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, isosporalone, 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 psoriasias, mycosis fungoides and eczema [4,5]. The plant is also used in indigeneous 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 antioxidantive 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 30 min. Then the seeds were disinfected with 0.1% (w/v) aqueous mercuric chloride exposure for 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.

### 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.

| 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                        |

| H2SO4 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                        |
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 possesses 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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References

[1] Jain, S.K, *Ethnobotany and the search for New Drugs*. Ethnobotany and research in medicinal plants in India. 1994, 185: 153-168.

[2] Bajwa, B.S, Pyare, L.K. and Seshadri, T.R, “A new chromenochalcone bavachromene from the seeds of *Psoralea corylifolia*”. *Car. Sci.*, 41 (22): 814-815. 1972.

[3] Bouque,V, Bourgaud, F, Nguyen, C. and Guckert, A, “Production of daidzein by callus cultures of *Psoralea* species and comparison with plants”. *Plant Cell, Tissue and Organ Culture*. 53: 35-40. 1998.

[4] Frank, S, Caffieri, S, Raffaelli, A, Vedaldi, D. and Dall’Acqua, F, “Characterization of psoralen-oleic acid cycloadducts and their possible involvement in membrane photo damage”. *J Photochem Photobiol B*. 44: 39-44. 1998.

[5] Yones, Ss., Palmer, RA., Kuno, K. and Hawk, JLM, “Audit of the use of Psoralen phototherapy (PUVA) and narrowband UVB phototherapy in the treatment of psoriasis”. *J Dermatol Treat* 16: 108-112. 2005.

[6] Rastogi and Mehrotra, In: Compendium of Indian medicinal plants, 1990, 1: pp. 322-333.

[7] Anonymous, In: The Wealth of India: A dictionary of Indian raw materials and industrial products. 1988, 116-118.

[8] Orient longman, In: Indian medicinal plants (Eds.) Orient Longman Ltd. Madras. 1996, 4: pp. 374.

[9] Baskaran, P and Jayabalan, N, “Rapid micropropagation and Psoralen production in *Psoralea corylifolia* L”. *Acta Physiol Plant* 30: 445-451. 2008.

[10] Faisal, M. and Anis, “Thidiazuron induced high frequency axillary shoot multiplication in *Psoralea corylifolia* L”. *Biologia Plantarum* 50 (3): 437-440, 2006.

[11] Baskaran, P and Jayabalan, N, “An improved protocol for adventitious shoot regeneration and plant formation in *Psoralea corylifolia* L”. *Scientia Hort* 123: 283-286. 2009.
[13] Sriram, Seed technological studies in selected medicinal crops. M. Sc. (Agri.) Thesis, Univ. Agric. Sci., Bangalore, Karnataka, India, 2004.

[14] Gupta, L. K. and Shah, S. C, “Effect of chemicals on germination of *Belladonna* seeds”. *Prog. Hort.* 3 (2): 17-20, 1971.

[15] Supari, M. R., Farooqui, A. A. and Prasad, T. G, “Influence of various pre-sowing treatments and growth regulators on seed germination in *Gloriosa superba*”. *Indian J. Forestry.* 16 (2): 123-126, 1993.

[16] Vivek Mittar, K, Srinivasan and Singh, B. M, “Overcoming hard seededness on *Psoralea corylifolia*”. *Seed Res.* 21 (1): 31-34, 1993.

[17] Rakesh Kumar and Saurabh Sharma, “Effect of light and temperature on seed germination of important medicinal and aromatic plants in north western Himalayas”, *Int.J.Arom.Plants.* Issn 2249-4340, Vol. 2, No. 3, pp. 468-475, 2012.

[18] Zahra Karimian Fariman, Majid Azizi and Samira Noori, “Seed germination and dormancy breaking techniques for *Echinacea purpurea*”, *J. Biol. Environ. Sci.* 5 (13), 7-10, 2011.

[19] Amira Sh, Soliman and Mohamed S. Abbas. “Effects of sulfuric acid and hot water pre – treatment on seed germination and seedlings growth of *Cassia fistula*, American–Eurasi”. *Agric. & Environ. Sci.*, 13 (1): 07-15, 2013.

[20] S.B. Hassani, Effects of temperature, GA3, and cytokinins on breaking seed dormancy of *Ferula Assa-foetida*, Iranian Journal of Science & Technology, Transaction A, Vol. 33, No. A1, 2009.