Revista Ciência Agronômica, v. 55, e20238762, 2024
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Phytase and alpha-amylase activity are positively associated with seed vigor in common bean seeds[1]

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ABSTRACT - Seed vigor is one of main attributes of the physiological quality from seed lot. Thus, identify which mechanisms are used by seeds of higher vigor that favor the formation of seedlings with better performance is a key aspect in agriculture. The enzyme activity of phytase and alpha-amylase favor the availability of phosphorus and sugars, respectively, during germination and can be determinants of seed vigor. Thus, the objective of the present work was to identify the association of phytase and alpha-amylase enzymes with the vigor of a seed lot of common beans. The results showed a positive correlation between seed vigor and the enzymes evaluated. Based on the findings, it can be concluded that seeds with high vigor have a high capacity to form seedlings with better performance, because they present high enzymatic activity. Also, in seed lots of the same genotype, they can be used to determine seed vigor during germination.

Key words: Reserve mobilization. Germination. Seedling growth. Enzyme markers.

DOI: 10.5935/1806-6690.20240014
Editor-in-Chief: Prof. Salvador Barros Torres - sbtorres@ufersa.edu.br
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Received for publication 16/03/2023; approved on 11/07/2023
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INTRODUCTION

Common beans are one of the main sources of carbohydrates, proteins, and minerals for the human diet in several countries in Latin America, Africa, and Asia. Over the years, common beans have become a crop of great commercial importance owing to the globalization of the market (BEEBE *et al*., 2013).

The production of common beans is influenced by many factors such as pests, diseases, weeds, mineral nutrition, environmental conditions, and plant density (MONDO; NASCENTE; CARDOSO NETO, 2016). In addition, the use of seeds with high physiological quality (i.e., germination and vigor) are determinant for the success of the production system, as they favor the emergence, seedling performance (MARCOS-FILHO, 2015), and increase in the productivity of common beans (MONDO; NASCENTE; CARDOSO NETO, 2016).

The growth parameters during germination are positively associated with physiological quality (MARCOS-FILHO, 2015). Seedling growth is determined by the capacity of mobilizing reserves, which favors the speed germination and the formation of vigorous seedlings (EHRHARDT-BROCARDO; COELHO, 2016).

Seed reserve mobilization is directly associated with the hydrolysis of the reserves to support the growth of the embryonic axis during germination (NONOGAKI, 2008). During germination, there is increased activity of enzymes associated with reserve mobilization, e.g., amylases, phytases, proteases, and lipases (GUZMÁN-ORTIZ *et al*., 2019). Among amylases, alpha-amylase is determinant during germination, as it is specifically associated with the hydrolysis of starch for subsequent availability of soluble sugars (BEWLEY *et al*., 2013). Similarly, phytases are specific in the degradation of phytic acid stored in seeds, providing phosphorus and minerals to be used by the embryonic axis (NKHATA *et al*., 2018). The availability of sugars and phosphate favors the germination and seedling formation (CHEN *et al*., 2019; DONG *et al*., 2020).

Common beans contain 30-50% of starch (MONTOYA *et al*., 2008; PADILHA; COELHO; EHRHARDT-BROCARDO, 2021). In many dicots, alpha-amylase activity increases during germination, indicating an association with starch degradation (BEWLEY *et al*., 2013). Starch degradation caused by alpha-amylase favors the formation of seedlings with better performance (SANTOS *et al*., 2015) in rice (WANG *et al*., 2016), and in cowpea (ZEID *et al*., 2019). This association occurs in a similar way in common bean seeds, although it depends on the influence of each genotype (PADILHA; COELHO; EHRHARDT-BROCARDO, 2021). However, these studies have not focused specifically on seed vigor, but the association of alpha-amylase and seed vigor needs to be determined.

Phosphorus is essential to biomolecules such as nucleic acids, phospholipids and intermediate metabolites that are used in many metabolic pathways (BAKER *et al*., 2015). In seeds, approximately 75% of phosphorus is allocated in the form of phytic acid (RABOY, 2009). During germination, phytase activity increases (BOUAJILA *et al*., 2020) and a there is positive association between the degradation of phytic acid and the formation of seedlings with high dry matter in soybean (DONG *et al*., 2020). Such finding indicates the importance of phytase during the germination process. It is suggested that high activity of the phytase enzyme during germination in common beans can favor seedling formation.

Considering the importance of alpha-amylase and phytase during germination, it can be hypothesized that these enzymes are associated with the high physiological quality of a seed lot when evaluated during germination. Thus, the objective of this work was to determine the association of alpha-amylase and phytase enzymes with seed vigor in common bean seeds during germination.

MATERIAL AND METHODS

Two experiments were conducted to determine the association between enzymatic activity and seed vigor. Seeds of eight genotypes were produced in the experimental field of the State University of Santa Catarina - Agroveterinary Sciences (UDESC/CAV) in Lages, Santa Catarina (27º48’58”S, 50º19’34”W, altitude 930 m) in the season 2020/2021 and, after harvesting,, they were dried and kept in a dry chamber (50% relative humidity and 20ºC temperature). The seeds were selected and a 1,000 g sample was used as a seed lot for quality analysis (BRASIL, 2009).

The first experiment used seeds of the genotypes BAF07, BAF13, BAF23, BAF42, BAF44, BAF55 and BAF102, as well as the commercial cultivar BAF112 (IPR-88 Uirapurú). Except for the cultivar (i.e., BAF112), the genotypes were sourced from the Bean Germplasm Active Bank (BAF) of the State University of Santa Catarina (UDESC). The genotypes were selected from previous works, in which they presented contrasting potential to produce seeds with differentiated physiological quality (GINDRI *et al*., 2017; MICHELS *et al*., 2014).

The second experiment used seeds of the BAF07 and BAF55 genotypes because they presented low and high vigor as compared to the seeds in the previous experiment. Seeds were subjected to artificial aging to produce a low-vigor seed lot for each genotype.

The original seed lot was aged using the accelerated aging procedure in saturated saline (i.e., NaCl) (JIANHUA; McDONALD, 1997) at 41 ± 1 °C for 168 hours. After this period, the seeds were dried in a forced air circulation oven at 35 ºC to 13% moisture. In this way, a group of high vigor seeds (i.e., not aged) and a group of low vigor seeds (i.e., aged) were obtained for each genotype.

The physiological quality of seed lots was determined by germination, accelerated aging, seedling length, seedling dry matter, reserve mobilization rate, and enzymatic activity of alpha-amylase and phytase.

Germination (G) was evaluated by germination tests carried out with three replications of 50 seeds. Seeds were distributed on Germitest® paper previously moistened with distilled water at 2.5 times the dry paper weight, and organized in rolls. The samples were kept in a Mangelsdorf germinator at 25 ºC (BRASIL, 2009).

The accelerated aging (AA) test was performed at 42 ºC for 72 h (SCAPPA-NETO *et al*., 2001). After this period, the seeds were submitted to the germination test as described above.

Seedling length (SL) and total seedling dry matter (SDM) were determined with three replications of 20 seeds. The seeds were distributed on the upper third of the paper sheets, and the paper was moistened with distilled water at 2.5 times the dry paper weight. The procedure was carried out at 25 ± 2 ºC, and normal seedlings were evaluated on the fourth day (NAKAGAWA *et al*., 1999). Seedling length was determined using a digital caliper and the data was expressed in millimeters per seedling (mm sl^{-1}). Seedling dry matter was determined after drying at 80 ºC for 24 h, and it was expressed as milligram per seedling (mg sl^{-1}) (NAKAGAWA *et al*., 1999). Seed reserve mobilization rate (RMR) was determined using the procedure described by Andrade, Coelho and Padilha (2019), which uses the ratio of seedling dry matter to initial seed dry matter, which was expressed in percentage.

Alpha-amylase activity was determined by the 3,5-dinitrosalicylic acid (DNS) method described by Miller (1959), using the procedure described by Monerri and Guardiola (1986) with modifications. Enzyme extraction was performed using 0.5 g of previously macerated fresh cotyledons, plus 10 mL of sodium acetate buffer 240 mmol L^{-1} pH 5.4 with 10 mmol L^{-1} of $CaCl_2$ and 0.005% of Triton X-100. The samples were stirred for 60 min in the presence of ice, and then centrifuged. The enzyme extract was incubated in a water bath for 10 min at 70 ºC and then 250 µL of enzymatic extract and 250 µL of 2.0% (w/v) starch solution were kept in a water bath for 20 min at 38 ºC. The reaction was stopped by adding 500 µL of DNS solution and the samples were kept in a water bath for 6 min at 95 ºC. At the end of this procedure, 4 mL of distilled water was added followed by homogenization. The readings were performed at 540 nm and the results were expressed in units of enzyme per milligram of protein (U mg^{-1}). One unit of enzyme was defined as the amount of enzyme to produce 1 µmol of maltose per minute. Protein content was determined as described by Bradford (1976).

Phytase activity was performed using the same extract used for alpha-amylase activity. For quantification, the procedure described by Engelen *et al*. (2001) was used with adaptations made by Ou *et al*. (2011). In each assay, 1.5 mL of enzyme extract and 0.5 mL of sodium phytate (5 mmol L^{-1}) were mixed and incubated at 38 ºC for 75 min in a water bath. The reaction was stopped with 2 mL of Vanadate-Molybdate reagent (80 mmol L^{-1} ammonium molybdate solution, 20 mmol L^{-1} ammonium vanadate and 5 mol L^{-1} HNO_3 in a 1:1:2 ratio, respectively). The samples were homogenized and centrifuged for 10 min. The reading was performed at 415 nm and the results were expressed in units of enzyme per milligram of protein (U mg^{-1}). One unit of enzyme was defined as the amount of enzyme to produce 1 nmol of phosphate per minute. Protein content was determined as described by Bradford (1976).

In experiment 2, the analyzes of total seedling length (PC), total seedling dry matter (SMS), reserve mobilization rate (RMR), alpha-amylase and phytase activity were carried out in two moments, at four days and at seven days of germination, using four repetitions, as previously described.

That first experiment was carried out in a completely randomized block design with eight genotypes and three replications. The data were submitted to the normality test when necessary, and the Analysis of Variance (ANOVA) was performed. Means were compared using the Scott-Knott test at 5% probability. The association between the variables was determined by Pearson's correlation analysis and Principal Component Analysis. For Pearson correlation analysis, the significance of the correlation coefficients (r) was determined by the t-test at 5% of probability. Statistical analyses were performed using the R Software (R CORE TEAM, 2020).

The second experiment was carried out in a completely randomized design with a 2x2 factorial arrangement, consisting of the combination of two vigor levels (high and low) and two germination times (4 and 7 days) with four replications for each cultivar. Data were submitted to Analysis of Variance (ANOVA) and the means were compared by the Scott-Knott test at 5% probability. Statistical analyses were performed using the R Software (R CORE TEAM, 2021).

RESULTS AND DISCUSSION

Experiment 01

The genotypes showed differences in terms of physiological quality as the genotypes BAF07 and BAF23 presented lower germination in comparison to the other genotypes. However, all the seed lots presented high germination percentage with values higher than 90% (Table 1).

Seed physiological potential was firstly evaluated by the accelerated ageing test (AA), according to which the seed lots were characterized in two groups (i.e., high vigor and low vigor). The high vigor seed lots (i.e., BAF13, BAF42, BAF55, BAF102 and BAF112) showed higher values for accelerated aging than the low-vigor seed lots (i.e., BAF07, BAF23 and BAF44). Also, the high-vigor group presented higher seedling length (SL) and mobilization rate of seed reserves (RMR) than the low-vigor one (Table 1). According to Marcos-Filho (2015), high vigor seeds can form vigorous seedlings, as shown by the findings.

For phytase, the genotypes BAF42 and BAF112 showed the highest activity, and high vigor and high capacity to mobilize reserves, indicating a possible association between vigor of the seed lot and phytase activity (Table 1). However, the low-vigor genotypes BAF07 and BAF44 showed the same enzymatic activity of genotype BAF55 (high vigor), and vigor could not be attributed to enzymatic activity when these results are found.

In this sense, the influence of the genotype on the synthesis capacity of this enzyme may explain this response. The effect of the genotype on phytase activity was found in barley (BOUAJILA *et al.*, 2020), soybean (DONG *et al.*, 2020) and wheat (SCHOLLENBERGER *et al.*, 2022) seeds. According to Nkhata *et al.* (2018), changes in mineral availability after germination depend on phytate content, phytase activity, the binding matrix between minerals, or the interaction between these factors. Thus, the evaluation of phytase during the germination of common beans is affected by the genotype.

Similar results were found for alpha-amylase activity as low-vigor seed lots of the BAF07 and BAF44 genotypes showed similar or statistically superior alpha-amylase activity in comparison to high-vigor seed lots (i.e., BAF13, BAF42, BAF55, BAF102 and BAF112). The effect of genotype on alpha-amylase activity was reported by Oliveira *et al.* (2013) in maize seeds. In barley seeds during germination, the modified structure of starch granules (e.g., morphology and topography) can affect carbohydrate mobilization from seeds owing to changes in the interactions between enzymes and starch degradation (SHAIK *et al.*, 2014). In common bean seeds, the presence of alpha-amylase inhibitor proteins varies in different genotypes (EHRHARDT-BROCARDO; COELHO, 2022). These findings may explain the differences in alpha-amylase activity in the study genotypes (Table 1).

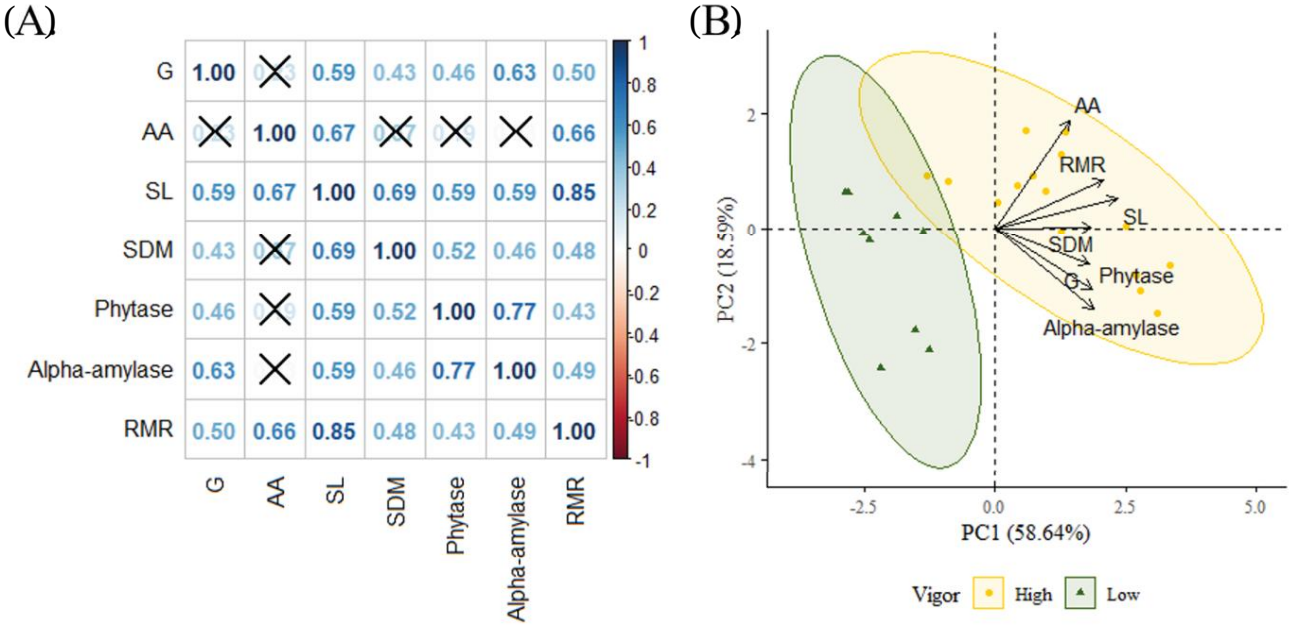
Pearson correlation analysis made it possible to identify how the study enzymes are associated with seedling formation. Except for accelerated aging (AA), enzyme activity was positively associated with seedling length (SL), seedling dry matter (SDM) and reserve mobilization rate (RMR) (Figure 1A).

Table 1 - Germination (G), accelerated ageing (AA), seedling length (SL), seedling dry matter (SDM) reserve mobilization rate (RMR), phytase activity and alpha-amylase activity evaluated four days after sowing for different common bean genotypes

Genotype	G	AA	SL	SDM	RMR	Phytase	Alpha-Amylase
	(%)	(%)	(mm sl^{-1})	(mg sl^{-1})	(%)	(U mg^{-1})	(U mg^{-1})
BAF07	92 b1	82 c	144.93 c	28.70 d	17.97 c	0.15 b	0.26 d
BAF13	95 a	88 b	159.89 b	32.52 c	19.32 c	0.20 b	0.19 e
BAF23	92 b	80 c	143.03 c	42.22 b	14.03 e	0.10 c	0.09 f
BAF42	97 a	86 b	172.84 a	43.74 b	25.65 a	0.37 a	0.50 b
BAF44	95 a	68 d	145.19 c	33.64 c	16.34 d	0.21 b	0.42 b
BAF55	95 a	92 a	174.54 a	41.40 b	25.38 a	0.19 b	0.26 d
BAF102	97 a	87 b	172.14 a	40.55 b	23.36 b	0.11 c	0.35 c
BAF112	98 a	88 b	182.62 a	47.45 a	21.92 b	0.48 a	0.63 a
C.V.	2.13	2.95	3.92	5.33	5.13	25.28	13.82

[1]Means followed by the same lower-case letter in the column do not differ statistically by the Scott-Knott test at 5% probability. C.V. Coefficient of variation

Figure 1 - Pearson correlation coefficients (A) found for the study variables and principal component analysis (B) demonstrating the association between the study variables and the previously defined vigor groups. In Figure A, coefficient of correlation (r) boxes marked with an "X" are non-significant by the t-test at 5% of probability



Seeds exhibiting high vigor showed greater capacity to produce vigorous seedlings (Table 1), and this relationship was found to occur in the correlation analysis by the parameters seedling length (SL) and reserve mobilization rate (RMR). Both are positively associated with the accelerated aging test (AA) (i.e., r = 0.67 and r = 0.66, respectively) (Figure 1A). The association between enzymatic activity and seed vigor (i.e., the high- and low-vigor groups) was shown to occur in the principal component analysis. The first principal component (PC1) explains 58.64% of the data variation, and this component shows an association between phytase and alpha-amylase with seedling performance parameters (i.e., SL, SDM and RMR), as well as the association of PC1 with the group of high-vigor seeds (Figure 1B).

Phytase and alpha-amylase were positively associated with all growth parameters (i.e., SL and SDM, which were the result of the high RMR) (Figure 1A, B). Phytase degrades the phytic acid stored in the seeds, which results in the availability of phosphorus and other minerals to the seedling (BOUAJILA *et al*., 2020; GUZMÁN-ORTIZ *et al*., 2019). Similarly, the activity of the alpha-amylase plays a decisive role during seedling growth because it hydrolyses the stored starch and provides soluble sugars for growth purposes (WANG *et al*., 2016). Alpha-amylase was positively associated with seed lot vigor in maize (OLIVEIRA *et al*., 2013; SANTOS *et al*., 2015) and common beans (PADILHA; COELHO; EHRHARDT-BROCARDO, 2021), favoring the formation of seedling with better performance. Considering the findings, it can be argued that during the germination of common bean seeds, high activity of phytase and alpha-amylase favors seedling growth, and these enzymes have an association with seed lot vigor (Figure 1A, 1B).

Accelerated aging (AA) was the only parameter that was not associated with the study enzymes (Figure 1A). The AA test is performed under a stress condition, but the study enzymes were determined under the absence of stress. For this reason, the association of these enzymes and accelerated aging could not be observed, especially with use of different genotypes, which had an influence on these conditions. However, RMR has a strong association with vigor of seed lots (ANDRADE; COELHO; PADILHA, 2019; PADILHA; COELHO; EHRHARDT-BROCARDO, 2021), and it evaluates the mobilization capacity that affects the performance of vigorous seedlings. There was an association of phytase and alpha-amylase with seed vigor of common bean seeds, since high-vigor seeds produce vigorous seedlings (Figure 1A, 1B).

Experiment 02

The results of the second experiment using two seed lots of the genotypes BAF07 and BAF55 demonstrate that artificially aged seed lots (i.e., low-vigor ones) presented low first germination count (FGC) for both genotypes (Table 2). The seeds of the high-vigor lot of these genotypes showed high values for seedling length (SL), seedling dry matter (SDM) and reserve mobilization rate (RMR) (Table 3).

The response of seed vigor was observed in both evaluation periods (i.e., 4 and 7 days) for each genotype (i.e., BAF07 and BAF55), and the differences found on the fourth day remained until the seventh day, demonstrating a connection between seed vigor and the physiological differences found in artificially aged and non-aged seeds (Table 3).

For the study enzymes, the same difference occurred in relation to the physiological parameters. Seeds with high vigor showed high activity of phytase and alpha-amylase enzymes at four and seven days after sowing and, as a result, high vigor seeds formed seedlings with high length (SL) and dry matter (SDM) (Table 3). Phytase activity increases with time (BOUAJILA *et al*., 2020; OU *et al*., 2011), and the same situation is reported for alpha-amylase in chickpea and common beans, i.e., increase in alpha-amylase synthesis is accentuated after germination, favoring starch hydrolysis and seedling development (BEWLEY *et al*., 2013).

The high activity of alpha-amylase, beta-amylase and protease enzymes during germination favored the better performance of cowpea seedlings owing to the greater availability of soluble sugars and soluble proteins when evaluation was performed at four days after sowing (ZEID *et al*., 2019). Thus, up to seven days, there were differences between seed vigor (i.e., SL, SDM, and RMR) and the study enzymes. These results confirm what was demonstrated in the previous experiment, which indicated the positive association of the study enzymes with the growth parameters. Thus, in the second experiment, there is not a genotype effect, and seeds with high vigor showed high activity of phytase and alpha-amylase enzymes in the evaluation periods (Table 3), thus favoring the availability of phosphorus, minerals, and soluble sugars for the formation of seedlings with higher vigor.

These results show the importance of reserve mobilization during seedling establishment and its direct relationship with seed lot vigor. Therefore, one needs to

Table 2 - First germination count and germination of common bean seed lots of genotypes BAF07 with low and high seed vigor and BAF55 with low and high seed vigor

	Genotype BAF07		Genotype BAF55	
Variables	High Vigor	Low Vigor	High Vigor	Low Vigor
FGC (%)	56 a	37 b	92 a	75 b
G (%)	89 a	77 b	94 a	90 b

[1]Means followed by the same lower-case letter in the row do not differ statistically by the Scott-Knott test at 5% probability

Table 3 - Physiological parameters, phytase and alpha-amylase activity in the germination of common bean seeds of the genotype BAF07 and BAF55 with contrast in seed vigor

Genotype BAF07				
Variables	4 days after sowing		7 days after sowing	
	High Vigor	Low Vigor	High Vigor	Low Vigor
SL (mm sl^{-1})	120.760 a	80.750 b	232.920 a	188.450 b
SDM (mg sl^{-1})	25.750 a	13.920 b	47.730 a	42.170 b
RMR (%)	12.090 a	8.710 b	29.870 a	26.390 b
Phytase (U mg^{-1})	0.210 a	0.110 b	0.860 a	0.580 b
Alpha-Amylase (U mg^{-1})	0.130 a	0.080 b	0.610 a	0.390 b
Genotype BAF55				
Variables	4 days after sowing		7 days after sowing	
	High Vigor	Low Vigor	High Vigor	Low Vigor
SL (mm sl^{-1})	161.510 a	128.470 b	301.810 a	248.820 b
SDM (mg sl^{-1})	35.930 a	26.270 b	78.890 a	62.560 b
RMR (%)	22.010 a	16.090 b	47.100 a	38.320 b
Phytase (U mg^{-1})	0.056 a	0.037 a	0.370 a	0.240 b
Alpha-Amylase (U mg^{-1})	0.217 a	0.113 b	1.150 a	0.770 b

[1]Means followed by the same lower-case letter in the row do not differ statistically by the Scott-Knott test at 5% probability

determine how this relationship occurs under conditions of abiotic stress, and to identify if these enzymes contribute in the same way under abiotic conditions. In addition, the findings indicate the need for genetic studies associated with regulation of the expression of phytase and alpha-amylase, with the identification of genes associated to them to advance the genetic control of seed vigor.

CONCLUSION

Seeds with high vigor have a greater capacity to synthesize phytase and alpha-amylase during germination, which favors the formation of seedlings with better performance, and they can be used to segregate seed lots of the same cultivar during germination.

ACKNOWLEDGEMENTS

The authors would like to thank the financial support from Programa de Bolsas Universitárias de Santa Catarina - UNIEDU and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting doctoral scholarships, and to FAPESC (FAPESC/2023TR332/UDESC) for the financial support. The corresponding author (Coelho, C.M.M) thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for productivity grant. This article is part of the doctorate obtained by Padilha, M. S.

REFERENCES

ANDRADE, G. C. D.; COELHO, C. M. M.; PADILHA, M. S. Seed reserves reduction rate and reserves mobilization to the seedling explain the vigour of maize seeds. **Journal of Seed Science**, v. 41, n. 4, p. 488-497, 2019.

BAKER, A. *et al.* Replace, reuse, recycle: improving the sustainable use of phosphorus by plants. **Journal of Experimental Botany**, v. 66, n. 2, p. 3523-3540, 2015.

BEEBE, S. E. *et al*. Phenotyping common beans for adaptation to drought. **Frontiers in Physiology**, v. 4, n. 35, p. 1-20, 2013.

BEWLEY, J. D. *et al.* **Seeds**: physiology of development, germination and dormancy. New York: Springer, 2013. 392 p.

BOUAJILA, A. *et al.* Changes in phytase activity, phosphorus and phytate contents during grain germination of barley (Hordeum vulgare L.) cultivars. **Agroforestry Systems**, v. 94, n. 4, p. 1151-1159, 2020.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1/2, p. 248-254, 1976.

BRASIL. **Ministério da Agricultura, Pecuária e Abastecimento**. *Regras para análise de sementes.* Brasília: MAPA/ACS, 2009. 395 p.

CHEN, L. *et al*. Metabolomic analysis of energy regulated germination and sprouting of organic mung bean (Vigna radiata) using NMR spectroscopy. **Food Chemistry**, v. 15, n. 286, p. 87-97, 2019.

DONG, Q. *et al.* Seedling growth, physiological characteristics, nitrogen fixation, and root and nodule phytase and phosphatase activity of a low-phytate soybean line. **Plant Physiology and Biochemistry**, v. 149, p. 225-232, 2020.

EHRHARDT-BROCARDO, N. C. M.; COELHO, C. M. M. Hydration patterns and physiologic quality of common bean seeds. **Semina: Ciências Agrárias**, v. 37, n. 4, p. 1791-1799, 2016.

EHRHARDT-BROCARDO, N. C. M.; COELHO, C. M. M. Mobilization of seed storage proteins is crucial to high vigor in common bean seeds. **Ciência Rural**, v. 52, n. 2, p. e20200894, 2022.

ENGELEN, A. J. *et al.* Determination of phytase activity in feed by a colorimetric enzymatic method: collaborative interlaboratory study. **Journal of AOAC International**, v. 84, n. 3, p. 629-633, 2001.

GINDRI, D. M. *et al*. Seed quality of common bean accessions under organic and conventional farming systems. **Pesquisa Agropecuária Tropical**, v. 47, p. 152-160, 2017.

GUZMÁN-ORTIZ, F. A. *et al*. Enzyme activity during germination of different cereals: a review. **Food Reviews International**, v. 35, n. 3, p. 177-200, 2019.

JIANHUA, Z.; McDONALD, M. B. The saturated salt accelerated aging test for small-seeded crops. **Seed Science and Technology**, v. 25, n. 1, p. 123-131, 1997.

MARCOS-FILHO, J. Seed vigor testing: an overview of the past, present and future perspective. **Scientia Agricola**, v. 72, n. 4, p. 363-374, 2015.

MICHELS, A. F. *et al.* Qualidade fisiológica de sementes de feijão crioulo produzidas no oeste e planalto catarinense. **Revista Ciência Agronômica**, v. 45, n. 3, p. 620-632, 2014.

MILLER, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. **Analytical Chemistry**, v. 31, n. 3, p. 426-428, 1959.

MONDO, V. H. V.; NASCENTE, A. S.; CARDOSO NETO, M. O. Common bean seed vigor affecting crop grain yield. **Journal of Seed Science**, v. 38, n. 4, p. 365-370, 2016.

MONERRI, C.; GUARDIOLA, J. L. Las amilasas del guisante: su estimación en extractos crudos. **Revista de Agroquímica y Tecnologia de Alimentos**, v. 26, n. 2, p. 219-233, 1986.

MONTOYA, C. A. *et al*. Susceptibility of phaseolin to in vitro proteolysis is highly variable across common bean varieties (Phaseolus vulgaris L.). **Journal of Agricultural and Food Chemistry**, v. 56, n. 6, p. 2183-2191, 2008.

NAKAGAWA, J. *et al*. Testes de vigor baseados no desempenho das plântulas. *In*: KRZYZANOWSKI, F. C.; VIEIRA, R. D.; FRANÇA NETO, J. B. (ed.). **Vigor de sementes**: conceitos e testes. Londrina: ABRATES, 1999. cap. 2, p. 1-24.

NKHATA, S. G. *et al*. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. **Food Science & Nutrition**, v. 6, n. 8, p. 2446-2458, 2018.

NONOGAKI, H. Seed germination and reserve mobilization. *In*: Encyclopedia of Life Sciences (ELS). New York: John Wiley & Sons, 2008.

OLIVEIRA, G. *et al*. Physiological quality and amylase enzyme expression in maize seeds. **Ciência e Agrotecnologia**, v. 37, n. 1, p. 40-48, 2013.

OU, K. *et al*. Phytase activity in brown rice during steeping and sprouting. **Journal of Food Science and Technology**, v. 48, n. 5, p. 598-603, 2011.

PADILHA, M. S.; COELHO, C. M. M.; EHRHARDT-BROCARDO, N. C. M. Vigor and alpha-amylase activity in common bean seeds under salt stress conditions. **Semina: Ciências Agrárias**, v. 42, n. 6, p. 3633-3650, 2021. Supplement 2.

R CORETEAM. **R**: a language and environment for statistical computing. Vienna, Austria, 2020. Available in: https://www.R-project.org/. Access in: February 12 th, 2023.

RABOY, V. Approaches and challenges to engineering seed phytate and total phosphorus. **Plant Science**, v. 177, n. 4, p. 281-296, 2009.

SANTOS, H. O. *et al.* Proteins expression and germination of maize seeds submitted to saline stress. **African Journal of Agricultural Research**, v. 10, n. 44, p. 4102-4107, 2015.

SCAPPA-NETO, A. *et al*. Efeito do teor inicial de água de sementes de feijão e da câmara no teste de envelhecimento acelerado. **Scientia Agricola**, v. 58, n. 4, p. 747-751, 2001.

SCHOLLENBERGER, M. *et al*. Storage duration and conditions change the phytate content and phytase activity of wheat grains. **JSFA Reports**, v. 2, n. 3, p. 100-106, 2022.

SHAIK, S. S. *et al*. Starch bioengineering affects cereal grain germination and seedling establishment. **Journal of Experimental Botany**, v. 65, n. 9, p. 2257-2270, 2014.

WANG, W. *et al.* Pre-sowing seed treatments in direct-seeded early rice: consequences for emergence, seedling growth and associated metabolic events under chilling stress. **Scientific Reports**, v. 6, n. 1, p. 1-10, 2016.

ZEID, I. M. *et al*. Promotive effect of ascorbic acid, gallic acid, selenium and nano-selenium on seed germination, seedling growth and some hydrolytic enzymes activity of cowpea (Vigna unguiculata) seedling. **Journal of Plant Physiology and Pathology**, v. 7, n. 1, p. 2, 2019.