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Methodology for Buckwheat Germination Test

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Abstract **– Buckwheat (*Fagopyrum esculentum*) despite its name, it is not related to common wheat, this comparison is due to the use of its grains that may be incorporated, in human nutrition in flours form. Among the factors responsible for the success of buckwheat cultivation, the quality of seeds is fundamental, especially regarding to germination and vigour levels. In Brazil, germination tests are guided by the Rules for Seed Analysis, a publication made by the Ministry of Agriculture, Livestock and Supply, however, not all plant species have already had a determined methodology. Thus, the objective of this research was to determine the suitable methodology protocol for conducting the buckwheat seed germination test. The experiment analyses were performed at the Laboratory of research, teaching and extension support at experimental farm of Agrarian Sciences of Grand Dourados Federal University (UFGD), in the municipality of Dourados, Mato Grosso do Sul state, Brazil, with buckwheat seed produced at the same farm. The experimental design used was completely randomized in a 4×3 factorial scheme, with four temperatures, three substrates and four replications with 50 seeds each. The temperatures were: 15°C, 25°C, 30°C and 35°C and the substrates were: on paper, between paper and paper roll. The treatments were placed to germinate in B.O.D with constant light. The counting of germinated seeds occurred daily until the germination stabilization date, verified when the number of normal seedlings remained stable for three successive counting days. The characteristics evaluated were: First germination count, percentage of germination, germination speed index, mean germination time, mean germination speed, seedling length and synchronization index. For buckwheat (*Fagopyrum esculentum*) germination test in the laboratory, the substrates on paper and between paper at temperatures of 25°C and 30°C are indicated with constant light. Furthermore, the first count must occur at four days and the final evaluation of the germination percentage at eight days after sowing.**

Keywords – Fagopyrum esculentum, **Rules for Seed Analysis, Germination Speed Index, Mean Germination Time.**

I. INTRODUCTION

Buckwheat, also known as black wheat (*Fagopyrum esculentum*), is a dicotyledonous plant belonging to the Polygonaceae family and despite its name, it is not related to common wheat (*Triticum aestivum* L.), which is a monocotyledonous belonging to the Poaceae family. This comparison is due to the use of its grains that may be incorporated, for example, in human nutrition in the form of flours with an advantage, it does not have gluten in its composition [1].

In addition to this use, buckwheat is sown as a ground cover plant in crop rotation systems aiming to promote the absorption of minerals that are in the deeper layers of soil, accumulating them in the aerial part of plants. Thus, when the crop senescence, these minerals will be available on the soil surface, so the later crop will have better development [2].

Among the factors responsible for the success of buckwheat cultivation and any other crop, the quality of seeds is fundamental, especially regarding to germination and vigour levels, in addition to genetic purity.

Alvarenga et al. [3] (2020) include that the success requires the use of high quality seeds, with the potential to produce vigorous and productive plants, uniformly and in the shortest possible time.

One of the ways to access this type of information and introduce material of good origin to the field is to perform germination tests in the laboratory.

The germination test is the most used to determine the physiological quality of the seeds, being performed under controlled conditions of temperature, substrate and light, allowing the seeds to express the maximum germinative power without undesirable external interferences [4].

In Brazil, germination tests are guided by the Rules for Seed Analysis [5], a publication made by the Ministry of Agriculture, Livestock and Supply of Federal Government standardized according to the international rules of seed analysis of the International Seed Testing Association - ISTA, with collaboration of several renowned Brazilian professionals in the field of seed technology.

Among its chapters, there is one specifically aimed at germination test methodologies whose objective is to standardize them. At the end of the chapter, a table is presented with several botanical species exemplifying the ways to proceed the tests indicating ideal substrate and temperature, as well as the duration of the test for each one.

However, the update of the species does not follow the scenario of Brazilian agriculture and crops that were not treated with much importance before, there may be changes and new methodologies for germination tests may be necessary, as is the case of buckwheat, which does not have a defined methodology in the Rules for Seed Analysis.

Thus, the objective of this research was to determine the suitable methodology protocol for conducting the buckwheat seed germination test.

II. MATERIAL AND METHODS

The experiment analyses were performed at the Laboratory of Research, Teaching and Extension Support at Experimental Farm of Grand Dourados Federal University (UFGD), located at latitude of 22° 13’ 52.4495”, longitude of 54° 59’ 10.5372”, altitude of 411.75 m, in the municipality of Dourados, Mato Grosso do Sul state, Brazil, with buckwheat seed produced at the same farm.

The experimental design used was completely randomized in a 4×3 factorial scheme, with four temperatures, three substrates and four replications with 50 seeds each. The temperatures were: 15°C, 25°C, 30°C and 35°C and the substrates were: on paper, between paper and paper roll. In all cases, the substrates were moistened with treatments 2.5 times their masses.

When the substrate was paper roll, they were packed in plastic bags to prevent moisture loss. When the substrate was on paper or between paper, they were placed in a plastic germination box (“gerbox” - 11×11×3.5 cm), needing substrate rehydration every two days. In both cases, they were placed to germinate in B.O.D with constant light and temperature according to the treatment.

The counting of germinated seeds occurred daily until the germination stabilization date, verified when the number of normal seedlings remained stable for three successive counting days and the results were expressed

as germination percentage. The first germination count was determined when the germination reached approximately 50% of that obtained at the end of the test [6, 7].

Germination speed index [8] in which the number of normal seeds or seedlings was counted during the test duration, according to the formula: GSI = G1/N1 + G2/N2 + ... Gn/Nn, where: G1, G2, ... Gn = number of germinated seeds at the day of observation and N1, N2, ... Nn = number of days after sowing.

Mean germination time, calculated using the formula presented by Edmond and Drapalla [9]: MGT = (N1 G1 + N2 G2 +...+ Nn.Gn) / (G1 + G2 +...+ Gn), where: G1, G2, ... Gn = number of germinated seeds at the day of observation and N1, N2, ... Nn = number of days after sowing, expressing the results in days.

Mean germination speed (MGS), calculated using the formula MGS = 1/MGT, where: MGT is mean germination time calculated previously. The results were expressed in $days^{-1}$.

Seedling length, determined using the mean of 10 plants per plot. They were measured from the apex of the largest primary root to the end of the primary leaves. The results were expressed in millimeters per seedlings.

Synchronization index, calculated according to the methodology cited by Labouriau and Pacheco [10]: $E = -\Sigma fi.\ \log_2 fi$, where: fi = relative germination frequency (RGF) and $\log_2$ = base 2 logarithm, expressing the results in bits.

Data were submitted to analysis of variance and, in case of significance at 5% of probability, means were compared by Tukey test by Sisvar computer program [11].

III. Results and Discussion

The percentage of germinated seeds in the first count was assessed four days after sowing, when the germination reached approximately 50% of that obtained at the end of the test, and the means are presented in Table 1. It is possible to notice that the paper roll substrate had the lowest percentage of germination at all evaluated temperatures and when evaluating the temperatures within this substrate, there was no statistically significant difference among them.

Comparing the substrates on paper and between paper, it is observed that at 15°C the seeds germinated in the first count are superior in the substrate between paper. At 25°C the substrate on paper presented a greater number of seeds germinated even four days after sowing, statistically differing from the substrate between paper and at 30°C and 35°C both substrates presented the highest germination averages and did not differ from each other.

Table 1. First germination count (%) of buckwheat (*Fagopyrum esculentum*) seeds submitted to the germination test in different substrates and temperatures four days after sowing.

First Germination Count – %											
	Substrate									**Overall Mean**	**CV(%)**
Temperature (°C)	**On Paper**			**Between Paper**			**Paper Roll**				
15	37.50	c	C	73.50	a	AB	57.50	b	A	56.17	11.58
25	87.00	a	A	70,5	b	B	61.00	b	A	72.83	

First Germination Count – %											
	Substrate									Overall Mean	CV(%)
Temperature (°C)	On Paper			Between Paper			Paper Roll				
30	80.50	a	B	88,5	a	A	63.50	b	A	74.50	
35	80.00	a	B	80.00	a	AB	63.50	b	A	77.50	
Overall Mean	71.25			78.13			61.38				

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other, at the level of 5% of probability, by the Tukey test (Source: the authors).

The final percentage of germinated buckwheat seeds was evaluated eight days after sowing, when there was consistency in the count for at least three consecutive days and the means were shown in Table 2.

The paper roll substrate maintained the behaviour analysed in the first count, presenting the lowest germination averages at all evaluated temperatures. In this case, at 25°C the roll paper substrate did not statistically differ from the substrate between paper, and at 30°C the roll paper substrate did not statistically differ from the substrate on paper.

Comparing the substrates on paper and between paper, that obtained the highest means of seed germination, it may be observed that at 15°C the germination was higher in the substrate between paper, differing statistically and at 25°C, 30°C and 35°C both substrates did not present means that were statistically different.

Table 2. Percentage of buckwheat (*Fagopyrum esculentum*) seeds germination submitted to the germination test in different substrates and temperatures eight days after sowing.

% of Germination											
	Substrate									Overall Mean	CV(%)
Temperature (°C)	On Paper			Between Paper			Paper Roll				
15	59.50	b	A	80.00	a	A	63.50	b	A	67.67	9.66
25	87.00	a	A	75.50	ab	A	69.50	b	A	77.33	
30	80.50	ab	A	88.50	a	A	70.00	b	A	79.67	
35	80.00	a	A	80.00	a	A	63.50	b	A	74.50	
Overall Mean	66.63			76.75			81.00				

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other, at the level of 5% of probability, by the Tukey test (Source: the authors).

The germination speed index was calculated at the end of the test with the number of seeds germinated daily and the averages are shown in Table 3. At temperatures 25°C and 35°C there was no statistical difference between any substrate, while at 15°C and 30°C, the substrates between paper and paper roll were the ones that presented the highest means, not differing from each other, however, differing statistically from the substrate on paper.

Table 3. Germination speed index – GSI of buckwheat (*Fagopyrum esculentum*) seeds submitted to the germination test in different substrates and temperatures eight days after sowing.

Germination Speed Index – GSI											
	Substrate									Overall Mean	CV(%)
Temperature (°C)	On Paper			Between Paper			Paper Roll				
15	26.78	b	B	40.39	a	C	45.81	a	C	37.66	11.80
25	72.15	a	A	60.35	a	B	64.32	a	B	65.60	
30	63.64	b	A	78.35	a	A	79.34	a	A	73.78	
35	66.76	a	A	71.55	a	AB	74.71	a	AB	71.00	
Overall Mean	57.33			62.68			66.04				

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other, at the level of 5% of probability, by the Tukey test (Source: the authors).

Table 4 shows the germination mean time of buckwheat seeds and it is possible to observe that at all temperatures the paper roll substrate presented the lowest mean germination time, statistically differing from the substrates on paper and between paper.

Comparing the substrates on paper and between paper, which presented higher percentages of germinated seeds in the first and final counts (Tables 1 and 2), the substrate between paper presented lower and statistically different means, at 15°C and 30 °C, in the other hand, at 25°C and 35°C the two substrates did not differ from each other.

Table 4. Mean germination time – MGT (days) of buckwheat (*Fagopyrum esculentum*) seeds submitted to the germination test in different substrates and temperatures eight days after sowing.

Mean Germination Time – GMT (Days)											
	Substrate									Overall Mean	CV(%)
Temperature (°C)	On Paper			Between Paper			Paper Roll				
15	4.98	a	A	4.76	b	A	4.23	c	A	4.65	1.50
25	4.05	a	B	4.07	a	B	3.90	b	B	4.01	
30	4.11	a	B	3.97	b	B	3.68	c	C	3.92	
35	4.04	a	B	3.97	a	B	3.67	b	C	3.89	
Overall Mean	4.29			4.19			3.87				

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other, at the level of 5% of probability, by the Tukey test (Source: the authors).

The mean germination speed was the highest in the paper roll substrate in any temperature evaluated (Table 5). The germination speeds at temperatures 25°C and 35°C were the same in substrates on paper and between paper and at temperatures 15°C and 30°C the mean speeds were lower in the substrate on paper compared to the substrate between paper in which there was a statistical difference.

Table 5. Mean germination speed – MGS (days^{-1}) of buckwheat (*Fagopyrum esculentum*) seeds submitted to the germination test in different substrates and temperatures eight days after sowing.

Mean Germination Speed – MGS (Days^{-1})											
	Substrate									**Overall Mean**	**CV(%)**
Temperature (°C)	**On Paper**			**Between Paper**			**Paper Roll**				
15	0.20	c	B	0.21	b	B	0.24	a	C	0.22	1.62
25	0.25	b	A	0.25	b	A	0.26	a	B	0.25	
30	0.24	c	A	0.25	b	A	0.27	a	A	0.26	
35	0.25	b	A	0.25	b	A	0.27	a	A	0.26	
Overall Mean	0.23			0.24			0.26				

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other, at the level of 5% of probability, by the Tukey test (Source: the authors).

Regarding the seedling length (Table 6), a fact that drew attention was the difference in length at the temperature 35°C in relation to the others in all substrates, in this case it was significantly smaller.

The paper roll substrate produced the longest seedlings in relation to the other substrates at 15°C, 25°C and 30°C. Comparing the two substrates that obtained the highest percentage of germination, on paper and between paper (Tables 1 and 2), the behaviour of seedling growth was the same, with no statistically significant differences between the values. However, in these same substrates, the largest seedlings may be highlighted at 25°C and 30°C.

Table 6. Seedling length – SL (cm) of buckwheat (*Fagopyrum esculentum*) seeds submitted to the germination test in different substrates and temperatures eight days after sowing.

Seedling length – SL (cm)											
	Substrate									**Overall Mean**	**CV(%)**
Temperature (°C)	**On paper**			**Between paper**			**Paper roll**				
15	11.42	b	B	12.02	b	B	20.94	a	A	14.79	13.38
25	17.07	b	A	15.84	b	A	23.47	a	A	18.82	
30	14.71	b	AB	14.45	b	AB	23.61	a	A	17.59	
35	4.53	a	C	5.58	a	C	4.85	a	B	4.98	
Overall Mean	11.93			11.97			18.24				

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other, at the level of 5% of probability, by the Tukey test (Source: the authors).

The synchronization index (Table 7) was the highest at temperatures of 15°C, 25°C and 30°C in relation to the paper roll substrate. In substrates on paper and between paper at 15°C the index was better on the substrate between paper, on the other hand, at 25°C and 30°C the synchronization index of both substrates was not statistically different, but they were lower than the roll paper substrate.

Table 7. Synchronization index – SI (bites) of buckwheat (*Fagopyrum esculentum*) seeds submitted to the germination test in different substrates and temperatures eight days after sowing.

Synchronization Index - SI (Bits)											
	Substrate									Overall Mean	CV(%)
Temperature (°C)	On Paper			Between Paper			Paper Roll				
15	1.56	a	A	0.80	b	A	1.23	ab	A	1.20	44.74
25	0.20	b	B	0.56	b	A	1.17	a	A	0.64	
30	0.40	ab	B	0.28	b	A	0.86	a	AB	0.51	
35	0.15	a	B.	0.56	a	A	0.49	a	B	0.40	
Overall Mean	0.58			0.55			0.94				

Means followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other, at the level of 5% of probability, by the Tukey test (Source: the authors).

The first count, germination speed index, mean germination time and mean germination speed are important diagnostic, which report the initial plant development, as in the soil is extremely important. After sowing, the first days are critical due to pests and diseases attack [12].

The germination speed index may be used to identify cultivars with faster emergence in field, thus minimizing the adverse conditions that occur during germination and seedling establishment [13, 14].

It was established that the first count would be the day when at least 50% of the seeds had germinated in relation to the end of the evaluations. As buckwheat does not have an established methodology, the test lasted eight days, because on the eighth day of evaluation it was noticed that germination had stabilized for three days, the first count was established at four days.

In this case, the paper roll substrate presented the lowest means, which determines that the establishment of this first stand took longer at all temperatures compared to the other substrates. On the other hand, after four days of evaluation, the substrate on paper at temperatures 25°C, 30°C and 35°C and the substrate between paper at temperatures 30°C and 35°C, have already showed a germination percentage above 80% (Table 1).

Following the first count, the final percentage of germination (eight days after sowing) also presented lower means for paper roll substrate at all temperatures compared to the others, however, analysing the two other substrates, it is noticed that there was no increment in the percentage of germination (Table 2).

The lowest temperature (15°C) negatively influenced the first count and the germination percentage only in the substrate on paper, as low temperatures reduce the germination percentage due to the reduction of enzymatic activities in seed metabolism [14]. The same temperature was placed on the substrate between paper and placed in the same germination box as the substrate on paper, in this way, the sheet of paper that covered the seeds served as a protection against excessive temperature loss or prevented the low temperature from affecting the seeds as they were not so exposed to the environment in which they were inserted.

Temperatures between 25°C and 30°C provided the highest germination averages for the substrates on paper

and between paper, as higher temperatures, as opposed to low ones, determine higher speed of water absorption process, accelerate the enzymatic activities and the germination process [15].

The mean speed of germination was the highest in the paper roll substrate in all temperatures, thus, this type of substrate may have already been discarded to evaluate the germination of buckwheat seeds. The highest speeds were detected in substrates on paper and between paper at a temperature of 15°C. As mentioned earlier, there were fewer seeds germinated at this temperature, so it was expected that germination would be faster. In relation to the other temperatures, in the substrate on paper and between paper, any temperature between 25°C and 35°C showed higher germination speed.

The germination speed index, in the comparison between substrates on paper and between paper, obtained the highest values for temperatures 30°C and 35°C in the second substrate (Table 3), in contrast to the mean germination speed (Table 5), disregarding the temperature of 15°C, which has already been discarded for presenting lower total germination, showed higher values for temperatures of 25°C and 35°C in both substrates (on paper and between paper).

The seedling length was categorical in showing that the largest seedlings were found on the paper roll substrate, but this was probably due to the greater contact surface that this substrate had, which favoured greater water absorption than the substrates on paper and between paper. Marcos Filho [16] states that, when there is not enough water availability, some processes may be harmed, causing damage to the embryo or even its death, in addition to lower development. This is a limiting factor when the species has a specific water demand, with its deficiency being able to reduce the germination percentage and seedling growth, through its negative effects on the seeds [17].

The synchronization index is a characteristic that demonstrates the level of organization or disorder of chemical reactions in the germination process. In the interpretation of the synchronization index, the lower its value, the more synchronized the germination will be, regardless of the total number of germinated seeds [18, 19]. In the present work, the highest values were presented by the paper roll at temperatures from 15°C to 30°C and the substrate on paper at a temperature of 15°C. The lowest synchronization indices were reported between substrate on paper at 25°C and between paper at 30°C. The loss of germination synchrony represents heterogeneity in seed physiology which may be harmful to the producer.

The shorter germination time is desirable when looking for a substrate definition. Under laboratory conditions, germination for prolonged periods may be harmful, and the action of pathogens that affect the germination process may occur [20], in addition to being undesirable for the laboratory routine.

IV. Conclusion

For buckwheat germination test in the laboratory, after analysis of all evaluated characteristics, the substrates on paper and between paper at temperatures of 25°C and 30°C are indicated with constant light. Furthermore, the first count must occur at four days and the final evaluation of the germination percentage at eight days after sowing.

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