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ACKNOWLEDGEMENTS. We thank Dr K. N. Shankara and Dr Pranav Desai for encouragement. We also thank Drs D. Sengupta, R. R. Rao and V. Sathyamurthy for stimulating discussions and Dr K. Satheesan, Mr Vihang Bhatt, Mr S. H. Rahman for help in data analysis. This work is supported by the Department of Ocean Development, New Delhi. We thank the Remote Sensing Systems, USA for providing TMI data on their website. The wavelet software was accessed from URL: paos.colorado.edu/research/wavelets.

Received 20 January 2004; revised accepted 29 May 2004

Effect of pre-sowing treatment on seed germination and seedling vigour in *Angelica glauca*, a threatened medicinal herb

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***Angelica glauca* (Apiaceae), endemic to the Himalayas, is an endangered medicinal plant for which *ex situ* cultivation has been recommended as a conservation strategy. However, seeds of this species show poor germination. Among the 14 pre-sowing treatments, KNO_3 (150 mM) and $NaHClO_3$ (30 min) significantly stimulated seed germination and reduced mean germination time under both laboratory and nursery trials, as well as developed seedling vigour under nursery conditions.**

DISTRIBUTED from temperate to alpine belts of Kashmir, Himachal Pradesh (HP), and Uttaranchal, *Angelica glauca* Edgew (Apiaceae), a high-value perennial medicinal herb endemic to the Indian Himalaya[1,2] is now endangered[3–5]. Locally known as 'Chora', it grows between 2000 and 3800 m altitudes in the Himalaya. For unique flavour and stimulating properties, local folk traditionally use its roots as a spice, in constipation and vomiting, and for its cardio-active, carminative and diaphoretic activities, yielding 1.3% essential oil[6]. Fruits are used in flavouring food items[7]. In recent years, high trade has threatened its natural populations. In 1999–2000, HP alone produced about 100 kg essential oil, 30% of which was exported (S. Mohan, pers. commun.). In addition, its natural populations in HP is very low (unpublished), which may be due to poor seed germination. Low seed germination in Apiaceae is known[8–10]. Using seeds of Uttaranchal population of *A. glauca*, Nautiyal *et al.*[9] reported only 8% maximum germination.

An experts' group prioritized *A. glauca* for conservation through *ex situ* cultivation for HP[4]. Enhancing seed germination and developing vigorous seedlings is crucial for this purpose. Pre-sowing chemical treatments have generally been used to enhance seed germination[10–15] and to increase seedling vigour[13,16,17]. The objectives of the present study include: (i) develop effective pre-sowing treatments to stimulate seed germination and seedling vigour and (ii) identify morphological traits for the assessment of healthy seedlings of *A. glauca*.

Seeds of *A. glauca* were collected in early October 2001 from moist, humus-rich riverine forests of upper Parvati valley, Kullu, HP (altitude 2500 m), dried for a week at room temperature (25 ± 2°C) and stored (4°C) before experimentation during the following April. Like many species of Apiaceae[18], seeds of *A. glauca* also experience heavy frost and very low winter temperatures in the wild. The fruits (schizocarp) consist of two one-seeded-mericarps that separate during ripening[19]; we refer to each mericarp as a seed. Thirty dried seeds were used to determine average fresh weight (8.0 ± 2.0 mg), dry weight (7.2 ± 1.8 mg), mean length (11.47 ± 1.12 mm), width (7.06 ± 0.97 mm) and thickness (1.06 ± 0.17 mm). Screening of 3000 seeds showed that 80% were healthy, 19% shrunk and 1% insect-damaged. Healthy seeds were used for all treatments. Thirty seeds were weighed and oven-dried at 80°C for 48 h. The moisture content was found to be 10%. Seed viability was observed both immediately after collection (67%) and at the time of germination tests (57%) using the tetrazolium test[20]. For germination tests, air-dried seeds were disinfected with 0.04% $HgCl_2$ (1 min), washed thoroughly with double distilled water (DW), and dipped in various pre-treatment solutions (24 h, 25 ± 2°C, dark). These include gibberelic acid (GA_3; 25 and 250 μM); 6-benzylaminopurine (BAP; 25, 100 and 250 μM, GA_3 25 μM + BAP 25 μM, GA_3 250 μM + BAP 250 μM, KNO_3 (50, 100 and 150 mM) and $NaHClO_3$ (5% available chlorine for 15, 30 and 45 min). Control was maintained using DW. Treated seeds were washed 2–3 times with DW and placed in petri dishes lined with Qualigens (615 Å) filter paper (three replicates/treatment, 50 seeds/replicate) in randomized design under laboratory conditions (average temperature: 29.02 ± 2.10°C max, 27.8 ± 2.20°C min) and monitored daily. The filter papers were moistened daily using DW. Seeds were considered germinated upon radicle emergence; the first germination was observed after

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four weeks. The final germination was obtained before nine weeks. The mean germination time was calculated by using the relation: $MGT = \sum(fx)/\sum x$, where x is the number of newly germinated seeds on each day, and f is the number of days after seeds were set to germinate[21].

During October 2002, the seeds from the same population were re-collected and stored until nursery tests in the last week of April. For nursery tests, only three best treatments from the laboratory experiment were conducted. Thirty seeds/replicate (three replicates/treatment) were treated with DW, GA_3 (25 μM), KNO_3 (150 mM) and $NaHClO_3$ (30 min) and sown (0.5 cm depth; distance between seeds 4.0 cm) in nursery pots (25 cm^3) in a mixture (pH 6.8) of farmyard manure and sandy-loam soil in equal volume. The experiment was conducted in a completely randomized design under a net-house (1200 m altitude; temperature: 24.71 ± 14.88°C max, 11.12 ± 5.05°C min; RH: 81.53 ± 1.93 max, 74.81 ± 4.09 min) with alternate-day watering. Daily observations recorded the first germination after five weeks of sowing; the first true-leaf emerged after eight-weeks and the cotyledons remained attached to seedlings until leaf maturation. After 15 weeks seedlings were thinned to mitigate competition, maintaining 8–10 cm distance. After 27 weeks, seedling growth was assessed by harvesting five individuals/treatment and different growth parameters, including fresh and dry weights were determined. In *A. glauca*, using seed-sowing, during the first-growing season, plants form only 2–4 compound leaves (three leaflets each), with long petioles arranged as a rosette; the shoot remains suppressed as a highly reduced structure confined to the leaf base, and the main shoot elongation occurs only during the second growing-season (authors' unpublished results). Therefore, the plant height was considered from root collar to leaf lamina, mainly accounting for long petioles, and the root length from collar to the tip of the primary root. The oven-dried weight was obtained by drying seedlings at 70°C to constant weight. Realizing the importance of dry-matter accumulation in seedling health and low MGT as indication of seed vigour and uniform seedling, a method was developed to determine the seedling vigour index as, SVI = dry weight per seedling/MGT × 100. Data were subjected to analysis of variance using SYSTAT program (Yale University, New Haven), correlation coefficient determined by pooling data from all the treatments and the relation amongst different seedling traits was examined.

Among the 14 laboratory treatments in the first year, five significantly stimulated seed germination and reduced MGT over control. However, lower concentration of GA_3 (25 μM) was effective, though only marginally non-significant (Figure 1). Higher concentration of KNO_3 (150 mM) was significantly effective, possibly through oxidized forms of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway[22], whereas $NaHClO_3$ (30 min) significantly increased germination (45.3%), thus confirming its role as a stimulatory agent[11,12,14]. These treatments may help germinating seedlings early, providing them higher competitive ability[23] and hence reducing chances of their mortality.

Under nursery conditions, all three treatments improved seed germination and reduced MGT (Table 1). Untreated seeds started germinating only when other treatments already reached the 90% stage of their final germination. This difference in treated seeds might be due to altered physiology of embryos and liberating enzymes, so that developmental processes occur more rapidly after sowing[13].

The above treatments were stimulatory to overall seedling growth (Table 2). KNO_3 and $NaHClO_3$ significantly enhanced plant height, whereas root length recorded significantly greater values for KNO_3 and GA_3. However, root elongation in other medicinal plants reported was positively influenced[13] by KNO_3 and negatively[24] by GA_3. All treatments enhanced below-ground dry weight, appearing highly influenced by the enhanced root diameter ($r = 0.938$; $P < 0.01$). The treatments significantly enhanced SVI. KNO_3 was the most effective (Table 2), which also stimulated seedling dry weight in *Withania somnifera*[13]. Negative correlation was obtained for seedling dry weight with that of 'days taken for first germination' ($r = -0.664$; $P < 0.01$) and MGT ($r = -0.506$; $P < 0.05$). Thus, early germinated seedlings should be healthier and may be retained during thinning to mitigate competition.

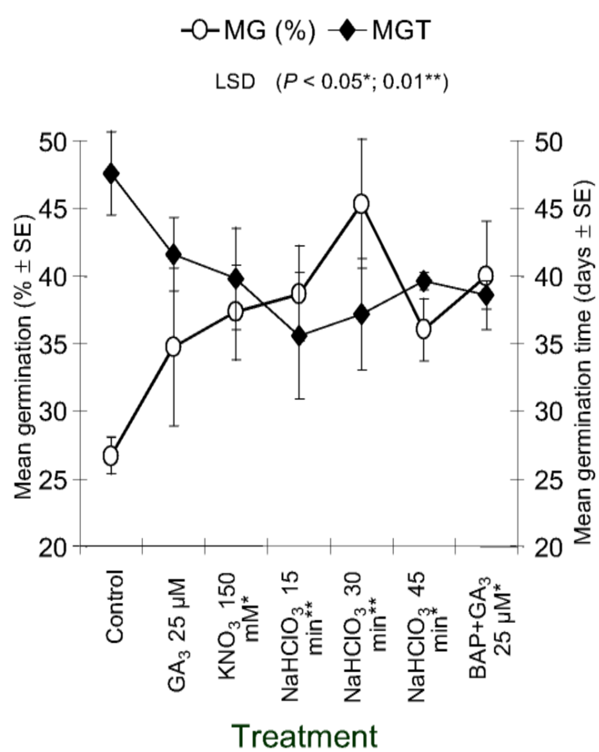

Figure 1. Mean seed germination (MS) and mean germination time (MGT) in *Angelica glauca* using various pre-sowing chemical treatments under laboratory condition, showing only best treatments (mean value ± standard errors).

Table 1. Seed germination in *Angelica glauca* using pre-sowing chemical treatments under nursery condition (net house)

Treatment	No. of days taken for first seed germination	Mean germination (%)	Mean germination (time)
Control	70.7 (5.5)	24.4 (15.8)	94.6 (19.44)
GA_3 (25 μM)	41.0 (1.7)^b	46.7 (0.0)^a	60.1 (6.6)^a
KNO_3 (150 mM)	38.7 (6.7)^c	48.9 (1.9)^a	61.0 (9.9)^a
$NaHClO_3$ (30 min)	44.0 (10.1)^b	50.0 (3.3)^b	74.41 (5.78)^{NS}
LSD			
$P < 0.05^a$	–	16.73	26.59
$P < 0.01^b$	19.75	25.31	–
$P < 0.001^c$	31.73	–	–
F	15.63	6.29	4.50

Values in parenthesis are standard deviation; NS, Non-significant.

Table 2. Effect of pre-sowing chemical treatments on seedling growth of *A. glauca* under nursery condition (net house) after 27 weeks of sowing

Treatment	Plant height (cm)	Root length (cm)	Root diameter (mm)	Above-ground fresh wt (g)	Below-ground fresh wt (g)	Above-ground dry wt (g)	Below-ground dry wt (g)	Seedling-vigour index
Control	26.2 (2.2)	12.0 (1.0)	5.59 (0.38)	1.822 (0.468)	1.016 (0.258)	0.367 (0.091)	0.325 (0.072)	0.733 (0.165)
GA_3 (25 μM)	28.9^{NS} (5.2)	16.0^b (1.3)	6.06^{NS} (0.76)	2.082^{NS} (0.527)	1.986^{NS} (1.085)	0.408^{NS} (0.114)	0.422^{NS} (0.119)	1.408^b (0.365)
KNO_3 (150 mM)	44.8^c (5.1)	16.4^c (1.8)	8.79^c (1.39)	5.463^c (0.483)	3.444^c (0.657)	1.102^c (0.119)	1.124^c (0.322)	3.661^c (0.603)
$NaHClO_3$ (30 min)	39.8^c (3.9)	12.7^{NS} (2.4)	7.93^c (1.02)	3.093^b (1.172)	2.571^a (0.882)	0.769^b (0.314)	0.923^b (0.347)	2.282^c (0.681)
LSD								
$P < 0.05^a$	–	–	–	–	1.13	–	–	–
$P < 0.01^b$	–	3.15	–	1.21	–	0.292	0.435	0.732
$P < 0.001^c$	12.67	4.46	2.24	1.71	2.24	0.410	0.617	1.037
F	18.12	9.482	17.132	35.133	7.705	25.937	14.497	55.541

Values in parenthesis are standard deviation; NS, Non-significant.

Plant height showed positive correlation ($P < 0.01$) with collar ($r = 0.617$) and root diameter ($r = 0.828$), above-ground fresh weight ($r = 0.835$), below-ground dry weight ($r = 0.780$) and SVI ($r = 0.775$). Root diameter ($r = 0.640$), above-ground fresh weight ($r = 0.762$) and below-ground dry weight ($r = 0.667$) were significantly correlated ($P < 0.01$) with SVI. Plant height appeared to be the strongest morphological trait and it was convenient to identify vigorous seedlings of *A. glauca*. During the study period, no insect/pest was seen.

The study suggests KNO_3 and $NaHClO_3$ treatments are economic and easily applicable by nursery workers and poor farmers in developing mass planting stock, over costly plant growth regulators and associated technicalities.

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ACKNOWLEDGEMENTS. We thank the Director of our institute for providing necessary facilities and the CBD Core Head, Dr U. Dhar for encouragement. Financial assistance from the Department of Biotechnology, New Delhi is acknowledged.

Received 1 March 2004; revised accepted 22 May 2004

Phytosociological observations on tree species diversity of Andaman Islands, India

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The Andaman Islands archipelago comprising 204 islands lies in the Bay of Bengal. Physical isolation of these islands has resulted in the evolution of unique floral and faunal components in this Indo-Malayan region. In the present study we have analysed the pattern of tree species diversity, diameter class distribution, species versus girth class relationship, evenness characteristic and similarity parameters of tree populations for different forest types of Andaman. The study reveals the importance of conservation of these biodiversity-rich islands.

THE Andaman group of islands is a treasure-house of distinct floral and faunal elements and home to three culturally unique tribal races, viz. Jarawas, Sentinels and Great Andamanese. Its unique geographical set up, lying in the lap of the Bay of Bengal, has resulted in the evolution of a variety of plant species with high degree of endemism through geological periods. The luxuriant flora shows affinity with Northeast India, Burma and Thailand in the Indo-Malayan region. A century-old cultural history of human occupation in these islands and the indiscriminate utilization of plant resources by humans in recent past, have posed a threat to the plant species. Sustainability of forest biodiversity is critical for the existence of tribal races and overall environmental conditions in these islands, in the face of increasing immigrant population and developmental pressure. Information on the distribution and abundance of tree species is of primary importance in the planning and implementation of biodiversity conservation. The diversity of trees is fundamental to total rainforest biodiversity, because trees provide resources and habitat structure for almost all other rainforest species[1]. The disappearance of tropical forests comes at a time when our knowledge on their structure and dynamics is woefully inadequate[2]. The results of quantitative inventory have enormous significance for the conservation and management of tropical forests[3]. Quantitative inventories help in identification of economically useful species as well as species of special concern, i.e. rare, uncommon and vulnerable species, and thus to quantify conservation worthiness of the candidate sites[4].

The flora of Andaman Islands still remains rather insufficiently known compared to those in other parts of the

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