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Research article

Seed dormancy testing and germination frequency determination of *Psoralea corylifolia* L., an endangered medicinal plant

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Abstract: Seed dormancy is one of important factor which prevent the cultivation of medicinally important plant *Psoralea corylifolia*. Mechanical dormancy is present in the seed due to hard seed coat. *P. corylifolia* is a slow growing species due to low germination percentage (5–7%). Long gestation period and delicate field handling discourages the commercial cultivation of the plant so different treatments were used to break dormancy in seeds. Out of five different methods used 98% concentrated H_2SO_4 for about one hour was found to be best method for seed dormancy breakage. Statistical analysis of data was performed by one way ANOVA using Sigma State Software version 4.

Keywords: *Psoralea corylifolia* - ANOVA - Dormancy - Gestation period - Dormancy.

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INTRODUCTION

Psoralea corylifolia L. (Indian bread root) is an endangered medicinally important plant (Fig. 1) distributed in tropical and subtropical region of world belonging to *Fabaceae* family (Jain 1994). In India it is seen along the road sides and waste place of the tropical regions. Especially in the semi-arid region of Rajasthan and Eastern district of Punjab, Bihar and Karnataka (Agrawal *et al*. 2013). The Hindi name of the plant is Babchi and bakuchi (Oudhiya 2001). It is an erect herb of height 30–180 cm that grows in winter season high. Leaves are broadly elliptical in shape, Flower shows indense axillary long-peduneled heads pods small 3.5–4.5 mm × 2.0–3.0 mm, seeds are compressed mucronate dark chocolate to almost black one, smooth, adhering to the pericarp (Uikey *et al*. 2010). The plant is harvested by drug industry when it sets in to flowering. *P. corylifolia* is propagated by seed germination.

Scientific classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family: *Fabaceae*
Genus: *Psoralea*
Species: *Psoralea corylifolia* L.

Figure 1. Plant of *Psoralea corylifolia* L. (Inset: Seeds)

Active constituent of *Psoralea corylifolia*

The major active constituents reported to be present in *P. corylifolia* seeds are corylifols a-c (prenylflavonoid) (Yin *et al.* 2004). Dried ripe fruit contains Psoralen, isopsoralen and neobavaislflavones

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(Rajput *et al*. 2008). Daidzein (4-7 dihydroxyisoflavonoid) and genistein (4, 5, 7 trihydroxy isoflavonoid) are present in natural plant as well as *in vitro* culture (Shinde *et al*. 2009).

Medicinal Uses

P. corylifolia has aromatic, antihelminthic, antibacterial and antifungal properties. It is used as a diuretic, diaphoretic, laxative and stimulant. The powdered seeds are applied externally to cure skin problems. It is valued as Chinese herbal medicine as a tonic remedy and is used to improve vitiligo. The roots are used for treating dental caries. Plant yields a useful medicinal oleoresin which treats kidney disorders, impotence and lumbago. The drug psoralen obtained from seed of *P.corylifolia* used for the treatment of vitiligo and leucoderma (Vaidya 2006).

Dormancy

Germination is prevented by dormancy mechanism during unsuitable ecological conditions, when the probability of seedling survival is low (Black *et al*. 2006). *P. corylifolia* has mechanical dormancy because its seed coats or other covering are too hard which prevent the embryo to expand during germination (Baskin & Baskin 1999). In, environment condition the seed coats of physically dormant seeds become water permeable over time through repeated heating and cooling over many months to years in the soil. Chemical or pigment that is present around the covering of embryo may be leached out of the tissue by specific chemical method (Bewley & Black 1994). *P. corylifolia* is a slow growing species due to low germination percentage (5–7%). Long gestation period and delicate field handling discourages the commercial cultivation of the plant (Pandey *et al*. 2013).

The objective of this paper was to determine the effective method for breaking dormancy of *Psoralea corylifolia* with the purpose of increasing cultivation of medicinally important plant.

MATERIAL AND METHODS

Seeds were collected form MFP-PARC located at coordinate 23.2086° N, 77.4731° E Madhya Pradesh. State Minor Forest Produce Trading & Development Co-operative Federation under the brand name "Vindhya Herbals" Located in Van Parisar, Barkheda Pathani, Near BHEL, *Bhopal, Madhya Pradesh.*

Methodology for seed dormancy

Five different treatments were performed to break seed dormancy.

a) Control
b) 98% Concentrated H_2SO_4 treatment for 1 hour and Hot water treatment 100ºC for 20 min (Siva *et al.* 2014).
c) 1 N NaOH treatment for 1 hour and Scarification by sand paper number 100 (Baes *et al*. 2002).
d) Cold water treatment Overnight incubation at 4ºC (Fariman *et al*. 2011).

Seeds were washed with running tap water for 5 min to remove surface dust than washed with Tween 20 for about 1 min and then seeds were given different treatment then under aseptic conditions seeds were washed with double distilled water for about three times. 70% alcohol treatment was given to the seeds for about 1 min again washed with double distilled water three times than 0.1% $HgCl_2$ treatment was given for about 2 min again then they were used with double distilled water for about three times. With the help of sterile forceps seeds were transferred in petridishes having layers of blotting paper soaked with distilled water. Each petridishes has 50 seeds shoot length was recorded at regular intervals and final result was recorded after 28 days.

According to International Seed Testing Association: ISTA (2004), the germination test was performed in total 400 seeds in 4 groups each having 100 seeds. Present experiment was carried out in 8 petridishes each having 50 seeds (8×50=400).

Methodology for seed germination

Germination percent and percent of germination speed were calculated according to Krishnaswamy & Seshu (1990).

$$1.\ \text{Germination}\ (\%) = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

2. Germination Speed (%) $= \frac{\text{Number of seeds germinated at 72 hrs}}{\text{Number of seeds germinated at 168 hrs}} \times 100$

3. Germination energy = Percentage of seed germinated at 72 hrs (Bam *et al.* 2006).

4. Vigor Index = Number of germinated seeds/day of first count + …… + Number of germinated seeds/days at final count.

5. Fresh and dry weight of seed ling, seedling were dried in oven 70ºC overnight.

RESULT AND DISCUSSION

Five different treatment performed for seed dormancy shows that 98% concentrated H_2SO_4 has maximum seedling length, germination frequency, germination energy, germination speed, germination vigor index, fresh weight (g), dry weight (g) as compared to control.

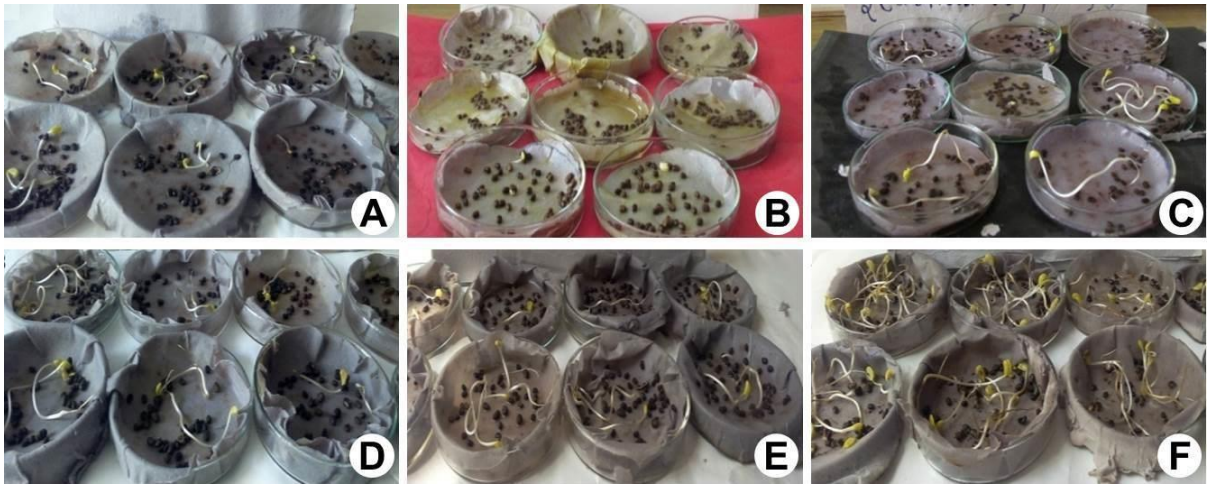

Figure 2. Seedling germination after 28 days: **A,** Control; **B,** Effect of 1 N NaOH treatment for 1 hour on seed germination; **C,** Effect of Scarification by sand paper number 100 P on seed germination; **D,** Effect of Hot water treatment 100ºC for 20 min on seed germination; **E,** Effect of Cold water treatment overnight incubation at 4ºC on seed germination; **F,** Effect of 98% Concentrated H_2SO_4 treatment for 1 hour on seed germination.

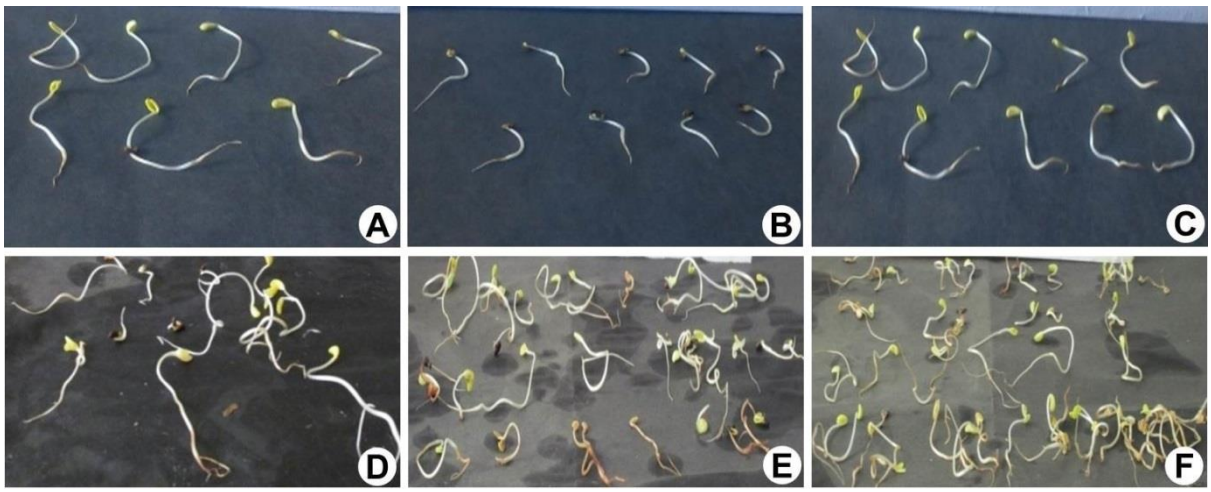

Figure 3. Shoot length determination after 28 days: **A,** Control; **B,** Effect of 1 N NaOH treatment for 1 hour on seed germination; **C,** Effect of Scarification by sand paper number 100 P on seed germination; **D,** Effect of Hot water treatment 100ºC for 20 min on seed germination; **E,** Effect of Cold water treatment overnight incubation at 4ºC on seed germination; **F,** Effect of 98% Concentrated H_2SO_4 treatment for 1 hour on seed germination.

Seed dormancy is a unique feature of family *Fabaceae*; in *P. corylifolia* mechanical dormancy is present. To overcome the dormancy and to increase the germination frequency five different treatments were used which breaks the dormancy. Sterile 400 seeds treated with different treatment for different time intervals were inoculated in petridishes having presoaked filter paper and they were incubated for 28 days at 28ºC (Figs. 2A–F). Subsequently the shoot length was calculated after 28 days (Figs. 3A–F).

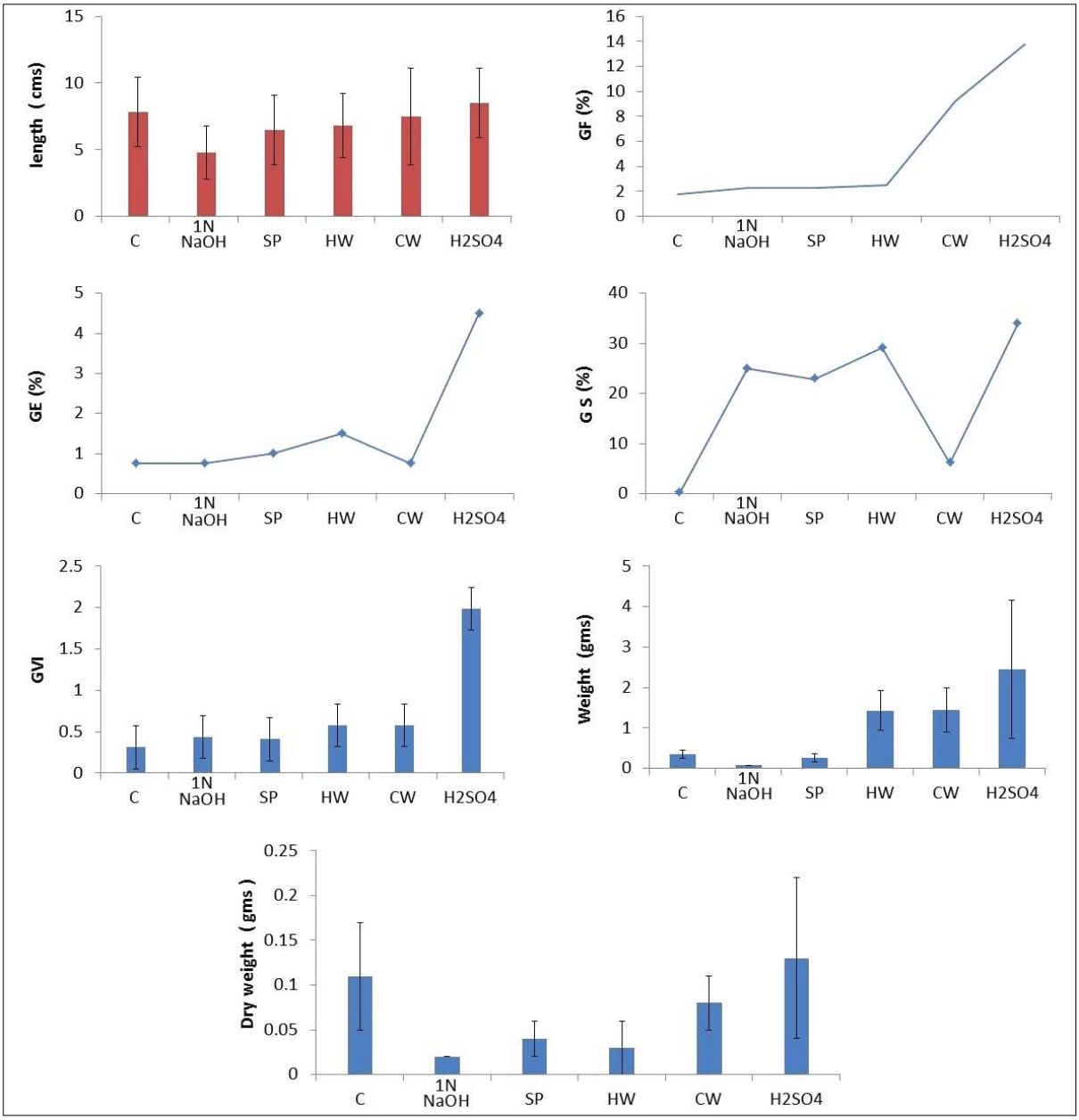

Figure 4. Effect of different treatment on dry weight of seedling after 28 days of germination.

Table 1. Effect of different treatment on germination parameter.

S. No.	Treatment	Number of Seeds (400)	Average Seedling length (cm)	Grm. frequency (%)	Grm. Energy (%)	Grm. Speed (%)	Grm. Vigor Index Average value	Fresh weight (g) Average value	Dry weight (g) Average value
1	Control	400	7.85±2.64	1.75	0.75	0.18	0.311±4.8	0.35±0.1	0.11±0.06
2	1N NaOH	400	4.78±1.99*	2.25	0.75	25	0.44±0.00 [a]	0.07±0.0[a]	0.02±0.00*
3	Scarification Sand Paper (100P)	400	6.5±2.58*	2.25	1	22.9	0.41±0.22 [a]	0.26±0.1[a]	0.04±0.02*
4	Hot water (100ºC)	400	6.80±2.40*	2.5	1.5	29.1	0.58±0.38 [a]	1.43±0.5[a]	0.03±0.03*
5	Cold water (4ºC)	400	7.35±3.60*	9.25	0.75	6.11	0.58±0.38 [a]	1.44±0.55[a]	0.08±0.03*
6	H_2SO_4	400	8.45±2.84*	13.75	4.5	33.9	1.99±1.4*	2.45±1.7*	0.13±0.09*

Note: Grm. = Germination; * = Significance indicates significant value compared with control at (P = <0.001); [a] = indicates non significant value with control at (P =<0.001).

Different treatments have been used for seed germination parameters study and breaking seed dormancy in *Psoralea corylifolia* medicinal plant and they were compared with the control (Table 1). In the present study sulfuric acid treatment for one hour was effective in all seed germination parameter after 28 days (Fig. 4). Mechanical injury to the seed coat or chemical treatment has been used for breaking the seed dormancy of

certain cultivated medicinal plants *Gloriosa superb, Echinacea purpurea, Belladonna* (Gupta & Shah 1971, Supari *et al.* 1993, Mittar *et al*. 1993, Kumar & Sharma 2012).

The present study was first which compare different treatments for seed dormancy breakage in *Psoralea corylifolia.* The plant is listed as an 'endangered species' mainly due to the destruction of its natural habitats. Sulfuric acid and hot water pre-treatment have been reported that to improve the seed germination and seedlings growth of *Cassia fistula* (Soliman & Abbas 2013). Primary exogenous dormancy due to physical factors present outside the embryo is present in most of the *Fabaceae* plants but in *P. corylifolia* mechanical dormancy is reported. Seed coats are too hard to allow the embryo to expand during germination. In *Coronilla varia* physical dormancy is present due to the low moisture level of seed, embryo gets quiescent. The outer macrosclereid and mucilaginous cell layer becomes impermeable to water. Or a hardened endocarp is three reasons that make seed coats impermeable to water. Such seed coats develop during the last stages of seed development. Whereas in *P. corylifolia*, acid breaks the seed layers and impermeable seed coat with helps in germination. Acid treatment was effective for breaking strong seed dormancy and impermeable seed coat which is the major cause low germination frequency in *P. corylifolia*. It is an effective, reliable and reproducible method to get high frequency seed germination in *P. corylifolia*, when compared to any other treatments, seeds Damaging and breaking possibilities are very low at this acid treatment. Thus, this method can be adopted as a good alternative than the other treatments for higher percentage of seed germination since; the aseptic seedlings were used as explants in large number of *in vitro* studies.

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