SEED DORMANCY AND PRE-TREATMENTS TO ENHANCE GERMINATION IN SELECTED *ALBIZIA* SPECIES

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KANNAN, C. S., SUDHAKARA, K., AUGUSTINE, A., & ASHOKAN, P. K. 1996. Seed dormancy and pre-treatments to enhance germination in selected *Albizia* species. An experiment was conducted to find out the best pre-treatment to improve the germination and vigour of *Albizia odoratissima, Albizia procera,* and *Albizia falcataria* seeds. Soaking the nicked seeds of *A. odoratissima* or mechanically scarified seeds of *A. falcataria* in flowing water gave the maximum germination percentage and vigour. Scarification with concentrated H_2SO_4 for 10 or 20 min gave the largest germination percentage and vigour in *A. procera.* Seedcoat extract of these seeds inhibited the germination of seeds of yardlong bean, okra, brinjal and rice. Thin Layer Chromatography showed the presence of a coumarin-like substance with a RF value of 0.22 in the seedcoat of *A. odoratissima.* The presence of yet another substance was also indicated.

Key words: Seed dormancy - germination - inhibitor - coumarin - *Albizia odoratissima, Albizia procera - Albizia falcataria*

KANNAN, C. S., SUDHAKARA, K., AUGUSTINE, A., & ASHOKAN, P. K. 1996. Kedormanan biji benih dan pra-rawatan untuk meningkatkan percambahan spesies-spesies terpilih *Albizia*. Satu eksperimen telah dijalankan untuk menentukan pra-rawatan terbaik untuk membaiki percambahan dan kesuburan biji benih *Albizia odoratissima, Albizia procera* dan *Albizia falcataria.* Merendam biji benih *A. odoratissima* yang tercalar atau biji benih *A. falcataria* yang dilelas secara mekanikal, di dalam air yang mengalir memberikan peratus percambahan dan kesuburan yang maksimum. Pelelasan dengan H_2SO_4 pekat selama 10 atau 20 min memberikan peratus percambahan dan kesuburan yang paling besar di dalam *A. procera.* Ekstrak dan kulit biji benih menghalang percambahan biji benih kacang panjang, bendi, terung dan padi. Lapisan Nipis Kromatografi menunjukkan kehadiran bahan seperti kumarin dengan satu nilai RF 0.22 di dalam kulit biji *A. odoratissima.* Kehadiran bahan-bahan lain juga turut dikesan.

Introduction

Growing multipurpose tree species (MPTS) is one of the ways to reverse tropical deforestation. Species like *Albizia odoratissima, Albizia procera* and *Albizia falcataria* of the family Fabaceae and sub-family Mimosoideae have received great attention because of their multiple uses and under-exploitation.

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Albizia odoratissima is widely distributed as a constituent of many types of mixed deciduous forests (Troup 1921). The dark brown heart wood of this specis is used for house building, carts, wheels and furniture. Leaves and twigs are lopped for fodder. *Albizia procera* is commonly found in alluvial soils along streams and swamps (Troup 1921). The heartwood of this tree is very durable and used for carts, furniture and house building; the wood also yields fine charcoal. *Albizia falcataria* is one of the fastest growing among the broad leaved tree species in the world. The tree is excellent for manufacturing cattamaram and for paper pulp.

Regeneration from seed is the most often used and cheapest method of propagation in these species, but the dormancy induced by the thick seedcoat is a great problem in achieving quick germination (Willan 1985). Natural regeneration of *Albizia* is unsatisfactory in its habitat (Rai 1978). Msanga and Maghembe (1986) observed that delayed and irregular germination in the nursery is a serious constraint in the large scale propagation of *Albizia.* Various treatments tried to break seed dormancy in *Albizia* were, soaking in cold or hot water of various temperatures for different durations (Valencia 1973, Gupta & Thapliyal 1974, Kemp 1975, Diangana 1985, Granfulah & El-Hadidy 1987, Khan & Tripathi 1987, Bahuguna *et al.* 1989), mechanical scarification like nicking and puncturing of seedcoat (Khan & Tripathi 1987), nicking and soaking in flowing water for 24 h (Sajeevukumar *et al.* 1995), filing and rubbing of seedcoat (Babeley *et al.* 1986), hot wire scarification (Sandiford 1988), hydrogen peroxide treatment (Carneiro 1975) and acid scarification (Rai 1978, Babeley *et al.* 1986, Rai *et al.* 1986, Granfulah & El-Hadidy 1987). Among these treatments, hot water scarification and acid scarification proved to be more successful. Sajeevukumar *et al.* (1995) reported the presence of a water soluble germinating inhibiting chemical in the seedcoat of *A. procera* and *A. falcataria.*

The present study was conducted to formulate effective and more practical pre-treatment to break the seed dormancy and improve the germination of *A. odoratissima, A. procera* and *A. falcataria* seeds. Another objective was to confirm the presence of chemical inhibitor in the seedcoat of these species with special reference to *A. odoratissima.*

Materials and methods

Healthy and middle-aged trees of *A. odoratissima, A. procera* and *A. falcataria* were selected for seed collection. Details of weather parameters and location of the mother trees are given in Table 1. Mature pods of *A. odoratissima, A. procera* and *A. falcataria* were collected directly from the crown by means of pole and hook on 26 January 1992, 10 May 1991 and 24 January 1992 respectively.

Pods were dried in the sun, beaten with a stick and the released seeds were cleaned by winnowing. Cleaned seeds were mixed, sealed in polythene bags, made air tight and then stored in a refrigerator at approximately 5 °C. The length and width of pod and number of sound seeds per pod were determined from samples of 25 pods in four replications. The weight and seed moisture content of a thousand seeds were also determined (ISTA 1985). The details are given in Table 2.

Table 1. Weather data and location of the mother trees

Species	Place of seed collection	Annual temperature (°C)		Annual rainfall (mm)	Latitude	Longitude	Altitude (m a.s.l.)
		Maximum	Minimum				
Albizia odoratissima	Peechi Reserve Forest	39.7	19.3	2955	10°32 N	76°32 E	100
A. procera	Peechi Reserve Forest	39.7 (March)	19.3 (JAN)	2955	10°32 N	76°32 E	100
A. falcataria	K.A.U. Main Campus, Vellanikkara	39.9	20.9	3623	10°32 N	76°10 E	22.3

Table 2. Pod and seed characteristics of *A.odoratissima, A. procera* and *A. falcataria*

Species	Mean pod length (cm)	Mean pod width (cm)	Mean number of seeds/pod	Weight of 1000 seeds (g) (%)	Moisture (wet weight basis) (%)
A. odoratissima	17.1	3.2	8.0	49.4	5.66
C.V. (%)	10.23	9.84	16.00		
A. procera	15.6	2.2	9.0	39.3	8.72
C. V. (%)	10.88	11.30	21.80		
A. falcataria	10.0	2.0	14.0	27.9	4.31
C.V. (%)	7.49	12.90	12.60		

Seed treatments

The following treatments were applied in samples of 100 seeds, 25 seeds each in four replications.

Water soaking

The seed samples to be treated were put in previously heated water (200 ml) at different temperatures and then allowed to cool for 24 h. The treatments were: T2 (40 °C), T3 (60 °C), T4 (70 °C), T5 (80 °C) and T6 (100 °C). Seeds soaked in water at ambient temperature for 24 h served as the control (T1).

Physical Methods

A small portion of seedcoat was removed from the end opposite the hilum (nicking) using a nail cutter and then soaked in still water (T7) or flowing water (T8) at ambient temperature for 24 h. In another treatment (T9), seeds were soaked in still water for 24 h followed by complete removal of the seedcoat.

Mechanical scarification was done by shaking seeds mixed with four times of its volume of sand in a tin container at a horizontal direction for 10 min (Approx. 160 shakes per minute). After scarification, seeds were separated from sand and soaked in still water (T10) or flowing water (T11) at ambient temperature for 24 h.

Chemical methods

The seeds were treated with the following chemicals of varying concentrations for different durations, washed thoroughly in running water and then soaked in water at room temperature for 24 h. In the case of concentrated sulphuric acid, 50 ml was taken and for all other chemicals 100 ml was used for treating the seeds.

Treatment	Chemical	Concentration	Duration
T12	Sulphuric acid	Concentrated	5 min
T13	Sulphuric acid	Concentrated	10 min
T14	Sulphuric acid	Concentrated	20 min
T15	Sulphuric acid	5 %	24 h
T16	Sulphuric acid	10 %	24 h
T17	Sulphuric acid	20 %	24 h
T18	Sulphuric acid	40 %	24 h
T19	Hydrogen peroxide	1 %	12 h
T20	Hydrogen peroxide	2 %	12 h
T21	Hydrogen peroxide	1 %	24 h
T22	Hydrogen peroxide	2 %	24 h
T23	Acetone	5 %	24 h
T24	Acetone	10 %	24 h
T25	Acetone	20 %	24 h
T26	Acetone	40 %	24 h
T27	Potassium nitrate	0.5 %	24 h
T28	Potassium nitrate	1.0 %	24 h
T29	Potassium nitrate	2.0 %	24 h
T30	Thiourea	0.5 %	24 h
T31	Thiourea	1.0 %	24 h
T32	Thiourea	2.0 %	24 h
T33	Gibberellic acid (GA3)	5 ppm	24 h
T34	Gibberellic acid (GA3)	10 ppm	24 h
T35	Gibberellic acid (GA3)	20 ppm	24 h

Treatments to test the presence of germination inhibitors

To test the presence of germination inhibiting chemicals in the seedcoat, seeds of *A. odoratissima, A. procera* and *A. falcataria* were dehusked separately in a grinder and the seedcoat material was sieved out. Ten grams of the powdered seedcoat was soaked in 400 ml of distilled water for 24 h and the whole suspension was considered as the seedcoat extract. One hundred seeds in four replications of 25 each of yardlong bean (*Vigna unguiculata*), okra (*Abelmoschus esculentus*), brinjal (*Solanum melongena*) and rice (*Oryza sativa*) were soaked in 100 ml of the seedcoat extract for 24 h and then kept for germination. The following were the treatments:

Seeds	Soaking in			
	Seed extract of			Dist. water
	A. odoratissima	*A. procera*	*A. falcataria*	
Yardlong bean	T36	T37	T39	T39
Okra	T40	T41	T42	T43
Brinjal	T44	T45	T46	T47
Rice	T48	T49	T50	T51

Thin Layer Chromatography (TLC) was employed to detect the inhibitor present in the seedcoat of *A. odoratissima.* Silica gel G and distilled water were placed in the ratio of 1:2 (by weight) in a stoppered flask. Plaster of Paris (20%) was added to it and shaken vigorously for about 5 min till total deaeration took place. Clean plates of size 10 × 20 cm were taken for 7 g silica gel G and placed over a dry clean platform. The well mixed deaerated slurry was poured over an applicator of 250 kept on the side of the plates. The applicator was moved evenly without interruption to ensure uniform thickness. The plates were air dried and kept in an oven at 110 °C for 20-25 min.

The seedcoat extract was spotted on the TLC plate before the development of chromatogram. Trials were conducted by using different solvent mixtures such as methanol, methanol: petroleum spirit (1:9) and acetic acid: methanol (1:9).

Germination methods

The treated seeds were kept for germination on a double layer of filter paper (Borosil No. 1) inside Petri dishes having a diameter of 9 cm. The filter paper was moistened with distilled water daily. However, for treatments T36 to T38, T40 to T42, T44 to T46 and T48 to T50, the respective seedcoat extracts were used to moisten the filter paper. The Petri dishes were covered and kept for germination in racks at room temperature (22.7 °C to 31.5 °C).

Germination was observed daily. A seed was considered as having germinated when the radicle reached about one centimetre length and the green hypocotyl became visible. All germinated seeds were recorded and removed at every assessment to prevent double counting. At the end of the tests, cumulative germination percentages were calculated for each treatment. Vigour parameters were calculated for *Albizia* species using germination value (GV), final mean daily germination (MDG) and peak value (PV) (Czabator 1962).

Statistical analysis

Standard deviation and coefficient of variation were determined for the data on pod characteristics of *Albizia* species and germination percentages of yardlong bean, okra, brinjal and rice seeds. In the case of *Albizia* species, treatment means for arc sine transformed germination percentage, final mean daily germination, peak value and germination value were analysed by applying techniques for analysis of variance for CRD (Snedecor & Cochran 1967). Differences among means were tested at $p \leq 0.01$ using Least Significant Difference (L.S.D.)

Results

Water soaking

Hot water treatment of 70 °C and above (T4 to T6) significantly increased the germination percentage of *A. odoratissima* compared to the control (Figure 1).

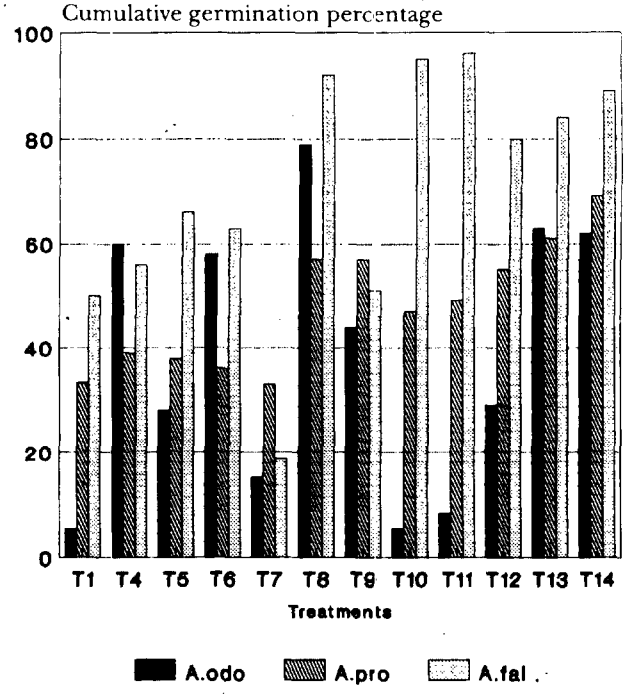

Figure 1. Cumulative germination percentage of *A. odoratissima*, *A. procera* and *A. falcataria*

In the case of *A. procera* and *A. falcataria,* hot water treatment did not have any added advantage. In general, the largest germination percentage was observed in *A. falcataria* and lowest in *A. odoratissima.* Vigour parameters like final MDG, PV and GV were also highest in the seeds of *A. falcataria* and lowest in *A. odoratissima* (Figures 2 - 4).

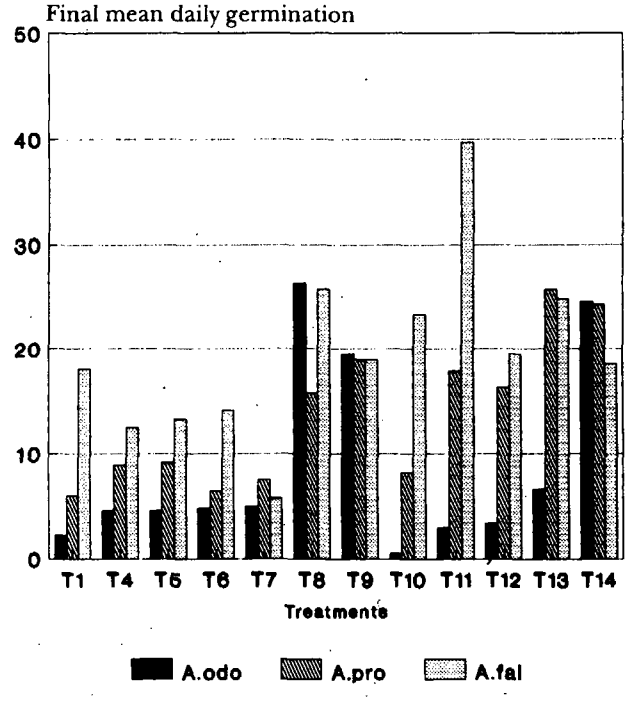

Figure 2. Final mean daily germination of *A. odoratissima, A. procera* and *A. falcataria*

Physical methods

Soaking the nicked seeds in flowing water (T8) gave the highest germination percentage in these three species (Figure 1). In the case of *A. falcataria,* soaking the mechanically scarified seeds in either still (T10) or flowing water (T11) also resulted in the highest germination percentage. Soaking the nicked seeds in still water (T7) did not have any influence on germination of these three species. Complete removal of the seedcoat (T9) resulted in significantly higher germination compared to soaking the nicked seeds in still water (T7).

Soaking the nicked seeds of *A. odoratissima* in flowing water (T8) gave the highest values in vigour parameters (Figures 2-4), with the next best method being complete removal of the seedcoat (T9). In the case of *A. procera,* soaking the nicked seeds or mechanically scarified seeds in flowing water and complete removal of seedcoat resulted in the highest MDG. A significant increase in GV was observed when the seedcoat was completely removed. In the case of *A. falcataria,* soaking the mechanically scarified seeds in flowing water (T11) gave the highest values in

vigour parameters, the next best treatment being soaking nicked seeds in flowing water (T8).

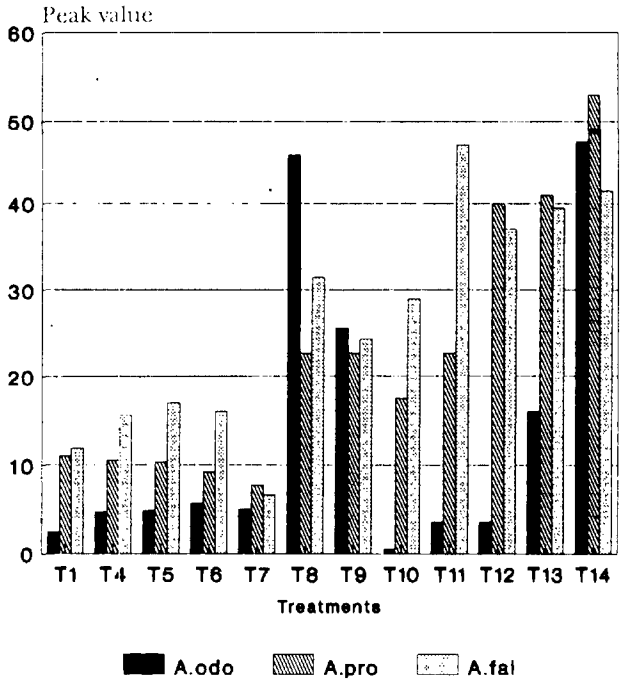

Figure 3. Peak value of *A. odoratissima*, *A. procera* and *A. falcataria*

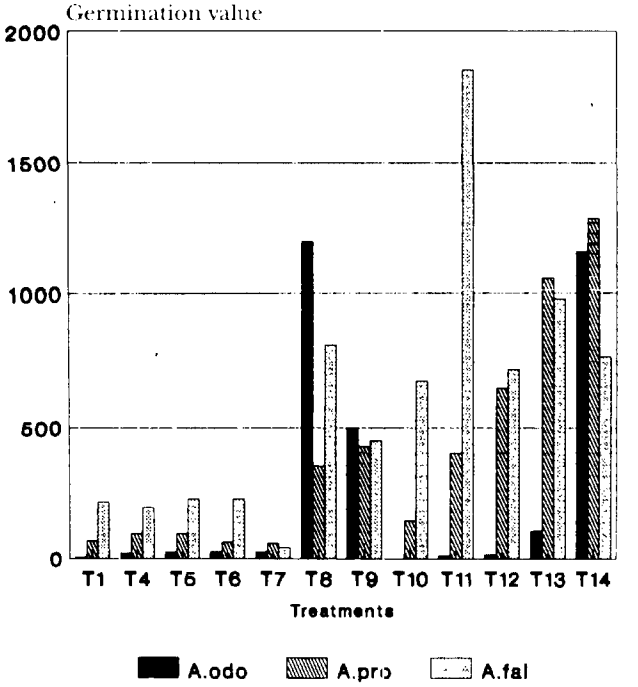

Figure 4. Germination value of *A. odoratissima*, *A. procera* and *A. falcataria*

Chemical methods

Scarification with concentrated sulphuric acid resulted in a significant increase in germination percentage in all the three species (Figure 1). Germination percentage of *A. odoratissima* more than doubled with increased duration of treatment up to 10 min (T13) compared to 5 min (T12). In *A. procera* and *A. falcataria*, scarification for 5 min (T12) gave a significant increase in germination percentage; no significant increase was observed with further increase in duration of scarification.

Concentrated sulphuric acid scarification for 20 min gave rise to the highest vigour values (Figures 2- 4), the increase being about 10 times in the case of MDG and 217 times in the case of GV. In general, acid scarification for 5 to 20 min resulted in significantly superior vigour parameters in *A. procera*. MDG and GV increased significantly when the seeds of *A. falcataria* were scarified for 10 min; however, duration of scarification did not have any influence on PV.

Dilute sulphuric acid treatment (T15-T18) completely inhibited the germination of these three species. But for some minor variations, soaking the seeds in hydrogen peroxide solution (T19-T22) also completely inhibited the germination of these species. In general, acetone treatment (T23-T26) did not show much influence on the germination and vigour parameters of these species. Potassium nitrate at different concentrations (T27-T29) completely inhibited germination of *A. odoratissima* seeds. Results were not conclusive in the case of other species. While thiourea at 0.5% conc. (T30) completely inhibited germination of *A. odoratissima*, at 1% and 2% (T31 and T32) it was similar with the control. Results were not conclusive in the case of other two species. Similarly, GA treatment (T33-T35) did not have any influence on *A. odoratissima* but results for the other two species were not consistent. Data are not shown for of these treatments.

Inhibiting action of seedcoat extract

Yardlong bean and okra seeds soaked in the seedcoat extract of the *Albizia* species failed to give any germination in all the four replications. In brinjal and rice seeds, germination percentages were significantly reduced when the seeds were treated with the seedcoat extracts of these species (Table 3).

In the TLC study a better resolution was obtained when acetic acid and methanol (1:9) was used as solvent. Spot development was made with iodine vapour. When the sample was run with coumarin, an RF value of 0.22 was obtained which indicated the presence of coumarin-like substance in the seedcoat. In addition to the coumarin spot, one more compound of a lower RF value (0.18) was obtained.

Table 3. Cumulative germination percentage of yardlongbean, okra, brinjal and rice seeds as affected by the seedcoat extract treatments

Species	*A. odoratissima*		*A. procera*		*A. falcataria*		Control	
	Cumulative germination (%)	C.V. (%)	Cumulative germination (%)	C.V. (%)	Cumulative germination (%)	C.V. (%)	Cumulative germination (%)	C.V. (%)
Yardlong bean	Nil	Nil	Nil	Nil	Nil	Nil	50.0	4.00
Okra	Nil	Nil	Nil	Nil	Nil	Nil	49.0	12.00
Brinjal	3.0	57.74	3.0	57.74	7.0	24.74	56.0	11.29
Rice	13.0	13.32	11.0	15.75	5.0	34.64	61.0	19.88

Discussion

The lower values in germination and vigour parameters of *A. odoratissima* compared to *A. procera* and *A. falcataria* in the control and a very high response at par with *A. procera* and *A. falcataria* to hot water scarification shows that inherent dormancy of *A. odoratissima* is greater than that of *A. procera* and *A. falcataria*. The fact that soaking the nicked seeds of *A. odoratissima* in flowing water gave rise to the highest germination and vigour values whereas soaking the mechanically scarified seeds in flowing water gave rise to the lowest germination shows that the seedcoat of *A. odoratissima* is the most hardy among the three species studied such as to render it impermeable even after mechanical scarification. This may be the reason for the lowest germination of *A. odoratissima* seeds in the control. The highest germination of *A. falcataria*, whether in the control or due to soaking the nicked or mechanically scarified seeds in flowing water, shows that the thickness of seedcoat and degree of dormancy are lowest in *A. falcataria*.

The poor germination in the control may be due to the poor imbibition caused by the impermeable seedcoat and micropylar plug. According to Jones (1963), Hendry and Staden (1982), Khan and Tripathy (1987) and Sniezko and Gwaze (1987), nicking or puncturing the seedcoat is very effective in removing the impermeability of seedcoat. Even though the seeds were fully imbibed, soaking the nicked seeds in still water did not improve the germination percentage. At the same time, soaking the nicked seeds in flowing water gave excellent results. In addition to the impermeability of seedcoat, the presence of a water soluble germination inhibitor in the seedcoat of *A. falcataria* and *A. procera* has been demonstrated by Sajeevukumar *et al.* (1995).

Scarifying the seeds with concentrated sulphuric acid resulted in a significant increase in germination percentage and vigour values of all the three species. This may be due to the ability of the acid to break the exogenous dormancy, (Hatano & Asakwa 1964, Babeley *et al.* 1986, Rai *et al.* 1986, Sur *et al.* 1987). Bhattacharya and Saha (1990) observed that disintegration of the seedcoat as well as the micropylar plug was the reason for increase in imbibition and subsequent

germination of *Cassia fistula* seeds after concentrated sulphuric acid treatment. Poor performance of all other treatments in these species indicate that they are ineffective to break the impermeability of the seedcoat.

It is clear from the studies that not only the impremeability of the seedcoat but the presence of chemical inhibitors in the seedcoat also contributes to the seed dormancy. The cumulative germination percentages of yardlong bean, okra, brinjal and rice seeds as affected by the seedcoat extract of *A. odoratissima*, *A. procera* and *A. falcataria* further confirm this finding. The chromatogram indicated the presence of a coumarin-like substance with a RF value of 0.22. The presence of yet another substance in the seedcoat extract is also indicated. Better techniques like HPLC, gas chromatography, etc. may be used to confirm the identification of this coumarin-like substance and also to quantify it. The presence of other inhibitors such as abscisic acid and maleic hydroxide needs to be checked.

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