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 2009. 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) (Ghaefarokhi 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 (Kaewnaree et al., 2011) and reduced synthesis of proteins involved in germination (DeL 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 (Syrkorová 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 T₄ (Table 1). In addition, each AA method allowed separation of barley cultivars into two vigor categories: T₁ separated MN 6021 and BRS Cauê seeds from BRS Brau seeds; T₂, MN 6021 and BRS Brau seeds from BRS Cauê seeds; T₃, MN6021 seeds from BRS Brau and BRS Cauê seeds; and T₄, 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 T₁ (control) and T₂ are minimal, allowed the use of T₂.

BRS Brau seeds aged at 41 °C for 72 h (T₁) 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 T₁ (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 (T₄) 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 T₁ (41 °C, 72 h), T₂ (42 °C, 48 h), and T₄ (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 T₁ (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 T₂ 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 T₁ (42 °C, 60 h), whereas total length measurements from methods T₃, T₄, and T₅ 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 T₃ (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 (T₄) when compare with the control (T₃), 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 T₃, 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.

<table>
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<tr>
<th>Method</th>
<th>Cultivar</th>
<th>MN 6021</th>
<th>FC (%)</th>
<th>LC (%)</th>
<th>V (%)</th>
<th>BRAU</th>
<th>FC (%)</th>
<th>LC (%)</th>
<th>V (%)</th>
<th>CAUÊ</th>
<th>FC (%)</th>
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<td>FC</td>
<td>LC</td>
<td>V</td>
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<td>T₁ (41 °C; 72 h)</td>
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<td>61.7 bA</td>
<td>74.8 abA</td>
<td>31.4 cB</td>
<td>31.4 bA</td>
<td>65.2 abB</td>
<td>59.9 aA</td>
<td>60.2 abA</td>
<td>74.0 aA</td>
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<td>BRAU</td>
<td>70.4 aA</td>
<td>75.5 aA</td>
<td>78.9 aA</td>
<td>64.5 aA</td>
<td>74.9 aAB</td>
<td>66.8 aabB</td>
<td>54.5 bAbB</td>
<td>66.7 aB</td>
<td>70.8 aB</td>
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<td>CAUÊ</td>
<td>70.7 aA</td>
<td>76.0 aA</td>
<td>70.0 bA</td>
<td>54.5 bB</td>
<td>61.7 bB</td>
<td>71.7 aB</td>
<td>48.5 bcB</td>
<td>53.9 bcB</td>
<td>67.2 aB</td>
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<td>T₂ (42 °C; 48 h)</td>
<td>MN 6021</td>
<td>36.4 cAB</td>
<td>38.9 cA</td>
<td>64.2 cAB</td>
<td>35.0 cB</td>
<td>40.6 cA</td>
<td>59.4 bB</td>
<td>43.0 cA</td>
<td>47.2 cA</td>
<td>69.8 aA</td>
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<td></td>
<td>BRAU</td>
<td>36.4 cAB</td>
<td>38.9 cA</td>
<td>64.2 cAB</td>
<td>35.0 cB</td>
<td>40.6 cA</td>
<td>59.4 bB</td>
<td>43.0 cA</td>
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<td>38.9 cA</td>
<td>64.2 cAB</td>
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<td>40.6 cA</td>
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<td>47.2 cA</td>
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<td>T₃ (42 °C; 60 h)</td>
<td>MN 6021</td>
<td>4.8 bB</td>
<td>7.6 bA</td>
<td>55.6 cAB</td>
<td>5.4 cA</td>
<td>6.2 cB</td>
<td>47.0 bC</td>
<td>5.3 aB</td>
<td>5.1 cC</td>
<td>65.7 bA</td>
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<td>BRAU</td>
<td>4.8 bB</td>
<td>7.6 bA</td>
<td>55.6 cAB</td>
<td>5.4 cA</td>
<td>6.2 cB</td>
<td>47.0 bC</td>
<td>5.3 aB</td>
<td>5.1 cC</td>
<td>65.7 bA</td>
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<td></td>
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<td>7.6 bA</td>
<td>55.6 cAB</td>
<td>5.4 cA</td>
<td>6.2 cB</td>
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<td>5.3 aB</td>
<td>5.1 cC</td>
<td>65.7 bA</td>
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<td>T₄ (43 °C; 48 h)</td>
<td>MN 6021</td>
<td>10.1 aA</td>
<td>62.6 aA</td>
<td>86.8 aA</td>
<td>10.9 aA</td>
<td>68.1 aA</td>
<td>7.3 aB</td>
<td>7.3 bB</td>
<td>78.8 aA</td>
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<td></td>
<td>BRAU</td>
<td>5.9 aB</td>
<td>9.7 aA</td>
<td>83.3 aA</td>
<td>6.8 aA</td>
<td>9.6 bA</td>
<td>67.4 aB</td>
<td>5.9 aB</td>
<td>6.9 bB</td>
<td>79.0 aA</td>
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<td></td>
<td>CAUÊ</td>
<td>5.0 bB</td>
<td>9.3 aA</td>
<td>72.3 bA</td>
<td>6.2 bA</td>
<td>8.8 aA</td>
<td>74.2 aA</td>
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<td>CV (%)</td>
<td>MN 6021</td>
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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.

<table>
<thead>
<tr>
<th>Method</th>
<th>Cultivar</th>
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<th>NR</th>
<th>SL (cm)</th>
<th>G (%)</th>
<th>BRAU</th>
<th>NR</th>
<th>SL (cm)</th>
<th>G (%)</th>
<th>CAUÊ</th>
<th>NR</th>
<th>SL (cm)</th>
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<td>T₁ (41 °C; 72 h)</td>
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<td>5.9 aB</td>
<td>10.1 aA</td>
<td>82.6 aA</td>
<td>7.3 aA</td>
<td>10.9 aA</td>
<td>68.1 aB</td>
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<td>78.8 aA</td>
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<td>BRAU</td>
<td>4.8 bC</td>
<td>9.7 aA</td>
<td>83.3 aA</td>
<td>6.8 aA</td>
<td>9.6 bA</td>
<td>67.4 aB</td>
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<td>CAUÊ</td>
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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 T1 (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\textsuperscript{®} 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\textsuperscript{®} index based on speed of growth and uniformity accurately identified normal lettuce seedlings.

Figure 2 shows the dispersion of SAS\textsuperscript{®} 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) (Krzyszanowski 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\textsuperscript{®}): 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\textsuperscript{®}, each image containing 10 seedlings, and a vigor\textsuperscript{1} index was calculated based on speed of growth\textsuperscript{2} and uniformity\textsuperscript{3} of barley shoot and radicle lengths averaged from two images totaling 20 seedlings per replicate as follows:

\[ \text{Vigor index} = w_g \text{growth index} + w_u \text{uniformity index} \]

\[ \text{Growth index} = \min (w_h \cdot l_h \cdot l_r \cdot 1000) \]

\[ \text{Uniformity index} = \max (1000 - (w_{S_h} \cdot s_h + w_{S_r} \cdot s_r + s_{total} + w_{S_{vh}} \cdot s_{vh}) - W_d) \]

Where:

- \( h \) and \( r \) are the means of the shoot and radicle lengths, respectively;
- \( s_h, s_r, s_{total}, s_{vh} \) are the standard deviations of the shoot length, radicle length, total length, and the ratio of the shoot and radicle lengths;
- \( w_g \) and \( w_u \) 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\textsuperscript{®} 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 T3 gave the best separation in seed lot vigor whereas T1 was the best method to evaluate seedling growth using the SAS index.

**References**


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