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# Physiological potential of seeds from different positions of the soybean plant at reduced seeding rates

# Potencial fisiológico de sementes de diferentes estratos da planta de soja em densidades reduzidas

André Sampaio Ferreira[1]*; Alvadi Antonio Balbinot Junior[2]; Flávia Werner[3]; Inês Cristina de Batista Fonseca[1]; Claudemir Zucareli[1]

## Highlights

The position of seeds from the soybean plant can affect the physiological potential.
Seeds from the upper stratum have greater physiological potential.
Drastic reductions in plant density reduce the physiological potential of seeds.
Reducing seeding rate by 20 to 40% results in greater physiological potential.

## Abstract

Reducing seeding rates is a technique that has been studied as a strategy to reduce soybean production cost without yield losses. However, this method can directly impact seed production and formation, because it alters plant morphology and modifies intraspecific competition, water use efficiency, and microclimates. This study investigated whether soybean cultivation utilizing reduced seeding rates alters the physiological potential of seeds at different stratum of plants in two cultivars with discrepant branching abilities. The experimental design was completely randomized, with split plots containing five seeding rates (100, 80, 60, 40, and 20% of the amount recommended by the breeder) and three seed positions of the plants (low, medium, and high) within the subplot. The cultivars BRS 1010 IPRO and NS 5959 IPRO were used. Germination, first count upon germination, seedling length, dry mass, electrical conductivity, accelerated aging, and sand emergence were evaluated. The physiological potential of the seeds was higher in the upper stratum of the plant compared to the lower stratum. Reducing the seeding rate to values up to 40% did not cause notable impacts on the physiological potential of the seeds. A drastic reduction of the seeding rate to only 20% resulted in decreased seed vigor. Seed germination of the cultivars NS 5959 IPRO and BRS 1010 IPRO was highest in seeding rates ranging from 40 to 80% of those recommended by breeders.

**Key words:** *Glycine max* (L. Merril). Minimal optimal seeding rate. Plant population. Physiological quality.

[1] Profs. Drs., Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: andresampaioferreira@uel.br, inescbf@uel.br, claudemircca@uel.br
[2] Researcher Dr., Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA, Londrina, PR, Brazil. E-mail: alvadi.balbinot@embrapa.br
[3] Profª. Drª., Universidade Alto Vale do Rio do Peixe, UNIARP, Caçador, SC, Brazil. E-mail: fla_werner@hotmail.com
* Author for correspondence

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## Resumo

A redução da densidade de semeadura tem sido estudada como uma estratégia para redução do custo de produção de soja, sem alterações na produtividade. Entretanto esta técnica pode impactar diretamente na produção e formação das sementes, pois altera a morfologia da planta e modifica a competição intraespecífica, a eficiência do uso da água e o microclima. O objetivo foi investigar se o cultivo de soja, em densidades reduzidas, altera o potencial fisiológico das sementes, em diferentes posições da planta, em duas cultivares com potencial de ramificação discrepante. O delineamento foi inteiramente casualizado, com parcelas subdivididas, considerando-se: cinco densidades de semeadura (100, 80, 60, 40 e 20% da densidade recomendada pelo obtentor) na parcela e três posições de sementes na planta (inferior, médio e superior) na subparcela. Utilizaram-se as cultivares BRS 1010 IPRO e NS 5959 IPRO. Avaliou-se germinação, primeira contagem da germinação, comprimento e massa seca de plântulas, condutividade elétrica, envelhecimento acelerado e emergência em areia. O potencial fisiológico das sementes é maior no estrato superior da planta em comparação com o inferior. A redução da densidade de semeadura para valores até 40% da densidade não provoca impactos significativos sobre o potencial fisiológico das sementes. A redução drástica da densidade de semeadura, para apenas 20% do recomendado, resulta em menor vigor de sementes. A germinação das sementes das cultivares NS 5959 IPRO e BRS 1010 IPRO é maior em densidades de semeadura variando entre 40 e 80% do recomendado pelos obtentores.

**Palavras-chave:** *Glycine max* (L. Merril). Densidade de plantas mínima ótima. População de plantas. Qualidade fisiológica.

## Introduction

The production of soybean seeds with a high physiological potential depends on environmental conditions during the seed formation period. Thus, plant density can directly impact soybean seed production, as it modifies intraspecific competition (Ferreira et al., 2016), water use efficiency, and microclimates (shading, air temperature, and humidity) (Gundel et al., 2014).

The soybean seeding rate (SR) reduction technique has been studied in Brazil (Corassa et al., 2018) and abroad (Carciochi et al., 2019; Thompson et al., 2015); these studies explored the phenotypic plasticity of the crop, combining high yield levels and reducing the cost of seeds and their treatment.

The main changes caused by low SRs, which confer soybean phenotypic plasticity, are the elevated number of pods on the main stem and greater branching resulting in increased production per plant (Ferreira et al., 2018). However, phenotypic plasticity is highly variable between cultivars, making it necessary to evaluate the feasibility of reducing SRs in cultivars with a low branching ability (Agudamu et al., 2016).

Seed formation depends on the supply of photoassimilates, and this can be influenced by the number of pods as well as the position of the pods on the plant (Huber et al., 2016). Therefore, reducing SR can affect the partition of photoassimilates by altering architecture and morphology (Gaspar & Conley, 2015), and these changes can alter the size and quality of the seeds produced (Baron et al., 2018; Ferreira et al., 2017; Werner et al., 2017). However, growing soybeans at a recommended SR can result in an excessive leaf area index, especially in years of high rainfall and conditions favorable to vegetative growth. These conditions can provide an unfavorable microclimate for grain filling. Therefore, we hypothesized that moderate reductions in SR are beneficial for seed production, while drastic reductions in density can result in lower vigor and germination.

The objective of the study was to investigate whether soybean cultivation at reduced SRs, alters the physiological potential of seeds in different positions of the plant, in two cultivars with discrepant branching abilities.

## Materials and Method

Two field experiments were conducted in Londrina, PR (23º 19' 54'' S, 51º 19' 99'' W, altitude 620 m) during the 2016/17 growing season (GS); the first tested the BRS 1010 IPRO cultivar and the other tested NS 5959 IPRO. These cultivars have an indeterminate growth type and branching ability that contrast with each other. The experimental design was randomized blocks with five replicates. The treatments consisted of five SRs (100, 80, 60, 40, and 20% of the rate recommended by the breeder of each cultivar).

The soil of the experimental area was classified as distroferric Red Latosol (Rhodic Eutrudox in the American classification). The soil texture is composed of 710, 82, and 208 g $kg^{-1}$ of clay, silt, and sand, respectively. Rainfall and air temperature data were collected at an agrometeorological station approximately 500 m away from the experimental area and are presented in Figure 1.

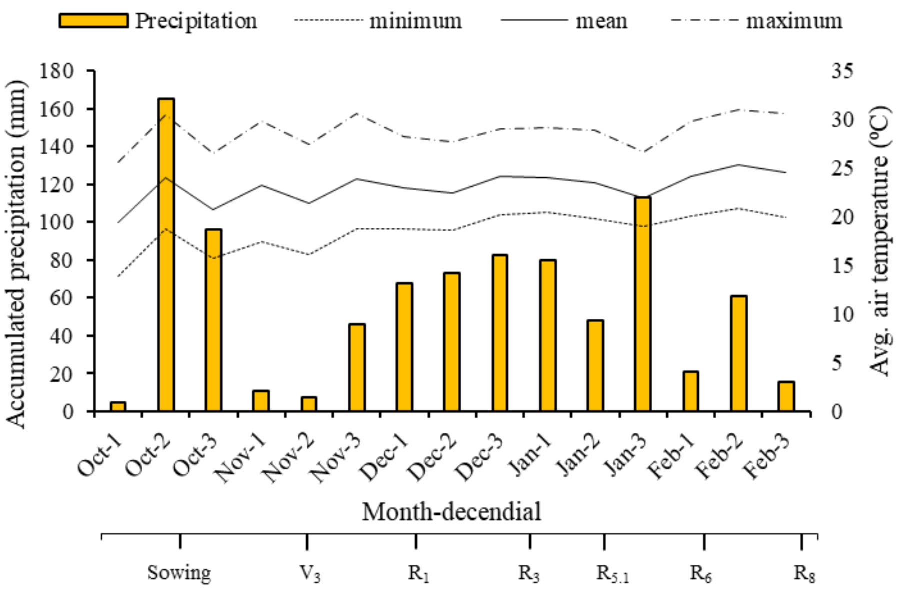



**Figure 1.** Accumulated precipitation and average air temperature, by ten-day period, and stages of soybean development according to Fehr and Caviness (1977) during the conduction of the experiments: October, 2016 through February, 2017. Londrina, Paraná, Brazil.

The recommended plant density ranges for the BRS 1010 IPRO and NS 5959 IPRO cultivars, in the region where the experiments were conducted, were 265 to 310 and 380 to 420 thousand plants $ha^{-1}$, respectively. The NS 5959 IPRO cultivar had an inferior branching ability and a more compact leaf architecture (smaller, more vertically inclined leaflets) and therefore the recommended density for this cultivar was higher than for BRS 1010 IPRO. Therefore, the treatments consisted of the following SRs: 310, 248, 186, 124, and 62 thousand viable seeds $ha^{-1}$ for the cultivar BRS 1010 IPRO and 420, 336, 252, 168, and 84 thousand viable seeds $ha^{-1}$ for the cultivar NS 5959 IPRO.

Sowing was performed on October 28, 2016. The plots measured 50 $m^2$ (5 m wide and 10 m long) with a row spacing of 0.45 m. Base fertilization was carried out according to the results of the soil chemical analysis and the fertilization recommendations for the crop. Linear samples of 2.0 m were investigated in each plot, shortly after the soybeans reached the harvest point. The plants were sectioned into three equal parts, referred to as the lower, middle, and upper stratum, with separation of the pods present in each. The samples were manually screened, and five replications of each treatment were grouped to obtain the composite sample and the volume of seeds necessary to evaluate the physiological potential. A completely randomized design was subsequently adopted, separately for each cultivar, with a split plot scheme incorporating five SRs in each plot and the three seed positions in the subplot.

When carrying out the tests, the water content of the seeds was determined using the oven method at 105 ºC (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009). The tests to assess physiological potential were determined according to the following methodologies:

Germination: determined based on eight replicates of 50 seeds on germitest paper moistened in a ratio of 2.5 to 1 (mL of distilled water per mass of dry paper in grams) and kept in a germination chamber at a controlled temperature of 25 ± 1 ºC for eight days.

First count of germination: recorded together with the germination test, and the number of normal seedlings germinated on the fifth day after sowing was computed (MAPA, 2009).

Seedling length: carried out according to the methodology proposed by Nakagawa (1999) on a roll of germistest paper with 10 seeds and an addition of 2.5 times the mass of water in relation to the mass of the paper, with four replicates. The seeds were positioned with the micropyle facing the bottom of the paper. After five days, the lengths of the shoot and root parts of the seedlings were measured.

Seedling dry mass: obtained by weighing normal seedlings obtained from the seedling length test, excluding cotyledons. The seedlings were dried in an oven with forced air circulation, maintained at a temperature of 80 ˚C for a period of 24 h (Nakagawa, 1999).

Electrical conductivity: determined with four replicates of 50 seeds for each treatment. The seeds were placed in plastic cups and weighed on a precision analytical balance. Subsequently, 75 mL of deionized water was added to the cups containing the seeds. These were kept in a germination chamber for 24 h at 25 ˚C. After the soaking period, the electrical conductivity of the solution was determined using a conductivity meter (Vieira & Krzyzanowski, 1999).

Accelerated aging: the seeds were distributed in a single layer on a metal screen and placed in plastic germination boxes (11 x 11 x 3.5 cm) containing 40 mL of distilled and deionized water at the bottom. Following the aging period of 48 h at 41 ˚C in an accelerated aging chamber, the seeds were placed to germinate, according to the methodology described for the germination test (Marcos, 1999).

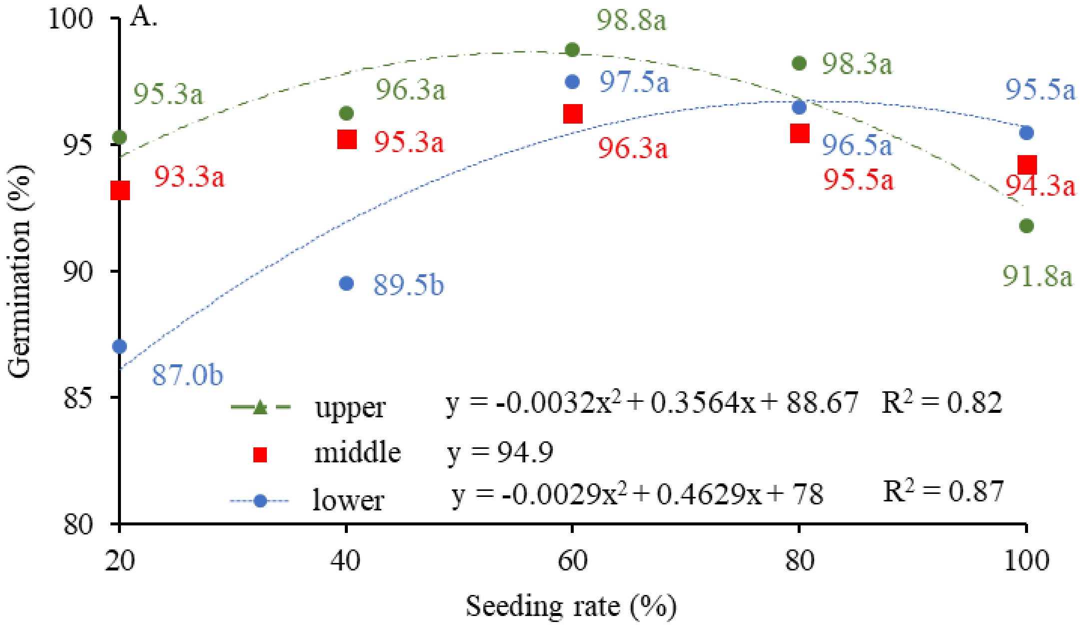
Seedlings emergence in sand: four replicates of 100 seeds from each treatment were sown in plastic boxes with sand in a greenhouse, keeping the soil moist throughout the test. The number of emerged plants was counted 21 d after sowing (Nakagawa, 1999).

The data were submitted to the Shapiro-Wylk test to verify normality and the Hartley test to verify the homoscedasticity of variances. After confirming these assumptions, an analysis of variance and regression analysis were applied to the SR factor and comparison of means using the Tukey test for the strata in the plant ($p < 0.05$).

## Results and Discussion

The seeds had a water content between 100 and 110 g $kg^{-1}$ before the implementation of the germination and vigor tests. This uniformity is important when conducting laboratory analyses to ensure standardized tests and reliable results.

Seed germination was influenced by the interaction between factors in the BRS 1010 IPRO cultivar. For the three highest SRs (100, 80, and 60%) no difference was observed in the germination potential of the seeds between the three strata. However, at the SRs of 40 and 20%, the seeds from the lower stratum showed lower germination rates compared to the other strata (Figure 2A). In both the lower and upper strata, there was a second-degree polynomial adjustment of the equation, with the highest percentage of germination observed in the intermediate SRs. However, in the lower stratum, the point of maximum germination occurred at a SR of 79% whereas in the upper stratum it occurred at a density of 56%; demonstrating that the seeds from the lower stratum of the plants produced at reduced SRs had a comparatively low viability (Figure 2A).



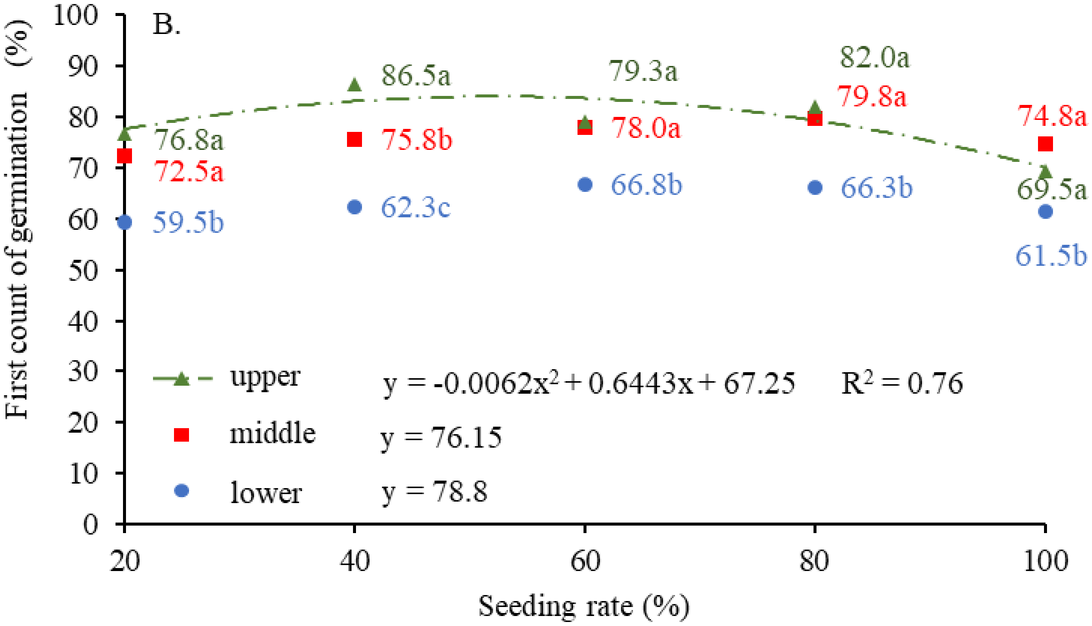




**Figure 2.** Germination of soybean seeds, cultivar BRS 1010 IPRO (A) and first count of germination, cultivar NS 5959 IPRO (B) in the lower, middle and upper strata of the plant in response to the seeding rate reduction (100, 80, 60, 40 and 20% of the recommended by the breeder).

The germination of NS 5959 IPRO was influenced separately by both factors. In analyzing the isolation effect of the seed position in the plant for the cultivar NS 5959 IPRO, for all SRs, the average germination rate was lower in the lower stratum than in the middle and upper strata (Table 1).

**Table 1**
**Isolated effect of the seed position in the plant (lower, middle and upper strata) on the physiological potential of soybean seeds of the cultivars BRS 1010 IPRO and NS 5959 IPRO (mean of five seeding rates)**

| BRS 1010 IPRO | Lower | Middle | Upper | CV(%) |
|---|---|---|---|---|
| First count of germination (%) | 87,5 b | 87,0 b | 92,8 a | 4,4 |
| Electrical condutivity (µS $cm^{-1}$ $g^{-1}$) | 84,3 a | 69,4 b | 62,3 c | 9,8 |
| Germination after accelerated aging (%) | 91,5 b | 86,1 c | 96,4 a | 3,7 |
| Seedling emergence in sand (%) | 94,7 b | 97,4 a | 98,0 a | 2,7 |
| NS 5959 IPRO | | | | |
| Germination (%) | 69,0 b | 82,8 a | 85,1 a | 5,3 |
| Electrical condutivity (µS $cm^{-1}$ $g^{-1}$) | 117,6 a | 107,2 a | 87,8 b | 13,2 |
| Germination after accelerated aging (%) | 53,5 c | 82,1 a | 73,0 b | 9 |
| Seedling emergence in sand (%) | 77,9 b | 82,5 b | 89,6 a | 7,9 |

* Means followed by the same letter in the line, are not significantly different according to the Tukey´s test at 5% significance.

The minimum standards for seed marketing stipulate that the germination of soybean seeds must be above 80%; however, the seeds from the lower stratum did not meet these standards. The factors that may have led to low seed germination in the lower stratum of the plants may have been; microclimate that was more favorable to the development of phytopathogenic microorganisms in the seeds, low penetration of agrochemicals in the lower parts of the canopy (Holtz et al., 2014), and low source strength of the leaves of the lower nodes. The seeds inserted in the lower nodes were adjacent to the leaves that have reduced light interception; which led to the decreased transport of photosynthates to the legumes, as transport occurs owing to the proximity between source and sink (Fioreze et al., 2022)

Furthermore, in cultivars with indeterminate growth, the seeds positioned in the lower stratum of the plants had a more advanced development than those above them. This signifies that the former group completed the period of seed formation/filling earlier, whereas soybeans maintained many leaves and were not ready for harvest (Thomas, 2018). Thus, it is possible that the longer that the seeds from the lower stratum remain in the field, the more their viability is influenced after harvest, owing to the ongiong deterioration processes associated with time, humidity, and consumption by bugs and microorganisms.

Regarding the isolation effect of SR for NS 5959 IPRO, there was a second-degree polynomial adjustment, with the highest germination percentages obtained with 60% of the recommended SR (Figure 3A), as occurred with BRS 1010 IPRO.

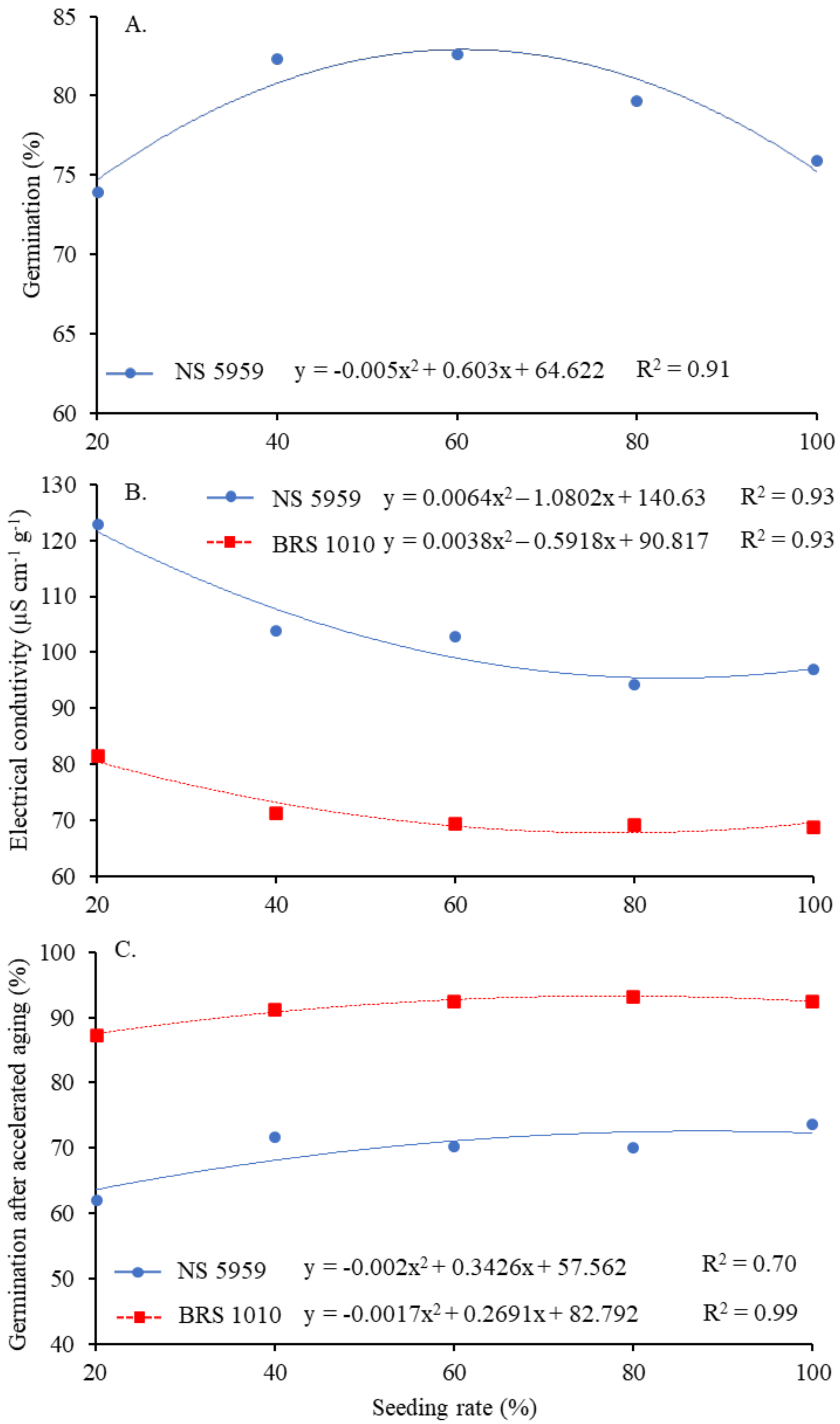



**Figure 3.** Germination (A) electrical condutivity (B) and accelerated aging (C) of soybean seeds, cultivars BRS 1010 IPRO and NS 5959 IPRO in response to seeding rate reduction (100, 80, 60, 40 and 20% of the recommended by the breeder). Means of lower, middle, and upper strata. Londrina, PR, 2016/17 growing season.

The low germination rate associated with the high SRs likely resulted from the microclimate caused by the greater number of plants per area, which results in greater shading and less aeration and penetration of agrochemicals inside the canopy; conditions that favor the occurrence of microorganisms in the seeds and can reduce germination. However, the low germination associated with the lowest SRs may have resulted from the greater number of seeds per plant (Ferreira et al., 2016). This increased seed number, being associated with lower interception of solar radiation (Werner et al., 2018) and reduced exploitation of the soil through the roots in the soybean reproductive phase (Balbinot et al., 2018), can compromise the partition of photoassimilates and adequate seed formation, causing them to have lower mass, viability and vigor (Schuch et al., 2009). According to Pádua et al. (2010) larger and denser seeds have greater germination and vigor, resulting in greater productivity.

Baron et al. (2018) also found that soybean seeds produced at lower plant densities had a lower viability. The authors also attributed this finding to low use of environmental resources, which can lead to the production of less dense and/or smaller seeds.

The first count of germination in the BRS 1010 IPRO cultivar was influenced by the position of the seeds on the plant, with no effect on SR. The seeds in the upper stratum had a higher germination rate in the first count than in the lower and middle strata, indicating superior vigor in this position (Table 1).

Interactions between factors occurred within NS 5959 IPRO. In all evaluated SRs, the seeds from the lower stratum had a lower germination rate in the first count than those from the other strata. The germination of seeds in the middle stratum differed from those in the upper stratum only at 40% SR (Figure 2B). A significant influence of SR was observed only in the upper stratum, with a second-degree polynomial adjustment indicating the point of maximum germination obtained with 52% of the recommended SR (Figure 2B).

Rossi et al. (2017) did not observe a reduction in germination and first count in response to a reduction in plant density from 170 to 70 thousand plants ha$^{-1}$ for soybean cultivars BRS 232 and BRS 284. However, these cultivars had a determined growth type and a stronger branching ability than the cultivars evaluated in the present study, providing a higher phenotypic plasticity.

Seedling length was influenced by the interaction between factors in both cultivars. Generally, in both cultivars, there was a greater length of seedlings from seeds in the upper strata, compared to the lower ones (Figures 4A and B). In BRS 1010 IPRO, SR only influenced the length of seedlings from the lower stratum, with a second-degree polynomial adjustment (Figure 4A). In NS 5959, there was an influence of SR only for the middle stratum, with an estimated linear reduction of 1.2 cm in seedling length for each 10% reduction in SR (Figure 4B). The low influence of SR on seedling length was corroborated by Ferreira et al. (2017), who did not observe an influence of SRs varying between 560 and 150 thousand viable seeds ha$^{-1}$ on seedling length and other characteristics of the physiological potential of seeds.

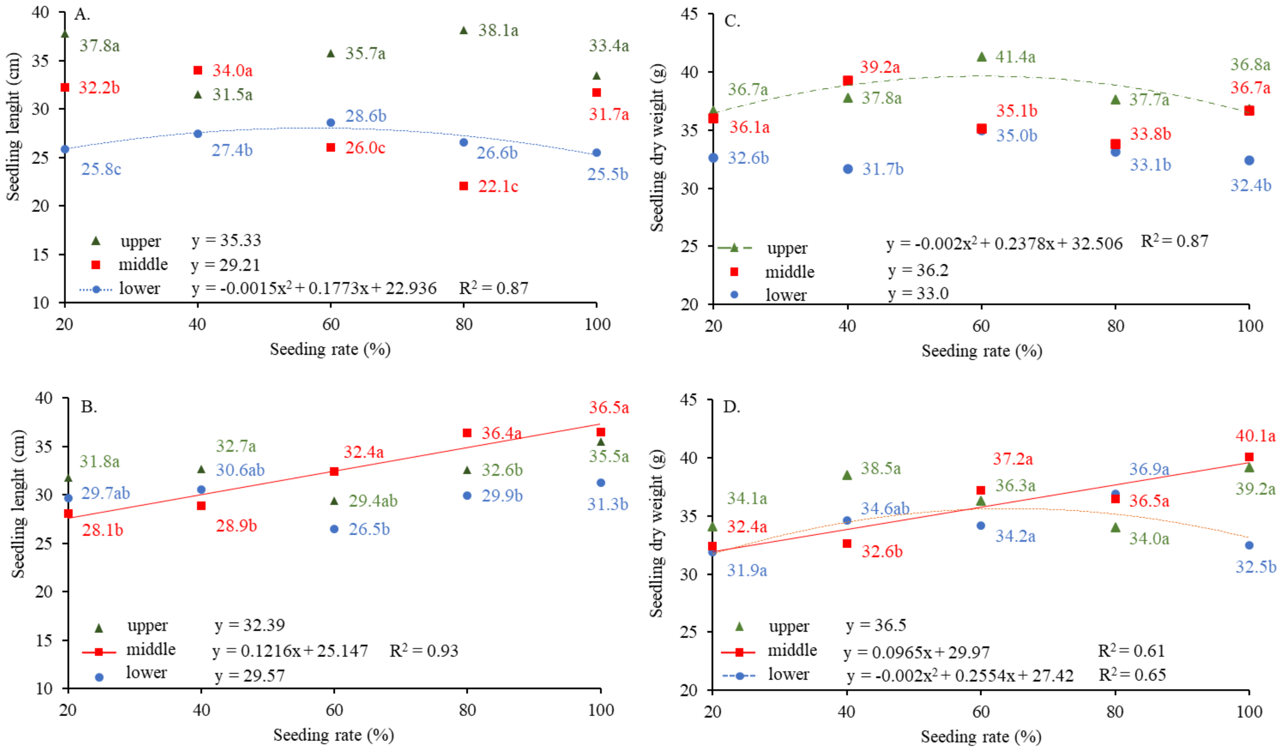



**Figure 4.** Seedlings length and dry mass of cultivar BRS 1010 IPRO (A and C) and cultivar NS 5959 IPRO (B and D) in the lower, middle and upper strata of the plant in response to seeding rate reduction (100, 80, 60, 40 and 20% of the recommended by the breeder). Londrina, PR, 2016/17 growing season.

For the seedling dry mass test in the present study, there was an interaction for the factors in both cultivars. In the BRS 1010 IPRO, a greater dry mass of seedlings was found in the upper stratum at the expense of its lower counterparts in all SRs (Figure 4C). In the NS 5959 IPRO cultivar, the upper stratum had a greater dry mass than the lower stratum, not differing from the middle stratum at 40% SR, whereas at 100% SR there was a decreased dry mass in the lower stratum (Figure 4D). In the lower stratum of NS 5959 IPRO and upper stratum of BRS 1010 IPRO there was a second-degree polynomial fit, indicating greater vigor of seeds of intermediate SRs (64 and 60% of the recommended rates, respectively). In the middle stratum of NS 5959 IPRO, there was a linear fit, with a reduction in the dry mass of seedlings at the lowest SRs (Figures 4C and D).

Therefore, vigor, as expressed by the dry mass of seedlings, was influenced by plant density so that reducing SR to approximately 60% of the recommended amount resulted in a stronger vigor of the seeds produced.

In the electrical conductivity test, both experimental factors showed influences without interacting with each other. In the BRS 1010 IPRO cultivar, seeds from the lower stratum showed a greater electrolyte loss than those from the middle stratum, which, in turn, demonstrated a greater loss than those from the upper stratum. In the NS 5959 IPRO cultivar, the seeds from the upper stratum also had a lower electrical conductivity, but those from the middle and lower stratum did not differ from each other (Table 1).

These results are another indication that seeds produced in the upper stratum have a stronger physiological potential than those produced in the lower portions of the canopy. Illipronti et al. (2000) and Flores (2016) evaluated the physiological potential of seeds produced in the lower, middle, and upper strata of different soybean cultivars and observed that seeds from the lower stratum showed lower germination and vigor, as expressed by electrical conductivity, field emergency, tetrazolium, and accelerated aging tests.

A drastic reduction in SR increased the electrical conductivity of the seeds in both cultivars, but with a greater intensity in NS 5959 IPRO (Figure 3B). However, Ferreira et al. (2017) and Rossi et al. (2017) did not obtain significant differences for electrical conductivity resulting from changes in SR. Notably, these studies did not evaluate plant densities as reduced as in the present work, which indicates that, the effect presented here may possibly not be repeated when the reduction in SR is less pronounced.

In the accelerated aging test, both experimental factors showed influences without interactions. The seeds of the BRS 1010 IPRO cultivar from the upper stratum had a higher germination rate following accelerated aging than the seeds from the lower and middle strata. In the cultivar NS 5959 IPRO, seeds from the lower stratum had the lowest percentage of germination after aging, followed by the upper and middle strata (Table 1). These results reinforce the results of other tests, indicating a clear trend towards the greater physiological potential of seeds originating from the upper stratum of the plant.

SR affected germination after aging in both cultivars, especially at the higher reduction. There was a second-degree polynomial fit for both cultivars, which again demonstrates that the extreme reduction in SR, to just 20% of the recommended rate, resulted in a lower physiological potential of the seeds produced (Figure 3C).

Notably, the adjustment of the polynomial equation only occurred owing to the lower SR, and the reduction of up to 40% in SR had a minimal influence on germination after accelerated aging. Additionally, Vazquez et al. (2008) and Ferreira et al. (2017) found no impact of SR, varying between 150 and 550 thousand viable seeds ha-1, on germination after accelerated aging; this confirms that the difference found in this study resulted from the drastic reduction.

Regarding the emergence test in sand, an influence was found only on the position of the seed on the plant for both cultivars. In the BRS 1010 IPRO cultivar, the seedling emergence was less for seeds from the lower stratum compared to those from the middle and upper strata (Table 1). In NS 5959 IPRO, seeds from both the lower and middle strata had a lower seedling emergence than those from the upper stratum (Table 1), which was consistent with Flores (2016). The seedling emergence values in sand were notably higher than the germination rates, which is common in soybean seeds considering that conducting the test on paper rolls in germination chambers may favor the occurrence of *Phomopsis sp.* (Schuab et al., 2008).

The lack of response of SR on seedling emergence in sand is also an important result that must be considered. Ferreira et al. (2017)observed no influence of SR on vigor tests based on seedling emergence. Therefore, although the other tests indicated the influence of SR reduction on seed vigor, this was not confirmed in the sand emergence test, thereby indicating that eventual reductions in SR may not interfere with seedling emergence in the field.

Although some tests showed an effect of SR on physiological potential, this influence was of a low magnitude and generally occurred at the ends of the SR range tested. Therefore, any reduction in SR to values between 40 and 60% of those recommended for cultivars BRS 1010 IPRO and NS 5959 IPRO should not cause significant changes in the physiological potential of the seeds produced. This result is highly important from a practical point of view, considering that many studies in Brazil and abroad have suggested the reduction of SR based on the phenotypic plasticity of soybeans, thereby resulting in a reduction in production costs without change in grain yields (Büchling et al., 2017; Carciochi et al., 2019).

## Conclusions

1. The physiological potential of seeds increases when progressing from the lower to the upper stratum of the plant, regardless of the seeding rate.

2. Reducing the seeding rate to values up to 60% of the recommended amount does not affect the physiological potential of the seeds in both cultivars.

3. Drastically reducing the seeding rate to 20% of the recommended amount results in a lower seed vigor.

4. Seed germination rates of cultivars NS 5959 IPRO and BRS 1010 IPRO were higher at seeding rates varying between 56 and 80% of that recommended by breeders.

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