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Comparative studies on seed germination and anatomy in *Withania somnifera* and *Sida cordifolia* (L.)

Narendra Choudhary* and P. C. Trived

Department of Botany, University of Rajasthan, Jaipur. Rajasthan

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ABSTRACT

The effects of dry-heat and seed soaking in distilled water on germination performance of W. somnifera and Sida cordifolia L. were studied. Dry-heat pretreatments for several times (1-60 min) significantly increase germination percentage. The highest germination was obtained at 1000 C (80% and 83%, respectively). A significant increase in germination rate was achieved under different pretreatments. The physiological dormancy caused by an impermeable seed coat can be overcome by dry-heat pretreatments. Further micrography and anatomical studies of both the plants were also made.

Introduction

Withania somnifera L. (Dunal) belongs to family Solanaceae and classically known for its rejuvenate benefits. It has been recently referred as Indian ginseng for its reputed restorative benefits. The wild plant is generally an erect perennial branched large herb, grows approximately up to a height of one meter. The plant is used for the treatment of tuberculosis, rheumatism, inflammation, and a potential antitumor agent (Suffness & Dourous, 1982) . *W. somnifera* L. was also used to inhibit the development of tolerance and dependence on chronic use of various phytotropic drugs (Gupta & Rana, 2007). The tribal especially *Bheel* and *Garasodia* give root powder orally to the male patients of asthma and bronchitis (Singh & Pandey, 1998). There are some reports of *in vitro* regeneration using shoot tip culture (Anand et al., 2011; Edward et al., 2012, Ambetkar, et al., 2012)

Sida cordifolia L. is native of tropical America and subtropical region of both hemispheres. The plant belongs to family Malvaceae. It is a small shrub, minute star shaped hairs are present all over the plant. The bark of the root is very efficacious in curing cases of facial paralysis and sciatica when caused by the inflammation of the nervous system. The roots are considered diuretic and depressive. They are given in the treatment of gonorrhoea and during worm infestation (Sharma et al., 2007).

Seed coat-imposed dormancy associated with hardness and impermeability to water has been suggested as the most important causes of the primary dormancy (Nadal et al., 2002). Stratification, scarification and gibberellins have a promotive effect on the germination of many species of angiosperms and gymnosperms (Tilki, 2004; Tilki & Cicek, 2005; Esen et al., 2007). Nevertheless, these methods vary from one species to the other, accentuating the need for formulating species-specific treatments. Modern hybrid seedlings are normally derived from crosses of two high-quality parental varieties. In general, breeding programs are designed to improve the size and color of the flowers, as well as other characteristics such as longevity, stalk length, leaf shape, ease of cultivation or disease resistance (Tang & Chen, 2007).

Hardness and impermeability to water of the seed coat were reported as the most important causes of dormancy in thegenus Cistus (Nadal et al., 2002). Dry heat treatment affects seed germination of various species (Perez-Fernandez et al., 2006) and is also effective to break dormancy, improve germination ability and reduce the mean germination time in various Cistus sp (Perez-Garcia, 1997). Probably dry-heat pretreatments crack the seed coats, particularly the internal layer, with strongly lignified cell wall (Corral et al., 1989).

Materials and Methods

The plant material was collected from the University of Rajasthan, Jaipur. The plant was identified from the Ayurveda Institute, Jaipur. Fresh plant materials were washed thoroughly and leaves were separated from the stem part of *W. somnifera.* Mature seeds of *W. sonmifera* and *S. cordifolia* were collected from wild populations. Collected seeds were cleaned and stored dry at 4^0C. Seeds were immersed in distilled water for 24 hr at room temperature (around 20^oC). To test the effects of dry-heat on breaking coat-imposed dormancy. Seeds were placed in oven at 50, 75 and 100^oC for 10, 20, 30, 40 and 50 min.

The seeds were enclosed in petri dishes on two layers of filter paper moistered with distilled water, then placed in the germination chamber at 15^oC in darkness (Nadal et al., 2002). The seeds were monitored every day and moistered when dry. The criterion for germination was root emergence. Germination percentages for each trial were calculated after 37 days (Nadal et al., 2002). Germination rate was calculated and expressed as peak value (PV), an index of germination speed which is the highest number obtained when germination percentage is divided by the number of days (Czabator, 1962).

Imbibition effect was also studied (Mansour, 1994)

Viability test

Embryo viability was estimated by three methods: In the tetrazolium chloride test (TTC), viable embryos were found to be red in colour after staining due to reduction of 2,3,5-triphenyltetrazolium chloride by respiratory activity in the cells (Nachlas et al., 1960). Individual embryos were incubated in TTC solutions diluted to 1 % and 0.1 % in phosphate buffer (pH 7) for 24 hours in the dark at room temperature.

Tele:

E-mail addresses: ptrivedi33@yahoo.com

Micrograph study was also studied. Analysis of variance (ANOVA) was carried out to determine whether significant differences were present among their treatment under laboratory condition. SEM(±), C.D. At 5%, CV(%) were performed to study the significance of different fluoride concentration on different parameters studied.

For estimating temporal pattern per day and total seed germination were analyzed. Equal number of seeds were taken for control and Colchicine treatment, seeds of each replicate were weighed, marked and then surface sterilized with 0.1 % mercuric chloride solution or soap water for 3 to 5 minutes and then thoroughly washed with distilled water. The seeds were added to the vial tubes and these were allowed to soak in various concentrations viz. 1.0 M, 0.5 M and 0.25 M of Colchicine for 6, 12 and 24 hrs and then kept equidistantly in petriplates

Results

Germination response varied significantly across the treatments. Germination response for different durations and temperatures of dry-heat pretreatments revealed a significant difference. The interaction between temperature and duration was also significant (Table 1 a and b).

Pretreatment of the seeds at three different temperatures for different durations increased germination percentages. In *W. somnifera* germination percentages reached to 39% at 50°C for 40 minutes treatment which was higher than *S. cordifolia* (37%). Germination percentages increased to 45% at 75°C for 30 minutes in *Withania* while it was 41% in *Sida* under same conditions. But the highest germination percentages in *W. somnifera* was reached with dry-heat pretreatment at 100°C for 20min .Maximum imbibitions was observed after 6 hours in both the plants. (Fig 1 a and b)

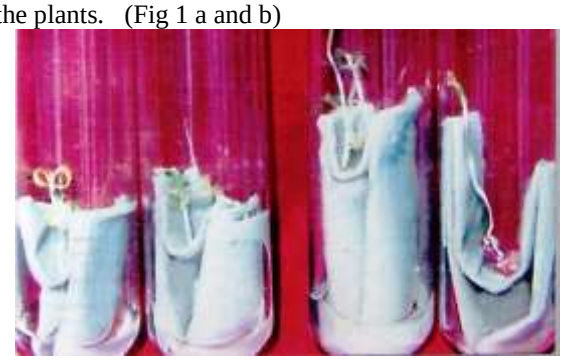

Fig. 1 a Germination of seeds of *W. somnifera*
b Germination of seeds of *Sida cordifolia*

Fig. 2 Anatomical histology of seeds of *W. somnifera*

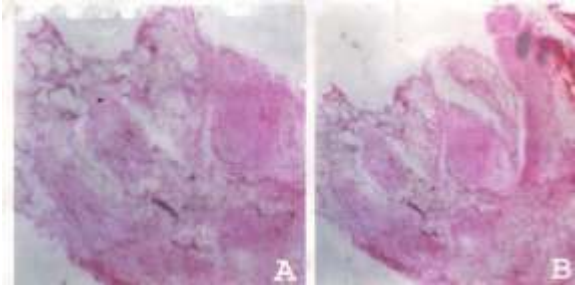

Fig. 3 Anatomical histology of seeds of *Sida cordifolia*

Effect of imbibition on seed germination was studied. In *Withania* maximum activity was at 6 hours which was 55% and in *Sida* it was 51% Table 2)

Seed viability was estimated clearly by the TTC test, but the percentage of doubtful embryos, especially from *W. somnifera* droppings, was significantly higher. In *W. somnifera* about 95% seeds were viable while 4% were non viable and 1 seed remained to be doubtful. In *Sida cordifolia* 2 % of seeds were doubtful and about 12% of the seeds were non viable.. (Table 3)Effect of colchicines on these plants was studied for their germination and survival at various concentrations. Control and Colchicine treated seeds of *W. somnifera* and *S. cordifolia* were studied for the seed characteristics. In control, smaller sized seeds were reported as compared to treated (0.50 M and 0.25 M) but in 1.0 M concentration, Colchicine was reported to have negative impact on seed germination and other Physiological / Cytogenetic characteristics. 1.0 M treated seedlings showed very low frequency of germination and died after six months of germination (Table 4 a and b)

It was observed that both germination and survival were best at 0.5 M dose while lowest at 1.0 M in both the experimental plants.

Discussion

Germination and seedling establishment are critical stages in the plant life cycle. In crop production, stand establishment determines plant density, uniformity and management options (Cheng & Bradford, 1999). In arid and semi-arid environments, the water needed for germination is available for a short period, and consequently, successful crop establishment depends not only on the rapid and uniform germination of the seed.

Soaking in distilled water of seed for 24 hr increased germination in C. ladanifer, C. albidus and C. laurifolius (Corral et al., 1989). In these species the promotive effect of washing can be associated to loosening and detachment of small pieces of the external waxy layer of seed coat (Corral et al., 1989). . Final germination percentages of the untreated seeds were relatively low (5%) and 5, 10 or 30 min dry-heat pretreatment increased germination to approximately 10%.

The present study shows that seeds have seed coat dormancy, in several species of the genus Cistus (Corral et al., 1989; Nadal et al., 2002). Hard seed coats behave as a barrier for water uptake which can be overcome by 1 or 5 min dry-heat pretreatment in C. creticus and 5 min in *C. laurifolius*. Natural forest fire can be an effective dormancy breaking treatment for the seed germination of *C. laurifolius* and *C. creticus.*
Variability may have resulted from different genes caused seeds variability.

Fully matured seeds of terrestrial orchid species from northern temperate regions are much more difficult to germinate in vitro than immature seeds. The main problem in seed germination of these terrestrial species have not been elucidated, various studies regarding germination at seed maturity have been advanced: induction of dormancy through accumulation of inhibitory substances and through increasing embryo impermeability (Stoutamire, 1974; Miyoshi & Mii, 1995)

Results of this study indicate that the TTC test is the simplest and most reliable method to determine viability in the seed dispersal system. The TTC and tests are accepted as methods of testing viability by the International Rules for Seed Testing (ISTA, 1999), although the TTC test is the most widely useful. This method has been successfully used to study germination and seedling growth of a wide range of species (Bhering et al., 2005). However, the majority of these studies are based on

plant species of agricultural and forestry interest. Thus, as far as we know, this is the first study to compare the three most common chemical methods for estimating seed viability from an ecological perspective on a particular seed dispersal system.

The inner integument has often been referred to as carapace' (Yeung et al., 1996). It has a putative role in the control of dormancy. In the present study, the 'carapace' was observed to comprise two layers with the anatomical histology and plant tissues were seen. Increased coloration of the carapace by safranine suggests the accumulation of lignin.

Table – 1a : Effect of heat pretreatments on germination percentage of W. somnifera

Temperature	Germination %					
	Control	10min	20 min	30 min	40 min	50 min
50	12	15	17	23	39	42
75	12	32	38	45	31	29
100	12	45	50	38	29	17

Means in the row followed by the same lowercase letter are not significantly different at p<0.05

Table – 1b : Effect of heat pretreatments on germination percentage of S.cordifolia

Temperature	Germination %					
	Control	10min	20 min	30 min	40 min	50 min
50	12	13	11	19	37	39
75	12	29	32	41	30	23
100	12	37	45	33	25	14

Means in the row followed by the same lowercase letter are not significantly different at p<0.05

Table-2 Effect of Imbibitions on Germination behavior of both plants

Imbibitions hour	Imbibitions% in *Withania*	Imbibitions% in *Sida*
2	12.3	10.1
3	23.4	18.3
4	34.5	31.8
5	45.6	42.4
6	55.6	51.1

Means in the row followed by the same lowercase letter are not significantly different at p<0.05

Similarly some researchers have indicated that the main reason for germination failure was the inhibition of seed water uptake due to a high salt concentration (Mansour, 1994), whereas others have suggested that germination was affected by salt toxicity. (Khajeh-Hosseini et al., 2003).

During laboratory conditions, the best effective concentrations of Colchicine in their various physiological ranges were studied on seed germination and survival percentage. Various workers in different plants have also reported late germination and less survival percentage in Colchicine treated plants. The results of the present findings are in conformity with the findings of Navarro et al. (2006). These findings are consistent with the premise that Colchicine inhibits the germination and survival percentage of this medicinal plant in higher percentage. Colchicine was probably the best materials for general use because they are non toxic to plants in low concentrations and effective in promoting big sized flowers and seeds, while higher concentration was reported toxic in case of armatum.

Table 3. Seed Viability test of W. somnifera and Sida cordifolia by Tetrazolium chloride

Treatments	No. of Viable seeds	No. of Non viable seeds	doubtful seeds
Control	100	0	-
W. somnifera	95	4	1
S. cordifolia	86	12	2

Means in the row followed by the same lowercase letter are not significantly different at p<0.05

Table 4a. Seed germination and survival test of W. somnifera by Colchicine

Concentration	Germination %	Survival %
1.0 M	14	16
0.5 M	26	47
0.25 M	15	43

Table – 4b Seed germination and survival test of Sida cordifolia by Colchicine

Concentration	Germination %	Survival %
1.0 M	13	18
0.5 M	27	46
0.25 M	14	44

Means in the row followed by the same lowercase letter are not significantly different at p<0.05

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