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Embryo maturation, dormancy and seed storage behaviour of *Gymnacranthera canarica* (King) Warb., a threatened endemic tree species of Southern Western Ghats

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ABSTRACT

Gymnacranthera canarica is a severely endangered endemic tree species found in the Myristica swamps of the Southern Western Ghats. Seed storage behaviour is an essential factor to consider when developing effective conservation methods for plant genetic resources whose *ex-situ* preservation is unclear. The seed storage behaviour, seed dormancy state, dormancy breaking treatments, germination, and phytohormonal analysis of *G. canarica* were explored in this work. *G. canarica* seed moisture at shedding was 28.86%, germinated to 34% at 25°C, and had a low germination rate in natural conditions. Germination was considerably aided by Gibberellic acid pretreatment and drying stratification. *G. canarica* seeds may have non-deep simple morphophysiological dormancy, as evidenced by the fact that their embryos grew at temperatures between 20 and 25°C (MPD). Seeds can be stored for up to 60 days at 20°C after being desiccated to 19.37% suggest that *G. canarica* seeds are recalcitrant, with non-deep simple morphophysiological dormancy, and that seed pretreatment with Gibberellic acid prior to germination could be a viable approach for mass propagation and long term *ex-situ* conservation could be the potential storage of this critically endangered species.

KEYWORDS: *Gymnacranthera canarica*, recalcitrant, Storage behaviour, Dormancy, Embryo immaturity, Gibberellic acid

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INTRODUCTION

Seed storage behavior is vital for preparing appropriate conservation strategies for plant genetic resources (Wyse & Dickie, 2017). Recalcitrant seed conservation is the most challenging issue in reducing *ex-situ* conservation in moist tropical forest trees (Pritchard, 2004). Identifying the seed storage behaviour and studies on germination helps to define the propagation of species and thereby plan appropriate conservation strategies. Moreover, such studies help to understand natural regeneration, what causes the species to decline and their response to climatic change and changing landscapes (Schütz, 2000; Tanaka-oda *et al*., 2009; Kleemann & Gill, 2013).

Recalcitrant seeds usually show a rapid germination response as they lack "metabolic arrest", especially species occurring in tropical regions or aquatic environments (Vertucci & Farrant, 2017). Moreover, higher seed moisture at shedding time also favours seed germination (Chin, 1989). But, *G. canarica* in natural conditions shows delayed and highly erratic germination (field observation) and takes almost 6-8 weeks to initiate germination in a humid environment (Howe, 1990; Pina-Rodrigues & Figliolia, 2005). These seeds require pretreatment for germination like stratification periods may indicate dormancy and could be due to immature embryos or the presence of germination inhibitors. Natural regeneration of this threatened tree species is less than 10% in the Myristica swamp habitat; seeds are naturally shedding in the first week of September having heavy rainfall, and most of the seeds are washed away in flooded forest grounds. There is a lack of knowledge regarding the dormancy, germination and regeneration capacity of this tree species, which is needed for conservation strategies. The objectives of the current work were to identify the seed storage behaviour and type of dormancy in *G.canarica* seeds through experimental approaches.

MATERIALS AND METHODS

Study Material, Study Site and Seed Collection

Gymnacranthera canarica of the family Myristicaceae (nutmeg family), is an endemic vulnerable tropical tree species limited to freshwater *Myristica* swamp forests (Chandran & Mesta, 2001). The best representatives of this habitat are found in the Sasthanada swamp of Sangili section, Kulathupuzha forest range (Latitude 8º 45 N, longitude 77º 10 E, altitude 155 MSL), a southern ecosystem of southern Western Ghats. *G. canarica* seeds were collected during September month of 2019 and 2020 from an elevation of 155 MSL, and mature seeds were identified by deep red-coloured lacinated aril. After carefully removing the aril, healthy seeds chosen by floatation method were washed in distilled water and surface dried for further experiments for a week before becoming non-viable.

Seed Morphology and Moisture Content

Randomly chosen, five replicates of 10 fresh seeds were selected to determine the length, width and thickness using a digital vernier calliper (Mitutoyo absolute digimatic). Seeds after immediate harvesting were used for determination of seed moisture content by Low constant temperature hot air oven method at 103ºC for 17 ± 1 h (ISTA, International Seed Testing Association 1993). Data from five replicates were calculated on a fresh weight basis.

Seed Storage Behaviour

To determine the seed storage behaviour of *G. canarica*, an experimental protocol proposed by Hong and Ellis (1996) was conducted. Five lots of 10 seeds each were randomly selected and each lot was initially weighed with a digital balance and air dried at ambient laboratory temperature (25±2ºC, 60% RH) in open plastic trays to the desired moisture content (23.82%, 19.37% and 12.94%). The initial moisture content of freshly harvested seeds was 28.86%. When seeds reached the target seed moisture content, they were tested for viability, germination percentage and rate, and subsequent seedling growth. Five replicates of 20 seeds from four seed moisture contents (initial mc 28.86%, 23.82%,19.37% and 12.94%) were sown in polythene nursery bag for germination experiments filled with vermiculate soil in ambient laboratory temperature (25±2ºC, 60% RH) and watered regularly. Seeds were monitored for germination (5mm radicle emergence) at everyday intervals for 60 days, and non-germinated seeds were cut open to check their viability. Viable seeds appeared with white endosperm and creamy white coloured embryo while seeds with brown shrunken endosperm with fungal attack and brown to black embryo were non-viable.

Effect of Low Temperature and Storage on Seed Viability and Germination

A completely randomized factorial design was used to evaluate the effect of temperature and storage. In this study, assigned five temperature conditions -ambient room temperature 25±3ºC, 20 ± 3ºC, 10ºC, 4ºC and 0ºC. Six storage duration of 10, 30, 45, 60, 75 and 90 days were assigned for 25 ± 3ºC, 20 ± 3ºC storage temperatures and three storage duration of 10, 30 and 60 days for 10ºC, 4ºC and 0ºC respectively. Each lot of seeds was stored hermetically in medium-sized polycarbonate bottles and polyethene covers. There were 42 treatments in total, resulting from the factorial combination of the two storage temperatures, six storage duration, three storage temperatures, and three storage duration in two different mediums. After the experimental storage, five replicates of 10 seeds for each treatment were assigned to viability check, germination percentage, speed of germination, time to the first germination, and mean germination time.

Seed Dormancy

Morphophysiological dormancy (MPD) shows seeds readily imbibe water but requires more than 30 days to germinate because of the underdeveloped embryo that is also physiologically dormant.

Water Imbibition Test

Twenty-five seeds were manually scarified by removing a small proximal portion of the seed coat using a razor blade, and the initial weight of each seed was measured. The weight of 25 non-scarified seeds also was noted. Both sets of seeds were placed on 9 cm Petri dishes lined with moist filter papers and covered under identical laboratory conditions. Each seed were reweighed after 2, 4, 8, 12, 24 and 48 hours; the changes in seed mass averaged from the 25 seeds from both intact and scarified lots.

Effect of Gibberellic Acid (Ga3) on Breaking Seed Dormancy, Germination, Root and Shoot Emergence Time and Development

To determine the effect of gibberellic acid (GA_3) on *G.canarica* seed dormancy breaking and the rate of germination rate, five replicates of 20 seeds were maintained for each treatment. Seeds were soaked separately in 100, 200, 300, 400, and 500 mg L^{-1} gibberellic acid (GA3) for 12 and 24 hours. Seeds without any treatment were taken as control, and both the (control and treated) were sown in polythene bags filled with vermiculate soil. Then transferred to the greenhouse with a photoperiod of 8 h and a constant temperature of 25±2 ºC. The germination of seeds in the greenhouse was monitored at everyday intervals after sowing for 60 days. The first day of germination (FDG), last day of germination (LDG), Germination percentage (GP), and Mean germination time (MGT) were recorded for each seed lot, and after 90 days of germination, plant height (PH), length of both root (RL) and shoot (SL) were measured using a digital vernier calliper to the nearest millimetre. The seedling vigour index (SVI) of treated seedlings was compared with control seeds using the method suggested by Abdul-Baki and Anderson (1973).

Embryo Length and Seed Length Measurement

Experiments were made to study the influence of dehydration on seeds and embryo growth. Mature seeds shed with 28.86% as control, and seeds were desiccated to 23.82% (3 days of dehydration), 19.37% (5 days of dehydration), 12.94 (12 days of dehydration) and 10.72% (15 days of dehydration) by air drying method in ambient laboratory conditions. Seeds at each stage of dehydration were dissected lengthwise with a razor blade along the long axis, and the seed embryos were observed and photographed. The embryo and seed length was measured in longitudinally sectioned seeds. Five replicates of 20 seeds from the control lot (28.86%) and seeds with critical moisture content lot (19.37%) were placed on filter paper moistened with distilled water and GA_3 100 mg L^{-1} in a 9 cm Petri dish and kept in the laboratory. The length of embryos and seeds for five seeds from each treatment was measured over a period of 60 days until all seeds were dead. To know the critical length at which the embryo grows maximum inside the seed before radicle emergence, embryo and seed length were measured with germinated seeds treated with GA_3 in both control seeds (28.86% mc) and dehydrated seeds (19.37% mc) The embryo length was measured up to the point where the radicle emerged from the seeds, and the ratio of embryo length to seed length (EL/SL) was measured.

Statistical Analysis

All the investigated parameters were analyzed using analysis of variance (ANOVA) and significance was determined at $P<0.05$, and the means were compared using Duncan's Multiple Range Test (DMRT). The Variability in data was expressed as mean± standard error.

Endogenous Phytohormones

To determine the endogenous phytohormones *viz.* abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), cytokinin (CK), auxin (IAA) and gibberllic acid (GA_3) content in dormant and nondormant *G. canarica* seeds, seed samples were weighed (250 mg), frozen with liquid nitrogen and kept at −80ºC until used. For phytohormone analysis, finely ground seed material was extracted with 1.5 mL of methanol containing 25 ng mL^{-1} D4-salicylic acid, D6-abscisic acid, jasmonic acid-[$^{13}C_6$] isoleucine conjugate, trans-[$^2H_5$] zeatin,[$^2H_5$]-indole-3-acetic acid ($^2H_5$-IAA) and [$^2H_2$]-GA_1 as internal standards. The homogenate was mixed for 30 min and centrifuged at 14,000 rpm for 20 min at 4ºC. After the supernatant was collected, the homogenate was re-extracted with 500 µL methanol, mixed and centrifuged, and the supernatant was pooled. The combined extract was evaporated in a speed vac at 30ºC and re-dissolved in 500 µL methanol. Phytohormones were quantified by liquid chromatography coupled with a SCIEX 6500+ 488 triple-quadruple-trap MS/MS as described (Vadassery *et al.*, 2012; Šimura *et al.*, 2018).

RESULTS

Seed Morphology and Moisture Content

Fresh *G. canarica* seeds at the time of shedding possess an average mass of 4.2 ± 0.09 g and seed length of 18.98 ± 0.19 mm, seed width of 20.58 ± 0.27 mm and seed thickness of 18.17 ± 0.25 mm, covered by bright red coloured deep lacinate aril (Figure 1). The seed moisture content (mc) was 28.86 ± 0.17%.

Seed Storage Behaviour

The viability of seeds was significantly reduced by desiccation (pv0.001) and was lost below 10.72 ± 0.17% seed moisture content within 15 days of open desiccation at ambient laboratory conditions (Figure 2). Surprisingly, dehydration positively affected seed germination ($p<0.001$) (Figure 2A). Maximum germination of 80% was observed in seeds with 19.37% mc on the fifth day of open desiccation; thereafter, germination percentage decreased. Seeds dehydrated to 10% MC with 15 days of desiccation became dead. Seed storage studies in *G. canarica* showed viability ranging from 0 to 82%. There was a highly significant ($p<0.001$) interaction effect between the temperature and duration in different storage mediums on the seed germination. Seed germination percentage decreased with a decrease in storage temperature and with increased storage duration. Seeds stored at ambient laboratory temperature in both hermetic and poly ethylene bags lost viability in 45 days of storage (Figure 2B). Data showed that bagged seeds stored at a temperature of 20 ± 3ºC retained 50% viability for 60 days, while in the same

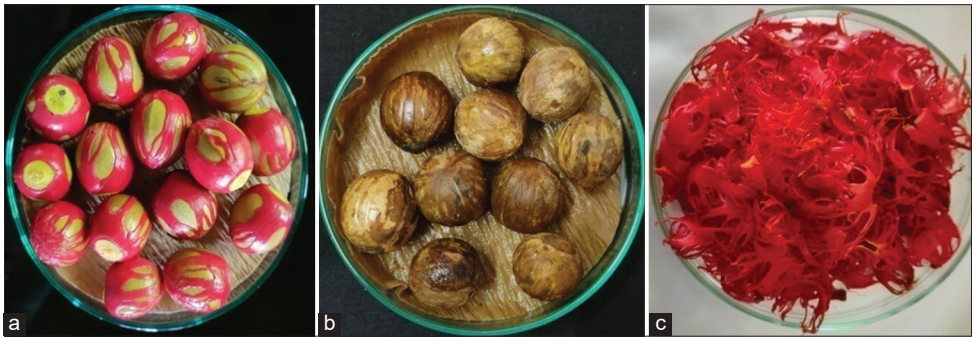

Figure 1: Protective coverings of *G. canarica* seeds. a) Fresh seeds covered by lacinated aril, b) Brown testa covered mature seeds, c) red coloured fleshy aril

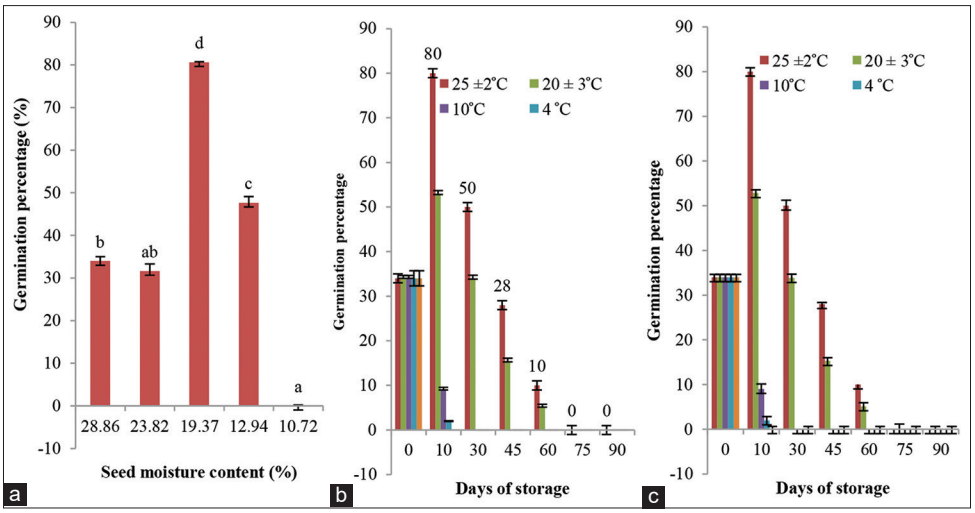

Figure 2: Effect of dehydration on germination percentage. a) Effect of storage temperature and duration on germination percentage in different storage medium, b) Hermetic storage, c) Seed storage cover

temperature, 33.85% of germination was maintained up to the 30 days in hermetic conditions. Storage for ten days at 4°C or 0°C was equally found to be detrimental. The highest germination percentage was observed in the bagged seeds stored at 20 ± 3°C for a maximum of 60 days.

Imbibition Test

The initial moisture content of scarified seed was 27.71 ± 0.21%, and the intact seed was 28.9 ± 0.17%. Both scarified and intact seeds absorbed water (Figure 3) and after 48 hours, moisture content of scarified seed becomes 29.52 ± 0.17% and intact seed, 28.92 ± 0.15%. The increase in the mass of seed from both treatments was nearly 6% at 48 hours in both scarified and intact seeds. There is no significant difference between scarified ($P < 0.5$) and non-scarified seed mass of this species. A Kruskal-Wallis H test showed the statistical difference in seed weight between the different duration of imbibitions as, $\chi^2 = 8.520$, $P=0.014$ in scarified seeds and $\chi^2 = 16.033$, $P = 0.014$ in intact seeds.

Embryo and Seed Length Measurements

Seed anatomy during dehydration showed continuously growing embryos of this species (Figure 4). At the time of shedding from the mother tree, seed embryo length was 3.87 ± 0.008 mm (28.86% m.c) which was increased to the maximum of 5.86 ± 0.009 mm on the fifth day of desiccation with 19.37% seed moisture content. Dehydrated seeds (19.37% m.c) pretreated with GA_3 had a significantly lengthier embryo: seed length ratio (0.32) at day 20 and those of fresh seeds (28.86% m.c) on GA_3 at day 25 (0.29). For fresh seeds, the ratio remained low (0.19) until the end of the experiment on day 50. In contrast, dehydrated seeds had a ratio (0.30) on day 40, significantly higher ($p = 0.000$) than fresh seeds and fresh seeds with GA_3 after germination on day 25 (Figure 5). The mean ratio in the germinated *G. canarica* seed was 0.32 ±0.002.

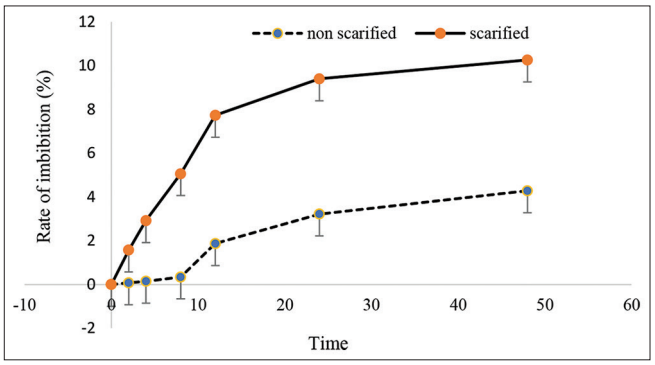

Figure 3: Imbibition curves for Scarified and Non scarified *G.canarica* seeds. Error bars

Germination Responses After Seed Pretreatment with Gibberellic Acid

The seed pretreatment with different concentrations of GA_3 significantly affected germination percentage and mean germination time. Primed seeds had higher GP than those in control, and a significant difference was observed among different concentrations of GA_3. The GP of control seeds was 34%, and seeds treated with 100ppm GA_3 for 24 hrs gave 100% germination with a mean germination time of 25.71 days (Table 1). Increased concentration of GA_3 (500 ppm) had an inhibitory effect on seed germination, and GP (26.33%) was lower than control seeds. Priming with GA_3 reduced the mean MGT in *G. canarica* seeds. Time to first germination (FDG) of control seeds was 47 days with an average of MGT 48.31±0.22 days, and the highest GP resulted in seeds treated with 100 ppm GA_3 for 24 hours shows 24 days FDG and 25.71 ± 0.33 MGT (Table 2). There was no significant difference in MGT among the different concentrations of GA_3 seed pretreatment. Priming with a lower concentration of gibberellic acid and longer duration had a stimulatory effect on seedling traits. The optimum concentration of gibberellic acid was 100 ppm GA_3 for 24 hours (Table 3). The seedling

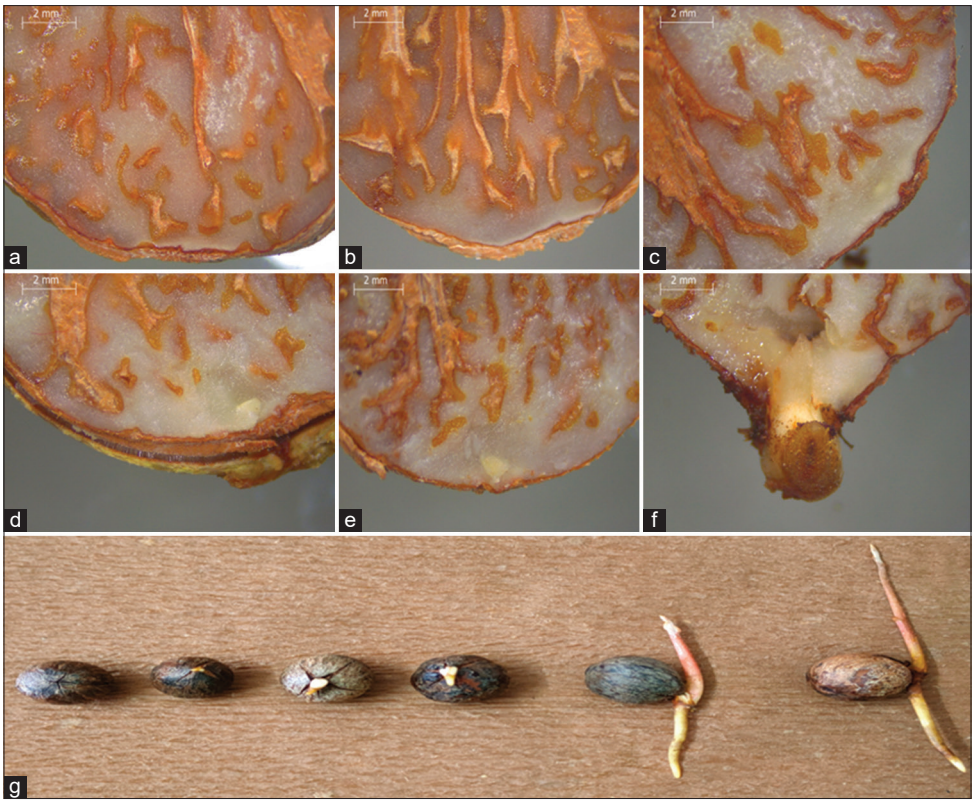

Figure 4: Embryo growth, plumule and radicle emergence in *G. canarica* seeds. a) fresh seeds (28.86% mc) with immature embryos, b) seeds after one week of warm stratification at 30ºC (19.37% mc) showing increase in embryo length, c) embryo growth began after 3 weeks of incubation, d) embryo after 4 weeks of incubation, e) embryo after 5 weeks of incubation, f) radicle emergence after 7 weeks of incubation, g) different stages of germination

Table 1: Effect of GA_3 treatment of *G. canarica* seed germination

GA_3 concentration (ppm)	Time duration	GP (%)	MGT (days)	FDG (days)	LDG (days)
0		34.00 ± 1.80^{a}	46.66 ± 0.33^{a}	49.31 ± 0.22^{e}	55.33 ± 0.84^{b}
100	12	84.33 ± 1.42^{d}	24.16 ± 0.54^{c}	26.00 ± 0.27^{a,b,c}	31.33 ± 0.66^{a}
100	24	99.83 ± 0.16f	24.00 ± 0.36^{b,c}	25.71 ± 0.33^{a,b,c}	31.00 ± 0.85^{a}
200	12	84.66 ± 1.20^{d}	22.66 ± 0.42^{a,b}	24.26 ± 0.56^{a}	31.00 ± 0.68^{a}
200	24	89.00 ± 0.96^{e}	23.33 ± 0.21^{a,b,c}	26.40 ± 0.57^{b,c}	34.00 ± 0.73^{a}
300	12	88.55 ± 1.17^{d,e}	22.33 ± 0.33^{a}	25.05 ± 0.76^{a,b}	33.33 ± 0.98^{a}
300	24	52.50 ± 2.14^{c}	23.16 ± 0.30^{a,b,c}	27.14 ± 0.90^{b,c}	32.66 ± 0.88^{a}
400	12	42.35 ± 2.14^{b}	24.16 ± 0.30^{c}	26.06 ± 0.82^{a,b,c}	33.83 ± 1.24^{a}
400	24	28.33 ± 0.84^{a}	26.83 ± 0.47^{b,c}	28.04 ± 1.32^{d}	32.66 ± 1.02^{a}
500	12	26.33 ± 0.13^{a}	24.81 ± 0.47^{b,c}	27.25 ± 0.13^{c}	33.00 ± 0.96^{a}
500	24	26.00 ± 0.84^{a}	23.80 ± 0.70^{b,c}	26.76 ± 0.35^{b,c}	33.33 ± 1.4^{a}
F value		448.75***	316.09***	94.23***	22.52***

GP – Germination percentage, MGT – Mean germination time, FDG- First day of germination, LDG – Last day of germination. Note: For each treatment, means followed by the same letter in each row do not differ significantly at $p<0.05$

vigour index was maximum for the seedling treated with a lower concentration of 100 ppm GA_3 (4010.50) for 24 hours duration, and higher concentration of gibberlic acid did not impose any significant enhancement in germination and seedling traits were similar to the control seedlings (Table 2).

Endogenous Phytohormones

Phytohormones were analyzed in fresh seeds (28.86% mc, embryo length - 4.06 mm) with immature embryos and warm stratified seeds (19.37% mc, embryo length- 6.88 mm) at 30ºC with mature embryos. The chromatograms of the analyzed phytohormones (Figure 6) were interpreted, and considerable variations were observed in the dynamics of endogenous hormone content between the immature embryo and mature embryo-bearing seeds. The ABA content decreased from 1172.09 ng/g (immature embryo seeds) to 519.18 ng/g (mature embryo seeds). IAA, CK, SA and JA contents decreased with warm stratification at 30ºC. Meanwhile, GA_3 showed an increasing trend from 116.32 ng/g (immature embryo seeds) to 832.45 ng/g (mature embryo seeds). The IAA content in immature embryo seeds was 98.43 ng/g, reduced to 72.13 ng/g

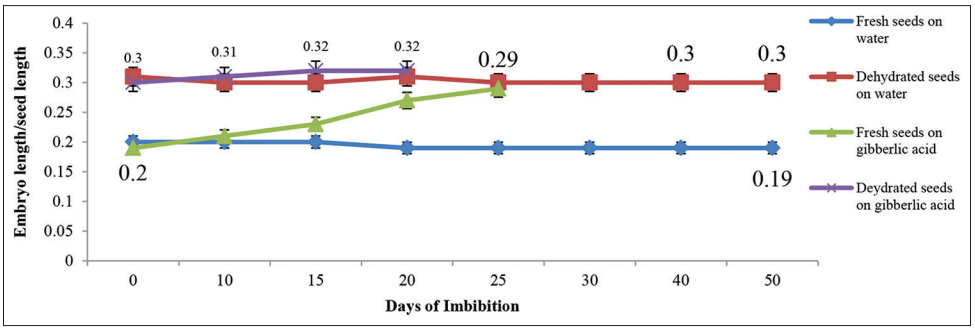

Figure 5: Mean embryo to seed length ratio of *G.canarica* seeds in response to different treatments – fresh seeds on water as control, dehydrated seeds, fresh seeds pretreated with gibberlic acid and dehydrated seeds on gibberlic acid under laboratory conditions

Table 2: Seedling growth parameters following gibberlic acid pretreatment in *G.canarica* seeds.

Treatment	Plant height (cm)	Root length (cm)	Shoot length (cm)	Seedling vigour index
Control	9.56 ± 0.11^{a}	3.86 ± 0.01^{a,b}	5.69 ± 0.11^{a}	287.07 ± 18.53^{a}
100 ppm GA_3 12hr	29.83 ± 0.83^{e}	5.28 ± 0.47^{b,c}	24.55 ± 1.20^{e}	2518.00 ± 94.46^{d}
100 ppm GA_3 24hr	40.16 ± 0.89^{f}	7.56 ± 0.54^{d,c}	32.60 ± 0.60^{f}	4010.50 ± 95.37^{f}
200 ppm GA_3 12hr	29.33 ± 0.90^{e}	5.09 ± 0.51^{b}	24.23 ± 1.07^{e}	2480.83 ± 48.12^{d}
200 ppm GA_3 24hr	38.00 ± 0.71^{f}	5.84 ± 0.65^{b,c}	32.15 ± 1.00^{f}	3383.83 ± 109.77^{e}
300 ppm GA_3 12hr	38.83 ± 1.03^{e}	5.91 ± 0.48^{b,c}	32.92 ± 0.70^{d}	3433.33 ± 80.49^{e}
300 ppm GA_3 24hr	25.33 ± 1.07^{c}	4.69 ± 0.57^{b,c}	20.63 ± 0.73^{c}	1330.00 ± 68.73^{c}
400 ppm GA_3 12hr	20.16 ± 0.76^{b}	4.97 ± 0.31^{b,c}	15.19 ± 0.66^{c}	860.00 ± 58.70^{b}
400 ppm GA_3 24hr	16.33 ± 0.54^{b}	4.01 ± 0.21^{a,b}	12.32 ± 0.70^{b}	462.23 ± 20.61^{a}
500 ppm GA_3 12hr	10.50 ± 061^{a}	2.94 ± 0.42^{a}	7.34 ± 0.58^{a}	286.83 ± 25.10^{a}
500 ppm GA_3 24hr	10.16 ± 0.76^{a}	3.00 ± 0.20^{a}	7.16 ± 0.58^{a}	276.16 ± 22.93^{a}
F value	219.200	8.30	176.38	467.663

For each treatment, means followed by the same letter in each row do not differ significantly at p<0.01

Table 3: Endogenous phytohormone content in *G. canarica* seeds with immature and mature embryos.

Phytohormone	Phytohormonal content (ng/g)	
	Immature embryo seeds	Mature embryo seeds
IAA	98.43	72.13
GA_3	116.32	832.45
CK	240.00	105.32
SA	26.07	17.09
ABA	1172.09	519.18
JA	338.38	101.39

IAA- Indole- 3- acetic acid, GA_3 – Gibberellic acid, CK- Cytokinin, SA- Salicylic acid, ABA- Abscisic acid, JA- Jasmonic acid

when the embryo got mature, and JA, one of the prominent phytohormones in G. *canarica* seeds, was higher in immature embryos having seeds (338.38 ng/g) reduced with embryo maturity (101.39 ng/g). The cytokinin content was 240 ng/g in dormant seeds was reduced to 105.32 ng/and the least abundant salicylic acid, 26.07 ng/g (immature embryo) was, declined to 17.09 with embryo maturity (Table 3).

DISCUSSION

G. *canarica* seeds, akin to most of the recalcitrant species with high moisture content on harvest, are also unable to resist desiccation below 12-10% moisture content (Hong & Ellis, 1996). The initial moisture content was found to be 28.86% which on becoming below 10%, seeds of G.*canarica* did not survive. Such severe viability loss followed by the moisture decline in G. *canarica* was also previously reported in other Myristica swamps like *Myristica malabarica* (Anilkumar *et al.*, 2002) and *Endocomia macrocoma* (Mathew *et al.*, 2016) and all of them are identified as recalcitrant seeded species. Moreover, recalcitrant seeds are reported to be relatively larger in size and seeds are covered by fleshy or juicy aril and hard testa (Chin, 1989) as in the case of G. *canarica* with large seeds covered by juicy red lacinated aril. G. *canarica* seeds were stored at room temperature for 30 days to reduce germination percentage. Certain tolerance to short-term storage of maximum 10 days at low temperatures *viz*. 10°C or 4°C was also observed. Stored seeds at 20 ± 3°C maintained seed viability for a prolonged duration of 75 days with 30% seed germination. This is in line with reports that seeds from tropical native species prefer to be stored at 10°C or above. The poor seed germination (34%) over a span of 50 days in control treatment conditions indicated dormancy. Hypogeal-type seed germination was observed in G. *canarica*, like most of the swamp species in seasonally flooded habitats having this type of germination. On water imbibition, seed mass of both scarified and non-scarified G. *canarica* seeds are equally enhanced as if the testa are permeable with no physical dormancy.

G. *canarica* possesses a small basal type embryo, and such embryo types are common in large-sized seeds with rudimentary cotyledons and ruminate endosperms (Martin, 1946). The growth of the embryo before radicle emergence indicates morphological dormancy. Thus the poor initial germination may be due to the immaturity of the embryo,

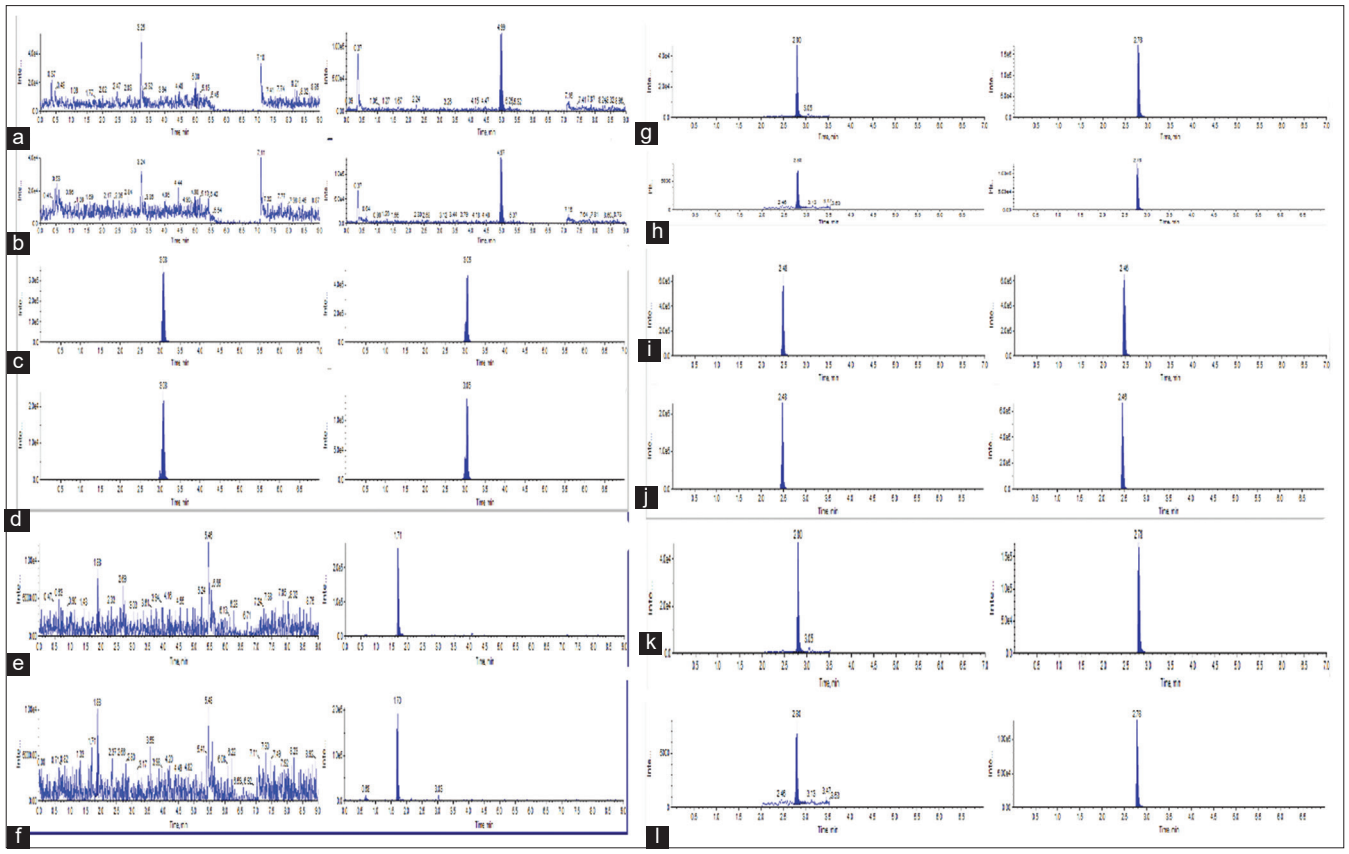

Figure 6: The chromatograms of analyzed phytohormones in the *G. canarica* seeds having mature and immature embryos. a) IAA of immature embryonic seed, b) IAA of mature embryonic seed, c) GA_3 of immature embryonic seed, d) GA_3 of mature embryonic seed, e) CK of immature embryonic seed, f) CK of mature embryonic seed, g) SA of immature embryonic seed, h) SA of mature embryonic seed, i) ABA of immature embryonic seed, j) ABA of mature embryonic seed, k) JA of immature embryonic seed and l) JA of mature embryonic seej

and an immediate radicle emergence would not be expected. Similar observations were seen in *Virola surinamensis*, another Myristicacean member (Pina-Rodrigues & Figliolia, 2005), *Magnolia champaca* (Fernando *et al.*, 2013), *Magnolia sinica* (Lin *et al.*, 2022). The embryo of *G. canarica* is relatively small at the time of dispersal (4.06±0.01mm), with an embryo: seed (E: S) ratio of 0.19. More germination in *G. canarica* (80%) is attained at 28-30ºC after five days of warm stratification period, and embryo growth was increased to 6.88±0.04 mm with 0.36 E: S ratio. An E: S ratio of 0.3 is similarly linked to an 80% late germination capability at 28ºC. The delayed embryo development beyond five weeks in these situations could also account for slower germination. The germination percentage of the seeds also increased (80.66%), concomitant with subsequent embryo growth as a need for attaining critical embryo size having a mean E: S ratio (0.32) to activate germination. Many species with endospermic seeds and tiny embryos, such as Ribes (Santo *et al.*, 2014) and Conopodium have been suggested to have a physical growth threshold (Blandino *et al.*, 2019). This could be due to a requirement for a physically large embryo enough to pierce the testa. The use of GA_3 to avoid seed dormancy in *G. canarica* could be a viable alternative to warm stratification, as they not only promote embryo growth but also gain more germination than those that were not pretreated. The permeability of the testa helps hormones to effectively pass through the seed coat and fasten germination even at lower concentrations. Nevertheless, a drop in germination percentage with increasing GA_3 concentration could be due to an excess of hormone content resulting GA_3 toxicity (Akbari & Salehi, 2008). Similar observations were reported in *Atropa belladona* (Al-Saedi, 2019), *Thymus satureioides* and *Lavandula dentate* (Chetouani *et al.*, 2017). Furthermore, seeds that remain non-germinated despite being kept moist for up to 60 days become dead. Observations align with Bahuguna *et al.* (1988) for reaching a conclusion on *G. canarica* seeds that they have both a morphological component (embryo growth) ands a physiological block defined as morphophysiological dormancy. Phytohormone analysis showed that the freshly harvested seeds with higher ABA concentration and lower GA_3 concentration, which become *vice versa* on dehydration of seeds in warm temperatures. This cause an increase in germination percentage but seeds remain dormant for some time. Such findings agree with the view that a reduction in ABA content in some dormant seeds alone is not sufficient to break dormancy (Trent & Walton, 1988). Exogenous application of GA_3 at lower concentrations could break dormancy effectively in *G. canarica* seeds. The effective application of low-concentration GA_3 reduced the mean germination time and time to the first germination and increased the speed of germination and seedling vigour index, which could be due to the decrease in abscisic acid

content responsible for dormancy (Hilhorst & Karssen, 2000). In the present study, we have shown the effect of dehydration (Warm stratification) on the seed germination of *G. canarica* and demonstrated that ABA and GA_3 might play a role in the regulation of seed dormancy and germination.

In conclusion, *G. canarica* seeds are of the recalcitrant type having non-deep simple morphophysiological dormancy. Seeds treated with 100 ppm GA_3 for 24 hrs were effective in breaking seed dormancy and increasing germination percentage and seedling vigour index. The ideal storage temperature was found to be 20 ºC as it showed the highest viability of 30.16% even after 75 days of storage. The outcome of this study will aid in the development of techniques for bulk seed propagation of the species. The findings would also aid in contributing to the cause of *ex-situ* conservation and rehabilitation programmes for this highly threatened species.

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