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# Standardization of Seed Germination Aspects in *Asparagus racemosus* Willd. and *Sida cordifolia* Linn. in Arid Region of India

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**ABSTRACT** *Asparagus racemosus* and *Sida cordifolia* are considered to be the most important medicinal plants of the Indian Thar desert. The present paper deals with seed germination studies of these plants under *in vitro* and *in vivo* conditions. Seed morphology, imbibition, germination (under different light regimes, growth regulators, acid and mechanical scarifications), vigour index, seedling quality index, sowing depth and soil mixture ratios experiments were conducted on these plants. Results revealed that seeds of *A. racemosus* exhibit 100% germination under controlled conditions, while those of *S. cordifolia* require conc. $H_2SO_4$ scarification for 15 min for maximum germination percentage. Maximum seedling quality index was obtained when the seeds were sown at $D_2$ (1.0 cm) depth with $R_1$ (1:1:1) soil mixture ratios of sand: clay: FYM in *A. racemosus*, while at $D_1$ (0.5 cmydepth in $R_3$ (2:2:1) ratio in *S. cordifolia*.

**Key words:** Germination, vigour index, imbibition, peak value, seedling growth

A seed will germinate or not, would depend on the conditions of seed and that of the environment in which it is placed for germination. Most important factors are temperature, available moisture and light conditions to which seeds are exposed. Each species has its own characteristic requirements for germination. Some seeds germinate immediately after dispersal from the fruit under favourable conditions of moisture, temperature and aeration. However, in others it does not take place even under favourable conditions. The seed sowing depth and soil mixture ratio also play an important role in seed germination behaviour and overall plant growth. Sowing at optimum depth is an important aspect to obtain higher germination and good quality seedlings [1]. Germination is a crucial phase in the life cycle of arid zone plants. For successful germination and cultivation practice, studies on standardization techniques for germination behaviour are necessary. Hence, the present study was carried out to study seed germination behaviour in *Asparagus racemosus* (Shatawar, Fam.: Asparagaceae) and *Sida cordifolia* (Bala, Fam.: Malvaceae). Emphasis was also given on morphological parameters of seeds, imbibition studies and different seed sowing techniques such as depth, soil mixture ratios, etc. to improve seed germination and seedling growth.

*A. racemosus* is considered to be of medicinal importance because of the presence of four steroidal saponins, viz. Shatavarin I to IV in its tuberous roots. Its roots have been referred as emollient, cooling, nervine tonic, constipating, galactogogue, aphrodisiac, diuretic, rejuvenating, carminative, stomachic, anti-septic and as a tonic. Beneficial effects of the roots are suggested in nervous disorders, dyspepsia, diarrhoea, dysentery, tumours, inflammations, hyperpiesia, neuropathy, hepatopathy cough, bronchitis, hyperacidity and certain infectious diseases. *S. cordifolia* contains the anti-asthmatic alkaloid, i.e. ephedrine. The plant exhibits anti-convulsant, anti-pyretic and anti-cancer activities. Seeds are used to treat urinary infections and are also aphrodisiac.

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The seeds of *A. racemosus* and *S. cordifolia* were collected from the natural habitats at Jodhpur in February-March for *A. racemosus* and April-May for *S. cordifolia* during 2005 and 2006. Seed output and reproductive capacity were calculated as per Salisbury [2]. The seeds were stored in plastic containers with insecticide/parad tablets. The seed viability was tested by the tetrazolium method [3, 4]. The seed size was measured with the help of vernier caliper and graph paper. The seeds volume and density were measured as per standard methods and calculated for 100 seeds in triplicate and confirmed twice. Imbibition studies in freshly collected seeds were conducted using the precise weight of seeds that were placed in distilled water and the increase in weight was quantified at specific intervals.

*In vitro seed germination behaviour*

The seed germination studies were performed in freshly collected seeds in sterilized Petri dishes lined with single layer of filter paper, moistened with distilled water. The germination experiments were carried out under CRD in alternate white light and dark (12 h) obtained from 3 fluorescent tubes of 40 watts each fitted at a height of half meter from the Petri dishes (1000 Lux) at 28°C in seed germinator. In *S. cordifolia*, the observations were recorded daily upto 10 days, while in *A. racemosus* upto 20 days because germination begins 8 days after setting the experiments. The experiments were performed in triplicate with each Petri dish containing 10 seeds and the experiments repeated thrice. At the end of experiments, the number of seeds germinated and roots and shoot lengths were measured.

The seedling vigour index (VI) was calculated as proposed by Abdul-Baki and Anderson [5]. Germination value of the seeds, which is an index combining the speed, and completeness of the germination was calculated for each treatment using the formula of Czabator [6]. MDG (mean daily germination) and PV (peak value) were calculated as per Czabator [6].

Seeds of *A. racemosus* were subjected to germination under different light regimes. The impact of different durations of light on germination and root-shoot developments was studied under the continuous dark, continuous light and alternate light (12 h) and dark conditions (12 h). In *S. cordifolia*, seeds were scarified with acid as well as mechanical scarifications. The mechanical scarification treatment was conducted by rubbing the seeds gently over emery stone. Acid scarification was done by treating the seeds with conc. $H_2SO_4$ for various durations (0-35 m). After the scarification, the seeds were washed in running tap water for 3-4 h and thereafter the treated seeds were placed for germination. To assess the role of different growth regulators on germination behaviour and root and shoot lengths, the seeds were also presoaked in different concentrations of $GA_3$ and IAA solutions and then kept for germination.

*In vivo seed germination behaviour*

Freshly harvested seeds of *A. racemosus* were sown without any treatment because they exhibited 100 per cent germination under *in vitro* conditions. However, seeds of *S. cordifolia* were pretreated for 15 min in conc. $H_2SO_4$ before sowing in polybags. Seeds of both species were sown in polybags with four different kinds of soil mixture ratios, i.e. sand: clay: FYM, viz. $R_1$ (1:1:1), $R_2$ (1:2:1), $R_3$ (2:2:1) and $R_4$ (1:2:2). Seeds of both the species were sown in polybags at four different depths, viz. $D_1$ (0.5 cm), $D_2$ (1.0 cm), $D_3$ (1.5 cm) and $D_4$ (2.0 cm) in 2:1:1 soil mixture ratio. Twenty replicates were maintained for both experiments and germination was recorded daily. Watering was done daily to maintain the soil moisture. Plant height, collar diameter, above and below ground biomass (dry weight) of seedlings were measured after one month of seed sowing. Seedling Quality Index (SQI) was estimated by using formula of Dickson *et al.* [7].

All the experiments were executed using CRD design separately during both years and the data were statistically analyzed by one-way ANOVA [8]. The mean values of both years are presented in tabular form.

Studies of seed morphology are considered essential to understand its variability, which is an important adaptation in the life of desert

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plants. It has an ecological significance for their long-term perpetuation in the area as well as introduction to new areas. 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. It is an important factor affecting the species composition of a community. The data of seed output and morphological parameters of both plants are presented in Table 1. In *A. racemosus* seeds showed 100 per cent germination under controlled conditions and the values of reproductive capacity and seed output were same, i.e. 820.8. The seeds are round, black having diameter and density of 3.16 mm and 1.326 g $cc^{-1}$, respectively. Seeds of *S. cordifolia* are brown, trigonous shaped, 2.43 mm long and 1.94 mm broad having 1.265 g $cc^{-1}$ density.

The first process, which occurs during germination, is the uptake of water by the seed, which is due to the process of imbibition. It depends upon the composition of the seed, the permeability of the seed coat or fruit to water and the availability of water. The results of imbibition studies of *A. racemosus* are shown in Fig. 1. The study showed that imbibition takes place very slowly. 50 and 100 per cent imbibitions were achieved after 12 and 30h, respectively. Seeds of *S. cordifolia* imbibed water rapidly at the initial stage and achieved 47 per cent imbibition within 10 min. Thereafter, the speed slowed down and 99.8 per cent imbibition was recorded at the end of 240 min (Fig. 2).

An important factor affecting germination of seeds is the availability of light. Sen *et al.* [9] and Kasera and Shukla [10], studied the effect of light pretreatment on seed germination behaviour of plants of Indian Thar desert. *In A. racemosus* the effect of different light treatments on various parameters such as seed germination, seedling growth, VI, GV, etc. is presented in Table 2. Results revealed that fresh seeds showed 100 per cent germination under different light pretreatments. The studies further showed that maximum R/S ratio was obtained in continuous dark, while seedling VI in alternate light/dark treatment. However, the maximum GV was obtained in continuous light and minimum in continuous dark treatment. The data were significant at ($P<0.01$) except for germination percentage and MDG.

In several important species germination process can be enhanced to some extent by appropriate pretreatment of seed. Saharan *et al.*

**Table 1. Various morphological parameters of seeds of *A. racemosus* and *S. cordifolia***

| Parameters | *A. racemosus* | *S. cordifolia* |
|---|---|---|
| Seed output $plant^{-1}$ | 820.8±29.89 | 657.6±85.49 |
| Reproductive capacity | 820.8±29.89 | 197.28±25.64 |
| Viability (%) | 100±0.0 | 95±5.0 |
| Colour | Black | Brown |
| Shape | Round | Trigonous |
| Length (mm) | 3.16±0.150 | 2.43±0.094 |
| Breadth (mm) | 3.16±0.150 | 1.94±0.107 |
| Wt. of 100 seeds (g) | 3.611±0.015 | 0.273±0.011 |
| Volume of 100 seeds (cc) | 2.723±0.030 | 0.216±0.015 |
| Density (g $cc^{-1}$) | 1.326±0.012 | 1.265±0.06 |

± = Standard deviation.

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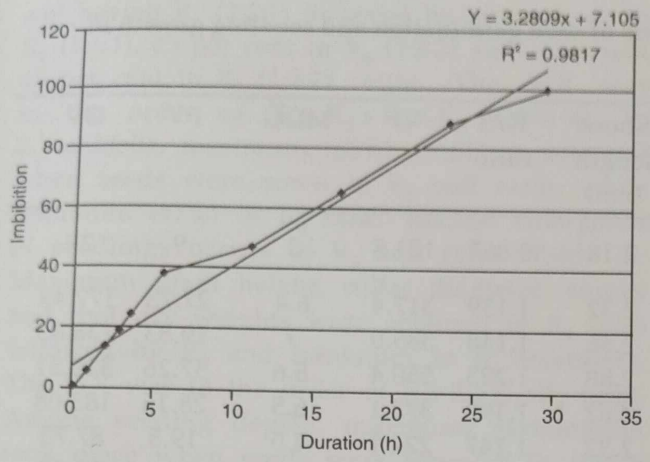


Fig. 1. Seed imbibition (%) in *A. racemosus* during different durations

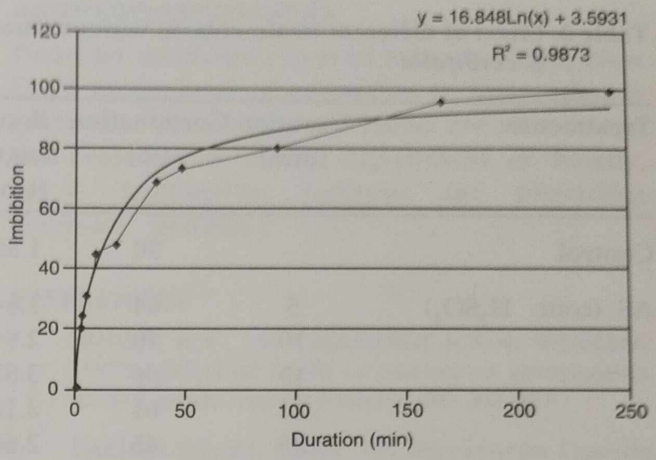


Fig. 2. Seed imbibition (%) in *S. cordifolia* during different durations

**Table 2. Effect of different light pretreatments on various parameters of seed germination and seedling growth in *A. racemosus***

| Light sources | Germination (%) | Root length (cm) | Shoot length (cm) | R/S ratio | Vigour index | MDG | PV | GV |
|---|---|---|---|---|---|---|---|---|
| Alternate light/dark | 100 | 3.46 | 3.86 | 0.897 | 732.0 | 5 | 15.29 | 76.48 |
| Continuous light | 100 | 2.39 | 4.35 | 0.549 | 674.0 | 5 | 16.61 | 83.09 |
| Continuous dark | 100 | 4.15 | 2.56 | 1.621 | 671.0 | 5 | 12.85 | 64.28 |
| CD | NS | 0.266** | 0.271** | 0.111** | | NS | 1.803** | 9.018** |

NS = Non-significant; and ** = Significant at (P<0.01) level.

[11], Gupta *et al.* [12] and Singh *et al.* [13] reported enhanced germination of seeds by providing acid and mechanical scarifications in *Evolvulus alsinoides*, *Thespesia populnea* and *Pongamia glabra*, respectively. Seeds of *S. cordifolia* exhibited 30 per cent germination under controlled laboratory conditions (i.e. without any treatment). Various pretreatments such as acid and mechanical scarifications, growth regulators ($GA_3$ and IAA), etc. were provided for enhancing germination percentage. No promising results were obtained in growth regulator pretreatments; however acid scarification increased the per cent germination significantly. Maximum germination was obtained in 15 min acid treatment and it decreased significantly by further increasing its duration. Highest values for R/S ratio, VI and GV were also observed by 15 min acid scarification (Table 3). The data were significant at (P<0.01) for all these parameters.

Soil is the natural physical and chemical environment of seeds. The depth of seed burial influences the rate and time of seedling emergence [14]. Bahuguna and Lal [15], Saharan *et al.* [16, 17] and Kasera and Shukla [10] observed the effect of different soil ratios and sowing depths on germination behaviour in seeds of *Mallotus philippensis*, *E. alsinoides*, *Commiphora wightii* and *Leptadaenia reticulata*, respectively. In the present investigations, 100 per cent emergence of seedlings were observed at $D_1$ (0.5) and $D_2$ (1.0 cm) depths in *A. racemosus*. Seeds sown at $D_2$ depth show maximum values of SQI followed by $D_1$ and minimum at $D_4$. Among soil ratios experiments, 100 per cent seedlings emerged in

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**Table 3. Effect of different treatments on various parameters of seed germination and seedling growth in *S. cordifolia***

| Treatments | Duration (min) | Germination (%) | Root length (cm) | Shoot length (cm) | R/S ratio | VI | MDG | PV | GV |
|---|---|---|---|---|---|---|---|---|---|
| Control | | 30 | 1.88 | 2.18 | 0.867 | 121.8 | 3 | 9 | 27 |
| AS (conc. $H_2SO_4$) | 5 | 64 | 2.64 | 2.32 | 1.139 | 317.4 | 6.4 | 27.73 | 177.49 |
| | 10 | 70 | 2.94 | 2.56 | 1.148 | 385.0 | 7 | 19.83 | 138.83 |
| | 15 | 86 | 3.52 | 2.88 | 1.223 | 550.4 | 8.6 | 37.26 | 320.51 |
| | 20 | 65 | 3.12 | 2.62 | 1.192 | 373.1 | 6.5 | 28.16 | 183.08 |
| | 25 | 45 | 2.66 | 2.32 | 1.147 | 224.1 | 4.5 | 19.5 | 87.75 |
| | 30 | 20 | 2.08 | 1.88 | 1.110 | 79.2 | 2.0 | 5.66 | 11.33 |
| | 35 | 5 | 1.92 | 1.76 | 1.095 | 18.4 | 0.5 | 2.33 | 1.16 |
| MS | | 40 | 2.88 | 2.62 | 1.101 | 220.0 | 4.0 | 17.33 | 69.33 |
| $GA_3$ ($10mg^{-1}$) | | 30 | 1.92 | 2.18 | 0.881 | 123.0 | 3.0 | 12.0 | 36.0 |
| IAA ($10mg^{-1}$) | | 30 | 1.96 | 2.12 | 0.928 | 122.4 | 3.0 | 13.0 | 39.02 |
| CD | | 0.364** | 0.127** | 0.131** | 0.092** | | 0.036** | 5.310** | 35.856** |

AS = Acid scarification; and MS = Mechanical scarification. **= Significant at (P<0.01) level.

**Table 4. Effect of different treatments on various seedling parameters in *A. racemosus* under nursery conditions**

| Treatments | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | Seedling emergence Days | Percent | Plant height (cm) | Collar diameter (mm) | Dry weight ($g\ plant^{-1}$) Shoot | Root | SQI |
| Soil mixtures: | | | | | | | |
| $R_1$ (1:1:1) | 8 | 80 | 12.06 | 2.88 | 0.466 | 0.116 | 0.0710 |
| $R_2$ (1:2:1) | 9 | 65 | 8.49 | 2.25 | 0.239 | 0.031 | 0.0237 |
| $R_3$ (2:2:1) | 7 | 100 | 13.24 | 2.32 | 0.507 | 0.063 | 0.0415 |
| $R_4$ (1:2:2) | 9 | 75 | 9.07 | 2.26 | 0.274 | 0.034 | 0.0252 |
| CD | 0.700** | 3.363** | 2.008** | 0.044* | 0.015** | 0.007** | 0.0039** |
| Sowing depths: | | | | | | | |
| $D_1$ (0.5 cm) | 6 | 100 | 11.04 | 2.41 | 0.251 | 0.042 | 0.0277 |
| $D_2$ (1.0cm) | 7 | 100 | 10.32 | ,2.47 | 0.212 | 0.075 | 0.0410 |
| $D_3$ (1.5cm) | 8 | 80 | 9.07 | 2.44 | 0.196 | 0.036 | 0.0253 |
| $D_4$ (2.0cm) | 10 | 75 | 8.18 | 2.26 | 0.144 | 0.028 | 0.0199 |
| CD | 1.037** | 2.996** | 0.970** | NS | 0.0236** | 0.0269** | 0.0116** |

NS = Non-significant; *&** = Significant at (P<0.05) & (P<0.01) levels, respectively.

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soil having $R_3$ (2:2:1) followed by 80 per cent in $R_1$ (1:1:1), 75 per cent in $R_4$ (1:2:2) and minimum 65 per cent in $R_2$ (1:2:1) ratio. The SQI was in the order of $R_1 > R_3 > R_4 > R_2$ (Table 4). In *S. cordifolia*, maximum (65%) seedlings emerged when seeds were sown in $R_3$ soil ratio, while minimum (47%) in $R_4$ ratio. Earliest emergence of seedlings occurs in $R_3$ soil ratio (Table 5). Maximum plant height, collar diameter, shoots and root dry weights were obtained in $R_3$ ratio followed by $R_2$ and minimum in $R_4$ treatment. The SQI was in the order of $R_3 > R_2 > R_1 > R_4$ ratio. Among seeding depths, maximum germination took place when seeds were sown at $D_1$ depth and minimum at $D_4$. Seedlings emerged earliest when the seeds were sown at $D_1$ depth. It decreased with increasing sowing depth. Maximum plant height, collar diameter, shoot and root dry weights and SQI were observed at $D_1$ depth. The data were significant at ($P<0.01$) level for all these parameters. Thus, it can be concluded that sowing of seeds at $D_2$ depth in $R_1$ soil ratio is optimum for *A. racemosus*, while $D_1$ depth with $R_3$ soil mixture ratio for *S. cordifolia*.

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**Table 5. Effect of different treatments on various seedling parameters in *S. cordifolia* under nursery conditions**

| Treatments | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | Seedling emergence Days | Percent | Plant height (cm) | Collar diameter (mm) | Dry weight (g plant$^{-1}$) Shoot | Root | SQI |
| Soil mixtures: | | | | | | | |
| R, (1:1:1) | 8 | 56 | 8.52 | 1.73 | 0.054 | 0.031 | 0.0128 |
| $R_2$ (1:2:1) | 7 | 61 | 8.83 | 1.84 | 0.061 | 0.034 | 0.0144 |
| $R_3$ (2:2:1) | 6 | 65 | 9.16 | 1.91 | 0.068 | 0.038 | 0.0161 |
| R4 (1:2:2) | 9 | 47 | 7.91 | 1.52 | 0.048 | 0.028 | 0.0110 |
| CD | 1.289** | 1.514** | 0.664** | 0.110** | 0.001** | 0.0006** | 0.0006** |
| Sowing depths: | | | | | | | |
| D, (0.5) | 5 | 70 | 8.97 | 2.01 | 0.052 | 0.031 | 0.0136 |
| $D_2$ (1.0) | 6 | 64 | 8.55 | 1.93 | 0.046 | 0.024 | 0.0110 |
| $D_3$ (1.5) | 7 | 58 | 7.94 | 1.84 | 0.041 | 0.018 | 0.0089 |
| $D_4$ (2.0) | 8 | 46 | 7.25 | 1.63 | 0.037 | 0.011 | 0.0062 |
| CD | 1.193** | 1.289** | 0.822** | 0.100** | 0.0006** | 0.0017** | 0.0012** |

**Significant at ($P<0.01$) level.

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