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CROP PRODUCTION

Intracultivar selection for seed quality of soybeans in an ultra-low-density selection model (Honeycomb Selection Designs)

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ABSTRACT. Selecting genotypes that combine high productivity with high seed quality is a challenge. The exploration of intracultivar genetic variation is an alternative to significantly increase the annual genetic gain and maximize the selection efficiency for both characteristics. The present study aimed to identify intracultivar variation to improve the seed quality of soybean genotypes derived from the commercial cultivar BRS 284, selected using the HSD (Honeycomb Selection Designs) model. Soybean genotypes selected for two years from two environments with contrasting edaphoclimatic characteristics, according to the principles of the HSD selection model, were cultivated under competition by using the experimental model in randomized blocks with four replicates and evaluated regarding the productivity and physiological quality of seeds. The results showed that genotype 284-3 presented a greater mass of 100 seeds, germination, vigour after accelerated ageing test, seedling emergence and emergence speed index than the other genotypes in both environments, with no significant difference in the standard cultivar regarding seed yield. The HSD method was an efficient selection method to identify intracultivar variation to improve cultivar performance.

Keywords: *Glycine max* (L.) Merrill; honeycomb breeding; intracultivar variation; single-plant selection; seed germination; breeder seed.

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Introduction

The process to develop elite cultivars is time-consuming and expensive, and the current cultivars present low longevity (Tokatlidis, 2015). An alternative to increase the useful life of the cultivars is the exploration of intracultivar variation, which, although important, has not been widely used because of the belief that elite cultivars are highly homogeneous. Despite the existing homogeneity, there are some residual heterozygosity and molecular mechanisms that can generate "de novo" variations due to mechanisms, such as intragenic recombination, transposons, crossing over, DNA methylation and gene amplification (Haun et al., 2011; Tokatlidis, Tsikrikoni, Lithourgidis, Tsialtas, & Tzantarmas, 2011; Silveira et al., 2013; Fasoula, 2012).

Considering this supposition, Fasoula (2013) proposed a new methodology, known as Prognostic Breeding, to significantly increase the annual genetic gain and maximize selection efficiency. Therefore, prognostic breeding consists of an integrated crop-improvement methodology that enables the selection of plants for their productive potential through the evaluation of two components: yield potential and stability (Fasoula, 2013, Greveniotis & Fasoula, 2016). Reliable estimates of these two components are ensured by the replicated moving complete block (MCB) obtained through Honeycomb Selection Designs (HSD) (Fasoulas & Fasoula, 1995). HSD methodology consists of selecting individual plants under null competition, comparing their production with that of their neighbours, reducing the effects of soil heterogeneity and maximizing selection efficiency.

Since the first studies involving soybean breeding to the present day, the main goal of breeding programmes has been to increase crop yield. However, productivity is considered a quantitative trait that typically presents low heritability, and annual genetic gains of soybean yield in Brazil have been low, ranging from 0.38 to 3.49% per year (Toledo, Almeida, Kiihl, & Menosso, 1990; Alliprandini, Toledo, Fonseca Junior, & Kiihl, 1993; Lange & Federizzi, 2009).

There are many factors that can contribute to increased crop yield. One factor of great importance, but with little focus in breeding programmes, is seed quality (Marcos-Filho, 2015; Martins, Unêda-Trevisoli, Môro, & Vieira, 2016, Pal, Sharma, Thakur, & Dogra, 2018). Seedling emergence and initial plant growth delay caused by reduced seed vigour have an indirect effect on crop productivity, since the plant population is affected by seed quality, either by non-germination or by non-plant survival until the reproductive phase (Scheeren, Peske, Schuch, & Barros, 2010; Carvalho & Nakagawa, 2012). The direct effects of seed vigour on crop yields have been reported by Marcos-Filho and França-Neto (2017), since vigourous seeds result in stand establishment with high performance plants that are more productive. Therefore, it is also important to consider seed quality aspects in the selection of new genotypes in soybean breeding programmes. However, it is difficult to select genotypes with both high seed yield potential and high seed quality.

There are several studies demonstrating the efficiency of the HSD method in the selection of soybean genotypes with high seed yield potential (Fasoula, 2012); however, there are no studies involving the application of this methodology to identify intracultivar variation to improve physiological seed quality. Therefore, the present study aimed to evaluate the physiological seed quality of four soybean genotypes derived from the commercial cultivar BRS 284, selected by using the ultra-low-density selection model Honeycomb Selection Designs and comparing these plants to standard cultivars.

Material and methods

The genetic material consisted of seeds of four soybean genotypes derived from the commercial cultivar BRS 284 selected by the ultra-low-density selection method Honeycomb Selection Designs (HSD) (Fasoulas & Fasoula, 1995). BRS 284 is a non-transgenic cultivar that presents early cycle, maturation group (GM) 6.4, indeterminate growth, high seed yield potential, resistance to the *Meloidogyne javanica* nematode, high lodging (Lodging index = 4.0) and low protein content (P = 36.3%).

Intracultivar selection trials to obtain the four genotypes were conducted at Embrapa Soybean in Londrina, Paraná State, Brazil (23’20” LS and 51’18” LW) and in Ponta Grossa, Paraná State, Brazil (25’17” LS, 50’09” LW) during two crop seasons (2014-15 and 2015-16). In the first crop season (2014-15), the selection of the individual plants in the absence of competition in Londrina and Ponta Grossa was performed using the unreplicated ultra-low-density method HSD, according to Fasoulas and Fasoula (1995). Four seeds were planted per pot, and 15 days after sowing, thinning was performed to leave only one plant at 1 m^{-2}. Each plot contained 400 plants, with a plant-to-plant space of 1.0 m (10,000 plants ha^{-1}) in an area of 400 $m^2$and no replicates. Selection was performed based on the *Plant Yield Index* [$PYI = (x/\bar{X}_r)^2$], where: x is the yield of each plant and $\bar{X}_r$ is the mean yield of the 12 surrounding plants. Comparison and ranking of the PYI values resulted in the selection of 20 plants, ten plants for each location (Fasoula, 2013; Greveniotis & Fasoula, 2016).

In the subsequent crop season (2015-16), the 20 plants selected in the previous cycle, including the standard cultivar, totalling 21 entries, were evaluated by using the replicated R-21 HSD model with 20 plants per entry. The genotypes were conducted under null competition (1.0 m plant-to-plant space), in a total of 420 plants per plot. For each location, the PYI values were estimated, considering the average yield of the 24 surrounding plants laid out within the moving replicate; and the Stability Index (SI) or coefficient of homeostasis [$SI = (\bar{x}_g/s)^2$]), where: $\bar{x}_g$ and s are the mean plant yield and standard deviation, respectively, of the correspondent sibling line. The estimated PYI and SI values were used as a base for the calculation of the prognostic equation [$pPE = (x/\bar{x}_r)^2 (\bar{x}_g/s)^2$], which measures the seed yield potential of the individual plants, ensuring the efficient selection of crop-yield “champion” plants (Fasoula, 2013; Greveniotis & Fasoula, 2016).

The four best genotypes derived from BRS 284, including the standard cultivar, were assessed under competition by using a randomized block design with four replicates in two locations (Londrina and Ponta Grossa, Paraná State, Brazil) during the crop season 2016-17. Each plot consisted of four 5.0 m rows spaced at 0.5 m and a density of 320,000 plants ha^{-1}. The genotypes were evaluated regarding seed yield, expressed as kg ha^{-1}, water content, corrected to 13%, and 100-seed mass by using a precision scale of 0.01 g and four replicates of 100 seeds per treatment.

Precipitation and temperature data as well as the soil analysis of Londrina and Ponta Grossa, Paraná State, Brazil, during the crop season 2016-17 are shown in Figure 1 and Table 1, respectively.

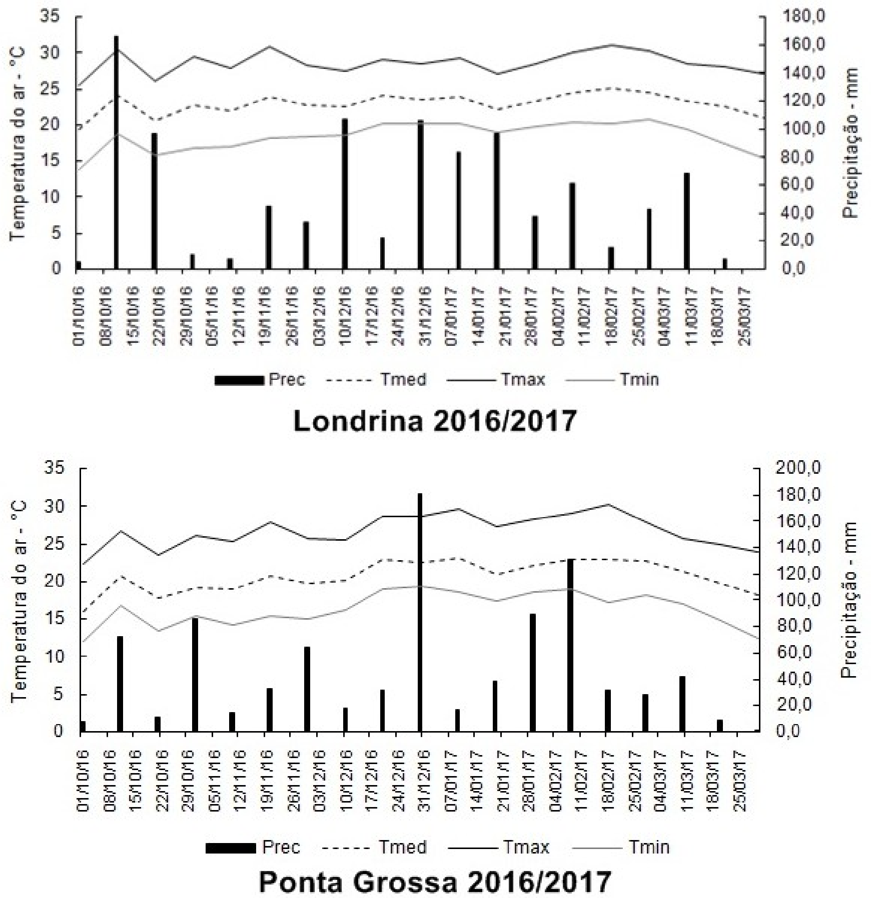

Figure 1. Maximum, minimum, and mean temperatures and precipitation during the crop season 2016-17 in Londrina and Ponta Grossa, Paraná State, Brazil.

Table 1. Altitude and soil chemical characteristics of the 0-20 cm layers in Londrina and Ponta Grossa, Paraná State, Brazil.

Locations	Altitude	pH	Al	H+Al	Ca	Mg	K	SB	CTC	V	P
	m		---------------------- cmol$_c$ dm^{-3} ----------------------							%	mg dm^{-3}
Londrina	585	5.0	0.0	4.1	4.1	1.4	0.8	6.5	10.2	59.3	16.2
Ponta Grossa	969	5.1	0.0	3.6	3.1	1.3	0.4	5.6	9.8	56.7	18.5

The physiological quality of the seeds was assessed in the Seed Analysis Laboratory of the State University of Londrina (Londrina, Paraná State, Brazil). Laboratory tests comprised seed water content determination, germination, first germination count, electrical conductivity, accelerated ageing, seedling emergence and emergence speed index as follows:

Water content: seeds were dried at 105 ± 3°C for 24h by using two sub-samples of 4.5 g of seeds per treatment, according to the Brazilian Rules for Seed Testing (Brasil, 2009), and the results are expressed as a percentage.

Germination and first germination count: conducted in eight sub-samples of 50 seeds per treatment distributed onto sheets of "Germitest" paper towel in a "Mangesdorf" germinator at 25 ± 2°C. The amount of water added to the substrate was equivalent to 2.5 times the mass of the dried paper. Germination counts were performed at five days (first count) and eight days after sowing, according to the Brazilian Rules for Seed Testing (Brasil, 2009; Smaniotto, Resende, Marçal, Oliveira, & Simon, 2014).

Electrical conductivity: four replicates of previously weighed 50 soybean seeds were placed in plastic cups (200 mL) with 75 mL of deionized water and maintained at 25°C for 24h (Vieira & Krzyzanowski, 1999). After embedding, the electrical conductivity of the solution was assessed by using a conductivity meter; the results are expressed as μs cm^{-1} g^{-1}.

Accelerated ageing: was performed by using gearbox-type plastic boxes with suspended stainless-steel screens. Forty millilitres of distilled water were added on the bottom of the boxes, and the seeds samples were distributed in a single layer on a canvas suspended in the plastic box. Thereafter, the plastic boxes containing the seeds were capped and maintained in a BOD incubator at 41°C for 48h (Marcos-Filho, 1999). After incubation of the seeds for 48 h, a germination test was performed as previously described. In parallel, the water content before and after ageing was determined by drying the seeds at 105 ± 3ºC for 24h to monitor the procedures used in the test.

Seedling emergence and emergence speed index: were performed in a greenhouse using four sub-samples of 50 seeds per treatment sown to 1 cm deep in plastic boxes containing coarse sand as a substrate. Seedlings with a shoot length of no less than 20 mm at 15 days after sowing were considered as emerged; results were expressed as a percentage of the emerged seedlings. The seedling emergence speed index was estimated according to the equation proposed by Maguire (1962), $SG= N1/T1 + N2/T2 + ... Nn/Tn$, where: SG is the speed of germination index (dimensionless); $N1, N2, ... Nn$ are the number of seedlings emerged in the first, second, and last count, respectively; and $T1, T2, ... Tn$ are the number of days from the first, second, to the last count.

Seed yield evaluation was conducted under a randomized complete block design with four replicates, while the evaluation of the 100-seed mass and physiological quality of the seeds were conducted under a completely randomized design with four replicates. Data obtained at the two locations were submitted to the joint analysis of variance; percentage data were transformed into arc-sine $(x/100)^{0,5}$. The ratio between the largest and the smallest mean square residual was verified by Hartley test to perform the experimental groups analysis (Banzatto & Kronka, 2015). The averages were compared by Tukey's test ($p \leq 0.05$). Data analysis was performed by using the "ExpDes.pt" package from R statistical software v. 3.4.1 (R Core Team, 2016).

Results and discussion

The joint analysis of variance revealed a significant interaction ($p \leq 0.05$) between locations and genotypes for all characteristics assessed, except seed yield (Table 2), demonstrating the influence of the environment on the physiological quality of the seeds.

Table 2. Joint analysis of variance summary regarding the seed yield and quality of four soybean genotypes derived from the BRS 284 cultivar by using the ultra-low density selection model HSD (Honeycomb Selection Designs) compared to the standard cultivars, totalling five genotypes grown in two locations (Londrina and Ponta Grossa, Paraná State, Brazil) during the crop season 2016-17.

FV	GL	Mean Square							GL	QM
		G	FG	EC	AA	EM	SG	M100		P
Location (L)	1	1.92**	1.34**	48,965**	1.31**	0.44**	0.15	106.28**	1	536,472
Genotype (G)	4	0.06**	0.06**	800**	0.03**	0.17**	237.40**	2.58**	4	1,107,036
L x G	4	0.05**	0.06**	735**	0.04**	0.03*	74.46**	0.43**	4	260,725
Error	30	0.003	0.005	123	0.004	0.008	15.87	0.099	27	414,124
Mean		65.4	56.83	107.86	51.27	68.58	26.96	15.13		5,100.70
CV (%)		6.44	8.27	10.30	7.82	9.18	14.78	2.08		12.62

G: germination (%); FG: first germination count (%); EC: electrical conductivity (µS cm^{-1} g^{-1}); AA: accelerated ageing (%); EM: seedling emergence (%); SG: speed of germination index; M100: 100-seed mass (g); P: seed yield (kg ha^{-1}). **$p \leq 0.01$ and *$p \leq 0.05$ by F-test.

The average seed yield of all soybean genotypes grown in Londrina and Ponta Grossa, Paraná State, Brazil, are presented in Table 3. On average, the genotypes showed higher productivity in Ponta Grossa (4,985 kg ha^{-1}), with no statistical difference from Londrina (4,642 kg ha^{-1}).

Table 3. Seed yield and 100-seed mass of the four soybean genotypes derived from the cultivar BRS 284 by using the ultra-low-density selection model HSD, compared to the standard cultivars grown in two locations (Londrina and Ponta Grossa, Paraná State, Brazil) during the crop season 2016-17.

Genotypes	Seed yield (kg ha^{-1})			100-seed mass (g)		
	LDA	PGA	Mean	LDA	PGA	Mean
BRS 284	4,784.80 Aa	5,467.18 Aa	5,125.99	12.87 Bc	16.65 Ab	14.76
284-1	4,326.04 Aa	4,933.46 Aa	4,629.75	12.91 Bc	15.85 Ac	14.38
284-2	4,730.08 Aa	4,589.33 Aa	4,659.71	13.60 Bb	16.88 Aab	15.24
284-3	4,641.26 Aa	5,488.83 Aa	5,065.05	14.45 Ba	17.12 Aab	15.79
284-5	4,725.83 Aa	4,445.64 Aa	4,585.74	13.69 Bb	17.33 Aa	15.51
Mean	4,641.60 A	4,984.89 A	4,813.25	13.5 B	16.77 A	15.14

Means followed by the same capital letter in each row, comparing locations, and the same lower-case letter in each column, comparing genotypes, do not differ by Tukey's test ($p \leq 0.05$).

Although no significant statistical difference among genotypes regarding seed yield was observed, the results in only two locations are not sufficient to evaluate yield performance. The same genotypes were tested in 14 locations during the 2016/17 season (data not published), and the results are promising, showing that selection using ultra-low density can be useful in breeding programmes. However, agronomic characteristics, such as plant height, days to 50% flowering, days to maturity, lodging and first pod height, revealed little intracultivar variation compared to the standard cultivar.

Line 284-3 presented the highest values of 100-seed mass (14.45 g) in Londrina, while in Ponta Grossa, line 284-5 presented a higher 100-seed mass (17.33 g) (Table 3). Perini, Fonseca Júnior, Destro, and Prete (2012) obtained similar values, showing a 100-seed mass around 14 g for cultivar BRS 284. We verified that the seeds produced in Ponta Grossa, Paraná State, Brazil, presented higher 100-seed mass values than those grown in Londrina on average. The selection of genotypes with greater 100-seed mass values, as well as the identification of environments that enhance the expression of this characteristic, is of great importance because, generally, larger seeds present a higher amount of reserves, and consequently, if they present greater germination and vigour, then these aspects may play an important role in the establishment of the crop and on the crop yield (Pádua, Zito, Arantes, & França-Neto, 2010). However, Limede, Zoz, Zuffo, Steiner, and Zoz (2018), studying the effect of seed size in the emergence of soybean, found that seed size had no influence in the emergence of soybean seedlings; nevertheless, the seeds with a higher size resulted in plants with a higher dry mass of stems, shoots and roots.

The water content, germination and first germination count data are presented in Table 4. We observed that the initial water content of the seeds was 10.9 ± 0.6%, indicating no interference in the seed quality results (Marcos-Filho, Kikuti, & Lima, 2009; Martins et al., 2016). According to the Normative Instruction No. 45 (MAPA, 2013), the minimum acceptable germination for basic seeds is 75 and 80% for seeds belonging to the categories C1, C2, S1 and S2. Therefore, all genotypes grown in Londrina, Paraná State, Brazil, did not reach the minimum germination required to produce basic seeds, while in Ponta Grossa, Paraná State, Brazil, all genotypes presented adequate germination values, except for BRS 284-1. The low germination values of BRS 284 have previously been reported. Carvalho, Coelho, and Souza (2014), evaluating the germination of BRS 284 seeds after seven months of storage, verified that the germination levels were below the minimum standard for commercialization.

Table 4. Water content (WC), germination (G) and first germination count (FG) of four soybean genotypes derived from cultivar BRS 284, by using the ultra-low-density selection model HSD compared to the standard cultivars grown in two environments (Londrina and Ponta Grossa, Paraná State, Brazil) during the crop season 2016-17.

Genotypes	WC (%)		G (%)			FG (%)		
	LDA	PGA	LDA	PGA	Mean	LDA	PGA	Mean
BRS 284	10.2	11.4	37 Bb	94 Aa	65	32 Bb	88 Aa	60
284-1	10.1	10.6	44 Bab	73 Ac	58	39 Bab	62 Ab	50
284-2	10.5	12.0	55 Ba	85 Ab	70	46 Ba	67 Ab	56
284-3	10.5	11.1	53 Ba	95 Aa	74	47 Ba	88 Aa	67
284-5	11.2	11.1	43 Bab	76 Abc	60	36 Bab	65 Ab	50
Mean	10.5	11.2	46 B	84 A	65	40 B	74 A	57

Means followed by the same capital letter in each row, comparing locations, and the same lower case letter in each column, comparing genotypes, do not differ by Tukey's test ($p \leq 0.05$).

Significant genotype-by-environment interactions regarding several characteristics of soybean seeds, such as yield, protein, oil and sugar content, isoflavone concentration, anti-nutritional factors, nodulation, and seed quality, have previously been reported (Rangel, Minuzzi, Braccini, Scapim, & Cardoso, 2007; Matsuo et al., 2008; Yan, Lauer, Borges, & de Leon, 2010; Tukamuhabwa, Asiimwe, Nabasirye, Kabayi, & Maphosa, 2012; Vasconcelos, Reis, Sediyama, & Cruz, 2012; Hemingway, Eskandari, & Rajcan, 2015; Agoyi et al., 2017; John, Khan, Luthria, Garrett, & Natarajan, 2017; Matei et al., 2017; Carter, Rajcan, Woodrow, Navabi, & Eskandari, 2018).

It is well known that temperatures above 30°C, mainly during seed filling and/or the final stages of maturation at pre-harvest, negatively affect seed quality (Carvalho & Nakagawa, 2012). We observed that Ponta Grossa, Paraná State, Brazil, presented lower average temperatures throughout the cycle, varying between 12.1 and 30.3°C (Figure 1), exhibited precipitation values closer to the soybean water requirement reported in the literature, at approximately 800 mm (Sediyama, 2009), and were better distributed, demonstrating a more favourable environment for the production of soybean seeds when compared to that of Londrina, Paraná State, Brazil. A well-distributed precipitation is one of the most important factors for maximizing soybean yield potential; in addition, precipitation and temperature variations play a key role in seed quality and may, in some cases, reduce their commercial quality (Minuzzi et al., 2010). Regarding soil chemical characteristics (Table 1), we verified that there was little divergence between the sites.

According to data from previous studies, high-vigour soybean seeds typically present electrical conductivity values lower than 70 - 80 μS cm^{-1} g^{-1} (Vieira & Krzyzanowski, 1999). Notably, we verified that the seeds obtained in Ponta Grossa/PR showed values within the expected range for high vigour seeds, except genotype 284-1, while in Londrina, Paraná State, Brazil, all genotypes presented high values of electrical conductivity (Table 5), indicating the potential degradation of the cell membranes and release of the solutes, deteriorating seed quality (Carvalho & Nakagawa, 2012; Carvalho et al., 2014; Castro, Oliveira, Lima, Santos, & Barbosa, 2016) and confirming that Ponta Grossa consists of an environment more favourable to the production of seeds of greater physiological potential than those of Londrina.

Table 5. Electric conductivity (EC), accelerated ageing (AA), seedling emergence (EM) and speed of germination index (SG) of four soybean genotypes derived from cultivar BRS 284, using the ultra-low-density selection model HSD, compared to the standard cultivar, grown in two environments (Londrina and Ponta Grossa, Paraná State, Brazil) during the crop season 2016-17.

Genotypes	EC (μS cm^{-1} g^{-1})			AA (%)		
	LDA	PGA	Mean	LDA	PGA	Mean
BRS 284	155.76 Aa	55.90 Bb	105.83	25 Bb	76 Aa	51
284-1	152.34 Aab	97.65 Ba	125.00	27 Bb	69 Aab	48
284-2	131.96 Ab	77.86 Bab	104.91	43 Ba	62 Ab	52
284-3	130.60 Ab	66.71 Bb	98.66	43 Ba	79 Aa	61
284-5	143.56 Aab	66.22 Bb	104.89	33 Aab	58 Ab	45
Mean	142.84 A	72.87 B	107.86	34 B	69 A	51
Genotypes	**EM (%)**			**SG**		
	LDA	PGA	Mean	LDA	PGA	Mean
BRS 284	46 Bc	79 Ab	62	21.38 Bb	30.49 Aa	25.94
284-1	51 Bc	71 Abc	61	21.95 Ab	19.88 Ab	20.92
284-2	69 Bab	84 Ab	76	30.91 Aa	30.22 Aa	30.57
284-3	77 Ba	94 Aa	86	33.33 Aa	35.19 Aa	34.26
284-5	58 Abc	59 Ac	58	26.91 Aab	19.32 Bb	23.12
Mean	60 B	77 A	69	26.90 A	27.02 A	26.96

Means followed by the same capital letter in each row, comparing locations, and the same lower case letter in each column, comparing genotypes, do not differ by Tukey's test ($p \leq 0.05$).

Similar results were found by Costa et al. (2005), who divided Paraná State into three ecological regions and investigated the seed production potential of these regions. The authors observed that region T3, comprising the cities Ponta Grossa, Castro, Guarapuava, Pato Branco, Cascavel and part of Marilândia do Sul, Paraná State, Brazil, presented seeds with greater germination on average, while region T2, comprising Londrina and region, showed an intermediate performance in terms of germination. According to these authors, this effect may have occurred because region T3 presents milder annual average temperatures, being more favourable to the seed production of early cultivars. In addition, in the regions located North of the parallel 24°S, such as Londrina (23'20" LS), the constant temperature oscillations, associated with precipitation during the maturation period, have a great impact on the physiological quality of the seeds.

Regarding the accelerated ageing test (Table 5), the means of each environment and genotype analysis showed that in Londrina/PR, genotypes284-2 and 284-3 presented the highest percentage values, while in Ponta Grossa, Paraná State, Brazil, genotype 284-3 showed the highest germination (79%) after exposure to accelerated ageing conditions, which did not significantly differ from the standard and line 284-1. We also verified that seeds presented a lower germination after ageing when cultivated in Londrina, Paraná State, Brazil, comparing to the seeds obtained from Ponta Grossa, Paraná State, Brazil. Similarly, Carvalho et al. (2014) evaluated the seed quality of the cultivar BRS 284, among other genotypes, and observed germination average values of approximately 52% after the accelerated ageing test — values close to the means of the standard cultivars in both locations in the present study. The germination reduction observed for seeds submitted to the accelerated ageing test, compared to the germination test, may be associated with lipid oxidation and a reduction in the synthesis of certain proteins, leading to a decrease in the fatty acid contents, as well as the content of phospholipids present in cell membranes, and an increase in antioxidant activity in the seeds, resulting in a reduction in germination (Ávila et al., 2012).

When assessing seeding emergence, we observed that the genotypes presented higher values in Ponta Grossa, Paraná State, Brazil, ranging from 59 to 94%. In both locations, genotype 284-3 presented the best performance (Table 5). Regarding the germination speed index (GS), the genotypes showed no significant differences between locations, but the genotypes differed from each other. In Londrina, Paraná State, Brazil, genotypes 284-3 and 284 -2 presented the highest IVE values, while in Ponta Grossa, Paraná State, Brazil, the genotypes 284-3, 284-2, and the standard cultivar showed the highest IVE.

It was observed differences in the germination results obtained between the tests on paper towel, under controlled conditions, and in sand, without environmental control. The low germination values obtained in the paper towel test can occur due to imbibition problems, as soybean seeds, under excess of water conditions, quickly absorb water and a rupture of the tissues can occur, adversely affecting seed quality. Another factor that may also be responsible for this difference is the higher incidence of fungi, such as *Phomopsis* spp. and/or *Fusarium semitectum*, that infect the seeds germinated in paper towel, which also have the highest humidity. Thus, the germination test should be performed on sand substrate or after pre-conditioning the seeds in a humid environment prior to performing the test in paper towel (Vanzolini, Araki, Silva, & Nakagawa, 2007).

In general, despite the low values of seed quality observed, we observed that genotype 284-3 presented better seed quality potential, confirming the existence of intracultivar genetic variation in elite cultivars. It has been previously reported that there are certain mechanisms, such as intragenic recombination, transposons, crossing over, DNA methylation and gene amplification, that can provide a continuous source of genetic variation (Haun et al., 2011; Tokatlidis et al., 2011; Silveira et al., 2013; Fasoula, 2012). Furthermore, these new variations may accumulate undesirable mutations that gradually contribute to the degeneration of a cultivar (Fasoula, 2012; Tokatlidis et al., 2011) but can be exploited by breeding programmes.

Intracultivar genetic variation was also investigated by Yates, Boerma, and Fasoula (2012). The authors, by using single-sequence repeat (SSR) markers to determine whether soybean single-plant lines selected under ultra-low-density conditions presented unique fingerprints, observed that the phenotypic variation of seed protein content and weight has a genotypic component, and most of the variation between the SSR bands resulted from the residual heterozygosity in the initial plant selected to become a cultivar.

Line 284-3 showed variability for characteristics related to seed quality. According to Martins et al. (2016), if genetic variability exists, then it is possible to improve seed quality through selection. According to these authors, the genetic gain for germination and seedling emergence is estimated as 20% per selection cycle by using conventional breeding methods.

Studying the agronomic characteristics of a genotype, as well as the characteristics regarding seed quality and the correlation between these characteristics, is important in breeding programmes, since these studies provide essential information to breeders to improve selection efficiency. According to Marcos-Filho and Kikuti (2006) and Marcos-Filho and França-Neto (2017), the use of seeds with high vigour is justified due to the assurance of an adequate plant stand, even if there is no consistent response in terms of seed yield. Therefore, the aim of the breeding programmes should not only be productivity but also other characteristics such as seed quality.

Conclusion

The commercial cultivar BRS 284 presents variability in seed quality and can be selected by using the Honeycomb Selection Designs (HSD) method.

Ponta Grossa, Paraná State, Brazil, comprises a more favourable environment for the production of soybean seeds of high physiological quality than that of Londrina, Paraná State, Brazil.

The genotype BRS 284-3, in general, shows higher germination and seed vigour compared to the standard cultivar.

The HSD ultra-low-density selection model is efficient for the intracultivar selection of the soybean cultivar BRS 284 in relation to the seed quality, demonstrating potential as an alternative method for use in breeding programmes.

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