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Tetrazolium test to evaluate the quality of *Dolichos lablab* seeds

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Abstract

The objective of this study was to verify the efficiency of different methods of pre-conditioning and concentrations of tetrazolium solutions to evaluate the physiological quality of lablab seeds. The seeds went through an accelerated aging process to make lots of 0, 24, 48 and 96 hours. The seeds were also submitted to pre-conditioning method: a) scarification with sandpaper and immersion in water at 25°C for 24 hours; b) immersion in water at 95°C and kept the seeds in the same water for 24 hours without heating. After pre-conditioning, the tegument was removed and the seeds immersed in 0.25; 0.5 and 1.0% tetrazolium solution at 25°C for 150 minutes. Germination tests

were carried out to compare the results obtained in the tetrazolium test, first counting and index of germination velocity. The pre-conditioning of the seeds with immersion in water at 95°C and the maintenance of the seeds in the same water at 25°C for 24 hours, seems to be the most indicate for lablab seeds. The immersion in 0.5% tetrazolium solution at 25°C for 150 minutes, showed the most effective in the evaluation of physiological quality of seeds of this species.

Index Terms: Legumes, *Lablab purpureus*, pastures.

Teste de tetrazólio para avaliação da qualidade de sementes de *Dolichos lablab*

Resumo

Este trabalho teve como objetivo verificar a eficiência de diferentes métodos de pré-condicionamento e concentrações da solução de tetrazólio na avaliação da qualidade fisiológica de sementes de Labe Labe. As sementes passaram pelo processo de envelhecimento acelerado para compor os lotes de 0, 24, 48 e 96 horas, e foram submetidas aos métodos de pré-condicionamento: a) escarificação manual com lixa e imersão em água a 25°C por 24 horas; b) imersão em água a 95°C e manutenção das sementes na mesma água por 24 horas, fora do aquecimento. Após o pré-condicionamento, o tegumento foi retirado e as sementes imersas em solução de tetrazólio a 0,25; 0,5 e 1,0% por 150 minutos a 25°C. Para comparação dos resultados obtidos no teste de tetrazólio, foram realizados os testes de germinação, primeira contagem e índice de velocidade de germinação. O pré-condicionamento das sementes com imersão em água a 95°C e manutenção das sementes na mesma água por 24h a 25°C parece ser o mais indicado para sementes de Labe Labe, e a imersão em solução de tetrazólio a 0,5% por 150 minutos a 25°C mostrou-se eficaz na avaliação da qualidade fisiológica das sementes desta espécie.

Termos para Indexação: Leguminosas, *Lablab purpureus*, pastagens.

INTRODUCTION

LabLab (*Dolichos lablab*) belongs to the family *Fabaceae* (legumes) and is believed to be from India, Southeast Asia or Africa. Currently, it has been cultivated and distributed in the tropics and subtropics (Aganga and Tshwenyane, 2003). It is planted by seeds, therefore it is important to know it's potential of storage in the seed lot and the marketing of this storage is based on the cultural value. Among the quality parameters used to determine the culture value of seed lots, only a percentage of germination can suffer alterations during the storage period, thus reducing the culture value of the seed lots (Usberti, 1982).

The development of rapid test aiming at determining the physiological seed quality has been one the main objective of seed technologists for several years, especially to make fast decision in programs of quality control. Among these tests, the tetrazolium test is notable by allowing the evaluation of seed physiological potential, which is quick and efficient when performed carefully (Cervi and Mendonça, 2009).

Tetrazolium test reflects the activity of enzymes dehydrogenases, involved in the respiratory process. For the hydrogenation of 2, 3, 5 triphenyl tetrazolium chloride, it is produced in living cells a red substance, stable and non-diffusible, the triphenyl formazan. This makes it possible to distinguish the live parts that are red from those that are dead, which are another color (Delouche et al., 1976). Procedures are indicated to perform the test, called pre-conditioning, such as cutting, scarification, and immersion in water (Oliveira et al., 2005). Other than pre-conditioning, it is fundamental the utilization of concentration of tetrazolium solution, time and temperature of conditioning and proper assessment of coloration.

The objective of this study was to evaluate the efficiency of different methods of pre-conditioning and concentration of tetrazolium solution in the evaluation of physiological quality of lablab seed lots.

MATERIALS AND METHODS

The experiment was held in the Seed Laboratory at the Center of Agriculture Science of the Federal University of *Espírito Santo, Alegre, ES*, from January to March 2011. Commercial cultivar "Rongai" lablab seeds were used (*Dolichos lablab*) produced in 2009. The experiment was conducted in a completely randomized factorial design 2x3 (2 pre-conditioning and 3 concentrations) with 4 replicates of 25 seeds.

For the tetrazolium test, 4 seed lots were used and to make these lots the seeds were commercially acquired in the harvest of 2009. The seeds were placed on wire gauze in a plastic *gerbox* box containing 40 ml of water in the bottom of the box. The seeds were submitted to 0, 24, 48 and 96 hours of accelerated aging in a ventilated dry oven at 45°C (Santos and Paula, 2007). The experiment was composed by two pre-conditioning: a) the seeds were filed until a small exposure of the cotyledons, with a water sandpaper no. 100, including scarification that were previously immersed in 25 ml of water at 25°C for 25 hours; b) the intact seed were immersed in 25 ml of water at 95°C and left at rest for 24 hours in the same water without heating.

During the resting period, the seed teguments were carefully removed with a scalpel blade and the seeds were put in plastic cups and were totally immersed in tetrazolium solution (pH 6.5) in the concentrations of 0.25; 0.5 and 1% and kept in the dark at 25°C for 150 minutes.

Once the coloring was done, the seeds were removed from the immersion and the cotyledons were separated in longitudinal direction to the center, including the cotyledons and embryonic axis. The two halves were individually examined and according to their length, intense red color, presence of milky-white areas, aspects of tissue and location of coloring in relation to the areas essential to growth. The seeds were individually placed in viable and unviable categories according to standard published by the International Seed Testing Association (ISTA) (1993), Figure 1.

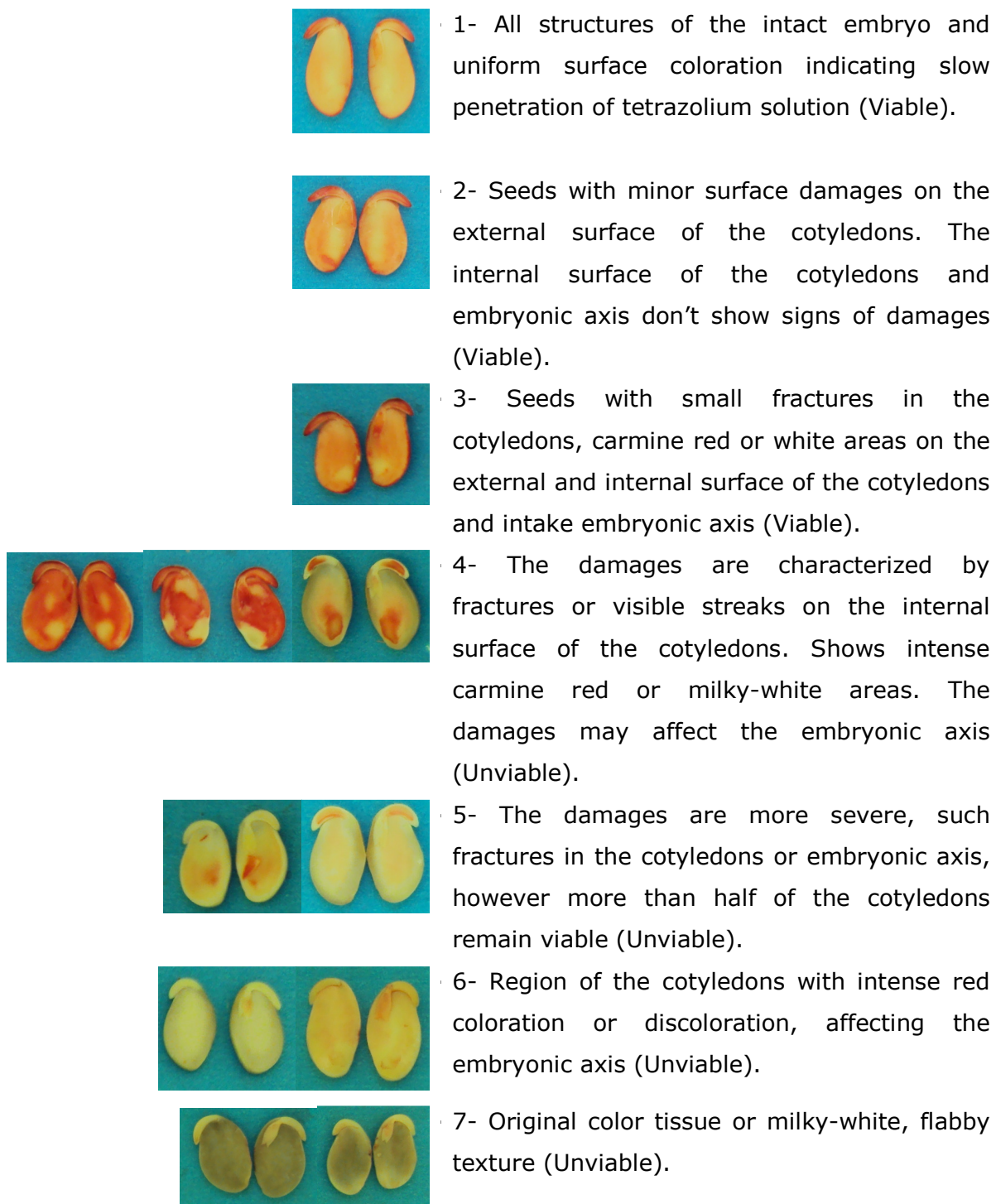


Figure 1. Categories of *Dolichos lablab* seeds submitted to the tetrazolium test (Adapted ISTA, 1993).

Germination test was completed between paper lasting 14 days, in the germinating chamber at 27°C with photoperiod of 18 hours, utilizing four replicated of 25 seeds adapted from Brazil (2009).

The index of germination velocity was calculated according to the adapted formula proposed by Maguire (1962): $IGV = (G_1/D_1) + (G_2/D_2) + (G_n/D_n)$, where G= number of plants germinated in first, second,..., last counting and D= number of days from sowing to first, second,..., last counting. The values of the first counting were obtained on the third day after the assembly of the germination test. The data obtained of the tratazolium test, germination, first counting, index of germination velocity were transformed into $\text{Arcsin } \sqrt{x}/100$ and submitted to analysis of variance. Means were compared by Tukey test at a level of 5% (Ferreira, 2000).

RESULTS AND DISCUSSION

According to the data presented in Table 1, it can be observed that there were no significant difference between the germination test and tetrazolium for the lot of 24 hours of aging, while the lots of 0 and 48 hours of aging in all concentration, the germination were underestimated probably because of the fact that indirect methods of evaluation is not always possible to identify which factors the test is being affected.

Tetrazolium test using concentrations of 0.5 and 1.0% made it possible to differentiate between the lots of 24 hours and 48 hours, consequently the results of this study corroborate with the results obtained by Fernandes et al. (2007), in which the authors verified that immersion in water for 24 hours, followed by the removal of the tegument and subsequent immersion in at a concentration of 0.5% tetrazolium solution for four hours permitting a better visualization of coloration of seed tissue of "coquinho-azedo" (*Butia capitata*).

Table 1. Results of the tetrazolium and germination test (GT), first counting of the germination test (1ST C) and index of germination velocity (IGV) in *Dolichos lablab* seeds (TZ 0.25 = 0.25% tetrazolium solution, TZ 0.5 = 0.5% tetrazolium solution and TZ 1.0 = 1% tetrazolium solution).

Tests	Lots				CV%
	0 hours	24	48 hours	96	
		hours		hours	
		72.34		0.00	
TZ 0.25	69.63 Ba	Aa	30.04 Bb	Ac	25.20
		64.08	33.28	0.00	
TZ 0.5	78.69 Aa	Aa	ABb	Ac	23.82
		64.91	56.69	0.00	
TZ 1.0	39.40 Cb	Aa	ABb	Ac	22.20
		72.00	58.00	0.00	
GT	88.00 Aa	Ab	ABb	Ac	12.89
		61.61		0.00	
1 ST C	74.00 Ba	Aa	38.00 Ab	Ac	20.81
IGV	5.18 a	4.28 a	3.08 b	0.00 c	14.96
CV%	8.44	17.17	31.45	0.00	

* Means followed by the same uppercase letter in columns and lowercase letters in rows do not differ among themselves, by Tukey Test at 5% probability.

It was found that the tetrazolium test at a concentration of 0.5% can be used to evaluate the viability of *Parkia velutina* seeds, as in addition in the germination test (Mendes et al. 2009). Evaluating *Jatropha* seeds (*Jatropha curcas*) found that hydration of the seeds between paper until they reach 30% of water, then by removing the tegument and coloration of the embryo in 0.5% of tetrazolium solution for 120 minutes in the dark at 40°C is

the most indicated procedure to do the tetrazolium test for the referred species (Pinho et al. 2009).

Assessing *peltophorum dubium* seeds it was found that the scarification and posterior immersion in water at 25°C for 14 hours and concentration of 0.1% tetrazolium solution at 25°C for 150 minutes, allows to evaluate the quality of seed lots of this specie (Oliveira et al. 2005). Evaluating the *guapuruvu seed (Schizolobium parahyba)* showed that the concentration of 0.05% tetrazolium solution allows to effectively evaluating the quality of seed lots of this specie (Ferreira et al. 2007).

Evaluating *clitorea ternatea* seeds, it was found that scarification using sandpaper and immersion in water at 25°C for 14 hours in concentration of 0.3% tetrazolium solution at 25°C for 2 hours and 30 minutes thus allowing to clearly evaluate the viability of seeds of this specie (Deminicis et al.2009). It is recommended to do the tetrazolium test of *leucena seeds (Leucaena leucocephala)* after pre-conditioning at 25°C for 18 hours, carry out the lateral cutting of the seeds followed by immersion of water at 30°C for one hour and later removing the tegument and for coloration should be used in 0.15% tetrazolium solution at 35°C for two hours (Costa and Santos 2010).

In the pre-conditioning with scarification with sandpaper no.100 and then immersion in water at 25°C, occurred a large water absorption by the seeds which caused the separation of the cotyledons, hence remaining united only by the embryo. In that way, the preparation of the seeds for interpretation of the tetrazolium tests was more intricate due to disruption of the embryo in some seeds.

Pre-conditioning before coloration is one the critical steps of the test, being that the slow absorption of water in controlled temperature is extremely desirable and necessary to prevent fractures of the embryo parts and stimulate enzyme activity which is one of the prerequisites of the respiratory process (Copeland et al. 1959). In this study, probably the slower absorption of water occurred when seeds were submitted to pre-conditioning of immersion of water at 95°C, facilitating the manipulation of seeds and test interpretation.

Nevertheless, there were no significant difference between the two methods of pre-conditioning ($P>0.5$), and thereby, found that because the treatment of immersion of water at 95°C is easier to perform, showed lower loss. It was observed that the treatment with hot water was effective as pre-conditioning of *senna marilandica* seeds and *Senna obtusifolia* for the tetrazolium test.

CONCLUSION

The pre-conditioning of immersion in water at 95°C and tetrazolium test using concentration of 0.5% tetrazolium solution at 25°C for 150 minutes is the most efficient method to evaluate the seed lots of *Dolichos lablab*.

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