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Ex situ conservation of *Aconitum heterophyllum* Wall.—an endangered medicinal plant of the Himalaya through mass propagation and its effect on growth and alkaloid content

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Abstract

The endangered alpine plant *Aconitum heterophyllum* was conserved under *ex situ* conditions (greenhouse and open) of Palampur by overcoming seed dormancy and plant establishment through hot water treatment (40–60 °C for 30–120 s) of seeds. High seed germination (88 and 76%) was obtained when seeds were treated with hot water at 45 and 50 °C for 90 s. Correlation studies also revealed that treatment enhanced vegetative growth and reproductive yield of the plants. The total alkaloid content of the roots and rhizomes of the plants growing under *ex situ* conditions was lower than the ones collected from the natural habitat in the 1-year-old plants. However, in the 2-year-old plants, it was almost at par with the ones collected from nature. The life cycle of the plants growing under *ex situ* conditions also did not vary largely from the plants growing under *in situ* conditions. Therefore, the present study indicates the successful adaptation of *A. heterophyllum* plants in conditions other than their natural habitat and hence its potential for sustainable commercialization.

Keywords: aconitin; *Aconitum heterophyllum*; *ex situ* conservation; hot water treatment of seeds; seed germination; seedling growth

Introduction

Aconitum heterophyllum (family *Ranunculaceae*) is an herbaceous, perennial, rhizomatous plant (Nayar and Sastry, 1990) of the alpine and sub-alpine Himalayan regions (2500–4300 m asl). The roots and rhizome of this plant yield di-terpenoid alkaloids including aconitine (Khorana and Murthy, 1968; Mori *et al.*, 1989) which is used in digestive tonics for children, curing dysentery, diarrhoea, vomiting, bilious complaints, periodic and intermittent fevers. The increasing demand for this compound in herbal medicines and health care compounds has led to over-harvesting of the tubers, resulting in rapid depletion of the natural stocks of this valuable plant (Pandey *et al.*, 2000). As a consequence, this plant is now on the list of rare and threatened species. The depletion of native populations is further aggravated by poor regeneration of this plant in nature.

Regeneration of *A. heterophyllum* in nature occurs through asexual and sexual means. Due to prevailing harsh climatic conditions, the flowering and fruiting pattern of this plant is erratic and limited numbers of viable seeds are produced, of which only a few germinate in an

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asynchronous manner (Nautiyal *et al.*, 1985; Pandey *et al.*, 2000). An essential step towards mass propagation and *ex situ* conservation is, therefore, to evolve strategies for high and synchronous germination, and plant establishment through better growth/thriving ability under conditions other than their natural habitat. Therefore, in the present study, attempts were made to germinate and establish *A. heterophyllum* plants in large numbers through appropriate treatments and also to establish them under *ex situ* conditions. The life cycle as well as secondary metabolite production in high altitude plants is bound to be affected when they are grown in conditions other than their natural habitats (Prasad, 2003). Therefore, the vegetative and reproductive cycle and the alkaloid content of the *A. heterophyllum* plants that were germinated and established under *ex situ* conditions were compared with that of the natural population since these are most crucial for sustainable utilization and conservation of endangered medicinal plants.

Materials and methods

Collection of seeds

Mature fruits of *Aconitum heterophyllum* were collected from the sub-alpine regions of Chamba (3000 m asl) Himalaya during the month of August for three successive years (2001–2003). Fresh seeds were separated from their fruits and soaked in distilled water for 24 h. Only the 'sinkers' or the seeds that sank to the bottom were treated with water heated to various temperatures ranging from 40 to 60°C at a difference of 5°C for 30, 60, 90 and 120 s.

Germination and raising of seed progenies

The above-treated and control seeds were germinated in plastic pots (15 × 20 cm) containing potting mixture of sand: soil: farmyard manure (1:1:1 w/w/w) under greenhouse conditions (13 h light/11 h dark, temperature min/max 23/28 °C, relative humidity 65% and irradiance 400 μmol/m^2/s). Data were recorded on percentage seedling emergence (hypocotyl bearing the two cotyledonary leaves) at regular intervals of 15 days until maximum emergence was attained. For each treatment 10 replicates were used with 100 seeds per replicate and each experiment was repeated for three consecutive years.

While about 300 seedlings were allowed to grow further under greenhouse conditions, the remaining 300 seedlings were transferred to the open, natural conditions of Palampur where there was no human interference, in six blocks with 50 seedlings per block. Each block comprised five rows with 10 seedlings in each row. The distance between plants and between rows was about 20 cm. Data were recorded at regular intervals on true leaf emergence, plant growth and time taken to complete its life cycle.

The life cycles of the seed progenies (plants raised from seeds) grown under greenhouse and open conditions were compared with those of the plants growing under natural conditions. The differences in the time taken for each of the vegetative and reproductive stages were recorded to (i) develop a time calendar for identifying the right time for the harvest of rhizomes/seeds and (ii) also for an exact time frame for the life cycle of plants domesticated under controlled conditions for sustainable utilization.

Growth and reproductive yield performance of the plants

In order to determine whether domestication under the controlled conditions of the greenhouse had any effect on growth and reproduction, different growth parameters with respect to emergence of functional or true leaves, plant height, leaf number, leaf area and plant biomass were recorded in plants obtained from both treated and control seeds prior to emergence of functional or true leaves, prior to flowering and fruit set and after fruit/seed set. The relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR) and leaf area duration (LAD) were calculated following the methods of Beadle (1993). Observations on reproductive yield in terms of the number of flowers, fruits, seeds and seed weight were noted. Moreover, the growth performance of the plants in the successive growth seasons after a dormancy period of 4 months was also assessed using parameters like RGR, NAR, LAR, LAD etc. The effect of treatment on the time taken for seed germination, seedling establishment, emergence of functional leaves, attaining maturity, flowering, anthesis, fruit/seed set, fruit dehiscence/seed maturity were noted. The seeds produced from the progenies growing under *ex situ* conditions were further tested for viability, germination and establishment of new populations.

Comparative alkaloid yield in roots/rhizomes of plants of different age growing under natural and ex situ conditions

The total alkaloid content of the roots and rhizomes of the 1- and 2-year-old seed progenies were compared with that of the plants growing under the natural

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conditions of the Chamba district of Himachal Pradesh. For the determination of total alkaloids, roots/rhizomes of 10 plants of different ages growing under greenhouse conditions were dried at 70°C till constant mass was attained. These dried samples were ground to fine powder and macerated in 90% ethanol for 4 h with occasional shaking and percolated twice. The pooled percolates were evaporated to dryness *in vacuo* at <60°C, residue was dissolved in 2% H_2SO_4 and fractionated with chloroform. The chloroform fraction was neutralized with sodium carbonate (pH 5.0) and dried *in vacuo* following the method of Prasad (2000). This was further dissolved in 0.05 N H_2SO_4 and titrated with sodium hydroxide using phenolphthalein as an indicator. The total alkaloid content was determined by deducting the number of millilitres of the alkaline solution required from 5.625 and multiplying the difference by 0.3127 (Remington and Horatio, 1918).

Statistical analysis

Differences in seed germination, growth and reproductive yield in response to different treatments were assessed using a randomized block design and differences among means were tested against critical difference $P < 0.01$ (Gomez and Gomez, 1984). Correlations between the vegetative and reproductive variables were assessed with a Pearson product moment correlation procedure (Thompson, 1992).

Results

Effect of hot water treatment on seed germination

Seed germination of *A. heterophyllum* was found to be responsive to specific temperature and duration of treatment as neither lower temperature of longer duration nor higher temperature of shorter duration seemed effective in enhancing seed germination (Table 1). The control seeds failed to germinate beyond 34% even after 60 days. Of the different treatments that were tested, only six were effective in enhancing seed germination (Table 1). Highest germination (88%) was obtained in the seeds treated with water at 45 °C for 90 s followed by 50 °C for 120 s (76%). In general, effective germination occurred after 45 days in almost all treatment combinations (Table 1). Statistical analysis also showed that germination was significantly higher (at $P < 0.01$) when treated with water at 45 °C for 90 s followed by 50 °C for 120 s as compared to control.

More than 70% survival and true leaf emergence was observed in the healthy, vigorously growing plantlets (seed progenies) in the open, natural conditions of Palampur (Fig. 1A), whereas about 95% survival and true leaf emergence was observed in the plants growing under greenhouse conditions (Table 2; Fig. 1B).

Table 1. Germination responses of *Aconitum heterophyllum* seeds treated with differentially heated water for different durations

Treatments	Germination % after	
	30 days	45 days
Control	24 ± 3.33	34 ± 3.09
40°C (30 s)	18 ± 2.10	26 ± 2.76
40°C (60 s)	18 ± 2.10	38 ± 5.10
40°C (90 s)	38 ± 4.20	52 ± 6.66
40°C (120 s)	10 ± 1.89	26 ± 4.33
45°C (30 s)	16 ± 2.90	36 ± 3.51
45°C (60 s)	16 ± 3.00	40 ± 4.77
45°C (90 s)	58 ± 5.20	88 ± 7.98
45°C (120 s)	12 ± 2.77	28 ± 2.00
50°C (60 s)	14 ± 3.10	24 ± 3.20
50°C (90 s)	28 ± 2.00	76 ± 6.87
50°C (120 s)	18 ± 2.87	40 ± 5.00
55°C (30 s)	36 ± 4.10	44 ± 4.19
55°C (60 s)	18 ± 2.00	34 ± 3.99
55°C (90 s)	16 ± 2.76	34 ± 3.66
55°C (120 s)	4 ± 0.99	18 ± 2.00
60°C (30 s)	18 ± 1.60	28 ± 2.56
60°C (60 s)	20 ± 2.54	46 ± 5.34
60°C (90 s)	12 ± 1.92	36 ± 4.00
60°C (120 s)	6 ± 1.55	30 ± 3.99
$P < 0.01$ Treatments = 7.66, Days = 6.55, Interaction = 11.45		

Values are mean ± SE ($n = 300$) and differences among treatment, days and interaction means are significant at $P < 0.01$.

Comparative life cycle of plants growing under ex situ conditions

Earlier emergence of functional leaves was observed in 80–85% of the progeny from treated seeds (Tables 3A and 4A) as compared to the control. In plants from treated seeds, the percentage flowering plants, and number of leaves, flower buds, flowers, fruits and seeds were higher than others and took less time to bud initiation, flower, fruit, seed set and germination (Table 4A). After the maturation of seeds and dehiscence of fruits, the aerial parts senesced and the rhizomes underwent dormancy (~4 months) both under greenhouse and open conditions. In the successive generation, the effect of the treatment prevailed (Table 3B) and hence there was early sprouting of shoots leading to healthy plants with greater numbers of leaves and higher percentage of flowering plants (Table 4A, B). Viability and germination percentage (<33%) of the seeds harvested from plants from treated seeds were also higher than the control (24%). The time taken to attain each of the growth stages in the plants growing in the open was shorter

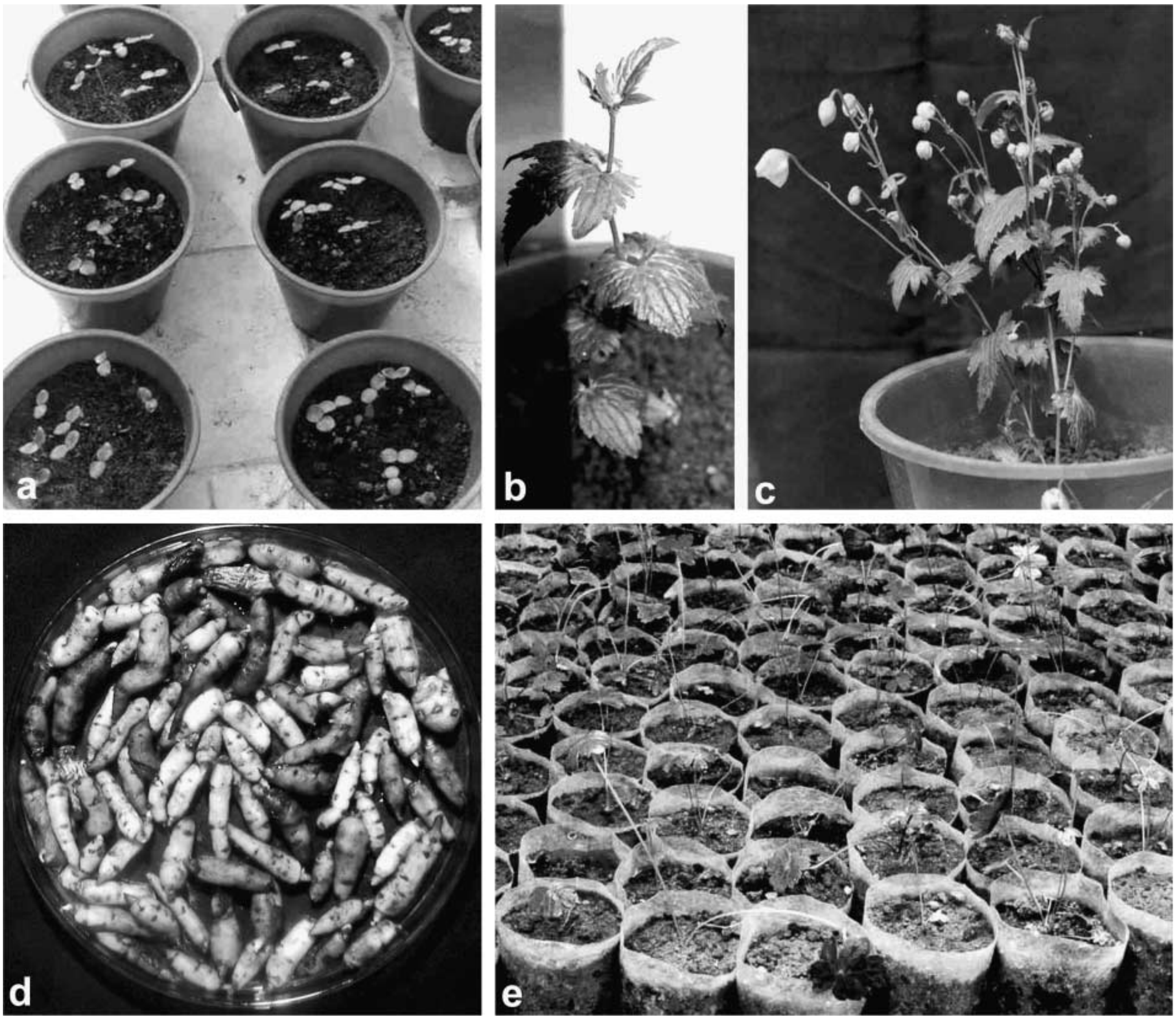

Fig. 1. Greenhouse-grown *Aconitum heterophyllum*: (a) seedlings with persistent cotyledonary leaves; (b) fully grown mature plants; (c) plants with flowers and mature fruits; (d) rhizomes from plants grown under greenhouse conditions; (e) mature plants growing in poly-sleeves in the open.

than the control but longer than those growing under greenhouse conditions (Table 4A, B).

Vegetative growth of seedlings

Under greenhouse conditions, control plants from untreated seeds had leaves with lesser area, minimum RGR, NAR, LAD and biomass as compared to those treated with hot water (45C and 50 °C) for 90 s. Growth and reproductive yield of the plants grown from hot water-treated (45 °C for 90 s) seeds were higher than others (Table 3A). In the successive year after a dormancy period of 4 months, growth as well as the reproductive yield improved significantly as compared to those established in the previous season prior to the onset of dormancy (Table 3A, B).

Table 2. Survival percentage of *Aconitum heterophyllum* plants under open and greenhouse conditions of Palampur

	Survival of vigorously growing plantlets with true leaves (%)	
Year	Open	Greenhouse
2002–2003	80	88
2003–2004	76	80

Vegetative growth versus reproductive yield

A significant positive correlation between leaf area and number of flowers ($r_s = 0.80$, $P < 0.01$) and fruits ($r_s = 0.86$, $P < 0.01$) produced per plant, total number of seeds ($r_s = 0.66$, $P < 0.01$), seed mass ($r_s = 0.67$, $P < 0.01$) and also between net assimilation rate (NAR) and number of flowers ($r_s = 0.81$, $P < 0.01$), fruits

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Table 3A. Growth performance of *Aconitum heterophyllum* plants (generated from seeds subjected to hot water treatment) during the first growing season

	Treatments					
Parameters	Control	40°C (90 s)	45°C (90 s)	50°C (90 s)	60°C (60 s)	$P < 0.01$
Plant with true leaves (%)	35.0 ± 2.10	45.0 ± 2.70	85.0 ± 2.20	88.0 ± 3.90	35.0 ± 2.12	6.76
Plant height (cm)						
Ss	1.82 ± 0.55	2.10 ± 0.88	2.82 ± 0.77	2.40 ± 0.50	2.29 ± 0.98	0.86
Js	3.80 ± 1.10	4.15 ± 1.94	6.36 ± 2.09	6.50 ± 2.11	4.14 ± 1.34	1.10
As	10.90 ± 2.20	11.05 ± 3.0	11.25 ± 3.09	11.60 ± 2.98	11.10 ± 1.21	1.12
No. of leaves per plant	6.00 ± 1.09	8.00 ± 2.12	9.00 ± 2.11	8.00 ± 1.09	7.00 ± 1.56	0.90
Leaf area (cm^2)	4.00 ± 1.33	4.00 ± 1.0	5.70 ± 1.02	6.00 ± 1.03	5.10 ± 2.01	0.88
RGR (mg/g/month)	115.00 ± 5.09	117.00 ± 5.65	118.00 ± 10.0	120.00 ± 10.98	116.00 ± 11.55	2.00
NAR (mg/m^2/month)	101.00 ± 3.98	103.00 ± 6.01	106.00 ± 9.09	109.00 ± 13.00	104.00 ± 7.90	2.20
LAD (m^2/month)	40.70 ± 2.60	41.20 ± 3.02	60.10 ± 8.99	64.70 ± 6.66	52.90 ± 4.78	2.90
Flowering plants (%)	40.00 ± 2.00	40.00 ± 4.44	50.00 ± 7.65	50.00 ± 2.50	44.00 ± 3.55	2.45
No. of flower buds per plant	17.50 ± 1.98	18.00 ± 1.99	24.00 ± 3.56	20.00 ± 2.20	16.50 ± 2.09	1.34
No. of flowers per plant	14.50 ± 2.78	15.00 ± 1.99	18.00 ± 2.32	18.50 ± 1.77	14.50 ± 0.67	1.22
No. of fruits per plant	6.50 ± 1.01	7.50 ± 0.98	9.00 ± 1.77	10.00 ± 1.90	7.50 ± 2.10	0.92
No. of seeds per fruit	65.00 ± 2.90	75.00 ± 5.09	90.00 ± 6.79	100.00 ± 9.40	75.00 ± 2.79	1.01
Seed viability and germination (%)	16.00 ± 2.72	26.70 ± 4.01	33.30 ± 2.94	33.30 ± 3.45	20.00 ± 1.09	1.12
Seed mass (mg) per 100 seeds	185.0 ± 4.99	188.0 ± 9.20	200.00 ± 11.04	200.00 ± 14.20	189.00 ± 9.20	8.54

Data represent mean ± SE ($n = 300$ plants per treatment) and differences among means are significant at $P < 0.01$. Ss, seedling stage; Js, juvenile stage; As, stage after anthesis.

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Table 3B. Growth performance of *Aconitum heterophyllum* plants in the successive growth season after a dormancy period of 4 months

	Treatments					
Parameters	Control	40°C (90 s)	45°C (90 s)	50°C (90 s)	60°C (60 s)	$P < 0.01$
Plant height (cm)	35.02 ± 2.65	39.16 ± 2.87	42.12 ± 3.32	39.80 ± 3.22	35.02 ± 1.65	1.56
No. of leaves per plant	12.0 ± 1.65	13.0 ± 1.32	14.0 ± 2.65	15.0 ± 3.22	14.0 ± 3.00	0.98
RGR (mg/g/month)	124.0 ± 12.0	126.0 ± 12.0	127.0 ± 9.76	126.0 ± 12.34	124.0 ± 11.67	2.01
NAR (mg/m^2/month)	111.0 ± 8.90	113.0 ± 11.65	116.0 ± 11.32	119.0 ± 11.09	114.0 ± 14.0	4.01
Flowering plants (%)	53.3 ± 2.65	60.0 ± 3.98	70.0 ± 4.98	63.3 ± 3.77	60.0 ± 7.01	3.99
No. of buds per plant	18.5 ± 2.78	20.0 ± 3.12	26.5 ± 2.09	28.5 ± 2.09	20.5 ± 3.65	2.28
No. of flowers per plant	16.0 ± 2.22	18.0 ± 2.55	22.0 ± 3.22	25.0 ± 1.55	16.0 ± 3.0	1.10
No. of fruits per flower	7.0 ± 1.88	9.0 ± 1.55	12.0 ± 1.45	13.0 ± 1.44	9.0 ± 2.01	1.66
No. of seeds per fruit	65.0 ± 4.32	85.0 ± 3.33	100.0 ± 7.45	120.0 ± 11.44	75.0 ± 6.76	12.98
Seed mass (mg) per 100 plants	185.0 ± 11.09	190.0 ± 5.98	205.0 ± 13.45	205.0 ± 13.77	190.0 ± 5.06	13.23
Seed viability (%)	20.0 ± 3.21	50.0 ± 4.02	58.0 ± 4.95	55.0 ± 3.79	30.0 ± 3.99	2.54

Data represent mean ± SE ($n = 300$ plants per treatment) and differences among means are significant at $P < 0.01$.

Table 4A. Calendar for physiological stages of development in *Aconitum heterophyllum* during the first growing season

	Treatments					
Parameters	Control	40°C (90 s)	45°C (90 s)	50°C (90 s)	60°C (60 s)	$P < 0.01$
Days to maximum germination	60.0 ± 2.43	55.0 ± 1.90	50.0 ± 2.00	50.0 ± 2.41	60.0 ± 1.86	4.52
Days to true leaf emergence	90.0 ± 3.10	85.0 ± 1.76	80.0 ± 1.99	83.0 ± 1.98	85.0 ± 2.07	3.85
Days to flower bud initiation	180.0 ± 1.99	175.0 ± 2.98	165.0 ± 1.76	161.0 ± 1.85	170.0 ± 2.83	4.98
Days to flowering (full blossom)	190.0 ± 2.00	190.0 ± 2.90	172.0 ± 3.06	172.0 ± 3.06	180.0 ± 2.00	7.80
Days to fruit set	210.0 ± 3.12	200.0 ± 3.	195.0 ± 3.90	190.0 ± 3.90	200.0 ± 4.32	4.79
Days to seed set	215.0 ± 3.20	205.0 ± 3.33	200.0 ± 4.38	196.0 ± 3.88	208.0 ± 4.02	3.99
Days to seed maturity	250.0 ± 3.87	245.0 ± 4.10	235.0 ± 4.22	230.0 ± 2.61	245.0 ± 4.0	9.11

Data represent mean ± SE ($n = 300$ plants per treatment) and differences among means are significant at $P < 0.01$.

Table 4B. Calendar for physiological stages of development in *Aconitum heterophyllum* during the second growing season after a dormancy period of 4 months

	Treatments					
Parameters	Control	40°C (90 s)	45°C (90 s)	50°C (90 s)	60°C (60 s)	$P < 0.01$
Days to sprouting of shoots	45.00 ± 2.22	35.0 ± 1.43	35.0 ± 1.23	40.0 ± 1.40	40.0 ± 1.41	4.88
Days to bud initiation	135.0 ± 4.30	118.0 ± 4.44	115.0 ± 4.10	115.0 ± 4.12	125.0 ± 4.45	6.88
Days to flowering	155.0 ± 3.98	147.0 ± 3.10	140.0 ± 3.90	145.0 ± 2.76	160.0 ± 4.32	6.90
Days to fruit set	180.0 ± 1.90	178.0 ± 2.65	168.0 ± 3.76	168.0 ± 2.65	175.0 ± 2.98	6.46
Days to seed set	183.0 ± 2.10	175.0 ± 2.20	165.0 ± 3.28	170.0 ± 3.09	188.0 ± 3.00	6.52
Days to seed maturity	228.0 ± 4.44	210.0 ± 3.27	205.0 ± 3.99	208.0 ± 3.67	232.0 ± 5.10	5.04

Data represent mean ± SE ($n = 300$ plants per treatment) and differences among means are significant at $P < 0.01$.

($r_s = 0.80$, $P < 0.01$), seeds ($r_s = 0.79$, $P < 0.01$) and seed mass ($r_s = 0.80$, $P < 0.01$) was observed irrespective of the germination treatments (Figs 1A, B and 2A, B). Significant positive correlation also existed between plant biomass and number of flowers ($r_s = 0.90$, $P < 0.01$), fruits ($r_s = 0.85$, $P < 0.01$), seed number ($r_s = 0.70$, $P < 0.01$) and seed mass ($r_s = 0.69$, $P < 0.01$).

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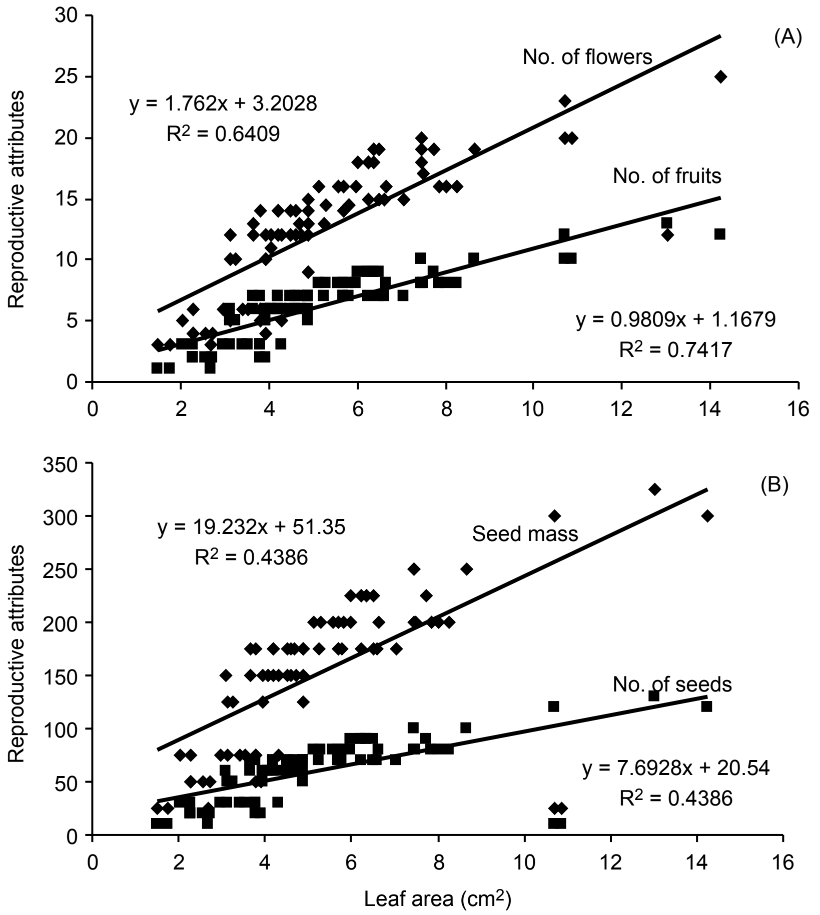

Fig. 2. Number of flowers and fruits (A) and seed number and mass per plant (B) as a linear function of leaf area in *Aconitum heterophyllum*. Slope values are significant at $P < 0.01$.

Comparative alkaloid yield in roots/rhizomes of plants of different age growing under natural and ex situ conditions

The total biomass of the roots/rhizome for 10 different plants after growth for 1 and 2 years was 77.72 and 90.56 g, respectively (Fig. 1D), whereas the average biomass was 7.77 and 9.56 g per plant (fresh mass), respectively. After drying, the biomass was 2.27 and 3.15 g per plant. The total alkaloid content of the dried roots/rhizomes was 1.67 and 2.4% in the 1- and 2-year-old plants, respectively, and this was almost on a par with the content of alkaloids present in the rhizomes collected from Chamba district of the Western Himalayan region (2.9%).

Discussion

Sexual reproduction in *A. heterophyllum* is generally restricted by erratic flowering and fruiting pattern, production of a limited number of viable seeds, long dormancy periods of 8–10 months and poor seed germination. Moreover, after germination, the two cotyledonary leaves of the seedlings further enter into a phase of dormancy and remain quiescent. As a result, the two cotyledonary leaves of the seedlings continue to persist for several months and even up to a few years, resulting in a delay in the emergence of functional leaves (Nautiyal *et al.*, 1985). The present study therefore provides a method for overcoming the above constraints on seed germination and establishment of a large number of genetically diverse plant populations under *ex situ* conditions. Among different temperature and time regimes tested, hot water treatment of seeds at 45 or 50°C for 90 s enhanced as well as synchronized the germination and subsequent plant vigour. Faster and higher germination of the treated seeds also further advanced the different physiological stages of plant development and in turn the reproductive yield. However, the alkaloid content of the rhizomes of the seed progenies growing under *ex situ* conditions (greenhouse or open) was not affected due to this treatment. This was especially important because retaining the original alkaloid content and synchronization of the vegetative growth and reproductive behaviour of the seed progenies under domesticated conditions are key to sustainable commercialization without compromising on the conservation of this endangered medicinal plant. Mass propagation of this plant under greenhouse conditions in a synchronous manner suggested the ease of cultivating these plants under controlled conditions for successful commercialization. This is in contrast to asynchronous germination in nature under *in situ* conditions, wherein a large number of seeds are produced but only a small number attain the ability to germinate progressively over long periods of time (Körner, 2003). This is an adaptive mechanism for maintaining population and survival fitness in plants growing in extreme alpine environments.

Besides bringing about synchrony in seed germination, the hot water treatment was found to be also effective in the initiation of early germination and faster development of *A. heterophyllum* plants. Hot water treatment probably eliminated the inhibitory substances in the seeds through leaching and/or sensitized the seeds towards a metabolic shift (Ross, 1984; Tran and Cavanagh, 1984; Pawlowski and Szczotka, 1997). Enhanced plant growth at a specific temperature was also speculated to be due to mobilization of food reserves towards the development of healthy leaves and for enhanced carbon assimilation. This seems true because the progenies obtained from hot water-treated seeds (Table 3A, B) exhibited higher vegetative growth, leaf area, RGR, NAR, LAD and reproductive yields (greater number of flowers and fruits per plant, seeds per fruit and seed mass). Availability of a good measure of stored resources for reproduction (Weiner, 1988) after hot water treatment and partitioning of photosynthates towards reproductive elements uniformly throughout the reproductive period was further indicated by the positive correlation between leaf area, NAR and reproductive yield (Figs 2 and 3; Table 3A, B). However, the leaves were more effective in producing flowers and fruits than seeds (Fig. 3). Moreover, only a few leaves (the ones in close proximity to the seeds) probably contributed towards providing food material to the developing seeds since the total leaf area supplying assimilates to flowers and fruits was further reduced during seed development.

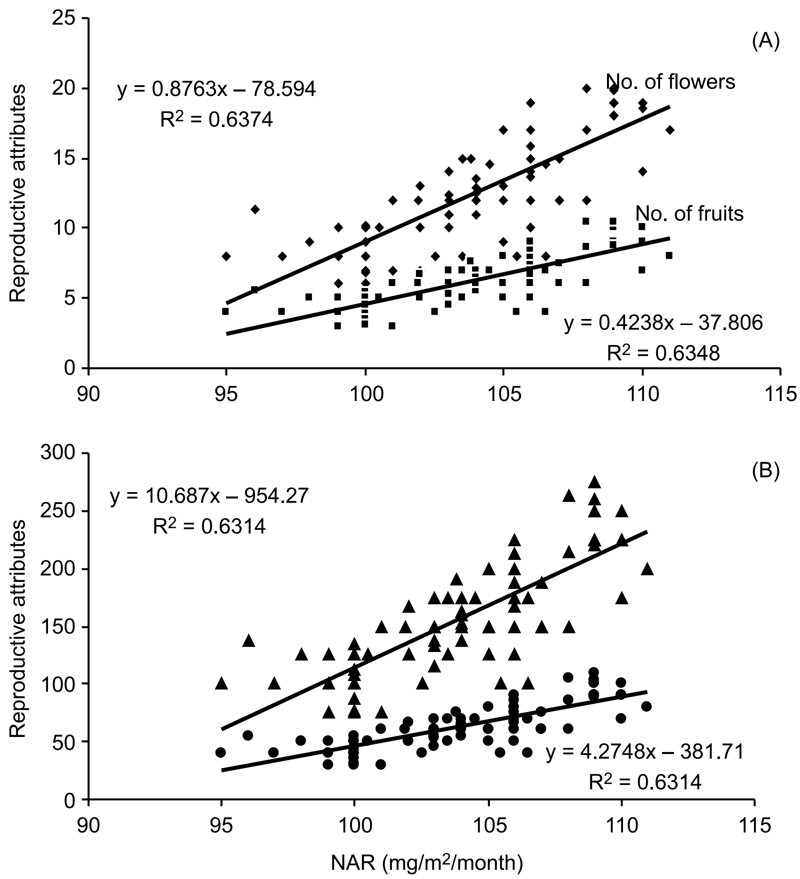

Fig. 3. Number of flowers and fruits (A) and seed number and mass per plant (B) as a linear function of the net assimilation rate (NAR) in *Aconitum heterophyllum*. Slope values are significant at $P < 0.01$.

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Generally, the reproductive behaviour of alpine plants is greatly affected when it commences in conditions other than their natural habitats (Stocklin and Favre, 1994). Thus, when the life cycles of the seed progenies growing under *in situ* and *ex situ* conditions were compared, the total time period required was more (9 months; Fig. 3) in the progenies obtained from treated seeds as compared to the plants growing under *in situ* conditions (about 6 months). This trend persisted in successive growing seasons even after a dormancy of 4 months under greenhouse conditions (Table 3B). The time taken for the completion of the life cycle of the plants growing under *ex situ* conditions of the open, natural conditions of Palampur was slightly longer than their greenhouse counterparts. Since growth and phenology of plants growing under *in situ* conditions (alpine regions) is generally different from that of plants grown under *ex situ* (greenhouse) conditions, the *in situ* plants of *A. heterophyllum* are smaller in size with small, thick leaves, have relatively higher root biomass and generally favour propagation through vegetative means as compared to their *ex situ* counterparts (data not shown).

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