RESEARCH NOTE

Controlled deterioration test for evaluation of sunn hemp seed vigor[1]

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ABSTRACT – The controlled deterioration test is efficient for seed vigor evaluation of several species. However, the procedures conditions are incipient, especially in respect to tropical forages such as sunn hemp, which still does not have a standardized procedure. The objective of this research was to adjust the procedures for a controlled deterioration test in *Crotalaria juncea* L. seeds. For this, five commercial seed lots were evaluated for water content and initial physiological potential (germination test, first germination count, germination speed index, saturated salt accelerated aging with NaCl, electrical conductivity and seedling field emergence). For controlled deterioration test, the initial seed moisture content was adjusted to 18, 21 and 24% and, subsequently, the samples were exposed at 45 °C during 24 hours. Seed germination test was conducted with the use of sand as substrate. Evaluations were performed on the fourth, fifth and sixth day after sowing. The controlled deterioration test for sunn hemp seeds should be performed with 24% seed moisture content, at 45 °C during 24 hours, and the test evaluation on the fourth day after sowing.

Index terms: *Crotalaria juncea* L., germination, physiological potential, test standardization.

Teste de deterioração controlada para avaliação do vigor de sementes de crotalária

RESUMO – O teste de deterioração controlada é eficiente para avaliar o vigor de sementes de diversas espécies. No entanto, as condições para realização desse teste são incipientes, principalmente quando se trata de forrageiras tropicais, como é o caso da crotalária, cujas informações sobre a adequação do teste ainda são escassas. Assim, o objetivo deste trabalho foi adequar os procedimentos para a execução do teste de deterioração controlada para sementes de *Crotalaria juncea* L. Para tanto, cinco lotes de sementes comerciais foram avaliados quanto ao teor de água e potencial fisiológico inicial (teste de germinação, primeira contagem, índice de velocidade de germinação, envelhecimento acelerado com solução saturada de NaCl, condutividade elétrica e emergência de plântulas em campo). Para o teste de deterioração controlada, o teor de água inicial das sementes foi ajustado para 18, 21 e 24% e, posteriormente, as amostras foram expostas a 45 °C durante 24 horas, postas para germinar entre areia, e avaliadas aos quarto, quinto e sexto dias após a semeadura. O teste de deterioração controlada em crotalária deve ser conduzido com sementes contendo 24% de teor de água, a 45 °C, durante 24 horas e a leitura realizada aos quatro dias após a semeadura.

Termos para indexação: *Crotalaria juncea* L., germinação, potencial fisiológico, padronização de teste.

Introduction

Sunn hemp (*Crotalaria juncea* L.) also known as brown hemp, Indian hemp or Madras hemp is a forage legume used as green manure, which has a high potential for biomass production in crop rotation or intercropping systems, promoting improvements in the chemical, physical and biological soil properties, and thus, becoming an important mechanism for recovery of degraded areas (Silva et al., 2012).

The use of high quality seeds is essential for implementation of uniform fields. Rapid and vigorous seedling establishment in the field are the keys factors to ensure the economic viability and sustainability in sunn hemp rotation and intercropping systems.

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Officially, only the germination test is required to attest the physiological potential of seed lots. However, the germination test is conducted under favorable environmental conditions such as oxygen, temperature and water availability, and sometimes, does not reflect the real seedling performance in the field. On the other hand, seed vigor tests are able to complement the determination of seed physiological potential because they can provide more sensitive information about this factor (Baalbaki et al., 2009).

Seed vigor can be defined as the set of characteristics that determine the ability of a commercial seed lot of acceptable germination to have a rapid and uniform seedling emergence under widely varying environmental conditions (Tekrony, 2003). In face of this, various methods have been developed for a safe evaluation of seed vigor.

Procedures of vigor testing based on stress tolerance have been used to determine the seed performance in the field (Marcos-Filho, 2015). High temperature and relative humidity can accelerate the seed deterioration process, and consequently, the seed vigor. Thus, the controlled deterioration test demonstrates to be promising to identify seed lots with different vigor levels. Because of its simplicity and efficiency, this test has drawn the attention of many seed technologists (Mendonça et al., 2003). Furthermore, the controlled deterioration test stands out due to better control over seed moisture content (Hu et al., 2012; Wang et al., 2015), differently of the accelerated aging test, where the seed moisture content may vary and can lead to inconsistent results (Santos et al., 2003).

The controlled deterioration test is recommended for brassica seeds (ISTA, 2014), and widely used for various seed crops, including bean (Santos et al., 2003), peanut (Rossetto et al., 2004), melon (Mavi and Demir, 2007), cotton (Dutra and Medeiros-Filho, 2008), maize (Zucareli et al., 2011), beet (Silva and Vieira, 2012), coriander (Torres et al., 2012), scarlet eggplant (Lopes et al., 2012), soybean (Cabral et al., 2012), eggplant (Lopes et al., 2013), forage turnip (Morais and Rossetto, 2013) and okra (Torres et al., 2013). However, for sunn hemp seeds, studies about seed vigor using the controlled deterioration test are still limited.

Therefore, the objective of this research was to adjust procedures for implementing of the controlled deterioration test in *Crotalaria juncea* L. seeds.

Material and Methods

The experiment was conducted at the Seed Analysis Laboratory of Sao Paulo State University (UNESP) of the Plant Production Department, Jaboticabal Campus, SP, Brazil, using five lots of sunn hemp seeds. For purposes of comparison and differentiation of the seed physiological potential, seeds were submitted to initial moisture content determination as well as evaluations of germination and vigor.

Seed moisture content was obtained by the oven method at 105 ± 3 °C for 24 hours (Brasil, 2009), with two subsamples of 25 seeds for each lot, and the data expressed as a percentage (fresh weight basis).

Germination test was conducted with four replications of 50 seeds distributed in plastic boxes containing moist sand with 60% of maximum water holding capacity. Thereafter, boxes were maintained in a growth chamber at 25 °C and an eight-hour photoperiod. The evaluations were performed at four (first germination count) (Nakagawa, 1999), and ten days after sowing (final germination count) (Brasil, 2009).

Germination speed index (GSI) was obtained by the daily counting of emerged seedlings (cotyledons surpassing the substrate level). GSI was obtained according to the formula proposed by Maguire (1962).

For electrical conductivity, four replications of 50 seeds were weighed with an accuracy of 0.01 g, and then placed in plastic cups (200 mL) containing 75 mL of deionized water. The containers were then kept at 25 °C for eight hours. The electrical conductivity of the solution was measured by conductivity meter and the results were expressed in relation to the weight ($\mu S.\ cm^{-1}.g^{-1}$) (Silva et al., 2012).

The saturated salt accelerated aging (SSAA) was carried out in a single layer on a stainless steel mesh inside transparent plastic boxes (11×11×3.5 cm), containing 40 mL of a saturated solution of NaCl (40 g/100 mL of water). The boxes were covered and kept in aging chambers at 41 °C for 96 hours (Silva et al., 2012). After the aging treatment, seeds were set to germinate following the methodology described for germination test, and assessment was carried out on the fourth day after sowing.

For seedling field emergence, four replications of 50 seeds were sown in 2 m long rows with 0.4 m between rows. Evaluations were made 14 days after sowing and the results were expressed as a percentage of emerged seedlings (Nakagawa, 1999).

After initial characterization of physiological potential of the seed lots, studies for standardization of methodologies were performed to the controlled deterioration test. Initially, seed moisture content was adjusted to three levels (18, 21 and 24%) by wet atmosphere method (Rossetto et al., 1995) using transparent plastic boxes. Then, seeds were kept in a bain-marie at 45 °C for 24 hours. After this period, seeds were put to germinate in sand substrate. Data were recorded at four, five and six days after sowing.

The results from each test were analyzed by analysis

of variance in a completely randomized design. For the controlled deterioration test, the treatments were arranged in a 5x3 factorial scheme (five seed lots x three seed moisture content levels), separately for each evaluation period. Data were tested for normality and homogeneity of variances and then subjected to analysis of variance. Means were compared by Tukey's test ($p \leq 0.05$) (Banzatto and Kronka, 2008).

Results and Discussion

All seed lots were homogeneous regarding to the initial moisture content, varying between 10.1 and 13.2% (Table 1). This small variation provides greater reliability in assessing vigor by the tests, since any difference noticed between the seed lots is attributed to their physiological characteristic and not due to the discrepant values in the seed moisture content.

The germination values of all the lots were acceptable for the commercialization of sunn hemp seeds, which is fixed at 60% by the Brazilian legislation (Brasil, 2008). The seed lots also presented similar germination, one of the pre-requirements for the application of vigor tests.

Lots showed differences in their physiological potentials, although the germination test, first germination count and germination speed index showed no differences (Table 1). Despite this, the electrical conductivity, saturated salt accelerated aging and the seedling field emergence were sufficient to stratify seed lots at different vigor levels. Lot 1 had low vigor, while lots 4 and 5 were the most vigorous.

Table 1. Initial seed moisture content (MC), germination (GE), first germination count (FC), germination speed index (GSI), electrical conductivity (EC), saturated salt accelerated aging (SSAA) and seedling field emergence (SFE) of five sunn hemp seed lots.

Lots	MC	GE	FC	GSI	EC	SSAA	SFE
	---------- % ----------				$\mu S.cm^{-1}.g^{-1}$	----------% ----------	
1	13.2	77 a*	70 a	9.1 a	108 c	51 c	65 b
2	14.2	79 a	76 a	9.7 a	99 b	60 b	66 b
3	13.8	80 a	78 a	9.9 a	92 ab	63 b	68 b
4	10.4	83 a	80 a	10.2 a	87 a	70 b	70 ab
5	10.1	79 a	85 a	10.7 a	81 a	72 a	75 a
LSD (5%)	–	22.4	18.7	2.6	7.9	6.5	5.1
CV (%)	–	10.5	11.4	10.3	12.7	9.8	11.5

*Means followed by the same letter do not differ at 5% by Tukey's test.

The controlled deterioration test was efficient in highlighting lots with low and high vigor, according to the classification indicated by electrical conductivity and saturated salt accelerated aging tests as well as seedling field emergence. According to specific procedures in the controlled deterioration test with 24% seed moisture content, the lot 1 also showed low vigor.

Regarding the seed performance after running test, the 18% seed moisture content had no effect on seed physiological behavior, because there was no separation of lots into vigor classes (Table 2). Similarly, in okra seeds, the controlled deterioration test also showed no sufficient sensitivity to evaluate the seed physiological potential with 18% seed moisture content (Torres et al., 2013).

The best separation of lots occurred in seed lots with 24% moisture content, because promoted comparable results with other tests which were used for physiological potential characterization, and also showed accordance with procedures for testing in seeds from other species (Table 2). The 24% seed moisture content was also the most suitable for controlled deterioration test in cotton (Dutra and Medeiros-Filho, 2008), scarlet eggplant (Lopes et al., 2012), and okra (Torres et al., 2013). On the other hand, the 20% seed moisture content was suitable for bean (Santos et al., 2003), melon (Mavi and Demir, 2007), and cauliflower (Kikuti and Marcos-Filho, 2008), whereas in peanut seeds, 15% moisture content was the most appropriate treatment (Rossetto et al., 2004).

There was a reduction in the controlled deterioration test results while seed moisture content increased (18, 21 and 24%). Seeds with higher moisture content have been more susceptible to unfavorable environmental conditions, so that, a higher moisture content was followed by a lower ability to the seeds to tolerate these stresses, which can affect the seed performance due to excessive intensification of metabolic activity.

The basic objective of a seed vigor test is to identify physiological differences among lots of marketable seeds with greater sensitivity compared to the germination test (Marcos-Filho, 2015). In addition, vigor tests should meet the requirements of objectivity, simplicity, low cost and

reproducibility as well as obtaining rapid results. Thus, the four-day period for the controlled deterioration test in sunn hemp seeds can be considered the most promising (Table 2), since the vigor rating among lots was the same to the indicated during initial physiological potential evaluation.

Table 2. Controlled deterioration of five sunn hemp seed lots due to the seed moisture content (MC) and evaluation periods (days).

Periods	Lots	MC		
		18%	21%	24%
		---------- % ----------		
4 days	1	66 aA*	76 abA	65 bA
	2	71 aA	65 bA	70 abA
	3	78 aA	73 abAB	65 bB
	4	75 aA	73 abA	65 bA
	5	78 aA	82 aA	83 aA
	CV (%)		8.8	
5 days	1	70 aA	77 abA	69 bA
	2	78 aA	70 bA	73 abA
	3	79 aA	75 abAB	67 bB
	4	79 aA	79 abA	68 bB
	5	79 aA	83 aA	83 aA
	CV (%)		8.0	
6 days	1	70 aA	77 abA	69 bA
	2	70 aA	70 bA	73 abA
	3	80 aA	75 abAB	68 bB
	4	80 aA	80 abAb	70 bB
	5	80 aA	84 aA	84 aA
	CV (%)		7.8	

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ at 5% by Tukey's test.

In researches about procedures standardization for seed vigor evaluation, studies have showed that during the test, a reduction in the period of time, up to certain limits seems to be more advantageous considering the speed of obtaining results. The proposal for evaluation at four days was confirmed in this study, being in agreement with recommendation, which suggests that the evaluation period of the controlled deterioration test should be the same of the first germination count. The decision to use the controlled deterioration test in seed quality control programs depends on the accessibility of the method for analysts as well as the obtaining quick results associated with data obtained during seedling field emergence. Vigor tests based on stress, such as controlled deterioration test can provide important information about the seed physiological potential when used with other tests, which helps during decision-making for seed storing and sowing (Barbosa et al., 2013).

Conclusions

The controlled deterioration test in *Crotalaria juncea* seeds should be carried out with 24% moisture content at 45 °C for 24 hours and the evaluation on the fourth day after sowing.

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