Development of vegetative and sexual multiplication protocol for commercialization of *Inula racemosa* Hook. f. – a critically endangered medicinal plant of N.W. Himalaya

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Abstract: *Inula racemosa* is an important and critically endangered medicinal plant with tremendous potential as an important natural resource. The development of conservation and commercialization technique is a priority at present. To assess this, rhizome splitting as a means of vegetative propagation and seed germination for sexual propagation were evaluated for mass multiplication of this potent medicinal herb of North Western Himalayas. Split rhizome cuttings treated with varying concentrations of IAA, IBA and GA_3 showed 88.89±0.95% sprouting and 77.78±1.42% of rooting in 100ppm of IAA. The studies undertaken on the seed germination of *I. racemosa* as a means of mass multiplication revealed that seeds show a broad range of pre-chilling requirements. Highest germination percentage- 90.00±0.30% were recorded when scarification and GA_3 (100ppm) were applied together. Mean germination time declined with higher concentrations of GA_3 applied to scarified seeds and also with increased duration of stratification. [Nature and Science 2010;8(10):246-252]. (ISSN: 1545-0740).

Key Words: *Inula racemosa*, vegetative propagation, germination, scarification, stratification, mass multiplication.

1. Introduction

North West Himalayas, one of the richest pools of biological diversity in the World, has been experiencing ruthless extraction of wild medicinal plants due to ever increasing global inclination towards use of herbal medicine and thus endangering many of its high value gene stock. The continued commercial over-exploitation of these valuable herbal gems has resulted in depleting the population of many such species in their natural habitats. In India, with an estimated 8000 species of medicinal plants (Joy *et al*., 1998), more than 90% of medicinal plants for herbal drug industries are drawn from natural habitats, thereby putting them to severe exploitation (Gupta *et al.,* 1998, and Ved *et al*., 1998). In addition to the over exploitation, destructive harvesting practices, habitat degradation and agricultural encroachment to wild habitats have also been recognized as contributing factors in the loss of our herbal gems.

Inula racemosa Hook. f., a critically endangered himalayan herb (Anonymous, 1998), commonly known as "Pushkarmoola" of family Asteraceae, was targeted for the present study. Distributed from temperate to alpine belts of Kashmir and Himachal Pradesh, this perennial herb, known for its potent medicinal properties, is facing ruthless over-exploitation and is at the verge of extinction. The chemical profiling of plant roots have shown the presence of alantolactones and isoalontolactones, sitisterol, daucosterol, inunolide, aplotexene, phenylacetonitrite and isoinunal (Kalsi *et al*., 1989, and Wang *et al.,* 2000). Allantolactone and isoalantolactone are the major constituents known to possess antifungal properties (Satyawati *et al.*, 1987). The diverse active ingredients of the *Inula* root are being utilized as therapeutic components since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal, native) form. The roots of *I. racemosa* have been used as folk medicine in East Asia and Europe (Kuda, 1986). Native Americans used this plant for treatment of tuberculosis (Moerman, 1986). The alantolactone obtained from roots of *I. racemosa* enhances insulin sensitivity and thus proves beneficial in fat reduction (Tripathi and Chaturvedi, 1995). Roots of *I. racemosa* are also useful in treating pulmonary and cardiovascular disorders (Miller, 1998, and Lokhande *et al.*, 2006)

Due to the great market demand, over and illegal exploitation from wild, and the endangered status of the species, the present study was carried out to ensure proper utilization, commercialization as well as conservation of the species. The plant propagation is generally regarded as the first phase of the complete package of productive technology of herbal medicine. The vegetative and sexual propagation have been recognized as efficient means of rapid multiplication and conservation of important taxa. Keeping in view all the above facts,

the present study was carried out to develop a mass multiplication protocol for conservation and commercialization of *Inula racemosa* and the information will also prove beneficial for the other related species.

Material and Methods:

Rhizome was used as the main means of vegetative propagation with an emphasis on its economic potential. In the month of April 2009, rhizomes of *I. racemosa* were collected from its natural habitat 'Drung Farozpur' at an altitude of 2150m (amsl). The freshly harvested rhizomes were washed thoroughly with running tap water followed by double distilled water to run off any contaminant. Each rhizome portion was split longitudinally with a sterilized razor blade/knife into 2, 4 or 8 pieces, according to the size of the parental rhizome, with each piece having at least one vegetative bud. The split rhizome cuttings were treated with different concentrations of hormonal viz. IAA, IBA and GA_3 (25ppm, 50ppm and 100ppm) for 48hours. One set in each case was treated with distilled water to serve as control. In each treatment three replicates with six cuttings each were used and subsequently sown in sandy loam textured soil in the Kashmir University Botanical Garden (KUBG), Srinagar, India. Percentage sprouting and rooting was recorded for each treatment.

Fresh seeds used for the study undertaken were collected from one population in natural habitats of *I. racemosa* in Oct. 2009 and were stored for two months at room temperature. The air dried seeds were surface sterilized by dipping in 0.01% aqueous solution of mercuric chloride ($Hgcl_2$) for 10 to 15 seconds to discourage fungal infection followed by washing 2-3 times with double distilled water. The disinfected seeds were placed in sterilized petriplates lined with a single layer of whattman filter paper and subjected to different physical and chemical treatments. Three replicates of seeds were used for each treatment and the control sets were maintained in each case using double distilled water. The germination count was made daily wherein the emergence of the radical was used as the criterion to determine germination. Mean germination time was calculated following Hartman and Kester (1989) i.e.:

$$\mathbf{MGT= \sum (nd)/N}$$

where '**n**' is number of newly germinated seeds after each incubation period in days '**d**', and '**N**' is total number of seeds germinated at the end of experiment. The seed vigour index was calculated using the formula of Abdul-baki and Anderson (1973) i.e.

$$\mathbf{VI=Ls\times Pg/100}$$

where, '**Ls'** is the mean of seedling length and '**Pg'** is percentage germination.

Data Analysis:

The data was analysed statistically using MS-Excel 2007. Data was analysed for Mean and standard deviation. ANOVA was used to interpret the variation and to identify the best treatments.

Results and Discussion:

Results of rhizome splitting as a means of mass multiplication for *I. racemosa* are shown in Table 1. Results indicate a maximum shoot sprouting and percentage survival (rooting) in 100ppm of IAA (88.89% and 77.78% respectively), as compared to control with 77.78% of sprouting and 44.45% of rooting. Both IAA as well as IBA were found to have a profound effect on sprouting as well as rooting percentage. Similar results have been reported by Butola and Badola, (2007) in *Angelica glauca* and *Heracleum candicans* and by Vashistha *et al*., (2009) in *Angelica glauca* and *Angelica archangelica*. Among different treatments IAA(100ppm) and IBA(100ppm) proved to be most effective ($p\leq0.05$) in increasing the sprouting and rooting percentage. GA_3 concentrations were found to be less effective particularly in the induction of adventitious root development.

Increased percentage of rooting after IAA and IBA treatments as compared to control shows the higher potential of IAA and IBA in the induction of adventitious root development as also reported earlier by Kuris et *al*., (1980), in *Origanum vulgare* L. Moreover, the chances of survival and growth performance of vegetatively propagated individuals are better implying that a reasonable number of plantlets could be raised and their survival could be ensured by using different concentrations of IAA and IBA. Thus the IAA and IBA in concentrations ranging from 50-100ppm can be recommended as promising treatments while carrying out vegetative mass multiplication of *I. racemosa*.

Table: 1 Effect of Growth hormones on vegetative propagation of *I. racemosa* using rhizome segments.

S. No	Treatments	Sprouting percentage	Rooting percentage
1.	Control	77.78±1.25	44.45±1.69
2.	IAA 25ppm	72.23±0.95	61.12±0.48
3.	IAA 50ppm	77.78±0.48	66.67±0.82
4.	IAA100ppm	88.89±0.95*	77.78±1.42*
5.	IBA 25ppm	61.12±0.95	50.00±0.82
6.	IBA 50ppm	66.67±0.82	55.56±0.95
7.	IBA 100ppm	83.34±1.42	61.12±0.48
8.	GA_3 25ppm	50.00±0.82	33.34±1.42
9.	GA_3 50ppm	66.67±1.42	50.00±1.64
10.	GA_3 100ppm	55.56±0.48	33.34±0.82
	LSD($p \leq 0.05$)	16.37	18.61

***=significant**

Figure 1. Parental rhizome used for propagation

Figure 2. Splitted rhizome cuttings wherein Vegetative each portion has part of apical meristem

Figure 3. One Rhizome segment showing

Figure 4. A progenial mature individual shoot regeneration

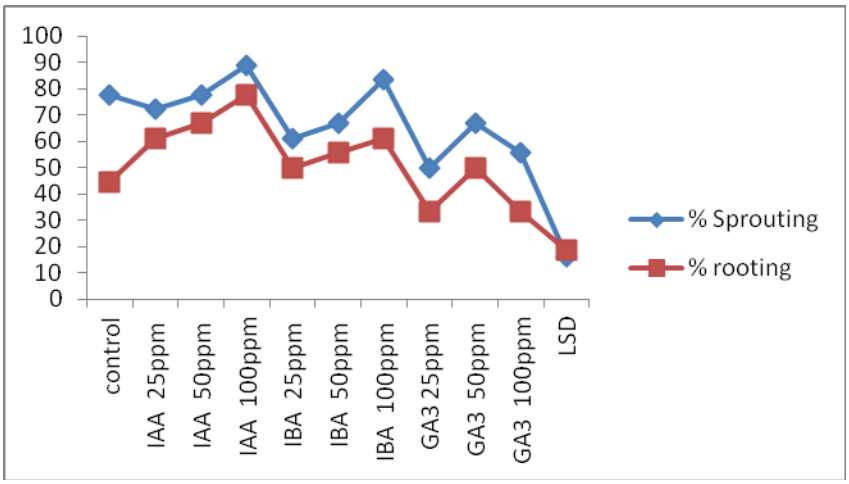

Figure 5. Graph showing effect of different growth hormones on sprouting and survival of vegetative cuttings of *I. racemosa*.

The effects of different pretreatments on *I. racemosa* seed germination are shown in Table 2. The results showed significant ($p \leq 0.05$) differences among method used for stimulation of *I. racemosa* seed germination and no germination was recorded on fresh seeds prior to onset of scarifaction or stratification. Among the pretreatments used, T10 (scarification + GA_3 100ppm) recorded the highest germination of 90.00±0.30% followed by T21 (wet stratification of 100 days) with 86.67± 0.34%. Germination was absent in control replicates. Seeds showed a broad range of prechilling requirement for germination. Dry and wet stratification for 40, 60, 80, and 100 days increased the percentage germination from 46.67±0.49% (dry stratification for 40 days) to 86.67±0.34% (wet stratification for 100 days). The stratification treatments for *I. racemosa* seed germination were also reported by Sharma *et al*., (2006) and Jabeen *et al*., (2007). The increased time of statification increased total seed germination and reduced mean germination time. Ren and Tao, (2004) also reported that cold stratification treatments significantly promoted overall germination in *Calligonium* species. The treatments T1-T6,T11-T13, and T22 proved to be ineffective and failed to stimulate the germination of *I. racemosa.* In comparision to mechanical scarification (removal of seed coat), acid scarification (Dip in H_2SO_4) was found ineffective in stimulating seed germination and GA_3 alone was also unable to induce germination in *I. racemosa* seeds which seems to be controvertial with the results obtained by Sharma *et al*., (2006). Scarification alone gave a good germination percentage but scarification and GA_3 together proved more effective and highest germination percentage were obtained when GA_3 in higher concentations were accompanied with scarification. Decline in mean germination time (MGT) upto 2.92±0.23 (Table 2 **)** and highest value of seed vigour index 2.74±0.27 was also observed when GA_3 (100ppm) and scarification were applied together. Appreciable reduction in MGT with increased concentrations of GA_3 were also reported by Pradhan *et al.,* (2010) in *Swertia chirayita*. GA_3 has been reported earlier also to mediate seed germination by inducing the production of hydrolytic enzymes in barley (Mozer, 1980). Other traits such as radicle length, plumule length, germination rate and vigour index were also affected by GA_3 concentrations (Table 2). MGT in *I. racemosa* seeds decreased in all effective pretreatments compared to control where germination is absent. Among the observed effective pretreatments, T10 shows the significant reduction in MGT (2.92±0.23 days). MGT also decreased with increasing duration of stratification. Similar results were reported by Bhatt *et al.*, (2000) in *Myrica esculanta*.

Table II. Effect of different pretreatments on *I. racemosa* seed germination.

S NO.	Treatment	Days for first germination	Percentage germination	MGT	Redicle length (cm)	Plumule length (cm)	Seed vigour index
T0	Control	×	×	×	×	×	×
T1	GA_3	×	×	×	×	×	×

T2	**Thiourea**	×	×	×	×	×	×
T3	**IAA**	×	×	×	×	×	×
T4	**IBA**	×	×	×	×	×	×
T5	**Dip in H_2SO_4**	×	×	×	×	×	×
T6	**KNO_3**	×	×	×	×	×	×
T7	**Scarification (mechanical)**	4.66±0.95	70.00 ±0.45	4.96±0.69	0.78±0.08	0.72±0.08	1.05±0.19
T8	**Scarification +GA_3 25ppm**	5.68±0.48	63.34±0.49	4.49±0.83	0.85±0.06	0.97±0.07	1.15±0.03
T9	**Scarification +GA_3 50ppm**	4.35±0.48	76.67±0.42	3.57±0.29	1.1±0.09	0.99±0.08	1.59±0.14
T10	**Scarification+ GA_3 100ppm**	1.35±0.47*	90.00±0.30*	2.92±0.23*	1.47±0.69*	1.56±0.09*	2.74±0.27 *
T11	**Kinetin**	×	×	×	×	×	×
T12	**Dry stratification (20 days)**	×	×	×	×	×	×
T13	**Wet stratification (20 days)**	×	×	×	×	×	×
T14	**Dry stratification (40 days)**	8.34±0.48	46.67±0.49	5.31±0.14	0.73±0.08	0.68±0.06	0.66±0.09
T15	**Wet stratification (40 days)**	7.67±0.29	53.34±0.49	4.89±0.27	0.82±0.09	0.83±0.07	0.89±0.11
T16	**Dry stratification (60 days)**	6.00±0.82	50.00±0.50	5.03±0.27	0.73±0.91	0.76±0.06	0.74±0.09
T17	**Wet stratification (60 days)**	6.34±2.13	46.67±0.48	4.86±0.46	0.88±0.90	0.89±0.06	1.06±0.12
T18	**Dry stratification (80 days)**	5.34±0.39	73.34±0.44	3.69±0.26	0.81±0.05	0.88±0.09	1.23±0.08
T19	**Wet stratification (80 days)**	4.34 ±0.48	80.00±0.40	3.57±0.11	0.75 ±0.03	0.86 ±0.08	1.29±0.13
T20	**Dry stratification (100 days)**	4.00 ±0.82	76.67 ±0.42	3.91±0.21	0.95±0.09	0.92 ±0.08	1.46±0.21
T21	**Wet stratification (100 days)**	3.67 ±1.74	86.67 ±0.34	3.81±0.52	1.01 ±0.16	0.99 ±0.16	1.61±0.12
T22	**Dark**	×	×	×	×	×	×
T23	**Dark+ scarification**	4.34 ±1.25	60.00 ±0.49	4.74±0.51	0.99 ±0.09	1.13 ±0.09	0.25±0.29
	LSD ≤0.05		1.51				

***Significant**

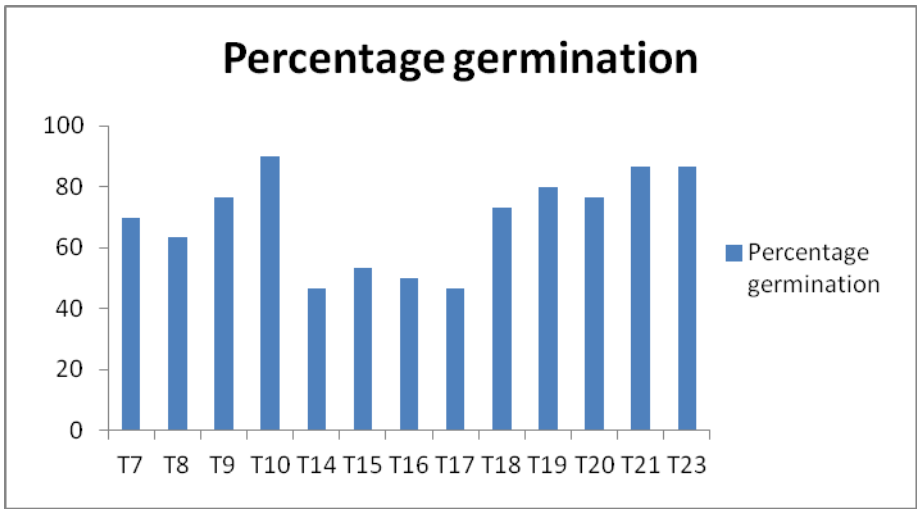

Figure 6. Graph showing percentage germination in different effective treatments, wherein T7–T23 represents- Scarification, Scarification+GA3 25ppm, scarification+GA3 50ppm, Scarification+GA3 100ppm, Dry Stratification 40 days, Wet stratification 40 days, Dry stratification 60 days, Wet stratification 60 days, Dry stratification 80 days, Wet Stratification 80 days, Dry Stratification 100 days, wet stratification 100 days and Dark+scarification.

Figure 7. Germination of seeds after 100 days of wet stratification.

Germination percentage of scarified seeds of *I. racemosa* were higher in light (70.00±0.45) than in darkness(60.00±0.49). Gulzar *et al*., (2007) also recorded an enhanced germination percentage in light compared to germination in dark in *Desmostachya bipinnata*.

Conclusion: Plant propagation multiplies plants in bulk and preserves their essential genetic charecteristics. The outcome of the present study can be gainfully utilized for multiplication of the species.Individuals raised through vegetative or sexual propagation could be utilized for revegetating the wild population and sustainable utilization of the species.The information will prove beneficial not only for conservation of species but also in boosting rural economy and the information will prove beneficial for other related species.

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