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Propagating for Survival: Conservation Strategies for the Endangered Himalayan Medicinal Plant *Picrorhiza kurrooa*

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Abstract— *Studies on propagation and conservation of an endangered medicinal herb - Picrorhiza kurrooa reveals that the seed germination did not occur in darker conditions and seem to be photoblastic. Among various treatments the species showed 93.33±2.88% germination when the seeds were germinated in a medium of 0.125mM GA_3 with mean germination time (MGT) of 8.0±0.866 days as against the MGT 18.66±0.57 days of controlled seeds (seeds without any treatment). In field conditions however, the seeds germinate only when placed on the upper layer of soil covered with a thin film of water exposed to light. Although the plants grew nicely on various soils, maximum plant survival and vigorous growth was obtained in loamy and sandy loam textured soils. The plants responded to various nutrient treatments and in loamy textured the maximum dry biomass was shown by the plants treated with nitrogen (500mg/kg soil) produced 9.50±2.50gm dry biomass and the plants treated with NPK (500mg/kg soil) produced 9.16±4.481gm dry biomass as against the control of 4.83±0.473gm in loam textured soils. In sandy loam (1:2) textured soils the plants treated with nitrogen and NPK (500mg/kg soil) produced the dry biomass of 13.66±0.581 and 10.16±0.763gm respectively as against the control of 5.06±1.401gm per individual. The experimental manipulations also reveal that the species has a potential to propagate through underground stem cuttings in sandy loam soils. The cuttings treated with IAA, IBA and GA_3 showed 93.33, 80.0 and 95.0% survival in top segments while as 60.0, 55.0 and 68.0% survival in basal segments respectively as against the control of 75.0% in top and 42.10% in basal segments.*

Keywords— Picrorhiza kurrooa, endangered medicinal plant, propagation, growth regulators, NPK treatments, seed germination, ex situ conservation.

I. INTRODUCTION

P. kurrooa (English: Hellabore; Hindi: Kutki; Punjabi: Karu; Kashmiri: Koad) is the major source of a multipurpose drug valued as a bitter tonic, antiperiodic, laxative and cathartic, when used in small and large doses respectively (Kirtikar and Basu, 1988) and hepatoprotective (Ansari et al., 1991). In the entire range of distribution the species is confined to alpine habitats and when viewed from a global perspective, the distribution range of the species turns out to be negligibly small. Even in this extremely narrow range of distribution, the species constitute small sized populations which are sparsely distributed and contain scant individuals. Justifiably therefore, the herb have been recognised as endangered (EN) (*P. kurrooa*) and accordingly listed in Red Data Book of BSI (Nayar and Shastry, 1998). Despite the poor and dismal populational picture of the herb in its range of distribution, the underground plant parts, which represent the main sources of its survival and multiplication over generations in natural setting, are ruthlessly and indiscriminately extracted from the soil as drugs. This practice has been going on for years together and continues unabated even today. The result is that most of the populations are bereft of this herb and that day is not

far when no single plant will be traceable to the posterity. There is an urgent need to rise to the occasion and come up with proactive strategies to salvage whatever is left and plan long term rescue measures not only to save this high potential herb from loss but also to rejuvenate the species, return to its natural ecosystem and ensure supply of raw material to the drug industry on sustained basis. In this backdrop the present studies have been conducted. The specific objectives of this study were: (i) to develop efficient seed germination protocols for *P. kurrooa*; (ii) to evaluate the species' performance on different soil types and nutrient regimes for ex situ cultivation; and (iii) to develop vegetative propagation techniques using stem cuttings for conservation and commercial multiplication.

II. MATERIALS AND METHODS

2.1 Study Sites and Source Material

Six alpine sites of Kashmir Himalaya ranging in altitude from 3,150 to 3,400m a.s.l. were monitored and used as a source material for seed, soil and propagule collection (Table 1). All populations were located in alpine habitats with soils rich in moisture, mosses and leaf litter. Threatened status of the species necessitated careful collection of material to avoid further depletion of natural populations.

TABLE 1
SALIENT FEATURES OF SOME KASHMIR HIMALAYAN SITES SELECTED FOR STUDIES ON *PICRORHIZA KURROOA*

Habitat character	Populations					
	Thajwas I	**Zaildar Dub**	**Arm Pathri**	**Thajwas II**	**Sangam Top**	**Razaka ka Paja**
Altitude (m)	3150	3150	3300	3350	3400	3400
Latitude/Longitude	34°30'N 75°30'E	34°30'N 75°30'E	34°02'N 75°30'E	34°30'N 75°30'E	34°30'N 75°30'E	34°30'N 75°30'E
Direction with reference to Srinagar	north east	north east	south east	north east	north east	north east
Climatic zone	alpine	alpine	alpine	alpine	alpine	alpine
Habitat	Rocky slope with partial shade	open grassy/ rocky slope	shady moist slope under *Betula utilis* cover	shady slope under *Betula utilis* cover	shady moist slope	moist shady slope
Threat factor	extraction, grazing, landslides	extraction, grazing	extraction, grazing	extraction, grazing	extraction, grazing	extraction, grazing

Note: In all populations soils are rich in moisture, mosses and leaf litter.

2.2 Seed Germination Studies

For seed germination, mature seeds of *Picrorhiza kurrooa* of the same age (seed cohort) were collected from a selected natural population, Thajwas I, 3150m a.s.l (Kashmir Himalaya). The seeds were treated with 0.1% mercuric chloride for 5 minutes and washed with double distilled water. Seeds were germinated in petriplates on Whatman filter paper (in vitro) using different media (Table 2). For each treatment three replicates were used each with a set of control. The mean germination time (MGT) was calculated following Joshi and Dhar (2003) as given below:

$$MGT = (n \times d) / N \quad (1)$$

where 'n' = number of seeds germinated on each day, 'd' = number of days from the beginning of the test, and 'N' = total number of seeds germinated at the termination of the experiment.

In field conditions, seeds were sown on the soil surface with a thin film of water, and at depths of 1 cm and 1.5 cm to assess the effect of burial on germination. The photoblastic nature of seeds was tested by germinating seeds in complete darkness.

TABLE 2
EFFECT OF DIFFERENT PHYSICAL AND CHEMICAL TREATMENTS ON SEED GERMINATION OF *PICRORHIZA KURROOA*

S. No.	Treatment	Mean germination time (MGT) days	% germination
1	**Lab. conditions (LC)**		
	Control	18.66 ± 0.57*	57.5 ± 3.53
	Chilling (days)		
	20	25.0 ± 5.0	6.66 ± 2.88
	40	-	-
	75	-	-
	90	-	-
	Conc. H_2SO_4 dip	16.83 ± 0.57	19.66 ± 0.57
	GA_3 (mM)*		
	0.125	8.0 ± 0.866	**93.33 ± 2.88****
	0.25	17.66 ± 0.288	65.0 ± 5.0
	0.5	16.66 ± 1.607	41.66 ± 17.55
	1	15.83 ± 1.04	57.66 ± 12.50
	1.5	17.16 ± 0.76	16.66 ± 2.88
	2	16.16 ± 1.119	70.00 ± 15.49
	Thiourea (mM)*		
	0.25	15.5 ± 1.322	**73.33 ± 2.88****
	0.5	16.33 ± 1.059	62.33 ± 7.50
	1	16.66 ± 0.763	69.33 ± 4.04
	1.5	16.0 ± 0.50	70.0 ± 5.0
	2	16.66 ± 0.763	40.0 ± 5.0
	Kinetin (mM)*		
	0.25	-	-
	0.5	17.16 ± 0.58	3.33 ± 2.88
	1	-	-
	1.5	-	-
	2	17.33 ± 0.812	15.0 ± 5.0
2	**Field conditions (FC)**		
	Seeds placed on upper surface of soil covered with thin film of water	14.33 ± 0.57	53.33 ± 8.50
	Seeds sown 1cm deep	-	-
	Seeds sown 1.5cm deep	-	-

***Mean ± Standard Deviation; **Maximum seed germination; Seed sample for each replica: LC = 20; FC = 200; Temperature range: LC = 15-20°C; FC = 18-25°C; ****Used as mediums; Seed germination did not occur in dark**

2.3 Development of Plant Production Technique (Ex Situ Conservation)

To make the cultivation of this herb possible in an effective way at lower altitudes, its response was observed in different soil textural classes and fertilizer applications (Table 3). The following soil textural classes were used: (i) Natural soil (collected from natural habitat). (ii) Loam : organic manure (2:1). (iii) Loam soil (garden soil) (iv) Sandy loam (2:1) (v) Sandy loam (1:2) (vi) Sand : silt : clay (1:2:2).

Young seedlings (of the same age) of *P. kurrooa* were collected from natural habitats in May and sown in the above mentioned soil textural classes in pots. Each set of pots (containing equal amount of soil) were irrigated at different intervals for the first year. In the next growing season the stabilized and adapted plants were treated with various inorganic fertilizers (Table 3) in different concentrations each with a set of control (Mohi-Ud-Din et al., 2022). The plants were kept in diffused light and harvested (at full bloom) to determine dry biomass (after oven dried at 80°C for 72 hours; Singh and Purohit, 2003).

TABLE 3
EFFECT OF VARIOUS TREATMENTS ON THE PRODUCTION OF *PICRORHIZA KURROOA* GROWN ON DIFFERENT SOIL TYPES

S. No.	Soil type & fertilizer applied	Dry weight per plant (gm)		Total dry biomass per plant (gm)
		below ground	above ground	
1	**Natural soil**	10.166 ± 2.020*	5.33 ± 0.763	**15.5 ± 2.783****
2	**Loamy soil**			
	N-300mg/kg soil	2.166 ± 1.040	5.0 ± 1.322	7.16 ± 2.084
	N-500mg	3.166 ± 0.763	6.33 ± 1.757	**9.50 ± 2.50****
	K-300mg	1.66 ± 0.766	3.833 ± 0.288	5.06 ± 1.914
	DAP-500mg	3.83 ± 1.755	4.166 ± 1.154	8.0 ± 2.179
	NPK-500mg†	4.33 ± 1.757	5.0 ± 2.783	**9.16 ± 4.481****
	Control	3.16 ± 0.763	1.66 ± 1.044	4.83 ± 0.473
3	**Loam with organic manure (2:1)**	3.0 ± 1.802	3.6 ± 0.60	**6.60 ± 2.260****
4	**Sandy loam (2:1)**			
	N-300mg/kg soil	1.833 ± 0.288	4.066 ± 2.328	5.9 ± 2.424
	N-500mg	3.33 ± 0.763	5.66 ± 1.040	**9.0 ± 0.866****
	K-300mg	1.67 ± 0.970	3.40 ± 1.153	5.06 ± 1.914
	DAP-500mg	2.80 ± 0.764	3.66 ± 0.763	6.5 ± 1.0
	NPK-500mg†	4.0 ± 0.50	5.066 ± 0.404	**9.066 ± 0.901****
	Control	1.66 ± 0.288	1.33 ± 0.577	3.0 ± 0.866
5	**Sandy loam (1:2)**			
	N-300mg/kg soil	5.33 ± 1.258	4.0 ± 1.50	**9.33 ± 1.258****
	N-500mg	8.0 ± 1.50	5.66 ± 2.02	**13.66 ± 0.581****
	K-300mg	3.30 ± 0.764	1.83 ± 0.577	5.0 ± 1.322
	DAP-500mg	1.33 ± 0.290	1.67 ± 0.763	3.0 ± 0.50
	NPK-500mg†	5.33 ± 1.040	4.833 ± 0.288	**10.16 ± 0.763****
	Control	1.83 ± 1.04	3.23 ± 0.461	5.06 ± 1.401
6	**Sand: Silt: Clay (1:2:2)**			
	N-300mg/kg soil	4.0 ± 0.50	5.5 ± 1.0	**9.50 ± 1.50****
	N-500mg	3.83 ± 1.154	2.80 ± 0.764	6.60 ± 1.260
	K-300mg	1.10 ± 0.360	1.0 ± 0.50	2.10 ± 0.692
	DAP-500mg	1.16 ± 0.288	1.0 ± 0.5	2.10 ± 0.583
	NPK-500mg†	3.83 ± 0.577	2.50 ± 1.0	6.16 ± 1.755
	Control	1.30 ± 0.36	1.0 ± 0.50	2.33 ± 0.763

***Mean* ± *Standard Deviation;* *Showed maximum dry biomass; †NPK (N-300mg + P-100mg + K-100mg) = 500mg/kg soil.**

2.4 In Vivo Propagation

The fresh underground as well as above ground stems of the species were collected from natural population Thajwas I, 3150m a.s.l (Kashmir Himalaya) in the first week of May and were cut horizontally into Basal (without shoot apex) and Top cuttings (with shoot apex). Both the types of cuttings were treated with 100ppm IAA, IBA and GA_3 for 48 hours and were sown in sandy loam soils under pots and trays at Herbal Garden (1495m), University of Kashmir, Srinagar (India). The pots and trays were kept in diffused light to monitor the leafy shoot generation, survival and comparison with the control (untreated cuttings) (Table 4).

TABLE 4
IN VIVO PROPAGATION OF *PICRORHIZA KURROOA*

Treatment	No. of cuttings studied		Days required for shoot regeneration		No. of plantlets survived till senescence		% survival	
	top*	basal**	top	basal	top	basal	top	basal
Control	12	19	8 to 12	10 to 12	9	8	75.0	42.10
IAA (100ppm)	15	20	6 to 9	8 to 15	14	12	93.33	60.0
IBA (100ppm)	15	20	7 to 10	8 to 15	12	11	80.0	55.0
GA_3 (100ppm)	20	25	4 to 10	6 to 14	19	17	95.0	68.0

**** cutting with shoot apex; ** cutting without shoot apex; *** Temperature Range = 21 to 27 Degree Centigrade***

2.5 Statistical Analysis

All experiments were conducted with three replications unless otherwise specified. Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by appropriate post-hoc tests where applicable. Differences were considered significant at $P < 0.05$. All statistical analyses were performed using [specify software, e.g., SPSS or GraphPad Prism].

III. RESULTS

3.1 Development of Agrotechniques

The studies on agrotechniques reveal that the total dry biomass per individual works to 15.5 ± 2.783gm in natural soil (soil collected from natural habitat). In loam and organic manure (2:1) the plants produce 6.60 ± 2.260gm dry biomass per individual. In the loam soil, among different fertilizers used nitrogen (N) (500mg/kg soil), DAP (500mg/kg soil) and NPK (500mg/kg soil) showed maximum dry biomass of 9.50 ± 2.50gm, 8.0 ± 2.179gm and 9.16 ± 4.481gm per individual respectively as against the control of 4.83 ± 0.473gm.

In the sandy loam (2:1) soil type among different fertilizers NPK (500mg/kg soil) and N - 500mg/kg soil showed good biomass production, which works to 9.066 ± 0.901gm and 9.0 ± 0.866gm respectively as against the control of 3.0 ± 0.86gm. In the sandy loam (1:2) soil type N - 500mg/kg soil and NPK - 500mg/kg soil showed the dry biomass production of 9.33 ± 1.258gm, 13.66 ± 0.581gm and 10.16 ± 0.763gm respectively as against the control of 5.06 ± 1.401gm.

In sand: silt: clay (1:2:2) soil type only the plants treated with nitrogen (300mg/kg soil) showed production of 9.50 ± 1.50gm of dry biomass per individual as compared to 2.33 ± 0.763gm of control.

The plants grown on the natural soil showed maximum belowground dry biomass (10.166 ± 2.020gm) per individual because of the loose aerated and moist soils full of decomposed litter.

FIGURES 1-6: Development of agrotechnology of *Picrorhiza kurrooa* growing in ex situ conditions under different fertilizer treatments showing differences in vegetative growth. 1. Control Plant; 2. DAP treated; 3. Nitrogen (300mg/kg soil) treated; 4. Nitrogen (500mg/kg soil) treated; 5. NPK (500mg/kg soil) treated; 6. Plant growing in soil (collected from natural habitat) - note the increased number of shoots.

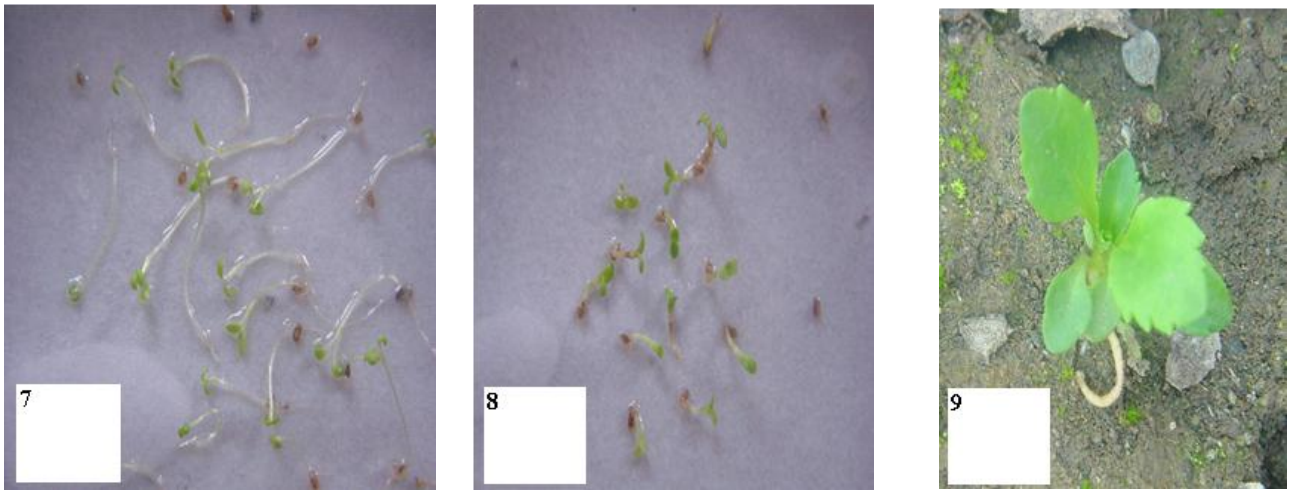
3.2 Seed Germination

The small perforated seeds of *Picrorhiza kurrooa* germinated nicely (57.5 ± 3.53% germination) without any treatment. However, the mean germination time (MGT) is higher which averages 18.66 ± 0.57 days.

The seeds fail to germinate when chilled at 4°C for varying periods (20 - 90 days) while as the seeds treated with GA_3 (0.125mM) and thiourea (0.25mM) germinate in large numbers (93.33 ± 2.88% and 73.33 ± 2.88% respectively) and relatively in lesser time compared to other treatments and control. The seeds kept for germination in different concentrations of kinetin also fail to germinate, however, the 2.0mM concentration showed 15.0 ± 5.0% germination.

In the field conditions, the seeds germinate only when placed on the upper layer of soil covered with thin film of water and exposed to light. The seeds fail to germinate when sown 1 - 1.5cm deep in the soil. The seedlings are too fragile and minute and show very less survival rate.

The seeds also germinate efficiently under laboratory conditions within the soil collected from natural habitats and covered with a thin film of water. In natural habitat the seeds sown after winter germinate in lesser quantity but mostly the seedlings fail to recruit while as the seeds sown before winter do not germinate at all.



FIGURES 7-9: In vitro seed germination of *Picrorhiza kurrooa*. 7 & 8: Small & fragile seedlings generated in Petri plates under laboratory conditions. 9: Transplanted seedling.

3.3 In Vivo Propagation

For the study of in vivo propagation the underground stem was transversely cut into top and basal segments. The study reveals that the cuttings without any treatment showed 75.0% and 42.10% survival in top and basal segments respectively in sandy loam soils. The number of days required for shoot regeneration is 8 - 12 days in case of top segments and 10 - 20 days in case of basal segments.

The cuttings treated with IAA (100ppm) showed 93.33% and 60.0% survival for top and basal segments respectively while as the cuttings treated with IBA (100ppm) showed 80.0% and 55.0% survival for the top and basal segments respectively. The number of days required for shoot generation in IAA treated cuttings is 6 - 9 days for top and 8 - 15 days for basal segments while as IBA treated cuttings require 7 - 10 days for top and 8 - 15 days for basal segments.

The GA_3 (100ppm) treated cuttings show best results and the number of days required for shoot generation was only 4 - 10 for top and 6 - 14 for basal segments and survival was 95.0% for top and 68.0% for basal segments.

FIGURES 10-12: In vitro propagation of *Picrorhiza kurrooa* through stem cuttings. 10: Regenerated cuttings treated with IAA. 11 & 12: Regenerated cuttings treated with GA_3, note increased number of shoots as compared to IAA treated cuttings.

IV. DISCUSSION

The alpine and sub-alpine medicinal plant species constitute an important natural bioresource. This wealth has been exploited extensively as a source of traditional herbal drugs and also in the manufacture of many allopathic drugs. The overexploitation of important species is the basic reason of their extinction. This has necessitated the development and standardization of agrotechniques for their cultivation and consequent conservation (Joshi et al., 1990). The major conservation strategies

recommended are development of germplasm centers, in situ and ex situ conservation of critically endangered species, establishment of high altitude nurseries, systematic collection and domestication etc. (Joshi and Rawat, 1997; Dwivedi, 1999).

Considering the increasing demand of herbal drugs in general and Himalayan medicinal plants in particular and consequent depletion of several species, it is important to urgently initiate proper steps for conservation (Nautiyal et al., 2001). In view of large-scale exploitation, biotic interferences and increasing demand for *Picrorhiza kurrooa* as herbal drug, its assessment as threatened species in nature, and consequently the need for its cultivation to salvage it from loss and also raise the germplasm and provide economic avenues for the poor, it becomes obligatory to initiate steps for its large scale cultivation and development of elementary agrotechniques at lower altitudes under ex situ conditions.

The plants performed well under shady conditions. Being a moisture loving plant, it requires good amount of moisture. Under shady conditions irrigation after 4 - 5 day interval resulted in highest survival rates and biomass production. Similar kind of responses has been reported by Nautiyal et al. (2001) while assessing the germinability of *Picrorhiza kurrooa*. *P. kurrooa* in its natural habitat grows on sandy, loamy silt and moisture rich humus soils while at the low altitude of Botanical Garden Kashmir University, 1495m, the plants got domesticated nicely on different textural classes. Thus, the plant enjoys and tolerates a wide range of soil texture and reaction.

Among different soil textural classes, the plants grown in natural soil (collected from its natural habitat) showed the best survival and performance with dry biomass of 15.5 ± 2.783gm per individual. On loamy soil among different nutrient trials nitrogen (500mg/kg soil) and NPK (500mg/kg soil) produced dry biomass of 9.50 ± 2.50gm and 9.16 ± 4.481gm per individual respectively as against the control of 4.83 ± 0.473gm.

The plants also showed good survival under sandy loam (2:1) textured soil and produced dry biomass of 9.066 ± 0.901gm on application of NPK (500mg/kg soil) as against the control of 3.0 ± 0.866gm and in sandy loam (1:2) textured soil, the NPK treated plants resulted in 10.16 ± 0.763gm as against the control of 5.06 ± 1.401gm per individual possibly because of the fact that the plant prefers sandy loam with organic matter in natural habitat. Such soils facilitate the underground rooting as the plants do have underground horizontal stem. In the loose aerated soil they grow better as compared to other textural soil classes. Similar kind of rooting and underground stem production was observed by Nautiyal et al. (2001) in *P. kurrooa* under sandy soils with litter treatments.

The species however, shows a poor performance in sand: silt: clay (1:2:2) soil. Possibly the rooting is difficult in clayey soils as the plant mostly prefers higher moisture content and loose soils. The plants also show best performance in loam soils grown together with many other grasses as compared to the plants growing in isolated populations. This perhaps is due to the fact that the combination of grasses taller than the species under discussion provides shade and protects the soil against exposure to direct sunlight due to which the moisture content remains higher than in the open beds where the species grows lonely. Under such conditions the plants of the *P. kurrooa* showed maximum number of vegetative shoots and thus propagate more efficiently as compared to the plants grown lonely.

The increasing demand for herbal drugs in general and Himalayan medicinal plants in particular and the depletion of important species from natural resources make it imperative to develop proper methods and strategies for their conservation and commercial production (Raina et al., 2003).

4.1 Seed Germination

Maximum viability and vigour of the seed coincide with the attainment of maximum dry weight or physiological maturity by it (Rasyad et al., 1990; Hay and Probert, 1995). However, some recent studies on a range of diverse crops have consistently shown that seed longevity, as well as other seed quality traits, continue to increase after the end of seed maturity phase (Kameswara et al., 1991; Zanakis et al., 1994).

Seeds of many plants fail to germinate immediately and pass through a phase of dormancy that may be caused by several factors thus delaying the life cycle of these plants. Some plants display a wide range of dormancy breaking and germination requirements (Leon, 1985). Besides, germination at the right time and in the right place largely determines the probability of seedling survival (Thompson, 1974). The dormancy characteristics and germination responses are considered to be the key elements in plant life history strategies (Meyer et al., 1990).

The dormancy state of seeds is determined by incubating populations of them on a moist substrate over a range of environmental conditions. Nondormant seeds germinate over the widest range of conditions possible for the species, while dormant ones fail

to germinate at any condition. Conditionally dormant seeds germinate over only a portion of the range of conditions possible for the species (Vegis, 1964; Baskin and Baskin, 1985). Buried seeds of many species change from one dormancy state to another and back again during the course of a year in response to seasonal cycles of environmental conditions (Baskin and Baskin, 1998).

The physiological and chemical treatments have been found to improve the seed germination, seedling growth and nitrogen metabolism in plants (Bose et al., 1982; Hay and Probert, 1995; Prassad, 1999; Bose and Sarma, 2000; Joshi and Dhar, 2003; Mohi-Ud-Din et al., 2005). Once the seed germinates successfully, establishment of the young seedlings as new recruits is a crucial demographic event and depends on the nutritional reserves within the seed (Thomas, 1966; Willson, 1983) and the ability of the seed to emerge from greater soil depths (Harper et al., 1970; Willson, 1983).

To understand the occurrence and distribution of a species in a certain habitat and its life history strategy, the timing of germination and the nature of dormancy breaking mechanisms assume great significance. The development of seed germination protocols is therefore, an important step (especially for threatened medicinal plants) to offset the increasing demand of the species in pharmaceutical industry (Joshi and Dhar, 2003). Such studies will also help in developing conservation strategies for the proper management and the maintenance of the bioresources especially economically important plants in general and medicinal plants in particular at lower and easy-to-approach habitats (Mohi-Ud-Din et al., 2005).

The species is endangered in status. Therefore availability of material for multiplication from the natural habitats is an arduous task (Nautiyal et al., 2001). The species produces a large quantity of small sized seeds, which mostly fail to recruit, with the result that the seedlings are rarely seen in natural habitat. The seed viability was tested by subjecting the seeds to different physical and chemical treatments (Table 2). The results reveal that under controlled conditions with a temperature range of 15°C to 20°C, the seeds germinate with ease without any treatment (control) and register 57.5 ± 3.53% germination.

GA_3 concentrations (0.125mM, 0.25mM and 0.5mM) showed 93.33 ± 2.88, 65.0 ± 5.0 and 41.66 ± 17.55% germination as against the control of 57.5 ± 3.53%. However, higher concentrations of GA_3 (1.5mM) resulted into only 16.66 ± 2.88% germination. Thiourea when given in 0.25mM, 0.5mM, 1.0mM, 1.5mM and 2.0mM concentrations resulted into 73.33 ± 2.88%, 62.33 ± 7.50%, 69.33 ± 4.04%, 70.0 ± 5.0% and 40.0 ± 5.0% germination.

Kinetin and conc. H_2SO_4 dip resulted into low germination, while the seeds chilled at 20 days resulted into 6.66 ± 2.88% germination and those at 40, 75 and 90 days suffered negative effects. In field conditions, however, the seeds germinate only when sown on the soil surface covered with thin film of water under exposed conditions. The seeds thus seem to be photoblastic in nature. The photoblastic nature of seeds has also been reported in a cofamilial taxon *Agalinis fasciculata* by Baskin et al. (1998).

The seedlings raised are too fragile and minute to survive after transplantation. If the species produces viable seeds, then why it fails to recruit in its natural home? To answer this question, the following manipulations were made:

1) Seeds were kept for germination within the soil (collected from natural habitat) and monitored under lab. conditions.
2) Seeds were sown in natural home (basic habitat) on pre-identified and selected spots before winter and after winter.
3) Seeds were sown in natural soil (in Lab. conditions) with a thin film of water.

These experiments reveal that in lab. conditions the seeds germinate in good quantity (60%) but only when kept on soil surface under a thin film of water. In natural habitat the seeds sown before winter fail to produce seedlings in the next growing season, while the seeds sown after winter (chilling) germinate but the seedlings fail to recruit because of seedling mortality.

From the above information, it is evident that the possible reasons for seed failure of the species in natural populations are:

1) Prolonged exposure to extreme chilly conditions in alpine sub-alpine habitats may lead to loss of viability in natural habitats.
2) The seeds are very small and photoblastic, thus non-availability of light due to burial of seeds below the leaf litter of the co-existing species may also inhibit germination.
3) Water seems to be essential for the germination both under lab. as well as field conditions. Thus due to non-availability of appropriate moisture in the natural habitat may lead to loss of seed's viability hence failure of germination.

4) The seedlings are too fragile and minute hence, may hardly survive in natural conditions as the smaller seedlings cannot compete with the larger seedlings and plants growing in the vicinity of the species.

Seeds of *P. kurrooa* are small in size. Such seeds are known to store less food material and thus have weaker ability to recover from competition and herbivory (Willson, 1983). Further, smaller seeds in general produce fragile seedlings with less survival potential (Edwards et al., 1934). The effects of seed size on seedling growth and survival have been studied for a variety of angiospermic taxa. The studies of Schaal (1980) reveal that the large sized seeds have a distinct advantage over the smaller seeds. Such seeds give the seedlings an attendant advantage in developing leaves earlier so as to meet the competition from co-occurring plants as compared to smaller seeds (Willson, 1983). Seeds also affects the time of seedling emergence which in turn is known to register a significant impact on seedling success (Ross and Harper, 1972). It is also important to know the extent to which seed size exerts an effect on seedling emergence.

From the above discussion, it becomes evident that the *Picrorhiza kurrooa* seed requires light and water to germinate. The seedlings being smaller and weak hardly survive in the harsh conditions of the alpine habitat. Thus in order to thwart this bottleneck, the species has established an efficient method of vegetative propagation.

4.2 Vegetative Propagation

Earlier, the micropropagation studies of the species have been carried out in vitro under tissue culture (Lal et al., 1988; Upadhyay et al., 1989). The present studies were undertaken to develop a simple and cost effective technique for the conservation and commercial production of the species. The studies reveal that the underground stem segments are most successful not only for multiplication but also for higher commercial production in a short period of time. Since multiplication through seeds is difficult (discussed earlier), this method can be used as an alternative for quick multiplication of the species under ex situ conditions.

The studies reveal that the top segments are efficient explants as compared to the basal segments. The segments treated with IAA, IBA and GA_3 (100ppm) show better growth, best survival and require lesser time to regenerate as compared to the control. Thus, the species demonstrates better regeneration and multiplication from the stems. Similar kind of trend has earlier been reported by Nautiyal et al. (2001) in the same species.

For the cultivation and conservation of *Picrorhiza kurrooa*, the methods developed during the present study can provide not only an alternate income generating resource but can also provide the opportunity for their sustainable management and long term supply of the drug.

V. CONCLUSION

The present study has successfully developed comprehensive propagation and conservation protocols for the endangered Himalayan medicinal plant *Picrorhiza kurrooa*. The key findings reveal that:

1) **Seed Germination**: The species is photoblastic, requiring light for germination. Maximum germination (93.33 ± 2.88%) was achieved with 0.125mM GA_3 treatment, with significantly reduced mean germination time (8.0 ± 0.866 days) compared to control (18.66 ± 0.57 days). The failure of natural recruitment is attributed to the combination of photoblastic nature, small seed size, fragile seedlings, and harsh alpine conditions.

2) **Ex Situ Cultivation**: The species performs best in loamy and sandy loam soils, with maximum dry biomass production (13.66 ± 0.581g) achieved in sandy loam (1:2) soil with nitrogen supplementation (500mg/kg soil). NPK supplementation also significantly improved biomass production.

3) **Vegetative Propagation**: Underground stem cuttings provide an efficient alternative for multiplication, with GA_3 (100ppm) treated top segments showing 95.0% survival and rapid shoot regeneration (4-10 days).

Recommendations for Conservation and Cultivation:

1) **Seed-Based Propagation**: For large-scale multiplication, seeds should be germinated in vitro with 0.125mM GA_3 treatment under light conditions at 15-20°C, followed by careful transplantation of seedlings.

2) **Vegetative Propagation**: For quick multiplication, underground stem cuttings (top segments) treated with GA_3 (100ppm) should be used, ensuring high survival rates and rapid regeneration.

3) **Cultivation**: Plants should be grown in sandy loam or loamy soils with adequate organic matter, under diffused light conditions, with nitrogen or NPK supplementation (500mg/kg soil).

4) **Conservation**: In situ conservation should focus on protecting natural populations from overexploitation, while ex situ cultivation should be promoted to meet the demand for raw material and reduce pressure on wild populations.

Limitations of the Study: The study was conducted at a limited number of sites in the Kashmir Himalaya, and the findings may not be directly applicable to other regions. The long-term survival and reproductive success of propagated plants in natural habitats were not assessed. Future research should include reintroduction trials, genetic diversity studies of propagated populations, and assessment of the economic viability of large-scale cultivation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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