

## PHYSIOLOGICAL PERFORMANCE OF CONVENTIONAL SOYBEAN CULTIVARS SEEDS AS A FUNCTION OF SIZE AND MOISTURE CONTENT DURING GERMINATION

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### ABSTRACT

Quality test protocols for soybean seeds recommend a period of soaking and preconditioning without detailing the size of the seeds to be evaluated, as seeds of different sizes reach different degrees of moisture in each period owing to differences in their initial moisture level and size. Determining the degree of seed moisture during the preconditioning period is important when conducting accelerated aging, tetrazolium, and primary root protrusion tests. The objective of this study was to evaluate the physiological performance of two conventional soybean cultivars (BRS 284 and BRS 511) with three seed sizes (6.0, 6.5, and 7.0 mm) and three moisture levels, adjusted to 10%, 12%, and 14% and a control condition with 9.0 % moisture. The experimental design was a randomized block scheme in a factorial arrangement of (3×3)+1 involving seed size and moisture. Regardless of the cultivar and moisture content, seeds with large diameters were more resistant to changes, such as accelerated aging, and resulted in larger seedlings and higher numbers of developed seedlings than seeds with small diameters.

**Keywords:** *Glycine max*, physiological quality, germination test.

### INTRODUCTION

Yield and quality are important characteristics of seeds that highlight the success of crops, including soybean (*Glycine max* (L.) Merrill) plants (COSTA et al., 2003; DAMETO et al., 2023a). To ensure high yield, seeds must have a high degree of purity, health, viability, and vigor (MINUZZI et al., 2010), as seeds with a low germination percentage result in low-quality yield under physical stress and adverse environmental conditions. Consequently, seeds with high

germination percentage are recommended in agriculture as they are more resistant to unfavorable weather conditions (FRANZIN; ROVERSI, 2010).

Furthermore, large seeds often have well-formed embryos with a large amount of nutrient reserves, and are potentially the most vigorous, having the greatest germination potential (PÁDUA et al., 2010). The larger nutrient reserves of large seeds increase the probability of successful establishment of seedlings and enables survival for prolonged periods under unfavorable environmental conditions (HAIG; WESTOBY, 1991). Presoaking seeds can lead to improvements in their development, with presoaked seeds showing uniform, rapid, and vigorous emergence with positive effects on grain yield (GUIMARÃES et al., 2013). Previous studies have recommended soaking and preconditioning seeds for vigor tests without detailing the size of the soybean seeds to be evaluated. Seeds of different sizes are known to reach different degrees of moisture in a certain period owing to their initial moisture level and size.

Several studies have been conducted to improve the quality of soybean seeds to meet the needs of producers (FRANÇA-NETO; HENNING, 1992; COSTA et al., 2003; MINUZZI et al., 2010, DAMETTO et al., 2023b). According to França-Neto and Krzyzanowski (2018), the physiological potential of seeds refers to the competence they must develop successfully under favorable or unfavorable conditions and encompasses viability and vigor. Vigor is the potential for rapid and uniform emergence of seeds followed by the development of normal seedlings under a wide range of field conditions and is considered an index of the degree of physiological deterioration and/or mechanical integrity of a high-germination seed lot, representing its ability to establish itself in the environment (AOSA, 2002). Germination is the process in which a quiescent seed resumes its metabolic processes, restarting the development of the embryo and giving rise to a seedling (FRANÇA-NETO; KRZYZANOWSKI, 2018). The objective of this study was to evaluate the physiological performance of the seeds of two soybean cultivars with different seed diameters and moisture contents using accelerated aging, primary root emission, and tetrazolium (seed vigor and viability) tests.

## **MATERIAL AND METHODS**

The experiment was carried out at the Seed Physiology Laboratory in the Seed and Grain Technology Center of Embrapa Soybean, Londrina, State of Paraná, Brazil (23°11'39" SL,

51°10'40" WL and altitude of 570 m), using seeds of the conventional soybean cultivars BRS 284 and BRS 511, which were of indeterminate growth type and different sizes (6.0, 6.5, and 7.0 mm). The moisture content of each size was adjusted to 10%, 12%, and 14% humidity on a wet basis, in addition to the control seed (9% moisture content).

After separation by size, the seeds were subjected to germination and vigor tests to compare the effects of moisture content and size. To obtain a moisture content of 10%, 12%, and 14% the samples were preconditioned in “Gerbox” type boxes with a wire mesh, filled with a single uniform layer of seeds, and 40 mL of water was added to the bottom of the boxes for humidity. The seeds were then stored in a germinator at 25 °C. The seed moisture content after certain period of incubation for each treatment was determined by the constant-temperature oven method (BRASIL, 2009).

After reaching the predefined degree of humidity, germination test was carried out with four subsamples of 50 seeds for each treatment and replication, totaling 192 subsamples. The seeds were set to germinate between three “Germitest” paper towels moistened with a volume of deionized water 2.5 times the weight of the paper. Then rolls of paper were prepared containing the subsamples of seeds and placed in a plastic box to maintain humidity and taken to a cabinet in the walking germination room regulated to maintain a constant temperature of  $25 \pm 1$  °C. The percentage of normal seedlings was evaluated on the fifth day after the beginning of the test, according to criteria established by the Rules for Seed Testing (BRASIL, 2009). To determine seedling length, five subsamples of 20 seeds each were used, for a total of a hundred seeds per replication per treatment. The seeds were distributed between three “Germitest” paper towels moistened with a volume of deionized water 2.5 times the weight of the paper. The seeds were oriented in such a manner that the micropyle faced the bottom of the paper, thus ensuring straight growth of the seedling. The rolls were placed vertically in the germinator at 25°C with no light for three days. After three days, the seedlings were removed from the germinator, scanned, and analyzed using the VIGORS® program (WENDT et al., 2017).

The accelerated aging test was conducted in plastic “gerbox” boxes, containing 40 mL of water at the bottom and a uniform layer of hundred twenty seeds arranged on top of the surface of the internal screen, maintained in a water jacket incubator (MARCOS FILHO, 1999). After the aging period at 41 °C for 24 h and 48 h, the samples were removed and four subsamples of 50 seeds each per treatment per replication were submitted to germination test. The evaluation was carried

out on the fifth day after sowing, calculating the percentage of the seedlings considered normal according to criteria established by the Rules for Seed Testing (BRASIL, 2009).

To determine the imbibition curve, the seeds were immersed in distilled water at 25°C. Every 4 h, the seeds were dried on absorbent paper and weighed (time weight, Pt). The test was conducted over the period of 48 h, and the imbibition (E) at each time point was calculated according to the equation  $E = [(Pt - Pi) / Pi] \times 100$ . The results are expressed as percentages.

The tetrazolium test was carried out with two subsamples of 50 seeds per treatment and replication, pre-conditioned on “Germitest” paper moistened with distilled water for a period of 16 h, in a germinator with the temperature adjusted to  $25 \pm 1$  °C. After this period, the seeds were transferred to plastic cups with a volume of 50 mL., totally submersed in a tetrazolium solution (2-3-5, triphenyl tetrazolium chloride) at concentration of 0,075%, and maintained at a temperature of 40 °C for 150 minutes in the interior of a oven. After the coloration process, the seeds were washed with running water and maintained submerged in water until the moment of evaluation in a refrigerator. Afterwards, the seeds were evaluated individually, sectioned longitudinally and symmetrically with the use of a scalpel blade, and classified in accordance with the criteria proposed by França-Neto and Krzyzanowski (2018). The viability was represented by the sum of the percentages of seeds belonging to classes 1 to 5, and the level of vigor was represented by classes 1 to 3.

The primary root emission test was performed using four subsamples of 50 seeds for each treatment and repetition. The seeds were set to germinate between three “Germitest” paper towels moistened with a volume of deionized water 2.5 times the weight of the paper. Then rolls of paper were prepared containing the subsamples of seeds and placed in a plastic box to maintain humidity and taken to a gabinet in the walking germination room regulated to maintain a constant temperature of  $25 \pm 1$  °C. After 48 h, the primary roots of the seedlings was evaluated based on parameter of at least 2.0 cm length to be classified as vigorous.

The data were subjected to normality and homogeneity tests, and analysis of variance and F-test were performed for each test conducted using a completely randomized design in a factorial scheme  $(3 \times 3) + 1$ , involving the size and degree of moisture content of soybean seeds; the means were compared using the Scott–Knott test at a 5% probability level.

## RESULTS AND DISCUSSION

The percentage of seed germination varied significantly between the cultivars (Table 1). Cultivar BRS 511 showed an increase in germination with an increase in seed moisture, whereas cultivar BRS 284 showed the highest percentages of germination after seed moisture treatments at sizes of 6.0-mm and 6.5-mm (Table 1). Despite the positive relationship between germination and size, the difference between cultivars was due to the presence of a significant amount of dead seeds in the BRS 284 cultivar at size 7.0-mm, indicating that regardless of seed size, harvest management is also essential for obtaining high-quality seeds.

**Table 1.** Germination test of two conventional soybean cultivars (BRS 284 and BRS 511) in response to moisture content and seed size.

Moisture %	Size (mm)			Mean
	6.0	6.5	7.0	
BRS 284				
	(%)	(%)	(%)	(%)
9	99.0aA	98.0aA	96.5aA	97.8a
10	98.0aA	98.0aA	97.0aA	97.7a
12	97.5aA	99.0aA	95.5aA	97.3a
14	100.0aA	99.0aA	96.0aA	98.3a
Mean	98.6A	98.5A	96.3A	97.8
BRS 511				
9	87.5bA	86.5aA	86.0bA	86.7b
10	85.5bA	88.0aA	87.0bA	86.8b
12	88.0bA	87.0aA	89.5bA	88.2b
14	94.0aA	88.0aA	93.0aA	91.7a
Mean	88.8A	87.4A	88.9A	88.4
Mean				
9	93.3aA	92.3aA	91.3aA	92.3a
10	96.8aA	93.0aA	92.0aA	92.3aa
12	92.8aA	93.0aA	92.5aA	92.8
14	97.0aA	93.5aA	94.5aA	95.0a
Mean	93.7A	93.0A	92.6A	93.1
CV%	6.23			

<sup>1</sup>Means followed by distinct lowercase letters in the same column and uppercase letters in the same line differ from each other, using the Scott-Knott test at 5% probability.

The positive effect of soybean seed size on germination percentage confirmed the initial hypothesis that large seeds have well-formed embryos with larger nutrient reserves, and that they were potentially the most vigorous (COELHO et al., 2019), which corroborates the results of Pádua

et al. (2010), who obtained the best results for the three soybean cultivar seeds retained on the 7.0-mm screen. It is noteworthy that in terms of the physiological performance of the seeds, size can be associated with several factors, such as cultivation conditions, planting density, mode, time of harvest, and edaphoclimatic conditions during the growth stages, as the size can vary depending on the cultivar, sampled lot, and harvest time (CANGUSSÚ et al., 2013). Thus, the relationship between seed size and physiological performance must be carefully analyzed because several factors can interfere with the results.

Aging evaluated 24 h after the beginning of the test also showed the importance of seed size regardless of humidity, as the two cultivars showed significant results, where the increase in size caused reduced aging, indicating that large seeds could be stored for a longer period without any loss of germination (Table 2), i.e., large seeds are less affected by high humidity and temperature than small seeds. Similar results were obtained by Pádua et al. (2010), wherein large seeds with a 7.0-mm diameter showed higher vigor than seeds with a 6.0-mm diameter. Among the cultivars, BRS 284 showed the best response to stress generated by the aging test (Table 2), indicating that soybean seeds with large diameters had high physiological capacity (VINHAL-FREITAS et al., 2011).

In the case of cultivar BRS 511, in the 48-h aging test, the interaction between the variables had a positive response with the highest values being observed for seeds with diameters of 6.5-mm and 7.0-mm and with 14% moisture (Table 2). Tomes et al. (1988) suggested that soybean seeds with an adjusted water content of 8–14% performed well in the accelerated aging test. Additionally, Beckert et al. (2000) reported that, owing to the ratio of the greater contact area per unit mass, small seeds reach imbibition faster than large ones, resulting in low performance.

In terms of root protrusion, in the cultivar BRS 511, the seed moisture  $\times$  seed size interaction was significant, with the best averages being observed for seeds measuring 6.5-mm and 7.0-mm, specifically, precisely because they are the most vigorous and with 10% moisture and in the control seed with 9 % moisture (Table 3). Large seeds develop better than smaller seeds because they have larger nutrient reserves and germinate faster. Additionally, an interaction was observed between moisture content and size in the BRS 284 cultivar (Table 3). The best averages were observed in the seeds with 10% moisture and in the control 9 % moisture as well as in the 6.0-mm and 6.5-mm seeds.

**Table 2.** Accelerated aging test of two conventional soybean cultivars (BRS 284 and BRS511) in response to moisture content and seed size.

Moisture %	Size (mm)			Mean
	6.0	6.5	7.0	
BRS 284				
	(%)	(%)	(%)	(%)
9	96.5aA	95.5aA	96.0aA	96.0a
10	100.0aA	96.0aA	94.5aA	96.8a
12	97.0aA	96.5aA	95.0aA	96.2a
14	97.0aA	97.0aA	97.5aA	97.2a
Mean	97.6A	96.3A	95.8A	96.6
BRS 511				
9	83.0bB	92.0aA	92.0aA	89.0a
10	67.0cB	90.5aA	88.5bA	82.0b
12	81.0bB	82.5bB	86.0bA	83.2b
14	90.5aB	93.0aA	94.5aA	92.7a
Mean	80.4B	89.3A	90.3A	86.7
Mean				
9	89.8bB	93.8aA	94.0aA	92.5a
10	83.5bB	93.3aA	91.5bA	89.4b
12	89.0bA	89.5bA	90.5bA	89.7b
14	93.8aA	95.0aA	95.8aA	95.0a
Mean	89.0A	92.8A	93.1A	91.7
CV%	4.35			

<sup>1</sup>Means followed by distinct lowercase letters in the same column and uppercase letters in the same line differ from each other, using the Scott-Knott test at 5% probability.

Seed vigor and viability were evaluated using the tetrazolium test. For cultivar BRS 511, a difference in vigor was observed as a function of moisture level, with the highest values at 14% (Table 4). In terms of seed viability, differences were observed between seeds of 7.0-mm and 6.5-mm sizes (Table 5). The low average observed in the 6.5-mm seeds was due to the large amount of mechanical damage that caused the death of the seeds. Cultivar BRS 284 also showed a difference in seedling size, with higher averages in seeds of 6.5-mm and 7.0-mm sizes at 10% moisture (Table 6). In terms of vigor, no significant differences in moisture or seed size were observed. Similar results were reported by Cangussú et al. (2013) in bean seeds (*Phaseolus vulgaris* L.).

Both cultivars showed significant interactions between variables in the length test. Large seeds produced large plants, consistent with the results of Lima and Carmona (1999), namely that when comparing soybean seeds of different sizes, small soybean seeds led to the emergence of

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small plants. Small plants, with a low height, have low agronomic potential and are more susceptible to environmental stress than large plants.

**Table 3.** Root protrusion test of two conventional soybean cultivars (BRS 284 and BRS 511) in response to moisture content and seed size.

Moisture %	Size (mm)			Mean
	6.0	6.5	7.0	
BRS 284				
	(%)	(%)	(%)	(%)
9	97.0aA	96.5aA	95.5aA	96.3a
10	94.0aA	99.0aA	92.0aA	95.0a
12	91.0aA	98.5aA	77.0bB	88.8b
14	93.5aA	86.5bA	78.5bB	86.2b
Mean	93.9A	95.1A	85.8B	91.6
BRS 511				
9	71.0bB	90.0aA	83.5aA	81.5a
10	82.5aB	79.0bB	88.0aA	83.2a
12	72.5bB	76.0bB	88.0aA	83.2a
14	71.3bB	81.0bA	75.0bB	75.8b
Mean	74.3B	81.5A	81.4A	79.1
Mean				
9	84.0bB	93.3aA	89.5aA	88.9a
10	88.3aA	89.0aA	90.0aA	89.1a
12	81.8bB	87.3bA	82.5bB	86.0b
14	82.4bA	83.8bA	76.8cB	81.0c
Mean	84.1B	88.3A	83.6B	85.4
CV%	5.50			

<sup>1</sup>Means followed by distinct lowercase letters in the same column and uppercase letters in the same line differ from each other, using the Scott-Knott test at 5% probability.



**Table 4.** Vigor test of two conventional soybean cultivars (BRS 284 and BRS 511) in response to moisture content and seed size.

Moisture %	Size (mm)			Mean
	6.0	6.5	7.0	
	BRS 284			
	(%)	(%)	(%)	(%)
9	95.0aA	97.0aA	98.0aA	96.7a
10	97.0aA	93.0aA	95.0aA	95.0a
12	97.0aA	95.0aA	96.0aA	96.0a
14	99.0aA	96.0aA	98.0aA	97.7a
Mean	97.0A	95.3A	96.8A	96.4
	BRS 511			
9	83.0aA	84.0aA	87.0aA	84.7a
10	84.0aA	82.0aA	86.0aA	84.0a
12	78.0aA	78.0aA	79.0aA	78.3a
14	86.0aA	83.0aA	86.0aA	85.0a
Mean	82.8A	81.8A	84.5A	83.0
	Mean			
9	89.0aA	90.5aA	92.5aA	90.7a
10	90.5aA	87.5aA	90.5aA	89.5a
12	87.5aA	86.5aA	87.5aA	87.2a
14	92.5aA	89.5aA	92.0aA	91.4a
Mean	89.9aA	88.6aA	90.7aA	89.7a
CV%	7.01			

<sup>1</sup>Means followed by distinct lowercase letters in the same column and uppercase letters in the same line differ from each other, using the Scott-Knott test at 5% probability.

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**Table 5.** Viability test of two conventional soybean cultivars (BRS 284 and BRS 511) in response to moisture content and seed size.

Moisture %	Size (mm)			Mean
	6.0	6.5	7.0	
	BRS 284			
	(%)	(%)	(%)	(%)
9	98aA	98aA	99aA	98.3a
10	98aA	99aA	95aA	97.3a
12	95aA	98aA	93bA	95.3a
14	100aA	98aA	98aA	98.7a
Mean	97.8A	98.3A	96.3A	97.4
	BRS 511			
9	92.0aA	85.0aB	90.0aA	89.0a
10	89.0aA	85.0aA	87.0aA	87.0a
12	87.0aA	84.0aA	89.0aA	86.7a
14	91.0aA	85.0aB	89.0aB	88.3a
Mean	89.8A	84.8A	88.8A	87.8
	Mean			
9	95.0aA	91.5aA	94.5aA	93.7a
10	93.5aA	92.0aA	91.0aA	92.2a
12	91.0aA	91.0aA	91.0aA	91.0a
14	95.5aA	91.5aA	93.5aA	93.5a
Mean	93.8A	91.5A	92.5A	92.6
CV%	6.39			

<sup>1</sup>Means followed by distinct lowercase letters in the same column and uppercase letters in the same line differ from each other, using the Scott-Knott test at 5% probability.

**Table 6.** Seedling size of two conventional soybean cultivars (BRS 284 and BRS 511) in response to moisture content and seed size.

Moisture %	Size (mm)			Mean
	6.0	6.5	7.0	
BRS 284				
	(%)	(%)	(%)	(%)
9	9.0bA	9.4bA	9.1bA	8.9b
10	13.8aB	17.0aA	17.3aA	16.3a
12	9.3bA	7.9bB	7.2bB	8.4b
14	8.9bA	7.8bA	7.8bA	8.2b
Mean	11.1A	10.3A	10.3A	10.6
BRS 511				
9	5.9aA	7.7bA	7.9bA	7.2b
10	6.6aB	14.6aA	17.0aA	12.8a
12	6.3aA	7.3bA	7.1bA	6.9b
14	6.6aA	7.5bA	6.7bA	7.0b
Mean	6.3B	9.3A	9.7A	8.4
Mean				
9	7.5bA	8.5bA	8.5bA	8.1b
10	10.2aB	15.8aA	17.2aA	14.5a
12	7.8bA	7.6bA	7.2bA	7.7b
14	7.8bA	7.7bA	7.3bA	7.6b
Mean	8.7bB	9.8A	10.0A	9.5
CV%	8.51			

<sup>1</sup>Means followed by distinct lowercase letters in the same column and uppercase letters in the same line differ from each other, using the Scott-Knott test at 5% probability.

## CONCLUSIONS

Soybean seeds with large diameters are less sensitive to the effects of accelerated aging and have higher germination rates than those with small diameters. In the germination test of the soybean cultivar BRS 511, seeds with 14% moisture showed a higher percentage of germination than the control seeds. In the root protrusion test, the large and vigorous seeds produced primary roots faster than the smaller and less vigorous seeds. The highest percentage of moisture negatively affected root emergence. In terms of growth, large plants produce large seeds that perform better under adverse planting conditions.

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