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RESEARCH NOTE

Reduced time for evaluation of the germination test for sunflower seeds[1]

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ABSTRACT – Establishing ideal conditions and speed in carrying out the germination test for sunflower seeds is of utmost importance in quality control programs of companies dedicated to the production of high technology seeds. In this regard, the aim of this study was to verify the possibility of reducing the evaluation time of the germination test of sunflower seeds in different substrates and temperatures. Six seed lots of the cultivar Helio 251 and six lots of the cultivar Helio 253 were used. As well as germination, determinations were made of first count germination and seed health. The seeds were kept in a germination chamber at 25 ºC, 30 ºC, and 20-30 ºC for the paper substrate, and 25 ºC and 30 ºC for the sand substrate. To verify the possibility of reducing the evaluation time of the germination test, the number of normal seedlings were counted daily, up to the tenth day after sowing. It was concluded that it is possible to conclude the germination test on the seventh day after sowing in sand at the temperature of 25 °C.

Index terms: *Helianthus annus* L., seed quality, temperature.

Redução do tempo de avaliação do teste de germinação de sementes de girassol

RESUMO - O estabelecimento de condições ideais e a rapidez para condução do teste de germinação de sementes de girassol é de suma importância nos programas de controle de qualidade das empresas que se dedicam a produção de sementes com alta tecnologia. Dentro deste contexto, objetivou-se com este trabalho verificar a possibilidade de redução do tempo de avaliação do teste de germinação de sementes de girassol em diferentes substratos e temperaturas. Foram utilizados seis lotes de sementes da cultivar Hélio 251 e seis lotes da cultivar Hélio 253. Além da germinação, foram efetuadas as determinações de primeira contagem de germinação e sanidade. As sementes foram mantidas em câmara de germinação regulada a 25, 30 e 20-30 ºC, para o substrato papel, e 25 e 30 ºC para o substrato areia. Para verificar a possibilidade de redução do tempo de avaliação do teste de germinação, as contagens do número de plântulas normais foram realizadas diariamente, até o 10° dia após semeadura. Concluiu-se que é possível a finalização do teste de germinação no sétimo dia após a semeadura em areia na temperatura de 25 °C.

Termos para indexação: *Helianthus annus* L., qualidade de sementes, temperatura.

Introduction

In Brazil, growing of sunflower has increased significantly in recent years due to its great capacity for adaptation to different edaphic and climatic conditions, which is reflected in agronomic traits such as resistance to drought, to cold, and to heat, and the limited effect of latitude, altitude, and photoperiod. It thus appears as an option for rotation and crop succession systems in various grain producing regions of Brazil (Ungaro, 2006). In addition, the sunflower crop has technical and environmental viability in biodiesel production (Silva et al., 2007). However, to meet the needs of a growing market, the use of good quality seeds is fundamental for establishing adequate populations in the field.

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One of the tests for determining seed quality level is the germination test (Brasil, 2009), which is performed under temperature and substrate conditions ideal for each species. The Rules for Seed Testing (Brasil, 2009) determine that the ideal conditions for the germination test of sunflower seeds are the temperatures of 20, 25, 30, and alternating 20-30 °C, and the substrates of rolls of paper or sand. Yet international guidelines (INTERNATIONAL SEED TESTING ASSOCIATION - ISTA, 2013) recommemd the temperature of 20 °C instead of 30 °C. However, definition of the type of substrate and ideal temperature, as well as the period of time for evaluation of the seedlings, without contamination from seedling to seedling in the roll, should be better established.

Among the environmental factors that affect seed germination, temperature has an effect on speed of germination and on germination potential. The temperature defined as optimum is that at which the highest germination percentage is obtained within the shortest period of time (Lopes et al., 2005). A temperature above the optimum increases the speed of germination (Marshall and Squire, 1996), whereas low temperatures delay emergence and lead to the formation of small seedlings (Szopińska et al., 2007). The substrate used in the germination test, in general, has the purpose of sustaining the seeds (Lopes and Pereira, 2005). The choice of substrate should take into account seed size, its requirements in relation to light, and ease in counting operations and seedling evaluation (Figliolia et al., 1993). The structure, aeration, water retaining capacity, and degree of infestation of pathogens in the substrate have a big effect on germination (Moraes et al., 2007). The substrate must be chosen in accordance with seed demands (Brasil, 2009).

For most species, the germination test requires a period considered to be long in order to obtain results and serve the commercial interests of seed producers. In addition, the seeds remain for a long time in the substrate, subject to attack from microorganisms, which could make test interpretation difficult. The RAS (Brasil, 2009) recommend evaluation of germination on the tenth day after setting up the test for sunflower seeds. Campos and Tillmann (1997) and Tomaz et al. (2010) concluded that it is possible to conclude the germination test for some species with single countings, emphasizing that there is the possibility of reducing the evaluation period of the germination test. This would be useful in quality control programs, allowing faster decision-making in respect to quality and the destination of seed lots.

Seed health is another aspect to be considered, with a view toward reducing evaluation time of the sunflower germination test. Reducing the time for carrying out the test reduces the time of exposure of the seeds to pathogen attack. Fungi associated with the seeds may cause seed deterioration and death, impeding evaluation of the germination test and considerably compromising germination (Faiad et al., 1997).

In this context, this study was carried out for the purpose of verifying the possibility of reducing the time of evaluation of the germination test of sunflower seeds in different substrates and temperature conditions.

Material and Methods

This study was carried out in the Seed Analysis Laboratory of the Agriculture Department of the Universidade Federal de Lavras (UFLA), Lavras, MG, Brazil. Six lots of recently harvested seeds from the cultivar Helio 251 and six lots from the cultivar Helio 253 were used.

In the germination test, the substrates of sand and paper were used, as well as the constant temperatures of 25 °C and 30 °C and the alternating temperature of 20-30 °C. Paper toweling was used, wetted with distilled water at the proportion of 2.5 times the weight of the substrate, with eight replications of 25 seeds per lot. Sowing was performed on two overlaid sheets and one used as a covering. For germination in sand, plastic trays were used with dimensions of 60 cm x 40 cm x 10 cm. Four replications of 50 seeds were sown within sand moistened to 40% water retention capacity. The seeds were kept in a germination chamber at 25 ºC, 30 ºC, and 20-30 ºC for the paper substrate, and 25 ºC and 30 ºC for the sand substrate. The substrates were rewetted as necessary.

To check the possibility of reducing the evaluation time of the germination test, the number of normal seedlings was counted daily, up to the 10th day. A control was used for the purpose of determining whether the seedlings were damaged by the daily opening of the rolls of paper and, in the control, there were only two countings, at four and ten days after setting up the test (Brasil, 2009). In addition to the normal seedlings, the number of abnormal infected seedlings up to the end of the test was evaluated. The results were expressed in percentage.

Seed health was evaluated by the incubation method in filter paper without freezing (Neergaard, 1979) with eight replications of 25 seeds per lot. The seeds were distributed in 15-cm diameter Petri dishes containing three sheets of filter paper previously sterilized and wetted in a 2,4-D solution. The seeds were incubated at 20 °C ± 2 °C in a incubation chamber with a 12 hour light/12 hour dark photoperiod for seven days. For identification of pathogens present in the seeds, a stereo microscope and an optical microscope were used. The incidence of pathogens was evaluated in percentage of fungi found.

An entirely randomized experimental design was used; the

data were interpreted statistically through analysis of variance in a 6 x 5 x 10 factorial arrangement with six seed lots, five treatments (paper/25 °C, paper/30 °C, paper/20-30 °C, sand/25 °C, and sand/30 °C) and daily evaluation of the germination test for 10 days. The effect of germination time, when significant, was evaluated through regression analysis. The mean values were compared by the Scott-Knott test at the 5% probability level.

Results and Discussion

It may be observed from Table 1 that the sand substrate led to greater speed of germination in relation to the paper substrate at the two temperatures tested for the two cultivars. Similar results have been found for various species and, according to Lopes et al. (2005) and Pacheco et al. (2006), this may be explained by the greater area of seed contact with the sand, which leads to greater speed in water absorption. For the paper substrate, the increase in temperature led to greater speed of germination. Neves et al. (2009) and Pacheco et al. (2008) observed that high temperatures lead to greater speed of germination as a result of faster imbibition and, consequently, acceleration of the metabolic reactions that occur during the germination process (Bello et al., 2008).

For the cultivar Helio 251, the results of germination were greater in the sand substrate, regardless of the temperature tested, while in the paper substrate at 30 °C, lower percentages of normal seedlings were observed. For the cultivar Helio 253, regardless of the time of evaluation, the results of germination were always greater when a sand substrate at 25 °C was used (Table 1).

Table 1. Mean values of normal seedlings (%) of sunflower (*Helianthus annus* L.) for the cultivars Helio 251 and Helio 253 on the different days of evaluation of the germination test in paper (P) and sand (S) substrate at the temperatures of 25 °C, 30 °C, and 20-30 °C.

Days	Helio 251					Helio 253				
	P25	P30	P20-30	S25	S30	P25	P30	P20-30	S25	S30
1	0 A	0 A	0 A	0 A	0 A	0 A	0 A	0 A	0 A	0 A
2	0 B	0 B	0 B	14 A	13 A	0 C	0 C	0 C	19 A	13 B
3	0 C	19 B	0 C	62 A	67 A	0 D	13 C	0 D	55 A	44 B
4	73 B	23 D	43 C	89 A	88 A	60 C	34 E	41 D	82 A	77 B
5	83 B	37 D	56 C	91 A	89 A	72 C	45 D	47 D	86 A	80 B
6	84 B	43 D	59 C	92 A	91 A	79 B	48 C	50 C	88 A	82 B
7	85 B	44 D	61 C	93 A	91 A	82 B	48 C	50 C	89 A	84 B
8	87 B	44 D	63 C	93 A	92 A	82 B	48 C	50 C	89 A	84 B
9	87 B	44 D	63 C	93 A	92 A	82 B	48 C	50 C	89 A	84 B
10	87 B	44 D	63 C	93 A	92 A	82 B	48 C	50 C	89 A	84 B
CV (%)			13.79					17.93		

Mean values followed by the same uppercase letter in the line do not differ among themselves by the Scott-Knott test at 5% probability.

In the paper substrate, although an increase in temperature led to greater speed in germination, this increase negatively affected the germination of sunflower seeds. At the alternating temperature of 20-30 °C and constant temperature of 30 ºC, two days after setting up the test, the seedlings exhibited necrotic lesions, a condition not observed at the temperature of 25 °C (Figure 1). Albuquerque and Carvalho (2003) found that high temperatures compromised germination of sunflower seeds. Germination in sand was greater than that obtained in paper, probably due to the greater occurrence of fungi in the paper substrate. Nery et al. (2009) and Novembre and Marcos-Filho (1999) obtained better germination results upon adopting sand as a substrate for some oilseed species, such as oilseed radish and cotton, with a high incidence of microorganisms. The fungus *Alternaria* sp. is one of the main microorganisms that may affect germination through its damage to seedlings and, moreover, reduce the oil content in the seeds (Humpherson-Jones, 1992). Microorganisms arising from storage, such as *Aspergillus* sp., are also causal agents of root collar rot and damping off of seedlings (Bellettini et al., 2005), and contamination from seedling to seedling in paper is facilitated by their closeness to one another.

Confirming the negative effect of the presence of microorganisms, associated with high germination temperatures, the percentage of abnormal infected seedlings was greater in the paper substrate at the temperature of 30 °C and 20-30 °C, with the lowest values observed in the sand substrate (Table 2).

Pacheco et al. (2006) and Pereira et al. (2000) found that germination of seeds with high rates of contamination by fungi in a sand substrate is less affected compared to a paper substrate. The authors affirmed that the fact of the fungus being

harbored in the seed coat facilitates its spread throughout the paper roll, which does not occur in sand because the seed coat does not remain adhered to the seedling. In sunflower seeds, the position of the achenes in the inflorescence and uneven maturation favor the appearance of fungi outside the seeds, which may affect seedling development (Bittencourt et al., 1991; Joker and Jepsen, 2003).

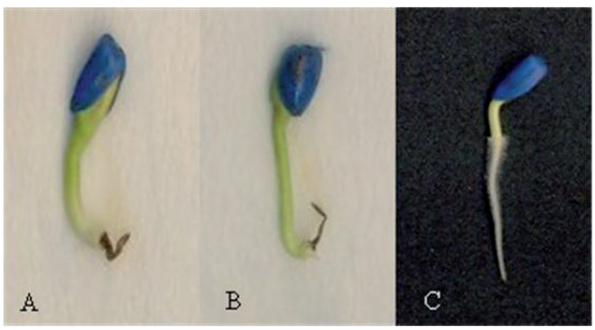

Figure 1. Sunflower (*Helianthus annus* L.) seedlings at two days of germination. (A) abnormal seedling observed in paper substrate at 30 °C; (B) abnormal seedling observed in paper substrate at 20-30 °C; (C) normal seedling observed in paper substrate at 25 °C.

From the mean results of the incidence of pathogenic fungi found in the seeds (Table 3), a greater percentage of contamination by fungi of the genera *Aspergillus* sp. and *Alternaria* sp. was observed. These species lower the quality of the seeds because they are able to invade and degrade the embryonic tissues, producing toxins that reduce germination capacity and lead to discoloring, rotting, and heating of the seed matter, characteristics which favor an increase in the speed of deterioration (Goulart, 2007). Although it is difficult to establish a direct relationship between the percentage of microorganisms and the quality of the seed lots of sunflower, it was possible to observe that there was the incidence of these microorganisms in the seeds through the results of the seed health test and through the number of abnormal infected seedlings.

Evaluation of the germination process in consecutive days allowed accumulated germination curves to be created (Figure 2). The data exhibit quadratic behavior, with rapid germination up to the 4th day and a trend toward stabilization after the 5th day. As of the 3rd day, the substrate + temperature factors show a drastic effect on seed germination.

It was observed that as of the 4th day, more than 50% of the seeds had already germinated, this period having been adopted as the date of the first count, in accordance with the procedure adopted by the RAS. On the 5th day, the germination curves began to stabilize. On the 7th day (the point of curve inflection), it was no longer possible to observe the appearance of new seedlings, which indicates that the period of seven days would be sufficient for final evaluation, without the need for counting at 10 days as recommended in the RAS (Brasil, 2009).

For tomato seeds, Campos and Tillmann (1997) found that it is possible to conclude the germination test with a single counting, allowing reduction in the period of evaluation of the germination test. Tomaz et al. (2010) found that it is possible to perform the final count of *Panicum maximum* seeds earlier. For cotton seeds, moving up the first count of germination allows greater accuracy in evaluation of seedlings (Novembre and Marcos-Filho, 1999).

Table 2. Mean values of abnormal infected seedlings – AI (%) of sunflower (*Helianthus annus* L.) for the cultivars Helio 251 and Helio 253 as a function of treatments (P25 – paper/25 °C; P30 – paper/30 °C; P20-30 – paper/20-30 °C; A25 – sand/25 °C and A30 – sand/30 °C).

Treatment	Helio 251	Helio 253
P25	10 c	13 b
P30	49 a	43 a
P20-30	33 b	41 a
A25	1 d	6 c
A30	2 d	3 c
CV (%)	39.14	42.67

Mean values followed by the same letter do not differ among themselves by the Scott-Knott test at 5% probability.

Table 3. Percentage (%) of the fungi *Aspergillus* sp. and *Alternaria* sp. in the different seed lots of sunflower (*Helianthus annus* L.) for the cultivars Helio 251 and Helio 253.

Lot	Helio 251		Helio 253	
	Aspergillus sp.	*Alternaria* sp.	*Aspergillus* sp.	*Alternaria* sp.
1	27	20	28	42
2	18	34	38	47
3	58	20	20	13
4	17	38	40	33
5	28	32	35	35
6	16	39	33	13

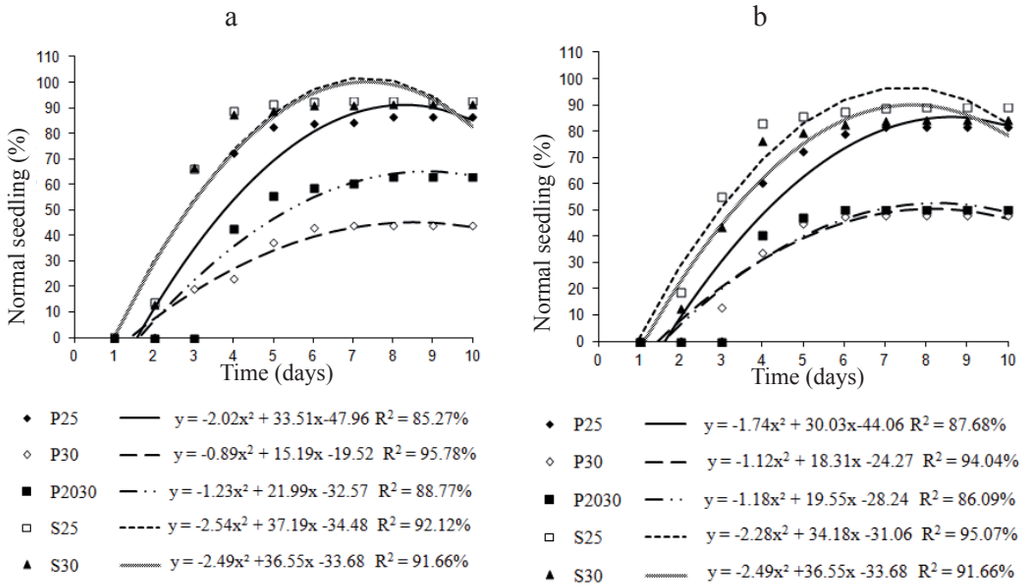

Figure 2. Mean values of normal seedlings (%) of sunflower (*Helianthus annus* L.) for the cultivars Helio 251 (a) and Helio 253 (b) as a function of days of evaluation of the germination test in paper (P) and sand (S) substrates at the temperatures of 25 °C, 30 °C, and 20-30 °C.

Conclusions

It is possible to conclude the germination test of sunflower seeds on the seventh day after sowing.

Use of the sand substrate and the temperature of 25 °C are adequate conditions for evaluation of sunflower seed germination.

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