Physiological quality of soybean seeds treated with different spray volumes¹

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ABSTRACT - The aim of this study was to define the influence of treatments with different seed spray volumes and their effects on the physical and physiological quality. The soybean seeds from cultivars NA 5909 RG and BMX Potência RR were used and three lots of each cultivar were selected in sieves of different diameters (5.5; 6.0 and 6.5 mm). The treatments consisted of different seed volumes: 1) non-treated seeds; 2) 600 mL.100 kg of seeds⁻¹; 3) 800 mL.100 kg of seeds⁻¹; 4) 1000 mL.100 kg of seeds⁻¹; 5) 1200 mL.100 kg of seeds⁻¹; 6) 1400 mL.100 kg of seeds⁻¹. The physical parameters evaluated were mechanical damage, water content and variations in the length and width of the seeds. The physiological seed quality was determined by germination, first count germination, length and dry weight of seedlings, field emergence and emergence speed index tests. In terms of use of the product in liquid form, it is possible to use the treatment in spray volumes up to 1400 mL.100 kg seed⁻¹, regardless the cultivar or seed size and without damaging the soybean seeds physically and physiologically.

Index terms: Glycine max L., seed treatment, seed size, germination.

Parâmetros físicos e qualidade fisiológica de sementes de soja tratadas com diferentes volumes de calda

RESUMO - O objetivo do trabalho foi determinar a influência de diferentes volumes de calda, via tratamento de sementes, nos parâmetros físicos e na qualidade fisiológica de sementes de soja. Foram utilizadas as cultivares NA 5909 RG e BMX Potência RR, sendo três lotes de cada cultivar, os quais foram classificados em peneiras de diferentes diâmetros (5,5; 6,0 e 6,5 mm). Os tratamentos consistiram na utilização de diferentes volumes de calda: 1) testemunha sem tratamento; 2) 600 mL.100 kg de sementes⁻¹; 3) 800 mL.100 kg de sementes⁻¹; 4) 1000 mL.100 kg de sementes⁻¹; 5) 1200 mL.100 kg de sementes⁻¹; 6) 1400 mL.100 kg de sementes⁻¹. Os parâmetros físicos avaliados foram dano mecânico, teor de água e variações no comprimento e largura das sementes. A qualidade fisiológica das sementes foi determinada pelos testes de germinação, primeira contagem da germinação, comprimento e fitomassa seca de plântulas, emergência a campo e índice de velocidade de emergência. Em condições de utilização dos produtos em forma líquida, independente da cultivar ou do tamanho da semente, é possível utilizar o tratamento em volumes de calda de até 1400 mL.100 kg de sementes⁻¹, sem que ocorram danos físicos e fisiológicos às sementes de soja.

Termos para indexação: Glycine max, tratamento de semente, tamanho de sementes, germinação.

Introduction

Soybean is the main oil seed cultivated worldwide and its production stands out in the national scenario, being one of the most important commodities of the Brazilian agribusiness. The use of soybean in the biofuel, oil and food industries requires productivity developments.

The soybean yield potential may be affected by several factors, including the attack of plagues and microorganisms. The use of phytosanitary products, such as fungicides and

insecticides applied in the seeds, guarantees the protection of seeds and seedlings in the early stage of germination (Ludwig et al., 2011; Pereira et al., 2011).

Thus, the seed treatment is a widespread technology, considering that, for soybeans, the volume of seeds treated with fungicides is approximately 95% (Henning et al., 2010). Several products have been applied directly in the seed, aiming at protecting them against fungus and bugs, diseases transmitted by seeds, micronutrients, growth regulators, inoculants, besides the using polymers for a greater adherence

¹Submitted on 05/02/2013. Accepted for publication on 08/19/2013.

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of products (Ávila et al., 2008; Avelar et al., 2011; Ludwig et al., 2011; Dourado-Neto et al., 2012). This variety of products causes the spray volume to be higher than 600 mL.100 kg⁻¹ of seeds. This value is indicated as maximum tolerated volume of liquid so there is no damage, such as rupture of the soybean seed skin, which may impair the germination and the physical properties of the seeds (Embrapa, 2011).

Nowadays, products used for the seeds treatment contain liquid formulas with greater permeation potential. Therefore, explanatory studies indicate the possibility of using larger spray volumes without the production of negative effect of the physiological performance of the seeds (Krzyzanowski et al., 2007).

The seeds vulnerability to damages occurred due to a larger use of spray volume in the seeds treatment may be influenced by factors such as their physiological quality, and also, the variations in seed sizes. According to a study conducted by Beckert et al. (2000), the water absorption is deeper in smaller soybean seeds; that may be related with their physiological potential. A faster water absorption process may cause damage due to impregnation. The classification of soybean seeds according to their sizes after the cleaning process has been adopted by several producers because it improves the sowing process (Camozzato et al., 2009).

Thus, this study aims at defining the influence of different spray volumes, through the seeds treatment, on the physical characteristics and the physiological potential of soybean seeds classified in sieves of different diameters.

Material and Methods

The research was held in Seed Laboratory of the Plant Science Department at Universidade Federal de Santa Maria. Two soybean cultivars were used, NA 5909 RG and BMX Potência RR, harvested on 2011/2012 and cultivated in the region of Santa Maria (RS). The lots of each cultivar were classified in sieves of three different sizes, 5.5; 6.0 and 6.5 mm. The seed quality was characterized based on the following tests:

Water Content: defined by oven method at 105 ± 3 °C, during 24 hours, with three subsamples from each lot, as recommended in the Rules for Seed Testing (Brasil, 2009).

Weight of one thousand seeds: eight subsamples of 100 seeds were used. The result was expressed in grams in accordance with the Rules for Seed Testing (Brasil, 2009).

Mechanical damage: the sodium hypochlorite test was performed, with four replications of 50 seeds for each cultivar and sieve size. The seeds were immersed in a solution with 5.25% sodium hypochlorite during 10 minutes; after that, the

number of swollen seeds was calculated. The results were expressed in percentage of swollen seeds or mechanically damaged seeds (Krzyzanowski et al., 2004).

Germination: four replications of 100 seeds were sowed over two towel paper sheets, covered with a third sheet and wrapped up. The germination paper was moistened with distilled water at a ratio of 2.5 times the weight of the dry paper. The rolls were kept in a germinator at 25°C. The evaluations were made five and eight days after the seeding, as established by Brasil (2009). The results were expressed in percentage of normal seedlings.

Field emergence: it was conducted with four replications of 100 seeds, sowed in 2.0 m long rows, with 0.20 m between them at 0.03 m depth. The evaluation of the seedling emergence percentage was made seven days after sowing.

Emergence speed index: in order to establish this characteristic, the seedlings emerged after the emergence tests were counted daily. The index was calculated with the equation described by Maguire (1962) and mentioned by Nakagawa (1994): IVE = $E_1/N_1+E_2/N_2+...E_n/N_n$, where IVE = emergence speed index; E_1 , E_2 e E_n = number of seedlings emerged on the first, second and umpteenth counting; N_1 , N_2 e N_n = number of days.

Tetrazolium test: four replications of 50 seeds for each cultivar and sieve size were used, in accordance with the method described by França-Neto et al. (1998). The samples were placed on paper towels, dampened with distilled water in a quantity that was 2.5 times more than their weight, during 16 hours at 25 °C. After that, the seeds were placed in plastic containers and kept in a solution with 0. 075% of chloride of 2, 3, 5 trifeniltetrazolium, at 40 °C in the dark, for 150 minutes. After this period, the seeds were washed in running water and analyzed one by one. The number of vigorous (vigour) seeds, included in classes 1 to 3, and the number of seeds that were likely to germinate (viability), included in classes 1 to 5, was accounted. The results were expressed in percentage of viability and vigor.

After the chacarcterization of the seeds quality, they were submitted to treatment with different spray volumes.

Initially, in order to obtain different spray volumes, the maximum volume was specified and the other volumes were obtained from it, by a reduction of the doses de bio-stimulating and by the removal of products that had not presented effect on the physiological seed quality in the laboratory, such as inoculants, micronutrients and liquid polymers (Pereira et al., 2009).

The products used were: fungicide Carbendazim + Thiram (Derosal Plus®); insecticide Imidacloprido + Tiodicarbe (Cropstar®), liquid fertilizer molybdenum (15%) and Cobalt (1.5%) (Agromix®), liquid polymer (Polifix Laborsan®), liquid inoculants with approximately 3 x 109 feasible cells

by millimeter (Nitragin Cell Tech HC®) and soluble biostimulating, cytokine 0.009% + gibberellin 0.005% + auxin

0.005% (Stimulate®). The respective dosages used are described in Table 1.

Table 1. Products used for the soybean seeds treatment and respective dosages, for attainment of different spray volumes. Fungicide (F), insecticide (I), bio-stimulating (B), micronutrient (M), polymer (P) e inoculant (I).

	F	I	В	M	P	I	Water	Total
Treatments	-			Dosages	s (mL. 100 kg	g ⁻¹)		
1	-	-	-	-	-	-	-	0
2	200	200	200	-	-	-	-	600
3	200	200	300	-	-	100	-	800
4	200	200	400	-	100	100	-	1000
5	200	200	400	200	100	100	-	1200
6*	200	200	400	200	100	100	200	1400

^{*}For the sixth treatment, the spray volume was complemented with 200 mL of water.

The seeds treatment had the following order: fungicide, insecticide, bio-stimulating, micronutrient, polymer and inoculants. It was made in 3 liter plastic bags, with 500 grams of seeds in each bag.

After the treatments, the seeds were submitted to germination, water content, field emergence and emergence speed index tests, as previously described, besides the additional specifications, described below, in order to verify the effect of spray volume on the physiological quality of seedlings.

Seedling length: four subsamples with 15 seeds were sowed on the upper part of the towel paper. The paper rolls were moistened with distilled water in a ratio of 2.5 times the weight of the dry paper, and then kept in a germinator at 25 °C. The evaluations were made seven days after the sowing, and the upper part, roots and total length of ten normal seedlings were measured. The results were expressed in cm.seedling⁻¹ (Nakagawa, 1999).

Dry weight of seedlings: the cotyledons of each seedling resultant from the evaluation of the seedlings length test were removed. After that, the seedlings were packed in paper bags and put to dry in an oven at 80 °C, during 24 hours. After this period, the samples were weighed in order to define the total dry weight of seedlings. The result was expressed in mg.seedling⁻¹ (Nakagawa, 1999).

Seeds length: measured with a digital caliper rule, in the embryo direction, with four replications of 25 seeds for each treatment. The results were expressed in milimiter (mm).

Seeds width: measured with a digital caliper rule, on the central part of the seed, perpendicular to the length measure, with four replications of 25 seeds for each treatment. The results were expressed in mm.

All the evaluations were made completely random desing. For the initial classification of the seeds, the treatments were organized in a factorial 2 x 3 (cultivars x sieve sizes), the variables were submitted to variance analyses and the means

were compared by the Scott Knott test at 5% of significance, with the software Sisvar (Ferreira, 2008). For the spray volume effect, the treatments were organized in a factorial 2 x 3 x 6 (cultivars x sieve size x spray volume) with four replications. The variance analysis was made by software Action, the means comparison with non-treated seeds was made by the Dunnett test, with 5% of probability. Additionally, the regression equations of the variables were adjusted, having as initial dosage the standard recommendation (600 mL.100 Kg⁻¹ of seeds), with the aid of the statistical program Sisvar.

Results and Discussion

By the initial classification of the seed lots (Table 2), it is possible to note that for both cultivars, the 5.5mm sieve presented a low germination percentage, even though they seemed feasible after the tetrazolium test. Such seeds did not reach the minimum percentage required for the commercialization of soybean seeds in Brazil, which is 80% (Brasil, 2005). Although the studies performed so far have presented conflicting results regarding the relation between seeds size and physiological quality, unfavorable climate conditions of the seeds production region during the harvest of 2011/2012 need to be considered. Thus, the seeds from the 5.5 mm sieve may present bigger problems during the maturation and reserves accumulation, causing a reduction in their physiological quality.

The soybean seeds from 6.0 and 6.5 mm sieves presented higher germination values for both cultivars (Table 2). These results are in agreement with Pádua et al. (2010), who observed the physiological quality differences regarding the seeds size; bigger seeds present higher germination and vigor percentages. The bigger or heavier seeds usually hold embryos that are steadily grown and have more reserves, being potentially more vigorous (Carvalho and Nakagawa, 2000).

Table 2.	Initial classification of the soybean seeds quality from two cultivars, classified in sieves of different diameters (mm).
	Median values of the results of water content (WC%) weight of one thousand seeds (OTS, g), mechanical damage (MD%),
	germination (G%), field emergence (FE%), emergence speed index (ESI), Viability (VIAB%) and vigour (V%).

Cultivar	Size	WC	OTS	MD	G	FE	ESI	VIAB	V
	5.5	11.14 a	127.99 с	4 a	57 c	57 a	9.26 a	90 a	80 b
NA 5909 RG	6.0	10.77 b	153.72 b	15 b	87 b	46 b	6.94 b	94 a	88 a
	6.5	10.35 c	180.64 a	15 b	94 a	55 a	8.67 a	92 a	76 b
	5.5	11.81 a	110.15 c	2 a	69 b	78 a	13.18 a	96 a	90 a
BMX Potência RR	6.0	11.06 b	139.29 b	4 b	93 a	78 a	12.52 a	100a	94 a
	6.5	10.78 c	170.42 a	6 b	96 a	71 b	11.53 a	95 a	96 a

^{*}Measures followed by the same letter in the column do not differ inwardly by the Scott & Knott test, p>0,05.

On the other side, this physiological quality variation among the different lots is important for the results obtained from different treatments to be used in lots with vigor level variations.

The water content values were similar among the cultivars and the sizes (Table 2). Even though there was a statistical difference, they were not higher than 1%, which does not wield influence over the other variables analyzed.

Regarding the weight of one thousand seeds, significant differences were observed among the sieve sizes. The heavier seeds were removed in the 6.5 mm sieve, followed by the seeds with 6.0 mm and 5.5 mm diameters, respectively. The relation between the size and weight observed was as expected; however, several aspects are responsible for this characteristic, such as environmental conditions and genetic differences (Camozzato et al., 2009; Egli, 2012).

The evaluation of mechanical damages in the seeds by the sodium hypochlorite test showed that less damage percentages were found in smaller sieves for both cultivars (Table 2). Also, in cultivar NA 5909 RG, the seeds from 6.0 and 6.5 mm sieves presented more than 10% of mechanical damage. Such values are considered to be critical (Krzyzanowski et al., 2004), which may significantly reduce the performance of soybean seeds (Obando Flor et al., 2004). Bigger seeds are more likely to suffer mechanical damage, especially during the mechanical harvest. Besides that, different results among cultivars are expected due to the physical characteristics of each cultivar. That explains the use of more than one cultivar in the studies.

The tetrazolium test indicated higher physiological quality of seeds from the 6.0 mm sieve, in cultivar NA 5909 RG. For cultivar BMX Potência RR there were no differences in the vigor and feasibility of seeds considering the sieve sizes evaluated.

Tables 3 and 4 show the physiological quality data of the seeds that were submitted to different spray volumes, compared with the non-treated seeds. Regardless the cultivar or the seed sizes, the treatments with different spray volumes not only did not affect the initial development of the seedlings negatively, but also improved most physiological characteristics analyzed (germination, first count germination, length and dry weight of seedlings), mainly in low quality lots (5.5 mm sieves).

The seeds treatment protects the seeds and their seedlings against diseases and bug plagues, maintaining the physiological and sanitary quality of the seed during the storage (Avelar et al., 2011), contributing to a proper initial establishment and the production increase (Gadotti et al., 2012). Moreover, Balardin et al. (2011) observed that treating seeds with insecticides and fungicides benefits the plants, increasing their tolerance to hydric stress, with a positive effect over the soybean yield potential.

Except for the non-treated seeds, in the other treatments the percentage of normal seedlings in the germination was higher, which means the treatment with larger spray volumes did not cause a phytotoxic effect on the seeds and no abnormalities occurred (Tables 3 e 4). According to Sediyama et al. (2012), the soaking of seeds during the germination in liquids, other than water, promotes a slow hydration, allows more time for membranes repair and rearrangement, lowers the lixiviation of solution by the seed and the risks of damage in the embryo caused by a fast soaking, promoting a significant influence on the seeds germination and vigor percentage.

Considering the field emergence, the treated seeds presented, mostly, a greater performance when compared with the non-treated seeds. According to Lima et al. (2006), the germination test is performed under ideal conditions of temperature and humidity unlike the field emergence. As Goulart (1998) describes, the soybean seed begins its germination process and emerges quickly on soils with adequate temperature and humidity. Whenever such conditions are not met, the process is slower, which causes the soil and seed funguses to attack and ruin the seeds or even kill the seedling. That explains the difference between the germination and field emergence test results.

Table 3. Means of cultivar NA 5909 RG for germination (G%), upper part length (UPL, cm), root length (RL, cm.seedling⁻¹) and total length (TL, cm.seedling⁻¹), dry weight of seedlings (DW, g.seedling⁻¹), field emergence (FE%) and emergence speed index (SEI), evaluated in three seed sizes and six spray volumes.

Size	Volume	G	UPL	RL	TL	DW	FE	SEI
	0	57	11.21	13.96	25.18	0.261	38	5.81
	600	88*	13.21	16.39*	29.60*	0.281	70*	11.4*
5.5	800	77*	12.64	17.88*	30.52*	0.305	61*	9.79*
3.3	1000	88*	11.55	18.47*	30.02*	0.270	53	8.89
	1200	92*	12.21	19.52*	31.73*	0.282	56*	8.30
	1400	95*	11.72	18.77*	30.49*	0.312	67*	11.4*
	0	87	9.87	16.74	26.61	0.338	43	6.91
	600	94*	11.44	17.47	28.91	0.351	65*	9.84
6.0	800	90	8.73	19.37	28.10	0.328	47	7.51
6.0	1000	91	6.72	14.53	21.25	0.306	48	6.64
	1200	92*	7.71	14.99	22.71	0.352	38	5.96
	1400	92*	10.40	17.84	28.25	0.340	36	4.81
	0	94	10.81	17.59	28.40	0.351	45	6.97
	600	94	9.76	15.63	25.39	0.337	50	7.29
(5	800	94	8.09*	11.37*	19.46*	0.296*	59	9.91
6.5	1000	91	11.31	14.58*	25.89	0.349	69	10.11
	1200	93	8.29*	14.16*	22.46*	0.309*	58	9.55
	1400	96	7.50*	11.97*	19.47*	0.288*	51	8.18

^{*}Significant with 5% of overshoot probability by Dunnett's test.

Table 4. Means of cultivars BMX Potência RR for germination (G%), upper part length (UPL, cm), root length (RL, cm.seedling¹) and total length (TL, cm.seedling¹), dry weight of seedlings (DW, g.seedling¹), field emergence (FE%) and emergence speed index (SEI), evaluated in three seed sizes and six spray volumes.

Size	Volume	G	UPL	RL	TL	DW	FE	SEI
	0	69	12.94	16.68	29.63	0.29	89	15.5
	600	84*	13.30	15.99	29.29	0.32	76	13.7
5.5	800	91*	11.16	16.74	27.90	0.29	76	13.1
5.5	1000	87*	12.74	16.64	29.38	0.30	75	12.5
	1200	93*	14.09	16.20	30.29	0.29	76	12.6
	1400	95*	12.96	17.40	30.36	0.27	76	11.8
	0	94	10.52	16.83	27.36	0.37	80	14.2
	600	96	7.01*	17.37	24.39	0.29*	77	10.6
(0	800	94	10.76	16.25	27.01	0.36	91	15.1
6.0	1000	95	10.18	17.58	27.77	0.35	80	12.2
	1200	94	7.08*	17.61	24.69	0.31*	74	12.5
	1400	92	15.06*	15.41	30.48	0.40	69	10.7
	0	96	11.69	16.79	28.48	0.37	77	13.9
	600	95	10.50	13.75*	24.25*	0.34	75	10.9
(5	800	94	13.08	15.00	28.09	0.39	72	11.3
6.5	1000	94	10.28	17.81	28.09	0.34	76	11.4
	1200	91*	11.25	12.82*	24.07*	0.34	65	11.1
	1400	94	11.74	14.87	26.61	0.36	62	10.7

^{*}Significant with 5% of overshoot probability by Dunnett's test.

Based on these results, it is possible to infer that for liquid products it is possible to use up to 1400 mL of spray volume for the treatment of 100 kg of soybean seeds without affecting

their physiological performance negatively, in laboratory as well as field studies. In this case, the evaluations refer to the treatment held before the sowing, without the storage aspect into consideration. These data are compatible with the one observed by Krzyzanowski et al. (2007), in a soybean seed research that enabled the complete treatment, with high spray volumes for evaluations in high and low vigor lots inside the laboratory or the greenhouse.

Regarding the seeds humidity (Table 5), for both cultivars and sieve sizes, the water content increased around 1% in the spray volumes considered. That shows the

treatments tested did not cause damage to this parameter, for, even with this increase, the seeds humidity remained under 13%, which is considered adequate for storage and sowing, without mechanical damages due to crush. On the other hand, in this study, the initial water content of the seeds was around 11% (Table 2). Whenever the initial humidity is close to 13%, the humidity increase may not be safe, especially for storage.

Table 5. Means of cultivars NA 5909 RG and BMX Potência RR for the characteristics of water content (WC, %), width (W, mm.seedling⁻¹) and length (L, mm.seedling⁻¹), evaluated in three seed sizes and six spray volumes.

Gi		NA 590	BMX Potênc	BMX Potência RR			
Size	Volume	WC	W	L	WC	W	L
	0	11.14	5.57	6.42	11.80	5.69	7.36
	600	11.30	5.55	6.51	12.09*	5.58	7.24
5.5	800	11.21	5.64	6.41	12.24*	5.63	7.34
3.3	1000	11.40	5.60*	6.46	12.07*	5.48	7.11
	1200	11.35	5.67*	6.49	12.06*	5.51	7.28
	1400	11.49	5.68*	6.66	12.26*	W 5.69 5.58 5.63 5.48	7.30*
	0	10.77	6.09	6.95	11.06	6.49	7.55
	600	11.19*	6.18*	6.88	11.58*	6.21	7.59
6.0	800	11.46*	6.14*	6.96	11.48*	6.18	7.54
6.0	1000	11.57*	6.17*	6.84	11.35*	6.20	7.57
	1200	11.54*	6.18*	6.86	11.01	6.21	7.60
	1400	11.70*	6.20*	6.93	11.21	W 5.69 5.58 5.63 5.48 5.51 5.48 6.49 6.21 6.18 6.20 6.21 6.20 6.56 6.59 6.64 6.61 6.64	7.61
	0	10.35	6.52	7.40	10.78	6.56	7.72
	600	10.46	6.51	7.31	11.15*	6.59	7.82
(5	800	11.02*	6.60	7.33	11.47*	6.64	7.96*
6.5	1000	11.20*	6.64	7.62	11.29*	6.61	7.90
	1200	11.16*	6.58	7.29	11.25*	6.64	7.81
	1400	11.15*	6.55	7.33	11.18*	6.57	7.79

^{*}Significant with 5% of overshoot probability by Dunnett's test.

Working with soybean seeds, Pereira et al. (2011) did not detect any relevant difference between the humidity degree of seeds treated with fungicides and polymer and the non-treated seeds. This effect may be related with the reestablishment of the seeds hygroscopic balance with the relative air humidity. Regarding the seeds width, for the higher spray volumes in some cases, the treated seeds presented greater width than non-treated seeds (Table 5). However, the values did not surpass the next sieve size tested. That means there is no need to change the seeder disc to sow the seeds treated with different spray volumes.

The regression analysis graphs (Figures 1 and 2) show that for most variables, no equation degree was in accordance with the data observed, considering as standard the maximum spray volume recommended (600 mL) (Embrapa, 2011). So, there was no difference between the spray volumes testes related with the physiological quality of the seeds. For the variables with adjustment, it was possible to note a quadratic behavior, without a behavior trend. Thus, it is not possible to say that increasing the spray volume based on the standard dosage (600 mL by 100 Kg of seeds) may improve or spoil the seeds quality.

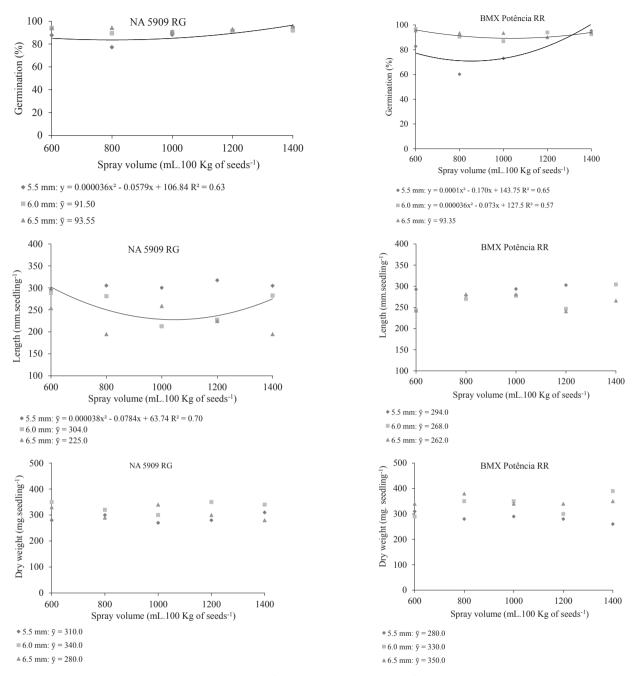


Figure 1. Germination (%), total length (mm.seedling⁻¹), dry weight (mg.seedling⁻¹) of cultivar NA 5909 RG and BMX Potência RR seeds, classified in three sieve sizes, submitted to five spray volumes in the seeds treatment (mL.100 Kg⁻¹ of seeds).

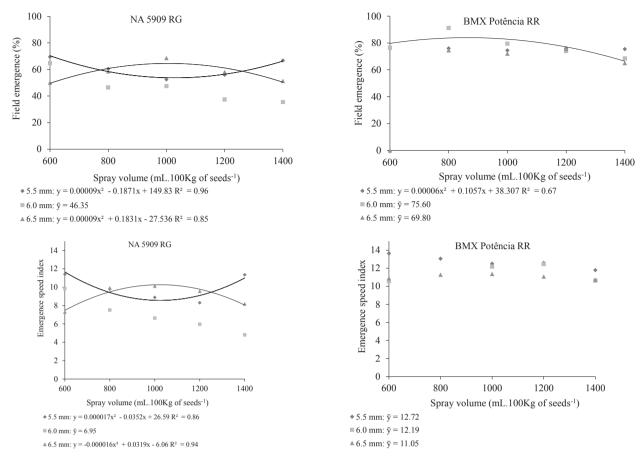


Figure 2. Field emergence (%) and emergence speed index of cultivar NA 5909 RG and BMX Potência RR seeds, classified in three sieve sizes, submitted to five spray volumes in the seeds treatment (mL.100 Kg⁻¹ of seeds).

Conclusion

For the product in liquid form, regardless the cultivar or seed size, it is possible to use the treatment in spray volumes up to 1400 mL per 100 kg of seeds, without causing physical and physiological damage to the soybean seeds.

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