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Effect of chemical treatment on dormancy and germination response of seeds of *Paeonia emodi*in Western Himalaya

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Abstract

*Paeonia emodi*Wall.exRoyle (family paeoniaceae) is commonly known as Himalayan Peony, or Chandra in Garhwal regions of Western Himalaya. The species is used to treat diarrhea, fever, stomach ache, backbone ache, colic and also maintaining the diabetic level the Himalayan region. The present study was conducted to find the germination percentage with respect to appropriate chemical treatment and understand the nature of seed dormancy in the laboratory conditions.Thiourea and Gibberellic acid ($GA_{3)}$with different concentration viz. 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm were used for breaking dormancy. In each treatment three replicates with 25 seeds were used.Maximum (81.33±1.33%) seed germination was found in seeds treated with Thiourea concentration of 50 ppm, followed by 100 ppm (76.00±2.31%), 250 ppm (61.33±3.53%), 500 ppm (58.67±4.81%) and 1000 ppm (44.00±4.62%).Minimum Mean Germination Time (29.89±0.43 days) were obtained for the seeds treated with Thiourea concentration of 50 ppm, followed by 100 ppm (31.33±0.65 days), 250 ppm (32.97±0.52 days), 500 ppm (33.89±0.26 days), while 1000 ppm concentration revealed the maximum (35.08±0.72 days) MGT for Thiourea treatment. Therefore, study revealed that Thiourea impact was more effective in the stimulating seed germination and reducing Mean Germination Time. The outcomes of the present study would be helpful to the local people, plant nursery growers and scientists to identify and propagation of *P. emodi* in large scale.

Keywords: *Paeonia emodi*,Seed dormancy, Thiourea,Gibberellic acid, Germination percentage, Mean Germination Time

1. Introduction

Seed is a sexual reproduction unit of flowering plants which is capable to develop a new plant. A developed seed contains a mature ovule, embryo, storage tissue and a protective outer covering which is the result of sexual fertilization (Hartmann et al., 2007). Variation in seed germination is due to a complex of environmental and genetic factors during seed formation and subsequent handling of treatments (Wang et al., 1982). Some seed sources showed better performance in certain locations than the other and the use of seed of a provenance in such areas having different climatic and edaphic conditions, often results in poor performance or failure (Rajasekharan and Ganeshan, 2002).Although, in recent years increased attention has been given to development of propagation methods for threatened MAPs, all over the world and large body of evidence has been collected to show immense potential of medicinal plants applied in various traditional systems (Arya et al., 2017). Conventional breeding and propagation methods in peony are very difficult and time consuming (Baskin, 2003). Like other herbaceous and tree species of *Paeonies,P. emodi* also reproduce by seeds, requires up to 3 years for germination due to presence of epicotyls and hypocotyls dormancy(Barzilay et al., 2002).

*Paeonia emodi*Wall. ex Royle is commonly known with different names such as Himalayan Peony, Chandra orDhandhuru in Garhwal regions of Western Himalaya while Ood-Saleeband Mamaikh in Pakistan and Afghanistan regions respectively. It is largely distributed in the cold climate of temperate areas of North Western India, Northern Pakistan, East Afghanistan, China and Western Nepal (Deyuan, 1997) and warm-temperate regions of Europe and Asia (Zargar et al., 2013). In India, it can be found from Kashmir to Uttarakhand (Chauhan, 1999; Ismail et al., 2003) from 2000 to 3000 m aslwhile in Grahwal and Kumaon regions with an altitudinal range of 1800 to 3000 m asl(Kumar and Rawat, 2011). The species grows and flourishes well in partial shady and moist places of deciduous mixed forest while associated with oak and other medicinally important species including *Quercusleucotrichophora, Q. floribunda, Rhododendron arboreum, Aesculasindica, Paris pollyphylla, Potentillafulgance and Geranium* species, etc. The plant preferred slope moist land area and well-drained sandy to loam soil for germination and growth. The species is used to treat diarrhea, fever, stomach ache, backbone ache, colic and also maintaining the diabetic level the Himalayan region.

Limited literature is available on seed germination of *P. emodi* in the laboratory conditions. Therefore,the present study was conducted to find the germination percentage

with respect to appropriate chemical treatment and understand the nature of seed dormancy in the laboratory conditions. This information could be useful for a large-scale propagation ofthe species.

2. Material and Methods

2.1. Seed collection and viability

Mature and dry seeds of *P. emodi* were collected (manually) from natural habitats of Uttarakhand (2000-2800 m asl) during mid of August, 2017. The seeds were collected from oval shaped-pods of the plant and they were rubbed well to remove unwanted materials and carried them into the perforation bags. To ensure that the seeds used for the experiment are viable and of high quality, the sample lots were proceeded to viability test using the tetrazolium technique (Peters, 2000). For this test, 100 seeds per condition (2 replicates having 50 seeds each) of *P. emodi*were soaked in distilled water for 24 hours and the seed coat was carefully removed. Decoated seeds were then completely submerged in 1,2,3-triphenyl tetrazolium chloride (TTC-1%, w/v; pH 7, 24 h, 25^{o}C, under dark condition), percent viability was determined by counting the total number of stained (red) portions of embryo and cotyledons in each replicate (ISTA, 1999) to find viability percentage of the seeds.The TTC solution was drained and sections were rinsed 2-3 times with water.

2.2.Experimental design

Experiment was designed in the herbal research and tissue culture laboratory of High Altitude Plant Physiology Research Centre (HAPPRC), Srinagar Garhwal, Uttarakhand. Seeds surface were sterilized by 0.2% aqueous solution of Mercuric chloride ($HgCl_2$) for two minutes followed by ethanol for two minutes and then rinsed thoroughly (3-4 times) with distilled water. Thiourea and Gibberellic acid (GA_3) were used for each treatment having three replicates with 25 seeds. In each replicate seeds dipped into different concentrations of chemical treatment for 48 hrs. After pretreatments, seeds were kept in glass Petri dishes (100×15 mm) on single layer of Whatman no.1 filter paper.**Each Petri dish was tagged with their respective pretreatment, replicate number (R_1, R_2, R_3), date and time of experiment.** Humidity was maintained by adding distilled water over filter paper in one day interval. All the observations were taken in 16 hrs light and 8 hrs dark at 25±2°C conditions. The whole experiment was monitored up to two months. Details of given pretreatments are presented in Table 1.

2.3. Germination Percentage and Mean Germination Time

The germination was recorded from the date of experiment designed and continued till the germination ceased while the germination percentage was calculated at the end of experiment. The seeds were considered germinated on emergence of radical about 1 cm long (Phartyal et al. 2009). Mean Germination Time (MGT) was calculated following (Ellis and Roberts 1981) as:

$$MGT = \Sigma D_n / \Sigma_n$$

Where n = number of germinated seeds on day; D = number of days since the sowing of seeds.

3. Data analysis

The values for each treatment, seed germinated in different days and growth performance parameters with time were calculated as the mean ± Standard Error (SE) using SPSS software (version 16.0).

4. Results

P. emodi seeds were tested and treated with various concentrations of Thiourea and Gibberllic Acid and compared with control (without treatment) for assessing the seed germination.The overall results for seed germination with respect to different concentrations shows the significant variations ($p<0.05$) among all the given treatment.

The seeds treated with different Thiourea concentration viz. 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm and results were recorded. Maximum (81.33±1.33%) seed germination was found in seeds treated with Thiourea concentration of 50 ppm, followed by 100 ppm (76.00±2.31%), 250 ppm (61.33±3.53%), 500 ppm (58.67±4.81%) and 1000 ppm (44.00±4.62%). Overall results were shown that maximum germination was obtained in lower concentration of Thiourea and it was found minimum in higher concentration. It means that decreasing concentration of Thiourea enhance the germination, while germination decreases with increasing the concentration of Thiourea. The seeds were also treated with GA_3 which was not much effective for the germination as compared to Thiourea. Maximum (24.00±4.00%) germination was recorded in 1000 ppm concentration followed by 250 ppm (14.67± 2.67%) while concentration of 50 ppm, 100 ppm and 500 ppm shows about similar (13.33±1.33%), germination percent. In the controlled seeds, there was no germination recorded till the end of germination experiment (Table 2& Figure 1).

Various treatments/concentrations of Plant Growth Regulators (PGRs) showed significant ($p<0.05$) results for Mean Germination Time (MGT). Minimum MGT (29.89±0.43 days) were obtained for the seeds treated with Thiourea concentration of 50

ppm, followed by 100 ppm (31.33±0.65 days), 250 ppm (32.97±0.52 days), 500 ppm (33.89±0.26 days), while 1000 ppm concentration revealed the maximum (35.08±0.72 days) MGT for Thiourea treatment. Similarly, overall minimum results for MGT exhibited in those seeds which were treated with GA_3, it was almost the same for 50 ppm, 100 ppm and 250 ppm (33.45±0.40 days, 33.50±1.32 and 33.80 ±0.61 days respectively), while maximum (38.40±0.87 days) for 500 ppm (Table 2& Figure1).

5. Discussion

In case of the herbaceous or tree peony seeds, they remain dormant for a period of time before germination due to double dormancy nature of seed. It requires both epicotyls and hypocotyls dormancy break treatments. Chilling period breaks the epicotyls dormancy while long worm period breaks the hypocotyls (Baskin and Baskin, 1998). In natural condition, peony seeds requires minimum two growing seasons for complete germination (Yu et al., 2014). Tetrazolium chloride test has been shown to be generally in close agreement with germination test results (Kandari et al., 2008). Fresh seed can be expected to have a better germination rates and require less time to begin their growth. This type of seed does not require any hydration steps before planting. Planting fresh seeds within a few days after harvest increases the chances of the first leaf being produced the following spring. If it is allowed to dry too much the seed may require another year to germinate (Barzilley et al., 2002). During the present task the viability of fresh harvested seeds of *P. emodi* as determined by the tetrazolium test. High percentage of viability (87%) was reported in fresh seeds is probably due to the short period between the harvesting and the trials of the seeds (5 weeks). However, a gradual decline in viability was observed on seeds which were storage for long period.

In present study, viability test indicated that seeds of *P. emodi*may not remain viable for extended periods at room temperature and even after storage at low temperature (4-6°C) for long. Loss of viability in stored seeds is a common phenomenon (Verma et al., 1996) and it increased with storage duration (Dell Aquilla, 1987). Further, seed moisture was slightly decreased after one year storage. Loss of viability as well as variation in seed viability among different natural populations may have the relation with growth and development of embryo which caused morpho-physiological dormancy as suggested by Walek and Hidayati (2004) in another Apiaceae species *Osmorhizadepauperata*.Singh and Gupta (2003) suggested that the seed storage at low temperature for long period does not affect the viability; however, storage under high humidity badly affects the seed germination. Present study also revealed that a presoaking treatment of about 48 hrs is sufficient to have imbibition of growth promoting hormones. Soaking of seeds promoted

germination by high percentage emergence at shorter period in the seeds of *Withaniasomnifera*(Ashwagandha) varieties and for all treatments (Chaudhary and Datta, 2014). The slow water absorbation by seeds of *P. emodi*in comparison to other MAPs in similar condition might be related to its hard seed coat characteristics. The present investigation on seed absorption indicated that there was a slow rate of water absorption in the seeds of *P. emodi*and took much time between 48-72 hours.

Under natural conditions, seeds of many species germinate only in the second year due to an underdeveloped embryo, but sometimes sprouting of peony seedlings may take up to five to seven years (Barzilley et al., 2002). Radical emergence in paeony seeds occurs readily, but the epicotyls requires specific growing conditions(BewleyandBlack, 1994).Similartomanyother species from temperate regions (e.g., *Trillium* spp., *Allium* spp. and *Hepatica* spp.), peony seeds have deep, simple epicotyl morpho-physiological dormancy. The epicotyl appears to be sensitive to cold stratification, only after the radical has elongated (Baskin and Baskin, 1998). For root emergence, a relatively high temperature of ~20°C in the dark is required. Following primary root emergence, dormancy release of the cotyledon leaf requires one to four months of low temperatures. Leaf sprouting and elongation occur when temperatures increase 15 to 20°C after the cold period (Baskin and Baskin, 1998). In general, dormancy is released at lower and induced at higher temperatures for the seeds those get matured in summer (Baskin and Baskin, 1998).

To overcome this condition, breaking of seed dormancy through different treatments can promote the germination in the laboratory also. In the present investigation, germination response of seeds subjected to different treatments showed significant variation ($p<0.05$) among the seeds collected from the natural habitats. While various pretreatment methods have been adopted to reduce dormancy and hasten germination (Sahoo, 2007), no single pretreatment technique has been found to be equally effective in all seed species (Amusa, 2011). During present investigation effect of Plant Growth Regulators (PGRs), namely; Thiourea and GA_3 (along with different concentrations) on seed germination was examined. Thiourea has long been known as a compound of marked biological activity. Thus, it is known to have bacteriostatic properties and dormancy breaker in plants (Sahoo et al., 2007). Maximum germination was observed in Thiourea (400 µL L-1) for all populations of *Angelica. archangelica* with germination percentage of 67.7% (Valley of Flower), 66.66% (Kedarnath), 63.54% (Rudranath) and 61.53% (Tungnath). In Kedarnath and Valley of Flower populations, excluding Thiourea (200 µL L-1), all treatments improved seed germination (Vashitha et al., 2006). Similar improved results were also seen in present study which showed the highest germination percentage in

all the concentrations of Thiourea than GA_3. The poor response of GA_3 may be due to the presence of naturally occurring inhibitors, which may not be suppressed by the application of GA_3 (Hartmann et al., 2007). Among all the concentration of Thiourea, concentration of 50 ppm showed the maximum (81.33±1.33%) percent germination which was 5.53% more than 100 ppm concentration while it was 37.33% more than 1000 ppm concentration of Thiourea.

GA_3 had a positive effect on promoting germination and embryo growth, while addition of 0.5 mg/L a-naphthalene acetic acid (NAA) to the culture medium significantly enhanced rooting in *Paeonia lactiflora*(Gao, 2010). Previous studies have shown that GA_3 enhances the germination of seeds exhibiting physiological, morphological or morpho-physiological dormancy (Ganai and Nawchoo, 2002; Kumar et al., 2006). The efficacy of GA_3 treatment in breaking dormancy depends on the concentration and length of incubation. Treatment with GA_3 (1000 ppm) showed highest and significant improvement among all the concentrations. In the present study, increased GA_3 concentrations (from 50 ppm to 1000 ppm) have positive effect on seed germination. Application of GA_3 is known to promote germination by breaking dormancy in a wide range of seeds (Bradbeer, 1988) and the efficacy of GA3 to enhance germination has been demonstrated in several studies (Nadeem et al., 2000; Pandey et al., 2000).

The Mean Germination Time (MGT) of *P. emodi* seeds was also affected by various treatments applied in the present study. The PGRs (CH_4N_2S and GA_3) have a significant impact over the MGT of seeds collected from natural habitats. Amongst all the different concentrations, treatment of Thiourea decreases the MGT for seed germination and it was increased with increasing level of concentration. It was significant ($p<0.05$) in both treatments as compared to control. MGT was reduced 3.56 days in 50 ppm concentration of Thiourea as compared to 50 ppm conc. of GA_3 While MGT was highest 35.08±0.72 days in Thiourea concentration of 1000 ppm and 38.40±0.87 days in 500 ppm concentration of GA_3 (Figure 1).

In control condition, no germination and MGT was recorded at the end of experiment. This shows the dormant nature of the *P. emodi* seed in natural condition.

6. Conclusions

The present study indicates that germination in *P. emodi*seed is very complicated and further research is required to understand the nature of germination. Study indicates the variation in seed viability andlow temperature about 4°C is compatible for long term viability. Although, fresh harvested seeds should be preferred for the seed germination study of this species. The experimental results revealed that 48 hrs duration for water

soaking or chemicals pretreatments in *P. emodi* seeds have the potential to enhance germination. The present investigations indicated that seeds of *P. emodi* require hormone application to break dormancy.In this context, the role of Thiourea and Gibberllic acid are helpful in breaking the dormancy. However, Thiourea impact was more effective in the stimulating seed germination and reducing Mean Germination Time (MGT). These treatments are quite simple and all the chemicals are cheaper as compared to PGRs, these can be widely used by the growers and nurserymen. The outcomes of the present study would be helpful to the local people, plant nursery growersand scientists to identify and propagation of *P. emodi* in large scale.

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Conflict of interest

Authors declare that they have no conflicts of interest.

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Table:

Table 1. Details of pre treatments given in laboratory conditions

S N	Treatments	Concentration	No. of seeds/ Replicates	Remarks
1.	Thiourea (CH_4N_2S)	50, 100, 250, 500 and 1000 (ppm)	25	Seeds in triplicate into petri dish at fixed Temperature 25±2°C in tissue culture lab.
2.	Gibberellic Acid (GA_3)	50, 100, 250, 500 and 1000 (ppm)	25	Seeds in triplicate into petri dish at fixed Temperature 25±2°C in tissue culture lab.
3.	Control		25	Seeds in triplicate into petri dish at fixed Temperature 25±2°C in tissue culture lab.

Table 2. Seed germination under Laboratory conditions

Concentration	GP			MGT		
	Thiourea	GA3	Control	Thiourea	GA3	Control
50 ppm	81.33±4.62	13.33±1.33	0.00±0.00	29.89±0.43	33.45±0.40	0.00±0.00
100 ppm	76.00±4.81	13.33±1.33	0.00±0.00	31.33±0.65	33.50±1.32	0.00±0.00
250 ppm	61.33±2.31	14.67±2.67	0.00±0.00	32.97±0.52	33.80±0.61	0.00±0.00
500 ppm	58.67±2.31	13.33±3.53	0.00±0.00	33.89±0.26	38.40±0.87	0.00±0.00
1000 ppm	44.00±1.33	24.00±4.00	0.00±0.00	35.08±0.72	36.75±1.02	0.00±0.00

± (Standard Error)

Figure:

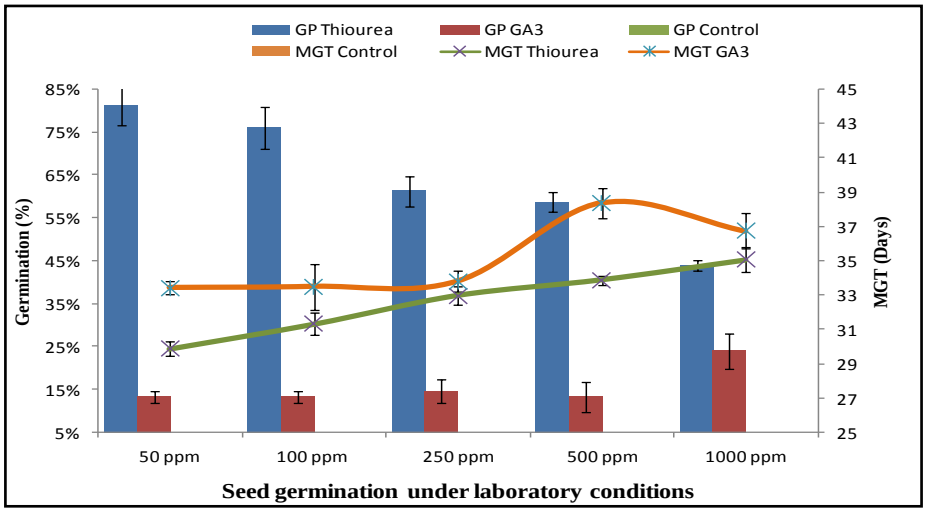

Fig. 1. Effect of chemical treatment (Thiourea and Gibberllic acid) on seed germination and MGT in laboratory condition