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Effect of seed priming on germination characteristics of seeds of *Podophyllum hexandrum* Royle: An endangered medicinal plant of western Himalaya

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Abstract
The present investigation was conducted to induce early, high and synchronized seed germination through priming in *Podophyllum hexandrum* Royle. The seeds of *Podophyllum hexandrum* were subjected to seventeen priming treatments and stored thereafter, for 0, 1and 2 months. The minimum number of days taken for onset of germination (19.67 days) was observed in seeds primed with GA_3 100 ppm for 48 hours followed by 2 months storage as compared to the control (26.33 days). Maximum germination percentage (68.38%) was observed with hydroprimng for 72 hrs + 2 months storage, which was closely followed by brassinolide 1.0 ppm for 48 hours + 2 months storage (60.89%), brassinolide 0.5 ppm for 72 hours + 2 months storage (60.78%), brassinolide 1.0 ppm for 48 hours + 2 months storage (56.67%), brassinolide 0.5 ppm for 72 hours + 2 months storage (52.22%), hydropriming for 24 hours + 2 months storage (47.56%), KNO_3 1% for 48 hours + 2 months storage (43.07%), GA_3 100 ppm for 48 hours + 2 months storage (40.41%) and KNO_3 1% for 72 hours + 1 month storage (40%) respectively as compared to the control i.e. 32.48, 32.29 and 35.89% for 0, 1 and 2 months storage respectively. Henceforth, it is concluded that the above seed treatments can pave the way for improving germination of *P. hexandrum* which otherwise shows late, low, and asynchronized germination.

Keywords: Seed priming, *Podophyllum hexandrum* Royle, seed germination

Introduction
Podophyllum hexandrum Royle, is an endangered medicinal plant endemic to the Western Himalayan region (Chaurasia *et al*., 2012) [4] and also enlisted as an endangered plant in the IUCN Red List. National Medicinal Plant Board, India has initiated efforts towards the conservation of rare, endangered, and threatened medicinal plants throughout the country (Ali and Sharma, 2013) [1]. In ancient times *Podophyllum hexandrum* was known as Aindri (a divine drug), but now it is commonly known as bantrapushi, Giriparpat, Himalayan Mayapple, Indian Mayapple, and bankakri. It belongs to family Berberidaceae and is a native to the lower elevations of Himalayan countries like Afghanistan, Pakistan, India, Nepal, Bhutan, and Southwest China. In India, *Podophyllum hexandrum* is mostly found in Alpine Himalayas (3000-4500 m) of Jammu and Kashmir, Himachal Pradesh, Sikkim, Uttaranchal, and Arunachal Pradesh. Despite its wider distribution in the entire Indian Himalayan range from Ladakh to Sikkim at an elevation of 3000-4200 m, the current status of *P. hexandrum* is 'endangered' (Nag *et al*., 2015) [10]. It grows in wet alpine meadows, in humus-rich and shaded localities or near stream banks as an undergrowth along with other herbs. It is 12 to 18 inches in height with deeply lobed leaves and fleshy stems, which rise straight up from the soil. In the spring, white or pale pink, 6-petaled flowers are borne at the ends of stout stems; these are followed by fleshy and oval fruit which is a large scarlet or reddish berry of 2.5-5 cm length with many seeds embedded in pulp.

Podophyllum hexandrum Royle is an important medicinal plant known for valuable drug podophyllotoxin which is effective against various diseases; purgative, laxative, cholagogue, polyps, alterative, emetic and also used against warts and tumorous growth of skin, anti-cancer. Podophyllotoxin, along with α-peltatin and β-peltatin, are aryltetralin liganins known

as anti-cancer, anti-fungal, anti-viral, anti-mitotic, and immunostimulatory properties (Chaurasia *et al*., 2012) [4].

Wild plant raw material is in great demand around the world for use by pharmaceutical companies and ethnomedicinal practitioners in a variety of traditional medicines. India is one of the world's major exporters of raw herbal drugs. The domestic and international demand is met mainly through unsustainable in-situ harvesting which has become a major threat to the survival of many Himalayan plant species (Badola, 2003) [2]. There is about 100 ton annual demand of Himalayan Mayapple from international sources, with only 50-80 tonnes of this being met (Ghimire *et al*., 2006) [6]. The estimated yield could range from 330 to 490 kg/acre and over 70 kg of podophyllotoxin extract could be produced per acre cultivated land. Etoposide, a derivative of podophyllotoxin, is currently in clinical use in the treatment of many cancers (Hameed, 2014) [7].

Due to its high medicinal importance and demand, *P. hexandrum* is overexploited rendering its survival at natural sites 'endangered'. The cultivation of this species may answer the growing needs of the plant material. In nature, *P. hexandrum* propagates vegetatively by rhizome and sexually by seeds. Therefore, a conservation strategy should be devised to increase its population number by propagating the plant through seeds in a short period, thereby compensating or reducing the harvesting pressure on the medicinally valuable rhizome. The plant raised from rhizome cutting takes 12 years to produces far-sized marketable rhizome, whereas the plants raised from seedling take an even longer time. Since it is subjected to harsh and extended winter conditions and perennates through underground parts for most of the year, the period of growth is confined only to summer months.

Seeds of *P. hexandrum* remain dormant for about 10 months under natural conditions. Since the difficulties of germination have not favored its domestication, this can certainly be explained by a difficult propagation due to late fruiting (after 3-4 years vegetation), very capricious germination, and very slow growth (Bhadwar and Sharma, 1963) [3]. Thus, major problems for the cultivation of this plant are its long juvenile phase and poor fruit setting ability (Qazi *et al*., 2011) [11]. Seeds of *P. hexandrum* show slow, low and asynchronous germination and poor seedling performance. The structure and arrangement of embryonic envelopes act as a barrier in seed germination has been elucidated. The uptake of water by *P. hexandrum* seeds during germination shows a triphasic pattern with a reduced rate of water uptake during Phase III, due to the presence of thick-walled endosperm cells which acts as a physical barrier to the protrusion of the radicle. The existence of the multi-layered endosperm and the thick testa appears to protect the embryo during extreme environmental conditions by preventing germination until conditions are more favourable (Sreenivasulu *et al*., 2009) [13].

There is an urgent need to develop and implement regeneration/conservation strategies for the exploitation of this medicinal plant. Simultaneous development of ex situ propagation of the species concerned would encourage their cultivation, thereby considerably easing the pressure on natural habitats (Sharma *et al*., 2006) [12]. Attempts have been made to conserve this plant through the artificial breaking of seed dormancy (Qazi *et al*., 2011) [11]. One of the effective techniques of seed enhancement is priming which can be used to enhance seed quality, rapid and uniform seed emergence, and improve overall germination (McDonald, 2000 and Farooq *et al*., 2007) [8, 5].

Therefore, keeping in view the problems of highly erratic, delayed, poor and asynchronous germination of *P. hexandrum* seeds, the proposed study was undertaken with aim to improve the germination potential and vigour of seeds by using multiple approaches of seed priming.

Methodology

The present study was conducted in Plant Physiology laboratory of the Department of Basic Sciences, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni Solan (H.P.) during the period 2014-16. The details of the experiments which were conducted to achieve the objectives and methodologies are given below:

T_1: Control
T_2: Hydropriming for 24 hours
T_3: Hydropriming for 48 hours
T_4: Polyethylene glycol (PEG 6000), -0.5 MPa for 48 hours
T_5: Polyethylene glycol (PEG 6000), -0.5 MPa for 72 hours
T_6: Polyethylene glycol (PEG 6000), -1.0 MPa for 48 hours
T_7: Polyethylene glycol (PEG 6000), -1.0 MPa for 72 hours
T_8: Gibberellic acid (GA_3), 100ppm for 48 hours
T_9: Gibberellic acid (GA_3), 100ppm for 72 hours
T_{10}: Polyethylene glycol (PEG 6000), -0.5 MPa + GA_3 100ppm for 48 hours
T_{11}: Polyethylene glycol (PEG 6000), -0.5 MPa + $GA_3$100ppm for 72 hours
T_{12}: Potassium nitrate 1% for 48 hours
T_{13}: Potassium nitrate 1% for 72 hours
T_{14}: Brassinolide 0.5 ppm for 48 hours
T_{15}: Brassinolide 0.5 ppm 72 hours
T_{16}: Brassinolide 1.0 ppm for 48 hours
T_{17}: Brassinolide 1.0 ppm for 72 hours

Time of Sowing – 3

1. Direct (0 storage after priming)
2. After storing primed seeds for 1 month
3. After storing primed seeds for 2 months

Number of treatments: 17
Number of treatment combinations: 51
Number of replications: 3 (30 seeds each)
Experimental design: CRD (Factorial)
The experiment was conducted in seed germinator at 20 ± 2^0C, 80% Relative humidity, and light.

Observations Recorded

Source of seeds

Freshly harvested seeds of *Podophyllum hexandrum* Royle were procured from Rohru, (2700 - 3400 m amsl) during the last week of September. Thereafter, seeds were cleaned and air-dried. Seed characteristics *viz*. moisture content, seed viability, and solute leakage were recorded. To study the priming physiology and its implication on seed germination in *Podophyllum hexandrum* Royle, seeds were surface sterilized with the help of 0.1% mercuric chloride for 1 minute. Thereafter, seeds were thoroughly washed with distilled water thrice to remove the strains of mercuric chloride completely.

Method of storage

The primed seeds were packed in sealed in plastic envelopes which in turn were stored in plastic containers at room temperature (25 ± 2 ºC).

Conduct of experiment

The seeds of *Podophyllum hexandrum* Royle species were allowed to germinate in Petri dishes using the top paper method in seed germinator at 20 ± 2ºC temperature, 80% relative humidity. The paper used for the experiment was Whatmann no.1.

Observational Procedure

Seed characteristics

Seed moisture content (%): The moisture content of seeds was determined by moisture meter according to the prescribed standard procedure and expressed as a percentage (%).

Seed viability (%)

The seeds of *Podophyllum hexandrum* were subjected to tetrazolium chloride (0.1%) pH 7.0 for 48 hours. Seeds were soaked in water first to allow imbibition, then seeds were excised and finally, embryos were exposed after 48 hours soaking (Moore, 1973) [9]. Darkly red embryos were considered as viable and viability was calculated as:

$$\text{Viability \%} = \frac{\text{No.of seeds with darkly stained embryo}}{\text{Total no.of seeds soaked in tetrazolium solution}} \times 100$$

Germination characteristics

Onset of germination (days)

Seeds kept for germination were observed daily and the day when the first seed showed germination was considered as time taken for onset of germination and it was expressed in days.

Completion of germination (days)

Daily count of seeds for germination was done and the day when the last seed showed germination was considered as the time taken for completion of germination and expressed in days.

Germination percentage (%)

Germination percentage was calculated according to the standard germination procedure (ISTA, 1965) using the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

Mean germination time (MGT) (days)

Mean germination time was calculated according to using Sfairi *et al*. (2012) the following formula:

$$\text{MGT (days)} = \sum (\text{ni x di}) / \text{N}$$

Where, ni is the number of germinated seeds on day i; d is the incubation time (day); N is the total number of seeds germinated.

Germination speed

The speed of germination was calculated according to Ellis and Robert (1981) by using the following formula:

$$\text{Speed of germination} = \frac{\text{No.of normal seedlings}}{\text{Days of first count}} + \ldots\ldots + \frac{\text{No.of normal seedlings}}{\text{Days of final count}}$$

Germination energy

Germination energy was calculated by using the following formula:

$$\textbf{Germination energy} = \frac{\text{Germination percentage}}{\text{Day of completion of germination}}$$

Results and Discussion

Seed characteristics

Seed characteristics: Seed moisture content, seed viability and solute leakage

Seed characteristics *viz*. moisture content, viability and solute leakage of Podophyllum hexandrum Royle were studied are exhibited in Table 1 which reveals that these seeds showed 6.9% moisture content and 78% seed viability. The Table also reveals that seeds showed 0.13 dSm^{-1} solute leakage after 24 hours and 0.75 dSm^{-1} after 48 hours.

Table 1: Seed characteristics of *Podophyllum hexandrum* Royle

Seed characteristics			
Moisture content (%)	**Seed viability (%)**	**Solute leakage (dS m^{-1}) after**	
		24 hrs	**48 hrs**
6.9	78.00	0.13	0.75

Germination characteristics

Onset of germination

The data presented in Table 2 indicates that priming treatments and storage of seeds had a significant effect on onset of germination. Untreated (control) seeds registered onset of germination after 25.44 days of sowing. Whereas, earliest onset of germination i.e. 21.33 days was registered for seeds pretreated with GA_3 100 ppm for 72 hrs (T_9), which was 4.11 days earlier as compared to control and statistically at par with T8 (GA_3 100 ppm for 48 hours) showing 21.56 days for onset of germination. However, onset of germination was delayed upto 58.44 days as recorded in T_5 i.e. PEG 6000 (-0.5 MPa) for 72 hours, which was significantly higher than all other treatments. Storage time also exerted a significant effect on onset of germination. The maximum number of days taken for onset of germination (34.14 days) was recorded in 2 months storage. However, the minimum number of days taken for onset of germination (31.18 days) was found in 0 month storage and this was followed by 32.10 days onset for 1 month storage period.

Table 2: Effect of seed priming and storage on onset of germination (days) in seeds of *Podophyllum hexandrum*

Priming treatments (T) \ **Storage Duration (D)**	**0 month**	**1 month**	**2 months**	**Mean**
T_1: Control	25.00	25.00	26.33	25.44
T_2: Hydropriming for 24 hours	25.33	27.33	27.67	26.78
T_3: Hydropriming for 48 hours	23.33	23.33	22.67	23.11
T_4: Polyethylene glycol (PEG 6000), -0.5 MPa for 48 hours	26.00	34.67	42.33	34.33
T_5: Polyethylene glycol (PEG 6000), -0.5 MPa for 72 hours	61.33	57.00	57.00	58.44
T_6: Polyethylene glycol (PEG 6000), -1.0 MPa for 48 hours	32.67	27.67	32.67	31.00
T_7: Polyethylene glycol (PEG 6000), -1.0 MPa for 72 hours	48.67	48.00	52.67	49.78
T_8: Gibberellic acid (GA_3), 100ppm for 48 hours	24.33	20.67	19.67	21.56
T_9: Gibberellic acid (GA_3), 100ppm for 72 hours	21.33	22.00	20.67	21.33

T_{10}: Polyethylene glycol (PEG 6000), -0.5 MPa + GA_3 100ppm for 48 hours	37.33	41.33	46.33	41.67
T_{11}: Polyethylene glycol (PEG 6000), -0.5 MPa + $GA_3$100ppm for 72 hours	36.33	35.00	38.00	36.44
T_{12}: Potassium nitrate 1% for 48 hours	20.67	22.67	25.67	23.00
T_{13}: Potassium nitrate 1% for 72 hours	28.00	42.67	42.33	37.67
T_{14}: Brassinolide 0.5 ppm for 48 hours	38.67	41.33	41.67	40.56
T_{15}: Brassinolide 0.5 ppm 72 hours	31.00	24.33	29.00	28.11
T_{16}: Brassinolide 1.0 ppm for 48 hours	24.33	25.33	26.67	25.44
T_{17}: Brassinolide 1.0 ppm for 72 hours	25.67	27.33	29.00	27.33
Mean	31.18	32.10	34.14	
$CD_{0.05}$				
T	1.45			
D	0.61			
T×D	2.52			

Completion of germination

The data presented in Table 3 shows that priming treatments and storage of seeds had a significant effect on completion of germination. Untreated (control) seeds registered completion of germination after 118.56 days of sowing. Whereas, earliest completion of germination i.e. 100.56 days was registered for seeds pretreated with T_8 i.e GA_3 100 ppm for 48 hrs, which was 18 days earlier as compared to the control. However, completion of germination was delayed upto 119.78 days as recorded in T_{11} i.e. PEG 6000 (-0.5 MPa) + GA_3 100 ppm for 72 hours, which was significantly higher than all other treatments. This was statistically at par with T10 i.e. PEG 6000 (-0.5 MPa) + GA_3 100 ppm for 72 hours showing 119.67 days for completion of germination. Storage time also exerted a significant effect on completion of germination. The minimum numbers of days taken for completion of germination (113.04 days) were recorded for 1 month storage. However, the maximum number of days taken for onset of germination (113.57 days) was found for 0 month storage and this was followed by 113.45 days for 2 months storage period.

Table 3: Effect of seed priming and storage on completion of germination (days) in seeds of *Podophyllum hexandrum*

Priming treatments (T) \ Storage Duration (D)	0 month	1 month	2 months	Mean
T_1: Control	117.00	118.67	120.00	118.56
T_2: Hydropriming for 24 hours	115.00	114.33	113.00	114.11
T_3: Hydropriming for 48 hours	115.00	114.00	114.00	114.33
T_4: Polyethylene glycol (PEG 6000), -0.5 MPa for 48 hours	111.67	111.00	111.33	111.33
T_5: Polyethylene glycol (PEG 6000), -0.5 MPa for 72 hours	114.00	114.00	115.00	114.33
T_6: Polyethylene glycol (PEG 6000), -1.0 MPa for 48 hours	112.67	112.00	112.00	112.22
T_7: Polyethylene glycol (PEG 6000), -1.0 MPa for 72 hours	112.33	114.33	115.00	113.89
T_8: Gibberellic acid (GA_3), 100ppm for 48 hours	100.00	100.33	101.33	100.56
T_9: Gibberellic acid (GA_3), 100ppm for 72 hours	101.33	104.67	108.33	104.78
T_{10}: Polyethylene glycol (PEG 6000), -0.5 MPa + GA_3 100ppm for 48 hours	120.00	119.00	120.00	119.67
T_{11}: Polyethylene glycol (PEG 6000), -0.5 MPa + $GA_3$100ppm for 72 hours	120.00	119.67	119.67	119.78
T_{12}: Potassium nitrate 1% for 48 hours	114.00	114.67	115.00	114.56
T_{13}: Potassium nitrate 1% for 72 hours	111.00	110.00	109.67	110.22
T_{14}: Brassinolide 0.5 ppm for 48 hours	113.67	114.00	114.00	113.89
T_{15}: Brassinolide 0.5 ppm 72 hours	115.00	113.00	111.67	113.22
T_{16}: Brassinolide 1.0 ppm for 48 hours	119.00	113.33	114.67	115.67
T_{17}: Brassinolide 1.0 ppm for 72 hours	119.00	114.67	114.00	115.89
Mean	113.57	113.04	113.45	
$CD_{0.05}$				
T	0.97			
D	0.39			
T×D	1.62			

Germination percentage

The data pertaining to germination percentage of seeds of Podophyllum hexandrum which were subjected to seventeen priming treatments and stored thereafter for 0, 1 and 2 months have been presented in Table 4. Untreated (control) seeds showed germination percentage of 33.35%. Among all seventeen treatments, germination percentage was maximum i.e. 58.93% observed for T_3 (hydropriming for 48 hours) as compared to the control. This was followed by T_{16} (brassinolide 1.0 ppm for 48 hours) i.e. 55.10% and other beneficial treatments showed higher germination were T_2 (hydropriming for 24 hours, 40.95%), T_{12} (KNO_3 1% for 48 hours, 42.48%), T_{14} (brassinolide 0.5 ppm for 48 hours, 40.94%), T_{15} (brassinolide 0.5 ppm for 72 hours, 52.06%) and T_{17} (brassinolide 1.0 ppm for 72 hours, 48.22%). Among treatments, the minimum germination percentage i.e. 13.12% was observed in T_{11} i.e. PEG 6000 (-0.5 MPa) + $GA_3$100 ppm for 72 hours. Storage time also exerted a significant effect on germination percentage. The maximum germination percentage (39.60%) was recorded for 2 months of storage and it was closely followed by 1 month storage (36.02%). However, the minimum germination percentage was 32.07% as found in 0 month storage.

Table 4: Effect of seed priming and storage on germination percentage (%) in seeds of *Podophyllum hexandrum*

Priming treatments (T) \ Storage Duration (D)	0 month	1 month	2 months	Mean
T_1: Control	32.48(34.73)	32.29(34.62)	35.89(36.79)	33.55(35.38)
T_2: Hydropriming for 24 hours	36.11(36.92)	39.19(38.74)	47.56(43.58)	40.95(39.75)
T_3: Hydropriming for 48 hours	48.88(44.34)	59.52(50.47)	68.38(55.76)	58.93(50.19)
T_4: Polyethylene glycol (PEG 6000), -0.5 MPa for 48 hours	31.11(33.89)	33.78(35.52)	35.44(36.52)	33.44(35.31)
T_5: Polyethylene glycol (PEG 6000), -0.5 MPa for 72 hours	15.26(22.98)	15.89(23.48)	13.85(21.84)	15.00(22.77)
T_6: Polyethylene glycol (PEG 6000), -1.0 MPa for 48 hours	25.37(30.23)	27.82(31.82)	30.00(33.20)	27.73(31.75)
T_7: Polyethylene glycol (PEG 6000), -1.0 MPa for 72 hours	18.00(25.09)	22.89(28.57)	27.89(31.87)	22.93(28.51)
T_8: Gibberellic acid (GA_3), 100ppm for 48 hours	34.89(36.19)	39.59(38.98)	40.41(39.45)	38.30(38.21)
T_9: Gibberellic acid (GA_3), 100ppm for 72 hours	30.18(33.31)	31.89(34.37)	31.30(34.00)	31.12(33.89)
T_{10}: Polyethylene glycol (PEG 6000), -0.5 MPa + GA_3 100ppm for 48 hours	17.00(24.34)	18.74(25.64)	20.00(26.56)	18.58(25.51)
T_{11}: Polyethylene glycol (PEG 6000), -0.5 MPa + $GA_3$100ppm for 72 hours	12.22(20.45)	14.89(22.69)	12.26(20.49)	13.12(21.21)
T_{12}: Potassium nitrate 1% for 48 hours	42.15(40.47)	42.22(40.51)	43.07(41.00)	42.48(40.66)
T_{13}: Potassium nitrate 1% for 72 hours	36.71(37.28)	40.00(39.22)	36.52(37.17)	37.74(37.89)
T_{14}: Brassinolide 0.5 ppm for 48 hours	34.41(35.90)	36.18(36.96)	52.22(46.25)	40.94(39.71)
T_{15}: Brassinolide 0.5 ppm 72 hours	42.11(40.44)	53.30(46.87)	60.78(51.20)	52.06(46.17)
T_{16}: Brassinolide 1.0 ppm for 48 hours	48.26(43.99)	56.15(48.51)	60.89(51.27)	55.10(47.92)
T_{17}: Brassinolide 1.0 ppm for 72 hours	40.00(39.22)	48.00(43.83)	56.67(48.81)	48.22(43.96)
Mean	32.07(34.10)	36.02(36.52)	39.60(38.57)	
$CD_{0.05}$				
T	0.16			
D	0.07			
T×D	0.28			

Mean germination time (MGT)

Table 5 reveals the data pertaining to mean germination time (MGT) of seeds of Podophyllum hexandrum which were subjected to seventeen priming treatments and stored thereafter for 0, 1 and 2 months. In untreated (control) seeds mean germination time was 24.62 days. The shortest mean germination time (MGT) i.e. 16.52 days was showed by T8 (GA_3 100 ppm for 48 hours)as compared to the control. Among treatments, the maximum mean germination time i.e. 28.45 days was recorded for T5 i.e. PEG 6000 (-0.5 MPa) for 72 hours. Storage time also exerted a significant effect on germination percentage. The minimum mean germination time was 22.75 days recorded for 0 month storage and this was closely followed by 22.79 days registered for 1 month storage. However, the maximum mean germination time was 23.00 days found for 2 months storage.

Table 5: Effect of seed priming and storage on mean germination time (days) in seeds of *Podophyllum hexandrum*

Priming treatments (T) \ Storage Duration (D)	0 month	1 month	2 months	Mean
T_1: Control	24.26	24.69	24.91	24.62
T_2: Hydropriming for 24 hours	20.30	22.09	21.74	21.38
T_3: Hydropriming for 48 hours	21.25	21.02	21.34	21.20
T_4: Polyethylene glycol (PEG 6000), -0.5 MPa for 48 hours	21.76	20.99	22.85	21.87
T_5: Polyethylene glycol (PEG 6000), -0.5 MPa for 72 hours	27.82	28.53	29.01	28.45
T_6: Polyethylene glycol (PEG 6000), -1.0 MPa for 48 hours	24.89	25.10	26.55	25.51
T_7: Polyethylene glycol (PEG 6000), -1.0 MPa for 72 hours	26.13	25.85	26.87	26.28
T_8: Gibberellic acid (GA_3), 100ppm for 48 hours	17.03	16.25	16.29	16.52
T_9: Gibberellic acid (GA_3), 100ppm for 72 hours	17.51	16.93	16.55	17.00
T_{10}: Polyethylene glycol (PEG 6000), -0.5 MPa + GA_3 100ppm for 48 hours	25.67	25.64	25.47	25.59
T_{11}: Polyethylene glycol (PEG 6000), -0.5 MPa + $GA_3$100ppm for 72 hours	27.24	26.70	23.76	25.90
T_{12}: Potassium nitrate 1% for 48 hours	21.75	21.19	21.10	21.35
T_{13}: Potassium nitrate 1% for 72 hours	20.67	21.21	23.36	21.75
T_{14}: Brassinolide 0.5 ppm for 48 hours	21.61	23.30	22.70	22.54
T_{15}: Brassinolide 0.5 ppm 72 hours	23.47	22.43	22.43	22.78
T_{16}: Brassinolide 1.0 ppm for 48 hours	21.52	22.19	23.35	22.35
T_{17}: Brassinolide 1.0 ppm for 72 hours	23.78	23.30	22.67	23.25
Mean	22.75	22.79	23.00	
$CD_{0.05}$				
T	0.33			
D	0.14			
T×D	0.58			

Germination speed

The data pertaining to germination speed of seeds of Podophyllum hexandrum which were subjected to seventeen priming treatments and stored thereafter for 0, 1 and 2 months have been presented in Table 6. Untreated (control) seeds registered germination speed as 0.23. Germination speed was maximum i.e. 0.35 which was found in T_3 i.e. hydropriming for 48 hoursas compared to the control. Among treatments, the minimum germination speed was 0.06 in T_{11} i.e. PEG 6000 (-0.5 MPa) + GA_3 100 ppm for 72 hours. Storage time also exerted a significant effect on germination speed. The maximum germination speed (0.26) was recorded for 2

months storage. However, the minimum germination speed (0.21) was registered for 0 month storage which was followed by 0.23 for 1 month storage.

Table 6: Effect of seed priming and storage on germination speed in seeds of *Podophyllum hexandrum*

Priming treatments (T) \ Storage Duration (D)	0 month	1 month	2 months	Mean
T_1: Control	0.220	0.220	0.250	0.230
T_2: Hydropriming for 24 hours	0.250	0.260	0.300	0.270
T_3: Hydropriming for 48 hours	0.290	0.330	0.430	0.350
T_4: Polyethylene glycol (PEG 6000), -0.5 MPa for 48 hours	0.230	0.240	0.260	0.240
T_5: Polyethylene glycol (PEG 6000), -0.5 MPa for 72 hours	0.120	0.130	0.160	0.140
T_6: Polyethylene glycol (PEG 6000), -1.0 MPa for 48 hours	0.110	0.110	0.120	0.110
T_7: Polyethylene glycol (PEG 6000), -1.0 MPa for 72 hours	0.100	0.110	0.110	0.110
T_8: Gibberellic acid (GA_3), 100ppm for 48 hours	0.190	0.310	0.310	0.270
T_9: Gibberellic acid (GA_3), 100ppm for 72 hours	0.270	0.320	0.310	0.300
T_{10}: Polyethylene glycol (PEG 6000), -0.5 MPa + GA_3 100ppm for 48 hours	0.080	0.090	0.100	0.090
T_{11}: Polyethylene glycol (PEG 6000), -0.5 MPa + $GA_3$100ppm for 72 hours	0.050	0.070	0.070	0.060
T_{12}: Potassium nitrate 1% for 48 hours	0.270	0.270	0.230	0.260
T_{13}: Potassium nitrate 1% for 72 hours	0.240	0.290	0.300	0.280
T_{14}: Brassinolide 0.5 ppm for 48 hours	0.250	0.280	0.320	0.280
T_{15}: Brassinolide 0.5 ppm 72 hours	0.280	0.330	0.350	0.320
T_{16}: Brassinolide 1.0 ppm for 48 hours	0.300	0.340	0.390	0.340
T_{17}: Brassinolide 1.0 ppm for 72 hours	0.270	0.280	0.340	0.300
Mean	0.210	0.230	0.260	
$CD_{0.05}$				
T	0.003			
D	0.001			
T×D	0.006			

Germination energy

Data pertaining to germination energy of seeds of Podophyllum hexandrum which were subjected to seventeen priming treatments and stored thereafter for 0, 1 and 2 months have been presented in Table 7. Germination energy in untreated (control) seeds was registered as 0.28. Maximum germination energy i.e. 0.51 was recorded in T_3 i.e. hydropriming for 48 hours as compared to the control. Among treatments, the minimum germination energy was 0.11 in T_{11} i.e. PEG 6000 (-0.5 MPa) + GA_3 100 ppm for 72 hours. Storage time also exerted a significant effect on germination energy. The maximum germination energy i.e. 0.35 was recorded for 2 months of storage. However, the minimum germination speed was 0.28, found for 0 month storage and this was followed by 0.32, for 1 month storage.

Table 7: Effect of priming and storage of seeds on germination energy in *Podophyllum hexandrum*

Priming treatments (T) \ Storage Duration (D)	0 month	1 month	2 months	Mean
T_1: Control	0.280	0.270	0.300	0.280
T_2: Hydropriming for 24 hours	0.310	0.340	0.420	0.360
T_3: Hydropriming for 48 hours	0.430	0.520	0.590	0.510
T_4: Polyethylene glycol (PEG 6000), -0.5 MPa for 48 hours	0.280	0.300	0.320	0.300
T_5: Polyethylene glycol (PEG 6000), -0.5 MPa for 72 hours	0.130	0.140	0.120	0.130
T_6: Polyethylene glycol (PEG 6000), -1.0 MPa for 48 hours	0.220	0.250	0.270	0.250
T_7: Polyethylene glycol (PEG 6000), -1.0 MPa for 72 hours	0.160	0.200	0.240	0.200
T_8: Gibberellic acid (GA_3), 100ppm for 48 hours	0.350	0.400	0.400	0.380
T_9: Gibberellic acid (GA_3), 100ppm for 72 hours	0.300	0.300	0.290	0.300
T_{10}: Polyethylene glycol (PEG 6000), -0.5 MPa + GA_3 100ppm for 48 hours	0.140	0.160	0.170	0.160
T_{11}: Polyethylene glycol (PEG 6000), -0.5 MPa + $GA_3$100ppm for 72 hours	0.100	0.120	0.100	0.110
T_{12}: Potassium nitrate 1% for 48 hours	0.370	0.370	0.370	0.370
T_{13}: Potassium nitrate 1% for 72 hours	0.330	0.360	0.340	0.340
T_{14}: Brassinolide 0.5 ppm for 48 hours	0.300	0.320	0.460	0.360
T_{15}: Brassinolide 0.5 ppm 72 hours	0.370	0.470	0.550	0.460
T_{16}: Brassinolide 1.0 ppm for 48 hours	0.410	0.500	0.530	0.480
T_{17}: Brassinolide 1.0 ppm for 72 hours	0.340	0.420	0.500	0.420
Mean	0.280	0.320	0.350	
$CD_{0.05}$				
T	0.003			
D	0.001			
T×D	0.006			

Conclusion

Based on the findings of present investigation it may be concluded that seeds of *Podophyllum hexandrum* subjected to hydropriming for 48 hrs resulted in maximum germination percentage (58.93%), followed by brassinolide 1.0 ppm for 48 hours (55.10%), brassinolide 0.5 ppm for 72 hours (52.06%),

brassinolide 1.0 ppm for 72 hours (48.22%), KNO_3 1% for 48 hours (42.48%), brassinolide 0.5 ppm for 48 hours (40.94%), hydropriming for 24 hours (40.95%), GA_3 100 ppm for 48 hours (38.30%) and KNO_3 1% for 72 hours (37.74%) as compared to control (33.55%). Higher germination with above treatments was coupled with shorter mean germination time, higher speed of germination and germination energy.

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