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RESEARCH ARTICLE

Antioxidant activity and seed vigor in germination of bean under salt stress conditions

Matheus Santin Padilha[1]; Cileide Maria Medeiros Coelho[1]*; Yasmin Pincegher Siega[1]

[1] Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Programa de Pós-Graduação em Produção Vegetal (PPGPV), Av. Luiz de Camões, 2090, Conta Dinheiro, 88520-000, Lages, SC, Brasil.

* Corresponding author: cileide.souza@udesc.br (C. M. M. Coelho).

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Abstract

Using seeds with higher physiological potential can help overcome saline stress, affecting many arable areas in tropical and subtropical regions. This study aimed to evaluate whether seed vigor contributes to overcoming saline stress, seeking to identify the association between the antioxidant system and seed lot vigor. Seeds of the BAF55 genotype with two levels of vigor were used. The seeds were germinated under no-stress conditions, with 75 and 150 mmol L^{-1} of sodium chloride in the solution during germination. After five days, morphological changes and changes in the enzyme's catalase, ascorbate peroxidase, guaiacol peroxidase, proline, malondialdehyde, and hydrogen peroxide were evaluated. An increase in antioxidant activity was observed with the imposed stresses and no significant difference was observed between the vigor level, except in the condition of 75 mmol L^{-1} in the hypocotyl of the seedlings and, for proline in the condition of 150 mmol L^{-1} in which the low-vigor presented greater activity. The stress of 150 mmol L^{-1} showed greater severity in seeds of low-vigor, resulting in greater lipid peroxidation in the seedlings formed and resulting in seedlings with lower performance.

Keywords: *Phaseolus vulgaris* L.; catalase; proline; peroxidase; malondialdehyde; seedling vigor.

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1. Introduction

The bean culture (*Phaseolus vulgaris* L.) is recognized for being a source of proteins, carbohydrates, vitamins, minerals, and antioxidants for human consumption. Considering that the crop is cultivated worldwide, one of the main aspects studied is the response to abiotic stresses, such as salt stress, present in several producing regions that result in losses in vegetative performance and, consequently, in production (Egea et al., 2023).

The use of seeds of higher physiological quality (i.e., germination and vigor) is crucial for the success of the production system, as it favors seedling stand and subsequent productivity (Reis et al., 2022). Among the aspects associated with the better performance of seeds with high-vigor, the mobilization and use of reserves is a determining point, because it favors the formation of seedlings with superior performance (Padilha et al., 2024).

Under salt stress, there is a reduction in germination speed, germination percentage, and lower performance of bean seedlings. This occurs due to osmotic restriction and the accumulation of Na+ and Cl^- ions in plant tissues, resulting in a toxic effect (Alharbi et al., 2022). Plants undergo several metabolic changes that are strategies of the plant organism to overcome or adapt to the stress present (Egea et al., 2023), with the plant antioxidant system being a vital component in this process.

This antioxidant system is composed of enzymes and non-enzymes that favor the balance between reduction and oxidation (redox) during stress conditions, seeking to maintain growth. Under abiotic stress conditions, there is an accumulation of reactive oxygen species, which results in damage to proteins, lipids, and genetic material (Wang et al., 2024). To achieve redox balance during abiotic stress conditions, plants synthesize enzymes (e.g., catalase, peroxidases, superoxide dismutase) and metabolites (e.g., proline, sugars, ascorbic acid) that totally or partially neutralize reactive oxygen species (Soares et al., 2019; Zulfiqar & Asharf, 2022).

The importance of the antioxidant system during saline stress conditions was verified by Alzahrani et al. (2019), in which *Vicia fava* L. plants with higher antioxidant activity (i.e., catalase, superoxide dismutase, proline) showed superior performance, this relationship being associated with genotypic tolerance. However, during the germination of bean seeds under salt stress, the higher proline content was not associated with seedlings produced by high-vigor seeds or genotypic tolerance (Padilha et al., 2022). Similar results were observed for *Vigna radiata* L. (Rohman et al., 2019) and beans (Taïbi et al., 2021) in which the genotypes tolerant to salt stress showed higher catalase and ascorbate peroxidase activity, but without correlation with proline content.

However, studies associated with this theme are generally directed at the genotypic response to stress, without highlighting the response of seed lot vigor under saline stress conditions or how the antioxidant system is associated with seed lot vigor under these conditions. Therefore, the objective of the present study was to determine the physiological response of beans with contrast in seed lot vigor under salt stress conditions, verifying the association between seed lot vigor and the antioxidant system of seedlings during germination.

2. Methodology

Plant material and obtaining lots with contrasting vigor

To experiment, seeds of the BAF55 genotype, belonging to the active bean germplasm bank of the Centro de Ciências Agroveterinárias (CAV) of the Universidade do Estado de Santa Catarina (UDESC), were used. The genotype used was selected considering its agronomic characteristics and tolerance to water stress (Padilha et al., 2022). The seed lot was produced in the 2020/2021 harvest in the municipality of Lages, Santa Catarina, Brazil, in the experimental area of CAV-UDESC. After harvesting, the seed lot underwent the cleaning and standardization process.

The obtained lot was segregated into two levels of vigor using artificial aging from the accelerated aging methodology in saturated saline solution (Jianhua & McDonald, 1997). The aged lot was kept for seven days in an aging chamber (Tecnal/TE-410/Brazil) at a temperature of 41 ºC. After each aging period, the lots were dried in an air circulation oven (Lucadema/LUCA-80/42/Brazil) until they reached 13% moisture content. This results in two seed lots of the same genotype, namely: The lot with high-vigor (HV), which was not aged, and the seed lot with low-vigor (LV), aged for seven days (Figure 1).

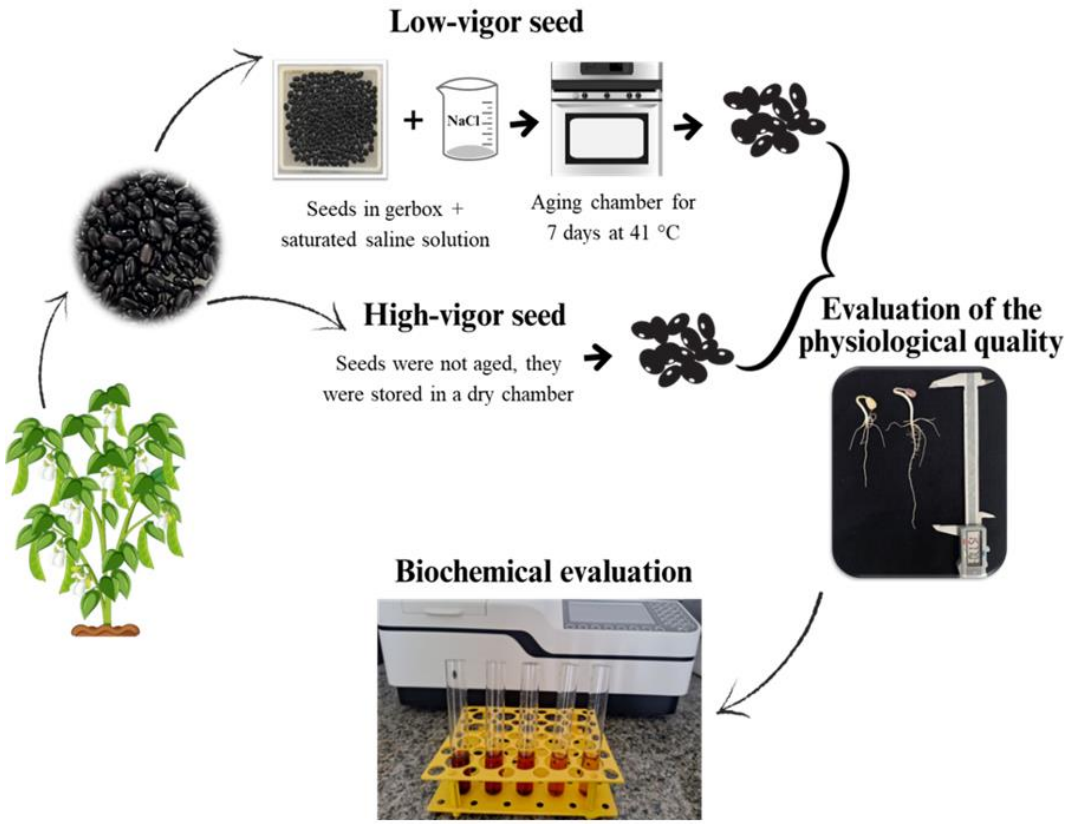

Figure 1. Steps of the study methodology. Created with canva.com

Evaluation of the physiological quality

The verification of physiological quality, as well as the physiological response of seeds to salt stress conditions, was determined using sodium chloride (NaCl, 99%, Sigma-Aldrich) saline solution as solute during germination. The germination test was conducted with four replicates of 50 seeds, which were distributed on filter paper in the form of a paper roll moistened with distilled water (i.e., 0 mmol L^{-1} of NaCl), NaCl solution with 75 mmol L^{-1} and 150 mmol L^{-1}. The paper rolls were kept in a germinator (J Prolab/Mangelsdorf/Brazil) at a temperature of 23 ± 2 ºC. The germination percentage was determined by evaluating normal seedlings after nine days.

The evaluation of seedling performance was performed five days after sowing using four replicates of 20 seeds. Using a digital caliper, the root length (RL), hypocotyl length (HL), and total length (TL) were measured. After evaluation, the seedlings and cotyledons were dried in an air circulation oven (Lucadema/LUCA-80/42/Brazil) at 80 ºC for 24 hours to determine the dry mass of root (DMR), hypocotyl (DMH), total (DMT) and dry mass remaining in cotyledons (DMRC). The results were expressed in centimeters per seedling (cm) and milligrams per seedling (mg).

The data were used to determine the seed reserve reduction (SRR), the root-hypocotyl ratio, and the seed reserve use efficiency (SRUE) (Soltani et al., 2006). The proportion of reserve mobilization to the roots (PRMR) and hypocotyl (PRMH) was determined from the data of DMR, DMH, and DMT and expressed as a percentage. These variables represent the proportion of dry mass mobilized to the roots and hypocotyl about the total dry mass of seedlings.

Biochemical evaluations

During the same period of physiological evaluation of seedling performance, normal seedlings obtained under conditions of absence and presence of salt stresses were collected and dissected into root, hypocotyl and cotyledons, frozen in liquid nitrogen and ground for analysis of ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT), malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline. For the cotyledons, starch content and alpha-amylase activity were also determined.

The enzymatic extract obtained from each biological replication was performed using 200 mg of fresh sample and macerated in a mortar with 5 mL of 100 mM potassium phosphate (KH_2PO_4, >98%, Dinâmica) buffer, pH 7.2; containing 1 mM of EDTA ($C_{10}H_{16}N_2O_8$, 99%, Biotec).

APX determination was performed as described by Nakano and Asada (1981), with modifications, in which the incubation medium was prepared using 700 µL of 50 mM potassium phosphate (KH_2PO_4, >98%, Dinâmica) buffer pH 7.2; 100 µL of 4 mM ascorbic acid ($C_6H_8O_6$, >99%, Neon) solution and; 100 µL of 1 mM hydrogen peroxide (H_2O_2, 20% v/v, Biotec) solution, with the reaction starting with the addition of 100 µL of enzymatic extract. The reading was performed in a spectrophotometer (Ion Lab/IL-0082-Y-BI/Brazil) at 290 nm for 60 seconds. One unit of APX was defined as the amount of enzyme required to oxidize 1 µM ascorbate per minute.

For GPX quantification, guaiacol was used as substrate (Nakano & Asada, 1981), performed according to the procedure of Simões et al. (2015) with modifications, in which the incubation medium was prepared using 700 µl of 200 mM sodium phosphate ($NaH_2PO_4H_2O$, >98%, Dinâmica) buffer, pH 6.0; 100 µL of 40 mM guaiacol ($C_7H_8O_2$, ≥99%, Sigma-Aldrich) and 100 µl of 10 mM hydrogen peroxide (H_2O_2, 20% v/v, Biotec), and the reaction was started by adding 100 µL of enzyme extract. The reading was carried out in a spectrophotometer (Ion Lab/IL-0082-Y-BI/Brazil) at 470 nm for 30 seconds. One unit of GPX was defined as the amount of enzyme required to perform the formation of 1 µM tetraguaiacol per minute.

For CAT quantification, the procedure proposed by Aebi (1984) was used with alterations, in which the incubation medium was performed using 2000 µl of 100 mM potassium phosphate (KH_2PO_4, >98%, Dinâmica) buffer, pH 7.2; 800 µL of 65 mM hydrogen peroxide (H_2O_2, 20% v/v, Biotec), and 200 µl of enzymatic extract. The reading was carried out in a spectrophotometer (Ion Lab/IL-0082-Y-BI/Brazil) at 240 nm for 120 seconds. One enzyme unit was defined as the amount of enzyme required to perform the degradation of 1 µM hydrogen peroxide per min.

MDA was determined according to the procedure described by Hodges et al. (1999). A total of 50 mg of sample macerated in 1 mL of 80% alcohol (v/v) was used, which was macerated in mortar and centrifuged (Solab/SL-706/Brazil). Quantification was performed using 1 mL of properly diluted extract and 1 mL of 20% (w/v) trichloroacetic acid (CCl_3COOH, 99%, Dinâmica) solution containing 0.65% (w/v) thiobarbituric acid ($C_4H_4N_2O_2S$, ≥98%, Sigma-Aldrich) and 0.01% (w/v) *butylated hydroxytoluene* ($C_{15}H_{24}O$, ≥99%, Sigma-Aldrich). The reaction was maintained in a water bath (Solab/SL-154/Brazil) at 95 ºC for 25 min and the readings were taken in a spectrophotometer (Ion Lab/IL-0082-Y-BI/Brazil) at 440, 532, and 600 nm,

and subsequently calculated as specified in Hodges et al. (1999).

The determination of H_2O_2 was performed as described by Alexieva et al. (2001) with modifications. 100 mg of previously macerated samples were homogenized in 4 mL of 0.1% trichloroacetic acid (w/v) and subsequently centrifuged (Solab/SL-706/Brazil). For the reading, 0.5 mL of supernatant was used, 0.5 mL of potassium phosphate (KH_2PO_4, >98%, Dinâmica) buffer, pH 7.0; 50 mM and 1 mL of potassium iodide (KI, >99%, Anidrol) were added. The samples were left to rest for 30 min in the dark and subsequently, the reading was performed in a spectrophotometer (Ion Lab/IL-0082-Y-BI/Brazil) at 390 nm. The H_2O_2 content was determined from a standard curve.

The proline content was determined as described by Bates et al. (1973) without modifications, from a standard curve with known proline values. The results were expressed in µmol of proline per gram of fresh mass.

The starch content of the cotyledon samples was determined as described by McCready et al. (1950). Starch extraction was performed using double extraction with 52% perchloric acid ($HClO_4$, 72%, Nuclear). Quantification was performed with 1 mL of the properly diluted extract and 3 mL of antrone ($C_{14}H_{10}O$, 99%, Dinâmica) reagent, followed by vortexing (Tecnal/AP-56/1/Bazil) for 3 s. The test tubes were kept in a water bath (Solab/SL-154/Brazil) at 95 ºC for 450 s. The reading was performed at an absorbance in a spectrophotometer (Ion Lab/IL-0082-Y-BI/Brazil) at 630 nm. With the results obtained, the starch reduction rate.

Alpha-amylase activity was determined by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959) using the procedure described by Monerri and Guardiola (1986) with modifications. To obtain the enzymatic extract, 300 mg of sample were extracted with 5 mL of sodium acetate ($C_2H_3NaO_2$, 99%, Sigma-Aldrich) buffer, pH 5.6; with the presence of 10 mmol of calcium chloride ($CaCl_2$, 99%, Sigma-Aldrich). The samples were kept in agitation (Solab/SL-222T/Brazil) for 60 min in the presence of ice and then centrifuged (Solab/SL-706/Brazil). The extract was incubated in a water bath (Solab/SL-154/Brazil) for 15 min at 70 ºC. Quantification was performed using 0.25 mL of enzyme extract and 0.25 mL of soluble starch ($C_6H_{10}O_5$, >99%, Synth) solution containing 2.0% (w/v). The samples were kept in a water bath (Solab/SL-154/Brazil) for 20 min at 38 ºC. The reaction was stopped with the addition of 0.5 mL of DNS solution and the samples were subsequently kept in a water bath (Solab/SL-154/Brazil) for 6 min at 95 ºC. The reaction was stopped in an ice bath, and, after cooling, 4 mL of distilled water was added. The reading was performed at an absorbance in a spectrophotometer (Ion Lab/IL-0082-Y-BI/Brazil) at 540 nm. One enzyme unit was defined as the amount of enzyme to produce one µmol of maltose per minute under the evaluated conditions. The results were expressed as enzyme units per milligram of protein (U mg^{-1}).

Statistical analysis

The experimental design used was completely randomized in a 2x3 factorial arrangement, with two lots (i.e., HV and LV) and three germination conditions (i.e., 0, 75, and 150 mmol L^{-1} of NaCl) with four replications. The data were submitted to a normality test when necessary and subsequently, analysis of variance (ANOVA) was performed, and the means were compared by Tukey's test at 5% probability using the Sisvar software (Ferreira, 2011).

3. Results and discussion

Evaluation of the physiological quality

The lots showed significant differences in their physiological quality, in which the seeds with high-vigor (i.e., not artificially deteriorated) showed a higher germination percentage than a lot of seeds with low-vigor under the conditions of water stress. In the absence of stress, both lots showed similar germination (Table 1). According to Marcos-Filho (2015), seed lots with high-vigor have a greater capacity to overcome abiotic stresses imposed during germination and emergence. In this sense, the seed lot used with greater vigor demonstrated this greater ability to form normal seedlings under the conditions of salt stress imposed during germination, expressed by its greater physiological potential.

Table 1
Germination of bean seed lots of the BAF55 genotype with high-vigor and low-vigor subjected to conditions of stress by sodium chloride (NaCl)

NaCl (mmol L^{-1})	Germination (%)	
	High-Vigor	Low-Vigor
0	88 aA	80 aA
75	85 aA	70 bAB
150	80 aA	65 bB
Coefficient of variation	7.88	

Means followed by the same lowercase letter in the row and uppercase in the column do not differ statistically from each other by Tukey's test at 5% probability.

The difference in the physiological quality of the lots can be observed in the growth parameters, in which the lot of seeds with high-vigor showed a greater capacity to form seedlings with greater length and dry mass of root, hypocotyl, and total in the condition of absence of salt stress (Table 2).

Table 2
Root length (RL), hypocotyl length (HL), total length (TL), dry mass of root (DMR), dry mass of hypocotyl (DMH), dry mass of total (DMT), reserve mobilization to the roots (PRMR), reserve mobilization to the hypocotyl (PRMH), root-hypocotyl ratio (RH), dry mass remaining in cotyledons (DMRC), seed reserves reduction (SRR) and seed reserve use efficiency (SRUE) of seed lots with high (HV) and low-vigor (LV) submitted to sodium chloride (NaCl) stress conditions

NaCl (mmol L^{-1})	RL (cm pl^{-1})		HL (cm pl^{-1})		TL (cm pl^{-1})		DMR (mg pl^{-1})	
	HV	LV	HV	LV	HV	LV	HV	LV
0	14.3 aA	13.1 bA	8.0 aA	6.4 bA	22.3 aA	19.9 bA	10.7 aA	8.2 bA
75	5.2 aB	4.6 bB	3.4 aB	3.0 bB	8.6 aB	7.6 bB	7.4 aB	7.1 aB
150	3.0 aC	2.7 bC	1.9 aC	1.1 aC	4.9 aC	4.4 aC	5.0 aC	4.1 bC
CV	5.33		4.21		3.70		5.97	
NaCl (mmol L^{-1})	DMH (mg pl^{-1})		DMT (mg pl^{-1})		PRMR (%)		PRMH (%)	
	HV	LV	HV	LV	HV	LV	HV	LV
0	46.2 aA	34.4 bA	56.9 aA	42.6 bA	18.9 aC	19.3 aB	81.2 aA	80.7 aA
75	22.3 aB	19.4 bB	29.7 aB	26.8 bB	25.0 aB	26.8 aA	75.0 aB	73.2 aB
150	11.3 aC	9.8 bC	16.3 aC	13.9 bC	30.9 aA	29.4 aA	69.2 aC	70.6 aB
CV	6.94		5.83		6.36		2.12	
NaCl (mmol L^{-1})	RH		DMRC (mg $seed^{-1}$)		SRR (mg $seed^{-1}$)		SRUE (mg mg^{-1})	
	HV	LV	HV	LV	HV	LV	HV	LV
0	1.8 bA	2.1 aA	95.8 bC	109.4 aC	75.6 aA	59.2 bA	0.8 aA	0.7 aA
75	1.5 aB	1.5 aB	128.9 aB	130.4 aB	39.5 aB	33.6 bB	0.8 aA	0.7 aA
150	1.6 aB	1.6 aB	147.2 aA	150.0 aA	23.3 aC	18.3 bC	0.7 aA	0.8 aA
CV	5,56		2,22		6,62		7,62	

Means followed by the same lowercase letter in the row and uppercase in the column do not differ statistically from each other by Tukey's test at 5% probability. CV: Coefficient of variation.

The best performance of seeds with high-vigor under salt stress conditions was also observed in the variables root length (RL), root dry mass (RDM), hypocotyl (RDH), and total (RDT) (Table 2), demonstrating the greater potential of seeds with superior vigor to overcome these conditions. In this context, Padilha et al. (2022) highlight that vigorous seeds under stress conditions have a greater capacity to mobilize reserves, which favors growth under these salt-stress conditions.

This relationship between reserve mobilization can be observed in the results of seed reserve reduction (SRR), in which the seeds with high-vigor showed a greater reduction in reserve, which favored the formation of seedlings with superior performance. According to Cheng et al. (2018), the greater reduction in seed reserves is a determining factor for the formation of seedlings with higher dry mass, and; in the present study, this relationship was verified for high-vigor seeds under conditions of abiotic stress (Table 2).

The response of seedlings formed during stress can be verified by the proportion of reserve mobilization and the root-hypocotyl ratio. In the absence of stress, the mobilization of seed reserves showed approximately 80% for hypocotyl (PRMH) and 20% for roots (PRMR). However, when subjected to salt stress conditions, PRMH was reduced according to the intensity of the stress and the PRMR increased in stress (Table 2). This association demonstrates that there is a change in the mobilization of reserves under saline stress conditions, with most of the mobilization going to the roots. According to Kakar et al. (2019), investment in the root system is a strategy to overcome salt stress. Considering that the genotype is the same, the response of the mobilization proportion for the structures was the same, indicating that there was no influence of vigor in changing the destination of the reserves during stress.

The root-hypocotyl ratio was reduced to 75 and 150 mmol L^{-1} stresses, indicating that the length of the root system was more affected than the shoot. In this context, deep growth was more severely affected and, for this reason, the greatest need to mobilize reserves for the root occurred, seeking to favor root performance to overcome stress (Table 2).

The seed reserve use efficiency (SRUE) did not present a significant difference between the lots used and was not influenced by the salt stress condition, indicating that the SRUE was not a determinant for seedling formation (Table 2). Thus, it is confirmed that the SRR is the determining factor for the best performance under conditions of abiotic stress.

Biochemical evaluations

Alpha-amylase activity and starch degradation showed significant differences in the control condition between vigor levels, but no difference was observed under saline stress conditions, indicating little relationship between the activity of this enzyme and starch hydrolysis under saline stress conditions. However, a reduction in enzymatic activity (Figure 2A) and starch hydrolysis (Figure 2B) was observed, corroborating the results of SRR (Table 2).

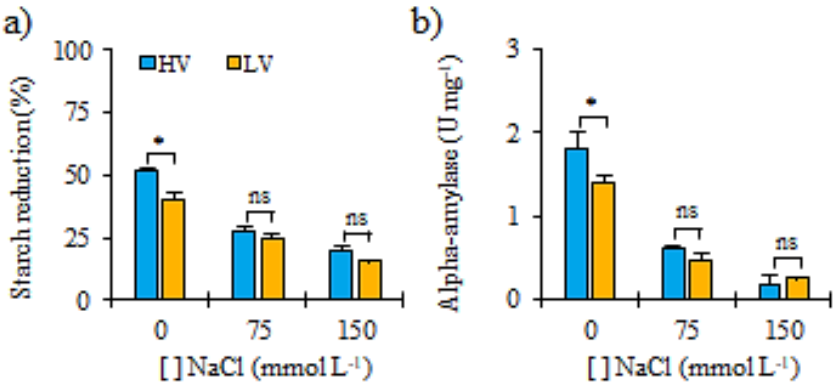

Figure 2. Starch reduction rate (a) and alpha-amylase activity (b) were evaluated in cotyledons for seed lots with high (HV) and low-vigor (LV) of the BAF55 genotype under saline stress conditions at five days of germination. *: indicates a significant difference between vigor levels.

The absence of a relationship between alpha-amylase activity, starch hydrolysis, and seed lot vigor under salt stress conditions was verified by Padilha et al. (2024), and this result is explained by the delay in the germination and hydrolysis process. According to Bewley et al. (2013) starch hydrolysis in dicotyledons occurs initially by other enzymes, such as starch phosphorylase and after the fifth day of germination, there is a marked increase in alpha-amylase synthesis. This relationship explains the absence of difference in alpha-amylase activity between vigor levels under stress conditions.

The proline content determined in seedlings and cotyledons showed an increase under salt stress conditions, in which both hypocotyl (Figure 3A) and cotyledons (Figure 3C) increased as the stress increased, and for the roots, the increase was observed only in the condition of 150 mmol L^{-1} (Figure 2B). Under the condition of 150 mmol L^{-1}, a significant difference was observed between the vigor levels when the proline content was determined in roots (Figure 3B).

Proline is an amino acid produced under abiotic stress conditions, and, under salt stress conditions, the highest concentration of proline is observed due to the need for osmotic regulation in cells (Nadeem et al., 2019). The results obtained highlight that seeds with lower vigor showed a greater need for proline synthesis in roots to maintain plant homeostasis, especially when under conditions of more severe stress (i.e., 150 mmol L^{-1}) (Figure 3).

The malondialdehyde (MDA) content increased under the stress conditions used when compared to the control, and this response was observed in both seed lots. The significant difference between the vigor levels was observed at 150 mmol L^{-1} in the hypocotyl of the seedlings (Figure 3D). Lipid peroxidation in roots (Figure 3E) and cotyledons (Figure 3F) showed an increase, however, without a difference between the vigor levels.

The MDA content can be used as an indicator of abiotic stress, in which it evaluates the damage to cell membranes suffered by a plant tissue during an abiotic stress condition (Soares et al., 2019). According to Alzahrani et al. (2019), cultivars of *Vicia faba* L. com greater tolerance to salt stress present lower lipid peroxidation (i.e., MDA) and consequently better performance during stress conditions. In this sense, seedlings produced by seeds of high-vigor under the stress condition 150 mmol L^{-1} showed lower lipid peroxidation than seedlings produced by seeds of low-vigor, indicating greater susceptibility of seedlings originated by seeds of low-vigor under severe conditions of salt stress.

The accumulation of H_2O_2 in the hypocotyl increased with stress levels, especially for the low-vigor lot, in which, in the condition of 75 mmol L^{-1}, the seeds showed a higher concentration of H_2O_2 (Figure 3G). The higher concentration of this reactive oxygen species indicates a greater quantity of this molecule to be metabolized or that can generate physiological damage by reacting with proteins and DNA in seedlings originating from low-vigor seeds (Mittler, 2017). The H_2O_2 determined in roots (Figure 2H) and cotyledons (Figure 3I) did not show a significant difference between vigor levels.

A higher concentration of H_2O_2 was observed in seedlings during the germination of cotton seeds under salt stress, which resulted in lower germination performance (Chen et al., 2020). Similar data were found in this study, in which the increase in H_2O_2 content in seedlings, especially in the condition of 150 mmol L^{-1} of NaCl, favored the loss of physiological performance (Table 2).

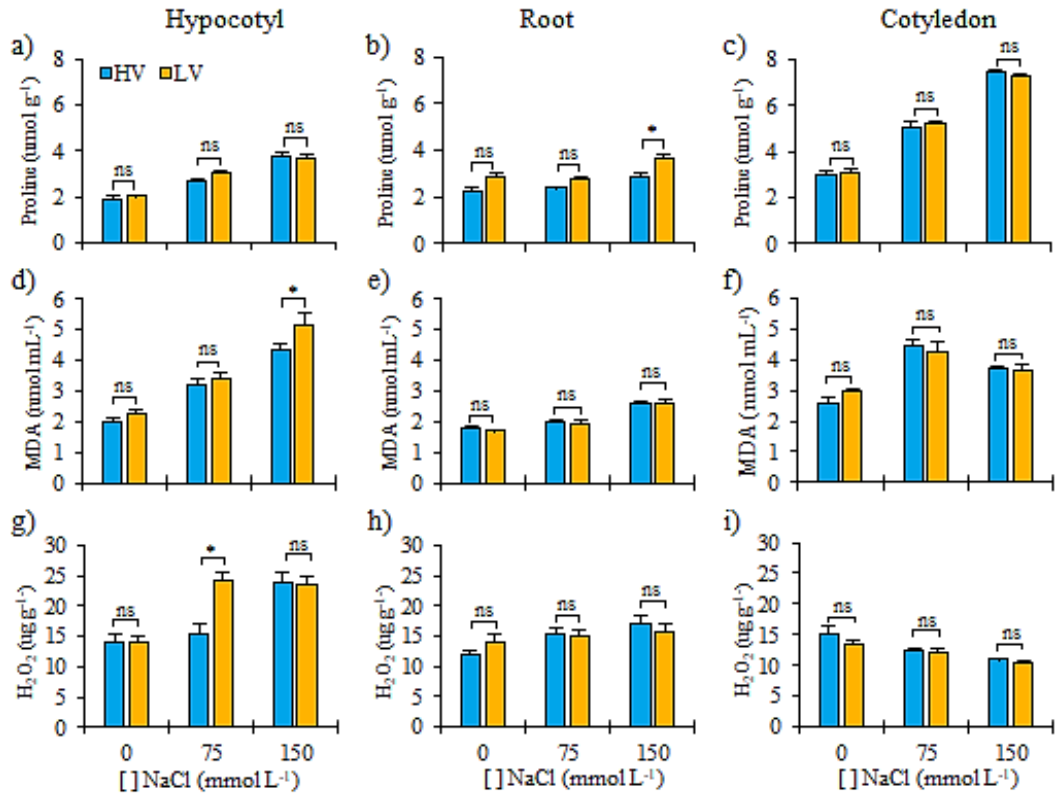

Figure 3. Proline, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) determined in hypocotyl (a, d, and g), root (b, e, and h), and cotyledons (c, f, and i) of seed lots with high (HV) and low-vigor (LV) under salt stress conditions.

The enzymes guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) showed a similar response to salt stress, in which their activity in the hypocotyl was higher than the control (Figure 4A, 4D). However, the significant difference between the vigor of the seed lot was observed only in the concentration of 75 mmol L^{-1} for the activity of the enzyme GPX low-vigor seeds showed higher activity. When evaluated in roots, the activity of these enzymes was higher than the control only at 150 mmol L^{-1}, and no significant difference was observed between the vigor of the seed lot in any germination condition (Figures 4B, 4E). In cotyledons, the activity of these two enzymes was lower under the stresses used, demonstrating that the response to salt stress is negative in this structure (Figures 4C, 4F).

The catalase enzyme (CAT) showed a reduction in all structures evaluated at both levels of stress and vigor (Figure 3G, 3H, 3I). Low-vigor seeds showed higher CAT activity in hypocotyl at 75 mmol L^{-1}. The higher activity of GPX and CAT at 75 mmol L^{-1} in hypocotyl (Figure 4A, 4G) for low-vigor seeds is associated with a higher concentration of H_2O_2 (Figure 2G). According to Nadeem et al. (2019) during salt stress conditions there is a greater need to produce enzymes associated with the antioxidant system to avoid the oxidative damage generated by reactive oxygen species and return to redox equilibrium. Thus, the higher enzymatic activity at 75 mmol L^{-1} demonstrated by low-vigor seeds is a response to avoid oxidative damage by H_2O_2 and maintain the vital conditions in the balance of the seedlings originated by these seeds.

On the other hand, under the 150 mmol L^{-1} condition, no significant difference was observed between the evaluated enzymes; however, the highest lipid peroxidation was verified for low-vigor seeds (Figure 3D), indicating that, even with no significant difference in the enzymatic activity of GPX, APX, CAT (Figure 4A, 4D, 4G) and proline concentration (Figure 3A), seedlings originated from lower-vigor seeds were more susceptible to the 150 mmol L^{-1} stress. This association indicates that other mechanisms may be associated with seedlings originating from more vigorous seeds, which prevented lipid peroxidation in the seedlings. According to Alzahrani et al. (2019), the content of antioxidant molecules (i.e., ascorbic acid) and enzymes (i.e., glutathione reductase and superoxide dismutase) favor the condition of overcoming stress by preventing lipid peroxidation.

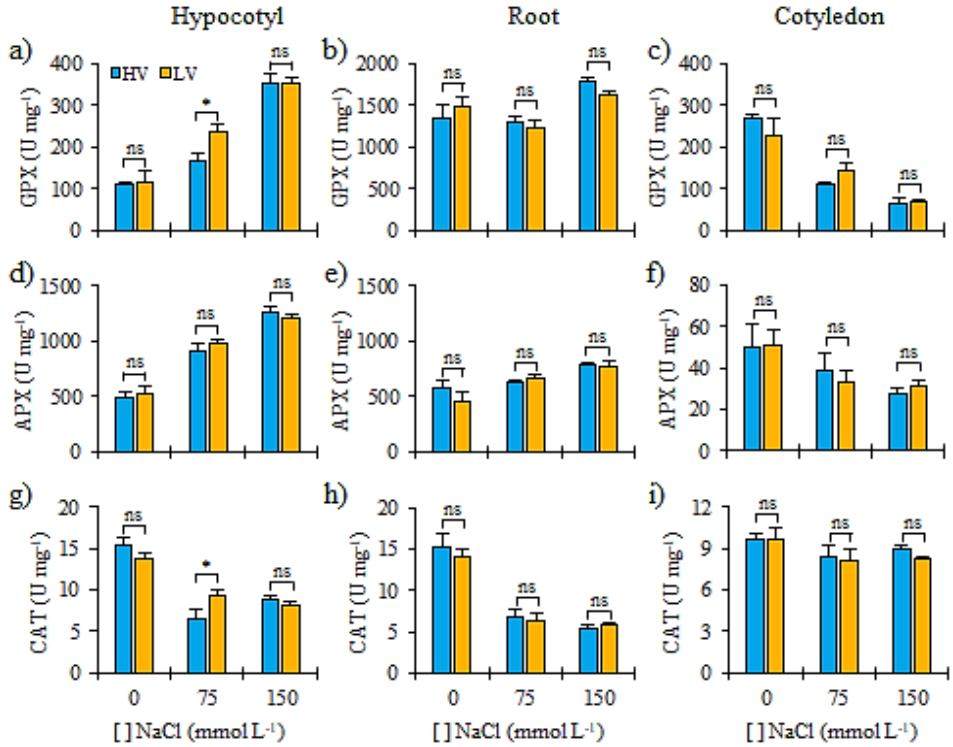

Figure 4. Guaiacol peroxidase (GPX), ascorbate peroxidase (APX), and catalase (CAT) activities in hypocotyls (a, d and g), roots (b, e, and h) and cotyledons (c, f, and i) of seed lots with high (HV) and low-vigor (LV) under saline stress conditions.

The increase in the level of salt stress results in a greater need for the synthesis of enzymes and molecules associated with the antioxidant system since it increases the intensity of the stress imposed on the seedlings (Taïbi et al., 2021). In this context, the stress of 150 mmol L^{-1} showed greater severity in low-vigor seeds, resulting in greater lipid peroxidation in the seedlings formed (Figure 3D) and in seedlings with lower performance (Table 2).

In this sense, the better in germination (Table 1) and seedlings (Table 2) performance demonstrated by seeds with high-vigor was not related to the higher activity of the antioxidant system evaluated in seedlings at 5 days of germination, and another mechanism may be associated with better performance. During the seed deterioration process, several biochemical changes occur, which result in loss of physiological performance during germination, among them, lipid peroxidation (Ebone et al., 2019); this relationship was verified in the seed lots used, with the highest lipid peroxidation in the embryonic axis and cotyledons of low-vigor seeds after deterioration and, no significant difference was found between the H_2O_2 concentration determined from the seeds used (Table 3). Thus, it is suggested that the higher initial lipid peroxidation favored the increase of abnormal seedlings in low-vigor seeds (Table 1), which generated greater sensitivity to the imposed stress.

Given this, the higher lipid peroxidation after the embryonic stress condition observed in the low-vigor lot, favored the greater susceptibility during germination under the saline stress conditions, being: in the condition of 75 mmol L^{-1} due to the greater accumulation of H_2O_2 (Figure 3G) CAT and GPX activity in hypocotyl (Figure 4A, 4G) and; in stress by 150 mmol L^{-1} by higher lipid peroxidation in the hypocotyl and proline concentration in roots (Figure 3B, 3D).

Table 3
Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) content determined in embryonic axis and cotyledons of high (HV) and low-vigor (LV) seed lots after artificial aging

Structure	MDA (nm mL^{-1})				H_2O_2 (ug g^{-1} MF)			
	HV	LV	*p*-valor	CV	HV	LV	*p*-valor	CV
Embryonic axis	4.72	5.9	0.013	8.99	5.74	5.17	0.687	34.76
Cotyledons	2.47	2.73	0.021	4.56	5.03	4.63	0.604	21.48

CV: Coefficient of variation.

In general, seed lots with a higher level of deterioration present a slower repair speed of structural membranes of the seeds during imbibition, resulting in loss of solutes, and slow and uneven germination; as well, they demonstrate lower protection capacity, greater lipid peroxidation in seeds, and damage to genetic material (Ebone et al., 2019).

Thus, the results indicate that low-vigor seeds, due to greater deterioration when subjected to saline stress conditions, have a greater need for enzyme synthesis and proline compared to the high-vigor lot, seeking to overcome the abiotic stress condition. In this context, the results highlight that the better performance of the high-vigor lot is partially explained by the greater SRR capacity even under salt stress conditions, as it favors seedlings' performance. The association of other molecules and enzymes associated with the antioxidant system during germination can help to understand the best response of seed lot vigor to germination and seedling performance under conditions of abiotic stress.

4. Conclusions

Seeds with high-vigor exhibit superior performance during germination under saline stress conditions. The greater capacity for hydrolyzing reserve components, especially soluble reducing sugars, is essential for the formation of viable seedlings in these adverse conditions, while antioxidant system activity does not appear to be the main differentiating factor between high and low-vigor seedlings. Low-vigor seeds, in turn, show greater susceptibility to saline stress due to increased lipid peroxidation in the embryonic axis, associated with the deterioration process.

Future studies are recommended to further explore the biochemical and physiological mechanisms related to reserve hydrolysis and lipid degradation, as well as to investigate other metabolic components that may contribute to saline stress tolerance. Additionally, research into the role of other non-enzymatic antioxidants and their impact on germination under stress conditions may offer new insights.

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Author's Contribution

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

ORCID

M. S. Padilha https://orcid.org/0000-0001-6622-9252
C. M. M. Coelho https://orcid.org/0000-0001-9528-7371
Y. P. Siega https://orcid.org/0000-0002-0484-4564

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