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Germination vigour of Himalayan alder seeds under controlled conditions

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Himalayan alder (*Alnus nepalensis* D. Don) is a native tree species prolifically colonizing the nutrient-deficient hostile habitats in eastern Himalaya and hills of Northeast India. It is an important species especially in terms of symbiotic nitrogen-fixation (Sharma & Ambasht 1984, Sharma 1988, Pradhan 1993), colonization of degraded landslide-affected areas (Sharma 1988), as nurse tree in large cardamom plantations (Sharma *et al*. 1994), alleviating soil N and other nutrients status, accelerating of N and P cycle and increasing stand productivity (Sharma *et al*. 1994). However, information relating to germination vigour and viability of seeds of this promising actinorhizal plant species either *in situ* or *in vitro* conditions is lacking. Therefore, this study was designed to investigate germination incidence of Himalayan alder seeds in various growth media under controlled laboratory conditions.

The female catkins of *A. nepalensis* bearing seeds were collected during February 1991 from Upper Shillong 25 years old Himalayan alder forest stand located at 1726 m amsl latitude 25°34 N and longitude 91°56 E in east Khasi hills, Meghalaya. Immediately they were husked manually and the mature and healthy seeds were stored at 4°C for various time intervals or were stored in polybags at room temperature. To evaluate germination incidence/vigour of seeds, various growth media such as sterile water agar of various concentrations and soils from Himalayan alder, Khasi pine stands, soil-sand mixtures in various ratios and pure sand were used. The seeds were withdrawn at 15, 30, 60, 90, 180, 365, 395 and 730 days intervals and were surface sterilized using 10% H_2O_2 for 15 minutes in dark. After thorough washing in sterile water they were allowed to stand overnight at room temperature, transferred individually to sterile cotton plugged 500 ml capacity conical flasks containing thrice steam-sterilised (at 15 psi for 30 min at 24 hours intervals) soil and soil-sand mixture from Himalayan alder and Khasi pine forests. The soils and soil-sand mixtures were stored for at least 15 days before they were used in the experiment in order to allow natural soil microflora to develop. Similarly sterile imbibed seeds were placed on plated solidified sterile agar. The conical flasks and Petridishes with seeds were then kept at controlled laboratory conditions with relative humidity ranging from 50-70%, ambient temperature at 25 1°C day and 19° night, and light intensity of 5000 lux with 12-14 h day and 10-12 h night cycles. To evaluate the effect of alternating temperatures on germination incidence, two sets of alternating temperatures, i.e., 25°C day and 19°C night, and 28°C day and 21°C night were used. Assessment of germination vigour was done after 3 weeks of seed culture. Mortality/ germination ratio of seedlings grown at different day-night alternating temperature cycles was also evaluated.

Data on germination incidence of Himalayan alder seeds presented in Table 1, suggests the importance of cold treatment or stratification of seeds to sustain higher percentage germination. Promotion of germination by chilling treatment may be due to increase in the level of gibberellins which results in overcoming seed dormancy (Frankland & Wereing 1962).

Himalayan alder seeds become fully mature during December-January in Eastern Himalayan and remain attached to mother plant till February. The seeds are then naturally exposed to chilling ambient temperature between 3-10°C during January to March, which is probably enough for breaking the seed dormancy (Strokes 1965). The seeds by virtue of possession of wing-like projections are then wind dispersed. The data suggest that under natural condition the germination potential of the fresh Himalayan alder seeds finding favourable sites within the forest vary between 39-

Table 1. Effect of storage time (days) on germination incidence of stratified and non-stratified Himalayan alder seeds grown on various growth media; First values indicate mean seed germination (%) of Himalayan alder seeds stored at 4°C for various experimental periods and values in parentheses indicate germination incidence of non-stratified seeds.

Growth media used	Percentage seed germination after days of stratification/storage							
	15	30	60	90	180	365	395	730
Himalayan alder forest soil (HA)	31 (24)	42 (20)	40 (20)	34 (10)	21 (6)	5 (0)	3 (0)	0 (0)
Khasi pine forest soil (KP)	36 (30)	45 (25)	47 (25)	35 (10)	25 (8)	5 (0)	2 (0)	0 (0)
Soil (HA) 1:1 sand	35 (26)	46 (27)	44 (27)	35 (11)	25 (6)	7 (0)	3 (0)	0 (0)
Soil (HA) 1:2 sand	36 (29)	49 (30)	49 (29)	43 (14)	29 (9)	8 (0)	4 (0)	0 (0)
Soil (HA) 1:3 sand	39 (30)	59 (28)	54 (30)	45 (17)	34 (17)	7 (1)	4 (0)	0 (0)
Soil (KP) 1:1 sand	36 (30)	41 (32)	39 (33)	36 (22)	24 (19)	9 (0)	3 (0)	0 (0)
Soil (KP) 1:2 sand	39 (37)	45 (39)	41 (35)	42 (21)	32 (18)	9 (0)	5 (0)	0 (0)
Soil (KP) 1:3 sand	37 (41)	60 (45)	49 (45)	47 (23)	38 (18)	11 (0)	5 (0)	0 (0)
Pure sand	65 (51)	67 (55)	63 (49)	51 (27)	47 (22)	36 (2)	20 (2)	3 (0)
Water agar (0.2%)	57 (55)	62 (41)	60 (40)	56 (35)	51 (29)	35 (2)	25 (2)	4 (0)
Water agar (0.4%)	67 (57)	70 (59)	72 (55)	68 (39)	60 (24)	39 (1)	30 (0)	1 (0)
Water agar (0.6%)	74 (63)	92 (62)	90 (57)	82 (31)	72 (25)	46 (2)	37 (2)	3 (0)
Water agar (0.8%)	61 (60)	70 (61)	70 (51)	64 (30)	56 (20)	42 (0)	26 (0)	0 (0)
LSD at 5% level	4.9 (5.2)	5.8 (6.3)	10.2 (4.8)	6.8 (3.9)	5.9 (3.2)	1.2 (1.1)	4.1 (0.2)	- (-)

Table 2. Estimates on effect of two alternating temperature regimes on seed germination incidence, seedling mortality and mortality to germination ratio of Himalayan alder.

Growth media used	Per cent germination 25°C day and 19°C night		Mortality/ germination ratio	Per cent germination 28°C day and 21°C night		Mortality/ germination ratio	Per cent seedling mortality			
	(Cycle A)			(Cycle B)			(Cycle A)		(Cycle B)	
Himalayan alder forest soil (HA)	42	3.1	0.97	39	4.8	1.0	41	2.2	39	3.2
Khasi pine forest soil (KP)	47	5.2	0.61	43	4.2	0.72	29	5.1	34	5.0
Soil (HA) 1:1 sand	45	8.1	0.77	37	7.2	1.0	35	3.9	37	5.2
Soil (HA) 1:2 sand	59	3.8	0.50	55	5.1	0.70	30	6.2	39	4.9
Soil (HA) 1:3 sand	62	3.0	0.39	60	7.4	0.46	24	6.0	28	5.5
Soil (KP) 1:1 sand	52	5.5	0.50	49	5.2	0.44	26	5.5	22	3.2
Soil (KP) 1:2 sand	57	6.2	0.29	55	4.9	0.34	17	5.0	19	2.2
Soil (KP) 1:3 sand	60	7.1	0.35	57	6.2	0.38	21	2.3	22	2.5
Pure sand	67	6.2	0.26	52	7.4	0.57	18	2.2	30	2.7
Water agar (0.2%)	62	5.1	0.50	60	5.9	0.66	31	6.1	40	3.9
Water agar (0.4%)	70	5.9	0.25	54	6.7	0.57	18	3.9	31	3.1
Water agar (0.6%)	92	9.0	0.17	72	10.3	0.40	16	4.1	29	4.2
Water agar (0.8%)	72	6.4	0.29	39	8.1	0.87	21	1.9	34	2.1

Seeds stratified for 30 days at 4°C; S.E.

49% and >60% if it finds freshly exposed open sites. Low seed germination may be attributed to presence of germination inhibiting factors such as polyphenols, and organic acids in Himalayan alder forest soil. Higher seed germination values on water agar and sandy medium substantiate above contention. Sharma *et al.* (1997) found that concentration of polyphenols in Himalayan alder litter is higher compared to other tree species and it inhibits decomposition in initial stages. High level of polyphenols and organic acids in mature Himalayan alder forest might therefore act as limiting ecological factor(s) on seed germination and seedling establishment consequently allowing other successional plant species tolerant to polyphenols to colonize and form climax vegetation.

It is also evident that irrespective of mode of seed storage, the germination vigour decreases exponentially with increasing time interval and reach lowest value after 395 days (Table 1). However, seeds stored continuously at 4°C showed higher percentage germination compared to those without stratification. Therefore, naturally stratified seeds show fair germination potential upto 60 days of harvest after which it decline substantially. Seeds stored at low temperature for 60 days, in contrast possess upto 2 fold higher germination capacity. Complete loss of germination vigour of long term (1 year) room temperature stored seeds may be attributed to deterioration in the quality or physiological state of the embryo present in the seed. However, continuous storage of *Alnus* seeds at low temperature prevent deteriorative process(s) to some extent to sustain higher germination.

An evaluation of alternating high and low temperature cycles on *A. nepalensis* seed germination indicated that temperature of 25°C day and 19°C night was optimal for seed germination. Percentage germination value was lower at 28°C day and 21°C night temperature cycle (Table 2). The promoting effect of alternating temperature cycle in comparison to constant day-night temperature on seed germination of some plant species was earlier reported by Toole *et al.* (1955). Higher percentage germination of Himalayan alder seeds at 25°C day and 19°C night depict lower alternating temperature cycle requirement of the species than those plant species growing in Siwalik and lower elevations. The specific temperature cycle of 25°C day and 19°C night is approximately attained naturally during spring in mid Himalayan range which immediately follows winter when seeds undergo natural stratification. Under the suitable conditions especially newly created habitats such as landslide-affected areas and other open and uncolonized sites, the seeds normally germinate and colonize as pioneer tree species. Thus, the natural abode Himalayan alder is higher altitudes of central and eastern Himalaya ranging from 1000 to 2500 m amsl (Sharma 1988).

The mortality to germination ratio showed a minimal value of 0.17 for 0.6% agar at 25°C day and 19°C night temperature cycle. Suitability of 0.6% sterile water agar as a medium for large scale seed germination of Himalayan alder may be exploited to raise seedlings for effective aforestation of marginal landslide-prone/affected sites.

Seeds of Himalayan alder cultured in dark or low diffused light did not germinate at stipulated experimental time. This indicate its photosensitive nature. Worthington *et al.* (1962), however, found enhanced germination and early survival of red alder on mineral soils in partial or full sunlight. This finding indicate that local alder's natural regeneration under dense canopy where light intensity is negligible, is very poor thus allowing other non-photosensitive plants to establish. Himalayan alder thus by virtue of possession of photosensitive seeds have ability to germinate and grow rapidly at newly-created exposed sites such as fresh landslide-affected sites in the region. Light stimulation of seed germination of Himalayan alder may be controlled by photochrome regulated system. The stimulatory effect of light on seed germination of red alder has been demonstrated previously under laboratory condition and probably results from the regulation of germination by photochrome (Bormann 1983).

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