

Enhanced Seed Germination of *Psoralea Corylifolia* L. by Heat Treatment

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Abstract *Psoralea corylifolia* is a medically important plant and it used for large scale level in pharmaceutical industries to cure various skin diseases. This plant is facing difficulties in propagation because of poor seed germination and high mortality of seedlings. Therefore, an efficient and simple protocol developed for seed germination of *Psoralea corylifolia* via hot water treatment for *in situ* and *ex situ* plant propagation and conservation. Different treatments such as hot water heat treatment (10°C to 100°C) and sulfuric acid (H₂SO₄) treatment (5 to 30 min) were used for seed germination. In which, hot water heat treatment with 70°C was produced the highest seed germination (70%) and survival rate. It is concluded that the hot water heat treatment favorably overcome the dormancy of seed. Developed protocol in this study will be helpful for mass propagation and *in situ* and *ex situ* conservations.

Keywords: *Psoralea corylifolia* L., seed germination, heat treatment, *ex situ*, *in situ*

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1. Introduction

Medicinal plants are naturally consists rich sources of different forms of alkaloids and chemical substances which are being used to cure a variety of diseases. *Psoralea corylifolia* Linn. is an important medicinal plant used in Folk, Siddha and Ayurvedic system of medicine. It is an endangered and rare herbaceous medicinal plant and distributed in the tropical region of the world [1]. From time to time the fruits, seeds and roots of *P. corylifolia* have been examined and a large number of compounds have been reported earlier [2]. The major compounds of this plant are psoralen, angelicin, psoralone, isopsoralone, bavachin, daidzein and so on [3]. Psoralen is a pharmaceutical interested compound because of their photosensitizing, photobiological and phototherapeutic properties which is used for the photochemotherapy of vitiligo and skin diseases such as psoriasis, mycosis fungoides and eczema [4,5]. The plant is also used in indigenous medicine as laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions [6]. It is specially recommended for the treatment of leucoderma, leprosy and inflammatory diseases of the skin and prescribed as both oral administration and external application in the form of a paste or ointment [7,8]. Also, it exhibits antitumour, antibacterial, antifungal and antioxidative activities [9,10]. Many Indian pharmaceutical industries have used *P. corylifolia* as a raw

material to produce medicines and Ayurvedic skin care soaps [9]. Propagation of *P. corylifolia* through seed is unreliable due to its poor germination rate and the high mortality of young seedlings under natural conditions [11]. The plant is listed as an 'endangered species' mainly due to the destruction of its habitats, as well as illegal and indiscriminate collection [1]. Although a number of *in vitro* regeneration protocols have been published for *P. corylifolia* [12], high frequency rapid mass propagation remains a major bottleneck. Efficient *in vitro* seed germination is therefore required for *in situ* and *ex situ* conservation and clonal propagation of *P. corylifolia*.

Many mechanisms involve for breaking seed dormancy such as mechanical injury to the seed coat or chemical treatment has been breaking the seed dormancy of certain cultivated medicinal plants [13], seed treatment with chemical or scarification or pre-soaking with hormone or hot water has been ideal for improving germination [14,15,16]. The present study aimed was to develop protocol with different treatment of acid and heat for high rate of seed germination of *P. corylifolia*.

2. Materials and Methods

2.1. Seed Material and Treatment

Psoralea Corylifolia seeds were collected from Department of Plant Physiology, Jawaharlal Nehru KrishiVishwaVidyalaya (JNKVV), Jabalpur, MP, India.

The seeds were used to treat with various temperatures (10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C and 100°C) of hot water for 10 to 15 minutes, and H₂SO₄ treated with 5, 10, 15, 20, 25, 30 min respectively. Each treatment repeated with 25 seeds per replicate and three replicates were used for entire experiments. In the entire experiment conducted, 50 - 70°C shows good results of seed germination (Table 1).

2.1.1. Aseptic Seed Germination

Seeds were washed thoroughly in tap water for 3 to 5 times, followed by soaking in soap solution (2% Teepol - commercial soap solution) for 5 min and then seeds were kept in running tap water for 30 min. Then the seeds were disinfected with 70% ethanol for 45 sec and rinsed with double distilled water for 3 times, followed by 0.1% (w/v) aqueous mercuric chloride exposure for 5 to 30 min. After decanting the mercuric chloride solution, the seeds were rinsed 5 times in sterile distilled water and the disinfected seeds were inoculated in sterilized 121°C for 15 minutes with 1.06 Kg cm⁻² pressure (15 lb)] test tubes containing moistened cotton for seed germination. Initially the cultures were maintained in dark condition for 48 h at 25±2°C and then under a 16 h light and 8 h dark photoperiod condition with the light intensity of 3000 lux. All the experiments were carried out under aseptic conditions. After 30 days of germination, healthy and vigorously growing seedlings were selected and used as the source of explants for *in vitro* regeneration and transformation studies.



Figure 1. Seed material of *Psoralea corylifolia* L.



Figure 2. *Psoralea corylifolia* L. plantlet

3. Results and Discussion

Heat and acid treatments were tested (Figure 3 & Figure 4) for seed germination and they were compared with the control (Table 1). Different treatments have been used for seed germination and breaking seed dormancy in many medicinal plant species [17,18,19]. In the present study, heat treatment with 50 and 60°C were showed above 50% of germination after one month of inoculation. However, 70°C heat treatment was produced the highest germination percentage (70%) and survival rate then the control and all other treatments (Table 1). For the improvement of the *Ferula assa-foetida* seed germination, the two temperature based experiments was conducted at 23°C and 4°C [20]. In the previous study, the highest seed germination was observed while the seeds of some plants were exposed in room temperature for 2 days and then placing at 20°C in continuous light [17]. Sulfuric acid treatment with different time period (minutes) was not effective in seed germination, whereas H₂SO₄ with 30 min promoted germination but the survival of plant rate was lower than the heat treatment (Table 2). Sulfuric acid and hot water pre-treatment have been reported (Figure 5 & Figure 6) that to improve the seed germination and seedlings growth of *Cassia fistula* [19]. In this study, we found that the seedling which is obtained at the 70°C heat treatment has grown well in the field (Figure 7). The aseptic seedlings were used as explants for *in vitro* regeneration and transformation studies.

Table 1. Effect of heat treatment on seed germination of *Psoralea corylifolia* L.

Heat treatment (°C)	Number of seeds germination	% of germination	Number of plant survival
0	0	0	0
10	1	10	0
20	2	20	0
30	4	40	2
40	3	30	3
50	6	55	5
60	5	65	6
70	7	70	6
80	5	50	3
90	4	40	3
100	5	50	4

Table 2. Effect of H₂SO₄ treatments on seed germination of *Psoralea corylifolia* L.

H ₂ SO ₄ Treatment (min)	Number of seeds germination	% of germination	Number of plant survival
0	0	0	0
5	0	0	0
10	0	0	0
15	1	10	0
20	2	20	1
25	2	20	1
30	3	30	2



Figure 3. Heat treatment



Figure 4. In vitro Seed germination of *Psoralea corylifolia* L.

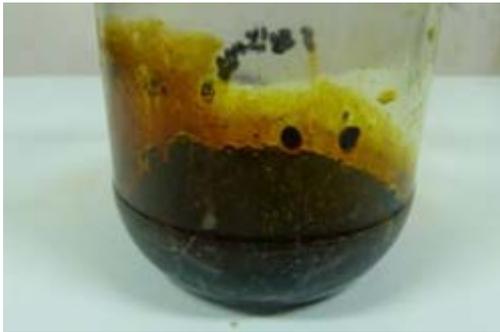


Figure 5. Acid treatment

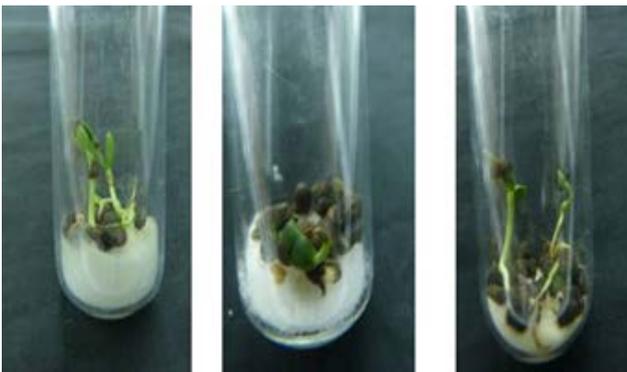


Figure 6. In vitro Seed germination of *Psoralea corylifolia* L.



Figure 7. In situ Seed germination of *Psoralea corylifolia* L.

4. Conclusion

The present study deals to overcome the lower percentage response of seed germination. Generally, seed germination is very difficult in *P. corylifolia* in *in vitro* conditions. This plant posses strong seed dormancy and impermeable seed coat which is the major cause of less frequency in seed germination. Hence, we used heat treatment method to break the seed dormancy for seed germination. The underlying mechanism of this method involves breaking seed dormancy and impermeable seed coat at 70°C for 15 min to break the seed layers and help the seed germination. It is a reliable and reproducible method to get high frequency seed germination. When compared to any other treatments, it is very simple and rapid one. Damaging and breaking possibilities of seeds are very low at this heat treatment and intensity as well as percentage of seed germination was higher. Thus, this method can be adopted as a good alternative than the other treatments for higher percentage of seed germination.

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