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Impact of gibberellic acid on seedling growth and enzymatic activity in bean cultivars with contrasting seed vigor

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ABSTRACT. Soaking seeds in gibberellin acid (GA) can reveal mechanisms controlling seed vigor. GA acts early in germination, stimulating the synthesis of hydrolytic enzymes that break down starch to provide the energy needed for radicle protrusion. This study investigated the effects of gibberellic acid (GA_3) on physiology, biochemistry, and molecular components in bean cultivars with different vigor levels. Two cultivars (BAF44, low vigor; and BAF55, high vigor) were soaked in water or 0.035 mM L^{-1} GA_3. The imbibition curve displayed a triphasic pattern; however, exogenous GA_3 accelerated root emergence only in cultivar BAF44. GA_3 increased root growth only in cultivar BAF44, but increased hypocotyl and epicotyl lengths in both cultivars. GA_3 treatment improved seedling length in the low-vigor cultivar, resulting in more vigorous seedlings. This is likely due to increased gene expression and activity of alpha-amylase, leading to greater starch and total soluble sugar reduction in cotyledons, providing more energy for growth points. The initial seed vigor of beans is crucial for how GA_3 affects reserve mobilization dynamics.

Keywords: *Phaseolus vulgaris* L.; seedling vigor; exogenous gibberellin; alpha-amylase.

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Introduction

Common beans (*Phaseolus vulgaris* L.) are a popular legume due to their high nutritional value (Uebersax et al., 2022). Farmers prefer vigorous seeds for better crop establishment in the field. Therefore, rapid and uniform emergence, is crucial for the initial growth and overall productivity of the crop (El-Sanatawy et al., 2021).

Seeds with high vigor efficiently hydrolyze and mobilize reserves to the embryonic axis, providing energy for vigorous seedling formation (Andrade et al., 2019). Germination involves tissue rehydration, increased respiration, and ultimately, energy for seedling development (Marinho et al., 2021).

Pre-sowing seed treatment with gibberellin acid (GA_3) is a recent practice to improve germination and seedling vigor (Azimi et al., 2022; Dhillon et al., 2021). GA stimulates germination by promoting the synthesis of hydrolytic and proteolytic enzymes (e.g., alpha-amylase and proteases), which break down reserves for plant growth and establishment (Damaris et al., 2019).

Existing research has focused on the role of GA in managing stress during early plant development (Saadat et al., 2020; Wang et al., 2018). However, genetic studies have primarily focused on mutations in key GA metabolism genes of *Arabidopsis* (Griffiths et al., 2006) and cereals (Tong et al., 2007), neglecting the effects of directly applying GA to seeds.

Despite reported benefits, knowledge gaps remain regarding how seed vigor affects the response to exogenous GA and how germination changes translate to seedling formation. Thus, this study investigated the effects of pre-soaking with GA_3 on physiology, biochemistry, and molecular components in contrasting bean vigor levels.

Material and methods

To determine the relationship between seed vigor and GA_3 use, two experiments were conducted with seed lots of BAF44 and BAF55 cultivars, produced in an experimental field of the Santa Catarina State University (UDESC/CAV) in Lages, Santa Catarina State, Brazil (27º48'58" S, 50º19'34" W, and 930-m altitude) during the 2021/2022 growing season. These cultivars belong to UDESC's Active Bean Germplasm Bank (BAF).

The first experiment aimed to characterize the physiological quality of the cultivars through germination and vigor tests. Germination (G, %) was evaluated with four replicates of 50 seeds in a Mangelsdorf germinator at 23 ± 2ºC. The seeds were placed on Germitest-type paper towels moistened with 2.2 times their weight in distilled water (Brasil, 2009). Counts were made on the 5th and 9th day after sowing, and data were expressed as a percentage of normal seedlings. Vigor was assessed using the accelerated aging (AA, %) test at 42ºC for 72 hours (Scappa-Neto et al., 2001), followed by the germination test as described.

The second experiment studied the interaction between cultivars and GA_3 application by immersing the seeds in 0.035 mM L^{-1} GA_3 or water (control) for 120 minutes. The study evaluated the imbibition curve, physiological performance, reserve mobilization, biochemical components, and alpha-amylase gene expression during germination and early seedling development.

The imbibition curve was determined at fixed intervals (every four hours up to 16 hours and every two hours thereafter) until 50% + 1 of the seeds showed radicle protrusion in each replicate. Four replicates of 50 seeds were analyzed on Germitest paper towels moistened with distilled water or GA_3 at a ratio of 2.2 to the mass of dry paper. The seeds were kept vertically in a Mangelsdorf-type germinator at 23 ± 2ºC.

Seedling evaluation involved four replicates of 20 seeds. After the imbibition period, seeds were placed on Germitest paper towels and kept in a Mangelsdorf-type germinator at 23 ± 2ºC for 3, 5, and 7 days. Measurements of root length (RL), hypocotyl length (HL), epicotyl length (EL), and seedling length (SL), were taken on 10 randomly sampled normal seedlings using a digital caliper, expressed as cm of seedling.

The cotyledons and measured structures were then separated and dried for 24 hours at 80ºC in a convection oven to determine the remaining cotyledon dry mass (RCDM) and seedling dry mass (SDDM), expressed as mg.

To evaluate seed reserve mobilization, the seed coat was removed from four replicates of 20 seeds, which were then oven-dried at 105ºC for 24 hours to obtain the seed dry mass (SDM) (Padilha et al., 2020). Using SDM and RCDM data, seed reserve utilization (SRU) was calculated with the following formula, and the results are expressed as mg $seed^{-1}$ (Soltani et al., 2006; Pereira et al., 2015).

$$SRU = SDM - RCDM$$

The seed reserve utilization rate (SRUR) evaluates of the actual utilization of the reserves without external factors. It is calculated as follows, with results expressed as a percentage (Soltani et al., 2006; Pereira et al., 2015).

$$SRUR = (SRU/SDM) \times 100$$

Biochemical component evaluation was conducted on the cotyledons after 0, 3, 5, and 7 days to determine changes during germination and seedling growth. After each period, the cotyledons were crushed with liquid nitrogen, and the resulting powder was stored in a freezer until testing.

Total soluble sugar (TSS) and starch contents were extracted and quantified according to the method described by McCready et al. (1950), with modifications. Cotyledon flours were dried in an oven at 60°C for 48 hours. After drying, 100 mg of the material was placed in a falcon tube. Soluble sugars were extracted in a two-step procedure with 80% ethanol, while starch from the residue was extracted in a two-step procedure with 52% perchloric acid.

Aliquots of 100 µL of the cotyledon extracts were diluted in 900 µL of distilled water and 3 mL of anthrone reagent, then vortexed for 5 seconds. The test tubes were placed in a water bath at 95°C for 450 seconds and then measured with a spectrophotometer at an absorbance of 630 nm. A standard curve for soluble sugar was prepared using a glucose solution with concentrations of 10, 20, 30, 40, and 50 µg mL^{-1}. Results were expressed as mg per seed.

Soluble protein was determined by homogenizing 250 mg fresh flour with 5 mL of Triton X^{-100} sodium acetate buffer (0.005%, pH 5.4 – 5.5) and 5 mmol L^{-1} $CaCl_2$. Samples were shaken on ice for one hour and then centrifuged at 6ºC for 5 minutes. Quantification followed the Bradford method (1976), using 20 µL of the diluted sample, 780 µL of distilled water, and 200 µL of Bradford reagent. Readings were taken with a spectrophotometer at 595 nm using plastic cuvettes. The standard curve was prepared with bovine serum albumin (BSA) at concentrations of 0.025, 0.05, 0.075, 0.100, 0.150, 0.200, and 0.250 mg mL^{-1}. Results were expressed in mg soluble protein per gram of fresh weight.

Alpha-amylase activity was determined using the dinitrosalicylic acid (DNS) method (Miller, 1959). Initially, 250 mg of fresh flour was homogenized with 5 mL of Triton X^{-100} sodium acetate buffer (0.005%, pH 5.4 – 5.5) and 5 mmol L^{-1} $CaCl_2$. Samples were shaken on ice for one hour and then centrifuged at 6°C for 5 minutes. The enzyme

extract was incubated in a water bath at 70°C for 15 minutes. Quantification used 250 µL of the enzymatic extract, 250 µL of sodium acetate buffer (100 mmol L^{-1}) containing 10 mmol L^{-1} $CaCl_2$, and 250 µL of 2% (w/v) starch solution. Samples were incubated in a water bath at 40°C for 20 minutes. The reaction was stopped by adding 500 µL of DNS solution, followed by incubation at 95°C for 6 minutes. Finally, 4 mL of distilled water was added and homogenized. Absorbance values were measured with a spectrophotometer at 540 nm, and results were expressed in mmol L^{-1} reduced sugar g^{-1} min^{-1}. Data were converted to enzymatic activity, calculated as a unit of activity (U) equivalent to 1 µmol of sugars produced per minute under the test conditions. Enzymatic activity results were expressed in U of enzyme per kg of seed.

Soluble starch and protein data were used to determine reduction rates during the evaluation days, with day 0 considered as 100%. The reduction rates of protein and starch were calculated based on the initial results obtained (I), and the remaining value of each component at the evaluated germination time point (R), using the following equation:

$$\text{Reduction Rate} = \left(\frac{\text{I (mg seeds g}^{-1}\text{) - R (mg seeds g}^{-1}\text{)}}{\text{I (mg seeds g}^{-1}\text{)}} \right) \times 100$$

Alpha-amylase gene expression was quantified in the cotyledons of 7-day-old seedlings from BAF44 and BAF55 cultivars with and without hormone administration. Initially, total RNA was extracted from the cotyledons (1 g) of the different vigor bean cultivars using TRIzol min.$^{-1}$ (Invitrogen, USA) and maceration in liquid nitrogen, following the manufacturer's recommendations. The RNA was treated with RNase-free DNase I (Invitrogen). RNA integrity was checked by 1% agarose gel electrophoresis, and quality and concentration were measured using a spectrophotometer (NanoDrop 2000, Thermo Scientific).

First-strand cDNA was synthesized from 3 µg of total RNA using oligo-dT (18) and MMLV-RT enzyme (Promega, USA), according to the manufacturer's recommendations. Gene expression was analyzed by qRT-PCR using primers designed with Primer Express 3.0 software (Applied Biosystems) after searching for gene sequences in the GenBank database (http://www.ncbi.nlm.nih.gov).

The gene of interest was alpha-amylase (forward primer: 5'- AAGGGGATAAAGTGCCTGGC -3', reverse primer: 5'- AATCAAGGCGTGCATCTGGA -3'; GenBank: AB015131.1), while actin (forward primer: 5'- GTTCCCTGGTATTGCGGACA -3', reverse primer: 5'- AGCCACCAATCCAGACACTG -3'; GenBank: KF033666.1) and tubulin (forward primer: 5'- TCGTGGGTTTCAGCAGTATGT -3', reverse primer: 5'- GCAAAGGCAGTCAAGTATCGG -3'; GenBank: MT292610.1) were used as endogenous controls. The quality and specificity of the primers were confirmed by monitoring the size of the amplification products through 1.5% agarose gel electrophoresis and analyzing the melting temperature (TM) of the amplification products in a dissociation curve.

Real-time PCR procedures, including pilot tests, validations, and experiments, followed the Applied Biosystems manual. Real-time RT-PCR assays were performed on an ABI 7500 instrument (Applied Biosystems) using SYBR Green PCR Master Mix (Applied Biosystems) with gene-specific primers describe above.

The thermal reaction conditions were 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Gene expression levels: were calculated using the comparative Ct method: ΔCt = Ct (sample) – Ct (average of endogenous controls), and ΔΔCt = ΔCt (sample) – ΔCt (calibrator). The expression level was calculated using the formula: $RQ = 2^{-\Delta\Delta Ct}$.

The first trial was conducted in a completely randomized design with two cultivars and eight replicates. Data were subjected to normality tests when needed, and an analysis of variance (ANOVA) was performed. Averages were compared using Tukey's test at a 5% probability level. To evaluate the imbibition curve, a regression analysis was performed to characterize water uptake by both cultivars in the absence or presence of GA_3 at hydration times.

The second experiment was conducted in a completely randomized design with a 2 x 2 factorial arrangement, consisting of both cultivars (BAF44 and BAF55) and two seed immersion types (water or 0.035 mM L^{-1} GA_3). Data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test at a 5% probability level. All statistical analyzes were performed using the SISVAR statistical program.

Results

Germination (G) did not differ between the cultivars (Figure 1A); however, accelerated aging (AA), results classified them as having contrasting vigor. Cultivar BAF55 was classified as high vigor with 90% normal seedlings, while BAF44 was classified as low vigor with 78% normal seedlings (Figure 1B).

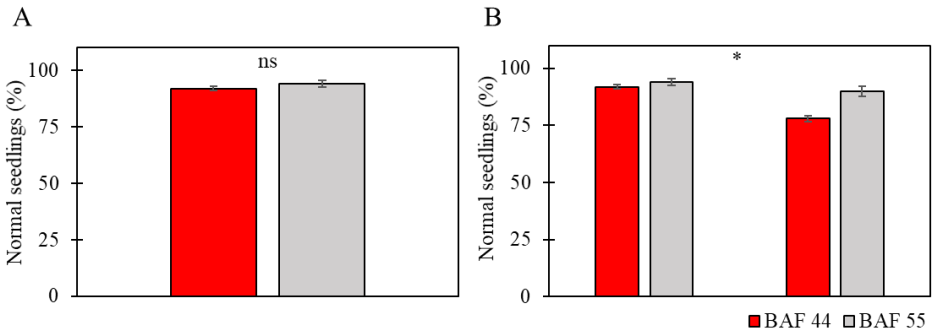

Figure 1. Germination (A) and vigor due to accelerated aging (B) of BAF44 and BAF55 cultivars during the 2021/2022 growing season. ns, *: non-significant, significant by Tukey's test at a 5% probability level, respectively.

The imbibition curve followed a triphasic pattern in both the absence and presence of GA_3 (Figure 2). However, the water uptake rate differed between the cultivars. The time required for radicle protrusion (T50) was higher in cultivar BAF44 than in BAF55, with 32 and 26 hours, respectively, in the control treatment (Figure 2A). Exogenous GA_3 resulted in faster radicle protrusion only in BAF44, reducing the time required to reach T50 to 30 hours (Figure 2C).

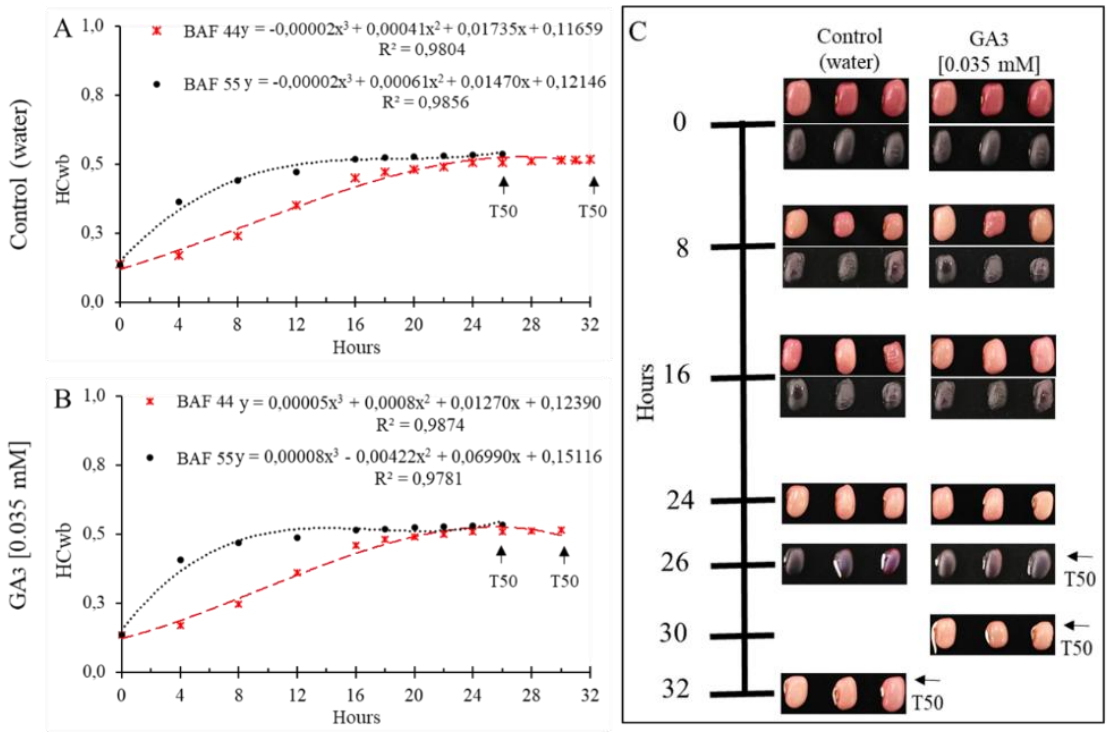

Figure 2. Imbibition curves without (A), and with (B) GA_3 application, and comparison of BAF44 and BAF55 cultivars (C), with values expressed as wet base humidity content (HCwb).

Regarding seedling length (SL), BAF55 had higher root, hypocotyl, and epicotyl values on the seventh day, resulting in a larger SL compared to BAF44 in the control treatment (Figure 3A, C, E, and G). The application of GA_3 promoted greater root length growth only in the low-vigor cultivar BAF44 (Figure 3B), compared to the control treatment (Figure 3A). The epicotyl length was consistently higher in cultivar BAF55 with or without hormone application throughout the germination process (Figure 3E and F).

GA_3 application increased epicotyl growth by 0.80 cm in BAF55 and 0.71 cm in BAF44 (Figure 3F). There were no differences in hypocotyl length between the cultivars after GA_3 application (Figure 3D), but significant elongation was observed in both cultivars compared to the control treatment values. Cultivar BAF44 showed greater seedling length when treated with the hormone (Figure 3H), in contrast to the control treatment (Figure 3G).

RCDM decreased in both cultivars in the absence (Figure 4A) and presence of GA_3 (Figure 4B), with BAF55 having the lowest values in both treatments. The application of GA_3 favored the variables SDDM (Figure 4D), SRU (Figure 4F), and SRUR (Figure 4H) during the seedling development for cultivar BAF44.

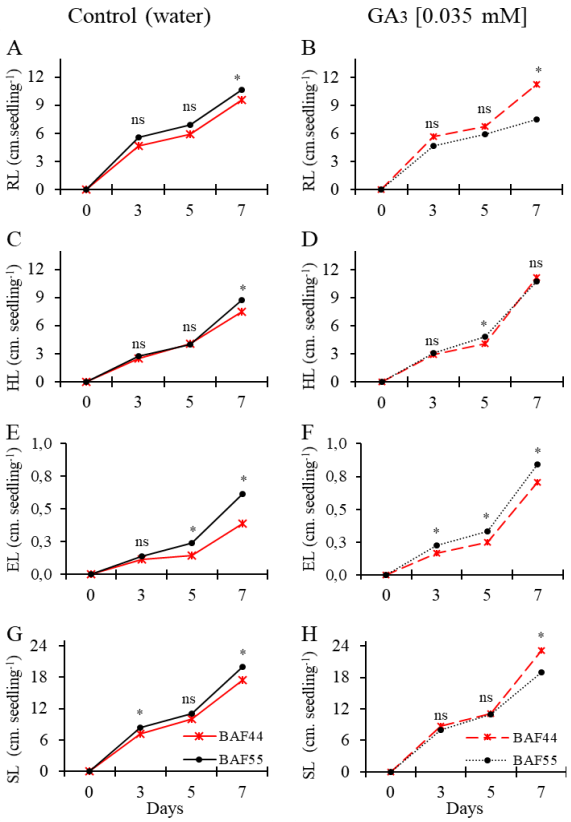

Figure 3. Root length (A and B), hypocotyl length (C and D), epicotyl length (E and F), and seedling length (G and H) in high- and low-vigor cultivars (BAF44 and BAF55) across evaluation days as a function of the GA_3 application. ns, *: non-significant and significant by Tukey's test at a 5% probability level, respectively.

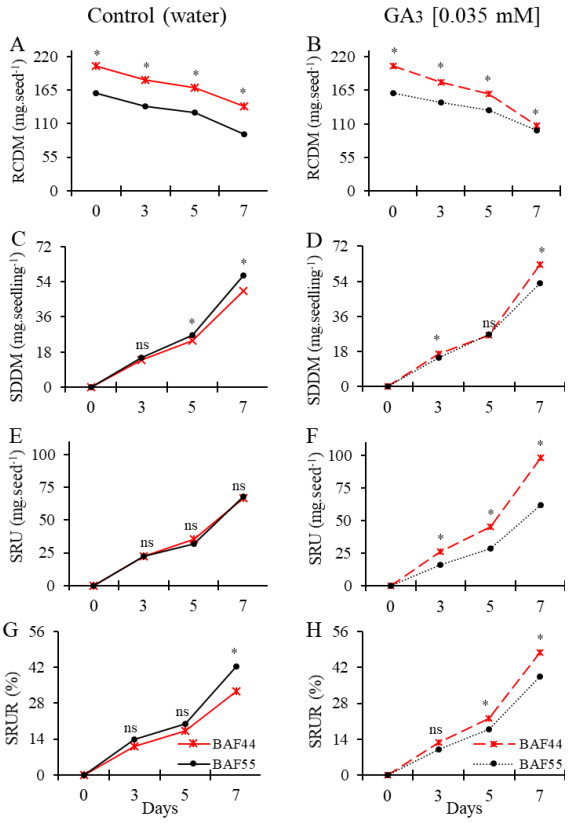

Figure 4. Remaining cotyledon dry mass (A and B), seedling dry mass (C and D), seed reserve utilization (E and F), and seed reserve utilization rate (G and H) of BAF44 and BAF55 cultivars during the seedling development as a function of the GA_3 application. ns, *: non-significant and significant by Tukey's test at a 5% error probability level, respectively.

Starch content decreased during germination, with cultivar BAF55 showing higher levels on day 7 (Figure 5A). However, hormone presence led to a greater starch reduction in BAF44 on days 5 and 7. For total soluble sugars, both cultivars peaked day 1, with cultivar BAF55 again having significantly higher contents (Figure 5C and D).

In the control condition, soluble sugar availability didn't differ between cultivars on days 3, 5, and 7 (Figure 5C). However, the GA_3 application increased sugar utilization for BAF44 on the seventh day (Figure 5D). Alpha-amylase increased during germination under both conditions. In the control, BAF55 showed higher enzyme activity on day 7 (Figure 5E); however, with GA_3 application, BAF44 exhibited higher activity during the same period (Figure 5F).

Protein reduction in the control was higher in cultivar BAF55 (Figure 5G), while GA_3 treatment led to greater protein reduction in BAF44 (Figure 5H).

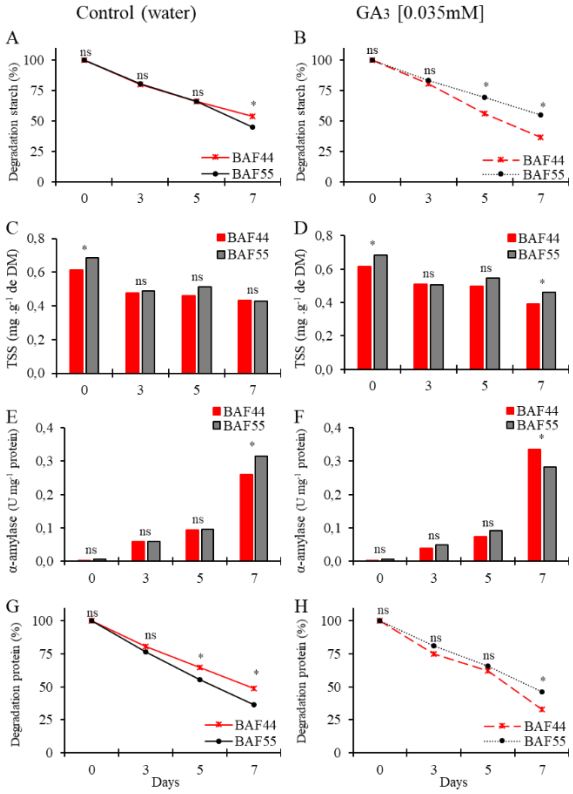

Figure 5. Degradation of starch content (A and B), total soluble sugar contet (C and D), alpha-amylase enzyme activity (E and F), and degradation protein (G and H) in BAF44 and BAF55 cultivars during the seedling development as a function of the GA_3 application. ns, *: non-significant and significant by Tukey's test at a 5% probability level, respectively.

Alpha-amylase gene expression was higher in BAF55 in the control (Figure 6A). With GA_3 application, alpha-amylase gene expression increased in BAF44 and decreased in BAF55 (Figure 6B).

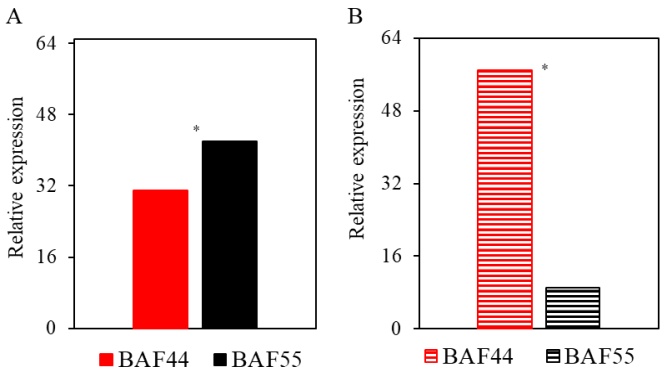

Figure 6. Relative expression of the alpha-amylase gene (GenBank: AB015131.1) for BAF44 and BAF55 cultivars on the seventh day of evaluation during the seedling development without (A) and with (B) GA_3 application. The expression of the alpha-amylase gene was monitored by qPCR. Gene expression was calculated using the $2^{-\Delta\Delta CT}$ method and the average of tubulin and actin mRNA as an endogenous control. *: significant by Tukey's test at a 5% probability level.

Discussion

Accelerated aging (AA) testing effectively measured the vigor of the cultivars, showing a clear contrast between BAF44 and BAF55, characterizing them as low and high vigor, respectively (Figure 1B). According to Marcos-Filho (2020), the AA test is widely used to measure vigor in various species, as it can differentiate lots with similar germination values by exposing seeds to unfavorable conditions that affect their metabolism and alter the percentage of normal seedlings.

The imbibition curve for both cultivars, whether in the absence or presence of GA_3, was triphasic (Figure 2), featuring an initial linear phase followed by stabilization until radicle protrusion, which marks the start of phase III (Bewley et al., 2013). However, the BAF55 cultivar demonstrated a higher water uptake rate (Figure 2A and B), attributed to its higher physiological potential. Seeds with high vigor require larger amounts of water to sustain metabolism (Ehrhardt-Brocardo & Coelho, 2016; Rohr et al., 2023).

Our results show that exogenous GA_3 reduced the time required for radicle sprouting only in the BAF44 cultivar (Figure 2B). Marinho et al. (2021) reported a similar result in sweet corn, where GA_3 application positively affected only the low-vigor batch, increasing the germination rate and physiological performance of the seed. This suggest that GA_3 enhances seed repair mechanisms from the onset of metabolic activities to the conversion of reserves into simpler compounds for germination, thus reducing the time needed to overcome phase II.

Seedling performance variables (Figure 3) confirm that these tests correlate highly with seed vigor (Marcos-Filho, 2015). The high-vigor cultivar produced seedlings with greater length and higher dry matter. The increased root length in BAF44 after GA_3 soaking (Figure 3B) is linked to improved GA signaling throughout seedling development. This can be explained by the interaction with auxin in the shoot apex, which promotes cell elongation in the root system (Fu & Harberd, 2003; Li et al., 2015).

GA_3 application increased hypocotyl (Figure 3D) and epicotyl growth in both cultivars (Figure 3F), suggesting that GA_3 alters plant architecture by expanding the aerial system through cell expansion and division (Carrera-Castaño et al., 2020). Another factor contributing to the elongation of these structures is the effect of expansins induced by GA_3 application (Cosgrove, 2015; Ragni et al., 2011).

Souza et al., (2010) found comparable results in bean cultivars, where GA_3 addition caused hypocotyl and epicotyl elongation by up to 9 cm, directly affecting the first pod. Equivalent results have been reported for rice (Wang et al., 2019), oat (Chauhan et al., 2019), chickpeas (Rafique et al., 2021), and wheat (Ibrahim et al., 2019), where exogenous GA_3 improved seedling vigor by increasing structure length.

However, these studies did not examine the relationship between initial seed vigor and hormone perception. Literature indicates that exogenous GA causes downregulation of GA20ox and GA_3ox genes (Wang et al., 2015), which catalyze the last steps in forming bioactive GAs (GA_1 and GA_4), reducing available GA. This study found that applying exogenous GA_3 to seeds with different vigor levels showed differences in physiological response, indicating unique signal transduction mechanisms after GA treatment.

Parameters related to reserve mobilization components that differed between cultivars during germination were used to explain the metabolic changes in seeds. Cultivar BAF55 showed a greater reduction in MSRC (Figure 4A), which was mobilized to form more vigorous seedlings, as confirmed by the increase in SDDM (Figure 4C) and SRUR (Figure 4G). Padilha et al. (2020), found that more vigorous bean varieties had lower cotyledon mass, longer seedlings, and higher dry matter, aligning with the results observed in BAF55.

The increases in SDDM (Figure 4D), SRU (Figure 4F), and SRUR (Figure 4H) after GA_3 application were observed only in the BAF44 cultivar. This is directly related GA's promotion of cotyledon reserve utilization, resulting in a lower RCDM (Figure 4B) compared to the treatment without GA_3. The increase in dry matter and greater reserve utilization is due to GA's role during germination, initially inducing the synthesis of hydrolytic enzymes like alpha-amylase and later activating the reserve mobilization system, essential for germination (Xiong et al., 2021).

GA_3 application increased sugar utilization in BAF44 on the seventh day (Figure 5D), which correlates with the reduction rate of starch on the fifth and seventh days (Figure 5B). Therefore, GA_3 promoted starch degradation and the subsequent utilization of soluble sugars in low-vigor seeds, resulting in more vigorous seedlings with greater dry matter.

This result, is supported by increased alpha-amylase activity in BAF44 (Figure 5F), which is responsible for starch hydrolysis and coordinates energy distribution to growth points (Sunmonu et al., 2016; Yu et al., 2015). Padilha et al. (2021) associated higher alpha-amylase activity with better initial seedling performance in bean cultivars with contrasting vigor.

In the control treatment, the rate of protein degradation was higher in the high-vigor cultivar (Figure 5G), while hormone application caused greater protein degradation in the low-vigor cultivar (Figure 5H). This difference may be related to the availability of the embryonic axis. Thus, the high-vigor cultivar in the control condition exhibited greater SL (Figure 3G), while GA_3 application resulted in greater seedling length in low-vigor seeds (Figure 3H).

Plants can store exogenously supplied hormones as reversible conjugate, releasing them as active hormones during the growing season when needed (Rafique et al., 2021). Mazid (2014) observed an increase in protein content and higher productivity in chickpea plants treated with GA before sowing.

This was followed by alpha-amylase gene expression evaluation. In the control treatment, alpha-amylase gene expression was higher in the high-vigor cultivar BAF55 (Figure 6A). This correlated with higher alpha-amylase activity (Figure 5E) and greater starch reduction (Figure 5A). Oliveira et al. (2015) found that higher vigor in maize is associated with increased alpha-amylase gene expression, which facilitates carbohydrates availability to the embryo, leading to faster germination and increased vigor.

This result is consistent with the higher enzymatic activity of alpha-amylase on the seventh day (Figure 5F) when GA_3 was applied, supporting that the reduction in RCDM (Figure 4B) is due to greater starch reduction (Figure 5B) and the use of available total soluble sugars in cotyledons (Figure 5D).

According to Wang et al. (2015), GA_3 treatment can increase GA accumulation, leading to various effects on plant growth and development due to complex interactions between hormones. Moreover, alpha-amylase gene expression is regulated in a tissue-specific manner, depending on the energy requirements from the embryo, and influenced by increased GA concentration, higher ABA concentration, and abiotic stress (Yu et al., 2015). Therefore, regulating alpha-amylase genes becomes a good model studying signaling mechanisms for reserve utilization and the interplay between GA and other hormones during germination.

Studies have established a link between hormone levels and alpha-amylase expression activity, particularly in cereals. Skadsen (1998) observed that in barley grains, GA induced mRNA transcription of alpha-amylase, but increased abscisic acid repressed the gene. Damaris et al. (2019) reported that GA treatment increases alpha-amylase production at the gene transcription level, with microarray studies showing upregulation of genes encoding many isoforms of alpha-amylase, as well as proteases and hydrolases.

Exogenous GA_3 directly benefited the low-vigor BAF44 cultivar qRT-PCR confirmed increased alpha-amylase gene expression, which aligns with improved physiological performance, reserve mobilization, and better utilization of the bean's main reserve component.

These results indicate that hormonal regulation of plant growth is highly complex, with various genes beyond alpha-amylases involved in controlling seedling vigor when treated with exogenous GA_3. For instance, genes related to starch hydrolysis, such as phosphorylases (Li et al., 2017), and those responsible for protein degradation, such as proteases and peptidases (Diaz-Mendonza et al., 2019) play significant roles. Additionally, monitoring potential regulators of gibberellin metabolism, such as the key genes GID1 and DELLA, can help correlate changes in these genes with physiological quality. Initial quantification of GA and MYB gene expression can also assess metabolic changes (Tan et al., 2019). Therefore, further studies should include a larger number of genes and gene families.

Conclusion

The conclusions of this study were that the initial vigor of bean seeds is crucial for GA effectiveness in promoting reserve mobilization mechanisms. In the cultivar BAF44 (low-vigor) GA_3 application is positively associated with higher alpha-amylase gene expression, increasing enzyme activity responsible for starch hydrolysis and leading to the formation of vigorous seedlings. In contrast, for cultivar BAF55 (high-vigor), GA_3 use was detrimental due to lower gene expression and alpha-amylase activity, negatively effecting on starch degradation and the availability of total soluble sugars for radicle development and seedling dry matter.

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