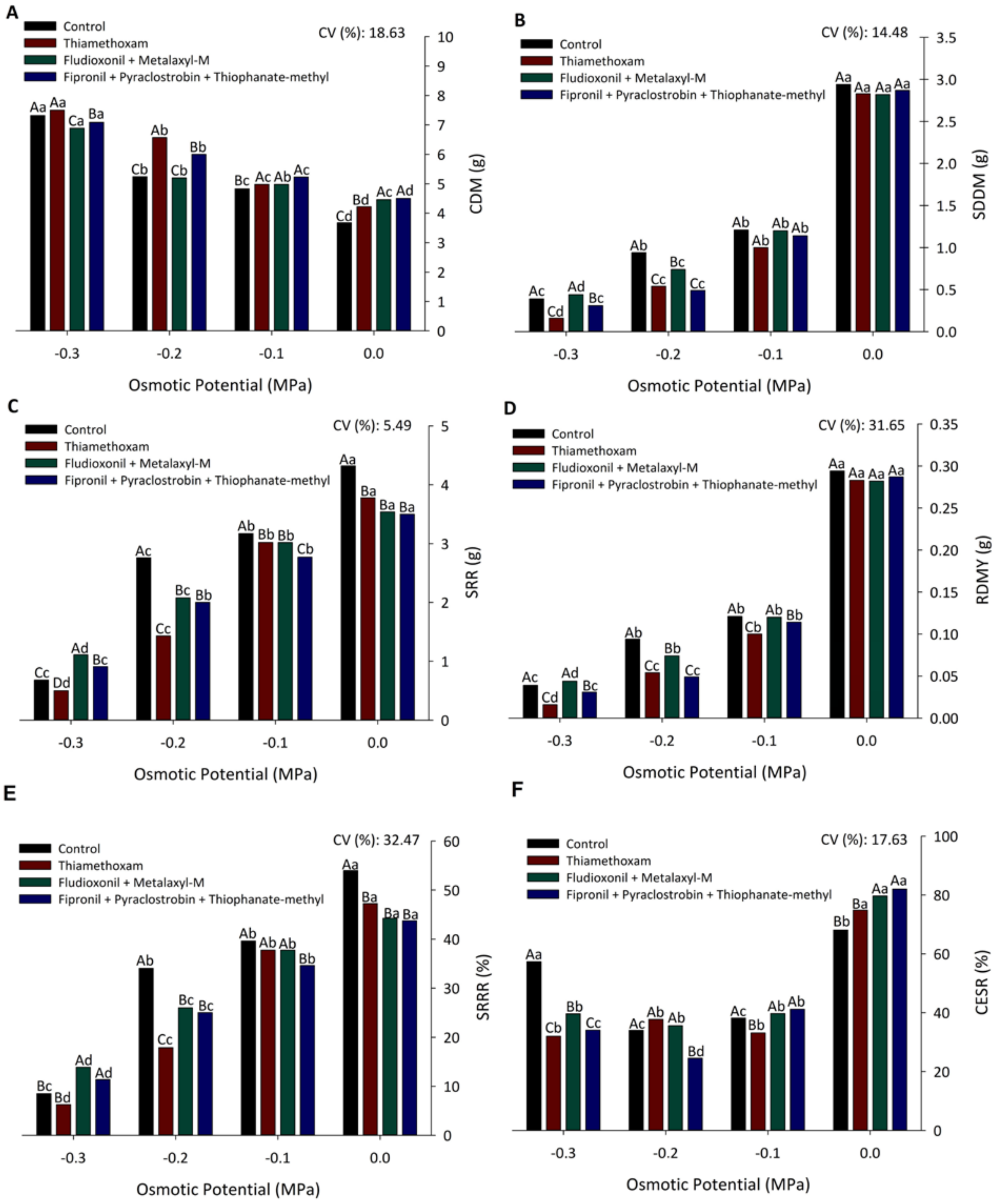

Figure 3. Estimated cotyledon dry matter (CDM) weight (in g) (A), seedling dry matter yield (SDDM) (in g) (B), seed reserve reduction (SRR) (C), relative dry matter yield (RDMY) (D), seed reserve reduction rate (SRRR) (E), and conversion efficiency of seed reserves (CESR) resulting from treated soybean seeds, cultivar Brasmax Desafio RR – 8473 RSF, under water deficit at different osmotic potentials. Mean values followed by the same uppercase letter within the osmotic potentials and by the same lowercase letter among the seed treatments of each potential do not differ statistically from each other by the Scott-Knott test at 5% probability.

rate than less vigorous seeds (Dranski et al., 2013; Venske et al., 2014; Santos et al., 2016). Thus, a seedling coming from a large seed with low mobilization of reserves is similar to a seedling from a small seed with high mobilization of reserves (Pereira et al., 2015). Furthermore, Dantas et al. (2017) reported that there is no correlation between the size of the seed and the characteristics related to reserve dynamics, not affecting the vigor.

The seed reserve reduction rate (SRRR) is shown in Figure 3E. At the osmotic potential of 0 MPa, the control treatment differed from the other treatments, just as at the osmotic potential of 0.2 MPa. Seed treatments with Fludioxonil + Metalaxyl-M and Fipronil + Pyraclostrobin + Thiophanate-methyl were better than the others at the osmotic potential of -0.3 MPa, with higher seed reserve reduction rates (SRRR). In the seed treatment with Thiamethoxam, there was a lower seed reserve reduction rate at the potentials of -0.2 MPa and -0.3 MPa. In general, as the osmotic potential declined, the seed reserve reduction rate decreased.

In relation to conversion efficiency of soybean seed reserves into seedling dry matter (CESR) (Figure 3F), at the osmotic potential of -0.3 MPa, there was greater efficiency of conversion of reserves than at the potentials of -0.1 and -0.2 MPa. Moreover, the conversion efficiency of seed reserves was compromised in the treatment with Thiamethoxam at the more negative osmotic potentials.

Pereira et al. (2015) reported that there is no positive and direct correlation between the seed reserve reduction rate (SRRR) and the conversion efficiency of seed reserves into seedling dry matter (CESR) in soybean seeds. Therefore, in more pronounced water deficit, there was an antagonistic relationship between the CESR and SRRR (Figures 3E and 3F). In this context, even if there is a low reserve translocation rate, there is efficiency in its conversion. Soltani et al. (2006) found in wheat seedlings under water and salt stress that the efficiency of reserve use is a conservative trait that does not result in decline in seedling dry weight, but rather in decline in the percentage of depletion of seed reserves. Therefore, the weight of the mobilized reserves of the seeds is the key to seedling development.

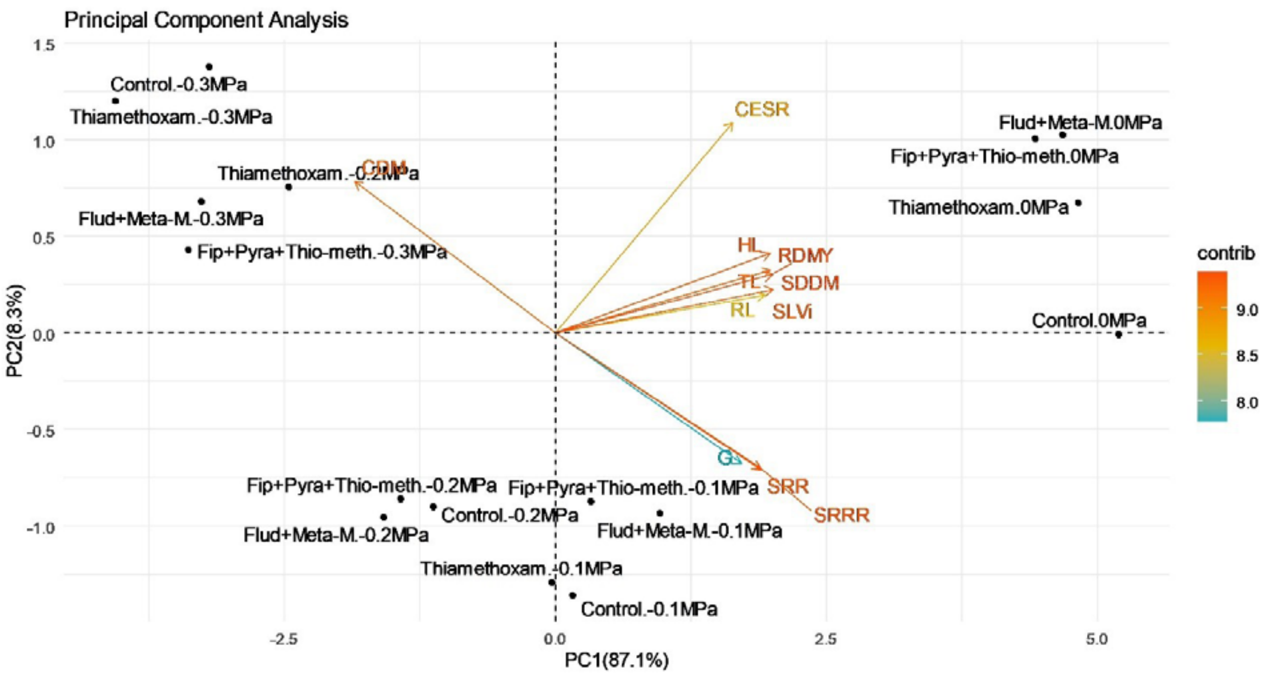

Figure 4. Principal component analysis (PCA) obtained by linear combination of the variables analyzed in soybean seeds under different chemical control and water deficit treatments. Principal component 1 (PC1), principal component 2 (PC2), hypocotyl length (HL), root length (RL), total seedling length (TL), seedling length vigor index (SLVi), estimated cotyledon dry matter weight (CDM), seedling dry matter yield (SDDM), seed reserve reduction (SRR), relative dry matter yield (RDMY), seed reserve reduction rate (SRRR), conversion efficiency of seed reserves (CESR).

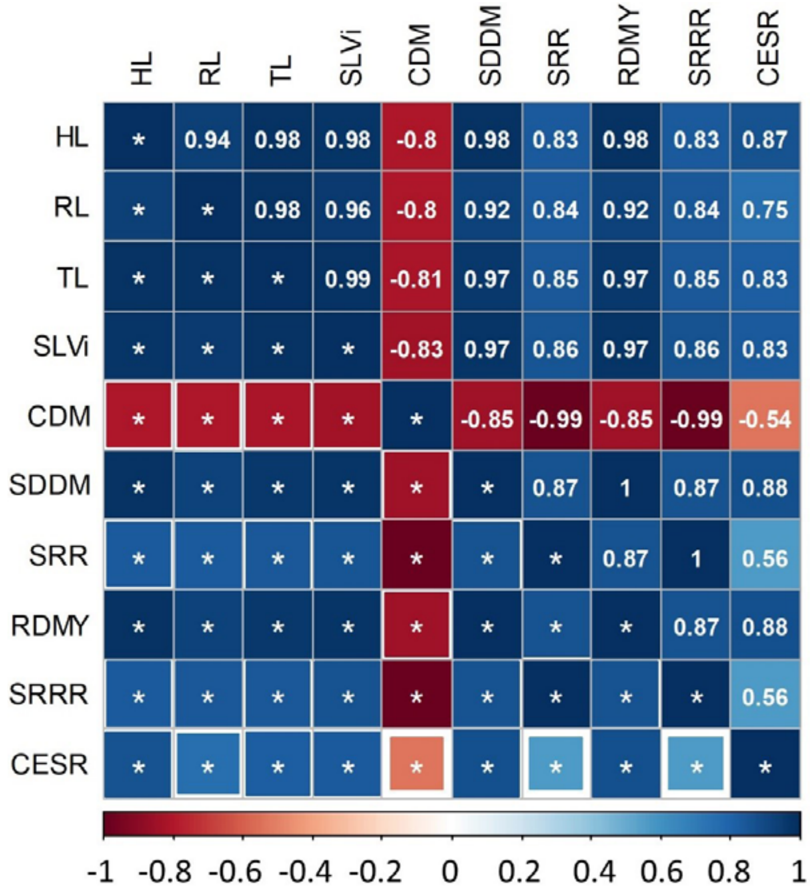

Figure 5. Pearson correlation (r) between all the variables analyzed in soybean seeds under different chemical control and levels of water deficit treatments. Hypocotyl length (HL), root length (RL), total seedling length (TL), seedling length vigor index (SLVi), estimated cotyledon dry matter weight (CDM), seedling dry matter yield (SDDM), seed reserve reduction (SRR), relative dry matter yield (RDMY), seed reserve reduction rate (SRRR), conversion efficiency of seed reserves (CESR). Quadrants marked with * represent significant correlation at 5% probability by the *t*-test.

By principal component analysis (PCA), component 1 (PC1) explained 87.1% of the variability, and component 2 (PC2) explained 8.3 % of the variability, for a total of 95.4% (Figure 4).

The variables CESR, HL, RL, TL, RDMY, SDDM, SLVi, G, SRRR, and SRR have positions in the positive scores of PC1, near the treatments without water deficit (0 MPa) or with moderate water deficit (-0.1 MPa). In contrast, the variable CDM is located in the negative scores of PC1, near the treatments with more severe water deficit (-0.2 MPa and -0.3 MPa). In relation to the chemical treatments, the treatment of the combination Fipronil + Pyraclostrobin + Thiophanate-methyl for seeds under water deficit (especially -0.2 and -0.3 MPa) is located nearer the vectors of physiological quality (such as germination). That indicates lower phytotoxicity to seeds under these conditions. In general, Pearson correlation confirmed the results previously observed in the means tests and in PCA, indicating negative and significant correlations of CDM with all the other variables (Figure 5). All of this reinforces the importance of evaluating CDM when considering both the effects of water deficit and the effects of toxicity by chemical treatment.

In short, as previously discussed, the results indicate that the lower the cotyledon dry matter, the greater the germination and vigor of the seedlings, due to greater translocation of the reserves in the treated soybean seeds.

CONCLUSIONS

Seed quality and seedling development decline in soybean under water deficit conditions. Phytotoxicity is lower in the treatment with Fipronil + Pyraclostrobin + Thiophanate-methyl than in the other treatments. Estimated cotyledon dry matter (CDM) weight is an indicator of phytotoxicity and water deficit. Mobilization of reserves is compromised in seeds treated with insecticides, as such treatments reduce the ability of converting reserves into dry matter. There is an inversely proportional relationship between the reduction in conversion of reserves and the efficiency of conversion of reserves in treated soybean seeds under water deficit.

REFERENCES

ABDUL-BAKI, A.A.; ANDERSON, J.D. Vigor determination in soybean seed by multiple criteria. *Crop Science*, v.13, n.6, p.630-633, 1973. https://doi.org/10.2135/cropsci1973.0011183X001300060013x

ABRASEM. Associação Brasileira de Sementes e Mudas. *Guia ABRASEM de Boas Práticas de Tratamento de Sementes*. 2015.

BALARDIN, R.S.; SILVA, F.D.L.; DEBONA, D.; CORTE, G.D.; FAVERA, D.D.; TORMEN, N.R. Tratamento de sementes com fungicidas e inseticidas como redutores dos efeitos do estresse hídrico em plantas de soja. *Ciência Rural*, v.41, n.7, p.1120-1126, 2011. http://www.scielo.br/pdf/cr/v41n7/a5711cr4207.pdf

BARBERO, A.P.P.; BARROS, F.; SILVA, E.A.; SUZUKI, R.M. Influência do déficit hídrico na germinação de sementes e no desenvolvimento inicial de três espécies de *Pleurothallidinae* (Orchidaceae). *Brazilian Journal of Botany,* v.34, n.4, p.593-601, 2011. https://doi.org/10.1590/S0100-84042011000400012

BEWLEY, J.D.; BRADFORD, K.; HILHORST, H. *Seeds: physiology of development, germination and dormancy*. New York: Springer Science & Business Media, 2013. 392p.

BIANCHI, M.C.; VILELA, N.J.D.; CARVALHO, E.R.; PIRES, R.M.O.; SANTOS, H.O.D.; BRUZI, A.T. Soybean seed size: how does it affect crop development and physiological seed quality? *Journal of Seed Science*, v.44, e202244010, 2022. https://doi.org/10.1590/2317-1545v44255400

BRACCINI, A.L.; RUIZ, H.A.; BRACCINI, M.C.L.; REIS, M.S. Germinação e vigor de sementes de soja sob estresse hídrico induzido por soluções de cloreto de sódio, manitol e polietileno glicol. *Revista Brasileira de Sementes*, v.18, n.1, p. 10-16, 1996.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Instrução Normativa nº 45, de 17 de setembro de 2013*. Padrões para a produção e a comercialização de sementes. Brasília (DF): Diário Oficial da União, 2013.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para Análise de Sementes*. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS, 2009. 399p. http:// www.agricultura.gov.br/arq_editor/file/2946_regras_analise__sementes.pdf

BRZEZINSKI, C.R.; HENNING, A.A.; ABATI, J.; HENNING, F.A.; FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; ZUCARELI, C. Épocas de tratamento de sementes no estabelecimento e desempenho produtivo da cultura da soja. *Journal of Seed Science*, v.37, n.2, p.147-153, 2015. https://doi.org/10.1590/2317-1545v37n2148363

CARVALHO, E.R.; ROCHA, D.K.; ANDRADE, D.B.D.; PIRES, R.M.O.; PENIDO, A.C.; REIS, L.V. Phytotoxicity in soybean seeds treated with phytosanitary products at different application times. *Journal of Seed Science*, v.42, e202042036, 2020. https://doi.org/10.1590/2317-1545v42237847

CASTRO, P.R.C.; PEREIRA, M.A. Bioativadores na agricultura. In: GAZZONI, D.L. (Ed.). *Tiametoxam: uma revolução na agricultura brasileira*. Petrópolis: Vozes, 2008. p.118-126.

CORTE, V.B.C.; BORGES, E.E.L.; PONTES, C.A.; LEITE, I.T.A.; VENTRELLA, C.M.; MATHIAS, A.A. Mobilização de reservas durante a germinação das sementes e crescimento das plântulas de *Caesalpinia peltophoroides* Benth. (Leguminosae-Caesalpinoideae). *Revista Árvore,* v.30, n.6, p.941-949, 2006. https://doi.org/10.1590/S0100-67622006000600009

DANTAS, S.A.G.; SILVA, F.C.S.; SILVA, L.; SILVA, L. Strategy for selection of soybean genotypes tolerant to drought during germination. *Genetics and Molecular Research*, v.16, n.2, gmr16029654, 2017. http://dx.doi.org/10.4238/gmr16029654

DRANSKI, J.A.L.; PINTO-JUNIOR, A.S.; HERZOG, N.F.M.; MALAVASI, U.C.; MALAVASI, M.D.M.; GUIMARÃES, V.F. Vigor of canola seeds through quantification of CO_2 emission. *Ciência e Agrotecnologia*, v.37, n.3, p.229-236, 2013. https://doi.org/10.1590/S1413-70542013000300005

FERRARI, E.; PAZ, A.; SILVA, A.C. Déficit hídrico no metabolismo da soja em semeaduras antecipadas no Mato Grosso. *Revista Nativa*, v.3, p.67-77, 2015.

FERREIRA, D.F. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, v. 35, n. 6, p. 1039-1042, 2011.

FERREIRA, T.F.; OLIVEIRA, J.A.; CARVALHO, R.A.D.; RESENDE, L.S.; LOPES, C.G.M.; FERREIRA, V.D.F. Quality of soybean seeds treated with fungicides and insecticides before and after storage. *Journal of Seed Science*, v.38, n.4, p.278-286, 2016. https://doi.org/10.1590/2317-1545v38n4161760

FRANÇA-SILVA, F., GOMES-JUNIOR, F. G., REGO, C. H. Q., MARASSI, A. G.; TANNÚS, A. Advances in imaging technologies for soybean seed analysis. *Journal of Seed Science*, v.45, e202345022, 2023. https://doi.org/10.1590/2317-1545v45274098

HENNING, F.A.; MERTZ, L.M.; JACOB JUNIOR, E.A.; MACHADO, R.D.; FISS, G.; ZIMMER, P.D. Composição química e mobilização de reservas em sementes de soja de alto e baixo vigor. *Bragantia*, v.69, n.3, p.727-734, 2010. http://www.scielo.br/pdf/brag/v69n3/26.pdf

IONOV, M.; YULDASHEVA, N.; ULCHENKO, N.; GLUSHENKOVA, A. I.; HEUER, B. Growth, Development and Yield of *Crambe abyssinica* Under Saline Irrigation in the Greenhouse. *Journal of Agronomy and Crop Science*, v.199, p.331-339, 2013. https://doi.org/10.1111/jac.12027

KRZYZANOWSKI, F.C.; FRANÇA-NETO, J.B.; GOMES-JUNIOR, F.G.; NAKAGAWA, J. Testes de vigor baseados em desempenho de plântulas. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B.; MARCOS-FILHO, J. (Eds.) *Vigor de sementes: conceitos e testes*. Londrina: Abrates, 2020. p.79-140.

LACERDA, M.P.; UMBURANAS, R.C.; MARTINS, K.V.; RODRIGUES, M.A.T.; REICHARDT, K.; DOURADO-NETO, D. Vigor and oxidation reactions in soybean seedlings submitted to different seed chemical treatments. *Journal of Seed Science*, v.43, e202143012, 2021. https://doi.org/10.1590/2317-1545v43247033

MEDEIROS, D.S.; ALVES, E.U.; SENA, D.V.; SILVA, E.O.; ARAÚJO, L.R. Desempenho fisiológico de sementes de gergelim submetidas a estresse hídrico em diferentes temperaturas. *Semina: Ciências Agrárias*, v.36, p.3069-3076, 2015. https://doi.org/10.5433/1679-0359.2015v36n5p3069

MEDEIROS, J.C.; CARVALHO, E.R.; ANDRADE, D.B.D.; MORAES, L.F.D.S.; LIMA, J.M.E.; MASSA, M.A.F. Quality of corn seed industrial seed treatment (IST) and on-farm treatment (OFT) in Brazilian agribusiness. *Journal of Seed Science*, v.45, e202345017, 2023. https://doi.org/10.1590/2317-1545v45268856

MUNNS, R.; TESTER, M. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, v.59, n.1, p.651-681, 2008. https://doi.org/10.1146/annurev.arplant.59.032607.092911

OLIVEIRA, T.F.; SANTOS, H.O.; CARVALHO, R.A.; SILVA, H.W.; PIRES, R.M.O.; CARVALHO, E.R. Reserve mobilization in soybean seeds under water restriction after storage. *Journal of Seed Science*, v.42, e202042024. 2020. https://doi.org/10.1590/2317-1545v42231384

PÁDUA, G.P.; ZITO, R.K.; ARANTES, N.E.; FRANÇA-NETO, J.B. Influência do tamanho da semente na qualidade fisiológica e na produtividade da cultura da soja. *Revista Brasileira de Sementes*, v.32, n.3, p.9-16, 2010. https://doi.org/10.1590/S0101-31222010000300001

PAIVA, E.P.D.; SÁ, F.V.D.S., TORRES, S.B.; BRITO, M.E.; MOREIRA, R.C.; SILVA, L.D.A. Germination and tolerance of cowpea (*Vigna unguiculata*) cultivars to water stress. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.22, n.6, p.407-411, 2018. https://doi.org/10.1590/1807-1929/agriambi.v22n6p407-411

PEREIRA I.C.; CATÃO H.C.R.M.; CAIXETA F. Seed physiological quality and seedling growth of pea under water and salt stress. *Revista Brasileira de Engenharia Agrícola*, v.24, n.2, p.95-100, 2020. https://doi.org/10.1590/1807-1929/agriambi.v24n2p95-100

PEREIRA, W.A.; PEREIRA, S.M.A.; DIAS, D.C.F.S. Dynamics of reserves of soybean seeds during the development of seedlings of different commercial cultivars. *Journal of Seed Science*, v.37, n.1, p.63-69, 2015. https://doi.org/10.1590/2317-1545v37n1142202

PEREIRA, W.A.; PEREIRA, S.M.A.; DIAS, D.C.F.S. Influence of seed size and water restriction on germination of soybean seeds and on early development of seedlings. *Journal of Seed Science*, v.35, n.3, p.316-322, 2013. https://www.scielo.br/j/jss/a/BxsdR5j6HvTdFfvJJv6yCWF/?lang=en

R CORE TEAM. R: *A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. 2022. https://www.R-project.org/

ROCHA, D.K.; CARVALHO, E.R.; PIRES, R.M.O.; SANTOS, H.O.; PENIDO, A.C.; ANDRADE, D.B. Does the substrate affect the germination of soybean seeds treated with phytosanitary products? *Ciência e Agrotecnologia*, v.44, e020119, 2020. https://doi.org/10.1590/1413-7054202044020119

RODRIGUES, O.; DIDONET, A.D.; LHAMBY, J.C.B.; BERTAGNOLLI, P.F.; LUZ, J.S.D. Quantitative response of soybean flowering to temperature and photoperiod. *Pesquisa Agropecuária Brasileira*, v.3, n.36, p.431-437, 2001. https://doi.org/10.1590/S0100-204X2001000300006

SANTOS, H.O.; VON PINHO, I.V.; VON PINHO, É.V.R.; PIRES, R.M.O.; SILVA, V.F.; CARVALHO, M.L.M.; OLIVEIRA, R.M.E. Physiological quality of hybrid maize seeds through respiratory and enzymatic activities. *African Journal of Agricultural Research*, v.11, n.20, p.1879-1886, 2016. https://ninho.inca.gov.br/jspui/handle/123456789/6202

SANTOS, S.F.D.; CARVALHO, E.R.; ROCHA, D.K.; NASCIMENTO, R.M. Composition and volumes of slurry in soybean seeds treatment in the industry and physiological quality during storage. *Journal of Seed Science*, v.40, n.1, p.67-74, 2018. https://doi.org/10.1590/2317-1545v40n1185370

SILVA, M.F.D.; ARAÚJO, E.F.; SILVA, L. J.; AMARO, H.T.R.; DIAS, L.A.S.; DIAS, D.C.F.S. Tolerância do crambe (*Crambe abyssinica* Hochst) à salinidade e estresse hídrico durante a germinação das sementes e crescimento inicial das plântulas. *Ciência e Agrotecnologia*, v.43, e025418, 2019. https://doi.org/10.1590/1413-7054201943025418

SOARES, C.M.; LUDWIG, M.P.; ROTHER, C.M.S.; DECARLI, L. Seed quality and crop performance of soybeans submitted to different forms of treatment and seed size. *Journal of Seed Science*, v.41, n.1, p.69-75, 2019. https://doi.org/10.1590/2317-1545v41n1210486

SOARES, M.M.; SANTOS JUNIOR, H.C.; SIMÕES, M.G.; PAZZIN, D.; SILVA, L.J. Estresse hídrico e salino em sementes de soja classificadas em diferentes tamanhos. *Pesquisa Agropecuária Tropical*, v.45, n.4, p.370–378. 2015. https://doi.org/10.1590/1983-40632015v4535357

SOLTANI, A.; GHOLIPOOR, M.; ZEINALI, E. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environmental And Experimental Botany*, v.55, n.1-2, p.195-200, 2006. https://doi.org/10.1016/j.envexpbot.2004.10.012

STEINER, F.; ZUFFO, A.M.; ZOZ, T.; ZOZ, A.; ZOZ, J. Drought tolerance of wheat and black oat crops at early stages of seedling growth. *Revista de Ciências Agrárias*, v.40, n.3, p.576-586, 2017. https://doi.org/10.19084/RCA16118

TAIZ, L.; ZEIGER, E. *Fisiologia vegetal*. Porto Alegre: Artmed, 2013. 918p.

TAIZ, L.; ZEIGER, E.; MOLLER, I.M.; MURPHY, A. *Fisiologia e desenvolvimento vegetal*. Porto Alegre: Artmed, 2017. 858p.

VENSKE, E.; JÚNIOR, J.D.S.A.; SOUSA, A.D.M.; MARTINS, L.F.; MORAES, D.M. Atividade respiratória como teste de vigor em sementes de algodão. *Revista Brasileira de Ciências Agrárias*, v.9, n.2, p.174-179, 2014. https://doi.org/10.5039/agraria.v9i2a3518

VIEIRA, R.D.; MARCOS-FILHO, J. Teste de condutividade elétrica. In: KRZYZANOWSKI, F.C.; VIEIRA, R.D.; FRANÇA-NETO, J.B.; MARCOS-FILHO, J. (Eds.). *Vigor de sementes: conceitos e testes.* Londrina: ABRATES, 2020.

VILLELA, F.A.; FILHO, L.D.; SEQUEIRA, E.L. Tabela de potencial osmótico em função da concentração de polietilenoglicol 6.000 e da temperatura. *Pesquisa Agropecuária Brasileira*, v.26, p.1957-1968, 1991.

WU, W.; YU, Q.; YOU, L.; CHEN, K.; TANG, H.; LIU, J. *Global cropping intensity gaps: Increasing food production without cropland expansion*. Land Use Policy, 76, 2018. p.515-525.