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Storage of Soybean Seeds and Addition of Insecticide and Micronutrients

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Abstract

The objective of this work was to evaluate the effects on the physiological attributes of soybean seeds submitted to the seed treatment with addition of insecticide, polymers and micronutrients throughout the storage. The experimental design was completely randomized in a factorial scheme, with four seed treatments per two seasons of storage of the seeds. The analysis of variance revealed a significant interaction among seed treatments and storage times for both cultivars at 5% of probability, referring to the characteristics of shoot length (SL), primary root length (RL), shoot dry mass (SDM) and dry mass of the primary root (RDM) for the cultivar Fundacep 37 RR. Addition of seed treatments influences the physiological performance of seedlings originated from soybean seeds stored for 240 days. The shoot and primary root lenghts, and shoot dry mass express the isoenzyme esterase through the aerial part and primary root of the seedling, the malate dehydrogenase is expressed in the primary root while in the peroxidase it is evident in the shoot of the seedlings.

Keywords: soybean, physiological potention, maneagent crops

1. Introduction

The soybean (*Glycine max* (L.) Merrill) belongs to the Fabaceae family being characterized as one of the main oleaginous plants produced worldwide, due to its economic importance and nutritional quality coupled with high crude protein concentration (Follmann et al., 2014). In Brazil, it is evidenced as the most cultivated species in the most varied agricultural regions, which provided an increase of 12.18% in national production for the 2016/2017 crop season (Conab, 2017).

These increases are due to the technological advances and seed quality used that directly influence the performance and establishment of the plants in the field (Ferrari et al., 2014; Meira et al., 2016). However, before the seeds are used in the field, they are submitted to certain periods of storage where some peculiar situations the seeds have been treated and exposed to stresses, that will contribute to reduce or maintain the physiological quality of the seeds. Among these treatments, the use of insecticides conjugated to polymers, fungicides and micronutrients (Pereira et al., 2005; Karam et al., 2007; Carvalho et al., 2015; Zanatta et al., 2018) stands out.

The use of insecticides in the seed treatment in combination with polymers and micronutrients may increase seedling uniformity, change the field emergence, provide conditions for the seeds to express their maximum vigor (Follmann et al., 2014), on the other hand, some products used may compromise the emergence of seedlings (Souza et al., 2015; Szareski et al., 2015).

The use of insecticides in the seed treatment in combination with polymers and micronutrients may increase seedling uniformity, change the field emergence, provide conditions for the seeds to express their maximum vigor (Follmann et al., 2014), in contrast, some (Sauzar et al., 2005). In addition, the use of seedlings has been shown to increase the number of seedlings. In general, insecticides are used to control insect pests and exert bioactivities that benefit agronomic interest attributes as well as soybean yield (Pelegrin et al., 2016; Ferrari et al., 2015). However, seeds treated and stored for up to 120 days may reflect positively on the dry matter accumulation of the seedling and on the leaf area (Ludwig et al., 2015), in contrast, storage prolongation may negatively affect the physiological quality of the seeds (Dan, et al., 2010, Souza et al., 2015; Gabriel et al., 2018).

There are certain cases that require longer storage periods of treated seeds, this may result in effects on physiological attributes such as seedling dry mass and activity while, the possibility of performing seed treatment in advance may facilitate the organizational logistics of the seed processing unit. In this context, the objective of this work was to evaluate the effects on the physiological attributes of soybean seeds submitted to the seed treatment with addition of insecticide, polymers and micronutrients throughout the storage.

2. Material and Methods

The work was conducted at the Seed Analysis Laboratory of the Postgraduate Program in Seed Science and Technology of the Eliseu Maciel Agronomy College, Federal University of Pelotas, located in the municipality of Capão do Leão, RS, Brazil. The soybean seeds used came from the cultivars Fepagro 37 RR and Nidera 6411 RR, which obtained initial germination of 81 and 82%. The treatments used were:

T_1: seeds without treatment;

T_2: seeds treated with micronutrients (2 mL kg^{-1} of commercial product based on 6.7% copper, 3.2% molybdenum, 15% zinc and 9.4% manganese) + insecticide (*thiametoxam* at the dose of 350 g L kg^{-1} of seeds) + polymer (0.5 mL kg^{-1} of seeds);

T_3: seeds treated with insecticide (*Thiametoxam* at a dose of 350 g L kg^{-1} of seeds) + polymer (0.5 mL kg^{-1} of seeds);

T_4: seeds treated with micronutrients (2 mL kg^{-1} of commercial product based on 6.7% copper, 3.2% molybdenum, 15% zinc and 9.4% manganese) + polymer (0.5 mL kg^{-1} of seeds).

Seeds were treated using a spray volume of 6 ml kg^{-1} of seeds using commercial concentrations of each isolated formulation, the seeds were distributed in individual plastic bags for each treatment. After the homogenization the seeds were transferred to paper bags and kept at room temperature for 24 hours, the rest of the seeds were stored in paper bags under controlled conditions of medium air temperature (15 °C) and relative humidity (50%) for 360 days.

The primary root and aerial part lengths of the seedlings, dry mass of the primary root and aerial part of the seedlings, expression of the isoenzymes: esterase, glutamate oxaloacetate transaminase, malatodesidrogenase, and peroxidase were measured, being these measured 240 days after the treatment of the seeds, to obtain the characters of interest the following methodologies were followed:

The length of the primary root and aerial part of the seedlings were obtained by the measurement of 10 seedlings at the end of the germination test, where the length between the basal insertion of the primary root to the apex of the shoot was measured, while the primary root length was measured between the apical distance and radicle base, results expressed in millimeters (mm). Dry matter of the primary root and aerial part were obtained by measuring 10 seedlings at the end of the germination test, where the seedlings were conditioned in brown paper envelopes and subjected to drying in forced ventilation oven at 70 °C until constant mass, results expressed in milligrams (mg).

The determination of the isoenzymes was performed by measuring 10 seedlings at the end of the germination test at 8 days after sowing (Brasil, 2009). The expression of the isoenzymes: esterase, glutamate oxaloacetate transaminase, malatodesidrogenase and peroxidase were obtained by vertical electrophoresis in polyacrylamide gel (Malone et al., 2007). The seedlings were separately macerated in porcelain gral and kept in an ice bath, 200 mg of the macerate from each sample were transferred to microcentrifuge tubes and added with extraction solution (0.2M Lithium Borate at pH 8.3 + Tris Citrate + 0.2M at pH 8.3 + 0.15% of 2-mercaptoethanol) in the ratio 1:2 (*m/v*).

Electrophoresis was performed on 7% polyacrylamide gels, applying 20 µL of each sample, and the staining systems used are described by Scandálios (1969) and Alfenas (1998). The interpretation of the results of the isoenzymes was based on the visual analysis of the electrophoresis gels, where the presence or absence, as well as the intensity of each of the electrophoretic bands for each measured isoenzymatic system was considered.

The experimental design was completely randomized in a factorial scheme, with four seed treatments x two seasons of storage of the seeds. The effects were performed for each soybean cultivar separately and the treatments were arranged in four replicates. The data were submitted to analysis of variance where the interaction among seed treatments and storage times (zero and 12 months) at of 5% probability was verified, the characters that showed interaction were dismembered to the simple effects, on the other hand, those that did not show interaction were dismembered to the main effects for each factor separately using the complementary analyzes by the Tukey test at 5% of probability.

3. Results and Discussion

The analysis of variance revealed a significant interaction among seed treatments and storage times for both cultivars at 5% of probability, referring to the characteristics of shoot length (SL), primary root length (RL), shoot dry mass (SDM) and dry mass of the primary root (MR) for the cultivar Fundacep 37 RR. The initial growth of the soybean seedlings before storage (Zero) presented different results compared to the treatments of seeds tested for both soybean cultivars (Table 1), being proved by the performance of the characters length (SL) and shoot dry mass accumulation (SDM).

The primary root lenght (RL) showed superiority through the use of *Thiametoxam* and polymer referring to Fepagro 37 RR cultivar, this insecticide may have stimulated the initial growth of these seedlings (Almeida et al., 2012; Kavalco et al., 2015). In contrast, NS6411 RR showed better performance through the absence of seed treatments and the use of polymer and micronutrients (Table 1), where it obtained superiority for radicular length, in this context, the use of micronutrients and polymers with the seeds of soybean can maintain and even contribute to the enhancement of the physiological quality of soybean seeds (Bays et al., 2007). For both cultivars there was a decrease in the primary root lenght in the period after storage (12 months), these results corroborate with Dan et al. (2010) where they determined that the seed treatment reduces the length of seedlings after prolonged storage.

The shoot length (CPA) was superior for seedlings from seeds treated with *Thimetoxan* + micronutrients + polymers referring to cultivar Fepagro 37 RR, in contrast, the cultivar NS6411 RR was superior when the seeds were treated with micronutrients + polymers (Table 1). The differential performance between the cultivars is due to the metabolic responses intrinsic to the genetic and morphological constitution, as well as the contact surface of the plasma membranes that allow to increase or decrease the effects of the treatment on the seeds (Moterle et al., 2011; Szareski et al., 2016c).

Table 1. Influence of seed treatments and storage times on primary root (RL) ands hootlenghts (SL) of seedlings from soybean seeds (Fepagro37 RR (F.37) and NS6411RR (NS6411))

Treatments	F.37		NS.6411		F.37		NS.6411	
	Zero	12 months	Zero	12 months	Zero	12 months	Zero	12 months
	RL (mm)				SL (mm)			
NoTreat.	89.55cA*	90.75aA	103.03aA	54.50aB	101.28bA	109.12aA	85.88bA	63.50aB
M+T+P	98.30bA	84.67abB	86.08cA	66.83aB	109.10aA	111.83aA	80.50bA	77.58aB
T+P	103.48aA	63.86bB	95.15bA	66.98aB	103.88abA	90.08bB	83.38bA	63.67aB
M+P	97.15bA	85.67aA	101.50aA	63.67aB	99.00bA	107.92aA	101.08aA	72.83aB
CV	5.90		7.90		3.10		7.50	

Note. * Means followed by the same letter, lower case in the column and upper case in the row, do not statistically differ for Tukey with 5% of probability.

After the seed storage, the shoot (SL) and primary root lenght (RL) with the use of *Thimetoxan* + polymer showed inferiority to the other treatments for the cultivar Fepagro 37 RR, in contrast, the shoot length (SL) of cultivar NS6411 RR (Table 1) reduced its magnitude after storage. In this way, the use of insecticides (Dan et al., 2010; Piccinin et al., 2013; Szareski et al., 2016a), polymers (Avelar et al., 2011) with seed treatment that will be maintained for a long period may negatively influence the physiological potential of soybeans.

The dry mass of the primary root (MR) and shoot (SDM) did not differ faced to the seed treatments (Table 2) used for both cultivars under conditions of absence of storage (Zero). Research has shown that the use of insecticide and bioregulator does not result in accumulation of dry matter in the seedlings (Dan et al., 2012; Moterle et al., 2011; Zimmer et al., 2016). However, the cultivar Fepagro 37 RR reduced the dry mass of the primary root after storage of the seeds.

Table 2. Influence of seed treatments and storage times on dry mass of the primary root (RDM) and shoot dry mass (SDM) of the seedlings from soybean seeds (Fepagro37 RR (F.37) and NS6411 RR (NS6411))

Treatment	F.37		NS.6411		F.37		NS.6411	
	Zero	12 months	Zero	12 months	Zero	12 months	Zero	12 months
	RDM (mg)				SDM (mg)			
NoTreat.	25.54aA*	21.66aB	27.98	26.93	11.08aB	45.55aA	15.54aB	46.60aA
M+T+P	24.60aA	21.70aB	28.41	27.44	11.05aB	40.58cA	14.62aB	42.94aA
T+P	24.65aA	21.42aB	29.02	28.62	11.58aB	44.37abA	14.30aB	41.06aA
M+P	24.50aA	22.99aB	29.77	30.39	10.77aB	43.23bA	16.11aB	44.04aA
CV	6.30		4.90		3.40		6.80	

Note. *Means followed by the same letter, lower case in the column and upper case in the row, do not statistically differ for Tukey with 5% of probability.

The dry mass of the primary root (MR) and shoot (SDM) did not differ faced to the seed treatments (Table 2) used for both cultivars under conditions of absence of storage (Zero). Research has shown that the use of insecticide and bioregulator does not result in accumulation of dry matter in the seedlings (Dan et al., 2012; Moterle et al., 2011; Zimmer et al., 2016; Rigo et al., 2018). However, the cultivar Fepagro 37 RR reduced the dry mass of the primary root after storage of the seeds. According to Ludwig et al. (2011), the quality of the soybean seeds when stored and covered by polymers and insecticides do not result in differentiation regarding the accumulation of dry matter of the seedlings.

The shoot dry mass (SDM) of the cultivar Fepagro 37 RR in the absence of seed treatment and the seeds treated with *Thimetoxan* + polymers obtained superiority to the other treatments, in contrast, the cultivar NS6411 RR was not statistically different (Table 3). According to Dan et al. (2011), it is possible that there is no differentiation of biomass accumulation in the seedlings due to the seed treatment used. However, this character compared to storage times showed an increase in seedlings from seeds that were exposed to storage. This increase may be due to the consumption of the available energy in the cotyledons, when its accumulation is higher than the biomass production of the shoot and the primary root is increased (Henning et al., 2010, Nardino et al., 2016; Dellagostin et al., 2016; Vargas et al., 2018).

In relation to the enzymatic profiles, the isoenzymatic expression was differentiated for both cultivars and after storage of the seeds (Figure 1). Therefore, esterase expression (EST) evidenced two bands for the seedling shoot (SL) for Fepagro 37 RR cultivar, being these more intense for the treatment with *Thimetoxan* + polymers, and micronutrients + polymer, in contrast, for the primary root (RL), the treatment with *Thimetoxan* + polymers showed only a band with higher intensity. In relation to the cultivar NS6411 RR the enzyme expressed higher intensity for the treatment with *Thimetoxan* + polymer in two bands, both in the aerial as well as radicular parts (Figure 1a). This enzyme acts on lipid metabolism reactions and esters hydrolysis (Peske et al., 2012), controlled deterioration (Padilha et al., 2001; Dubal et al., 2016), which culminate in the reduction of the physiological quality of the seeds.

Glutamate oxaloacetate transaminase (GOT) did not show intensity variation for both shoot (SL) and root (RL) bands for both cultivars (Figure 1b). The increase in metabolic activity may lead to deterioration due to the increase in the expression of the enzyme glutamate oxaloacetate transaminase, which is responsible for the oxidation of amino acids, reduction of α-ketoglutarate for the synthesis of new amino acids, minimizing the energy supply for the Krebs cycle and to the developing embryo (Tunes et al., 2010; Strobel et al., 2016).

The expression of malate dehydrogenase (MDH) showed only one band for both cultivars (Figure 1c) in both shoot (SL) and root (RL). This enzyme acts on the cellular respiratory processes (Satters et al., 1994), catalyzes the malate and transforms it into oxaloacetate in the Krebs cycle, which results in the production of NADH, acts in the conversion of the stored triacylglycerols in the form of glucose and provides energy to the processes of germination and initial growth of the seedlings, in this way, when expressed, can minimize the seed vigor (Pedó et al., 2006).

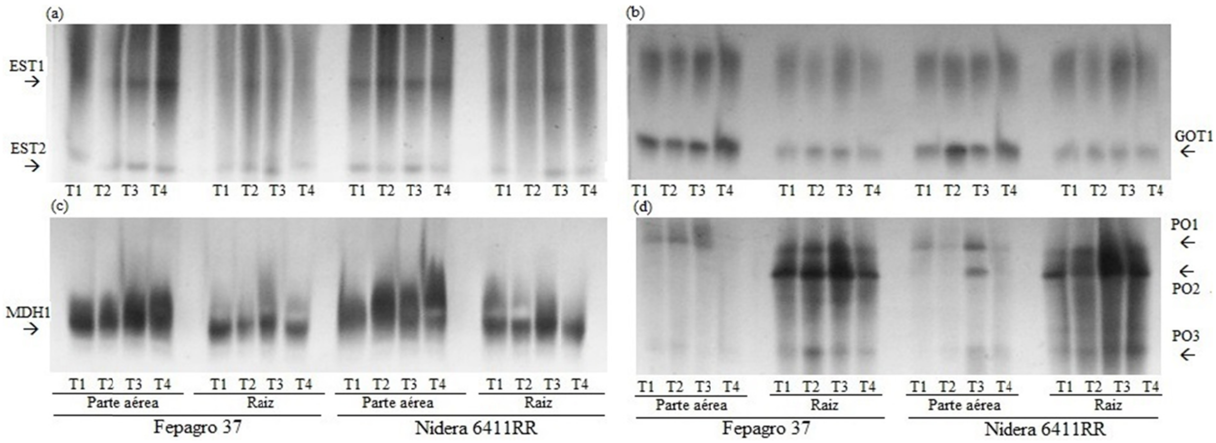

Figure 1. Isozyme expression of esterase (a), glutamate oxaloacetate transaminase (b), malate dehydrogenase (c) and peroxidase (d) for the primary root (RL) and shootlengths (SL) traits of seedlings from soybean cultivars (Fepagro 37 RR and Nidera 6411 RR) submitted to different seed treatments

In relation to the peroxidase enzyme (PO), the aerial part of the soybean seedlings of the cultivar Fepagro 37 RR expressed only a low intensity band, referring to the treatment with micronutrients + polymers, in contrast, for the primary root, evidenced the formation of three bands of greater intensity. One of the bands referred to the treatment with *Thimetoxan* + micronutrient + polymer, however, for the NS6411 RR cultivar, in aerial part it was possible to visualize the formation of three bands with greater expression for the treatment with *Thimetoxan* + polymer (Figure 1d). This behavior did not allow differentiation among treatments for both cultivars. This isoenzyme can be used to evaluate the physiological potential of the seeds that are submitted to certain periods of storage (Costa et al., 2008), since current as polysaccharide binding, regulates cell elongation, scarring of pathogenic damage (Rossi & Lima, 2001; Szareski et al., 2016b). The increase in its expression may be indicative of a reduction in the level of hydrogen peroxide (free radical) and its accumulation causes the lipid peroxidation, where it modifies the permeability of the cell membranes.

4. Conclusions

Addition of seed treatments influences the physiological performance of seedlings originated from soybean seeds stored for 240 days. The shoot and primary root lenghts, and shoot dry mass express the isoenzyme esterase through the aerial part and primary root of the seedling, the malate dehydrogenase is expressed in the primary root while in the peroxidase it is evident in the shoot of the seedlings.

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