Journal of Medicinal and Aromatic Plant Sciences 37(1-4) (2015) 41-45

Pre-treatments for overcoming dormancy in *Sida alnifolia* L.

VEERESH NETEKAL*[1] • ALICE K[1] • ASHA SM[2] • GIRIJA T[2]

Article History

Received: 21st November, 2014
Accepted: 18th July, 2015

Key words

Dormancy
Germination
Pre-treatments
Scarification
Sida alnifolia

ABSTRACT

Seeds of Sida species are reported to exhibit seed coat imposed dormancy posing serve problems in germination. In the present study some Pre-treatments for overcoming dormancy in Sida alnifolia L. were standardized. Among the eighteen pre-treatments tried for improving germination, chemical scarification using concentrated H_2SO_4 was significantly superior and recorded higher germination percentage and speed. Untreated seeds failed to germinate. Scarification of seeds with concentrated H_2SO_4 for higher duration of 30 minutes was selected as the best pre-treatment considering improvement in water imbibition, germination percentage, speed of germination, growth and vigour of the seedlings alongwith significant reduction in the intensity of dormancy.

INTRODUCTION

The medicinal plant known as 'Bala' in Sanskrit belongs to the genus *Sida* of the family Malvaceae. *Sida alnifolia* (Syn. *Sida rhombifolia* ssp. *retusa*), is a woody herb or sub shrub with strong branching. Flowers are solitary with orange-yellow petals, seeds are black with two awns. It is commonly seen in plains and hills of Southern peninsular India and occurs along roadsides and forest clearings as secondary growth in lateritic hill slopes and occasionally as a weed in upland cultivation [15]. It is reputed as a remedy for curing neurological disorders, as an anti-rheumatic and anti-pyretic agent. *Sida* species are a source of indoloquinoline alkaloids, principally cryptolepine, which produce many pharmacological effects such as anti-microbial, anti-hyperglycemic and cytotoxic effects and as leads in the design of new anticancer drugs [6].

*Correspondence author, Email: veeresh650@gmail.com
[1]College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India.
[2]Dept. of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Kerala Agricultural University, Thrissur 680 656.

In recent years, the increasing market demand for *Sida* species makes it difficult to rely on harvesting the plant material from the wild for its supply. Out of 230 species, *Sida alnifolia* ranks top in procurements of raw drugs by Ayurvedic industries, with an annual consumption of 1193.47 tons per year [12]. *Sida alnifolia*, the principal source of Bala in Kerala, has reached a stage of rarity due to habitat destruction, over exploitation and destructive harvesting for collection of roots. Low reproductive capacity, seed output and seed viability also adds to its rarity [7, 17]. *Sida alnifolia* is one among the 35 (short duration) medicinal plants promoted for cultivation by National Medicinal Plant Board, New Delhi.

Seeds of *Sida* species are reported to exhibit seed coat imposed dormancy posing severe problems in germination [5, 13]. Though few attempts have been made to standardize treatments for improving germination in *Sida* species [3, 4], detail studies are warranted to identify the effect of pre-treatments on growth and vigour of seedlings.

MS-1169

In this context, the present study was taken up to optimise certain germination pre-treatments for overcoming dormancy in *Sida alnifolia.*

MATERIALS AND METHODS

Seeds of *Sida alnifolia* were collected from Kerala Agricultural University campus, Vellanikkara, Thrissur. Moisture content of fresh seeds was brought down to four per cent and the seeds were stored in self sealing polythene bags at ambient condition.

Three months old seeds were subjected to a set of 19 pre-treatment before sowing in sterilized petriplates and kept under laboratory conditions (Table 1). Seeds of treatments T_1 to T_{14} were subjected to overnight water soaking after the pre-treatment. In the soaking treatments, constant seed water ratio was maintained. The petriplates were moistened daily using hand sprayer and kept under observation for 45 days and germination percentage and speed of germination was noted. The experiment was laid out in completely randomized design (CRD), with 19 treatments, replicated four times with 25 seeds per replication.

Table 1. Pre-treatments employed in the present study

T_1	Abrasion with hard sand paper
T_2	Conc. H_2SO_4 treatment for 05 min
T_3	Conc. H_2SO_4 treatment for 10 min
T_4	Conc. H_2SO_4 treatment for 20 min
T_5	Conc. H_2SO_4 treatment for 30 min
T_6	Boiling water treatment for 05 min
T_7	Hot water treatment at 90° C for 05 min
T_8	Hot water treatment at 80° C for 05 min
T_9	Hot water treatment at 70° C for 05 min
T_{10}	Hot water treatment at 60° C for 05 min
T_{11}	Hot water treatment at 50° C for 05 min
T_{12}	Hot water treatment at 40° C for 05 min
T_{13}	Treatment with boiling water for 05 min followed by freezing (0° C) for 12 h
T_{14}	Abrasion with hard sand paper followed by soaking in one per cent KNO_3 for 12 h
T_{15}	Soaking in water for 48 h
T_{16}	Making pin pricks followed by soaking in water for 48 h
T_{17}	Overnight soaking in cow dung slurry
T_{18}	Overnight water soaking
T_{19}	Control (untreated seeds)

From the above experiment the best four treatments *viz.* treatment with Conc. H_2SO_4 for 05 min, 10 min, 20 min and 30 min were compared with untreated control. Seeds were subjected to the respective treatments with four replications and 25 seeds each were sown in plastic trays containing sand. Trays were moistened daily and kept under observation for 14 days. Observations were recorded in terms of germination per cent, speed of germination [8], dormancy index, intensity of dormancy at seven and 14 days [16], seedling growth, vigour index-1 [1], vigour index-2 [2] and water imbibition rate. The data were analyzed statistically using Stastical Packages, MSTAT and multiple comparisons among the treatments were done using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Germination per cent and speed of germination varied significantly among the various pre-treatments (Table 2). Pre-treatment of seeds with Conc. H_2SO_4 proved significantly superior to rest of the treatments. Increase in the duration of scarification showed gradual increase in germination percentage and speed peaking in 30 min treatment with Conc. H_2SO_4. Similar to the present findings, Packa *et al*. [9] also found that scarification for 30 minutes with 95.00 per cent sulphuric acid was most effective in breaking the physical dormancy of seeds of *Sida hermaphrodita*, resulting in imbibition without impairing embryo viability. Lissy [7] reported beneficial effect of acid scarification in four *Sida* species with the optimum duration to be 10-20 min as well as variation in germination per cent, with the highest in *Sida cordifolia* and lowest in *Sida rhombifolia*. The favourable influence of Conc. H_2SO_4 in improving germination was strongly supported by Chauhan and Johnson [3] who suggested that seeds of *Sida* treated with sulphuric acid for 120 min resulted in 65.00 per cent germination compared with five per cent, for non-scarified seeds and predicting the response to scarification indicates that a hard seed

Table 2. Effect of pre-treatments on seed germination in *Sida alnifolia*

Treatments	Germination per cent	Speed of germination
T_1	26.00^{c} (5.05)	10.20^{d} (3.21)
T_2	54.00^{b} (7.37)	36.08^{c} (6.03)
T_3	78.00^{ab} (8.84)	72.33^{b} (8.51)
T_4	72.00^{ab} (8.48)	69.33^{b} (8.32)
T_5	94.00^{a} (9.72)	89.96^{a} (9.51)
T_6	0.00^{e} (0.70)	0.00^{e} (0.70)
T_7	0.00^{e} (0.70)	0.00^{e} (0.70)
T_8	2.00^{de} (1.41)	0.14^{e} (0.79)
T_9	0.00^{e} (0.70)	0.00^{e} (0.70)
T_{10}	0.00^{e} (0.70)	0.00^{e} (0.70)
T_{11}	0.00^{e} (0.70)	0.00^{e} (0.70)
T_{12}	6.00^{d} (2.12)	0.22^{e} (0.84)
T_{13}	2.00^{de} (1.41)	0.06^{e} (0.75)
T_{14}	6.00^{d} (2.51)	0.74^{e} (1.07)
T_{15}	0.00^{e} (0.70)	0.00^{e} (0.70)
T_{16}	2.00^{de} (1.41)	0.14^{e} (0.79)
T_{17}	2.00^{de} (1.41)	0.15^{e} (0.80)
T_{18}	0.00^{e} (0.70)	0.00^{e} (0.70)
T_{19}	0.00^{e} (0.70)	0.00^{e} (0.70)

coat is the primary mechanism restricting germination.

Mechanical scarification with sand registered 26.00 per cent germination, less than 60.00 per cent which is minimum mandatory germination requirement. In contrast, beneficial effect of mechanical scarification was reported by Pedroso et al. [10] in *Sida rhombifolia* and Shooshtharian and Salehi [14] in *Alcea aucheria*; probably because the agents used and extent of scarification may be different. Sand paper rubbing followed by soaking in one per cent KNO_3 for 12 h was not very effective giving only six per cent germination. Oxidants like KNO_3 help to break dormancy in seeds of many species [11]. As per the findings of Bewley and Black [2], KNO_3 enhances germination by enhancing the O_2 level. However, in the case of *Sida*, dormancy may not be related to oxygen availability, since soaking seeds for 12 h in KNO_3 could not improve germination percentage.

Germination percentage in case of hot water boiling and soaking treatments, ranged from zero to six per cent. Hot water boiling followed by freezing, overnight soaking in cow dung slurry and making pin pricks followed by soaking in water for 48 h recorded only two per cent germination. Soaking in water for 48 h, overnight water soaking and untreated seeds failed completely to soften the seed coat and none of the seeds germinated.

Among the eighteen pre-treatments tried to soften the seed coat, scarification treatments with Conc. H_2SO_4 were the best. The effect of best four treatments of acid scarification with Conc. H_2SO_4, treatment for 05 min, 10 min, 20 min and 30 min compared with untreated control on growth and vigour of seedlings was studied further. Acid scarification treatments of all durations were significantly superior compared to untreated control seeds. Scarification for 30 min recorded highest germination percentage, speed of germination, dormancy index, seedling growth and vigour parameters and lowest intensity of dormancy, which is inversely related to germination (Tables 3 and 4). Scarification for 10 min and 20 min were at par but superior than scarification for duration of five minutes. Untreated seeds recorded highest intensity of dormancy (99.00 per cent), exhibiting only one per cent germination. The rate of water imbition was faster for the treated seeds than untreated seeds (Fig. 1). Irrespective of treatments, the maximum mass increase was noticed within

Table 3. Effect of acid scarification treatments on germination and dormancy behaviour

Treatments	Germination per cent	Speed of germination	Dormancy index	Intensity of dormancy at 7 days (%)	Intensity of dormancy at 14 days (%)
Conc. H_2SO_4 05 min	30.00^{bc}	6.77^{b}	0.024^{b}	70.00^{b}	70.00^{b}
Conc. H_2SO_4 10 min	60.00^{ab}	17.18^{a}	0.042^{a}	51.00^{c}	40.00^{c}
Conc. H_2SO_4 20 min	54.00^{abc}	18.12^{a}	0.041^{a}	51.00^{c}	46.00^{c}
Conc. H_2SO_4 30 min	72.00^{a}	19.33^{a}	0.047^{a}	48.00^{c}	28.00^{c}
Control	1.00^{d}	0.14^{c}	0.001^{c}	99.00^{a}	99.00^{a}

Values having common superscript are not significantly different from each other.

Table 4. Effect of acid scarification treatments on growth and vigour of seedlings

Treatments	Shoot length (cm)	Root length (cm)	Total length (cm)	Fresh weight (g)	Dry weight (g)	Vigour index-I	Vigour index-II
Conc. H_2SO_4 05 min	6.75^{a}	1.09^{a}	7.84^{a}	0.024^{a}	0.0025^{b}	235.20^{d}	0.075^{b}
Conc. H_2SO_4 10 min	7.24^{a}	1.37^{a}	8.62^{a}	0.026^{a}	0.0028^{b}	517.20^{b}	0.168^{a}
Conc. H_2SO_4 20 min	7.15^{a}	1.25^{a}	8.40^{a}	0.028^{a}	0.0031^{a}	453.30^{c}	0.167^{a}
Conc. H_2SO_4 30 min	7.19^{a}	1.13^{a}	8.32^{a}	0.028^{a}	0.0030^{ab}	590.90^{a}	0.216^{a}
Control	1.02^{b}	0.25^{b}	1.27^{b}	0.004^{b}	0.0005^{c}	1.27^{e}	0.0005^{c}

Values having common superscript are not significantlydifferent from each other.

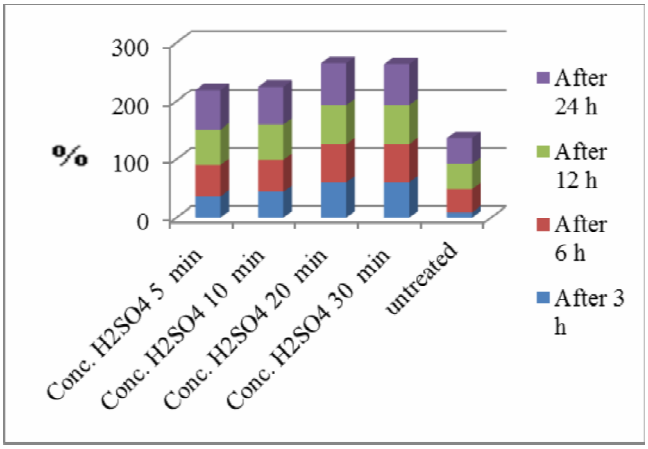

Fig. 1 Effect of acid scarification treatments on water imbibition per cent.

three hours of soaking for the scarification treatments, but untreated seeds absorbed maximum water after six hours of soaking. With increase in duration of scarification, rate of imbition was also higher. However, increase in water imbition rate after 6h, 12 h and 24 h was low for the treated seeds than the untreated seeds and after the 24 h of soaking in water, per cent of mass increase of treated seeds was 30.73 to 37.71 per cent higher than the untreated seeds.

Therefore, it is concluded that the difference in germination percentage between acid scarification treatments, mechanical and hot water treatments and other treatments might be due to differences in the rates of seed coat softening and water imbibition as achieved by the pre-treatment techniques. The acid scarification treatments are not hampering the seedling growth and vigour also.

REFERENCES

1. Abdul-Baki AA, Anderson JD. 1972. Physiological and biochemical deterioration of seeds. In Seed Biology (Ed. TT Kozlowski), Academic Press, New York, pp. 283-309.

2. Bewley JD, Black M. 1994. Seed Physiology of Development and Germination II Edition. Springer Verlag, New York, pp. 1-18.

3. Chauhan BS, Johnson DE. 2008. Dormancy, germination and emergence of *Sida rhombifolia* L. *Indian J Weed Sci* **40**: 1-2.

4. Egley GH. 1976. Germination of developing prickly *Sida* seeds. *Weed Sci* **24**: 239-243.

5. Egley GM. 1989. Water-impermeable seed coverings as barriers to germination. In Recent Advances in the Development and Germination of Seeds (Ed. RB Taylorson), New York Plenum Press, New York, USA, pp. 207-223.

6. Karou D, Savadogo A, Canini A, Yamegol S, Montesano C, Simpore J, Colizzi V, Traore SA. 2005. Anti bacterial activity of alkaloids from *Sida acuta*. *Afr J Biotechnol* **4**: 1452-1457.

7. Lissy KP. 2004. A comprehensive investigation into some selected species of *Sida* Linn. with reference to their substitutes and adulterants. Ph.D. Thesis, Mahatma Gandhi University, Kottayam, Kerala, India, 125p.

8. Maguire JD. 1962. Speed of germination aid in selection and evaluation for seedling emergence and vigour. *Crop Sci* **2**: 176-177.

9. Packa D, Kwiatkowski J, Graban L, Lajszner W. 2014. Germination and dormancy of *Sida hermaphrodita* seeds. *Seed Sci Technol* **42**(1): 1-15.

10. Pedroso DC, Menezes VO, Lopes SJ, Silva ACF, Tedesco SB. 2007. *In vitro* germination tests of *Sida rhombifolia* L. seeds. *Agrociencia* **11**: 90-96.

11. Roberts EH, Smith RD. 1977. Dormancy and the pentose phosphate pathway. In Physiology and Biochemistry of Seed Dormancy and Germination (Ed. AA Khan), Elsevier Press, Amsterdam, pp. 385-411.

12. Sasidharan N, Muraleedharan PK. 2009. The raw drugs requirement of Ayurvedic medicine manufacturing industry in Kerala. KFRI Research Report No. 322. Kerala Forest Research Institute, Peechi, Thrissur, Kerala, India.

13. Seal S, Gupta K. 1998. Germination behaviours of two species of *Sida*, a medicinal plant. *J Trop Ecol* **39**: 279-287.

14. Shooshtarian S, Salehi H. 2010. Enhancing *Alcea aucheri* (Boiss.) Alef. seed germination by application of some scarification treatments. *Adv Environ Biol* **4**: 216-219.

15. Sivarajan VV, Pradeep AK. 1996. Malvaceae of Southern Peninsular India: A Taxonomic Monograph. Daya Publishing House, New Delhi, pp. 224-287.

16. Swain SK, Sahoo P, Patnaik MC. 2001. Seed dormancy in groundnut (*Arachis hypogea* L.) -variability for intensity and duration. *Seed Res* **29**: 13-17.

17. Veeresh N. 2014. Seed production potential, dormancy and seed storage behaviour in *Sida* spp. M.Sc (Hort) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India, 119p.