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Vigor and reserve mobilization of common bean seed during germination under salt stress conditions

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ABSTRACT: The mobilization of reserves determines the formation of seedlings during germination, being influenced by seed vigor and adverse stress conditions. Seeds with higher vigor have greater potential for mobilization of reserves, and it can favor performance under conditions of salt stress. The objective of this study was to identify how the mobilization of reserves stored in common bean seeds contributes to the overcoming of salt stress, seeking to identify the difference in hydrolysis and mobilization of reserves among seeds with superior vigor. Seeds from two genotypes contrasting in vigor (BAF44 and BAF55) were used. Salt stress was simulated using sodium chloride, and evaluations were performed at one, three, five and seven days of germination. The variables analyzed in seedling was length, dry mass, and metabolic expenditure, while biochemical analyses of total starch, total protein, total soluble sugars, total soluble proteins, and alpha-amylase activity were evaluated in the embryonic axis and/or cotyledons. The salt stress condition negatively affected the performance of the seedlings, resulting in a decrease in length, mass accumulation, and reduced use of seed reserves, associated with a lower hydrolysis of starch and proteins. Seeds with higher vigor showed higher dry mass and seedling length even when subjected to salt stress conditions, because of greater hydrolysis capacity and use of soluble proteins and soluble sugars, which result in seedlings with better performance under control and salt stress conditions.

Key words: *Phaseolus vulgaris* L., physiological quality, globulins, alpha-amylase, soluble sugars.

INTRODUCTION

Grain production occurs all over the world. For this reason, there are several production conditions to which agricultural crops are subjected. In this sense, the presence of abiotic stresses are the main limiting factors in the production of a crop, resulting in a large decrease in productivity (Sindhu et al. 2020). Among the abiotic stresses, cultivation in saline soil presents a great challenge for producers, considering that its effect can occur in all stages of plant growth, resulting in a negative effect on germination, seedling formation, growth and development, culminating in the loss of productivity (Yadav et al. 2019). In this context, saline soil usually occurs due to the accumulation of natural sodium chloride or due to irrigation (Nadeem et al. 2019).

Common beans (*Phaseolus vulgaris* L.) are produced and consumed mainly in developing countries in Latin America, Africa, and Asia, and are one of the most important legumes in the world due to their use in human food as a source of starch, protein, fiber, and minerals (Los et al. 2018, Mukankusi et al. 2019). Bean cultivation is carried out all over the world, and its main producing regions are located in arid and semi-arid regions, susceptible to abiotic stresses (e.g., salinity and water restriction) or natural salinization of soils (Farooq et al. 2017, Nadeem et al. 2019).

With the negative effects resulting from salt stress, the loss of seedling emergence can be partially avoided by using seeds of higher physiological quality (germination and vigor) (Padilha et al. 2022a). In addition, the use of seeds with greater vigor favors the formation of seedlings with better performance (Marcos-Filho 2015).

In order to resume the seed metabolism and the germination process to be established, the reserves stored in the cotyledons must be hydrolyzed and mobilized to the embryonic axis, in order to be used for the formation of new tissues (Nonogaki 2008). Greater hydrolysis and mobilization of reserves can allow a better performance of seedlings under stress conditions, favoring their overcoming (Verma et al. 2015). The main reserves stored in bean seeds are starch and protein, making up more than 70% of their mass (Los et al. 2018). The higher hydrolysis of starch favors the availability of soluble sugars for use during germination (Wang et al. 2016). Similarly, protein catabolism favors the availability of soluble proteins and amino acids, which can be used during germination (Nonogaki 2008, Guzmán-Ortiz et al. 2019).

Seed reserve mobilization is an important parameter in the assessment of abiotic stress, since seeds with superior performance (germination, seedling length, and dry matter accumulation) under stress conditions have higher reserve utilization during these conditions (Wang et al. 2016, Padilha et al. 2022b). In this sense, the greater hydrolysis and mobilization of these stored components can favor the best performance in a condition of salt stress, and this mobilization is superior in seeds of greater vigor. Thus, the objective of this study was to evaluate the mobilization of reserves in bean seeds contrasting in physiological quality, seeking to determine the importance of this process during the formation of vigorous seedlings under conditions of salt stress.

MATERIAL AND METHODS

Plant material and growth conditions

The genotypes used were BAF44 and BAF55, originating from the Bean Active Germplasm Bank (BAF) of the Universidade do Estado de Santa Catarina (UDESC). The seeds were produced in the UDESC-CAV experimental area in the municipality of Lages, Santa Catarina, Brazil, in the 2020/2021 harvest, and, after harvest, the seeds were dried and kept in a dry chamber (10 ± 2°C and 50 ± 5% relative humidity) until the moment the analyses were carried out. The genotypes used were selected from previous works, considering the genotypes that contrasted in their seed production potential with statistical differences for physiological quality (Zilio et al. 2013, Gindri et al. 2017).

Evaluation of the physiological quality

For the physiological characterization of seed lots, a completely randomized experimental design was used, in a 2×2 factorial scheme, consisting of two bean genotypes (BAF44 and BAF55) and saline stress conditions (NaCl 0 e 100 mmol·L^{-1}), in six repetitions.

The weight of 1,000 seeds (TSW, g) was determined according to the procedure described in the rules for seed analysis (Brasil 2009), using six replications.

The germination percentage (G, %) was determined by the test conducted in a *Mangelsdorf* germinator at the temperature of 25 ± 2°C, and the sowing was carried out on paper substrate using three sheets arranged in the form of a roll, with the first germination count (FGC, %) performed on the fifth day and the final count on the ninth day (Brasil 2009). Distilled water (control) and saline solution (salt stress) containing 100 mmol·L^{-1} of sodium chloride (NaCl) in the proportion of 2.5 mL of distilled water or NaCl solution per gram of dry paper were used to moisten the paper.

Seedling performance was carried out using 15 seeds distributed in the upper third of paper sheets (Nakagawa 1999). The paper sheet was moistened with distilled water or saline solution in the proportion of 2.5 mL·g^{-1} of dry paper. At the fifth day, 10 normal seedlings were measured with the aid of a digital caliper for their total seedling length (TSL, cm), and, subsequently, seedling and cotyledons were separated for drying in an oven at 80°C for 24 h to obtain seedling dry mass (SLDM, mg) and remaining dry mass in the cotyledons (RDMC). The seed reserves reduction (SRR, mg) was achieved by the difference between the initial seed dry mass (SDM) and the RDMC (Soltani et al. 2006).

Field emergence (FE, %) was performed in the experimental field of UDESC/CAV in the city of Lages (27°78'S, 50°30'W, and 930 m altitude). According to the Köppen's classification, the climate of the region is Cfb, humid mesothermal, constantly humid with mild summer, and the soil is classified as Humic aluminic clayey Cambisol, moderate horizon A. Sowing occurred in November, in a completely randomized experimental design, with six replicates. In each replicate, 50 seeds were sown at the depth of 5 cm. Emergence counting was performed on the 15th day after sowing.

Biochemical evaluations

To evaluate the biochemical variables, a completely randomized experimental design was used, in a 2×5×2 triple factorial scheme, consisting of two bean genotypes (BAF44 and BAF55), five evaluation times during germination (0–dry seed, one, three, five and seven days) and two saline stress conditions (NaCl 0 e 100 mmol·L^{-1}), with the evaluations determined in three biological replicates at each evaluation period. The biochemical components evaluated during seedling formation were total starch (mg·g^{-1}), total protein (mg·g^{-1}), total soluble sugars (mg·g^{-1}), total soluble proteins (mg·g^{-1}), and alpha-amylase activity (U·mg^{-1}), which were carried out in cotyledons and/or embryonic axis.

Starch evaluation was performed as described by McCready et al. (1950), and double extraction with 52% perchloric acid was used. Quantification was performed on the cotyledon with 1 mL of properly diluted extract and 3 mL of antrone reagent, followed by vortex stirring for 3 seconds. The test tubes were kept in a water bath at 95ºC for 450 seconds. The reading was performed in a spectrophotometer at the absorbance of 630 nm, and the results were expressed in mg·g^{-1} of dry mass.

The total protein was determined by the Kjeldahl method (AOAC 1995) from the total nitrogen content of the sample, using 200 mg of cotyledon sample. For each sample, 1 mL of 30% hydrogen peroxide, 2 mL of concentrated sulfuric acid and 0.7 g of catalyst (containing 90% sodium sulfate, 9% copper sulfate, and 1% selenium) were added, and digestion was performed in a digester block. The total protein content was determined by titration, and the nitrogen obtained from the sample was multiplied by 6.25. The results were expressed in mg·g^{-1} of dry mass.

From the data obtained from starch and total protein, the rate of content of these components was determined. Thus, the remaining value was measured based on the values obtained in each evaluation period (one, three, five, and seven days) in relation to the initial one, and expressed as a percentage. The starch and protein content rate were determined from the results obtained initially (I), and the remaining value of each component at the germination time evaluated (F), being calculated by the Eq. 1:

$$\text{Content rate} = \left(\frac{\text{I (mg·seed}^{-1}) - \text{F (mg·seed}^{-1})}{\text{I (mg·seed}^{-1})}\right) \times 100 \tag{1}$$

The extraction and quantification of total soluble sugars (TSS) was performed on embryonic axis and cotyledons as proposed by Clegg (1956). One hundred mg of dry and ground sample in mortar was used, and double extraction with 80% ethyl alcohol was performed. The quantification reaction was performed using 1 mL of diluted extract and 5 mL of antrone reagent, followed by vortex stirring for 3 seconds. The test tubes were kept in a water bath at 95°C for 480 seconds, after which the reading was performed in a spectrophotometer at the absorbance of 630 nm. The results were expressed in mg·g^{-1} of dry mass.

For the extraction of total soluble proteins (TSP), samples of 100 mg macerated in mortar and 2 mL of potassium phosphate buffer pH 7.0 were used and then centrifuged. The TSP was quantified in a spectrophotometer at the wavelength of 595 nm, as described by Bradford (1976), and the results were expressed in mg·g^{-1} of dry mass.

The activity of alpha-amylase was evaluated by the dinitrosalicylic acid method (Miller 1959) using the procedure of Monerri and Guardiola (1986) with adaptations. To obtain the enzyme extract, 5 mL of sodium acetate buffer pH 5.6 was added to 500 mg of sample, together with 4 mmol of CaCl2 and 0.005% of Triton X^{-100}. The samples were kept in agitation for 1 h on ice and then centrifuged. The extract was subjected to the temperature of 70°C for 15 min. Quantification was performed using 0.5 mL of the enzyme extract and 0.5 mL of soluble starch solution containing 2.0% (w/v). The samples were kept in a water bath for 30 min at 38ºC. Afterwards, 1 mL of 3,5-Dinitro-2-hydroxybenzoic acid (DNS) solution was added, and the samples were kept in a water bath for 6 min at 95°C. After cooling, 8 mL of distilled water was added. The reading was performed in a spectrophotometer at the absorbance of 540 nm. One unit of enzyme was considered as the amount of enzyme required for the production of 1 μmol of maltose per minute in the evaluated condition. The results were expressed in enzyme units per milligram of protein (U·mg^{-1}).

The electrophoresis of the proteins obtained during germination was performed in a vertical system at the concentrations of 12% (resolving gel) and 4% (packing gel), containing sodium dodecyl sulfate (SDS). Electrophoresis was performed with soluble protein extract from cotyledons, according to Laemmli's (1970) methodology. After quantification, 15 µg of sample was added to the buffer (Tris-HCL pH 6.7; Glycerol; 10% SDS; 0.5% bromophenol blue; and 2-mercaptoethanol in a 1:1 ratio). The electrophoresis system containing the samples was packed in a pH 8.3 buffer solution (25 mM Tris, 192 mM Glycine and 1% SDS) and subjected to 140V at room temperature (20°C) for two hours. After electrophoresis, the gel was washed with distilled water and kept for three hours in Coomassie Brilliant Blue R-250 dye solution. Electrophoresis was performed twice (two repetitions) under similar ambient conditions in order to verify reproducibility.

Statistical analysis

The collected data were subjected to analysis of variance with the Sisvar software. When significant by the F test ($p < 0.05$), the comparison between means and interactions between factors were compared using the Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

Germination (G) did not show a significant difference between the seed lots of the genotypes, but the differences in vigor were observed for the variables FGC, FE, germination under salt stress (GNaCl), TSL, and SLDM, in which the seed lot of the BAF55 genotype showed the best performance, indicating greater vigor (Table 1), and the association of these variables with the vigor of the seed lot was highlighted by Finch-Savage and Bassel (2016).

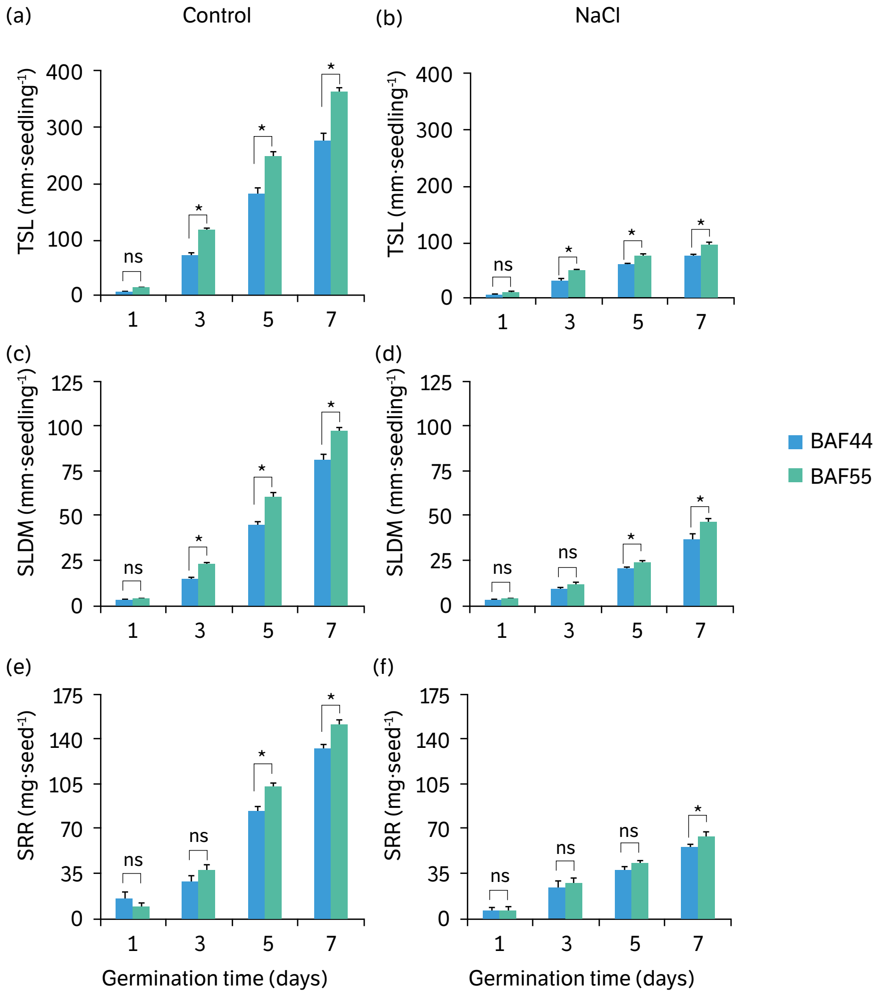
Table 1. Mean and *p*-value for the variables analyzed 1,000-seed weight (TSW), germination (G), first germination count (FGC), field emergence (FE), total seedling length (TSL), seedling dry mass (SLDM), starch (mg·g^{-1} dry mass), protein (mg·g^{-1} dry mass), and saline germination at 100 mmol·L^{-1} (GNaCl).

Attribute	BAF 55	BAF 44	F-calc	P-value
TSW (g)	223.88	271.45	370.302	0.0000
G (%)	97.00	95.00	4.623	0.0571
G (NaCl) (%)	94.00	88.00	23.442	0.0007
FGC (%)	96.00	81.00	19.220	0.0014
FE (%)	91.00	86.00	12.712	0.0051
TSL (cm)*	245.72	182.67	61.549	0.0000
SLDM (mg)*	58.87	45.55	41.250	0.0001
Starch (mg.g^{-1})	429.32	450.07	1.097	0.3197
Protein (mg.g^{-1})	238.17	250.74	2.127	0.1754

*Assessed at five days after sowing.

The potential for seed production with higher physiological quality of the BAF55 genotype compared to the BAF44 genotype is an already established trait that has been verified under different production conditions (Ehrhardt-Brocardo and Coelho 2016, Gindri et al. 2017). This behavior was also verified in the present study, using seeds produced in the same environment in the 2020/2021 harvest. Thus, the BAF55 genotype seed lot has higher vigor in contrast to the BAF44 lot with lower vigor. Regarding the biochemical composition of the seeds, there was no significant difference between the genotypes for the starch and total protein components (Table 1).

The salt stress condition negatively affected the performance of the seedlings, resulting in less growth in seedling length, accumulation of dry mass and reduction of seed reserves. However, the response of seed vigor on the mobilization of reserves during germination was verified, in which the best seedling performance was observed from the third day of germination for seeds of genotype BAF55, generating in both germination conditions (i.e., absence and presence of salt stress) seedlings with greater length (Figs. 1a and 1b) and dry matter (Figs. 1c and 1d).



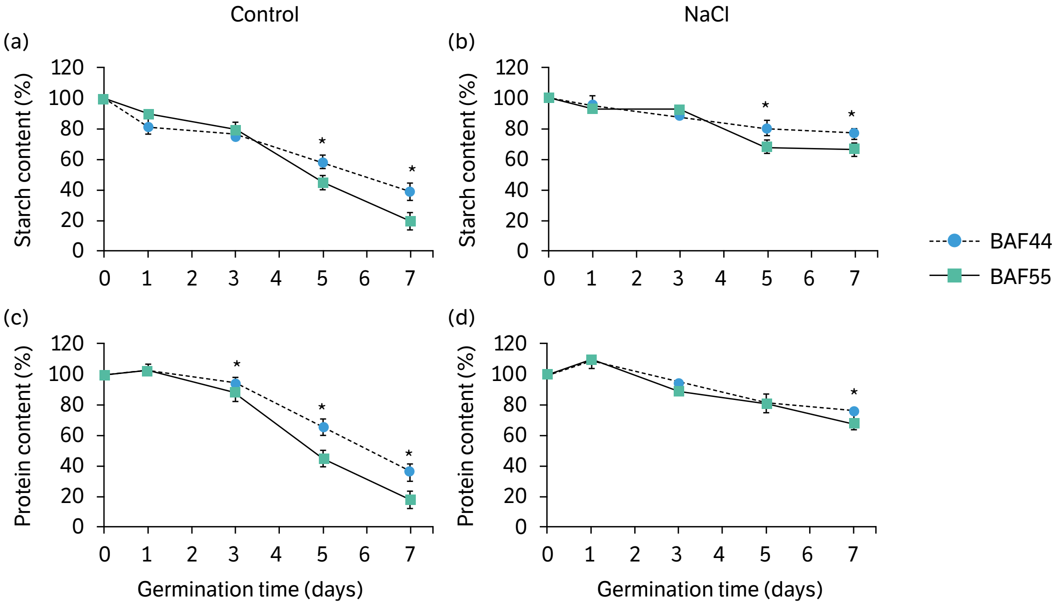
ns: non-significant difference between lots within the evaluation time by Tukey's test at 5% probability; *significant difference between lots within the evaluation time by Tukey's test at 5% probability.

Figure 1. Total seedling length (TSL), seedling dry mass (SLDM), and seed reserve reduction (SRR) evaluated in the periods of one, three, five and seven days during germination under conditions of absence of stress (control) and presence of salt stress (NaCl) for the lots of genotypes BAF44 and BAF55.

Considering the SRR, the seeds with higher vigor showed greater utilization of stored reserves, a difference observed on the fifth day of germination in the absence of stress and only on the seventh day in salt stress (Figs. 1e and 1f). Similarly, Padilha et al. (2021) found that seeds with higher vigor have a greater capacity to mobilize reserves under conditions of salt stress, favoring the formation of better performing seedlings (TSL and SLDM).

The highest SRR is the result of the degradation of the reserves stored in the seed and that during germination were hydrolyzed and mobilized to the growth points. This relationship can be seen by the reduction of starch and total proteins content during seed germination. In starch, the reduction at five and seven days was significantly higher in BAF55 in both germination conditions, demonstrating the highest hydrolysis in seeds with higher vigor (Figs. 2a and 2b). Similarly, the hydrolysis of total proteins in seeds with higher vigor was superior in the control condition, observed from the third day of germination (Fig. 2c). In salt stress, this difference was observed on the seventh day of germination, results that corroborate the RSS (Figs. 1e and 1f).

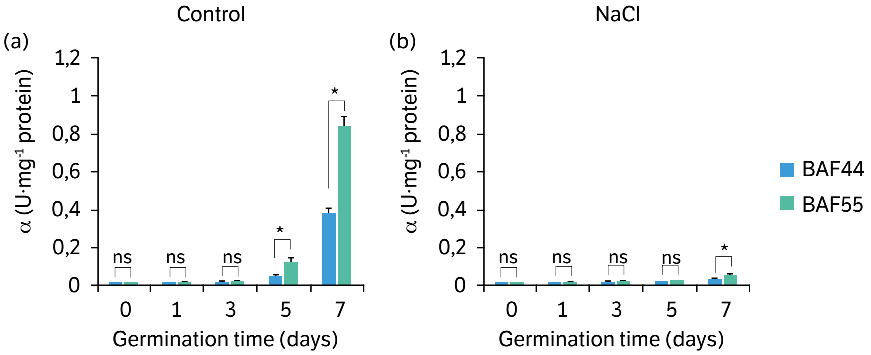
Seeking to associate greater starch degradation with seedling formation and performance, Wang et al. (2016) worked with rice, Shaik et al. (2014) with barley, and Zeid et al. (2019) with cowpea. This association was also observed for bean seeds in this study, in which seeds with higher vigor showed greater potential for starch degradation, favoring the formation of seedlings with better performance in both germination conditions (Fig. 1). According to Wang et al. (2016), the higher starch degradation capacity is important for survival and rapid growth in a wide range of environmental conditions. Thus, the higher utilization of reserves allowed the best performance in salt stress, considering that the seeds with higher vigor had a higher capacity for starch hydrolysis (Figs. 2a and 2b) and better seedling performance in salt stress (Fig. 1).



*significant difference between batches within the evaluation time by Tukey's test at 5% probability.

Figure 2. Content of (a and b) starch and (c and d) protein evaluated during germination under conditions of absence of stress (control) and presence of salt stress (NaCl) for batches of genotypes BAF44 and BAF55.

In the evaluation of the activity of the enzyme alpha-amylase, an increase in the course of germination was observed in both conditions. The results showed that, at five and seven days, seeds with higher vigor showed higher enzyme activity in the absence of stress, indicating greater potential to hydrolyze starch (Fig. 3a). However, when considering the results obtained during salt stress, a significant difference was observed only on the seventh day of germination, and the activity was significantly compromised in relation to the control condition (Fig. 3b).



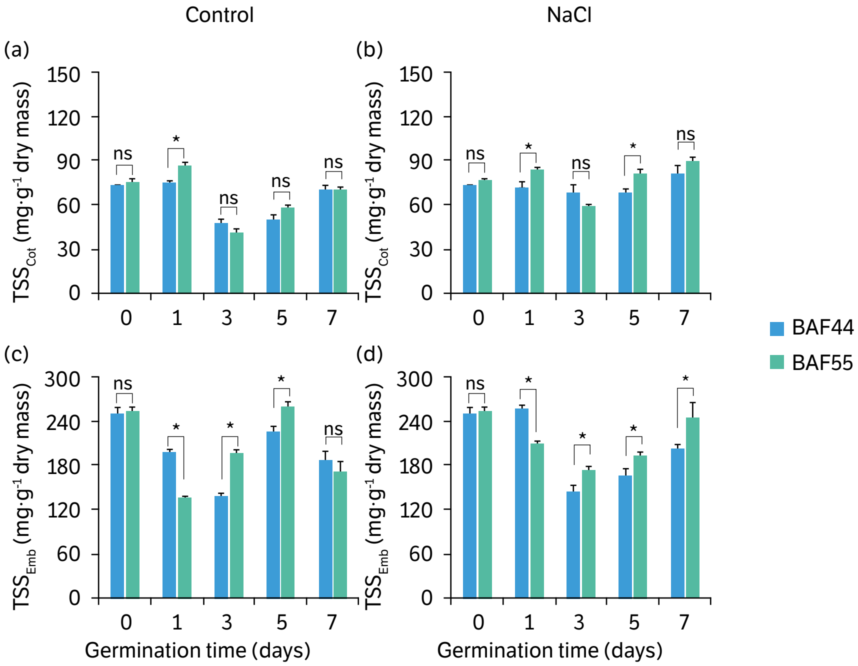
ns: non-significant difference between lots within the evaluation time by Tukey's test at 5% probability; *significant difference between lots within the evaluation time by Tukey's test at 5% probability.

Figure 3. Alpha-amylase (α) activity evaluated during germination under conditions of (a) absence of stress (control) and (b) presence of salt stress (NaCl) for batches of genotypes BAF44 and BAF55.

Among the enzymes responsible for starch degradation, alpha-amylase activity is one of the main ones (Bewley et al. 2013). During germination, alpha-amylase contributes to starch hydrolysis and the formation of seedlings with greater length and dry mass (Wang et al. 2016). Thus, the higher alpha-amylase activity observed for the seeds with higher vigor in the absence of stress contributed to the higher starch hydrolysis.

Considering salt stress, alpha-amylase activity is lower in relation to the condition of absence of stress (Liu et al. 2018, Padilha et al. 2021). This reduction in enzymatic activity resulted in lower starch hydrolysis (Fig. 2b) and lower seedling performance (Figs. 1b and 1d) in both seed lots used. Although alpha-amylase activity did not show significant difference between lots at five days, seeds with higher vigor showed higher performance (Figs. 1b and 1d). This had already been observed by Padilha et al. (2021) in bean seeds with contrast in the initial vigor under conditions of salt stress. The seeds showed similar alpha-amylase activity, and the authors indicated that other enzymes associated with starch hydrolysis are important in this condition until the fifth day of germination.

In relation to TSS, it is possible to identify similarities in the behavior of this variable in the two germination conditions, and this response depends on the structure evaluated (cotyledons or embryonic axis). In both germination conditions, the seeds with higher vigor showed higher availability of TSS in cotyledons on the first day of germination, however, at three, five and seven days no difference was observed in the availability of TSS between lots in the control condition (Fig. 4a). On the third day, there was a decrease in TSS in the control and under stress conditions, followed by an increase on the fifth and on the seventh day (Figs. 4a and 4b).



ns: non-significant difference between lots within the evaluation time by Tukey's test at 5% probability; *significant difference between lots within the evaluation time by Tukey's test at 5% probability.

Figure 4. Total soluble sugars (TSS) evaluated in (a and b) cotyledons and (c and d) embryonic axis during germination under conditions of absence of stress (control) and presence of salt stress (NaCl) for batches of genotypes BAF44 and BAF55.

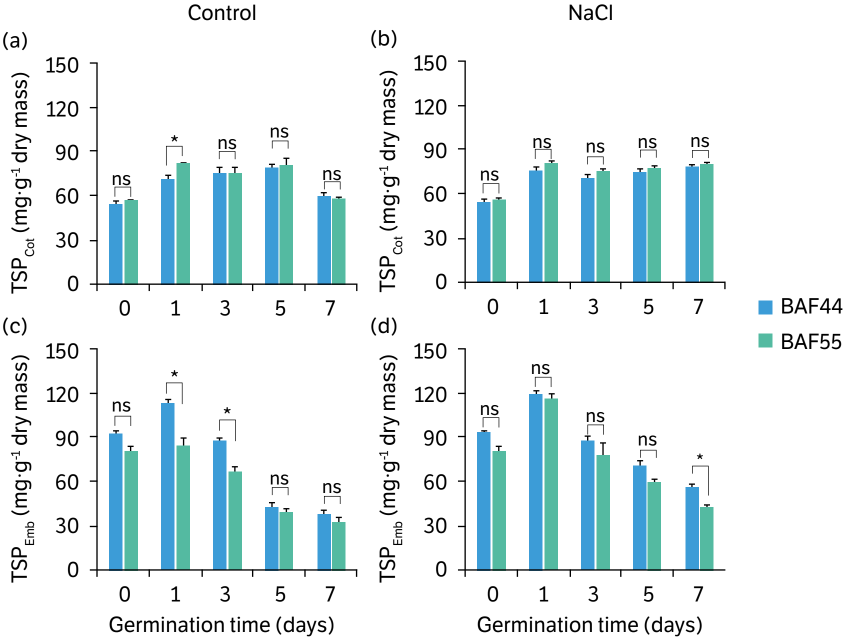
The availability of organic substances is important for the formation of seedlings during germination, being mobilized to the growing embryonic axis (Bewley et al. 2013). Thus, the results obtained showed respectively the hydrolysis and mobilization of TSS for the axis, in which there was an increase on the first day followed by a decrease on the third day. As already reported by Ehrhardt-Brocardo and Coelho (2016), phase II of germination, which comprises the active transport of previously unfolded substances from the reserve tissue to the meristematic growth points, varied in bean cultivars from 21 to 27 hours after hydration, similar with increased TSS hydrolysis and then mobilization to growth points.

The increase at five and seven days may be associated with increased amylase activity (Fig. 3) in starch hydrolysis (Figs. 2a and 2b). Considering the increased availability of soluble sugars in the embryonic axis, Zeid et al. (2019), evaluating germination in cowpea seeds, identified that seeds that present greater physiological performance have greater amylolytic activity (i.e., alpha and beta-amylase) and provide a greater quantity of soluble sugars for the embryonic axis.

Considering the TSS evaluated in embryonic axis, it is possible to observe that the seeds with higher vigor presented lower values on the first day of germination and later (3.5 days) presented a higher amount of TSS available in both germination conditions (presence and absence of salt stress) (Figs. 4c and 4d). These results demonstrate that the dynamics of soluble sugar mobilization is dependent on their hydrolysis and utilization. The reduction of TSS observed at a specific time in relation to the previous one is due to the use of starch, the subsequent increase is dependent on starch hydrolysis, since the enzymatic activity increases during germination (Fig. 3).

The hydrolysis of starch and the availability of TSS as a carbon source for mass accumulation are decisive during seedling formation (Guzmán-Ortiz et al. 2019, Zeid et al. 2019). Thus, seeds with higher vigor showed rapid use of TSS, which favored higher TSL (Figs. 1a and 1b) and SLDM (Figs. 1c and 1d). The higher hydrolysis of starch ensured a greater availability of TSS in the embryonic axis for use from the third day of germination (Figs. 4c and 4d), resulting in the highest performance throughout the remainder of the germination period evaluated (Fig. 1). Thus, the degradation of starch and its use by the embryonic axis is fundamental for the formation of seedlings under conditions of salt stress.

Total protein decreased during germination, indicating hydrolysis of stored proteins and having less reduction during salt stress (Figs. 2b and 2d). In the work of Shaik et al. (2014) with barley seeds, it was observed that during germination there was reduction in the total proteins of the seed reserve tissues, indicating their hydrolysis and use during this process. On the other hand, the TSP in cotyledons did not show significant differences between the lots, but there was an increase in soluble proteins on the first day of germination in the control condition (Figs. 5a and 5b).



ns: non-significant difference between lots within the evaluation time by Tukey's test at 5% probability; *significant difference between lots within the evaluation time by Tukey's test at 5% probability.

Figure 5. Total soluble proteins (TSP) evaluated in (a and b) cotyledons and (c and d) embryonic axis during germination under conditions of absence of stress (control) and presence of salt stress (NaCl) for batches of genotypes BAF44 and BAF55.

For TSP evaluated in embryonic axis, in both germination conditions and for both seed lots, an increase in the first day of germination and a subsequent decrease in soluble proteins were observed in subsequent times, indicating that the proteins were used by the embryonic axis (Figs. 5c and 5d).

The qualitative evaluation of soluble proteins evaluated in cotyledons can be verified by the electrophoresis gel, which demonstrates the gradual reduction of soluble proteins present in the bands between 40 and 54 kDa for both batches evaluated and under both germination conditions. The seed lot with higher vigor showed lower intensity of the 40 to 54 kDa bands from the third day of germination in the control condition (Fig. 6a) and only from the fifth day after salt stress, indicating a delay in the use of soluble proteins during the stress condition (Fig. 6b).

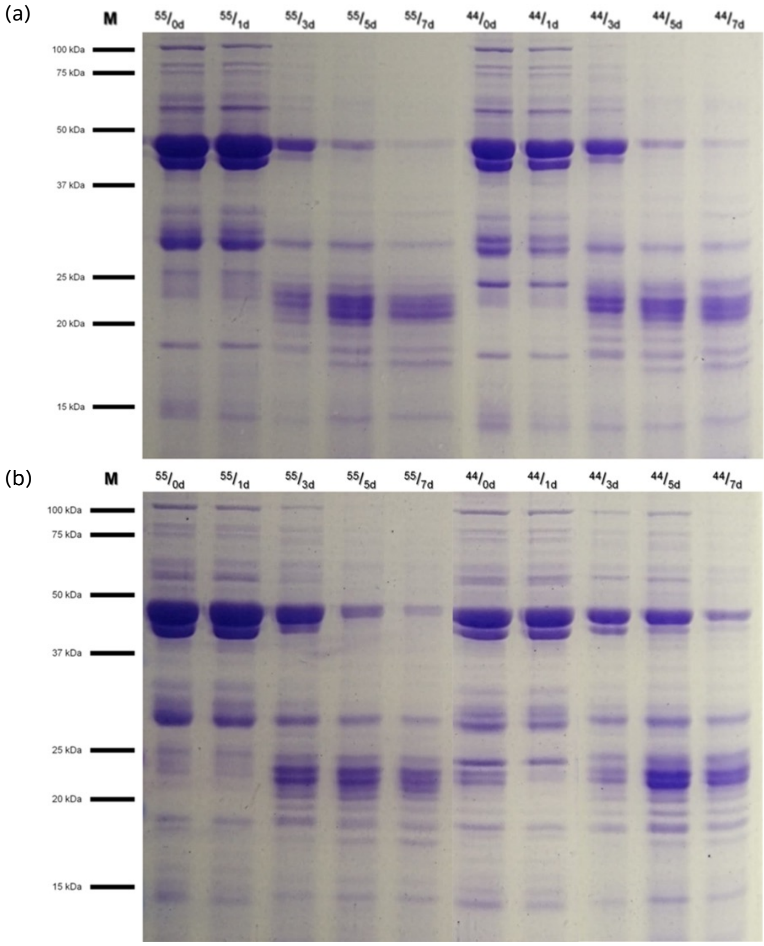


Figure 6. Polyacrylamide gel electrophoresis (SDS-PAGE) of cotyledons during germination under conditions of (a) absence of stress and (b) presence of salt stress for batches of genotypes BAF44 and BAF55 at germination times of zero (0d), one (1d), three (3d), five (5d) and seven days (7d).

The main reserve proteins in *Phaseolus vulgaris* L. are globulins, which can be observed by means of the polyacrylamide gel electrophoresis technique (SDS-PAGE) (Brown et al. 1981) with greater emphasis on bands with molecular weight between 41.1 and 54.7 kDa (Montoya et al. 2008). In Karmous et al. (2011), a decrease in the intensity of globulin bands was observed during the germination process of common bean seeds and an increase in seedling dry mass associated with this reduction. Thus, the reduction in the intensity of the cotyledon-soluble protein bands, observed in the high and low vigor lots, is a result of the hydrolysis and mobilization of these proteins during germination.

The lower intensity of bands observed in low vigor seeds indicates the greater action of proteases in protein hydrolysis. In cowpea, Zeid et al. (2019) found that higher protease activity favored germination and seedling formation. Under conditions of salt stress, Punia et al. (2020) observed that tolerant sorghum varieties had a higher quantity of proteases, which culminated in a greater reduction in proteins. Proteases are determinant enzymes during the hydrolysis of reserve proteins; they increase during germination for the availability of soluble proteins (Guzmán-Ortiz et al. 2019). This higher protein availability can be observed in the increase of soluble proteins in the cotyledons (Figs. 5a and 5b), which may have contributed to a better performance, considering the lower intensity of bands from the fifth day of germination in both germination conditions (Fig. 6).

The seeds with higher vigor showed greater capacity for hydrolysis and mobilization of the stored reserves during the germination process. This catabolic activity of the reserve components associated with higher protein synthesis resulted in the formation of higher performance seedlings and. Except for the delay in the process, these results occurred in a similar way in the presence or absence of salt stress. Thus, the mobilization process as a whole favored the overcoming of salt stress, which is one of the main reasons why the seeds with higher vigor showed better performance under these conditions.

CONCLUSION

Seed lots with higher vigor have higher hydrolytic capacity of the reserve components and demonstrate rapid utilization of TSS and TSP, resulting in seedlings of higher vigor even under conditions of salt stress.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Padilha, M. S. and Coelho C. M. M. **Formal analysis:** Padilha, M. S., Coelho, C. M. M., Siega, Y. P. and Ehrhardt-Brocardo, N. C. M. **Investigation:** Padilha, M. S., Siega, Y. P. and Ehrhardt-Brocardo, N. C. M. **Methodology:** Padilha, M. S., Coelho, C. M. M., Siega, Y. P. and Ehrhardt-Brocardo, N. C. M. **Writing – original article:** Padilha, M. S. **Writing – review & editing:** Padilha, M. S., Coelho, C. M. M., Siega, Y. P. and Ehrhardt-Brocardo, N. C. M.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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REFERENCES

[AOAC] Association of Official Analytical Chemists (1995). Vitamins and other nutrients. In AOAC (Ed.). Official methods of analysis (p. 58-61). Arlington: AOAC.

Bewley, J. D., Braford, K. J., Hilhorst, H. W. M. and Nonogaki, H. (2013). Seeds: Physiology of development, germination and dormancy (3. ed). New York: Springer.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Brasil. Ministério da Agricultura, Pecuária e Abastecimento (2009). Secretaria de Defesa Agropecuária. Regras para análise de sementes. Diário Oficial da União.

Brown, J. W. S., Ma, Y., Bliss, F. A. and Hall, T. C. (1981). Genetic variation in the subunits of globulin-1 storage protein of French bean. Theoretical and Applied Genetics, 59, 83-88. https://doi.org/10.1007/BF00285895

Clegg, K. M. (1956). The application of the anthrone reagent to the estimation of starch in cereals. Journal of the Science of Food and Agricultural, 7, 40-44. https://doi.org/10.1002/jsfa.2740070108

Ehrhardt-Brocardo, N. C. M. and Coelho, C. M. M (2016). Hydration patterns and physiologic quality of common bean seeds. Semina: Ciências Agrárias, 37, 1791-1800. https://doi.org/10.5433/1679-0359.2016v37n4p1791

Farooq, M., Gogoi, N., Hussain, M., Barthakur, S., Paul, S., Bharadwaj, N. and Siddique, K. H. (2017). Effects, tolerance mechanisms and management of salt stress in grain legumes. Plant Physiology and Biochemistry, 118, 199-217. https://doi.org/10.1016/j.plaphy.2017.06.020

Finch-Savage, W. E. and Bassel, G. W. (2016). Seed vigour and crop establishment: extending performance beyond adaptation. Journal of Experimental Botany, 67, 567-591. https://doi.org/10.1093/jxb/erv490

Gindri, D. M., Coelho, C. M. M., Souza, C. A., Heberle, I. and Prezzi, H. A. (2017). Seed quality of common bean accessions under organic and conventional farming systems. Pesquisa Agropecuária Tropical, 47, 152-160. https://doi.org/10.1590/1983-40632016v4745189

Guzmán-Ortiz, F. A., Castro-Rosas, J., Gómez-Aldapa, C. A., Mora-Escobedo, R., Rojas-León, A., Rodríguez-Marín, M. L. and Román-Gutiérrez, A. D. (2019). Enzyme activity during germination of different cereals: A review. Food Reviews International, 35, 177-200. https://doi.org/10.1080/87559129.2018.1514623

Karmous, I., El-Ferjani, E. and Chaoui, A. (2011). Copper excess impairs mobilization of storage proteins in bean cotyledons. Biological Trace Element Research, 144, 1251-1259. https://doi.org/10.1007/s12011-011-9115-9

Laemmli, U. K. (1970). Preparation of slab gels for one-or two-dimensional Polyacrylamide sodium dodecyl sulfate gel electrophoresis. Nature, 227, 680-685.

Liu, L., Xia, W., Li, H., Zeng, H., Wei, B., Han, S. and Yin, C. (2018). Salinity inhibits rice seed germination by reducing αamylase activity via decreased bioactive gibberellin content. Frontiers in Plant Science, 9, 275. https://doi.org/10.3389%2Ffpls.2018.00275

Los, F. G. B., Zielinski, A. A. F., Wojeicchowski, J. P., Nogueira, A. and Demiate, I. M. (2018). Beans (*Phaseolus vulgaris* L.): whole seeds with complex chemical composition. Current Opinion in Food Science, 19, 63-71. https://doi.org/10.1016/j.cofs.2018.01.010

Marcos-Filho, J. (2015). Seed vigor testing: an overview of the past, present and future perspective. Scientia Agricola, 72, 363-374. https://doi.org/10.1590/0103-9016-2015-0007

McCready, R. M., Guggolz, J., Silviera, V. and Owens, H. S. (1950). Determination of starch and amylose in vegetables. Analytical Chemistry, 22, 1156-1158. https://doi.org/10.1021/ac60045a016

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31, 426-428. https://doi.org/10.1021/ac60147a030

Monerri, C. and Guardiola, J. L. (1986). Estudio electroforético de las amilasas del guisante. Revista Agroquímica y de Tecnologia de Alimentos, 26, 424-434.

Montoya, C. A., Leterme, P., Victoria, N. F., Toro, O., Souffrant, W. B., Beebe, S. and Lallès, J. P. (2008). Susceptibility of phaseolin to in vitro proteolysis is highly variable across common bean varieties (*Phaseolus vulgaris*). Journal of Agricultural and Food Chemistry, 56, 2183-2191. https://doi.org/10.1021/jf072576e

Mukankusi, C., Raatz, B., Nkalubo, S., Berhanu, F., Binagwa, P., Kilango, M. and Beebe, S. (2019). Genomics, genetics and breeding of common bean in Africa: A review of tropical legume project. Plant Breeding, 138, 401-414. https://doi.org/10.1111/pbr.12573

Nadeem, M., Li, J., Yahya, M., Wang, M., Ali, A., Cheng, A., Wang, X. and Ma, C. (2019). Grain legumes and fear of salt stress: Focus on mechanisms and management strategies. International Journal of Molecular Sciences, 20, 799. https://doi.org/10.3390/ijms20040799

Nakagawa, J. (1999). Testes de vigor baseados no desempenho das plântulas. In F. C. Krzyzanowski, R. D. Vieira and J. B. França Neto (Eds.). Vigor de sementes: conceitos e testes (p. 1-24). Londrina: Abrates.

Nonogaki, H. (2008). Seed germination and reserve mobilization, In J. Wiley (Ed.). Encyclopedia of Life Sciences. J. Wiley. https://doi.org/10.1002/9780470015902.a0002047.pub2

Padilha, M. S., Coelho, C. M. M. and Ehrhardt-Brocardo, N. C. M. (2021). Vigor and alpha-amylase activity in common bean seeds under salt stress conditions. Semina: Ciências Agrárias, 42, 3633-3650. https://doi.org/10.5433/1679-0359.2021v42n6SUPL2p3633

Padilha, M. S., Coelho, C. M. M. and Sommer, Â. S. (2022a). Seed vigor, genotype and proline in common bean seedling formation under drought and saline stress. Revista Ciência Agronômica, 53, e20228350. https://doi.org/10.5935/1806-6690.20220056

Padilha, M. S., Coelho, C. M. M., Andrade, G. C. and Ehrhardt-Brocardo, N. C. M. (2022b). Seed vigor in reserve mobilization and wheat seedling formation. Revista Brasileira de Ciências Agrárias, 17, e1477. https://doi.org/10.5039/agraria.v17i3a1477

Punia, H., Tokas, J., Malik, A., Singh, S., Phogat, D. S., Bhuker, A. and Sheokand, R. N. (2020). Discerning morpho-physiological and quality traits contributing to salinity tolerance acquisition in sorghum [*Sorghum bicolor* (L.) Moench]. South African Journal of Botany, 140, 409-418. https://doi.org/10.1016/j.sajb.2020.09.036

Shaik, S. S., Carciofi, M., Martens, H. J., Hebelstrup, K. H. and Blennow, A. (2014). Starch bioengineering affects cereal grain germination and seedling establishment. Journal of Experimental Botany, 65, 2257-2270. https://doi.org/10.1093/jxb/eru107

Sindhu, S., Dahiya, A., Gera, R. and Sindhu, S. S. (2020). Mitigation of abiotic stress in legume-nodulating rhizobia for sustainable crop production. Agricultural Research, 9, 444-459. https://doi.org/10.1007/s40003-020-00474-3

Soltani, A., Gholipoor, M. and Zeinali, E. (2006). Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. Environmental and Experimental Botany, 55, 195-200. https://doi.org/10.1016/j.envexpbot.2004.10.012

Verma, G., Mishra, S., Sangwan, N. and Sharma, S. (2015). Reactive oxygen species mediate axis-cotyledon signaling to induce reserve mobilization during germination and seedling establishment in *Vigna radiata*. Journal of Plant Physiology, 184, 79-88. https://doi.org/10.1016/j.jplph.2015.07.001

Wang, L. L., Chen, X. Y., Yang, Y., Wang, Z. and Xiong, F. (2016). Effects of exogenous gibberellic acid and abscisic acid on germination, amylases, and endosperm structure of germinating wheat seeds. Seed Science and Technology, 44, 64-76. https://doi.org/10.15258/sst.2016.44.1.09

Yadav, S. P., Bharadwaj, R., Nayak, H., Mahto, R., Singh, R. K. and Prasad, S. K. (2019). Impact of salt stress on growth, productivity and physicochemical properties of plants: a review. International Journal of Chemical Studies, 7, 1793-1798.

Zeid, I. M., Gharib, F. A. E. L., Ghazi, S. M. and Ahmed, E. Z. (2019). Promotive effect of ascorbic acid, gallic acid, selenium and nano-selenium on seed germination, seedling growth and some hydrolytic enzymes activity of cowpea (*Vigna unguiculata*) seedling. Journal of Plant Physiology and Pathology, 7, 1. https://doi.org/10.4172/2329-955X.1000193

Zilio, M., Souza, C. A., Coelho, C. M. M., Miquelluti, D. J. and Michels, A. F. (2013). Cycle, canopy architecture and yield of common bean genotypes (*Phaseolus vulgaris*) in Santa Catarina State. Acta Scientiarum. Agronomy, 35, 21-30. https://doi.org/10.4025/actasciagron.v35i1.15516