Special Supplement: Pulses

Antioxidant response of bean seeds with contrasting vigor to cold stress[1]

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ABSTRACT

Seed germination is a critical part of plant emergence and establishment in the field, and cold stress can affect the emergence potential and seedling formation. The present study aimed to determine the differences in the plant antioxidant system among common bean seeds with contrasting vigor and assess the relationship between seed lot vigor and antioxidant system. Six seed lots of common bean genotypes (BAF42, BAF44 and BAF55) with contrasting vigor levels (high and low vigor) were submitted to germination and cold tests. Lipid peroxidation, hydrogen peroxide, catalase, guaiacol peroxidase and ascorbate peroxidase were evaluated after cold stress. The high-vigor seeds were better able to overcome the cold stress, with the catalase enzyme activity more associated with vigor. Low-vigor seeds are more susceptible to stress, with high physiological damage in seedlings, due to the greater hydrogen peroxide accumulation in the embryonic axis. In order to maintain metabolic homeostasis, the low-vigor seeds increase the production of antioxidant enzymes in the seedlings, thus exhibiting a greater antioxidant enzymatic activity and hydrogen peroxide accumulation in response to cold stress.

KEYWORDS: *Phaseolus vulgaris* L., antioxidant system, physiological seed quality, seedling performance.

RESUMO

Resposta antioxidante de sementes de feijão com vigor contrastante ao estresse por frio

A germinação de sementes é uma parte crítica da emergência e estabelecimento de plantas no campo, e o estresse por frio pode afetar o potencial de emergência e a formação de mudas. Objetivou-se determinar as diferenças no sistema antioxidante da planta entre sementes de feijão comum com vigor contrastante e avaliar a relação entre o vigor do lote de sementes e o sistema antioxidante. Seis lotes de sementes de genótipos de feijão comum (BAF42, BAF44 e BAF55) com níveis de vigor contrastantes (alto e baixo vigor) foram submetidos a testes de germinação e frio. Peroxidação lipídica, peróxido de hidrogênio, catalase, peroxidase de guaiacol e peroxidase de ascorbato foram avaliados após o estresse por frio. As sementes de alto vigor foram mais capazes de superar o estresse por frio, com a atividade da enzima catalase mais associada ao vigor. Sementes de baixo vigor são mais suscetíveis ao estresse, com alto dano fisiológico nas mudas, devido ao maior acúmulo de peróxido de hidrogênio no eixo embrionário. Para manter a homeostase metabólica, as sementes de baixo vigor aumentam a produção de enzimas antioxidantes nas mudas, exibindo, assim, maior atividade enzimática antioxidante e acúmulo de peróxido de hidrogênio em resposta ao estresse por frio.

PALAVRAS-CHAVE: *Phaseolus vulgaris* L., sistema antioxidante, qualidade fisiológica de sementes, desempenho de plântulas.

INTRODUCTION

Bean is an essential crop for the human diet and an important source of proteins, carbohydrates and minerals (Nadeem et al. 2021). The global common bean production has reached 12 million metric tons, with India, Myanmar and Brazil standing out as the main producing countries (FAO 2023). However, several production challenges, mainly associated with abiotic stresses, result in reduced crop performance and grain losses (Lone et al. 2021).

Seed quality is an important trait in bean production, with high-quality seeds ensuring a better establishment in the field and higher grain yields (Mondo et al. 2016). Seed quality is determined by physical, physiological, genetic and health parameters. Because of their direct impact on production system success, physiological parameters (i.e., germination and vigor) are some of the most widely studied in the literature (Marcos-Filho 2015). Vigor is a key characteristic in seed lots, since it favors the formation of normal seedlings during

[1] Received: Nov. 06, 2024. Accepted: Feb. 28, 2025. Published: Mar. 27, 2025. DOI: 10.1590/1983-40632025v5580995.

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Editor: Luis Carlos Cunha Junior

germination and emergence in the field under a wide range of environmental conditions (Finch-Savage & Bassel 2016).

Abiotic stresses, such as salinity, drought, nutrient deficiency and extreme temperatures, compromise the performance during seedling emergence in the field (Ali et al. 2022). Cold stress is among those that cause yield loss, with some of the physiological, biochemical and molecular responses observed during vegetative growth linked to the plants' ability to tolerate cold stress (Bhat et al. 2022).

In corn crops under cold stress, Sbrussi & Zucarelli (2014) observed a decline in the germination speed and percentage, as well as seedlings performing poorly, whereas high-vigor seeds performed better under these conditions. Additionally, the superior cold-tolerance of high-vigor seeds has been reported in bean (Gindri et al. 2017), soybean (Kaya et al. 2016) and corn (Sbrussi & Zucarelli 2014). However, these studies do not identify which mechanisms favor the optimal performance in high-vigor seeds under these conditions.

One of the main responses associated with better plant performance under cold stress is greater antioxidant enzyme activity (Ali et al. 2022). Changes that may occur during cold stress include the reduction of the plasma membrane function due to increased rigidity, loss of membrane integrity and the accumulation of reactive oxygen species (e.g., hydrogen peroxide and superoxide radical) (Bhat et al. 2022, Lone et al. 2021). Thus, an increased antioxidant activity is needed to restore homeostasis and, consequently, normal metabolic activity (Mittler 2017).

The higher antioxidant activity is associated with a greater germination capacity in cold-tolerant corn lines, demonstrating its relation with tolerance to this stress (Meng et al. 2022). Similar results were obtained by Freitas et al. (2019), whereas rice seeds with greater tolerance to cold stress exhibited lower lipid peroxidation and higher antioxidant activity (e.g., catalase and guaiacol peroxidases), favoring their performance under these conditions.

The superior physiological response of high-vigor seeds to cold stress may therefore be associated with a higher antioxidant activity, which favors a better performance. Thus, the present study aimed to evaluate the physiological response of common bean seeds with contrasting vigor to cold stress conditions, and identify the association between seed lot vigor and plant antioxidant system.

MATERIAL AND METHODS

The experiment was conducted using the common bean genotypes BAF42, BAF44 and BAF55, obtained from the active bean germplasm of the Universidade do Estado de Santa Catarina. The genotypes were selected based on their physiological quality traits (Ehrhardt-Brocardo & Coelho 2016).

The genotype lots were produced in the 2020/2021 growing season in Lages, Santa Catarina state, Brazil, at the Universidade do Estado de Santa Catarina experimental area. After harvesting, the seed lots were cleaned and standardized. For each genotype, the obtained seed lot was separated considering two vigor levels via artificial aging, using plastic germination boxes (Gerbox®) measuring 11 x 11 x 3.5 cm. The seeds were evenly distributed on stainless steel screens and 40 mL of saturated saline solution [40 % (w/v) of NaCl] deposited at the bottom of each box (Jianhua & McDonald 1997). The aged lot was kept in an aging chamber at 41 ºC with approximately 60 % of humidity for 7 days, then removed from the boxes and dried (35 ºC) in a forced-air oven until reaching a moisture content of 12-13 %.

The other lot was kept in a dry chamber (10 ± 2 ºC and 50 ± 5 % of relative humidity) until the analyses, being considered high-vigor (control), while the lot submitted to artificial aging deemed low-vigor. This resulted in two seed lots for each genotype: 42L1, 44L1 and 55L1 as high-vigor lots, which were not aged, and 42L2, 44L2 and 55L2 as the aged low-vigor seed lots.

Physiological quality was determined by germination and cold tests. The germination test was conducted with four replicates of 50 seeds, which were placed on Germitest paper moistened with distilled water at 2.5 mL of water per gram of dry paper and rolled up. The paper rolls were placed in a Mangelsdorf germinator at 23 ± 2 ºC in the dark. The first germination count was performed four days later, and the final germination percentage, number of abnormal seedlings and dead seeds after 7 days (Brasil 2009).

For the cold test, four replicates of 50 seeds were placed on filter paper previously moistened with distilled water at 2.5 mL of water per gram of dry paper. The paper rolls were placed in a BOD chamber at 5 ± 0.3 ºC, for 7 days. After the cold incubation period, the germination rolls were relocated to a Mangelsdorf

germinator at 23 ± 2 ºC in the dark (Soliman et al. 2018). The counts were performed using the same process adopted for the germination testing.

The physiological seed vigor response after cold stress was analyzed under the same conditions as the cold test, at the end of the cold stress period (7 days after incubation in the cold test) and 4 days after relocation to the Mangelsdorf germinator, as previously described.

To evaluate the seedling performance, 4 replicates of 15 seeds from each treatment (control and artificially-aged seed of each genotype) were placed on the upper third of Germitest paper previously moistened with 2.5 mL of water per gram of dry paper, rolled up and placed inside a BOD chamber at 5 ± 0.3 ºC, for 7 days. After the cold incubation period, the rolls were relocated to a Mangelsdorf germinator at 23 ± 2 ºC in the dark and, on the fourth day, the formed root, hypocotyl and total length of the normal seedlings (seedlings that presented all essential structures and absence of anomalies) were evaluated. Seedling length was measured using a digital caliper. Next, the seedlings were dried in an oven at 80 ºC, for 24 h, to determine the total dry mass (Nakagawa 1999).

The ascorbate peroxidase, guaiacol peroxidase, catalase, malondialdehyde and hydrogen peroxide (H_2O_2) in the embryonic axis were analyzed at the end of the cold stress exposure, and at 4 days after the submission to the cold stress in normal seedlings. The enzymatic extract was obtained from each biological replicate, using 200 mg of fresh sample ground in a mortar and pestle with 5 mL of 100 mM potassium phosphate buffer (pH 7.2) containing 1 mM of ethylenediamine tetraacetic acid (EDTA), 3 mm of dichlorodiphenyltrichloroethane (DTT) and 1 % of polyvinylpyrrolidone (PVPP) (Azevedo et al. 1998).

The ascorbate peroxidase was determined as described by Nakano & Asada (1981), with modifications. The incubation medium was prepared using 700 µL of 50 mM potassium phosphate buffer (pH 7.2), 100 µL of 5 mM ascorbic acid solution and 100 µL of 1 mM hydrogen peroxide solution, with the reaction started by adding 100 µL of enzymatic extract. Reading was performed at 290 nm, for 60 s. One ascorbate peroxidase unit was established as the amount of enzyme required to oxidize 1 µM min^{-1} of ascorbate.

Guaiacol peroxidase was quantified using guaiacol as substrate (Nakano & Asada 1981) and in accordance with Simões et al. (2015), with some changes. The incubation medium was prepared using 700 µL of 200 mM sodium phosphate buffer (pH 6.5), 100 µL of 40 mM guaiacol and 100 µL of 10 mM hydrogen peroxide, and the reaction started by adding 100 µL of enzymatic extract. Reading was performed at 470 nm, for 30 s. One guaiacol peroxidase unit was determined as the amount of enzyme required to form 1 µM min^{-1} of tetraguaiacol.

For catalase quantification, the procedure proposed by Aebi (1984) was used, with some modifications. The incubation medium was prepared using 2000 µL of 100 mM potassium phosphate buffer (pH 7.2), 800 µL of 65 mM hydrogen peroxide and 200 µL of enzymatic extract. Reading was performed at 240 nm, for 120 s. The amount of enzyme required to degrade 1 µM min^{-1} of hydrogen peroxide was one catalase unit. The evaluated enzymes were expressed in enzyme units per milligram of protein (U mg^{-1}) (Bradford 1976).

Malondialdehyde was determined using the procedure described by Hodges et al. (1999). A total of 50 mg of sample was macerated in a mortar in 1.5 mL of 80 % (v/v) alcohol and centrifuged. The quantification was performed using 1 mL of properly diluted extract and 1 mL of 20 % (w/v) trichloroacetic acid (TCA) solution containing 0.65 % (w/v) thiobarbituric acid (TBA) and 0.01 % (w/v) butylated hydroxy toluene (BHT). The reaction was kept in a water bath at 95 ºC, for 25 min, and readings were performed at 440, 532 and 600 nm. The malondialdehyde content was calculated as specified by Hodges et al. (1999).

Hydrogen peroxide was determined according to Alexieva et al. (2001), with modifications. A previously macerated 50 g sample was homogenized in 2.5 mL of 0.1 % TCA (w/v) and then centrifuged. For reading, 0.5 mL of supernatant was added with 0.5 mL of 50 mM potassium phosphate buffer (pH 7.0) and 1 mL of potassium iodide (KI). The samples were allowed to rest for 30 min in the dark, followed by reading at 390 nm. The H_2O_2 content was determined from a standard curve.

The experimental design was completely randomized, in a 2 x 3 factorial arrangement, consisting of two vigor levels (high and low vigor) and three genotypes (BAF42, BAF44 and BAF55), with four replicates. The data were submitted to normality testing when needed, followed by analysis of variance (Anova) and means comparison by the

Tukey test at 5 % of probability. In the second stage, the seed lots were characterized for vigor by cluster analysis, and principal component analyses (PCA) were performed based on the obtained clustering. The statistical analyses were carried out using the R software (R Core Team 2022).

RESULTS AND DISCUSSION

The high-vigor seed lots of all genotypes displayed a superior physiological quality during the germination test, with higher first germination count percentage at the first count, and only the BAF55 genotype obtained percentages similar to those of the low-vigor lot (Table 1).

A similar result was found for seed lot vigor in the cold test, whereas the high-vigor seed lots showed a higher germination speed and percentage after the cold test. Thus, the low-vigor seed lots showed a higher percentage of abnormal seedlings than their high-vigor counterparts (Table 1).

The physiological response of seed lot vigor under different germination conditions generally performs better with high-vigor seeds, exhibiting higher germination speeds and percentages (Finch-Savage & Bassel 2016). This was confirmed in the present study, with lower physiological quality in all the artificially aged lots, in relation to their unaged counterparts. According to Xing et al. (2023), vigor is closely linked to seed deterioration, which results in different morphological, physiological, metabolic and genetic changes that reduce germination and vigor. In this respect, artificial aging simulated deterioration and allowed germination to remain above 80 %, with more pronounced differences in seed lot vigor (Table 1). Thus, the study model is acceptable, since, according to Marcos-Filho (2015), it is important that seed lots used in vigor studies have a similar germination, but effective differences in vigor, assessed by physiological tests.

Considering the observed genotype effect, BAF44 showed lower first germination count percentage for high and low-vigor in both germination conditions, demonstrating a lower initial physiological quality for the BAF44 seed lot (Table 1). These results were expected according to Ehrhardt-Brocardo & Coelho (2016) and Gindri et al. (2017). BAF44 showed a low potential for quality seed production. As such, the genotypic effect is associated with the observed lower initial physiological quality.

Higher coefficients of variation were recorded for the abnormal plant and dead seed variables, what can be attributed to the nature of these characteristics. According to Gomes (2009), variables related to predicted vigor tend to display a greater variability due to factors such as physical heterogeneity of seed lots, interaction with the environment and influence of multiple metabolic processes during germination. In addition, because these lots showed a high segregation for physiological quality, low-vigor lots obtained higher values. As such, the observed considerable variation does not necessarily compromise the data reliability, but reflects the sensitivity of these changes to stress.

The influence of the seed lot vigor of the analyzed genotypes is also evident in the assessment of the seedlings' performance after the cold test, whereby all the high-vigor (control) lots showed

Table 1. First germination count (FGC), germination percentage (GP), abnormal seedlings (AS) and dead seeds (DS) in seed lots of the BAF55, BAF44 and BAF42 common bean genotypes with contrasting vigor, without cold conditions and after cold stress.

Lots	FGC	GP	AS	DS	FGC_{Cold}	GP_{Cold}	AS_{Cold}	DS_{Cold}
	%							
BAF55								
High-vigor (55L1)	85 a*	95 a	6 a	0	87 a	90 a	10 b	1 a
Low-vigor (55L2)	66 b	91 a	9 a	0	54 b	71 b	27 a	2 a
BAF44								
High-vigor (44L1)	58 a	89 a	11 b	0	51 a	68 a	17 b	16 b
Low-vigor (44L2)	45 b	84 b	17 a	0	21 b	39 b	37 a	25 a
BAF42								
High-vigor (42L1)	81 a	96 a	5 b	0	85 a	88 a	12 b	0 a
Low-vigor (42L2)	54 b	88 b	12 a	0	54 b	73 b	25 a	3 a
CV (%)	7.42	3.14	29.11	-	14.78	8.83	24.28	45.76

* Means followed by the same lowercase letter in the column do not differ statistically according to the Tukey test at 5 % of probability ($p \geq 0.05$). CV: coefficient of variation.

a better seedling performance (root and hypocotyl length and seedling dry mass) than their artificially aged counterparts (Table 2).

The relationship between high-vigor seed lots and formation of better performing seedlings is explained by the greater capacity of high-vigor seeds to mobilize reserves (Padilha et al. 2020). The grater deterioration of low-vigor seeds results in more delayed membrane, DNA and RNA repair, slower germination and, consequently, reduced seedling performance (Marcos-Filho 2015). This behavior was confirmed in the present study even after cold stress, with high-vigor seeds showing a better reserve mobilization capacity and producing taller seedlings with higher dry mass (Table 2). Based on the obtained physiological results, the high-vigor seeds were better able to overcome the imposed cold stress, generating a larger number of normal (Table 1) and high-performance seedlings (Table 2).

For the variables catalase, ascorbate peroxidase, guaiacol peroxidase and malondialdehyde evaluated in the embryonic axis at the end of cold stress, only vigor effect was observed for the catalase enzyme in the genotype 55 seed lots (Table 3).

The high-vigor BAF55 genotype (55L1) showed a higher catalase activity at the end of cold stress, indicating a possible association between seed lot vigor level and ability to overcome cold stress (Table 1). For BAF44 and BAF42, there were no differences in the catalase activity, regardless of vigor level.

High-vigor BAF55 and BAF44 seeds showed a lower H_2O_2 concentration after stress, with no significant difference in vigor for BAF42, indicating that the genotype affected the H_2O_2 accumulation during cold stress (Table 3).

Among the enzymes associated with the antioxidant system, catalase is decisive during abiotic stress, because it degrades H_2O_2 and prevents its toxic effect (Mittler 2017). In this respect, the greater catalase activity observed at the end of cold stress in the 55L1 lot favored the H_2O_2 degradation, explaining the lower values obtained and demonstrating the possible importance of this enzyme under cold stress conditions.

The seedling assessment after cold stress indicated that those produced by low-vigor seeds of

Table 2. Seedling performance at four days after cold stress of seven days at 5 ºC, in terms of root (RL_{Cold}), hypocotyl (HL_{Cold}) and total length (TL_{Cold}) and total dry mass (TDM_{Cold}) of seedlings for the lots of BAF55, BAF44 and BAF42 genotypes with contrast in vigor.

Lots	RL_{Cold}	HL_{Cold}	TL_{Cold}	TDM_{Cold}
	—— mm seedling^{-1} ——			mg seedling^{-1}
			BAF55	
High-vigor (55L1)	87.42 a*	42.02 a	129.64 a	29.79 a
Low-vigor (55L2)	48.48 b	29.90 b	78.38 b	18.96 b
			BAF44	
High-vigor (44L1)	37.62 a	28.25 a	65.88 a	20.78 a
Low-vigor (44L2)	27.76 b	27.68 a	55.44 b	17.75 a
			BAF42	
High-vigor (42L1)	71.94 a	37.39 a	109.34 a	22.85 a
Low-vigor (42L2)	55.34 b	33.03 b	88.37 b	17.96 b
CV (%)	17.29	10.75	14.00	18.65

* Means followed by the same lowercase letter in the column do not differ statistically according to the Tukey test at 5 % of probability ($p \geq 0.05$). CV: coefficient of variation.

Table 3. Catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in the embryonic axis after 7 days of cold stress at 5 ºC (after stress) and at 4 days of germination after 7 days of cold stress at 5 ºC (4d after stress), in high and low-vigor seed lots of the BAF55, BAF44 and BAF42 genotypes.

Lots	After stress					4d after stress				
	CAT	APX	GPX	MDA	H_2O_2	CAT	APX	GPX	MDA	H_2O_2
	—— U mg^{-1} of protein ——			nmol mL^{-1}	ug g^{-1}	——— U mg^{-1} protein ———			nmol mL^{-1}	ug g^{-1}
					BAF55					
High-vigor (55L1)	5.08 a*	13.92 a	0.60 a	0.83 a	3.55 b	7.37 a	642.12 b	834.58 b	0.94 b	6.96 b
Low-vigor (55L2)	4.08 b	14.55 a	0.57 a	0.78 a	5.11 a	7.86 a	861.81 a	1,012.05 a	1.36 a	9.19 a
					BAF44					
High-vigor (44L1)	4.46 a	12.67 a	0.58 a	0.81 a	4.86 b	7.89 b	948.67 b	1,175.58 a	1.35 b	11.48 a
Low-vigor (44L2)	4.05 a	13.32 a	0.59 a	0.80 a	5.90 a	9.18 a	1,193.42 a	1,154.04 a	1.63 a	13.90 a
					BAF42					
High-vigor (42L1)	4.47 a	15.78 a	0.59 a	0.77 a	5.13 a	7.59 b	709.90 b	938.86 b	1.09 b	8.98 a
Low-vigor (42L2)	3.88 a	13.33 a	0.62 a	0.83 a	4.89 a	9.81 a	906.96 a	1,112.34 a	1.31 a	11.01 a
CV (%)	12.40	28.40	36.15	6.21	7.69	7.60	12.65	9.48	13.15	17.23

* Means followed by the same lowercase letter in the column do not differ statistically according to the Tukey test at 5 % of probability ($p \geq 0.05$). CV: coefficient of variation.

the BAF44 and BAF42 genotypes exhibited a higher catalase activity and no significant difference from the BAF55 lots. In regard to ascorbate peroxidase, seedlings from the low-vigor seed lot of all the studied genotypes showed a greater activity for this enzyme, while the highest guaiacol peroxidase activity was recorded in seedlings from the low-vigor BAF55 and BAF42 seed lots (Table 1).

The highest observed lipid peroxidation (malondialdehyde) was in seedlings obtained from low-vigor seeds of all the genotypes, and the highest H_2O_2 concentration was recorded in seedlings from low-vigor BAF55 seeds (Table 3). This result may be associated with the greater susceptibility of low-vigor seeds to abiotic stress. According to Xing et al. (2023), deteriorated seed lots display greater reactive oxygen species accumulation, which damages proteins, lipids and genetic material and results in loss of vigor and, consequently, performance. In this context, the greater susceptibility of low-vigor seeds to stress increased the lipid peroxidation after cold stress (Table 3).

In general, the higher antioxidant activity observed in seedlings obtained from low-vigor seeds after exposure to cold conditions at 5 ºC may be due to the increase in the H_2O_2 concentration at the end of cold stress (Table 3), whereby a greater synthesis of these enzymes was needed to prevent oxidative damage when the seeds were submitted to adequate temperature (23 ± 2 ºC). According to Bhat et al. (2022), the increase in reactive oxygen species under cold stress requires plant metabolism to synthesize antioxidant enzymes, in order to reestablish homeostasis.

Thus, because seedlings obtained from low-vigor seeds were more damaged by H_2O_2 accumulation, a greater catalase, ascorbate peroxidase and guaiacol peroxidase synthesis was needed (Table 3) to control H_2O_2 and perform vital functions, even under optimal temperature conditions (23 ± 2 ºC). However, the resulting seedlings displayed poor performance, since, depending on the duration of stress exposure and extent of the resulting damage, the antioxidant enzymes were unable to improve plant response (Table 2).

Considering the genotype effect observed in the physiological results (Table 1), the grouping of seed lots according to physiological quality, regardless of genotype, indicated low-vigor in the BAF44 lot (44L1) and in relation to the BAF55 (55L1) and BAF42 (42L1) genotypes. As such, the characterization of physiological quality via cluster analysis produced two vigor groups, with 55L1 and 42L1 characterized as high-vigor and 42L2, 44L1, 44L2 and 55L2 as low-vigor (Figure 1).

The total variance explained by the two principal components was 73.91 %, being 54.34 and 19.57 % for the components 1 (PC1) and 2 (PC2), respectively (Figure 2). According to Varmuza & Filzmoser (2009), if in a score plot, using the first two PCs, more than about 70 % of the total variance is preserved, the scatter plot gives a good picture of dimensional data structure.

The principal component analysis (PCA) of the vigor groups obtained for the seed lots demonstrated a positive association between first germination count and germination percentage in the cold test, hypocotyl length, root length, total length and total dry mass, in relation to low-vigor seeds. Additionally, the high-vigor seeds obtained lower dead seed and abnormal plant values and H_2O_2 concentration in the embryonic axis at the end of cold stress (Figure 2a).

Due to their higher H_2O_2 concentration, when compared to high-vigor seeds, the low-vigor seeds produced seedlings with a larger number of anomalies after cold stress, with a correlation between these variables (Figure 2a). The rise in the H_2O_2 concentration under cold stress can be toxic, causing damage to proteins and genetic material (Freitas et al. 2019, Lone et al. 2021). The molecule

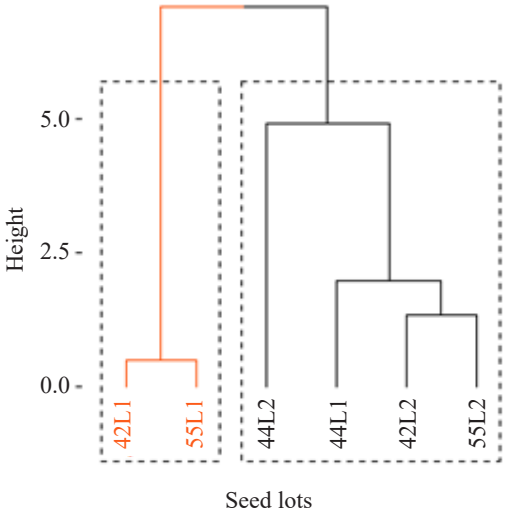

Figure 1. Grouping of the seed lots based on the assessed physiological parameters.

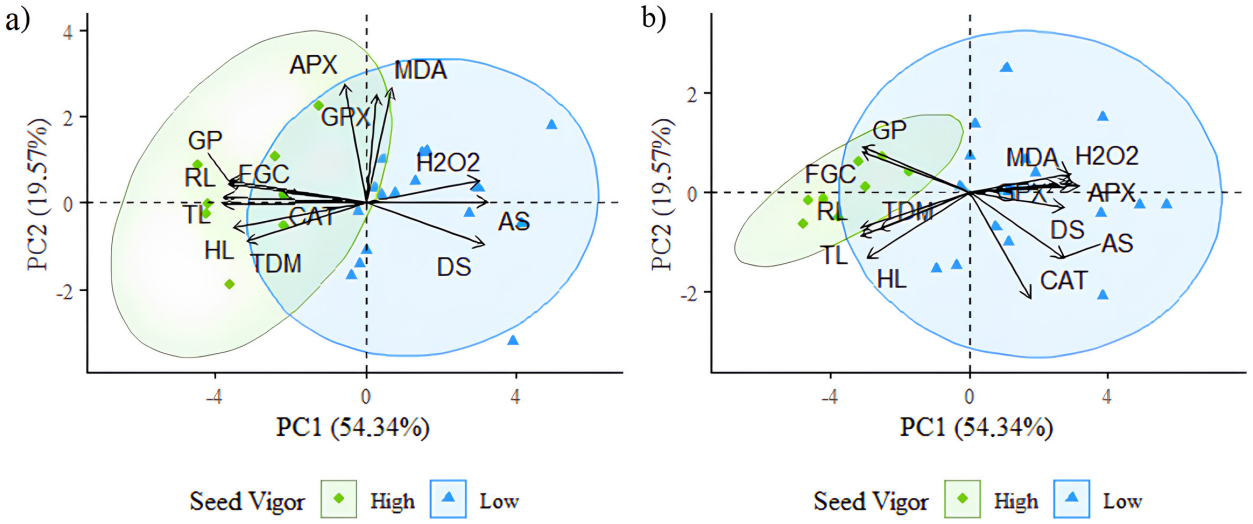

Figure 2. Principal component analysis showing the association between physiological (first germination count - FGC; germination percentage - GP; abnormal seedlings - AS; dead seeds - DS; root length - RL; hypocotyl length - HL; total length - TL; and total dry mass - TDM) and biochemical (catalase - CAT; ascorbate peroxidase - APX; guaiacol peroxidase - GPX; malondialdehyde - MDA; and hydrogen peroxide - H_2O_2) parameters in vigor groups formed by cluster analysis, assessed in the embryonic axis at the end of cold stress (a) and in seedlings at four days after cold stress (b).

is also a precursor to the hydroxyl radical, which results in lipid peroxidation and other cellular damage (Mittler 2017). Thus, the damage caused by high H_2O_2 concentration at the end of stress resulted in seed mortality and abnormality at the temperature of 23 ºC (Table 1).

There was no association between the enzymes ascorbate peroxidase and guaiacol peroxidase seed lot vigor at the end of cold stress, indicating that these enzymes are not related to the better seed and seedling performance observed in high-vigor seeds (Figure 2a). However, catalase showed a positive association with vigor level, whereby the high-vigor lots (55L1 and 42L1) performed better and exhibited H_2O_2 contend in the embryonic axis (Figure 2a). The enzymes ascorbate peroxidase, guaiacol peroxidase and catalase degrade H_2O_2, what favors overcoming abiotic stresses; however, this is dependent on the species, intensity of the imposed stress and evaluated structure (Dreyer & Dietz 2018, Van Doorn & Ketsa 2014). Thus, during the cold period at 5 ºC, catalase proved to be the most important enzyme for overcoming this stress.

The PCA highlights the increased need for catalase, ascorbate peroxidase and guaiacol peroxidase synthesis by seedlings obtained from low-vigor seeds, what is negatively associated with greater seed lot vigor (Figure 2b). The greater susceptibility of the low-vigor seeds to the previously imposed cold stress produced a larger number of abnormal seedlings and dead seeds, with greater lipid peroxidation and H_2O_2 in the seedlings, generating the need for catalase, ascorbate peroxidase and guaiacol peroxidase synthesis to maintain metabolic activity and growth (Figure 2b). Similarly, Freitas et al. (2019) observed an increase in H_2O_2 under cold stress in rice crops, and, consequently, a greater catalase and guaiacol peroxidase activity.

Thus, the better performance of high-vigor seedlings after cold stress is evident in the positive correlation between vigor and growth parameters (root length, hypocotyl length, total length and total dry mass), as well as increased germination and germination speed (Figure 2b). The evaluated antioxidant system was not associated with growth in high-vigor seedlings after exposure to cold stress conditions, indicating less need for enzyme synthesis to maintain homeostasis.

Seed lots with lower vigor have a greater need for antioxidant action after stress. Furthermore, the better seedling performance and tolerance to cold stress of high-vigor seeds are associated with greater reserve mobilization and catalase activity, respectively.

CONCLUSIONS

1. The catalase activity in the embryonic axis is associated with high-vigor seeds under cold stress;
2. Low-vigor seeds exhibited a greater H_2O_2 accumulation in the embryonic axis under cold stress, prompting a greater physiological damage and the need for antioxidant enzyme production in the resulting seedlings to recover metabolic homeostasis after stress;
3. After exposure to cold stress, low-vigor seeds show increased antioxidant enzyme activity and H_2O_2 accumulation, in response to stress.

ACKNOWLEDGMENTS

To the Universidade do Estado de Santa Catarina for support during the research, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Finance Code 001), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (Grant Nº 2023TR332) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (Grant Nº PQ1C).

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