

# Physiological quality of seeds in conventional and glyphosate-resistant soybean produced by foliar application of manganese

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## Abstract

Soybean seeds with high physiological quality are essential to achieve high yields, one of the factors affecting seed production is mineral nutrition. The aim of this study was to evaluate the effect of foliar application of manganese, with different doses and stages of application, on the physiological quality of soybean seeds in conventional cultivars and GR descendants with different lignin content in the seed coat. The experiment was conducted in randomized blocks with three replications and a 4 x 4 x 2 factorial arrangement consisting of four soybean cultivars, two conventional and their GR descendants (BRS Celeste and BRS Baliza RR; BRSGO Jataí and BRS Silvânia RR), four doses of Mn via foliar application (0; 200; 400 and 600 g Mn ha<sup>-1</sup>) and two stages of application (R<sub>1</sub> and R<sub>2</sub>). Before and after six months of storage, the physiological quality of seeds was estimated by testing: germination, accelerated aging, seedling emergence, electrical conductivity and tetrazolium (Viability, vigor and mechanical damage). Lignin contents were determined in the seed coats. The foliar application of Mn provides increased physiological quality in soybean seeds produced. Seeds of the soybean cultivars Celeste and Baliza RR have better physiological quality when compared with Jataí and Silvânia RR. Seeds of soybean cultivars with higher lignin content in the seed coat do not necessarily have better physiological quality, thus seed quality is related to other factors intrinsic to the genotype.

Key words: *Glycine max*, micronutrients, Roundup Ready soybean, vigor.

## Qualidade fisiológica de sementes de soja convencional e transgênica RR produzidas sob aplicação foliar de manganês

### Resumo

Sementes de soja com alta qualidade fisiológica são essenciais à obtenção de produtividade elevada e um dos fatores que afetam a produção de sementes é a nutrição mineral. O objetivo neste trabalho foi avaliar o efeito da aplicação foliar de manganês, em diferentes doses e estádios de aplicação, na qualidade fisiológica de sementes de soja, em cultivares convencionais e em suas derivadas transgênicas RR, com distintos teores de lignina do tegumento. O ensaio foi conduzido em blocos casualizados, com três repetições e esquema fatorial 4 x 4 x 2, sendo quatro cultivares de soja, duas convencionais e suas derivadas transgênicas RR (BRS Celeste e BRS Baliza RR; BRSGO Jataí e BRS Silvânia RR), quatro doses de Mn via foliar (0; 200; 400 e 600 g Mn ha<sup>-1</sup>) e dois estádios de aplicação (R<sub>1</sub> e R<sub>2</sub>). A qualidade fisiológica das sementes foi estimada, antes e após seis meses de armazenamento, por meio dos testes: Germinação, Envelhecimento acelerado, Emergência de plântulas, Condutividade elétrica e Tetrazólio (Viabilidade, vigor e danos mecânicos). Realizou-se também a quantificação de lignina no tegumento das sementes. A aplicação foliar de Mn proporciona incrementos na qualidade fisiológica nas sementes de soja produzidas. Sementes das cultivares de soja Celeste e Baliza RR apresentam melhor qualidade fisiológica quando comparadas às de Jataí e Silvânia RR. Sementes de cultivares de soja com maior teor de lignina no tegumento não apresentam necessariamente melhor qualidade fisiológica, sendo a qualidade das sementes relacionada a outros fatores intrínsecos ao genótipo.

Palavras-chave: *Glycine max*, micronutrientes, soja Roundup Ready, vigor.

## 1. INTRODUCTION

In soybean, the achievement of high yields may be limited by the quality of seeds used. Scheeren et al. (2010) reported that the yield of high vigor seeds may be 9% higher than that of low vigor ones.

Despite its importance, studies are lacking on the influence of mineral nutrition for yield and physiological quality of seeds. The recommendation of fertilizers for the establishment of crops intended for seed production

is usually similar to that used for the production of grain (Carvalho and Nakagawa, 2000). In most cases, these recommendations emphasize the effect of mineral nutrition on yield, not correlating with seed quality. Mondo et al. (2012) performed soil and seed samplings at georeferenced sites for analysis of soil fertility and physiological potential of soybean seeds, and concluded that the correlation between these factors is very important for seed production technology.

Among the nutrients, the importance of manganese (Mn) is related to its function in chlorophyll synthesis and enzyme activation, such as, the enzymes related to the formation of lignin in the cell wall (Malavolta, 2006), a compound that influence the impermeability and resistance of the seed coat (Capeleti et al., 2005). The limited number of studies have still controversial results as to the action of Mn on the quality of soybean seeds.

When evaluated the influence of different sources and modes of application of Mn, on soil and leaves ( $V_4$  and  $R_1$ ), on soybean seeds production, Melarato et al. (2002) concluded that Mn application had a positive influence on the weight of soybean seeds and that the nutritional status of the plants, in relation to Mn, had no influence on the physiological potential of seeds. In turn, Mann et al. (2002) examined the influence of manganese applied to leaves ( $V_4$ ,  $V_8$  and  $V_{10}$ ) and to soil on the yield and quality of soybean seeds, and reported increased yield, germination, vigor, protein and oil content in seeds and Mn content in the plant, with both modes of application of Mn, with greater efficiency of foliar application.

Moreover, it has been raised the hypothesis that seeds of glyphosate-resistant transgenic soybean (RR, Roundup Ready®, GTS 40-3-2) have lignin content superior to conventional soybean seeds (Gris et al., 2010). This can affect the resistance to mechanical damage and physiological quality of soybean seeds, considering their relationships with the lignin content in the seed coat, as reported by Capeleti et al. (2005), Panobianco et al. (1999) and Santos et al. (2007). There are also reports that glyphosate-resistant transgenic soybean plants could be less efficient in the accumulation of Mn than conventional soybean, so they would be more responsive to the use of this micronutrient (Gordon, 2007), but the results are still inconclusive and conflicting as to productivity (Andrade and Rosolem, 2011; Basso et al., 2011; Loecker et al., 2010; Zobiolo et al., 2010). Regarding the scarcity of studies on physiological quality of seeds, there is a need for further researches like that of Carvalho et al. (2012) who worked with the CD 206 and CD 206 RR cultivars and observed that conventional soybean seeds showed a greater physiological potential compared with to the glyphosate-resistant cultivar (Roundup Ready®, RR). It is noteworthy that these authors used only a pair of conventional and RR for this observation.

Given this context, the aim of this study was to evaluate the effect of foliar application of manganese at different

doses and stages of application on the physiological quality of soybean seeds in conventional (non-genetically modified) cultivars and their glyphosate-resistant (Roundup Ready®, RR) transgenic descendants, with different contents of lignin in the seed coat.

## 2. MATERIAL AND METHODS

The experiment was developed in Lavras, southern region of Minas Gerais State, Brazil, latitude 21°14'S, longitude 45°00'W and 918 m altitude. The climate, according to the Köppen classification, is Cwa, rainy temperate (mesothermal) with dry winter and rainy summer, subtropical, with the highest rainfall in December and January, when the monthly average may reach 321 mm. The average annual rainfall is 1460 mm (Dantas et al., 2007). In the experimental area (Dystriferic Red Latosol – Rhodic Eutrudox), a chemical analysis of soil (0.00 to 0.20 m depth) indicated a pH in  $H_2O$  of 5.4 and Mn content of 5.0 mg  $dm^{-3}$  (Mehlich 1), a value classified as low, since the critical level considered by Ribeiro et al. (1999) is 8.0 mg  $dm^{-3}$ . Soil preparation consisted of plowing and disking. Mechanical traction was used to make sowing furrows. The fertilizer was applied in the furrow, with 400 kg  $ha^{-1}$  of NPK 04-30-10 (Ribeiro et al., 1999).

Soybean cultivars tested in this study were two conventional (non-genetically modified) and their glyphosate-resistant (Roundup Ready®, RR) transgenic descendants, with the pairs of cultivars: BRS Celeste and BRS Baliza RR, BRSGO Jataí and BRS Silvéria RR. Seeds were treated with the fungicide Carbendazim + Thiram and inoculated with *Bradyrhizobium japonicum* (peat-based inoculant at 1,200,000 bacteria per seed). Seeds were sown in the first fortnight of November and the thinning at 15 days after seedling emergence, leaving fifteen plants per meter, 300,000 plants per hectare. We used four sowing rows, five meters long, spaced at 0.5 m, with the two central rows as working area, excluding 0.5 m from each end. For weed control, glyphosate was not used, only fluzifop-P-butyl + fomesafen. The control of diseases and pests were carried out uniformly in all plots with the fungicides Azoxystrobin + Cyproconazole and Thiophanate-methyl and the insecticides Gamma-chalothrin and Beta-cyfluthrin + Imidacloprid.

Four doses were used in the foliar application of Mn: 0; 200; 400 and 600 g  $Mn ha^{-1}$ , in a single application, along with plant adjuvant. The source of Mn was a commercial product with Mn soluble in water of 137.50 g  $L^{-1}$ . For the dose 0 g  $Mn ha^{-1}$ , only water and vegetable adjuvant was applied, for all cultivars and stages. Manganese was applied to leaves when the plants were at stage  $R_1$ , early flowering, and  $R_3$ , beginning of pod formation (Fehr et al., 1971). We used a CO<sub>2</sub> pressurized (2.8 kgf  $cm^{-2}$ ) backpack sprayer

with spray solution consumption of 200 L ha<sup>-1</sup>. During application, the plot was encircled with plastic canvas.

Plants at R<sub>7</sub>, physiological maturity, and at R<sub>8</sub>, full maturity (Fehr et al., 1971), were harvested manually. The material was dried naturally (sun) until the seeds reached moisture content 13% (wet basis). The threshing was performed by a stationary threshing machine (track system consisting of cylinder and concave). For the analyses and determinations, we used seeds retained on circular sieves of 5.5 and 6 mm. A portion of the seeds was separated for the initial analysis of physiological quality and the remaining were packed in paper bags and stored in conventional storage (uncontrolled conditions) for six months, between June and November (mean temperature: 19.1 °C, mean relative humidity: 65.4%).

To assess the quality before and after storage, seeds were subjected to the following tests: **Germination**, according to Brasil (2009) and assessment performed five days after sowing; **Accelerated aging**, with the use of Gerbox plastic boxes adapted with suspended aluminum screen. To each gerbox, we added 40 mL water and a single layer of seeds on aluminum screen. They were then kept in a BOD chamber at 41 °C for 48 hours (Marcos Filho, 1999). After this period, seeds were subjected to germination test; **Seedling emergence**: sowing was performed in plastic trays containing soil + sand at a 2:1 proportion, moistened to 60% retention capacity. Trays were kept in a chamber at 25 °C and 12 hours photoperiod with daily assessments as to the emergence of normal seedlings and final count at 14 days after sowing. We considered the final emergence percentage (%E) and the emergence speed index, ESI (Maguire, 1962); **Electrical conductivity** was measured according to Vieira and Krzyzanowski (1999), using a conductivity meter Digimed CD-21 and results expressed in  $\mu\text{S cm}^{-1} \text{g}^{-1}$ ; **Tetrazolium**, the seeds were placed between moist paper for 16 h at 25 °C and then immersed in tetrazolium solution (2, 3, 5 triphenyltetrazolium chloride) at 0.075%, in which the seeds remained for 3 h at 40 °C in the absence of light. The result was expressed as the percentage of viability, vigor and mechanical damage (at levels 1-8) according to França Neto et al. (1998). Complementarily, we quantified lignin content in the seed coat as proposed by Capeleti et al. (2005), with modifications. In laboratory, four replicates were used for all tests with 50 seeds for each block.

The experiment was a randomized block design (RBD) with three blocks. Treatments were arranged in a factorial 4 x 4 x 2, involving four cultivars of soybean, four doses of Mn, via foliar application, and two stages of application. Statistical analyzes were run separately for the physiological quality before and after storage. Data were subjected to analysis of variance with the aid of the software Sisvar® (Ferreira, 2011), at 5% probability by F-test ( $p < 0.05$ ). When the response variable was significant (F-test), means were compared using the Scott-Knott test at 5%, or was

performed polynomial regressions, with the selection of mathematical models significant at 5%, with the highest coefficient of determination.

### 3. RESULTS AND DISCUSSION

Regarding the physiological quality before storage, the analysis of variance evidenced differences between cultivars for germination, accelerated aging, electrical conductivity, emergence speed and mechanical damage. In relation to doses, differences were found for germination, viability, vigor and mechanical damage, as to the stage of application of Mn a significant difference was detected only for viability. For the interactions, there was a significant effect cultivar\*dose\*stage for emergence speed index and cultivar\*dose for electrical conductivity and incidence of mechanical damage.

For quality after storage, considering the source of variation cultivar a significant effect was observed for germination, emergence speed and electrical conductivity. For the doses, a significant difference was detected only for germination, and for stage of application, no significant effect was observed. The interactions cultivar\*stage and dose\*stage were significant for germination and cultivar\*stage for electrical conductivity.

#### Germination

Before storage, the percentage of germination of seeds of the cultivar Baliza RR, averaging 93%, was higher than the others, which were not different to each other, with values of 90%, 90% and 89% for Celeste, Jataí and Silvânia RR, respectively. Gris et al. (2010) found that seeds of the cultivar Celeste (95%) and Baliza RR (91%) showed germination percentages higher than of Silvânia RR (82%) and Jataí (76%).

With respect to the doses, we observed a quadratic effect (Figure 1a). With the use of 0 g Mn ha<sup>-1</sup> the germination was 88.61%, but with increasing doses there was an increase in the germination of soybean seeds, up to the dose 335 g Mn ha<sup>-1</sup>, which led to the maximum value (91.97%). This emphasizes the importance of mineral nutrition with Mn to obtain high quality seed. Mann et al. (2002) found increases in germination in both modes of application of Mn, on soil and on leaf, with higher efficiency of foliar application, but the same was not observed by Melarato et al. (2002). Conversely, Mondo et al. (2012) registered a negative correlation between Mn content in the soil and germination of soybean seeds.

From the dose 335 g Mn ha<sup>-1</sup>, the use of Mn applied to leaves had a negative (Figure 1a), possibly due to the phytotoxic effect caused by the micronutrient at higher doses.

For germination after storage, in relation to the interaction cultivar\*stage, with the foliar application of Mn at the R<sub>3</sub> stage, seed of the cultivars Celeste and Baliza RR with average germination of 86% and 84%, respectively, were superior to Jataí (82 %) and Silvânia RR (80%). With the application at the R<sub>1</sub> stage, the result was similar, Celeste (88%) and Baliza RR (86%) had higher averages in relation to others, Silvânia RR (80%) and Jataí (75%) differed from each other. This result partially corroborates with that observed before the storage, i.e., the cultivar Baliza RR showed an average higher than the others. Among the stages of application of Mn in each of soybean cultivars, the only difference was detected in the cultivar Jataí, with average superior to the application implemented at R<sub>3</sub> of 82%, compared with the R<sub>1</sub>, with 75%.

The results of the interaction dose\*stage (Figure 1b) indicated a linear effect of Mn doses when applied at R<sub>1</sub> and quadratic at R<sub>3</sub>, on seed germination. When foliar Mn was applied at R<sub>1</sub>, the average germination without using Mn was 79%, while with the use of foliar application of Mn, the germination percentage increased linearly, reaching 86% with 600 g Mn ha<sup>-1</sup> (Figure 1b), validating the influence of Mn in increasing soybean seed germination. Considering the application at R<sub>3</sub>, with 0 g Mn ha<sup>-1</sup> the average value was 79%, but the germination was higher in seeds produced with the use of Mn, reaching 86% and 87% when applied 200 and 300 g Mn ha<sup>-1</sup>, respectively, being the maximum value, 87.5%, provided by the dose of 323.94 g Mn ha<sup>-1</sup>, from this dose, the effects were decreasing (Figure 1b).

One possibility for the differential behavior between R<sub>1</sub>, early flowering, and R<sub>3</sub>, beginning of pod formation, is the interval between the stages of application of foliar Mn and the reserve accumulation in seed. The application at R<sub>3</sub> is closer to seed filling, thus a greater amount of the micronutrient was possibly translocated to the seed, and up to the dose of 323.94 g Mn ha<sup>-1</sup>, the effect was positive on germination and from this, negative (Figure 1b), probably due to the phytotoxic effect caused by greater accumulation

at higher doses. However, with the application at R<sub>1</sub>, the effect was linear, obtaining increases in germination even at higher doses.

Regarding the stages of application according to doses of foliar Mn, significant differences were observed at doses of 200 and 600 g Mn ha<sup>-1</sup>. With 200 g Mn ha<sup>-1</sup>, the average germination observed in R<sub>3</sub>, 87%, was higher than in R<sub>1</sub>, 81%. However, an opposite situation was found with 600 g Mn ha<sup>-1</sup>, the average observed at R<sub>1</sub>, 86%, was higher than that observed in R<sub>3</sub>, 82%.

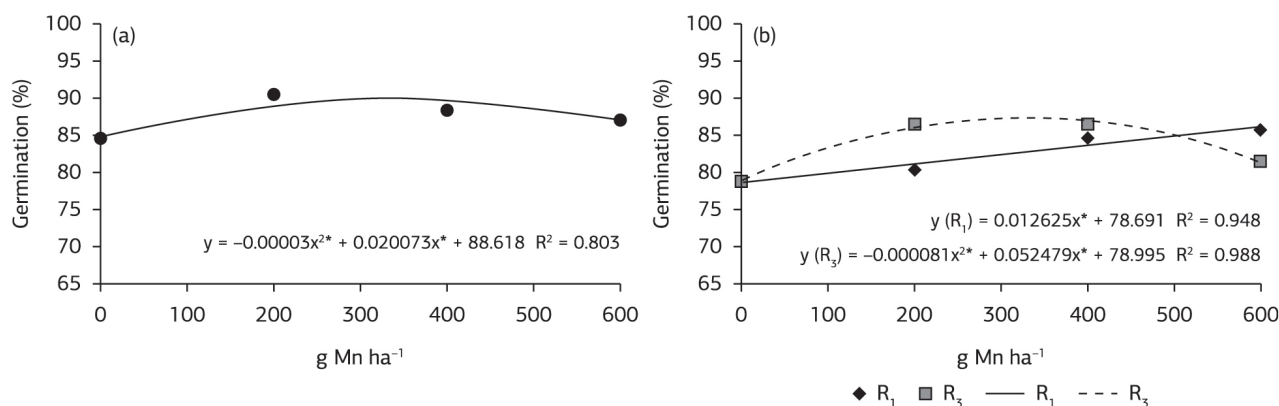
### Accelerated aging

As for vigor before storage, estimated by the accelerated aging test, seeds of the cultivar Baliza RR showed a greater vigor (94%) than the other cultivars which had no differences from each other, with values of 91, 91 and 90% for Celeste, Jataí and Silvânia RR, respectively, which were similar to that of germination. There is a difference in vigor of cultivar seeds, being a characteristic inherent to each genotype. Gris et al. (2010) observed that during germination and in accelerated aging, with delaying harvest, seeds of the cultivars Celeste and Baliza RR obtained higher averages in relation to Jataí and Silvânia RR.

Doses and stages of application of Mn had no influence on seed vigor, estimated by accelerated aging. The same was reported by Melarato et al. (2002), but, Mann et al. (2002) verified a greater vigor with the use of Mn, applied to soil and leaves for the cultivar Garimpo.

### Emergence percentage

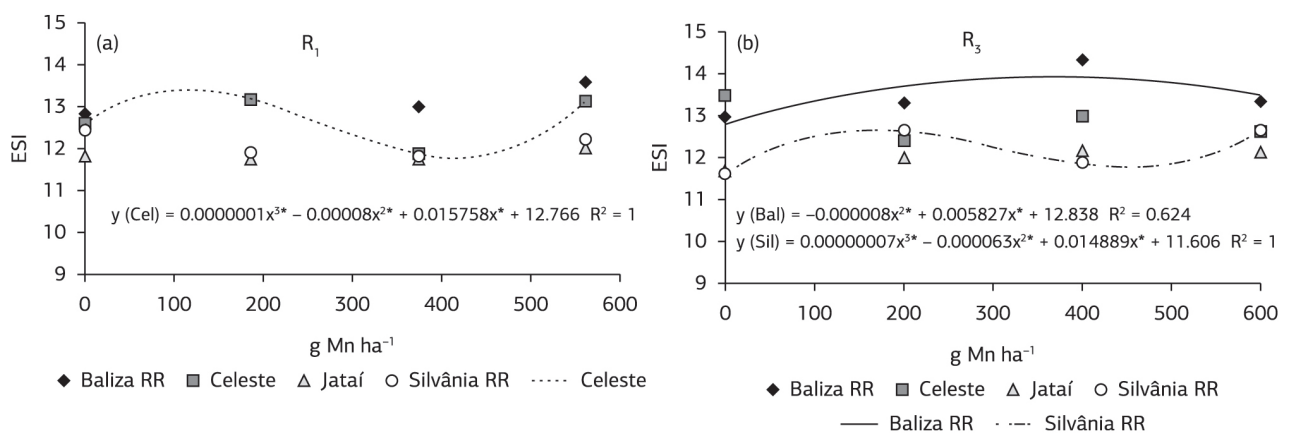
The percentage of seedling emergence was not affected by any of the sources of variation, both before and after storage, with overall averages of 98% and 97%, respectively. Mann et al. (2002) also found no differences between treatments with Mn for emergence percentage.



**Figure 1.** Regression equation for data of soybean seed germination, percentage of normal seedlings at five days, before storage (a) according to doses of foliar Mn and after storage (b) according to doses and stage of application of foliar Mn. \*significant at 5% by F-test.

## Emergence speed index

The emergence speed index (ESI) before storage, figure 2, indicated that foliar application of Mn at the R<sub>1</sub> stage resulted in significant differences only in seedlings of the cultivar Celeste. A cubic model fitted the data; the highest ESI (13.68) was achieved with 130.34 g Mnha<sup>-1</sup>, demonstrating that the application of Mn with doses near this value can increase the seed vigor, since without the use of this micronutrient, the ESI of seedlings of this cultivar was 12.76 (Figure 2a). At the R<sub>3</sub> stage (Figure 2b), the effect of Mn doses was significant for Baliza RR and Silvânia RR, but with different trends. For Baliza RR, the model was quadratic, starting from 12.83 and reaching the maximum ESI, 13.89, with 364.18 g Mn ha<sup>-1</sup>. At R<sub>1</sub>, Mn doses were significant for Celeste and, at R<sub>3</sub>, for Baliza RR, these are descendant cultivars, suggesting a possible relationship of response to Mn conditional on genotype. The effects of Mn were also significant for Silvânia RR seedlings with the application held at R<sub>3</sub>, with cubic polynomial regression. Without the application of Mn, ESI was 11.60, but with the application, it reached the maximum of 12.66 with 162.79 g Mn ha<sup>-1</sup> (Figure 2b).



**Figure 2.** Regression equation for data of emergence speed index (ESI) of soybean seedlings, before the storage, according to doses of foliar Mn applied to seed-producing plants at different stages and in different cultivars.\*significant at 5% by F-test.

**Table 1.** Emergence speed index (ESI) of seedlings of different soybean cultivars, before storage, according to stages and doses of foliar Mn applied to seed producing plants

Stages	Cultivars	Doses of foliar Mn*			
		0	200	400	600
R <sub>1</sub>	Baliza RR	13.01 a	13.38 a	13.21 a	13.85 a
	Celeste	12.76 a	13.37 a	12.01 b	13.35 a
	Jataí	11.96 a	11.86 b	11.92 b	12.21 b
	Silvânia RR	12.60 a	12.03 b	12.00 b	12.36 b
R <sub>3</sub>	Baliza RR	12.97 a	13.27 a	14.31 a	13.34 a
	Celeste	13.45 a	12.39 b	12.97 b	12.62 a
	Jataí	11.71 b	11.98 b	12.15 c	12.12 a
	Silvânia RR	11.60 b	12.62 b	11.88 c	12.65 a

\*Means followed by the same letter in the column are not significantly different at 5% probability by Scott-Knott test.

Higher emergence speed is a relevant component of seed vigor, for providing a rapid seedling establishment. Seeds with higher ESI have better performance and thus greater ability to resist stress that might occur by chance (Dan et al., 2010).

For seedlings of the cultivar Celeste, with the Mn application at the R<sub>1</sub> stage, the lowest ESI (12.66) was provided by 402.99 g Mn ha<sup>-1</sup> (Figure 2). With the application held at R<sub>3</sub>, for Silvânia RR, the minimum ESI (11.92) was achieved with 438.20 g Mn ha<sup>-1</sup>, and for Baliza RR, doses greater than 364.18 g Mnha<sup>-1</sup> caused negative effects on the ESI (Figure 2b). Thus, in general, higher doses, above 400 g Mnha<sup>-1</sup> are unnecessary and have detrimental effect on the vigor of soybeans seeds.

Regarding cultivars depending on the doses and stages of application (Table 1), for application at R<sub>1</sub>, with 0 g Mn ha<sup>-1</sup>, there was no difference among cultivars. Seedlings of the cultivars Baliza RR and Celeste were superior to Jataí and Silvânia RR with 200 and 600 g Mn ha<sup>-1</sup>, with 400 g Mn ha<sup>-1</sup>, the cultivar Baliza RR was superior. In relation to the application at R<sub>3</sub>, with 200 g Mn ha<sup>-1</sup>, seedlings of Baliza RR achieved the highest ESI. This was also observed with 400 g Mn ha<sup>-1</sup>, followed by Celeste, superior to Jataí and Silvânia RR. In general, seedlings of Baliza RR and Celeste

presented ESI values higher than those of Silvânia RR and Jataí.

Taking into account the stages of application according to soybean cultivars and doses of foliar Mn, significant differences were observed for Baliza RR with 400 g Mn ha<sup>-1</sup>, with the ESI with the application at R<sub>3</sub> (14.31) higher than at R<sub>1</sub> (13.21). With this same dose, for Celeste seeds, the average in R<sub>3</sub> (12.97) was higher than in R<sub>1</sub> (12.01), but with 200 g Mnha<sup>-1</sup>, the ESI in R<sub>1</sub> (13.37) was higher than in R<sub>3</sub> (12.39).

After storage, the emergence speed index (ESI) varied among cultivars. The highest value was found for seedlings of the cultivar Baliza RR (14.02), followed by Celeste (13.31), Silvânia RR (12.66) and Jataí (11.96), which differed from each other. These results corroborate that observed before storage, as to the difference in vigor between the genotypes.

## Electrical conductivity

The results of electrical conductivity before storage considering the interaction cultivar\*dose indicated a statistical difference only for Celeste seeds (Figure 3a). The effect was a cubic trend, and the lowest value (46.91 μS cm<sup>-1</sup> g<sup>-1</sup>) was obtained with 120.89 g Mn ha<sup>-1</sup>. For this cultivar, the use of foliar Mn favored the production of high vigor seeds, as also observed for the ESI with the maximum value obtained with 130.34 g Mn ha<sup>-1</sup> (Figure 2a). Melarato et al. (2002) reported lower values of electrical conductivity in treatments with foliar application of Mn.

Seeds of the cultivar Celeste showed higher electrical conductivity at high doses of Mn, with maximum value observed with 536.88 g Mn ha<sup>-1</sup> (Figure 3a). This is related to a lower quality of these seeds at higher doses, once the lowest ESI for this cultivar was found with 402.99 g Mn ha<sup>-1</sup> (Figure 2a). The higher conductivity at higher doses, 400-600 g Mnha<sup>-1</sup>, may be due to an excess of this

micronutrient in leachates, or to a possible disturbance caused by this amount of Mn. These observations may be related to lower quality of seeds obtained at high doses, as seen in the physiological testing.

As for electrical conductivity of seed cultivars depending on the doses, significant differences were observed between the materials for all levels of foliar Mn (Table 2). The cultivar Baliza RR had lower electrical conductivity in all doses, except at 200 g Mn ha<sup>-1</sup>, in which Celeste had the lowest value, followed by Baliza RR, Jataí and Silvânia RR, which were not different from each other. This fact is associated with physiological seed quality of the cultivar Baliza RR, which was generally higher.

Considering the interaction cultivar\*stage (Table 3) after storage, when applications were performed at R<sub>1</sub>, seeds of the cultivar Baliza RR shower greater vigor, with lower electrical conductivity compared with the others, which differed from each other. With the applications at R<sub>3</sub>, the lowest electrical conductivity was also observed in seeds of

**Table 2.** Electrical conductivity (μS cm<sup>-1</sup> g<sup>-1</sup>) of soybean cultivar seeds at different doses of foliar Mn

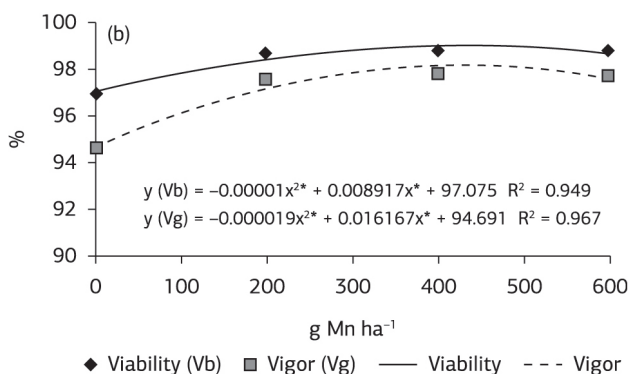
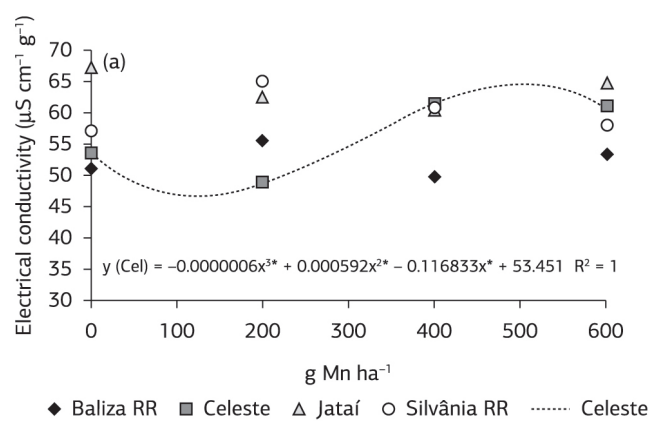
Cultivars	Doses of foliar Mn*			
	0	200	400	600
Baliza RR	50,98 a	55,55 b	49,68 a	53,31 a
Celeste	53,45 a	48,74 a	61,26 b	60,87 b
Jataí	67,07 b	62,41 c	60,28 b	64,72 b
Silvânia RR	57,02 a	64,99 c	60,65 b	57,95 a

\*Means followed by the same letter in the column are not significantly different at 5% probability by Scott-Knott test.

**Table 3.** Electrical conductivity (μS cm<sup>-1</sup> g<sup>-1</sup>) of soybean cultivar seeds produced with the application of Mn at different stages, after storage

Stage	Baliza RR	Celeste	Jataí	Silvânia RR
R <sub>1</sub>	59.48 Aa	70.35 Ba	74.31 Ca	78.69 Da
R <sub>3</sub>	64.87 Ab	70.19 Ba	73.82 Ca	75.96 Ca

\*Means followed by the same uppercase in the row and lowercase in the column are not significantly different at 5%, by Scott-Knott test.



**Figure 3.** Regression equation for data of electrical conductivity (a) and viability (Vb) and vigor (Vg) obtained in the tetrazolium test (b), before storage, according to doses of foliar Mn applied to seed-producing plants. \*significantat 5% by F-test.

the cultivar Baliza RR, differently from Celeste, whose values were lower than those registered for Jataí and Silvânia RR.

In general, as for the difference between cultivars, seeds of the cultivar Baliza RR had lower electrical conductivity values, followed by Celeste, Jataí and Silvânia RR, with higher values (Table 3). This finding corroborates the different physiological qualities of seeds observed between cultivars for germination and ESI. Baliza RR showed better physiological quality of seeds, followed by Celeste, and superior to Jataí and Silvânia RR, demonstrating the relationship between the lowest electrical conductivity, a result of more organized membranes, and thus lower amounts of leachate released into the external environment, and the highest physiological quality of seeds.

Greater vigor of seeds of the cultivars Baliza RR and Celeste in relation to Jataí and Silvânia RR, even after storage, were independent of lignin content in seed coats. This result confirms the relationship of seed physiological quality and factors inherent to each genotype, not related to lignin content in the seed coat.

The only statistical difference between the stages of application in each cultivar, for electrical conductivity, was found in the cultivar Baliza RR, with the value at  $R_1$  lower than in  $R_3$  (Table 3).

According to Vieira and Krzyzanowski (1999), for batches of seeds with great vigor, electrical conductivity should be at maximum  $70-80 \mu\text{S cm}^{-1} \text{g}^{-1}$ , but with a strong tendency to present medium vigor. In the present work, in all cultivars, the values obtained were below  $80 \mu\text{S cm}^{-1} \text{g}^{-1}$ , even after storage, but it is interesting to note that in the cultivar Silvânia RR, whose seeds had lower vigor, values were near  $80 \mu\text{S cm}^{-1} \text{g}^{-1}$  after storage (Table 3), which agrees with the values observed for Jataí. This supports the observation that seeds with electrical conductivity between  $70$  and  $80 \mu\text{S cm}^{-1} \text{g}^{-1}$  have a strong tendency to medium vigor. For seeds of Baliza RR, whose vigor was high, electrical conductivity before storage did not exceed  $55.55 \mu\text{S cm}^{-1} \text{g}^{-1}$  and after storage  $65.00 \mu\text{S cm}^{-1} \text{g}^{-1}$ , suggesting that values within or below this range may be associated only with high vigor seed.

## Tetrazolium

Figure 3b shows the results of viability and vigor obtained in the tetrazolium test before storage. Regarding the dose of Mn, the model that best fit the data, both for viability and vigor, was the second degree model. The maximum values of viability and vigor were 99.06% and 98.13%, respectively, with doses 445.85 and 425.44  $\text{g Mn ha}^{-1}$ . These maximum viability and vigor depending on the Mn doses were not related to the other characteristics, where, in general, doses above  $400 \text{g Mn ha}^{-1}$  did not improve the physiological quality of seeds.

We observed a steeper slope of the curve between 0 and  $200 \text{g Mn ha}^{-1}$ , indicating a larger difference between these doses, both to viability and vigor (Figure 3b). From  $200 \text{g Mn ha}^{-1}$ , slope was less marked due to minor differences between the other doses. The slope between 0 and  $200 \text{g Mn ha}^{-1}$  was more prominent as to the vigor in relation to viability. The increased seed vigor using this dose of foliar Mn is correlated to that observed in other vigor tests, such as ESI and electrical conductivity, besides the results of Mann et al. (2002).

For viability, no significant difference was detected between the stages of application, the value observed in  $R_3$ , 98.70%, was higher than in  $R_1$ , 97.87%. Regardless of the stage, seeds showed high viability.

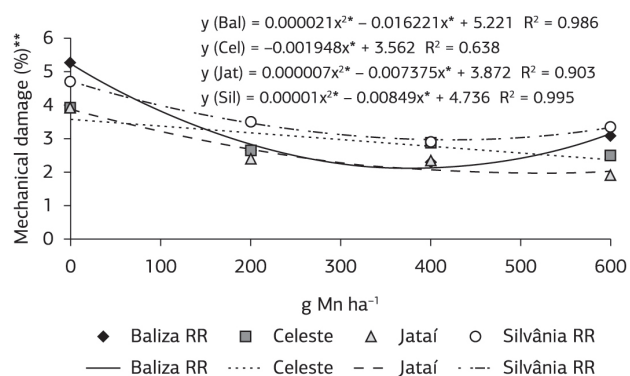
It was not possible to set a relationship for the difference in seed quality between conventional cultivar and its glyphosate-resistant (RR) transgenic descendant, as Carvalho et al. (2012) found.

## Mechanical damage

The results of mechanical damage estimated by the tetrazolium test, referring to the interaction cultivars\*doses (Figure 4), pointed out a significant effect of doses on all cultivars, with a quadratic effect for Baliza RR, Jataí and Silvânia RR, and a linear effect for Celeste. In the absence of foliar Mn, in the seeds of the cultivar Baliza RR, the incidence of mechanical damage was 26.24%, but with the use of foliar Mn, the values reduced. With  $200 \text{g Mn ha}^{-1}$ , the value dropped to 6.89%, and the minimum value (3.32%) was found with  $386.21 \text{g Mn ha}^{-1}$ . A similar behavior was registered for Jataí and Silvânia RR, whose values started from 13.97% and 21.37%, respectively, with the application of Mn, and reached 6.12% and 10.76%, with the dose of  $200 \text{g Mn ha}^{-1}$ , and minimum incidence of 2.72% and 7.58%, with 526.78 and  $424.5 \text{g Mn ha}^{-1}$ , respectively. It is noteworthy that mechanical threshing favors the incidence of mechanical damage.

Greater steepness of the curves was found between 0 and  $200 \text{g Mn ha}^{-1}$  due to a larger difference between these doses for Baliza RR, Jataí and Silvânia RR, and a less marked slope between subsequent doses. For the cultivar Celeste, we verified a linear effect, i.e., with the highest dose of foliar Mn, the lower the incidence of mechanical damage (Figure 4), thus indicating the effect of foliar application of manganese on the incidence of mechanical damage in soybean seeds. This fact possibly influenced the increments of germination and vigor previously reported with the use of this micronutrient.

Among the cultivars, the highest averages of mechanical damage were observed in Silvânia RR seeds (Table 4), which contributed to the impairment of quality, since, in general, the seeds of this cultivar had lower quality, as previously discussed.



**Figure 4.** Regression equation for data of mechanical damage observed in soybean seeds in the tetrazolium test (1-8) according to doses of foliar Mn applied to seed-producing plants of different cultivars.\*significant at 5% by F-test. \*\*transformed into  $\sqrt{x+1}$ .

**Table 4.** Percentage of mechanical damage in soybean cultivar seeds at different doses of foliar Mn, verified by the tetrazolium test

Cultivars	Doses of foliar Mn*			
	0	200	400	600
Baliza RR	27.00 b	6.33 a	5.00 a	8.66 b
Celeste	14.33 a	6.66 a	7.33 a	6.33 a
Jataí	15.00 a	5.00 a	5.33 a	2.66 a
Silvânia RR	21.66 b	11.66 b	7.66 a	10.33 b

\*Means followed by the same letter in the column are not significantly different at 5% by Scott-Knott test. Raw mean values are presented, but data were compared with transformed data  $\sqrt{x+1}$ .

### Lignin content in the seed coat

The average content of lignin in the seed coat differed among cultivars, being higher in Silvânia RR with 0.5954 g%, followed by Jataí with 0.5364 g%, Baliza RR with 0.4171 g% and Celeste with 0.3132 g%.

The cultivar with the highest lignin content in the seed coat, Silvânia RR, showed, in general, a higher incidence of mechanical damage (Table 4). In the cultivar Celeste, with the lowest lignin content, the incidence of mechanical damage in seeds was always among the lowest. Nevertheless, regarding the relationship between lignin content in the seed coat and resistance to mechanical damage, Capeleti et al. (2005) found a direct relationship between these two characteristics, suggesting that lignin content in the seed coat above 0.4 g% may be a reasonable indicator of resistance to mechanical damage, but they estimated this resistance by means of the pendulum test, this test proposed by Carbonell and Krzyzanowski (1995).

Considering the direct relationship between lignin content in the seed coat and physiological quality of soybean seeds, it was not observed in the present study, because the cultivar that presented the highest content of lignin, Silvânia RR

(0.5954 g%), showed the lowest physiological quality of seeds, in general, both before and after storage. In the cultivar Baliza RR, which had the seeds of better quality before and after storage, the lignin content was 0.4171 g%, lower than that found in Silvânia RR and Jataí. In this way, only the lignin content in the seed coat is not enough to infer the quality of soybean seeds of a given cultivar. The quality is thus influenced by other factors inherent to the genotype, but, additional studies are necessary to prove this statement.

Meantime, other studies have reported an association between high physiological quality of soybean seeds with high lignin content in the seed coat, like Panobianco et al. (1999) and Santos et al. (2007). Menezes et al. (2009) found positive correlations between the lignin content and the percentage of normal seedlings in the aging test, and a negative correlation for germination speed.

In this way, Panobianco et al. (1999) verified higher values of electrical conductivity in soybean seeds with low lignin content in the seed coat. Nevertheless, this relationship was not observed herein, since the seeds of the cultivars Baliza RR and Celeste, whose electrical conductivity values were lower, also showed lower content of lignin in relation to Jataí and Silvânia RR.

The high lignin content in the seed coat may not have been related to the high quality of seeds, because in the present study, the harvest was made when plants were between R<sub>7</sub>, physiological maturity, and R<sub>8</sub>, full maturity, thus not suffering from stress that could occur with a late harvest, like rainfall events in the pre-harvest, considering that one of the characteristics of lignin is the impermeability (McDougall et al., 1996). Meanwhile, even with a delayed harvest (R<sub>8</sub> + 20), Gris et al. (2010) found higher physiological quality in seeds of the cultivars Baliza RR and Celeste compared with Silvânia RR and Jataí, which strengthens the relationship between physiological quality and genotype, previously reported.

### 4. CONCLUSION

The foliar application of Mn provides increased physiological quality of soybean seeds produced.

Seeds of the soybean cultivars BRS Celeste and BRS Baliza RR show better physiological quality compared with those of BRSGO Jataí and BRS Silvânia RR.

Seeds of the soybean cultivars with higher lignin content in the seed coat do not necessarily have better physiological quality, thus seed quality is related to other factors intrinsic to the genotype.



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