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Seed germination potential in *Sida cordifolia* Linn. – A medicinal flannel weed from Indian arid zone

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ABSTRACT

Sida cordifolia is an important medicinal plant of the Indian Thar desert. The present study deals with the evaluation of seed germination and seedling growth behaviours of S. coedifolia under controlled (in-vitro) and natural (in-vivo) conditions. Results revealed that seeds require a scarification pretreatment of 30 minutes with concentrated H_2SO_4 to obtain maximum germination. Maximum seedling quality index (SQI) values were obtained when seeds were sown at 0.5 cm depth in 2:2:1 soil mixture ratio of sand: clay: FYM.

INTRODUCTION

Sida cordifolia Linn. (Family: Malvaceae), commonly known as Bala, is an erect, perennial branched undershrub, which grows as wasteland weed. Seed germination starts in July-August after 1-2 showers of rain under natural field conditions due to presence of a hard seed coat. The plant has been used as depressant, analgesic, anti-inflammatory, hypotensive, and hepatoprotective agent. Presence of ephedrine has highlighted its utility in various Ayurvedic preparations for asthma and for increasing energy [11]. Seed germination is one of the most important phases in the life cycle of a plant and is highly responsive to existing environment [3]. The dormancy may primarily be due to under-developed embryo or decreased metabolic activity in the seeds. The scarification treatment enables the induction/activation of enzymes, hormones and other soluble metabolites needed by the developing embryo for germination and growth [7]. Acid scarification leads to partial or complete removal of inhibitory substance and weakening of the hard seed coat or pericarp [13]. According to Barmukh and Nikam [2] concentrated H_2SO_4 pretreatment for 30 minutes was suitable for seed germination and seedling growth in *Pterocarpus marsupium*. Acid scarification treatments can also improve the germination by making seed coats more permeable to water and gases [4] by hydrolyzing the organic components of the fruit or seed coat. H_2SO_4 brings down the pH across membrane, to cause H^+ pumping and the radicle emergence may be due to imbibitions and acid growth effect [9]. This process has been shown to give significant improvement in germination in many plant species [18].

The present investigation was therefore carried out to know if the temporary dormancies in *S. cordifolia* can be overcome by scarification treatments. The emphasis was also given on morphological parameters of seeds and different seed sowing techniques such as depth, soil

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mixture ratio, etc. to improve germination and seedling growth parameters under controlled (*in-vitro*) and natural (*in-vivo)* conditions.

MATERIALS AND METHODS

The mature fruits of *S. cordifolia* were collected from Old Campus of JNV University, Jodhpur in July-September. The seeds were cleaned and stored in plastic containers with insecticides/parad tablets under laboratory conditions before use.

Seed morphological parameters

Seed output and reproductive capacity was calculated as suggested by Salisbury [17]. The seed viability was tested by the tetrazolium method [15]. The seed size was measured with the help of vernier caliper and graph paper. The weight of 100 seeds was taken by electronic balance. All parameter were taken in triplicate and confirmed twice.

Seed germination behaviour under in vitro conditions

In *S. cordifolia,* hard seed-coat-imposed dormancy is prevalent. The seeds were first cleaned and treated with 0.1% $HgCl_2$ for 30 seconds and then kept under running tap water for 3-4 hours. Afterwards, below-mentioned different pretreatments were provided to seeds for enhancing germination percentage and improvement in seedling quality as well as seedling growth. The pretreatments given were: conc. H_2SO_4 (100%; AR Grade) acid scarification (0-30 minutes); mechanical scarification; GA_3 (presoaking for 24 and 48 h in 2, 5, 10, 25 and 50 mg L^{-1}); and presoaking for 24 and 48 h in 10, 25, 50 and 100 mg L^{-1} concentrations of $Ca(NO_3)_2$, KNO_3, NH_4NO_3 and $Co(NO_3)_2$. Significant results were only obtained in acid and mechanical scarification pretreatments. Hence, only these results are mentioned here. The acid scarified seeds were tied in muslin cloth and kept under running tap water for 3-4 h. For mechanical scarification, seeds were treated with sand paper/ emery stone at micropylar end to enhance germination percentage. Afterwards, pretreated seeds were kept for germination studies. The experiments were performed in alternate white light and dark (12h each) photoperiod given through three fluorescent tubes of 40 watts each fitted at a height of half meter from the Petri-dishes (1000 Lux) at 28 °C in a seed germinator. Seeds were placed in sterilized Petri-dishes lined with single layer of filter paper. The filter paper was moistened with the required volume of distilled water as and when needed. After one week of incubation, seed germination percentage and root & shoot lengths of seedlings were recorded. The experiments were performed in triplicate and repeated thrice. The vigour index (VI) was derived from the formula given by Abdul-Baki and Anderson [1] as follows: VI= Percentage of germination × Seedling length (cm); Where, seedling lengths are the sum of root and shoot lengths. Germination values (GV) of seeds were calculated for each treatment as per Czabator [5] as follows: GV = PV × MDG; Where, PV= Peak value of germination; and MDG = Mean daily germination. PV was calculated with the formula: PV= Final germination percentage / No. of days required to reach the peak germination.

Seed germination behaviour under In vivo conditions

The nursery experiments with different soil mixture ratios and sowing depths were carried out during the month of July. Seeds (100 seeds for each treatment with 20 replicates) were pretreated with 30 min of conc. H_2SO_4 before sowing in polybags. The pretreated seeds were sown at four different types of soil mixture ratios of sand: clay: FYM, *i.e.* R_1 (1:1:1), R_2 (1:2:1), R_3 (2:2:1) and R_4 (1:2:2). The seeds sowing depths used were: D_1 (0.5 cm), D_2 (1.0 cm), D_3 (1.5 cm) and D_4 (2.0 cm). The measured quantity of water was provided on each alternate day to keep the soil moist. Germination percentage was recorded daily. Plant height, collar diameter and above and below ground biomass (dry weight) of the seedlings was measured after every one month, till the survival of plants. SQI was estimated by using following formula given by Dickson *et al*. [6]: SQI = Total weight of the plant (g plant^{-1} d.wt.) / Plant height (cm)/Root diameter (mm) + Shoot wt. /Root wt. (g plant^{-1} d.wt.)

All experiments were executed using CRD design separately during both the two consecutive years of study and the data were statistically

Table 1. Morphological antributes of *S.cordifolia* seeds

Parameters	Values
Seed output plant^{-1}	640.0 ± 85.40
Reproductive capacity	190.21 ± 24.63
Viability (%)	95.0 ± 5.031
Colour	Light brown
Shape	Trigonus
Length (mm)	2.217± 0.109
Breadth (mm)	2.002± 0.109
Wt. of 100 seeds (g)	0.260± 0.016

± = Standard deviation

analyzed as suggested by Gomez and Gomez [8]. The mean average values of two years for each parameter was tabulated.

RESULTS AND DISCUSSION

Seed morphological parameters

The data on various seed morphological parameters for *S. cordiflora*, *viz*. seed output, reproductive capacity, colour, weight, size and viability of the seeds are given in Table 1. The potentiality of a species to colonize, perpetuate and establish itself, mostly depends on the seed output. The seed output of a plant is defined as the number of seeds produced by an individual plant. The values of seed output and reproductive capacity for *S. cordiflora* was calculated to be 640.0 and 190.21, respectively. The seeds were trigonous-shaped and light brown in colour. The mean length and breadth of seeds were 2.22 and 2.00 mm, respectively.

In vitro seed germination behavior

The data on effect of acid and mechanical scarification pretreatments on various parameters of seed germination are provided in Table 2. It is evident from this table that the freshly collected seeds recorded only 8.83% germination under controlled conditions. The H_2SO_4 treatment for different durations as well as mechanical scarifications increased the percent germination significantly. The highest germination percentage, seedling growths, R/S ratios, germination value (GV) and vigour index (VI) were observed in treatment comprising of 30 minutes of sulphuric acid-scarification. Improvement in germination of seeds soaked in conc. H_2SO_4 might be due to dissolution of funicles and formation of deep fissures in hilum and cracked palisade cells, which ultimately helped to absorb water and gases to initiate germination. Heit [10] reported that in fragrant sumac and skunkbush plants, seed dormancy is caused by a hard seed coat and required scarification with sulfuric acid for 1 hour to obtain optimum germination. Meginnis [14] stated that sulphuric acid treatment is favourable for increasing seed germination of Black Lucost. Sheikh [19] reported that 30-45 minutes of sulphuric acid treatment was minimum for getting maximum germination in tree seeds. Sahoo and Kasera [16] also observed that germination percentage and seedling growth parameters in *Citrullus colocynthis* can be enhanced significantly when seeds were pretreated with 2 minutes of concentrated H_2SO_4. The present findings also support results observed by these earlier workers. The seed coat softening caused by H_2SO_4 supported the unrestricted expansion of embryonic parts.

In vivo seed germination behavior

It is evident from Table 3 that maximum values of seedling emergence percentage (70%) and SQI (0.0207) were observed in D_1 treatment where in the seeds were sown at 0.5 cm depth. Among various soil ratio treatments, 82% seedlings emerged in soil mix R_3 (2:2:1). The SQI was in the order of $R_3>R_1>R_4>R_2$. Maximum values for plant height, collar diameter, shoot and root dry weights were also obtained in R_3 (2:2:1) soil mix. Among sowing depths, maximum germination took place when seeds were sown at D_1 depth and minimum at D_4. Sowing depth and soil mixture are known to play an important role on seed germination, seedling emergence, establishment, vigour and overall plant growth. Pre-soaking of seeds of *Berberis aristata* with sulphuric acid was very effective in induction of early germination with 40-140 percent improvement in germination and

seedling vigour [20]. Kedia *et al.* [12] observed maximum plant height and collar diameter in *Eclipta alba* and *Phyllanthus fraternus* at 0.5 cm depth. The present results also confirm these studies. In the present studies, all the parameters showed statistical differences among different treatments ($p<0.05$), except for seedling emergence (days), emergence percent, plant height and collar diameter in soil mix ratio experiments, which showed non-significance variations. The data for seedling emergence (days) and collar diameter in depth experiments were also non-significant.

The results on seed germination parameters in the present investigation are suggestive of the hard seed-coat-imposed dormancy in *S. cordifolia*. The method used here for scarifying the seeds with conc. H_2SO_4 for 30 minutes was an effective strategy for breaking this dormancy and can be used for conservation of this medicinal herb. Also, for obtaining the optimal seedling growth the practice of sowing the scarified seed at a depth of 0.5 cm in a soil mix ration of 2:2:1 of sand: clay: FYM could be followed for this species.

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Table 2. Effect of different pretreatments on seed germination and seedlings growth parameters in *S. cordifolia*

Pretreatments		Germination (%)	Root length (cm)	Shoot length (cm)	R/S ratio	VI	GV
Control		8.83	1.45	1.70	0.852	27.8	3.52
H_2SO_4	5min	53.33	2.36	2.83	0.833	276.7	141.29
	10min	56.66	2.71	2.77	0.978	310.4	158.64
	15min	70.00	2.47	2.94	0.840	378.7	163.31
	20min	73.33	2.27	3.00	0.756	386.4	267.61
	25min	80.00	2.80	2.54	1.102	427.2	213.28
	30min	98.33	3.75	3.25	1.153	688.3	481.16
MS		76.66	2.56	2.60	0.984	395.5	291.30
CD		8.263*	0.379*	0.427*	0.429^{ns}	42.32*	2.45*

MS =Mechanical scarification; VI= Vigour index; GV= Germination value; R/S ratio = Root and shoot lengths; NS = Non-significant; and * = Significant at (P<5%) probability levels

Table 3. Effect of different sowing depth and soil mix ratio treatments on seedling parameters in *S. cordifolia* under nursery conditions

Treatments	Seedling emergence (days)	Emergence percent	Plant height (cm)	Collar diameter (mm)	Dry weight (g plant^{-1})		SQI
					Shoot	Root	
Soil mix ratios:							
R_1 (1:1:1)	7	75.00	13.9	1.62	0.182	0.042	0.0254
R_2 (1:2:1)	8	80.00	15.0	1.59	0.130	0.015	0.0151
R_3 (2:2:1)	5	82.00	15.6	1.91	0.269	0.045	0.0376
R_4 (1:2:2)	9	77.00	10.0	1.50	0.134	0.035	0.0243
CD	4.565^{ns}	11.168^{ns}	0.555^{ns}	0.216 *	0.0306^{ns}	0.0417*	0.0106 *
Sowing depths:							
D_1 (0.5 cm)	6	70.00	14.0	1.97	0.115	0.040	0.0207
D_2 (1.0 cm)	7	65.00	13.4	1.90	0.110	0.022	0.0182
D_3 (1.5 cm)	8	62.00	12.7	1.86	0.105	0.020	0.0178
D_4 (2.0 cm)	9	52.00	12.2	1.70	0.041	0.010	0.0068
CD	3.825^{ns}	6.058*	0.0812*	0.280^{ns}	0.00987*	0.0675*	0.00427*

SQI= Seedling quality index; NS = Non-significant; and * = Significant at (P<5%) probability levels; Data was recorded after 4 months of sowing

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