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Accelerated ageing test for vigour assessment of pigeon pea *Cajanus cajan* (L.) Millsp. seeds

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Abstract

Pigeon pea [*Cajanus cajan* (L.) Millsp.] is widely cultivated in tropical areas of the world and it is used as a green manure and forage. The accelerated ageing test is one of the most frequently used tests to evaluate the physiological potential of seeds, however, accurate and adjusted procedures for this test are still missing for pigeon pea seeds. Thus, the aim of the present study was to establish the best combination of conditions to submit pigeon pea seeds to the accelerated ageing test. Five pigeon pea seed lots (cv. Mandarim) were subjected to germination (paper and sand substrate), vigour (first count of germination and emergence speed index) and seedling emergence in the field tests. For the accelerated ageing test procedure, seeds were placed uniformly in a single layer on a fine-mesh tray suspended over 40mL of water (traditional procedure) or saturated salt solution (40% of NaCl), being kept at 42 and 45 °C for 24, 48, 72 and 96 hours. After each period of exposure to the accelerated ageing test, seeds were submitted to the germination test (paper substrate) with seedling evaluation at four, six, eight and ten days after sowing in order to identify the best time for seedlings evaluation. The data were subjected to analysis of variance and treatments were distributed in a completely random design (CRD) with four repetitions. Means were compared with the Tukey test at the 5% level of probability. The results indicated that the traditional accelerated ageing method for pigeon pea seeds should be carried out using the combination of 24 hours and 42 °C with the seedlings evaluation after six days of germination. The accelerated aging test with saturated salt solution should be accomplished using the combinations of 48 hours and 42 °C, with seedlings evaluation after six days of germination and 24 hours and 45 °C with evaluation after four days of germination.

Keywords: *Cajanus cajan* (L.) Millsp.; physiological potential; procedure adjustments; seed quality; vigour.
Abbreviations: AA_Accelerated Ageing; AASS_Accelerated Ageing with Saturated Salt; CV_Coefficient of Variation; FC_First Count of Germination; G_Germination in Paper Substrate; GS_Germination in Sand Substrate; NaCl_Sodium Chloride; SE_Seedling Emergence in the Field; SEI_Speed of Emergence Index; SWC_Seed Water Content.

Introduction

Pigeon pea [*Cajanus cajan* (L.) Millsp.], a member of the Fabaceae family, is popularly known in Brazil as "feijão-guandu", "andu", "guandu" and "guando" (Souza et al., 2007). With its origin in Africa, Asia and some southern islands, pigeon pea has become largely cultivated in tropical and sub-tropical regions of the world where temperatures between 20 and 40 °C are likely to occur (Whyte et al., 1968; Eltayeb et al., 2010). Its ever growing use as a green manure either in rotational or intercropping systems is due to its high biomass production which results in significant increment in soil organic matter content, in high amounts of atmospheric nitrogen symbiotically fixed and in high amounts of feed for domestic livestock (protein bank), particularly during the dry season (Souza et al., 2007). The protein rich grains are also used for human consumption in some countries (Azevedo et al., 2007). Due to all these favourable characteristics, pigeon pea cropping has been growing steadily in the last few years all over the world. However, information on how to properly evaluate pigeon pea seed vigour is still scarce (Nakagawa et al., 2009), a situation hard to explain since the value of the seed as one of the most important agricultural input is largely recognized. The seeds are considered the foundation of the agricultural productivity and getting uniform plant stand in the field is essentially a result of using high vigour seeds.

The evaluation of seeds physiological potential is an essential tool in seed industry since it permits to obtain consistent and reproducible results for the detection and solution of problems during the productive process (Fessel et al., 2010). Therefore, using quick, trustworthy and reliable tests to evaluate seeds physiological potential is of extremely importance for seed companies to make decisions about seed lots. The accelerated ageing test is one of the most traditional methods used to evaluate seed vigour. It is based on the fact that high vigour seeds slowly deteriorate when exposed to sub-optimal and stressful conditions such as high temperature and air relative humidity (Marcos-Filho, 1999a). On the other hand the accelerated ageing test have certain limitations when small seeds are used, once they absorb water too fast and in a non uniform way (Ramos et al., 2004; Freitas and Nascimento, 2006). The substitution of pure water by a saturated salt solution is an alternative to the traditional procedure since it permits to work with lower relative humidity values thus imposing a slower absorption of water by the seeds (Jianhua and McDonald, 1996). For pigeon pea seeds, no technique capable of estimating seed vigour is available. Therefore, the aim of the present research was to establish the best combination of temperature, ageing period and seedling evaluation day to submit pigeon pea seeds during the accelerated ageing test using the traditional and saturated salt solution (NaCl) procedures.

Results

Characterization of the physiological potential

The initial evaluation of the physiological potential of pigeon pea seeds, cv. Mandarim, showed that the seed lots had a moisture content from 10.2 to 12.0%, i.e., their moisture variation was within the expected limits that is around two percent points. Seed water uniformity is essential if consistent results are to be expected (Marcos-Filho, 1999a) (Table 1). The germination tests (sand and paper) and the first count of germination results permitted a satisfactory differentiation among seed lots. The first count of germination results varied between 27 and 88%, the germination results were between 36 and 95% when the substrate was paper and from 44 and 90% when sand was the substrate (Table 1). These tests together with the speed of emergence index characterized lots 1 and 4 as inferior to the other ones. On the other hand, the presence of hard seeds was detected in those two lots and they did not germinate, which may have interfered in the results such as was verified in a previous study with pigeon pea stored seeds (Nakagawa et al., 2009) (Table 1). The seedling emergence in the field results permitted the classification of the lots in different vigour levels, in which lots 3 and 5 were of superior performance and lot 1 showed the poorest performance (Table 1). Probably this higher ranking of the lots was due to the time the test was carried out since the environmental conditions were favourable and this made clearer the discrepancy between lots (Table 2).

Accelerated ageing test: traditional and saturated salt solution methods

The seeds after the exposure to the conventional and the saturated salt solution accelerated ageing methods showed different water absorption patterns as indicated by the values in Table 3. The variation in water content after the traditional accelerated ageing method exceed the recommended limits of 3 to 4 percent points between samples (Marcos-Filho, 1999a), mainly when the temperature was of 42 °C (Table 3). The results of the traditional accelerated ageing method using two temperatures and four ageing periods are in Table 4. The used combinations of temperature and exposition time permitted the separation between lots except the combination of 96 hours and the temperature of 42 °C with seedling evaluation taking place on the fourth day and also the combination of 72 hours, temperature of 45 °C and seedling evaluation on the eighth day (Table 4). The superior vigour performance of lots 3 and 5 as determined by the combination of 42 °C, 24 hours and seedling evaluation on the sixth, eighth and 10^{th} days in the traditional accelerated ageing method corroborated the results of the first count of germination test and those of the germination test carried out both in sand and paper substrates (Table 4). The use of the combination 96 hours and 45 °C, independently of the day in which seedling evaluation was carried out, resulted in a lot classification, which was the opposite of that found in the initial classification (Table 4). The lots classified as vigorous (lots 2, 3 and 5) underwent a vigour reduction whereas lots 1 and 4 showed good results. This reclassification of lots started from the combination of 72 hours and 45 °C (Table 4). During the tests, it was verified that the number of hard seeds in lots 1 and 4 decreased since the first ageing period (24 hours) to the last one (96 hours) at the temperature of 45 °C. At the initial combinations, lot 1 showed around 30% of hard seeds whereas when combinations was of 72 hours and 45 °C that number dropped to 12%. Lot 4, which at first showed 25% of hard seeds, had that value reduced to 15% when the seeds were exposed to conditions of 96 hours and 45 °C (Table 4). The accelerated ageing test with saturated salt solution also permitted to detect differences among the seed lots (Table 5). When the ageing temperature was of 42 °C, most of the ageing periods permitted a lot classification similar to that verified at the initial evaluation (Tables 1 and 5). When the ageing temperature was of 45 °C, the exposure period of 24 hours with seedlings being evaluated on the fourth or on the sixth day were the combinations that permitted to separate lots in different vigour levels and these showed the lots 1 and 4 to be those of inferior performances. These combinations were the ones making possible the earliest evaluation of seed lots quality (Table 5).

Discussion

The presence of hard seeds may explain the difference in seed water content shown by the lots after the traditional ageing method (Table 3). The amount of hard seeds in a lot is determined by the year and the location when and where the seeds were produced (Pietrosemoli, 1997; Souza et al., 2007). The lots used in this experiment were produced in different crop years and it was verified that the ones with the highest amounts of hard seeds were those more recently produced. The non-uniformity in water absorption by the seeds during the accelerated ageing test as observed in this work may cause modifications in seed physiology expressed as differences in deterioration levels from one seed to the other. These results are likely to reduce the efficiency of the ageing test (Baalbaki et al., 2009) (Table 3). In this research though, the presence of hard seeds even if causing non-uniformity in seed water content was not capable of reducing the test efficiency. Regarding the accelerated ageing method using saturated salt solution, seed water contents showed little variation and were lower than those observed when the ageing method was the traditional one (Table 3). These results are consequence of the substitution of pure water in the traditional method by the saturated salt solution, which is capable of reducing the relative humidity in the interior of the plastic boxes (approximately 76%). According to Jianhua and McDonald (1996) and Lopes et al. (2012), this condition controls the water absorption by the seeds and permits uniform results in the evaluating tests. The saturated salt solution also reduces the seeds deterioration rate so that the results are less drastic and uniform in addition to making more likely a better identification of the lots as to different physiological potential, as observed in broccoli and lentil seeds (Fessel et al., 2005; Freitas and Nascimento, 2006). In general, pigeon pea seeds submitted to the traditional accelerated ageing method had not their deterioration process affected by the seeds water content since the lots with higher water content were the ones with the highest vigour level (Tables 3 and 4). These results are in disagreement with those reported by Alves et al. (2012) who found that scarlet eggplant seed submitted to the same accelerated ageing method deteriorated most when they had the highest water contents. Similar results to the ones herein reported showed that *Brachiaria brizantha* (cv. Marandu) dormant seeds had their dormancy overcome by exposure to the accelerated ageing method as well as reduction of the physiological

Table 1. Initial seed water content, germination in paper and sand substrate, first count of germination, seedling emergence in the field and speed of emergence index of five pigeon pea seed lots, cv. Mandarim.

Lots	SWC	G	GS	FC	SE	SEI
		...............................%................................				
1	11.5	40 c	44 c	28 c	31 d	3.4 c
2	10.2	72 b	66 b	50 b	58 b	5.4 b
3	11.9	95 a	90 a	88 a	80 a	7.4 a
4	10.4	36 c	46 c	27 c	42 c	3.6 c
5	12.0	79 ab	80 ab	69 a	78 a	6.4 ab
CV (%)	-	14.7	12.24	16.47	7.45	12.39

Means in the same column followed by the same letter are not significantly different at the level of 5% of probability, according to the Tukey test. CV (%): Coefficient of Variation. SWC: Seed Water Content. G: Germination in Paper Substrate. GS: Germination in Sand Substrate. FC: First Count of Germination. SE: Seedling Emergence in the Field. SEI: Speed of Emergence Index.

Table 2. Maximum and minimum temperatures, pluvial precipitation and air relative humidity from December 3 to 17 of 2011, in Jaboticabal, state of São Paulo, during the seedling emergence in the field test.

Day/Month	Temperature		Pluvial precipitation (mm)	Air Relative Humidity (%)
	Maximum (°C)	Mínimum (°C)		
12/03	30.4	15.1	0.0	65.2
12/04	31.8	17.0	0.0	63.5
12/05	31.7	19.0	0.0	64.8
12/06	30.5	20.3	3.7	75.8
12/07	24.9	18.0	0.0	74.7
12/08	29.3	20.2	7.7	80.9
12/09	25.0	20.5	26.9	90.7
12/10	25.8	18.2	1.0	84.2
12/11	29.5	16.9	0.0	66.8
12/12	31.2	16.8	0.0	56.6
12/13	32.9	19.3	0.0	58.0
12/14	30.6	19.9	3.5	72.3
12/15	27.0	19.8	1.6	83.5
12/16	30.1	19.3	0.0	64.3
12/17	31.8	18.0	0.0	61.9

potential of non-dormant seeds, due to their high rate of seed deterioration (Meschede et al., 2004) (Table 4). In various leguminous species, seed dormancy decreases as the seeds age and high temperatures, temperature alternation and a humid atmosphere favor this reduction (Godoy and Souza, 2004). The long exposition of the seeds to high temperature and relative humidity promoted hard pigeon pea seed dormancy overcoming thus making possible a new classification of the seed lots (Table 4). When the accelerated ageing method with saturated salt solution was used, the different combinations of the temperature of 42°C and time of exposition resulted in the lots being classified the same way they were classified by the initial evaluation (Tables 1 and 5). However, the characteristics searched in seed vigour tests are rapidity, objectivity and efficiency, so the exposition period of 24 hours and seedling evaluation on the eighth day or an exposition period of 48 hours and seedling evaluation on either the sixth or the eighth day are the most appropriate combinations (Table 5). Souza et al. (2009) and Cantos et al. (2011), working with seeds of bristle oat and common oat, respectively, also reported the combination of 48 hours and 42 °C as the most adequate for the artificial ageing of their seeds. It was observed that either too high temperatures or too prolonged exposition periods may difficult or totally prevent the detection of significant differences in the physiological potential of seed lots and this leads to the lots being classified either as of high or low vigour, that is, the intermediate vigour level lots are not detected. This problem was verified for several ageing periods in this accelerated ageing method. Lopes et al. (2010) also reported the same observation in a research work with okra seeds, considering the traditional accelerated ageing test. The use of saturated salt solution was not capable of overcoming seed dormancy such as observed for the combination of 96 hours and 45 °C in the traditional method (Tables 4 and 5). The relative humidity of the air inside the plastic boxes in the saturated salt solution method may have been insufficient to permit the dormancy overcoming process to occur (Table 5). Thus, the traditional accelerated ageing method for pigeon pea seeds should be carried out using the combination of 24 hours and 42 °C with the seedlings being evaluated on the sixth day after the starting of the test. Equivalent efficiency resulted from the accelerated aging test with saturated salt solution using the combinations of 48 hours and 42 °C, with seedlings evaluation on the sixth day and 24 hours and 45 °C with evaluation on the fourth day.

Materials and methods

Seeds

Five pigeon pea [*Cajanus cajan* (L.) Millsp] seed lots, cultivar Mandarim, were identified and stored in a cold room chamber (10 °C and ± 60% of RH). The seeds received no chemical treatment until they were taken for the experiment. Before being used, they were treated with carbendazin + thiram (2mL kg^{-1} of seeds).

Table 3. Seed water content of five pigeon pea seed lots, cv. Mandarim, after being exposed to the traditional ageing method and to the saturated salt solution method during four ageing periods (24, 48, 72 and 96 hours) and two temperatures (42 and 45 °C).

Test	Lots	42 °C				45 °C			
		24 h	48 h	72 h	96 h	24 h	48 h	72h	96 h
	...%								
AA	1	11.0	21.6	21.9	30.4	16.3	24.1	28.2	29.6
	2	14.6	21.0	23.7	27.1	18.3	21.4	28.2	29.1
	3	15.8	26.6	30.0	31.2	22.1	24.2	32.8	33.6
	4	13.0	19.3	25.6	31.6	17.4	24.4	27.3	31.0
	5	23.3	29.2	30.3	35.3	21.4	28.7	32.6	34.0
AASS	1	9.8	8.8	11.1	14.6	9.0	10.0	11.0	11.4
	2	11.6	10.6	12.3	11.5	11.5	11.3	12.0	13.2
	3	12.3	12.4	13.0	13.4	12.7	13.0	13.5	14.2
	4	9.8	8.8	9.4	11.1	9.4	9.9	11.0	11.7
	5	11.7	12.7	12.0	14.3	12.5	13.5	13.7	14.2

AA: Accelerated Aging Test – Traditional Method. AASS: Accelerated Aging Test with Saturated Salt Solution (NaCl).

Table 4. Vigour assessed by traditional accelerated ageing test of five pigeon pea seed lots, cv. Mandarim, following exposition to conditions of different temperatures (42 and 45 °C) and periods (24, 48, 72 and 96 hours) and determination of the best day for seedling evaluation (4, 6, 8 and 10 days after ageing).

Ageing period	Lots	Temperature/Assessment day 42 °C				45 °C			
		4	6	8	10	4	6	8	10
	...%								
24	1	36 d	44 c	50 c	54 c	44 b	50 b	54 b	58 b
	2	56 bc	67 b	75 b	79 b	57 ab	76 a	82 a	87 a
	3	80 a	89 a	94 a	94 a	76 a	85 a	87 a	88 a
	4	41 cd	48 c	52 c	54 c	46 b	54 b	59 b	64 b
	5	70 ab	82 a	83 b	85 ab	62 ab	74 a	78 a	82 a
	CV (%)	13.2	8.2	6.1	8.9	15.5	10.1	9.8	7.5
48	1	46 b	50 b	55 b	59 b	34 b	42 b	47 b	52 b
	2	59 ab	69 ab	77 a	80 a	61 a	68 a	77 a	82 a
	3	82 a	88 a	91 a	92 a	60 a	68 a	68 ab	68 ab
	4	46 b	50 b	54 b	56 b	43 ab	48 ab	52 b	54 b
	5	63 ab	74 a	77 a	78 a	53 a	58 ab	60 ab	62 ab
	CV (%)	18.1	13.6	11.2	11.1	17.3	18.2	17.7	17.7
72	1	46 b	52 bc	60 bc	62 bc	42 a	60 a	66 a	69 a
	2	58 ab	73 ab	78 a	84 a	37 a	54 a	61 a	62 ab
	3	68 a	80 a	86 a	86 a	30 ab	50 ab	53 a	54 ab
	4	40 b	46 c	50 c	51 c	34 a	47 ab	56 a	56 ab
	5	61 ab	70 ab	73 ab	76 ab	18 b	34 b	46 a	46 b
	CV (%)	18.0	15.1	11.8	11.1	21.8	17.8	16.3	16.8
96	1	45 a	49 b	52 b	53 b	38 a	50 a	54 a	56 a
	2	55 a	70 a	76 a	76 a	18 b	26 b	28 b	28 b
	3	67 a	74 a	76 a	78 a	2 c	5 c	6 c	6 c
	4	52 a	59 ab	62 ab	64 ab	33 a	55 a	58 a	58 a
	5	44 a	60 ab	64 ab	64 ab	5 c	8 c	9 c	9 c
	CV (%)	20.6	13.3	12.2	12.7	23.0	21.6	21.3	20.0

Means in the same column and in each ageing period followed by the same letter are not significnantly different at the 5% level of probability, according to Tukey test. CV (%): Coefficient of Variation. AA: Accelerated Aging Test – Traditional Method.

Determination of seed water content

Determined with two repetitions of 25 seeds by the oven method at 105 ± 3 °C for 24 hours (Brasil, 2009). Results are expressed as percentages on a fresh weight basis.

Germination test

Four repetitions of 50 treated seeds per lot were distributed over germination paper, which had been previously moistened with an amount of deionized water equivalent to three times the dry weight of the paper. After this, they were kept in a germination chamber at 25 °C. Seedling evaluations were performed at four and 10 days after sowing; results are expressed as percentage of normal seedlings (Brasil, 2009). This test was also executed using sand substrate. In this case, four repetitions of 50 treated seeds per lot were distributed at a depth of 3 cm in plastic boxes (26.0 × 16.0 × 9.0 cm) in sieved sand which was previously sterilized and moistened with distilled water to 60% of its water holding capacity. The

Table 5. Vigour assessed by accelerated ageing test with saturated salt solution of five pigeon pea seed lots, cv. Mandarim, following exposition to conditions of different temperatures (42 and 45 °C) and periods (24, 48, 72 and 96 hours) and determination of the best day for seedling evaluation (4, 6, 8 and 10 days after ageing).

Ageing period	Lots	Temperature/Assessment day 42 °C				45 °C			
		4	6	8	10	4	6	8	10
		....%..							
24	1	38 b	45 b	49 c	52 b	39 c	45 c	54 b	60 b
	2	58 a	70 a	76 b	84 a	57 b	68 b	76 a	88 a
	3	77 a	89 a	92 a	92 a	75 a	83 a	87 a	89 a
	4	35 b	42 b	48 c	55 b	38 c	48 c	54 b	58 b
	5	65 a	76 a	85 ab	89 a	66 ab	76 ab	82 a	87 a
	CV (%)	17.2	13.5	10.4	8.5	10.91	8.15	8.26	7.4
48	1	38 c	48 c	54 c	56 c	34 b	46 b	52 b	59 b
	2	48 c	66 b	74 b	79 b	60 a	73 a	81 a	90 a
	3	78 a	90 a	92 a	92 a	68 a	87 a	88 a	90 a
	4	39 c	50 c	58 c	62 c	37 b	45 b	50 b	56 b
	5	62 b	74 b	84 a	86 ab	61 a	75 a	82 a	86 a
	CV (%)	12.0	8.4	5.4	6.6	18.3	11.0	8.51	6.76
72	1	30 c	44 c	51 c	53 c	30 b	55 c	60 b	64 b
	2	46 ab	70 b	78 b	81 b	40 ab	70 b	82 a	84 a
	3	50 a	92 a	94 a	95 a	46 a	86 a	90 a	91 a
	4	32 bc	50 c	59 c	60 c	31 b	49 c	56 b	58 b
	5	47 ab	79 b	86 ab	88 ab	36 ab	75 ab	82 a	83 a
	CV (%)	17.4	7.4	7.6	7.7	14.03	7.55	7.33	6.48
96	1	27 c	40 c	48 c	54 b	33 b	47 c	56 b	60 b
	2	44 b	68 b	76 b	82 a	54 a	71 ab	82 a	86 a
	3	64 a	85 a	88 a	90 a	56 a	87 a	90 a	90 a
	4	39 bc	47 c	52 c	54 b	38 b	50 c	54 b	58 b
	5	59 a	78 a	86 ab	88 a	44 ab	69 b	76 a	78 a
	CV (%)	13.3	7.1	7.5	7.3	16.15	12.54	10.0	9.78

Means in the same column and in each ageing period followed by the same letter are not significantly different at the 5% level of probability, according to Tukey test. CV (%): Coefficient of Variation. AASS: Accelerated Aging Test with Saturated Salt Solution (NaCl).

plastic boxes were kept under laboratory conditions (20 – 30 °C and ± 45% of RH). Seedling evaluation was performed 14 days later. The number of normal seedlings was used to calculate the percentage of germination (Brasil, 2009).

Vigour tests

The following vigour tests were used to evaluate pigeon pea seeds: first count of germination and speed of emergence index. The first count of germination was accomplished simultaneously with the germination test on paper substrate. The number of normal seedlings found on the fourth day of the germination test was considered the result of this specific test (Brasil, 2009). The speed of emergence index was determined simultaneous with the germination test in sand. It consisted in the daily count of normal seedlings emerging from the fourth through the 14th day of the germination test. After the test was completed, the speed of emergence index was calculated by means of the formula proposed by Maguire (1962):

$$SEI = \left(\frac{G1}{N1}\right) + \left(\frac{G2}{N2}\right) + \cdots + \left(\frac{Gn}{Nn}\right)$$

Where the SEI is the speed of emergence index, G is the number of normal seedlings found in the several counts and N is the number of days from sowing to the first, second until the last evaluation.

Seedling emergence in the field test

The experiment was conduct in a Haplorthox Sandy Soil, medium texture with irrigation. Four repetitions of 50 seeds per lot were manually distributed in 2 m long rows at 3 cm depth and 0.04 m spacing between rows. Emerged seedlings were counted 15 days after sowing. The seedlings to be counted were those whose leaves originated from the embryo (simple leaves) were developed and their margins were no longer touching each other (Nakagawa, 1999). The maximum and minimum temperatures, pluvial precipitation and air relative humidity observed during the experimental period are shown in Table 2.

Accelerated ageing test – traditional method

Seed samples of approximately 250 treated seeds each lot were arranged in a single layer on top of an stainless steel screen adjusted to a transparent plastic box (11 × 11 × 3.5 cm) containing 40 mL of deionized water on its bottom so as to have an atmosphere of 100% of relative humidity (Marcos-Filho, 1999b). The boxes were kept inside a germination chamber at 42 and 45 °C for 24, 48, 72 and 96 hours. After each ageing period, two repetitions of 25 seed were used to determine seed water content and four repetitions of 50 seeds were submitted to the germination test on paper substrate with seedlings evaluation on the fourth, sixth, eighth and 10th day after sowing, in order to determine the most appropriate day for the evaluation of the seedlings. The results are expressed in percentage of normal seedlings.

Accelerated ageing test with saturated salt solution (NaCl)

The procedure was similar to that identified as the traditional one except that the water in the bottom of the plastic box was replaced by a saturated salt solution, which resulted from the solving of 40 g of NaCl in 100 mL of water. The relative humidity resulting in the interior of the plastic box was of 76%, as described by Jianhua and McDonald (1996).

Statistical procedure

The experimental data were tested for normality (Shapiro-Wilk test) and homogeneity of the variances (Cochran test), and subjected to analysis of variance. The distribution of the treatments was in a completely random design (CRD) with four repetitions. Means were compared with the Tukey's test at the 5% level of probability (Banzatto and Kronka, 2006).

Conclusion

The results indicated that the traditional accelerated ageing method for pigeon pea seeds should be carried out using the combination of 24 hours and 42 °C with the seedlings evaluation on the sixth day. The accelerated aging test with saturated salt solution should be accomplished using the combinations of 48 hours and 42 °C, with seedlings evaluation on the sixth day and 24 hours and 45 °C with evaluation on the fourth day.

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References

Alves CZ, Godoy AR, Candido ACS, Oliveira NC (2012) Qualidade fisiológica de sementes de jiló pelo teste de envelhecimento acelerado. Cienc Rural. 42:58-63.

Azevedo RL, Ribeiro GT, Azevedo CLL (2007) Feijão guandu: uma planta multiuso. Revista Fapese. 3:81-86.

Baalbaki R, Elias S, Marcos-Filho J, McDonald MB (2009) Seed vigor testing handbook, 1st edn. AOSA, Ithaca, p 341

Banzatto DA, Kronka SN (2006) Experimentação agrícola, 4th edn. Funep, Jaboticabal.

Brasil (2009) Ministério da Agricultura, Pecuária e Abastecimento – Secretaria de Defesa Agropecuária. Regras para análise de sementes. Mapa/ACS, Brasília, p 344.

Cantos AA, Tunes LM, Barbieri APP, Tavares LC (2011) Avaliação de testes de vigor em sementes de aveia branca. Revista FZVA. 18:1-11.

Eltayeb ARSM, Ali AO, Haron R (2010) The chemical composition of pigeon pea (*Cajanus cajan*) seed and functional properties of protein isolate. Pak J Nutr. 9:1069-1073.

Fessel SA, Panobianco M, Souza CR, Vieira RD (2010) Teste de condutividade elétrica em sementes de soja armazenadas sob diferentes temperaturas. Bragantia. 69:207-214.

Fessel SA, Silva LJR, Galli JA, Sader R (2005) Uso de solução salina (NaCl) no teste de envelhecimento acelerado em sementes de brócolis (*Brassica oleracea* L. var. *itálica* Plenk). Científica. 33:27-34.

Freitas RA, Nascimento WM (2006) Teste de envelhecimento acelerado em sementes de lentilha. Rev Bras Sementes. 28:59-63.

Godoy R, Souza FHD (2004) Dormência em sementes de guandu (*Cajanus cajan* (L.) Millsp). Rev Bras Zootecn. 33:2201-2205.

Jianhua Z, McDonald MB (1996) The saturated salt accelerated aging test for small-seeded crops. Seed Sci Technol. 25:123-131.

Lopes MM, Barbosa RM, Vieira RD (2012) Methods for evaluating the physiological potential of scarlet eggplant (*Solanum aethiopicum*) seeds. Seed Sci Technol. 40:86-94

Lopes MM, Sader R, Paiva AS, Fernandes AC (2010) Teste de envelhecimento acelerado em sementes de quiabo. Biosci J. 26:491-501.

Maguire JD (1962) Speed of germination: aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2:176-177.

Marcos-Filho J (1999a) Testes de vigor: importância e utilização. In: Krzyzanowski FC, Vieira RD, França-Neto JB (eds) Vigor de sementes: conceitos e testes, Abrates, Londrina.

Marcos-Filho J (1999b) Teste de envelhecimento acelerado. In: Krzyzanowski FC, Vieira RD, França-Neto JB (eds) Vigor de sementes: conceitos e testes, Abrates, Londrina

Meschede DK, Sales JGC, Braccini AL, Scapim CA, Schuab SRP (2004) Tratamentos para superação da dormência das sementes de capim-braquiária cultivar Marandu. Rev Bras Sementes. 26:76-81.

Nakagawa J (1999) Testes de vigor baseados no desempenho das plântulas. In: Krzyzanowski FC, Vieira RD, França-Neto JB (eds) Vigor de sementes: conceitos e testes, Abrates, Londrina.

Nakagawa J, Cavariani C, Toledo MZ (2009) Germinação de sementes armazenadas de guandu. Rev Bras Sementes. 31:49-48.

Pietrosemoli S (1997) Efecto del almacenamiento y la escarificación sobre la germinación de la semilla de quinchoncho *Cajanus cajan* (L.) Millsp. Arch Latinoamericano de Prod Animal 1:25-27

Ramos NP, Flor EPO, Mendonça EAF, Minami K (2004) Envelhecimento acelerado em sementes de rúcula (*Eruca sativa* L.). Rev Bras Sementes. 26:98-103.

Souza FHD, Frigeri T, Moreira A, Godoy R (2007) Produção de sementes de guandu. Embrapa Pecuária Sudeste, São Paulo, p 67.

Souza SA, Nakagawa J, Machado CG (2009) Teste de envelhecimento acelerado em sementes de aveia preta. Rev Bras Sementes. 31:155-163.

Whyte RO, Nilsson-Leissner G, Trumble HC (1968) Las leguminosas en la agricultura. FAO – Food and Agriculture Organization, Yugoslavia, p 405.