The Pharma Innovation Journal 2023; 12(2): 2137-2141

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 2137-2141

www.thepharmajournal.com
Received: 20-12-2022
Accepted: 29-01-2023

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Improving germination and dormancy breaking in *Gleditsia triacanthos* L. seeds by Presowing treatments

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Abstract
Gleditsia triacanthos L. belongs to family Fabaceae is reported to flourish in urban environments and tolerate wide range of conditions. Seedling production rely upon suitable pre-sowing treatments for seeds with physical dormancy. The present investigation carried to explore different pre-sowing treatments for enhancing seed germination in *G. triacanthos* was arranged in a completely randomized design with thirteen treatments and three replications. The results indicate that seed dipped in concentrated sulfuric acid for 20 minutes and 30 minutes, followed by soaking in water for 12 h each, increased germination percentage of 84.50% and 83.50% respectively. The study recommends sulfuric acid scarification for hard, impenetrable seed coat with the aim of obtaining the best treatment for breaking dormancy.

Keywords: *Gleditsia triacanthos*, pre-sowing treatment, germination, seed dormancy

Introduction
Tree cover is an essential component of urban infrastructure as they provide numerous environmental, economic, social and aesthetic values which has been well recognized universally. The efforts at enriching trees on the sites that can withstand urban stress should be considered when planting to the landscape. *Gleditsia triacanthos* L. (Fabaceae) is a deciduous shade-intolerant tree, growing to 20-25 m tall, native to United States of America and exotic to India. The tree's generic name, *Gleditsia*, was named after the German botanist Johann Gottlieb Gleditsch and *triacanthos*, is from the Greek word *acantha* meaning thorn or spines in reference to the large three-branched thorns usually clustered on the trunk or base of the tree's branches (Herman *et al*. 1996; Orwa *et al*. 2009) [20, 26]. A number of cultivars and 'thornless' variants of this species have been developed (Bradley 1978; Rowell 1991) [5, 33]. The tree is commonly known as honey locust describes the sweet flavour; aromatic honey-like pulp found in its young unripe pods, and the pulp in older pods turns bitter (Herman *et al*. 1996) [20]. Honeylocust is recognized for rehabilitation of drought conditions, sterile lands and can grow on broad range of soils (Putod 1982; Kacimi 1996) [30, 22]. The tree is well adapted to stress environments, atmospheric pollution, salinity, poor drainage, erosion control, windbreaks, highways, and urban forestry (Detwiler 1947; Blair 1990; Gilman and Watson 1993; Gold and Hanover 1993; Preston and Braham 2002; Schnabel and Wendel 1998; Ertekin and Kirdar 2010; Gailing *et al*. 2017) [11, 3, 17, 18, 29, 35, 13, 16]. The tree has open, rounded and attractive crown with shiny green pinnately compound leaves and permits light to pass through the canopy (Moore 1948; Gold and Hanover 1993) [24, 18]. The young bark is thin and smooth while the older bark is fissured into vertical furrows and into long, scaly ridges. The indehiscent 30-40 cm long dark brown pods bulging at seeds are strap-shaped, somewhat twisted and attracts animals (endozoochory) to overcome seed dormancy in nature through the digestive tract of dispersers (Colombo and De Viana 2000; Ferreras and Galetto 2010) [9, 14]. The lustrous brown seeds have hard, impenetrable coats (Vines 1960; Herman *et al*. 1996; Nesom 2003) [40, 20, 25]. The fragrant flowers appear in hanging clusters during late spring (May-June) are attractive to bees; leaves are source of fodder; the pod pulp are used as sweetener, fermented to energy alcohol and used in traditional medicine by native Americans (Herman *et al*. 1996) [20].The wood is strong, hard, durable, resistant to soil decay and produces good timber (Blair 1990; Herman *et al*. 1996; Cassens 2007; Orwa *et al*. 2009) [3, 20, 7, 26]. The physical dormancy imposed due to impermeable hard seed coat in *G. triacanthos* may be treated by mechanical or chemical scarification to make seed permeable before germination can occur (Heit 1942; Bonner *et al*. 1974; Roleston 1978; Burton and Bazzaz 1991; Ertekin and Kirdar 2010; Asl *et al*. 2011; Tecco *et al*. 2012) [19, 4, 32, 6, 13, 2, 39].

Scarified seed resulted enhanced germination percentage to unscarified seed, but at the same time soaking duration in acid must be determined due to variation in seed coat hardness (Bonner *et al*. 1974) [4]. In this respect, the objective focusing on effect of mechanical, chemical scarificators and boiling water was designed for enhancing germination rates in seeds of *G. triacanthos* L. with the purpose of increasing seedling production in forest nurseries.

Materials and Methods

The research was conducted in laboratory of the department of silviculture and agroforestry at the College of Horticulture and Forestry, Central Agricultural University (I), Pasighat, East Siang District, Arunachal Pradesh, India situated at 28°04′43″ N latitude and 95°19′26″ E longitude with an altitude of 163 msl. to investigate the influence of different seed pre-treatment on the germination of *Gleditsia triacanthos* L. For present investigation, mature pods of *G. triacanthos* was collected during the month of November 2021, from Pasighat (28°3′58.68″N latitude; 95°19′40.44″E longitude at 155 msl) East Siang District, Arunachal Pradesh. The pods were air-dried in shade for four days at room temperature, after which the seeds were separated manually from pods and all the wrinkled, infected or diseased seeds were discarded.

The experiment was laid out in a completely randomized design (CRD) subjected to thirteen treatments with four replications each, and one hundred seeds per replication to investigate the effect of different dormancy breaking treatments. Prior to seed germination study, the matured fresh seeds were subjected to thirteen pre-sowing treatments *viz*., T_1 -control (untreated seed); T_2 - soaked in hydrogen peroxide (H_2O_2 4%) for 12 h; T_3 - soaked in hydrogen peroxide (H_2O_2 8%) for 12 h; T_4 -dipped in concentrated sulfuric acid (98% H_2SO_4) for 20 minutes, followed by washing and soaking in water at ambient temperature for 12 h; T_5 - dipped in concentrated sulfuric acid (98% H_2SO_4) for 30 minutes, followed by washing and soaking in water at ambient temperature for 12 h; T_6 - dipped in concentrated sulfuric acid (98% H_2SO_4) for 60 minutes, followed by washing and soaking in water at ambient temperature for 12 h; T_7 - dipped in concentrated hydrochloric acid (HCl) for 10 minutes, followed by washing and soaking in water at ambient temperature for 12 h; T_8 - dipped in concentrated hydrochloric acid (HCl) for 20 minutes, followed by washing and soaking in water at ambient temperature for 12 h; T_9 - dipped in concentrated hydrochloric acid (HCl) for 30 minutes, followed by washing and soaking in water at ambient temperature for 12 h; T_{10} - soaked in water at ambient temperature for 24 h; T_{11} - soaked in hot water (4 times the volume of seeds) and left to cool at ambient temperature for 12 h; T_{12} - soaked in lukewarm water and left to cool at ambient temperature for 12 h; T_{13} - seeds nicked at distal end and soaked in water at ambient temperature for 24 h. The plastic germination trays (48×35×8 cm) were filled with a mixture of soil, sand and farm yard manure in the ratio 3:1:1 and the fertile seeds were sown at a depth of 1.0 cm to 1.5 cm in the laboratory condition in the month of December, 2021. The trays were irrigated as per need to maintain suitable moisture. The germination response was scored daily, and seeds were counted germinated as when visible cotyledon was seen emerged on the surface. Germination was recorded from 9th day after sowing to a maximum of 24th day until all germination process was over. The germination variables studied at the end of the experiment were calculated using the following methods:

1. Germination percentage was calculated using the formulae (ISTA 2003) [21]:

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

2. Peak value was calculated as the maximum mean daily germination (MDG) reached at any time during the period of test.
3. Germination value and germination speed: Germination value is a composite value combining both germination speed and total germination providing an objective means of evaluating the results of germination test was calculated using the formula of Czabator (1962) [10].

Germination Value = Final DGS x Peak value; where DGS is (Daily Germination Speed)

$$\text{Germination Speed} = N_1/d_1+N_2/d_2+N_3/d_3+ \ldots\ldots\ldots\ldots\ldots + N_n/d_n$$

Where, N- number of germinated seeds, d- number of days.

4. Germination energy and energy period: Germination energy (GE) was calculated on the basis of percentage of total number of seed that had germinated when germination reached its peak, and the Energy Period was taken up to the day of peak germination (Seward 1980; Willan 1987) [36, 41].

$$\text{GE} = \frac{\text{Number of seed germinated upto the time of peak germination}}{\text{Total number of seeds sown}} \times 100$$

Data analysis: The results of the germination studies were subjected to an analysis of variance (ANOVA) procedure for the significance of the treatments following the model suggested by Panse and Sukhatme (1985) [27] and the means of the germination parameters was tested using Duncan's Multiple Range Test (DMRT) at 0.05 level (Gomez and Gomez 1984) [28] among the means of various treatments. Result data lying beyond the range (in per cent) were transformed using the arcsine values before statistical analysis.

Results

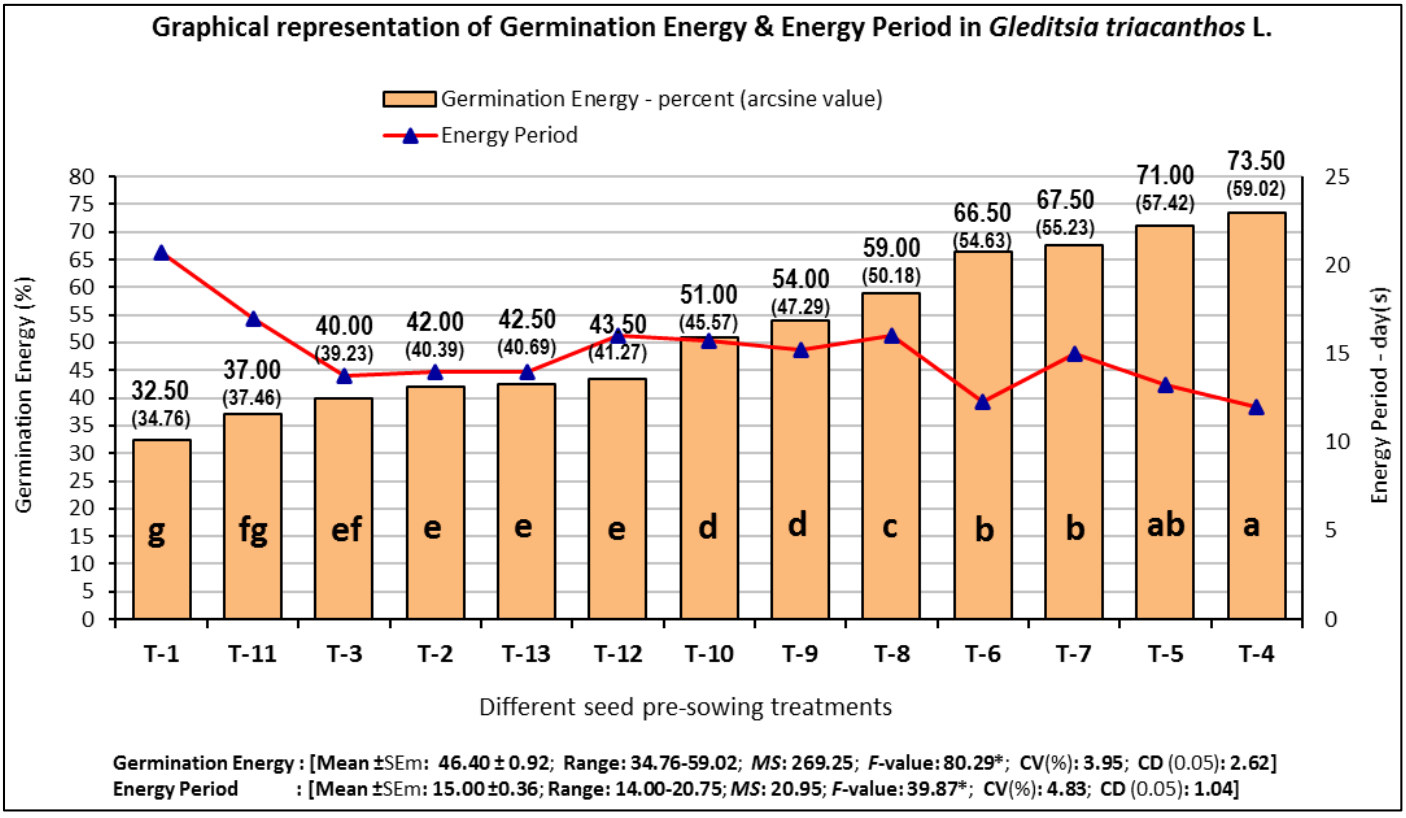
All the pre-sowing treatments had varying impacts on germination behaviour of *G. triacanthos* as evident from the summary results appended in table 1. Significant differences ($p \leq 0.05$) were observed among the different treatments for germination percentage, peak value, germination speed, germination value and energy period. Chemical scarification using sulfuric acid and hydrochloric acid has shown a radical effect on germination percentage as comparable to hot water T_{11} (35%) and control T_1 (37%). The study revealed significantly highest (84.50%) germination percentage observed in T_4 and 83.50% in T_5 followed by 78% in T_6. Peak value varied from 1.78 to 5.92 and the highest value was calculated in T_4, T_5, T_6 and lowest in control (T_1). The results illustrated similar trend of superlative germination value and germination speed in treatments T_4, T_5, T_6 and was statistically at par, with lowest value found in untreated seeds (control).

Table 1: Effect of seed pre-sowing treatment on germination parameters of thirteen treatments under laboratory conditions.

Treatments	Germination percent (arcsine value)	Peak value	Germination value	Germination speed
T_1	37.00 (37.47) ^{g}	1.78 ^{h}	03.14 ^{g}	1.14 ^{h}
T_2	49.00 (44.43) ^{f}	3.00 ^{efg}	08.18 ^{ef}	2.06 ^{fg}
T_3	48.50 (44.14) ^{f}	2.91 ^{fg}	07.85 ^{ef}	2.03 ^{fg}
T_4	84.50 (66.27) ^{a}	5.92 ^{a}	29.07 ^{a}	3.89 ^{a}
T_5	83.50 (66.04) ^{a}	5.55 ^{b}	27.60 ^{a}	3.82 ^{a}
T_6	78.00 (62.07) ^{b}	5.42 ^{b}	24.90 ^{b}	3.60 ^{b}
T_7	74.00 (59.37) ^{c}	4.50 ^{c}	18.52 ^{c}	2.97 ^{c}
T_8	64.50 (53.46) ^{d}	3.70 ^{d}	13.33 ^{d}	2.58 ^{d}
T_9	59.50 (50.48) ^{e}	3.54 ^{d}	11.73 ^{d}	2.41 ^{e}
T_{10}	58.00 (49.61) ^{e}	3.24 ^{e}	09.41 ^{e}	2.18 ^{f}
T_{11}	35.00 (36.27) ^{g}	1.92 ^{h}	03.54 ^{g}	1.28 ^{h}
T_{12}	49.00 (44.42) ^{f}	2.72 ^{g}	07.06 ^{f}	1.91 ^{g}
T_{13}	49.50 (44.71) ^{f}	3.04 ^{ef}	08.36 ^{ef}	2.05 ^{fg}
Mean ± S.Em (Range)	50.67 ± 0.77 (49.00 - 84.50)	3.63 ± 0.10 (1.78 - 5.92)	13.28 ± 0.63 (3.14 - 29.07)	2.46 ± 0.06 (1.14 - 3.89)
MS	410.69	7.15	316.83	3.16
CV (%)	3.06	5.56	9.57	4.64
F- value	170.54 *	174.81*	195.72*	243.63 *
CD ($p \leq 0.05$)	2.22	0.29	1.82	0.16

Note: Value denoted with the same letter(s) are not significantly different at $p \leq 0.05$ probability level according to Duncan's Multiple Range Test (DMRT). Values with (*) are significantly different at $p \leq 0.05$

The effectiveness of various pre-sowing treatments was significant with relation to germination energy and energy period in all the treatments (fig.1). Scarification with sulfuric acid and hydrochloric acid increased germination energy dramatically between 66.50 to 73.50% in T_4, T_5, T_7 and T_6 and the minimum value obtained by untreated seeds T_1 (32.50%). The shortest energy period in 12 days showed highest germination energy for T_4 following an ascending trend in energy period for T_5 (13.25 day), T_7 (15 day) and maximum days exhibited in control T_1 (20.75 day).



Note: Bars with same letter(s) for germination energy are not significantly different at $p \leq 0.05$ probability level according to Duncan's Multiple Range Test (DMRT).Values with (*) are significantly different at $p \leq 0.05$; ±SEm, standard error of the mean; *MS*, mean square value; *F*-value, ratio of variances (*Fisher* analysis of variance); CV, coffiecient of variation; CD, critical difference at 0.05 level, and the bars exhibit on percent value.

Fig 1: Germination energy and energy period for different pre-sowing treatments under laboratory conditions.

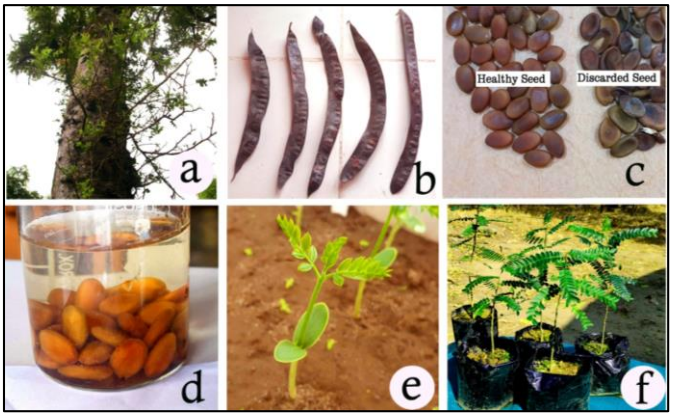


Plate 1: Thorns clustered on lower trunk of adult *Gleditsia triacanthos* L. (a), strap-shaped matured pods in variable lengths (b), grading: healthy and discarded seeds (c), pre-sowing treatment: chemical scarificator (d), germinated seedlings (e) seedling in polybag (f).

Discussions

The prevalence of physical dormancy in seeds of *Gleditsia triacanthos* L. is well-recognized, and the present study attempted to reduce the germination time, as well increasing the germination percentage which are both important pre-requisites for production of large planting stock for any nursery grower. The results of this study showed impressive germination percentage in T_4 (84.50%), T_5 (83.50%), T_6 (78%), followed by T_7 (74%) pre-treated with sulfuric acid and hydrochloric acid as compared with other treatments in our investigation. The findings indicate that chemical scarification had stimulatory effect and is an effective method of softening impermeable seed coats when treated under stipulated time of freshly collected mature seeds. The findings on enhanced seed germination percentage of *G. triacanthos* are in accordance with Heit (1942) [19], Asl *et al*. (2011) [2], Zoghi *et al*. (2011) [42], Sabina and Dorin (2015) [34]. However, the results obtained in T_{11} treated with hot water revealed a low germination percentage as compared to all other treatments probably due to the high temperature hindered germination in seeds, and this argument are in line with Fordham (1965) [15] and Singh *et al*. (2019) [37]. The comparison analysis (DMRT) were significantly different among the means of various treatments ($p \leq 0.05$) for peak value, germination value and germination speed and all these parameters emulates parallel trends to the germination percent for all the treatments (table 1). The maximum peak value, germination value and speed of germination attained in T_4, T_5, T_6, followed by T_7 reflected integrated measure of seed quality and completeness of germination (Czabator 1962; Dunlap and Barnett 1983) [10, 12]. Similar trends of knowledge support the findings on other species by Asiedu *et al*. (2011) [1], Masoodi *et al*. (2014) [23], Chettri and Singh (2022) [8], Rinaldi *et al*. (2022) [31] and Tamuk and Singh (2022) [38].

Furthermore, germination energy calculated to remark the speed of germination under 13 pre-sowing treatment varied between 32.50-73.50% shown in figure-1 exhibited maximum percentage in T_4, T_5, T_7, and T_6 expressed within 12 to 15 days of energy period attribute for those seeds which germinate rapidly and prove itself potential of producing good characteristics of seedling (Willan 1987) [41], and delayed germination of highest imbibition period (20.75 days) was found in T_1 (control). The results revealed that soaking seeds in sulfuric acid and hydrochloric acid solutions significantly increased germination, and these pre-treatments are useful for a good nursery production.

Conclusion

Different pre-sowing treatments of honeylocust seeds were evaluated for improving germination percent. The results obtained in this study entail the important role of chemical scarification prior to sowing for enhanced germination in seeds of *G. triacanthos*. The superlative pre-treatment with concentrated sulfuric acid, followed by concentrated hydrochloric acid at specified duration is recommended for production of nursery seedling stock of honeylocust.

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