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Full Length Research Paper

Assessment of inter-population variability in *Heracleum candicans* wall with emphasis on seed characteristics and germination behavior

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Heracleum candicans **wall, a Himalayan native and cosmopolitan medicinal plant species being a potential source of Xanthotoxin, has great demand in drug industries. Rampant unsustainable commercial harvesting coupled with other biotic pressures synergistically posed a severe threat to its existence in natural habitats and thus, registered as endangered species of Himalayan region. We assessed this species for its occurrence and availability in natural habitats and especial emphasis was given to understand inter-population variability in this plant with regard to morphological characteristics, biomass and seed germination behavior. Six populations lying along attitudinal gradients (2200 - 3700 m above mean sea level) in two geographically distinct valleys (Parvati and Kullu) in district Kullu, Himachal Pradesh, India, were assessed. Results revealed that the species occurs in different habitats, most preferably in rocky crevices, open dry slopes, humus rich moist areas and along water channels, indicating its wide adaptability and thus, arouses high possibilities for its cultivation in a range of agro-climatic conditions. In spite of endangered status, the plant density is well enough in study area. Further, the species exhibits a high degree of inter-populations variation ($P < 0.05$) prominently in seed size (seed length and width), biomass and germinability which ranged from 14.67 - 82.33% across populations. Based on the assessment, elite population was identified for mass multiplication and cultivation. Overall, the implications of these results in strengthening existing propagation protocol and agro techniques and in formulating effective conservation measures for overall sustainability of the species have been suggested.**

Key words: *Patrala,* medicinal herb, occurrence, availability, conservation, seed, morphology, biomass, germination and Himalaya.

INTRODUCTION

Heracleum candicans wall. (Family-Apiaceae), a Himalayan native medicinal plant species, is found growing in montane and alpine zones of west Pakistan, Nepal, Bhutan, Afghanistan, south-west China and India (Table 1). It is one amongst the rare Himalayan resources and the most valued species of genus *Heracleum* which produces optimum quantity of Xanthotoxin (Kaul, 1989). Almost every part of this plant has unique properties to cure various diseases and its medicinal efficacy is well recognized by indigenous communities as well as modern systems of medicine (Table 2). Being a major source of Xanthotoxin, it has constant demand in pharmaceutical industries, mainly in foreign countries (Europe, America, etc.) and thus, exported from India (BCIL, 1996). The demand of raw

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Table 1. Distribution of *H. candicans* wall.*

World	West Pakistan, Nepal, Bhutan, Afghanistan, South-west China and India along 1800-5000 m asl
India	Himachal Pradesh, Jammu and Kashmir, Sikkim and Uttarakhand
Himachal Pradesh	
Distribution range (m)	1800 - 5000
Average density (ind./m^2)	1 - 1.65
Districts (Locality)	Kullu (Rohtang pass, Banjar valley (Jalodi pass, Hirb and Shojha catchments), Parvati valley (Kanawar Wildlife Sanctuary, Malana), Manali Wildlife Sanctuary, Great Himalayan National Park, Khokan Wildlife Sanctuary); Mandi (Jaidevi forest division, Kamrunag, Jwalapur, Churag valley, Nargu Wildlife Sanctuary); Lahaul and Spiti (Khoksar, Mulling, Trilokinath, Hinsha, Gosal, Tindi, Jalma, Yangla, Gondla, Nalda, Raape, Rasil, Bihadi, Dasrath, Jahlma, Sansha, Pin valley); Chamba (Pangi valley, Bharmour, Dharwas valley); Kinnaur (Sangla valley, Rispa, Sarahan-Chora); Shimla (Rohru forest division and Mahaso) and Kangra (Treuend and Titarcha hills)
Uttarakhand	
Distribution range (m)	2000 - 4000
Average Density (ind./m^2)	1 - 2
Districts (Locality)	Chamoli (Kunwari pass, Rudranath, Bednibugyal, Nanda Devi Biosphere Reserve (Valley of Flowers, Lata), Bakkibugyal, Auli, Garpak, Dronagiri); Rudraprayag (Madmaheswar, Vashukital); Pouri (Bharsar, Binser forest (Chakisain); Tehri (Panwali Kantha); Uttarkashi (Harkidun); Nainital (Mukteshwar, Tipin Top; Bageshwar (Pindari valley); Almora (Morunala Reserve Forest, Near Bhimtal, Doonagiri); Pithoragarh (Ghandhuru) and Dehradun (Mussoorie)
Jammu and Kashmir	
Distribution range (m)	1700 - 3100
Average density (ind./m^2)	2 - 3
Districts (Locality)	Jammu (Khan et al., 2009) reported its distribution between 800 and 1500 m in Sewa river catchment area which is an exception over other reports on distribution ranging from 1700-5000 m); Leh (Drass, Ladakh); Kargil (Kargil); Gandbral (Boniyar, Harwan, Drang, Dachigam Sanctuary); Kupwara (Lolab valley, Machile, Sadhna valley, Budinimal, Mudhum-Zurhuma, Jumgund, Bungus, Keran, Rajwar); Baramulla (Uri, Tangmerg, Gulmerg) and Bandipora (Guraz valley)
Sikkim	
Distribution range (m)	2000 - 3500
Average density (ind./m^2)	1 - 2
Locality	Kangchenzonga Biosphere Reserve

*Based on available reports and extensive surveys of the authors on threatened medicinal plants in Himachal Pradesh, J and K and Uttarakhand.

materials is met exclusively through destructive harvesting of its natural populations. At present, rare and sparse populations of this species have become the worse victim of unsustainable commercial harvesting, changing climatic conditions and other biotic pressures (invasion of alien/weed species, habitat loss, fragmentation, grazing pressure, etc.) prevailing in Himalayan region. The plants, being larger in size with bearing attractive umbels, are easier to locate and identify in the wild habitats and unfortunately, the whole plant is harvested just before seed set which poses major threat on plant survival. Moreover, long vegetative growth phase coupled with low seed viability or deep dormancy in this species (Butola and Badola, 2004; 2006a, b) offer less chances to regenerate and shift in new habitats. An international union for conservation of nature (IUCN) criterion based assessment of Himalayan medicinal plant species has considered and enlisted this species under endangered category for north-west Himalaya (CAMP, 2003). To formulate *in-situ* conservation strategy for a threatened plant species, the information on ecological features mainly distribution range, phytosociology, occurrence, habitat preference, availability, phenology and susceptibility of the species to abiotic and biotic factors (harvesting, grazing, trampling etc.) is essentially required. However, for medicinal plant species having high market demand, *ex-situ* cultivation has been realized as one of the priority steps, both supporting *in-situ*

Table 2. Ecological characteristics, taxonomic description and uses of *H. candicans* Wall.

Local/ trade name	Tukar, Sukar, Chhetaro, Folla, Hakh Bul, Radara, Padara, Tunak, Gojihwa, Tukar, Rasal, Padara, Patrala, Patishan, Patlain, Kakriya, Raswal, Arwa, Hirakali, Gurkrandal, Patrali, Hirwi, Ramthianthen. Hogweed and *Heracleum* in English.
Synonymous or taxonomically similar species	*H. lanatum* Michx., *H. nepalense* D. Don and var. *H. obtusifolium* Wallich ex de Candolle
IUCN-Threat status	Endangered for the Indian Himalayan Region, particularly in northwest Himalaya. The reduction in population of at least 50% over the last 10 years.
Habitats	Open slopes, open meadows, rocky crevices, along water channels, riverbeds, shrubberies, slopy marshy lands and near cultivated fields.
Taxonomic description	Perennial, erect, robust herb; stem 0.5-2 m high, fistular, striate, pubescent. Leaves large, 20-40 cm long, glabrous to pubescent, 1-pinnate or pinnatifid; lobes oblong-lanceolate, 8-12 cm long, irregularly sharply toothed, upper surface usually glabrous, lower densely white tomentose; petioles 6-12 cm long. Flowers pale-white, polygamous, in terminal, 8-15cm across, compound umbels. Rays 15-40, unequal, pubescent, 8.5 cm long. Bracts 0; bracteoles 5-8, linear to lanceolate. Calyx teeth minute, linear. Petals 5, ovate, deply notched, incurved. Fruits 4-12 mm long, pyriform or obovate, minutely pubescent; dorsal and intermediate ridges filiform, lateral winged. Fruit (a schizocarp) consists of two one seeded mericarps that separate during ripening. Each mericarp is a seed.
Medicinal and other uses	Xanthotoxin extracted from roots is widely used in the treatment of leucoderma as a component of sun-tan lotion and in treating skin diseases (eczema, itches, *etc.*), stomach disorders (liver complaints), arthritis and toothache. Fruits are used as aphrodisiac and nerve tonic, and for intestinal parasites. Other uses are for phlegm and wind disorders, earache, bleeding, leprosy, fever due to wounds, and blood pressure. Leaves are used as a good fodder and tender shoots are edible.

conservation in-directly as well in reaching directly to a sustainable supply of raw material (IUCN, 1993). Seeds commonly used as the best and most successful means of cultivation and to produce large numbers of plants inexpensively. It is more pertinent to threatened species in which destructive harvesting of underground parts is considered one of the major threats. If the seeds of target species are introduced artificially, information on seed morphology, germination phenology, dormancy, response to storage and pre-treatments are essential to increase the likelihood of seedling success, crucial for economically viable cultivation. However, it is well known fact that various environmental and edaphic factors are responsible for genotypic and phenotypic variations among populations of a plant species. Inter-population variability in germination percentage invariably limits extensive applicability of standardized seed germination protocol.

Numerous attempts have been made to assess distribution (occurrence, habitat preference, etc.), phytosociology, availability and status of Himalayan threatened medicinal herbs in wild habitats. These species showed a high degree of inter-population variation in morphology, biomass and most prominently in germination behavior of seeds (Bhadula et al., 1996; Singh et al., 1999; Kuniyal et al., 2002, 2003; Nautiyal et al., 2003, 2009a,b: Bhatt et al., 2005, 2006, 2007a,b; Vashistha et al., 2006; Chauhan and Nautiyal, 2007; Bisht et al., 2008; Purohit et al., 2008;) (Table 3). Although, comprehensive packages of practices for propagation and cultivation of *H. candicans* are available (Kaul, 1989; Joshi and Dhar, 2003; Butola and Badola, 2004, 2006a,b; Butola, 2009) but, no attempt has been made to assess such variability in this species. Moreover, there is a paucity of information on occurrence, availability and status of the species in natural habitats and suitable seed storage conditions. Therefore, the present study was carried out on these lines.

MATERIALS AND METHODS

Assessment of populations: occurrence, availability and performance

During April-October 2001, six populations (Madi, Bhandag Thach, Suajani Nala, Chouki God, Dohara Thach and Khod Nala) of *H. candicans* lying along altitudinal gradient from 2200 to 3700 m above mean sea level (amsl) in two valleys, viz., Parvati and Kullu, district Kullu, Himachal Pradesh, India, were assessed (Table 4 and Figure 1). The district is situated in 31° 25` and 32° 35` N latitude and 76° 09` and 77° 09` E longitude along 900 m to >6000 m amsl in the central part of the State. For quantitative assessment of the species, a representative sample plot of approximately 1 ha was selected in each population and 50 random quadrates of 1 x 1 m size were placed. The quadrate was laid only in the place where the selected species was seen. In each population, the total number of mature individuals was also recorded. The populations were identified on the basis of earlier surveys of these areas under medicinal plant conservation programme of our Institutions. Fundamental characteristics of the populations including altitude, habitat type, aspect, slope and associated plant species were recorded (Table 4). The soil samples (composite) from each population were taken to examine their physico-chemical properties following Allen (1974). The density was calculated using the equation:

Table 3. High altitude Himalayan threatened medicinal herbs assessed for inter-population variability in morphology and biomass including germination behavior of seeds.

Species	Study area	Altitudinal range (m)	References
Aconitum atrox (Bruhl) Muk.	Uttarakhand (Tungnath, Valley of flowers, Dayara and Bharnala)	3300 - 3500	(Kuniyal et al., 2002, Kuniyal et al., 2003)
	Uttarakhand (Tungnath, Kilpur, Dayara and Panwali Kantha)	2800 - 3600	(Nautiyal et al., 2009a)
Aconitum heterophyllum Wall.	Uttarakhand (Tungnath, Kilpur, Dayara and Panwali Kantha)	3400-3600	(Nautiyal et al., 2009b)
Angelica archangelica L.	Uttarakhand (Tungnath, Kunwari Pass, Panwali Kantha, Kedarnath, Valley of flowers and Dayara)	2800 - 4000	(Vashistha et al., 2006)
Angelica glauca Edgew.	Uttarakhand (Bharnala, Tungnath, Kunwari Pass, Panwali Kantha, Rudranath, Kedarnath, Valley of flowers and Dayara)	2650 - 4000	(Vashistha et al., 2006)
	Uttarakhand (Sunderdunga and Phurkiya) and Himachal Pradesh (Oonch, Naumor, Solang, Dorah, Fojal and Pulga)	2500 - 3740	(Bisht et al., 2008)
	Himachal Pradesh (Bhandag Thach, Suajani Nala, Tindrabhan, Shat Nala, Khod Nala, Tosh Nala, Tahuk Nala, Chandrabhan and Rawali Dhar)	2200 - 3040	(Butola, 2009)
Arnebia benthamii (Wall. Ex G. Don) Johnston	Uttarakhand (Sainikharak and Kathalia)	3420 - 3875	(Manjkhola et al., 2003)
Nardostachys jatamansi (D. Don) DC.	Uttarakhand (Pindari, Sunderdhunga and Kaphani)	3100 - 4000	(Airi et al., 2000)
	Uttarakhand (Hari Ki Dun, Panwali Kantha, Tungnath, Valley of flowers, Dayara, Kunwari Pass)	3000 - 4000	(Nautiyal et al., 2003)
	Uttarakhand (Tungnath, Valley of flowers, Panwali Kantha, Har Ki Doon, Dayara and Kunwari Pass)	3200 - 3600	(Chauhan and Nautiyal, 2007)
Picrorhiza kurrooa Royle ex. Benth	Uttarakhand (Tungnath, Kilpur, Valley of flowers, Kunwari Pass and Panwali Kantha)	2700 - 3800	(Purohit et al., 2008)
Podophyllum hexandrum Royle	Uttarakhand (Kedarnath, Tungnath, Valley of flowers, Chopta, Ghangaria and Dayara)	2300 - 3700	(Bhadula et al., 1996)
	Uttarakhand (Pindari, Sunderdhunga and Kaphani)	2790 - 3350	(Airi et al., 1997)
	Uttarakhand (Kedarnath, Ghangaria, Tungnath, Chopta, Valley of flowers and Dayara)	2500 - 3700	(Singh et al., 1999)
Swertia angustifolia Ham. Ex D. Don	Uttarakhand (Snow view, Killburry, Jageshwar, Jalna, Deenapani, Majhkhali and Katarmal)	1200 - 2210	(Bhatt et al., 2005, 2007a)
Swertia chirayita (Roxb. ex Fleming) Karsten	Uttarakhand (Kanchula, Kalaseer, Duggalbitha) and Himachal Pradesh (Pullaga and Dohra)	2280 - 2650	(Bhatt et al., 2006, 2007b)

Density = Total number of individuals / Total number of quadrates studied

During September-October, five plants having reproductive stems were randomly harvested from each population and taken to the

Table 4. Ecological features and other details of *H. candicans* populations.

Parameters	Populations					
	Madi	Bhandag Thach	Suajani Nala	Chouki God	Dohara Thach	Khod Nala
Altitudinal range (m amsl)	3200 - 3700	3040 - 3120	2950 - 3020	2680 - 2800	2500 - 2620	2200 - 2470
Aspect	North - east	North - east	North - east	North	South - west	North - east
Slope (°)	40 - 65	20 - 60	45 - 65	45 - 60	20 - 55	45 - 70
pH	6.5	6.7	6.2	5.8	6.3	6.5
Organic carbon (%)	3.05 (0.02)	6.06 (0.06)	7.16 (3.15)	2.09 (0.44)	2.31 (0.07)	3.76 (0.97)
N (%)	0.25 (0.05)	0.44 (0.14)	0.68 (0.12)	0.61 (0.02)	0.14 (0.05)	0.61 (0.09)
Average density (ind./m^2)	1.65 (0.49)	1.40 (0.49)	2.05 (0.57)	1.20 (0.47)	1.25 (0.51)	1.00 (0.52)
No. of mature individuals	121	70	110	87	74	62
Habitat characteristics	Open dry slopes, rocky crevices, between boulders and along roadside	Gaps within scrub, humus rich moist area	Humus rich moist slope along water channels	Open marshy slopes and dominated by herbaceous plants	Moss covered moist rocks, under canopy and shrubberies	High slopes, rocky crevices and along water channels
Major associated species	*Acer acuminatum*, *Betula utilis*, *Quercus semecarpifolia* and *Selinum tenuifolium*	*Abies pindrow*, *Picea smithiana*, *Cedrus deodara* and *Indigofera heterantha*	*Abies pindrow*, *Picea smithiana* and *Pinus wallichiana*	*Quercus semecarpifolia*, *Abies pindrow*, *Polygonum affine*, *P. amplexicaule* and *Viburnum cotinifolium*	*Picea smithiana, Quercus semecarpifolia, Acer acuminatum* and *Aesculus indica*	*Syzygium cumini, Acer acuminatum, Polygonum affine, P. amplexicaule* and *Aralia cachemirica*

*Values in parenthesis are standard deviation.

laboratory. In each population, the plants were selected from all the micro-habitats and subsequently, pooled them representing the whole population. Plant height, number of leaves per plant and number of umbel per plant were recorded. Subsequently, inflorescence of the plants were carefully detached and observed for physical parameters including number of umbellet per umbel and number of seeds per umbellet, and mature seeds intact with the inflorescence were extracted (Figure 2). The underground parts (rhizome and roots) were carefully washed and wiped with tissue paper. Afterward, these parts were chopped into uneven-sized pieces and oven dried at 70°C to constant weight (Butola and Badola, 2006a). Total biomass was obtained by multiplying individual dry weight with stand density of the species (Airi et al., 1997).

Seed physical characteristics: morphology and biomass

Fruits (schizocarp) of the species consist of two one-seeded-mericarps that separate during ripening (Butola and Badola, 2004). Hereafter, each mericarp refer as a seed. One hundred seeds (in triplicate) were randomly selected from each population to determine their morphological characteristics through an electronic Vernier caliper (Model CD-8'C, Mitutoyo, Japan) and biomass through a micro-electronic balance (ANAMED, model MX7301A). To obtain dry weight, seeds were oven-dried at 70°C to constant weight (Butola and Badola, 2006a). Colour of seeds of each population was determined through naked eyes. ISTA (1976) guidelines were followed to determine the seed moisture content using equation:

Moisture content = (Weight of fresh seed – Weight of dry seed) / Weight of fresh seed x 100

Seed storage condition

To determine suitable storage conditions, a lot of 2000 seeds per population were stored in hermetic plastic containers and placed in two different conditions, that is, in refrigerator at 4°C and in room temperature ranging from 20 - 25°C. The seed viability and moisture content (%) were determined immediately after collection and during the time of germination test (after six months) in spring using 100 seeds per replicate (in triplicate) following ISTA (1976). Wings of seeds were excised before immersing in Tz solution (0.5% of 2,3,5, triphenyl tetrazolium chloride). Dark red stained embryos were considered viable. Viability of ungerminated seeds was also examined to know the cause of variable germination among populations.

Seed germination test

The seeds stored at low temperature (4°C) were taken and sort out to remove damage and infested seeds. Fifty seeds (in triplicate) from each population were surface sterilized with 0.04% $HgCl_2$ (1 min) and subsequently, washed thoroughly with double distilled water. These were placed in petridishes (9 cm diameter) lined with Qualigens (615 A) filter paper. The petridishes were placed in BOD (Biochemical Oxygen Demand) incubator at 25 ± 2°C under alternate photoperiods (16 h light and 8 h dark). Distilled water was

Figure 1. Map of study area showing Parvati and Kullu valleys, district Kullu, Himachal Pradesh, India. (Source: www.mapsofindia.com by Compare Infobase Ltd., 2007).

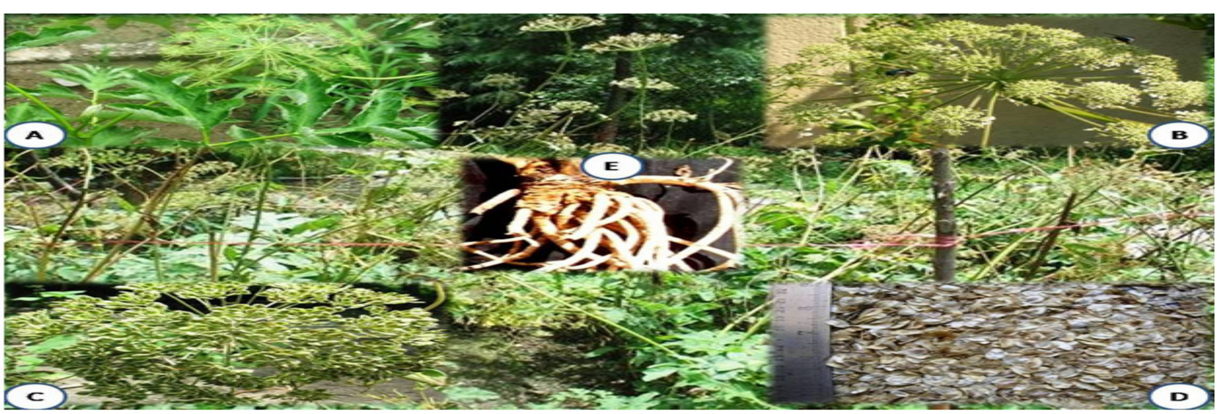

Figure 2. *H. candicans* under cultivation: (A) mature individual (B) inflorescence (C) fruiting (D) mature seeds and (E) rhizome and roots.

used to maintain optimum moisture in petridishes. The seed germination was monitored and recorded on the daily basis. Seeds were considered geminated upon radicle emergence (1 mm). Tiny seedlings were transplanted inside polyhouse for optimum growth and development ahead. Experiment was terminated when no germination recorded up to four weeks. The ungerminated seeds containing a viable embryo and healthy endosperm were considered as dormant seeds.

Data analysis

Data were analyzed statistically using (micro soft) MS excel, 2007. One way analysis of variance (ANOVA) and Fisher's least significant differences (F-LSD; P < 0.05) was employed to calculate significant difference among different populations using means of different parameters (Snedecor and Cochran, 1967). Data in percentages were subjected to arcsine transformation before analysis of variance and then converted back to percentage for presentation. Correlation coefficient was calculated between various parameters.

RESULTS AND DISCUSSION

Ecological characteristics and other details of *H. candicans* populations are presented in Table 4. Barring Chouki God and Dohara Thach, other populations were occurred in north-east facing slopes. Across the populations, the degree of steepness of slope ranged from 20 - 70°. However, the nature of soils was medium to slightly acidic as it ranged from 5.8 to 6.7. Suajani Nala population had maximum organic carbon (7.16%) and nitrogen (0.68%), which otherwise ranged from 2.31 - 6.06% and 0.14 - 0.68%, respectively. Average plant density ranged from 1.0 to 2.05 ind./m^2, which maximized in Suajani Nala. However, projected plant density ranged from 10,000 to 20,500 plants/ha (Figure 3). Maximum numbers of mature individuals were found in Madi (#121) and Khod Nala had least numbers (#62). These results are comparable with Uniyal et al. (2006). They have recorded average density of the species between 1.0 and 1.4 ind./m^2 in steep slopes and undulating meadows of Chhota Bhangal area of Himachal Pradesh.

As many as 11 habitat types were noted across populations, wherein the species preferred to thrive well with rocky crevices, open dry slopes, humus rich moist areas and along water channels. In all populations, the plants were found growing singly and rarely occurred in clumps or patchy form. Fifteen species were found growing as dominant associates, in which *Abies pindrow, Acer acuminatum*, *Picea smithiana, Polygonum affine, P. amplexicaule* and *Quercus semecarpifolia* were common associates among populations. There was no other species of genus *Heracleum* in the study area. The occurrence of the plant in different habitats and soil conditions indicates its wide adaptability and thus, suitable for cultivation in a range of agro-climatic zones. Inter-population variability in morphological characteristics and biomass in *H. candicans* is presented in Table 5. Plant height, number of leaves, number of umbel per plant, above ground and below ground biomass ranged from 66.0 - 120.0 cm (Av. 89.47 cm), 5.0 - 8.20 (Av. 6.43) and 2.7 - 4.7 (Av. 3.50), 23.20 - 53.80 g (Av. 40.77 g) and 18.40 - 57.0 g (Av. 39.88 g), respectively. Maximum plant height and above ground biomass were observed in Khod Nala, however, number of leaves and below ground biomass were observe in Dohra Thach. Plant height, number of leaves and above ground biomass did not vary significantly (P < 0.05) among populations. On the contrary, number of umbel per plant was maximum and significantly (P < 0.05) higher in Suajani Nala as compared to that in other populations. Likewise, the same population stood significantly higher (P < 0.05) than other populations pertaining to number of umbellet per umbel (45.3) and number of seed per umbellet (55.7), which otherwise ranged from 30.3 - 45.3 (Av. 37.89) and 40.3 - 55.7 (Av. 47.17), respectively, across populations. These variations are supposed to be beneficial as it is defined that the visible characteristics of plant species resulting from the interaction of genotype and habitat are evolutionary in nature and may contribute to the maintenance of genetic diversity in plant populations (Allard, 1997).

Availability of projected root biomass in different populations ranged from 220.8 (Chouki God) to 877.7 kg (Dohra Thach), with mean value of 549.6 ± 246.5 kg/ha (Figure 3). These estimations are comparable with Uniyal et al. (2002, 2006) for *Rheum australe*, a threatened medicinal herb resemblance to *H. candicans* in terms of size. Destructive harvesting of underground parts mainly marketable roots is one of the major threats causing rapid declination in populations of Himalayan medicinal plants (Bhadula et al., 1996; Airi et al., 2000). For *H. candicans*, Kaul (1989) has estimated that during 1980 - 85, about 150 tonnes of fresh roots of the plant was harvested every year from the wild sources in Kashmir Himalaya. More recently in Himachal Pradesh (H.P.), 186.0 tonnes in 2004 - 05 and 101.0 tonnes in 2005 - 06 were extracted which was fairly higher than that of 68.3 tonnes in 2002 - 03 (Source: Forest Department, H.P.). In H.P., local people have rights to harvest minor forest produce by paying a certain amount of money as royalty, it is only Rs. 25 per quintal for *H. candicans*.

The seeds of different populations showed significant (P < 0.05) variation in size and biomass (Table 5). Seed size in Suajni Nala with respect to width, thickness and biomass was greater than that of other populations. Seed length, width, thickness, fresh and dry weights among populations ranged from 8.77 - 11.04 mm (Av. 9.70 mm), 6.13 - 7.63 mm (Av. 6.99 mm), 0.60 - 0.74 mm (Av. 0.66 mm), 4.80 - 6.90 mg (6.07 mg) and 4.07 - 6.12 mg (Av. 5.19 mm), respectively. The seed colour differed slightly between the populations with pale-yellowish being common in majority of populations. Among seed traits, seed weight is most important which reflects potential food reserves available to subsequent seedlings and determines the success of individual plant (Westoby et al., 1990). Variations in seed weight between or within plant species are due to evolutionary responses of plants

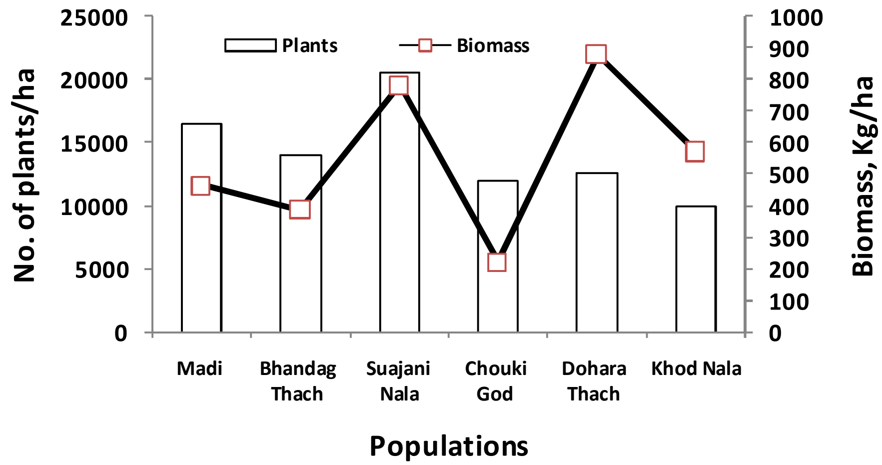

Figure 3. Available root biomass (dry wt.) in different populations of *H. candicans.*

Table 5. Inter-population variability in morphological characteristics and biomass of different parts of *H.. candicans* plants.

Populations	Plant						Inflorescence				Seed			
	Plant height (m)	No. of leaves	No. of umbel/ plant	Above ground dry weight (g/plant)	Below ground dry weight (g/plant)	Total biomass (g/plant)	No. of umbellet/ umbel	No. of seeds/ umbellet	Length (mm)	Width (mm)	Thickness (mm)	Fresh weight (mg)	Dry weight (mg)	Colour
Madi	69.40 (25.46)	7.60 (4.77)	4.3 (0.58)	52.40 (21.42)	28.0 (6.63)	80.40 (26.02)	43.0 (1.00)	52.4 (6.45)	9.80 (0.30)	7.37 (0.11)	0.66 (0.03)	6.10 (0.17)	5.13 (0.15)	Pale-yellowish
Bhandag Thach	66.0 (24.85)	5.0 (1.41)	2.7 (0.58)	37.0 (16.25)	27.60 (14.72)	64.60 (16.30)	40.3 (2.52)	40.3 (1.53)	9.15 (0.16)	6.38 (0.46)	0.60 (0.15)	5.96 (0.25)	5.37(0.33)	Spotted-greenish
Suajani Nala	70 .0 (12.75)	5.60 (0.89)	4.7 (0.58)	31.02 (5.46)	38.09 (19.28)	69.10 (19.41)	45.3 (2.52)	55.7 (3.79)	8.77 (0.45)	7.63 (0.76)	0.74 (0.07)	6.90 (0.98)	6.12 (0.65)	Pale-whitish
Chouki God	96.0 (6.52)	5.20 (2.68)	2.7 (1.15)	23.20 (9.20)	18.40 (11.70)	41.60 (19.30)	35.7 (6.66)	47.0 (6.56)	11.04 (0.16)	7.39 (0.12)	0.69 (0.02)	6.33 (0.29)	5.63 (0.32)	Pale-yellowish
Dohara Thach	115.4 (54.2)	8.20 (2.86)	3.0 (0.00)	47.20 (28.13)	70.20 (28.90)	117.4 (50.22)	32.7 (3.06)	45.0 (9.00)	10.21 (0.05)	6.13 (0.08)	0.66 (0.01)	6.30 (0.17)	4.83 (0.06)	Pale-yellowish
Khod Nala	120.0 (54.31)	7.0 (2.24)	3.7 (1.15)	53.80 (39.69)	57.0 (25.36)	110.8 (54.52)	30.3 (0.58)	42.7 (5.69)	9.25 (0.43)	7.02 (0.16)	0.62 (0.07)	4.80 (0.50)	4.07 (0.55)	Pale-yellowish

Abbreviations used: *Significant at P < 0.05 level; NS=Non-significant; Seed width included wings of the seeds; Values in parenthesis are standard deviation.

Table 5. Cont'd.

Av.	89.47 (24.41)	6.43 (1.35)	3.50 (0.86)	40.77 (12.36)	39.88 (19.84)	80.65 (28.90)	37.89 (5.95)	47.17 (5.85)	9.70 (0.83)	6.99 (0.60)	0.66 (0.05)	6.07 (0.70)	5.19 (0.70)	-
Range	66.0-120	5.0-8.20	2.7-4.7	23.2-53.8	18.4- 70.2	41.6-117.4	30.3-45.3	40.3- 55.7	8.77-11.0	6.13-7.63	0.60-0.74	4.80-6.90	4.07-6.12	-
LSD F	45.71 2.43^{NS}	3.61 1.18^{NS}	1.39 3.65*	30.16 1.43^{NS}	25.26 5.26*	58.32 3.49*	5.97 9.41*	10.64 2.87^{NS}	0.55 25.46*	0.42 15.20*	0.14 0.65^{NS}	0.50 21.28*	0.71 9.300*	-

*Significant at P < 0.05 level, NS = Non-significant, Seed width included wings of the seeds, Values in parenthesis are standard deviation.

to obtain high level of potential fitness by producing seed mass and increase the chances of seedling establishment through a great allocation of maternal resources to individual seed (Westoby et al., 1990, 1992).

Effects of different storage conditions on seed moisture contents (Figure 4) and viability (Figure 5) in *H. candicans* populations were observed. Freshly collected seeds of different populations showed significant variation (P < 0.05) in moisture contents (range: 9.92 - 23.2%; Av. 14.46%) and viability (range: 75 - 100%; Av. 90%), wherein 100% viable seeds were found in Suajani Nala and Khod Nala. After six months of storage, seeds of different populations stored at low temperature (4°C) showed a minor reduction in viability percentage (range: 67.3 - 93.3%, Av. 82.8%). However at room temperature, a significant (P < 0.05) loss in the same was recorded (range: 56.5 - 80.5%, Av. 68.9%). Similar results were found in case of moisture contents. Long term seed storage is one of the important *ex-situ* conservation strategies for rare and threatened plant species. Long term seed storage at low temperature for retaining optimum viability in Himalayan medicinal herbs have been suggested by different workers (Butola and Samant, 2006; Chauhan and Nautiyal, 2007). Low temperature storage maintains seed viability and physiological potential and also may reduce seed metabolism including respiration, decreasing oxidation and minimize tissue damage. The loss of germinability at room temperature might be due to wide temperature fluctuation, activity of mycoflora and fauna present in the seeds and depletion of food reserves (Abdul Baki, 1980), rendering the seeds as non-viable. Seed of *H. candicans* require dry chilling to initiate germination (Butola, 2009) and this may be substituted through low temperature storage of the seeds.

Seed germination percentage in *H. candicans* varied significantly (P < 0.05) among populations (Figure 6), which ranged from 14.67 to 82.33%, with maximum germination in Suajani Nala and least in Dohra Thach. As many as 20 - 30% ungerminated seeds were found viable. On finding ungerminated seeds as viable, it is very clear that the low germination in this species is due to dormancy very well known phenomenon in family *Apiaceae*. Such response in germination may also be due to variation in dormancy level among populations (Milberg et al., 1996) and in seed size and weight (Baskin and Baskin, 2001). Besides these factors, latitude and elevation also play an important role in variable germination responses among different populations. Plant to plant variation in fruit mass and percentage germination is also reported in *Heracleum mantegazzianum* (Moravcova et al., 2005). Seed germinability not only determines natural regeneration and long term sustainability of a plant species but also indicates superiority of germplasm desirable for mass multipli-cation. On the basis of higher germinability, Suajani Nala may be considered as elite population then other studied populations.

Plant traits are commonly inter-correlated (Schlichting, 1986). In present study, plant height (r = 0.714), number of leaves (r = 0.711) and above ground biomass (r =0.588) showed positive and significant (P < 0.05) correlation with the below ground biomass. The strong correlation of plant height and other aboveground parts to belowground biomass suggested that these traits be of great use in making pre-assessment of the crop productivity. In most of Himalayan medicinal herbs below ground parts contribute major significance in their medicinal value. Further, the seed width (r = 0.739), seed thickness(r = 0.603), seed weight (r = 0.582) and plant density (r = 0.822) had positive correlation to seed germination percentage. Positive correlation of seed size and seed weight to seed germination indicates that the healthy seeds of *H. candicans* yield optimum germination. However, a negative correlation (r = -0.535) between plant height and seed germination was noticed.

The altitude showed positive correlation to those of plant density (r = 0.704), seed length (r = 0.422), seed weight (r = 0.481) and germination (r = 0.483) and negatively correlated with the plant

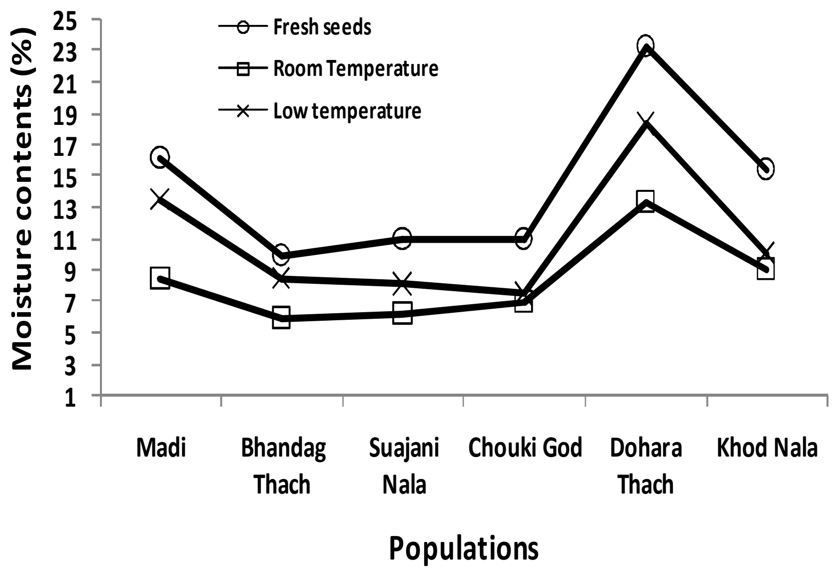

Figure 4. Effect of storage conditions (room temperature: 20 - 25°C and low temperature: 4°C on seed moisture contents in different populations of *H. candicans.*

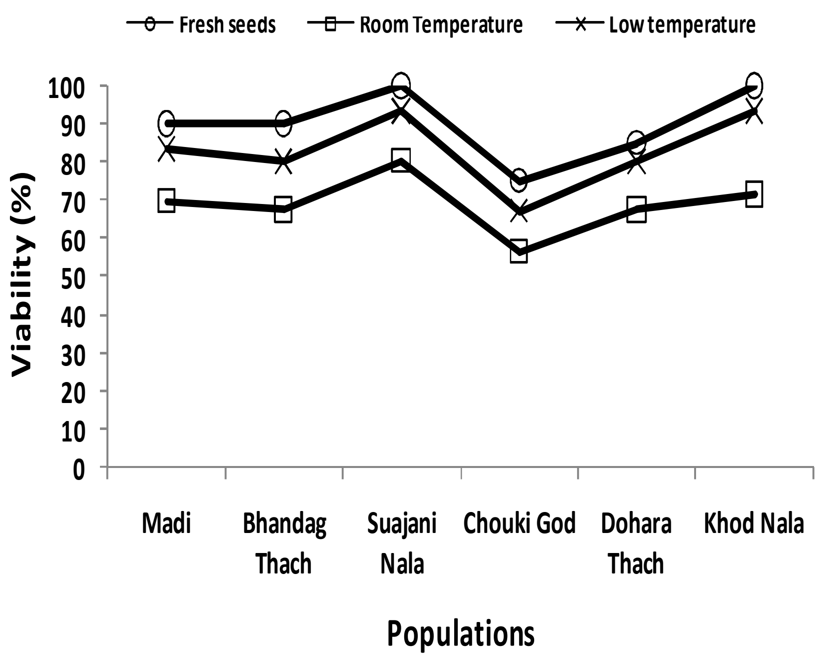

Figure 5. Effect of storage conditions (room temperature: 20 - 25°C and low temperature: 4°C on seed viability in different populations of *H. candicans.*

height (r = -0.958) and below ground biomass (r = -0.674). Ongoing heavy *in situ* harvesting of this species may be one of the reasons of low density at low altitudes being easy accessible to commercial collectors. On the contrary, negative correlation of all morphological parameters to the increasing altitudes indicates better growth performance of this species at lower elevations. Airi et al. (2000) have observed similar trend in *Nardostachys jatamansi*.

Conclusions

In view of threatened status and industrial demand, the

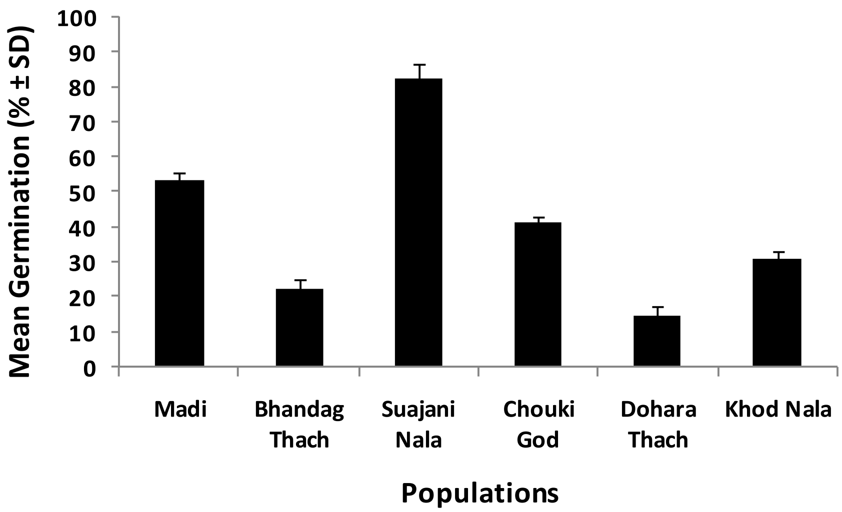

Figure 6. Inter-population variability in seed germination in *H. candicans* (significant variation at $P < 0.05$ level, LSD = 6.15, F = 59.55).

following points emerged from the present investigations, are crucial for conservation management and sustainable utilization of *H. candicans*:

1. The occurrence of *H. candicans* in different habitats, most preferably in rocky crevices, open dry slopes, humus rich moist areas and along water channels, indicates its wide adaptability to grow in different set of environmental conditions and thus, can be cultivated in a range of climatic zones.
2. In spite of endangered status, density of this species is well enough in study areas. This is due to remoteness of these areas, inaccessible to commercial collectors and low industrial demand of the species at local level. However, it is right time to initiate attempts for its *in-situ* conservation and sustainable utilization and to reduce *in-situ* harvesting pressures through commercial cultivation.
3. The species exhibits a high degree of inter-population variation which appeared throughout the ontogeny; however, these variations are more visible in seeds with regard to morphology and germination behavior. These variations are closely associated to varying environmental and edaphic conditions.
4. Besides morphological variations, seeds of majority populations possess dormancy which results in delay, erratic and low germination. Tested propagation protocols should be used for mass production of homogenous lot of quality seedlings (Butola, 2009).
5. Low temperature storage is suitable practice to maintain seed viability till spring. From the field-sowing point of view, the spring time sowing suits majority of middle temperate and high altitude zones in the Himalayan region, which otherwise remain covered with snow till April last.
6. Based on overall performance, the population of Suajani Nala may be considered as elite and therefore, marked for conservation and regular monitoring as a potential source of superior germplasm for cultivation. However, further studies on exploration of potential habitats supporting elite populations and their quantitative and qualitative (Phyto-chemical) analysis should be initiated.
7. Overall, the results of present study would assist in strengthening existing propagation protocol and agro-techniques of the species and form basis for formulating strategies for conservation and sustainable utilization the species.

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