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Journal of Threatened Taxa

The international journal of conservation and taxonomy

www.threatenedtaxa.org

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

SHORT COMMUNICATION

SEED GERMINATION STUDIES ON *GYMNACRANTHERA CANARICA* (KING) WARB. - A VULNERABLE TREE SPECIES OF A HIGHLY THREATENED *MYRISTICA* SWAMP ECOSYSTEM

K. Keshavachandra & G. Krishnakumar

26 July 2016 | Vol. 8 | No. 7 | Pp. 9009–9013

10.11609/jott.2152.8.7.9009-9013



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ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

SEED GERMINATION STUDIES ON *GYMNACRANTHERA CANARICA* (KING) WARB. - A VULNERABLE TREE SPECIES OF A HIGHLY THREATENED *MYRISTICA* SWAMP ECOSYSTEM

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Abstract: *Gymnacranthera canarica* (King) Warb. is an exclusive *Myristica* swamp species endemic to the Western Ghats. The *Myristica* swamp is a Critically Endangered ecosystem. Studies were carried out to assess the viability, germination and storage behaviour of *Gymnacranthera canarica* seeds. In the present study, it was observed that seeds have shown an initiation of germination after two weeks. A maximum of 90% germination was recorded when the initial moisture content was 38.04 ± 1.75 %. A decreased percentage (3%) was observed when the moisture content reached 14.26 ± 2.3 after 70 days of storage. Seeds failed to germinate beyond this moisture level. A desiccation study showed recalcitrant behaviour and seeds can be stored in lab conditions for up to two and half months.

Keywords: *Gymnacranthera canarica*, germination, moisture content, *Myristica* swamps, polyembryony, seed storage, viability.

The genus *Gymnacranthera* Warb. belongs to the family Myristicaceae with seven species in the Indo-Malayan region (Mabberly 1987). *Gymnacranthera canarica* (King) Warb. is an endemic and Vulnerable tree species (WCMC 1998) exclusively found in *Myristica* swamp ecosystems of evergreen forests in the Western Ghats. The *Myristica* swamp ecosystem was first reported by Krishnamoorthy (1960) from Kulathupuzha region of the Western Ghats, Kerala, which is a forest patch in the perennial swampy regions in the evergreen forests dominated either by *Gymnacranthera canarica* (King) Warb. or *Myristica fatua* Houtt. var. *magnifica*

(Bedd.) Sinclair. or both. These trees possess a modified root system, knee roots (Image 1A) with prominent lenticels on the surface for respiration in order to survive in the swamp. These ecosystems are virtually living museums of ancient life. These relic forests are often considered as ancient forest patches (Chandran et al. 2010). Champion & Seth (1968) classified this type of vegetation patch as tropical fresh water swamp forests (4C/FSI).

The occurrence of swamps depends on non biotic conditions like flat bottomed or gently sloping valleys in between heavily forested hills, deep soil in the adjoining hills with rock allowing water storage above the rock layer, slow and continued water seepage into the valley and an average of 3,000mm rain fall (Chandran et al. 1999; Varghese et al. 1999; Nair et al. 2007). Hence, these swamps are highly restricted in distribution and occurrence and are considered as critically endangered ecosystems (Roby et al. 2014).

The seed of *Gymnacranthera* conforms to the fruit with testa and bright red coloured aril (Image 1B). Seeds are used in the manufacture of candles (Gamble 1921). Seeds contain much fat, which is probably an adaptation for dispersal and survival under swampy conditions (Tambat et al. 2007). The present study was made to

DOI: <http://dx.doi.org/10.11609/jott.2152.8.7.9009-9013>

Editor: A.J. Solomon Raju, Andhra University, Visakhapatnam, India.

Date of publication: 26 July 2016 (online & print)

Manuscript details: Ms # 2152 | Received 16 July 2015 | Final received 20 May 2016 | Finally accepted 03 July 2016

Citation: Keshavachandra, K. & G. Krishnakumar (2016). Seed germination studies on *Gymnacranthera canarica* (King) Warb. - a Vulnerable tree species of a highly threatened *Myristica* swamp ecosystem. *Journal of Threatened Taxa* 8(7): 9009–9013; <http://dx.doi.org/10.11609/jott.2152.8.7.9009-9013>

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Funding: UGC Major Research Project No.: F.No.39-376/2010 dated 6-1-2011.

Conflict of Interest: The authors declare no competing interests.

Acknowledgements: We thank University Grants Commission for the financial assistance in the form of a major research project.





Image 1. (A) *Myristica* swamp - Knee Roots; (B) *Gymnacranthera* seeds with aril; (C) Seeds without arils; (D) Experimental set up - seeds sown on sand bed; (E) Polyembryony: Twin seedlings with separate roots and shoots

assess the germination behaviour, storage and viability in seeds of *Gymnacranthera canarica*.

MATERIALS AND METHODS

Seeds of *Gymnacranthera canarica* (King.) Warb. were collected from the *Myristica* swamp forest in Belthangady Taluk of Dakshina Kannada District, Karnataka in August 2012. The aril was removed from the collected seeds (Image 1C) and washed with water. Seed samples without any insect infestation and physical damage were selected for the experiment. To assess the viability and germination behaviour, initially the seeds were sown in a sand bed twice with 10 days interval. Later, they were sown at an interval of five days up to 100 days in two replicates. Sixty seeds were taken in each experiment. The remaining seeds were used to test moisture content and for a viability test.

Germination test

The germination percentage was determined by using the standard sand bed method (International Seed Testing Association 1985). Sand was taken in clean plastic trays of required size. The seeds were pressed onto the surface of sand; seeds were placed at a distance of 4 cm on the sand bed (ISTA 1991). Seeded sand beds were watered daily. Germination was scored on the emergence of radicle (2cm in length) and the results are expressed as percentage of seeds germinated

(Image 1D).

Seed viability

Seed viability was evaluated by taking seeds randomly from the seed lot, treating dissected seed with embryo with 1% 2, 3, 5 - triphenyl tetrazolium chloride solution (TZ) for 17 hours in darkness (ISTA 1999).

Moisture content

The Moisture content (MC) of the whole seed was determined following a low constant oven drying method (ISTA 1991). Randomly selected seeds were cut into pieces of definite quantity (5g). Then dried at 103°C for 17 hours in a hot air oven. This experiment was done in triplicate. Seed MC was calculated on a fresh mass basis.

$$\text{Moisture percentage} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

M_1 - is the weight of Petri plates; M_2 - is the weight of Petri plates with seeds before drying; M_3 - is the weight of Petri plates with seeds after drying.

Desiccation study

After procuring the seeds, clean and pure seeds were selected for the study. The initial fresh moisture content of the seeds was first determined using the above method; viability is checked by the Tetrazolium test

and the seeds were desiccated up to 10–12 % moisture content (Hong & Ellis 1996). Desiccation was performed by mixing equal amounts of self indicating silica gel in glass desiccators. The seeds were mixed twice a day to get proper aeration (IPGRI 2000; Varghese et al. 2002). The data obtained on seed germination was used for the following parameters.

Cumulative daily total number of germination of seeds (CDTG)

Germination speed index (GSI): Germination speed was estimated according to the method prescribed by Maguire (1962)

$$GSI = \frac{G_1}{T_1} + \frac{G_2}{T_2} + \frac{G_3}{T_3} + \dots + \frac{G_n}{T_n}$$

Where, G_1 - Number of seeds germinated on first day

G_2 - Number of seeds germinated on second day

G_3 - Number of seeds germinated on third day

T_1 - Day one; T_2 - Day two; T_3 - Day three

Germination energy (G.E.): It is the number of days required to attain 50% of germination (Allen 1958).

Germination percent: It is the ratio of number of seeds germinated to the seeds kept for germination

$$\text{Germination percent \%} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds kept for germination}} \times 100$$

Statistical analysis: Statistical analysis was done by using analysis of variance (Anova) in Sigma Plot 11.0

RESULTS AND DISCUSSION

Seeds of *Gymnacranthera canarica* are solitary and enclosed in a dark red coloured fleshy aril. Embryos are very small. Hypogeal germination is observed. This type of germination is most prevalent in species associated with seasonally flooded habitats (Smith et al. 2009). Germination started at the end of two weeks and the first leaf occurrence started after seven weeks (Table 1). Based on the germination period, these seeds can be assigned to the 'rapid' category (Rajeshwari 2001).

Tambat et al. (2006) reported a minimum of 40% of germination in controlled conditions, i.e. seeds without any treatment and a maximum of 79% of germination, in theseedstreatedwithgibberelicacidwhileworkingonthe enhancement of seed germination in *Gymnacranthera canarica*. However, in the present study, a maximum of 90% and a minimum of 3% germination were observed without any treatment. Fresh seeds subjected to Tetrazolium test showed 98% viability. The maximum seed germination recorded was 90%. This difference in seed viability and percentage of germination were seen

Table 1. Beginning of germination and leaf occurrence in *Gymnacranthera canarica* seeds.

Observations	Beginning of germination	First leaf occurred
	14–59 days (2–8 weeks)	53–68 days (7–9 weeks)

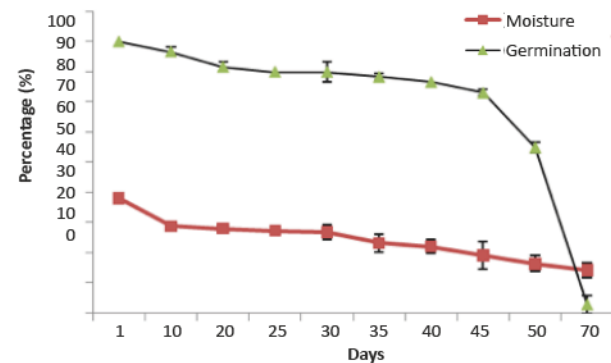


Figure 1. Germination percentage and moisture content in *Gymnacranthera canarica*

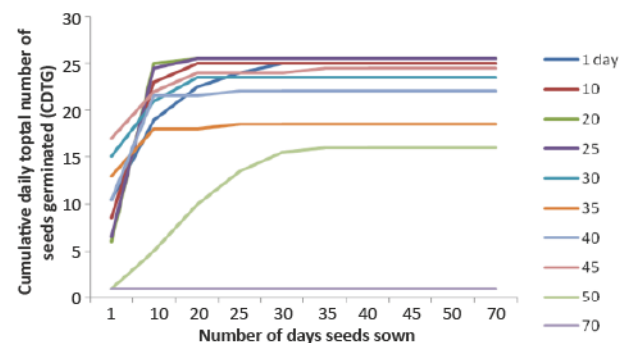


Figure 2. Cumulative daily total number of seeds germinated in *Gymnacranthera canarica*

in earlier studies also by Tambat et al. (2006). This is attributed to the presence of dehydrogenase enzyme in the other living cells in immature seeds which reduces 2,3,5 triphenyl tetrazolium chloride, forming bright red, stable, triphenyl formazan. These immature seeds fail to germinate. It is also possible in seeds infected with fungi which react with tetrazolium forming a red colour (R.K. Mittal pers. comm. 1997).

Initial moisture content of the seed was 38.04 ± 1.7 % which was significantly decreased on the 10th day (28.82 ± 1.56 %). A steady decrease in the moisture content was observed on subsequent days. On the 50th day the moisture content was 16.44 ± 2.57 %. When the MC content reached 14.26 ± 2.3 (seeds kept after 70 days of storage), there was no germination. Only, one seed germination was observed on the seeds sown on

Table 2. Deterioration in seed germination, germination energy and germination speed index of *Gymnacranthera canarica* (*p<0.05)

Days	Germination speed	Germination energy	Germination speed Index
1	76.34±2.8 ^a	4.5±3.5 ^a	20.79±1.21 ^{b*}
10	64.34±1.5 ^a	6.5±0.5 ^a	18.91±0.66 ^a
20	62.93±2.5 ^a	10±0.2 ^a	12.85±0.24 ^a
25	62.57±1.2 ^a	10±2 ^a	12.7±1.4 ^a
30	59.3±0.15 ^a	10.5±0.5 ^a	12.3±1.5 ^a
35	55.63±1.29 ^a	11±1 ^a	7.9±0.26 ^a
40	54.63±0.16 ^a	11.5±1.5 ^a	8.5±0.97 ^a
45	53.95±1.83 ^a	14.5±0.5 ^a	6.4±1.92 ^a
50	25.20±2.6 ^{b*}	23±1 ^{b*}	4.8±1.1 ^a
55	-	-	-
60	-	-	-
65	-	-	-
70	0.51±0.5 ^{b*}	0	0.01±0.01 ^a

the 70th day (Fig. 1). On the other hand, no germination was observed in seeds sown on the 55th, 60th and 65th days. Hence, the above mentioned moisture percentage may be considered as the critical level of moisture (16.44 ± 2.57% – 14.26 ± 2.3%) for seed germination since, below this level seeds failed to germinate. This result exactly corroborates with the result obtained in the desiccation study.

It has been observed that seeds that reached below 15% of moisture content after 40 days of desiccation resulted in seed quality deterioration, which clearly indicates the recalcitrant behavior (Roberts 1973; Chin et al. 1989; Chin 1995). Characteristically, desiccation sensitive seeds have been shown to be damaged if desiccated below critical moisture content (Chaitanya et al. 1998; Varghese et al. 2002). Many recalcitrant seeds die when stored at <15% of moisture (Mng'omba et al. 2007). Seeds with fleshy or juicy arilloid layers are generally recalcitrants (Chin et al. 1989).

In the present study, it is clearly observed that seeds readily germinate for the first one and half months and can be stored up to two and half months. It has been observed that, as the moisture content decreases over days, the time required for germination increases. A similar kind of observation was also reported in *Lophopetalum wightianum*, an invariable component of *Myristica* swamp ecosystem (Chandra et al. 2014), *Myristica malabarica* (Kumar et al. 2006) and *Knema attenuata* (Vinayachandra & Chandrashekar 2011).

Percentages of moisture and germination have been shown to have an effect on germination energy

and germination speed index. Decrease in germination energy and germination percent is due to the seed deterioration (Falleri 1994). The germination speed significantly decreased (p<0.05) at 50–70 days and germination energy also followed a similar trend. A significantly high germination speed index was observed on the first day (Table 2). The speed of germination is an expression of seed vigour and it is one of the seed quality parameters (Ahmadloo et al. 2011). High vigour seeds germinate faster than the low vigour seeds under any conditions (Schmidt et al. 2000). The Cumulative Daily Total number of seeds germinated supports this (Fig. 2).

Polyembryony

Out of the 800 seeds sown, 10 seeds showed twin seedlings indicating polyembryony (Image IE). Bhat & Kaveriappa (2002) have reported polyembryony in *Gymnacranthera canarica*. In Myristicaceae, polyembryony was first reported by Kannan (1971). Recently, Gunaga & Vasudeva (2011) have reported twin seedlings in *Semecarpus kathalekanensis* which is an obligatory *Myristica* swamp species.

CONCLUSION

Gymnacranthera canarica seeds are classified as recalcitrants. The seeds can be stored up to two and half months in lab conditions (28±4 °C) with critical moisture of 16.44±2.57 % – 14.26±2.3 %. Below this seeds fail to germinate. This germination studies will complement the conservation efforts of this obligate swamp species.

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ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

July 2016 | Vol. 8 | No. 7 | Pages: 8953–9052

Date of Publication: 26 July 2016 (Online & Print)

DOI: 10.11609/jott.2016.8.7.8953-9052

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