ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 03, pp. 28031-28035, March, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

VIABILITY OF CANAFISTULA (*CASSIA GRANDIS* L.F.) SEEDS BY THE TETRAZOLIUM TEST

[1,*]Heloisa Oliveira dos Santos, [2]Renata Silva-Mann, [2]Robério Anastácio Ferreira, [1]Edila Vilela de Resende Von Pinho, [1]Carla Massimo Caldeira, [1]Raquel Maria de Oliveira Pires and [1]Valkiria Fabiana da Silva

[1]FederalUniversity of Lavras, Department of Agriculture, P.O.Box 3037, 37200-000, Lavras, Minas Gerais, Brazil
[2]FederalUniversity of Sergipe, Department of Florest, 49100-000, São Cristóvão, Sergipe, Brazil

ARTICLE INFO

Article History:
Received 15th December, 2015
Received in revised form
24th January, 2016
Accepted 28th February, 2016
Published online 31st March, 2016

Key words:
Speed Tests,
Vigor,
Forest Seeds.

ABSTRACT

Among the speed tests that allows to determine the viability and vigor of seeds, the tetrazolium test is one of the most promising alternative. However, it is necessary to establish a specific methodology for each species studied. Thus, this present work was realized with the aim to adequate the methodology of tetrazolium test to evaluate the viability of canafistula(Cassia grandis L. F.). Were evaluated the following procedures to the removal of seeds coat: moistening in paper towel and immersion in water by 24 hours 30°C in an incubator type BOD. Then, the embryos were immersed in 0,075%, 0,5% and 1% of tetrazolium solution at 30°C in the dark for 4, 8 and 12 hours for the color development. Between the tested procedures to the removal of seeds coat, the immersion of seeds in water at 30°C for 24 hours allows better imbibition of seeds. Uniform coloration was obtained when seeds were immersed in tetrazolium solution at 0,5% for 8 hours at 30°C in the dark. The vigorous tissue was colored by brilliant pink, while the tissues with some deterioration or with damages presented the coloration of red-carmine intense. It was possible to establish three viable classes of seeds (class 1, 2 and 3) and three non-viable and dead (class 4, 5 and 6).

Citation: Heloisa Oliveira dos Santos, Renata Silva-Mann, Robério Anastácio Ferreira, Edila Vilela de Resende Von Pinho, Carla Massimo Caldeira, Raquel Maria de Oliveira Pires and Valkiria Fabiana da Silva, 2016. "Viability of canafistula (cassia grandis l.f.) seeds by the tetrazolium test", *International Journal of Current Research*, 8, (03), 28031-28035.

INTRODUCTION

The evaluation of physiological quality is an important parameter to be considered in a program of seeds production, and nowadays, physiological tests which provide results in a period of time relatively short are the most required to become faster the decision-making at different stages in the production process in the companies (Bhering *et al*., 2005). The tetrazolium test has been shown to be a promising alternative by the quality and speed in determining the viability and vigor of seeds. Is a test that is based in the activity of dehydrogenases enzymes, particularly the dehydrogenase of malic acid that reduces the tetrazolium salt in alive tissues of seeds, where ions H^+ are transferred to tetrazolium salt. When seeds are immersedin tetrazolium solution, occurs the reaction of reduction in alive cells resulting in the formation of a red compound, not diffusible, known as triphenyl formazan, indicating respiratory activity in mitochondrias and consequently, the viability of tissue (alive) (França Neto, 1999). Dead tissues (no viable) did not react with solution conserving their natural color (Delouche, 1976; Ferreira, 2003). It is a test based in the observation of coloration obtained in different parts of seeds that allows to determine the presence, the localization and the nature of alterations in the tissues of seeds (Copeland, 1974;França-Neto, 1999;Schatral, 1994), and also, to identify many times, the causes of losses of viability and vigor. The efficiency of the test in evaluate the vigor and the viability of seeds is related to the development of adequate methodology for each specie, in a way to define the most appropriate conditions for preparation, pre-conditioning and coloration of seeds. With this, the preparation and the pre conditioning of seeds before the coloration, are decisive factors (Bhering *et al*., 2005). The tetrazolium test it has been used successfully to evaluate the quality of forest seeds (Ferreira *et al*., 2007;Oliveira *et al*., 2005a;Oliveira *et al*., 2005b; Ferreira *et al*., 2004; Ferreira *et al*., 2001; Mendonça, *et al*., 2001). However, for seeds which presents impermeable tegument to water, pre germinative methods, like immersion in water, perforations or scarifications, must be used previously

****Corresponding author: Heloisa Oliveira dos Santos,***
FederalUniversity of Lavras, Department of Agriculture, P.O.Box 3037, 37200-000, Lavras, Minas Gerais, Brazil.

to the immersion of these seeds in tetrazolium solution. This procedure is known as pre conditioning of seeds. In studies with *Platycyamusregnellii* (Davide *et al*, 1995), and *Solanum lycocarpum* (Malavasi *et al*., 1996), was realized manual scarification of seeds and posterior imbibition for 24 hours, to remove the seed coat that prevented the absorption the tetrazolium solution. However, the use of concentration of tetrazolium solution and time of conditioning and adequate time of evaluation, are also fundamental to the obtainment of reliable results about the quality of seeds. Embryos of *Cassia sieberiana*can be submitted to 1% of tetrazolium solution by 9 hours at 35 ºC in the realization of test (Todd-Bockarie *et al*., 1993). Already to *Dalbergiamiscolobium,* was used the same concentration, however by 24 hours, at 30ºC (Sassaki, 1992). Being this, in this work the objective was to verify the efficiency of different methods of pre conditioning and concentrations of tetrazolium solution in the evaluation of viability and vigor of lots of canafistula seeds.

MATERIALS ANDMETHODS

The experiment was realized in the Central Laboratory of Seeds in the Department of Agriculture at Universidade Federal de Lavras, using a lot of *Cassia grandis* L.f seeds provided of many individual from the region of Lower São Francisco at Sergipe. All seeds were scarified in the opposite extremity to embryonic axis, to overcome the dormancy coats with sandpaper nº60, being in after, submitted to the following methods of pre conditioning for the removal of seeds coat: imbibition in moistened towel paper with quantity of water equivalent to 2,5 times the weight of the dry paper, keeping the seeds in BOD at 30ºC by 24 hours and direct immersion in water at 30ºC by 24hours in BOD. After each treatment, the seeds coat was removed with aim of a stylet.

The embryos were immersed for coloration in solution of 2,3,5 triphenyl tetrazolium chloride at 0,075%, 0,5% and 1% for 4, 8 and 12 hours in dark at 30° C in BOD. After each period, were so, washed in running water and immersed in water for evaluation. For each treatment were used four replications of 25 embryos, totalizing 100 embryos per treatment. After the evaluation of each treatment and defined based on levels, the datas were submitted to the analyzes of variance, using the Scott-Knott at 5% of probability, being used to realize these analyzes, the statistical program SISVAR® (Ferreira, 2003). The embryos were individually evaluated, external and internally, after their longitudinal sectioning, between the cotyledons, with the care to not cut the embryonic axis in the middle. Was observed the occurrence of damages in the intern and extern parts of cotyledons and of embryonic axis.

The lots of seeds were submitted to preliminary tests to evaluation of viability, by germination test. For this test, were used 80 seeds (four replication of 20 seeds), distributed in gerboxes, containing sand of medium texture (washed and sterilized in oven with air circulation at 125ºC, for 24hours), being kept in temperature of 25ºC, on continuous light, in germination type Mangelsdorf. The evaluations were realized daily according the criteria established by the Regras para Análise de Sementes (BRASIL, 2009), considering the emergence of normal seedlings. The results were expressed in percentage. The moisture content of lots was determined by the oven method at 105ºC ±3ºC for 24 hours, using four replications of 10 seeds per treatment sectioned with the help of pruning shears (Souza *et al*., 2005). The water content was calculated based on wet weight (BRASIL, 2009).

RESULTS AND DISCUSSION

The lot presented percentage of germination medium of 85% and moisture content of 14,36% in the occasion of the realization of test. Between the procedures tested to the removal of seeds coat, the immersion of seeds in water at 30ºC per 24 hours was the pre conditioning that allows the better imbibition of seeds. The moistening in towel paper at 30 ºC per 24 hours, did not show to be adequate, cause did not allow the removal of seed coat with facility, once the seeds did not absorb sufficient water to soften the coat, interfering with this, in the penetration of the tetrazolium solution into seed (Figure 1). The pre-conditioning before the coloration consists one of the critical steps of the tetrazolium test. These author still added that the slow absorption of water, in controlled temperature, is extremely desirable and necessary to prevent damages in parts of embryo and stimulate the enzymatic activity, that is one of the prerequisites of the respiratory process (Costa *et al*., 1994).

Was verified yet, that the coloration more uniform and coherent with the recommendation of (Moore, 1972) was obtained when seeds were immersed in tetrazolium solution of 0,5% per 8 hours at 30°C in dark. The most vigorous tissues, were colored for brilliant pink (Figure 2a), while, the tissues with same deterioration or with damages presented the coloration red carmine intense (Figure 2b). The use of tetrazolium solution of 1% did not show to be the most adequate to the evaluation of viability of seeds, once the viable tissues, instead of pink, presented with red coloration, a little less intense than the tonality of red observed in tissues with damages, what difficult the observation of the damages presented in seeds, especially in vital regions of the embryo (Figure 3). In seeds, the vital area includes the hypocotyl-radicle axis and the region of insertion between the cotyledon and the axis.

Figure 1. Canafistula seeds (Cassia grandisL.f.) after the pre-conditioning: imbibition in paper (A) and imbibition with immersion (B)

Figure 2. Embryo of canafistula seeds (Cassia grandisL.f) treated with solution of tetrazolium showing the viable tissues with pink coloration (A) and tissues with deterioration with red-camine coloration (B)

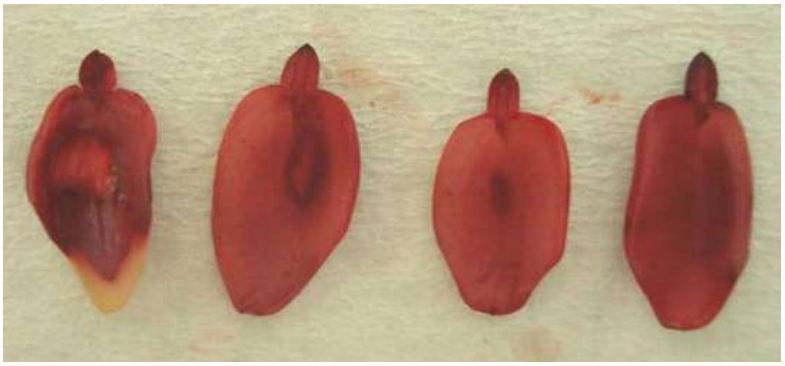

Figure 3. Embryos of canafistula seeds (Cassia grandis L.f.) treated with tetrazolium solution of 1%

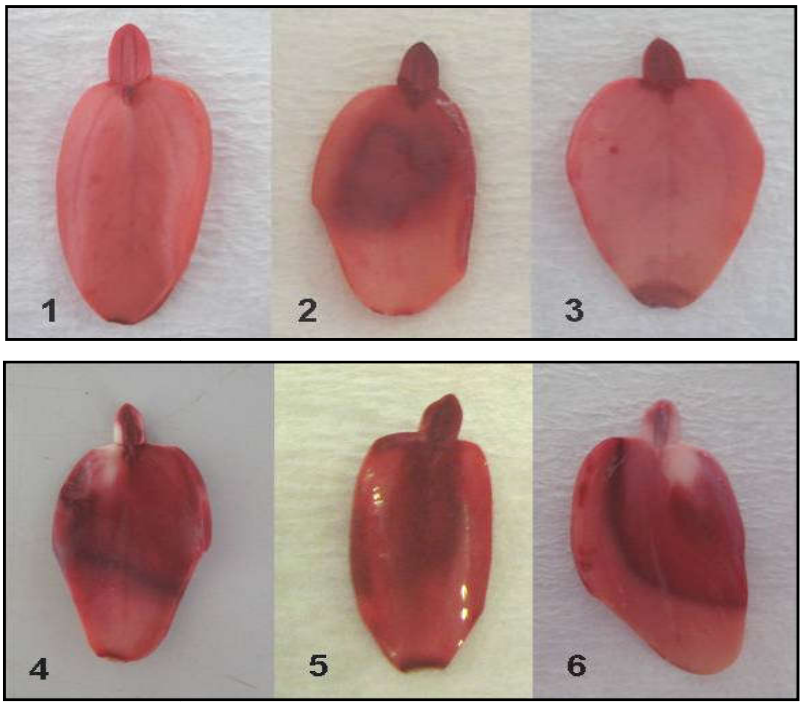

Figure 4. Class of viability obtained in the standardization of tetrazolium test for canafistula seeds (Cassia grandisL.f.)

Table 1. Seeds of canafistula(Cassia grandisL.f.), submitted to evaluation of viability by tetrazolium test under different periods of imbibition and saline concentrations

Treatments (Period x conc.)	*Viable Seeds(%)*	*Inviable Seeds (%)*	*Withoutdefinition (%)*
8h a 1%	97,50 a	2,50 b	0,00 b
4h a 1%	92,75 a	6,25 b	0,00 b
12h a 1%	93,75 a	5,00 b	1,25 b
12ha 0,075%	91,25 a	3,75 b	5,00 b
12h a 0,5%	91,25 a	3,75 b	5,00 b
4h a 0,5%	85,00 b	5,00 b	10,00 a
8h a 0,5%	82,50 b	17,50 a	0,00 b
8h a 0,075%	81,25 b	8,75 b	10,00 a
4h a 0,075%	72,50 b	8,75 b	18,75 a
CV(%)	8,84	59,57	31,31

Means followed by the same letter in the column do not differ by Scott-Knott test at 5% probability.

Based in the observations of the intensity of coloration, depth, and localization of damages were stablished six classes of viability. The potential of vigor was determined by the sum of number of seeds from classes 1 to 3 and the seeds non-viable by the sum of seeds from classes 4 to 6 (Figure 4).

These classes were described like

Class 1 (viable): embryo with coloration pink or darker and tissues with appearance normal and firm;

Class 2 (viable): less than 50% of cotyledons without color, not affecting the region of connection with the embryonic axis. The other regions with pink coloration or darker and firm tissues;

Class3 (viable): embryos with coloration red-intense and firm tissues;

Class 4 (inviable): more than 50% of cotyledons with coloration red- intense, presenting flabby tissues;

Class 5 (inviable): embryo with coloration red-intense and flabby tissues, indicating the deterioration process;

Class 6 (inviable): embryonary axis completely without color, presentig flabby tissues.

Based on datas presented on Table 1, it can be inferred that the treatment relative to the concentration of 0,5% and period of imbibition of 8 hours (82,50% of viable seeds) present the results equivalent to the percentage of germination of lot (85%) when the test was realized. Even other concentrations presenting higher percentages of viable seeds, these datas could have been masked due to the higher coloration of tissues, what can be occasioned a super estimative in the interpretation of datas referred to viable and inviable seeds. Must be considered that despite of the treatment relative to the concentration of 0,5% and time of imbibition of 4 hours have presented 85% of viable seeds, it was not appropriate, once this condition presented 10% of seeds classified like without definition, due to the inefficient imbibition of seeds by the short period of these seeds in solution.

The coloration obtained when was used the concentration of 0,5 % with imbibition per 8 hours in solution, facilitates the evaluation of embryos of canafistula when compared to the others concentrations. The choice of the adequate methodology to the use of tetrazolium test must be based on the facility to differentiate viable tissues from the inviable tissues and based on the capacity of differentiate lots with distinct physiological quality.

Conclusion

The tetrazolium test using the concentration of 0,5%, with imbibition in solution per 8 hours can be used to differentiate lots of *Cassia grandis* L.f seeds of different physiological quality. The method using imbibition by twenty four hours presents efficiency in the pre conditioning of canafistula seeds at 30ºC in the dark.

Acknowledgement

To the National Research Council (CNPq), the Personnel Improvement Coordination of Higher Education – (CAPES) and ETENE/FUNDECI/BNB, for the financial support and the scholarships awarded to the project.

REFERENCES

Bhering, M. C., Dias, D. C. F. S. and Barros, D. I. 2005. Adequação da metodologia do teste de tetrazólio para avaliação da qualidade fisiológica de sementes de melancia. Rev. Bras. Sementes, vol.27, no.1, p.176-182.

Brasil. 2009. Ministério da Agricultura. Regras para Análise de Sementes. Brasília. 365p.

Copeland, L.O. 1976. Principles of seed science and technology. Minneapolis: Burges Publishing Company,. 369p.

Costa, N.P. and Marcos Filho, J. O 1994. Emprego do teste de tetrazólio na avaliação da qualidade da semente de soja. Informativo ABRATES, Londrina, v. 4, n. 2, p.53-62.

Davide, A.C., Botelho, S.A., Malavasi, M.M. and e Oliveira, L.M. 1995. Avaliação da viabilidade de sementes de pau-pereira (Platycyamusregnellii). In: Congresso Brasileiro de Sementes, 5., 1995, Florianópolis. Anais... Florianópolis: ABRATES. p.178.

Delouche, J.C. 1976. Standardization of vigor tests. Journal of Seed Technology, v.1, p.75-85.

Ferreira, D.F. 2003. Sisvar versão 4.2. DEX/UFLA.

Ferreira, M.M.P. 2003. "Desenvolvimento, caracterização e secagem de sementes com cobertura artificial". Doutorado, PPG-EQ/UFSCar, São Carlos SP, 235p.

Ferreira, R.A., Davide, A.C. and Motta, M.S. 2004. Vigor e viabilidade de sementes de Senna multijuga (Rich.) Irwin et Barn. e Senna macranthera (Collad.) Irwin et Barn., num banco de sementes em solo de viveiro. Revista Brasileira de Sementes, Brasília, v.26, n.1, p.24-31.

Ferreira, R.A., Oliveira L.M., Tonetti, O.A.O. and Davide, A.C., Comparação da viabilidade de sementes de Schizolobiumparahyba (vell.) blake – leguminosaecaesalpinioideae, pelos testes de germinação e tetrazólio, Revista Brasileira de Sementes, Brasília, v.29, n.3, 2007.

Ferreira, R.A., Vieira, M.G.G.C., Von Pinho, E.V.R., Tonetti, O.A.O. 2001. Morfologia da semente e de plântulas e avaliação da viabilidade da semente de sucupira-branca (PterodonpubescensBenth. – Fabaceae) pelo teste de tetrazólio. Revista Brasileira de Sementes, Brasília, v.23, n.1, p.108-115.

França Neto, J.B. 1999. Teste de tetrazólio para determinação do vigor de sementes. In: KRYZANOWSKI, F.C.; VIEIRA, R.D. e FRANÇA NETO, J.B. Vigor de sementes: conceitos e testes. ABRATES: Londrina.

Malavasi, M.M., Davide, A.C., Oliveira, L.M., Botelho, S.A. and Tonetti, O.A.O. 1996. Aplicação do teste de tetrazólio na avaliação da viabilidade de sementes de Solanumlycocarpum St. Hill.-Solanaceae- (fruta-de-lobo). In: Seminário Panamericano de Semillas, 15.; Workshop sobre Marketing em Sementes e Mudas, 3., 1996, Gramado. Anais...Gramado: CESM/FELAS.

Mendonça, E.A.F., Ramos, N.P. and Paula, R.C. 2001. Viabilidade de sementes de Cordiatrichotoma (Vellozo) ArrabidaexSteudel (louro-pardo) pelo teste de tetrazólio. Revista Brasileira de Sementes, Brasília, v.23, n.2, p.64-71.

Moore, R.P. 1972. Interpretation of color differences in tetrazolium testing. SeedTechnologist News, v.44, n.3, p.22-24.

Oliveira, L.M., Carvalho, M.L.M. and Davide, A.C. Teste de tetrazólio para avaliação da qualidade de sementes de Peltophorumdubium (Sprengel) Taubert – leguminosaecaesalpinioideae. Revista Cerne, Lavras, v.11, n.2., p.159-166. 2005a.

Oliveira, L.M., Carvalho, M.L.M. and Nery, M.C. 2005b. Teste de tetrazólio em sementes de Tabebuia serratifoliaVahlNich. e T. impetiginosa (Martiusex A. P. de Candolle) Standley – Bignoniaceae. Revista Ciência Agronômica, Fortaleza, v.36, n.2.

Sassaki,M.R. and Felippe, G.M. 1992. Viabilidade de sementes de Dalbergiamiscolobium Bentham (Fabaceae). RevistaBrasileiraBotânica, São Paulo, v.15, n.1, p.1-3, jul.

Schatral, A. and Fox, J.E.D. 1994. Quality and viability of seeds in the genus Hibbertia. Seed Science and Technology, Zurich, v.22, n.2, p.273-284.

Souza, V. C., Bruno, R. L. A., Andrade, L. A. 2005. Vigor de sementes armazenadas de Ipê amarelo (Tabebuia serratifolia (Vahl.) Nich. RevistaÁrvore, Viçosa-MG, vol.29, no.6.

Todd-Bockarie, A.H., Duryea, M.L., West, S.H., White, T.L. 1993. Pretreatment to overcome seed coat dormancy in Cassia sieberiana. Seed Science and Technology, Zurich, v.21, n.2, p.383-398.

****