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Full Length Research Paper

Effect of pre-sowing treatments on seed germination in *Hedychium spicatum:* An important vulnerable medicinal plant of Indian Himalayan region

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The objective of Present study was to examine the effect of various pre-sowing treatments on seed germination of an important and vulnerable high value medicinal plant, *Hedychium spicatum* with different plant growth regulators (PGRs) and nitrogenous compounds under different conditions. Out of various used treatments, gibberellic acid (GA_3) and potassium nitrate (KNO_3) were significantly effective treatments regarding seed germination of this species. This simple cost effective technique could be implemented by the nursery growers and by the commercial laboratories because KNO_3 is cost effective (low in price) and easily available rather than other plant growth regulators. Using this simple and significant study we could propagate a large number of seedlings of this species under laboratory as well as nursery conditions.

Key words: Pre-sowing treatment, gibberellic acid (GA_3), potassium nitrate (KNO_3), seed germination, medicinal plant.

INTRODUCTION

The Indian Himalayan Region (IHR) is a rich reservoir of biological diversity in the world. Over 1748 species of medicinal and aromatic plants (MAPs) reported from IHR are used in different systems of medicine (Samant and Joshi, 2005). *Hedychium spicatum* Buch- Ham ex Sm (Family Zingiberaceae) is one of the most important aromatic and medicinal plant of IHR. Commonly it is known as Kapoor Kachri or Ginger lilly. It is distributed in subtropical Himalaya in the state of Assam, Arunanchal Pradesh and Uttarakhand within an altitudinal range of 1000 to 3000 m. The root stock (rhizome) is also useful in asthma, pain, foul breath, hiccough, vomiting and laxative, stomachic, carminative, stimulant tonic to the brain, in liver complaints, diarrhea and pains (Kirtikar and Basu, 1987). Rhizome extracts possess antimicrobial activity against both Gram-positive and Gram-negative bacteria (Bisht et al., 2006). Essential oil contains cineole, terpenes, limonene, phellandrene, p-cymene, linalool and terpeneol as major constituents (Bottini et al., 1987). Recently the crude extract of the rhizome has been used in the preparation of an anticancerous drug, PADMA-28 (Nayab et al., 2004). Phenolics carotenoids and vitamins are well known for its antioxidant activity (Kahkonen et al., 1999; Javanmardi et al., 2002) and repeatedly been used as natural antioxidant in fruits, vegetables and other plants. Latest study showed that *H. spicatum* can also used as a hepatoprotective agent (Joshi and Mishra, 2011).

According to the threat status of IUCN criteria, *H. spicatum* has become vulnerable (Samant and Pant, 2006), due to reduction in population of over 20.0% in last ten years. It is also listed in near threatened category of the essential oil bearing plants (Kemp, 2003; Samant and Palni, 2000). Samant et al. (2007) reported that *H spicatum* is commercially exploited from its natural area. Giri et al. (2010) reported that due to high value medicinal

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plant, prioritization needs to be done for propagation and cultivation of this species at large scale.

Plant growth regulators including auxin, gibberelin, cytokinin, ethylene and abscisic acid (ABA) may control nucleic acids performance, stimulation of seed germination and contribute to dormancy (Chiwocha et al., 2005). Pre-sowing chemical treatments were used to enhance and improve seed germination in many Himalayan medicinal plants and reported by several workers (Pandey et al., 2000; Nadeem et al., 2000; Joshi and Dhar, 2003; Gupta, 2003; Shivkumar et al., 2006, Ghimire et al., 2006; Kandari et al., 2008; Ali et al., 2010; Zare et al., 2011). On the basis of our knowledge and available literatures there is no previous report on seed germination study of *H. spicatum* by using different pre-sowing treatments. Present study was conducted to see the effect of pretreatments of various PGRs and nitrogenous compound on seed germination (percent seed germination and time taken for germination) of *H. spicatum* under different conditions.

MATERIALS AND METHODS

Seeds of *H. spicatum* were collected in bulk (Plate 1A) in the month of October from Nainital catchment forest (1990 m District Nainital, Uttarakhand, India). After collection these seeds were brought to the laboratory and air dried for few days at room temperature. These were stored in air tied polythene bags at 4°C until used. Seeds were thoroughly washed with a detergent (labolene; 1% v/v; 15 min). Following washing (3×) with double distilled water these were placed in beakers containing 25 ml of test solutions for 24 h at 25°C under dark condition. The test solutions included, gibberelic acid (GA_3, 25.0, 125.0 and 250.0 µM), 6-benzyladenine (BA, 25.0, 125.0 and 250.0 µM), thidiazuron (TDZ, 25.0, 125.0 and 250.0 µM), GA_3 + BA (25.0 + 25.0, 125.0 + 125.0 and 250.0 + 250.0 µM), TDZ + BA (25.0 + 25.0, 125.0 + 125.0 and 250.0 + 250.0 µM), and potassium nitrate (KNO_3, 25.0, 50.0 and 100.0 mM). For control, seeds were soaked in distilled water for 24 h at 25°C under dark condition.

Following treatment with test solutions, seeds were placed in petri plates (95 × 17 mm) containing moistened filter paper (Whatman No.1) and kept in the growth chamber at 25±2°C in dark (laboratory condition) and in thermocole cups containing non-forest soil at 1.5 cm depth. These cups were placed inside Polyhouse at Nursery Department (1990 m) (nursery condition) and watered weekly. The mean temperature ranged from 15 - 20°C inside the poly house during the experiment.

Seeds were considered germinated upon the emergence of radical. Seed germination started after third weeks of seed sowing. Observations on germination were recorded at two days interval (or every day) and seed germination was found to be completed by the end of twelve weeks of seed sowing. For each treatment, total of 36 seeds were used in three replicates. Data were subjected to statistical analysis; standard error (SE), dunnett t test (2-sided) and analysis of variance (ANOVA- multivariate) were calculated following Snedecor and Cochran (1967).

RESULTS

The percent seed germination and time required for initiation of germination were varied among various treatments in different conditions (Table 1). In laboratory condition, 25.0 µM GA_3 induced maximum seed germination (61.11%) within 29 days followed by 125.0 µM GA_3 (52.77% within 33 days). TDZ (25.0 µM) alone induced only 19.44% seed germination which was minimum but in conjunction with GA_3 (25.0, 125.0 µM) it stimulates a better germination response that was, 52.77 and 49.99%, respectively. In nursery condition the maximum seed germination (49.99%) was stimulated by KNO_3 (50.0 mM) after 57.66 days. In both conditions the TDZ treatment alone did not respond well but in conjunction with GA_3 , it was able to increase the mean germination percentage. Dunnett-t test showed that percent seed germination was significant (at the 0.05 level) among used treatments in only lower concentration of GA_3 (25.0, 125.0 µM) alone and in conjunction with TDZ (25.0, 125.0 µM) (Table 1). All the treatments significantly reduced (at the 0.05 level) the mean germination time in comparison to control except higher concentration of KNO_3 (100.0 mM) in both conditions (Table 1). Analysis of variance (ANOVA- multivariate test) showed that percent seed germination and time taken for germination were significant ($p<0.01$ at 1%) among the used concentrations of different treatments. Time taken for seed germination also showed a significant variation ($p<0.01$ at 5%) between two conditions (laboratory and nursery) and concentrations of used treatments (Table 2).

DISCUSSION

Pre-sowing treatments of seeds with various chemicals improved the percent germination and reduced the mean germination time in comparison to control; it might be due to altered physiology of embryos and liberating enzymes so that the development process occurs more rapidly after sowing (Kattimani et al., 1999). In the present study out of twenty two used pretreatments, the lower concentration of GA_3 (25.0 µM) and lower concentration of KNO_3 (50.0 mM) showed best germination response in laboratory and nursery conditions, respectively (Plate 1B and C). Similar results were reported in *Heracleum candicans* in which KNO_3 and GA_3 pretreatment improved seed germination (Joshi and Dhar, 2003). Butola and Badola (2004) reported that the lower concentration of GA_3 was effective treatment. It was also reported by several researchers that GA_3 enhances the germination of seeds exhibiting physiological, morphological or morph physiological dormancy (Ganai and Nawchoo, 2002; Shivakumar et al., 2006). Ali et al. (2010) reported that treatment of lower percent of KNO_3 (0.3%) was effective in stimulating seed germination of *Descurainia sophia.* Besides plant growth regulators used in this experiment, nitrogenous chemical treatments (KNO_3) were also an effective pre-treatment for seed germination in both conditions. This may be due to the higher concentration of KNO_3 that was effective, possibly via oxidized form of nitrogen which causes shift in respiratory metabolism to

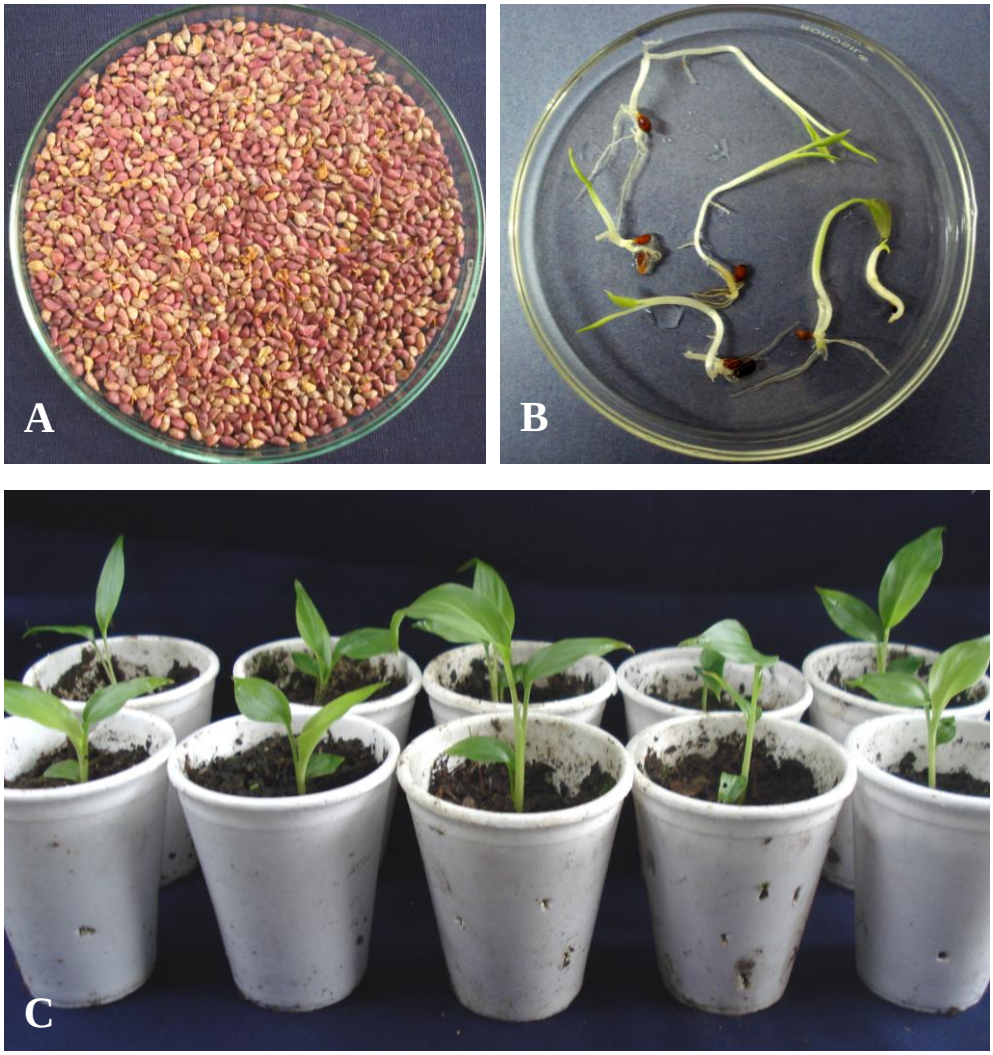

Plate 1. Seed germination study in *H. spicatum.* (A) Mature seeds collected from the natural habitat in the month of October. (B) Germinated seeds under laboratory condition after six weeks of GA_3 (25.0 µM) treatment. (C) Well grown seedlings under nursery conditions after eight weeks of KNO_3 (50.0 mM) treatment.

Table 1. Effect of pre-sowing treatments on seed germination of *H. spicatum* under different conditions.

S/N	Treatment	Concentration (µM)	Laboratory condition		Nursery condition	
			% seed germination	Time taken for germination (in days)	% seed germination	Time taken for germination (in days)
1	Control		24.99±4.81	62.66±2.48	27.77±2.77	58.66±2.02
2	BA	25.0	27.77±2.77	34.00±2.51*	30.55±2.77	43.00±1.52*
3		125.0	33.33±4.80	33.33±2.33*	27.77±5.58	45.33±2.18*
4		250.0	19.44±2.75	36.00±1.15*	16.66±4.81	46.00±3.60*
5	GA_3	25.0	61.11±2.77*	29.00±1.15*	44.44±2.78*	41.66±3.71*
6		125.0	52.77±2.77*	33.33±1.20*	41.66±4.81*	45.66±3.52*
7		250.0	44.44±7.34	36.00±1.52*	33.33±4.80	42.66±2.33*
8	TDZ	25.0	19.44±2.74	34.00±2.51*	19.44±7.34	47.00±0.57*
9		125.0	30.55±2.77	32.00±0.57*	27.77±2.77	44.33±3.17*
10		250.0	22.21±5.55	43.00±1.52*	27.77±5.55	45.66±3.17*

Table 1. Contd.

11	GA_3 + BA	25.0+25.0	38.88±2.77	36.66±0.88*	33.33±4.80	44.66±2.02*
12		125.0+125.0	30.55±5.53	39.66±0.88*	33.33±4.80	52.66±2.90*
13		250.0+250.0	27.77±2.77	37.33±1.20*	27.77±2.77	37.33±2.33*
14	TDZ+ BA	25.0+25.0	24.99±4.81	26.00±1.52*	27.77±5.55	44.00±3.05*
15		125.0+125.0	38.88±2.77	36.66±2.40*	36.10±2.77	45.66±5.17*
16		250.0+250.0	24.99±4.81	41.00±2.30*	22.22±7.32	45.00±4.16*
17	GA_3 + TDZ	25.0+25.0	52.77±5.55*	32.67±3.28*	44.44±2.75*	46.00±2.30*
18		125.0+125.0	49.99±4.81*	32.00±3.21*	44.44±2.78*	41.66±2.33*
19		250.0+250.0	33.33±4.80	31.66±1.85*	27.77±2.77	42.33±4.37*
20	KNO_3	25.0 mM	44.44±2.78*	50.66±5.04*	44.44±7.34*	54.33±2.40*
21		50.0 mM	47.22±5.55*	48.00±2.08*	49.99±4.81*	57.66±1.20*
22		100.0 mM	25.00±4.82	52.66±2.33	27.77±2.77	57.66±2.33

Values are mean ± standard error (SE), 36 seeds (in three replicate) in each treatment. * The mean difference is significant at the 0.05 level (Dunnett-t test treat one group as a control and compare all other groups against it).

Table 2. ANOVA summary.

Source	Dependent variable	df	Mean square	F-value
Condition	% seed germination	1	231.928	3.833^{Ns}
	Time taken for germination (in days)	1	2487.341	120.798*
Concentration	% seed germination	21	634.499	10.487*
	Time taken for germination (in days)	21	284.741	13.828*
Condition × Concentration	% seed germination	21	47.929	0.792^{Ns}
	Time taken for germination (in days)	21	38.928	1.891**

*Significant at 1% ($p<0.01$), **5% ($p<0.01$) and Ns = not significant.

the pentose phosphate pathway (Roberts and Smith, 1977). Another reason for the positive effect KNO_3 on seed germination is related to creating a balance between hormonal ratios in seed and reducing the growth preventable materials, like ABA (Ali et al., 2010).

Conclusions

On the basis of the present study, in control treatment, percent seed germination was below 30.0%, however it could be enhanced up to 61.11% by applying pre-sowing treatments with reduced mean germination time. In our opinion this is the significant and first report on seed germination study in this species by applying various PGRs and nitrogenous chemical treatments under different conditions. This methodology is simple; a cost effective technique and could be easily implemented by the nursery growers and commercial laboratories because KNO_3 is economically low in price and easily available rather than other plant growth regulators. Using this simple and significant methodology we could propagate a large number of seedlings for commercial purpose and reduce the mean germination time.

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