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CONSERVATION OF *PODOPHYLLUM HEXANDRUM* THROUGH SEEDS

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SUMMARY

***Podophyllum hexandrum* Royle, inhabitant of north-western Himalayas, is an endangered and prioritized medicinal herb for conservation purpose. This herb shows delayed and low seed germination with poor seedling survival. The aim of this study was to develop a complete package for attaining higher and early germination percentage and to innovate suitable storage environment for prolonging germination potential of seeds, which is otherwise lost quickly. The findings revealed that germination percentage, onset of germination and vigour index can be improved by subjecting seeds to presowing chilling, hydropriming for 8 days or chemical treatments with petroleum ether, triacontanol or sodium hypochlorite. Storage of seeds at -10° C in plastic jars was most suitable for maintaining germination ability, even after one year, which otherwise is lost in six months at room temperature. Sowing of seeds in mixture of soil + FYM + coco peat (2:1:1) resulted in maximum seedling emergence and survival. The study will be useful for conserving this herb species.**

Keywords: **Seed germination, seedling vigour, seedling emergence, storage environment, vigour index**

INTRODUCTION

North-West Himalayas are the richest source of diversity of medicinal and aromatic herb species. Among these, *Podophyllum hexandrum* Royle is a herbaceous species belonging to family Podophyllaceae; commonly known as 'Bankakri, kanda-ri-mokri or Indian *Podophyllum'*.

It is distributed in inaccessible far flung areas of temperate Himalayas between 2600-4500 m amsl. The herb species is perennial, succulent with knotted rhizomes, which are considered hepatic, stimulant, cholagogue, purgative and alterative. Rhizome and roots of *Podophyllum* consist of podophyllotoxins, podophyllic acid, podophylloresin and quercetin (Chauhan 1999, Singh *et al.* 1999*)*. The Indian *Podophyllum* yields 7-15% resin as compared to American *Podophyllum*, which yields only 4-8% resin. Recently, podophyllotoxin has acquired great importance and high medicinal status due to its effectiveness as antimitotic and anticancerous activity, especially for curing of uterine tumors (Macrae and Towers 1984, Richter *et al.* 1987). This high value gene stock has been put at risk and acquired endangered status (Badola and Pal 2002). The reasons are several; the main cause of declining population of this precious herb is the unscientific and excessive exploitation and over harvesting of rhizomes and roots to meet the ever increasing demand by pharmaceutical industries. Beside this, habitat destruction and inter-annual climate change coupled with inadequate efforts towards replenishment have led to depletion of herbal population from natural

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habitats (Thakur *et al.* 2008). According to Red Data Book (Nayar and Sastry 1990), *Podophyllum hexandrum* is the prioritized species that needs immediate conservation. *Podophyllum* can be propagated through seeds and rhizomes. Seed propagation is recommended in order to protect the valuable rhizome. However, seed germination in *Podophyllum* is highly erratic, delayed and low with low rate of seedling survival (Nautiyal *et al.* 1987, Bhadula *et al.* 1996). Though, some studies have been conducted on seed germination behaviour and viability of different populations of *Podophyllum hexandrum* (Singh *et al.* 1999, Thakur *et al.* 2004). Information is not available on seed storage behaviour and seedling establishment. Our earlier studies have suggested the significance of osmopriming treatments in achieving uniform germination in other temperate medicinal herbs (Thakur 2008). Therefore, the objectives of the present investigation were to develop complete package for the i) identification of simple and practically adoptable treatments to induce early onset of germination along with higher germination percentage, ii) suitable storage conditions i. e.temperature and containers and iii) proper soil media to attain maximum seedling survival.

MATERIALS AND METHODS

Seed collection and germination test

Mature fruits of *Podophyllum hexandrum* Royle were collected from their natural populations in Kinnaur district of Himachal Pradesh in the months of July and August. Seeds were extracted from these fruits, cleaned, sorted and dried in shade for one week at room temperature (22 ± 2 °C). Thereafter, seeds were surface sterilized by dipping in aqueous solution of 0.1 per cent $HgCl_2$ for 10 min, washed with distilled water thoroughly and repeatedly to remove $HgCl_2$, surface dried and used for three separate experiments i.e. presowing chilling, hydropriming and chemical treatments. Presowing chilling was performed by placing seeds in contact with moist substratum at -5, 2 and 5 °C for 15 , 30, 60 and 90 days in separate chambers of refrigerator. Hydropriming was done by soaking seeds in distilled water for 1, 2, 4, 8 and 12 days at room temperature (20 ± 2^{0}C).

Chemical treatments

Chemical and other treatments included mechanical scarification, acid scarification with H_2SO_4 for 5 and 10 min, GA_3 50 and 100 ppm for 24 h and BA + GA_3 (Benzyl adenine + gibberellic acid; procured from Lobachem, each 25 ppm for 24 h); petroleum ether for 3 h, sodium hypochlorite (NaOCl, 10/1000 v/v for 15 min) and triacontanol (Miraculan manufactured by NOCIL BOMBAY, 4/1000 v/v for 3 h). Mechanical scarification was done by shaking seeds with coarse sand vigorously. Thereafter, all sets of seeds which were subjected to presowing treatments along with the control were surface dried on filter paper and kept for germination in Petri plates (7.5 inch diameter) in seed germinator at 20 ^{0}C, 80 per cent RH and at 16 h light and 8 h dark period. The experiment was conducted in CRD with 4 replicates of 100 seeds each. Germination test was performed according to ISTA; germination energy was studied according to Czabator (1966). Field emergence of chemically pretreated seeds was tested in the nursery.

Storage treatments

In order to standardize the optimum storage conditions, seeds were kept in polythene envelope at -20, -10, -5, 0, and 5 ^{0}C temperature in deep freezer and refrigerator for 2, 4, 6, 8, 10 and 12 months. Seeds stored at room temperature (22±2 °C) served as control. Treated and control seeds were tested for germination in seed germinator as above. After the completion of above experiment, another experiment was conducted to work out best storage container out of four containers i.e. cloth bags, paper bags, plastic jars and sealed polythene bags. Seeds were stored in above containers at -10 ^{0}C for 5 months, since results of the storage experiments revealed comparatively higher germination in seeds stored at -10 °C. These seeds, in the month of March were sown in nursery for testing their field emergence. Survival per cent of seedlings after 10 months was also recorded. Germination tests for storage treatments were performed according to ISTA. Vigour index for all experiments was calculated as per the equation by Baki and Anderson (1973). Since seedling emergence, establishment and survival in the field is low, therefore, in order to improve these parameters, the soil

media was worked out. Seeds were stored at -10°C for 6 months because this storage treatment was found to be the best treatment for *Podophyllum*. Thereafter, seeds were sown in three types of soil media i.e. Soil + Cocopeat (1:1), Soil + FYM + Cocopeat (2:1:1) and soil + FYM (1:1) in black protrays.

RESULTS

Presowing chilling treatments of seeds resulted in substantial improvement in germination percentage, vigour index and early onset of germination (Fig. 1. A&B). Presowing chilling treatment of seeds at -5 ^{0}C for 30 days was the best treatment for achieving maximum germination (70 %) and higher vigour index (4467). These prechilled seeds took 22 days to emerge whereas, control seeds took longer and emerged after 38 days of sowing thereby decreasing germination time by 16 days. Presowing chilling at 2 ^{0}C for 60 days resulted in 66.6 % germination.

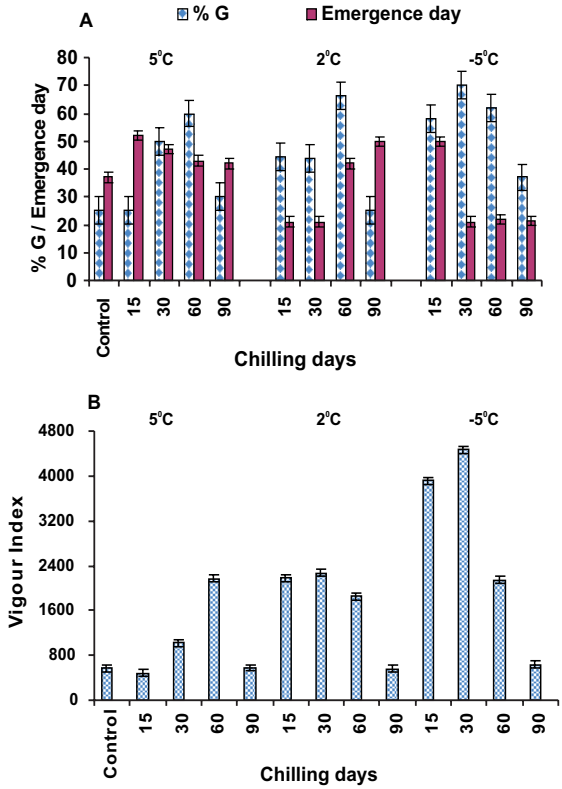

Fig. 1. Effect of presowing chilling treatments on germination characters (A) and vigour index (B). F values for germination (12.13), emergence day (10.38), vigour index (19.21)

Hydropriming of seeds for 1, 2, 4 and 8 days resulted in substantial improvement in germination percentage, advancement in emergence, germination energy and vigour index (Fig. 2. A&B). Hydropriming for 8 days led to maximum (60 %) germination and earliest emergence (35th day) against 25 % germination and 87th day emergence in control (Fig. 2. A). Maximum germination energy (60) and vigour index (1080) was observed in seeds subjected to 8 days of hydropriming (Fig. 2. A & B).

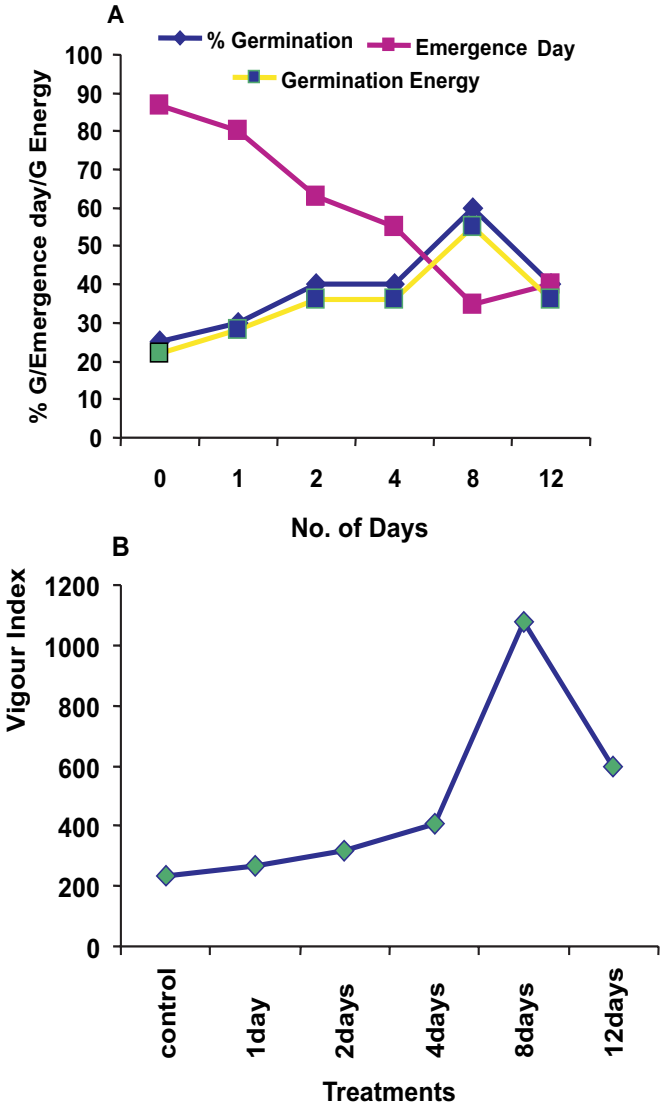

Fig. 2. Effect of hydropriming on seed germination characters (A) and vigour index (B). F values for germination (8.64), emergence day (6.95), germination energy (8.32), vigour index (21.86).

All presowing treatments resulted in substantially higher germination percentage compared to 30% in control (Fig. 3. A). Maximum germination (84%) was observed in seeds treated with petroleum ether for 3 h, followed by triacontanol (80%) for 3 h and 75% in NaOCl (10/100 v/v) for 15 min. Scarification with H_2SO_4 for 10 min, mechanical scarification, GA_3 50 ppm and BA + GA_3 (25 ppm each) resulted in 60% germination against 30% germination in control. Petroleum ether was most effective in inducing early emergence within 8 days against 30 days in control. Maximum vigour index was observed in seeds treated with petroleum ether, followed by triacontanol (Fig. 3. B).

Based on the above findings some selected beneficial treatments were tested for field emergence. Table 1 indicates that overall emergence percentage in field was low i.e. 11.9% in control, however, all tested treatments resulted in higher emergence percentage than control. Maximum 43.8% emergence was registered by seeds treated with petroleum ether for 3 hours, followed by 40.4% by seeds treated with triacontonol (4/1000 v/v) for 3 h and 35% in NaOCl (10/100 v/v) for 15 minutes and seeds chilled at -5 ^{0}C for 30 days.

Storage of seeds at low temperatures i. e. -20, -10 and -5 ^{0}C was effective in maintaining the germination ability of seeds (Fig. 4) whereas, majority of seeds stored at room temperatures lost this ability quickly i.e. within 6 months. Germination percentage of stored seeds declined with increased duration of storage. The best option for storing seeds was at -10 ^{0}C, since this could induce maximum 20% germination even after 1 year of storage. Seeds stored at -20 and -5 ^{0}C showed 10 & 5% germination ability, respectively after 1 year of storage. Field testing of seeds stored under conditions mentioned above revealed almost similar pattern (data not given).

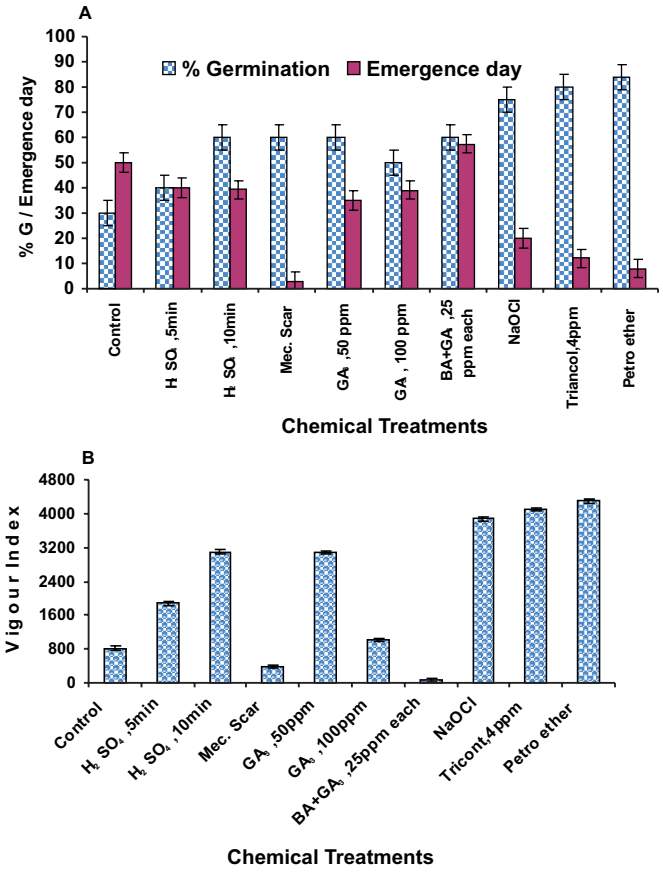

Fig. 3. Effect of chemical treatments on germination characters (A) and vigour index (B). F values for germination (7.38), emergence day (8.2), vigour index (16.58)

Storage of seeds in various containers, namely cloth bags, paper bags, plastic jars and polythene bags, showed significant variation in field emergence and survival percentage of seedlings (Fig. 5 A). The best viable option for storing seeds was plastic jar stored at -10 ^{0}C for 6 months as this treatment resulted maximum (47%) field emergence and 38 % seedling survival. Soil media i. e. Soil + FYM + Cocopeat (2:1:1) resulted in 66.6% field emergence, 42% seedling survival (Fig. 5 B) along with earliest field emergence on 25th day (data not given).

Table 1. Effect of presowing treatments on field emergence of *P. hexandrum* seeds.

Control	Chilling at 2^{o}C for 60 days	Chilling at -5^{o}C for 30 days	NaOCl 10/100 v/v for 15min.	Triacontanol 4/1000 v/v for 3 hrs	Petroleum ether for 3 hrs	CD (5%)
11.9	32.0	35.0	35.4	40.4	43.8	2.11

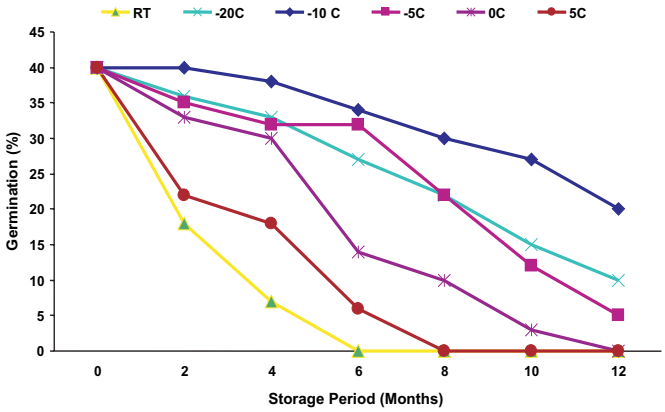

Fig. 4. Effect of storage conditions on seed germination

DISCUSSION

The results of various experiments indicated that germination percentage, onset of germination and seedling vigour was improved by subjecting seeds to prechilling and other presowing treatments like scarification, hydropriming, plant growth regulators and other chemicals. *Podophyllum hexandrum* is a shy seed bearer with low seed set, poor seed viability and high dormancy (4-24 months) in different populations. Seeds show asynchronized, late and low germination percent even after removal of dormancy (Thakur and Mehta 2006). Work experience of authors with *Podophyllum* seeds have shown that late and poor germination in *Podophyllum hexandrum* is due to i) a post harvest ripening requirement and ii) a hard seed coat, especially in temperate populations, which acts as a barrier for imbibition and gaseous exchange. Same observations have earlier been made by Bhadula *et al*. (1996). Scarification treatments were very effective in inducing germination by improving seed coat permeability and removal of the blockage of gaseous exchange. Similar hypothesis was put forth by Francis and Coolbear (1987). Cold treatment can promote germination by inducing GA biosynthesis (Thakur *et al.* 2005) or by increasing GA sensitivity (Pullock and Toole 1961). Chilling on the other hand, exerts a stimulating effect on seed germination promoting factors other than GAs, such as increase of abscisic acid degradation (Egley and Paul 1982) or may instigate changes in membrane permeability as also reported by Francis and Coolbear (1987), which are pivotal in seed germination. Hydropriming induced

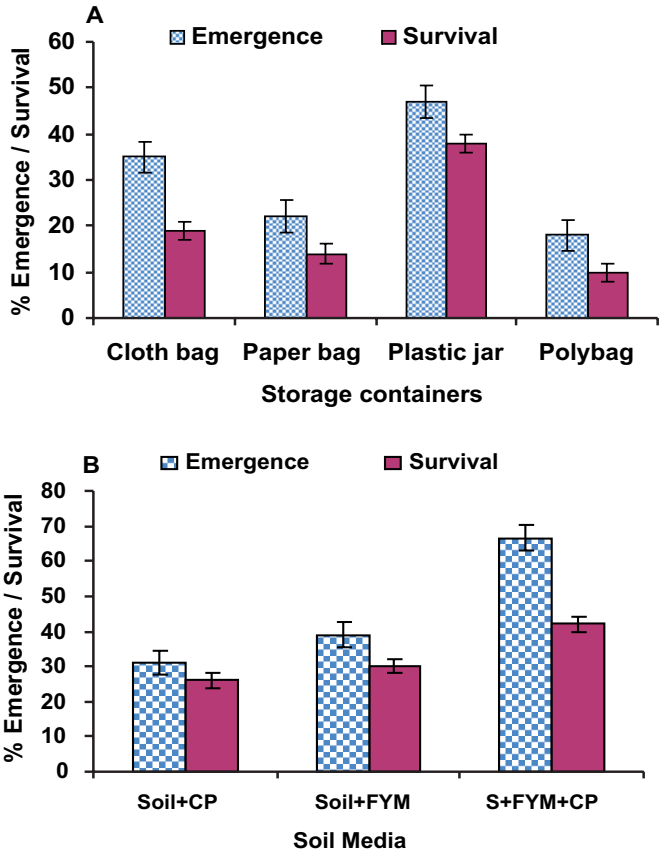

Fig. 5. Influence of storage containers (A) and soil media (B) on field performance. S (soil), CP (Coco peat)

substantial improvement in seed germination was possibly by softening of thick and hard seed coat by prolonged imbibition, which could facilitate the imbibition, a prerequisite for germination along with the leaching of germination inhibitors. The presence of inhibitors in seeds has also been reported earlier (Douglas 1995). Early onset of germination alongwith higher germination in seeds treated with GA_3 might be due to enhanced activity of hydrolases. This is also reported in certain temperate species (Nautiyal and Chauhan, 2007). Successful induction of seed germination by petroleum ether and triacontanol treatments may be assigned to break the permeability barrier probably of organic nature for water absorption as also reported by Verma *et al.* (1996). Sodium hypochlorite treatment to seeds was beneficial on one hand by providing sterilization and inducing germination on the other, since *Podophyllum* seeds take long imbibition period i.e. 1 to few months to germinate, which makes seeds susceptible for fungal and other

infections and may result in seed deterioration. Field testing of seeds subjected to above presowing treatments clearly demonstrate the effectiveness of above treatments in early onset of germination, higher germination percentage with enhanced seedling vigour. However, values for field studies were lower as compared to laboratory studies indicating that the results of germination studies in laboratory almost invariably overestimate the field germination as it is reported in other species too (Dell Aquilla 1987).

Several plant species namely *Salix setchellina* and *Myristica malabarica* indicate the loss of viability and germination ability when stored at room temperature (Douglas 1995, Kumar *et al.* 2002). Storing seeds in plastic jars was the best viable option, since moisture resistant containers could conserve the moisture in seeds (Thakur *et al.* 2007*)*. Loss of germination ability and viability of seeds depend upon the time span and storage conditions (Dell Aquilla 1987). Moisture loss is an important factor for seed deterioration during storage (Copeland and McDonald 2005). Above storage condition can be practiced for storing seed for 1 year and such seeds can be used for propagation when availability of seeds is low during the next year. Maximum seedling emergence was obtained in Soil + FYM + Cocopeat (2:1:1), which seems to be due to porous and light soil media; enriched with organic matter and offers least resistance for seedling emergence. This situation resembles with the soil atmosphere in natural habitat i.e. forest, where seeds are covered with forest litter. This is in concurrence with reports on other endangered medicinal species of temperate Himalayas (Chauhan and Nautiyal 2007).

Our earlier studies with other endangered medicinal species namely *Dioscorea deltoidea, Gentiana kuroo* and *Berberis aristata* also indicated the significance of above presowing treatments in enhancing germination and vigour ((Thakur 2008). These presowing treatments probably induce an increase in germination by exploiting the seeds own resources to the maximum and providing an opportunity for low vigour seeds to cope with the more vigorous ones, which may further result in achieving higher germination percentage and vigour. The findings suggest that presowing seed treatments like chilling, hydropriming and seed treatment with petroleum ether and sodium hypochlorite are of great significance for improving germination, vigour and reducing the time for field emergence. Storing the seeds in plastic jars at -10^{o} C, followed by sowing in soil medium composed of soil +FYM+cocopeat (2:1:1) will be beneficial for better germination ability, field emergence and survival.

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