

Reserve mobilization and seedling development of common bean seeds pre-soaked in gibberellic acid¹

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ABSTRACT - Soaking seeds in gibberellic acid can alter seedling performance by directly affecting reserve mobilization and the formation of vigorous seedlings. The aim of this study was to assess whether common bean seeds with contrasting vigor pre-soaked in different doses of exogenous gibberellic acid (GA₃) exhibited changes in reserve mobilization and seedling development. The experimental design was completely randomized, with a 2 x 5 factorial scheme (two cultivars and five GA₃ concentrations). The BAF55 and BAF44 cultivars were characterized as high and low vigor, respectively. The parameters assessed exhibited cultivar-dose interaction, demonstrating genetic variability for sensitivity to the hormone. An increase in GA₃ doses compromised reserve mobilization in BAF55, with the larger amount of remaining dry mass in the cotyledons indicating that the reserves were not used during seedling development, resulting in lower dry mass. On the other hand, higher hormone doses favored seed reserve use, promoting greater seedling length. GA₃ doses up to 0.10 mM L⁻¹ resulted in greater hypocotyl, epicotyl and seedling length and lower cotyledon mass, demonstrating use of the energy reserves previously synthesized in the cotyledon. Thus, while applying the hormone to seeds directly influences seedling architecture, it promotes more efficient use of cotyledon reserves in cultivar BAF44, producing taller, more vigorous seedlings with higher dry mass.

Key words: *Phaseolus vulgaris* L. Gibberellic acid. Seed vigor. Cultivars.

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INTRODUÇÃO

The common bean (*Phaseolus vulgaris* L.) is the most important legume for human consumption worldwide as a source of plant protein, minerals, antioxidants and bioactive compounds (Karavidas *et al.*, 2022). Beans are a staple and symbol of Brazilian cuisine, consumed by 70% of the population, especially low-income families, with average consumption reaching 17 kg inhabitant⁻¹ year⁻¹ (Brasil, 2018; Carneiro; Paula Júnior, 2015).

The use of high-quality seeds is essential to ensure better plant establishment in the field and agronomic performance. Germination, seed vigor and longevity are particularly important quality-related physiological traits related to quality are particularly important because of their relationship with seeds' ability to perform vital functions (Krzyzanowski; França-Neto; Henning, 2018).

Vigor plays a vital role in rapid uniform emergence, directly influencing dry matter accumulation during the growth cycle, resulting in higher grain yields. Cultivar is one of the factors that can affect this parameter, making it essential to identify possible differences in seed lot vigor in order to assess the correlation between vigor and cultivar during seedling growth (Nerling; Coelho; Brümmer, 2018; Padilha; Coelho; Andrade, 2020).

The germination process and early development of vigorous seedlings can be influenced by different factors, such as the seed production conditions (Marcos-Filho, 2015), stored energy reserves (Bewley *et al.*, 2013), reserve mobilization dynamics (Padilha; Coelho; Sommer, 2022), genetic expression (Tuan *et al.*, 2018) and hormonal adjustment (Carrera-Castaño *et al.*, 2020).

Hormones act in coordination in development-related metabolic processes, stimulating or inhibiting growth (Atroch *et al.*, 2020). Absciscic (ABA) and gibberellic acid (GA) are the most prominent hormones in the germination process, acting to prevent or promote germination. GA regulates the synthesis of hydrolytic enzymes, particularly alpha and beta-amylase, which promote starch degradation and increase the availability of soluble sugars for initial seedling development (Li *et al.*, 2020; Xiong *et al.*, 2021). Given that cereals use starch as their main energy source, studies that investigate exogenous GA₃ application focus largely on crops such as rice (Wang *et al.*, 2019; Yano *et al.*, 2015), corn (Akter *et al.*, 2014; Shahzad *et al.*, 2021) and wheat (Mares *et al.*, 2022).

The chemical composition of some legumes, such as beans, consists of 50 to 60% carbohydrates. Thus, given the effect of GA on alpha-amylase activity and, consequently, starch degradation, exogenous application of this hormone may favor seedling performance. Padilha, Coelho and Ehrhardt-Brocardo (2021) reported that

greater alpha-amylase activity is associated with the vigor of common bean seeds, which showed better seedling performance during germination.

Additionally, studies that analyze seed vigor metabolism aim to differentiate between cultivars according to their ability to mobilize seed reserves under stress (Padilha; Coelho; Andrade, 2020); however, there are no reports of investigations into changes caused by applying exogenous hormones to seeds. As such, the aim of this study was to assess whether common bean seeds with contrasting vigor pre-soaked in different doses of exogenous gibberellic acid (GA₃) exhibited changes in reserve mobilization and seedling development.

MATERIAL AND METHODS

The experiment was conducted with two cultivars from the Common Bean Active Germplasm (BAF) of the Agricultural and Veterinary Sciences Center of Santa Catarina State University (UDESC-CAV). The BAF44 and BAF55 cultivars were multiplied in the 2021/2022 growing season in the UDESC-CAV experimental area in Lages, Santa Catarina state, Brazil (27°78'S, 50°30'W, 930 m.a.s.l.). These cultivars are the result of self-selection over the last 12 growing seasons, producing considerable homogeneity and stability within the population (Ehrhardt-Brocardo; Coelho, 2022; Gindri *et al.*, 2017; Michels *et al.*, 2014; Padilha; Coelho; Andrade, 2020).

The seeds were manually harvested and taken to the UDESC Seed Analysis Laboratory for standardization by drying in an oven at 35 °C until reaching 13% moisture content, and then classified using an elongated 12-mesh sieve. After standardization, one average and one working sample were obtained for each genotype, according to the Brazilian Regulations for Seed Analysis (Brasil, 2009).

Experiment 1 Physiological analysis of seeds from the 2021/2022 growing season

The germination percentage (GE) of seeds of each cultivar was assessed in a Mangelsdorf germination chamber at 25 ± 2 °C. The seeds were distributed on Germitest® paper moistened with distilled water at 2.5 times its dry weight (Brasil, 2009), using four repetitions of 50 seeds. Counts were performed 5 and 9 days after sowing (DAS) and the data expressed in percentage of normal seedlings.

Vigor was evaluated based on accelerated aging (AA), seedling performance (SP) and the vigor index (VI). AA was performed using four repetitions of 50 seeds, distributed on a steel mesh screen suspended inside clear polystyrene Gerboxes®, with 40 mL of distilled

water on the bottom of the box. The boxes were sealed and placed in an accelerated aging chamber for 72 at 42 °C (Marcos-Filho, 1999). Next, the seeds were removed from the boxes and submitted to germination testing as previously described.

The SP test was carried out using four repetitions of 20 seeds, distributed on Germitest paper (Nakagawa, 1999) and moistened with water at 2.5 times its dry weight, rolled up and placed in a *Mangelsdorf* germination chamber at 25 ± 2 °C for seven days. Next, total seedling length (shoot and roots) was measured in normal seedlings only, using a digital pachymeter, and the results expressed in cm seedling⁻¹ (Nakagawa, 1999).

The VI was calculated in accordance with Abdul-Baki and Anderson (1973), using Equation 1:

$$VI = \text{germination}(\%) \times \text{seedling performance}(\text{cm}) \quad (1)$$

This first experiment used a completely randomized design with two genotypes and four repetitions. The data were submitted to normality testing when needed, followed by analysis of variance (ANOVA), and means were compared by Tukey's test at 5% probability. The statistical analyses were performed using SISVAR software (Ferreira, 2011).

Experiment 2: Soaking common bean seeds in gibberellic acid (GA₃)

For exogenous GA₃ application, seeds of each cultivar were soaked in different GA₃ concentrations (i.e., 0.025; 0.05; 0.10; and 0.20 mM L⁻¹) or water (control) for 120 min, and then submitted to germination testing as previously described.

The SP test was conducted after soaking, with four repetitions of 20 seeds rolled up in Germitest paper and placed in a *Mangelsdorf* germination chamber at 25 ± 2 °C for 7 days in the dark. After this period, only normal seedlings were randomly collected and the following measured with a digital pachymeter: root (RL), hypocotyl (HL), epicotyl (EL) and seedling length (SL), expressed in cm seedling⁻¹. Next, the cotyledons were separated from the seedling axis and dried at 80 °C for 24 h in a forced-air oven to determine the remaining dry mass in the cotyledons (RDMC) and seedling dry mass (SDM), expressed in mg seedling⁻¹ (Nakagawa, 1999).

To assess the mobilization of seed reserves, the seed coat was removed from four repetitions of 20 seeds, which were then dried in an oven at 105 °C for 24 h to obtain seed dry mass (SEDM) (Padilha; Coelho; Andrade, 2020).

The SEDM and RDMC data were used to calculate seed reserve use (SRU), as per Equation 2, and the results expressed in mg seed⁻¹ (Pereira; Pereira; Dias, 2015; Soltani; Gholipoor; Zeinali, 2006).

$$SRU = SEDM - RDMC \quad (2)$$

Seed reserve use efficiency (SRUE), that is, initial seed dry mass and how much of this mass was converted into seedling dry mass, was calculated using Equation 3 and the result expressed in mg mg⁻¹ (Pereira; Pereira; Dias, 2015; Soltani; Gholipoor; Zeinali, 2006).

$$EUSR = \frac{SDM}{USR} \quad (3)$$

The seed reserve use rate (SRUR) is used to assess actual reserve use, without the influence of external factors, in accordance with Equation 4, and the results expressed as percentage (Pereira; Pereira; Dias, 2015; Soltani; Gholipoor; Zeinali, 2006).

$$SRUR = \frac{SRU}{SEDM} \times 100 \quad (4)$$

The amount of reserves mobilized per seedling was determined via Equation 5 using the seed reserve mobilization rate (SRMR) and expressed in percentage (Andrade; Coelho; Padilha, 2019).

$$SRMR = \frac{SDM}{SEDM} \times 100 \quad (5)$$

The experimental design was completely randomized, with 2 x 5 factorial scheme and four repetitions, consisting of two cultivars (BAF44 and BAF55) combined with five GA₃ concentrations (0; 0.025, 0.05; 0.10; and 0.20 mM L⁻¹). The data obtained were submitted to normality testing and analysis of variance (ANOVA) and, when significant, means were compared for the qualitative factors (cultivars) and regression applied for the quantitative factor (GA₃ doses) at 5% significance, in SISVAR® statistical software (Ferreira, 2011).

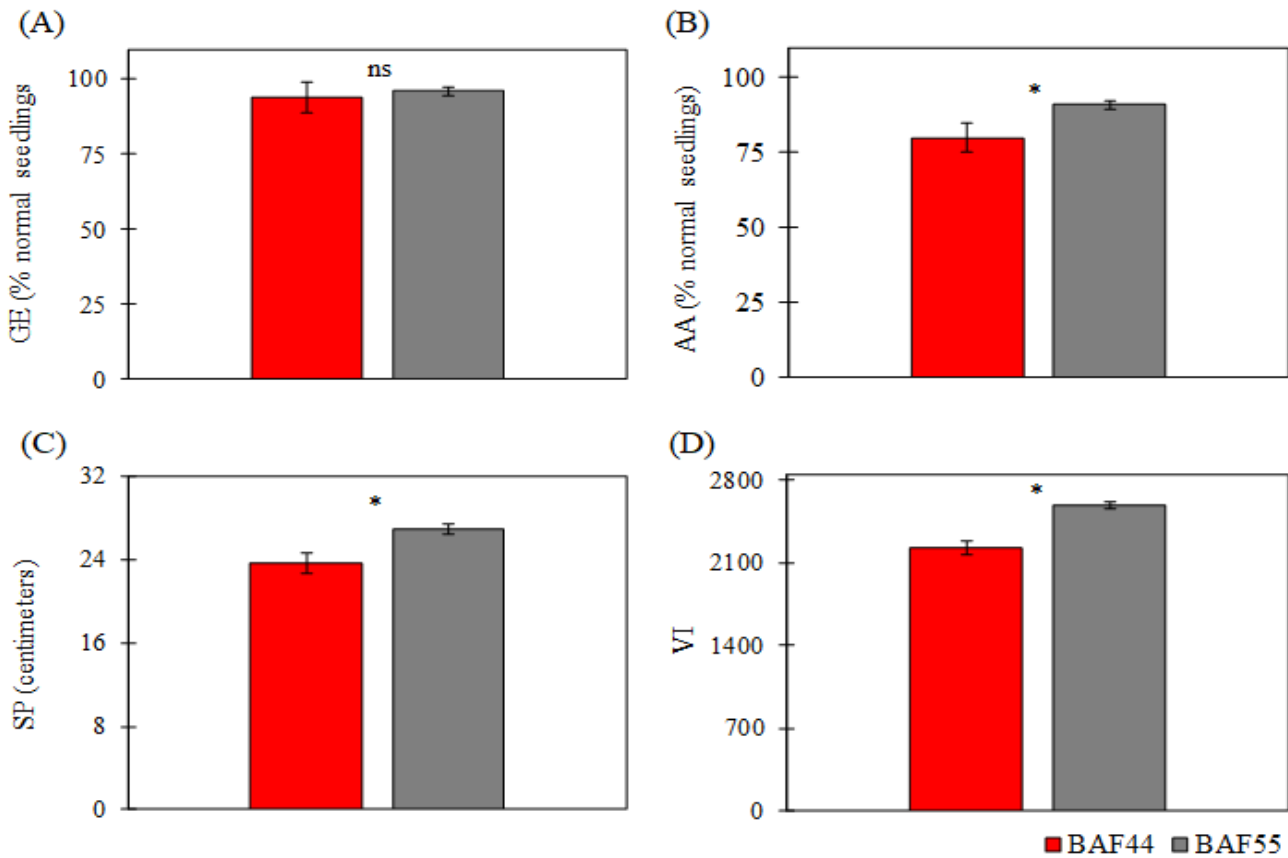
RESULTS AND DISCUSSION

Experiment 1

There was no statistical difference between the cultivars for GE percentage (Figure 1A). The similar cultivar GE behavior observed is likely due to the drying process, which maintained seed physiological quality by reducing metabolic activity that could compromise normal seedling formation after germination testing (Zucareli *et al.*, 2015).

However, the tests performed to determine vigor indicated a difference between cultivars. BAF55 obtained higher values than those of BAF44 and was classified as high vigor, exhibiting significantly superior performance under both adverse (Figure 1B) and ideal conditions (Figure 1C and 1D). The findings of Bertolin, Eustáquio de Sá and Moreira (2011) in common bean cultivars corroborate those obtained here, whereby germination testing did not distinguish between cultivars despite their different vigor levels.

Figure 1 - Physiological characterization of common bean cultivars BAF44 and BAF55 via (A) germination (GE) and (B) accelerated aging tests (AA), (C) seedling performance (SP) and (D) vigor index (VI)



ns, *: not significant and significant according to Tukey's test at 5% probability, respectively

According to Marcos-Filho (2015b), the accelerated aging test and seedling performance parameters show potential for determining seed vigor. These tests are vital indicators of greater performance potential in the field, since vigorous seeds have physiological and biochemical traits that enable faster and more uniform seedling establishment under a wide range of environmental conditions that result in higher crop yield (Ebene *et al.*, 2020; Reis *et al.*, 2022).

The results obtained indicate that seed physiological quality is a cultivar-based trait, given that the seeds were sown, collected and analyzed under the same conditions but differed in terms of the variables assessed. Another important aspect is that only seed vigor tests were able to identify differences in physiological quality, largely due to their greater sensitivity in detecting these differences in seed lots with similar germination capacity (Marcos-Filho, 2015a).

Experiment 2

Exogenous GA₃ application to seeds showed cultivar-dose interaction (Table 1) for the variables

hypocotyl (LH) and seedling length (SL), remaining dry mass in the cotyledons (RDMC), seedling dry mass (SDM), seed reserve use (SRU), seed reserve use rate (SRUR), and seed reserve mobilization rate (SRMR). The difference between cultivars in terms of seedling growth, dry mass, and reserve mobilization characteristics due to the GA₃ doses indicate genetic variability for sensitivity to this hormone.

For BAF44, an increase in the GA₃ dose to 0.05 mM L⁻¹ stimulated hypocotyl growth, which in turn influenced seedling growth (Figure 2A and 2B). Longer HL is related to the greater specificity of the hormone, since exogenous application induces the synthesis of enzymes involved in cell wall weakening, such as alpha-amylase (Cosgrove, 2015; Pio *et al.*, 2011), and expansins (Choi *et al.*, 2003), which act in cell wall elongation. Ragni *et al.* (2011) studied external GA₃ application in *Arabidopsis* and found that GA stimulates xylem fiber differentiation and elongation.

The increase in GA₃ prompted a declining trend for RDMC in BAF44 (Figure 2C), directly affecting reserve mobilization metabolism. This reduction

indicates greater use of reserves for growth regions, explaining the higher HL and SL values in this cultivar. Research on soybean (Oliveira *et al.*, 2020) and corn (Andrade; Coelho; Padilha, 2019), demonstrated that less remaining dry mass in the cotyledons or endosperm is associated with greater seedling vigor. This relationship explains the superior performance of BAF44 cultivar seeds under GA₃ application, which favored the hydrolysis capacity of the reserve components in the cotyledons.

However, BAF55 exhibited an increasing trend for RDMC with the rise in GA₃ dose, demonstrating less use of the preexisting reserves in the cotyledon. This resulted in a decline in SDM and SRMR after GA₃ application (Figure 2D and 2G). Seedlings with lower dry mass values also obtained lower reserve use rates, indicating a direct correlation between these variables (Padilha *et al.*, 2022). Similar results were reported by Jaques *et al.* (2019), whereby bean seedling dry mass was negatively affected by an increase in the GA dose applied to seeds.

In BAF55, exogenous GA₃ application compromised SRU and SRUR, whereas in BAF44, higher hormone doses prompted increases in both traits. SRU is a key factor in the development of high-vigor seedlings because of its relationship with the hydrolysis of seed reserves into small molecular compounds, such as amino acids and soluble sugars, which are used for germination immediately after seed imbibition (Cheng *et al.*, 2018).

GA₃ improved reserve use only for BAF44 (low vigor), resulting in greater mobilization and use of the reserves applied for germination and subsequent seedling development. These results indicate that exogenous application of this hormone benefits the metabolism of low-vigor seeds.

Analysis of the cultivar effect alone showed that BAF55 obtained a shorter RL and higher average HL and EL (Table 1). Studies reveal that interaction between GA and ethylene has contrasting effects in the shoots and roots, whereby ethylene inhibits root elongation by not allowing RGA (repressor de GA1⁻³) degradation but favors this process in the hypocotyl (Fleet; Sun, 2005).

However, the data obtained in the present study for BAF44 showed no decline in RL, indicating a difference between cultivars for sensitivity to this hormone, which may be directly related to differences in gene expression during renewed respiration, which ultimately leads to seedling development. BAF44 also displayed higher SRU values, demonstrating that the previously mobilized reserves were used for seedling formation, resulting in higher SDM values (Table 1).

Average EL and SRUE values showed a significant difference between the GA₃ doses studied (Figure 3). According to Espíndula *et al.* (2010), application of this hormone and plant tissue sensitivity determine the seedling growth and development response.

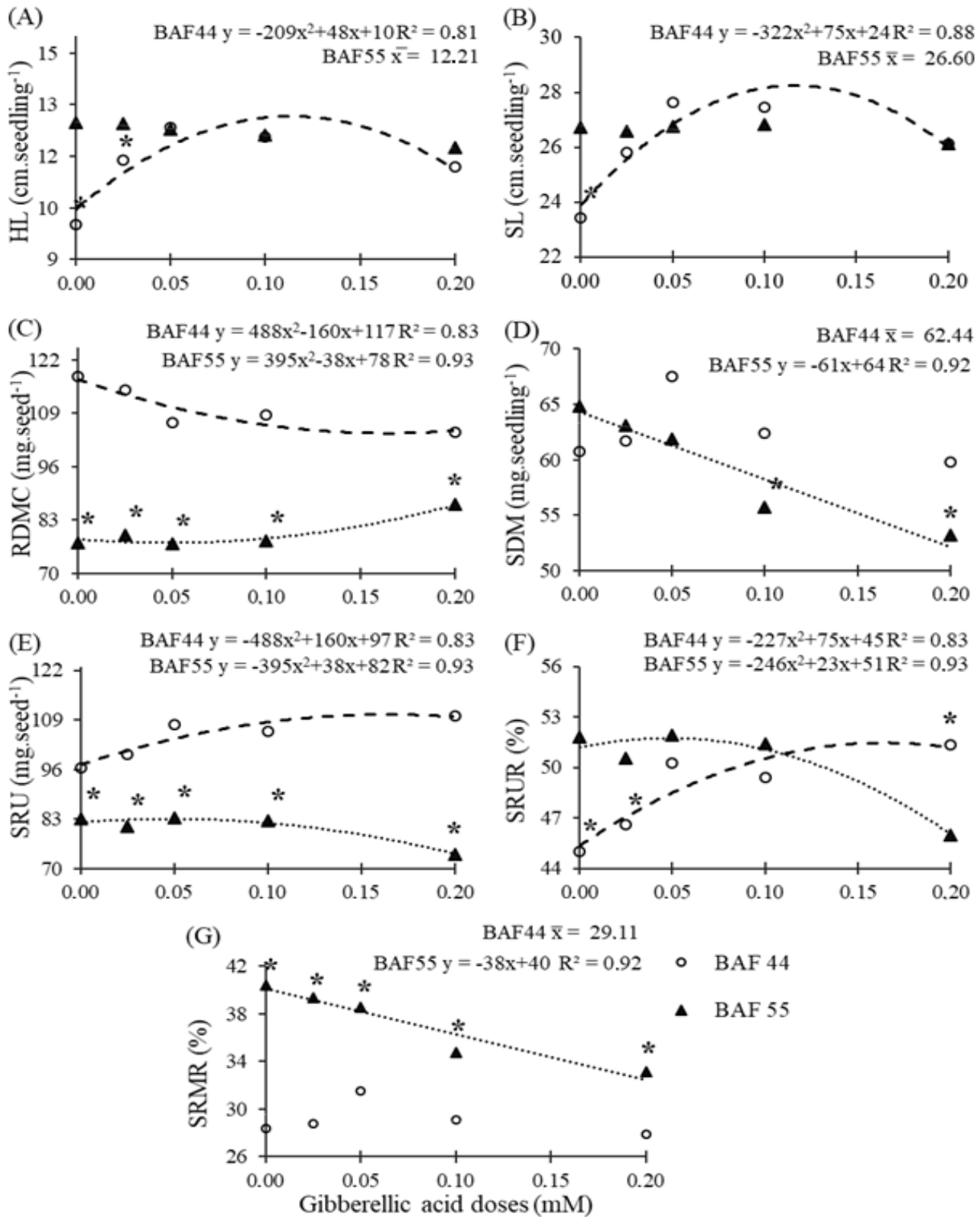
Higher GA₃ doses increased EL, indicating greater sensitivity to the hormone in this structure (Figure 3A). In a pioneering study, Garcia-Martinez e Rappaport (1984) found that GA application in black-eyed pea increased epicotyl length by up to 13 times when compared to the control. Moreover, under scanning electron microscopy, the authors observed that this effect was entirely due to cell elongation; however, GA is also known to increase cell division (Jusoh *et al.*, 2019).

Table 1 - F test and mean comparison for the variables germination (GE), root (RL), hypocotyl (HL), epicotyl (EL) and seedling length (SL), remaining dry mass in the cotyledon (RDMC), seedling dry mass (SDM), seed reserve use (SRU), seed reserve use efficiency (SRUE), seed reserve use rate (SRUR) and seed reserve mobilization rate (SRMR)

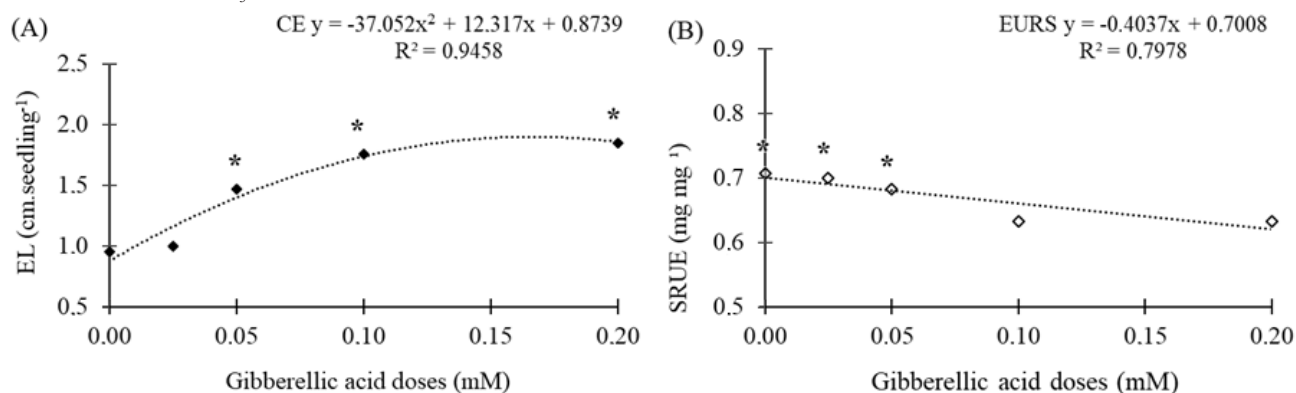
| F Test (p-value) | | | | | | | | | | | |
|-------------------------|---------|---------|---------|--------|---------|----------|---------|----------|--------|---------|---------|
| Source of variation | GE | RL | HL | EL | SL | RDMC | SDM | SRU | SRUE | SRUR | SRMR |
| Cultivar | 0.485 | 0.000 | 0.000 | 0.000 | 0.080 | 0.000 | 0.039 | 0.000 | 0.000 | 0.002 | 0.000 |
| Dose | 0.178 | 0.203 | 0.005 | 0.000 | 0.000 | 0.005 | 0.001 | 0.005 | 0.005 | 0.008 | 0.002 |
| Cultivar x Dose | 0.943 | 0.351 | 0.001 | 0.395 | 0.000 | 0.000 | 0.023 | 0.000 | 0.358 | 0.000 | 0.023 |
| CV (%) | 1.88 | 4.05 | 5.93 | 13.94 | 3.41 | 3.37 | 6.45 | 3.46 | 7.06 | 3.47 | 7.11 |
| Tukey's test (p < 0.05) | | | | | | | | | | | |
| Cultivars | GE | RL | HL | EL | SL | RDMC | SDM | SRU | SRUE | SRUR | SRMR |
| BAF44 | 95.00 a | 13.58 b | 11.30 b | 1.20 b | 26.08 a | 110.42 a | 62.44 a | 104.07 a | 0.60 b | 48.51 b | 29.11 b |
| BAF55 | 96.00 a | 12.78 a | 12.21 a | 1.60 a | 26.60 a | 79.71 b | 59.76 b | 80.78 b | 0.73 a | 50.33 a | 37.23 a |

Means followed by the same letter in the column do not differ according to Tukey's test at 5% probability

Figure 2 - Interaction of cultivars BAF44 and BAF55 as a function of different GA3 doses for the variables (A) hypocotyl (B) and seedling length, (C) remaining dry mass in the cotyledon, (D) seedling dry mass, (E) seed reserve use, (F) seed reserve use rate, and (G) seed reserve mobilization rate



*: significant according to Tukey's test at 5% probability

Figure 3 - Effect of GA₃ doses on (A) epicotyl length and (B) seed reserve use efficiency

*: significant according to Tukey's test at 5% probability in relation to the control dose

This result is associated with bioactive GA accumulation regions in the seedling, which are typically located in the elongation zone of the seedling, promoting cellular growth in this region (Binenbaum; Weinstein; Shani, 2018). Souza *et al.* (2010) studied bean cultivars and demonstrated that treating seeds with GA₃ resulted in longer epicotyls, which can be explained by the fact that these structures act as carbohydrate sinks at the expense of leaf development (source). The increase in these traits directly affects the architecture of bean plants, making them taller.

SRUE exhibited a decreasing trend and was negatively affected by increased GA₃ doses from 0.10 and 0.20 mM L⁻¹ onwards (Figure 3B). This behavior may be associated with its interaction with other hormones, such as auxin and ethylene (Saibo *et al.*, 2003), where a hormonal imbalance may compromise reserve mobilization dynamics.

In research with rice seeds, Liu *et al.* (2018) found that exogenous GA favors the use of seed reserves by activating hydrolytic enzymes (alpha-amylase), since it increases the availability of soluble sugars for the embryonic axis, resulting in taller seedlings and higher dry mass. Thus, it is believed that higher GA₃ doses altered the respiration rate and the way seed reserves were used, directing them to other metabolic pathways rather than seedling formation.

According to results obtained by Saadat *et al.* (2020), treating bean seeds with GA₃ favors physiological performance, which is associated with less gene expression of antioxidant enzymes, indicating that using this hormone strengthens bean seedlings. The fact that GA₃ improved early seedling development in BAF44 (low vigor) indicates more efficient reorganization and greater mobilization of the previously synthesized reserves. Further research is

needed to determine whether biochemical changes in the composition of seed reserves affect the reserve use rate during seedling development, and if seeds with different vigor levels exhibit genetic alterations after gibberellic acid treatment to generate vigorous seedlings.

CONCLUSIONS

1. The better hydrolysis of cotyledon reserves after GA₃ application in the low-vigor cultivar generated taller, more vigorous seedlings with higher dry mass when compared to the high-vigor cultivar;
2. GA₃ application negatively affects reserve mobilization dynamics in the high-vigor cultivar due to the lower reserve mobilization rate, which compromises seedling dry mass.

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