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Methodology of the Tetrazolium Test for Evaluating Physiological Quality in Pitaya (*Hylocereus undatus*) Seeds

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Abstract

The germination test is routinely used for evaluating physiological quality of seeds, but it has not been satisfactory, since it requires relatively long periods to obtain results. In this sense, there is the possibility to resort to vigor tests, although the absence of standardized methodologies has hindered your applicability and reproducibility. The goal of the present study was to establish a methodology for the tetrazolium test that is effective for evaluating physiological quality in pitaya seeds. So, we used five seed batches obtained from mature fruits of pitaya (*Hylocereus undatus*), harvested in the years of 2008, 2009, 2010, 2011, and 2012. The experimental design was the completely randomized design, with four replicates of 50 seeds. The tetrazolium test was conducted in a 5 × 4 × 3 factorial plot, corresponding to five batches of pitaya seeds (2008, 2009, 2010, 2011, and 2012), four concentrations of the tetrazolium solution (0.075%, 0.1%, 0.5%, and 1.0%) and three immersion periods (2, 3, and 4 h). In addition, the germination test for the seed batches was carried out and the analyzed variables were: percentage of germination (PG), germination speed index (GSI) and mean germination time (MGT). Data were submitted to ANOVA and means were compared by the Scott-Knott test ($p \leq 0.05$). Tetrazolium test conducted at a solution concentration of 0.5% and an immersion period of 3 h proved to be efficient for evaluating physiological quality of pitaya seeds, in order to stratify the seed batches into more viability levels compared to the germination test.

Keywords

Cactaceae, Germination, *Hylocereus undatus*, Vigor Test, Viability

1. Introduction

Pitaya [*Hylocereus undatus* (Haworth) Britton & Rose], fruit plant of the Cactaceae family occupies a growing niche in the exotic fruit market [1]. Due to its organoleptic characteristics, your fruits have become an accepted product in consumer markets [2] [3]. On the other hand, the rusticity of the plant and the commercial value of the fruits have aroused the interest of the producers [4] [5]. In this sense, methods to optimize the cultivation of this species are indispensable.

Pitaya reproduction can be performed both sexually and asexually. Seed propagation is particularly useful in breeding programs, in which genotypes are obtained with desirable genetic information, as characteristics related to the yield, external appearance, pulp color and best adaptation to different edaphoclimatic conditions [6]. Thus, it is necessary to determine methodologies in order to evaluate the physiological quality of seed batches of this species.

The physiological quality of the seed is the ability to perform vital functions, characterized by germination, vigor and longevity [7]. Routinely, the physiological quality of the seeds is determined by the standard germination test, but it has not been satisfactory, since it requires relatively long periods to obtain results, especially when considering the commercial interest of the seed producers, which may require days, weeks or even months, depending on the species [8].

The main challenge of research about vigor tests is the identification of parameters related to the deterioration of the seeds that precede the loss of germinative capacity. In this way, the use of tests that allow the detection of the initial stages of the deterioration, related to the membrane system, enzymatic activity and reduction of the energetic mechanisms becomes important [9].

Among the currently available methods, the tetrazolium test has been shown a promising alternative [10]. The use of this test has assumed importance in the quality control of seeds of some species, mainly due to the agility and the precision in the estimation of the germination potential and in the determination of vigor and viability of seed batches. Still, it is able to provide the diagnosis of the factors that affect the quality, indicating the damages with greater precision; identify different levels of viability and not be affected by microorganisms [11] [12] [13].

The supply of this diagnosis has been responsible for the high rate of application of the test, since, in addition to pointing out the problems of the seed quality reduction, when applied in the stages of the production system, it also can identify the origin of these problems, allowing take corrective actions and obtain a production of high-quality seeds [12]. In addition, the tetrazolium test has the advantages of focusing on the physical quality of the embryo and the physiological conditions of each seed, besides requiring simple and low cost equipments [14].

Solution concentration, period and temperature of preconditioning and immersion may affect the efficiency of the tetrazolium test [15]. In this sense, seeds

of several species have already been researched and many of them have a standardized methodology for the tetrazolium test [16]. Due to the importance of determining the methodology for each species, other studies have already been developed for those that do not present standardized methodology in the Rules for Seed Analysis, such as in *Ceiba speciosa* (A. St.-Hil.) Ravenna [17], *Cupania vernalis* Camb. [18], *Hordeum vulgare* L. [10], *Parkia velutina* Benoist [8] and *Tabebuia roseoalba* (Ridl.) Sandwith [19]; since the efficiency of tetrazolium test depends on a series of factors that must be determined for each species, in order to define the most appropriate conditions for applying [11] [20].

Therefore, the use of tetrazolium solutions of different concentrations, period and temperature of preconditioning and immersion in order to obtain coloration uniform and suitable for a safe and an efficient interpretation is fundamental to access reliable results about the quality in certain species [11] [19] [20]. By estimating the vigor and/or viability of the seed in less than 24 hours, farmers and nursery owners can benefit from the information provided by the test, since the use of faster tests has benefited growers considering providing accurate and rapid information regarding the seed performance in the field or greenhouse [20].

For reason that the research does not highlight standardized methodologies for the determination of pitaya seed vigor, there is a necessity to standardize the methodology that favors the applicability and reproducibility of the results in order to obtain reliable information. Therefore, in view of the efficiency and practicality of the vigor tests and the possibility of using them for different species, the goal of the present study was to establish a methodology for the tetrazolium test that is effective for evaluating physiological quality in pitaya seeds.

2. Material and Methods

The research was performed in the Laboratory of Seed Technology and Production at State University of Londrina (Universidade Estadual de Londrina-UEL), Londrina, Brazil. The seeds were obtained from ripe fruits of white-fleshed red pitaya (*Hylocereus undatus*) mother plants, aged approximately 10 years. The plants were cultivated in the experimental area of the Department of Agronomy at UEL, located at 23˚23'S and 51˚11'W, at an average elevation of 566 m. Pitaya plants were grown in soil area classified as RED NITISOL Eutroferric latosol [21], planted every 2.0 × 3.0 m, and supported by 2.5-m-high sticks, with two plants per stick.

Seeds were harvested in the years 2008, 2009, 2010, 2011, and 2012, which characterizes the five seed batches used, which were extracted according to the methodology proposed by [22]. As the seeds were harvested, they were packed in polyethylene tubes and stored in a refrigerator (10˚C) until the tests were set up, which took place in February and March of 2013.

Tetrazolium test was conducted in a completely randomized experimental design with four replicates of 50 seeds in a 5 × 4 × 3 factorial scheme (batches of pitaya seeds, concentrations of the solution tetrazolium, and immersion pe-

riods). First, the seeds were pre-conditioned, where they were wrapped in Germitest-type paper towel, moistened with distilled water in the proportion of two and a half times your dry mass. Then, the material was packed in polystyrene boxes (Gerbox® type) and placed in germinators at 25˚C for 16 h.

Afterwards, with the aid of a scalpel, the seeds were longitudinally sectioned along the embryo and ¾ of the endosperm, placed in polystyrene boxes (Gerbox® type) containing a sheet of blotter paper, moistened with tetrazolium solution (2,3,5-Triphenyl-tetrazolium chloride) in the proportion of two and a half times the dry mass of the paper, with the concentrations corresponding to each treatment: 0.075%, 0.1%, 0.5%, and 1.0% of tetrazolium. Subsequently, the material was arranged in germinators with temperature of 30˚C for 2, 3, or 4 h (immersion periods), according to the treatment, for the coloring of the seeds.

After each immersion period, the seeds were washed in tap water and kept submerged in water until evaluated individually with a bench magnifier. When the tissues presented carmine red coloration it was considered alive and vigorous, classifying it as viable seed, but when presented dark carmine red and milky white colorations, they were considered in deteriorating and dead tissue, respectively, characterizing non-viable seeds.

For the germination test, four subsamples of 50 seeds were used for each lot, in a completely randomized design. The seeds were placed in boxes of polystyrene (Gerbox® type), lined with blotter paper, moistened with distilled water in the proportion of two and a half times your dry mass. The germination test was conducted in germinators with a temperature of 25˚C.

The evaluation was performed daily, for 18 days, when the germination process was stabilized. We considered germinated seeds those with root extension equal to or greater than 2.00 mm. The variables analyzed from this test were: germination (G), in percentage; germination speed index (GSI), calculated according to the methodology of [23] and mean germination time (MGT), in days, according to [24].

Previously, the data were submitted to analysis of normality of errors and homoscedasticity of variances, complying these assumptions, they were submitted to analysis of variance (ANOVA), with means compared by the Scott-Knott test ($p \leq 0.05$).

3. Results and Discussion

The viability of pitaya seeds through the tetrazolium test under the three factors [batch (Ba), concentration of the tetrazolium solution (CTS), and immersion period (IP)] showed a significant triple interaction ($p \leq 0.05$) (**Table 1**).

Table 2 shows the interaction effects of the "IP" within each "CTS" and "Ba", and the interaction effects of the "Ba" within each "CTS" and "IP" for the tetrazolium test in pitaya seeds. Only at the concentration of 0.1%, the "IP" did not present a significant difference for the evaluated "Ba", showing that this concentration was detrimental to the conduction of the test, in view of the non-stratification

Table 1. ANOVA of the tetrazolium test in five batches (Ba in year) of pitaya seeds as a function of the concentration of the tetrazolium solution (CTS in %) and of the immersion period (IP in hour).

Source	DF[1]	SS[2]	MS[3]	F[4]	*p*-value (*p*)
L	4	309357.56	77339.39	1282.676	0.0000*
CST	3	3123.08	1041.03	17.265	0.0000*
PC	2	2857.23	1428.62	23.694	0.0000*
L * CST	12	9595.74	799.64	13.262	0.0000*
L * PC	8	3593.07	449.13	7.449	0.0000*
CST * PC	6	1854.42	309.07	5.126	0.0001*
L * CST * PC	24	11929.43	497.06	8.244	0.0000*
Error	180	10853.16	60.30		
Total	239	353163.69			
CV (%)	15.08				

[1]Degrees of freedom, [2]Sum of squares, [3]Mean sum of squares and [4]*F*-statistic. *Significant ($p \leq 0.05$).

Table 2. Interactive effects of the immersion period within each concentration of the tetrazolium solution and batch, and interactive effects of the batch within each concentration of the tetrazolium solution and immersion period on the tetrazolium test of *Hylocereus undatus* seeds.

Concentration of the tetrazolium	Batch	Immersion period		
		2 h	3 h	4 h
0.075%	2008	0.00 a C	2.62 a C	1.03 a C
	2009	4.05 a C	5.67 a C	4.00 a C
	2010	95.50 a A	98.49 a A	98.00 a A
	2011	71.91 a B	71.00 a B	75.28 a B
	2012	2.53 b C	77.86 a B	83.46 a B
0.1%	2008	0.00 a C	2.04 a C	9.71 a C
	2009	3.51 a C	4.53 a C	5.54 a C
	2010	97.49 a A	97.47 a A	99.00 a A
	2011	70.00 a B	72.23 a B	69.50 a B
	2012	67.47 a B	80.00 a B	76.35 a B
0.5%	2008	9.08 b D	19.38 a D	25.59 a D
	2009	6.74 a D	4.55 a E	9.03 a E
	2010	99.00 a A	97.46 a A	97.00 a A
	2011	65.74 a C	67.77 a C	67.35 a C
	2012	87.27 a B	82.89 a B	81.87 a B
1.0%	2008	8.50 b D	37.66 a D	39.37 a C
	2009	6.53 a D	7.00 a E	6.00 a D
	2010	95.49 a A	95.48 a A	95.50 a A
	2011	60.50 a C	64.32 a C	65.35 a B
	2012	81.22 a B	83.15 a B	76.39 a B

Averages within each row that are followed by different lowercase letters, for interactive effects of immersion period within each concentration of the tetrazolium solution and batch, and averages within each column that are followed by different uppercase letters, for interactive effects of batch within each concentration of the tetrazolium solution and immersion period, differ significantly, according to the Scott-Knott test ($p \leq 0.05$).

of the batches according to viability. The "IP" stratified in two viability levels the batch "2008" in the concentrations of 0.5% and 1.0% and the batch "2012" in 0.075%. The immersion period should be taken into account in the evaluation of seed viability through the tetrazolium test [25].

As for the "Ba", it was verified that when using the concentration of 0.075%, with 2 h, it is already possible to differ batches according to viability, stratifying in only 3 levels. However, it has been observed that the increase in both the "CTS", from 0.5%, and the "IP", from 3 h, have proved advantageous in order to defer the batches to more viability levels. Previous study suggested the use of seed batches with different physiological quality, for the development of methodology of a given test, is of fundamental importance due to the different responses found between batches for the same treatment [26].

To validate the methodology of the tetrazolium test for a particular species, batches of different quality levels should be tested to ensure that the tested method is indeed reliable. Therefore, it is very important that a batch of lower physiological quality is included among the batches tested, since it is in these that the greatest difficulties and doubts arise in the interpretation of the living and non-vigorous tissues/regions [27]; it is worth noting that this requirement is fulfilled in the present study.

Thus, the tetrazolium test for pitaya seeds could be developed at "0.5% and 3 h", "0.5% and 4 h" and "1.0% and 3 h", for stratifying the batches into five viability levels; shown to be these efficient methodologies for evaluating physiological quality of seeds of this species (**Table 2**). Recent study, adapting the methodology for the tetrazolium test for seeds of *C. speciosa*, in two immersion periods (3 and 4 h), three concentrations of the tetrazolium solution (0.1%, 0.5%, and 1%) and two batches, also observed significant interaction between "IP" and "CTS"; concluding that the best methodology was provided when the test is conducted in 0.5% tetrazolium solution for 4 h [17].

Research with *P. velutina* seeds, submitted to the concentration of 0.1% tetrazolium, observed that did not color enough for the correct interpretation; already concentrations of 0.5% and 1.0% were sufficient to color the embryos of this species. The authors concluded that the concentration of 0.5% of the tetrazolium solution allowed a more uniform staining of the embryos, which facilitated the visual analysis of the viability, and the seeds submitted to the concentration of 1.0% showed dark staining, making interpretation difficult [8]. In the present study, both concentrations (0.5% and 1.0%) were satisfactory for conducting the tetrazolium test in pitaya seeds, however, the lower concentration is chosen to reduce the cost with the product.

On the other hand, in a previous study evaluating two concentrations (0.05% and 0.1%) and five batches (0, 6, 12, 18, and 24 months of storage) in *T. roseoalba* seeds, the authors observed that the lowest concentration was able of stratifying the seed batches in more vigorous levels [19]. It can be observed that for each species, whether forest, ornamental, medicinal or agricultural, there are

variations regarding the procedure for standardization of the tetrazolium test [20].

The lower stratification of the batches at concentrations of 0.075% and 0.1% may be justified because the smaller concentrations were not able to color adequately the viable seeds, or the light staining may have made it difficult to classify as viable or unviable, resulting in lower stratification.

Table 3 shows the interactive effects of the "CTS" within each "IP" and "Ba" for the tetrazolium test in pitaya seeds. Thus, it was verified that the "CTS" presented significant difference only for the batch "2012" in 2 h and "batch "2008" in 3 and 4 h, stratifying all in three viability levels; no effect of the "CTS" on the other "Ba" and "IP" evaluated.

Table 3. Interactive effects of the concentration of the tetrazolium solution within each immersion period and batch on the tetrazolium test of *Hylocereus undatus* seeds.

Concentration of the tetrazolium	Immersion period 2 h Batch				
	2008	**2009**	**2010**	**2011**	**2012**
0.075%	0.00 a	4.05 a	95.50 a	71.91 a	2.53 c
0.1%	0.00 a	3.51 a	97.49 a	70.00 a	67.47 b
0.5%	9.08 a	6.74 a	99.00 a	65.74 a	87.27 a
1.0%	8.50 a	6.53 a	95.49 a	60.50 a	81.22 a
Concentration of the tetrazolium	**Immersion period 3 h Batch**				
	2008	**2009**	**2010**	**2011**	**2012**
0.075%	2.62 c	5.67 a	98.49 a	71.00 a	77.86 a
0.1%	2.04 c	4.53 a	97.47 a	72.23 a	80.00 a
0.5%	19.38 b	4.55 a	97.46 a	67.77 a	82.89 a
1.0%	37.66 a	7.00 a	95.48 a	64.32 a	83.15 a
Concentration of the tetrazolium	**Immersion period 4 h Batch**				
	2008	**2009**	**2010**	**2011**	**2012**
0.075%	1.03 c	4.00 a	98.00 a	75.28 a	83.46 a
0.1%	9.71 c	5.54 a	99.00 a	69.50 a	76.35 a
0.5%	25.59 b	9.03 a	97.00 a	67.35 a	81.87 a
1.0%	39.37 a	6.00 a	95.50 a	65.35 a	76.39 a

Averages within each row that is followed by different uppercase letters, for interactive effects of concentration of the tetrazolium solution within each immersion period and batch, differ significantly, according to the Scott-Knott test ($p \leq 0.05$).

Due to the germination test being the standard test for the physiological quality of seeds, the variables percentage of germination (PG), germination speed index (GSI) and mean germination time (MGT) were analyzed for comparative criteria with the treatments tested for the tetrazolium test, in order to establish an effective methodology for evaluating physiological quality of pitaya seeds.

Table 4 shows the variance analysis of the variables PG, GSI and MGT, where it can be verified that the three variables were significant ($p \leq 0.05$) for the cause of variation analyzed (Table 4). From the germination test, it was possible to obtain four viability levels when evaluating batches of pitaya seeds. The batch "2010" was the most vigorous, followed by the batches "2012" and "2011". The batches "2008" and "2009" did not differ statistically from each other, being those of less vigor (Table 5).

Table 4. ANOVA of the percentage of germination (PG %), germination speed index (GSI) and mean germination time (MGT days) of batches of pitaya seeds.

Source	DF[1]	SS[2]	MS[3]	F[4]	*p*-value (*p*)
PG					
Batch	4	28031.20	7007.80	114.882	0.0000*
Error	15	915.00	61.00		
Total	19	28946.20			
CV (%)	17.09				
GSI					
Batch	3	524.58	174.86	38.039	0.0000*
Error	12	55.16	4.60		
Total	15	579.74			
CV (%)	24.00				
MGT					
Batch	3	184.09	61.36	79.948	0.0000*
Error	12	9.21	0.77		
Total	15	193.30			
CV (%)	14.58				

[1]Degrees of freedom, [2]Sum of squares, [3]Mean sum of squares and [4]*F*-statistic. *Significant ($p \leq 0.05$).

Table 5. Percentage of germination (PG %), germination speed index (GSI) and mean germination time (MGT days) of batches of pitaya seeds.

Batch	PG	GSI	MGT
2008	4.50 d	0.19 c	11.71 a
2009	0.00 d	-	-
2010	95.00 a	10.67 b	5.18 b
2011	58.00 c	8.75 b	4.27 b
2012	71.00 b	16.12 a	2.87 c

Averages followed by different uppercase letters in the column differ significantly according to the Scott-Knott test ($p \leq 0.05$).

Regarding GSI and MGT, it was not possible to calculate both variables for the batch "2009", due to the absence of germination. Therefore, for both GSI and MGT, only four batches were compared, and the maximum possible stratification would be four vigor levels. When analyzing both variables, the vigor expressed from these variables was similar, since the batch "2012" was the most vigorous and the batch "2008" was the least vigorous. There was no significant difference in vigor between the batches "2010" and "2011". Despite the same behavior regarding the vigor of the seed batches when analyzing the GSI and MGT variables, and this differs from that observed from the PG, it was decided to use the variable PG as the basis for the comparison of the results obtained from the tetrazolium test, in order that be frequently used in the evaluation of the physiological quality of seeds.

Thus, it was found that the behavior similar to PG was obtained by the tetrazolium test, but only at the "concentration of 0.5% and period of 2 h" and at the "concentration of 1.0% and periods 2 and 4 h", resulting in same stratification of batches (four viability levels). The batch "2010" was the most vigorous and the batches "2008" and "2009" were the less vigor.

Two batches and two concentrations of tetrazolium solution (0.1% and 0.5%) were tested in *C. vernalis* seeds and the authors observed that the highest concentration gave results similar to the one obtained by the germination test, allowing the differentiation of the batches [18]; as observed in the present study. The concentration of 0.1% did not provide the coloration of the seed tissues of *C. vernalis*, which made it difficult to characterize them as viable or non viable, not expressing statistical difference between seed batches. As in the present study we worked with a larger number of batches, it was possible to obtain statistical differences between them, even in the lowest concentrations, however, the higher concentrations (0.5% and 1.0%) provided a greater stratification as to the viability of batches, resembling stratification from germination.

Evaluating physiological quality of *Citrullus lanatus* Thunb. Mansf. through the tetrazolium test, from five batches, three concentrations of the tetrazolium solution (0.075%, 0.5% and 1.0%) and two immersion periods (3 and 4 h), the authors observed that the viability depends on the concentration and period [15]; in the same manner observed for the pitaya seeds of the present study. Thus, these ones indicated the methodology "0.075% and 4 h", being the one that most resembled the batches stratification obtained by the germination test.

However, when the tetrazolium test was conducted at a "concentration of 0.5% and time 3 and 4 h" and at a "concentration of 1.0% and time 3 h", the seed batches were stratified into five viability levels, showing that these methodologies are favorable for the species under this study. However, it is recommended to perform the tetrazolium test of pitaya seeds in a concentration of 0.5%, by economy of product used (salt), aiming at cost reduction and for 3 h, in order to obtain the results with greater quickness; providing advantages in the evaluation, such as the possibility of selecting and discarding batches, according to their physiological quality, in less time.

In this way, it was possible to combine fundamental characteristics attributed to a test of excellence, such as economy and speed, considering that they are two relevant characteristics in establishing the most adequate methodology of the test, because both the reagents saving in the laboratory and the time saving in obtaining the result may favor the research in question [28].

The lower concentrations are more indicated because they present a lower cost with the salt and allow better visualization of the coloration disorders and identification of different types of injuries [29]. However, caution should be exercised when considering such an assertion, since the species under study must be taken into account in conjunction with the factors tested. Under the conditions which the present study was conducted, it is observed that concentrations below 0.5% were not adequate for the conduction of the tetrazolium test in pitaya seeds, given that it did not present the same efficiency provided by the germination test in stratification of seed batches in terms of viability. Such results may be due to the concentrations of 0.075% and 0.1% being insufficient to color the tissues. The low concentration prevented the promotion of adequate staining in order to distinguish living tissues from dead and/or deteriorated tissues.

The choice of the appropriate methodology for the use of the tetrazolium test should be based on the facility for the differentiation of viable and unviable tissues and on the ability to differentiate batches of different physiological qualities [30]. Therefore, the methodology with concentration of the tetrazolium solution of 0.5% and immersion period of 3 h, allows recommending the tetrazolium test as a reliable substitute of the germination test for evaluation of the physiological quality of pitaya seeds. The tetrazolium test should represent the germination test, that is, to give an approximate idea of the germination of a particular seed batch [17]. This requirement was observed in the present study, since the recommended methodology proved to be efficient for evaluating physiological quality of pitaya seeds, in order to stratify the seed batches into more viability levels compared to the germination test.

The solution concentration should be enough to allow tissue staining to accurately differentiate living, deteriorated and/or dead tissues and to identify the nature of injury [31]. These same authors reported that higher concentrations tend to lead to increased staining, making it difficult to recognize the injuries and increasing test costs. Short periods of seed/solution contact may cause poor staining in healthy tissues, hindering interpretation. On the other hand, excessive periods allow the appearance of darker colors in both healthy and deteriorating tissues.

As can be observed, several factors are susceptible to variation in the determination of methodology of the tetrazolium test, especially when it intends to evaluate the physiological quality of seeds of any species. For this reason, for each species the variations of each factor must be evaluated, in order to adapt the methodology to the desired species.

Due to the inexistence of standardized methodology for pitaya seeds, the

present study makes it possible to recommend a methodology able of stratifying batches with different viability levels, in view of the results obtained by the tetrazolium test to corroborate those of germination, which is a test routinely used in the seed production chain, especially when it aims at the discard of the batches with physiological quality lower than those allowed for each class of seed.

4. Conclusion

Tetrazolium test conducted at a solution concentration of 0.5% and an immersion period of 3 h proved to be efficient in assessing the physiological quality of pitaya seeds, in order to stratify the seed batches into more viability levels compared to the germination test.

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References

[1] Le Bellec, F., Vaillant, F. and Imbert, E. (2006) Pitahaya (*Hylocereus* spp.): A New Crop, a Market with a Future. *Fruits*, **61**, 237-250. https://doi.org/10.1051/fruits:2006021

[2] Silva, M.T.H., Martins, A.B.G. and Aparecida de Andrade, R. (2006) Enraizamento de estacas de pitaya vermelha em diferentes substratos. *Revista Caatinga*, **19**, 61-64.

[3] Andrade, R.A., Martins, A.B.G. and Silva, M.T.H. (2007) Influência da fonte de material e do tempo de cura na propagação vegetativa da Pitaya Vermelha (*Hylocereus undatus* Haw). *Revista Brasileira de Fruticultura*, **29**, 183-186. https://doi.org/10.1590/S0100-29452007000100039

[4] Ortiz-Hernández, Y.D., Livera Muñoz, M., Colinas León, M.T. and Carrillo Salazar, J.A. (1999) Estrés hídrico y intercambio de CO_2 de la pitahaya (*Hylocereus undatus*). *Agrociencia*, **33**, 397-405.

[5] Bastos, D.C., Pio, R., Scarpare Filho, J.A., Libardi, M.N., Almeida, L.F.P., Galuchi, T.P.D. and Bakker, S.T. (2006) Propagação de pitaya "vermelha" por estaquia. *Ciência e Agrotecnologia*, **30**, 1106-1109. https://doi.org/10.1590/S1413-70542006000600009

[6] Andrade, R.A., Oliveira, I.V.M., Silva, M.T.H. and Martins, A.B.G. (2008) Germinação de pitaya em diferentes substratos. *Revista Caatinga*, **21**, 71-75.

[7] Popinigis, F. (1985) Fisiologia da semente. Agiplan, Brasília.

[8] Mendes, A.M.S., Bastos, A.A. and Melo, M.G.G. (2009) Padronização do teste de tetrazólio em sementes de *Parkia velutina* Benoist (Leguminosae-Mimosoideae). *Acta Amazônica*, **39**, 823-828. https://doi.org/10.1590/S0044-59672009000400010

[9] Lamarca, E.V., Leduc, S.N.M. and Barbedo, C.J. (2009) Viabilidade e vigor de sementes de *Caesalpinia echinata* Lam. (pau-brasil-Leguminosae) pelo teste de

tetrazólio. *Brazilian Journal of Botany*, **32**, 793-803. https://doi.org/10.1590/S0100-84042009000400017

[10] Grzybowski, C.R.S., Ohlson, O.C., Silva, R.C. and Panobianco, M. (2012) Viability of Barley Seeds by the Tetrazolium Test. *Revista Brasileira de Sementes*, **34**, 47-54. https://doi.org/10.1590/S0101-31222012000100006

[11] 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. *Revista Brasileira de Sementes*, **27**, 176-182. https://doi.org/10.1590/S0101-31222005000100022

[12] Costa, N.P., França Neto, J.B., Krzyzanowski, F.C. and Henning, A.A. (2007) Metodologia alternativa para o teste de tetrazólio em semente de soja: Série Sementes. Circular técnica 39. Embrapa, Londrina.

[13] Tunes, L.M., Bandinelli, P.G., Olivo, F. and Barros, A.C.S.A. (2009) Tratamento para superação da dormência em sementes de cevada. *Scientia Agrária*, **10**, 15-21. https://doi.org/10.5380/rsa.v10i1.12519

[14] França Neto, J.B. (1999) Teste de tetrazólio para determinação do vigor de sementes. In: Krzyzanowski, F.C., Vieira, R.D. and França Neto, J.B., Eds., *Vigor de sementes*: *Conceitos e testes*, Abrates, Londrina, 1-7.

[15] Nery, M.C., Moreira de Carvalho, M.L. and Oliveira, L.M. (2007) Teste de tetrazólio para avaliação da qualidade fisiológica de sementes de melancia. *Semina*: *Ciências Agrárias*, **28**, 365-372. https://doi.org/10.5433/1679-0359.2007v28n3p365

[16] Brasil (2009) Regras para análise de sementes. Mapa/ACS, Brasília.

[17] Lazarotto, M., Piveta, G., Muniz, M.F.B. and Reiniger, L.R.S. (2011) Adequação do teste de tetrazólio para avaliação da qualidade de sementes de *Ceiba speciosa*. *Semina*: *Ciências Agrárias*, **32**, 1243-1250. https://doi.org/10.5433/1679-0359.2011v32n4p1243

[18] Oliveira, L.M., Nicoletti, M.F. and Gomes, J.P. (2010) Qualidade de sementes de *Cupania vernalis* (Sapindaceae). *Revista de Ciências Agroveterinárias*, **9**, 195-197.

[19] Abbade, L.C. and Takaki, M. (2014) Teste de tetrazólio para avaliação da qualidade de sementes de *Tabebuia roseoalba* (Ridl.) Sandwith Bignoniaceae, submetidas ao armazenamento. *Revista Árvore*, **38**, 233-240. https://doi.org/10.1590/S0100-67622014000200003

[20] Azerêdo, G.A., Paula, R.C. and Valeri, S.V. (2011) Viabilidade de sementes de *Piptadenia moniliformis* Benth. pelo teste de tetrazólio. *Revista Brasileira de Sementes*, **33**, 61-68. *https://doi.org/10.1590/S0101-31222011000100007*

[21] Embrapa (2013) Sistema brasileiro de classificação de solos. Embrapa, Brasília.

[22] Ortiz, T.A., Gomes, G.R., Takahashi, L.S.A., Urbano, M.R. and Strapasson, E. (2014) Water and Salt Stress in Germinating Seeds of Pitaya Genotypes (*Hylocereus* spp.). *African Journal of Agricultural Research*, **9**, 3610-3919.

[23] Maguire, J.D. (1962) Speed of Germination-Aid in Selection and Evaluation for Seedling Emergence and Vigor. *Crop Science*, **2**, 176-177. https://doi.org/10.2135/cropsci1962.0011183X000200020033x

[24] Lima, J.D., Almeida, C.C., Dantas, V.A.V., Silva, B.M.S. and Moraes, W.S. (2006) Efeito da temperatura e do substrato na germinação de sementes de *Caesalpinia férrea* Mart. ex Tul. (Leguminosae, Caesalpinoideae). *Revista Árvore*, **30**, 513-518. https://doi.org/10.1590/S0100-67622006000400003

[25] Camargos, V.N., Carvalho, M.L.M., Araújo, D.V. and Magalhães, F.H.L. (2008)

Superação da dormência e avaliação da qualidade fisiológica de sementes de *Sesbania virgata*. *Ciência e Agrotecnologia*, **32**, 1858-1865. https://doi.org/10.1590/S1413-70542008000600026

[26] Oliveira, L.M., Carvalho, M.L.M. and Davide, A.C. (2005) Teste de tetrazólio para avaliação da qualidade de sementes de *Peltophorum dubium* (Sprengel) Taubert-Leguminosae Caesalpinoideae. *Cerne*, **11**, 159-166.

[27] Deminicis, B.B., Vieira, H.D. and Silva, R.F. (2009) Teste de tetrazólio para avaliação da qualidade de sementes de *Clitorea ternatea* L. *Revista Brasileira de Sementes*, **31**, 54-62. https://doi.org/10.1590/S0101-31222009000200006

[28] Corte, V.B., Borges, E.E.L. and Pereira, B.L.C. (2010) Adequação da metodologia do teste de tetrazólio para avaliação da viabilidade de sementes de *Melanoxylon brauna* Schot. *Cerne*, **16**, 415-421. https://doi.org/10.1590/S0104-77602010000300018

[29] França Neto, J.B., Krzyzanowski, F.C. and Costa, N.P. (1998) O teste de tetrazólio em sementes de soja. Documentos, 116. Embrapa, Londrina, PR.

[30] Krzyzanowski, F.C., Vieira, R.D. and França Neto, J.B. (1999) *Vigor de sementes: conceitos e testes*. Abrates, Londrina.

[31] Añez, L.M.M., Coelho, M.F.B., Albuquerque, M.C.F., Mendonça, E.A.F. and Dombroski, J.L.D. (2007) Padronização da metodologia do teste de tetrazólio para sementes de *Jatropha elliptica* M. Arg. (Euphorbiaceae). *Revista Brasileira de Plantas Medicinais*, **9**, 82-88.