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Research Article

REGENERATION OF TWO MEDICINALLY IMPORTANT GARCINIA SPECIES OF ASSAM, NE INDIA

Bhuyan L., Sharma C. L., Singh B., Sharma M and Bharali P

Department of Forestry, NERIST, Nirjuli- 791 109 Arunachal Pradesh

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ABSTRACT

The present study was carried out to find out the most effective treatments for seed germination of *Garcinia pedunculata* Roxb. and *G. morella* (Gaertn.) Desr. The material for the study was collected from Dullung Forest Reserve, Assam, India. Different treatments were applied to both coated and decoated seeds of *G. pedunculata* and *G. morella*.GA 250 ppm was the most effective treatment in decoated seeds of *G. pedunculata* with 100% seed germination and highest seedling vigor index (21.23) while H_2O_2 (30%) treatment was the best treatment in *G. morella* with 90% seed germination and highest seedling vigor (37.85).

INTRODUCTION

As natural vegetation is depleting very fast, some areas are converted to plantation forest to meet the growing human needs (Hansen *et al*., 1991; Pandey and Shukla, 1999). Cultivation and management of plants particularly economically or medicinally useful species are important aspects of biodiversity conservation. Accordingly, a large number of useful species are selected and cultivated. The success of the productive cultivation fully depends on the understanding the reproductive biology, phenology, regeneration capacity and climatic adaptability of the species.

Garcinia is a tropical plant with number of important economic species in Africa, America and Asia (Rai, 2003; Eyog-Matig *et al*., 2007; Joseph *et al*., 2007). The species are medicinally important due to their antimicrobial activities (Anonymous, 2002; Baruah and Borthakur, 2012). *Garcinia* L. has about 350 species estimated worldwide (Whitemore, 1973) and about 200 species are widely distributed southern parts of the Thailand and Peninsular Malaysia to Indonesia and South East Asian region (Sharma and Sanjappa, 1993; Mabberley, 2005; Baruah and Borthakur, 2012). Anderson (1874) reported 30 *Garcinia* species from India and later 35 species were reported by Maheswhari (1964) of which 15 species occur in North-East India (Baruah and Borthakur, 2012).

Garcinia pedunculata Roxb. and *G.morella* (Gaertn.) Desr. are two economically important multipurpose tree species. Both species are useful for medicine, fruits, vegetables or other domestic uses. The species are also attached with the socio cultural aspects of some of the indigenous communities in Assam (Baruah and Borthakur, 2012). However, the natural regeneration of the species is poor and the seedlings are hardly found in the forest areas. Over the years, the natural population of these species has reduced drastically due to their over exploitation and poor regeneration status. Several scientists have studied the germination behaviours of different *Garcinia* species and observed major difficulty in their propagation due to seed dormancy problem (Aduse-Poku *et al*., 2003; Eyog-Matig *et al*., 2007). Though some investigations were carried out in laboratory conditions but the part of the outcomes couldn't be implemented by small farmers (Nzegbule and Mbakwe, 2001; Liu *et al*., 2005; Eyog-Matig *et al*., 2007). Since, no study has been conducted on seed germination of *Garcinia pedunculata* and *G. morella* so far, the aim of present study is to find out the most effective treatment for seed germination of these two species.

Corresponding author:* **Bhuyan, L
Department of Forestry, NERIST, Nirjuli- 791 109 Arunachal Pradesh

MATERIAL AND METHOD

Seed germination

Ripe fruit of *Garcinia pedunculata* and *G. morella* were collected from the natural forest areas of Dullung Reserve Forest, Assam and were carefully brought to the laboratory in Department of Forestry, NERIST, Arunachal Pradesh for further processing. Seeds were separated from the fruits by rubbing softly with hands and washed thoroughly in running water to remove the pulp (Plate 1A-C). After washing, the seeds were allowed to dry. The viability of seeds was determined by TZ (Tetrazolium) test before testing them for germination.

The germination tests were selected based on information available in literature (Eyog-Matig *et al*., 2007; Nzegbule and Mbakwe, 2001). Both coated and decoated seeds were taken for pre germination treatments. The study on germination phenology was conducted in a shade (50%) nursery. The seeds were sown in polyethylene containers. Three substrata were used in nursery i.e. sand mixed with garden soil and decomposed cow dung (1:2:1). The containers were 10cm in diameter, 18cm in height with three rows of small holes for drainage.

The mixed media were treated with fungicide Carbendazim solution and left for three days before sowing to prevent damage to the seeds due to chemical content of the fungicide. One seed was sown in each container and watered regularly to keep the sand moist. Three replicates with 20 seeds were taken for each treatment. Germination was recorded on every alternate day for six months and weekly later on. Seeds were considered germinated when the length of the radicle was about 2mm.

The following treatments were given to seeds of *G. pedunculata* and *G. morella*.

Table 1 Seed treatments of *G. pedunculata* and *G. morella*.

Sl. No.	Types of seed	Treatments	Duration
1.	Coated seeds	Normal water at 30^0C(T_1)	24 h
2.	Coated seeds	Sulphuric acid 20% (T_2)	20 min
3.	Coated seeds	Nitric acid 20% (T_3)	20 min
4.	Coated seeds	H_2O_2 30% (T_4)	30 min
5.	Coated seeds	Hot water at 70^0C (T_5)	24 h
6.	Coated seeds	GA 250 ppm (T_6)	18 h
7.	Coated seeds	GA 500 ppm (T_7)	18 h
8.	Decoated seeds	Normal water at 30^0C (T_8)	24 h
9.	Decoated seeds	GA 250 ppm (T_9)	18 h
10.	Decoated seeds	GA 500 ppm (T_{10})	18 h
11.	Decoated seeds	NAA 500 ppm (T_{11})	18 h

The germination percentage and mean germination time were calculated using the following equations:

Germination percentage(%): $GR_g = (n_g/N) \times 100$;
Where N is the total number seeds; n_g is the number of germinated seeds of the treatment g and GR_g is the germination percentage of the treatment g.

Mean germination time (days): $MGTg = \sum (t_f \times n_{gf}) / n_g$;

Where MGTg is the mean germination time of the treatment g; t_f is the f^{th} day from sowing; n_{gf} is the number of germinated seeds of the treatment g at the f^{th} day from sowing and n_g is the total number of germinated seeds of the treatment g.

Seedling vigor index

The seedling vigor index (SVI) was calculated by the formula given by Hangarter (1997)

Seedling vigor index (SVI) = Seedling length (cm) × Germinated percentage/100

Seedling growth

The growth of the seedling was determined after one-year from the date of emergence. For growth measurement, six seedlings of each species were excavated from the plots of each treatment.

Survival of the seedlings was studied at interval of two months for one year. Mortality rate and survival of the seedlings were also calculated. Growth performance of the seedlings was assessed in terms of shoot elongation, collar diameter, leaf number and leaf area. Leaf area was measured with the help of a leaf area meter.

RESULTS

A significant effect of pre-germination treatments was observed during germination of seeds. The time taken for seed germination varied among most of the treatments. In case of *G. pedunculata*, H_2O_2 was highly effective seed treatment resulting early seed germination. The germination started after 05 days for coated seeds soaked in H_2O_2 (T_4). Decoated seeds treated with GA 250 ppm (T_9) and GA 500 ppm (T_{10}) germinated after 18 days. On the other hand, decoated seeds germinated after 26 days in case of seeds soaked in normal water (T_8), 29 days for coated seeds in GA 500 ppm (T7), 33 days for coated seeds in GA 250 ppm (T6). Coated seeds inT_2, T_3, T_5 and T_{11}failed to germinate. Seeds treated with normal water (T_1) took maximum time to germinate in T_1 (Table 2, Plate 1D-E).

Mean germination time (MGT) varied according to seed germination tests. The mean germination time was lowest (9.25 days) for coated seeds in H_2O_2 (T_4) followed by decoated seeds in GA 500 ppm (T_{10}), decoated seeds in GA 250 ppm (T_9), decoated seeds in normal water (T_8), coated seeds in GA 500 ppm (T_7) and coated seeds in GA 250 ppm (T_6). The MGT of coated seeds in normal water treatment (T_1) was much higher than the other mentioned treatments (Table 2).

Germination percentage varied from 20% (Coated seeds in normal water: T_1) to 100% (Decoated seeds in GA 250 ppm: T_9) in *G. pedunculata* seeds. Both the treatments with decoated seeds in GA 500 ppm (T_{10}) and coated seeds in GA 250 ppm (T_6) showed better response having 90% seed germination in each treatment. Dormancy breaking treatments, coated seeds in GA 500 ppm (T_7) and decoated seeds in normal water (T_8) showed equal germination rate (80% and 75% respectively). Treatments with coated seeds in H_2O_2 (T_4) and coated seeds in normal water (T_1) had comparatively low germination rates (40% and 20% respectively) against the above mentioned dormancy-breaking methods (Table 2).

In *G. morella*, germination was earliest (60 days) in decoated seeds in GA 500 ppm(T_{10}) followed by decoated seeds with

normal water T_8 (73 days) and decoated seeds in GA 250 ppm treatment T_9 (80 days). Other dormancy breaking methods took more than 160 days for initiation of seed germination. Coated seeds with normal water treatment (T_1) took the longest period (182 days) for seed germination. No seed germination was found in coated seeds treated with Nitric acid (T_3), Hot water (T_5), GA 250 ppm (T_6), GA 500 ppm (T_7) and decoated seeds in NAA 500 ppm treatment (T_{11}).

Mean germination time (MGT) was lowest (83 days) in decoated seeds treated with normal water (T_8), followed by decoated seeds in T_9 (90 days). Only the treatment with decoated seeds in GA 500 ppm (T_{10}) showed intermediate MGT (118.71 days) and the remaining all three treatments i.e. T_1, T_2andT_4have MGT more than 200 days. The highest MGT (244.91 days) was found in T_1(Fig. 1).

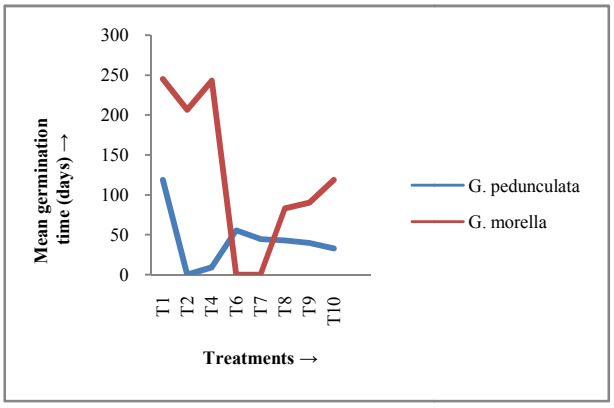

Fig 1 Frequency distribution of mean germination time of *Garcinia pedunculata* and *G. morella*.

Germination percentage of *G. morella* with different treatments showed varying results. Seeds treated with H_2O_2 (T_4) achieved maximum germination rate (90%). The germination percentages were much lower for coated seeds T_2 (10%) and decoated seeds in T_8 (45%). Decoated seeds treated in GA 250 ppm (T_9) had better results (65%) than coated seeds in normal water (55%) and decoated seeds in GA 500 ppm treatment (55%), with same germination rates (Fig. 2).

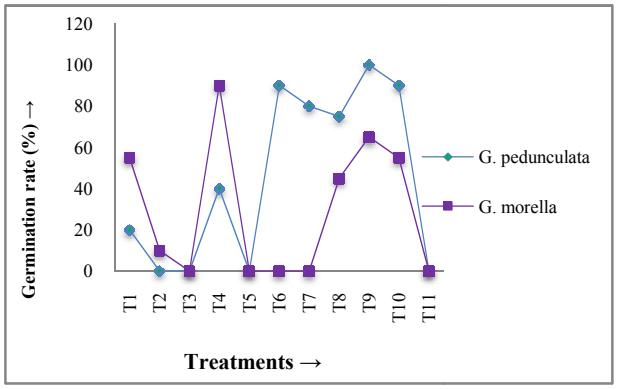

Fig 2 Germination percentage of *G. pedunculata* and *G. morella* in different treatments.

Seedling vigor index (SVI)

Seedling vigor index varied among the treatments. However, in *G. pedunculata* seed treatment, the highest seedling vigor index (21.21) in decoated seeds was recorded in T_9 followed by the treatment T_6 and T_{10}. While, the lowest seedling vigor index (1.45) was recorded in T_1.On the other hand, in *G. morella* seed treatments, highest seedling vigor index (37.85) was recorded in seeds with T_4 and lowest SVI (3.5) with treatment T_2. Decoated seeds treated with $T_9$and T_1 were much better result of SVI than the rest treatments (Fig. 3).

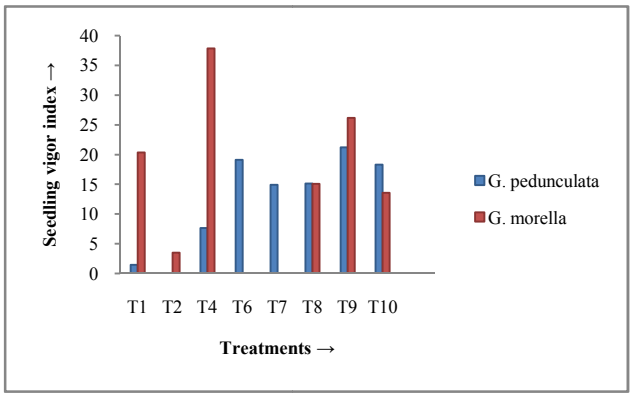

Fig 3 Seedling vigor index in *G. pedunculata* and *G.morella*.

Seedling survival

In case of *Garcinia pedunculata*, five treatments T_4(coated seeds in H_2O_2), T_6 (coated seeds inGA 250 ppm), T_7 (coated seeds in GA 500 ppm), T_8(decoated seeds in normal water:, T_9(decoated seeds in GA 250 ppm) and T_{10}(decoated seeds in GA 500 ppm) showed more or less similar results of seedling growth. Coated seeds treated with normal water (T_1) showed the lowest seedling growth performance. However, in *G. morella*, seedling survival was observed highest in treatment T_9. Seedling survival varied randomly in all other treatments in *G. morella* (Table 3).

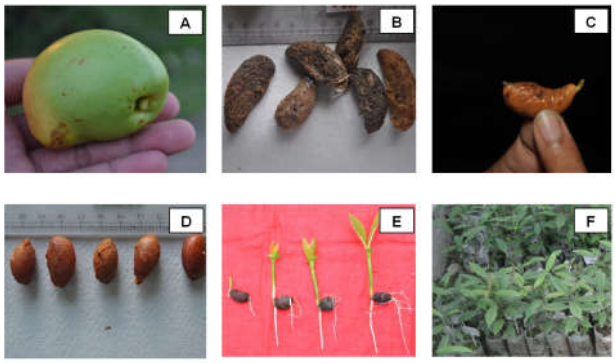

Plate 1 Fruit of *Garcinia morella* (A); Coated seeds of *G. morella* (B); Decoated seed germination of *G. morella* (C); Seeds of *G. pedunculata*(D); Seedlings of *G. pedunculata*(E); Seedlings of *G. morella*(F).

DISCUSSION

The germination of seeds shows variation of spread over time due to differences between dormancy depths (Bewley, 1982). Removal of seed coat and application of growth hormones are the important factors to affect the germination rate.The present study on seed germination and seedling survival of *Garcinia pedunculata* and *G. morella* reveals that *G. pedunculata* seeds treatment with H_2O_2 was highly effective in early seed germination. The seed germination took place after 05 days from seed sowing in coated seeds soaked in H_2O_2. The treatments treated with H_2SO_4 (T_2), nitric acid (T_3), hot water (T_5) and NAA (T_{11}) were failed to germinate seeds.

Table 2 Seed germination (%), period required to commence (days), mean germination time (days) and seedling vigor index of *G. pedunculata* and *G. morella*. The values are mean ± SD.

Treatments	Period (days) required to commence		Mean germination time (days)		Final germination (%)		Seedling vigor index (SVI)	
	GP	GM	GP	GM	GP	GM	GP	GM
T_1	112±1.73	182±3.46	118.5 ± 9.19	244.91±55.78	20 ± 5	55 ± 10	1.45	20.35
T_2	N/G	166±2.65	-	206.5 ± 35.12	-	10 ± 5	-	3.5
T_3	N/G	N/G	-	-	-	-	-	-
T_4	05±1.0	178±10.54	9.25 ± 3.06	242.81 ± 32.59	40±5	90 ± 5	7.66	37.85
T_5	N/G	N/G	-	-	-	-	-	-
T_6	33±2.65	N/G	55.11 ± 17.79	-	90±5	-	19.1	-
T_7	29±2.65	N/G	44.25 ± 9.57	-	80±5	-	14.89	-
T_8	26±1.73	73±2.65	43 ± 14.26	83 ± 13.61	75±13.23	45 ± 8.66	15.15	15.05
T_9	18±1.73	80±1.73	39.73 ± 32.33	90 ± 7.91	100±0	65 ± 8.66	21.23	26.19
T_{10}	18 ± 2.65	60±1.73	32.78 ± 14.64	118.71 ± 51.46	90±8.66	55 ± 0.00	18.32	13.58
T_{11}	N/G	N/G	-	-	-	-	-	-

N/G = Not Germinated, GP = *Garcinia pedunculata*, GM = *G. morella*, SD = Standard deviation.

Table 3 Seedling survival in *G. pedunculata* (GP) and *G. Morella* (GM). The values are mean ± SD.

Dormancy breaking method	Height (cm)		Collar diameter (mm)		Leaf number per seedling		Leaf area (cm^2) per seedling	
	GP	GM	GP	GM	GP	GM	GP	GM
T_1	7.23 ± 2.86	37 ± 7.36	3.92 ± 0.61	4.41 ± 0.87	9 ± 1.1	22.0 ± 4.0	1244.51 ± 47.18	720.38 ± 125.77
T_2	-	34.1 ± 8.86	-	3.82 ± 1.53	-	15 ± 2.58	-	523.58 ± 121.93
T_4	18.18 ± 4.52	40.92 ± 4.46	4.55 ± 0.66	4.84 ± 1.1	10.67 ± 1.03	21.83 ± 4.02	1531.56 ± 234.47	726.19 ± 121.67
T_6	21.22 ± 3.71	-	5.0 ± 0.53	-	10.67 ± 1.03	-	1463.78 ± 199.22	-
T_7	18.6 ± 3.68	-	4.85 ± 0.87	-	10.00 ± 0	-	1338.26 ± 136.44	-
T_8	20.83 ± 3.68	32.6 ± 7.76	5.77 ± 0.73	4.95 ± 0.74	10.33 ± 0.82	18.67 ± 3.27	1562.78 ± 205.99	605.67 ± 122.88
T_9	21.23 ± 2.8	41.9 ± 3.46	5.49 ± 0.76	5.57 ± 0.55	10.33 ± 0.82	30.8 ± 3.63	1468.68 ± 105.87	986.65 ± 125.19
T_{10}	20.35 ± 5.9	25.22 ± 13.26	5.74 ± 0.57	3.76 ± 1.17	10.33 ± 0.82	19.67 ± 7.31	1583.45 ± 242.13	646.12 ± 236.95

GP = *Garcinia pedunculata*, GM = *G. morella*, SD = Standard deviation.

The percentage of seed germination varied significantly from 20% (Coated seeds in normal water) to 100% (Decoated seeds in GA 250 ppm) among seed treatments in *G. pedunculata*. Both decoated seeds treated with GA 500 ppm and coated seeds in GA 250 ppm also gave good response each with 90% seed germination. An examination of literature reveals that soaking the decoated seeds of *G. gummi gutta* in GA (500ppm) for 12 hrs increase germination (Nazeema, 1992; Rema, 1997). Our experiment also supports this finding as maximum seed germination was observed in decoated seed treatment with hormonal application in *G. pedunculata* seeds. On the other hand, Joseph *et al*. (2007) reported that seed germination was earlier in H_2O_2 and cow milk treatment in *G. cowa* and *G. gummi-gutta*, and seed germination was also higher than the other treatments which were much similar with our pre germination treatments. They further reported that seed treatments of *G. tinctonia* with H_2SO_4, H_2O_2, Gibberellic acid and kinetin also gave good results. In the present study, H_2O_2 treatment for *G. pedunculata* and GA (500 ppm) for *G. morella* were highly effective as seed germination took place after 05 days in *G. pedunculata* and after 60 days in *G. morella*. Hence, it is in agreement with the findings of Joseph *et al*., 2007; Nazeema, 1997 and Rema 1997. There was no seed germination in seeds treated with H_2SO_4, HNO_3 and NAA 500 ppm in *G. pedunculata*. Also *G. morella* did not show any germination of seeds treated with HNO_3, Hot water, GA 250 ppm, GA 500ppm and NAA 500 ppm. In *G. morella*, highest seed germination percentage (90%) was found in coated seeds with H_2O_2 treatment and lowest (20%) in normal water treatment. The failure of germination of both species in hot water treatment may be due to embryo destruction.

The germination behaviour of seeds in *Garcinia* species is very unique (Chandler, 1958; Vogel, 1980). Chandler (1958) reported that the seed viability of *G. mangostana* can be retained for 3-5 weeks within the fruits. The viability of seed can be increased for 3-5 weeks by storing seeds in moist charcoal, peat moss or coconut fibres (Gonzalez and Anoos, 1951; Winters and Rodriquez-Colon, 1953; Chandler, 1958). Mathew and George (1995) reported the highest germination rate in *G. gummi-gutta* seeds with Gibberellic acid treatment. George *et al*. (2002) conducted studies on seed propagation and softwood grafting in *G. indica*. Liu *et al*. (2005) proved that thick seed coat acted as a mechanical barrier to water permeability and radical protrusion in *Garcinia cowa* seeds. Osman and Rahman (2006) have suggested that pre germination treatment is helpful in increasing of seed germination rate in *G. mangostana* and seeds prefer to germinate under high humidity and shaded condition. The present study shows that seeds of *Garcinia pedunculata* lost its viability within 2 months after extracting from the fruit which

may be due to decrease in moisture content of seed after extraction. However, the seed viability of *G. morella* was more than 1 years due to presence of thick seed coat as reported in other *Garcinia* sps. also (Liu *et al*., 2005).

Seed germination also depends on seed quality and seedling survival rate. Nzegbule and Mbakwe (2001) observed decrease in seed viability of *G. kola* due to desiccation and increase in seed germination percentage due to presence of high moisture content.They also failed to germinate *G. kola* seeds in hot water (60°C) treatment for 8 hours. On the other hand, Eyog-Matig *et al*. (2007) reported lower rate of seed germination due to poor seed quality. The present study shows that coated seeds of *G. morella* treated with H_2O_2 showed 90% germination where as decoated seeds of *G. pedunculata* treated with GA 250 ppm showed 100% germination and 20% only with normal treatment.In case of *G. pedunculata* the seeds took less time for germination than *G. morella* due to thin seed coat. But the seeds of *G. pedunculata* are surrounded by some yellowish gummy substanceswhich may be the reason for delay in seed germination resulting lower germination percentage. Higher germination percentage and faster germination in decoated seed indicate that the hard seed coat and the gummy substances act as barrier in germination process and hamper in proper changing of physiochemical activities to favour seed germination.

CONCLUSION

Seed germination is much easier and cheaper way of propagation. Proper cultivation and management is highly beneficial to the mankind and also in conservation of medicinal plants. Decoated seeds with GA 250 ppm treatment in *Garcinia pedunculata* and H_2O_2 treatment in *G. morella* are the best as these treatments showed maximum germination percentage and seedling vigour index.

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