

Accelerated aging test and image analysis for barley seed

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Abstract

Barley (*Hordeum vulgare* L.) planted area has increased in Brazil after a decline from 2005–2009, but the country continues to import malt for the brewing industry. The physiological quality of the seed lot is essential for plant establishment and for screening for high yield grain. However, a uniform accelerated aging (AA) procedure has not been developed for testing barley seeds. Thus, this study aimed to determine the best AA method for measuring barley seed vigor. Seeds of three barley cultivars (BRS Cauê, BRS Brau, and MN 6021) were subjected to four AA treatment (T₁: 41 °C for 72 h; T₂: 42 °C for 48 h; T₃: 42 °C for 60 h; and T₄: 43 °C for 48 h). The following variables were evaluated using traditional vigor tests: seed viability in the first and last count of the germination test, number of seminal roots, shoot length, and radicle length. In addition, digital images of seedlings were acquired with the Seed Analysis System (SAS[®]) to compute seedling vigor, growth, and uniformity. AA methods T₂ and T₃ were effective in separating barley cultivars into vigor categories at day four of the germination test. Computer analysis of digital images of barley seedlings is a valuable tool for testing barley seed vigor. Accelerated aging methods T₂ and T₃ also gave the best separation in seed lot vigor and seedling growth using the SAS[®] index, respectively.

Keywords: *Hordeum vulgare* L., software, seedling uniformity, vigor, temperature.

Abbreviation section: AA_accelerated aging, FC_first count of viable seeds, LC_last count of viable seeds, SAS[®]_Seed Analysis System,

Introduction

In 2017, worldwide barley (*Hordeum vulgare* L.) production amounted to 147 million tons over a harvested area of 48.2 million ha (USDA, 2017). In Brazil, barley area planted for harvest in 2009 was 77,500 ha, which is significantly reduced compared to the 142,900 ha in 2005. Even though planted area increased compared to 2009 and is currently estimated at 109,200 ha, the country continues to import malt (CONAB, 2017).

Crop success is largely dependent on seed quality, which can only be accurately measured using tests that are able to detect differences in physiological quality between seed lots. The accelerated aging (AA) vigor test is used to determine the level of variability that occurs within and among seed lots in various crops such as soybean (*Glycine max* L. Merrill) (Tomes et al. 1988; Torres, Vieira and Panobianco, 2004), beans (*Phaseolus vulgaris* L.) (Demir and Mavi, 2008), wheat (*Triticum aestivum* L. Thell) (Ghahfarokhi et al., 2014), and corn (*Zea mays* L.) (Arisnabarreta et al., 2012).

One of the early proponents of the concept of seed vigor, Isely (1957) defined vigor as “the sum total of all seed attributes which favor stand establishment under unfavorable conditions”. The concept has been refined over decades of research to include rapid and uniform emergence of plants under a wide range of field conditions (Marcos-Filho, 2015; Kazim and Ibrahim, 2007) and the rate and uniformity of seed germination and seedling growth (Zhang

et. al., 2015) for maximizing potential yield (Han et al., 2014).

The effects of accelerated aging include damage to cell membranes by lipid peroxidation (Kaewnaee et al., 2011) and reduced synthesis of proteins involved in germination (Dell’ Aquila, 1994; McDonald, 1999), especially hydrolytic enzymes, in particular α -amylase, and enzymes that protect against oxidative stress such as catalase (Kapilan, 2015).

Seed testing laboratories generally use for barley the AA test described by Krzyzanowski and Vieira (1999). Although originally developed for wheat seeds, the AA test can also be used for seeds from other species. Its use for barley seed is supported by the physicochemical similarities of the two species (Sýrkorová et al., 2009; Yücel et al., 2009; Koehler and Wieser, 2013).

In the last decades, image processing computer applications that measure germinating seedlings have been developed. Seedlings are represented by digital images and the computer can calculate a vigor index from those measurements for different species (Silva et al., 2012; Gomes Junior et al., 2014; Peñaloza et al., 2015; Pinto et al., 2015). Image processing computer solves the main limitations of traditional vigor tests as it takes rapid, objective, and highly accurate and standardized measurements that yield reproducible vigor assessments

(Sako et al., 2001; Marcos-Filho et al., 2009; Kikuti and Marcos-Filho, 2013).

This study aimed to determine the best AA method for separating barley seed lots into vigor categories using the standard germination test and an automated vigor assessment index.

Results and Discussion

Vigor

This study evaluated the effect of four AA methods on seed vigor of three barley cultivars. In general, cultivar MN 6021 recorded the highest percentage of viable seeds in the first count (FC) across AA methods, with the exception of T_4 (Table 1). In addition, each AA method allowed separation of barley cultivars into two vigor categories: T_1 separated MN 6021 and BRS Cauê seeds from BRS Brau seeds; T_2 , MN 6021 and BRS Brau seeds from BRS Cauê seeds; T_3 , MN 6021 seeds from BRS Brau and BRS Cauê seeds; and T_4 , BRS Cauê and MN 6021 seeds from BRS Brau seeds. Thus, the result show that all the methods can separate the cultivar in two groups, and the differences of T_3 (control) and T_2 are minimal, allowed the use of T_2 .

BRS Brau seeds aged at 41 °C for 72 h (T_1) had the smallest number of viable seeds and the lowest vigor (Table 1), but also had a large number of seminal roots, long shoots, and rapid growth typical of vigorous seeds.

Kim, Bin and Choe (1989) found the highest correlation coefficients between AA germination and field emergence and grain yield in barley seeds aged at 41 °C for 48 h. A shorter aging time in T_1 (41 °C, 72 h) could possibly improve vigor in BRS Brau seeds to levels similar to those of MN6021 and BRS Cauê seeds, but would reduce the accuracy of the AA test in separating seed lot vigor.

No significant differences were detected in seed vigor between cultivars aged at 43 °C for 48 h (T_4) in the last count (LC) (Table 1), indicating that FC is a better predictor of seed vigor than LC due to the rapid embryo growth and, predictably, rapid emergence in the field. The FC could be recorded at day four instead of day seven of the germination test thus saving time and costs for the seedsman.

There was a positive correlation between seed vigor in the FC (day 4) and the SAS[®] index (day 7). In fact, FC and the SAS[®] index gave identical separation in seed vigor for AA methods T_1 (41 °C, 72 h), T_2 (42 °C, 48 h), and T_4 (43 °C, 48 h), (Table 1).

Growth

In wheat seed exposed to AA, RNase expression was significantly reduced in the embryo but was not affected in the endosperm (Spanò et al., 2007). Similarly, Abdul-Baki and Anderson (1970) found that AA up to 12 days did not affect the leaching of sugars, found primarily in the endosperm, from germinating barley seeds. Thus, complex responses to stress occur at the seedling level and seedling measurements provide sensitive measures of seed vigor.

The number of seminal roots can affect the photosynthetic capacity of seedlings and is an important aspect of vigor assessment (Nepomuceno et al., 2009). However, in the current study, the number of seminal roots in barley

seedlings germinated from artificially aged seeds was not a good predictor of seed vigor, because MN 6021 barley seeds had the highest germination percentage in the FC and LC (Table 1) but a smaller number of seminal roots than BRS Brau and BRS Cauê seeds (Table 2). This result supports the observation that AA methods sorted barley cultivars into different vigor categories; thus, BRS Brau seeds should not be considered the least vigorous. Similarly, AA method T_1 (41 °C for 72 h) was unable to separate seed lot vigor both at first count and final count.

Shoot growth is essential for seedling vigor, because the slow emergence of a small or uneven number of seedlings may result in stand failure, delayed development, weed control problems, and affect harvest-related traits (Marcos-Filho, 2013). Conversely, fast seedling growth in barley has been used in breeding programs to improve water use efficiency (Bort et al., 1998).

Shoot measurements showed that even though BRS Cauê seeds had the largest number of viable seeds in some AA methods, shoot length (cm) was shorter and initial establishment was slower, contrary to the rapid emergence expected for seedlings germinated from vigorous seeds.

Thus, even though BRS Cauê seeds should at first be considered the least vigorous, shoot length was significantly higher in T_3 than in the other AA methods (Table 2), and this difference is likely due to a genetic response to stress. Similarly, Jatoi et al. (2001) found a positive correlation between shoot length and aging temperature in pea (*Pisum sativum* L.) seedlings, whereas Kappor et al. (2011) argued that the lower content of soluble proteins found in artificially aged rice seeds might be associated with a reduction in shoot length and impaired seedling growth.

The SAS[®] index correlated significantly (1%) with shoot length, and similar results were reported for wheat seed (Silva et al., 2012; Brunes et al., 2016). In this study, there were no significant differences in seedling growth and shoot length between barley cultivars in T_3 (42 °C, 60 h), whereas total length measurements from methods T_1 , T_2 , and T_4 could not unequivocally validate the SAS[®] index for classifying viable seeds into separate vigor categories.

Radicle length varied considerably among barley cultivars and AA methods and this variation may be inherent to the species. Nevertheless, the results show that T_4 (43 °C, 48 h) was not the best AA method for testing barley seed vigor. It should be noted that the significant reduction in radicle length across cultivars in seeds aged at 43 °C for 48 h (T_4) when compare with the control (T_3), this could result from gross DNA damage. In fact, it has been shown that artificially aged seeds may exhibit chromosomal abnormalities, including chromosome breaks and micronuclei formation (Menezes et al., 2014).

DNA damage is more extensive in root cells. According to Radha et al. (2014) "only when a critical proportion of aberrant dividing cells occur will root growth ceases (sic) with subsequent seedling death". Abdalla and Roberts (1968) have demonstrated the increased susceptibility of roots to heat-induced chromosome damage. In the current study, no significant difference in radicle length was detected between barley cultivars aged in T_4 , and this AA method was not effective in separating seed lot vigor.

Table 1. Seed viability in the first count at day four of the germination test (FC) and in the final count at day seven of the germination test (LC), and seedling vigor index (V%) values computed by the Seed Analysis System (SAS[®]) for three barley seed varieties subjected to four accelerated aging (AA) methods.

Method	Cultivar								
	MN 6021			BRAU			CAUÊ		
	FC (%)	LC (%)	V (%)	FC (%)	LC (%)	V (%)	FC (%)	LC (%)	V (%)
T ₁ (41 °C; 72 h)	61.2 bA	61.7 bA	74.8 abA	31.4 cB	31.4 Cb	65.2 abB	59.9 aA	60.2 abA	74.0 aA
T ₂ (42 °C; 48 h)	70.4 aA	75.5 aA	78.9 aA	64.5 aA	74.9 aAB	66.8 abB	54.5 abB	66.7 aB	70.8 aB
T ₃ (42 °C; 60 h)	70.7 aA	76.0 aA	70.0 bcA	54.5 bB	61.7 bB	71.7 aA	48.5 bcB	53.9 bcB	67.2 aA
T ₄ (43 °C; 48 h)	36.4 cAB	38.9 cA	64.2 cAB	35.0 cB	40.6 cA	59.4 bB	43.0 cA	47.2 cA	69.8 aA
CV (%)	FC = 8.5			LC = 8.9			V = 6.0		

Means followed by the same uppercase letter within a row and lowercase letter within a column are not significantly different by the Tukey's test ($P = 0.05$).

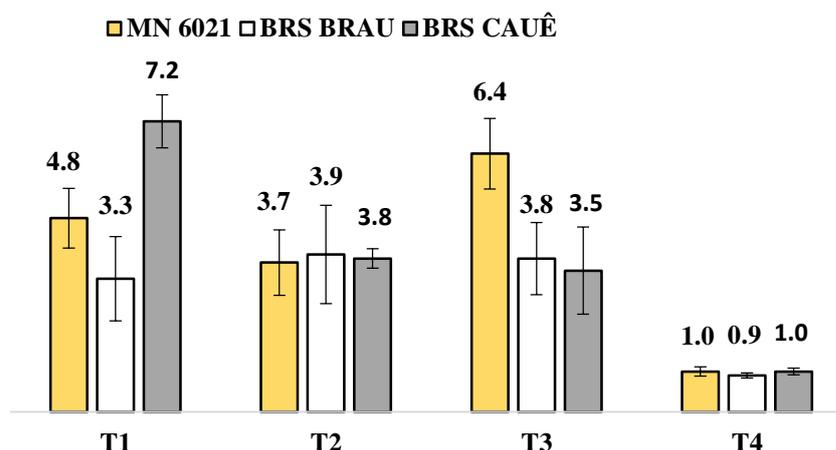


Fig 1. Longest radicle length (cm) at day seven of the germination test in seedlings germinated from seeds of three barley cultivars (MN 6021, BRS Brau and BRS Cauê) exposed to the following accelerated aging (AA) methods: T₁ (41 °C, 72 h), T₂ (42 °C, 48 h); T₃ (42 °C, 60 h); and T₄ (43 °C, 48 h).

Table 2. Number of seminal roots (NR), shoot length (SL), and growth (G) values computed by the Seed Analysis System (SAS[®]) at day seven of the germination test for three barley seed varieties subjected to four accelerated aging (AA) methods.

Method	Cultivar								
	MN 6021			BRAU			CAUÊ		
	NR	SL (cm)	G (%)	NR	SL (cm)	G (%)	NR	SL (cm)	G (%)
T ₁ (41 °C; 72 h)	5.9 aB	10.1 aA	82.6 aA	7.3 aA	10.9 aA	68.1 aB	5.9 aB	7.3 bB	78.8 aA
T ₂ (42 °C; 48 h)	4.8 bC	9.7 aA	83.3 aA	6.8 aA	9.6 bA	67.4 aB	5.9 aB	6.9 bB	79.0 aA
T ₃ (42 °C; 60 h)	5.0 bB	9.3 aA	72.3 bA	6.2 bA	8.8 bA	74.2 aA	5.9 aA	8.6 aA	71.5 abA
T ₄ (43 °C; 48 h)	4.8 bB	7.6 bA	55.6 cAB	5.4 cA	6.2 cB	47.0 bC	5.3 aAB	5.1 cC	65.7 bA
CV (%)	NR = 5.7			SL = 7.4			G = 6.4		

Means followed by the same uppercase letter within a row and lowercase letter within a column are not significantly different by the Tukey's test ($P = 0.05$).

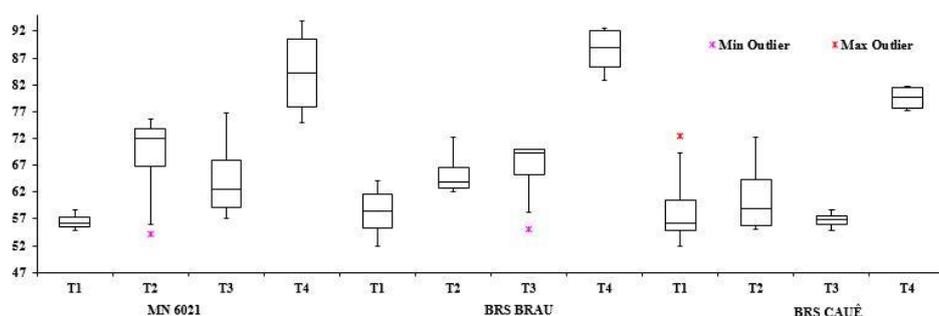


Fig 2. Seedling uniformity (%) values computed by the Seed Analysis System (SAS[®]) at day seven of the germination test for of three barley cultivars (MN 6021, BRS Brau and BRS Cauê) exposed to the following accelerated aging (AA) methods: T₁ (41 °C, 72 h), T₂ (42 °C, 48 h), T₃ (42 °C, 60 h); and T₄ (43 °C, 48 h).

Uniformity

Seedling uniformity is one of the attributes used to determine the physiological quality of a seed lot, and uniform seed lots are more vigorous (Pinto et al., 2015). In our study, the uniformity index was similar among cultivars in T₄ (43 °C, 48 h), and the higher aging temperature may have caused a reduction in protein synthesis leading to a more uniform radicle length between cultivars (Fig. 1). Thus, uniformity was not a reliable indicator of vigor because of the discrepancy in radicle lengths observed between AA methods. Similarly, Pinto et al. (2015) found contrasting results between the SAS[®] uniformity index and standard germination and vigor tests when screening maize hybrids for seed vigor, unlike Peñaloza et al. (2015), who reported that the SAS[®] index based on speed of growth and uniformity accurately identified normal lettuce seedlings.

Figure 2 shows the dispersion of SAS[®] uniformity index values within and between seedling replicates for each AA method. The dispersion of the data provides useful information about the number of seedlings per replicate. Even though uniformity index values were not highest in T₂ (42 °C, 48 h), there was a smaller variance in the index between seedlings. In addition, the box plot identified outliers among the replicates in AA methods T₁, T₂, and T₃ (cultivar × treatment). Thus, future studies should increase the number of seedlings per replicate to improve the power of the tests.

Materials and Methods

Plant material and treatment

Barley seeds from three varieties (cultivars BRS Brau, BRS Cauê, and MN 6021) with similar germination potential (85–87%) were exposed to the following AA conditions (M): T₁ (controlled aging at 41 °C for 72 h) (Tunes et al., 2010), T₂ (42 °C for 48 h) (Samarah and Al-Kofahi, 2008), T₃ (control) (42 °C for 60 h) (Krzyszczak and Vieira 1999), and T₄ (43 °C for 48 h) (Ohlson et al., 2010).

Analyzed variables

Eight seed vigor parameters were evaluated in the study, of which five were determined using traditional vigor tests: first count of viable seeds (FC), final (Last) count of viable seeds (LC), shoot length, radicle length, and number of seminal roots; and three using the Seed Analysis System (SAS[®]): vigor, uniformity, and growth.

Methods

For AA tests, barley seeds were distributed uniformly on a wire mesh tray suspended over 40 ml of water in a plastic box (Tunes et al., 2010). Seeds were then placed in four aging chambers set up to maintain different temperatures (± 0.5 °C) for different aging times (T₁–T₄). Samples of 50 seeds from each cultivar were then placed on germination paper wrapped in plastic bags and stored at 5 ± 4 °C for seven days to overcome dormancy. The seeds were then placed in a Mangelsdorf-type germination chamber and maintained at 20 °C for seven days (Brasil, 2009).

The number of viable seeds, defined as those that developed normal seedlings according to Association of Official Seed Analysts (AOSA, 1983), was recorded at days four (first count, FC) and seven (last count, LC) of the germination test. After the LC, five normal seedlings from each replicate (Nakagawa, 1999) had their longest shoot length, radicle length, and number of seminal roots recorded. An image of these seedlings was acquired using the SAS[®], each image containing 10 seedlings, and a vigor¹ index was calculated based on speed of growth² and uniformity³ of barley shoot and radicle lengths averaged from two images totaling 20 seedlings per replicate as follows:

$$^1Vigor\ index = wg_{growth\ index} + wu_{uniformity\ index}$$

$$^2Growth\ index = \min(w_h * l_h * l_r * 1000)$$

$$^3Uniformity\ index = \max(1000 - (w_{Sh} * S_h + w_{Sr} * S_r + S_{total} + w_{Srh} * S_{rh}) - W_d)$$

Where:

l_h and l_r are the means of the shoot and radicle lengths, respectively;

S_h , S_r , S_{total} , and S_{rh} are the standard deviations of the shoot length, radicle length, total length, and the ratio of the shoot and radicle lengths;

wg and wu are weights of the growth and uniformity indices; and the w 's represent associated weights with parameters being multiplied.

Experimental design and statistical analysis of data

Data from traditional vigor tests and the SAS[®] were arranged in a completely randomized factorial design (temperature-time vs cultivars) with four replicates and analyzed using the F test (1% and 5%) followed by the Tukey's test for means comparison (5%). The relationship between vigor and first count, growth, and shoot length was analyzed using correlation analysis (1% and 5%).

Conclusion

The best accelerated aging (AA) methods for measuring the physiological quality of barley seeds were T₂ (42 °C, 48 h) and T₃ (43 °C, 60 h) with the first count of normal seedlings made at day four of the germination test.

Computer analysis of digital images of barley seedlings is a valuable tool for testing barley seed vigor. Because the method is easy to perform and seedling measurements can be taken earlier at day four of the germination test, accelerated aging method T₂ gave the best separation in seed lot vigor whereas T₃ was the best method to evaluate seedling growth using the SAS index.

References

- Abdalla FH, Roberts EH (1968) Effects of temperature, moisture and oxygen on the induction of chromosome damage in seeds of barley, broad beans and peas during storage. *Annals of botany (New Series)*. 32:119-136.
- Abdul-Baki AA, Anderson JD (1970) Viability and leaching of sugars from germinating barley. *Crop Sci*. 10:31-34.
- Association of Official Seed Analysts [AOSA] (1983) Seed vigor testing handbook. Ithaca, AOSA.
- Arisnabarreta S, Eslava ET, Cannon P (2012) Screening method and response surface design for drying hybrid maize to maintain seed quality. *Crop Sci*. 52:1298-1305.

- Bort J, Araus JL, Hazzan H, Grando S, Ceccarelli S (1998) Relationships between early vigour, grain yield, leaf structure and stable isotope composition in field grown barley. *Plant Physiol Bioch.* 36:889–897.
- Brasil (2009) Regras para análise de sementes, Brasília, Ministério da Agricultura, Pecuária e Abastecimento.
- Brunes AP, Araújo AS, Dias LW, Villela FA, Aumonde TZ (2016) Seedling length in wheat determined by image processing using mathematical tools. *Rev Cienc Agron.* 47:374-379.
- Companhia Nacional de Abastecimento [CONAB] (2017) Séries históricas. Available from: <http://www.conab.gov.br/conteudos.php?a=1252&Pagina_objcmsconteudos=2#A_objcmsconteudos>
- Dell' Aquila A (1994) Wheat seed ageing and embryo protein degradation. *Seed Sci Res.* 4:293-298.
- Demir I, Mavi K (2008) Controlled deterioration and accelerated aging tests to estimate the relative storage potential of cucurbit seed lots. *HortScience.* 43:1544-1548.
- Ghahfarokhi MG, Ghasemi E, Saeidi M, Kazafi ZH (2014) The effect of accelerated aging on germination characteristics, seed reserve utilization and malondialdehyde content of two wheat cultivars. *Journal of Stress Physiology & Biochemistry.* 10:15-23.
- Gomes-Junior FG, Chamma HMCP, Cicero SM (2014) Automated image analysis of seedlings for vigor evaluation of common bean seeds. *Acta Sci-Agron.* 36: 195-200.
- Han Z, Ku L, Zhang Z, Guo S, Liu H, Zhao R, Ren Z, Zhang L, Su H, Dong L, Chen Y (2014) QTLs for seed vigor-related traits identified in maize seeds germinated under artificial aging conditions. *PLoS ONE.* 9:e92535.
- Isely, D (1957) Vigor tests. *Proceedings of association of official seed analysts.* 47: 176-182.
- Jatoi SA, Afzal M, Nasim S, Anwar R (2001) Seed deterioration study in pea, using accelerated ageing techniques. *Pakistan Journal of Biological Sciences.* 4:1490-1494.
- Kaewnaree P, Vichitpham S, Klanrit P, Siri B, Vichitpham K (2011) Effect of accelerated aging process on seed quality and biochemical changes in sweet pepper (*Capsicum annuum* Linn.) seeds. *Biotechnology.* 10:175-182.
- Kapilan R (2015) Accelerated aging declines the germination characteristics of the maize seeds. *Scholars Academic J Biosciences.* 3:708-711
- Kappor N, Mohd AA, Siddiqui A, Kumar H, Amir A (2011) Physiological and biochemical changes during seed deterioration in aged seeds of rice (*Oryza sativa* L.). *American Journal of Plant Physiology.* 6:28-35.
- Kazim M, Ibrahim D (2007). Controlled deterioration and accelerated aging tests predict relative seedling emergence potential of melon seed lots. *HortScience.* 42:1431-1435.
- Kikuti ALP, Marcos-Filho J (2013) Seedling imaging analysis and traditional tests to assess okra seed vigor. *J S Sci.* 35:443-448.
- Kim SH, Bin YH, Choe ZH (1989) The use of multiple seed vigor indices to predict field emergence and grain yield of naked and malting barley. *Korean Journal of Crop Science.* 34:134-141.
- Koehler P, Wieser H (2013) Chemistry of cereal grains. In: Gobbetti, M. and Gänzle, M. (ed.), *Handbook on sourdough siotechnology.* New York, NY, Springer Science+Business Media.
- Kryzanowki FC, Vieira RD (1999) Deterioração controlada. In: Kryzanowki, FC, Vieira, RD and França-Neto, JB. (ed.). *Vigor de sementes: conceitos e testes.* Londrina, PR, Abrates.
- Marcos-Filho J, Kikuti ALP, Lima LB (2009) Métodos para avaliação do vigor de sementes de soja incluindo a análise computadorizada de imagens. *Rev Bras Sementes.* 31:102-112.
- Marcos-Filho J (2013) Importância do potencial fisiológico da semente de soja. *Informativo Abrates.* 23:21-24.
- Marcos-Filho J (2015) Seed vigor testing: an overview of the past, present and future perspective. *Sci Agr.* 72:363-374.
- McDonald MB (1999) Seed deterioration: physiology, repair and assessment. *Seed Science Technology.* 27:177-237.
- Menezes VO, Lopes S, Tedesco SB, Henning FA, Zen HD, Mertz LM (2014) Cytogenetic analysis of wheat seeds submitted to artificial aging stress. *J Seed Sci.* 36: 71-78.
- Møller B, Munck L (2002) Seed vigour in relation to heat sensitivity and heat resistance in barley evaluated by multivariate data analysis. *J I Brewing.* 108: 286–293.
- Nakagawa J (1999) Testes de vigor baseados no desempenho das plântulas. In: Kryzanowki FC, Vieira RD, França-Neto JB. (ed.). *Vigor de sementes: conceitos e testes.* Londrina, PR, Abrates.
- Nepomuceno CF, Rios APS, Domingos SRO, Pelacani CR, Santana JRF (2009) Respostas morfofisiológicas in vitro de plântulas de *Anadenanthera colubrina* (Vell.) Brenan var. cebil (Griseb) Altschul. *Revista da Árvore.* 33:481-490.
- Peñaloza P, Ramires-Rosales G, McDonald MB, Bennet MA (2015) Lettuce (*Lactuca sativa* L.) seed quality evaluation using seed physical attributes, saturated salt accelerated aging and the seed vigour imaging system. *Electron J Biotech.* 8.
- Pinto CAG, Carvalho MLM, Andrade DB, Leite ER, Chalfoun I (2015) Image analysis in the evaluation of the physiological potential of maize seeds. *Rev Cienc Agron.* 46:319-328.
- Ohlson OC, Kryzanowski FC, Caieiro JT, Panobianco M (2010) Teste de envelhecimento acelerado em sementes de trigo. *Rev Bras Sementes.* 32:118-124.
- Radha BN, Channakeshava BC, Bhanuprakash K, Gowda KTP, Ramachandrapa BK, Munirajappa R (2014) DNA damage during seed ageing. *Journal of Agriculture and Veterinary Science.* 7:34-39.
- Sako Y, McDonald MB, Fujimura K, Evans AF, Bennett MA (2001) A system for automated seed vigor assessment. *Seed Science Technology.* 29:625-636.
- Samarah NH, Al-kofahi S (2008) Quality Tests to field emergence of artificial aged barley seeds in the semiarid mediterranean region. *Jordan Journal of Agricultural Sciences.* 4: 217-229.
- Silva VN, Júnior FGG, Cicero SM (2012) Computerized imaging analysis of seedlings for assessment of physiological potential of wheat seeds. *Rev Bras Sementes.* 34:589-596.
- Spano C, Buselli R, Castiglione MR, Bottega S, Grilli I (2007) RNAses and nucleases in embryos and endosperms from naturally aged wheat seeds stored in different conditions. *J Plant Physiol.* 164:4487–495.
- Sýkorová A, Sárka E, Bubník Z, Schejbal M, Dostálek P (2009) Size Distribution of Barley Kernel. *Czech J Food Sci.* 27:249–258.

- Tomes LJ, Tekrony DM, Egli DB (1988) Factors influencing the tray accelerated aging test for soybean seed. *Journal of Seed Technology*. 12:24-36.
- Torres RM, Vieira RD, Panobianco M (2004) Accelerated aging and seedling field emergence in soybean. *Sci Agr*. 61:476-480.
- Tunes LM, Barros ACSA, Bandinelli PG, Garcia DC (2010) Diferentes épocas de colheita e qualidade fisiológica de sementes de cevada. *Rev Bras Sementes*. 32:42-48.
- United States Department of Agriculture [USDA] (2017) World Agricultural Production. Available from: <<http://apps.fas.usda.gov/psdonline/circulars/production.pdf>>
- Yücel C, Baloch FS, Özkan H (2009) Genetic analysis of some physical properties of bread wheat grain (*Triticum aestivum* L.). *Turk J Agric For*. 33:525-535.
- Zhang H, Wang WQ, Liu SJ, Moller IM, Song SQ (2015) Proteome analysis of poplar seed vigor. *PLoS ONE*. 10:e0132509.