*Plant Archives* Vol. 17 No. 2, 2017 pp. 1697-1706 *ISSN 0972-5210*

# EVALUATION OF VARIOUS PHYSICO-CHEMICAL TREATMENTS ON SEED GERMINATION OF *RHEUM AUSTRALE* D. DON. AND *PODOPHYLLUM HEXANDRUM* (ROYLE.) : TWO ENDANGERED MEDICINAL PLANT SPECIES OF THE KASHMIR HIMALAYA, INDIA

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## Abstract

In this study, an attempt was made to overcome seed dormancy and enhance germination percentage of seeds of the two endangered and medicinally important species namely *Rheum australe* D. Don (syn. *Rheum emodi* Wall. ex Meissn.)/ rhubarb and *Podophyllum hexandrum* Royle for their effective conservation. The propagation of these species through seeds takes lot of time, due to dormancy and poor germination rate. Germination of seeds in *R. australe* was monitored up to 25$^{th}$ day of the treatment. The best results were recorded in 50 days chilled seeds with 92% germination;; followed by 86 and 82 percent germinations in 40 and 30 day chilled seeds, respectively. Treatment $CHl_3$ (50 day chilled seeds) was statistically different from all other treatments including chilling treatments $chl_1$ (30 day chilled seeds) and $chl_2$ (40 day chilled seeds). Treatments $GA_3$1 (Gibbrellic acid $10^{-3}$ M) and $GA_3$2 (Gibbrellic acid $10^{-4}$ M) with 80% and 75% germination respectively, were at par with each other. Coefficient of variation for all the treatments was 43%, indicating that the treatments were variable with respect to each other. Across days, highest mean (± standard deviation) (60.45±26.38) and range (0-92) was observed on 14$^{th}$ day. In *P. hexandrum* germination started on 27$^{th}$ day and was completed by 90$^{th}$ day. Maximum germination percentage of (66%) was observed in treatment Comb1 ($H_2SO_4$ treatment for 8 minutes, $GA_3$1 = $10^{-3}$ M) and the least (8%) in treatment $S_2$ ($H_2SO_4$ treatment for 8 minutes). No germination was observed in all the three chilling treatments (Ch1; Ch2; Ch3) as well as in potassium nitrate ($K_1$ =0.2%: $K_2$= 0.3%) and control treatments. All the treatments were varied statistically from each other. The coefficient of variation and mean (±SD) across days varied from 48.41 to 230.86 and 1.18±2.72 to 51.36±24.84, respectively. Highest mean (±S.D) (16.63±23.39) across days was recorded in 73-81 day interval and least (0.36±1.14) in 19-27 day interval.

***Key words :*** Seed germination, acid scarification, chilling, conservation, endangered species.

## Introduction

Medicinal plants are the marvelous source of life saving drugs for the most of the people throughout the Globe. They enjoy an inherent and prominent role in general health services of the people. In india, there are 1814 threatened plant species, out of which 113 taxa occur in Indian Himalaya (Nautiyal *et al*., 2001; Wawrosch *et al*., 2003). The demand for medicinal plant species has increased throughout the world due to the rising or renewal of curiosity and attention in belief of herbal medicine for treatment of routine ailments without any side effects. In India, as per the recent estimate, over 7000 species of plants posses medicinal properties and more than 70% Indian people rely on plant based medicines (Holley and Williams, 1996). India is the pioneering country in the trade of medicinal plants and their derivatives. The Indian Herbal drug industry has a turnover of Rs. 23,00 crores per annum and it is expected to touch Rs. 40,00 crores per annum by the end of the century (Handa, 1997). Over exploitation, natural calamities, road construction,

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ruthless uprooting and overgrazing has pushed many species towards endangered and threatened status (Kumar *et al*., 2011). Kashmir Himalayan region is situated within the North-western region of Himalayas that has been currently declared as one of the Global Biodiversity Hotspot (Mitttermier *et al*., 2005). The State of Jammu and Kashmir possesses varied flora and fauna due to great differences in altitude and presence of different climatic zones. The valley of Kashmir harbours about 500 medicinal plant species found in different high altitude and low land areas. The forests of the State have been damaged, like in other parts of the country, due to intense pressure from the ever increasing human and cattle population and over-exploitation. Due to the increasing demand and depletion in supply of herbal drugs it is necessary to conserve these pharmaceutically valued medicinal plant species (Airi *et al*., 2000: Srivastava *et al*., 2010). The medicinal wealth of Kashmir is depleting at a fast rate. Currently 11 of the Indian medicinal plants are enlisted in the appendices of CITES which include *P. hexandrum* and *R. australe* as well. International Union for Conservation of Nature (IUCN) committee for threatened plant species has listed endangered medicinal plant species and *Rheum emodi* is unfortunately present at the top of that list. Therefore, it has been described as the species of paramount importance for conservation and propagation (Parveen and Wani, 2013). Export of these herbs has been banned vide public notice number 47PN (92-97), dated March 30th 1994 and they have been enlisted in negative list of exports and imports policy 1997-2002 of the Govt. of India. Being confined to stringent alpine-sub alpine habitats, which register near arctic severity for a certain period of a year coupled with high humidity round the year, these herbs endure extremely specific ecological niches in this part of the Himalayas. Alterations in these specialised habitats are bound to be disastrous for the very survival of this precious natural resource.

*Rheum australe* (Polygonaceae) is a perennial herb with healthy rhizomes. The roots of *R. australe* are widely used in Ayurvedic and Chinese folk medicine. This plant species produce diverse phenolic metabolites. Different active components like flavonoids, stilbenes, oxanthrone, anthrones ethers and esters, lignans, chromones, anthraquinones, carbohydrates, sterols and phenols have been isolated and characterized from the rhizomes of this plant species (Zargar *et al*., 2011; Rokaya *et al*., 2012). Some of these isolated compounds possess a wide range of pharmacological and biological properties. They are used to lower cholesterol levels, used against infections, for relieving pain, against tumor growth, astringent, as laxative and tonic (Xiang *et al*., 2005). The active component rhein of the species possesses anticarcinomic, antiseptic, antitumor, antiviral, bactericide and viricide properties (You *et al*., 2013; Gupta *et al*., 2014). In addition to the curative properties, its rhizomes yield a red dye used for colouration of silk and wool (Debaish and Bhattacharya, 2008).

*P. hexandrum* (Banvangun), a perennial, rhizomatous herbaceous species, belonging to family Podophyllaceae, is distributed from temperate to alpine regions of Kashmir Himalaya. It has an endangered status in India and occurs at an altitudinal range of 2000 to 4000 m above mean sea level (amsl) (Bhadula *et al*., 1996: Nautiyal *et al*., 2003). The rhizomes and roots of *P. hexandrum* contain both resin and toxin contents (lignans) (Purohit *et al*., 1998). Among the lignans, podophyllotoxin is the most important for its use in the synthesis of anti-cancer drugs- etoposide and teniposide (Choudhary *et al*., 1998), which are used to treat lung and testicular cancer, neuroblastoma, hepatoma and other tumours. Therefore, this plant over the past several decades has been extensively exploited through extraction of crude drug.

There is thus an urgent need to develop and implement regeneration for these economically important medicinal plant species. Propagation under natural habitat of the species concerned would encourage their cultivation, thereby minimising the pressure on natural habitat. The common means of regeneration of medicinal plants are either through seeds, cuttings or micropropagation techniques. Regeneration through seeds is the most promising, ideal and appropriate means for most of the medicinal plant species, as seed is the effective and suitable material for developing *ex-situ* conservation strategies (Hawkes *et al*., 2000; Ganai and Nawchoo, 2002). Therefore, current experiment was conducted to study the effect of various presowing treatments on the enhancement of seed germination on two economically important medicinal plant species *P. hexandrum* and *R. australe* of Kashmir Himalaya. The present experiment is an attempt to standardize some basic techniques of seed germination to overcome seed dormancy and to enhance seed germination that will subsequently help in effective conservation of the species.

## Materials and Methods

Seeds of *Podophyllum hexandrum* and *Rheum australe* were collected at maturity from different alpine natural populations *viz*, Kokernag, Pahalgam and Sonamarg of Kashmir (3000–3850 m asl) during August to September 2014. The seeds were air-dried for a fortnight at room temperature (15±2°C) and then were stored at

room temperature (15±2°C). Seeds were washed with 0.1% mercuric chloride for 5-7 minutes and then with 70% alcohol for 1 minute and thoroughly rinsed with double distilled water and divided into groups of 50 seeds each. The seeds were subjected to various treatments and placed on a moist filter paper in Petri- plates.

**Physical treatment**

**Stratification/Chilling :** The surface sterilized seeds (using mercuric chloride) were soaked in distilled water for 24 hours and then subjected to chilling at low temperature (3-4ºC) for different durations [(Chilling treatment (Ch): $Ch_1$ = 30 days, $Ch_2$ = 40 days and $Ch_3$ = 50 days)] using Refrigerator (Make L.G.).

**Acid scarification/Sulphuric acid ($H_2SO_4$) treatment :** the seeds were treated with concentrated sulphuric acid for varying durations depending upon the species [(Sulphuric acid treatment: $S_1$= 1 min (*Rheum australe*) and $S_2$= 8 min (*Podophyllum hexandrum*)] followed by thorough washing in distilled water. Sulphuric acid (Sigma) used was 99.9% pure.

**Chemical treatment**

**Potassium nitrate ($KNO_3$) :** Surface-sterilized seeds were moistened with different concentrations of aqueous solution potassium nitrate ($K_1$ = 0.2%, $K_2$= 0.3%) for 24 hours followed by germination on substratum moistened with different concentrations of aqueous solution potassium nitrate ($K_1$= 0.2%, $K_2$= 0.3%).

**Gibbrellic acid treatment :** The surface sterilized seeds were kept submerged in different concentrations ($GA_3$1= $10^{-3}$ M, $GA_3$2= $10^{-4}$ M) of aqueous solution of $GA_3$ for 24 hours followed by germination on substratum moistened with different concentrations ($GA_3$1 = $10^{-3}$ M, $GA_3$2= $10^{-4}$ M) of $GA_3$ solution.

**Combined treatment :** Seeds were treated with $H_2SO_4$ for 1 min (*Rheum australe*) and 8 min (*Podophyllum hexandrum*) followed by through washing with distilled water and then soaking in different concentrations of $GA_3$ ($GA_3$1= $10^{-3}$ M, $GA_3$2= $10^{-4}$ M) solution for 24 hours and subsequent germination on substratum moistened with in different concentrations of $GA_3$ solution ($GA_3$1= $10^{-3}$ M, $GA_3$2= $10^{-4}$ M).

(Combined treatment (Com) $H_2SO_4$/$GA_3$: Com 1= $H_2SO_4$/$GA_3$1, Com 2= $H_2SO_4$/$GA_3$2). Different concentrations ($GA_3$1= $10^{-3}$ M, $GA_3$2= $10^{-4}$ M) of aqueous solution of $GA_3$. One treatment was kept as control (Cont). There were eleven treatments, fifty seeds in triplicate were used for each treatment. The experiment was laid in complete randomized design (CRD) with 3 replications each. After the treatment, the seeds were subjected to germination test by allowing them to germinate on the moistened filter paper. The germination of the seeds was monitored over the next three-month at an average temperature of 15-20°C. Observation on the no. of days taken for the first seed to germinate, total no of days for complete germination and the total no of seeds germinated were noted on regular basis. The dispersion around the average germination in each treatment was measured by standard deviation. The relative effectiveness of different physio-chemical and hormonal treatments in dormancy removal and germination improvement was calculated and the seedlings were transferred to the pots.

## Results and Discussion

Treatment of seeds with acid is regarded as the most useful method to overcome the seed dormancy of a species with hard seed coats (Youssef, 2008). *Rheum australe* and *Podophyllum hexandrum* are perennial stout herbs, distributed in the temperate and subtropical regions of Himalaya *viz*. Afghanistan, Pakistan, Kashmir, North India, Nepal; North America and Western China (Nautiyal *et al*., 2002; Li *et al*., 2009). Current estimates by the Threatened Plant Species Committee of the Survival (TPSSC) of International Union for Conservation of Nature (IUCN) indicate that one in ten species of ferns, conifers and flowering plants on earth possess a threat of extension because of excessive commercial utilization and trade with other countries. It has been pointed out that nearly 60,000 species of plants will be in verge of extinction leading to genetic erosion and vulnerability to various biotic and abiotic stresses due to change in climate in next 30–40 years. *Rheum* is present at the top of that list. Exploitation of *P. hexandrum* from the wild is also prohibited for export from India under Convention on International Trade in endangered species of wild flora and fauna (CITES).

***R. australe***

The seed dormancy may be due to unfavorable environmental conditions or sometimes, some seeds may not germinate because of some inhibitory factor of the seed itself. Inactiveness of seed to germinate may be because of certain inhibitory factors such as tough seed coat (external) or physiological conditions of the interior of the seed. Seed dormancy in *R. australe* and *P. hexandrum* belongs to the first type. Both these species reproduce sexually through seeds and asexually by rhizome cuttings. Since the final and the last effort or result of the sexual reproduction in plants is the production of seed which in addition to division control some processes; serving as an agent of perrennation, generation of variation and dispersal (Ganaie *et al*., 2011).

In *R. australe* seed germination was monitered up to 25th day. It started and also completed on different dates in different treatments (table 1). In three chilling treatments *i.e.* chl1, chl2 and chl3, it started on 2nd day and completed by 14th day. So it took about thirteen days for complete germination (table 1). Hence, analysis was done for data collected up to 13th day of germination. The best results were obtained in 50 days chilled seeds with 92% germination followed by 86% and 82% germinations in 40 and 30 day chilled seeds, respectively. As chilling performs a pivotal role in inducing the stimulus that is needed to surmount dormancy. It is regarded to initiate an increase in the concentration of gibberllic acid (Bretzloff and Pellett, 1979). Chilling is useful to relieve primary inactiveness of many Northern hemisphere species (Baskin, 2001). It has been commonly used as a pre-sowing treatment to overcome dormancy and enhance percentage of germination of dormant seeds of many different species (Fang *et al*., 2006). The pre-chilling treatment conditions may actually be simulating the events that occur during the winter season just before the appearance of summer. Nabaei *et al*. (2011) assessed effective methods in dormancy break and increase of seed germination of *Rheum ribes* and found the highest germination (96%) in integrated treatment of moist chilling (25 days at 2°C and $GA_3$ 50 mg/L). However, in the present study, chilled seeds took only thirteen days for complete germination. Treatment $chl_3$ (50 day chilled seeds) was statistically different from all other treatments including chilling treatments $chl_1$ and $chl_2$, which were at par with each other. Treatments $GA_3$1 and $GA_3$2 with 80% and 75% germination respectively, were at par with each other and with chilling treatments $chl_1$, $chl_2$ and chl3, but were statistically different from treatments $K_1$, $K_2$, Comb1, Comb2, S1 and control treatments (table 2). Gibberellins surmount seed and bud dormancy in many species, thus serving as a substitute for low temperatures, long days or red light (Salisbury and Ross, 1992). Dormant seeds, which demand cold temperature treatment, dry storage following maturation as initiator or stimulator of germination are mostly treated with $GA_3$ to surmount their dormancy (Nadjafi *et al*., 2006). In the present study, this response to germination was influenced by proporition of applied $GA_3$. At lower concentrations ($GA_3$2= $10^{-4}$ M), germination was lower (75%) and at higher concentration ($GA_3$1= $10^{-3}$ M), it was higher (80%) (table 1). Plant growth hormones are chemicals which in small quantities can regulate various plant processes in addition to seed dormancy. Different plant hormones can control different plant processes including seed dormancy and germination, growth and development of various plant parts (Agraeber *et al*., 2012). Gibberellins are mostly employed to destroy the low temperature requirements of some plant seeds and enhance their germination percentage (El-Dengawy, 2005). It plays a role in inducing enhancement of enzyme synthesis that changes stored nutrients carbohydrates, which are required for quick cell respiration during germination (Bakrim *et al*., 2007). Increase in germination per centage was reported by other workers from studies carried out on other species, such as *Ferula gummosa* (Nadjafi *et al*., 2006), *Sesamum indicum* (Kyauk *et al*., 1995) and *Rumex dentatus* (Ali *et al*., 1996). The percentage germination in potassium nitrate ($KNO_3$, 0.1% and 0.2%) treated treatments in the present investigation was 65 and 60 per cent, respectively. However, these treatments were at par with each other. Similar results were reported by Butola and Badola (2004) in *Angelica glauca* (Apiaceae), an endangered medicinal plant species wherein they reported significant increase in the rate of seed germination and promotion in mean germination time in treatments treated with $KNO_3$ (150 mM) and $NaHClO_3$ (30 min). Farajollahi *et al*. (2014) also reported increase in percentage germination by the application of potassium nitrate ($KNO_3$ 0.1%) in seeds of *Calotropis persica*. Further, Roberts and Smith (1977) reported, higher concentration of $KNO_3$ (150 mM) was significantly effective, possibly through oxidized forms of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway. The lowest germination (30%) was observed in the control treatment. However, no germination was observed in seeds treated with conc. $H_2SO_4$, indicating that acids have negative influence on seed germination. Similar results were observed by Nasiri and Eisavand (2001) while studying the influence of acidic treatments on germination of *Ceratonia siliqua*. Contrary to the present findings, Saied *et al*. (2008) on *Ziziphus*, Khaleghi *et al*. (2009), on Tamarind, Nasiri and Eisavand (2001), on *Albizia julibrissin* and Hojati *et al*. (2007), on *Cycas revolute* had introduced sulfuric acid as the best treatment.

All treatment means, except treatment $S_1$ revealed significant differences when compared with control (table 3). Further CD values revealed that the treatment means varied significantly from each other. It was further observed that the rate of germination of unchilled seeds treated with gibberlic acid, potassium nitrate and combination of $H_2So_4$/ gibberlic acid was enhanced in comparison to the control treatment (fig. 1). On 14th day coefficient of variation for all the treatments was 43%, which indicated that the treatments are variable with respect each other (table 4). Across days, highest mean ±Standard deviation (60.45±26.38) and mean range (0-

**Table 1 :**Seed germination studies of *R. australe* using physico-chemical treatments.

| Treatments | No. of days taken for I$^{st}$ seed to germinate | No. of days taken for last seed to germinate | Total no. of days taken for complete germination | Percentage of seed germination |
|---|---|---|---|---|
| $Chl_1$ | 2 | 14 | 13 | 82 |
| $Chl_2$ | 2 | 14 | 13 | 86 |
| $Chl_3$ | 2 | 14 | 13 | 92 |
| $S_1$ | - | - | - | - |
| $K_1$ | 4 | 21 | 18 | 70 |
| $K_2$ | 4 | 22 | 19 | 64 |
| $GA_3$1 | 3 | 19 | 17 | 83 |
| $GA_3$2 | 3 | 19 | 17 | 78 |
| Comb 1 | 4 | 21 | 18 | 54 |
| Comb 2 | 4 | 21 | 18 | 49 |
| Cont. | 7 | 25 | 19 | 34 |

{Chilling treatment (Ch),: Ch1= 30 days, Ch2= 40 days, Ch3= 50 days: Potassium nitrate (K) with K1= 0.2 %, $K_2$= 0.3%: Gibbrellic acid ($GA_3$) with $GA_3$1= $10^{-3}$ M and $GA_3$2= $10^{-4}$ M: Combination of sulphuric acid and $GA_3$ (Com), Com 1= $H_2SO_4$/ $GA_3$1, Com 2= $H_2SO_4$/$GA_3$2: Sulphuric acid treatment,S1=1 min.: Control = Cont.}.

**Table 2 :**Analysis of variance for seed germination in *R. australe*.

| Source | d.f. | M.S. | F-Ratio | CD (5%) | C.V. |
|---|---|---|---|---|---|
| Treatments | 10 | 2309.75 | 194.95 | 5.82 | 5.69 |
| Error | 22 | 11.84 | | | |

**Table 3 :** Treatment means on 14$^{th}$ day in *R. australe*.

| S. no. | Treatment | Mean |
|---|---|---|
| 1. | $Chl_1$ | 82.00 |
| 2. | $Chl_2$ | 86.00 |
| 3. | $Chl_3$ | 92.66 |
| 4. | $S_1$ | 0.00 |
| 5. | $K_1$ | 65.00 |
| 6. | $K_2$ | 60.00 |
| 7. | $GA_3$1 | 80.00 |
| 8. | $GA_3$2 | 75.00 |
| 9. | Comb 1 | 50.00 |
| 10. | Comb 2 | 45.00 |
| 11. | Cont. | 30.00 |

92) was observed on 14$^{th}$ day. The percentage of germination in all the treatments ranged from 0 to 92 on 14$^{th}$ day (table 4). Duration of time taken by a particular treatment prior to germination revealed that treatment of seeds with gibberllic acid seems to be the best treatment for seed germination as it took only few days for germination as compared to the time (30, 40 and 50 days) taken by chilled seeds.

### *P. hexandrum*

No germination was obtained in all the three chilling treatments as well as in $K_1$, $K_2$ and control treatments

**Table 4 :**Mean, range and coefficient of variation of different treatments across days in *R. australe*.

| Day no. | Mean±SD (Days) | Range | Coefficient of variation |
|---|---|---|---|
| 1. | 0 | 0 | 0 |
| 2. | 2.45 ± 4.88 | 0-15 | 199.18 |
| 3. | 5.36 ± 8.46 | 0-26 | 157.83 |
| 4. | 10.90 ± 11.08 | 0-37 | 101.66 |
| 5. | 13.36 ± 11.45 | 0-39 | 85.77 |
| 6. | 17.09 ± 12.01 | 0-44 | 70.28 |
| 7. | 25.18 ± 15.00 | 0-55 | 59.55 |
| 8. | 32.18 ± 18.26 | 0-67 | 56.74 |
| 9. | 37.09 ± 20.08 | 0-73 | 54.13 |
| 10. | 42.72 ± 21.29 | 0-78 | 49.83 |
| 11. | 46.72± 21.79 | 0-82 | 46.63 |
| 12. | 53.54 ± 24.40 | 0-86 | 45.57 |
| 13. | 58.09± 25.78 | 0-89 | 44.37 |
| 14. | 60.45± 26.38 | 0-92 | 43.63 |

(fig. 2). All the treatments were statistically different from each other (table 6). The coefficient of variation and mean ±standard deviation across days varied from 48.41 to 230.86 and 1.18±2.72 to 51.36±24.84, respectively (table 8). Highest mean ± standard deviation (16.63±23.39) across days was observed in the day interval of 73-81 and least (0.36±1.14) in 19-27. Further treatment means revealed significant differences when compared with control (table 7). The coefficient of variation for day interval of 82-90 for all the treatments was 153.96%, depicting that the treatments were highly variable with respect each other (table 8).

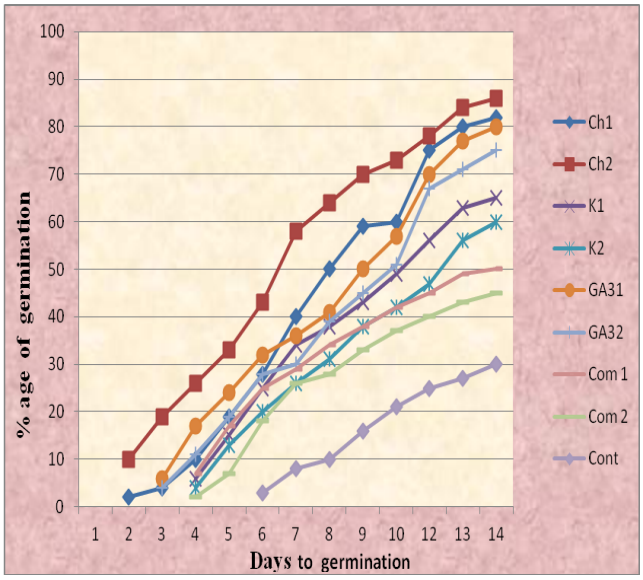


**Fig. 1 :** Time course of seed germination of *R. australe*.

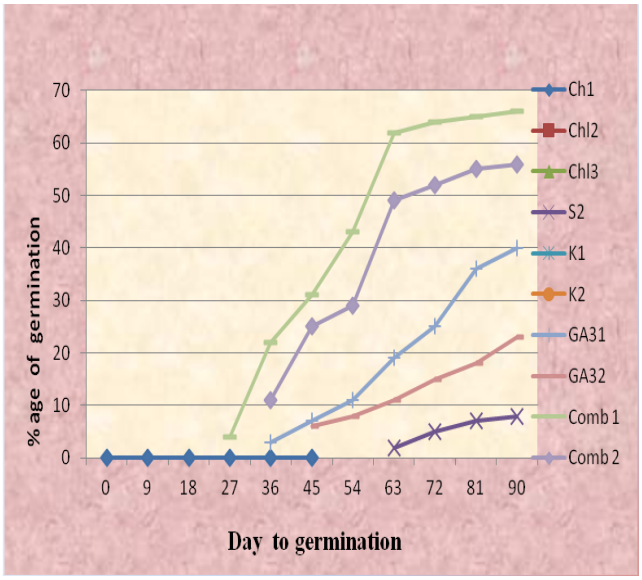


**Fig. 2 :** Time course of seed germination of *P.hexandrum*.

**Table 5 :** Seed germination studies of *P. hexandrum* using physico-chemical treatments.

| Treatments | No. of days taken for I$^{st}$ seed to germinate | No. of days taken for last seed to germinate | Total no. of days taken for complete germination | Percentage of seed germination |
|---|---|---|---|---|
| $Chl_1$ | - | - | - | - |
| $Chl_2$ | - | - | - | - |
| $Chl_3$ | - | - | - | - |
| $S_2$ | 60 | 90 | 27 | 8 |
| $K_1$ | - | - | - | - |
| $K_2$ | - | - | - | - |
| $GA_3$1 | 36 | 86 | 47 | 40 |
| $GA_3$2 | 45 | 88 | 43 | 23 |
| Comb 1 | 27 | 81 | 55 | 66 |
| Comb 2 | 32 | 84 | 52 | 56 |
| Cont. | - | - | - | - |

{Chilling treatment (Ch),: Ch1= 30 days, Ch2= 40 days, Ch3= 50 days: Potassium nitrate (K) with $K_1$= 0.2 %, $K_2$= 0.3%: Gibbrellic acid ($GA_3$) with $GA_3$1= $10^{-3}$ M and $GA_3$2= $10^{-4}$ M: Combination of sulphuric acid and $GA_3$ (Com), Com 1= $H_2SO_4$/ $GA_3$1, Com 2 = $H_2SO_4$/$GA_3$2: Sulphuric acid treatment, $S_1$= 8 min.: Control = Cont.}.

**Table 6 :** Analysis of variance for seed germination in *P. hexandrum*.

| Source | d.f. | M.S. | F-Ratio | CD (5%) | C.V. |
|---|---|---|---|---|---|
| Treatments | 10 | 1889.61 | 296.94 | 4.27 | 14.38 |
| Error | 22 | 6.36 | | | |

Difference in the rate of seed germination has been described in various populations and variants of the similar populations of *P. hexandrum* (Bhadula *et al*., 1996). In the present investigation, seed germination in *P. hexandrum* started on 27$^{th}$ day and completed by 90$^{th}$ day at a room temperature of 15-20$^0$C. Highest percentage of germination (66%) was observed in combination treatment (Comb1: $H_2SO_4$/$GA_3$1) and the least (8%) in treatment $S_2$. Beigh *et al*. obtained 50% germination in scarified seeds of *P. hexandrum*. This is in contradiction to the present study where in only 8% germination was recorded in scarified seeds (treated with $H_2SO_4$ for 8 minutes). Treatment of seeds is regarded as highly decisive treatment in enhancing germination of those plant species with hard seed coats (Youssef, 2008). Seed germination with acid treatment was described by many other workers as well in different plant species (Manzoor and Bhat, 2013, in *Datura quercifolia*, Bisht and Kediyal, 1995 in *Atropa belladona*). Honey-locust (*Gleditschia triacanthos*) seeds have hard and impermeable coats, so germination percentage is increased when seeds are scarified or treated with hot water or concentrated $H_2SO_4$

**Table 7 :** Treatment means across 82-90 days in *P. hexandrum*.

| S. no. | Treatment | Mean |
|---|---|---|
| 1. | $Chl_1$ | 0.00 |
| 2. | $Chl_2$ | 0.00 |
| 3. | Chl3 | 0.00 |
| 4. | $S_2$ | 8.00 |
| 5. | $K_1$ | 0.00 |
| 6. | $K_2$ | 0.00 |
| 7. | $GA_3$1 | 40.00 |
| 8. | $GA_3$2 | 23.00 |
| 9. | Comb 1 | 66.00 |
| 10. | Comb 2 | 56.00 |
| 11. | Cont. | 0.00 |

**Table 8 :** Mean, range and coefficient of variation of different treatments across days in *P. hexandrum*.

| Day no. | Mean ± SD (Days) | Range | Coefficient of variation |
|---|---|---|---|
| 0-9 | 0 | 0 | 0 |
| 10-18 | 0 | 0 | 0 |
| 19-27 | 0.36±1.14 | 0-4 | 319.00 |
| 28-36 | 3.27±6.71 | 0-22 | 205.00 |
| 37-45 | 6.45±10.6 | 0-31 | 164.90 |
| 46-54 | 9.00±14.21 | 43 | 157.90 |
| 55-63 | 13.45±21.22 | 0-62 | 157.79 |
| 64-72 | 15.09±22.24 | 0-64 | 147.43 |
| 73-81 | 16.63±23.39 | 0-65 | 140.70 |
| 82-90 | 15.72±24.20 | 0-66 | 153.96 |

(sulfuric acid). Babashpour *et al*. (2011) reported significant ($P<0.05$) enhancement in percentage of germination in honey-locust seeds when treated with acid in comparison to hot water and control treatments. Also they found that the acid scarification treatments significantly ($P<0.05$) increased length of roots (mm) and number of days taken by seeds to 50% germination (T50) of honey-locust seeds in comparison to hot water and control treatments. Nadeem *et al*. (2000) reported a marked promotion in germination of seeds of *P. hexandrum* with $GA_3$ treatment. In the present study, lower concentration of $GA_3$ strongly relieved the seeds from dormancy and resulted in 40% germination. Kandari *et al*. (2007) while studying the influence of presowing treatments on the seed germination of medicinal herbs of the Himalaya also reported a significant ($P<0.05$) enhancement in germination of *Angelica glauca*, seeds treated with $GA_3$ at 100 ppm. However, they do not found any effect of $GA_3$ on seed germination of *Pleurospermum angelicoides* in comparison to control at $25^0$C under light conditions. To overcome physiological dormancy in *Arnebia benthamii*, Kandari *et al*. (2008) also reported maximum seed germination (100%) by treating seeds with 100 ppm $GA_3$ for a period of 24 h and incubation at 25°C in 12 h light photoperiod conditions. They also reported that combination of pre-soaking treatment and temperature had highest germination even in continuous dark conditions and it also lowered the mean germination time. $GA_3$ effect was further enhanced when seeds were acid-scarified prior to applying the hormone. The seed coat tissues were first softened by acid treatment to enable entry of more quantity of gibberelline. Gibberellins are most directly connected in the enhancement of seed germination. $GA_3$ is regarded to enhance the formation of hydrolases particularly α amylase in the endosperm of cereal grains. Its breakdown is generally assumed to be an essential process of germination (Kolumbina *et al*., 2006). Further, Finch-Savage and Leubner (2006) are of the opinion that gibberellins stimulate seed germination through amylase synthesis. Enhancement in seed germination has also been reported by other workers (Sharma *et al*., 2006 in *P. hexandrum* and Ganaie *et al*., 2011 in *Arnebia benthamii*). Similarly, Manzoor and Bhat (2013) in *Datura quercifolia*, reported that seed scarification followed by a $GA_3$ (200ppm) treatment as a very effective treatment wherein they observed 87% germination. Apparently, indicating that seed coat hardness is involved in imparting dormancy to seeds besides a requirement for after-ripening. During the after-ripening of seeds, $GA_3$ synthesis might be stimulated to an extent necessary for dormancy removal (Sharma *et al*., 2006). However, there was no effect of chilling treatment (chl1, chl2 and chl3) and different concentrations of potassium nitrate ($K_1$= 0.2%, $K_2$= 0.3%) on the seed germination of *P. hexandrum,* in which no germination was obtained as in control treatment. All the treatments were statistically different from each other (table 6). The coefficient of variation and mean ±standard deviation across days varied from 48.41 to 230.86 and 1.18±2.72 to 51.36±24.84, respectively (table 8). Highest mean ±standard deviation (16.63±23.39) across days was observed in the day interval of 73-81 and least (0.36±1.14) in 19-27. Further treatment means revealed significant differences when compared with control (table 7). The coefficient of variation for day interval of 82-90 for all the treatments was 153.96%, depicting that the treatments were highly variable with respect each other (table 8).

Seed germination can also be regulated by the conditions prevailing during seed formation as well as by hereditary factors (Gutterman, 1995). In all cases increase

in germination percentage reduces mean germination time. The present study reveals enhanced effect of $GA_3$ together with acid scarification on *P. hexandrum*. Good response of $GA_3$ may be due to the presence of physiological factors, which may suppressed by the application of $GA_3$.

## Conclusion

In this study, an attempt was made to overcome seed dormancy and to enhance germination of seeds of the two endangered medicinal plant species *viz*. *R. australe* and *P. hexandrum.* It can be recommended that the most practical and useful pretreatment for propagation of *R. australe* is the chilling treatment for a period of 50, 40 and 30 days at low temperature (3-4ºC) wherein 92, 86 and 82 per cent germination was obtained. Similarly in case of *P. hexandrum,* treatment of seeds with acid followed by gibberelline seem to be the most practical pretreatment method for propagation of species on a large scale. As highest percentage of germination (66%) was obtained in treatment Comb1 ($H_2SO_4$ treatment for 8 minutes, $GA_3 1 = 10^{-3}$ M).

## References


Agraeber, K., K. Nakabayashi, E. Miatton, G. Leubner-Metzger and W. J. Soppe (2012). Molecular mechanisms of seed dormancy. *Plant Cell Environment*, **35(10)** : 1769-1786.

Airi, S., R. S. Rawal, U. Dhar and A. N. Purohit (2000). Assessment of availability and habitat preference of *Jatamansi* – A critically endangered medicinal plant of West Himalaya. *Current Science*, **79** : 1467-1471.

Ali, A. and A. Helal (1996). Studies on germination of *Rumex dentatus* L. seeds. *Journal of Arid Environments*, **33** : 39–47.

Babashpour, M., A. Rahbari and R. Sharivivash (2011). Effect of Different Treatments on Seed Germination of Honey Locust (*Gleditschia triacanthos*). *Modern Applied Science*, **5** : No. 1.

Bakrim, A., M. Lamhamdi, F. Sayah and F. Chib (2007). Effects of plant hormones and 20-hydroxyecdysone on tomato (*Lycopersicum esculentum*) seed germination and seedlings growth. *African Journal of Biotechnology*, **6(24)** : 2792-2802.

Baskin, C. C. (2001). Seeds : Ecology, biogeography and evolution of dormancy and germination. *Nordic Journal of Botany*, **20(5)** : 598.

Bhadula, S. K., A. Sing, H. Lata, C. P. Kuniyal and A. N. Purohit (1996). Genetic resources of *Podophyllum hexandrum* Royle, an endangered medicinal species from Garhwal Himalaya, India. *Plant Genetic Resource Newsletter*, **106** : 26-29.

Bisht and Kediyal (1995). Effect of chemical treatments on germination and survival of the seedlings in *Atropa belladomna* Linn. *Indian Journal Forestry*, **11(3)** : 208-210.

Bretzloff, L. V. and N. Pellett (1979). Effect of stratification and gibberllic acid on the germination of *Carpinus caroliniana* Walt. *Hort Science*, **14** : 621–622.

Butola, J. S. and H. K. Badola (2004). Effect of pre-sowing treatment on seed germination and seedling vigour in *Angelica glauca*, a threatened medicinal herb. *Current Science*, **87(6)** : 796-799.

Choudhary, D. K., B. L. Kaul and S. Khan (1998). Cultivation and conservation of Podophyllum hexandrum - an overview. *Journal of Medicinal and Aromatic Plant Science*, **20(4)** : 1071-1073.

Debaish, D. and S. C. Bhattacharya (2008). Colouration of Wool and Silk. *Ind. Journal of Fibre Textile*, **33** : 14-119.

El-Dengawy, E. R. F. (2005). Promotion of seed germination and subsequent seedling growth of loquat (*Eriobotrya japonica* Lindl) by moist-chilling and $GA_3$ applications. *Scientia Horticulturae*, **105(3)** : 331-342.

Fang, S., J. Wang, Z. Wei and Z. Zhu (2006). Methods to break seed dormancy in *Cyclocarya paliurus* (Batal) Iljinskaja. *Scientia Hrticulturae*, **110(3)** : 305-309.

Farajollahi, A., B. Gholinejad and H. J. Jafari (2014). Effects of Different Treatments on seed germination improvement of *Calotropis persica*. *Advances in Agriculture*, **2014** : 1-5.

Finch-Savage, W. E. and G. Leubner-Metzger (2006). Seed dormancy and the control of germination. *New Phytolology*, **171(3)** : 501-523.

Ganaie, K. A. and I. A. Nawchoo (2002). *In vitro* seed germination studies on *Arnebia benthamii*. *Indian Journal of Plant Physiology,* **7(3)**: 252-255.

Ganaie, K. A., N. S. Aslam and I. A. Nawchoo (2011). No chilling obligation for germination in seeds of *Arnebia benthamii* : A critically endangered alpine medicinal plant of north-west Himalayas. *International Journal of Biodiversity and Conservation*, **3(5)** : 155-159.

Gupta, R. K., G. B. Bajracharya and R. N. Jha (2014). Antibacterial activity, cytotoxicity, antioxidant capacity and phytochemicals of *Rheum australe* rhizomes of Nepal. *Journal of Pharmacognosy and Phytochemistry*, **2 (6)** : 125-128.

Gutterman, Y. (1995). Seed germination in desert plants. *Springer-Verlag*, Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong, 1993. **5(1)** : 59-60.

Handa, S. S., A. S. Chawla and A. Maninder (1989). Hypoglycemic plants: a review. *Fitotherapia*, **60** : 195.

Hawkes, J. G., N. Maxted and B. V. Ford-Lloyd (2000). *The ex-situ conservation of plant genetic resources*, Kluwer Academic publishers, Dordrecht.

Hojati, Y., R. Naderi, A. Faramarzi and J. Gholipour (2007).

Evaluation the effect of Sulfuric acid, $GA_3$ and temperature treatments on seed germination of *Cycas revolute*. *Journal of Agricul. Modern Science*, **9** : 13-22.

Holley, J. and J. T. Williams (1996). India uniquely positioned to capitalize on abundant, medicinal plant heritage. *Diversity*, **12** : 35-37.

Kandari, L. S., K. S. Roa, K. Chauhan, R. K. Maikhuri, V. K. Purohit, P. C. Phondani and K. G. Saxena (2007). Effect of Presowing Treatments on the Seed Germination of Two Endangered Medicinal Herbs of the Himalaya (*Angelica glauca* Edgew and *Pleurospermum angelicoides* (Wall. Ex DC.) Benth. Ex C.B. Clarke). *Proceedings of the Indian National Science Academy*, **73** : 11-16.

Kandari, L. S, K. S. Rao, R. K. Maikhuri and K. Chauhan (2008). Effect of pre-sowing, temperature and light on the seed germination of *Arnebia benthamii* (Wall. ex G. Don): An endangered medicinal plant of Central Himalaya, India. *African Journal of Plant Science*, **2(1)** : 5-11.

Khaleghi, A., A. Dehghan and N. Moalemi (2009). Effects of Sulfuric acid and hot water treatments on germination properties of Tamarind and Acacia seeds. *Journal of Iranian Hort. Science*, **40(3)** : 71-77

Kolumbina, M., M. Blesing and D. J. Mares (2006). α-Amylase and programmed cell death in aleurone of ripening wheat grains. *Journal of Experimental Botany*, **57(4)** : 877-885.

Kumar, G. P., R. Kumar and O. P. Chaurasia (2011). Conservation status of medicinal plants in Ladakh: Cold arid zone of Trans-Himalayas. *Research Journal of Medicinal Plant*, **5** : 685-694.

Kyauk, H., N. W. Hopper and R. D. Brigham (1995). Effects of temperature and pre-soaking on germination, root, length and shoot length of sesame (*Sesamum indicum* L.). *Environmental and Experimental Botany*, **35** : 345–351.

Li, W., M. Li, D. Yang, R. Xu and Y. Zhang (2009). Production podophyllotoxin by root culture of *Podophyllum hexandrum* Royle. *Electronic Journal of Biology*, **5(2)** : 34-39.

Manzoor, E. and B. A. Bhat (2013). *In vitro* Propagation of *Datura quercifolia*. *International Journal of Green and Herbal Chemistry*, **2(2)** : 301-305.

Mitttermier, R. A., P. R. Gil, M. Hoffmann, J. Pilgrim, T. Brooks, C. G. Mittermeier, J. Lamoreux and G. Da-Fonseca (2005). Hot spots revised: Earth's biologically richest and most threatened terrestrial eco-regions. *Conservation International* (http://www. Biodiversity hot spots. Org).

Nabaei, M., P. Roshandel and A. Mohammadkhani (2011). Effective techniques to break seed dormancy and stimulate seed germination in *Rheum ribes* L. *Iranian Journal of Medicinal and Aromatic Plants*, **27(2)** : 212-223. (In Persian).

Nadeem, M., L. M. S. Palni, A. N. Purohit, H. Pandey and S. K. Nandi (2000). Propagation and conservation of *Podophyllum hexandrum* Royle : an important medicinal herb. *Biological. Conservation*, **92** : 121-129.

Nadjafi, F., M. Bannayan, L. Tabrizi and M. Rastgoo (2006). Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. *Journal of Arid Environment*, **64** : 542–547.

Nasiri, M. and H. R. Eisavand (2001). Evaluation the effect of Sulfuric acid on dormancy break and germination of *Albizia julibrissin* and *Ceratonia siliqua* seeds. *Iranian Journal of Genetic and Breeding Researches of Pasture and Forest Plants*, **8** : 95-111.

Nautiyal, B. P., V. Prakash, R. S. Chauhan, H. Purohit, M. C. Nautiyal, V. Prakash and H. Purohit (2001). Assessment of germinability, productivity and cost benefit analysis of *Picrorhiza kurroa* cultivated at lower altitudes. *Current Science*, **81** : 579-585.

Nautiyal, B. P., V. Prakash, R. S. Chauhan, H. Purohit, M. C. Nautiyal, V. Prakash and H. Purohit (2001). Assessment of germinability, productivity and cost benefit analysis of *Picrorhiza kurroa* cultivated at lower altitudes. *Current Science*, **81** : 579-585.

Nautiyal, M. C., V. Prakash and B. P. Nautiyal (2002). Cultivation techniques of some high altitude medicinal herbs. *Annals of Forestry*, **10** : 62–67.

Parveen, S. and N. A. Wani (2013). Germanibility, conservation and cultivation of *Rheum emodi* wall ex. Meissner – a threatened medicinal plant. *International Journal of Life Sciences Biotechnology and Pharma Research*, **2(3)** : ISSN 2250-3137.

Purohit, A. N., H. Lata, S. Nautiyal and M. C. Purohit (1998). Some characteristics of four morphlogical variants of *Podophyllum hexandrum* Royle. *Plant Genetic Resource Newsletter*, **114** : 51-52.

Roberts, E. and R. D. Smith (1977). Dormancy and the pentose phosphate pathway. In : *The Physiology and Biochemistry of Seed Dormancy and Germination* (ed. Khan, A.), North-Holland Publ. Co, Amsterdam, pp. 385–411.

Rokaya, M. B., P. Marsík and Z. Münzbergová (2012). Active constituents in *Rheum acuminatum* and *Rheum australe* (Polygonaceae) roots: A variation between cultivated and naturally growing plants. *Biochemical Systematics and Ecology*, **41** : 83-90.

Saied, A. S., J. Gebauer and A. Buerkert (2008). Effects of different scarification methods on germination of *Ziziphus spina-christi* seeds. *International Seed Testing Association*, **36(1)** : 201-205

Salisbury, F. B. and C. W. Ross (1992). *Plant Physiology*, fourth ed. Wadsworth Publishing Company, Belmont, CA,p. 682.

Sharma, K., S Shama and S. S. Sharma (2006). Seed germination behavior of some medicinal plants of Lahaul and spiti cold desert (Himachal Pradesh) : implications for conservation and cultivation. *Current Science*, **25** : 1113- 1118.

Srivastava, N., V. Sharma, B. Kamal, A. K. Dobriyal and V. S. Jadon (2010). Advancement in research on *Aconitum* sp. (Ranunculaceae) under different area : A review. *Biotechnology*, **9** : 411-427.

Wawrosch, C., P. Zeitlhofer, B. Grauwald and B. Kopp (2003). Effects of rooting chemicals on the establishment of micropropagated *Picrorhiza kurroa* plantlets in the greenhouse. *Acta Horticultura*, **616** : 271-273.

Xiang, L., F. Lei, D. Xing, W. Wang and J. Zheng (2005). Neuron protective constituents from *Rheum nanum* and *Rheum sublanceolatum*. *Tsinghua Science and Technology*, **10** : 426–429.

You, X., S. Feng, S. Luo, D. Cong, Z. Yu, Z. Yang and J. Zhang (2013). Studied on a *rhein* producing endophytic fungus isolated from *Rheuk palmatum* L. *Fitoterapia*, **85** : 161-168.

Youssef, A. M. (2008). Adaptive responses of some desert plants from different ecosystems of Suez road, *Egyptian Research Journal of Agriculture and Biological Science*, **4(5)** : 595-603.

Zargar, B. A., M. H. Masoodi, B. Ahmed and S. A. Ganie (2011). Phytoconstituents and therapeutic uses of *Rheum emodi* wall. ex Meissn. *Food Chemistry*, **128** : 585–589.