

## Seed germination and seed storage behaviour of *Nardostachys jatamansi* DC., an endangered medicinal herb of high-altitude Himalaya

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**The present communication describes seed germination and storage behaviour of *Nardostachys jatamansi* DC., an endangered medicinal herb of high-altitude Himalaya. The results showed that germination percentage, onset and final germination can be improved with optimum conditions of light, dark, temperature, hormonal treatment, soil composition, depth of seed sowing in soil and sowing months. Under laboratory conditions, 15°C temperature with continuous light and GA<sub>3</sub> @ 100 ppm treatment favoured higher germination. Seed-sowing depth of 0.5 cm in soil, sand and FYM @ 1 : 1 : 1 and 1 : 2 : 1 proportion during October and February in the middle altitude (1800 m) and in May at higher altitudes (3600 m) was found suitable. Seeds stored at room temperature exhibit viability of less than a year, whereas low-temperature storage enhanced it more than two times. It is interesting to note that germination percentage in the seeds collected from different populations showed little difference. Further studies on these populations would be useful to understand the implications.**

**Keywords:** Endangered herb, *Nardostachys jatamansi*, seed germination, seed storage.

*NARDOSTACHYS jatamansi* DC. is a small herbaceous species of family Valerianaceae, commonly known as jatamansi, Indian nard, balchar or spikenard. It is a perennial, dwarf, hairy, rhizomatous medicinal herb and grows in steep, moist, rocky, undisturbed grassy slopes between 3000 and 5000 m asl in random forms. It has a long history of use in ethnomedicine, perfume, incense and modern medicine<sup>1-4</sup>. The species has become endangered due to over-exploitation for medicinal use, habitat degradation and other biotic interferences in its distribution ranges. It has been identified as an endangered species of Northwest Himalaya<sup>5-7</sup>. Due to the high level of threat, Convention on International Trade of Endangered Species (CITES) has notified *N. jatamansi* DC. in its schedule for care. Though studies have been conducted on different aspects of *N. jatamansi*, no information is available on seed germination and seed storage aspects of this species<sup>1,4,6</sup>.

Due to endangered status and poor natural regeneration in *N. jatamansi*, seed germination was tested to propagate the species in its natural habitat at Tungnath (3600 m), in a

nursery at Tala (1800 m) and under laboratory conditions at Srinagar Garhwal (550 m), Uttarakhand (29°26'–31°28'N and 77°49'–80°6'E), India. Different treatments were used with a view to develop complete information on germination, seed viability and vigour of the species under storage conditions. These results will assist in the development of a suitable location-specific cultivation package for this species.

Mature seeds were collected from different alpine regions, viz. Tungnath (TN, 3600 m), Valley of Flowers (VF, 3400 m), Panwali Kantha (PK, 3200 m), Hari Ki Doon (HKD, 3400 m), Dayara (DR, 3500 m) and Kunwari Pass (KP, 3500 m) in October, dried in shade and stored at room temperature. Further, seeds of all populations were divided into two lots. The first lot of all populations was pooled together and seeds of the second lot of each population were stored separately. Moisture content of seeds was determined by oven-dry method<sup>8</sup>, i.e. 103°C for 17 h, and seed viability using viability test<sup>9</sup>. Seed germination was studied both under controlled and field conditions. Pooled seeds were surface-sterilized by dipping them in 0.5% aqueous solution of HgCl<sub>2</sub> for 2 min to discourage fungal infection, washed with 10–15 ml distilled water three times and placed in glass petri dishes (90 mm) on a single layer of Whatman No. 1 filter paper in a seed germinator under laboratory conditions. Every treatment has three replicates of 20 seeds each. Seed germination was observed in (i) continuous, light and dark at different temperature regimes (10, 15, 25 and 30°C), (ii) alternate temperatures (25°C in light for 12 h and 10°C in dark for another 12 h), and (iii) germination in different hormonal treatments, i.e. seeds were dipped in GA<sub>50</sub>, GA<sub>100</sub>, GA<sub>200</sub>, IAA<sub>100</sub> and IBA<sub>100</sub> ppm solutions for 24 h, washed with distilled water and then sown at 15°C with 16 h light and 8 h dark condition. Observations on germination of seeds kept in dark were taken in dull green light.

Under field conditions, observations on seed germination were also carried out to standardize best population and season along with appropriate sowing depth for maximum germination potential. Here, each treatment contained 40 seeds with triplicates in Styrofoam seedling trays inside a polyhouse to standardize best treatments/conditions and season for maximum germination. Pooled seeds from different populations were sown at two soil depths, i.e. 1.0 and 0.5 cm during October in Tala (1800 m) using different soil compositions, viz. soil, sand and FYM @ 1 : 1 : 1, 2 : 1 : 1, 1 : 2 : 1 and 1 : 1 : 2 proportion. These seeds were also sown at Tala during different months, i.e. October, November, January, February and May to test the suitable season for germination. In addition, seeds were sown during October immediately after seed maturation, and during April at the time of commencement of favourable growth season in alpine habitat (Tungnath) under polyhouse and nursery bed conditions. To determine/identify superior population having maximum germination potential as well as better seedling producing capability, seeds of different

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populations, i.e. TN, VF, PK, HKD, DR and KP were sown at 0.5 cm depth in a mixture of soil, sand and FYM @ 1 : 1 : 1 proportion. For this experiment, seeds were sown during October at Tala. A variation in germination potential under different treatments as well as populations was tested using ANOVA.

To observe the impact of storage environment on viability and germination, pooled seeds were divided in two lots (each with two treatments). In the first lot, seeds were stored in a refrigerator (0–4°C) to increase longevity in airtight polythene bags and tin container. The second lot was stored at room temperature (10–35°C) in airtight polythene bags and tin container. Germinability of these seeds was tested (at 15°C temperature with 16 h light and 8 h dark) after every two months up to 2 years to estimate viability and germination loss.

Results indicate that continuous light condition enhanced percentage germination, decreased time of onset of germination and delayed final germination under 10, 15 and 25°C compared to continuous dark. A temperature of 15°C was found optimum and showed 83.33% germination, with 6 days for onset and 22 days for final germination under light condition. It was found optimum even under dark for seed germination and required 8 days for onset and 24 days for final germination (81.66%). However, at 30°C, seed germination considerably decreased and recorded 61.66% in both light and dark condition. Alternate temperature (25°C in light for 12 h and 10°C in dark for

another 12 h) was suitable to achieve maximum (86.66%) germination along with minimum time (18 days) for completion of germination among all treatments (Table 1). Further, variation in germinability in different light and dark conditions and at different temperatures was significant ( $F = 6.86$ ;  $P = 0.05$ ).

Hormonal treatment (Table 2) enhanced germination percentage compared to control (63.33). Highest 90% germination was observed in GA<sub>3</sub>-100 ppm and required 6 days for onset and minimum time of 20 days for completion of germination. Five per cent reduction was observed in germination when the concentration of GA<sub>3</sub> increased or decreased as compared to GA<sub>3</sub>-100 ppm. Treatments of IAA-100 and IBA-100 ppm also increased percentage of germination (88.33) compared to control. However, on the basis of ANOVA, no significant variation was found in germination due to these hormonal treatments.

Among all the soil compositions (Table 3), sowing depth of 0.5 cm showed highest germination with less time required for onset and final germination compared to 1.0 cm sowing depth. Maximum (80%) germination was observed at 0.5 cm depth in soil, sand and FYM in equal proportions. However, germination percentage and onset of germination were almost similar in soil, sand and FYM in 1 : 1 : 1 and 1 : 2 : 1 proportions with 0.5 cm soil depth, but completion of germination took less time in soil, sand and FYM in 1 : 2 : 1 proportion. As stated above,

**Table 1.** Seed germination at different temperatures in continuous light and dark conditions

Temperature/ light or dark	Germination percentage	Days required for onset of germination	Days required for completion of germination
Control	63.33 ± 7.63	6	22
10°C, Dark	78.33 ± 7.63	10	24
10°C, Light	80.0 ± 5.0	8	28
15°C, Dark	81.66 ± 2.88	8	24
15°C, Light	83.33 ± 7.63	6	22
25°C, Dark	76.66 ± 5.77	6	22
25°C, Light	78.33 ± 2.88	6	18
30°C, Dark	61.66 ± 7.63	8	20
30°C, Light	61.66 ± 7.63	6	20
Alternate temperature (25°C in light and 10°C in dark for 12 h)	86.66 ± 7.63	6	18

$F = 6.86$  (significant at  $P = 0.05$ ).

**Table 2.** Effect of different hormonal treatments on seed germination

Treatment	Germination percentage	Days required for onset of germination	Days required for completion of germination
Control	63.33 ± 7.63	6	22
GA <sub>3</sub> -50 ppm	85.0 ± 8.66	6	24
GA <sub>3</sub> -100 ppm	90.0 ± 5.0	6	20
GA <sub>3</sub> -200 ppm	85.0 ± 5.0	6	22
IAA-100 ppm	88.33 ± 7.63	6	22
IAA-200 ppm	88.33 ± 2.88	6	22

$F = 0.48$  (non significant at  $P = 0.05$ ).

**Table 3.** Seed germination at different sowing depths and soil compositions

Proportion of soil : sand : FYM	Soil depth (cm)	Germination percentage	Days required for onset of germination	Days required for completion of germination
S : S : FYM, 1 : 1 : 1	1.0	64.16 ± 20.96	14	30
S : S : FYM, 1 : 1 : 1	0.5	80.0 ± 4.33	12	24
S : S : FYM, 2 : 1 : 1	1.0	63.33 ± 11.54	16	30
S : S : FYM, 2 : 1 : 1	0.5	68.33 ± 16.26	14	24
S : S : FYM, 1 : 2 : 1	1.0	77.5 ± 2.50	13	22
S : S : FYM, 1 : 2 : 1	0.5	79.16 ± 5.20	12	22
S : S : FYM, 1 : 1 : 2	1.0	76.66 ± 18.76	15	24
S : S : FYM, 1 : 1 : 2	0.5	78.33 ± 3.81	14	22

$F = 0.93$  (non significant at  $P = 0.05$ ).

**Table 4.** Germination and viability of seeds of different populations

Site of seed collection	Viability percentage	Germination percentage	Days required for onset of germination	Days required for completion of germination
Tungnath	98.33 ± 2.88	80.0 ± 4.33	11	25
Valley of Flowers	95.66 ± 4.04	76.0 ± 5.65	8	23
Panwali Kantha	95.66 ± 4.04	74.0 ± 2.82	8	21
Har Ki Doon	95.66 ± 4.04	74.0 ± 2.82	8	25
Dayara	95.0 ± 5.0	75.0 ± 3.25	10	24
Kunwari Pass	98.33 ± 2.88	78.33 ± 4.33	10	25

$F = 0.43$  (non significant at  $P < 0.05$ ).

**Table 5.** Seed germination during different months under field condition at Tala (1800 m)

Month of seed sowing	Germination percentage	Days required for onset of germination	Days required for completion of germination
October	80.0 ± 4.33	12	24
November	48.33 ± 10.40	104	128
January	56.66 ± 20.20	58	78
February/March	80.0 ± 13.22	12	32
May	63.33 ± 2.88	10	22

$F = 4.30$  (significant at  $P < 0.05$ ).

germination was almost similar in different depths and no significant variation was found.

Seeds of different populations (Table 4) did not show much variation in germination percentage. Germination percentage was maximum (80) in seeds collected from TN population and minimum (74) in seeds from PK and HKD populations. Minimum 8 days was required for onset and 25 days for completion of germination in most of the populations. Similarly, seed viability among different populations was almost similar (95.0–98.33%). Observations clearly indicated that there was a slight variation in germination of seeds collected from different populations and no significant variation on the basis of ANOVA was detected. Therefore, pooled seeds from all populations were used for germination study in hormonal, light/dark as well as in nursery conditions during the study.

Seed germination during different months (Table 5) at Tala showed that maximum 80% seeds germinated in October and February, with minimum time for onset and final germination. Germination percentage decreased, whereas onset and completion of germination were delayed during other months. There was significant level of variation in

seed germination during different months ( $F = 4.30$ ;  $P = 0.05$ ).

In Tungnath (Table 6), germination was only 11.16% in nursery beds and 20.83% under polyhouse condition if seeds were sown during October, while there was a slight increase in germination (15.83%) in seeds sown during April under nursery condition. Maximum (61.66%) seeds germinated if sown in April under polyhouse condition. However, in nursery bed seeds germinated when temperature increased during May. Onset and final germination were much delayed in seeds sown during October. Further variation in germination were found significant under these conditions ( $F = 4.5$ ;  $P = 0.05$ ).

Seed storage study indicated that moisture content, viability and germination percentage of stored seeds gradually decreased with increase in storage period. Initial viability (98.33%), moisture content (9.40%) and germination percentage (83.33) decreased during storage with different rates depending on storage temperature and the container used (Table 7). Loss of seed viability was faster at room temperature and gradual at 0–4°C (in refrigerator). Loss of seed viability and germination was higher during

**Table 6.** Seed germination under field condition at Tungnath (3600 m)

Month of seed sowing	Germination percentage	Days required for onset of germination	Days required for completion of germination
October (polyhouse)	20.83 ± 3.81	225	245
October (nursery)	11.16 ± 3.81	235	260
April (polyhouse)	61.66 ± 1.44	42	70
April (nursery)	15.83 ± 3.81	54	76

$F = 4.5$  (significant at  $P = 0.05$ ).

**Table 7.** Periodical germination studies of seeds stored under different treatments

Treatment	Percentage	Period after storage (in months)								
		0	3	6	9	12	15	18	21	24
Room (polythene)	Moisture content	9.40 ± 2.69	9.0 ± 1.61	8.60 ± 2.35	7.66 ± 2.31	6.58 ± 1.37	–	–	–	–
		Viability	98.33 ± 2.88	90.47 ± 8.25	78.0 ± 5.0	53.33 ± 7.63	43.58 ± 4.43	–	–	–
	Germination	83.33 ± 7.63	81.8 ± 15.2	70.73 ± 7.63	42.0 ± 4.0	12.5 ± 3.0	–	–	–	–
		Moisture content	9.40 ± 2.69	8.82 ± 1.68	8.43 ± 1.65	7.11 ± 2.11	5.41 ± 0.72	–	–	–
	Viability	98.33 ± 2.88	90.0 ± 2.88	70.0 ± 7.63	53.33 ± 11.5	28.19 ± 4.43	–	–	–	–
		Germination	83.33 ± 7.63	81.11 ± 4.43	66.66 ± 7.66	41.43 ± 7.63	7.69 ± 2.33	–	–	–
Refrigerator (polythene)	Moisture content		9.40 ± 2.69	9.12 ± 1.68	9.0 ± 1.38	8.66 ± 1.65	7.58 ± 2.11	7.32 ± 1.12	7.18 ± 2.26	7.0 ± 1.68
		Viability	98.33 ± 2.88	96.66 ± 2.88	96.66 ± 5.77	93.93 ± 2.88	86.66 ± 11.5	84.33 ± 7.63	83.33 ± 5.77	80.0 ± 5.0
	Germination	83.33 ± 7.63	82.66 ± 2.82	82.11 ± 5.77	80.66 ± 2.30	78.66 ± 5.77	78.33 ± 7.63	75.0 ± 5.77	70.86 ± 8.66	63.33 ± 11.5
		Moisture content	9.40 ± 2.69	9.10 ± 1.50	8.88 ± 3.32	8.68 ± 2.88	7.37 ± 2.27	6.66 ± 1.61	6.33 ± 1.33	5.88 ± 1.02
	Viability		98.33 ± 2.88	96.66 ± 5.77	91.66 ± 5.77	88.88 ± 8.66	82.0 ± 7.63	77.77 ± 5.77	70.0 ± 10.0	62.37 ± 3.15
		Germination	83.33 ± 7.63	80.0 ± 5.0	78.11 ± 5.77	76.66 ± 5.77	70.33 ± 7.63	68.8 ± 8.88	53.33 ± 11.5	46.95 ± 5.78

summer months. Under room temperature, germination percentage decreased up to 7.69% in tin container and 12.5% in polythene bags after one year of storage. Majority of seeds stored at room temperature became nonviable after one year of storage, whereas refrigerated (0–4°C) seeds were viable even after two years of storage. In the seeds stored in the refrigerator, 63.33% germination in polyethylene bag-stored seeds and 36.66% germination in tin container-stored seeds was observed at the end of the second year.

The results of various treatments showed that germination percentage, onset and final germination can be improved with optimum conditions of light, dark, temperature, hormonal treatment, soil composition, depth of seed sowing in soil and sowing months. Seeds germinated within a week of sowing under favourable temperature condition, thus showing no intrinsic dormancy in them. Thus seeds can be sown immediately after collection in October.

Increase in germination percentage with continuous light proved that the seeds are positively photoblastic. In positively photoblastic seeds, light (red light) converts

phytochrome to active Pfr form, which stimulates GA biosynthesis<sup>10</sup>. Earlier onset and higher percentage germination in GA<sub>3</sub> treated seeds may be due to increased activity of hydrolytic enzymes, as reported earlier in few plant species<sup>11,12</sup>.

Under field condition higher seed germination was recorded in soil composition of sand, soil and FYM @ 1 : 1 : 1 and 1 : 2 : 1 with 0.5 cm sowing depth, probably because such a soil mixture was porous with sufficient organic matter, thus minimizing pressure on germinating cotyledons, whereas deep sowing (1.0 cm) decreased germination due to the formation of a thick layer of soil on small seeds. Similar results were also reported for *Commiphora wightii* seeds<sup>13</sup>. In the present investigation germination percentage in the seeds collected from different populations had little difference, whereas difference in the seed germination behaviour of various populations of the same species had been reported earlier<sup>14,15</sup>. The data indicated that October and February/March were favourable for seed sowing in the field at the middle altitude regions

and May at high altitude regions. During these months day–night temperature of the experimental sites varied between 10 and 25°C, probably meeting the optimum temperature requirement of this species and resulting in higher germination with less time for completion of germination. Seeds sown in October at Tungnath could not germinate till May and showed poor germination. This may be due to low temperature conditions prevailing during this period, as the germination increased under polyhouse condition due to a combined effect of increased temperature, moisture and low light intensity. Low germination percentage under natural conditions may be responsible for poor distribution of the species in nature. Use of polyhouse was also supported earlier for seed germination at high altitudes in *Picrorhiza kurrooa*, a high-altitude medicinal plant species<sup>16</sup>. In the present study, percentage of seed germination, days required for onset and final germination showed differences under nursery and laboratory conditions. Such differences were common for many mountain tree species<sup>17</sup>. It has been reported that results of germination in the laboratory almost invariably overestimate field germination<sup>18</sup>.

In several plant species seed viability is lost within a few months of storage at room temperature<sup>19,20</sup>. Seeds of *N. jatamansi* stored at low temperature could maintain viability and germination for more than two years, whereas room temperature-stored seeds become nonviable within a year of storage. Loss of viability of seeds depends upon the time-span and storage condition<sup>21</sup>. Seeds stored in polythene bags showed superiority over tin container in both room and refrigerated condition due to imperviousness against moisture loss. Loss of moisture content is the chief cause of deterioration of seeds under storage condition<sup>22,23</sup>. Superiority of moisture impervious containers was also advocated earlier<sup>20</sup>.

Observations revealed that seeds of different natural populations of *N. jatamansi* have more or less similar germination potential. Under laboratory condition, 15°C, light condition and alternate temperature regimes favoured high germination as well as early onset of germination. Seeds can be stored at low temperature as they remain viable for more than two years. Further, the study also revealed that germinability was maximum at the time of harvesting during October and inside polyhouse during February–March at lower altitude, thus suggesting sowing of seeds during these months. In nursery, under different compositions of soil, sand and FYM, germination was achieved up to 80% and therefore can easily be practised by farmers for raising seedlings for commercial cultivation.

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