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Seed Viability and Influence of Presowing Treatments on Germination and Seedling Development of *Uraria picta* (Jacq.) DC.

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ABSTRACT

Uraria picta (Jacq.) DC. (Pithvan), Fabaceae, is an important medicinal woody herb used in an Ayurvedic preparation "Dashmula". The utilization of roots by uprooting the entire plants and the problems associated with the seed germination leads to shortage of root material for Ayurvedic preparation. The aim of the present study was to assess seed viability and the influence of presowing treatments on the seed germination and seedling development in *U. picta*. Application of 2,3,5-triphenyl tetrazolium chloride test showed that 100% of embryos were viable. The treatments applied singly and in combination include acid scarification (H_2SO_4), cold, hot and boiling water, rubbing with sand paper, presoaking in gibberellic acid (GA_3) and application of cut to the seed coat followed by presoaking in distilled water. Significantly highest (100%) rate of seed germination and higher proportion of strong seedlings were observed in the seeds soaked in distilled water for 12 hr after application of cut to the seed coat. This was evident by the significantly higher emergence index, germination speed, germination value and vigor index when compared with the control and other treatments. The protocol developed for the seed germination and seedling development can be applied for raising a large number of plants of *U. picta* which will help in the conservation of plant and availability of root material for medicinal purposes.

Keywords: dormancy, Fabaceae, gibberellic acid, stratification, scarification

INTRODUCTION

Uraria picta (Jacq.) DC. (syn. *Doodia picta* Roxb.) a medicinal woody herb of the family Fabaceae, commonly known as *Pithvan*, has traditionally been used in the Ayurvedic preparation known as 'Dashmula', within which the roots of 10 different plants are used (Kasar *et al*. 2007). Dashmula is the best formula traditionally used for treating fatigue, worries, poor sleep due to the ill-effect on the entire nervous system. It is used as a hormone, cures post delivery problems, all sorts of inflammations, and detoxifies the entire body. It is an excellent sedative used in balancing tri doshas like *vata*, *pitta* and *kapha* (Kasar *et al*. 2007). The whole plant is of medicinal importance and is used by certain adivasis and native tribes (Jain and Defilipps 1991). Leaves are a good antiseptic and used against gonorrhoea. The fruits and pods are effective against oral sores in children and the roots have use against cough, chills and fever (Rehman *et al*. 2007). The roots are used medicinally for invigorating the liver and spleen, as a sedative, produces strength to the nervous system, cures all sorts of inflammations; detoxify the entire body, used in the treatment of skin diseases and to heal the fracture wound (Anonymus 1976). Traditionally, the plant is used as an antidote against the bites of certain Indian vipers (Allen and Allen 1981). The root isolates of the plant show antimicrobial activity against fungi and both Gram-positive and -negative bacteria (Osazuwa and Igboechi 1988; Rehman *et al*. 2007).

The propagation of *U. picta* via seeds is too slow because of poorly viable seeds and dormancy of several months (Anand *et al*. 1998). On the other hand, since the root is important for Ayurvedic preparation, the plants are uprooted on a mass scale from their natural habitats, which is leading to a depletion of resources and it is increasingly become rare and endemic (Anand *et al*. 1998; Tuli 2006). Several pretreatments have been proven efficient to overcome dormancy of leguminous seeds and other seeds (Baskin and Baskin 1998; Schmidt 2000). Micropropagation of *U. picta* was achieved through axillary bud culture (Anand *et al*. 1998). However, the micropropagation method is cost intensive and sensitive compare to the seed propagation method.

Therefore, the objective of this study was to evaluate the seed viability and germination of seeds by applying physical treatments, acid scarification and application of gibberellic acid (GA_3) and to develop a viable method for large-scale plantation towards the availability of root biomass for Ayurvedic and other medicinal preparations.

MATERIALS AND METHODS

Seed source and surface sterilization of seeds

The seeds of *Uraria picta* (Jaqc.) DC. were obtained from the Medicinal Plants Conservation Centre-Rural Communes (MPCC-RC) - Queen Mary's technical Institute (QMTI), Pune, India. The surface sterilization of seeds was conducted using a 0.1% (w/v) aqueous mercuric chloride solution for 5 min. After rinsing 4-5 min with sterile distilled water (SDW), the seeds were used for the treatments and germination trials.

Seed viability and germination conditions

The viability of seeds was tested by the 2,3,5-triphenyltetrazolium chloride (TTC) test (Hartman *et al*. 1997). A 0.5% (w/v) TTC solution was prepared in sodium phosphate buffer (pH 7.0). 50 seeds, after a slight cut to the seed coat at the micropylar end and 50 intact seeds were separately soaked in TTC solution for 24 h in the dark. Then the seeds were washed three times with SDW and the embryos were dissected out by removal of seed coat and sepa-

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rating the cotyledons by using needle and forceps under a dissecting microscope and observed for change in colour to red. The seeds were also sown in moistened soil and Petri dishes and observed for germination; only germinated seeds were considered to be viable and only those seeds producing about 0.5 mm or more radical growth were counted as germinated seeds.

The control (seeds without treatments) and treated seeds were germinated on blotting paper (1 mm thick, Modern Paper Ltd., India) overlying a layer of about 5 mm thick absorbent cotton in a transparent plastic Petri dish (90 × 15 mm). Each Petri dish contained 50 seeds. The substrates were moistened initially with 10 ml SDW. Later, 2 ml SDW was added at two days' intervals. The Petri dishes were maintained at 25 ± 2°C under an 8-h photoperiod and light intensity of about 30 µmol $m^{-2}s^{-1}$ provided by cool white fluorescent tube lights (Champion 40W, Philips Electronics. India Ltd.).

Physical treatments

***Sandpaper scarification*:** The surface sterilized seeds were directly transferred to Petri dishes for the control experiments. The seeds were placed in a 0-grade sandpaper flap and slight friction force for 15 sec was applied manually to scarify the seeds. Then the damaged seeds were discarded by visual observations and undamaged seeds were used for germination test.

***Wet heat treatment*:** The seeds were soaked separately in hot water (60°C) for 5, 10 and 15 min and boiling water (100°C) for 1 and 2 min. After the specific duration treatment the hot water was removed and then the seeds were washed with SDW at room temperature (25 ± 4°C) three times. The treated seeds were placed for germination in Petri dishes.

***Cold water treatment*:** For low temperature treatment the seeds were immersed in cold water (4°C) and placed in a refrigerator at 4°C for 6, 12, 18 and 24 h, after which germination was observed.

Chemical treatments

***Acid scarification*:** The seeds were immersed in concentrated H_2SO_4 and HCl (Sisco Research Laboratories Pvt. Ltd. (SRL), India) separately for 2, 5 and 10 min. After treatment the seeds were washed 5 times with SDW and used for the germination test.

***Gibberellic acid treatment*:** Randomly selected seeds were subjected to a presoaking treatment of GA_3 (Hi Media, India) at 1.25, 2.50, 3.75 and 5.00 mM for 12, 24 and 36 h separately. In another set of experiments, a superfine cut to the seed coat at the micropylar end was applied by using a surgical blade (Surgeon Blade No. 21, Kehr Surgical Pvt. Ltd., Kanpur, India) and were subjected to GA_3 treatments at the concentrations and time intervals described above. The seeds soaked in SDW for a similar time duration were used as control. After treatment the seeds were washed twice with SDW, placed in Petri dishes and observed for germination.

Data collection on germination

***Daily and cumulative germination counts*:** The seeds in which 0.5 mm or more radical growth occur were counted as germinated seeds. The primary data on seed germination was collected daily and continued until the completion of germination (maximum up to 30 days). The final germination percentage (FGP) was calculated from the total seeds that germinated on the day of completion. Daily germination percentages were summed up to obtain cumulative germination percentage (CGP) for each treatment.

***Germination speed and germination value*:** Germination speed and germination values were calculated using the formula described by Aldhous (1972) and Djavanshir and Pourbeik (1976):

Germination speed = Final Germination percentage/Day of completion of germination.

$$GV = (\Sigma DGs / N) GP / 10.$$

where GV = germination value, GP = germination percentage at the end of the test, DGs = daily germination speed obtained by dividing the cumulative germination percentage by the number of days since sowing, Σ DGs = the total germination obtained by adding every DGs value obtained from the daily counts, N = the total number of daily counts, starting from the date of first germination, 10 = constant.

***Emergence index*:** Emergence index were calculated by using the formula as follows:

Emergence index (EI): dn/n,

where dn = emergence (number of seeds germinated on a particular day) and n = day of emergence (Thakur *et al*. 2004).

***Seedling vigor*:** Seedling vigor Index (SVI) was calculated as per the recommendations of ISTA (1976):

SVI = Germination percent × Shoot length.

For seedling vigor normal seedlings from standard germination tests were further classified as strong or weak seedlings by visual observations and expressed as percentage. The erect and sturdy seedlings with well developed seedling parts were kept in the category of strong seedling (ISTA 1976). Root length and shoot length of the seedlings were recorded and root to shoot ratio was calculated.

Seedling development

The seedlings which sprouted in the Petri dishes during experiments using different treatments were transferred to polythene bags containing moist garden soil during the evening. As needed watering was carried out to maintain moisture and seedlings in polythene bags were maintained in a shade-net (Green Net India Pvt. Ltd., Ahemdabad, Inida) house (equipped with the net that can cut 50% of the incident light) in the Botanic Garden of the Department of Botany, University of Pune, Pune.

Seedling moisture content

Fresh weight of seedlings was recorded and the seedlings were dried in an oven at 60°C until constant weight; dry weight of the seedlings was recorded (Sestak 1971). Moisture content of seedlings was calculated using the formula:

[Fresh weight-Dry weight/Fresh weight] × 100.

Assessment of seedling quality

Morphological data such as height, weight of the aerial part of the seedling, diameter of the seedling at soil level and weight of the root system and data on moisture content in the entire seedling and root/shoot ratio were used to evaluate seedling quality. The quality index (QI) was determined by following the method described by Dickson *et al.* (1960):

QI = seedling dry weight (g) / [height (cm) + shoot weight (g) / diameter (mm) + root weight (g)].

Statistical analysis of data

The experiment was carried out in a completely randomized design with three replicates. Data were analyzed by analysis of variance (ANOVA) to detect significant differences between means. Means differing significantly were compared using Duncan's (1955) multiple range test (DMRT) at the 5% probability level. Variability in data has been expressed otherwise as mean ± standard error.

Table 1 Effect of physical treatments and acid scarification on intact seed germination in *U. picta*.

Treatments	Germination (%)	EI	GS	GV	VI	Root/Shoot ratio	SS (%)
Control	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 h	0.0 ± 0.0 j
PS	0.0 ± 0.0 g	14.4 ± 0.6 c	4.9 ± 0.7 c	11.4 ± 0.9 d	1151.1 ± 15.6 a	1.3 ± 0.1 g	71.3 ± 5.0 g
HW 5 min.	44.4 ± 5.9 c	0.0 ± 0.0 g	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 h	0.0 ± 0.0 j
HW 10 min.	0.0 ± 0.0 g	5.2 ± 0.2 e	4.0 ± 0.4 d	7.2 ± 0.7 e	786.7 ± 10.2 c	0.9 ± 0.1 ab	75.0 ± 0.0 f
HW 15 min.	40.0 ± 3.9 c	2.9 ± 0.2 f	2.2 ± 0.4 fg	1.9 ± 0.6 gh	68.5 ± 8.0 h	1.5 ± 0.2 cde	21.6 ± 1.7 i
BW 1 min.	20.0 ± 3.8 de	15.1 ± 0.3 c	6.2 ± 0.3 b	17.8 ± 0.7 c	956.0 ± 4.0 b	1.7 ± 0.2 fg	100.0 ± 0.0 a
BW 2 min.	55.6 ± 2.2 b	5.2 ± 0.3 e	3.2 ± 0.3 de	2.6 ± 0.3 fg	349.1 ± 12.3 e	1.7 ± 0.1 bc	70.0 ± 5.0 g
H_2SO_4 2 min.	22.2 ± 2.2 d	7.0 ± 0.2 d	3.3 ± 0.3 ef	3.9 ± 0.5 f	482.3 ± 13.5 d	1.0 ± 0.1 cde	93.3 ± 3.3 c
H_2SO_4 5 min.	24.5 ± 2.2 d	22.3 ± 0.3 a	8.4 ± 0.3 a	38.1 ± 0.4 a	943.3 ± 13.5 b	1.1 ± 0.2 cd	96.7 ± 3.3 b
H_2SO_4 10 min.	75.6 ± 2.2 a	16.7 ± 0.4 b	6.2 ± 0.3 b	20.5 ± 0.3 b	922.2 ± 19.0 b	1.0 ± 0.1 efg	90.0 ± 0.0 d
HCl 2 min.	55.6 ± 2.2 b	0.0 ± 0.0 g	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 h	0.0 ± 0.0 j
HCl 5 min.	0.0 ± 0.0 g	2.1 ± 0.4 f	1.5 ± 0.0 gh	1.2 ± 0.1 hi	213.4 ± 7.7 f	2.1 ± 0.2 a	81.7 ± 1.6 e
HCl 10 min.	13.3 ± 0.0 ef	4.4 ± 0.3 e	1.1 ± 0.0 h	0.6 ± 0.1 hi	154.1 ± 12.9 g	1.9 ± 0.2 ab	66.7 ± 3.3 h

EI: emergence index; GS: germination speed; GV: germination value; VI: vigour index; SS: strong seedling; PS: physical scarification using sand paper; HW: hot water (60°C); BW: boiling water (100°C). Values are mean ± SE of three independent experiments each with 3 replicates. Means followed by the same letters within columns are not significantly different at the 5% level (DMRT).

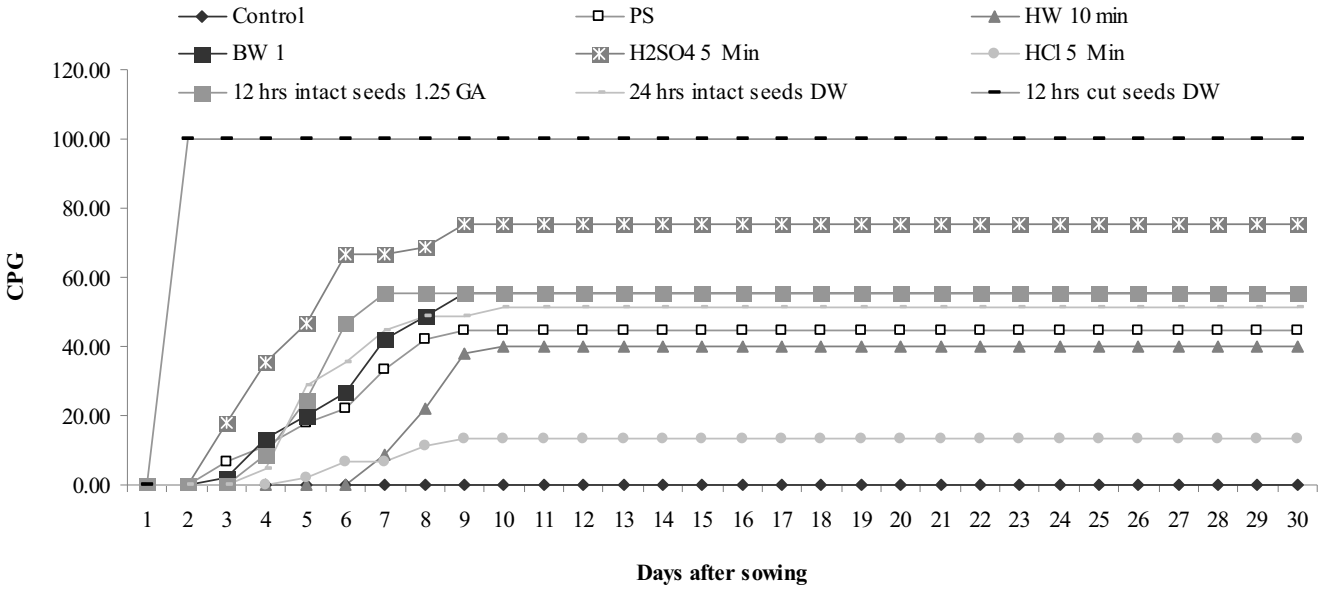

Fig. 1 Effect of physical treatments, acid scarification and gibberellic acid on cumulative germination percentage in intact seeds of *U. picta*.

RESULTS AND DISCUSSION

Seed viability

The TTC test is used primarily to obtain rapid results on seed viability for both non-dormant and dormant seeds (Hartman *et al*. 1997). In the present investigation intact seeds were soaked in TTC solution for 24 h in the dark; those that did not stain red was due to the impermeability of the solution in the seeds through the seed coat. However, the embryos of seeds with a seed coat cut at the micropylar end soaked in TTC solution for 24 h turned dark red. This indicated that all the seeds (100%) were viable. In contrast, Anand *et al*. (1998) reported that the seeds of *U. picta* were poorly viable (although what tests were used were not specified or quantified). This variation in seed viability might be due to differences in seed source usage. The viability might be affected by the nutrient status of the mother plant and environmental factors and also the age of the seeds (Baskin and Baskin 1998). However, seeds placed on moist germination paper in Petri dishes and the seeds sown in moist soil did not shown any sign of germination at 30 days of incubation. These results indicate that some kind of dormancy exists in the seeds of *U. picta*.

Effect of physical treatments on seed germination

About 44.4% of FGP was observed in the seeds scarified with zero-grade sand paper and incubated on moist germination paper in Petri plates (**Table 1**). The observations on CGP showed that germination started on the third day and increased up to the 8th day and later remained constant (**Fig. 1**). Most of the germinated seeds showed damaged cotyledons. The remaining seeds that failed to germinate might have been due to excessive damage cause to the embryonal parts by scarification. The seeds are very small in size (about 2 mm × 1 mm), non-endospermic with a very thin seed coat and therefore the embryo is easily damaged. Therefore the sand paper scarification method did not help to improve germination and healthy seedling formation.

The treatment of hot water for 5 min was not effective to induce germination. However, the hot water treatment for 10 min resulted in 40% FGP while a longer duration (15 min) resulted in a decline in FGP to 20% (**Table 1**). A improved FGP (55.6%) was observed in intact seeds treated with boiling water for 1 min while this value declined to 22.2% with treatment of 2 min boiling water (**Table 1**). In all the physical treatments CGP was higher in the seeds treated with boiling water for 1 min, which increased from 3rd day up to 9th day and remained constant (**Fig. 1**). The intact seeds presoaked in cold water for 6, 12, 18 and 24 h completely failed to germinate within 30 days of the incubation period (data not shown), which indicates that the cold water treatment was not effective for stimulation of seed germination in *U. picta*. These results indicate that hot water and boiling water treatments were slightly effective in promoting germination in *U. picta*. Similar observations were recorded in other legume species viz. in *Dimorphandra mollis* Benth. seeds immersed in water at 100°C for 2 min attained 64% germination, while germination of un-

treated seeds was 12% (Hermansen *et al*. 2000). The seeds of *Dinizia excelsa* Ducke scarified with water at 80°C for 10 min, show ~62% germination against 7% in the control (Vastano Júnior *et al*. 1983). *Mimosa scabrella* Benth. seeds immersed in water at 90°C showed 79% germination, while in non-scarified seeds germination was 17% (Bianchetti 1981). 42.9% germination was reported in *Parkia biglobosa* seeds treated with hot water for 4 sec (Aliero 2004). Hot water treatment at 60°C for 30 min increased germination percentage significantly from 44 to 100%, showing an increase of 56% over control in *Pongamia pinnata* (Kumar *et al*. 2007). The hot water treatment (60°C) for 24 hrs give an average of 24.10% germination in *Stylosanthes* species and 55% germination in *Stylosanthes scabra* (Bhatt *et al*. 2008). The promoting effect of boiling water treatment on seed germination (57%) has been reported in *Pterocarpus marsupium* (Barmukh and Nikam 2008). The present data suggest that the seeds of *U. picta* exhibit some level of dormancy and benefit from a dormancy breaking treatment of hot or boiling water similar to other legume species (Baskin *et al*. 2000).

Effect of chemical treatment on seed germination

The data in **Table 1** reveals that with an increase in the duration of concentrated H_2SO_4 scarification from 0 to 10 min, a progressive improvement was noticed in germination; best FGP (75.6%) was observed in seeds scarified for 5 min. Treatment for 10 min decline the FGP of the seeds to 55.6% (**Table 1**). Scarification for more than 10 min was deleterious and completely inhibited germination. An increase in CGP was observed from the 3rd to the 7th day and later remained constant (**Fig. 1**). These results indicate that the seed coat may contain or produce an inhibitory substance(s) or it may be impermeable to oxygen and water. Acid scarification leads to partial or complete removal of inhibitory substance(s) and weakening of the hard and impermeable seed coat (Mayer and Poljakoff-Mayber 1963). In the present investigation, this process has been shown to significantly improve *U. picta* germination and earlier in many genera (Baskin and Baskin 1998). The treatments of concentrated H_2SO_4 hydrolyze and increase the permeability of the seed coat in several leguminous species such as *Acacia* species (Kumar and Purkayastha 1972; Natarajan and Rai 1988; Rana and Nautiyal 1989; Gunn 1990), *Parkia biglobosa* (Aliero 2004), and *Astragalus adsurgens* (Kondo and Takeuchi 2004). In the present investigation, 5 min treatment of H_2SO_4 might be sufficient to hydrolyze the seed coat constituents and further improve germination.

The treatment of concentrated HCl for 2 min was not found to be effective for stimulating germination. About 13.3% FGP was observed in intact seeds treated with HCl for 5 min. Treatment of HCl for 10 min reduced FGP to 8.89% (**Table 1**). In seeds treated with concentrated HCl for 5 min the CGP rose from the 5th to the 8th day (**Fig. 1**), indicating that the treatment of concentrated H_2SO_4 for 5 min was superior than other of H_2SO_4 treatments and all HCl treatments. This variation in germination with acid treatments was probably due to the very thin protective seed coat.

Effect of gibberellic acid on seed germination

Among the intact seeds presoaked in distilled water for 12, 24 and 36 hours, 22.2, 51.1 and 46.7%, respectively of the seeds germinated within 30 days of incubation (**Table 2**). In contrast, in the GA_3 treatments, presoaking intact seeds with 1.25 mM GA_3 for 12 hrs was slightly superior than the other GA_3 treatments, i.e. FGP was enhanced (55.6 ± 5.9) (**Table 2**). CGP increased from the 3rd up to the 9th day and remained constant. The CGP of seeds treated with 1.25 mM GA_3 was higher in comparison to other treatments (**Fig. 1**). These observations were supported by a higher emergence index, germination speed, germination value and seedling vigor index (**Table 2**). As per the guidelines of ISTA (1976) (described in materials and methods) the erect and sturdy seedlings with well developed parts were classified as strong seedlings whereas the seedlings with stunted growth and poorly developed parts were categorized as weak seedlings. The percentage of strong seedlings was also higher from the seeds treated with 1.25 mM GA_3. Seed germination improved slightly in intact seeds after GA_3 treatment compared to the seeds presoaked in distilled water but it was not significantly different (**Table 2**).

The application of a superfine cut to the seed coat at the micropylar end followed by 1.25, 2.50, 3.75 and 5.00 mM GA_3 for 12 and 24 hrs treatment and the presoaking treatment of distilled water for 12, 24 and 36 hrs induce 100% CGP (**Table 3, Fig. 2A**). However, the strong seedling percentage (**Table 3**) and root/shoot ratio (**Table 3**) was higher in the seeds treated with distilled water than those treated with GA_3. Further, the emergence index, germination speed and germination value were also higher in the cut seeds presoaked in distilled water for 12 hrs. However, a prolong treatment of 36 hrs presoaking in 1.25, 2.50, 3.75 and 5.00 mM GA_3 inhibited germination (**Table 3**). The FGP of the seeds treated with GA_3 decreased as the concentration of GA_3 increased (**Table 3**). Other germination parameters such as emergence index, germination speed and germination value also declined as the concentration of GA_3 increased. Germination energy is the criterion used to measure the speed of germination. The interest in emergence index and germination speed is based on the theory that only those seeds which germinate rapidly and vigorously under controlled conditions in the laboratory are capable of producing vigorous seedlings under field conditions while weak and delayed germination is fatal (Aldhous 1972;

Table 2 Effect of presoaking treatments [distilled water and gibberellic acid] on intact seed germination characteristics of *U. picta*.

Treatments	Germination (%)	EI	GS	GV	VI	Root/Shoot ratio	SS (%)
12 hrs DW	22.2 ± 8.9 c	6.5 ± 0.6 e	0.7 ± 0.1 c	2.4 ± 0.5 j	577.7 ± 29.2 k	0.6 ± 0.1 ab	90.7 ± 4.9 b
12 hrs 1.25 mM GA_3	55.6 ± 5.9 a	15.2 ± 0.6 a	8.0 ± 0.8 a	18.7 ± 0.5 a	1557.8 ± 21.1 c	0.6 ± 0.1 ab	93.0 ± 3.5 a
12 hrs 2.50 mM GA_3	46.7 ± 10.2 ab	12.5 ± 0.6 bc	6.7 ± 1.5 a	12.5 ± 0.6 cd	1468.9 ± 17.4 d	0.6 ± 0.1 ab	78.7 ± 2.4 d
12 hrs 3.75 mM GA_3	53.3 ± 6.7 a	14.5 ± 0.4 a	6.7 ± 0.8 a	16.1 ± 0.6 b	1720.0 ± 20.0 b	0.6 ± 0.1 ab	75.9 ± 4.9 efg
12 hrs 5.00 mM GA_3	40.0 ± 7.7 ab	10.6 ± 0.5 d	5.0 ± 1.0 ab	9.4 ± 0.5 h	1240.0 ± 23.0 g	0.6 ± 0.1 a	75.0 ± 4.8 efg
24 hrs DW	51.1 ± 9.7 a	13.6 ± 0.5 ab	5.7 ± 1.1 ab	15.3 ± 0.6 b	1826.6 ± 20.3 a	0.6 ± 0.1 ab	76.7 ± 1.7 e
24 hrs 1.25 mM GA_3	40.0 ± 11.6 ab	10.5 ± 0.6 d	5.7 ± 1.7 ab	9.6 ± 0.6 h	1393.3 ± 29.1 e	0.7 ± 0.1 a	78.7 ± 2.5 d
24 hrs 2.50 mM GA_3	44.5 ± 9.7 ab	12.3 ± 0.5 c	6.4 ± 1.4 a	12.2 ± 0.7 cde	1211.2 ± 25.6 g	0.6 ± 0.1 ab	74.7 ± 1.8 g
24 hrs 3.75 mM GA_3	46.7 ± 7.7 ab	12.5 ± 0.6 bc	6.7 ± 1.1 a	13.2 ± 0.5 e	1482.2 ± 17.7 b	0.6 ± 0.1 ab	76.4 ± 2.6 ef
24 hrs 5.00 mM GA_3	42.2 ± 7.7 ab	12.4 ± 0.5 c	5.3 ± 1.2 ab	10.7 ± 0.4 efg	1311.2 ± 16.1 f	0.5 ± 0.1 ab	78.7 ± 2.5 d
36 hrs DW	46.7 ± 6.7 ab	15.6 ± 0.5 a	7.8 ± 1.1 a	13.3 ± 0.6 c	1037.7 ± 15.6 i	0.7 ± 0.2 ab	88.9 ± 5.5 c
36 hrs 1.25 mM GA_3	42.2 ± 5.9 ab	12.7 ± 0.4 bc	5.3 ± 0.7 ab	11.5 ± 0.7 cde	1142.2 ± 16.0 h	0.7 ± 0.1 ab	76.7 ± 1.7 e
36 hrs 2.50 mM GA_3	40.0 ± 7.7 ab	12.2 ± 0.7 c	6.7 ± 1.3 a	10.4 ± 0.7 gh	1466.7 ± 15.4 d	0.5 ± 0.1 ab	79.4 ± 2.4 d
36 hrs 3.75 mM GA_3	42.2 ± 11.8 ab	11.7 ± 0.6 cd	4.2 ± 1.2 ab	11.2 ± 0.5 efg	1135.6 ± 27.9 h	0.6 ± 0.1 ab	74.8 ± 4.1 fg
36 hrs 5.00 mM GA_3	26.7 ± 0.0 ab	7.4 ± 0.4 e	2.7 ± 0.0 bc	4.3 ± 0.5 i	826.8 ± 15.4 j	0.4 ± 0.1 a	63.9 ± 7.4 h

EI: emergence index; GS: germination speed; GV: germination value; VI: vigour index; SS: strong seedling; DW: distilled water; GA_3: gibberellic acid. Values are mean ± SE of three independent experiments each with 3 replicates. Means followed by the same letters within columns are not significantly different at the 5% level (DMRT).

Table 3 Effect of presoaking treatments [distilled water and gibberellic acid] on cut seed germination characteristics in *U. picta*.

Treatments	Germination (%)	EI	GS	GV	VI	Root/Shoot ratio	SS (%)
12 hrs DW	100.0 ± 0.0 a	50.0 ± 0.0 a	50.0 ± 0.0 a	99.8 ± 0.0 a	2620.0 ± 11.5 l	1.1 ± 0.2 a	97.8 ± 2.2 a
12 hrs 1.25 mM GA_3	100.0 ± 0.0 a	50.0 ± 0.0 a	50.0 ± 0.0 a	99.8 ± 0.0 a	4523.3 ± 14.5 d	0.5 ± 0.1 ab	95.6 ± 4.4 ab
12 hrs 2.50 mM GA_3	100.0 ± 0.0 a	50.0 ± 0.0 a	50.0 ± 0.0 a	99.8 ± 0.0 a	4320.0 ± 11.5 f	0.4 ± 0.1 ab	93.3 ± 3.8 bc
12 hrs 3.75 mM GA_3	100.0 ± 0.0 a	50.0 ± 0.0 a	33.3 ± 0.0 b	99.5 ± 0.0 b	4716.7 ± 12.0 c	0.4 ± 0.1 ab	91.1 ± 4.4 cd
12 hrs 5.00 mM GA_3	100.0 ± 0.0 a	50.0 ± 0.0 a	33.3 ± 0.0 b	99.1 ± 0.0 c	4513.3 ± 13.3 d	0.3 ± 0.1 c	84.5 ± 2.2 f
24 hrs DW	100.0 ± 0.0 a	50.0 ± 0.0 a	50.0 ± 0.0 a	99.8 ± 0.0 a	2986.7 ± 13.3 k	0.8 ± 0.1 b	95.6 ± 2.2 ab
24 hrs 1.25 mM GA_3	100.0 ± 0.0 a	50.0 ± 0.0 a	33.3 ± 0.0 b	99.1 ± 0.0 c	5533.3 ± 24.0 a	0.3 ± 0.1 c	86.7 ± 0.0 ef
24 hrs 2.50 mM GA_3	100.0 ± 0.0 a	50.0 ± 0.0 a	33.3 ± 0.0 b	99.5 ± 0.0 b	4133.3 ± 20.3 g	0.5 ± 0.1 ab	88.9 ± 2.2 de
24 hrs 3.75 mM GA_3	100.0 ± 0.0 a	50.0 ± 0.0 a	33.3 ± 0.0 b	98.7 ± 0.0 d	5133.3 ± 20.3 b	0.3 ± 0.1 c	86.7 ± 6.7 ef
24 hrs 5.00 mM GA_3	100.0 ± 0.0 a	50.0 ± 0.0 a	33.3 ± 0.0 b	99.1 ± 0.0 c	4430.0 ± 20.8 e	0.4 ± 0.1 ab	86.7 ± 3.9 cde
36 hrs DW	100.0 ± 0.0 a	50.0 ± 0.0 a	33.3 ± 0.0 b	99.1 ± 0.0 c	3736.7 ± 27.3 h	0.6 ± 0.1 ab	88.9 ± 4.4 de
36 hrs 1.25 mM GA_3	100.0 ± 0.0 a	50.0 ± 0.0 a	50.0 ± 0.0 a	99.8 ± 0.0 a	3543.3 ± 20.3 i	0.4 ± 0.1 ab	80.0 ± 3.9 g
36 hrs 2.50 mM GA_3	97.8 ± 2.2 b	48.9 ± 0.0 b	32.6 ± 0.0 c	91.1 ± 0.0 e	3487.7 ± 17.5 j	0.4 ± 0.1 ab	73.3 ± 0.0 h
36 hrs 3.75 mM GA_3	93.3 ± 2.2 c	46.7 ± 0.0 c	19.6 ± 0.0 e	87.0 ± 0.0 f	4548.8 ± 24.4 d	0.4 ± 0.1 ab	66.7 ± 3.9 i
36 hrs 5.00 mM GA_3	91.1 ± 2.2 d	45.6 ± 0.0 d	29.6 ± 0.0 d	76.5 ± 0.0 g	3703.5 ± 17.7 h	0.6 ± 0.1 ab	73.3 ± 3.9 h

EI: emergence index; GS: germination speed; GV: germination value; VI: vigour index; SS: strong seedling; DW: distilled water; GA_3: gibberellic acid. Values are mean ± SE of three independent experiments each with 3 replicates. Means followed by the same letters within columns are not significantly different at the 5% level (DMRT).

Alamgir and Hossain 2005). The concept of germination value aims to combine in a single figure an expression of total germination at the end of the test period with an expression of germination energy or speed of germination (Djavanshir and Pourbeik 1976).

The results of the present investigation indicate that the application of exogenous GA_3 treatment was ineffective in promoting germination percentage and germination characteristics. Thus, the results show that the seeds of *U. picta* might contain sufficient endogenous GA_3 for germination and therefore there is no need to apply exogenous GA_3 for germination.

The seeds which do not need exogenous gibberellins for germination presumably contain enough endogenous gibberellin to mediate germination (Khan 1971). In contrast, *Sapindus trifoliatus* seed germination was enhanced to 70-89% when treated for 10-50 hrs at GA_3 concentrations ranging from 250-1500 ppm, whereas without gibberellin treatment only 5.2% seeds were germinated (Naidu *et al*. 2000). The seeds of *Stylosanthes scabra* cv. 'Seca' pretreated with GA_3 (300 ppm) increased germination up to 55% (Bhatt *et al*. 2008).

Growth of seedlings

On transfer of strong seedlings obtained from different treatments to polythene bags kept in a shade net house, the seedling survival rate was 90%. All seedlings of different treatments showed similar morphological characters. The morphological characters of seedlings obtained from 12 hrs presoaking treatment to the seeds to which a cut was applied to the seed coat at micropylar end are presented in **Table 4**. After 6 months of growth the seedlings showed an average height of 62.3 ± 3.0 cm, while 3-month-old seedlings were 15.8 ± 2.2 cm tall (**Fig. 2B**).

The seedlings obtained with the different treatments to the seeds and/or developed in nursery may not perform growth equally good on transfer to the field conditions (Duryea 1984; Hartman *et al*. 1997). This results in failure of many reforestation and plantation projects. In such situations careful selection and planting of only viable and vigorous seedlings is crucial to ensure the growth and establishment in the field. For the future seedling performance, the seedling quality index is one of the useful predictor (Dickson *et al*. 1960; Bayala *et al*. 2009). In the present investigation, the results on evaluation of seedling quality are depicted in **Table 4**. Shoot length increased as the age of the seedlings increased while a similar increase in length was not observed in roots. This resulted in a decreased in the value of the root/shoot ratio as the age of the seedling increased. The quality index of the seedlings also increases as the age of seedlings increase.

Table 4 Morphological characters of seedlings obtained from the 12 hrs presoaking treatment to the seeds after application of cut to the seed coat at micropylar end of *U. picta*.

Characters \ Age of seedlings		1 month	3 months	6 months
Height (cm)		5.3 ± 0.5 c	15.8 ± 2.2 b	62.3 ± 3.0 a
Leaves/seedlings		5.9 ± 0.5 c	6.8 ± 0.8 b	11.1 ± 0.7 a
Nodes/seedlings		7.1 ± 0.6 c	9.5 ± 0.6 b	11.9 ± 0.6 a
Internodal distance (cm)	Base	0.6 ± 0.1 c	1.1 ± 0.1 b	1.7 ± 0.3 a
	Top	1.2 ± 0.1 c	3.9 ± 0.5 b	11.6 ± 1.3 b
Seedling fresh weight (g)		0.2 ± 0.1 c	7.0 ± 2.3 b	42.0 ± 4.3 a
Seedling dry weight (g)		0.07 ± 0.1 c	7.0 ± 0.6 b	15.0 ± 0.9 a
Shoot fresh weight (g)		0.09 ± 0.1 c	9.0 ± 0.3 b	25.0 ± 1.0 a
Shoot dry weight (g)		0.04 ± 0.1 c	4.0 ± 0.2 b	9.0 ± 1.2 a
Root Fresh weight (g)		0.1 ± 0.1 c	8.0 ± 0.7 b	17.0 ± 2.0 a
Root dry weight (g)		0.03 ± 0.1 c	3.0 ± 0.8 b	6.0 ± 0.3 a
Stem diameter (mm)		1.0 ± 0.1 c	3.0 ± 0.4 b	4.0 ± 0.1 a
Moisture content (%)		65.0 ± 2.3 a	58.8 ± 1.7 b	64.3 ± 3.1 ab
Root/shoot Ratio		1.2 ± 0.2 a	0.8 ± 0.3 b	0.3 ± 0.3 c
Quality index (QI)		0.01 ± 0.1 c	1.1 ± 0.2 b	2.1 ± 0.1 a

Values are mean ± SE. Means followed by the same letters within rows are not significantly different at the 5% level (DMRT) (in other treatments the seedling quality index has low as compare to the other treatments mentioned in materials and methods).

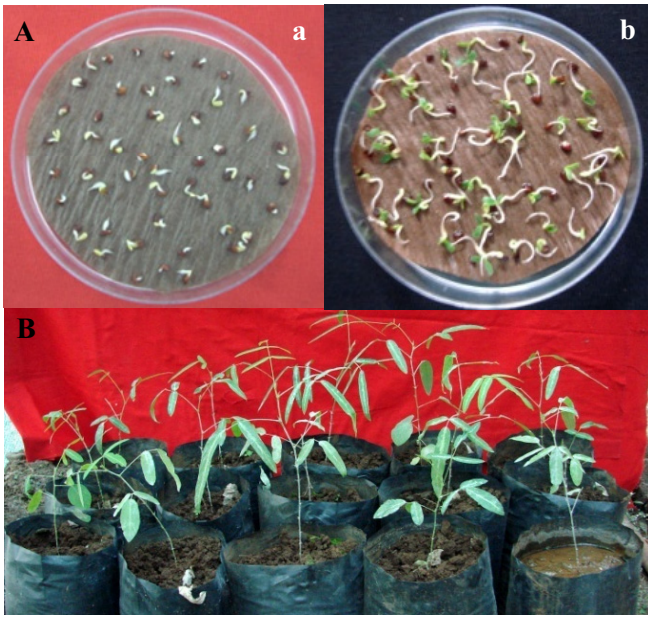

Fig. 2 Germination and seedling development in *Uraria picta* (Jacq.) DC. (**A**) Germinated seeds after application of cut to the seed coat followed by 12 hr presoaking in distilled water. a) Two days after sowing, b) 7 days after sowing. (**B**) Three-months-old seedlings in polythene bags.

CONCLUSIONS

In the present investigation, the application of superfine cut to the seed coat followed by soaking in distilled water resulted in 100% germination. As the seeds are small in size, the application of superfine cut to the seed coat is tedious time consuming manual work. Therefore, the alternative is the treatment of concentrated sulphuric acid for 5 min which results about 75% germination. These results indicate that the seeds of *Uraria picta* might have the physical dormancy imposed by the impermeable seed coat which could be overcome either by application of cut to the seed coat or the treatment of concentrated H_2SO_4. Physical dormancy is one of the two most common classes of seed dormancy in legume seeds (Baskin 2003). In legumes, seeds have hard, thin and impermeable seed coat which plays a significant role in the process of germination and seedling vigor mainly by restricting the water absorption to the embryo and gaseous exchange (Koirala *et al.* 2000; Alamgir and Hossain 2005). Legume seeds with hard, impermeable seed coats have been reported to enhance the germination with presowing treatments (Alamgir and Hossain 2005). The results of the present investigation on TTC test and 100% germination on application of slight cut to the seed coat at the micropylar end and earlier reports in other legume species indicates that the thin membranous seed coat of the seeds of *U. picta* might be impermeable to water and gases causes the unpredictable germination. The protocol describe in the present investigation can be utilized for large-scale cultivation of *Uraria picta*: a medicinally important woody herb.

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