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Seed Germination Studies in Andaman Kokum (*Garcinia dhanikhariensis* S.K. Srivastava): An Endemic Species from Bay Islands, India

Sneha Horo[1], Roopa Ranjani[1], Ajit Arun Waman[2]*, Santosh K. Jha[1] and Pooja Bohra[2]

[1]*Jawaharlal Nehru Rajkeeya Mahavidyalaya, Port Blair-744101, Andaman and Nicobar Islands, India*

[2]*Division of Horticulture and Forestry, ICAR-Central Island Agricultural Research Institute, Port Blair- 744105, Andaman and Nicobar Islands, India*

***Corresponding author's E-mail:** *ajit.hort595@gmail.com*

Abstract

Garcinia dhanikhariensis is an endemic species distributed in the warm and humid tropical South Andaman Island in the Bay of Bengal. Natural populations of this species are dwindling due to anthropogenic activities and natural calamities. Lack of awareness among the local people about its potential uses is adding to the gravity of the situation. Considering the endemic nature and very low natural population of the species, urgent efforts on conservation are required. Effect of retention of seed coat (presence/ absence), seed division (whole/ fragmented) and seed size (small/ large) was studied on seed germination and seedling growth parameters. Results revealed that removal of seed coat hastened the germination process and 95.9% germination was noticed in such seeds. Division of seeds into two pieces gave rise to plants from both the segments, although with variable success. Higher germination percentage of 70.7% was obtained when large seeds were used for sowing. Seedling growth parameters were also influenced due to the studied treatments. Thus, the present findings could be helpful in large scale multiplication of this endemic genetic resource of the fragile island ecosystem.

Key words: *Seed coat removal, seed division, seed size, seedling vigour*

Introduction

Garcinia dhanikhariensis S.K. Srivastava is an endemic species of the Clusiaceae family distributed in the Andaman Islands in the Bay of Bengal (Srivastava, 1994). Limited natural populations of this species are found scattered around the Dhanikhari, Chouldari, Lal Pahad and Kalatang villages of South Andaman Island. Locally it is known as *Lal Kau Phal* while it was christened as Andaman Kokumowing to some similarities of the species with *Garcinia indica* of the Western Ghats (Bohra et al., 2021a). The tree grows slender with narrow canopy. Fruits are generally ready to harvest during January-February and the harvesting continues until May (Bohra et al., 2021a). Traditionally, only a few people in and around the area are aware about the species and they consume the sour sweet pulp of the fruits, while rind is largely discarded.

Even though the species was discovered in 1994, it remained largely understudied till the recent past. Systematic studies were initiated at ICAR-Central Island Agricultural Research Institute, Port Blair since 2016 to conserve, characterize and utilize this species (Bohra et al., 2021a). Rind of the species has been identified as a natural source of anthocyanins, and antioxidant with potential as a natural colorant, and pharmaceutically important hydroxycitric acid (Bohra and Waman, 2022). Further, seeds of the species have been identified as a potential source of fatty acids *viz*. stearic acid and oleic acid of industrial significance (Bohra et al., 2021b). Considering these characteristics, the species has been identified as a novel crop for commercial cultivation in the warm humid tropical regions of Andaman Islands (Bohra et al., 2021a).

Even though the species has potential for commercial utilization in the islands, lack of planting material is a major issue in promoting it. As the species is endemic and only a few natural populations are available, development of efficient multiplication protocol is important. Seed germination is one of the easy yet efficient means for mass multiplication of species, especially the endemic ones. However, a number of aspects need to be studied to have a reliable mass multiplication protocol. Presence of prolonged dormancy (8-11 months) has been reported in species of *Garcinia* (Liu et al., 2005). In earlier study, we studied the effect of seed soaking treatments and seed source for obtaining superior germination in this species (Bohra et al., 2021).

Earlier studies involving different *Garcinia* species suggested that removal of seed coat (Joshi et al., 2006; Liu et al., 2005; Cardoso et al., 2021) and seed size (Florent et al., 2021) significantly influenced the seed germination and subsequent plant growth. *Garcinia* type germination, which is characterized by primary root and shoot emergence from opposite ends of the seed (de Vogel, 1980), has also been reported in this species recently (Bohra et al., 2021). In Malabar tamarind (*G. gummi-gutta*), use of seed fragments was found to give rise to seedlings from each segment of the seed (Joshi et al., 2006). This could be of great practical help in multiplication of endemic species such as Andaman Kokum, where the seed availability is rare. Considering these aspects, the present study was undertaken with three objectives: 1. to study the effect of seed coat removal on seed germination and seedling growth, 2. to explore the possibility of using seed pieces for propagation and 3. to study the effect of seed size on seed germination and seedling growth.

Materials and methods

Collection of seed samples

Fully ripe fruits of *Garcinia dhanikhariensis* S.K. Srivastava were collected from an identified collection (GDH/SA/RUP) from Lal Pahad village of South Andaman Island during 2021. Seeds were extracted manually from the fruits and washed with water to remove the adhering pulp. After washing, seeds were divided into three experiments as detailed below. After appropriate treatments, the seeds were sown in pro-trays (49 cavities) filled with soil + vermicompost (1:1, v/v) as substrate. Each treatment was replicated thrice and there were 49 seeds in each replication.

Experiment 1: Effect of removal of seed coat

In this experiment, washed seeds were divided into two lots. In first lot, seed coats were manually removed from the seeds and used for sowing (T_1), while in the second lot, seed coat was kept intact (T_2).

Experiment 2: Effect of seed division

In order to know the effect of division of seeds, seed coats were removed manually from all the seeds. Seeds in the first lot were cut into two pieces (T_1) before sowing while seeds in other lot were used intact for sowing in the aforesaid medium (T_2).

Experiment 3: Effect of size of seeds

Seeds after washing were air dried and then sorted into two groups based on seed weight. Individual seeds were weighed using analytical balance and those weighing less than 0.5 g were graded as small sized seeds (T_1), while those weighing more than 0.5 g were graded as large sized seeds (T_2). Such graded seeds were sown in the substrate as mentioned above.

Record of observations

Number of seeds germinating every day was counted manually. Seedlings with minimum 2 mm of epicotyls elongation above the substrate were considered as germinated. Germination percentage was determined at 30, 60, 90 and 120 days after sowing. Seedling growth parameters were recorded at the end of experiment (120 days) using 20 seedlings in each treatment. Parameters such as seedling length (cm), shoot length (cm), root length (cm), number of roots per seedling, length of longest root (cm), number of leaves per seedling, leaf length (cm), leaf width (cm) and collar thickness (cm) were recorded using standard procedures.

Mean time taken for initiation of germination (days) was determined as time taken for start of germination in each replication / number of replications, whereas mean time taken for completion of germination (days) was calculated as time taken for completion of germination in each replication / number of replications. Seedling vigour index was calculated as mean seedling length × cumulative germination percentage (Abdul Baki and Anderson, 1973).

Data analysis

Collected data from various experiments was subjected to *t*-test using Web Agri Stat Package 2.0 (ICAR-CCARI, Ela, Goa).

Results and discussion

Standardization of seed germination technique is one of the basic steps for mass multiplication of any species. Standardized nursery techniques could help in habitat enrichment activities in species which are endemic or facing conservation issues, whereas for commercial/ potential crops, it helps in providing rootstocks/ propagules for area expansion activities. Andaman Kokum belongs to both the categories as it is endemic and also has potential for cultivation as a novel crop (Bohra et al., 2021). Hence, series of experiments were undertaken to standardize its mass multiplication protocol.

Experiment 1: Effect of removal of seed coat

Effect of removal of seed coat on germination percentage and characteristics was studied. Germination percentage over a period of 120 days showed significant influence of the treatment. After 30 days, no germination was noticed in seeds wherein seed coat was retained (Fig. 1), while 19.05% seeds could germinate when the coat was removed. After 60, 90 and 120 DAS also, this treatment showed significantly higher germination of 89.80, 95.24 and 96.60%, respectively as against 36.73, 74.15 and 82.31%, respectively in seeds wherein seed coat was retained. Presence of seed coat induced dormancy has also been reported in *G. gummi-gutta, G. brasiliensis, etc.* (Joshi et al., 2006; Cardoso et al., 2021).

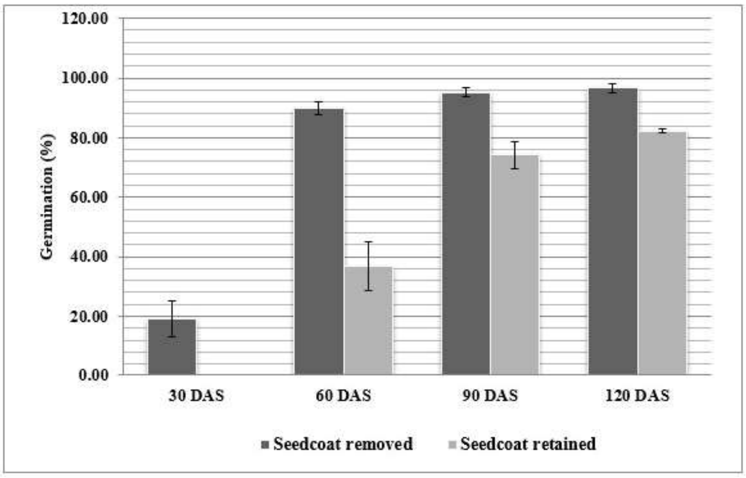

Fig. 1. Effect of seed coat removal on germination percentage over the period of 120 DAS

Removal of seed coat quickened the initiation of germination (Fig. 2a) and about 39.71 days were required in this treatment as against 65.68 days for seeds in which seed coat was retained. This finding is in conformity with earlier report on *G. kola* (Matig et al., 2007). Similarly, the completion of germination (Fig. 2b) was also achieved within 79.33 days when seed coat was removed as against 111.33 days in seeds, where it was not removed. Imbibition of water is the prime step in seed germination process and removal of this barrier is known to quicken the moisture absorbance in the seed (Cardoso et al., 2021) and hence, the results obtained here could be expected. Thick seed coat (testa) has been considered as one of the major hindrances in germination of *Garcinia* species (Liu et al., 2005). Presence of hydrophobic suberin layer has been reported in the seeds of *G. prainiana*, which serves as a mechanical barrier to movement of air, restricts imbibition process and emergence of radicle (Normah et al., 2016). Seed coat removal has been reported to hasten the germination process in *G. gummi-gutta,* wherein Joshi et al. (2006) reported germination time of three weeks as against four months in intact seeds. Removal of seed coat could significantly reduce the mean germination time from 120 days to just 13 days in *G. cowa* (Liu et al., 2005).

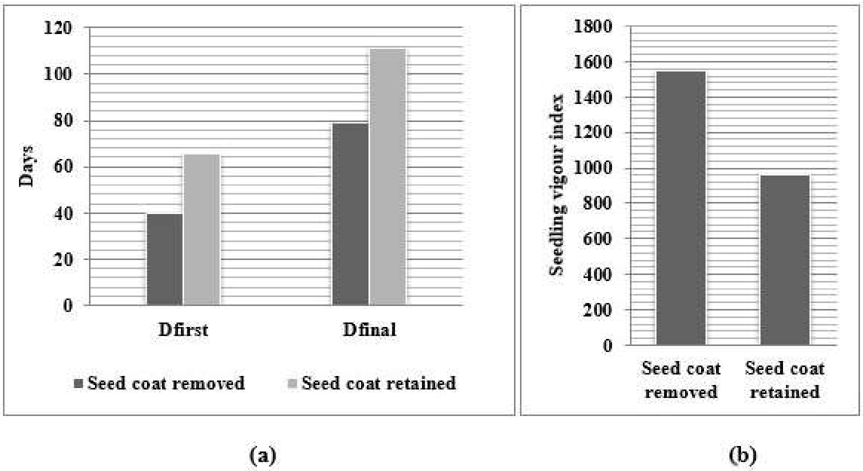

Fig. 2. Effect of seed coat removal on (a) days for first and final germination and (b) seedling vigour index

The *t*-test analysis was carried out for the seedling growth parameters. Highly significant superiority of seedling characteristics such as seedling length (16.14 cm), shoot length (6.68 cm), root length (9.46 cm), number of roots per seedling (18.90), number of leaves per seedling (12.80) and leaf length (5.56 cm) was observed in the treatment in which seed coat was removed. Leaf width was significantly superior in this treatment (1.06 cm) over seeds receiving seed coat retention treatment (0.83 cm) (Table 1, Fig. 3). Length of longest root and petiole length showed no significant differences between the treatments. Seedling vigour index is one of the important seedling characteristic, which could determine the further establishment under field conditions. In present study, higher SVI of 1547.7 was observed in seeds in which seed coat was removed as against 965.25 in treatment wherein seed coat was retained.

Table 1. Seedling morphological parameters as influenced by retention or removal of seed coat in *G. dhanikhariensis*

Parameters	Seed coat removed	Seed coat retained	t- test
Seedling length (cm)	16.135	11.535	**
Shoot length (cm)	6.680	4.970	**
Root length (cm)	9.455	6.415	**
Number of roots per seedling	18.900	9.350	**
Length of longest root (cm)	4.030	2.480	NS
Number of leaves /Seedling	12.800	11.200	**
Leaf length (cm)	5.560	4.140	**
Leaf width (cm)	1.055	0.830	*
Petiole length (cm)	0.205	0.185	NS

NS: non significant; **: significant at 1% level of significance, *: significant at 5% level of significance

As the germination started earlier in the seeds wherein seed coat was removed, the regenerated seedlings got more time to grow than those which germinated later and hence, superior seedling growth characteristics were observed in this treatment. As the SVI is a product of seedling length and percent germination, higher SVI in this treatment could be justified. These findings are in accordance with the earlier reports on endemic wild banana of the islands (Bohra et al., 2020).

Fig. 3. Seedlings obtained from seeds without seed coat (left) and seeds with seed coat intact

Experiment 2: Effect of seed division

Occurrence of *Garcinia* type germination has been reported in *G. dhanikhariensis* (Bohra et al., 2021) and hence, to explore the possibility of use of fragmented seeds for propagation, the present study was carried out. Germination was significantly influenced by division treatment (Fig. 4). In general, fragmented seeds showed higher germination percentage than that observed in intact seeds throughout the study period. As high as 84.35% seeds showed germination at 120 DAS in seed fragmented treatment as against 55.78% germination in intact seeds. Joshi et al. (2006) have reported that even small segments of seeds could give rise to seedlings in Malabar tamarind, which they proposed as a possible strategy for exploiting mammalian frugivory for seed dispersal. However, in the present study, only 7.48% of the fragmented seeds gave rise to two plants, one from each segment, while the intact seed gave single seedling per seed.

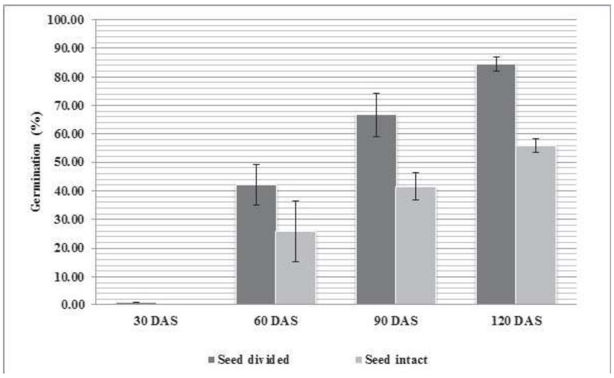

Fig. 4. Effect of seed division on germination percentage over the period of 120 DAS

Results revealed that fragmented seeds germinated (Fig. 5a) earlier (55.97 days) than the intact seeds (67.87 days), while the completion of germination took almost similar time (110.0 and 111.0 days) in both the treatments (Fig. 5b). None of the seedling growth parameters were influenced by the division treatments (Table 2, Fig. 6). Seedling vigour index was found to be higher (700.55) in fragmented seeds than the intact ones (577.71).

Experiment 3: Effect of size of seeds

Effect of size of seed on germination characteristics was studied, which showed significant differences among the treatments. Irrespective of the seed size, no germination was noticed at 30 DAS, which increased with passage of time (Fig. 7). Use of large sized seeds resulted in significantly higher germination of 7.48, 47.62 and 65.99% after 60, 90 and 120 DAS, respectively as against 4.08, 21.09 and 44.22%, respectively in the small seeds.

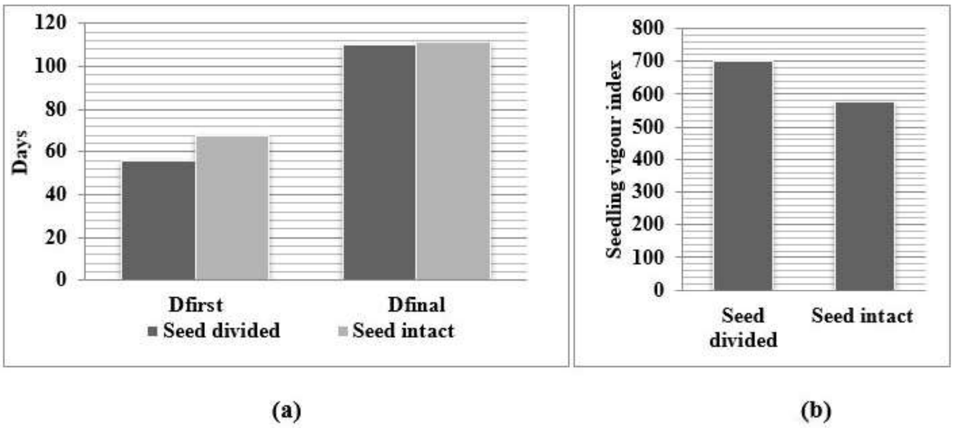

Fig. 5. Effect of seed division on (a) days for first and final germination and (b) seedling vigour index

Table 2. Seedling morphological parameters as influenced by seed division or non-division in *G. dhanikhariensis*

Parameters	Seed fragmented	Seed intact	t- test
Seedling length (cm)	9.715	10.355	NS
Shoot length (cm)	4.785	5.320	NS
Root length (cm)	4.930	5.020	NS
Number of roots per seedling	11.450	9.800	NS
Length of longest root (cm)	2.760	2.280	NS
Number of leaves /Seedling	11.500	10.900	NS
Leaf length (cm)	4.390	4.430	NS
Leaf width (cm)	0.800	0.795	NS
Petiole length (cm)	0.215	0.180	NS

NS: non significant; **: significant at 1% level of significance, *: significant at 5% level of significance

Fig. 6. Seedlings derived from seed segments (left) and intact seeds (right)

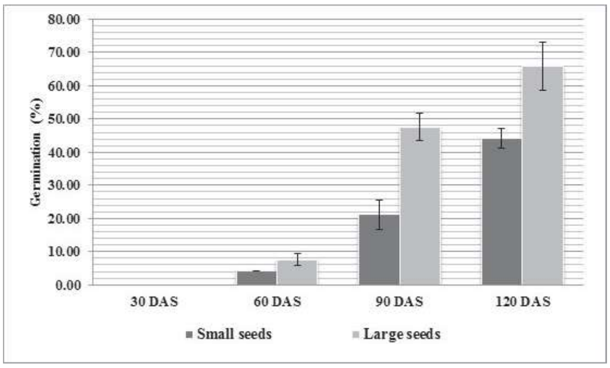

Fig. 7. Effect of seed size on germination percentage over the period of 120 DAS

Results suggested that the initiation (Fig. 8a) and completion (Fig. 8b) of germination process took marginally lesser time in large seeds (84.61 days and 117.33 days, respectively) than that observed in seeds of small size (88.58 days and 118.67 days, respectively). Seedling growth parameters were influenced due to the seed size used for experimentation (Table 3). Use of large sized seeds resulted in highly significant superiority for seedling length (9.76 cm) and root length (5.21 cm), when compared with use of small seeds (7.935 cm and 3.835 cm, respectively). Shoot length (4.55 cm), number of leaves per seedling (10.60) and leaf width (0.76 cm) were significantly superior in seedlings raised from large seeds; whereas other parameters were not influenced by the size of seeds.

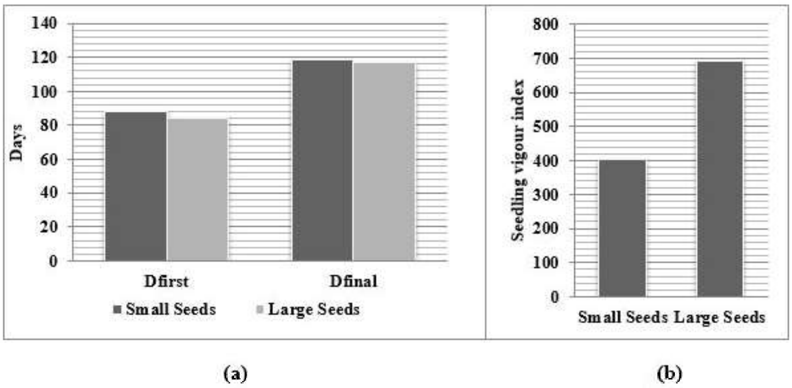

Fig. 8. Effect of seed size on (a) days for first and final germination and (b) seedling vigour index

Table 3. Seedling morphological parameters as influenced by seed size in *G. dhanikhariensis*

Parameters	Small seed	Large seed	t- test
Seedling length (cm)	7.935	9.760	**
Shoot length (cm)	4.115	4.550	*
Root length (cm)	3.835	5.210	**
Number of roots per seedling	6.650	6.300	NS
Length of longest root (cm)	1.495	1.325	NS
Number of leaves /Seedling	9.600	10.600	*
Leaf length (cm)	3.365	3.635	NS
Leaf width (cm)	0.650	0.755	*
Petiole length (cm)	0.155	0.165	NS

NS: non significant; **: significant at 1% level of significance, *: significant at 5% level of significance

SVI was observed to be higher (690.42) in seedlings regenerated from large seeds than those from smaller ones (404.84). Seed size is known to vary within a fruit and also within different genotypes/ collections of a species (Okonkwo et al., 2020). Seed size determines the amount of stored reserves and moisture content available in the seeds (Normah et al., 2016, Florent et al., 2021). Principal component analysis suggested that seed sizes in *G. kola* are positively correlated with germination and growth parameters (Agwu et al., 2018). Similar results have been reported in *G. mangostana* and thus, use of large sized seeds has been recommended for obtaining superior quality seedlings (Florent et al., 2021). Maturity and favourable physiological predisposition of the large seeds might have resulted in improvement of the germination characteristics.

Conclusion

The present study helped in understanding various germination features of this lesser known endemic species. Removal of seed coat before sowing and large sized seeds facilitated improving the germination percentage, seedling vigour and germination characteristics of the regenerated seedlings. Further, use of fragmented seeds improved the germination process apart from improving the possibility of getting two seedlings from each segment. These standardized nursery practices could be of great practical utility for mass multiplication of this endemic species.

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