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BIOLOGY

Accelerated aging as vigor test for sunn hemp seeds

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ABSTRACT: *This study aimed to determine the most appropriate method to assess the sunn hemp (**Crotalaria juncea** L.) seed vigor in the accelerated aging test. Five seed lots from harvest 2007/2008 were evaluated for germination, vigor and seedling emergence in the field. Accelerated aging test was performed at 41°C during 48, 72 and 96 hours, with and without sodium chloride saturated solution. Then, the promising procedure was also performed for 2008/2009 and 2009/2010 harvests. In the traditional accelerated aging test, the no-uniform water acquisition by the seeds adversely affected results. Accelerated aging test with sodium chloride saturated solution is the most appropriate method to evaluate the physiological potential of sunn hemp seeds, and the combination of 41°C and 96 hours is efficient for separating of seed lots in different vigor levels.*
Key words: ***Crotalaria juncea*** *L., forage crop, physiological potential, germination, standization.*

Envelhecimento acelerado como teste de vigor para sementes de crotalária

RESUMO: *Esta pesquisa objetivou determinar os procedimentos mais adequados para avaliação do vigor de sementes de crotalária (**Crotalaria juncea** L.) pelo teste de envelhecimento acelerado. Para tanto, cinco lotes de sementes foram avaliados quanto à germinação, vigor e emergência de plântulas em campo. O procedimento do teste de envelhecimento acelerado foi testado com 41°C durante 48, 72 e 96 horas, com e sem o uso de solução saturada de cloreto de sódio. O envelhecimento acelerado com o uso de solução saturada de cloreto de sódio é o método mais adequado para avaliação do potencial fisiológico de sementes de crotalária, sendo a combinação 41°C e 96 horas eficiente na classificação de lotes em diferentes níveis de vigor.*
Palavras-chave: ***Crotalaria juncea*** *L., forrageira, potencial fisiológico, germinação, padronização.*

INTRODUCTION

The use of vigorous seeds is essential for ensuring economic viability and sustainability of agro-ecosystems, specially for species such as sunn hemp (***Crotalaria juncea*** L.), which has contributed to performance of various crops: coffee (RICCI et al., 2005), rice (JESUS et al., 2007), sugarcane (DUARTE-JUNIOR & RABBIT, 2008) and maize (AMBROSANO et al., 2009). Sunn hemp also contributes to control weeds and nematodes (COLLINS et al., 2008) in addition to reduction of agricultural inputs used, because it can provide considerable amount of nitrogen for other crops (DINIZ et al., 2014).

Seed vigor tests and the concept of vigorous seed are used during seed production programs as an important strategy for seed quality control as well as marketing tools, since help to ensure a good stand of plant population in the field. Seed vigor tests give additional information provided by the germination test, which facilitates decision-making on seed lots destination according their physiological potential.

The accelerated aging test was developed and further refined to evaluate the seed vigor of several species such as cowpea (DUTRA & TEÓFILO, 2007), wheat (PEDROSO et al., 2010), lettuce (BARBOSA et al., 2011), okra (TORRES et al., 2014), crambe (LIMA et al., 2015), arugula (VIEIRA et al., 2015), and coriander (PEREIRA et al., 2015).

During artificial aging of seeds, the rate of seed deterioration is increased with the increase

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in temperature and relative humidity, which leads to damage on membranes, proteins and nucleic acids (FUJIKURA & KARSSEN, 1995), and so, deleterious effects on germination, particularly for low vigor seeds lots. Procedures based on stress tolerance to evaluate the seed vigor have been widely used to determine its performance after sowing (MARCOS-FILHO, 2015).

The rate and intensity of water uptake by seeds can influence the accelerated aging test results (MARCOS-FILHO, 2015). Accordingly, replacement of distilled water with saturated salt solutions have been studied for testing conduction, which control the seed hydration without compromising on the efficiency of testing (JIANHUA & McDONALD, 1997).

For evaluation of the physiological potential of sunn hemp seeds, some vigor tests have been standardized and validated such as electrical conductivity and Seed Vigor Imaging System - SVIS® (SILVA et al., 2012a), tetrazolium (SILVA et al., 2012b) and controlled deterioration (SILVA et al., 2015) tests. However, studies on accelerated aging test in sunn hemp seeds are still incipient.

Based on these facts, this study aimed to determine the most appropriate procedures of the accelerated aging test for evaluation of sunn hemp seed vigor.

MATERIALS AND METHODS

Experiments were conducted at the Seed Analysis Laboratory of Department of Crop Science, UNESP/FCAV, Jaboticabal Campus, SP. Seventeen commercial lots of sunn hemp seeds from three harvest years were studied: five seed lots from 2007/2008, seven seed lots from 2008/2009 and five seed lots from 2009/2010. Seed lots were kept in multi-layered Kraft paper containers and stored in a cold chamber at 10°C and 60% relative air humidity during the experimental period.

The research was conducted in three stages: characterization; results achieving and validation. (1) Evaluation of initial physiological potential (first germination count, cold test and seedling emergence in the field); (2) Study of different procedures during accelerated aging test in seed lots from 2007/2008 harvest; (3) Assessment of the accelerated aging test using the procedure considered promising in the previous stage by use of seed lots from 2008/2009 and 2009/2010 harvests.

Seed water content was determined by oven method, at 105±3°C for 24h (BRASIL, 2009), before and after aging, with two replicates for each lot. Results were expressed as a percentage on a fresh weight basis.

For germination test, four replications of 50 seeds each per lot were placed on sand in plastic boxes (28.5×18.5×10.0cm), and kept at 20-30°C. Data were recorded at four days after sowing, corresponding to the first germination count (NAKAGAWA, 1999), and at ten days after sowing; results were expressed as a percentage of normal seedlings (BRASIL, 2009).

Cold test was conducted with four replications of 50 seeds per lot, using plastic boxes (28.5×18.5×10.0cm) and a mixture of soil and sand in the ratio of 2:1. Boxes were covered to minimize water loss, and maintained in a chamber for seven days. Thereafter, covers were removed from the boxes and the seeds were kept at 25°C for five days (BARROS et al., 1999); results were expressed as a percentage of normal seedlings.

To evaluate the seedling emergence in the field, four replications of 50 seeds per lot were sown in seed beds, in 1.5m long rows with 0.25m between rows. Evaluations were made 10 days after sowing and the results expressed as a percentage of emerged seedlings (NAKAGAWA, 1999).

For traditional procedure of the accelerated aging (AA) test, 18g of seeds per lot were distributed in a single layer on a stainless steel mesh inside transparent acrylic boxes (11×11×3.5cm), containing 40mL of distilled water, resulting in an internal relative air humidity of approximately 100%. Boxes were covered and kept in a water-jacketed aging chamber at 41°C, for 48, 72 and 96h. After each aging period, seed water content was determined to observe the uniformity of test conditions. Following the aging treatment, four replications of 50 seeds each per lot were set to germinate. The evaluation was made four days after sowing by calculating the percentage of normal seedlings.

The saturated salt accelerated aging (SSAA) test was conducted in a way similar as described for the traditional procedure, except that each plastic box received 40mL of saturated NaCl solution (40g of NaCl 100mL^{-1} water), resulting in an internal relative air humidity of approximately 76% (JIANHUA & McDONALD, 1997).

Data from each test were previously tested for normality and homoscedasticity, and subsequently analyzed separately by analysis of variance in a completely randomized design. Means were compared by Tukey's test (P≤0.05) (BANZATTO & KRONKA, 2008).

RESULTS AND DISCUSSION

Initial seed moisture content ranged from 11.5 to 12.3% (2007/2008 harvest), 9.6 to 11.2% (2008/2009 harvest), and 10.9 to 11.9% (2009/2010 harvest). This small variation among results gives more precision to the tests; thus, any difference among lots can be attributed to the physiological characteristics of seeds and not due to differences among initial moisture content of seed lots.

All seed lots have an initial germination higher than the minimum required for the commercialization of sunn hemp seeds, regardless of harvest season, which is fixed at 60% in Brazil (BRASIL, 2008) (Table 1). This indicates high potential of the seed lots to germinate and provides high seedling density under unfavorable field conditions, demonstrating only a lower performance in lot 5 (2007/2008).

Table 1 - Germination - GE and vigor (first germination count - FG, cold test - CT and seedling emergence in the field - SE) of five sunn hemp seed lots from 2007/2008, 2008/2009 and 2009/2010 harvests.

Lot	GE	FG	CT	SE
	--------------------------- % ---------------------------			
	-----------------------2007/2008---------------------			
1	93 a	82 a	83 a	85 a
2	90 ab	79 ab	84 a	84 a
3	89 ab	74 ab	81 a	78 a
4	93 a	80 a	84 a	89 a
5	82 b	69 b	81 a	74 a
CV (%)	4.5	6.2	8.0	8.8
	-----------------------2008/2009---------------------			
1	89 a	87 a	83 a	82 a
2	89 a	85 a	87 a	91 a
3	87 a	84 a	82 a	81 a
4	79 a	78 a	77 a	81 a
5	92 a	86 a	80 a	89 a
6	88 a	84 a	80 a	85 a
7	88 a	83 a	88 a	84 a
C.V. (%)	6.5	6.7	6.9	5.8
	-----------------------2009/2010---------------------			
1	95 a	84 a	84 a	91 a
2	87 a	84 a	72 b	70 b
3	91 a	87 a	77 ab	76 b
4	90 a	90 a	80 ab	72 b
5	92 a	92 a	75 b	75 b
C.V. (%)	5.1	5.1	5.1	6.0

Any two means followed by the same letters within a column indicate no statistically significant difference (Tukey's test; P≤0.05).

Seed vigor assessment (Table 1) of lots from 2007/2008 harvest at first germination count, showed lots 2 and 3 as intermediate vigor, and lot 5 the lowest performance. Conversely, cold test and seedling emergence in the field do not detected significant difference among seed lots.

The stress conditions during the AA test (high temperature and relative air humidity) increased the seed moisture content, and consequently the seed deterioration occurred at faster rate (Table 2). For birds foot trefoil seeds, also a forage legume, the seed vigor was also drastically reduced during AA test at 41°C and relative humidity of approximately 95% (ARTOLA & CARILLLO-CASTAÑEDA, 2005). Under these conditions, there are limitations to evaluate the seed vigor, because differences in seed moisture content before or after AA test, can cause variability in the results (MARCOS-FILHO, 2015).

Conversely, during the SSAA test, moisture values were markedly lower (Table 2). However, among the aging periods, only the period of 96 hours was able to classify seed lots in accordance with the vigor level, which identified the lowest vigor of lot 3 in addition to confirmation of low physiological potential of lot 5. The SSAA for 96 hours was also efficient to detect differences among okra seed lots (TORRES et al., 2014) and wheat seed lots (PEDROSO et al., 2010). The SSAA during 48 and 72h were not considered suitable for sunn hemp seeds (Table 2), whereas 48 and 72h were the SSAA period recommended for other legume such as cowpea (DUTRA & TEÓFILO, 2007) and coriander (PEREIRA et al., 2015) seeds, respectively.

First germination count, cold test and seedling emergence in the field did not detect differences among seed lots produced in 2008/2009 harvest (Table 1). However, for the 2009/2010 harvest, the cold test as well as the seedling emergence in the field were able to separate the seed lots in different vigor levels, with greater sensitivity for seedling emergence in the field. In addition, seeds from both harvest seasons (2008/2009 and 2009/2010) showed uniformity during process of water absorption in the SSAA test, where the variation in the seed moisture content was 1.4 percentage points for 2008/2009 harvest, and 1.5 percentage points for 2009/2010 harvest (Table 3).

Table 2 - Seed moisture content, traditional accelerated aging - AA and saturated salt accelerated aging - SSAA of five sunn hemp seed lots from 2007/2008 harvest: 48, 72 and 96 hours after aging.

Lot	AA			SSAA		
	48h	72h	96h	48h	72h	96h
	%					
	Seed moisture content					
1	36.2	40	43.9	12.5	13.3	12.4
2	37.3	39.1	42.9	13.2	12.9	13.3
3	36.4	40.9	43.7	13.9	14.2	14.3
4	35.4	41.1	43.4	13.5	12.9	12.9
5	36.4	41.1	44.1	13.5	14.3	13.8
	Accelerated aging					
1	71 a	37 b	3 b	81 a	82 a	91 a
2	69 a	36 b	6 b	87 a	80 a	87 ab
3	53 b	43 ab	55 a	76 a	76 a	70 c
4	65 ab	31 b	6 b	81 a	86 a	84 ab
5	61 ab	57 a	56 a	77 a	80 a	79 bc
C.V. (%)	10.7	18.7	20.7	9.0	7.2	5.2

Any two means followed by the same letters within a column indicate no statistically significant difference (Tukey's test; P≤0.05).

The SSAA test during 96 hours in seeds from 2008/2009 and 2009/2010 harvest seasons (Table 3) demonstrated potential for the seed vigor classification. In the 2008/2009 harvest, SSAA indicated the highest vigor level for lot 2, and the lowest level for lot 4. For 2009/2010 harvest, lot 1 showed the best performance while seeds from lot 2 the worst performance, corroborating the results during initial evaluations (Table 1).

From the moment that there is a resumption of the seed development towards germination, degenerative physiological changes in response to decrease of seed vigor can slow down the biomass accumulation by plants, and so, interfering negatively on crop rotation or intercropping systems. The decline in seed vigor implies slow germination and low uniformity of seedlings in the field (MARCOS-FILHO, 2015). Thus, the

Table 3 - Seed moisture content – MC and seed vigor after saturated salt accelerated aging – SSAA at 41°C for 96 hours of five sunn hemp seed lots from 2008/2009 and 2009/2010 harvests.

Lot	MC		SSAA	
	2008/2009	2009/2010	2008/2009	2009/2010
	%			
1	13.7	12.8	84 ab	86 a
2	12.8	14.3	91 a	64 d
3	13.6	14.1	77 b	66 cd
4	14	13.4	82 ab	82 ab
5	13.8	13.7	87 ab	75 bc
6	14.2	-	88 ab	-
7	14	-	82 ab	-
CV (%)	-	-	5.6	6.7

Any two means followed by the same letters within a column indicate no statistically significant difference (Tukey's test; P≤0.05).

correct classification of seed lots according to the physiological potential is an important stage in seed production systems.

CONCLUSION

The accelerated aging test with NaCl saturated solution at 41°C and 96 hours is efficient for classifying seed lots of ***Crotalaria juncea*** L. in different vigor levels.

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