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EFFECT OF PRE-SOAKING IN ORGANIC SOLVENTS ON SEED GERMINATION AND SEEDLING GROWTH OF CASHEW

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ABSTRACT

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Pre-soaking of cashew (*Anacardium occidentale* L.) seeds (which are actually nut-fruits) in chloroform or acetone for 2 h hastened and partially synchronised germination as well as field emergence. The organic solvents removed the waxy layer of the pericarp and thereby facilitated water imbibition and phenol exudation. The rapid germination further resulted in a significant increase in seedling vigour expressed as total dry weight of the seedling.

INTRODUCTION

The use of organic solvents to improve seed germination was reported long ago (Verschaffelt, 1912; Barton, 1947). These solvents have also been successfully employed to introduce growth regulators, pesticides, antibiotics and bactericides into the seed (Milborrow, 1963; Papavizas and Lewis, 1976; Maneendranath and Funk, 1978). Recently, it has also been reported that ethanol, acetone and other anaesthetics break seed dormancy in fall panicum and other weedy grasses (Taylorson and Hendricks, 1979). Although the practical advantages have been well established, the technique has not been fully exploited. In the present study, I attempted to use the technique in cashew, an important plantation crop of South India.

MATERIALS AND METHODS

Materials. — In a pilot study using acetone, chloroform, carbon tetrachloride and petroleum ether, all solvents accelerated germination. Only acetone and chloroform were used for detailed studies because of their superiority. The seeds were of A.R. grade from the British Drug House Ltd. Seeds from accession numbers VTH 1, 11, 49 and 62 of the cashew germplasm collection, maintained at our station, were employed.

Germination. — The seeds were pre-soaked in distilled water, acetone or chloroform for 2 h. Seeds without any pre-treatment served as control. Germination studies were conducted in enamel trays lined with wet filter paper pads, kept in the dark at $25 \pm 5^\circ\text{C}$. The protrusion of the radicle about 0.5 cm out of the shell was reckoned as germination. The rate of water absorption (g of water/seed) was gravimetrically determined on each day. Each treatment consisted of 4 replicates with 25 seeds in each replicate. The results are the averages of 3 experiments.

To determine the rate of CNSL (Cashew nut shell liquid) exudation, a single seed from each treatment was placed in a wide-mouthed culture tube and covered with 10 ml of distilled water. Each treatment was replicated 4 times. Half a ml of water was aliquoted following 2, 7 or 24 h of soaking and total phenols were determined using Folin—Ciocalteu reagent (Farkas and Kiraly, 1962). Phenol content was expressed on seed⁻¹ as well as g⁻¹ dry weight basis. To understand the mode of action of organic solvents, 25 seeds were soaked in each solvent to which a grain of saffranin or fast green was added. Five seeds were removed following 2, 4, 8, 10 and 24 h of soaking and cut open to track the passage of the stain into the seed through the shell.

Further, a few cross sections of the pericarp from treated fruits were microscopically compared with those of the control after staining the sections with Sudan Black to locate cuticular lipids. The amounts of wax extractable by acetone or chloroform were determined gravimetrically (Silva Fernandes et al., 1964) and their composition by preparative thin layer chromatography (Baker, 1974).

Field emergence. — The experiment was conducted in a net-house located at the Water Technology Centre, Indian Agricultural Research Institute, New Delhi. Three uniform plots, 4×3 m, were divided into 4 equal sub-plots. The pre-soaking treatments were as described in the germination studies. Each treatment occupied 3 sub-plots distributed randomly. In each sub-plot 50 seeds were sown 20 cm apart and 5 cm deep with the stalk end upward (Rao et al., 1957) and watered regularly. The appearance of the epicotyl above ground was considered as "emergence". One-month-old plants were sampled to determine seedling heights and weights. Ten seedlings from each sub-plot were uprooted at random and thoroughly washed. All the linear growth parameters and fresh weights were determined. Then the plants were dried at 50°C in an electric oven for 30 h and their dry weights were taken.

RESULTS AND DISCUSSION

The pilot study showed that pre-soaking of cashew seeds in an organic solvent accelerated germination. The effect was evident even with 2 h of treatment, though there was a marginal improvement by further soaking. At this time pre-soaking appeared to affect the percentage

germination. Hence in the main study, the treatment was limited to 2 h.

The germination experiment showed that seeds soaked in organic solvents began their germination on the 2nd day, while the germination was completed within a week after incubation (Table I). However, in the control and water-soaked seeds, it took about 2 weeks for the germination to be completed, though the earliest seed germinated on the 3rd or 5th day. Thus, the organic solvents not only accelerated germination, but also synchronised it to a large extent. In fact, there was a distinct advantage in soaking in water only, where the germination was a little more synchronised than that in the control. Of the 4 organic solvents that were tested in the pilot study, chloroform was superior in its effect. It behaved as well in the main experiment, but the main disadvantage was that the seeds would float in the solvent. Hence, the treatment would not be complete unless the seeds were made to sink with the help of a weight. Therefore the seeds were weighted down in these experiments.

TABLE I

Effect of pre-soaking treatments on % germination of cashew seeds. Figures in parentheses are mean values after arcsine transformation

Days of ambition	Treatments			
	Dry (control)	Water	Chloroform	Acetone
	0 (0)	0 (0)	6.7 (15)	10 (18.4)
	0 (0)	1 (6.1)	30 (33)	23 (28.5)
	1 (6.1)	10 (18.4)	71 (57.8)	68 (55.8)
	16 (23.4)	25 (30)	93 (75)	91 (72.8)
	50 (45)	61 (51)	95 (77.7)	91 (72.8)
	60 (50.8)	73 (59)	No further germination	
	73 (59)	80 (63.9)		
	93 (75)	88 (70)		

Critical difference for transformed values at 5% level = 17.21

The treatments gave similar results in the field experiment (Fig. 1). Chloroform pre-soaking was distinctly superior to others, with 50% of the seeds germinating on the 5th day.

The effect of soaking in organic solvents could be only on the surface of the fruit because the dye (safranin) showed negligible penetration into the fruit within the first 2 h of soaking. It required about 24 h of soaking for the dye to enter the kernel through the pericarp. Microscopic examination of the pericarp sections showed a very thick cuticle and a waxy layer. The treatment with organic solvents resulted in the loss of the waxy layer, as evidenced by the weak staining of the cuticle with Sudan Black in contrast to the sections from untreated seeds. Gravimetric estimation of the wax removed showed that chloroform was more effective in removing the wax (0.8 mg

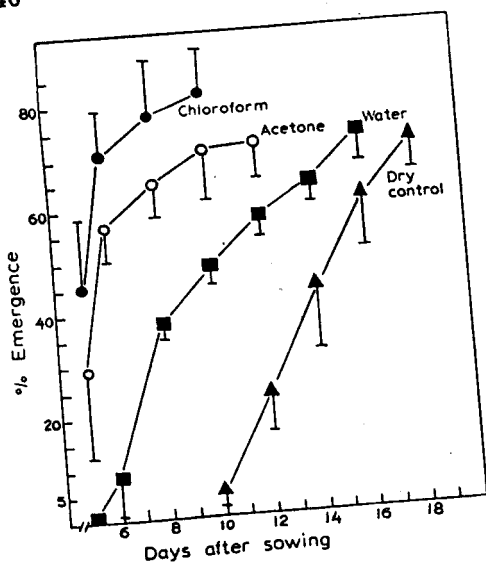


Fig. 1. Effect of pre-soaking treatments on field emergence of cashew seeds. Treatments are as indicated. The standard deviation values represented by bars are given only on one side to retain the clarity of the figure.

seed⁻¹) than acetone (0.5 mg seed⁻¹). The surface wax of cashew fruit mainly consisted of hydrocarbons, β -diketones and fatty acids as revealed by thin layer chromatography. The first 2 components are known to be highly hydrophobic and offer water repellent properties to the epicuticular waxes (Freeman et al., 1979). There was no difference in the composition of wax extracted by acetone and chloroform. The loss of the waxy layer due to organic solvent pre-treatment clearly facilitated water imbibition (Table II), as well as the loss of cashew nut shell liquid out of the shell (Table III). The latter effect might further promote water uptake because cashew nut shell liquid is a hydrophobic phenol.

The early germination of pre-soaked seeds resulted in significantly higher dry-matter accumulations than those from untreated ones (Table IV). The

TABLE II

Effect of pre-soaking treatments on water imbibition (g/seed)

Imbibition time (h)	Treatments			
	Dry (control)	Water	Chloroform	Acetone
24	0.68	0.95	2.55	1.81
48	1.37	1.63	3.61	2.93
72	2.06	2.05	3.86	3.60
96	2.78	3.05	4.86	4.69

Critical difference at 5% level 0.256

TABLE III

Effect of pre-soaking treatments on the exudation of phenols (mg/seed and mg/g.d. wt.)

Imbibition time	Treatments			
	Dry (control)	Water	Chloroform	Acetone
2 h				
Seed ⁻¹	0.24	0.16	0.48	0.42
g ⁻¹ d.wt.	0.032	0.024	0.059	0.059
7 h				
Seed ⁻¹	0.40	0.30	1.35	1.12
g ⁻¹ d.wt.	0.054	0.044	0.168	0.170
24 h				
Seed ⁻¹	1.35	1.25	7.67	7.13
g ⁻¹ d.wt.	0.180	0.180	0.950	1.100

Critical difference seed⁻¹ at 5% level 1.302
 Critical difference g⁻¹ d.wt. at 5% level 0.331

TABLE IV

Effect of pre-soaking treatments on the growth parameters of 1-month-old seedlings

Parameters	Treatments				Critical difference at 5% level
	Dry (control)	Water	Chloroform	Acetone	
Seed fresh wt. (g)	4.49	6.00	7.46	6.22	2.6
Seed d.wt. (g)	1.14	1.84	2.41	2.11	0.684
1st leaf fresh wt. (g)	1.38	1.65	2.29	1.64	0.505
1st leaf d.wt. (g)	0.29	0.47	0.67	0.52	0.137
2nd leaf fresh wt. (g)	1.82	1.82	1.70	1.63	NS
2nd leaf d.wt. (g)	0.36	0.41	0.52	0.51	NS
3rd leaf fresh wt. (g)	1.66	2.54	3.72	2.94	1.11
3rd leaf d.wt. (g)	0.56	0.96	1.22	1.00	0.34
4th leaf length (cm)	12.10	18.00	20.44	16.10	3.613
5th leaf length (cm)	11.84	17.25	18.25	19.19	3.348
6th leaf length (other than 5 embryonic)	2.53	4.63	11.88	4.63	3.154

Differences were mainly due to increased top growth rate which persisted at for the next 3 months. Data are not shown because it was not possible to continue the observations further (Table IV).
 In some annual crops like celery, the acceleration in field emergence induced by pre-treatment was found to result in higher yields (Biddington et

al., 1975). Such an extrapolated effect is difficult to anticipate in a perennial crop like cashew, and seed germination as such is not a problem in this crop, but if the pre-soaking technique is adopted by nursery men, it should result in earlier and more uniform seedlings. The absence of any adverse effect due to treatment with organic solvents highlights it further as a prospective technique to infuse growth regulators, pesticides and other chemicals into the dry seed. Moreover, the reduction in the pre-germination period by this technique may offer an avoidance-mechanism to the seed against soil-borne pathogens. Research along these lines is in progress.

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EFFECT OF EXOGENOUS GROWTH REGULATORS ON FLOWERING AND CYTOKININ LEVELS IN AZALEAS

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ABSTRACT

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'Alaska' and 'Redwing' azaleas having dormant flower buds were sprayed with gibberellins (GA₁ or GA₃, + K) alone and in combination with thiourea, N⁶ benzyl adenine (BA) and kinetin weekly for 3 or 4 weeks to test the efficacy of these materials in breaking bud dormancy. Additional plants received 6 weeks of cold storage at 4.5°C or glasshouse day temperatures of 21°C and above. The 2000 and 3000 mg l⁻¹ GA₁ and GA₃ sprays were more effective than 1000 mg l⁻¹ in promoting flowering, with 'Redwing' responding better than 'Alaska'. GA-treated plants flowered in fewer days than those receiving cold storage. Flower diameter and pedicel length increased with higher levels of GA, and flower uniformity was comparable to cold-stored plants on most GA-treated 'Redwing'-plants. Thiourea, BA and kinetin applied alone had no effect and considerable cytokinin activity was highest in GA-treated buds 14—21 days after treatment application. No increase in cytokinin activity occurred on plants not receiving GA.

INTRODUCTION

The increased popularity of potted, flowering azaleas over the last few years has spurred research efforts in year-round scheduling. Several workers (Post and Straitton, 1966; Nell and Larson, 1974) hastened uniform flowering by bud initiation and development with favorable photoperiods, temperature and growth regulator applications. Marketable azalea plants were produced, and timing was shortened, by combined chemical pinching and manual pruning rather than a system of manual pruning only (Joiner and Nell, 1974).

Breaking of dormancy in azalea flower buds has been accomplished since Post (1943) primarily by 4—6 weeks of cold storage at 4—10°C. Such a method is expensive in time, labor and energy, and requires the grower to make a large investment in cold-storage rooms. Exogenously applied gibberellins (GA₃) has been used to hasten flowering of azaleas (Joiner and Nell, 1974).