

Effects of Presowing Treatment on Germination and Seedling Vigour in *Dioscorea deltoidea*

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ABSTRACT *Dioscorea deltoidea*, an endangered medicinal perennial climber is recommended for propagation by seeds to save the medicinally rich rhizome. The seeds of this species show poor and erratic germination. Seeds showed photoblastic nature with maximum germination recorded at 25°C. Presowing chilling at 2°C for 60 days resulted in 95 per cent germination, besides registering 5 days early germination. Other treatments like, 2 cycles of wetting and drying, osmoconditioning with -0.8 MPa, permeation with 50 ppm IAA and 100 ppm kinetin to improve seed germination and vigorous seedlings. Hence, presowing treatment of seeds is recommended for enhancing germination and seedling vigour.

Key words: Germination, seedling vigour, presowing treatment, pre-chilling

Dioscorea deltoidea (Dioscoreaceae) is a perennial vine of medicinal importance predominately growing in north-western Himalayas at 1000-3500 m altitude [1]. The rhizome of this herb contain the steroid sapogenin and diosgenin, which are used for preparation of medicines for curing rheumatic diseases, ophthalmic disorders and allergies. *D. deltoidea* is in high demand by pharmaceutical industries. Though, both seeds and rhizomes are used for propagation, but propagation by seeds is recommended to save the medicinally valuable rhizome.

Reports regarding the germination behaviour of some medicinal plant species are available [2-4], however, the work on germination behaviour and vigour of *Dioscorea deltoidea* is scarce. The study on germination behaviour of some related endangered medicinal herbs have indicated the late, erratic and poor germination with substantial loss of viability of seeds [5]. Most of the temperate medicinal and aromatic species are shy seed bearer, therefore, their natural regeneration is poorly affected. In order to encourage the successful commercial cultivation of this endangered species

by seeds, a systematic study on germination behaviour and vigour is essential. Since, presowing treatments have been found very effective in inducing germination and vigour in seeds of various species [4, 6, 7]. Various presowing treatments need to be investigated to optimise its germination and vigour. Therefore, present study was undertaken to understand the germination behaviour of *Dioscorea deltoidea*.

MATERIALS AND METHODS

Fruits of *Dioscorea deltoidea* were collected from its natural habitat i.e., moist dense forests during the months of Oct.-Nov. 2004. Seeds were extracted from the fruits, cleaned, air dried for two weeks, sorted and stored in plastic jars at room temperature (19°C). Uniform and healthy seeds of *D. deltoidea* were disinfected with 0.15 per cent HgCl₂ for 5 min, washed thoroughly with double distilled water to remove traces of HgCl₂, if any. Thereafter seeds were tested for germination and vigour. Germination test was carried out according to ISTA rules [8]. Germination behaviour of seeds was compared in light and dark and at three

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temperatures, i.e., 20°, 25° and 28°C. The procedures for computing emergence index, germination speed and vigour index was adopted as described elsewhere [5]. Root length, shoot length, and fresh and dry weight of seedlings were recorded after 30 days of seed sowing.

Presowing treatments: To stimulate seed germination and seedling vigour, surface sterilized seeds were subjected to various presowing treatments.

Chilling: Seeds were subjected to presowing chilling treatment at -5°, 2° and 5°C for 15, 30, 60 and 90 days.

Hot water: Seeds were immersed in hot water (50°C) for 10 minutes.

Hydration: Seeds were hydrated by soaking in distilled water at room temperature (19°C) for 24 hours.

Wetting and drying: One and two cycles of hydration for 12 hours at 20°C followed by air-drying of seeds.

Osmo-conditioning: Seeds were osmo-conditioned with various concentrations i.e., -0.4, -0.8, -1.0 MPa, each of sucrose, polyethylene glycol, KH_2PO_4 and KNO_3 for 2 days at 15°C. Unconditioned seeds served as the control.

Permeation: Seeds were subjected to hydration with plant growth regulators IAA, IBA, GA_3 and Kinetin separately @ 25, 50 and 100 ppm for 24 hours. Seeds soaked in distilled water served as control.

Each set of experiments was conducted in a completely randomized block design with four replications of 100-seeds each. Treated seeds were allowed to germinate in dual chamber (MAC make) seed germinator in contact with moist substratum at 25°C and 80 per cent RH.

RESULTS AND DISCUSSION

Seeds of *Dioscorea deltoidea* showed hypogeal germination and registered highest germination percentage (80), emergence index (20) and germination speed (5.71) at 25°C (Table 1). Emergence started earliest at 25°C i.e., 4th day from

Table 1. Comparison of germination percentage of seeds of *Dioscorea deltoidea* at different temperatures

Character	Temperature			
	20°C	25°C	28°C	CD _{0.05}
Germination (%)	20.00	80.00	60.00	11.0
Day of emergence	14	4	13	2.01
Day of completion	14.50	14	23	1.00
Emergence index	1.43	20.00	4.60	2.51
Germination speed	1.42	5.71	2.61	0.25
Root length (mm)	25.20	21.66	10.67	1.07
Shoot length (mm)	68.00	60.00	93.67	3.56
Seedling dry wt (mg)	27.00	21.16	21.00	0.89
Vigour index	1360.00	4800.00	5620.00	51.45

seed sowing in comparison to 14th and 15th day from seed sowing at 20° and 28°C, respectively. *Dioscorea* seeds were found to be photoblastic in nature and showed an absolute requirement of light. A significant variation for standard germination, day of emergence, day of completion of germination, emergence index, vigour index and seedling vigour was registered due to presowing chilling treatments (Table 2). Presowing chilling treatments resulted in 43-95 per cent higher germination over the control. Maximum germination (58.33%) was registered by seeds chilled at 2°C for 60 days, which had 95 per cent higher germination as compared to untreated seeds. Advancement (up to 5 days) in germination was induced by presowing chilling treatment. Higher emergence index, vigour index and germination speed was registered by prechilled seeds. Seedling vigour characteristics viz., root and shoot length and seedling dry weight were significantly higher in seedlings obtained from prechilled seeds as compared to control. Further, these seedlings registered 1.5 times higher dry matter accumulation than that of control seedlings. Among the three temperatures 2°C was the most effective prechilling temperature. Maximum vigour index (4055) was observed for seeds prechilled at 2°C for 60 days and at -5°C for 90 days, whereas the minimum vigour index (1440) was registered by control seeds (Table 2).

Table 2. Effects of presowing treatments on germination and seedling vigour of *Dioscorea deltoidea*

Treatment	Germination (%)	Day of emergence	Day of completion	Emergence index	Mean germination time	Vigour index	Root length (mm)	Shoot length (mm)	Seedling dry weight	Germination energy	
A. Presowing chilling treatments											
5°C	Control	30.00	19.67	23.00	1.53	1.32	1440	14.53	48.67	19.91	25.00
	15 days	43.33	16.00	26.00	2.72	1.67	2197	18.00	50.67	19.47	40.00
	30 days	46.67	18.00	28.00	2.60	3.25	2717	23.33	58.33	20.93	45.00
	60 days	55.00	19.00	28.33	2.89	2.89	3288	26.67	60.00	22.67	50.00
	90 days	48.33	19.00	28.33	2.55	1.94	2650	20.67	54.67	20.33	45.00
2°C	15 days	46.67	15.33	25.67	3.04	2.24	2860	25.00	59.33	23.79	45.00
	30 days	51.67	15.67	25.67	3.29	2.01	3350	28.67	65.00	23.77	50.00
	60 days	58.33	14.67	24.67	3.98	2.36	4055	30.00	69.67	29.83	55.00
	90 days	55.00	16.67	26.67	3.30	2.46	3392	23.67	62.67	24.73	50.00
-5°C	15 days	43.33	16.00	25.00	2.72	1.78	2430	20.33	56.00	20.00	40.00
	30 days	45.00	15.33	25.00	2.93	1.80	2540	20.73	56.00	21.23	40.00
	60 days	50.00	16.00	26.00	3.10	1.93	3392	22.50	62.83	23.10	45.00
	90 days	46.67	17.00	26.67	2.74	1.76	4055	20.87	63.07	21.10	42.00
CD _{0.05}	9.21	1.47	1.56	NS	1.43	523.96	7.01	8.51	4.53	6.30	
B. Presowing other treatments											
Hot water 10 min dip	30.00	21.00	23.33	1.44	1.28	1693	19.00	56.67	20.53	30.00	
Distilled water soaking 24 hrs	26.67	14.33	16.00	1.84	1.61	1683	20.67	62.40	25.67	25.00	
GA ₃ 50 ppm	30.00	19.67	23.00	1.52	1.28	1981	18.87	65.73	23.33	30.00	
GA ₃ 100 ppm	50.00	18.67	19.67	2.68	2.53	4217	18.53	85.00	27.42	50.00	
Control	20.0	21.00	21.00	0.95	0.95	1107	14.53	55.33	22.13	20.00	
CD _{0.05}	NS	1.40	NS	NS	0.61	1094.8	NS	7.72	3.12	NS	

Similar findings with the presowing chilling, as a pre-requisite, for the germination in *Gentiana lutea* seeds at 4°C for 30-50 days have been reported [9]. Seeds subjected to presowing chilling will be independent of subsequent temperature conditions [10], and will be able to germinate rapidly with an enhanced germination percentage and seedling vigour. Low temperature influences biomembrane metabolism. Seeds during prechilling would become physiologically advanced by carrying out some of the initial steps of germination, thus improving the subsequent germinability [11].

Table 2 shows the impact of presowing seed treatments on seed germination and vigour. The germination i.e., 50 per cent was registered by seeds subjected to 2 cycles of wetting and drying followed by 30 per cent by 1 cycle, and the hot water treatment for 10 minutes. Above treatments were found effective in inducing early germination and improving the germination speed and energy (Table 2). The beneficial effects of seed pretreatments have been reported earlier [12, 13]. The promoting effects of these presowing treatments on germination percentage may be due to the enhanced uniform hydration of all seed parts

and thus reducing the damage of embryonic axis [14].

Osmoconditioning with different osmolytes proved to be beneficial for improving germination and seedling vigour. Maximum promotion (154% over the control) was registered by seeds conditioned with -0.8 MPa. Osmoconditioning resulted in higher emergence index, however, the days to emergence was not affected. The maximum emergence index (5.28) was registered by seeds

osmo-conditioned with -0.4 MPa KNO_3 , whereas the maximum vigour index (1573.33) was registered by seeds osmoconditioned with -1.0 MPa sucrose (Table 3). The similar findings were reported by Kattimani et al. [15] in *Withania somnifera*, where osmopriming resulted in enhanced germination, vigorous seedlings and higher dry matter accumulation. Osmopriming induced the pregerminative metabolic changes based upon controlled hydration of seeds to a level that prevents actual emergence of radicle [16].

Table 3. Influence of osmoconditioning of seed and permeation with plant growth regulators on germination and seedling vigour in *Dioscorea deltoidea*

Conditioning agent	Conc.	Germination (%)	Day of emergence	Day of completion	Emergence index	Speed of germination	Vigour index	Root length (cm)	Shoot length (cm)	Seedling dry wt (mg)
Sucrose	-0.4 MPa	33.33	12.00	27.00	2.78	1.35	756.67	13.00	23.00	19.00
	-0.8 MPa	43.33	13.33	19.33	3.21	2.24	1373.33	12.67	31.33	16.33
	-1.0 MPa	50.00	11.33	24.67	4.56	1.99	1573.33	13.67	28.67	21.67
PEG	-0.4 MPa	30.00	17.67	20.67	1.73	1.37	866.67	11.33	22.67	15.67
	-0.8 MPa	53.33	11.67	19.67	4.52	2.64	1013.33	11.00	19.00	14.33
	-1.0 MPa	23.33	17.67	18.33	1.36	1.31	600.00	11.33	25.00	14.00
KH_2PO_4	-0.4 MPa	30.00	15.33	24.33	1.94	1.15	733.33	10.67	20.00	22.33
	-0.8 MPa	13.33	16.33	23.33	0.81	0.59	293.33	10.00	20.00	15.00
	-1.0 MPa	53.33	15.00	22.33	3.69	2.46	983.33	10.67	18.67	20.33
KNO_3	-0.4 MPa	63.33	12.00	18.33	5.28	3.46	1516.67	7.67	22.00	23.67
	-0.8 MPa	66.67	13.00	21.00	5.15	3.23	1453.33	13.00	20.67	25.33
	-1.0 MPa	53.33	12.00	17.00	4.44	3.11	1110.00	11.33	22.10	25.00
Control		43.33	14.33	20.00	3.54	2.17	620.00	7.33	13.00	18.67
$\text{CD}_{0.05}$		7.26	1.02	5.47	0.84	0.56	115.00	1.24	2.54	1.11
IAA	25 ppm	73.33	8.67	17.33	8.58	4.24	1251.00	5.54	17.04	14.17
	50 ppm	93.33	8.00	19.67	11.67	5.00	2079.00	6.81	22.36	19.53
	100 ppm	66.67	7.33	15.00	9.17	4.69	1756.00	6.74	25.79	21.75
IBA	25 ppm	73.33	8.00	15.33	9.17	5.02	1248.00	6.70	17.06	16.60
	50 ppm	70.00	10.00	16.67	7.00	4.24	944.60	6.72	13.63	17.96
	100 ppm	63.33	9.33	18.00	6.83	3.55	1486.00	7.61	23.33	17.03
GA_3	25 ppm	76.67	7.67	15.33	10.00	5.19	2015.00	7.00	25.95	25.06
	50 ppm	90.00	7.67	11.33	11.73	8.00	2043.00	5.28	22.85	20.23
	100 ppm	86.67	7.00	16.00	12.70	5.47	2047.00	5.17	23.56	14.67
Kinetin	25 ppm	86.67	7.00	10.67	12.38	8.12	2100.00	7.17	24.58	21.92
	50 ppm	90.00	7.33	15.00	12.32	6.19	2227.00	8.67	24.75	18.17
	100 ppm	93.33	8.00	13.00	11.67	7.38	2035.00	7.00	22.01	17.17
Control		56.67	10.67	18.67	5.33	3.34	840.90	5.49	14.70	14.00
$\text{CD}_{0.05}$		15.67	1.26	NS	2.50	2.06	819.79	NS	NS	4.86

Permeation of seeds with various concentrations of plant growth regulators improved seed germination and seedling vigour, besides inducing early germination (Table 3). The maximum germination (93.33%) was registered by seeds treated with 50 ppm of IAA and 100 ppm of kinetin as compared to 56.7 per cent germination registered by control seeds. Kinetin treated seeds showed significantly higher emergence index i.e., 12.38 as compared to the control (5.33), which was followed by GA₃, IAA and IBA treatments. The seedlings obtained from permeated seeds accumulated higher dry weight as compared to untreated ones. The maximum dry weight was registered by seedlings raised from seeds permeated with 25 ppm GA₃. The growth regulators play a key role in metabolism and enhanced the germination potential of seeds [17, 18]. Enhancement in germination of *Podophyllum hexandrum* by GA₃ and IBA has been reported earlier [2, 19]. The enhanced germination percentage due to GA₃ treatment may be attributed to the enhanced secretion of hydrolases and thus, mobilizing the food reserves from cotyledons to the emerging seedlings and enabled the seeds to germinate early and in higher number.

The study suggests that presowing chilling at 2°C for 60 days; 2 cycles of 12 hrs of wetting and drying at 20°C and osmoconditioning treatments with -0.8 MPa KNO₃ for 2 days at 15°C are economic and practically viable for mass multiplication by seeds. This will be helpful for successful conservation of this endangered species of great medicinal importance.

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