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Research Article

Effect of plant growth regulators on germination of seed of *Podophyllum hexandrum* in high altitude region of Ladakh, India.

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Abstract: This study shows an effective but simple method of conserving characterised populations through seed germination modification and treatment with plant growth regulators. It is tolerant to cold temperature, as would be expected of a Northern Himalayan plant, but not tolerant to dry conditions. *Podophyllum hexandrum* seed dormancy has been considered to be a major constraint and most of the earlier reports recommended dormancy breaking pre-treatments such as chilling, gibberellic acid (GA_3). However, in this study, we showed that the pre-treatment of seeds with IAA, IBA and GA_3 plant growth regulators at high altitude (Leh - Ladakh) to analyse the effects on germination percentage of seeds and morphological characteristics of the treated seeds over control seeds. The pre-treatment helps the seeds to give high germination percentage than control seedlings. The treated seedling showed a high degree of growth especially the seedlings treated with GA_3. These seedlings in the greenhouse showed very prominent growth in height and leaf as compared to the control seedling plants.

Keywords: Germination; High Altitude; May apple-*Podophyllum hexandrum* and Seed Treatment

Introduction

Podophyllum hexandrum is an endangered species in India and Northen Himalaya (Jain SK and Shastry ARK 1990, IUCN 2001). The term *Podophyllum* is derived from ancient Greek word 'podos' means a foot and 'phyllos' means a leaf. The name refers to the resemblance of leaves to duck's foot. *P. hexandrum* is a herbaceous plant belongs to family Berberidaceae. The genus *Podophyllum* is generally represented by two species, *hexandrum* and *peltatum*. *P. hexandrum* commonly called as Himalayan May apple because of ripening of fruits in spring and *P. peltatum* commonly known as American Mayapple (Shaista *et al.,* 2014).

P. hexandrum growing at an altitude of 2500-4500 m from mean sea level in trans Himalayan region of Jammu & Kashmir and also found in some part of Sikkim, Shalai hills Shimla, higher altitude of Kangra, Kullu, Rohtang and Chamba of Himachal Pradesh (Sharma, 2013). It attains a height of about 12 to 18 inches. Leaves are lobed and stems are fleshy. Leaves are umbrella-like lobed 2-3 arise on its few stiff branches, it 10-25 cm long and divided into 3 lobes. In spring season flowers are white or pale pink with 6-petaled, borne at the ends of stout stems; these are followed by fleshy, oval fruit or reddish berry 2.5-5 cm in length, with many seeds embedded in pulp. The flower in May-August has six petals and six stamens, which inspired its species name *hexandrum*, meaning six stamens. It is an important source of various biological metabolites and possesses antioxidant, anti-inflammatory, antifungal, cytotoxic and radioprotection activity (Prakash H *et al.,* 2005; Ganie *et al.,* 2011). It can be propagated by seed or by dividing the rhizome (Qazi *et al.,* 2011). The rhizomes of this plant species are the source of podophyllotoxin, which is used for the synthesis of anticancer drugs and also used for various medicinal applications (Rajesh, 2012). It is effective against various diseases including warts and tumours growth of skin and also possesses different types of properties such as purgative, laxative, cholagogue and emetic (Chaurasia *et al.,* 2000). It is the producer of abundant quantities of lignin. It is believed that *P. hexandrum* Royle, (syn. *P. emodi*) to be originated from the Himalayan region. Some Researcher reported that Indian Podophyllum (*P. hexandrum*) contains three-times more resin and podophyllotoxin (4.3%) than the American Podophyllum (*P. peltatum*) species contain only 0.25%, which additionally contains α- and β peltatins (Sharma *et al.,* 2006**).**

However, our study on *P. hexandrum* aimed at Northern Himalayas, Leh-Ladakh, Jammu and Kashmir, India. Moreover, the impact of geographical distance/altitude and phytochemical analysis of podophyllotoxin content and their relationship with each other in the diminishing wild populations of *P. hexandrum* of these study areas has not been reported so far, which we feel is very important for plants conservation aspect and also for herbal formulation point of view.

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Materials and Methods

The present study was conducted at Defence Institute of High Altitude Research (DIHAR-DRDO), Leh-Ladakh in the year 2012. The mature seeds of *P. hexandrum* were collected last week of August 2012 from Trans Himalayan region of Jammu & Kashmir. Collected seeds were surface sterilised in sodium hypochlorite (1% NaOCl) for 3 minutes and then washed three times with sterile distilled water prior to an experimental procedure to prevent fungal contamination. Physical scarification was carried out by soaking intact seeds in distilled water for 24 hours at ambient temperature (25°C) and then after treated with hot distilled water (80°C) for 2 min. After completion of hot water treatments seeds were removed from the water and left to cool for 10 min. Again, the seed was soaked in Concentrated H_2SO_4 for 10 seconds. Chemical scarification was accomplished using two different techniques. After this four different groups formed each group contained hundred (100) numbers of seeds. In these groups, one group of seeds were controlled, and other three were treated with different plant growth regulators before sowing in the trench of the controlled greenhouse. As per the International Seed Testing Association (ISTA) protocol 1985, seeds were treated for 10 min with three different type of plant growth regulator for seed treatment, IAA@100ppm, IBA@100ppm and GA_3@100ppm concentration. Control seeds were treated with only double-distilled water (ddH_2O). After Seed treatment of *P. hexandrum* were sown in the trench of polycarbonate greenhouse in the month of October 2012. The plant growth was under observation and the data were collected in the interval of 60 days. The treatment of plant growth regulators only to seeds laters plants was not treated with it.

Table 1. Seed Germination percentage after treatment of different type Plant Growth Regulators (PGR).

	Treatment			
	Control	**IAA**	**IBA**	**GA_3**
Germination percentage (%)	30	53	55	67

Table 2. Effect of Plant growth regulators on the morphology of plants after 60 days of sowing.

Parameter	Control	IAA	IBA	GA_3
Plant height (cm)	2.50	3.60	4.00	4.50
Leaf length (cm)	2.49	2.51	2.54	2.68
Leaf width (cm)	1.48	1.47	1.38	1.53
Leaf area (cm)	2.61	2.84	2.64	2.98

Table 3. Effect of Plant growth regulators on the morphology of plants after 120 days of sowing.

Parameter	Control	IAA	IBA	GA_3
Plant height (cm)	3.00	4.40	4.20	4.80
Leaf length (cm)	4.72	4.21	2.42	4.70
Leaf width (cm)	2.33	2.11	3.34	2.13
Leaf area (cm)	7.14	6.04	5.98	6.08

Table 4. Effect of Plant growth regulators on the morphology of plants after 180 days of sowing.

Parameter	Control	IAA	IBA	GA_3
Plant Height (cm)	28.00	30.00	31.50	33.50
Leaf Length (cm)	3.66	06.99	06.20	8.20
Leaf width (cm)	4.80	06.42	08.68	11.01
Leaf area (cm)	15.33	32.56	41.96	59.98

Results

Irrespective of the different accessions tested, substratum had no effect on the percent germination of freshly collected seeds at 25ºC. However, the total time taken by the seeds to germinate was found to vary (Table 1). Seed germination of the different accessions commenced within 23–36 days on moist sand (Table 1, Fig. 1). The days took to germinate on moistened filter paper in Petri plates, however, was prolonged to 32–53 days (Table 1). About 17–20% of seedling emergence was observed only after a prolonged period of 11 months at Great Himalayan National Park (GHNP), where the mean maximum temperatures ranged between 6ºC-16ºC and the minimum between 1ºC-13ºC during September and March. The seeds underwent with the chemical scarification and treatment with plant growth regulators to check the dormancy.

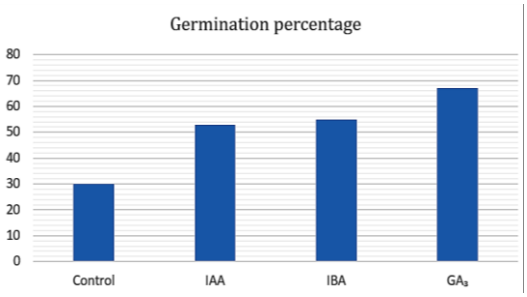

Figure 1. Germination percentage of *P. hexandrum* seeds with PGRs.

It has been observed that the seed treated with IAA, IBA and GA_3 showed more germination percentage than control. The germination percentage of both control and treated seeds were poor. Control seeds germination percentage is only 30% which was treated with ddH_2O then the IAA treated seeds showed 53% germination then IBA treated seeds shown 55% germination the little satisfactory germination is shown by the GA_3 which is 67%.

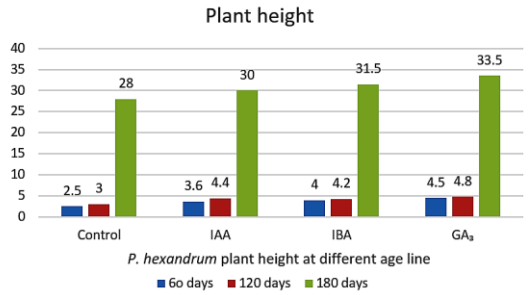

Figure 2. Plant height comparison between treated plants and control.

The germinated plants were under observation to check the effect of IAA, IBA and GA_3 on plant height, leaf length, and leaf width and leaf area. Initially, 60 days when observations were made it was observed that the controlled and treated seedlings growth were all most same [Table 2]. But as the time of observation interval increases the PGRs treated plants showed the significant growth than control. The GA_3 treated seed plants have the highest growth and the seeds treated with IBA and IAA are also showed good growth as compared to control seeds but relatively less than the GA_3 treated plants [Table 3 and 4].

Discussion

P. hexandrum is an endangered species but high medicinal value plant from the cold climatic zone of the world. Its over-exploitation poses serious threat toward its extinction. It is the need of the hour to save this highly important medicinal plant. The different conservation methods should be used to avoid the extinction of supreme plant species by protecting plants in their habitats and by cultivating them and again re-establish them in the natural environment.

To overcome these problem different methods of propagation were adopted. Propagation through vegetative means only may result in loss of valuable representative genetic diversity, whereas dormancy, poor or erratic seed germination and poor seedling establishment are major blocks in propagation through seeds (Troup, 1995; Badhwar *et al.,* 1963; Kharkwal *et al.,* 2004). Whereas GA_3 after acid scarification could strongly relieve the seeds from dormancy (Sharma *et al.,* 2013), its lone application failed to bring about optimal germination (Chaudhary *et al.,* 1996; Nadeem *et al.,* 2000). In our experiment, the GA_3 treated seeds showed the highest percentage of seedlings germination and further the growth of the plants were also significant and satisfactory as compared to those seeds which were treated with IAA and IBA. As the GA_3 can stimulate rapid stem and root growth induce mitotic division in the leaves of some plants, and increase seed germination rate.

Auxin increased the number of bud emergence and root growth as compared with control. In this study, the sets of seed treated with IAA and IBA, as both are auxins and have the similar properties. The two different auxins are compared here to check the better plant growth option in a single hormones derivative. But we found that both the derivatives worked well in this treatment. So it concluded that among Auxin the IAA will be the better option for the plant growth and germination.

Conclusion

The present study showed that this method can be a good alternative for the conservation of *P. hexandrum* in the habitat region only. This concluded that the GA_3 showed maximum germination percentage than that of IAA and IBA. And GA_3 treated seeds also shown comparatively better morphological growth in the areas like plant height, leaf length, leaf width and leaf area. So GA_3 treated seeds can be used at high altitude to flourish the valuable medicinal plant i.e. *P. hexandrum* population and can be used to save the other endangered plants at high altitude.

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