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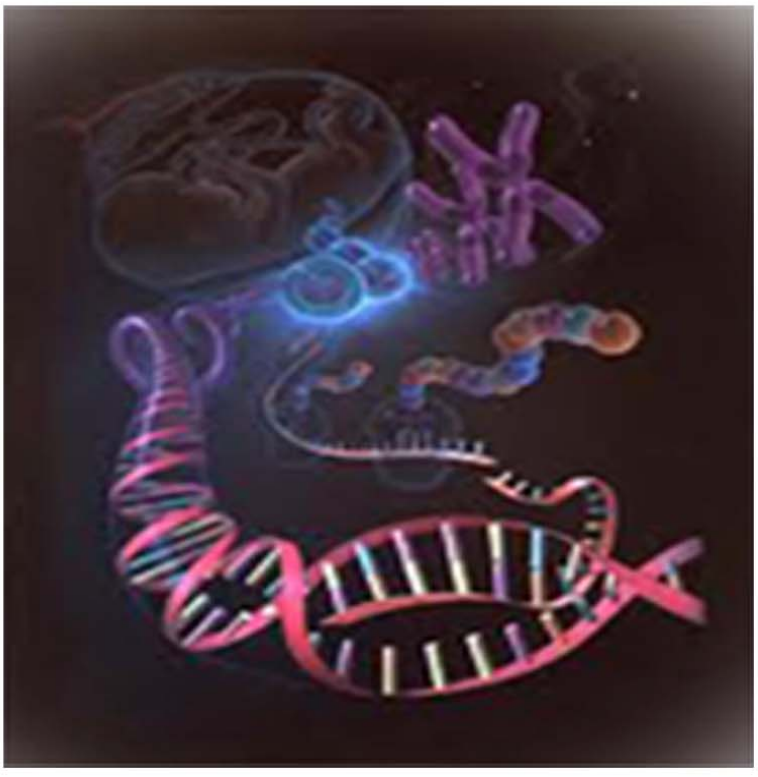

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Research Paper

GERMANIBILITY, CONSERVATION AND CULTIVATION OF *RHEUM EMODI* WALL EX. MEISSNER – A THREATENED MEDICINAL PLANT

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International Union for Conservation of Nature (IUCN) committee for threatened plant species indicates that one in ten species of vascular plants on the earth is endangered or threatened due to commercial exploitation and international trade. This may lead to gene erosion in next 20-30 years. *Rheum emodi* is among the top of that list. Therefore, it has been identified top priority species for conservation and cultivation. Seeds of *Rheum emodi*, a threatened medicinal plant of North-western Himalaya collected from natural populations at the different regions of high altitude were germinated under different conditions by giving different treatments to form seedlings. It was observed that pre-chilling treatment of seeds for four weeks and seed coat removal proved most effective in enhancing seed germination. This process ensures mass propagation and establishment of *Rheum emodi* at easily approachable altitudes which determines the success of survival strategy of this species.

Keywords: Chilling, Conservation, Germination, Gibberellic acid, *Rheum emodi*

INTRODUCTION

Seed germination is basic requirement for life cycle of any spermatophytic plant species. Seed germination is the process which causes sudden transformation of a dry dormant seed into a young plant (Tahir and Qamer 2009). For which basic requirement are oxygen, moisture, temperature and type of soil. In most of spermatophytic plants, seeds pass through phase of dormancy caused by several physical and physiological factors there by delaying seed germination (Koul, 1997). The age factor of *Rheum emodi* seeds show great affect on their germination. Older seeds are more prone to infection and show less germination, therefore decline in viability and vigour of the seeds (Debaish and Bhattacharya, 2008). The understanding of these eco-physiological constraints is fundamental to development of a protocol of seed based conservation cum multiplication programme (Dev and Singh, 2003).

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Rheum emodi is a perennial stout herb found between 2,800-3,600 m in alpine zone of rocky soil. It is endemic to western and central Himalayan region. It is an important medicinal herb of Himalaya with its rhizome being valued for its purgative and astringent properties. In China and Mediterranean region, it was highly popular Laxative drug and a general tonic. It is a stimulant and useful drug in dyspepsia (Agarwal, 1986). Powdered roots are sprinkled over ulcers for healing and also used for cleaning teeth. Leaf stalks are eaten either raw or boiled sprinkled with salt and pepper. Roots also yield red dye that is used for coloration of silk and wool (Debaish and Bhattacharya, 2008). The affinity of color is found to be more for silk than wool.

Rheum emodi possess polyphenolic contents like ferulic acid, coumaric acid, chlorogenic acid, Gallic acid, and catechin (Mayer and Mayber, 1982). These polyphenols show greater anti-oxidant, cardio protective, immunomodulatory and anti-microbial potential (Zarger, 2010). Besides these it is hepatoprotective (Sen, 1977), anti-helminthic, purgative, appetite stimulants, diuretic, curative for sore eyes and used for treating appendicitis and hypertension, etc. Therefore, this plant over the past several decades has been extensively exploited through extraction of crude drug and also through impact of various anthrogenic pressures like grazing, uncontrolled deforestation, selective extraction, rapid urbanization and industrialization. Now the situation is such that a single plant of *Rheum emodi* is seen after hours of trekking in higher regions of Himalaya (Zarger, 2010). The species is categorized as threatened (Maithani, 2001). In order to reverse the trend of its extinction, it is essential that a protocol for its ex situ conservation be developed to regenerate the germ plasm for industrial utilization. This study is based on efforts to enhance the seed germination through overcoming period of prolonged dormancy; aims at developing a protocol for seed based cultivation of species at low and easily approachable altitudes in order to ensure its conservation. Economic viability of cultivation of *Rheum emodi* near the vicinity of villages generates self employment to local people and also generates extra income and helps in conservation of this threatened species and its natural habitat (Wafai and Nawchoo, 2001).

MATERIALS AND METHODS

Seed of *Rheum emodi* were collected from populations in natural habitat of sonamarg (2,800 m), kokernag (2,590 m) and Gulmarg (3,000 m) of Kashmir region. The seeds were washed with 0.1% mercuric chloride for 5-7 min and then 70% alcohol for 1 min. These seeds were thoroughly rinsed with distilled water. The seeds were divided into 5 sets of 50 seeds each.

First set of seeds was subjected to various treatments without prior chilling. Various treatments were given which includes (A) Acid wash treatment using H_2SO_4, (B) Hot water treatment for 5 min, (C) Scarification with sand paper, (D) GA_3 treatment with 50 ppm and 100 ppm solution, (E) Seed coat removal and (F) Control, i.e., without any treatment. Second, third, fourth and fifth sets were subjected to chilling treatment at 0 ^oC for one week, two weeks, three weeks and four weeks, respectively followed by above mentioned treatment. For each set, two replicates were made one kept in ordinary light and one in complete dark. For each sample about 25 numbers of seeds were used and after treatments results were monitored on daily basis for two months.

RESULTS AND DISCUSSION

Seed Germination was observed for 2 months with data being recorded on daily basis. The number of days taken for seed germination to begin, total seeds germinated and total number of seedlings survived to senescence was noted for each set of seeds kept in ordinary light and complete darkness. The seed germination results are presented in Tables 1 to 5. From the result it was clear that hot water, acid wash treatment and scarification do not show any proper impact on seed germination. Exposure to ordinary light and complete darkness also did not show significant effect in increasing rate or % age of seed germination nor made seedling survival appreciable, though in some cases its impact was moderate. The seed coat removal seems to be major factor as it enhances seed germination but the survival of seedlings is on the lower side. The results observed on application of gibberellins

Table 1: Seeds Subjected to Various Treatments Without any Prior Chilling

Treatment	In Ordinary Light			In Complete Darkness		
	No. of Days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived	No. of Days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived
Control	15	8	5	17	3	1
Acid Wash	0	0	0	x	0	0
Hotwater	x	0	0	x	0	0
Scarification	x	0	0	x	0	0
GA3 50 ppm	10	9	5	14	6	4
GA3 100 ppm	8	12	7	10	10	4
Seed Coat Removal	6	11	3	12	7	2

Table 2: Seeds Subjected to Various Treatments After one Week Pre-Chilling At 0 °C

Treatment	In Ordinary Light			In Complete Darkness		
	No. of Days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived	No. of Days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived
Control	12	10	10	10	10	0
Acid Wash	x	0	0	x	x	x
Hotwater	x	0	0	x	x	x
Scarification	x	0	0	x	0	0
GA3 50 ppm	9	12	8	12	10	2
GA3 100 ppm	6	16	14	6	12	3
Seed Coat Removal	5	14	5	9	11	0

Table 3: Seeds Subjected to Various Treatments After Two Week of Chilling at at 0 °C

Treatment	In Ordinary Light			In Complete Darkness		
	No. of Days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived	No. of Days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived
Control	6	12	10	10	10	0
Acid Wash	x	0	0	x	x	x
Hotwater	x	0	0	x	x	x
Scarification	x	0	0	x	0	0
GA3 50 ppm	10	14	8	12	10	2
GA3 100 ppm	8	19	14	6	12	3
Seedcoat Removal	5	12	5	9	11	0

Table 4: Seeds Subjected to Various Treatments After Three Week of Chilling at 0 °C

Treatment	In Ordinary Light			In Complete Darkness		
	No. of Days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived	No. of Days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived
Control	14	6	0	6	12	0
Acid Wash	x	0	0	x	0	0
Hotwater	x	0	0	x	0	0
Scarification	4	10	0	6	5	0
GA3 50 ppm	12	8	4	14	3	0
GA3 100 ppm	10	10	10	12	8	0
Seedcoat Removal	4	11	7	14	6	0

Table 5: Seeds Subjected to Various Treatments After Four Week of Chilling at 0 °C

Treatment	In Ordinary Light			In Complete Darkness		
	No. of Days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived	No. of days Taken to Germinate	No. of Seeds Germinate	No. of Seedling Survived
Control	14	8	3	10	5	0
Acid wash	14	2	0	8	5	0
Hotwater	x	0	0	x	0	0
Scarification	x	0	0	8	2	0
GA3 50ppm	12	16	12	10	5	0
GA3 100ppm	10	20	20	8	9	0
Seedcoat removal	8	20	16	8	6	0

were appreciable but the best results were obtained when seeds were subjected to four week chilling at 0 °C followed by treatment with 100 ppm solution of GA_3 and kept in ordinary light (Figure 1). In this treatment 80% seed germination and seedling survival was seen. In seeds subjected to four week chilling at 0 °C followed by treatment with 50 ppm solution of GA_3 and kept in ordinary light about 64% seed germination and 75% seedling survival was observed, these two treatments were by far the best and most productive. Among other treatment two week chilling followed by treatment with 50 ppm and 100 ppm solution of GA_3 shows 56% seed germination and seedling survival and 76% seed germination and seedling survival respectively when kept in ordinary light, 40% seed germination and 20% seedling survival, 48% seed germination and 25% seedling survival respectively when kept in complete darkness. Further, in case of seeds

Figure 1: Seed Germination (A) and Seedling Formation (B)

Figure 2: Development of Seedlings in Pots (A) and transplantation (B)

with no prior chilling, the application of GA_3 (50 ppm or 100 ppm) do enhances the % age of seed germination by 40% or 50% respectively. The seed viability was recorded as 50-60% for 1 year when seeds were stored in cotton bags or plastic bags at 4-5 ^oC temperature under dry condition.

CONCLUSION

These findings reveal that seeds of *Rheum emodi* show maximum seed germination, when seeds were subjected to 4 week chilling at 0 ^oC followed by application of 100 ppm solution of GA_3, seeds were kept in ordinary light and placed in sandy soil treated with forest litter in 1:2 ratio during October with higher moisture and optimum temperature of 15-30 ^oC. In October seed viability was maximum and seedlings were well developed in February for transplantation. Watering/irrigation was done frequently to decrease the mortality rate of seedlings after transplantation (Figure 2). The growth rate at lower altitude was fast, vigorous in comparison to natural habitat and yield recorded was three times higher as recorded in natural population. Therefore mass propagation and wide spread cultivation of this threatened medicinal plant at lower altitude would facilitate availability of raw material to the drug industry as it is known for its active metabolites like emodin, rutin, chrysophanol, chrysophenic acid, etc.

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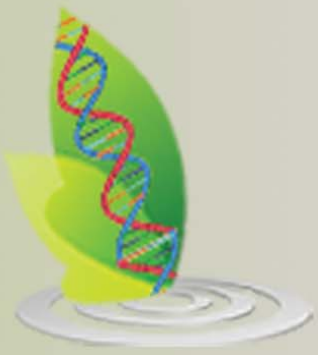

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