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Enhancement in Seed Germination through Pre-sowing Treatments in *Cinnamomum glaucescens* (Nees) Hand.-Mazz., A Native Tree of Eastern Himalaya, India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Cinnamomum glaucescens (Nees) Hand.-Mazz. belongs to family Lauraceae, is an aromatic evergreen tree, prized for its scented timber and fruits which are rich in essential oil commercially. The key challenges of forest establishment are indiscriminate collection methods of fruit and poor seed germination. The aim of this study was to enhance the germination response of *C. glaucescens* seeds to various pre-sowing treatments. The seeds were collected from mature and

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healthy trees and sixteen different pre-sowing seed treatments, arranged in a randomized complete block design in nursery bed were applied to analyse their effect on germination. Depulped seeds soaked in 4% hydrogen peroxide in dark for 12 hours significantly (*P*<.05) enhanced the germination percentage (66.67 %), peak value (0.55), germination value (0.31), germination speed (0.66) followed by seeds treated in 8% H_2O_2 with 60.56% germination, as compared to others. Therefore, it is suggested to use 4% hydrogen peroxide to promote seed germination rates, that could be useful to propagate *C. glaucescens* seedlings to satisfy the demands of planting stock.

Keywords: Cinnamomum glaucescens; pre-sowing treatment; hydrogen peroxide; germination.

1. INTRODUCTION

The genus *Cinnamomum* Schaeff. comprises about 250 species, widely distributed throughout tropical and subtropical Asia, South America, Australia and the Pacific [1-5]. *Cinnamomum glaucescens* (Nees) Hand.-Mazz. (syn. *C. cecidodaphne*) recognized as large-sized evergreen tree belonging to the family Lauraceae and also known by the vernacular name Sugandhakokila, Malagiri (Nep.), Gonorai (Manip.), Gonsorai (Ass.) native to the Eastern Himalayan regions of India from Sikkim eastward, Assam, Manipur, Mizoram, Meghalaya ascending up to an altitude of 1330 m [6-12] The tree seed at intervals of 2-3 years, in October-November [13] so the production of seeds is also very limited. The fragrant fruits are about 3 cm long, surrounded by an enlarged perianth at the base enclosed in a cup 10-12 mm across [10,14]. When young, seeds are usually dark green and turn black as they ripen. *C. glaucescens* produces high-quality scented timber which is valued for furniture, wardrobes, boxes or cabinet making [10]. The wood oil has a persistent camphoraceous odour and resistant to insect attack. The timber in Assam is considered to be first-class for furniture and boat-building [7]. The wood on distillation yields about 1 to 1.25% of essential oil with a good source of safrole [8,11,15]. The plant possesses medicinal properties, although the aromatic fruit is highly prized commercially which is rich in essential oils with insecticidal, antifungal, antibacterial, antiaflatoxin, antioxidant activities have been reported [16-22]. Natural germination of this species is typically low in the forests [23], although over-exploitation and indiscriminate collection methods of aromatic fruit for market demand make it challenging to proliferate and stands at the risk of existence in nature [24-27] Seeds of *C. glaucescens* lose their viability within two months [14,28]. As seed dormancy occurs in many tropical tree species to varying degrees with some mechanism for delaying germination after seed has been dispersed while at the same time seed viability being a crucial factor for successful germination [29-31]. The specific conditions required to break dormancy and initiate germination can vary significantly among species or within a species [32]. Proper nursery growing techniques are not available for *C. glaucescens* and the germination is reported just over 30% in nursery conditions [33]. There are challenges involved in establishing this species and special attention is needed through nursery production to promote cultivation and consequently enhancing conservation initiatives [27]. Hence, the experiment was thus conducted to find out the appropriate pre-sowing treatments that maximize total germination response of *C. glaucescens.*

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was performed in the research field, Department of Silvicultural and Agroforestry, College of Horticulture and Forestry, Central Agricultural University (I), Pasighat, Arunachal Pradesh (elevation: 153 m, latitude: N28°04’43" and longitude: E95°19’ 26").

2.2 Seed Source and Treatments

Mature fruits of *Cinnamomum glaucescens* (Nees) Hand.-Mazz., were collected from healthy mother trees (avg. ht. 27 m and dbh of 25.50 cm) during the month of November 2022, from Gopur (26°52’48.2772"N, 93°36’29.9880"E), Sonitpur District, Assam (India). The fruits were brought to the laboratory and were depulped, dried in shade for 1 day, and finally some external anomaly and damaged seeds were discarded. Selected seeds were subjected to sixteen pre-sowing treatment combinations and were denoted as follows: T_1-control (with pericarp); T_2-soaking in tap water for 24h at ambient temperature; T_3-nicking micropylar and soaking in tap water for 24h at ambient temperature; T_4-dipping in lukewarm water (38°C) and left to cool at ambient temperature 12h; T_5-dipping in warm water (45°C), and left to cool at ambient temperature

12h; T_6-dipping in warm water (75°C) and left to cool at ambient temperature 12h; T_7-dipping in 4% hydrogen peroxide in dark for 12h; T_8-dipping in 8% hydrogen peroxide in dark for 12h; T_9-15 days cold stratification at 5°C in moist cotton; T_{10}-15 days cold stratification at 5°C in moist cotton and, followed by dipping in 15% hydrogen peroxide in dark for 30 min.; T_{11}-dipping in gibberellic acid (0.05%) for 12h; T_{12}-dipping in gibberellic acid (0.01%) for 12h; T_{13}-dipping in indole-3 Acetic Acid (500 ppm) for 12h; T_{14}-dipping in indole-3 Acetic Acid (1000 ppm) for 12h; T_{15}-dipping kinetin 25 mg/L for 12h and T_{16}-dipping kinetin 50 mg/L for 12h.

2.3 Experimental Design and Parameters

Sixteen different pre-sowing seed treatments were sowed in raised bed (upper layer prepared with soil, sand and farmyard manure planting mixture of ratio 2:1:1 respectively) arranged in Randomized Complete Block Design with four replications consisting of 100 seeds per replication. The germinated seeds were recorded on daily basis starting from the 8th day until the 23rd day. Germination was defined as emergence of plumule on nursery bed. The following germination parameters were evaluated:

(i) Germination percentage was calculated using the formulae as ISTA [34].

Germination percentage = Number of seeds germinated / Total number of seeds sown *100

(ii) Peak value was calculated as the maximum mean daily germination (MDG) reached at any time during the period of test.

(iii) Viability loss (expressed in percent/arcsine) was calculated as: total number of seeds sown-total number of seeds germinated.

(iv) 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 [35].

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

(v) Germination Speed = $N_1/d_1+N_2/d_2+N_3/d_3+$ ……$+ N_n/d_n$

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

(vi) 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 [36].

GE = Number of seed germinated upto the time of peak germination / Total number of seeds sown * 100

Data analysis: The results of the germination experiments were analysed for statistical significance (ANOVA) and the differences between the means were compared by Fisher's Least Significant difference (LSD) test at 0.05 level following the model suggested by Panse and Sukhatme [37]. Statistical significance was set at $P<.05$. Results were subjected to analysis of variance ANOVA and the means were compared by Ducan's Multiple Range Test [38]. Result data (in percent) were transformed by arcsine prior to analysis in order to unify the variances as per the standard rules.

3. RESULTS AND DISCUSSION

The comparative effect of various pre-sowing treatments on germination parameters have been given in Table 1. The germination parameters under different treatments showed significant variations. Maximum germination percent (66.67) was noticed in T_7 and T_8 (60.56) followed by T_4 (45.83%), T_2 (43.61%) and other treatments($T_3>T_{14}>T_9>T_{13}>T_{12}>T_{15}>T_6>T_{10}>T_5>T_{16}>T_{11}$) ranging between 23.06 - 38.33% and lowest in T_1-control (22.22%). However, this significant difference ($P<.05$) in germination rate with 4 and 8% use of H_2O_2 as compared to rest of other pretreatment was reported to be more pronounced, and this may be characterised by its highly oxidative reactivity. Presently, the findings of enhanced germination with application of H_2O_2 could not be supported enough with available literature but similar research are in accordance with Ching [39] in (*Pseudotsutga menziesii*); Ogawa et al. [40] in *Zinnia elegans*; Ghildiyal et al. [41] in *Pinus roxbughii*; Joseph et al. [42] in *Garcinia* spp.; Omokhua et al. [43] in *Maesobotrya barteri*; Chen et al. [44] in *Cinnamomum migao*; Rinaldi et al. [45] in *Michelia champaca*; Lorenzo-Barrera [46] in *Eysenhardtia polystachya* and Puwein et al. [47] in *Canarium strictum*.

Table 1. Effect of seed pre-sowing treatment on germination parameters under nursery conditions

Treatments	Germination % arcsine	Peak value	Germination value	Germination speed	Energy Period (days)
T_1	28.11^{k} (22.22)	0.20^{i}	0.04^{i}	0.22^{i}	109.25^{bc}
T_2	41.31^{d} (43.61)	0.39^{c}	0.15^{c}	0.43^{d}	111.25^{b}
T_3	38.26^{e} (38.33)	0.40^{c}	0.16^{c}	0.42^{d}	95.75^{i}
T_4	42.60^{c} (45.83)	0.45^{b}	0.20^{b}	0.49^{c}	100.75^{fgh}
T_5	31.47^{j} (27.22)	0.28^{f}	0.07^{gh}	0.29^{g}	95.25^{i}
T_6	32.52^{hi} (28.89)	0.27^{fg}	0.07^{gh}	0.30^{g}	103.75^{ef}
T_7	54.75^{a} (66.67)	0.55^{a}	0.31^{a}	0.66^{a}	120.00^{a}
T_8	51.10^{b} (60.56)	0.54^{a}	0.30^{a}	0.61^{b}	110.25^{bc}
T_9	35.92^{f} (34.44)	0.34^{d}	0.11^{d}	0.37^{e}	99.75^{gh}
T_{10}	31.64^{ij} (27.50)	0.28^{f}	0.08^{fg}	0.29^{g}	98.00^{hi}
T_{11}	28.67^{k} (23.06)	0.24^{h}	0.06^{h}	0.26^{h}	95.75^{i}
T_{12}	34.40^{g} (31.94)	0.33^{d}	0.11^{d}	0.35^{e}	97.25^{hi}
T_{13}	34.57^{g} (32.22)	0.31^{e}	0.09^{ef}	0.33^{f}	104.50^{de}
T_{14}	36.60^{f} (35.56)	0.33^{d}	0.10^{de}	0.36^{e}	107.50^{cd}
T_{15}	33.21^{h} (30.00)	0.28^{f}	0.08^{fg}	0.31^{g}	102.00^{efg}
T_{16}	31.11^{j} (26.67)	0.26^{g}	0.07^{gh}	0.26^{h}	102.75^{efg}
Mean ± SEm (Range)	**36.63 ± 0.34 (28.11 - 54.75)**	**0.34 ± 0.06 (0.20 - 0.55)**	**0.13 ± 0.005 (0.04 - 0.30)**	**0.37 ± 0.007 (0.22 - 0.66)**	**103.36 ± 1.19 (95.25 - 120)**
CV (%)	**1.85**	**3.50**	**7.70**	**3.57**	**2.29**
***F*- value**	**496.85***	**286.49***	**275.91***	**342.11***	**33.66***
CD (*P*<.05)	**0.967**	**0.017**	**0.014**	**0.019**	**3.39**

Note: Value denoted with the same letter(s) are not significantly different at P<.05 according to Duncan's Multiple Range Test (DMRT). Values with (*) are significantly different at P<.05 probability level. Values within parenthesis are in percentage for germinarion %

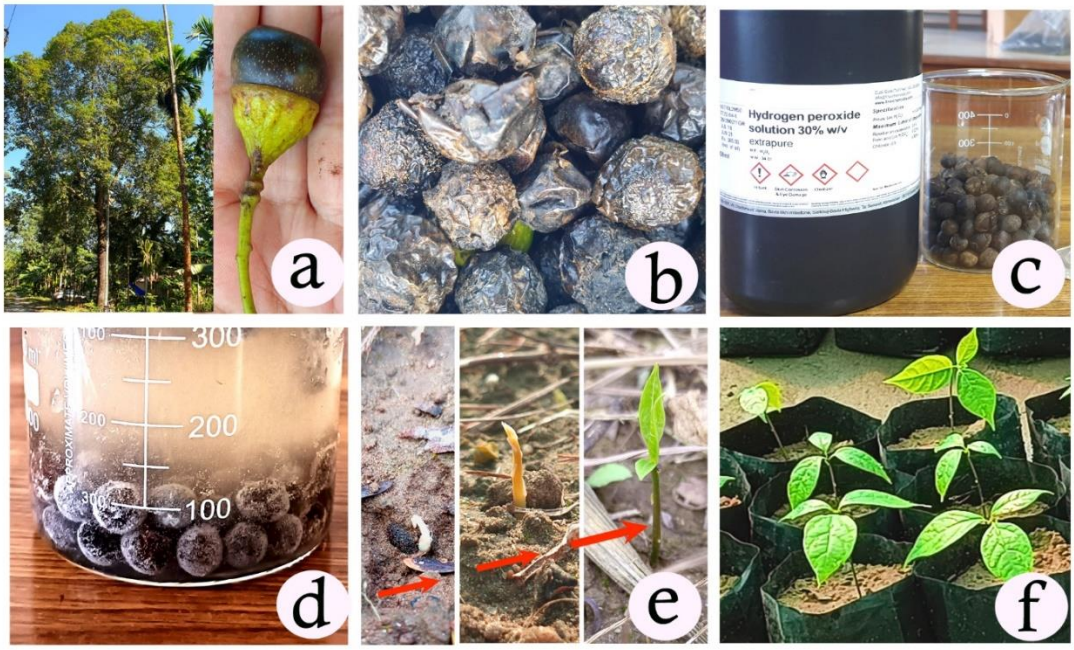

Plate 1. Ripened fruit of adult *Cinnamomum glaucescens* (Nees) (a), fruit collection with pericarp (b), depulped fruit ready for pre-treatments and pre-sowing treatment: hydrogen peroxide (c), treated seeds (d), hypogeal germination stages (e) and seedling in polybag (f)

The germination value captures the speed and completeness of seed germination [48,35] also varied significantly amongst different treatments, the highest value was recorded in T_7 (0.31) and T_8 (0.30) followed by T_4(0.20) and the other treatments showing decreasing trends. It was also observed that maximum germination speed observed in T_7 and T_8 making hydrogen peroxide suitable therapy for triggering seed germination of *C. glaucescens.* The lowest energy period (95.25 days) was recorded for the seeds subjected to T_5 with an ascending sequence of T_3>T_{11}>T_{12}>T_{10}>T_9> T_4>T_{15}>T_{16}>T_6>T_{13}>T_{14}>T_1> T_8>T_2 and reaching maximum duration in T_7 (120 days), contrariwise representing enhanced germination percentage and reduced duration. Peak value indicates the maximum germination rate in a particular day, and the highest peak was recorded in T_7 (0.55) and was statistically at par with T_8 (0.54), although this trend was more or less similar to the germination energy and the highest value (66.39%) was calculated for T_7 followed by T_8 (60.28%) were significantly better than other pre-treatments. The viability loss inversely varied with respect to germination energy as demonstrated in Fig. 1, and the treatments exhibiting higher germination energy could maintain their viability for an extended period of time and results in lower viability losses, hence the higher germination energy depicts the germination potential and vigourness of the seeds [36]. As compared to 16 pretreatments examined in our experiment, 4% H_2O_2 acts best and may contribute in 'vigouration' benefits for seedling productions in forest nurseries. There may be few indication in reverence to the unfavourable effects of hydrogen peroxide treatments to tree seeds, while this elevated germination responded due to H_2O_2 has been noted in many investigations on tree species in understanding the germination expressions beyond the sanitation benefits viz., *Pinus elliottii* (1%) [49]; *Pseudotsuga menziesii*, *Pinus ponderosa*, *P. lambertiana* (1%) [50]; *Cinnamomum camphora* (15%) [51]; *Tectona grandis* (0.5 to 5%) [52]; *Pinus Palustris* (30% & 3%) [53] and *Fagus orientalis* (1%, 2%, 5% and 30%) [54].

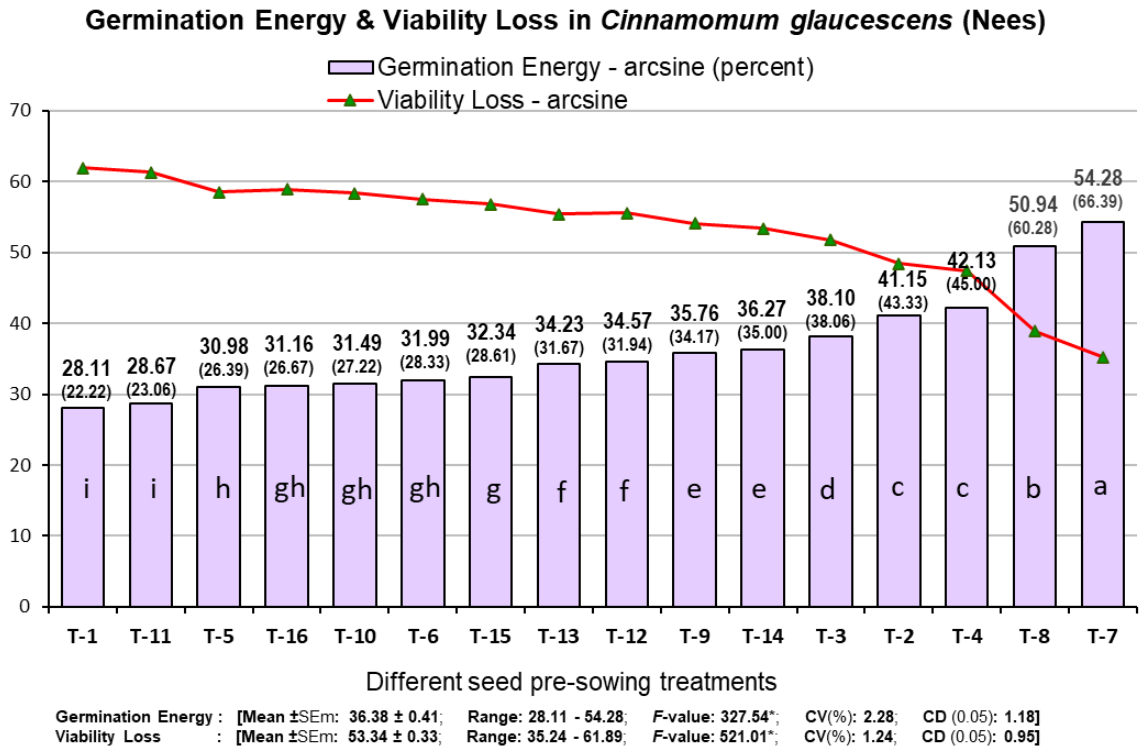

Fig. 1. Germination energy and viability loss of different pre-sowing treatment of *Cinnamomum glaucescens* (Nees) under nursery conditions

Note: Bars with same letter(s) for germination energy are not significantly different at P<.05 according to Duncan's Multiple Range Test (DMRT).Values with (*) are significantly different at P<.05; ±SEm, standard error of the mean; F-value, ratio of variances (Fisher analysis of variance); CV, coffiecient of variation; CD, critical difference at 0.05 level. Bars (ascending) and graphical line (descending) depicts arcsine value

4. CONCLUSION

Suitable pretreatment applied to dormant seeds ensures successful propagation to get additional level of seed germination. The findings of the experiment concluded that seeds dipped in 4% hydrogen peroxide for 12h in dark (T_7) significantly enhanced the germination percentage among different pre-sowing treatments sowed in nursery conditions. Hence, this treatment can be recommended for large scale production of nursery seedlings of *C. glaucescens* for promoting commercial plantation and support conservation efforts. The mechanism of seed breaking dormancy of this species also needs further study.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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