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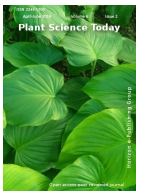

Research Article

Conservation of *Garcinia imberti* Bourd. through seeds

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Abstract
Garcinia imberti seeds were collected during 2015-2017 from Shangili, Cheenikkala and Bonaccord evergreen forests of Agasthyamala Biosphere Reserve, the only abode of this endangered endemic species. Germinability of seeds were analysed through decoating, Gibberellic acid (GA_3) and light inductive pre-treatments on fresh (62.8 % moisture content; MC) and desiccated (fast; 23.3% MC and slow; 30.5 % MC) seeds. The seed germination with impermeable coat (0.7-1.2 mm) was restricted which on decoating got enhanced. Application of GA_3 along with exposure to light breaked dormancy within 4-6 days compared to non-treated seeds that took 238-254 days to germinate. Stored seeds behaviour revealed that seed moisture content and rate of germination were negatively correlated. Seed storage was found to be more efficient only up to 80 days at controlled seed banking conditions (20 ± 2^0C, 20 % relative humidity; RH). Both fast and slow desiccated seeds stored for 60 days in seed bank conditions exhibited 50.4 and 43.4 % of germination compared 39.4% germination of non-desiccated seeds. Hence fast desiccated and decoated *G. imberti* seeds pre-treated with GA_3 on subsequent exposure to light alleviated dormancy. For seed banking, fast desiccated seeds with MC in between 40-20% are found to be promising.

Keywords: Agasthyamala Biosphere Reserve; Desiccation; Dormancy, Endangered; Seed storage practices.

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Introduction

Seed germination is a complex physiological process that responds to environmental signs such as light, temperature, water potential etc. Seed germination failure and dormancy is the main limiting factor of threatened plants for large scale production. Bewley and Black (1) reported that certain seeds require treatment both for eliminating hard resistant seed coat and endogenous dormancy. Exogenous dormancy imposed seeds have rigid pericarp/seed coat that are impermeable to water and oxygen. The seed coat or testa of many plants comprises significant amount of germination inhibitor, which prevent seed germination (2). Nearly 95% dormancy of seeds are physiological or biochemical in nature, while for the rest of dormant seeds enforced dormancy is due to an impermeable seed coat (3). The seed coat inhibitory effects on seed germination are caused by some possible process such as mechanical constraint, prevention of water and oxygen uptake and production of chemical inhibitors (4). Certain process like hot or cold water scarification, chemical

scarification, seed decoating, etc. are sufficient to overcome this coat imposed on dormancy. The endogenous dormancy imposed seeds are in need of after-ripening, either high or low temperature and moisture, or both in sequence to break dormancy. Seed dormancy may be able to overcome by using a number of processes which stimulate germination by physical or physiological treatments. GA_3 is used as a stimulator to enhance seed germination credited by the mobilization of stored food reserves (5). This case is akin to the effect of light in using the nutrients for photosynthesis (6).

Garcinia imberti is an evergreen, endangered tree species (7) growing up to 10 m height bearing recalcitrant seeds with high moisture content. The niche specificity is due to its patchy, specific altitudinal (600-1200 m asl) distribution, recruitment failure due to high fruit/seed predation and a prolonged period of seed dormancy (8). The major difficulty in *G. imberti* propagation as for many species of *Garcinia* genus is related to its seeds germination, dormancy and storage aspects. Because of seed dormancy, *G. imberti* seeds can take 14 months in its niche to germinate due to the physical barrier of the seed coat (9,8). Niche based cues on the ecological requirements preferred by *G. imberti* seedlings indicated the presence of balancing nature of sunlight and misty shaded canopy. Natural occurrence of more *G. imberti* seedlings at the forest floor where canopy gaps formed by uprooted trees or shrubs indicated the azimuthal proactive effect on seed germination. Germination performance induced by light are likely to fluctuate among habitats, for example in shaded areas, the occurrence of strong light can be associated with a canopy gap that enhance chances for developing seedlings (10). These observations validated confirmatory experiments on the light enhanced *G. imberti* seed germinability.

The importance of seed storage in plants especially endangered as well as plants at the brink of extinction has been practised ever since humans initiated to domesticate plant species. However, the successful seed storage depends on knowledge concerning the seed's behaviour during storage, which allows the use of applicable environments that maintain their sustainability (11). *Ex-situ* seed conservation is 100 times economic than the *in-situ* protection of individual trees (12). Storage possibilities of tree seeds are vastly species-specific and huge variation has been faced across the tree species (13). The seed storage life-span of *G. imberti* is very limited as other species of *Garcinia* due to its recalcitrant nature and elevated moisture contents. The aim of the study was to find out suitable solutions to overcome seed dormancy and to extend seed storage of *G. imberti.*

Materials and Methods

G. imberti fruits were collected during 2015-2017 November with 25 accessions each from Shangili (1185 m, N 08°47'48.90", E 077°11'45.44"), Cheenikkala (1124m, N 8°47'46", E 77°9'2") and Bonaccord (643 m, N 8°45'25", E 77°11'20") evergreen forests of Agasthyamala Biosphere Reserve. Mature ripened fruits were recognized by their yellowish-green colour and these fruits were directly harvested from the mother trees. After collecting, the fruits were brought to the laboratory and the rinds removed by manual pressing. These seeds were thoroughly washed with running water and thereafter allowed to surface dry in laboratory conditions.

The following aspects were analysed in order to evaluate

(1) Overcome seed dormancy

1. Coated seeds + dark (control)
2. Coated seeds + light
3. Coated seeds + light + GA_3 (regular intervals of 0-5000 ppm concentration)
4. Decoated seeds + dark
5. Decoated seeds + light
6. Decoated seeds + light + GA_3 (regular intervals of 0-5000 ppm concentration)

Impermeable seed coat (0.7-1.2 mm) of *G. imberti* seeds restricts the germination to a certain extent. Even though various methods like seed decoating, light and GA_3 treatments were applied to reduce the dormancy and enhance/induce the percentage germination. For that purpose, the hard outer and thin inner seed coat was removed mechanically. For GA_3 treatments, seeds were pre-treated with GA_3 solutions of different concentrations (0, 50, 250, 500, 1000, 2000, 3000, 4000 and 5000 ppm) for both coated and decoated seeds. Seeds submerged in distilled water for 24 hours were considered as control. The all tested seeds (12 seeds/ 10 lots) were placed over filter paper wetted with distilled water in light transparent petri dishes, kept in seed germinator (30 ± 2°C, 80% RH) with light (provided by Philips daylight lamps (15.4 Lux)) and without light.

(2) Seed storage aspects

Seed were kept in six different storage temperature units for analysing the optimum storage conditions for long term seed conservation aspects. The seeds were stored in closed polycarbonate bottles; half portions of the bottles were kept empty to enhance seed respiration. Additional experiments were conducted to assess the viability of both fast and slow desiccated stored seeds for long term storage or conservation programmes. For the storage aspects (7) two different moisture contents above the limit of

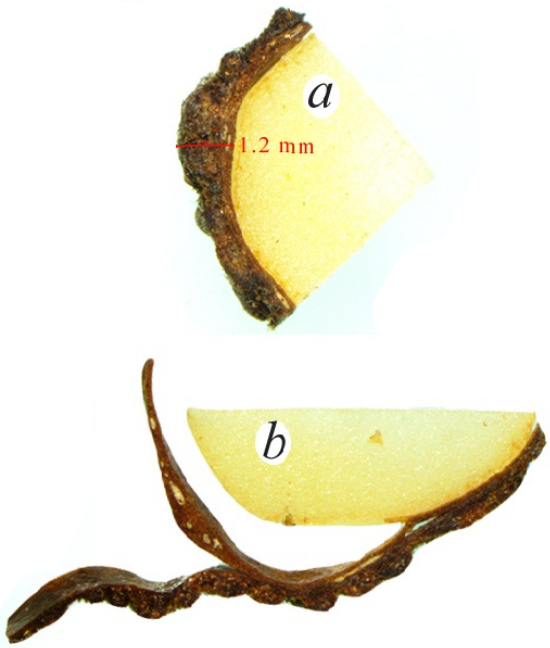

Fig. 1. Seeds with 0.7-1.2 mm thickened seed coat (***a.*** seed section with hard seed coat; ***b.*** showed its double layer

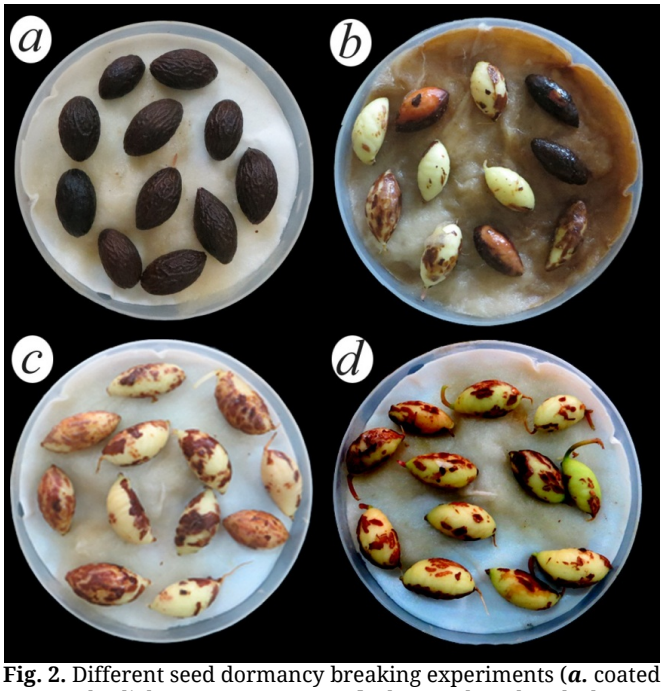

Fig. 2. Different seed dormancy breaking experiments (***a.*** coated seeds+ light + GA_3 1000 ppm, ***b.*** decoated seeds + dark, ***c.*** decoated seeds + light, ***d.*** decoated seeds + light + GA_3 1000 ppm)

critical moisture contents (CMCs), 23.3 % (fast desiccated seed) and 30.5 % (slow desiccated seeds) both registered above 50 % seed germination were considered for storage experiment. For cryopreservation, both desiccated and fresh seeds were covered with aluminium foils before directly dipped to liquid nitrogen for one week; and subsequently defrosted for 5 minutes in water bath (40°C).

The following storage conditions were used:

1. Seeds stored at laboratory conditions (28 ± 2°C, 60 % RH)
2. Seeds stored at controlled seed banking conditions (20 ± 2°C, 20 % RH)
3. Seeds stored at 10°C
4. Seeds stored at 0°C
5. Seeds stored at -20^{0}C
6. Cryopreservation (-196^{0}C)

Seed germination

Seeds were taken at regular intervals (10 days) for MC analysis and germination tests with ten replicates of 10 seeds/lot rolled in an acid free moist germination paper kept in a seed germinator without light (30 ± 2°C, 80% RH). The viability was tested on daily basis on the percentage of germinated seed when the radicle reaches 5 mm. Germination parameters like speed of germination (SPG), mean germination time (MGT) (14), mean daily germination (MDG), peak value (PV) and germination value (GV) (15) were calculated.

Results

G. imberti seeds are the sole medium of propagation with 62.8 ± 4.7 % initial moisture content. The 0.7-1.2 mm thick seeds coat (Figure 1*a* and *b)* enforces deep dormancy for 12-14 months with only 38 ± 1.3 % of germination in *in-situ* and 6-8 months in controlled germinator conditions (30 ± 2°C, 80% RH, without light) with 12.6 % of germination *ex-situ*. The seed coat is impermeable to water and gases to certain extent and decoated seeds are devoid of physical dormancy along with enhanced germination.

The present experiments related to different dormancy breaking methods revealed that both coated and decoated seeds on pre-treatment with GA_3 exhibited significant variation in germination and associated parameters *viz.* days taken for germination, speed of germination, germination value. The seeds with higher concentration of GA_3 (1000-4000 ppm) registered cent percentage of germination along with good rate of germination value. Seed decoating enhances germination, however decoated seeds with GA_3 registered higher rate and speedy germination (Table 1). The overall results showed that decoated seeds in the presence of GA_3 especially 1000 ppm displayed a good and speedy germination and enhanced germination value. This treatment combination took only 09-12 days for its complete germination compared to 238-254 days by the delayed germination over non-treated coated seeds (Table 1).

The results of different dormancy breaking methods (Fig. 2) indicated that light and GA_3 are the main factor that breaks the physiological dormancy. Decoated seeds contacted with light (15.4 Lux) and GA_3 (1000 ppm) showed a promising value of cent percentage germination along with speedy germination (1.63), peak value (0.75) and

Table 1. Effect of GA_3 in both coated and decoated seeds without light (in germinator) (*G. imberti* seeds; *n*=25).

Treatments (Germinator, 30 ± 2°C, 80% RH, without light)	Days taken for initiation to completion of germination	Germination Percentage	Speed of Germination	Germination Value
Coated seeds + 0 ppm GA_3 (Control)	238- 254	12.6 ± 0.4^{a}	0.0028 ± 0.0^{a}	0.0006 ± 0.0^{a}
Coated seeds + 50 ppm GA_3	218-232	13.2 ± 0.8^{a}	0.0042 ± 0.0^{ab}	0.0002 ± 0.0^{a}
Coated seeds + 100 ppm GA_3	214-221	14.4 ± 1.2^{a}	$0.0071 \pm 0.0^{b**}$	0.0019 ± 0.0^{a}
Coated seeds + 250 ppm GA_3	210-219	$35.6 \pm 1.9^{b**}$	$0.0188 \pm 0.0^{cd**}$	0.0021 ± 0.0^{cd}
Coated seeds + 500 ppm GA_3	196-209	$36.2 \pm 3.1^{b**}$	$0.0183 \pm 0.0^{cd**}$	0.0053 ± 0.0^{ab}
Coated seeds + 1000 ppm GA_3	140-166	$62.2 \pm 0.6^{cd**}$	$0.0168 \pm 0.0^{c**}$	0.0004 ± 0.0^{a}
Coated seeds + 2000 ppm GA_3	118-126	$38.0 \pm 0.9^{b**}$	$0.0185 \pm 0.0^{cd**}$	0.0004 ± 0.0^{a}
Coated seeds + 3000 ppm GA_3	98-105	$55.4 \pm 3.8^{c**}$	$0.0185 \pm 0.0^{cd**}$	0.0007 ± 0.0^{a}
Coated seeds + 4000 ppm GA_3	95-102	$69.2 \pm 3.1^{d**}$	$0.0185 \pm 0.0^{cd**}$	0.0002 ± 0.0^{a}
Coated seeds + 5000 ppm GA_3	79-86	$63.6 \pm 0.8^{cd**}$	$0.0207 \pm 0.0^{d**}$	0.0001 ± 0.0^{a}
Decoated seeds + 0 ppm GA_3	15-18	$100 \pm 0.0^{e**}$	$0.3150 \pm 0.0^{l**}$	$0.0087 \pm 0.0^{bc**}$
Decoated seeds + 50 ppm GA_3	13-18	$100 \pm 0.0^{e**}$	$0.1538 \pm 0.0^{h**}$	$0.0256 \pm 0.0^{fgh**}$
Decoated seeds + 100 ppm GA_3	13-24	$100 \pm 0.0^{e**}$	$0.0769 \pm 0.0^{f**}$	$0.0104 \pm 0.0^{bc**}$
Decoated seeds + 250 ppm GA_3	14-20	$100 \pm 0.0^{e**}$	$0.1429 \pm 0.0^{g**}$	$0.0214 \pm 0.0^{efg**}$
Decoated seeds + 500 ppm GA_3	11-24	$100 \pm 0.0^{e**}$	$0.0485 \pm 0.0^{e**}$	$0.0174 \pm 0.0^{de**}$
Decoated seeds + 1000 ppm GA_3	09-12	$100 \pm 0.0^{e**}$	$0.4317 \pm 0.0^{m**}$	$0.0714 \pm 0.0^{j**}$
Decoated seeds + 2000 ppm GA_3	11-18	$100 \pm 0.0^{e**}$	$0.1984 \pm 0.0^{i**}$	$0.0278 \pm 0.0^{gh**}$
Decoated seeds + 3000 ppm GA_3	10-12	$100 \pm 0.0^{e**}$	$0.2833 \pm 0.0^{j**}$	$0.0502 \pm 0.0^{i**}$
Decoated seeds + 4000 ppm GA_3	12-22	$100 \pm 0.0^{e**}$	$0.3084 \pm 0.0^{k**}$	$0.0303 \pm 0.0^{h**}$
Decoated seeds + 5000 ppm GA_3	14-20	$100 \pm 0.0^{e**}$	$0.1429 \pm 0.0^{g**}$	$0.0204 \pm 0.0^{ef**}$

The data were statistically assessed by one-way ANOVA, *significant at $P = 0.05$, **significant at $P = 0.01$ level, ± SE: Standard Error of the mean and means followed by the same letter in the column do not differ significantly by Tukey's post hoc test of Honest Significant Difference.

germination value (0.86) compared to non-treated seeds of only 12.6 % of germination and 0.0009 germination value (Table 2, Fig. 2 *d*). These experiments revealed that only 4-6 days were taken for initiation to completion of germination compared to coated seed germination without light in a span of 238-254 days. The GA_3 (1000 ppm) pre-treatment with decoated seeds in petri dishes without light encouraged seeds contamination and reduced germination percentage compared to decoated seeds germinated in the presence of both light and GA_3 (1000 ppm) (Fig. 2. *b)*.

The present investigation also gives an idea about optimum seed storage conditions of *G. imberti.* The rate of germination is highly correlated with seed moisture content and storage conditions. During the course of seed storage, the moisture content and germination percentages were negatively correlated and finally the rate of germination became reduced. An interesting observation is that, seed storage was possible both at controlled seed bank conditions (20°C/20%RH) and laboratory ambient (28 ± 2°C, 60 % RH) conditions with above 50% germination. However, the former method enhanced storage up to 50-60 days but latter showed only 30-40 days of seed storage (Table 3). These results clearly indicated that the seed storage was more efficient in seed bank conditions up to 80 days. All the other tested storage condition below 10°C found to be futile (Table 3).

Moisture content of seeds negatively affect the storage especially of recalcitrant seeds, which may be overcome through appropriate desiccation levels. Two different moisture contents above the limit of critical moisture contents (CMCs) *i.e.,* 23.3 % (fast desiccated seed) and 30.5 % (slow desiccated seeds) with 50 % seed germination were selected to test the extension of seed storage in *G. imberti*. These desiccated seeds with reduced moisture content showed higher percentage of germination for long term storage practices compared to storage aspects of fresh seeds with higher moisture content. The 60 days stored fast and slow desiccated seeds in controlled seed bank conditions exhibited 50.4 and 43.4 % of germination respectively compared to fresh, highly moisturized seed storage of 39.4%. Whereas both desiccated 30 days stored seeds registered 84.4 and 78.4 % of germination compared to 76.9 % of fresh seed storage. The controlled seed bank conditions (20^0C/20%RH) favoured *G. imberti* seeds for better storage programmes especially fast desiccated seed storage (Table 4).

Discussion

When considering the seed germination process, awareness about the mechanisms linked to seed dormancy assumes a significant role (16). Nonogaki (17) said that seed dormancy has an ecological relevance, since it maintains the survival mechanism of the seeds, confirming its viability till the ecological conditions are suitable for seedling growth and development. Seed dormancy breaking methods are essential to identify the factors that imposed seed dormancy. The rate and speed of seed germination has been linked with temperature and it affects particularly the rate of water absorption, reactivate metabolic and reserve mobilization processes and seedling growth (1).

Table 2. Effect of seed decoating, GA_3 pre-treatment and light induction (15.4 Lux) (*G. imberti* seeds; *n*=25).

Treatments	Coated seeds + Dark (Control)	Coated seeds + Light	Coated seeds + GA_3 + Light 1000 ppm	Decoated seeds + Dark	Decoated seeds + Light	Decoated seeds + GA_3 + Light 1000 ppm
Days taken for initiation to completion of germination	238-254	196-208	136-147	15-19	6-9	4-6
Germination Percentage	12.6 ± 0.4^{a}	15.8 ± 1.1^{a}	24.4 ± 1.6^{b**}	100 ± 0.0^{c**}	100 ± 0.0^{c**}	100 ± 0.0^{c**}
Speed of Germination	0.0028 ± 0.0^{a}	0.0046 ± 0.0^{a}	0.0082 ± 0.0^{b**}	0.3150 ± 0.0^{c**}	0.9607 ± 0.0^{e**}	1.6262 ± 0.0^{f**}
Mean Germination Time	274 ± 6.1^{e}	242 ± 14.8^{d*}	208 ± 3.61^{c**}	76.0 ± 0.89^{b**}	9.52 ± 0.02^{a**}	5.12 ± 0.0^{a**}
Mean Daily Germination	0.0038 ± 0.0^{a}	0.0038 ± 0.0^{a}	0.0046 ± 0.0^{a}	0.2097 ± 0.0^{b**}	0.4706 ± 0.0^{d**}	1.1429 ± 0.0^{e**}
Peak Value	0.0030 ± 0.0^{a}	0.0031 ± 0.0^{a}	0.0041 ± 0.0^{a}	0.0418 ± 0.0^{b*}	0.5200 ± 0.0^{d**}	0.7502 ± 0.0^{e**}
Germination Value	0.0009 ± 0.0^{a}	0.0010 ± 0.0^{a}	0.0020 ± 0.0^{b}	0.0087 ± 0.0^{c**}	0.2353 ± 0.0^{e**}	0.8571 ± 0.0^{f**}

The data were statistically assessed by one-way ANOVA, *significant at P = 0.05, **significant at P = 0.01 level, ± SE: Standard Error of the mean and means followed by the same letter in the column do not differ significantly by Tukey's post hoc test of Honest Significant Difference.

Table.3. Effect of seed storage (fresh seeds with 62.8 % MC; *n*=25).

Storage conditions	Storage period (Days)	Moisture contents (%)	Germination (%)
Laboratory ambient condition (28 ± 2^{0}C, 60 % RH)	0	62.8 ± 4.7^{b}	100.0 ± 0.0^{i}
	10	66.2 ± 3.6^{d**}	82.4 ± 5.6^{h**}
	20	66.8 ± 2.9^{e**}	74.5 ± 6.3^{g**}
	30	67.3 ± 3.9^{f**}	63.5 ± 5.4^{f**}
	40	67.8 ± 4.1^{g**}	39.6 ± 5.3^{e**}
	50	68.2 ± 2.4^{h**}	33.6 ± 4.7^{d**}
	60	68.8 ± 5.1^{i**}	26.1 ± 3.9^{c**}
	70	64.5 ± 4.7^{c**}	13.7 ± 3.6^{b**}
	80	61.8 ± 5.6^{a**}	0.0 ± 0.00^{a**}
Controlled condition (Seed bank) (20^{0}C/20%RH)	0	62.80± 4.7^{a}	100.0 ± 0.0^{i}
	10	64.8 ± 3.6^{b**}	86.5 ± 3.6^{h**}
	20	66.5 ± 3.4^{d**}	71.6 ± 3.5^{f**}
	30	66.9 ± 4.5^{d**}	76.8 ± 4.6^{g**}
	40	68.3 ± 2.9^{f**}	65.8 ± 3.2^{e**}
	50	69.5 ± 3.2^{g**}	51.4 ± 3.6^{d**}
	60	67.5 ± 3.3^{e**}	39.4 ± 4.7^{c**}
	70	65.5 ± 4.3^{c**}	19.6 ± 3.2^{b**}
	80	65.3 ± 4.6^{c**}	3.3 ± 0.10^{a**}
10^{0}C	0	62.80± 4.7^{a}	100.0 ± 0.0^{h}
	10	67.8 ± 5.3^{b**}	39.4 ± 2.3^{g**}
	20	69.5 ± 6.3^{c**}	38.4 ± 2.6^{f**}
	30	70.2 ± 4.7^{d**}	32.5 ± 2.4^{e**}
	40	70.6 ± 4.2^{e**}	30.2 ± 3.1^{d**}
	50	74.4 ± 3.6^{f**}	17.5 ± 1.2^{c**}
	60	73.6 ± 3.2^{e**}	6.8 ± 0.12^{b**}
	70	72.5 ± 4.7^{c**}	0.0 ± 0.00^{a**}
0^{0}C	0	62.80± 4.7^{a}	100.0 ± 0.0^{d}
	10	64.3 ± 1.2^{b**}	18.6 ± 3.8^{c**}
	20	65.3 ± 3.5^{c**}	6.3 ± 2.6^{b**}
	30	66.2 ± 2.9^{d**}	0.0 ± 0.00^{a**}
-20^{0}C	0	62.80± 4.7^{a}	100.0 ± 0.0^{c}
	10	66.3 ± 3.3^{b**}	13.6 ± 0.13^{b**}
	20	66.8 ± 2.9^{c**}	0.0 ± 0.00^{a**}

The data were statistically assessed by one-way ANOVA, *significant at P = 0.05, **significant at P = 0.01 level, ± SE: Standard Error of the mean and means followed by the same letter in the column do not differ significantly by Tukey's post hoc test of Honest Significant Difference.

The seeds of *G. imberti* are the only part of propagation that recorded 62.8 ± 4.7 % initial moisture content. According to Anto and Anilkumar (8) *G. imberti* seeds exhibited 1.2 ± 0.2 years of dormancy with only 38 ± 1.3 % of germination in *in-situ* and 6-8 months of dormancy in controlled *ex-situ* germinator conditions (30 ± 2°C, 80% RH, without light). The seed with 0.7 - 1.2

Table 4. Effect of seed storage (both fast and slow desiccated seeds with 23.31 and 30.53% MC; *n*=25).

Storage condition		Storage duration (days)	Germination (%)
Fresh decoated seeds		0	100 ± 00
Fast desiccated decoated seeds (MC; 23.31 %)		0	54.6 ± 3.6
Slow desiccated decoated seeds (MC; 30.53 %)		0	54.4 ± 4.1
Laboratory ambient (28 ± 2^{0}C, 60 % RH)	Fresh decoated seeds (MC; 62.8 %)	30	63.5 ± 3.2^g
		60	26.1 ± 3.2^a
	Fast desiccated decoated seeds (MC; 23.31 %)	30	72.9 ± 5.1^i
		60	32.8 ± 2.2^c
	Slow desiccated decoated seeds (MC; 30.53 %)	30	70.5 ± 3.1^h
		60	28.4 ± 2.6^b
Controlled (Seed bank) condition (20^{0}C/20%RH)	Fresh decoated seeds (MC; 62.8 %)	30	76.8 ± 2.5^j
		60	39.4 ± 4.2^d
	Fast desiccated decoated seeds (MC; 23.31 %)	30	84.4 ± 6.1^l
		60	50.4 ± 4.3^f
	Slow desiccated decoated seeds (MC; 30.53 %)	30	78.4 ± 4.6^k
		60	43.4 ± 2.9^e
Cryopreservation (-196 ^{0}C)	Fresh coated seeds (MC; 62.8 %)	7	-
	Fast desiccated decoated seeds (MC; 23.31 %)	7	-
	Slow desiccated decoated seeds (MC; 30.53 %)	7	-

The data were statistically assessed by one-way ANOVA, *significant at P = 0.05, **significant at P = 0.01 level, ± SE: Standard Error of the mean and means followed by the same letter in the column do not differ significantly by Tukey's post hoc test of Honest Significant Difference.

mm thick hard coat enforces deep dormancy. Baskin and Baskin (18) reported that seed dormancy is usually allied with intrinsic factors linked to the seed itself, such as rigid and impermeability of the seed coat to gases and water, immature embryos, inhibitors, and abiotic factors like temperature, humidity, light and substrate. The *G. imberti* seeds when subjected to decoating, dormancy were also reduced (8). The seed decoating was efficient for avoiding the inhibitory phyto-compounds present (19). These results revealed that the *G. imberti* seeds possess mainly physical dormancy along with mild physiological dormancy. Dormancy breaking methods tried with *G. kola* seeds reported by Eyog-Matig *et al.* (20) suggested that seed coat removal followed by dipping in cold water (25^{0}C) reduces the dormancy. Total removal of *G. cowa* seed coat was considered as the most efficient dormancy-breaking methods (21) and (22).

The present study showed that, decoated *G. imberti* seeds with GA_3 displayed significant enhancement of germination and associated parameters. The higher concentration of GA_3 (1000-4000 ppm) applied on seeds showed cent percentage germination and improved germination values. The GA_3 treatment is used for releasing inhibitory phyto-compounds present in seeds (23). Kouakou *et al.* (19) reported that scarification along with GA_3 application on *G. kola* seeds offer an alternative procedure to progress the seed germination and seedling vigour for a large-scale seedling production for species conservation.

The *G. imberti* decoated seeds applied with GA_3 of 1000 ppm showed cent percent germination with germination value of 0.07 ± 0.00 against 0.009 ± 0.00 of non-treated decoated seeds. A similar case of best percentage of germination was reported with *G. kola* scarified seeds treated with GA_3 of 10^{-2} to 10^{-4} g L^{-1} for 24 to 48 hours respectively (19). Germination rates of *G. kola* seeds were 0.012 ± 0.00 and 0.013 ± 0.00 for 10^{-4} and 10^{-3} g L^{-1} of GA_3, respectively, compared to scarified seeds untreated with GA_3 as control of 0.010 ± 0.00 (19). These treatment combination (Decoated seeds + GA_3 1000 ppm) taken only 09-12 days for its complete germination compared to non-treated coated *G. imberti* seeds of 238-254 days.

The results of different dormancy breaking methods in *G. imberti* indicate that light along with GA_3 are the major factors to release the physiological dormancy and enhance germination. Especially decoated seeds contacted with light (15.4 Lux) and GA_3 (1000 ppm) showed a promising value of cent percent germination and superior germination value. These experiments revealed that only 4-6 days taken for the completion of seed germination in the presence of light compared to 238-254 days for the coated seeds without light. The GA_3 applied decoated seeds in petri dishes without light exhibited seeds contamination and reduced rate of germination. These experiments clearly indicated the influence of both light and decoating for initiation of germination. The light intensity, which greatly influences the seed germination is responsible for the perception and translation of the shining stimulus (17). Seed

dormancy is overcome by exogenous application of GA_3 and light in *Rheumemodi* seeds reported by Kandari *et al.* (24). Several cultivated species are uninterested to light to sprout; but, the light is very essential and it stimulates seeds of various wild species (25). Alternating temperature treatments with light dramatically improved the germination process of *Cuphea viscosissima* dormant seeds reported by Widrlechner and Kovach (26).

The present study also gives a clue about optimum seed storage conditions of *G. imberti*. Storage environment is very important for extending seed's life and the ideal metabolic rate in storage will conserve as much of the stored food reserves in the seeds and maintains the integrity of the embryos (27). King and Roberts (28) clarified storage life span of hydrated (recalcitrant) seeds from days or weeks for some tropical species and reported that rate of seed germination is highly associated with seed moisture content and storage conditions. Seed morphology, chemical composition, maturity, storage environments which includes temperature, moisture, storage facilities of cold storage, containers and moisture regulators plays an important role on successful seed storage (29,30). An interesting observation is that, *G. imberti* seeds stored at controlled (Seed bank 20°C/20%RH) conditions registered nearly 50% germination up to 50-60 against 30-40 days at laboratory ambient (28 ± 2°C, 60 % RH) conditions. The general objective of seed storage is to lessen the metabolism of the seeds as much as possible without harm them and to avoid attack by microorganisms (29). The results clearly indicated that the *G. imberti* seed storage was more effective in seed bank conditions up to 80 days.

Extreme cold storage was not efficient for *G. imberti* seed conservation since low temperatures like 0°C and -20°C exhibited very less rate of germination in 10 days. At this chilling temperature, seeds respire and produce moisture content which could not be used for the on-going metabolism which eventually recedes and henceforth the initially formed respired moisture content become condensed in the hermitic storage. Seed storage temperature below 15°C has been reported to be dangerous for most tropical recalcitrant seeds (29,31). Chitra (32) reported that fresh desiccated seeds of *Coscinium fenestratum* stored with 6.8 % MC at 30 and 20°C retained 80% viability. This loss of viability is generally due to the physical, chemical and metabolic changes which can destruct both the cell membrane and cytoplasm (1). Bonner (29) reported that tropical-recalcitrant seeds like *Quercus* acorns seeds registered higher lethality in low temperature and chilling damage below 12 to 20°C. Yong *et al.* (22) reported that *G. cowa* seed viability was depleted rapidly when seeds were stored at 4°C, which registered only 2% germination after 1 week. Similarly, *G. subelliptica* seeds in < 4°C storage temperature were also registered very fewer viability loss when the moisture became 30% (33).

Seed moisture content negatively affect the stored seeds especially recalcitrant seeds, though proper desiccation overcome this difficulty linked with viability retention. During the study, a clear difference was observed in germination rate between fresh and stored seeds. Previous results on both fast and slow seed desiccation with two different moisture contents (MCs) *viz*. 23.3 and 30.5 % respectively registered 50.5 and 43.0% germination (7). The present study was with these two MCs, (without exceeding the critical moisture contents) standardized *G. imberti* seed storage practices. It was also revealed that the CMCs may also vary with the method of desiccation (34).

The seed storage behaviour of *G. imberti* is determined after drying the seeds to two different moisture levels (fast and slow desiccated seeds) and assessing the corresponding rate of germination. The 60 days stored fast and slow desiccated seeds in controlled seed bank conditions exhibited 50.4 and 43.4 % of germination compared to fresh highly moisturized seed stored with 39.4 % germination. Whereas both desiccated 30 days stored seeds with 23.3 and 30.5 % MC registered 84.4 and 78.4 % of germination compared to 76.8 % of fresh seed storage. The fast desiccation was more effective than slow desiccation for *G. imberti* seeds (8) and fast desiccated seeds stored in controlled seed bank conditions (20°C/20%RH) favoured better storage programmes. The fast desiccated seeds exposed to intermediary levels of hydration only for a short-term period which in turn shortened the possible damage linked with desiccation (33). Goncalves *et al.* (35,36) point out that, fast dried seeds of *Citrus reshni* were more beneficial and it provided more root projection along with lesser percentage of anomalous seedlings. The knowledge on optimal seed germination and seed storage conditions are vital factors for any effort towards the maintenance of a plant species (37). Drew *et al.* (38) reported that recalcitrant seeds with reduced quality weaken the storage life span, which was confirmed with the storage behaviour of *Trichilia dregeana* and *Inga vera*. Seeds of *T. dregeana* with poor quality dropped viability from 100 to 20% when stored wet over three weeks at 16°C. The desiccated, cryo stored *G. imberti* seeds were also futile due to its recalcitrant nature. Pammenter and Berjak (39) pointed out that the main possible problem in cryopreservation of seeds is the formation of intracellular ice crystals that can be fatal to the tissue.

Conclusion

Seed decoating and GA_3 pre-treatments along with exposure to light are the most suitable methods to eliminate complete physical and physiological

dormancy of *G. imberti* seeds. Seed storage was more efficient in seed bank conditions though for a limited period up to 80 days due to recalcitrant nature. The desiccated seeds especially fast desiccated seeds in 20°C/20% RH registered maximum life span compared to other storage practices.

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Conflict of interest

The authors declared that they have no conflict of interest.

Authors' Contribution

AM and AM collected the seed samples from the field, carried out the work, analysed the data. JPS and AC interpreted the data and helped in manuscript writing.

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