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Seed Germination Enhancement in High Altitude Medicinal Plants of Garhwal Himalaya by Some Pre-sowing Treatments

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ABSTRACT

High value and threatened medicinal plants of Western Himalaya needs attention for conservation. Three medicinal plants were studied to find out the technique for enhancement of germination. Seeds were collected to observe the germination potential of the selected plant species. Seeds of *P. rumicifolium* were excised and treated with phytohormone (GA) while the seeds of both the *Polygonatum* species were kept at low temperature and then treated with Gibberellic Acid. In *P. rumicifolium*, GA₃ (100 ppm) treated excised and intact seeds showed 85 and 50% germination, respectively at 25±1°C under alternate light conditions. *P. cirrhifolium* showed 70% germination in seeds without any treatment and 80% in seeds treated with GA100 ppm at 25±1°C while seeds of *P. verticillatum* required low temperature (-20°C) along with GA 100 ppm treatments for germination and showed 60% germination after the same. In both the species of *Polygonatum*, seed germination was followed by thickening of radicle which was an interesting phenomenon. Single leaf like structure emerged from this swollen portion after prolonged time in the subsequent growing season. The study revealed that the Scarification, GA and chilling treatments enhanced the germination of experimental highly valuable medicinal plants of Garhwal Himalaya.

Key words: Dormancy, low temperature, gibberellic acid, excised seeds, germination

INTRODUCTION

According to a recent estimate, over 7000 plant species are known to be used for medicinal purposes in India where more than 70% people rely on plant based medicines (Holley and Williams, 1996). India is the pioneering country in the trade of medicinal plants and their derivatives. Herbal drug industry of India has a turn over of Rs. 23,00 Crores per annum and it is expected to touch Rs. 40,00 crores per annum by the turn of the century (Handa, 1997). Unscientific exploitation, natural calamities road construction, uprooting and overgrazing has pushed many species towards endangered and threatened (Kumar *et al.*, 2011). Change in size and density of populations over a period of time may indicate rare, threatened and endangered status of the plant species (Maikhuri *et al.*, 1998).

Due to the increasing demand and depletion in supply of herbal drugs there is an urgent need to conserve these pharmaceutically valued species (Airi *et al.*, 2000; Srivastava *et al.*, 2010). According to local collectors and traders of medicinal plants from North Kashmir Himalaya, the demand and supply is not in equilibrium for some medicinal plants, indicating poor presence in natural provenances and absence of cultivation of these medicinal plants (Malik *et al.*, 2011). Uttarankhand is a store house of many important MAP's but due to lack of appropriate cultivation technology and improper management of important medicinal plants of Himalaya, the future of herbal state Uttarakhand could not assess. Cultivation of the medicinal plants may restrict the collection from natural provenances and also conserve naturally and fulfill the demand of market (Prakash *et al.*, 2011).

In view of the above, Three economically important and threatened plant species used as Astvarga components viz. *Polygonum rumicifolium* Royle ex Bab, *Polygonatum verticillatum* All and *Polygonatum cirrhifolium* Royle were selected to workout cultivation technology and to develop strategies for *in situ* conservation measures. The young parts of *Polygonum rumicifolium* are acidic, eaten like rhubarb and rhizomes are useful in joint pain, skin diseases and act as blood purifier. *Polygonatum verticillatum* is used in fever, burning sensation and phthisis and it contains a digitalis glucoside. *Polygonatum cirrhifolium* leaves of the plant are eaten as vegetable as well as reported to be used as a tonic and vulnerary. In Tibet, *Polygonatum cirrhifolium* is used for loss of vigor, pain in kidneys and hips, swelling and fullness in the abdominal region, accumulation of fluids in bone joints, skin eruptions and bronchitis (Singh, 2006).

MATERIALS AND METHODS

Seeds of *Polygonum rumicifolium* were collected from Tungnath (3600 masl) and both the species of *Polygonatum* from Maggu (2800 masl) near Trijuginarayan on the way of Panwali kantha site situated in Rudraprayag District of Uttarakhand in 2010. Seed germination studies play key role in maintaining the cultivation and conservation measures for the desired plant species. So, the attempts were made in this direction to find out the best suited treatments for enhancement in seed germination. For the germination, seeds of *Polygonum rumicifolium* were placed in triplicate in petriplates lined with Whatman No. 1 filter paper under different temperatures with pretreatment of 100 ppm gibberellic acid for 24 h. Seeds of both the *Polygonatum* species were divided in three lots and a lot was kept at 4°C and another lot was at -20°C for 15, 30, 60 and 120 days to determine the effect of low temperature on seed germination and a remaining a lot was kept in room conditions. In the beginning seeds without any treatment kept for germination at 25°C and after that seeds from all the conditions were tested as per the planning to observe the germinability. Each replicate comprised of twenty seeds. Filter papers were moistened regularly with distilled water and daily observations were made. Seeds were placed in alternate (light 16 h/dark 8 h) conditions and continuous dark condition to see the influence of light and dark on the germination.

RESULTS

Maximum germination (85%) was recorded when excised seeds were kept at 25°C under alternate light and dark conditions, treated with GA3 (100 ppm). This is the appropriated temperature (25°C) required for germination, low temperature and dark conditions are not advisable for germination (Table 1).

Table 1: Germination potential of *Polygonum rumicifolium*

Type of seeds	Soaking medium	Light/Dark conditions	Germination (%)	
Intact seeds 25°C	Water	Alternate	35	
	Water	Dark	20	
	GA100 ppm	Alternate	50	
	GA100 ppm	Dark	30	
	12°C	Water	Alternate	20
		Water	Dark	10
		GA100 ppm	Alternate	25
		GA100 ppm	Dark	20
Excised seeds 25°C	Water	Alternate	80	
	Water	Dark	70	
	GA100 ppm	Alternate	85	
	GA100 ppm	Dark	75	

Table 2: Seed germination of both the *Polygonatum* species at 25°C along with alternate light after low temperature treatment

Species	Temperature (°C)	GA treatments	Mean germination (%) after below mentioned treatment days			
			15	30	60	120
<i>Polygonatum cirrhifolium</i>	-20	Control	70	70	60	20
		GA100 ppm	75	75	60	40
	4	Control	70	60	50	10
		GA100 ppm	75	60	55	25
		Control	70	65	65	60
<i>Polygonatum verticillatum</i>	-20	GA100 ppm	80	80	75	70
		Control	35	45	50	45
	4	GA100 ppm	45	50	60	45
		Control	20	25	35	40
		GA100 ppm	30	40	40	40
		Control	15	20	20	20
GA100 ppm	20	25	25	25		

Seed germination studies were also carried out in both the *Polygonatum* species. Seeds of *Polygonatum cirrhifolium* showed 70% germination without any treatment while 10% enhancement in germination (80%) was observed after GA₃ 100 ppm treatment at 25°C. It is noticeable to investigate that the seeds of *Polygonatum cirrhifolium* passed through different experimental trials like cold temperature (-20 and 4°C) treatments did not show any remarkable variation in comparison to control. Seeds of both the *Polygonatum* species were also tested at complete dark conditions at 25°C but not showed in table and text form due to very low percent of germination.

Seeds of *Polygonatum verticillatum* showed very low percent (15) of germination in control condition while enhancement in germination (60%) was observed after the chilling at -20°C for 60 days along with GA 100 ppm treatment to the seeds. To overcome the dormancy seeds of *Polygonatum* species were also scarified and treated with different concentration of GA₃ but seeds were failed to germinate in all these conditions. Seeds of *Polygonatum verticillatum* kept at 4 and -20°C for 120 days, showed only 40 and 45% germination, respectively (Table 2).

In natural conditions, all these experimental species seed shedding followed by cold winter and to protect the seedlings from cold and frost damage, seeds exhibit dormancy to increase adaptability to stress environment.

An interesting phenomenon observed in both the species of *Polygonatum* is that seed germination is followed by thickening of radicle. Most of the germinated seeds remain in this condition till the cold conditions remain. Single leaf like structure emerged from this swollen portion after prolonged time in the subsequent growing season. During past two years seedlings showed only single leaf like structure throughout the growing season and after active growing period, swollen portion of radicle again become dormant. It can be presumed that this swollen portion of radicle requires some specific treatments for seedling emergence and may be an adaptive strategy to overcome severe winter under its natural conditions where such conditions prevail subsequent to its germination.

DISCUSSION

The demand for medicinal plant based raw materials is growing at the rate of 15 to 25% annually and according to an estimate of WHO, the demand for medicinal plants is likely to increase more than US \$5 trillion in 2050. In India, the medicinal plant-related trade is estimated to be approximately US \$1 billion per year (Joshi *et al.*, 2004).

There are many other potential causes of rarity in medicinal plant species, such as habitat specificity, narrow range of distribution, land use disturbances, introduction of non-natives, habitat alteration, climatic changes, heavy livestock grazing, explosion of human population, fragmentation and degradation of population, population bottleneck and genetic drift (Weekley and Race, 2001; Oostermeijer *et al.*, 2003; Kala, 2005). In case of *Polygonum rumicifolium* seeds were excised and treated with different concentration of GA₃ to enhance the germination capability of seeds. Seeds of *Polygonatum verticilatum* kept at 4°C for 120 days, showed only 40% germination. In all these experimental species seed shedding is followed by cold winter and to protect the seedlings from cold and frost damage, seeds exhibit dormancy to increase adaptability to stress environment. In *Bauhinia divaricata*, scarification was found an effective and practical method for breaking seed dormancy (Alderete-Chavez *et al.*, 2011).

Seeds of *Viburnum* species containing embryos described as underdeveloped are dormant at maturity and may need warm ($\geq 15^{\circ}\text{C}$) and/or cold (0-10°C) stratification before embryo growth and germination can take place (Baskin and Baskin, 1998). This class of dormancy is called morphophysiological and eight types are distinguished (Baskin and Baskin, 2004).

Domestication plays a major role in cultivation of high altitude medicinal plants, it may provide more benefits to developmental, social and economic sides of the state. Cultivation of medicinal plants as a renewable resource and non-food crops will provide stable supply of raw materials and could lower the pressure on natural habitats (Kinghorn and Eun-Kyong, 1996). Supply of herbal raw plant materials, extensively used by the pharmaceutical industry as well as the traditional practitioners, becoming a major problem due to very less production and high demand. So, cultivation of these plants is urgently needed to ensure their availability to the industry as well as to people associated with traditional system of medicine. If timely steps are not taken for their conservation, cultivation and mass propagation, they may be lost from the natural vegetation forever.

Polygonatum verticilatum showed enhancement in germination percentage from 15 up to 60% by giving the chilling (-20°C) for 15 to 60 days and exogenous application of GA 100 ppm to the

seeds. Otrshy *et al.* (2009) observed the exogenous application of gibberellic acid and chilling used to overcome the problem of dormancy and increased the germination in seeds of *Asafoetida* (*Ferula assafoetida* L.). It has earlier been reported by Sondheimer *et al.* (1968) that the most common cause of seed dormancy in some alpine plants is a requirement for chilling of the hydrated seeds. Such seeds commonly germinate promptly and uniformly only after they have become hydrated and exposed to low temperature (0-10°C) for a few weeks and same happening was recorded in *P. verticillatum* also. Seed germination enhanced in ex situ produced seeds of *Swertia chirayita* (Pradhan and Badola, 2010), *Coffea arabica* L. (Gebreselassie *et al.*, 2010) and *Aconitum heterophyllum* Wall (Srivastava *et al.*, 2011) by using various pre sowing chemical treatments.

An interesting phenomenon observed in both the species of *Polygonatum* is that seed germination is followed by thickening of radicle. Single leaf like structure emerged from this swollen portion after prolonged time in the subsequent growing season. It can be presumed that this swollen portion of radicle requires some specific treatments for seedling emergence and may be an adaptive strategy to overcome severe winter under its natural conditions where such conditions prevail subsequent to its germination. Chien *et al.* (2002) demonstrated that cold stratification alone overcame dormancy in seeds of a *Taiwanese* species, *V. odoratissimum*, in the section *Solenotinus* (Thyrsoma). It seems that *Polygonatum* species showed epicotyls dormancy might be an adaptation to temperate climates. Similar results were also reported in the case of *Viburnum* (Kollmann and Grubb, 2002).

CONCLUSION

Cultivation process needs to be adopted to overcome the illegal and overexploitation of rare and endangered medicinal plants. On the basis of result obtained it was concluded that excised seeds placed in alternate light and dark conditions favoured the germination of *P. rumicifolium* at 25°C with or without GA₃ treatment. *Polygonatum cirrhifolium* did not show the dormancy as reported in results, species germinated well without any treatment at 25°C. Seeds of *Polygonatum verticillatum* showed the dormancy which often found in alpine plant species and required low temperature along with gibberellic acid treatment. Both the species of *Polygonatum* showed swelling in radicle and required very long time to produce complete seedlings. To overcome the above mentioned problem, there is a scope to researcher in future to find out the solution.

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