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Pre-storage treatments to improve viability in *Casuarina equisetifolia* seeds

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Abstract : ***Casuraina equisetifolia*** **is an important plantation tree which is propagated through seed. Seeds loose their initial viability of 40-50% rapidly and reach 5% at the end of one year. Keeping these in view, an attempt was made to develop a technology to slow down the speed of seed deterioration using chemicals and botanicals viz., neem (*Azadirachta indica*), turmeric (*Curcuma longa*), acorus (*Acorus calamus*), arappu (*Albizzia amara*), acetyl salicylic acid and halogenation. Evaluations were made at bimonthly intervals on physiological potential and enzyme activity of seeds viz., amylase, catalase, peroxidase and superoxide dismutase. The results indicated that neem (*Azadirachta indica*) leaf powder and acetyl salicilic acid is capable of sustaining the activity of the enzymes during the storage period which can inturn maintain the seed germination potential and seedling vigour as well.**

Key words : Casuarina equisetifolia, pre-storage treatments, enzyme activity, germination

Introduction

Casuarina equisetifolia is an important multipurpose tree and is propagated mainly through seed. However, the seeds are short lived and can germinate only up to 5 per cent at the end of the year. Free radicals have been implicated as a major cause for ageing of seeds. Cells have the capacity for repair and protection against such deleterious reactions. The protection mechanism includes scavenger enzymes *viz*., catalase, peroxidase and superoxide dismutase (SOD) . *The electron transport chain leaks electrons to oxygen forming superoxide radicals*. Superoxide dismutase, catalyses, the disproportion of the superoxide radical to H_2O_2 and dioxygen. SOD scavenges free radicals and subsequently reduces the free radical induced damage. The toxicity of H_2O_2 concentrations in plants leads to many degenerative processes involving photo-oxidation. Catalase and peroxidase act as protectants against accumulation of peroxide (Woodstock, 1967). They cause the decomposition of H_2O_2 into water and oxygen as per the following equation.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Amylase is the enzyme which hydrolyses starch to give reducing sugars like glucose, maltose and dextrins. Decreased amylase activity results in reduced food supply causing poor seedling growth.

Leaves of *Azadirachta indica* (neem), rhizome powder of *Curcuma Tonga* (turmeric) and *Acorus calamus* (vasambu) have been reported to have anti-insect properties (Georage Usher, 1984). Leaf powder of *Albizzia amara* (arappu) has been reported to improve the germination of stored seeds (Ravichandran, 1991). Halogenation with Iodine helps to stabilize the lipo-protein memberanes (Basu and Rudrapal, 1980).

Keeping these in view, graded seeds of *Casuraina equisetifolia* were subjected to pre-storage treatments and evaluated at regular intervals throughout the storage period to trace the effect on the endogenous enzyme systems and the germination potential of the seeds.

Materials and methods

Seeds (samaras) were extracted from cones of *Casuarina equisetifolia* collected from Coimbatore District (11.00°N, 77.00°E) of Tamil

Table 1. Effect of pre-storage treatments on germination (%) of *C. equiserifolia*

Treatments	Months of storage						Mean
	0	1	3	5	7	9	
Control	41	36	33	30	28	15	30
	(39.8)	(36.8)	(35.0)	(34.4)	(31.6)	(22.87)	(33.5)
Halogenation	41	36	34	30	29	17	31
	(39.8)	(36.7)	(35.7)	(35.5)	(32.8)	(24.50)	(34.2)
A. indica	41	36	37	36	32	32	36
	(39.8)	(36.9)	(34.6)	(36.6)	(34.1)	(34.41)	(36.84)
C. longa	41	36	35	31	29	17	31
	(39.8)	(36.9)	(36.0)	(33.9)	(32.4)	(24.65)	(34.1)
A.calamus	41	36	34	31	28	23	32
	(39.8)	(36.9)	(35.6)	(33.6)	(31.9)	(28.59)	(34.5)
Acetyl Salycilic acid	41	36	36	33	29	26	34
	(39.8)	(36.8)	(36.7)	(34.9)	(33.5)	(30.33)	(35.4)
A.amara	41	36	37	31	29	23	33
	(39.8)	(36.9)	(37.34)	(33.8)	(32.6)	(28.87)	(34.9)
Mean	41	36	35	32	29	22	
	(39.8)	(36.8)	(36.2)	(34.3)	(32.7)	(28.23.)	

CD (P=0.05)	Treatments (T)	Period (P)	T x P
	1.44	1.33	3.52

(Figures in parantheses indicate arcsine values)

Table 2. Effect of pre-storage treatments on root length (cm) of *C. equiserifolia*

Treatments	Months of storage						Mean
	0	1	3	5	7	9	
Control	2.32	2.20	2.24	2.13	2.08	1.50	2.07
Halogenation	2.32	2.21	2.25	2.15	2.10	1.70	2.12
A. indica	2.32	2.19	2.29	2.20	2.14	1.91	2.17
C. longa	2.32	2.21	2.25	2.17	2.10	1.62	2.11
A.calamus	2.32	2.19	2.28	2.16	2.13	1.70	2.13
Acetyl Salycilic acid	2.32	2.21	2.29	2.17	2.13	1.89	2.17
A.amara	2.32	2.20	2.26	2.19	2.14	1.80	2.15
Mean	2.32	2.20	2.26	2.17	2.11	1.73	

CD (P=0.05)	Treatments (T)	Period (P)	T x P
	0.33	0.31	0.08

Table 3. Effect of pre-storage treatments on shoot length (cm) of *C. equiserifolia*

Treatments	Months of storage						Mean
	0	1	3	5	7	9	
Control	2.38	2.32	2.27	2.14	2.16	1.50	2.13
Halogenation	2.38	2.32	2.29	2.15	2.18	1.70	2.17
A. indica	2.38	2.33	2.34	2.18	2.24	1.90	2.23
C. longa	2.38	2.31	2.30	2.15	2.17	1.72	2.17
A.calamus	2.38	2.32	2.29	2.15	2.17	1.65	2.16
Acetyl Salycilic acid	2.38	2.33	2.34	2.18	2.24	1.90	2.23
A.amara	2.38	2.32	2.32	2.17	2.24	1.85	2.21
Mean	2.38	2.32	2.30	2.16	2.20	1.74	
CD (P=0.05)	Treatments (T)		Period (P)		T x P		
	0.034		0.031		0.083		

Table 4. Effect of pre-storage treatments on vigour index of *C. equiserifolia*

Treatments	Months of storage						Mean
	0	1	3	5	7	9	
Control	192	162	148	129	74	45	125
Halogenation	192	162	154	130	83	58	129
A. indica	192	163	172	155	94	131	151
C. longa	192	163	157	133	79	58	130
A.calamus	192	163	155	131	77	76	132
Acetyl Salycilic acid	192	162	165	142	83	96	140
A.amara	192	163	158	160	89	85	142
Mean 1292	192	163	160	140	82	77	
CD (P=0.05)	Treatments (T)		Period (P)		T x P		
	4.31		3.99		10.55		

Table 5. Effect of pre-storage treatments on protein content (%) of *C. equiserifolia*

Treatments	Months of storage						Mean
	0	1	3	5	7	9	
Control	0.250	0.248	0.238	0.160	0.123	0.114	0.188
Halogenation	0.250	0.249	0.238	0.181	0.147	0.137	0.200
A. indica	0.250	0.250	0.250	0.193	0.160	0.162	0.210
C. longa	0.250	0.248	0.239	0.183	0.145	0.132	0.199
A.calamus	0.250	0.249	0.240	0.183	0.147	0.130	0.199
Acetyl Salycilic acid	0.250	0.249	0.249	0.192	0.181	0.162	0.214
A.amara	0.250	0.249	0.239	0.182	0.160	0.143	0.204
Mean 1292	0.250	0.249	0.242	0.182	0.152	0.140	
CD (P=0.05)	Treatments (T)		Period (P)		T x P		
	0.0035		0.0032		0.008		

Table 6. Effect of pre-storage treatments on carbohydrate content (%) of *C. equiserifolia*

Treatments	Months of storage						Mean
	0	1	3	5	7	9	
Control	8.85	8.83	8.58	8.43	7.20	6.00	7.98
Halogenation	8.85	8.85	8.72	8.58	7.42	6.22	8.11
A. indica	8.85	8.87	8.83	8.83	7.74	6.40	8.25
C. longa	8.85	8.83	8.72	8.58	7.42	6.22	9.10
A.calamus	8.85	8.85	8.72	8.72	7.60	6.22	8.16
Acetyl Salycilic acid	8.85	8.87	8.83	8.83	7.74	6.40	8.25
A.amara	8.85	8.85	8.83	8.72	7.60	6.22	8.17
Mean	8.85	8.85	8.75	8.67	7.53	6.24	

CD (P=0.05)	Treatments (T)	Period (P)	T x P
	0.126	0.116	0.NS

Table 7. Effect of pre-storage treatments on reducing sugar content (%) of *C. equiserifolia*

Treatments	Months of storage						Mean
	0	1	3	5	7	9	
Control	0.283	0.285	0.312	0.406	0.416	0.432	0.355
Halogenation	0.283	0.283	0.301	0.345	0.330	0.398	0.323
A. indica	0.283	0.283	0.298	0.301	0.315	0.329	0.301
C. longa	0.283	0.285	0.303	0.343	0.345	0.412	0.328
A.calamus	0.283	0.283	0.305	0.347	0.356	0.412	0.331
Acetyl Salycilic acid	0.283	0.2832	0.297	0.300	0.314	0.325	0.300
A.amara	0.283	0.285	0.330	0.313	0.350	0.365	0.316
Mean 1292	10.283	0.284	0.302	0.336	0.346	0382	

CD (P=0.05)	Treatments (T)	Period (P)	T x P
	0.0051	0.0048	0.0012

Table 8. Effect of pre-storage treatments on oil content (%) of *C. equiserifolia*

Treatments	Months of storage						Mean
	0	1	3	5	7	9	
Control	15.27	15.26	14.80	14.42	14.00	14.00	14.33
Halogenation	15.27	15.26	14.90	14.42	14.40	14.00	14.71
A. indica	15.27	15.27	15.00	14.71	14.40	14.40	14.84
C. longa	15.27	15.27	14.90	14.42	14.00	14.00	14.64
A.calamus	15.27	15.26	15.00	14.41	1 11.00	14.00	14.65
Acetyl Salycilic acid	15.27	15.26	15.00	14.74	14.20	14.40	14.81
A.amara	15.27	15.27	15.00	14.40	14.40	14.20	14.70
Mean	15.27	15.26	15.00	14.50	14.20	14.10	

CD (P=0.05)	Treatments (T)	Period (P)	T x P
	NS	0.209	NS

Nadu, India during the year 1998. Seeds were upgraded in a specific gravity separator (WESTRUP, LA-K No. 89036) with a vertical height, horizontal height and air blow rate adjustments of 2, 0 and 3, respectively with an rpm of 390-410 (Umarani, 1999) in TamilNadu Agricultural University, Coimbatore. The first grade seeds were subjected to the following dry pre storage treatments using $CaCO_3$ as the filler @ 3g kg^{-1} of seed.

i) Untreated seed served as control

ii) Halogenation mixture @ 2g kg^{-1} of seed

iii) Neem (*Azadirachta indica*) leaf powder @ 20g kg^{-1} of seed

iv) Turmeric (*Curcuma longa*) rhizome powder @ 2g kg^{-1} of seed

v) Vasambu (*Acorus calamus*) rhizome powder @ 20g kg^{-1} of seed

vi) Acetyl salicylic acid @ 2 g kg^{-1} of seed

vii) Arappu (*Albaizia amara*) leaf powder @ 20g kg^{-1} of seed.

The halogenation mixture of 1 kg consisted of Iodine (1g), *Curcuma longa* rhizome powder (2g), *Albaizia amara* leaf powder (2g), Acetyl salicylic acid (1g), *Acorus calamus* rhizome powder (1g) and 993 g of dehydrated $CaCO_3$.

The seeds thus treated were divided into ten parts and sealed air tight in polythene bags (700 guage) in order to facilitate periodical sampling without interrupting the sealed storage environment. Seed samples were drawn at bimonthly intervals and the following observations were recorded.

1. Germination test

The seeds were surface sterilized with 0.01% (W/V) Mercuric chloride for 3 min. washed thoroughly with distilled water and 100 seeds of each grade class were set in roll towel method (ISTA, 1985). Fourteen days after sowing, counts were made and germination expressed as the percentage of seeds which produced normal seedlings. After germination count, ten random seedlings were measured for their root and shoot length and vigour index (Abdul-Baki and Anderson, 1973) was derived.

$$\text{Vigour Index} = \text{Germination (\%)} \times \text{Seedling lengh (cm)}$$

The experiment was completely randomized and replicated thrice.

2. Amylase

100 mg of seed material after 48h of imbibition was homogenized in 1.8 ml of 0.02 M Sodium phosphate buffer (pH 6.0). Amylase activity was estimated by the amount of starch hydrolysed, determined by the change in the optical density from time zero to the end of the reaction at 620nm (Paul *et al.*, 1970).

3. Catalase

250 mg of seed material after 48 h of imbibition was homogenized in 1.8 ml 0.066 M sodium phosphate buffer (pH 6.8). Catalase activity was estimated by the permanganate method (Povolotskaya and Sedenka, 1956).

4. Peroxidase

250 mg of seed material after 48 h of imbibition was homogenised in 1.8 ml 0.25 M Tris buffer (pH 6.0). Peroxidase activity was estimated by the colorimetric method at 420nm (Malik and Singh,1980).

5. Superoxide dismutase

250 mg of seed material after 48 h of imbibition was homogenised in 1.8 ml of Sodium phosphate buffer (pH 7.8). The activity of SOD was assayed by measuring its ability to inhibit the

photochemical reduction of Nitroblue tetrazolium at 560nm, adopting the method of Beauchamp and Fridorich (1971).

The results were subjected to analysis of variance and tested for significant differences (Panse and Sukhatme, 1967).

Results and discussion

The results of the present study revealed that there was a progressive decline in *C. equisetifolia* seed viability from 41 per cent (initial stage) to 22 per cent (ninth month). The rate of deterioration was faster in the untreated control seeds which recorded the lowest germination per cent of 15. All the seed treatments recorded higher germination per cent compared to control, after nine months of storage . The percentage increase was found to vary, which was 108.6, 68.8, 54.3, 51.6, 15.2 and 13.9 per cent for neem, acetyl salicylic acid, arappu, acorus, turmeric and halogenation, respectively (Table 1). Seedling growth and vigour, were measured by the root length and shoot length and vigour index (Table 2-4). All the treatments showed superiority over the untreated control seeds, though the percentage of increase varied. The neem leaf powder treated seeds registered superiority over rest of the treatments followed by acetyl salicylic acid .

The enzyme activity assayed in the seeds revealed that magnitude of decrease in amylase and superoxide dismutase activity was lesser until the fifth month of storage, whereas catalase and peroxidase registered a steep decline after the third month of storage. The magnitude of decrease in amylase activity was 0.23, 3.45, 3.10, 10.34 and 25.0 per cent in first, third, fifth, seventh and ninth month of storage, respectively. Similarly in superoxide dismutase it was 1.0, 6.9, 35.0, 34.7 and 53.8 per cent, respectively. In catalase, the per cent decrease in activity was 0.3, 0.6, 10.7, 27.9 and 43.4, respectively. Likewise, in peroxidase it was 4.4, 4.6, 22.2, 64.4 and 74.4, respectively (Table 5-8).

By definition, a free radical is any atomic species, capable of independent existence, which contains one or more unpaired electrons. A free radical combines with most molecules present in living tissues, including nucleic acids, proteins and lipids with extremely high reaction causes mutation or strand breaks in DNA by combining with bases. It directly or indirectly initiates lipid peroxidation and the inactivation of enzymes. The superoxide dismutase scavenges superoxide which is cytotoxic by causing photooxidation. Only by the action of catalase and peroxidase this cytotoxic hydrogen peroxide is converted into water and oxygen, which is harmless.

In the present study, with respect to the treatments, all of them recorded higher enzyme activity when compared to the control through out the period of storage. As in other biochemical parameters, for enzyme activity also neem and acetyl salicylic acid reigned superiority over the other treatments by recording higher enzymatic activity . The per cent increase over the control recorded at the ninth month of storage was 28.2, 33.0, 53.1 and 22.3 by the neem leaf treated seed, for amylase, catalase, peroxidase and superoxide dismutase activities, respectively. Acetyl salicylic acid recorded a per cent increase of 26.1, 33.0, 25.0 and 18.9, respectively. Szezotka (1974) found that amylolytic activity was very high at the beginning of storage but quickly declined to non-detectable levels by eight months in *Quercus robus* and *Q. borealis*. Through over the storage period the germination per cent decreased significantly. Ghosh *et al.*, (1978) reported that percentage of germination and seedling vigour of rice decreased with increasing storage time. α-amylase and ATPase activity could be detected upto 3 or more year old seeds but peroxidase activity could not be detected in seeds more than

one year old. Rame Gowda (1992) reported a decrease in the activity of enzymes viz., a-amylase, catalase and peroxidase, coupled with progressive ageing. He further authenticated that amylase and peroxidase enzymes are more directly involved in the maintenance of better germination of differentially aged seeds. The decreased enzyme synthesis may be because of impairment of post-ribosomal supernatant and ribosomal fractions. (Bryan *et al.,* 1973) . Impairment of transcription mechanism which inturn is a consequence of damage to nuclear DNA can also reduce the capacity for protein synthesis (Bewly and Black, 1982).

In the present study, the increased activity of the free radical scavenging enzyme system, should have lead to removal of the damaging free radicals in cells. Thereby, the structural integrity of the cells could have been maintained. The increased synthesis of the enzyme amylase, stands proof for the intactness of the *invivo* enzyme synthesis system of the cells. This might have resulted in higher seed viability and seedling vigour in seeds treated with neem leaf powder. Against the results of the experiment , it is concluded that presowing seed treatment of *Casuarina equisetifolia* with neem (*Azadirachta indica*) leaf powder is capable of sustaining the activity of the enzymes viz., amylase, catalase, peroxidase and superoxide dismutase, which can inturn maintain the seed germination potential and seedling vigour as well.

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