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Seed Fatty Acid Composition and Germination Studies in *Garcinia dhanikhariensis* S.K. Srivastava (Clusiaceae): A Novel Tropical Fruit Species from Bay Islands, India

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ABSTRACT

Garcinia dhanikhariensis is an endemic tropical species distributed in the Bay Islands, India. The species has been found to be of horticultural significance owing to its edible fruits and potential as natural colorant. Being an endemic species, identifying the potential uses and standardization of nursery techniques of *G. dhanikhariensis* could be helpful in conserving and popularizing it. The present investigation aimed at determining fatty acid composition of seed fat using GC-MS and standardization of seed germination technique for mass multiplication. Results revealed that seeds had higher saturated fatty acids, dominant being stearic acid (43.62%) and oleic acid (42.40%). Among the pretreatments studied, soaking of seeds in potassium nitrate (0.1%) or gibberellic acid (500 mg/L) was recommended based on superior germination percentage and seedling growth parameters. The effect of seed source on germination characteristics was also studied, which revealed considerable variability among the collections. Regenerated seedlings were successfully transplanted without any mortality. Thus, seeds of *G. dhanikhariensis* were found to be novel sources of industrially important stearic acid and oleic acid. Nursery protocol discussed here would be useful for mass multiplication of this novel fruit species.

KEYWORDS

Conservation; fatty acids; GC-MS; seedling vigor index

Introduction

Members of Clusiaceae family, especially of the genus *Garcinia*, are known to produce edible fruits and have been valued for their medicinal properties. About 800 species of *Garcinia* have so far been reported to occur in tropical regions of the world (Osman and Milan, 2006). Species such as mangosteen (*G. mangostana* L.) have attained commercial status, whereas kokum [*G. indica* (Thouars) Choisy], Malabar tamarind [*G. gummi-gutta* L. (Roxb.)], Mysore gamboge (*G. xanthochymus Hook. f. ex T. Anderson*), mundu [*G. dulcis* (Roxb.) Kurz.], Cowa mangosteen (*G. cowa* Roxb. Ex Choisy) and kydia mangosteen (*G. kydia* Roxb.) are in semi-domesticated state in different parts of the tropics (Bohra et al., 2019; Parthasarathy et al., 2013). Most of the remaining species still occur in wild or semi-wild state (Bansude et al., 2013; Manikandan and Ramasubbu, 2017) and could hold promise as potential sources of industrially important compounds (Bohra et al., 2021, 2019).

Garcinia dhanikhariensis is an endemic species distributed in warm and humid tropical South Andaman Island in the Bay of Bengal, India (Srivastava, 1994). Fruits are dark red to purple with smooth skin (Figure 1). Fruit weight ranges between 11.6 and 59.2 g. Fruit dimensions are 2.5–3.8 cm × 2.7–5.3 cm with rind of 1.7–4.0 mm thickness. Each fruit has 1–6 developed seeds of 1.2–2.0 cm ×

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Figure 1. Harvested fruits of *G. dhanikhariensis*.

0.6–1.3 cm × 0.3–0.6 cm size. Pulp is light yellow, sour-sweet and is eaten by settler communities of the Islands. So far, no systematic research work has been attempted in this species after its discovery.

Seeds of *Garcinia* species have been identified as sources of industrial fats. *Garcinia* fat/butter could be utilized as a substitute to cocoa butter and as an ingredient in personal care products, pharmaceuticals, biofuels, edible oils and other products (Ananthakrishnan and Rameshkumar, 2016; Parthasarathy and Nandakishore, 2014). Though *Garcinia* butter has considerable potential, only a few species such as *G. andamanica* King, *G. indica, G. gummi-gutta* and *G. xanthochymus* have been studied (Bohra et al., 2021; Manohar et al., 2014; Patil et al., 2016), while other Indian *Garcinia* species are yet to be explored (Parthasarathy and Nandakishore, 2014; Patil et al., 2016). During the present study, fatty acid composition of *G. dhanikhariensis* was carried out for the first time.

Recently, we have identified potential of this species as a source of natural colorant (Bohra and Waman, 2020). Production of quality planting material is necessary to facilitate conservation and cultivation of this endemic species. Considering this, present paper is the first attempt to standardize chemical pretreatments and seed source on germination in *G. dhanikhariensis*. Soaking of seeds with gibberellic acid, kinetin, cow milk and hydrogen peroxide have been reported to improve germination process in *G. cowa, G. gummi-gutta* and *G. tinctoria* (Joseph et al., 2007). Suitable pretreatments could not only improve the germination percentage, but also improve the vigor of regenerated seedlings, which can facilitate better field establishment (Bohra et al., 2018). Further, source of seed has been reported to influence germination characteristics in *Garcinia* species and identification of superior seed source plays an important role in conservation and breeding programs (Bansude et al., 2013; Zobel and Talbert, 1984).

The present report concerns determination of seed fatty acid composition in this lesser known species and standardizing seedling production technique for its mass multiplication.

Materials and Methods

Collection of Samples

Fruits of *G. dhanikhariensis* were collected from three locations of South Andaman Island, India (GDH/SA/LP, GDH/SA/KT and GDH/SA/DK). During ripening, fruit color changes from green to dark red to purple. Fully mature fruits fall down naturally from the trees, which is a maturity index for this species. Such tree fallen fruits were collected from the ground and washed with tap water. Fruits were allowed to ripe at room temperature (27 ± 2°C) till they turned soft. Fruits were cut opened and

seeds were manually extracted by rubbing the pulp between hands under running tap water. After complete removal of pulp, seeds of collection GDH/SA/DK were used for determination of fatty acid composition, while seeds of GDH/SA/LP were used for imparting different germination pretreatments as described in the section below. For studying the seed source, seeds of all the three collections were used.

Fatty Acid Profiling

Lipid Fractionation and Esterification

Procedure for lipid fractionation and esterification as described in Bohra et al. (2021) was used. Seed sample was accurately weighed to 5 g, followed by homogenization in 10 mL of chloroform: methanol (3:1, v/v) and filtration through filter paper. The residue was re-extracted thrice using the said solvent and resultant filtrate was pooled. Water was then removed by using a separating funnel. Sodium sulfate was added to it, volume was made up to 50 mL and 5 mL of extract was taken for drying in a conical flask. For esterification, BF_3/methanol (2 mL) was transferred in a conical flask through a condenser and then heated for 3 min at 45–50 °C. Heptane (2 mL) was then added through a condenser, followed by heating for 3 min. After heating, 1 mL of heptane was added to it, mixed well and then was allowed to cool. Saturated sodium chloride (2 mL) was added to the flask, followed by vigorous shaking. After this, all the contents were taken into test tube and the heptane layer was separated to another test tube containing sodium sulfate. After about 3 min, the heptane layer was filtered through nylon syringe filters of 0.2 µm size and filtrate of 2 µL was used for injecting in the GC-MS.

as Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry analysis was carried out using Varian-3800 (Varian, USA) Gas Chromatograph along with Varian 4000 GC-MS-MS ion trap mass selective detector as detailed in Bohra et al. (2021). Separation of fatty acids was carried out using fused silica capillary column (VF-5 MS) with dimension: 30 m × 0.25 mm id and 0.25 µm film thickness. Helium at the rate of 1 mL/min was used as carrier gas with injector temperature of 260 °C. Temperatures maintained in the assembly were 220 °C for ion source, 200 °C for trap and 260 °C for transfer line. Mass detector was used with scan range of 50–450 amu in EI-mode (70 eV). For identification of fatty acid compounds, relative retention times of FAME peaks were compared with those of reference standards (Sigma-Aldrich, USA) along with spectral comparison with references viz. Wiley and NIST-2007libraries.

Seed Germination Studies

Effect of Pre-treatments on Seed Germination

Seeds of GDH/SA/LP collection were subjected to different pretreatments, viz. T_1: soaking in water for 24 h (control); T_2: soaking in 0.1% potassium nitrate (KNO_3) for 24 h; T_3: soaking in thiourea (2%) for 24 h and T_4: soaking in 500 mg/L gibberellic acid (GA_3) for 24 h. Treated seeds were sown in plastic protrays filled with soil: vermicompost (1:1, v/v) as substrate. The experiment was laid in completely randomized design with four replications and each treatment had hundred seeds. Observations on seed germination were recorded after 2, 3 and 4 months of sowing. Emergence of plumule above the substrate was used as an indication of germination. Seedling growth parameters such as shoot length (cm), root length (cm), number of roots and number of leaves were recorded after 128 days of sowing in ten randomly selected seedlings from each treatment. Germination (%) was calculated as (number of germinated seeds/ number of seeds sown) × 100, while seedling vigor index (SVI) was calculated as per formula described earlier, i.e. germination (%) × seedling length (Abdul Baki and Anderson, 1973).

Effect of Seed Source on Germination

To determine the effect of seed source on germination, the best treatment obtained in the germination experiment was applied to seeds from GDH/SA/DK and GDH/SA/KT locations. Data obtained from the retained treatment in the previous experiment were used for comparison. Final germination percentage was recorded after 128 days of sowing and germination (%) and SVI were calculated as described above.

Analysis of Data

Data collected from the various experiments was used for calculation of mean and standard error of mean (SEm) using Microsoft Excel software and values were presented as mean ± SEm in respective tables/figure. Further, data pertaining to seedling growth parameters and SVI was subjected for analysis of variance (ANOVA) using Web Agricultural Statistical Package (WASP ver. 2.0) (ICAR-CCARI, Ela, India) and mean separation was done using least significant difference.

Results and Discussion

Fatty Acid Composition in Seeds

Profiling of fatty acids of seeds suggested presence of seven compounds (Table 1), which contributed to 98.80% of the total composition. Of these seven compounds, three were saturated fatty acids (SFAs), three were monounsaturated fatty acids (MUFAs), while one was polyunsaturated fatty acid (PUFA). In general, higher amounts of saturated fatty acids (52.69%) were present than unsaturated ones. Dominance of SFAs has been reported in other species such as *G. hanburyi* Hook. f. (53.11%) and *G. gaudichaudii* Planch. & Triana (61.15%) (Phuong et al., 2018). Of the unsaturated fatty acids observed, amounts of MUFAs were higher than that of PUFAs, which is in line with that found in *G. andamanica* (Bohra et al., 2021), *G. multiflora* Champ. ex Benth. and *G. hanburyi* (Phuong et al., 2018).

Stearic acid was found to be the most abundant compound (43.62%). In general, stearic acid is present in lesser frequencies in fats derived from plant sources. However, this compound has been reported to be present to a tune of about 28–45% in industrially important cocoa and shea butters (Beare-Rogers et al., 2001); while *G. gaudichaudii* (50.12%), *G. hanburyi* (58.87%) and *G. andamanica* (11.83%) are other species containing stearic acid (Bohra et al., 2021; Phuong et al., 2018). Stearic acid is a common constituent in the manufacture of detergents, soaps, shaving creams and similar cosmetic products (Parthasarathy and Nandakishore, 2014). *G. dhanikhariensis* with significant amount of this compound could be utilized as potential alternative for these industries. Botanical relatedness might result into similarity or dissimilarity in the chemical composition of *Garcinia* species. For example,

Table 1. Fatty acid composition of *G. dhanikhariensis* seeds.

Fatty acid methyl ester	C number	Class of compound	Peak area (%)
Palmitic acid	16:0	Saturated	8.21 ± 1.127
Stearic acid	18:0	Saturated	43.62 ± 0.494
Arachidic acid	20:0	Saturated	0.86 ± 0.347
Σ Saturated fatty acid			52.69
Palmitoleic acid	16:1	Monounsaturated	0.08 ± 0.040
Oleic acid	18:1	Monounsaturated	42.40 ± 1.009
11-Eicosenoic acid	20:1	Monounsaturated	0.75 ± 0.052
Σ Monounsaturated fatty acid			43.23
Linoleic acid	18:2	Polyunsaturated	2.88 ± 0.041
Σ Polyunsaturated fatty acid			2.88
Σ Unsaturated fatty acid			46.11
Total			98.80

G. dhanikhariensis is botanically close to *G. gaudichaudii* (Srivastava, 1994) and both species exhibited dominance of stearic acid.

Oleic acid was second dominant compound present in the seed fat to the tune of 42.40%. Oleic acid has been regarded to have the ability to reduce the low density lipoproteins cholesterols (Grundy, 1989) and hence, is considered as a beneficial compound. It is one of the dominant compounds in *G. andamanica, G. multiflora, G. hanburyi, G. gummi-gutta, G. morella* (Gaertn.) Desr., *G. indica, G. xanthochymus* and *G. mangostana* (Ajayi et al., 2007; Bohra et al., 2021; Choppa et al., 2015; Patil et al., 2016; Phuong et al., 2018). Vietnamese *Garcinia* species rich in oleic acid have been identified as healthy oil sources for commercial utilization (Phuong et al., 2018). Further, fractionation, counter-current distribution and hydrophilization techniques could also improve the recovery of this component from seed oil of *G. dhanikhariensis* as reported in *G. gummi-gutta* (Choppa et al., 2015).

Further, palmitic acid (8.21%) was also present in seeds of *G. dhanikhariensis*. Palmitic acid is the most common constituent in soaps, cosmetics and skin care products due to its positive effects on skin including softening and healing effects (Parthasarathy and Nandakishore, 2014). Apart from the major compounds, linoleic acid, palmitoleic acid, arachidic acid and 11-eicosenoic acid were also present in the studied species, which have been reported at varied concentrations in other *Garcinia* species as well (Bohra et al., 2021; Manohar et al., 2014; Patil et al., 2016).

Seed Germination

Sexual propagation is known to provide opportunity for creation and maintenance of natural diversity in a species. This is highly relevant for conservation of endemic species as seedling progenies support the evolution process in nature and created diversity could be used for identification of desirable types (Bohra et al., 2018). Chemical seed pretreatments have been reported to improve germination process and seedling growth. However, the kind of treatment is generally species specific and hence, identification of appropriate treatment is required (Butola and Badola, 2004).

During the present investigation, effect of pretreatments on germination process of *G. dhanikhariensis* was studied, which revealed significant differences among the treatments. Data recorded 2 months after sowing (MAS) revealed lowest germination (42%) in seeds soaked in water (control), followed by 44% in thiourea treatment. Potassium nitrate (KNO_3) and GA_3 significantly improved the germination as 71 and 69% germination was noticed in these treatments, respectively (Figure 2). Similar trend was recorded after 3 and 4 MAS also. At 3 MAS, germination varied from 68% in control to 86% in KNO_3 treatment. Final observations recorded after 4 months of sowing revealed that lowest germination (72%) was observed in control. Both KNO_3 and GA_3 retained their superiority with 87 and 86% germination, respectively.

Seedling development was found to be normal and healthy in all the treatments throughout the experimental period (Figure 3). Seedling growth parameters recorded 128 days after sowing revealed significant differences for shoot length, number of roots per seedling and SVI (Table 2). Shoot length varied significantly from 9.0 cm in control to 10.9 cm in GA_3. Number of roots per seedling was lowest (11.9) in seeds treated with KNO_3. It remained statistically similar with values reported in water soaking control and treatment with thiourea. As high as 17.9 roots were produced per seedling in case of seeds treated with GA_3. A typical type of germination, commonly called as '*Garcinia*-type' germination, was seen during the present study (Figure 3). It is characterized by development of radicle and plumule from opposite ends of seeds (Joshi et al., 2006). In such cases, embryo is present in the seed in the form of elongated hypocotyl that connects the two poles though a vasculature connection (Joshi et al., 2006). However, the adventitious roots arising at the plumular end develop into main root system for the rest of plant life. Such phenomenon has been reported in a number of *Garcinia* species such as *G. gummi-gutta, G. indica, G. xanthochymus, G. mangostana, G. prainiana, G. atroviridis* and *G. hombroniana* (Joshi et al., 2006; Malik et al., 2005; Normah et al., 2016). Recently, we have reported occurrence of this phenomenon in another endemic species of these Islands i.e. *G. andamanica* (Bohra et al., 2021).

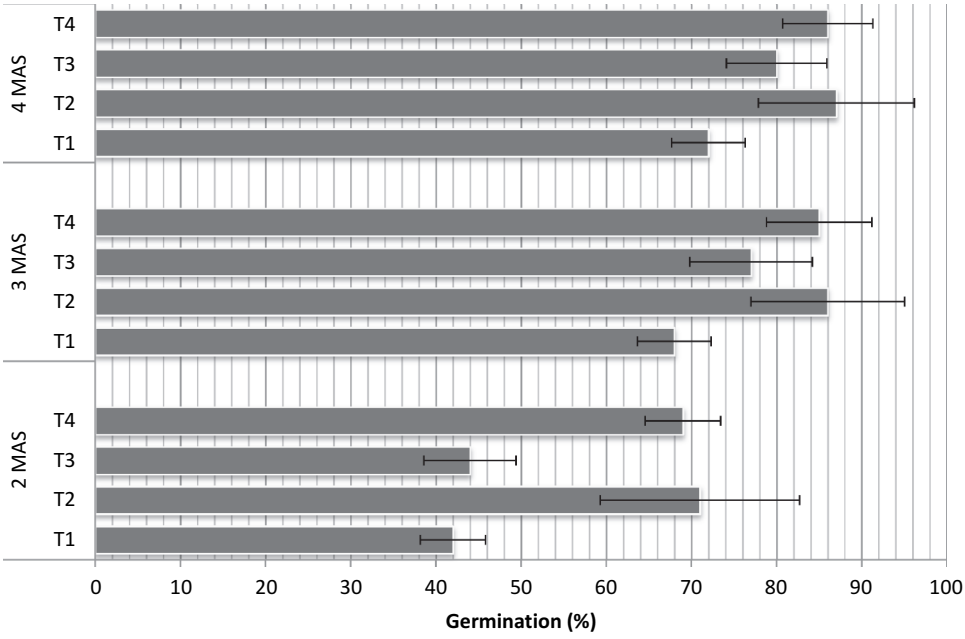

Figure 2. Seed germination (%) as influenced by seed pretreatments [T_1: soaking in water for 24 h (control); T_2: soaking in 0.1% KNO_3 for 24 h; T_3: soaking in 2% thiourea for 24 h and T_4: soaking in 500 mg/L GA_3 for 24 h] after 2, 3 and 4 months of sowing (MAS).

Figure 3. Healthy seedlings of *G. dhanikhariensis* (GDH/SA/LP) in KNO_3 treatment (left) and seedling exhibiting *Garcinia*-type germination (right) (PR: primary root; AR: adventitious root).

Seedling vigor index is a measure to determine the vigor of regenerated seedlings. The poorest SVI (1111.7) was reported in seedlings obtained from control. All the chemical treatments studied improved SVI significantly and the highest SVI of 1535.1 was reported in seeds treated with GA_3. This value, however, remained statistically similar with that of KNO_3 treatment (1412.9). Seed pretreatment had no influence on root length and number of leaves per seedling, and their values ranged between 5.8–6.9 cm and 6.6–8.2 cm, respectively.

In general, use of KNO_3 at 0.1–0.2% concentration has been recommended for improving seed germination by Association of Official Seed Analysts and International Seed Testing Association (Saini

Table 2. Seedling parameters as influenced by seed pretreatments after 128 days of sowing.

Treatment	Shoot length (cm)	Root length (cm)	No. of leaves	No. of roots	Seedling vigor index
T_1 (water soaking)	9.0 ± 0.12b	6.4 ± 0.37a	7.4 ± 0.45a	14.3 ± 1.12ab	1111.7 ± 28.64 c
T_2 (KNO_3 0.1%)	9.5 ± 0.31b	6.8 ± 0.45a	7.8 ± 0.47a	11.9 ± 0.89b	1412.9 ± 45.19ab
T_3 (thiourea 2%)	10.1 ± 0.30ab	5.8 ± 0.27a	6.6 ± 0.43a	15.0 ± 1.48ab	1272.0 ± 28.62b
T_4 (GA_3 500 mg/L)	10.9 ± 0.70a	6.9 ± 0.59a	8.2 ± 0.47a	17.9 ± 1.66a	1535.1 ± 78.11a

Values presented as mean ± SEm. Values followed by a similar alphabet in a column do not differ significantly at $p< 0.05$ using least significant difference.

Table 3. Seed germination parameters as influenced by seed source after 128 days of sowing.

Seed source	Germination (%)	Seedling length (cm)	No. of leaves	No. of roots	Seedling vigor index
GDH/SA/DK	96.0	18.5 ± 0.53 a	7.6 ± 0.34 a	9.0 ± 1.07 b	1777.0 ± 51.21 a
GDH/SA/KT	54.1	13.1 ± 0.41 c	4.9 ± 0.31 b	2.6 ± 0.50 c	708.7 ± 22.23 c
GDH/SA/LP	87.0	16.2 ± 0.52 b	7.8 ± 0.47 a	11.9 ± 0.89 a	1412.9 ± 45.19 b

Values presented as mean ± SEm. Values followed by a similar alphabet in a column do not differ significantly at $p< 0.05$ using least significant difference.

et al., 1984). Nitrates are known to augment germination by varying K^+/Na^+ ratio (Zheng et al., 2009) and by providing nutrients to germinating seedlings (Hegazi et al., 2011). The utility of KNO_3 in improving seed germination has been reported in *G. andamanica* as well (Waman and Bohra, 2019). The efficacy of gibberellins in improving germination process has been reported by several researchers in tropical fruit species (Khan, 2015; Samir et al., 2015; Waman et al., 2018). The main role of gibberellic acid is to increase the production of hydrolase enzyme (Prasad and Prasad, 2009). It also supports mobilization of starch for respiration process (Shah, 2007) and increases mineral availability thereby aiding cell elongation and germination process.

To study the effect of seed source on seed germination parameters, results of KNO_3 treatment of GDH/SA/LP were compared with two other collections (Table 3). In general, collection GDH/SA/KT showed the poorest germination parameters, mainly due to delayed start of germination in GDH/SA/KT than the other two collections. Germination percentage varied between 54.1% (GDH/SA/KT) to 96.0% (GDH/SA/DK). Seedling length (18.5 cm) and seedling vigor index (1777.0) were significantly highest in GDH/SA/DK, whereas it was the lowest in GDH/SA/KT (13.1 cm and 708.7, respectively). Statistically similar number of leaves per seedling (7.8 and 7.6) were produced in GDH/SA/LP and GDH/SA/DK. The number of roots per seedling varied significantly from one source to another. Seeds collected from GDH/SA/LP produced as high as 11.9 roots per seedling against 2.6 roots per seedling produced in GDH/SA/KT collection. Identification of superior seed source has been reported to be a viable tool for assisting utilization of wild *G. kola* (Kanmegne and Omokolo, 2007). The vigorous seedlings of GDH/SA/DK could be used for establishment of conservation blocks and also for establishing orchards for regular supply of fruits and seeds to various industries.

Findings of the present study would be helpful in conserving this valuable genetic resource apart from unraveling its potential for area expansion in other humid tropical regions.

Conclusion

G. dhanikhariensis was found to be a promising crop for cultivation in the humid tropical regions due to its multifaceted uses. Seeds were found to be novel source of industrially important stearic acid and oleic acid. Considering the improved germination percentage and seedling vigor, soaking of seeds for 24 h in KNO_3 (0.1%) or GA_3 (500 mg/L) could be recommended for production of seedlings in large number in this species. Furthermore, the source of seeds influenced germination parameters and hence, the use of seeds from selected mother plants could help in obtaining seedlings of desirable quality in large numbers.

Considering the potential uses of the species, the present findings would be of great help for promoting its cultivation in humid tropical regions across the world.

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Disclosure Statement

The authors declare that they have no conflict of interest.

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