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Standardising methods of quick viability test for Pungam (*Pongamia pinnata* L. Pierre) seeds

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ABSTRACT

The viability of seeds can be determined with the use of 2,3,5-triphenyl tetrazolium chloride in a shorter period of time. Tetrazolium staining is a dependable and accepted biochemical method for testing the inherent germination of a seed lot. This is especially important in forest seeds which take much longer time to germinate or may exhibit varying types of dormancy. To standardize the preconditioning methods, soaking duration and concentration of the tetrazolium solution for pungam *(P. pinnata*) seeds, an experiment was conducted at the laboratory with different methods of precondition, soaking duration and concentration of the TZ solution.

The bulk seeds of Pungam collected from the plus trees after cleaning and grading were used for this study. With these seeds, the following preconditioning methods were followed.

1. Presoaking of the seeds in water for 4h, followed by removal of the seed coat without injuring the embryo-M_1

2. Presoaking of the seeds in water for 4h, followed by removal of the seed coat and splitting of the cotypledons with careful retention of the enbryo in one of the cotyledons-M_2.

3. Presoaking of the seeds in water for 4h, followed by removal of the seed coat and cutting of cotyledons horizontally-M_1.

The seeds preconditioned with the above methods were soaked is 0.5 (CN_1) and 1 per cent (CN_2) solutions of 2,3,5 triphenyl tetrazolium chloride. The pre conditioned seeds in the solution were kept in the Fischer hot air oven maintained at 40°C for different durations viz., 3 h (D_1), 4h (D_2) and 5 h (D_3) for developing the colour complex. After the development of the colour, the seeds were evaluated as the seeds with full red, partial red and no colour and the number of seeds in each category were counted after the specific period. Those seeds with full red colour of the cotyledons and embryo were classified as viable in the seeds subjected to the three preconditioning methods. The observed data were analysed statistically as per the procedure of panes and Sukhatme (1978).

For carrying out the quick viability test, preconditioning of the seed and standardization of the concentration of the tetrazolium solution and the duration of soaking the seeds are necessary for the proper penetration of the solutions into the viable tissue of the seed in order to develop a perfect colour complex.

The tetrazolium test is the accepted biochemical test which differentiates the living and dead tissues of a seed by the presence or absence of a red stain, known as formazan. The intensity of the stain and its distribution are the criteria used to evaluate the potential germination of the seed.

From the inception of use of tetrazolium chloride for viability testing by Lakon (1942), numerous workers have successfully used it for the topographic determination of seed viability. Standard procedures for particular species have been worked out in various parts of the world (Gopal and Thapliyal, 1969; Guta and Raturi, 1975). A standardized procedure for *P. pinnata* is necessary as a reliable indication of viability. When an ISTA certificate has not been issued, the

analyst is free to choose a concentration suited for the purpose. In fact, the concentration of the solution is not conclusive for the practicability of the method and concentrations of 0.1% to 1.0% may be used successfully (Overaa, 1979).

In the present study, percentage of fully stained seeds, and non stained seeds differed significantly due to seed conditioning methods, duration of treatment and concentration of solutions (Table 1 and 3) whereas the partially stained seed showed significant differences only for first two factors (Table 2).

Table 1. Effect of durations of treatment, concentrations of TZ solution and seed conditioning methods on fully stained seeds (%).

Seed Conditioning methods (M)		Cn_1			CN_2	
	D_1	D_2	D_3	D_1	D_2	D_3
M_1	32	44	44	40	48	50
M_2	35	47	47	36	50	50
M_3	24	32	32	29	36	35
Mean	CN_1	CN_2				
	37	41				
Mean	M_1	M_2	M_3	D_1	D_2	D_3
	43	44	31	32	42	43

CD (P=0.05)	M	D	CN	MxD	DxCN	MxCN	MxDxCN
	1.51	1.54	1.26	2.67	NS	NS	NS

Among the three methods of seed preconditioning, M_2 - recorded the highest percentage of fully stained seeds (44%) irrespective of durations and concentration followed by M_1 (43%), but both the methods did not differ significantly. So, both are equally superior. The duration of D_3-recorded the highest value and between the two concentrations, CN_2 recorded more number of fully stained seeds (Table 1, Fig.1). For partially stained seeds, M_3 recorded the highest value (60%) and in the duration, D_1 was found to be superior (Table 2). Likewise, different authors have worked with different crops to standardize the methods of conducting the quick viability test. According to Babeley and Kandya (1986), the seeds of *Lagerstroemia parviflora* bisected longitudinally and stained with 0.1% triphenyl tetrazolium chloride for 5 hr, were in agreement with germination test. The *Chloroxylom, sweitinia* seeds cut and soaked in 0.1% tetrazolium solution were used to differentiate the viable and non-viable seeds (Yadav *et al*., 1986). In *Dendrocalamus strictus*, examination of longitudinal section stained with 0.1 per cent tetrazolium chloride for 6 hr showed several abnormalities of the embryo (Karivaratharaju *et al.*, 1987).

Table 2. Effect of durations of treatment, concentrations of TZ solution and seed conditioning methods on partially stained seeds (%).

Seed Conditioning methods (M)		CN_1			CN_2	
	D_1	D_2	D_3	D_1	D_2	D_3
M_1	56	47	48	50	46	44
M_2	58	47	47	60	48	48
M_3	52	61	62	58	62	62
Mean	CN_1	CN_2				
	53	53				
Mean	M_1	M_2	M_3	D_1	D_2	D_3
	48	51	59	55	51	51

CD (P=0.05)	M	D	CN	MxD	DxCN	MxCN	MxDxCN
	1.31	1.31	NS	2.27	NS	1.85	3.20

Table 3. Effect of durations of treatment, concentrations of TZ solution and seed conditioning methods on nonstained seeds).

Seed conditioning methods (M)		CN_1			CN_2	
	D_1	D_2	D_3	D_1	D_2	D_3
M_1	10	9	7	11	6	6
M_2	7	6	6	4	3	2
M_3	24	6	6	13	3	3
Mean	CN_1	CN_2				
	9	5				
Mean	M_1	M_2	M_3	D_1	D_2	D_3
	8	4	9	11	5	5

CD (P=0.05)	M	D	CN	MxD	DxCN	MxCN	MxDxCN
	2.06	2.06	1.68	3.57	NS	NS	NS

In *Dendrocalamus strictus*, examination of longitudinal section stained with 0.1 per cent tetrazolium chloride for 6 hr showed several abnormalities of the embryo (Karivaratharaju et al., 1987). Natarajan (1999) reported that in *Albizia lebbeck* nipping off the seed coat followed by 12 h water soaking and longitudinal splitting of cotyledon were standardized as the best suited preconditioning and preparation methods respectively for tetrazolium test. He also reported that 1.0% solution was the optimum to test the viability percentage of the seeds. The results of the present study indicates that presoaking of pungam seeds in water for 4 h and removal of seed coat without damage to the embryo or splitting the cotyledons with careful retention of the embryo in any one of the cotyledgons to facilitate the penetration of tetrazolium solution. The subsequent soaking in 1 per cent tetrazolium solution for 5 hr resulted in perfect staining of the seeds which could be followed for assessing the viability of pungam seeds based on the staining pattern of the seeds

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