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Physiological quality of soybean seeds and the influence of maturity group

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Elaine Cristina Batista[1], Gabriel Mendes Villela[2], Raquel Maria de Oliveira Pires[1]*, Heloisa Oliveira dos Santos[1], Everson Reis Carvalho[1], Adriano Teodoro Bruzi[1]

ABSTRACT: Productive soybean genotypes is one of the main objectives of soybean breeding programs. However, there are few studies on the selection of characteristics associated with the quality and performance of soybean seeds. The objective in this study was to predict physiological and genetic parameters associated with seed quality in segregating soybean populations derived from parents of differing maturity groups. Eight genotypes of the $F_{2:3}$ segregating soybean population (numbered 163, 24, 57, 108, 164, 169, 157, and 81) cultivated in the 2018/19 harvest were evaluated. To conduct the experiment, a completely randomized design was used. The physiological quality of the seeds was evaluated by germination test, tetrazolium test, and image analysis. Statistical analyses were performed by using a mixed model approach. Greater seed vigor was observed in the genotypes 108, 164, and 169, whose results were among the best for most of the analyzed parameters. The greater mechanical damage and damage due to stink bugs can explain the lower vigor of some seeds, as observed in genotypes 57 and 163, while weathering damage did not correspond to the results of the seed vigor tests. The analyses indicated a greater influence of genetic characteristics than of relative maturity group on the responses of the genotypes. No effect of relative maturity group on soybean seed vigor was evident.

Index terms: *Glycine max* L. Merrill, plant breeding, REML/BLUP, seed physiology.

RESUMO: Genótipos mais produtivos da cultura da soja é um dos principais objetivos dos programas de melhoramento. Existem poucos estudos quanto à seleção de caracteres associados à qualidade e desempenho de sementes de soja. Objetivou-se predizer parâmetros fisiológicos e genéticos sobre a qualidade de sementes produzidas em populações segregantes de soja, provenientes de genitores com diferentes grupos de maturidade. Foram avaliados oito genótipos da população segregante $F_{2:3}$ de soja (163, 24, 57, 108, 164, 169, 157 e 81), cultivados na safra 2018/19. Para a condução do experimento foi utilizado o delineamento inteiramente casualizado. Avaliou-se a qualidade fisiológica das sementes por meio dos testes de germinação, tetrazólio e análise de imagem. As análises estatísticas foram realizadas utilizando-se uma abordagem de modelos mistos. Foi possível observar maior vigor nas sementes dos lotes 108, 164 e 169, cujos resultados estiveram entre os melhores na maioria dos parâmetros analisados. Maior incidência de dano mecânico e por percevejos pode ser usado para explicar o menor vigor, como observado nos genótipos 57 e 163, enquanto o dano por umidade não corroborou com os testes de vigor das sementes. A resposta dos genótipos às análises realizadas indicou maior influência de características genéticas destes do que efeito do grupo de maturidade relativa. Não foi possível observar evidente efeito do grupo de maturidade relativa e o vigor das sementes de soja.

Termos para indexação: *Glycine max* L. Merrill, melhoramento de plantas, REML/BLUP, fisiologia de sementes.

***Corresponding author**
E-mail: raquelmopires@ufla.br

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[1]Departamento de Agricultura, Universidade Federal de Lavras (UFLA), Caixa Postal 3037, 37200-000 - Lavras, MG, Brasil.

[2]Departamento de Biologia, Universidade Federal de Lavras (UFLA), Caixa Postal 3037, 37200-000 - Lavras, MG, Brasil.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a cultivated species of great importance in the global agricultural market, with an annual production of more than 363 million tons of grain (USDA, 2021). In the national context of Brazil, its relevance is even greater, since it is the main agricultural export product, accounts for much of the agribusiness in the country and occupies more than 50% of the total area, which corresponds to more than 38.5 million planted hectares; and an annual production of more than 135 million tons is expected (CONAB, 2021).

The performance of soybean crops is directly related to the quality of seeds that are sown (Pereira et al., 2015; Oliveira et al., 2020), therefore the production and utilization of high quality seeds are important and basic keys for the success of crop production (França-Neto and Krzyzanowski, 2019). Studies carried out in soybean breeding, establish benefits to the producer in terms of grain yield, considering that the seed with high physiological quality acts as a precursor to superior characteristic cultivars (Peske et al., 2012; Marcos-Filho, 2015). However, even seeds with superior quality are subject to deterioration due to postharvest techniques and long periods of water deficit, affecting the physiological quality of seeds, interfering with the emergence of seedlings, and resulting in decreased uniformity of plants in the field (Marcos-Filho, 2015; Bakhshandeh and Gholamhossieni, 2018; Oliveira et al., 2020).

Furthermore, the physiological processes of seeds, which are related to the factors that affect germination, are genetically determined. Seed performance and metabolism vary among species and cultivars, although the environment and cultivation conditions also have decisive influences (Lin et al., 2014). The quality and the chemical composition of soybean seed are related to genetic factors and can also be influenced by the environment (Santos et al., 2007; Westgate et al., 1995). According to Gris et al. (2010) genetic traits and environmental effects during the stages of development, harvesting, processing and storage are key factors for the seed viability period, directly influencing seed quality.

Seed quality can be investigated early based on the performance of the parent lineage, which can be evaluated based on agronomic, morphological, physiological, and molecular characteristics, among others. The characteristics related to the physiological quality of seeds are genetically inherited, so different lineages may show high variation in vigor, germination, and field emergence (Bakhshandeh and Gholamhossieni, 2018).

Plant breeding is important for not only developing cultivars with desirable agronomic performance and resistance and obtaining genetic gains but also eliminating factors unconducive to high productivity, such as poor seed quality. However, evaluations of the parameters associated with seed quality remain lacking. Such evaluations are unquestionably important for increasing productivity and accelerating the safe recommendation of elite lineages in final tests. Thus, seeds form the basis of any genetic breeding program of plants and it is through them that the technology generated is established. The selection of superior genotypes for traits related to seed quality is therefore important (Pereira et al., 2017). For this reason, the involvement of seed technologists in genetic improvement programs has been fundamentally important to the success of these programs (Vasconcelos et al., 2009)

In soybean breeding programs, the physiological quality of seeds is usually evaluated in the more advanced phases of the programs, such as in cultivation and use value (CUV) tests. However, the seed physiological quality of the lineages is of great interest to breeders to assist in decision-making. Currently, soybean cultivars that have an earlier cycle, i.e., that belong to lower maturity groups (MGs) (Alliprandini et al., 2009), are being sought to allow the cultivation of corn as a second crop (Nóia-Júnior and Sentelhas, 2019). In view of this, it is necessary to verify whether there is an influence of MG (precocity) on the characteristics associated with the physiological quality of seeds (França-Neto et al., 2016).

With this, the aim of this present study was to predict physiological and genetic parameters associated with seed quality in segregating soybean populations derived from parents of differing maturity groups.

MATERIAL AND METHODS

The study was conducted at the Central Laboratory of Seed Analysis of the *Universidade Federal de Lavras* (UFLA). Eight soybean $F_{2:3}$ genotypes (163, 24, 57, 108, 164, 169, 157, and 81) that differ in days to absolute maturity (DTM) and relative maturity group (RMG) were evaluated (Table 1); they were grown in the 2018/2019 harvest and planted on 10/21/2018 at the Center for Scientific and Technological Development of Agriculture—“Muquém” Experimental Farm (located at 21°12’ S, 45°58’ W and 918 m altitude) of the UFLA in the municipality of Lavras, Minas Gerais state, Brazil.

The following tests were performed to determine seed quality:

Germination test: Four replications of 25 seeds per treatment were used. Seeds were sown in “Germitest” paper rolls and stored at 25 °C. Water was added to an amount 2.5 times the weight of the paper, aiming at adequate wetting and standardization of the test. Evaluations and counts of normal seedlings were performed on the third, fifth, and eighth days after sowing according to the Rules for Seed Testing (RAS) (Brasil, 2009). The results are expressed as the percentage of seeds with protruding radicles on day 3, the percentage of normal seedlings on day 5, and the percentages of normal seedlings, abnormal seedlings, and dead seeds on day 8. Seedling length was also determined by image analysis (item 3).

Tetrazolium test: In the tetrazolium test, 100 seeds (four subsamples with 25 seeds) of each genotype were used. The samples were preconditioned between moistened paper towels, which held an amount of water equivalent to 2.5 times the mass of the paper, for 16 hours at 25 °C. Then, the seeds were placed in plastic containers and submerged in a 0.075% solution of 2,3,5-triphenyl tetrazolium chloride at 40 °C in the dark for 150 minutes. Then, the seeds were washed under running water and analyzed individually. The results are expressed as the percentages of seeds with damage (mechanical damage, weathering damage, or stink bug damage), viable seeds, and vigorous seeds.

Image analysis: The image analyses were performed with GroundEye® L800 equipment consisting of an information capture device composed of a conveyor belt and real-time video analysis software. A total of 15 seeds/seedlings were used to obtain the images; each seed/seedling was considered a replicate. Each seed was sown onto a “Germitest” paper roll moistened with an amount of water equivalent to 2.5 times the mass of the nonhydrated paper and maintained in a germinator at 25 °C. The rolls were removed to image the seeds/seedlings at three, five, and eight days after sowing. The size of the radicle and shoot and the radicle/shoot ratio were evaluated. The results are expressed in centimeters.

Experimental design and Statistical analysis: The experimental design was completely randomized, consisting of eight soybean $F_{2:3}$ genotypes with four replications in each test. The data were analyzed according to the mixed model methodology (Henderson, 1984); the genetic parameters were estimated via restricted maximum likelihood (REML), and the genotypic values, or genotypic means, were estimated by the best linear unbiased predictor (BLUP) procedure of R software (R Core Team, 2017). Mixed linear models have the advantage of using genotypic values instead of phenotypic values, generating more accurate results (Lopes et al., 2014), and are still appropriate for the present experiment, as the data are measures repeated in time. Such measurements are taken on plots that have been randomly allocated treatments as fixed effects such as crop variety with different replications as random effects (Akbas et al., 2001).

The statistical model, in which underlined variables are considered random variables, can be written as follows:

$$\underline{y}_{ij} = \mu + \underline{G}_{ij} + \underline{\varepsilon}_{ij}$$

Table 1. Days to absolute maturity (DTM) and relative maturity group (RMG) of eight soybean genotypes.

Genotype	DTM	RMG
163	117	5.4
24	126	6.2
57	128	6.4
108	139	7.5
164	139	7.5
169	147	8.2
157	150	8.5
81	148	8.3

where $\underline{y}_{ij}$ is the observed value for genotype i (with i = 1, ..., n), μ is a constant, $\underline{G}_{ij}$ is the effect of genotype i in replicate j, and $\underline{\varepsilon}_{ij}$ is the experimental error.

The methodology developed by Nunes et al. (2005) was used to generate the figures.

Estimates of broad-sense heritability (h_C^2) for the characteristics were obtained from the variance components according to the method developed by Cullis et al. (2006).

RESULTS AND DISCUSSION

The analyses of the seed data from the eight soybean genotypes revealed significant differences in physiological potential among genotypes in all evaluation periods for most of the parameters evaluated. This finding indicates that the tests were sensitive to differences in seed performance, even among genotypes with high germination power, i.e., within the established limit (> 85%) for the commercialization of soybean seeds. Figure 1 presents the results of the germination test, showing the seed/seedling counts on the (a) third, (b) fifth, (c) and eighth days and the percentages of (d) abnormal seedlings and (e) dead seeds.

Regarding the germination potential, the genotypes with the highest percentages of root protrusion were genotypes 169, 164, 157, and 81, and all these genotypes had percentages of seeds with protruding radicles on the third day of greater than 85% (Figure 1a). During the first count (Figure 1b), genotypes 81 and 157 had the highest numbers of seedlings, with corresponding percentages above 90%. On the eighth day (Figure 1c), genotypes 24 and 108 had the highest percentages of normal seedlings, above 96%. It was observed that there was convergence between the results of percentages of abnormal seedlings (Figure 1d) and dead seeds (Figure 1e) because the genotypes exhibited similar performance for these two characteristics, and genotypes 163 and 57 presented the highest percentages of abnormal seedlings and dead seeds, which were above 20%. Genotype 108 had percentages close to the mean, while the remaining genotypes had values below the overall mean (Figures 1d and e).

Consistent with the germination results for the same day (Figure 1a), a greater primary root length three days after sowing was observed for the seeds of genotype 169. The highest values of hypocotyl length and primary root length-to-hypocotyl length ratio were also observed for this genotype (Table 2).

Five days after sowing, the highest values for primary root length, hypocotyl length, and the ratio of these two characteristics were observed for genotype 157, followed by genotype 81 (Table 3). As observed for the results on the third day, the results on the fifth day corroborated the germination results on the same day (Figure 1b).

On day 8, the highest values of primary root length and hypocotyl length were observed for genotypes 108 and 169. The highest value of the ratio of primary root length to hypocotyl length was observed for genotype 164 (Table 4). Moreover, on day 8, genotype 108 had the highest percentage of normal seedlings (Figure 1c), as well as the highest primary root length and hypocotyl length values (Table 4).

The tetrazolium test (Figure 2) revealed differences in the different types of damage among the genotypes. For mechanical damage, the highest values were observed for genotype 57, and the lowest values were observed for genotype 164 (Figure 2a). Genotypes 164, 169, and 108 had the greatest weathering damage, while genotype 157 had the lowest weathering content (Figure 2b). Stink bug damage was greatest in genotype 163 and lowest in genotypes 169 and 108 (Figure 2c).

The tetrazolium test revealed greater seed vigor of genotypes 164, 24, and 81 (Figure 2c) and lower vigor for genotypes 57 and 163. Seed viability was generally similar among the genotypes; genotypes 57 and 163 had the lowest viability values, whereas the remaining genotypes had values above the average (Figure 2e).

The heritability estimates of the characteristics are shown in Table 5. The highest percentages were observed for hypocotyl length on the third day and for primary root length on the eighth day.

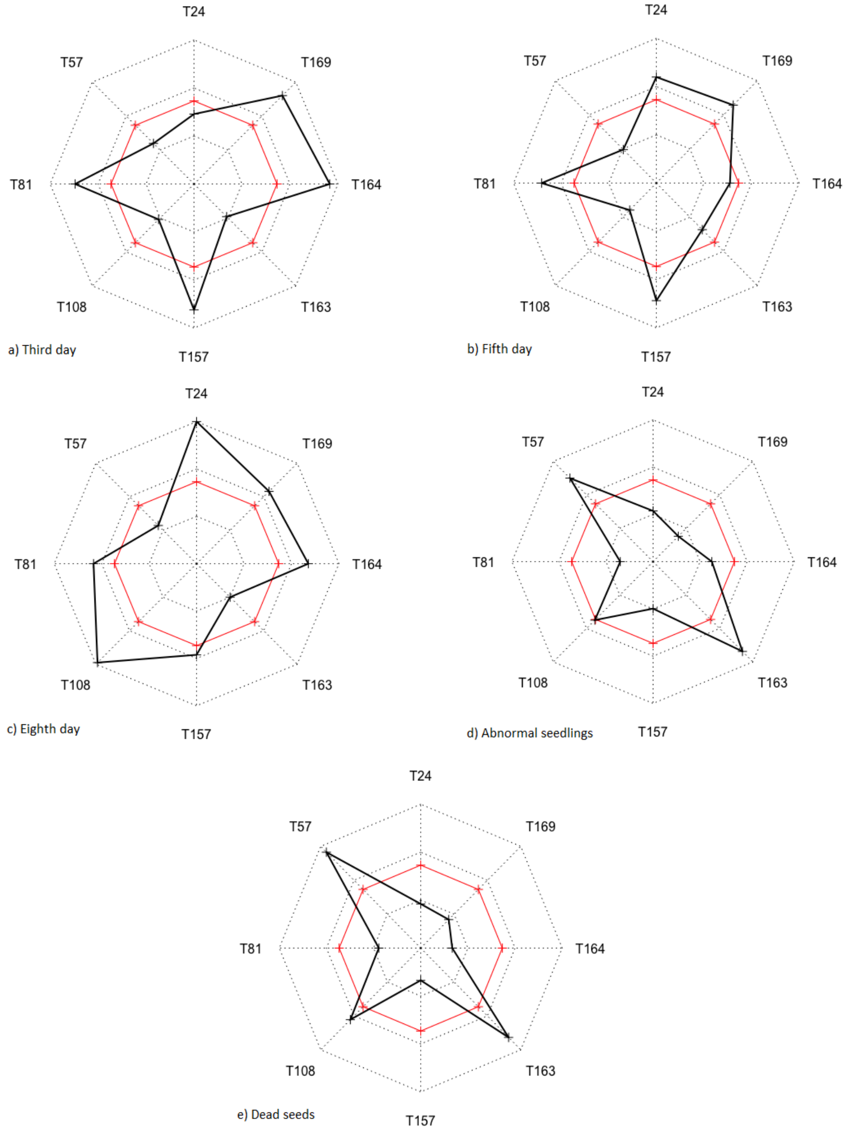

Figure 1. Results of the germination test of eight soybean genotypes: (a) counts on the third, (b) fifth, (c) and eighth days, (d) percentages of abnormal seedlings and (e) dead seeds.

Table 2. Mean predicted values of primary root length, hypocotyl length, and the ratio of primary root length to hypocotyl length on day 3 for eight soybean genotypes. Data in cm.

Genotype	Primary root length	Hypocotyl length	Primary root length/hypocotyl length ratio
24	3.67	5.92	2.27
57	3.04	4.94	1.96
81	4.66	7.95	3.27
108	4.49	7.85	3.55
157	4.33	7.66	3.33
163	4.45	6.68	2.41
164	4.82	7.75	2.94
169	4.88	8.93	4.01

Table 3. Mean predicted values of primary root length, hypocotyl length, and the ratio of primary root length to hypocotyl length on day 5 for eight soybean genotypes. Data in cm.

Genotype	Primary root length	Hypocotyl length	Primary root length/ hypocotyl length ratio
24	6.73	15.52	8.81
57	6.42	13.79	7.46
81	7.20	17.66	10.41
108	6.44	14.89	8.52
157	8.56	21.82	12.99
163	5.99	12.26	6.42
164	6.77	15.86	9.10
169	7.14	17.30	10.11

Table 4. Mean predicted values of primary root length, hypocotyl length, and the ratio of primary root length to hypocotyl length on day 8 for eight soybean genotypes. Data in cm.

Genotype	Primary root length	Hypocotyl length	Primary root length/hypocotyl length ratio
24	16.41	29.74	13.20
57	16.61	28.83	12.57
81	17.44	31.40	13.57
108	28.36	40.82	12.88
157	20.14	33.79	13.41
163	15.94	26.90	11.84
164	17.76	33.13	14.38
169	23.10	36.00	13.04

Similarities were observed between the results obtained for the analyzed characteristics of the soybean seeds. On days 3, 5 and 8, the values of primary root length and hypocotyl length (Tables 2, 3 and 4) were consistent with those observed for germination (Figures 1a, b, and c). Furthermore, the genotypes with the highest values of the analyzed characteristics varied with evaluation day. For example, for primary root length, the highest values were observed in genotypes 169, 157, and 108 on days 3, 5, and 8, respectively. Similar variation was observed for the other characteristics (Tables 2, 3 and 4).

By using the image analysis technique on soybean seeds, Monteiro et al. (2021) revealed reductions in germination and vigor with increasing damage. In the present study, the greatest mechanical damage was observed in genotype 57, and genotype 163 exhibited the greatest stink bug damage (Figures 2a and c). These same genotypes had lower values for vigor and viability (Figures 2d and e) as well as germination (Figures 1a-c) than the other genotypes. The results for weathering damage in the present study are inconsistent with those of Monteiro et al. (2021). Genotypes 108, 164, and 169 had the highest weathering damage values but were among the genotypes with higher vigor and viability (Figures 2d and e) and germination success (Figure 1). Thus, for the data analyzed in the present study, the presence of weathering damage may not be a useful indicator of quality in soybean seeds. Contrary results were found in studies carried out by de Forti et al. (2013), Huth et al., (2016) and Pinheiro et al. (2021), and these indicated that severe climatic damage influenced the viability and vigor of soybean seeds, affecting the physiological potential, and could be one of the most harmful factors affecting soybean seed quality.

Figure 2. Results of the tetrazolium test of eight soybean genotypes for (a) mechanical damage, (b) weathering damage, (c) stink bug damage, (d) vigor, and (e) viability.

Table 5. Estimates of broad-sense heritability (h^2) (shown as percentages).

Trait	Day 3	Day 5	Day 8
Primary root length	84%	76%	89%
Hypocotyl length	92%	84%	88%
Primary root length/Hypocotyl length	80%	83%	59%

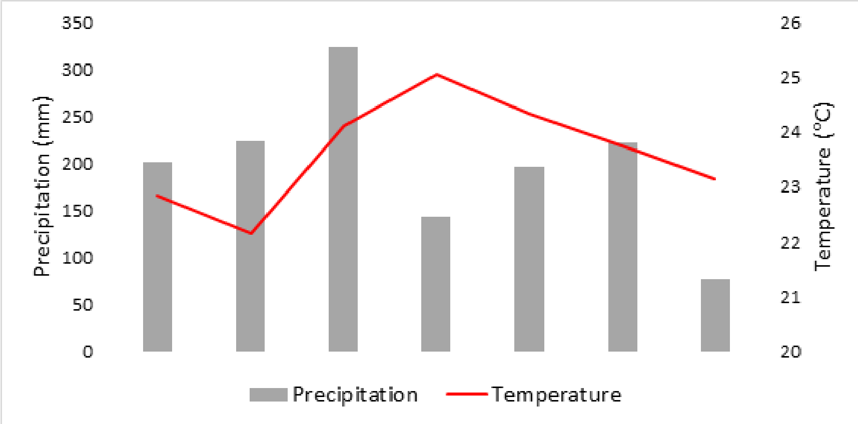

Figure 3. Rainfall and mean temperature from October 2018 to April 2019.

Pinheiro et al. (2021), studying the deterioration of weathering in the pre-harvest of soybean seeds, verified that the effects of humidity and air temperature were reinforced by the tetrazolium test, which showed decayed and dead tissue in seeds under deterioration by weathering, confirming that the damage caused by weathering resulted in the reduction of the physiological potential of the seeds and influenced the integrity of the tissues.

Figure 3 shows the temperature and precipitation data for the seed development period. Zambiazzi et al. (2017) found that soybean genotypes subjected to different cultivation environments showed variation in the quality of seeds produced. Obtaining seeds with high physiological quality and productivity depends on the adaptive capacity of the genotype studied and the production environment (França-Neto et al., 2016).

Seed vigor is associated with the genetic characteristics of seeds lot, as observed in soybean by Feliceti et al. (2020). Those authors found that the vigor of soybean seeds was more strongly associated with the genetic characteristics of the cultivar than to their RMG. Similarly, in the present study, cultivars 108 and 164 were classified into the same RMG (7.5). However, the analyzed parameters were not always similar between these genotypes, such as germination on the third day (Figure 1a), the value of which was above the mean for genotype 164 and below the mean for genotype 108. Similarly, large differences between these genotypes were observed for germination on the eighth day (Figure 1c); the values for dead seeds (Figure 1e), primary root length and the ratio of primary root length to hypocotyl length on days 3 (Table 2) and 5 (Table 3); and the three seedling measurements on day 8 (Table 4).

The physiological quality of seeds is characterized by their germination potential and vigor (Silva et al., 2016). These authors also emphasize that the use of high-vigor seeds is essential for greater plant production. This relationship was observed by Scheeren et al. (2010), who studied different soybean lots and found up to 9% higher production for plants from high-vigor seeds than for those developed from low-vigor seeds.

The evaluation of seed vigor is a fundamental step for the success of soybean seed production, which is recognized for its sensitivity to seed deterioration and inadequate management practices after seed maturity. Tests realized in stress conditions and those which evaluate the germination speed are the main methods used to analyze seed vigor. Other evaluations can be of great value for obtaining complete information about the vigor of a lot, for example, the tetrazolium test is useful not only to verify other indicators of seed vigor, but also to evaluate the presence of damage, which may be responsible for low seed vigor (França-Neto and Krzyzanowski, 2018). Similar to the present study, other studies have used vigor tests to analyze differences among soybean seeds. Yagushi et al. (2014) used germination and tetrazolium tests to classify the vigor of soybean cultivars, whereas Vanzolini et al. (2007) used seedling length analyses for such classification.

Studies that have analyzed the seed vigor of plants from different MGs are scarce, which hinders comparison between the present results and those in the literature. However, the comparison of the vigor tests performed here for soybean with those reported by other studies on soybean (Santos et al., 2012), as well as other species such as sorghum

(Oliveira and Gomes-Filho, 2009), and beans (Michels et al., 2014), indicated higher and lower vigor for each genotype and/or cultivar. This observation highlights the effects of genetic characteristics on seed vigor and, consequently, on productivity. In the present study, differences in the analyzed parameters were observed; although an effect of RMG was not clearly established, an effect of genotype on these parameters was observed.

CONCLUSIONS

No effect of maturity group and the physiological quality of the soybean seeds, as high or low germination percentages were found in the different maturation groups studied.

The results indicate that the genetic characteristics of the analyzed genotypes influenced their performance, which was more relevant in terms of physiological seed quality than the maturity group.

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