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An alternative procedure for evaluating the quality of castor seeds by the tetrazolium test

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The use of fast technologies, which also permit efficient decisions on seeds lots quality, is fundamental to the seed industry. Five seeds lots of "IAC 80"and five seeds lots "AL Guarani 2002" cultivars were used to test an alternative procedure for evaluating the seed quality of castor seeds by the tetrazolium test. Seeds lots characteristics were determined by tests of germination, first count germination, seedling emergence, speed emergence index and initial stand. The seeds were imbibed in water at 30°C for 3 h for the tetrazolium test and submitted to three preparation methods: (1) a bevel cut in the region opposite to the caruncle; (2) removal of the coats and a bevel cut in the region opposite to the caruncle; (3) removal of the coats and lateral cuts in the seed. After preparation, the seeds were immersed in 0.5 and 1.0% tetrazolium solutions for 6 h at 30°C. Imbibitions in water at 30°C for 3 h, followed by removal of the coats and lateral cuts in the seeds, with immersion in a 1% tetrazolium solution at 30°C for 6 h, is a suitable methodology for evaluating castor seed quality.

Key words: *Ricinus communis* L., seed technology, seed analysis.

INTRODUCTION

The launching of the Probiodiesel program, which aims at the substitution of 2% of the diesel produced from petroleum by biodiesel from vegetable oils, has resulted in Brazil relaunching castor seed production (*Ricinus communis* L.- Euphorbiaceae) in bunches. This production will bring in foreign exchange for Brazil and play an important social role in the development of family agriculture (Holanda, 2004). To supply this new demand, castor seeds have become scarce and expensive, consequently favoring a market of poor quality "pirate" seeds.

Research on castor seed quality is essential for crop establishment and is justified by the potential of the plant and the scarcity of information on the seed production technology. Considering that the seed is the basic input in agricultural production and its quality the starting point for a successful crop, it is necessary to develop technologies which permit a rapid and efficient evaluation of seeds lot quality.

Among the available methods for evaluating seed viability is the tetrazolium test, which is based on the activity of dehydrogenase enzymes in tissue respiratory

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processes. During respiration, hydrogen ions are liberated, which react with the 2,3,5 triphenyl chloride salt of tetrazolium forming an insoluble, red compound, called formazam (Delouche et al., 1976). The test has some specific characteristics: It is unaffected by conditions which can alter the results of the germination test, such as seed dormancy; evaluation of the viability and vigor at different levels is fast; a diagnosis of the causes of seed viability losses is possible (Delouche et al., 1976; França-Neto et al., 1998; França-Neto, 1999). Tissues dead or damaged presented discolored. The pattern of staining of tissues can be used to identify viable seeds, non-viable and within the viable category of the high and low vigor (Vieira and Von-Pinho, 1999).

The speed with which the tetrazolium salt is absorbed by seed tissues depends on the physical barriers present (Piña-Rodrigues and Santos, 1988). Many species require preparation of the seeds to allow penetration of the solution and activation of the respiratory system. Among the preparation methods most used are puncturing, cuts and removal of the coats (Brasil, 2009).

Apart from the preparation, other factors, such as solution concentration and staining time, may influence test efficiency and the methodology may have to be adjusted for each species. *Amburana cearensis* seeds, for example, require a 0.05% tetrazolium solution for 3 h (Guedes et al., 2010); whereas Jatropha curcas only needs a 0.5% tetrazolium solution for 120 min to evaluate seed quality (Pinto et al., 2009).

The rules for seed analysis - RSA (Brasil, 2009) provide information on the methodologies for evaluating the seeds of many species. Castor seeds require an 18 h period for seed preparation and 6 to 24 h immersion in a 1% tetrazolium solution, totaling around 42 h for a test evaluation. However, due to the need for a rapid analysis of seed quality, this period may be considered too long. Also, the possibility of reducing the concentration used would be an advantage since lower concentrations would have a lower salt cost and provide a better observation of staining differences to identify different types of injury (França-Neto et al., 1998).

The objective of this study was to develop an alternative methodology for evaluating the quality of castor seeds (*R. communis* L.) with the tetrazolium test.

MATERIALS AND METHODS

The analysis was conducted at the Central Laboratory of Seed Analysis Laboratory of the Federal University of Lavras (Universidade Federal de Lavras). Five seeds lots of “IAC 80” and five seeds lots “AL Guarani 2002” were produced during the 2005/2006 crop cycle. The seeds were collected from different plants, cleaned and naturally dried in a warehouse, after which the seeds lots were sent to the Seed Laboratory for evaluation. The physiological potential of the seed lots was evaluated using the: Seed Water Content (SWC): Determined by the oven method at 105°C for 24 h, using two replications of approximately 5 g per sample kept in aluminium capsules. The data were expressed as a percentage (Brasil, 2009). Germination test (G) was done with four replications of 50 seeds, in a rolled paper towel, moistened with distilled water 2.5 times the weight of the dry substrate. The rolls were kept in a germinator at 20 to 30°C (16 to 8 h) and counts were made seven (First Count Germination - FCG) and 14 days after the test was initiated, according to criteria established by the Rules for Seed Analysis-RAS (Brasil, 2009). Seedling Emergence Test (SE) was done in nursery beds with an earth-sand substrate (1:1) with four replications of 50 seeds. Seedling emergence was evaluated at seven days (Initial Stand- IS) and 21 days after test initiation. The Speed Emergence Index (SEI) was calculated according to Maguire’s (1962) formula with data from the daily counts of the emergence test.

For the tetrazolium test (TZ) were used four replications of 25 seeds for each treatment. The seeds from the different castor seeds lots were imbibed between paper moistened in water at 30°C for three hours before being tested with the three different preparation methodologies: (1) With coats: The seed coats were maintained and a bevel cut was made in the opposite region to the caruncle; (2) Lateral cuts: The seed coats were removed and lateral cuts were made in the endosperm; (3) Bevel cut: The seed coats were removed and a bevel cut was made in the opposite region to the caruncle (Figure 1).

After preparation, the seeds were immersed in 0.5 and 1% tetrazolium solutions in plastic recipients and kept in the dark in a BOD chamber at 30°C, for six hours, constituting six treatments (three preparation methodologies x two concentrations of tetrazolium solution). The seeds were examined individually and according to the extension and intensity of the red staining and presence of milky-white areas, tissue appearance and location of the these colorations with respect to the essential growing areas, they were classified as viable or unviable, according to the standards published by Grabe (1976) and Moore (1972), for various agricultural and forest species.

All the data were submitted to an analysis of variance and the means compared using the Tukey test at the 5% probability level. The results in percentages were transformed into arc sine $\sqrt{x/100}$ and those of the emergence speed index into log(x+5). Pearson’s simple correlation was calculated between the results of the germination and tetrazolium tests.

RESULTS

The mean values for the seed water content were 7.8% for the “IAC 80”and 6.5% for the “AL Guarany 2002” cultivars. The coats were observed to hinder penetration of the tetrazolium solution, independently of the concentration used, masking the evaluation of seeds lot quality for both cultivars. This was confirmed by the lower percentage of viable seeds obtained from these treatments (TZ1 and TZ4) compared to the other treatments and tests (Tables 1 and 2). The main objective of the preparation was to facilitate penetration of the tetrazolium solution, and although only imbibition and cuts are sufficient for some species, such as *Jatropha elliptica* seeds, which should be imbibed in water followed by removal of the caruncle and a longitudinal cut of the seeds (Añez et al., 2007), for castor seed complete integument removal is necessary to stain the viable organs. Similar results were obtained by Gaspar-Oliveira et al. (2009a) for castor seeds, who said that for the evaluation of their physiological potential with the tetrazolium test, the recommended preparation is the removal of the coats followed by posterior longitudinal

Figure 1. Methods of preparation in castor bean seeds for tetrazolium testing. A, With coats; B, lateral cuts; C, bevel cut.

Table 1. Speed emergence index (SEI), Seedling emergence (SE), initial stand (IS), First count germination (FCG) Germination (G) and tetrazolium (TZ1 - With coats and 0,5% tetrazolium solutions; TZ2 - Lateral cuts and 0,5% tetrazolium solutions; TZ3 - Bevel cut and 0,5% tetrazolium solutions; TZ4 - With coats and 1% tetrazolium solutions; TZ5 - Lateral cuts and 1% tetrazolium solutions e TZ6 - Bevel cut and 1% tetrazolium solutions) of seed lots of castor "Guarani AL 2002" cultivar.

Lots	SEI	SE	IS	FCG	G	TZ1	TZ2	TZ3	TZ4	TZ5	TZ6
		----------%----------									
1	43.64^{b}	84^{a}	18^{a}	72^{b}	84^{a}	02^{b}	49^{b}	45^{b}	04^{a}	58^{a}	48^{a}
2	44.80^{b}	87^{a}	16^{a}	89^{a}	94^{a}	03^{b}	60^{ab}	41^{b}	03^{a}	74^{a}	66^{a}
3	52.42^{a}	90^{a}	30^{a}	81^{ab}	87^{a}	01^{b}	78^{a}	55^{ab}	03^{a}	70^{a}	65^{a}
4	53.43^{a}	91^{a}	33^{a}	82^{ab}	90^{a}	26^{a}	71^{ab}	55^{ab}	03^{a}	74^{a}	67^{a}
5	53.88^{a}	90^{a}	32^{a}	87^{a}	95^{a}	01^{b}	71^{ab}	69^{a}	00^{b}	62^{a}	61^{a}
CV (%)	1.62	4.70	27.88	7.19	8.44	34.98	14.94	13.84	18.73	18.16	20.77

*Comparison of means within each column (Tukey test, P ≤ 0.05); CV (%) = coefficient of variation.

Table 2. Speed emergence index (SEI), Seedling emergence (SE), initial stand (IS), First count germination (FCG) Germination (G) and tetrazolium (TZ1 - With coats and 0,5% tetrazolium solutions; TZ2 - Lateral cuts and 0,5% tetrazolium solutions; TZ3 - Bevel cut and 0,5% tetrazolium solutions; TZ4 - With coats and 1% tetrazolium solutions; TZ5 - Lateral cuts and 1% tetrazolium solutions e TZ6 - Bevel cut and 1% tetrazolium solutions) of seed lots of castor "IAC 80" cultivar.

Lots	SEI	SE	IS	FCG	G	TZ1	TZ2	TZ3	TZ4	TZ5	TZ6
		----------%----------									
1	3.58^{b}	23^{c}	01^{b}	04^{c}	07^{b}	01^{c}	12^{b}	11^{b}	05^{b}	09^{b}	07^{b}
2	17.38^{a}	52^{b}	00^{b}	10^{c}	55^{a}	04^{b}	38^{a}	39^{a}	04^{b}	36^{a}	20^{a}
3	17.89^{a}	51^{b}	01^{b}	13^{bc}	22^{b}	11^{a}	34^{a}	29^{ab}	18^{a}	23^{a}	28^{a}
4	29.88^{a}	81^{a}	07^{a}	27^{ab}	59^{a}	06^{ab}	45^{a}	37^{ab}	05^{b}	38^{a}	18^{ab}
5	19.62^{a}	58^{b}	01^{b}	31^{a}	58^{a}	02^{bc}	36^{a}	17^{b}	26^{a}	35^{a}	21^{a}
CV(%)	8.99	12.48	83.18	25.55	24.36	28.49	12.23	10.84	14.66	15.74	20.08

*Comparison of means within each column (Tukey test, P ≤ 0.05); CV (%) = coefficient of variation.

and median cuts in the embryo and endosperm. However, Costa and Santos (2010) observed that the tetrazolium test is efficient for evaluating leucaena seed viability using a lateral cut followed by the seed coat removal.

Similarly, staining was only observed in the region close to the cut in some seeds, which had had their coats removed and were bevel cut, due to the greater exposure of this region to the tetrazolium solution (Figure 2). This result agrees with that of Gaspar-Oliveira et al. (2009a), who observed that only the peripheral regions of castor seeds, which had direct contact with the tetrazolium solution, were stained. Considering that the embryo is located in the internal part of the seed and is the principal structure to be analyzed in quality evaluation in the tetrazolium test, this preparation method may be considered inefficient.

On the other hand, the preparation method involving the removal of the coats and lateral cuts permitted the penetration of the tetrazolium solution up to the

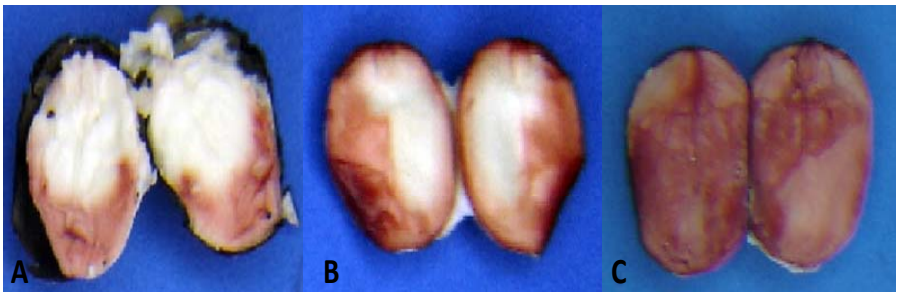

Figure 2. Staining of castor bean seeds submitted to the tetrazolium test. A, With coats; B, bevel cut; C, lateral cuts.

Table 3. Simple correlation coefficients between the results of the germination (G) and tetrazolium (TZ1 - With coats and 0,5% tetrazolium solutions; TZ2 - Lateral cuts and 0,5% tetrazolium solutions; TZ3 - Bevel cut and 0,5% tetrazolium solutions; TZ4 - With coats and 1% tetrazolium solutions; TZ5 - Lateral cuts and 1% tetrazolium solutions e TZ6 - Bevel cut and 1% tetrazolium solutions) in the seed lots of castor "AL Guarani 2002" (GUA) and "IAC 80"(IAC) cultivars.

Tests/cultivars	TZ1	TZ2	TZ3	TZ4	TZ5	TZ6
G (GUA)	-0.018^{NS}	0.354^{NS}	0.407^{NS}	-0.775^{NS}	0.340^{NS}	0.595^{NS}
G (IAC)	-0.069^{NS}	0.8594*	0.572^{NS}	0.131^{NS}	0.976*	0.362^{NS}

NS = not significant; * Significant at P ≤ 0.01 level.

embryonic axis and cotyledons, which facilitated quality analysis due to contact of the whole seed with the solution. Seed imbibition followed by seed coat removal and cuts was also an efficient preparation method for the seeds of other species, such as *J. curcas* (Gris et al., 2007) and Albizia hasslerii (Zucareli et al., 2001).

The numerical results of the germination tests, first germination count, initial stand, emergence and emergence speed index, showed that the seeds lots from the "AL Guarany 2002" cultivar had a higher physiological quality compared to those from the "IAC 80"cultivar (Tables 1 and 2). There were no significant differences between the seeds lots of the AL Guarani 2002 cultivar for emergence, initial stand and germination tests. Similar results were obtained from the treatments with lateral cuts and the 1% solution (TZ5) and the bevel cut and the 1% solution (Table1). However, as mentioned previously, the difficulty of making an evaluation should be noted when the seeds are submitted to a bevel cut. For the "IAC 80"cultivar, the IAC1seeds lot can be classified as having a worse quality compared to the rest based on the numerical results of the tests. The inferior quality of the seeds lot compared to the others may also be observed in the lateral cut and 0.5% (TZ2) and 1% (TZ5) treatments (Table 2). Therefore, the removal of the coats and lateral cuts in a 1% solution (TZ5) permitted a differentiation of the seeds lots of castor seeds corresponding to the results obtained in the other tests for both cultivars (Tables 1 and 2). The results from this treatment were also correlated with those from the germination test for the "IAC 80"cultivar, but this was not observed for the AL Guarani 2002 cultivar (Table 3).

Although the 1% tetrazolium solution intensely stained the seed tissues of some species, as observed by Wetzel et al. (1992) in rubber tree seeds and by Añez et al. (2007) in *J. elliptica* seeds, this concentration can be recommended for castor seeds, as verified in the present study and according to the description in the Rules for Seed (Brasil, 2009). Other methodologies have been recommended for castor seeds, such as that of Gaspar-Oliveira et al. (2009b), who observed that to evaluate the physiological potential using the tetrazolium test, the castor seeds should be immersed in a 0.2% tetrazolium solution for 120 min at 35°C. Three categories of viable and five of unviable seeds were found and described from the tetrazolium test (Table 4). The classification of the seeds submitted to the tetrazolium test into viable and unviable classes facilitates future seed quality evaluations. Another important factor to be considered when doing the tetrazolium test on seeds is the time taken since a rapid evaluation has advantages, such as the possibility of discarding seeds lots of unsuitable quality. The germination test needs 14 days for castor beans whereas the results of the tetrazolium test were available in only one day.

Thus, to be able to differentiate seed seeds lots in a similar way to most of the tests used, to facilitate test evaluations and correlate with the results of the germination test, the methodology using seed imbibitions in water at 30°C for three hours, followed by removal of

Table 4. Categories of castor seeds submitted to the tetrazolium test.

Category	Description
1 (viable)	Embryo completely stained with firm tissues
2 (viable)	Embryo with damage to the embryonic axis without affecting the central cylinder. Cotyledons with a normal color or with less than 50% of the tissues affected
3 (viable)	Embryonic axis with a normal color and less than 50% of the cotyledons stained an intense red.
4 (unviable)	Embryo with damage to the reserve translocation region
5 (unviable)	Embryo with damage to the embryonic axis reaching the central cylinder. Cotyledons with a normal color or with more than 50% of the cotyledons damaged
6 (unviable)	Embryonic axis intensely stained and less than 50% of the cotyledons with intense staining
7 (unviable)	More than 50% of the cotyledons with an intense red stain, embryonic axis with a normal color
8 (unviable)	Embryo completely discolored or with more than 50% discolored, with flaccid tissues

Source: Adapted Moore (1972) and Grabe (1976).

the coats and lateral cuts and the immersion in a 1% tetrazolium solution at 30°C for six hours was suitable for evaluating castor seed quality.

Conclusions

Imbibition in water at 30°C for three hours followed by the seed coat removal and lateral cuts in the seeds with immersion in a 1% tetrazolium solution at 30°C for six hours is a suitable methodology for evaluating castor seed quality.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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