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Alan Mario Zuffo
Jorge González Aguilera
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Rua Abaete, 83, Sala B, Centro. CEP: 78690-000.
Nova Xavantina – Mato Grosso – Brasil.
Telefone (66) 99682-4165 (Whatsapp).
https://www.editorapantanal.com.br
contato@editorapantanal.com.br

Apresentação

As áreas de Ciências Agrárias e Ciências Ambientais são importantes para a humanidade. De um lado, a produção de alimentos e do outro a conservação do meio ambiente. Ambas, devem ser aliadas e são imprescindíveis para a sustentabilidade do planeta. A obra, vem a materializar o anseio da Editora Pantanal na divulgação de resultados, que contribuem de modo direto no desenvolvimento humano.

O e-book "Pesquisas Agrárias e Ambientais Volume XI" é a continuação de uma série de volumes de e-books com trabalhos que visam otimizar a produção de alimentos, o meio ambiente e promoção de maior sustentabilidade nas técnicas aplicadas nos sistemas de produção das plantas e animais. Ao longo dos capítulos são abordados os seguintes temas: fatores físico-químicos que interferem no processo de compostagem; ácido húmico e microrganismos promotores de crescimento na germinação de sementes e desenvolvimento inicial de plantas de pepineiro; bioatividade de extratos de laranja e alho no desempenho germinativo de sementes de cenoura; paradigmas associados ao cultivo do eucalipto no cerrado; accelerated aging, cold, and electrical conductivity tests as parameters to analyze wheat seed vigor; germinação de sementes de espécies da flora brasileira ameaçadas de extinção: uma revisão; desempenho agronômico de híbridos de milho em segunda safra no Mato Grosso do Sul; agricultura 4.0: desenvolvimento, aplicações e impactos sociais; uso do biossólido como substrato para a produção de mudas; atributos físicos de uma topossequência de Luvissolos Crômicos (TC) no Semiárido paraibano; três espécies de Senecio (Asteraceae) proibidas na composição de produtos tradicionais fitoterápicos no Brasil; censo de roedores por consumo de alimentos no município de Paranaguá; uso da programação linear para estimar ganhos econômicos em sistemas de integração lavoura-pecuária: o caso da combinação da ovinocultura com atividades agrícolas no estado do Paraná, Brasil; comparação da presença de *Cryptococcus* ssp. em área verde urbana antes e após processo de revitalização; dificuldades e estratégias na comercialização de produtos da feira livre da Quatorze de Março em Capanema, Pará; análise dos impactos ambientais causados pela urbanização no Igarapé Sajope no município de Igarapé-Açu – Pará; aspectos Sobre a Produção e Comercialização de Tomate Orgânico; produção de Brássicas na Região Serrana do Estado do Rio de Janeiro. Portanto, esses conhecimentos irão agregar muito aos seus leitores que procuram promover melhorias quantitativas e qualitativas na produção de alimentos e do ambiente, ou melhorar a qualidade de vida da sociedade. Sempre em busca da sustentabilidade do planeta.

Aos autores dos capítulos, pela dedicação e esforços sem limites, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos na área de Ciência Agrárias e Ciências Ambientais Volume XI, os agradecimentos dos Organizadores e da Pantanal Editora. Por fim, esperamos que este ebook possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias e avanços para as áreas de Ciências Agrárias e Ciências Ambientais. Assim, garantir uma difusão de conhecimento fácil, rápido para a sociedade.

Os organizadores

Sumário

Capítulo 5

Accelerated aging, cold, and electrical conductivity tests as parameters to analyze wheat seed vigor

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Cristina Batista de Lima[1*]
Júlio César Altizani Júnior[2]
Jean Vitor Coutinho[3]
Victor Matheus Martins[4]

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second cereal with the largest production volume in the world, approximately 761 million tons in 2020 (FAO, 2021). In Brazil, to supply the domestic market, it is estimated that in the 2021 harvest alone, 6.4 million tons will be imported, representing over 50 % of demand (Conab, 2021). In addition to the economic point of view, the higher wheat production in Brazil is essential from the agricultural perspective, given its use as a cover crop in the summer (Kelh et al., 2016).

Commercial crops that result in higher yields depend on seed quality and sowing density. However, in the case of wheat, it is a common assumption that problems related to seed physiological quality have a less negative impact on the crop field because tillage can minimize losses caused by crop stand problems (Henning et al., 2019). In fact, crop problems related to seed quality are largely irremediable because increased sowing density may not compensate for the losses caused by underdeveloped plants originating from low vigor seeds, even if the crop stand is adjusted.

The minimum germination percentage required by the Ministry of Agriculture, Livestock, and Supply (MAPA) to market wheat seeds is 80 % (Brasil, 2013). However, the germination percentage is not always verified after seedling emergence, thereby making it essential to complement the information obtained in the germination test with additional vigor tests capable of detecting important differences in lots with similar germination and that meet established standards for marketing (Marcos-Filho, 2020a).

There are several vigor tests to evaluate seed lots, and the efficiency of each test depends on applying the appropriate method according to the species and intended purpose. Some vigor tests are

[1] Prof. Associado da Universidade Estadual do Norte do Paraná, Campus Luiz Meneghel - UENP/CLM, Departamento de Produção Vegetal, Bandeirantes, PR, Brasil.
[2] Mestrando da Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz - USP/Esalq, Curso de Mestrado em Fitotecnia, Piracicaba, SP, Brasil.
[3, 4] Graduando da Universidade Estadual do Norte do Paraná, Campus Luiz Meneghel, - UENP/CLM, Curso de Graduação em Agronomia, Bandeirantes, PR, Brasil.
[*] Autor correspondente: crislima@uenp.edu.br

validated by international organizations such as the International Seed Testing Association (ISTA) and the Association of Official Seed Analysts (AOSA), and among them, soybean (accelerated aging test), pea (electrical conductivity test), and maize (cold test) seeds have specific and standardized methods. The difficulty in establishing procedures for a vigor test for seeds of a particular species, according to Marcos-Filho (2020a), occurs because vigor is a complex and unmeasurable characteristic involving factors associated with the performance of seed lots.

The germination test for wheat seeds is standardized and performed under the ideal substrate, temperature, and humidity conditions and provides the maximum germination of the sample analyzed (Brasil, 2009). There are no specific procedures for vigor analysis of wheat seeds; hence, to reduce the possibility of incorrect interpretation of results, it is paramount to consider that the ranking of seed lots may vary depending on the genotype and the characteristic evaluated when selecting a method.

Research aimed at evaluating and/or comparing the efficiency of vigor tests is scarce for wheat seeds; the most recent study was by Ohlson et al. (2010), who studied the accelerated aging test. Given this scenario, this study was performed to compare the efficiency of accelerated aging, cold, and electrical conductivity tests to detect the differences in the physiological quality of wheat seed lots.

MATERIAL AND METHODS

This study was carried out in the Seed Analysis Laboratory of the Universidade Estadual do Norte do Paraná, Campus Luiz Meneghel (UENP-CLM), Bandeirantes, Paraná State. Nine wheat seed lots (category S2) of the cultivars 'Toruk', 'Sossego', and 'Mestre' (three lots of each cultivar) were analyzed. The seeds were obtained without chemical treatment from a seed trading company. All consumables, equipment, and utensils used were previously disinfected, as recommended in the Rules for Seed Analysis (RAS) (Brasil, 2009).

The seeds were analyzed for water content (WC) by the oven method at 105 ± 3 °C for 24 h, with two 5-g samples of seeds from each lot/cv (Brasil, 2009). The results were presented in percentage without statistical analysis because it is a parameter for the initial characterization of the lots.

For the germination test (GE), the seeds were distributed equally on sheets of filter paper previously moistened with distilled water at 2.5 times the mass of dry paper. The rolls were placed in transparent plastic bags and kept in a germination chamber at a constant temperature of 20 °C for eight days with a 16-h photoperiod. Evaluations occurred on the fourth and eighth day after the experiment began by considering the percentage of normal seedlings according to the criteria established by the RAS (Brasil, 2009). The first count of the germination test (FGE) refers to the percentage of normal seedlings on the fourth day after the germination test. Four repetitions with 50 seeds were used for each lot/variety.

Seedling emergence (SE) consisted of one seed/cell being sown 1.0 cm deep in polyethylene trays with 128 cells filled with a commercial substrate (MecPlant®). After sowing, the trays were irrigated and maintained on screened benches and under greenhouse conditions and irrigated daily in the morning and late afternoon; water was provided until the beginning of the excess was visible through the drainage hole. The percentage of normal seedling emergence was obtained on the eighth day after sowing. Four repetitions with 32 seeds per lot/crop were used.

The accelerated aging (AA) was determined using seed samples from each lot uniformly distributed on metal screens placed over transparent acrylic plastic boxes containing distilled water (40 mL). The covered containers were kept in a germination chamber at 43 °C for 48 h (Ohlson et al., 2010). The seeds were then similarly evaluated by the germination test as described above, and the counting of the percentage of normal seedlings was performed on the fourth day. The samples with fungi at the end of aging were disinfected with a 1 % (v/v) sodium hypochlorite solution before the germination test. Before and after accelerated aging, the water content of the seeds was determined in a similar way as described above, tolerating a maximum initial variation of 3 % between lots (Marcos-Filho, 2020b). Four repetitions with 50 seeds were used for each lot/cv.

For the soil-free cold test (CT), the seeds were distributed equidistantly on two sheets previously moistened with distilled water at 2.5 times the mass of dry paper to prepare the filter paper rolls, which were then placed in transparent plastic bags and kept at a constant temperature (10 ºC) for seven days with a 16-h photoperiod. The plastic bags with the rolls were then transferred to a germinator and kept at a constant temperature of 20 ºC for four days with a 16-h photoperiod (Cicero; Vieira, 2020). Four repetitions with 50 seeds were used for each lot/cv.

The electrical conductivity (EC) test consisted of the mass of the seeds being determined on a 0.0001 (g) precision scale and placed in 200-mL plastic cups containing 75 mL of deionized water. The containers were kept in a germination chamber at a constant temperature of 25 ºC for 24 h (Vieira; Marcos-Filho, 2020). Then, the EC of the solutions was measured with a conductivity meter calibrated with a standard solution at 146.9 $\mu S\ cm^{-1}$ (constant K = 1). Before each measurement, the conductivity electrode was cleaned with deionized water. The measurement result of each sample ($\mu S\ cm^{-1}$) was divided by its initial mass (g); therefore, the EC values for each lot were presented in $\mu S\ cm^{-1}\ g^{-1}$ of seed. Four repetitions with 50 seeds were used for each lot/cv.

The experimental design was entirely randomized with four repetitions for each lot/cv/test. The original data obtained in each test were submitted to variance analysis, and the Scott-Knott test grouped the means at 5% ($p < 0,05$). The association degree between results was analyzed by Pearson's correlation coefficient (r). The analyses were performed with the statistical software Sisvar® (Ferreira, 2019).

RESULTS AND DISCUSSION

The initial water content among the lots ranged from 7.3 to 8.3 % (Table 1) and is in accordance with MAPA guidelines, which covers the storage of wheat seeds in Brazil and mandates moisture percentages below 13 % (Brasil, 2011). In the case of seed storage for extended periods, Carvalho and Nakagawa (2012) recommended values between 4 to 8 %, provided they are under favorable environmental conditions. Nonetheless, excessive water removal during drying may cause irreversible damage because the remaining water in dehydrated tissues (below 7.5 % on a wet basis) has practically no mobility (Marcos-Filho, 2015). In this situation, rehydration after sowing in the field must be performed carefully to avoid damage during soaking, which could lead to lower seedling emergence. For wheat seeds, Eichelberger (2011) suggested that long-term storage should be carried out at 11-13 % because if drying is inadequate, the seeds will be subject to immediate mechanical damage and significant germination reductions.

Table 1. Means of initial water content (WC), water content after accelerated aging (WCA) and first germination count (FGE), germination (GE), seedling emergence (SE), accelerated aging (AA), cold test (CT), and electrical conductivity (CE) tests of wheat seeds. Source: author's own.

Cultivar	Lot	WC	WCA	FGE	GE	SE	CT	AA	EC
		%							μS cm^{-1} g^{-1}
'Toruk'	1	7.6	37.7	74.0 a	89.0 a	95,4 a	94.5 a	82.0 b	50.8 a
	2	7.5	37.6	77.0 a	92.0 a	95.3 a	94.5 a	76.5 b	49.2 a
	3	7.3	38.6	76.5 a	94.5 a	96.1 a	94.5 a	89.0 a	52.1 a
CV (%)		---	---	6.4	5.1	2.8	4.4	6.2	7.4
'Mestre'	1	7.7	36.0	67.5 a	96.5 a	96.1 a	95.5 a	70.5 a	33.3 a
	2	8.2	36.7	49.5 b	80.5 b	70.0 b	86.6 b	27.0 b	33.4 a
	3	8.3	36.2	50.0 b	80.0 b	78.9 b	83.0 b	13.5 c	36.7 a
CV (%)		---	---	16.0	7.1	7.3	5.4	18.8	8.1
'Sossego'	1	7.3	36.3	69.5 a	90.0 a	91.4 a	82.5 b	8.5 c	45.8 a
	2	7.4	38.7	70.5 a	94.0 a	92.2 a	87.5 b	45.0 b	43.0 a
	3	7.8	36.0	74.5 a	94.0 a	93.0 a	95.0 a	74.5 a	45.0 a
CV (%)		---	---	12.3	3.2	4.6	4.6	17.6	5.2

Means followed by the same latter in the column within each cultivar do not fifer by the Scott-Knott test at 5% ($p < 0.05$); CV = coefficient of variation.

The FGE and GE tests identified differences between the 'Mestre' seed lots, with Lot 1 having the highest viability; however, the averages of Lots 2 and 3 were greater than or equal to 80 % (Table 1), which is the minimum percentage required for marketing wheat seeds according to MAPA (Brasil, 2013). The FGE can be an indication of the physiological quality of the lot because samples with seeds that germinate quickly (i.e., have a higher normal seedling percentage in the first count of the germination test) are classified as having greater vigor (Carvalho; Nakagawa, 2012; Marcos-Filho, 2015).

In the SE test, the average percentages of emersed seedlings were above 90 % except in Lots 2 and 3 of 'Mestre', in which the percentages were below 80 % (Table 1). The results of the SE, FGE, and GE tests coincided in separating Lots 2 and 3 of this cultivar. The environmental conditions that occurred during the seedling emergence test were appropriate for wheat seed germination; favorable environmental conditions at the time of sowing allow the correspondence between the germination and seedling emergence percentages to be observed (Marcos-Filho, 2020a). Despite no standardized method, the SE test is useful for obtaining more information and evaluating the real viability of seed lots (Carvalho; Nakagawa, 2012).

Although the results of the CT allowed us to classify the 'Mestre' and 'Sossego' lots in two vigor levels, the average normal seedling percentages of the lower quality lots were above 80 % and, in some cases, exceeded the averages verified in the GE test (Table 1). According to Marcos-Filho (2015), the minimum temperature for wheat seed germination is 4 ºC; RAS guidelines mandate pre-cooling at 5-10 ºC as one of the treatments to overcome wheat seed dormancy (Brasil, 2009). Considering that the seeds remained below 10 ºC in the CT, this temperature may not have been enough to promote adverse effects, which is characteristic of a vigor test that assesses stress resistance. It is worth noting that despite the CT being employed at a large scale to evaluate the vigor of seed lots, its use is exciting when one intends to evaluate the effects of the industrial chemical treatment of seeds (Cicero; Vieira, 2020).

The maximum variation in water content between the lots evaluated after the AA was 2.7 % (Table 1), which supports the results obtained because the AA test is the only one in which there is the possibility of using a criterion that indicates the adequacy of the procedures adopted by comparing the initial and post-AA moisture content, which according to Marcos-Filho (2020b), should not surpass 3.0%. If the variation in the final moisture content between the lots is above the reference value, the test must be redone as the higher the water content of a lot, the greater its sensitivity to elevated temperatures and relative humidity that occur during the test. The AA test results allowed us to identify two vigor levels among the 'Toruk' lots and, in the 'Mestre' and 'Sossego' lots, it was possible to observe three levels (Table 1). Although there is no formal proposal to characterize vigor levels in the interpretation of the results presented herein, Marcos-Filho (2020b) suggested the classification of high vigor for lots with germination above or equal to 85%, medium between 65 and 85%, and low for values below 65%. Thus, Lot 3 of the 'Toruk' cultivar can be classified as high vigor because it obtained a percentage of 89% (Table 1).

Wheat is a winter crop and benefits from temperatures between 18 and 24 ºC, and temperatures above 30 ºC significantly decrease yield and grain quality (Oliveira-Neto; Santos, 2017); hence, it was assumed that the high temperature and humidity conditions imposed by the AA test would significantly reduce the normal seedling percentage in all lots. Nevertheless, it was possible to verify at least one lot with data above 70% in all three cultivars, thus indicating that the better quality lots maintained good germination percentages even after stress. This finding demonstrates that the AA test can be used to estimate the vigor of wheat seeds.

Based on the results of the EC test, it was not possible to identify significant differences between the lots of each cultivar (Table 1). The absence of reference values to classify the vigor of wheat seed lots by the EC test, as occurs with pea (Matthews and Powell, 1981) and soybean (Prado et al., 2019) seeds, makes the interpretation of the results restricted to statistical differences, thereby giving room to incorrect deductions. According to Vieira and Marcos-Filho (2020), lower vigor seeds have higher levels of cell membrane deterioration during soaking and consequently release higher amounts of solutes. Based on this principle, Lots 2 and 3 of the 'Mestre' cultivar can be classified as high vigor because they had the lowest EC values and did not differ from Lot 1; nevertheless, these lots were identified as lower quality by the FGE, GE, SE, CT and AA tests (Table 1).

The available methods for analyzing the physiological quality of seeds do not describe specific EC test procedures for wheat seeds, making it difficult to precisely explain why the data of this test were discrepant from the others. One plausible explanation may be the structure of the wheat seed and the resistance of its membranes to the oxidative processes inherent to deterioration, causing solute leaching to occur at lower speeds, or that it is not among the first noticeable events. Marcos-Filho (2015) reported that several modalities of cellular alterations take place during seed deterioration, and the models proposed to demonstrate the sequence in which these processes occur briefly and do not necessarily obey a pre-defined and easily identified succession; moreover, the relative importance of each event or its set may vary according to each lot or species. In this context, the indication of the electrical conductivity test to evaluate the vigor of wheat seed lots requires this method to be adapted for these seeds to make it appropriate to obtain safe and reproducible results capable of predicting their actual physiological condition.

When correlating the test results, it is possible to verify a strong and positive (>0.7) correspondence between the SE and FGE and AA and CT tests (Figure 1). The correlations verified between the FGE test with the GE and EC tests as well as the GE test with the SE test, even though they were significant and above 0.65, cannot be considered strong because they were below 0.7 and, therefore, have a lower probability of occurring in the field or storage environments. The correlation analysis should not be the only parameter to estimate the efficiency of vigor tests, and, according to Marcos-Filho (2020a), a significant correlation indicates a tendency of similar variation between two tests. However, this does not mean that there is a corresponding precision in estimating the physiological quality of the lot because the conditions imposed during each test promote different stress levels and reactions on the seeds. In this context, Wendt et al. (2017) reported that the correspondence between the results of different vigor tests increases when comparing tests that use conditions capable of causing similar stress to those that occur when field circumstances are unfavorable. For Marcos-Filho (2020a), the ability to separate lots based on statistical comparison of means has greater efficiency in distinguishing the physiological potential of seed lots compared to correlation analysis, which favors incorrect or incomplete interpretations. Data can correlate positively or negatively only because they present similarities regarding variation trends, which is not enough to evaluate the sensitivity of each test to detect differences between lots.

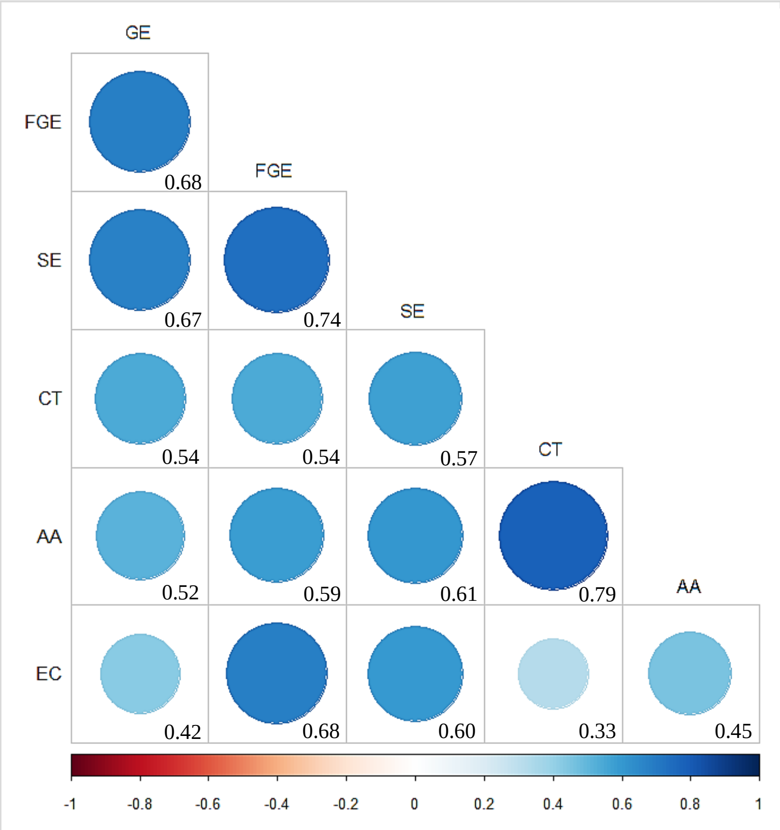

Figure 1. Pearson's correlation coefficient (r) among the tests used to analyze the physiological quality of wheat seed lots of the cultivars 'Toruk', 'Mestre', and 'Sossego'. Ns = not significant at 5%; GE = germination; FGE = first germination count; SE = seedling emergence; CT = cold test; AA = accelerated aging; EC = electrical conductivity. Source: author's own.

The temperatures of the germination (20 °C), cold (10 °C), and accelerated aging (41 °C) tests affected seedling development. To exemplify, images of 'Sossego' seeds were selected (Figure 2), in which the lots did not differ in the GE tests while being classified in three levels of vigor in the AA test (Table 1). When comparing the appearance of the seedlings of Lots 1 and 3 (Figure 2), it is possible to observe that they were visually similar in the germination test in both lots. The Lot 3 seedlings, after the AA test, developed roots and aerial part, while the growth of the roots seems to have been favored over the aerial part in the CT. The seedlings from Lot 1 after the CT were similar to Lot 3 after the AA test. Thus, there is the possibility that the temperature imposed by the CT did not provide enough stress to drastically reduce the percentage of germinated seeds, although it acted directly on seedling quality. Temperature affects the speed of water absorption and biochemical reactions, which determine the germination process and change seedling emergence. According to Taiz et al. (2017), under heat stress, water absorption by the seed decreases, impairing the subsequent growth of the seedling. The ability of a seed to germinate under a wide range of conditions is used as a parameter to estimate the manifestation of its vigor. According to Carvalho and Nakagawa (2012), minimum temperatures reduce germination speed and interfere in emergence uniformity, while under maximum temperatures, only the seeds of greater vigor germinate.

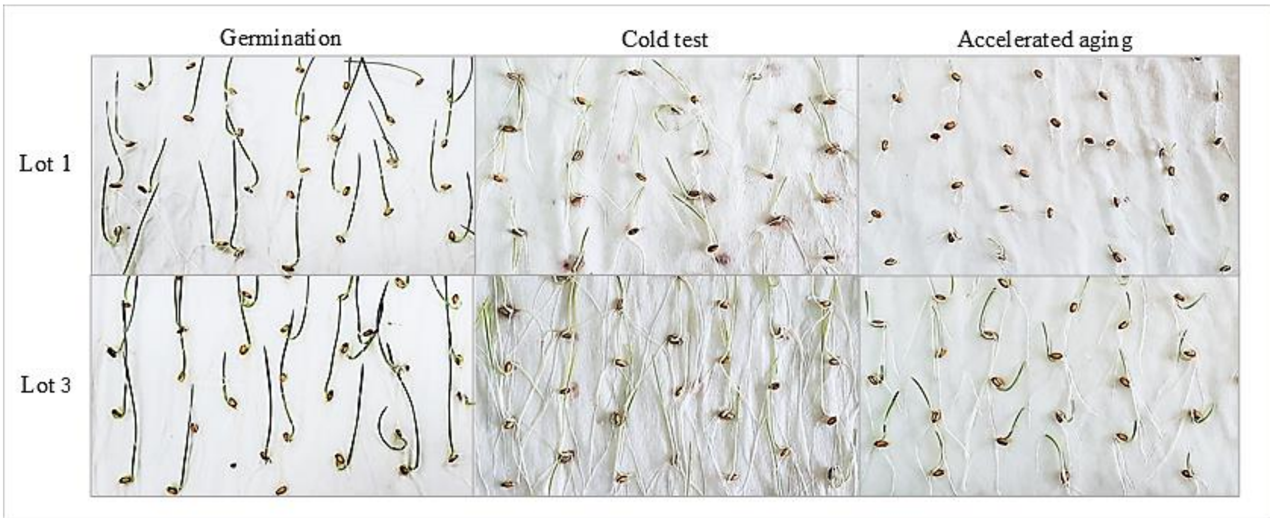

Figure 2. The appearance of lots 1 and 3 of 'Sossego' wheat seeds during the evaluation of the germination, cold, and accelerated aging tests. Source: author's own.

The differences between 'Mestre' lots were identified in all the tests, in the CT and AA tests for the 'Sossego' lots, and only in the AA test for the 'Toruk' lots (Table 1). The AA test was the only one to reveal differences between the lots of all three cultivars without the need to provoke excessive stress because in the lots with better physiological quality, the averages reached percentages above 70 %. This result demonstrates the importance of adopting vigor analysis as a routine for wheat seeds. According to Marcos-Filho (2020a), lots that present high viability (i.e., high germination percentage) may have different performance in other tests depending on stress intensity and vigor level.

The development of new cultivars, soil and climate differences generated by the regions where wheat is grown, and the sowing seasons reinforce the need for further research on the effects of environmental factors on seedling germination, development, and establishment. In fact, Marcos-Filho (2015) reported it is unlikely that a single vigor test can satisfactorily and precisely evaluate the quality of seed lots, which may result in incomplete and/or insufficient data. Given this context, the joint analysis of the results of two or more tests guarantees consistent and reliable information. Notably, the findings of vigor tests only allow comparisons between lots to be made and not predictions about seedling emergence in the field. According to Marcos-Filho (2020a), stress after sowing in the field can be severe enough to drastically reduce seedling emergence, even for lots with high germination percentage and vigor, making it crucial to avoid predictions about seedling emergence solely based on the results of vigor tests. The actual superiority of a seed lot will always depend on the environment after sowing or during storage.

CONCLUSION

The accelerated aging test allowed us to clearly and objectively classify wheat seed lots as to vigor, and its inclusion in the routine of companies that sell wheat seeds is highly recommended. The stress caused during the cold test acted qualitatively on seedling development, although it did not make it possible to accurately identify differences in vigor among wheat seed lots.

The lack of reference values to help interpret the data of the electrical conductivity test to evaluate the vigor of wheat seed lots makes the classification of the lots highly subjective, making it difficult to obtain reliable and reproducible results capable of predicting the actual physiological conditions of wheat seeds.

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Índice Remissivo

Sobre os organizadores

Alan Mario Zuffo

Engenheiro Agrônomo, graduado em Agronomia (2010) na Universidade do Estado de Mato Grosso (UNEMAT). Mestre (2013) em Agronomia - Fitotecnia (Produção Vegetal) na Universidade Federal do Piauí (UFPI). Doutor (2016) em Agronomia - Fitotecnia (Produção Vegetal) na Universidade Federal de Lavras (UFLA). Pós - Doutorado (2018) em Agronomia na Universidade Estadual de Mato Grosso do Sul (UEMS). Atualmente, possui 165 artigos publicados/aceitos em revistas nacionais e internacionais, 127 resumos simples/expandidos, 66 organizações de e-books, 45 capítulos de e-books. É editor chefe da Pantanal editora e revisor de 18 revistas nacionais e internacionais. Professor adjunto na UEMA em Balsas. Contato: alan_zuffo@hotmail.com.

Jorge González Aguilera

Engenheiro Agrônomo, graduado em Agronomia (1996) na Universidad de Granma (UG), Bayamo, Cuba. Especialista em Biotecnologia (2002) pela Universidad de Oriente (UO), Santiago de Cuba, Cuba. Mestre (2007) em Fitotecnia na Universidade Federal do Viçosa (UFV), Minas Gerais, Brasil. Doutor (2011) em Genética e Melhoramento de Plantas na Universidade Federal do Viçosa (UFV), Minas Gerais, Brasil. Pós - Doutorado (2016) em Genética e Melhoramento de Plantas na EMBRAPA Trigo, Rio Grande do Sul, Brasil. Professor Visitante na Universidade Federal de Mato Grosso do Sul (UFMS) no campus Chapadão do Sul (CPCS), MS, Brasil. Atualmente, possui 74 artigos publicados/aceitos em revistas nacionais e internacionais, 29 resumos simples/expandidos, 49 organizações de e-books, 37 capítulos de e-books. É editor da Pantanal Editora e da Revista Agrária Acadêmica, e revisor de 19 revistas nacionais e internacionais. Contato: j51173@yahoo.com, jorge.aguilera@ufms.br.

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Pantanal Editora
Rua Abaete, 83, Sala B, Centro. CEP: 78690-000
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