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Efficient propagation of an endangered medicinal plant *Jurinea dolomiaea* Boiss in the North Western Himalaya using rhizome cuttings under *ex situ* conditions

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Jurinea dolomiaea **is an important medicinal and aromatic plant species of Kashmir Himalaya. Due to its tremendous overexploitation the species has been listed as endangered for Himalayan region. In this study we carried out the propagation of *J. dolomiaea* using rhizome cuttings. Propagation through rhizome cuttings is a means towards conserving the species and making available planting material of this species for cultivation. Bringing more species under large-scale cultivation helps reduce the pressure on the wild stocks. We investigated the sprouting ability and percentage survival of rhizome cuttings under *ex situ* conditions including soil textures, moisture contents and different concentrations of Indole acetic acid (IAA), Indole butyric acid (IBA) and Gibberellic acid (GA_3) treatments. A better rooting response ($p \leq 0.05$) was observed with GA_3 25 ppm treatment when compared to zero hormone soaked.**

Key words: Rhizome cuttings, field capacity, Indole acetic acid (IAA), Indole butyric acid (IBA) and Gibberellic acid (GA_3), vegetative propagation.

INTRODUCTION

Jurinea dolomiaea Boiss., commonly known in Kashmir as dhup, of family Asteraceae was selected for present study. It is endemic to Himalaya and is distributed from Pakistan to East Nepal between 3000 and 4300 m in open slopes (Chauhan, 1999). It is an important medicinal and aromatic herb of North Western Himalaya and is being exploited because of its medicinal values. A decoction of the roots is cordial. It is given in the treatment of colic and puerperal fever. The juice of the roots is used in the treatment of fevers, diarrhoea and stomachache. The crushed root is applied as a poultice to eruptions (Chopra et al., 1956). The root extract is used as incense (Manandhar, 2002). In India, *J. dolomiaea* has been used as aphrodisiac (Sekar and Srivastava, 2005). In Jammu and Kashmir, the plant is used for treatment of eye infection and it aromatic oil

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from roots is useful in gout and rheumatism (Kumar et al., 2009). Due to its tremendous overexploitation, the species has been listed as endangered for Himalayan region (IUCN, 2003; Pant and Pant, 2011; Siwach et al., 2013).

Vegetative propagation is one of the potential and useful means of propagating those species which are economically important and difficult to raise through seeds. Plant propagation through vegetative means multiplies these plants and preserves their essential genetic characters. This is an easy and effective technique for multiplication and conservation of plant species. Sexual reproduction is considered less important than vegetative propagation for arctic and alpine species (Bliss, 1971). Further, one of the most appropriate actions for safeguarding over exploited species is to improve propagation techniques and to encourage cultivation. The mode of regeneration of *J. dolomiaea* in nature is by seed as well as by rhizome. Any improvement in seed germination and vegetative multiplication can substantially help in propagation. The present study was carried out to develop an efficient method of propagation using rhizome cuttings so as to facilitate mass multiplication and conservation of species under *ex situ* conditions.

MATERIALS AND METHODS

Plant material

Rhizome section is the best planting material for cultivation other than seed because the planting material raised by the seeds takes more time for crop maturity as well as production (Nautiyal and Nautiyal, 2004; Anonymous, 2008). In this context, rhizomes were used for carrying out the mass propagation of *J. dolomiaea*. In the first week of June, rhizomes were collected from natural habitat Apharwat: an alpine zone (3378 m asl) located between 34°01'N Lat. and 74°21'E Long, in Kashmir Himalaya, India.

Effect of soil texture and field capacity on vegetative propagation

Before the collection of plant material the field capacity of different soil textures was determined using Buckner's funnel. Different planting trays were prepared by putting weighed amount of soil and sand in it as per the selected combination. Depending upon the size of parental rhizome, each rhizome was splitted longitudinally into a number of pieces but care was taken to ensure that each piece contains a portion of shoot apex. These cuttings were sown in the prepared plastic trays. In each case, three replicates with four cuttings each were used. Different moisture levels were ensured by adding the measured quantity of water as per the field capacity of each soil texture. The experiment was carried out under controlled conditions in green house of Kashmir University Botanical Garden (KUBG). Total sprouting was recorded at the culmination of the experiment in respect of all the treatments.

Effect of growth hormones on vegetative propagation

The rhizomes were washed thoroughly with running tap water so as to remove soil particles. Each rhizome was cut longitudinally, with a sterilized razor blade, into 2, 4, or 8 pieces, according to the size of the parental rhizome. The split rhizome cuttings were treated with different hormonal concentrations by placing them in sterilized petri-plates containing 25, 50, and 100 ppm concentrations of IAA, IBA and GA_3. One set in each case was treated with distilled water to treat as control. The hormone solutions were made using deionized water and analytical grade chemicals. In each treatment, three replicates with four cuttings each were used. After 48 h of treatment control and segments treated with the plant growth regulators (PGRs) were planted in earthen pots containing sandy loam soil in Kashmir University Botanical Garden (KUBG). The pots were irrigated and monitored regularly throughout the course of experiment. Days taken for first sprouting, percentage sprouting and rooting were recorded for each treatment.

Statistical analysis

The data was analysed statistically using MS-Excel 2007. Data was analysed for Mean and Standard Deviation. The analysis of variance (ANOVA) procedures were used to test for significant effect of treatments, followed by Duncan's Multiple Range Test (DMRT) for comparisons of different means of different treatments.

RESULTS

Effect of soil texture and field capacity on vegetative propagation

Sprouting percentage of rhizome cuttings of *J. dolomiaea* varied in different soil textures and moisture content of soil (Table 1). Maximum sprouting of 80.5±4.2% was recorded in soil texture having sand : soil in 1 : 1 ratio, followed by 63.8±4.8% sprouting in soil texture having sand : soil in 1 : 2 ratio (Figure 1). However, no sprouting occurred in pure sand. It was observed that irrespective of the soil texture, the rhizomes showed higher percentage of sprouting at ½ field capacity, followed by full field capacity and least when ¼ field capacity was used.

Effect of growth hormones on vegetative propagation

Effect of different hormonal treatments on the sprouting of rhizome cuttings of *J. dolomiaea* are shown in Table 2. The rhizome cuttings treated with GA_3 25 ppm took minimum days (8±1) for sprouting. Maximum shoot sprouting and percentage survival (rooting) was observed in GA_3 25 ppm (94.45±0.24% and 83.34±0.38% respectively), as compared to control treatments with 77.78±0.43% of sprouting and 44.45±0.51% of rooting. IAA 25 ppm, 50 ppm and 100 ppm treatments showed lesser percentage of sprouting and rooting than control. Among different treatments, GA_3 treatments proved to be most effective ($p \leq 0.05$) in increasing the sprouting and rooting percentage. However, IBA treatments were found to be ineffective as no shooting and rooting was observed in these treatments (Figure 2).

Table 1. Effect of soil texture and field capacity on sprouting percentage of rhizome cuttings of *J. dolomiaea*.

Soil texture	Field capacity-F.C (ml)	Percentage sprouting
Soil	Full F.C	27.7^{ae} ±9.6
	½ F.C	38.8^{ab} ±4.8
	¼ F.C	19.4^{a} ±4.8
Sand	Full F.C	0
	½ F.C	0
	¼ F.C	0
Sand : Soil 1 : 1	Full F.C	55.5^{bc} ±4.9
	½ F.C	**80.5^{d}** ±4.2
	¼ F.C	22.22^{ag} ±3.6
Sand : Soil 1 : 2	Full F.C	44.4^{beghi} ±4.8
	½ F.C	63.8^{cdfh} ±4.8
	¼ F.C	33.33^{ab} ±0
Sand : Soil 1 : 3	Full F.C	27.7^{ai} ±2.9
	½ F.C	38.8^{ab} ±4.6
	¼ F.C	0
Sand : Soil 2 : 1	Full F.C	33.33^{ab} ±5.4
	½ F.C	52.7^{bf} ±4.8
	¼ F.C	0
Sand : Soil 3 : 1	Full F.C	36.1^{ab} ±4.1
	½ F.C	0
	¼ F.C	0

*Mean values followed by the same letter are not significantly different by DMRT at $p < 0.05$. Each treatment consisted of twelve rhizome cuttings. Data was recorded after ninety days of setting the experiment.

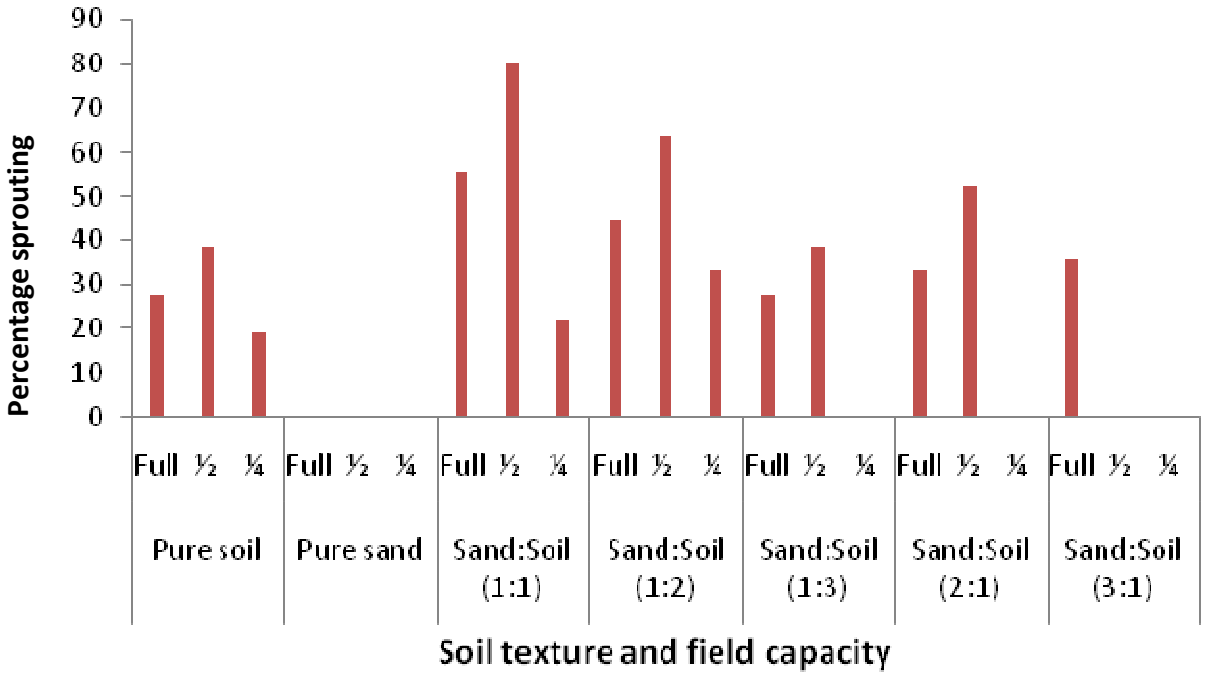

Figure 1. Effect of soil texture and moisture content on sprouting of rhizome cuttings of *J. dolomiaea*.

Table 2. Effect (Mean±S.D.) of different growth hormones on vegetative propagation of *J. dolomiaea* using rhizome segments.

S. No.	Treatments (ppm)	Days taken for first sprouting	Sprouting percentage	Rooting percentage
1	Control	10±1.73	77.78±0.43	44.45±0.51
2	IAA 25	14.6±1.15	44.45±0.51	27.78±0.46
3	IAA 50	11.3±1.52	55.56±0.51	38.89±0.50
4	IAA 100	9.6±0.57	66.67±0.49	50.00±0.51
5	IBA 25	-	-	-
6	IBA 50	-	-	-
7	IBA 100	-	-	-
8	GA_3 25	8±1	94.45±0.24	83.34±0.38
9	GA_3 50	10.6±0.57	83.34±0.38	72.73±0.46
10	GA_3 100	10.6±1.15	72.23±0.46	61.12±0.50
	p-value	0.03	0.02	0.01

*Each treatment consisted of twelve rhizome cuttings. Sprouting percentage was recorded after 30 days of planting the cuttings & rooting percentage after 100 days of setting the experiment.

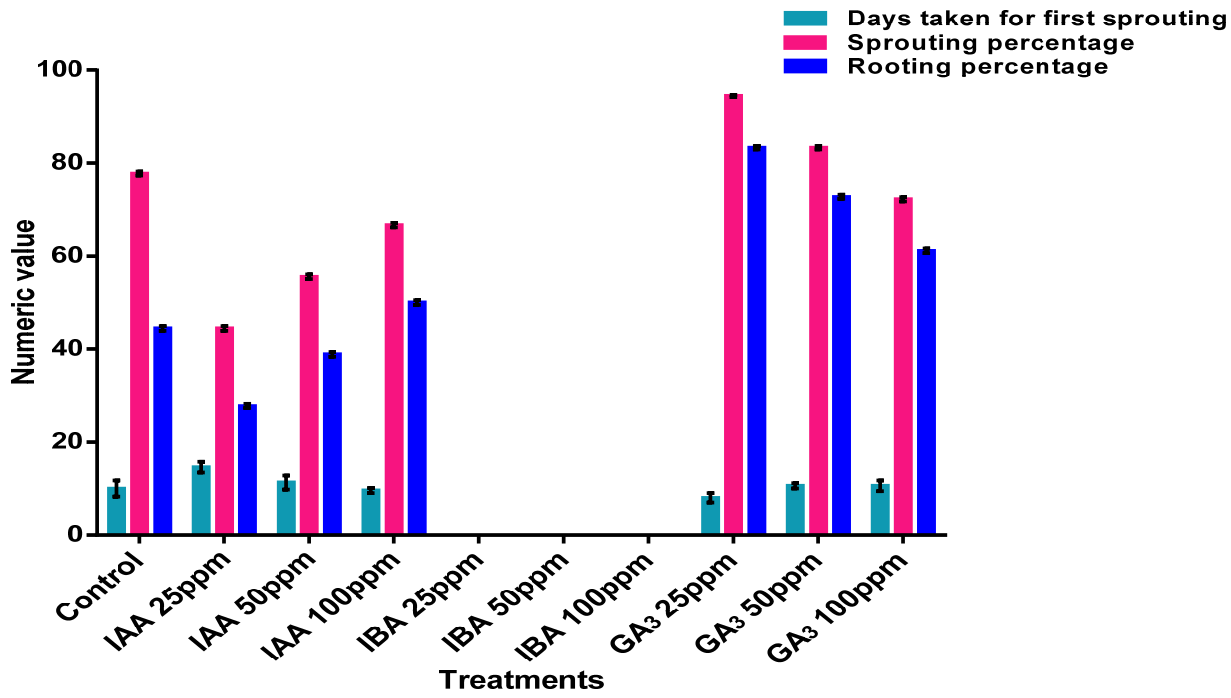

Figure 2. Effect of different growth hormones on vegetative propagation of *J. dolomiaea*.

DISCUSSION

Vegetative propagation can be used as an efficient tool for mass scale propagation of tuberous roots of medicinally important species as in case of *Aconitum atrox*, the species fails to establish through seeds under natural conditions in an alpine environment (Kuniyal, 1999). In *Picrorhiza kurrooa*, vegetative propagation using stolon segments was found successful for cultivation up to 1800 m altitude with high moisture regime and proper aeration (Nautiyal et al., 2001).

The results revealed that sprouting percentage decreased with the increase in sand content and no sprouting occurred in soil texture having only sand. Furthermore, it was evident that irrespective of the soil texture the rhizomes showed higher percentage of sprouting at ½ field capacity, followed by full field capacity and least when ¼ field capacity was used. This indicates that the species prefers less moisture for its better growth and survival. For successful cultivation of *Jurinea*, deep

sandy porous soil is best as the plant develops a thick rootstock (Chauhan, 1999).

Vegetative propagation through splitting of roots was found successful in *Nardostachys jatamansi* and observed as better for multiplication as well as higher production within a short period than cultivation through seedlings (Nautiyal and Nautiyal, 2004). Plant growth regulators and other chemicals have been widely used in vegetative propagation to improve rooting and subsequent growth of cuttings (Nadeem et al., 2000; Butola and Badola, 2007a). The results revealed that the rhizome cuttings treated with GA_3 25 ppm took minimum days (8±1) for sprouting. Maximum shoot sprouting of 94.45% and percentage survival of 83.34% was observed in GA_3 25 ppm, as compared to control with 77.78% of sprouting and 44.45% of rooting. The results contrast with those of Butola and Badola (2007b) who reported increased percentage of rooting after IAA and IBA treatments compared to control in *Angelica glauca* and *Heracleum candicans*. Further, the results were also contrary to Shabir et al. (2010) who reported that GA_3 concentrations are less effective particularly in the induction of adventitious root development in *Inula racemosa*. Chances of survival and growth performance of vegetatively propagated individuals were better implying that a reasonable number of plantlets could be raised from a single rhizome and their survival could be ensured by treating with GA_3 25 ppm.

Conclusion

Although *J. dolomiaea* has been considered as an endangered species, large scale removal (generally during the peak flowering) of its rhizomes still continues at an increasing rate. Therefore, special attention needs to be given for its propagation and conservation; systematic cultivation would go a long way in achieving its conservation. Propagation through rhizome cuttings is a convenient and cost effective method for large scale multiplication and conservation of species. We found that longitudinal cuttings treated with GA_3 25 ppm resulted in maximum sprouting and survival of species. Further, the species showing preference for habitats with less moisture, and thus maximum survival of seedlings resulted in soil texture of sand : soil (1 : 1) at half field capacity. Thus, splitting of rhizomes is a cheap and convenient way for ensuring large scale cultivation as well as conservation of species under *ex situ* conditions.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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