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Relationship between size and physiological potential of soya bean seeds under variations in water availability

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Abstract

This research was performed to evaluate the effect of size grading on the physiological potential of soya bean seeds and the influence of water availability on the germination of different size classes. Two seed lots of 'M7739IPro' were classified by thickness in metal sheet screens with rectangular openings. The seed size classes (control and seeds held in the M + 0.8 mm, M, M - 0.8 mm and < M - 0.8 mm openings, M representing medium seed size) were evaluated by germination, vigour (saturated salt accelerated aging, seedling growth, seedling computerised image analysis and tetrazolium staining), seedling emergence and 1000 seed weight tests. The imbibition and germination performance of sized seeds under variations in substrate water potential (-0.04, -0.10, -0.20 and -0.40 MPa) was evaluated. Results showed that the establishment of medium size as a reference is fundamental for identifying relations between seed size and physiological potential. Seeds smaller by more than 0.8 mm than the medium size of the lot have lower physiological potential but are more tolerant to water deficit. Thus, it is recommendable to use seeds within the size limits of M ± 0.8 mm, to constitute soya bean seed lots of higher physiological potential.

Keywords: germination, *Glycine max*, seed handling, vigour, water deficit

Introduction

Seed physiological potential is determined by germination (viability) and vigour. The reliable evaluation of physiological potential allows the identification of seed lots with greater probability to better perform during storage and stand establishment in the field. Various factors can influence physiological potential, including seed size (Barbosa, 2010; Pádua *et al.*, 2010; Ambika *et al.*, 2014). Seed size can vary both among cultivars, under the influence of genotype, and among lots of the same cultivar, in response to the action of various other factors, such as climate, seed health, soil fertility, water availability and degree of maturation (Vinhal-Freitas *et al*., 2011; Liu *et al*., 2012; Ambika *et al*., 2014). Consequently, seed grading is an important step in seed processing, to obtain lots composed of seeds with uniform dimensions and thus assuring better performance of sowing machines and adequate stand establishment.

Seed size comprises three dimensions, i.e., length, width and thickness. Lots are usually sized for width by using round-hole screen openings and differences in seed thickness are separated by rectangular (oblong) screen openings designated by two dimensions, the width and the length. The width of each hole is measured in 64ths of an inch or in millimetres, while the length does not change (usually ¾" or 19.5 mm) among holes in this type of screen (Vaughan *et al.*, 1968). For corn, soya bean, groundnut and sunflower, separation based on thickness is useful in separating split and poorly-formed seeds.

There is a general belief, both in the scientific community and in the seed industry, that "large" seeds have better performance with respect to germination and vigour, since they store larger quantities of reserves. Nevertheless, various studies have shown that this premise does not always hold true (Beckert *et al*., 2000). This diversity of opinion is associated with different criteria to define what are "large" or "small" seeds, which makes it difficult to correctly interpret and compare results. Consequently, it is very important to establish reference standards to identify the size classes of seeds that compose a lot and, consequently, to produce precise information to define what would be considered as "large" or "small" seeds.

A valuable contribution to clarifying this matter was provided by Wetzel (1979), who evaluated the size distribution of seeds from three isogenic soya bean lines. Wetzel (1979) showed that, regardless of genotype and environment effects, the percentage distribution of different sizes within a soya bean seed lot approximates to a normal curve, with the majority of seeds being medium-sized. For that reason, Wetzel (1979) considered the identification of the medium width or thickness of the seeds that compose a lot as a more effective reference parameter to establish relationships between size and physiological potential.

Aguiar (1979) worked with six lots of soya bean seeds classified by differences in width using round screen openings and found variations in medium-sized seeds within cultivars and within seed lots. However, for all the materials studied, the seeds held in openings corresponding to the medium size showed better physiological potential than the other size classes. He considered that seeds of greater physiological potential are within the limits of M ± 0.8 mm (2⁄64"), in which M represents the medium width or thickness of seeds that compose the lot; similar findings were reported by Wetzel (1979), Lima and Carmona (1999), Beckert *et al*. (2000), Rezapour *et al*. (2013) and Adebisi *et al*. (2013) in soya bean; by Silva and Marcos-Filho (1979) in maize; and by Marcos-Filho and Avancine (1983) in field bean.

Differences in the expression of the physiological potential of soya bean seeds differing in size may depend on conditions of water availability during the germination process (Beckert *et al*., 2000; Pereira *et al*., 2013; Soares *et al*., 2015). For that reason, in addition to evaluating the effect of soya bean seed size on physiological potential, it is important to clarify whether the water availability influences the germination and vigour of different sized seeds, to obtain results that allow the estimation of seed performance in the field after sowing. The literature does not document detailed information on this subject. The objective of this study was therefore to evaluate the effect of seed size on soya bean seed physiological potential and to verify the influence of water availability on germination and manifestation of vigour of different sized seeds.

Materials and methods

Two seed lots (3.0 kg each) of soya bean cultivar M7739IPro (semi-determinate growth habit) produced by Sementes Adriana were used. Initially, four 3-kg samples of seeds from each lot were classified by differences in thickness (Vaughan *et al.*, 1968), using a set of perforated metal sheet screens with oblong (rectangular) openings with a constant length of ¾" and variations of 0.4 mm (1⁄64") in opening width. The percentage of seed retention was determined by recording the weight of seeds held on top of each screen (table 1). The medium size (M) was considered as a reference, corresponding to the highest percentage of retention of seeds from each lot. In addition, seeds held in screens with openings of M + 0.8 mm, M - 0.8 mm and those retained in screens with openings immediately under the size of M - 0.8 mm were collected. Seeds of the original samples (not classified) represented the control and seeds retained in 5.95 × 19.5 mm (M + 0.4 mm) and 5.15 × 19.5 mm (M - 0.4 mm) were discarded and their performance was not evaluated. An image representing the seeds of each size constituting the size classes is shown in figure 1.

Table 1. Retention of soya bean seeds in perforated metal screens with variation of 1⁄64" (0.4 mm) in the width of the rectangular openings.

Screen (inches)	Screen (mm)	Size class	Proportion of seeds retained (%)	
			Lot 1	Lot 2
16⁄64 × ¾	6.35 × 19.5	M + 0.8	1.3	2.0
15⁄64 × ¾	5.95 × 19.5	–	21.6	26.6
14⁄64 × ¾	5.55 × 19.5	M*	41.1	40.3
13⁄64 × ¾	5.15 × 19.5	–	27.0	24.6
12⁄64 × ¾	4.77 × 19.5	M - 0.8	7.0	5.2
11⁄64 × ¾	4.37 × 19.5	< M - 0.8	2.1	1.2

* Medium size of seeds for the respective lot.

After seed size classification, the following tests were performed:

Water content: determined by the oven method at 105°C (± 3°C) in two subsamples for each lot and size class (Brasil, 2009). Results are expressed as a percentage (fresh weight basis) for each lot and size class.

1000-seed weight: evaluated according to Brasil (2009), with eight replicates of 100 seeds per lot and size class.

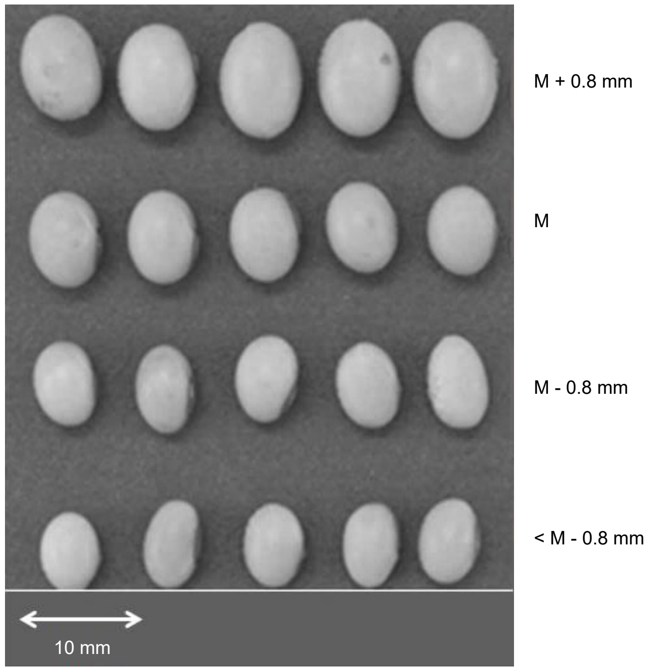

Figure 1. Images of 'M7739IPro' soya bean seeds graded by differences in thickness in screens with rectangular openings.

Physical characterisation of seeds: performed in GroundEye S120 equipment, composed of an image-capturing module, a tray for seed placement and image analysis software. For calibration of the background colour of the image, the CIEL *a*b colour system was used, with variation in brightness from 0.0 to 100.0, variation of the *a dimension from -20.7 to 39.3 and variation in the brightness from -594 to -2.9.

Four replicates of 50 seeds per lot and treatment were evaluated. Seeds were fastened to a sheet of acetate with double-sided tape, with the hilum facing upward to obtain seed images by the software component of the equipment. Reports were generated containing the mean values of area (mm^2), maximum diameter (mm), minimum diameter (mm) and perimeter (mm) of the seeds from each lot and size class.

Germination: four replicates of 50 seeds each per lot were germinated in rolled paper towel moistened with water equivalent to 2.5 times of the weight of the paper, at 25°C. Seedling evaluations were performed at four (germination first count) and eight days after sowing according to the Rules for Seed Testing (Brasil, 2009). Results are expressed as percentage of normal seedlings per lot and size class.

Saturated salt accelerated ageing (SSAA): seed samples from each lot and treatment were distributed in single layers on the surface of a wire mesh screen suspended over 40 mL of saturated NaCl solution (40 g per 100 mL of water) inside a plastic accelerated ageing box (110 × 110 × 35 mm). This established an inner environment of approximately 76% relative humidity in each box. The boxes were covered with a lid and kept at 41°C for 48 hours (Marcos-Filho *et al.*, 2000). After this period, the germination test was conducted according to Brasil (2009) as described above and results are expressed as mean percentage of normal seedlings for each lot and size class.

Seedling emergence: the test was conducted with four replications of 50 seeds per size class and lot sown in plastic boxes (470 × 300 × 110 mm) containing a mixture of sand and soil at the proportion of 3:1 (in volume); the substrate was moistened with water quantity corresponding to 60% of retention capacity. The plastic boxes were then kept in a normal laboratory environment and seedling emergence was recorded at the same time daily, computing the number of emerged seedlings. Mean percentage and speed of seedling emergence for each treatment and lot were assessed. Speed of emergence index was calculated by the sum of the quotients of number of emerged seedlings registered at each daily count and the respective number of days after sowing (Maguire, 1962) as follows:

$$SSE = \frac{N_1}{D_1} + \frac{N_2}{D_2} + \ldots + \frac{N_n}{D_n}$$

where SSE is the speed of emergence index, N_1, N_2, …N_n are the numbers of seedling emerged on each daily count and D_1, D_2, …D_n are the respective number of days after sowing.

Tetrazolium: two replications of 50 seeds per treatment and lot were pre-conditioned between three sheets of moistened paper towels at 25°C for 16 hours. The samples were then transferred to 50 mL plastic cups containing a solution of 0.075% 2,3,5 triphenyl tetrazolium chloride and kept in a laboratory oven at 40°C for 180 minutes. The seeds were then washed in running water and immersed in water until interpretation. Evaluation of staining was conducted by sectioning each seed lengthwise. Interpretation was performed according to the criterion described by França-Neto *et al.* (1999). Results are expressed as mean percentage of vigorous seeds (classes 1 to 3) and viable seeds (classes 1 to 5) for each treatment and lot.

Seedling image analysis (SVIS®): conducted with four replicates of 25 seeds each per treatment and lot. The seeds were germinated in paper towel rolls at 25°C for three days. After this period, the seedlings were scanned and analysed by the SVIS® software, according to Marcos-Filho *et al.* (2009). Data regarding seedling length (mm) and vigour index were expressed as mean values for each treatment and lot.

Seedling dry weight: five replicates of 20 seeds each were evaluated per treatment and lot. The paper towel substrate was first moistened with water equal to 2.5-times its dry weight, and the seeds were distributed on the upper third of the substrate, with the hilum turned toward the lower part of the paper. The rolls were then kept in plastic bags and placed vertically in the germinator for seven days at 25°C. At the end of this period, after removal of the cotyledons, the seedlings were packed in a paper bag and dried in a forced air circulation oven at 80°C for 24 hours (Nakagawa, 1999). Mean values of seedling dry weight were obtained with accuracy of 0.001 g, for each lot and treatment.

Germination under variations of water availability: the performance of seeds exposed to different water potentials was evaluated, using paper towel substrate previously moistened with polyethylene glycol 6000 (PEG 6000) solutions at concentrations sufficient to obtain each potential. The amounts of solute were defined based on Villela *et al.* (1991)

at 25°C. Solutions were prepared at the concentrations of 35.5, 78.5, 119.3 and 178.3 g $PEGL^{-1}$ to obtain water potentials of -0.04, -0.10, 0.20 and 0.4 MPa, respectively. Initially, as the two seed lots had similar germination percentage, the seed imbibition curve under each water potential was determined for Lot 1 according to Bewley and Black (1994) and Rossetto *et al*. (1997), with three replicates of 25 seeds each for each size class and water potential.

Seeds of each replication, after the determination of the initial seed water content and initial seed weight, were distributed between two sheets of paper towel moistened with 20 mL of PEG 6000 solution corresponding to each water potential previously planned. Seed samples were then placed on a wire mesh screen inside an AA box containing 40 mL of distilled water and transferred to a germinator at 25°C. The amounts of water taken up were determined by changes in weight (0.01 g) at 60-minute intervals up to primary root emergence; it was identified when occurred in at least five seeds for each size class. Results are expressed as percentage water content for each imbibition period.

The germination test under variations in water availability was conducted with four replicates of 50 seeds each distributed in paper towel rolls moistened with PEG 6000 solution at -0.04, -0.10, -0.20 and -0.40 MPa, following the same procedure described for the germination test above. Seeds were moved to new papers moistened with the respective PEG solution each 12 hours during test to ensure the maintenance of the desired water potentials. Results were expressed as mean percentage of primary root protrusion (three days after sowing) and of normal seedlings (five days after sowing), and the mean speed of primary root protrusion (Maguire, 1962) for each size class and water potential.

The experiment was conducted using a completely randomised design for each test separately in a lot × size factorial arrangement or treatment × water potential factorial arrangement, depending on the type of study; transformation of data was provided when necessary. The mean values were compared by the Scott-Knott test ($P \leq 0.05$); the data regarding the seed imbibition curve were subjected to polygonal regression analysis with the assistance of SISVAR software (Ferreira, 2003).

Results

The variation in initial seed water content 'M7739IPro' was 0.1% (from 11.0% to 11.1%), showing the uniformity among seed lots and size classes before the tests carried out in this study.

Physical characterisation of the seeds held in the top of different screens showed proportionality among the parameters evaluated. For example, the minimum diameter corresponding to each size was close or equal to the width of the equivalent screen opening (table 2). The values obtained for the control (unclassified seeds) were also similar to those determined for the medium-sized seeds (M). In general, the area, maximum diameter, minimum diameter and the perimeter of the seeds were directly proportional to the sizes separated (table 2); the same was true for 1000-seed weight (table 3).

For both lots, germination of the medium-sized seeds (M) was higher than seeds of < M - 0.8 mm size (table 3). This superiority was also found in most of the evaluation of

Table 2. Area (A), maximum diameter (DMax), minimum diameter (DMin) and perimeter length (P) of seeds from two soya bean seed lots sorted by differences in thickness.

Size class	A (mm^2)		DMax (mm)		DMin (mm)		P (mm)	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
Control	32	31	7.2	7.0	5.6	5.4	21.9	21.0
M + 0.8	41	41	7.8	8.3	6.1	6.1	24.7	25.7
M	32	32	7.3	7.3	5.6	5.5	21.3	21.8
M – 0.8	23	23	6.2	6.2	4.7	4.7	18.2	18.4
< M – 0.8	23	21	6.2	6.0	4.3	4.3	15.8	17.8

Table 3. 1000-seed weight, germination (G), saturated salt accelerated ageing germination (SSAA), percentage emergence (E) and emergence speed index (ESI) of seedlings from two soya bean seed lots classified by differences in thickness.

Size class	1000-seed weight (g)		G (%)		SSAA (%)		E (%)		ESI (%)	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
Control	163.25*	165.62	97 Aa	93 Aa	95 Aa	91 Aa	91 Aa	92 Aa	9.2 Aa	9.3 Aa
M + 0.8	240.12	241.61	95 Aa	88 Bb	92 Aa	84 Ab	93 Aa	91 Aa	9.6 Aa	9.3 Aa
M	177.12	178.05	93 Aa	94 Aa	92 Aa	87 Aa	93 Aa	95 Aa	9.5 Aa	9.5 Aa
M – 0.8	109.55	109.38	88 Ba	92 Aa	92 Aa	89 Aa	92 Aa	88 Aa	9.4 Aa	9.0 Ba
< M – 0.8	97.43	96.30	88 Ba	90 Ba	75 Bb	82 Aa	81 Ba	76 Ba	8.5 Ba	8.5 Ba

* Uppercase letters refer to mean comparisons within each column; lowercase letters refer to comparisons within each row (Scott-Knott test, $P \leq 0.05$).

vigour and seedling emergence, although in the saturated salt accelerated aging test, this occurred only for Lot 1; in Lot 2, there were no statistical differences, although the lowest numerical values were also found for the treatment < M - 0.8 mm.

No significant variation in seed viability of the different sized seeds were revealed by the tetrazolium test (TZ 1-5, table 4). Nevertheless, in evaluation of vigour (TZ 1-3), the lower performance of smaller seeds (< M - 0.8 mm) was identified. This trend was also evident in computerised seedling image analysis, with respect to seedling length at three days and the vigour index values. In the tetrazolium test, variations were not found among different sized seeds regarding occurrence of mechanical or stink bug injuries and field weathering deterioration (data not shown).

In contrast, there were differences in the root, hypocotyl and seedling dry weight from different sized seeds (table 5). In both lots, there was significant reduction in the dry weight of seedlings from < M - 0.8 mm seeds.

Table 4. Tetrazolium test 1-5 (viability) tetrazolium 1-3 (vigour), seedling length at three days after sowing (SL 3rd day) and vigour index (VI) determined by the SVIS® of seeds from two lots of soya bean seeds sorted by differences in thickness.

Size class	Tetrazolium				SVIS®			
	Viability (%)		Vigour (%)		SL 3rd day (mm)		VI	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
Control	98 Aa*	84 Bb	90 Aa	79 Bb	68 Ba	55 Cb	707 Ba	614 Bb
M + 0.8	95 Aa	95 Aa	90 Aa	87 Aa	58 Ba	67 Ba	603 Ca	669 Ba
M	93 Aa	91 Aa	88 Aa	86 Aa	77 Aa	72 Ba	769 Aa	761 Aa
M – 0.8	89 aBb	96 Aa	88 Aa	91 Aa	6.5 Bb	81 Aa	674 Bb	669 Ba
< M – 0.8	94 Aa	90 Aa	82 Ba	79 Ba	59 Bb	75 Aa	516 Db	651 Ba

* Uppercase letters refer to mean comparisons within each column; lowercase letters refer to comparisons within each row (Scott-Knott test, $P \leq 0.05$).

Table 5. Seedling dry weight of seeds from two soya bean seed lots sorted by differences in thickness.

Size class	Hypocotyl (mg)		Root (mg)		Seedling (mg)	
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2
Control	112 Aa*	118 Ba	40 Aa	42 Aa	152 Aa	160 Aa
M + 0.8	123 Ab	142 Ab	43 Aa	45 Aa	166 Ab	187 Aa
M	131 Aa	116 Ba	37 Ab	50 Aa	168 Aa	166 Aa
M – 0.8	86 Ba	87 Ca	32 Ba	30 Ba	118 Ba	117 Ba
< M – 0.8	78 Ba	50 Cb	26 Ba	15 Cb	104 Ba	65 Ba

* Uppercase letters refer to mean comparisons within each column; lowercase letters refer to comparisons within each row (Scott-Knott test, $P \leq 0.05$).

Water uptake by different sized seeds followed the triphasic pattern (figure 2), and showed reduction in imbibition rate as water potential decreased. This resulted in a delay in the protrusion of the primary root. For example, in medium-sized seeds (M), the protrusion of the primary root started after 34 hours hydration at -0.04 MPa, but only after 40, 50 and 61 hours at -0.1, -0.2 and -0.4 MPa, respectively. In addition, water uptake and primary root emergence were faster in the smaller (M - 0.8 mm and < M - 0.8 mm) seeds than the medium-sized (M) or larger seeds under all water potentials, indicating an indirect relationship between seed size and imbibition rate.

The percentage of normal seedlings (table 6) also decreased as water potential declined. The seeds larger than medium size exhibited a sharper reduction in germination percentage and in speed of root protrusion as decreases in water availability intensified.

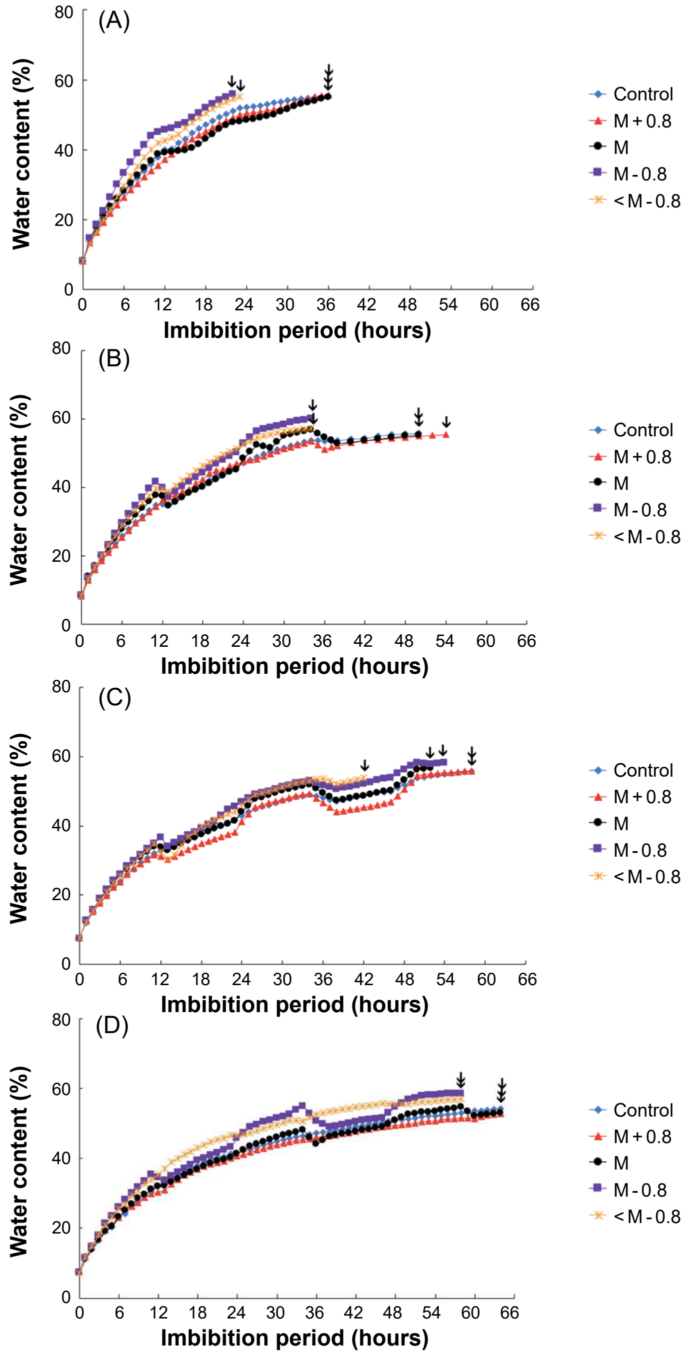

Figure 2. Progression of water uptake by 'M7739IPro' soya bean seeds classified by differences of thickness, under water potentials of (**A**) -0.04, (B), -0.10, (**C**) -0.20 and -0.40 (**D**) MPa. Down arrows indicate the timing of root protrusion.

Table 6. Germination (%) and speed of root emergence (index) under variations in water availability of seeds from two soya bean seed lots sorted by differences in thickness.

Treatment	Germination (%)				Speed of primary root protrusion (index)			
	Water potential (MPa)				Water potential (MPa)			
	-0.04	-0.10	-0.20	-0.40	-0.04	-0.10	-0.20	-0.40
Control	93 Aa*	86 Aa	75 Ab	33 Cc	33.3 Aa	20.1 Ab	14.8 Ac	10.3 Bd
M + 0.8	90 Aa	77 Bb	30 Bc	10 Dd	28.8 Ca	17.1 Bb	8.9 Cc	2.9 Cd
M	90 Aa	84 Aa	66 Ab	32 Cc	30.9 Ba	18.9 Ab	13.5 Bc	9.4 Bd
M - 0.8	91 Aa	88 Aa	74 Ab	68 Ab	32.0 Aa	20.5 Ab	15.5 Ac	13.4 Ad
< M - 0.8	86 Aa	84 Aa	60 Bb	47 Bc	29.9 Ba	19.5 Ab	15.8 Ac	12.2 Ad

* Uppercase letters refer to mean comparisons within each column; lowercase letters refer to comparisons within each row (Scott-Knott test, $P \leq 0.05$).

Discussion

Germination of the two lots of 'M7739IPro' was higher than the minimum standard (80%) established for soya bean seed marketing in Brazil (Brasil, 2013). This is important since the seed company needs to compare the vigour of seed lots with acceptable germination (ISTA, 2017) and emphasises the possibility for applying the results of our study in seed quality control programmes.

There were differences in seed performance of both lots in the germination and vigour (saturated salt accelerated ageing, tetrazolium, speed and seedling growth) tests; the efficiency of SVIS® analyses to identify differences in vigour of high germination seed lots as emphasised by Hoffmaster *et al*. (2003), Marcos-Filho *et al*. (2009) and Yagushi *et al*. (2014) was also confirmed. The seeds that were smaller than the medium size of each lot (M - 0.8 and < M - 0.8) clearly showed reduced physiological potential as reported by Wetzel (1979), Beckert *et al*. (2000), Rezapour *et al*. (2013) and Adebizi *et al*. (2013). This happened despite the relatively small proportion (less than 10%) of those seeds, which contributed for the reduction of the overall physiological quality of both seed lots.

The lower physiological potential of the "smaller" seeds (< M - 0.8) can be explained by the fact that this fraction of the lot contains a greater number of poorly formed seeds (Wetzel, 1979; Beckert *et al*., 2000; Krishnan *et al*., 2014). Differences in size and dry matter of soya bean seeds are usually related to the efficiency of transfer of plant reserves to seeds during maturation. In this process, larger seeds generally receive a greater amount of dry matter than smaller seeds. Soya bean has a relatively long flowering period and the smaller seeds of a population are generally formed from fertilisation of ovules coming from later flowering. Therefore, the period of transfer of dry matter during development of these seeds would be shortened compared with seeds formed from the initial flowers (Marcos-Filho, 2016). As a result, they usually accumulate a smaller amount of dry matter.

Other factors can contribute to reduced seed size during maturation, such as injuries caused by stinkbugs and field weathering deterioration.

The data of physical characteristics (area, diameter, perimeter) were directly proportional to seed size and, within each size, both seed lots showed similar values for all the parameters evaluated. Consequently, the determinations of area, diameter and perimeter confirmed the accuracy of seed lot grading in different sizes using rectangular screen openings.

Determinations of seedling dry weight identified the higher vigour in seedlings from medium-sized (M) or larger (M + 0.8) seeds, compared with "smaller" seeds (M - 0.8 and < M - 0.8), showing, therefore, a direct association between seed size and seedling weight. These "larger" seeds have a greater amount of stored reserves in the cotyledons and, consequently, produce more developed seedlings than those from "smaller" (M - 0.8 and < M - 0.8) seeds, under adequate water availability (Carvalho and Nakagawa, 2012). These results, considering the roots, hypocotyl and the whole seedling (table 5), were comparable with those obtained in the germination and vigour tests, in both lots, which also identified the lower performance of seeds of < M - 0.8 mm size.

Some authors have emphasised the influence of water availability and of seed size on field seedling emergence (Costa *et al*., 2004; Pereira *et al*., 2013). The lower physiological potential of smaller seeds has a particular impact when seeds are subjected to water stress. The initial seed water content and the level of water availability influence the whole water uptake process of soya bean seeds as the imbibition prior to primary root protrusion is usually slower as the water potential of the substrate decreases (figure 2).

The association between the speed of primary root protrusion and the germination percentage with the water potential of the substrate was confirmed. Decreases in water potential retards metabolic activities for mobilisation of reserves and for transport and assimilation of the metabolised products (Costa *et al*., 2004; Pereira *et al*., 2013; Soares *et al*., 2015).

At -0.04 MPa water potential, corresponding to soil field capacity, there were no differences in germination of different sized seeds. Results also showed decreases in the speed of primary root emergence as the water potential decreased, regardless of seed size, because under water restriction, seeds need a longer period to reach the water content necessary for primary root development. According to Rossetto *et al*. (1997), the speed of germination of soya bean seeds is usually reduced only under water potentials less than or equal to -0.20 MPa. This was confirmed here as seed performance of the two lots was reduced only under water potentials of -0.20 and -0.40 MPa.

The smaller seeds took up water more rapidly and showed faster primary root protrusion than those of medium size or larger. Small seeds (M - 0.8 and < M - 0.8) have a larger area per unit of weight (Beckert *et al*., 2000; Costa *et al*., 2004) and, consequently, need less time and to take up less water amount to reach the water content necessary for germination. Therefore, germination percentage was less affected by reductions in water potential in seeds of size lower than the medium.

In conclusion, this study provided relevant information regarding the establishment of the medium size of the seeds that compose the lot as a parameter of reference for seed grading and confirming the relationship between the size and physiological potential of soya bean seeds. It also identified the influence of variations in water availability of the substrate on the speed and percentage of germination of soya bean seeds of different sizes.

Soya bean seeds smaller than medium size have lower physiological potential, but under water deficit during the germination process, larger seeds (M + 0.8 mm) are more negatively affected. Consequently, it was demonstrated that medium sized soya bean seeds have higher physiological potential than seeds outside the limits M ± 0.8 mm or M ± ²⁄₆₄". This knowledge constitutes the basis for soya bean and other crop seed grading during processing, i.e., adequate seed grading leads to higher physiological potential of the seed lot.

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