

Physiological quality of soybean seeds coming from cultivation with application of biostimulant

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Abstract

Several studies have proved the efficiency of the application of biostimulant to increase soybean yield. However, little is known about the influence of these products on the seeds quality coming from cultivation with its application. Thus, the objective of this study was to evaluate the physiological quality of soybean seeds coming from the cultivation in function of periods and doses of biostimulant application. The study was performed in two phases. The first phase was carried out in field condition, at the Experimental Station of MT Foundation during the 2012/13 crop. The experimental design was a randomized block with four replications and nine treatments arranged in an incomplete factorial scheme $2 \times 4 + 1$, i.e. two periods of foliar application (V_5 and R_2) and four doses of biostimulant (250, 500, 750 and 1,000 mL ha⁻¹) and one control treatment (without application of product). The second phase of the study was performed in the Seed Analysis Laboratory. In the cold test, the treatments with application of biostimulant in V_5 with 750 mL ha⁻¹ and in the R_2 with 250, 500 and 1,000 mL ha⁻¹ presented higher percentage of normal seedlings than the control. The fresh biomass and accelerated aging also presented positive results. However, most of tests did not affect seed physiological quality. It was concluded that the foliar application of biostimulant with doses of 0, 250, 500, 750 and 1,000 mL ha⁻¹ in V_5 and R_2 on soybean plants does not influence the quality of the seeds produced.

Keywords: Germination; *Glycine max*; growth regulator; plant hormones; seed vigor.

Abbreviations: a.i_active ingredient; BOD_biochemical oxygen demand; CoMo_product containing cobalt and molybdenum; GSI_germination speed index; K₂O_potassium oxide; P₂O₅_phosphorus pentoxide; Ca_calcium; S_sulfur; PRL_primary root length; SFB_seedling fresh biomass.

Introduction

The soybean is one of the most important crops in the world, especially as a source of protein and vegetable oil (Bezerra et al., 2015). Brazil has a prominent position in the world production. It is the second biggest soybean producer, being only behind the United States (USDA, 2015). Good climate conditions associated with the intensive use of technology have boosted soybean production in several producer states, especially in Mato Grosso, Paraná and Rio Grande do Sul (Conab, 2015a). In 2014/15 crop year, the estimated increment of area, productivity and production were 1.8 million ha (5.9%), 157.7 kg ha⁻¹ (5.5%) and 10,082.7 thousand tons (11.7%), respectively, compared to the previous year (2013/14) (Conab, 2015b). The Brazilian soybean productivity and production capacity increase is due to scientific advances and availability of technologies in the productive sectors. One of the advances is the use of plant growth regulators and/or biostimulants (Klahold et al., 2006). The plant growth regulators are synthetic substances that, when exogenously applied present actions similar to the known groups of plant hormones, such as auxin, gibberellin, cytokinin, the retardants, the inhibitors and the ethylene. The plant stimulants or biostimulants refer to the plant growth regulators mixture or regulators with other compounds of

different biochemical nature (amino acids, nutrients, vitamins, etc.). For example, the Stimulate[®] has the composition of: 0.009% of kinetin (cytokinin), 0.005% of gibberellic acid (gibberellin) and 0.005% of indolebutyric acid (auxin) (Castro and Vieira, 2001). The use of plant growth regulators as agronomic technique to optimize production in several crops has increased in the last years (Klahold et al. 2006). Many authors studied the application effects of Stimulate[®] in soybeans and noticed increase in the number of pod, grains (Klahold et al. 2006, Batista-Filho et al. 2013) and productivity (Klahold et al., 2006; Batista-filho et al., 2013; Bertolin et al., 2010; Albrecht et al., 2012). However, Klahold et al. (2006) and Batista-Filho et al. (2013) reported that the grain weight was lower. It is noticed that many works report increase in pod number, grains and production of soybean with biostimulants; however, little is known about its effect on the quality of seeds coming from cultivation with biostimulants application".

Because it affects the productivity and the seeds weight, the application of Stimulate[®] may result in effects on their physiological quality. Vinhal-Freitas et al. (2011) noticed that the soybean seedlings coming from small or lighter seeds

were less vigorous in relation to the seedlings from big and heavier seeds.

The Stimulate[®] is composed of plant hormones such as auxin, cytokinin and gibberellin, which acts as mediators of physiological processes and tend to increase the root system, by stimulating the cellular division, differentiation and lengthening. Application of Stimulate[®] results in higher water and nutrients absorption potential by the plants (Garcia et al., 2009), ensuring a faster allocation of substances to the preferred sinks in the plant, like the seeds (Dourado-Neto et al., 2014). This also may lead to higher physiological quality of the seeds produced by these plants. Among the factors that affect the vigor during the seed formation are the water supply and nutrients availability (Carvalho and Nakagawa, 2012). Lack of water may cause reduction of seeds weight and affect significantly their performance, since it speeds up the foliar senescence and reduce the reserve accumulation period (Marcos-Filho, 2005). In relation to nutrients, a well-nourished plant has the conditions to produce more well-formed seeds, because the nutrient availability interferes in the good formation of the embryo and reserves organ, as well as in the chemical composition, metabolism and seed vigor (Carvalho and Nakagawa, 2012). The aim of this work was to evaluate the physiological quality of soybean seeds coming from cultivation in function of periods and doses of application of biostimulant Stimulate[®].

Results and Discussion

F-test

The F-test results of the variance analysis for the physiological quality tests of soybean seeds according to periods and doses of Stimulate[®] application are shown in Table 1. It is noticed that the F-test was significant (5%) to: factorial versus control in the accelerated aging and interaction between periods and doses on the primary root length. The significance of 1% occurred in: interaction between doses x periods and factorial versus control for the cold test and seedling fresh biomass.

Physiological quality of seeds

The average values of water content in the seeds, germination, first count of germination, germination speed index, accelerated aging, cold test and electrical conductivity of soybean seeds are shown in the Table 2. For the water content, the statistical analysis was not made, because this test was performed only to verify the uniformity between treatments. It is noticed that the water content values are adequate, because according to Vieira and Krzyzanowski (1999), uniform water contents provide uniform results for the electrical conductivity test and also for the accelerated aging test (Marcos-Filho, 2005). Besides, the accelerated aging test must be installed with samples whose water content does not present variation higher than 2%, because the most humid seeds present higher sensibility to the test conditions (Marcos-Filho, 1999). The tests of germination, first count of germination and germination speed index (GSI) were not influenced by the treatments. The average germination value (86%) is within the commercial patterns recommended to soybean seeds, on which the minimum germination required is 80% (Brasil, 2013). In similar way, Ávila et al. (2008) did not report differences in germination of seeds produced by plants that received the biostimulant foliar application between the stages V₅ and V₆. Bertolin (2008) researching the effect of biostimulant application in the seeds and foliar spray on stages V₅, R₁ and R₅ of soybean did not observe influence on germination, first count and GSI

of seeds from these treatments either. It is highlighted that as the tests of first count and germination speed index are made together, they can present many times a similar behavior. However, according to what was highlighted by Marcos-Filho (2005) the germination speed decrease is among the first events that indicate the loss of seeds quality and; therefore, lower vigor. The first count shows the seeds germination uniformity, to achieve uniform stands and development of plants. According to the data obtained for these parameters, a good uniformity in germination is observed (first count) and also in the germination speed, that indicates a good seed vigor level, independent on the biostimulant application.

For the accelerated aging, the treatment with foliar application in V₅ with dose of 1,000 mL ha⁻¹ of biostimulant presented increase of 22% in normal seedlings number, compared to the control. On the other hand, Bertolin (2008) reported that there was no difference between the control and the treatments with the application of Stimulate[®] on soybean. According to Marcos-Filho (2005), the accelerated aging test is one of the most sensitive and efficient to the vigor evaluation of several species, considering that the seeds are submitted to stressful conditions of high temperature and humidity. Bertolin (2008) also stated that seeds with higher normal seedlings values in accelerated aging, present higher storage potential. The cold test is a vigor test, which evaluates the answers of seed samples subjected to stressful condition of low temperature and high humidity level (Marcos-Filho, 2005). In this test, the treatments with biostimulant foliar application in V₅ with dose of 750 mL ha⁻¹, in R₂ with doses of 250, 500 and 1,000 mL ha⁻¹ produced seeds with higher percentage of normal seedlings comparing to the control. It is important to highlight that the benefits resulting from the biostimulant application on the seed vigor obtained by the cold test, may be associated with the physiological effect on the plants as higher rooting system development, providing higher water and nutrients uptake (Dantas et al., 2012; Dourado-Neto et al., 2014) and consequently more vigorous seeds. Besides, this effect of the product application in R₂ may have happened due to the hormone capacity in interfering in the source/sink relation. In other words, in the control of interaction between the areas of supply and demand of photosynthates according to what was reported by Taiz and Zeiger (2013). According to the referred authors, the chemical messengers which include hormones and nutrients, are important for signaling the state of an organ to another in the plant. According to Mortelet et al. (2008), when the stimulant is applied on the reproductive stage, it serves as a sink for the liberation and/or remobilization of carbohydrates. At 250 mL ha⁻¹ of biostimulant dose, the application in R₂ provided higher percentage of normal seedlings on cold test in comparison to its application in V₅. On the other hand, in the dose of 750 mL ha⁻¹ of biostimulant, the application in R₂ presented lower percentage of normal seedlings in comparison to the application in V₅. As to the doses of biostimulant, it is verified that in the application made in V₅, there was data adjustment to a positive linear equation at 5% significance (Fig 1). In other words, the higher the biostimulant dose the higher the normal seedlings percentage in the cold test. The seeds electrical conductivity was not influenced by the treatments (Table 2).

Seedling growing

The length of aerial part, the total seedling length and the dry seedling biomass were not influenced by the treatments (Table 3), highlighting that the biostimulant application did

Table 1. Significance levels obtained in the variance¹ analysis and variation coefficient for germination (G), first count of germination (FCG), germination speed index (GSI), accelerated aging (AA), cold test (CT), electrical conductivity (EC), primary root length (PRL), aerial part length (APL), seedling total length (STL), seedling fresh biomass (SFB) and seedling dry biomass (SDB) of soybean, cultivar TMG 1176 RR, according to periods and doses of Stimulate[®] application. Itiquira – MT, Brazil (2012/13).

Variation Source	G	FCG	GSI	AA	CT	EC	PRL	APL	STL	SFB	SDB
Period (P)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dose (D)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P x D	ns	ns	ns	ns	**	ns	*	ns	ns	**	ns
Fact. vs control	ns	ns	ns	*	**	ns	ns	ns	ns	**	ns
VC (%)	5.94	6.34	5.77	7.77	8.24	9.34	10.03	28.84	20.71	7.71	14.76

¹F Test: **, * and ns – significant at 1%, 5% of significance and not significant, respectively, VC: variation coefficient.

Table 2. Average values of water content in the seeds, germination, first count of germination, germination speed index, accelerated aging, cold test and electrical conductivity of soybean seed, TMG 1176 RR cultivar, according to periods and doses of Stimulate[®] application. Itiquira – MT, Brazil (2012/13).

Period	Control	Stimulate [®] Doses (mL ha ⁻¹)				Average
		250	500	750	1,000	
Water content in the seeds (%)						
V ₅	-	9.7	9.8	9.8	9.5	9.7
R ₂	-	9.9	9.9	10.2	9.6	9.9
Average	-	9.8	9.6	10.0	9.6	-
Control	9.3	-	-	-	-	-
Germination (%)						
V ₅	-	86.5	84.0	87.0	86.5	86.0
R ₂	-	85.5	89.5	85.5	88.5	87.2
Average	-	86.0	86.7	86.3	87.5	-
Control	86.0	-	-	-	-	-
First count of germination (%)						
V ₅	-	84.0	81.5	84.0	82.0	82.9
R ₂	-	82.5	85.5	83.5	83.5	83.7
Average	-	83.2	83.5	83.7	82.7	-
Control	82.0	-	-	-	-	-
Germination speed index						
V ₅	-	10.6	10.2	10.7	10.5	10.5
R ₂	-	10.4	10.8	10.5	10.7	10.6
Average	-	10.5	10.5	10.6	10.6	-
Control	10.5	-	-	-	-	-
Accelerated aging (%)						
V ₅	-	79.5	79.0	81.0	88.5(*)	82.0
R ₂	-	82.5	79.0	73.5	83.5	79.6
Average	-	81.0	79.0	77.2	86.0	-
Control	79.5	7	-	-	-	-
Cold Test (%)						
V ₅	-	66.5 b	68.5 a	75.5 a(*)	73.0 a	70.9
R ₂	-	76.5 a(*)	74.5 a(*)	56.5 b	75.0 a(*)	70.6
Média	-	71.5	71.5	66.0	74.0	-
Controle	62.0	-	-	-	-	-
Seeds electrical conductivity (µS cm ⁻¹ g ⁻¹)						
V ₅	-	61.3	54.9	60.7	62.5	59.9
R ₂	-	66.9	60.0	64.6	63.3	63.7
Average	-	64.2	57.4	62.7	62.9	-
Control	67.4	-	-	-	-	-

Averages followed by the same letter in the columns do not differ from each other by the Tukey test at 5% significance; (*) Averages followed by asterisk differ from the control treatment by the Dunnett bilateral test at 5% significance.

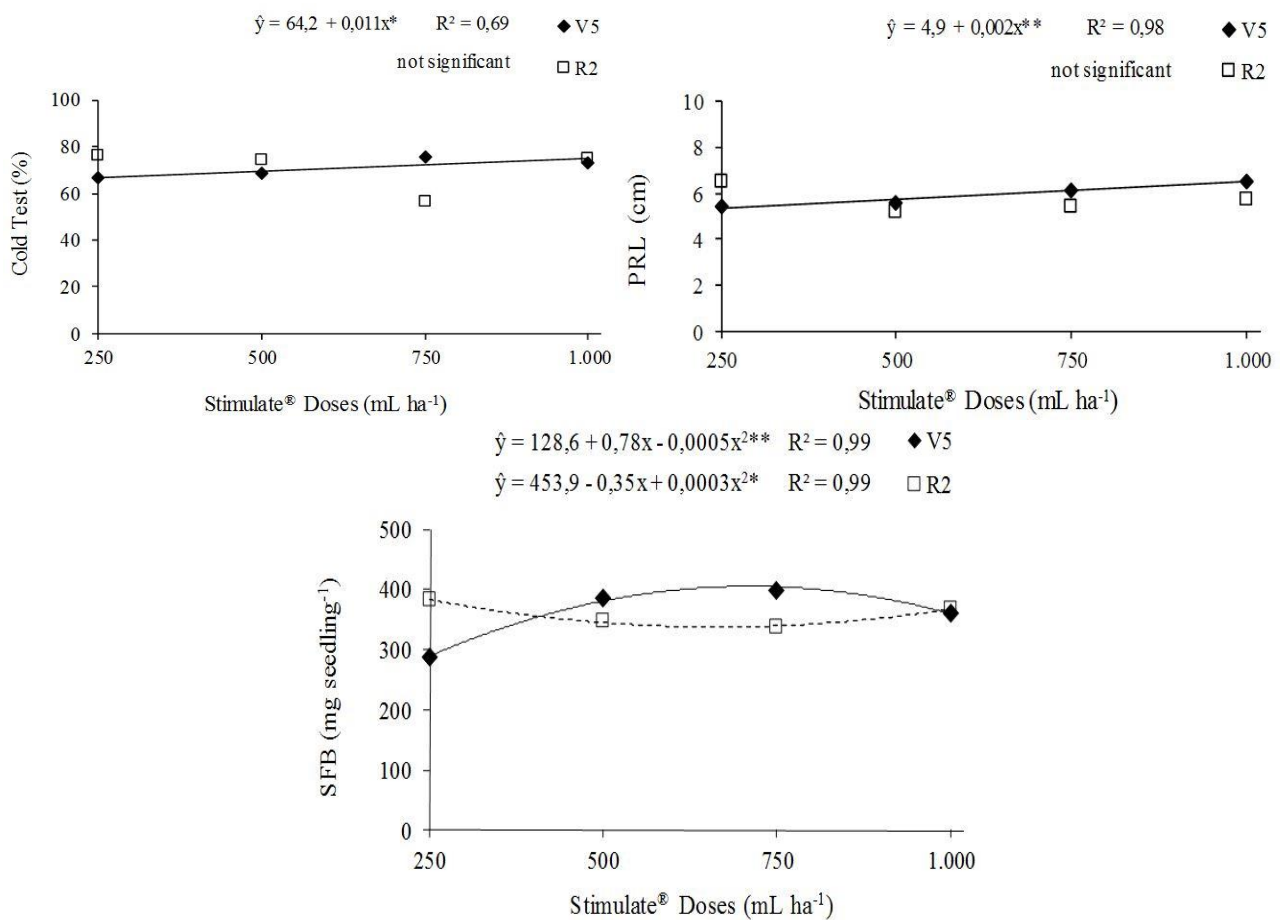


Fig 1. Seeds cold test, primary root length (PRL) and seedling fresh biomass (SFB) of soybean, TMG 1176 RR cultivar, according to periods and doses of Stimulate[®] application. Itiquira – MT, Brazil (2012/13). F Test: ** and * – significant at 1% and 5% significance, respectively.

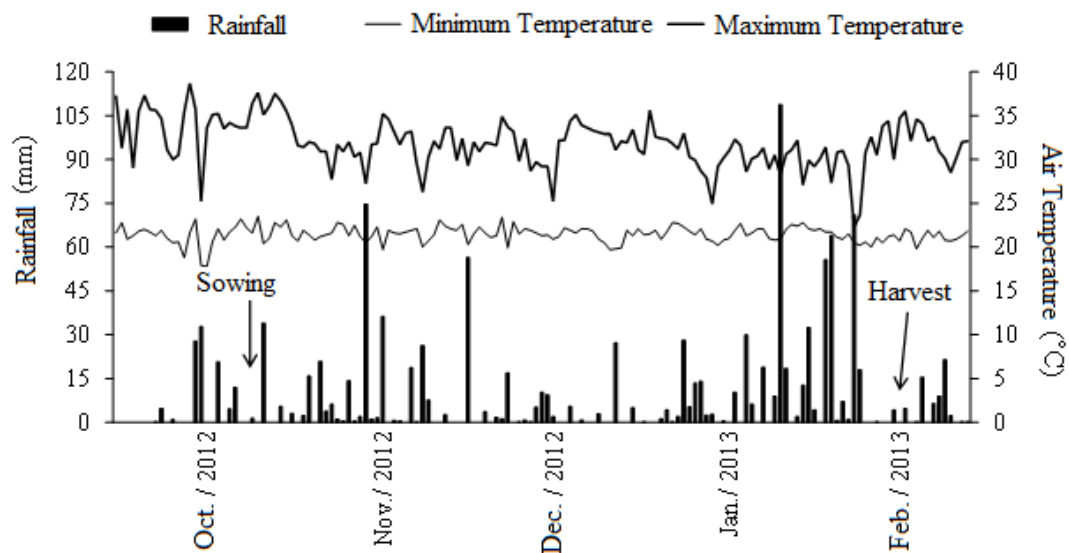


Fig 2. Rainfall daily data and air minimum and maximum temperature during the experimental period. Itiquira, MT, Brazil (2012/13).

Table 3. Average values of primary root length, aerial part length, total seedling length, seedling fresh biomass and seedling dry biomass of soybean, TMG 1176 RR cultivar, according to periods and doses of Stimulate® application. Itiquira – MT, Brazil (2012/13).

Period	Control	Stimulate® Doses(mL ha ⁻¹)				Average
		250	500	750	1,000	
Primary root length (cm)						
V ₅	-	5.4 b	5.6 a	6.1 a	6.5 a	5.9
R ₂	-	6.5 a	5.2 a	5.4 a	5.7 a	5.7
Average	-	6.0	5.4	5.8	6.1	-
Control	5.2	-	-	-	-	-
Aerial part length (cm)						
V ₅	-	10.1	12.7	12.3	12.2	11.8
R ₂	-	13.5	11.7	11.7	16.8	13.4
Average	-	11.8	12.2	12.0	14.5	-
Control	11.5	-	-	-	-	-
Total seedling length (cm)						
V ₅	-	15.6	18.3	18.4	18.7	17.8
R ₂	-	20.0	16.8	17.1	22.5	19.1
Average	-	17.8	17.6	17.8	20.6	-
Control	16.7	-	-	-	-	-
Fresh Biomass (mg seedling ⁻¹)						
V ₅	-	286.8 b	387.0 a(*)	398.2 a(*)	361.8 a(*)	358.4
R ₂	-	382.2 a(*)	347.2 a	337.2 b	368.0 a(*)	358.7
Average	-	334.4	367.1	367.7	364.9	-
Control	03.1	3	-	-	-	-
Dry Biomass (mg seedling ⁻¹)						
V ₅	-	25.7	30.6	30.5	30.2	29.3
R ₂	-	29.7	24.6	28.3	26.7	27.4
Average	-	27.7	27.6	29.4	28.4	-
Control	27.2	-	-	-	-	-

Averages followed by the same letter in the columns do not differ from each other by the Tukey test at 5% significance; (*) Averages followed by asterisk differ from the control treatment by the Dunnett bilateral test at 5% significance.

not interfere with the seeds quality for these evaluations. The length of seedling primary root, in seeds coming from the cropping with application of 250 mL ha⁻¹ of biostimulant in R₂ was higher in relation to the application of the same dose in V₅. In relation to the biostimulant doses applied in V₅ (Fig 1), the data were adjusted to a positive linear equation at 1% significance, on which with the dose increment there was increase of the soybean seedling root length.

The treatments with biostimulant application in V₅ with doses of 500, 750 and 1,000 mL ha⁻¹ and in R₂ with doses of 250 and 1000 mL ha⁻¹ provided greater fresh biomass compared to the control. This may also be related to the physiological effect of the biostimulant on the soybean plants. Regarding to the deployment of biostimulant application period within the dose, it is verified that the application in R₂ in the dose of 250 mL ha⁻¹ and in V₅ in the dose of 750 mL ha⁻¹ provided higher fresh biomass values. The influence of doses application on the seedlings fresh biomass occurred in both periods with quadratic effect (Fig 1), in V₅ with maximum point with dose of 780 mL ha⁻¹ and in R₂ with minimum point of 583 mL ha⁻¹ of biostimulant.

In general, in most tests performed there was no difference in seeds quality, because the physiological quality of seeds produced by plants which received the biostimulant Stimulate® in V₅ and R₂ with doses of 0, 250, 500, 750 and 1,000 mL ha⁻¹ was not influenced by the treatments. However, this is a warning that, in determined situations effects of this type of treatment can be observed. Furthermore, the reduction in the seeds quality was not observed due to application of product, it is believed that its application for other purposes would not cause any problem in a seed production field.

Materials and Methods

Description of the area and installation of field experiment

The study was performed in two phases. The first was performed in field conditions, in Itiquira, state of Mato Grosso, Brazil, at the Experimental Station of MT Foundation (latitude of 17° 09' S, longitude of 54° 45' W and altitude of 490 m), during the 2012/13 crop. The soil of the experimental area is classified as dystrophic Red Oxisol and very clayey texture (Embrapa, 2006). The region is under Cerrado biome, whose prevailing climate, according to Köppen classification, is the Aw type (Ribeiro and Walter, 1998). The average annual rainfall is between 1,200 and 1,800 mm and the average annual temperature is between 22-23 °C. The rainfall daily data and air minimum and maximum temperature, were registered during the experimental period (Fig 2).

In the agricultural year previous to conduction of this experiment, 2011/12 crop, the experimental area was cropped with soybean system (first crop) / late season corn (second crop). The soybean cultivar used was the TMG 1176 RR (maturation group 7.6) and the aimed population was 350,000 plants ha⁻¹. The seeds were treated with pyraclostrobin+ thiophanate-methyl+fipronil (0.025+0.225+0.25 g kg⁻¹ of a.i.), carbendazim+thiram (0.3+0.7 g kg⁻¹ of a.i.), CoMo (3.0 mL kg⁻¹) and liquid inoculant, with strains of *Bradyrhizobium japonicum*, on the sowing day. A 120 kg ha⁻¹ of K₂O was applied in pre-sowing via potassium chloride (broadcasting). The 50, 42 and 30 kg ha⁻¹ of P₂O₅, Ca and S, respectively, were applied in the sowing furrow, via single superphosphate (280 kg ha⁻¹).

The sowing was made on 10/24th/2012, in no-tillage system, using a sowing machine equipped with furrower rods

mechanism (“botinha” type) and pneumatic seed distribution system. The other phytotechnical practices, during the crop development were made according to the necessities.

Experimental design and biostimulant treatments

The experimental design was a randomized block with four replications and nine treatments arranged in incomplete factorial scheme $2 \times 4 + 1$, which means, two application periods (V_5 and R_2 – Ritchie et al., 1994) and four doses of Stimulate® (250, 500, 750 e 1,000 mL ha⁻¹) and 1 control treatment (without product application). The product was applied with CO₂ pressurized backpack sprayer at constant pressure of 3 Kgf cm⁻², equipped with bar with hollow cone spray nozzles, spaced at 0.45 m. The applications were made in the afternoon and the volume of the mixture was 150 L ha⁻¹.

The Stimulate® product is a biostimulant highly used in Brazil in order to increase soybean productivity. This product has a hormonal effect, with composition of: 0.009% of kinetin (cytokinin), 0.005% of gibberellic acid (gibberellin) and 0.005% of indolebutyric acid (auxin).

The plots were formed by 14 lines with 10 m length each, spaced at 0.45 m. Right after the harvest, which was on 02/14th/2013, the second phase of the study was started, which was performed at the Seed Analysis Laboratory in the Plant Science, Food Technology and Socioeconomy Department, Ilha Solteira Engineering College, Unesp, in Ilha Solteira – SP.

Variables analyzed: Seeds physiological quality

The seeds physiological quality was evaluated through the following tests:

1. Water content in the seeds: determined with four replications of 4.5±0.5 g of seeds per treatment, using the greenhouse method at 105±3 °C, for 24 hours (Brasil, 2009).
2. Germination: performed with four replications of 50 seeds per treatment, conditioned in germitest paper towel rolls moistened with distilled water equivalent to 2.5 times their dry weight. The rolls were placed in germinator, regulated to keep constant temperature of 25 °C. The counting was performed on the fifth and the ninth days after the test installation, and the results were expressed in normal seedlings percentage (Brasil, 2009).
3. First count of germination: evaluated along with the germination test, computing the percentage of normal seedlings on the fifth day after the test installation (Brasil, 2009).
4. Germination speed index (GSI): It was also evaluated along with the germination test. The GSI calculation was made according to the methodology proposed by Maguire (1962). This way, the first counting and the GSI formed an indicative of the seeds vigor, while the final count, their viability.
5. Accelerated aging: performed with four replications of 50 seeds per treatment, which were distributed in an only layer on stainless steel screen, fixed inside plastic boxes of gerbox type (11.0 x 11.0 x 3.0 cm), containing 40 mL of distilled water at the bottom. The boxes were capped and kept at temperature of 41 °C for 72 hours, in BOD germination chamber (model SL-224) (Marcos-Filho, 1999). After this period, the seeds were submitted to the germination test (Brasil, 2009), with evaluation of normal seedlings percentage on the fifth day after the test installation.
6. Cold test without soil: made with four subsamples of 50 seeds per treatment, which were distributed on germitest paper previously moistened, the same way it was made for the germination test (Brasil, 2009). The rolls were put inside

plastic bags, and closed with Scotch tape and kept in BOD incubator chamber (model MAQIB-F340) regulated at 10 °C, during five days (Barros et al., 1999). After that, the rolls were transferred to the germinator with regulated temperature of 25 °C, where they stayed for five days, when the normal seedlings count was then made (Brasil, 2009).

7. Electrical Conductivity: according to Vieira and Krzyzanowski (1999), made after the weight determination of four subsamples with 25 seeds per treatment in precision analytical balance (0.001 g), where seeds were subjected to soaking in plastic cups with 75 mL of deionized water. The cups were put in germinator with regulated temperature of 25 °C, for 24 hours. After this period, the seeds were gently shaken and the electrical conductivity measurement was made in the soaking solution, using a conductivimeter model mCA-150. The electric conductivities of the soaking solutions were calculated, with values expressed in $\mu\text{S cm}^{-1} \text{g}^{-1}$ of seeds;

Variables analyzed: Seedling growth

The seedling growing was evaluated through the following tests:

1. Length of primary root, of aerial part and total of seedlings: The substrate was prepared the same way as described for the germination test. The rolls were kept in vertical position in germinator with regulated temperature of 25 °C, for five days. Four subsamples of 20 seeds per treatment were used; however, to determine the length of the primary root and the aerial part, 10 normal seedlings per subsample were randomly considered. The total seedling length was obtained through the sum of length measures of the primary root with the aerial part, in each repetition. The evaluation was made using a millimeter ruler and the average results were expressed in cm.
2. Fresh and dry seedling biomass: they were determined after evaluating the first count of germination. Four subsamples of 10 normal seedlings per treatment randomly were chose and weighted with precision analytical balance (0.001 g). The cotyledons were removed and the total biomass in each subsample obtained and was divided by the number of seedlings, obtaining the average fresh biomass per seedling (in mg). After this determination, the embryonic axes were put in paper bags and taken to dry in a forced air circulation greenhouse, with regulated temperature of 80±2 °C for 24 hours. After that, the material was weighted again, obtaining the dry biomass with precision of 0.001 g. The total biomass obtained in each subsample was divided by the number of component embryonic axes, resulting in the average dry biomass per seedling (in mg) (Nakagawa, 1999; Brasil, 2009).

Statistical analysis

The results were submitted to the F-test after analysis of variance and the degrees of freedom of the treatments were deployed in period, doses, interaction between period and doses of Stimulate® and a factorial contrast versus control. When they were meaningful, the factorial component treatments were compared to each other. The averages of the factor period were compared through the Tukey test at 5% significance, and the dose averages were analyzed through regression, adjusting models of significant equations through the F-test. Through the bilateral test of Dunnett, the orthogonal contrasts of the factorial treatments were compared to the control at 5% significance. The ASSISTAT software was used for the analyses (Silva and Azevedo, 2002).

Conclusion

The dose of 1,000 mL ha⁻¹ applied in V₅ increased the percentage of normal seedlings in accelerated aging test compared to control. In the cold test, the treatments with application of biostimulant in V₅ with 750 mL ha⁻¹ and in the R₂ with 250, 500 and 1,000 mL ha⁻¹ presented higher percentage of normal seedlings than the control. The fresh biomass was higher than the control with the application of 500, 750 and 1,000 mL ha⁻¹ in V₅ and 250 and 1,000 mL ha⁻¹ in R₂. However, most treatments did not show beneficial results. It was concluded that the foliar application of biostimulant Stimulate[®] with the doses of 0, 250, 500, 750 and 1,000 mL ha⁻¹ in V₅ and R₂ on soybean plants does not influence the physiological quality of the seeds produced, since the seeds presented high quality either with or without the biostimulant application. Meantime, although no increase in physiological quality was observed, this result is an important indicative for seed producers, because they may use this product on the plants for other purposes, as for the increase of the productivity.

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