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Primary root emission as a vigor test in soybean seeds[1]

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ABSTRACT - The objective was to verify if the primary root emission can be recommended as a vigor test for soybean seeds. For this, five lots of varieties ANTA 82, NA7337 and BRS1074IPRO were evaluated by first count, germination, emergence, emergence speed index and tetrazolium tests. In the root protrusion test at 20, 25 and 30 ºC, two evaluation criteria were adopted: primary root emission precocity index (PREPI) and percentage of seeds that emitted the primary root (PRE). The seeds counted were those with a primary root equal to or greater than 2 mm, every 12 h from the installation of the test until 120 h. The design was completely randomized and means were compared, within each evaluation time, using the Scott-Knott test at 5% probability. Pearson's linear correlation was performed for PREPI and PRE with emergence. PREPI was not considered efficient. For the PRE, at 20 ºC, there was a correlation at 72 h, for the three varieties. At 25 ºC there was a correlation for a period of 36 h for ANTA 82 and NA7337 and 60 h for BRS1074IPRO. At 30 ºC there was a correlation in the time of 36 h for the three cultivars. Considering that speed in obtaining results is important in vigor tests, it is concluded that primary root emission for 36 h after installing the test at 30 ºC is recommended as a vigor test in the quality control of soybean seeds.

Key words: *Glycine max* L. Merrill. Protrusion. Quality Control.

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INTRODUCTION

Using vigorous seeds is directly correlated to successful establishment of a crop in the field, which is key to ensuring satisfactory production. Therefore, selecting seed lots with high vigor is important in crop implantation. Vigor tests are used to analyze the quality of seed lots and assist the producer in decision-making.

Vigor tests must be simple, accurate, fast, objective, low-cost, and be directly correlated with the in-field behavior of seedlings. Germination tests, which provide concise data on viability, are among the most commonly used tests in seed analysis laboratories. However, germination tests may overestimate the physiological potential of the batch because they are conducted under optimal conditions of water and temperature; additionally, the results are obtained after substantial delay.

Seeds of various species have been subjected to different vigor tests, and deteriorated lots showed slow germination, which is verified by an increase in time from the beginning of water absorption to emergence of the primary root (MARCOS-FILHO, 2015b). Therefore, late root protrusion is an initial sign of seed deterioration following physiological maturity. In this sense, Khajeh-Hosseini, Lomholt, and Matthews (2009) proposed the evaluation of corn seed vigor as a function of primary root protrusion at six and three days at 13 and 20 ºC, respectively, and the tests evaluated emergence and accelerated aging. The International Rules for Seed Analysis (INTERNATIONAL SEED TESTING ASSOCIATION, 2011) include this evaluation as a supplementary method for analyzing the vigor of corn seeds. Although the Rules for Seed Analysis (RAS) (BRASIL, 2009) have not set defined criteria for this test, studies have been developed for some crops.

Recently, studies have validated the efficiency of the primary root protrusion test in seeds of pepper (DEMIR *et al*., 2008), pumpkin (SOUSA; VILLELA; AUMONDE, 2013), super sweet corn (ALVARENGA; MARCOS-FILHO; TIMÓTEO, 2013), leeks (ERMIS *et al*., 2015; OZDEN *et al*., 2017), tomatoes (ERMIS; OZDEN; DEMIR, 2015; OLIVEIRA *et al*., 2021), coffee (TRUJILLO; GOMES-JUNIOR; CICERO, 2019), and chia (OLIVEIRA *et al*; 2019); however, with the exception of maize, the validity of this test in seeds of important crops has not been explored sufficiently. Therefore, further studies on economically important crops, such as soybeans, are essential.

This study was aimed at verifying whether primary root protrusion can be used as a vigor test for soybean seeds.

MATERIAL AND METHODS

This study was conducted at the Seed Laboratory of the Federal University of Mato Grosso do Sul, Campus of Chapadão do Sul (CPCS/UFMS), using five commercial lots of soybean seeds: cultivars ANTA 82, NA7337, and BRS1074IPRO. The water content and germination states were determined, and vigor tests (first count, emergence, emergence speed index, and tetrazolium) were performed.

The water content was determined using two repetitions per lot of seeds, kept in an oven for 24 h at 105 ± 3 ºC, according to the Rules for Seed Analysis (BRASIL, 2009) and the results were expressed in average percentage per lot.

In the germination test, four subsamples of 50 seeds per lot were distributed over two sheets of paper towel, previously moistened with distilled water in a volume equivalent to 2.5 times the weight of the dry substrate (BRASIL, 2009) and kept in a germinator at 25 °C. The evaluations were performed at five (first germination count) and eight days (final germination) after the start of the test.

For emergence, four repetitions of 25 seeds per lot were used, being sown in styrofoam trays at a depth of 0.5 cm, using the commercial substrate Plantax® and irrigated thrice a day. On day 10, the number of normal seedlings that emerged was counted. In conjunction with this test, daily counts were performed until stand stabilization to determine the emergence speed index, as was recommended by Maguire (1962).

For the tetrazolium test, soybean seeds were pre-soaked in paper towels with distilled water and kept in a germinator at 25 ºC for 16 h. Subsequently, they were placed in a solution of 2,3,5 triphenyltetrazolium chloride and kept in the dark at 35 ºC for 3 h. Seeds were individually evaluated and classified according to the method described by França-Neto and Krzyzanowski (2022).

Primary root protrusion test was performed in the same manner as the germination test. Temperatures of 20, 25, and 30 °C were used and two evaluation criteria were adopted: a) primary root emission precocity index (PREPI) - the data being calculated by the formula proposed by Maguire (1962): $ESI = E1/N1 + E2/N2 + ... En/Nn$; where PREPI = primary root issuance precocity index. E1, E2,... En = number of seeds that emitted the primary root (> 2 mm) in the second count up to the nth count; N1, N2,... Nn = period in hours that was performed at the first, second to the nth count; and b) primary root emission (PRE) – percentage value of the seeds with primary roots of lengths equal to or greater than 2 mm, every 12 h from the installation of the test up to 120 h.

The statistical design employed was completely randomized with four replicates. Means were compared within each evaluation period using the Scott-Knott test at 5% probability. Pearson’s linear correlation analysis between PREPI, PRE, and emergence was also performed.

RESULTS AND DISCUSSION

Seed lots showed a water content of 11.7–12.4% and this small variation ensured greater reliability of the results obtained in this work (MARCOS-FILHO, 2015a). Analysis of variance of the initial data obtained from the seed lots of the three soybean cultivars revealed significant differences in their physiological potential (Table 1). The lots showed germination values within the standard for the commercialization of soybean seeds (BRASIL, 2013). In general, lot 1 showed the greatest potential for germination and vigor in the three cultivars analyzed, while lots 2, 3, and 4 showed intermediate values; lot 5 had the lowest physiological potential for the three cultivars, showing unsatisfactory values for all analyzed variables.

Regardless of the temperature, cultivar lots could be ranked at different vigor levels (Table 2) using PREPI. However, when correlating the PREPI values with emergence, no significance was observed for any of the cultivars at the tested temperatures, with the exception of ANTA82 at 20 ºC. Therefore, this criterion was not efficient for classifying soybean seed lots in terms of vigor.

Seed lots of the ANTA82 and NA7337 cultivars took 48 h to initiate PRE when subjected to a temperature of 20 °C (Table 3). For BRS1074IPRO, protrusion started within 36 h, but at a lower percentage than when compared to the performance of the same batches at other temperatures. For cultivars ANTA82 and NA7337, root emission stabilization occurred at 108 h, whereas for BRS1074IPRO, stabilization occurred at 96 h.

Table 1 - Characterization of the initial quality of five soybean seed lots, cultivars ANTA 82, NA7337, and BRS1074IPRO, as a function of the first germination count (FGC), germination (G), emergence (E), emergence speed index (ESI), and tetrazolium (TZ-vigor)

Lots	FGC	G	E	ESI	TZ-vigor %
	----------	%	----------		
			ANTA82		
1	79 a	94 a	94 a	4.73 a	93 a
2	67 a	85 b	84 b	4.39 a	81 c
3	63 b	80 b	77 c	3.77 b	80 c
4	62 b	81 b	76 d	3.51 b	85 b
5	65 b	78 b	71 e	3.28 b	81 c
F	11.1*	7.74*	34.98*	13.32*	54.37*
CV (%)	6.1	5.3	3.7	7.0	1.7
			NA7337		
1	95 a	97 a	93 a	4.79 a	98 a
2	85 b	95 a	92 a	4.94 a	95 b
3	82 b	90 b	89 a	4.61 a	92 c
4	76 c	88 b	85 b	4.31 a	87 d
5	62 d	81 c	78 b	3.57 b	82 e
F	43.84*	15.27*	6.58*	3.74*	61.50*
CV (%)	4.6	3.6	5.4	12.6	1.8
			BRS1074IPRO		
1	88 a	99 a	96 a	5.18 a	96 a
2	81 b	94 a	96 a	5.28 a	95 a
3	74 c	90 b	86 b	4.73 a	89 b
4	71 c	85 b	78 c	3.94 b	85 c
5	67 c	83 b	78 c	3.73 b	82 d
F	19.63*	11.11*	20.30*	8.37*	79.93*
CV (%)	5.1	4.4	4.6	10.7	1.5

Means followed by the same letter in each column do not differ according to the Scott–Knott test at 5% probability. (ns): not significant according to the F test at 5% probability. CV – coefficient of variation

Table 2 - Primary root emission precocity index (PREPI), at three temperatures for five lots of three soybean cultivars and Pearson (r) correlation with emergence

Lots	20	25	30
		ºC	
		ANTA82	
1	60.51 a	55.53 b	59.68 a
2	60.20 a	53.96 b	57.84 a
3	53.75 b	58.52 a	52.59 b
4	55.74 b	61.20 a	59.24 a
5	54.18 b	57.22 b	55.73 b
F	9.02*	4.19*	7.07*
CV (%)	3.8	4.7	3.8
r	0.88*	-0.57^{ns}	0.54^{ns}
		NA7337	
1	60.06 a	59.21 a	52.44 b
2	58.78 a	56.99 a	52.18 b
3	56.33 a	59.07 a	52.86 b
4	55.53 a	59.50 a	53.04 b
5	56.73 a	59.10 a	55.40 a
F	1.64^{ns}	0.47^{ns}	3.98*
CV (%)	5.1	5.0	2.4
r	0,66^{ns}	-0.41^{ns}	-0.95^{ns}
		BRS 1074 IPRO	
1	61.35 b	52.94 b	52.50 b
2	67.88 a	58.95 a	50.97 b
3	71.28 a	53.51 b	51.63 b
4	65.58 b	53.23 b	54.24 a
5	69.87 a	53.66 b	55.62 a
F	4.07*	13.95*	7.20*
CV (%)	5.8	2.5	2.7
r	-0.43^{ns}	0.50^{ns}	-0.81^{ns}

Means followed by the same letter in each column do not differ according to the Scott–Knott test at 5% probability. (ns): not significant according to the F test at 5% probability, *= r significant at 5% probability; ns = r not significant

At temperatures of 25 and 30 ºC, protrusion occurred in shorter periods (Tables 4 and 5). Protrusion began 36 h after the start of the test for all cultivars, and stabilization occurred at 84 h. This meets one of the principles of vigor testing, which is the speed at which results are obtained. According to the Rules for Seed Analysis (BRASIL, 2009), the germination test can be conducted both at 25 and 30 ºC, resulting in rapid and uniform germination, which corroborates the results obtained when these temperatures were used.

The optimum temperature for the germination of soybean seeds is 32 ºC, and the speed at which germination occurs is associated with the speed of imbibition (MARCOS-FILHO, 2015a). This is directly related to the temperature to which the seed is subjected, whose lower values reduce the kinetic energy of the water, leading to reduction in metabolic reactions that control germination.

Primary root emission at 20 ºC resulted in a significant correlation (P > 0.05) for the 72 h period with the emergence test for the three cultivars (Table 6). However, at 25 °C, the correlation was significant post the 36 h period for ANTA82 and NA7337, and post the 60 h period for BRS1074IPRO. Under 30 ºC, all cultivars showed a significant correlation with emergence after 36 h. At the lowest temperature, the test correlated with emergence over a period twice as long (72 h) compared to other temperatures tested.

Table 3 - Percentage of seeds that emitted the primary root (PRE), evaluated every 12 h, at 20 ºC, for five lots in three soybean cultivars

Lots	12	24	36	48	60	72	84	96	108	120
ANTA82										
	h									
1	0 a	0 a	0 a	24 a	69 a	81 a	88 a	91 a	91 a	91 a
2	0 a	0 a	0 a	24 a	67 a	76 a	79 b	81 b	82 b	82 b
3	0 a	0 a	0 a	11 b	45 c	61 b	69 c	80 b	81 b	81 b
4	0 a	0 a	0 a	15 b	56 b	66 b	77 b	80 b	81 b	81 b
5	0 a	0 a	0 a	12 b	40 c	53 c	62 c	72 c	77 b	77 b
F	-	-	-	8.72*	11.04*	19.33*	13.75*	13.90*	9.63*	9.11*
CV (%)	-	-	-	26.3	14.2	7.7	7.3	4.5	4.1	4.1
NA7337										
1	0 a	0 a	0 a	23 a	72 a	90 a	94 a	96 a	96 a	96 a
2	0 a	0 a	0 a	21 a	62 a	78 b	88 a	92 b	92 a	92 a
3	0 a	0 a	0 a	16 a	63 a	82 a	89 a	91 b	91 a	91 a
4	0 a	0 a	0 a	14 a	59 a	72 b	80 b	83 c	83 b	83 b
5	0 a	0 a	0 a	17 a	56 a	72 b	77 b	80 c	80 a	80 a
F	-	-	-	1.59*	2.06*	6.13*	12.49*	29.32*	29.32*	29.32*
CV (%)	-	-	-	33.0	13.8	7.7	4.6	2.7	2.7	2.7
BRS1074IPRO										
1	0 a	0 a	51 a	76 a	85 a	93 a	95 a	95 a	95 a	95 a
2	0 a	0 a	34 b	72 a	81 a	91 a	94 a	94 a	94 a	94 a
3	0 a	0 a	32 b	77 a	85 a	88 a	89 b	89 b	89 b	89 b
4	0 a	0 a	34 b	68 a	75 b	81 b	84 c	84 c	84 c	84 c
5	0 a	0 a	14 c	57 b	66 c	76 c	79 d	82 c	82 c	82 c
F	-	-	13.35*	6.59*	12.73*	18.57*	66.11*	56.92*	56.92*	56.92*
CV (%)	-	-	21.6	9.0	5.7	3.7	1.9	1.8	1.8	1.8

Means followed by the same letter in each column do not differ according to the Scott–Knott test at 5% probability. (ns): not significant according to the F test at 5% probability

Table 4 - Percentage of seeds showing PRE, evaluated every 12 h, at 25 ºC, for five lots in three soybean cultivars

Lots	12	24	36	48	60	72	84	96	108	120
ANTA82										
	h									
1	0 a	0 a	69 a	82 a	90 a	92 a	93 a	93 a	93 a	93 a
2	0 a	0 a	65 a	75 b	79 b	82 b	84 b	84 b	84 b	84 b
3	0 a	0 a	50 b	70 b	76 b	79 b	79 b	79 b	79 b	79 b
4	0 a	0 a	46 b	71 b	78 b	80 b	80 b	80 b	80 b	80 b
5	0 a	0 a	40 c	57 c	70 c	76 b	78 b	78 b	78 b	78 b
F	-	-	49.0	9.68	22.22	9.01	9.54	9.54	9.54	9.54
CV (%)	-	-	6.5	8.1	3.8	4.9	4.7	4.7	4.7	4.7

Continuation Table 4

					NA7337					
1	0 a	0 a	64 a	85 a	90 a	93 a	94 a	94 a	94 a	94 a
2	0 a	0 a	64 a	80 a	89 a	83 a	93 a	93 a	93 a	93 a
3	0 a	0 a	49 b	70 b	81 b	87 a	88 a	88 a	88 a	88 a
4	0 a	0 a	45 b	66 b	75 c	80 b	85 b	85 b	85 b	85 b
5	0 a	0 a	26 c	47 c	62 d	73 c	80 b	80 b	80 b	80 b
F	-	-	33.46*	21.58*	28.76*	18.40*	11.21*	11.21*	11.21*	11.21*
CV (%)	-	-	11.2	9.2	5.4	4.7	3.8	3.8	3.8	3.8
					BRS1074IPRO					
1	0 a	0 a	82 a	89 a	95 a	96 a	97 a	97 a	97 a	97 a
2	0 a	0 a	60 b	80 b	91 a	95 a	95 a	95 a	95 a	95 a
3	0 a	0 a	67 b	76 b	84 b	86 b	86 b	86 b	86 b	86 b
4	0 a	0 a	65 b	74 b	81 b	84 b	84 b	84 b	84 b	84 b
5	0 a	0 a	59 b	69 c	77 c	81 b	81 c	81 c	81 c	81 c
F	-	-	14.12*	18.50*	18.82*	29.06*	35.52*	35.52*	35.52*	35.52*
CV (%)	-	-	7.3	4.6	4.0	2.9	2.7	2.7	2.7	2.7

Means followed by the same letter in each column do not differ according to the Scott–Knott test at 5% probability. (ns): not significant according to the F test at 5% probability

Table 5 - Percentage of seeds showing PRE, evaluated every 12 h at 30 ºC, for five lots in three soybean cultivars

					ANTA82					
Lots	12	24	36	48	60	72	84	96	108	120
	------------------- h -------------------									
1	0 a	0 a	61 a	83 a	91 a	93 a	93 a	93 a	93 a	93 a
2	0 a	0 a	54 b	72 b	81 b	84 b	84 b	84 b	84 b	84 b
3	0 a	0 a	52 b	66 c	70 d	75 d	75 d	75 d	75 d	75 d
4	0 a	0 a	45 c	60 d	76 c	79 c	81 c	81 c	81 c	81 c
5	0 a	0 a	43 c	57 d	67 d	73 d	75 d	75 d	75 d	75 d
F	-	-	11.96	23.25	28.05	33.08	40.90	40.90	40.90	40.90
CV (%)	-	-	8.3	6.4	4.6	3.3	2.8	2.8	2.8	2.8
					NA7337					
1	0 a	0 a	83 a	89 a	94 a	95 a	95 a	95 a	95 a	95 a
2	0 a	0 a	82 a	88 a	91 a	93 a	93 a	93 a	93 a	93 a
3	0 a	0 a	76 a	84 a	89 a	91 b	91 b	91 b	91 b	91 b
4	0 a	0 a	71 b	79 b	83 b	86 c	88 c	88 c	88 c	88 c
5	0 a	0 a	61 c	74 b	79 b	80 d	82 d	82 d	82 d	82 d
F	-	-	12.13*	11.06*	17.34*	40.92*	34.11*	34.11*	34.11*	34.11*
CV (%)	-	-	6.7	4.7	3.3	2.1	2.0	2.0	2.0	2.0
					BRS1074IPRO					
1	0 a	0 a	84 a	90 a	94 a	95 a	95 a	95 a	95 a	95 a
2	0 a	0 a	86 a	89 a	93 a	94 a	94 a	94 a	94 a	94 a

Continuation Table 5

3	0 a	0 a	79 a	84 a	87 b	88 b	88 b	88 b	88 b	88 b
4	0 a	0 a	68 b	78 b	82 c	85 b	85 b	85 b	85 b	85 b
5	0 a	0 a	54 c	68 c	77 d	83 b	83 b	83 b	83 b	83 b
F	-	-	14.93*	11.57*	18.25*	15.02*	15.02*	15.02*	15.02*	15.02*
CV (%)	-	-	9.2	6.6	3.9	3.2	3.2	3.2	3.2	3.2

Means followed by the same letter in each column do not differ according to the Scott–Knott test at 5% probability. (ns): not significant according to the F test at 5% probability

Table 6 - Pearson's linear correlation between PRE and emergence test for five lots of three soybean varieties

					20 ºC					
	12	24	36	48	60	72	84	96	108	120
ANTA82	0^{ns}	0^{ns}	0^{ns}	0.87^{ns}	0.87^{ns}	0.93*	0.92*	0.94*	0.96*	0.95*
NA7337	0^{ns}	0^{ns}	0^{ns}	0.73^{ns}	0.81^{ns}	0.88*	0.82^{ns}	0.86^{ns}	0.78^{ns}	0.76^{ns}
BRS1074IPRO	0^{ns}	0^{ns}	0.72^{ns}	0.70ns	0.75^{ns}	0.93*	0.96*	0.99*	0.99*	0.99*
					25 ºC					
ANTA82	0^{ns}	0^{ns}	0.95*	0.92*	0.96*	0.98*	0.98*	0.98*	0.98*	0.98*
NA7337	0^{ns}	0^{ns}	0.99*	0.99*	1.00*	0.88*	0.98*	0.98*	0.98*	0.98*
BRS1074IPRO	0^{ns}	0^{ns}	0.47^{ns}	0.87^{ns}	0.96*	0.97*	0.98*	0.98*	0.98*	0.98*
					30 ºC					
ANTA82	0^{ns}	0^{ns}	0.95*	0.98*	0.96*	0.97*	0.95*	0.95*	0.95*	0.95*
NA7337	0^{ns}	0^{ns}	1.00*	0.99*	0.98*	0.99*	1.00*	1.00*	1.00*	1.00*
BRS1074IPRO	0^{ns}	0^{ns}	0.89*	0.89*	0.96*	0.98*	0.98*	0.98*	0.98*	0.98*

*= r significant at 5% probability; ns = r not significant

At 25 ºC, no correlation was observed for the three cultivars in the common time period. Only when the test was conducted at 30 ºC was there a significant correlation with all cultivars, which also resulted in the ranking of the lots most similar to the initial tests, mainly compared with seedling emergence (Table 1).

The direct correlation between vigor tests and field emergence is crucial. From a phytotechnical point of view, early germination allows for the rapid establishment of seedlings, in addition to countering the adverse conditions to which seeds are subjected to on-field during the initial establishment phase (NERLING; COELHO; BRÜMMER, 2022). Therefore, lots with greater vigor are more efficient at using reserves to form normal seedlings (Andrade *et al*., 2019; EHRHARDT-BROCARDO; COELHO, 2016).

Several authors have demonstrated the effectiveness of primary root emission tests for evaluating seed vigor. Alvarenga *et al*. (2013) adopted the precocity of the emission of a primary root 2 mm in length and found that seed lots of supersweet corn could be differentiated in terms of vigor. Prazeres and Coelho (2017) also observed rapid root protrusion in maize hybrids with high germination and vigor. Studies on millet (MACHADO *et al*., 2012), white wattle (ROCHA *et al*., 2016), tomato (ERMIS; OZDEN; DEMIR, 2015; OLIVEIRA *et al*., 2021), and leek (ERMIS *et al*., 2015) also corroborated the results obtained in this study. This demonstrates the effectiveness of this test for distinguishing lots, proving that it is a promising method that can be utilized in seed quality control programs.

Although primary root emission is still not considered a valid test according to the RAS precepts, it proved to be viable and efficient for classifying the vigor levels of soybean seed lots. In addition, it is advantageous because of the ease of execution and the provision of faster results compared to the first germination count.

CONCLUSION

Examining primary root emission for 36 h after test installation at 30 ºC is recommended as a vigor test for soybean seeds.

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