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Seed vigor, genotype and proline in common bean seedling formation under drought and saline stress[1]

Vigor de sementes, genótipo e prolina na formação de plântulas de feijão sob estresse hídrico e salino

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ABSTRACT - Abiotic stresses, especially water and saline stress, are the main causes of reduced field emergence percentage. Several studies have associated the higher concentration of proline with the tolerance of cultivars to these stresses. However, one needs to determine how genotype and seed vigor act during seedling formation in the presence of stresses and how proline concentration interacts with these factors. The objective of this work was to evaluate the relationships of proline with genotype and seed vigor in seedling formation under drought and saline stress. The genotypes BAF07, BAF13, BAF23, BAF42, BAF44, BAF55 and BAF112 were used. They were subjected to germination tests under water and saline stress. At ten days after sowing, the following parameters were assessed: length, seedling dry mass and capacity to mobilize reserves, as well as the free proline content of the seedlings. Seed vigor and tolerant genotypes may help overcome water and saline stresses, and they should be used as a strategy during sowing. Also, higher proline concentrations are not associated with higher either vigor of the seed lot or tolerant genotypes.

Key words: Germination. Physiological quality. Stress tolerance.

RESUMO - As principais causas da redução na porcentagem de emergência a campo é a ocorrência de estresses abióticos, especialmente o estresse hídrico e salino. Diversos estudos associam a maior concentração de prolina com a tolerância de cultivares a esses estresses. Entretanto, é necessário determinar como o genótipo e o vigor atuam durante a formação de plântulas, e como a concentração de prolina interage com esses fatores. O objetivo deste trabalho foi avaliar a relação da prolina com o genótipo e o vigor de sementes durante o estabelecimento das plântulas em condições de estresse hídrico e salino. Foram utilizados os genótipos BAF07, BAF13, BAF23, BAF42, BAF44, BAF55 e BAF112, as quais foram submetidas a germinação em estresse hídrico e salino. Aos 10 dias após a semeadura foram avaliados o comprimento, massa seca das plântulas e capacidade de mobilização de reservas, assim como o teor de prolina livre das plântulas. O uso de sementes de alto vigor e genótipos tolerantes influenciam na superação dos estresses hídrico e salino e devem ser utilizados como estratégia durante a semeadura e, a maior concentração prolina não apresentou associação com o maior vigor do lote de sementes ou aos genótipos tolerantes.

Palavras-chave: Germinação. Qualidade fisiológica. Tolerância a estresses.

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INTRODUCTION

Common beans are one of the most cultivated crops worldwide, and it is an important source of carbohydrates, protein and minerals to human populations (MOROSAN *et al*., 2017). Among the main factors limiting the production of this culture, drought and saline stresses cause severe loss in plant performance, which result in osmotic stress, germination inhibition, oxidative stress, photosynthetic inhibition, nutritional imbalance and reduced growth (EL-MOUKHTARI *et al*., 2020; PER *et al*., 2017).

The effects of these abiotic stresses can be found in the entire plant cycle, and seedling emergence is the most critical moment for successful production. Losses in germination percentage and, consequently, in plant stand, have been reported in the literature during water and saline stress conditions (ARAUJO-NETO *et al*., 2020; JOVOVIĆ *et al*., 2018). Likewise, negative effects occur during seedling formation, e.g., decreased dry mass and length (AVCI; İLERI; KAYA, 2017; PANTOLA; BARGALI; BARGALI, 2017).

To maintain the potential of seedling emergence in the field, the use of seeds with higher vigor is crucial. These seeds are well-known for their ability to express higher percentages of germination, field emergence and seedling performance under conditions of abiotic stress, and such potential of seeds with higher vigor must be utilized (FINCH-SAVAGE; BASSEL, 2016; MARCOS-FILHO, 2015). However, the mechanisms used by higher seed vigor under abiotic stresses to provide better germination and seedling performance, are not fully elucidated.

As with vigor, genotypes have a great influence on responses to abiotic stresses, and they can be determinant during seed germination and seedling formation (AFLAKI *et al*., 2017). In this context, further research is needed on the interaction between genotype tolerance to abiotic stresses and the ability of seeds with higher vigor to perform better under these conditions, with a view to clarify the role played by genotype and seed vigor during the germination and seedling formation of common bean.

Strategies to overcome abiotic stresses, e.g., the action of the antioxidant system, accumulation of solutes, hormonal regulation and others, are important to help plants to have a better response during stresses (NADEEM *et al*., 2019). Therefore, proline is one of the cumulative solutes that occur in different organs of the plant, e.g., leaves, stem and roots; such accumulation can be higher with increased stress intensity (CHEN *et al*., 2016). Proline accumulation causes an osmotic adjustment of cells, favoring the maintenance of metabolic activities during these stresses (MANSOUR; ALI, 2017); also, it regulates physiological, biochemical and enzymatic processes during stresses that are positive to plant growth (EL-MOUKHTARI *et al*., 2020). Proline has been used in several studies to determine genotype tolerance to stresses; also, it can be utilized as a marker of drought and saline stresses (MOROSAN *et al*., 2017; PER *et al*., 2017). However, the association of proline with seed vigor in early stages of seedling formation have not been reported in the literature.

The present study was based on the hypotheses that the common bean seed lots with higher seed vigor have greater capacity to overcome conditions of osmotic stresses by presenting a higher concentration of proline in seedlings; the different genotypes show differences in proline accumulation and stress tolerance when evaluated at the initial stage of development. Moreover, these factors contribute to seedling formation under the study conditions. Thus, the objective of this work was to evaluate the relationships of proline with genotype and seed vigor in seedling formation under drought and saline stress.

MATERIAL AND METHODS

Seed lots of genotypes BAF07, BAF13, BAF23, BAF42, BAF44, BAF55 and commercial cultivar BAF112 (IPR-88-Uirapurú) were used. Except for BAF112, these genotypes are originally from the Bean Active Germplasm Bank (BAF) of the Universidade do Estado de Santa Catarina. Genotypes underwent successive self-selection that favored greater homogeneity and stability within the population that had been previously characterized and studied, and selected on their seed physiological quality (EHRHARDT-BROCARDO; COELHO, 2016). These genotypes have been utilized in many previous studies in different years and were studied for physical, physiological, morphological and agronomic characteristics (GINDRI *et al*., 2017; MICHELS *et al*., 2014; ZILIO *et al*., 2013).

The seeds were produced in the municipality of Lages - SC, Brazil, in the season 2019/2020. After harvesting, they were homogenized and 1000 g of seeds were separated to determine the average sample, and the working sample for further analysis was obtained later (BRASIL, 2009). The seeds were stored in a dry chamber (10 ± 2 °C and 50 ± 5% relative humidity) for four months until analysis.

Physiological quality of seed lots was determined on the basis of germination and vigor index.

Vigor index was determined with three replicates of 20 seeds distributed in the upper third of paper moistened with a volume of distilled water in the proportion of 2.5 mL g^{-1} of dry paper. The rolls were kept in a germinator at 25 ± 2 °C for three days (SILVA; MEDEIROS; OLIVEIRA, 2019). After this period, hypocotyl length (HL_{3days}), root length (RL_{3days}) and total seedling length (TSL_{3days}) were measured with the aid of a digital caliper and expressed in centimeters per seedling (cm sl^{-1}). The vigor index (VI) was calculated using the growth index (G_{3days}) (SAKO *et al.*, 2001) and the uniformity index (U_{3days}) (CASTAN; GOMES-JUNIOR; MARCOS-FILHO, 2018), in the software R (R CORE TEAM, 2020) with the SeedCalc package (SILVA; MEDEIROS; OLIVEIRA, 2019).

In the germination test, first germination count (FGC) and germination percentage (G) were determined on the fifth and ninth days after sowing, respectively (BRASIL, 2009) for control, drought and saline stress conditions. Drought stress was determined using a solution of polyethylene glycol 6000 equivalent to -0.2 MPa (113.63 g of polyethylene glycol 6000 per liter of water) (VILLELA; DONI-FILHO; SEQUEIRA, 1991). Salt stress was assessed using a sodium chloride solution with a concentration of 150 mmol L^{-1} with electrical conductivity of 14.6 dS m^{-1}. The water used for control and to dilute the stress treatments was distilled and deionized.

The germination test was carried out in a Mangelsdorf germinator at a temperature of 20 ± 2 ºC, and the seeds were sown on paper towel rolls, with three replications of 50 seeds. The paper was moistened with distilled water, polyethylene glycol 6000 or sodium chloride solution in the proportion of 2.5 mL g^{-1} of dry paper. The paper rolls were placed vertically inside the germinator, and wrapped with plastic bags to avoid loss of humidity and maintain the desired stress.

Seedling performance was evaluated in three replicates at 10 days after sowing. The test was conducted in the same way as for the vigor index described above at a temperature of 20 ± 2 ºC. The resulting normal seedlings were used for the evaluations and to determine root length (RL), hypocotyl length (HL), and total seedling length (TSL), measured with the aid of a digital caliper and expressed in centimeters per seedling (cm sl^{-1}). The measured seedlings were used to determine root dry mass (RDM), hypocotyl dry mass (HDM) and total seedling dry mass (TSDM), dried at 80 ºC for 24 h; the results were expressed in milligrams per seedling (mg sl^{-1}) (KRZYZANOWSKI *et al.*, 2020). With the results, the water content (WC) of the seedlings was determined and expressed as a percentage. The number of secondary roots was determined using the measured seedlings, and the roots larger than 0.5 cm (NSR) were counted. The reserve mobilization rate (RMR) was determined according to Andrade, Coelho and Padilha (2019) and expressed as percentage and; the seed reserve reduction rate (SRRR) was determined according to (PEREIRA; PEREIRA; DIAS, 2015) and expressed as a percentage.

Stress tolerance was determined with the data collected at 10 days, using the calculation results of individual responses of each parameter to stresses, based on the methodology used by Kakar *et al.* (2019), which was adapted to germination conditions, according to the variables being used. The interpretation of the calculation is based on the fact that the genotypes with the highest index have greater tolerance to particular stresses. The cumulative response index to salt stress (CRISS) and the cumulative response index to drought stress (CRIDS) were calculated using the individual response values of the parameters germination (G), root length (RL), hypocotyl length (HL) total length (TSL), root dry mass (RDM), hypocotyl dry mass (HDM), total seedling dry mass (TSDM), reserve mobilization rate (RMR) and number of secondary roots (NSR). The variable SRRR was not considered for tolerance as it is not a direct evaluation parameter in the formed seedlings. The parameters evaluated in salt stress (S), drought stress (D) and control (C) were placed in the Equations 1 and 2 below:

$$CRISS = \left(\frac{G_S}{G_C}\right) + \left(\frac{RL_S}{RL_C}\right) + \left(\frac{HL_S}{HL_C}\right) + \left(\frac{TSL_S}{TSL_C}\right) + \left(\frac{RDM_S}{RDM_C}\right) + \left(\frac{HDM_S}{HDM_C}\right) + \left(\frac{TSDM_S}{TSDM_C}\right) + \left(\frac{RMR_S}{RMR_C}\right) + \left(\frac{NSR_S}{NSR_C}\right) \quad (1)$$

$$CRIDS = \left(\frac{G_D}{G_C}\right) + \left(\frac{RL_D}{RL_C}\right) + \left(\frac{HL_D}{HL_C}\right) + \left(\frac{TSL_D}{TSL_D}\right) + \left(\frac{RDM_D}{RDM_C}\right) + \left(\frac{HDM_D}{HDMc}\right) + \left(\frac{TSDM_D}{TSDM_C}\right) + \left(\frac{RMR_D}{RMR_C}\right) + \left(\frac{NSR_D}{NSR_C}\right) \quad (2)$$

Proline concentration was determined in three biological replicates of seedlings originated in each condition and evaluated at 10 days using the method of Bates, Waldren and Teare (1973) without modifications, and conducted in duplicate.

That experiment was conducted to determine the two main characteristics in the study that can affect proline concentration; in other words, seed vigor and genotype. Each genotype has a CRISS and a CRIDS, as well as, a seed vigor index. The experimental design used for characterization of seed vigor and tolerance was completely randomized with seven genotypes and three replicates. The experimental design for evaluation under stress conditions was completely randomized in a 7 x 3 factorial arrangement, with seven genotypes and three germination conditions with three replicates. Analysis of variance and Pearson correlation analysis were performed using the R software (R CORE TEAM, 2020). The data underwent analysis of variance (F-test), and comparison between means was performed using the Scott-Knott test at 5% probability of error. For the correlation analysis, significant correlations were determined at 1% or 5%, and non-significant ones, by the t-test.

RESULTS AND DISCUSSION

The results of variance analysis showed significant differences between genotypes and their physiological quality and tolerance to drought and saline stress (Table 1).

The variables root length (RL_{3days}), hypocotyl length (HL_{3days}), total length (TSL_{3days}), growth index (G_{3days}), uniformity index (U_{3days}), and vigor index (VI) showed significant differences and the seed lots of genotypes BAF13, BAF42, BAF55 and BAF112 showed higher physiological quality when compared to genotypes BAF07, BAF23 and BAF44 (Table 1).

One of the basic principles for research on vigor is to determine differences in vigor between the materials used, classify into groups and subsequently associate them with the characters of interest. According to Finch-Savage and Bassel (2016), seeds with greater vigor present greater speed, uniformity and seedling growth during germination, and these responses were observed in the findings (Table 1). The seed vigor index is strongly associated with the physiological quality of seed lots (CASTAN; GOMES-JUNIOR; MARCOS-FILHO, 2018; SILVA; MEDEIROS; OLIVEIRA, 2019).

The summary of the analysis of variance for evaluation in control, saline and drought stress demonstrates a significant effect for the factors alone and the interaction of the factors for all the study variables, except for first germination count (FGC), which did not show an interaction between the factors (Table 3).

First germination count (FGC) in all germination conditions (i.e., control, drought stress and saline stress) was used for confirmation of seed vigor, and the results are the same result as those found for the vigor index (Table 2). The seeds of genotypes BAF07, BAF23 and BAF44 demonstrate lower FGC; such results complement the segregation between the seed lots of genotypes used previously (Table 4). Thus, the parameters associated with seed vigor were used to define two distinct vigor groups, the group with high seed vigor (i.e., BAF13, BAF42, BAF55 and BAF112), and the group with low seed vigor (i.e., BAF07, BAF23 and BAF44).

The germination results under drought stress and saline stress conditions showed a significant effect among genotypes. Under drought stress, only the BAF44 genotype with less vigor showed lower germination percentage. However, under saline stress, genotypes BAF07, BAF23 and BAF44 showed lower germination percentage compared to genotypes with high seed vigor (Table 4). The negative effect of these stresses on germination percentage is known in the literature (AVCI; İLERI; KAYA, 2017;

Table 1 - Summary of variance analysis for Root length (RL_{3days}), hypocotyl length (HL_{3days}), total length (TSL_{3days}), growth index (G_{3days}), uniformity index (U_{3days}), vigor index (VI), cumulative response to saline stress (CRISS) and cumulative response to drought stress (CRIDS) of common bean genotypes evaluated

SV	DF	RL_{3days}	HL_{3days}	TSL_{3days}	G_{3days}	U_{3days}	VI	CRISS	CRIDS
Genotype	6	39.70*	26.27*	48.71*	40.91*	28.54*	45.69*	4.80*	4.88*
Error	14								
Total	20								
CV	-	6.06	4.52	4.91	5.88	3.04	4.42	5.68	11.35

Legenda: SV: Source of variation; DF: degrees of freedom; CV: Coefficient of variation; ^{ns} and *, not significant and significant at 5%, respectively by the F test

Table 2 - Root length (RL_{3days}), hypocotyl length (HL_{3days}), total length (TSL_{3days}), growth index (G_{3days}), uniformity index (U_{3days}), vigor index (VI) of common bean seed lots evaluated

Genotype	RL_{3days}	HL_{3days}	TSL_{3days}	G_{3days}	U_{3days}	VI
BAF07	5.69 c	2.02 c	7.72 d	533 c	747 c	597 c
BAF13	7.85 b	2.44 a	10.30 b	731 b	846 b	772 b
BAF23	6.04 c	2.18 b	8.23 c	566 c	823 b	643 c
BAF42	7.79 b	2.46 a	10.26 b	726 b	879 a	772 b
BAF44	4.50 d	1.65 d	6.16 d	422 d	691 d	502 d
BAF55	8.73 a	2.44 a	11.17 a	810 a	898 a	836 a
BAF112	7.93 b	2.32 b	10.25 b	737 b	882 a	781 b

Means followed by the same lowercase letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability

JOVOVIĆ *et al*., 2018; PANTOLA; BARGALI; BARGALI, 2017). The reduction in germination percentage was due not only to reduced availability of water (which is necessary for the germination process), but also to an ionic process caused by saline conditions that impose toxicity on the protoplasm of plant cells under salt stress (ARAUJO-NETO *et al*., 2020).

There was significant variation for the evaluated parameters. In general, this variation shown in the boxplot is associated with the genetic response of the genotypes when under different stress conditions, as well as associated with vigor differences (Figure 1).

Saline stress affected seedling growth more severely when compared to drought stress. Among the most affected variables, there is a greater reduction in root length (RL) (Figure 1a) and number of secondary roots (NSR) (Figure 1c). RL was reduced by 76% under salt stress and by 37% under water stress. For NSR, there was a decrease by 81% in salt stress and 56% when it was under drought stress. Under water stress conditions, root growth

Table 3 - Summary of variance analysis for root length (RL), hypocotyl length (HL), total length (TSL), root dry mass (RDM), hypocotyl dry mass (HDM), total seedling dry mass (TSDM), number of secondary roots (NSR), reserve mobilization rate (RMR), seed reserve reduction rate (SRRR) and proline concentration of different common bean genotypes submitted to saline and drought stress at 10 days of germination

SV	DF	FGC	G	RL	HL	TSL	RDM
F1	6	23.02*	7.27*	15.88*	9.59*	21.07*	17.97*
F2	2	145.95*	40.96*	888.84*	350.63*	1021.70*	40.07*
F1 x F2	12	1.47^{ns}	2.31*	3.96*	2.12*	4.32*	3.70*
Error	42						
Total	62						
CV	-	8.64	3.59	9.37	16.74	8.71	9.21
SV	DF	HDM	TSDM	NSR	RMR	SRRR	Proline
F1	6	18.43*	21.10*	18.94*	49.33*	43.79*	60.69*
F2	2	1128.12*	736.58*	1225.99*	770.69*	1083.85*	318.26*
F1 x F2	12	17.06*	12.31*	5.78*	3.66*	3.66*	17.67*
Error	42						
Total	62						
CV	-	8.95	7.87	10.09	7.41	6.48	15.23

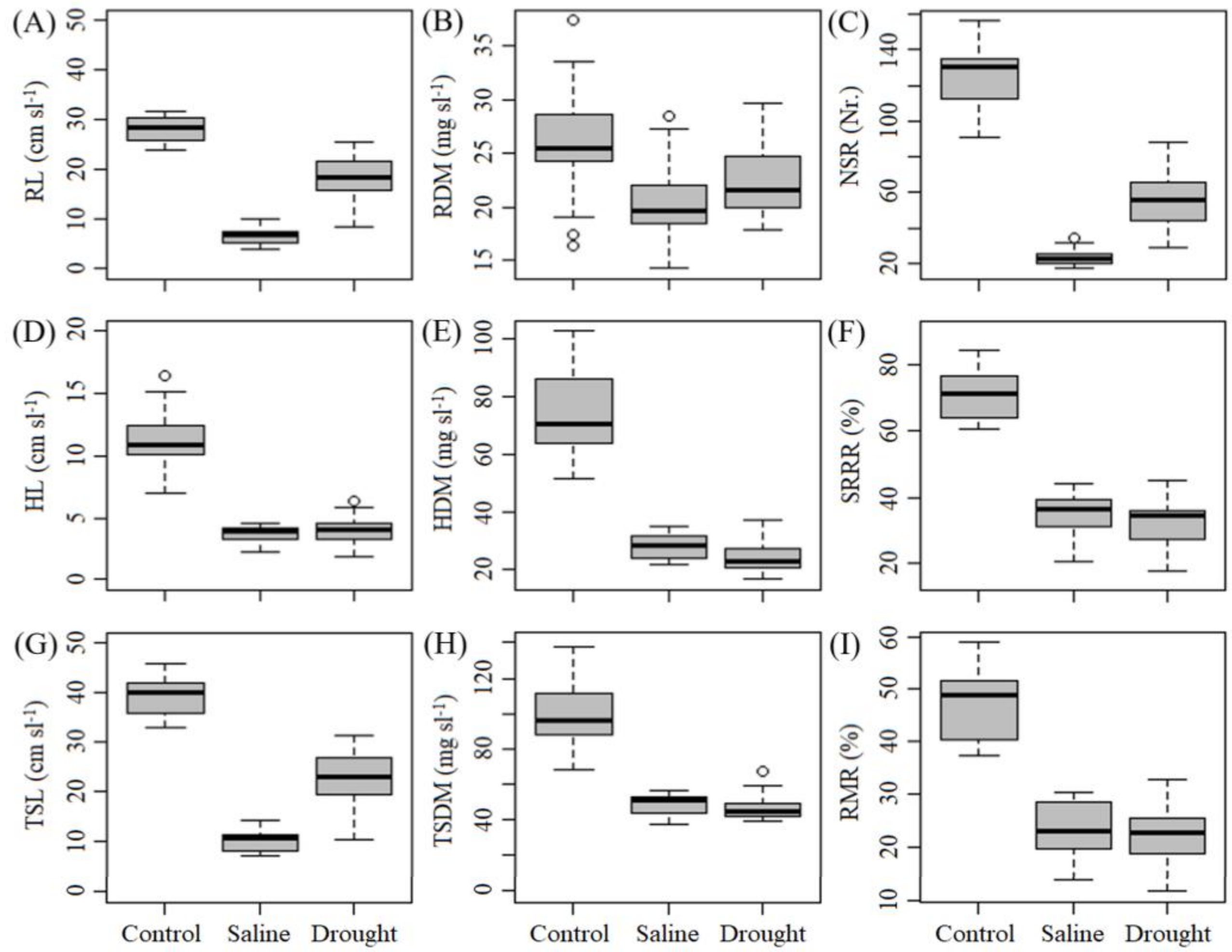
F1: Factor 1 (Genotype); F2: Factor 2 (Stress conditions); SV: Source of variation; DF: degrees of freedom; CV: Coefficient of variation; ^{ns} and *, not significant and significant at 5%, respectively, by the F test

Table 4 - First germination count (FGC) and germination percentage (G) under conditions without stress (Control), saline stress and drought stress for the evaluated genotypes

Genotype	FGC (%)			G (%)		
	Control	Saline	Drought	Control	Saline	Drought
BAF07	82 bA	50 bB	53 bB	95 aA	87 bB	87 aB
BAF13	90 aA	67 aB	69 aB	96 aA	89 aB	91 aB
BAF23	79 bA	55 bB	47 cB	95 aA	83 bB	88 aB
BAF42	95 aA	74 aB	65 aB	97 aA	91 aB	91 aB
BAF44	79 bA	47 bB	43 cB	95 aA	85 bB	77 bC
BAF55	97 aA	73 aB	72 aB	97 aA	93 aB	91 aB
BAF112	90 aA	73 aB	56 bC	97 aA	92 aB	90 aB

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically from each other by the Scott-Knott test at 5% probability

Figure 1 - Boxplot demonstrating the variation and influence of saline and drought stresses on root length (RL), hypocotyl length (HL), total length (TSL), root dry mass (RDM), hypocotyl dry mass (HDM) and total seedling dry mass (TSDM) of the number of secondary roots (NSR), seed reserve reduction rate (SRRR), reserve mobilization rate (RMR) of common beans genotypes at 10 days after sowing



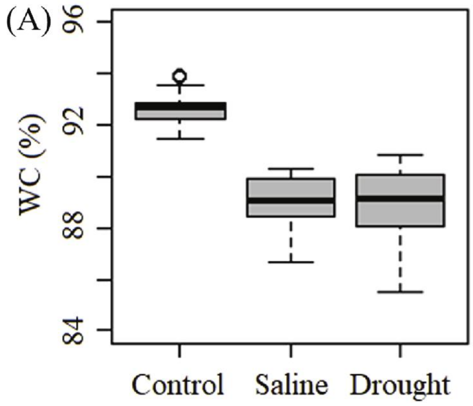
was higher than when under saline stress (Figure 1a); similar results were found by Maia *et al*. (2015), who attributed this phenomenon to the death of the apical meristem in salt stress, thus limiting the differentiation and, consequently, root growth in depth.

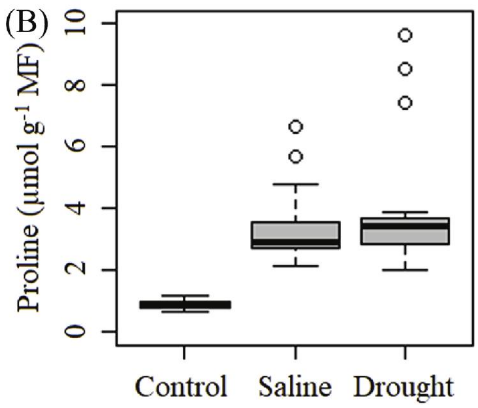
For both stresses, hypocotyl dry mass (HDM) and total seedling dry mass (TSDM) did not differ between the stresses (Figure 1e, 1h). Root dry mass (RDM) showed a significant difference between the evaluated conditions; in drought stress and saline stress, RDM was lower than in the control. There was a reduction in RDM by 22% in salt stress and 14% in water stress. RDM was less affected in comparison to the values found for HDM, which showed a reduction of 61% under saline stress and 67% under drought stress. These results demonstrate that the seedlings, when stressed, prioritized the mobilization of reserves for root growth, seeking to overcome the imposed stress condition (Figure 1b).

The reserve mobilization rate (RMR) and the reserve reduction rate (SRRR) showed a similar behavior between the imposed stresses; there was a reduction in the volume of reserves being used and mobilized for the embryonic axis (Figure 1f, 1i). Water restriction and/or the presence of ions (i.e., Na^+ and Cl^-) cause a reduction in cellular turgor and toxicity, respectively, resulting in a reduction in the degradation capacity and mobilization of stored reserves (DANTAS *et al*., 2017; LIU *et al*., 2018), which decrease seedling performance (Figure 1g, 1h).

There was a reduction in the water content (WC) of the plants (Figure 2a) and an increase in the free proline content (Figure 3b). In both stress situations, seedling moisture percentage is reduced and, consequently, there was an increase in the proline content of the plant tissue of the seedlings. Similar results were found by Arteaga *et al*. (2020) in common bean plants.

Figure 2 - Boxplot showing the variation and influence of saline stress and drought stress on water content (a) and free proline content of common bean seedlings at 10 days after sowing





Several studies have reported genotypic differences regarding tolerance to abiotic stresses, which makes it possible to determine the tolerance of a genotype using physiological parameters (AFLAKI *et al*., 2017; DANTAS *et al*., 2017; KAKAR *et al*., 2019). A significant difference was found in the cumulative response index to salt stress (CRISS) and the cumulative response index to drought stress (CRIDS), indicating differences between genotypes in relation to tolerance to saline and drought stress. The genotypes BAF07, BAF42 and BAF55 showed greater tolerance to salt stress. For drought stress, the highest tolerance was found for genotypes BAF07 and BAF55 (Table 5).

The genetic response of genotypes to stresses indicated differences between stress tolerance (Table 5) and initial vigor of the seed lot (Table 2). In this case, these findings indicate the genotypes with seeds of high and low vigor that are more tolerant to stresses (e.g., BAF55 and BAF07), as well as the genotypes with seeds of high and low vigor that are less tolerant (e.g., BAF112 and BAF44). This is important to determine the relationship between genotype tolerance and vigor during seedling formation.

Under control conditions, RL, HL and TSL showed the highest lengths for the genotypes BAF55 and BAF112. However, under water stress conditions, the RL and HL of genotype BAF112 were affected; they were lower than the values found for genotype BAF07 (Table 6). According to Krzyzanowski *et al*. (2020), seeds with greater vigor are able to originate seedlings with greater length. Thus, the results indicate the influence of the genotype during the formation of seedlings under water stress, as genotype BAF112 has greater seed vigor and shorter length. This result corroborates the difference in water stress tolerance found for these genotypes (Table 5). Under saline stress, the genotypes with higher vigor presented higher TSL, which indicates the influence of seed vigor under stress conditions (Table 6).

Under control conditions, the NSR of the high-vigor seed group (i.e., BAF13, BAF42, BAF55 and BAF112) was the highest. Under saline stress, there was no significant difference between the genotypes, possibly owing to the severity found for this stress in terms of growth parameters (Figure 1). Under water stress conditions, the highest NSR was found for genotype BAF55 (high vigor with greater tolerance), and the lowest values were found for genotypes BAF23 and BAF44 (low vigor with less tolerance); under these conditions, the tolerance and the vigor of the genotype enhance the formation of a greater number of secondary roots, thus favoring the formation of more vigorous seedlings during water stress (Table 6).

Genotypes BAF07 and BAF55 are tolerant to water stress (Table 5) and have contrasting vigor (Table 2); it was found that the genotype BAF55 that had high seed vigor had higher RL, TSL and NSR. This finding confirms the influence of vigor on these parameters during water stress. In the same way, for RDM, HDM and TSDM, genotype BAF55, under saline and drought stress, had the highest dry mass regardless of the condition (Table 6). This result demonstrates the importance of vigor during seedling formation under stress conditions. The same association can be found for TSL and TSDM of less tolerant genotypes (i.e., BAF112 with higher vigor and BAF44 with low vigor).

Considering the genotypes with high vigor, the tolerant genotypes BAF42 and BAF55 showed greater capacity of digestion of reserves (SRRR) and mobilization to the seedling (RMR) compared to genotypes BAF13 and BAF112 (Table 6). The greater capacity for hydrolysis and mobilization of reserves favors the formation of seedlings with better performance (ANDRADE; COELHO; PADILHA, 2019; PEREIRA; PEREIRA; DIAS, 2015). These results demonstrate the influence of genotype during seedling formation under drought and saline stress.

Table 5 - Cumulative response index to salt stress (CRISS) and the cumulative response index to drought stress (CRIDS) of evaluated genotypes of common bean

Genotype	CRISS	CRIDS
BAF07	4.30 a	5.96 a
BAF13	4.07 b	5.19 b
BAF23	3.66 b	3.85 b
BAF42	4.47 a	5.01 b
BAF44	3.85 b	4.89 b
BAF55	4.37 a	5.98 a
BAF112	3.99 b	4.75 b

Means followed by the same lowercase letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability

Table 6 - Root length (RL), hypocotyl length (HL), total seedling length (TSL), root dry mass (RDM), hypocotyl dry mass (HDM) and total seedling dry mass (TSDM), number of secondary roots (NSR), reserve mobilization rate (RMR) and seed reserve reduction rate (SRRR) in control conditions, drought and saline stress evaluated 10 days after sowing

Var	T	Genotype						
		BAF07	BAF13	BAF23	BAF42	BAF44	BAF55	BAF112
RL (cm sl^{-1})	Control	26.24 b	27.54 b	27.50 b	30.24 a	25.76 b	31.23 a	28.81 a
	Saline	5.02 c	7.07 b	6.36 b	9.34 a	4.84 c	7.18 b	6.56 b
	Drought	18.67 a	21.09 a	11.53 c	22.27 a	14.41 b	20.16 a	16.34 b
HL (cm sl^{-1})	Control	12.33 b	11.28 c	8.18 d	10.64 c	9.69 d	12.00 b	14.42 a
	Saline	3.74 a	3.81 a	2.43 c	4.25 a	3.10 b	4.97 a	4.03 a
	Drought	5.08 a	4.29 a	2.13 b	4.05 a	3.15 b	5.48 a	3.71 b
TSL (cm sl^{-1})	Control	38.58 b	38.81 b	35.76 b	40.89 a	35.46 b	43.23 a	43.27 a
	Saline	10.04 b	12.46 a	10.14 b	15.29 a	91.68 b	12.97 a	12.46 a
	Drought	21.72 c	26.76 a	14.79 d	27.65 a	18.59 c	30.57 a	23.57 b
RDM (mg sl^{-1})	Control	17.57 c	27.89 b	33.23 a	28.07 b	24.35 b	24.42 b	26.57 b
	Saline	16.47 c	18.59 c	26.23 a	21.13 b	17.69 c	19.82 b	21.60 b
	Drought	19.51 b	20.77 b	24.58 a	20.36 b	24.40 a	24.12 a	23.33 a
HDM (mg sl^{-1})	Control	57.18 e	69.77 c	98.64 a	69.88 c	63.59 d	71.43 c	89.56 b
	Saline	24.50 b	29.75a	24.90 b	30.95 a	23.12 b	33.60 a	31.58 a
	Drought	25.38 b	24.21 b	18.58 b	22.99 b	20.18 b	32.57 a	26.41 b
TSDM (mg sl^{-1})	Control	74.75 e	97.67 c	131.8 a	97.95 c	87.85 d	95.85 c	116.14 b
	Saline	40.98 b	48.34 a	51.14 a	52.09 a	40.81 b	53.42 a	53.18 a
	Drought	44.89 b	44.99 b	43.17 b	43.36 b	44.58 b	56.70 a	49.74 a
NSR (Nr.)	Control	110 b	142 a	127 a	135 a	100 b	138 a	129 a
	Saline	21 a	25 a	23 a	29 a	18 a	23 a	23 a
	Drought	57 b	64 b	35 c	56 b	36 c	79 a	56 b

Continuation Table 6

RMR (%)	Control	43.39 c	48.19 b	38.82 c	55.25 a	40.33 c	53.10 a	50.62 b
	Saline	23.26 b	23.62 b	15.15 c	29.57 a	18.95 c	29.34 a	22.96 b
	Drought	25.73 b	21.93 c	12.84 d	24.44 b	20.79 c	31.55 a	21.66 c
SRRR (%)	Control	64.23 c	71.42 b	62.93 c	76.63 a	63.51 c	79.62 a	76.89 a
	Saline	34.63 b	34.13 b	22.94 d	41.83 a	30.45 c	41.54 a	37.68 b
	Drought	37.41 a	32.79 b	19.46 d	35.08 b	28.41 c	43.03 a	31.59 b
Proline (µmol g^{-1} MF)	Control	0.87 a	0.78 a	1.09 a	0.91 a	0.85 a	0.78 a	0.91 a
	Saline	3.01 c	2.71 c	5.71 a	3.05 c	3.85 b	2.39 c	2.75 c
	Drought	3.10 b	3.57 b	8.51 a	2.94 c	3.51 b	2.53 c	3.33 b

Means followed by the same lowercase letter in the row do not differ statistically from each other by the Scott-Knott test at 5% probability; Var: variables; T: treatments

The importance of seed vigor and tolerance can be observed in the Pearson correlation analysis. VI showed a negative correlation with tolerance indexes. Vigor and genotype act during seedling formation, but no significant correlation between these indexes is desirable. The results show how vigor and tolerance favored seedling formation during each of the study stresses (Table 7).

The evaluated growth parameters and the vigor of the seed lot show a positive association during seedling formation (ANDRADE; COELHO; PADILHA, 2019; MARCOS-FILHO, 2015), and a similar association was found even under water and saline stress conditions (Table 7). There was a positive correlation of seed vigor with RL, HL, TSL, RDM, TSDM, NSR, RMR and SRRR under saline stress. Under drought stress conditions, the vigor of the seed lot positively influenced the parameters RL, TSL, RDM, NSR and SRRR. Considering these positive correlations, the seeds with greater vigor have greater capacity of hydrolysis (i.e., SRRR) under drought and saline stress, which favors seedling growth and root dry mass. However, considering the observed correlations, genotype tolerance was important for seedling formation under saline and drought stress, and with the exception of RDM, all the physiological parameters were positively associated with genotype tolerance (Table 7).

Under water stress conditions, there was a strong association of genotype (i.e., CRIDS) for all variables, except for RDM (Table 7). This relationship can be observed in the results of genotype BAF07 (low vigor with greater tolerance) and genotype BAF112 (high vigor with less tolerance). The performance of genotype BAF07 in the control situation was inferior in all physiological parameters because it presented lower seed vigor. However, under water stress, RL, HL, TSL, RMR and SRRR were higher in that genotype (Table 6). Common bean genotypes with greater tolerance to water or saline stress have better physiological performance when compared to less tolerant genotypes (ARTEAGA *et al*., 2020), considering that the parameters RL, HL, TSL, RMR and SRRR are positively associated with tolerance to water stress under germination conditions.

Proline concentrations are negatively correlated with seed vigor under saline stress, and they are non-significant under drought stress. These results suggest that seeds with higher vigor do not need a high concentration of proline to maintain their metabolic activity at an acceptable level, probably because other mechanisms act during stress at the same time. Plants that perform better under water and saline stress conditions have an efficient cellular antioxidant defense mechanism, in addition to a great ability to transport water and ions, solute accumulation for osmoprotection to re-establish cellular homeostasis in order to protect membranes and proteins, thus preventing cell death and maintaining growth and development of seedlings (MAIA *et al*., 2015; NADEEM *et al*., 2019). Such aptitudes can be associated with genotypic tolerance and seed vigor in early stages of germination and seedling development; these mechanisms need to be elucidated in future work, especially when they are associated with seed vigor.

Proline concentration in seedlings was negatively associated with tolerance to salt stress and drought stress. Similar results were found by Arteaga *et al*. (2020). Proline accumulation is one of the main responses to salt and water stresses, indicating the presence of these stresses in plants (PER *et al*., 2017). This response was found (Figure 2); however, there was no association with tolerance to these stresses, since the genotypes with the lowest tolerance (i.e., BAF23 and BAF44) (Table 5) had the highest concentrations of proline in both conditions

(Table 6). The same finding was reported by Morosan *et al*. (2017): the common bean genotypes with lower tolerance had higher proline concentration in the tissues. Morosan *et al*. (2017) and Arteaga *et al*. (2020) described proline as just a marker of drought and saline stress during the vegetative stages of common bean, and there was no association with genotype tolerance.

The association of proline concentration with the evaluated physiological parameters was, in general, negative or non-significant, which reinforces the absence of a relationship between high proline concentration and better seedling formation (Table 8).

Proline concentration can mitigate osmotic stresses (NADEEM *et al*., 2019). However, the results of Arteaga

Table 7 - Pearson's correlation coefficient (r) for the variables and their association with the seed vigor and seedling parameters when evaluated in saline and drought stress

Variable	Saline		Drought	
	VI	CRISS	VI	CRIDS
RL	0.66*	0.58*	0.55**	0.60*
HL	0.61*	0.75*	0.39^{ns}	0.94*
TSL	0.74*	0.71*	0.63*	0.81*
RDM	0.80*	-0.14^{ns}	0.48**	-0.01
HDM	0.22^{ns}	0.54*	-0.14^{ns}	0.81*
TSDM	0.71*	0.31^{ns}	0.33^{ns}	0.65*
NSR	0.55*	0.58**	0.69*	0.76*
RMR	0.64*	0.84*	0.38^{ns}	0.88*
SRRR	0.57*	0.82*	0.45**	0.90*
Proline	-0.50**	-0.69*	-0.27^{ns}	-0.59*
CRISS	0.39^{ns}	-	-	-
CRIDS	-	-	0.17^{ns}	-

Not significant (^{ns}), Significant at 1% (*) and 5% (**) probability by t test. Root length (RL); Hypocotyl length (HL); Total length (TSL); Root dry mass (RDM); Hypocotyl dry mass (HDM); Total seedling dry mass (TSDM); Number of secondary roots (NSR), Reserve mobilization rate (RMR); Seed reserve reduction rate (SRRR); Proline content (Proline), Cumulative response index to salt stress (CRISS); Cumulative response index to drought stress (CRIDS); n = 21

Table 8 - Pearson correlation coefficients (r) for parameters associated with seedling performance and proline concentration in different common bean genotypes

Variable	Proline (µmol g FW^{-1})	
	Saline	Drought
RL	-0.32^{ns}	-0.61*
HL	-0.85*	-0.67*
TSL	-0.53**	-0.68*
RDM	0.48**	0.33^{ns}
HDM	-0.59*	-0.43^{ns}
TSDM	-0.16^{ns}	-0.21^{ns}
NSR	-0.23^{ns}	-0.57*
RMR	-0.79*	-0.73*
SRRR	-0.84*	-0.77*

Not significant (^{ns}), Significant at 1% (*) and 5% (**) probability by t test. Root length (RL); Hypocotyl length (HL); Total length (TSL); Root dry mass (RDM); Hypocotyl dry mass (HDM); Total seedling dry mass (TSDM); Number of secondary roots (NSR), Reserve mobilization rate (RMR); Seed reserve reduction rate (SRRR); n = 21 *et al*. (2020) showed that proline had a significant negative correlation with the growth parameters of different common bean genotypes under drought and saline stresses, and they demonstrate that higher concentrations of proline negatively affect growth and mass accumulation. This decrease in performance may be associated with the synthesis of this amino acid, which requires high metabolic energy costs (TAIZ *et al*., 2017), thus limiting the growth and development of new tissues, consequently forming less vigorous seedlings. This association can be seen in the results of genotypes BAF23 and BAF44, both with lower vigor (Table 2) and lower tolerance (Table 5). They presented higher proline content in seedlings and presented a greater decrease in TSDM accumulation during both stresses (Table 6). These results indicate that during early seedling formation, higher proline accumulation is not associated with higher tolerance or high seed vigor of common bean – it is just a marker of these stresses.

CONCLUSIONS

Seed vigor favors germination and seedling formation under drought or saline stress conditions, but better performance is not associated with a higher concentration of proline in the seedlings originated by these seeds. Genotypes show differences in their tolerance to drought and saline stress; tolerance can be identified during the early stages of development and favors seedling formation in the establishment phase.

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