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RESEARCH ARTICLE

Epicotyl morphophysiological dormancy and a rare case of epigeal cryptocotylar seed germination in *Goniothalamus wynaadensis* (Bedd.) Bedd., a tropical threatened endemic tree species of the Western Ghats

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Abstract

Goniothalamus wynaadensis (Bedd.) Bedd. is a threatened indigenous tree species found in the Western Ghats and possesses significant medicinal value. This species belongs to the primitive family Annonaceae. However, there is a lack of information regarding seed germination in this species. We conducted experiments on seed germination using various media and found that the species exhibits epicotyl morpho-physiological dormancy (eMPD), marking the first such record in Annonaceae. GA_3 at a concentration of 500 ppm and warm stratification at 20 °C for one week proved effective in enhancing embryo growth and radicle emergence. The epicotyl emergence was observed only at 30±2 °C. Because warm stratification promotes both radicle and shoot emergence in *G. wynaadensis,* the level of eMPD is non-deep and simple. Hence, dormancy in *G. wynaadensis* can be described as $C_{1b}B_b$ (radicle)- $C_{1b}B_b$ (epicotyl); i.e., the embryo is underdeveloped and grows before radicle emergence and epicotyl emergence under warm temperatures (B_b) and both the radicle and epicotyl have non-deep simple physiological dormancy broken by warm temperatures (C_{1b}). Consequently, *G. wynaadensis* seed dormancy is phenologically well- adapted to the seasonal climate of Wayanad. Additionally, the species was found to display epigeal cryptocotylar seed germination—a rare occurrence in the development of angiosperm seedlings and this is the first record of such a phenomenon in the genus *Goniothalamus*.

Keywords

endemic; seed dormancy; epicotyl morphophysiological dormancy; seed germination

Introduction

An important stage in the life cycle of a plant is reproduction (1). Plants can be seen as achieving 4 major objectives through the production of seeds: the utilization of their genetic material, dispersal mechanisms, multiplication mechanisms and survival mechanisms (2). Therefore, genes that regulate seed germination and dormancy are particularly favoured in natural plant populations. This can lead to the evolution of complex mechanisms to balance the timing of germination with environmental conditions, ultimately contributing to the fitness and success of plant species in their specific habitats.

Seeds are classified as either dormant or nondormant (1). Nikolaeva classified seed dormancy into endogenous or exogenous dormancy (3). Endogenous dormancy includes physiological, morphological and morpho-physiological dormancy. In seeds with 'morphological dormancy', the embryo remains underdeveloped at maturity, and a dormant phase occurs as the embryo continues to grow post-shedding. Annonaceae, Arecaceae, Degeneriaceae, Lactoridaceae, Monimiaceae, Myristicaceae and Winteraceae are just a few of the families with seeds that have underdeveloped embryos (4).

Morpho-physiological dormancy (MPD) is a type of seed dormancy in which the embryo is both underdeveloped (i.e., small and must grow inside the seed before the radicle can emerge) and physiologically dormant. MPD occurs in primitive gymnosperms and in basal to advanced angiosperms (5). MPD is found in many plant families, including Apiaceae, Aquifoliaceae, Araceae, Arahaceae, Aristolochiaceae, Berberidaceae, Fumariaceae, Illiciaceae, Lardizabalanceae, Liliaceae, Magnoliaceae, Papaveraceae, Ranunculaceae and Schisandraceae (4). Although the vast majority of MPD cases have been reported in temperate regions, a few cases of nondeep MPD have been reported in tropical or subtropical species, such as *Stangeria eriopus*, *Zamia furfuracea* and *Gymnacranthera canarica* (6, 7). According to one report, *Goniothalamus tortilipetalus* seeds exhibit delayed germination (8). Later, this delayed dormancy was noted as in *G. tortilipetalus* as morphophysiological dormancy (6). Reports are on some seeds with morphophysiological dormancy that also exhibited a delay in shoot (epicotyl) emergence immediately after radicle emergence. The term "epicotyl-morphophysiological dormancy" is used to describe this type of dormancy.

Goniothalamus wynaadensis is endemic to the evergreen forests of Southern Western Ghats. The plant is locally referred to as "Anavalli" or "Anapanal" in Malayalam language (9). The occurrence of this species is restricted to a relatively small area of 6261 km^2, with its actual habitat covering only 40 km^2. There are 11 known locations, 10 of which are situated around Wayanad and one in Anamalai in the Western Ghats. Its conservations status is "near threatened" (9). Reports are on the leaf extracts of *G. wynaadensis* in ethyl acetate and water were cytotoxic to various cancer cell lines and effective against bacteria such as *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* (10). Further purification of the extract using column chromatography yielded the known cytotoxic molecule goniodiol-7-monoacetate, containing α, β-unsaturated δ-lactone (11). The bark juice of *G. wynaadensis* is used by the tribes of Wayanad as a component of arthritis medications (10, 12). Previous reports have highlighted the medicinal important of this species, which is also categorized as in the near-threatened. Therefore, there is an urgent need to develop necessary conservation methods to safeguard this threatened, yet medicinally important species.

While numerous examples (6) highlights the importance of seed germination traits in temperate species for synchronising germination time with the ideal conditions for seedling development, very few studies have focused on tropical and subtropical species. To date, only a few studies on seed germination and dormancy in the genus *Goniothalamus* have been published. Understanding seed germination and dormancy- breaking requirements is crucial for implementing suitable conservation methods. In this context, we conducted experiments to determine the seed dormancy and germination behaviour of *G. wynaadensis* . This study aims to provide information on seed dormancy and the requirements for breaking seed dormancy in this threatened plant species, which is crucial for implementing suitable conservation strategies.

Materials and Methods

Study area

The species is predominantly confined to Wayanad and its surrounding areas, as indicated by previous research. Wayanad district is situated in the Bayalu Seeme region of the Nilgiri Biosphere Reserve, characterised by hilly area. Geographically, it bears resemblance the neighbouring districts such as Nilgiris of Tamil Nadu and Kodagu of Karnataka and Mysore. Wayanad enjoys a salubrious climate, with the district's mean average rainfall recorded at 2608 mm. Areas like Lakkidi, Vythiri and Meppadi are known for high-rainfall, with annual precipitation ranging from 3000 to 4000 mm. During the southwest monsoon, high-velocity winds are common, while dry winds prevails during March–April. Regions at high-altitude experience severe cold. Over the last 5 years, the mean maximum and minimum temperatures in Wayanad were recorded at 29 °C and 18 °C respectively (13).

The present study was conducted on seeds of *G. wynaadensis* collected from Thamarassery Ghats Pass (N 11° 29′54.0" E 75°1′20.0") and the Periya reserve forest (N 75°48′ 25.0", E 11° 50′50.0") from 2019 to 2023 (Fig. 1). The species has been observed at elevation ranging from 350 to 900 m above sea level.

Periya Reserve Forest

The Periya reserve forest is an evergreen forest patch cov-

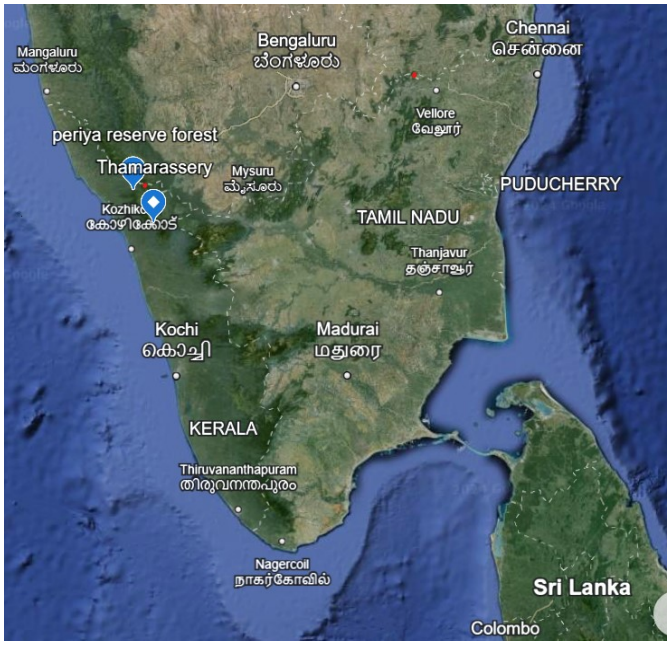


Fig. 1. The *G. wynaadensis* study area in the Western Ghats.

ering an area of 85.12 km^2 on the western slope of the North Wayanad Western Ghats ecoregion (N 11°50'43.0" E 75° 48'27.8"). Wayanad is located within the moist deciduous forest eco region of the south western Ghats and Periya is one of the very few blocks of old-growth evergreen forest remaining in the region. Several small, perennial seasonal streams and rivers in this area join the Kabini River. More than 80 % of the species in this region are categorised as rare and threatened (13). In this area, both the southwest and northeast monsoons bring rain, with the majority coming from the southwest monsoon, which typically begins with pre-monsoon showers by mid-April. The months with the highest precipitation are June and July, while the Northeast monsoon occurs in September and October, with sporadic rainfall in November. Precipitation is exceedingly rare between December and March. The observed annual rainfall ranges from 3000 to 3500 mm in this area. The soil type in this region is lateritic soil, a common byproduct of humid tropical conditions. The average summer temperature is 30 °C, the average monsoon temperature is 20 °C and the average winter temperature is 19 °C in this area. The soil is acidic and inadequate in readily available nitrogen, phosphorus, potassium and organic matter.

Thamarassery Ghat Pass

The Wayanad Pass or Thamarassery Pass (N 11°30'13.7" E 76° 01'41.0") is located in the Kozhikode district. The Ghat is also known as Wayanad Churam because it leads to Wayanad. As the altitude increases, the diversity of species also increases. The region becomes quite cold during the rainy season. The first hairpin area contains very few trees. From the third to the ninth hairpin, it is misty in the afternoon and evening, and the canopy is dense. The candidate species are found at the 8th hairpin bend of this pass. Water flows freely through the rocky patches on all hairpins. This region typically experiences pleasant, cooler weather, similar to that of the Periya Reserve Forest. The driest month is January and the warmest month is March. Each year, this region receives approximately 2900 mm of rainfall. The average annual temperature in this area is 22 °C. The soil is rich in readily accessible potassium and calcium and is slightly acidic (14).

Seed collection and processing

Dispersal units or ripe fruits (orange to red and fleshy) were collected from each site at the fruiting peak (November to December). Mature naturally dehisced fruits were collected, placed in polythene bags, tagged and transported to the Department of Conservation Biology, KSCSTE-JNTBGRI, Palode, Thiruvananthapuram, where laboratory experiments were conducted. Seeds were extracted from the fruits by hand, removing the pulp in water. The cleaned seeds were sterilised with 0.1 % $HgCl_2$ for 5 min and washed again with distilled water. Subsequently, the seeds were air-dried at room temperature, after which germination tests were immediately performed (15).

Seed morphology

The dimensions of the seeds, such as length and breadth, were measured using a digital Vernier calliper (Mitutoyo Absolute Digimatic, Japan) and the values were recorded.

Seed purity

The seed purity test denotes the % of seeds (by weight). The following formula was used to determine the purity of the seeds on a weight basis:

Purity% = [weight of pure seed (g) ÷ total weight of working sample (g)] 100

Seed imbibition test

The initial weight of the seeds was measured, and the seeds were placed on moist filter paper at room temperature. After 3 h, 6 h, 9 h, 12 h, 15 h, 18 h, 21 h and 24 h, the seeds were removed from the wet paper, blotted dry and weighed. The percentage of seed imbibition was calculated using the following formula:

$$\%Ws = [(Wi – Wd)/Wd] 100$$

where Ws is the increase in seed mass, Wi is the seed mass after a given interval of imbibition and Wd is the initial seed mass at 0 h (16).

Seed moisture content

The moisture content was measured using the International Seed Testing method. The following formula (17) was used to calculate the moisture content, which was then expressed as a %.

% of seed moisture content = [(Fresh weight of the seed – Dry weight of the seed)/Fresh weight of the seed] 100

Seed viability

Tetrazolium test

Tetrazolium testing is the most widely adopted biochemical method for examining seed viability. This method is also called the topographical Tetrazolium test (TTZ). In this method, a 1 % TZ solution was used. Three replicas of fresh seeds, each containing 25 seeds, were bisected longitudinally before the test. The collected seeds were then immersed in TZ solution for 48 h and the staining pattern was evaluated after washing the seeds in distilled water (18). The TZ test quickly determined the viability of the seeds. Seeds with fully and partially stained endosperms were considered viable, whereas those with unstained endosperms were considered nonviable. The accuracy of the test was assessed by comparing the viability % obtained from the tests with the germination % obtained through a simultaneous seed germination test.

Seed germination

Seed germination was examined using 4 different treatments. For these experiments, fresh, mature, naturally dehisced fruits were used. Seed germination on Petri dishes (treatment 1), on germination paper (treatment 2), on soil from the JNTBGRI (treatment 3) and in a natural environment (treatment 4) was studied. When the radicle measures 0.5 cm in length, the seeds are deemed to have germinated (19). The following parameters were considered for this study:

The speed of seed germination (SPG) was calculated using the following formula:

$$SPG = n1/d1 + n2/d2 + n3/d3+\ldots\ldots$$

where n1 is the number of seeds germinated on day 1 of germination, n2 is the number of seeds germinated on day 2 and d is the number of days taken for seed germination (20, 21).

The mean germination time (MGT) was calculated by the following formula:

$$MGT = ((n1\times d1) + (n2 \times d2) + (n3 \times d3) + (n4 \times d4) +\ldots.) \div (\text{total number of germinated seeds})$$

where n1 is the number of seeds germinated on day 1 (d1), n2 is the number of seeds germinated on day 2 (d2) and d is the number of days taken for seed germination (20).

The mean daily germination (MDG) was calculated using the following equation:

MDG = Number of seeds germinated in total ÷ Total number of days taken for seed germination (20).

Peak value (PV) of seed germination

PV = highest number of seeds germinated ÷ number of days (21)

Germination energy (GE)

Germination energy = peak value (PV)/mean daily germination (MDG)

Mean germination value (GV) = peak value (PV) × mean daily germination (MDG)

Data Analysis

The research data were analysed using SPSS version 25. One-way analysis of variance was used to compare the mean values of germination %, emergence index, germination speed, germination energy, mean germination value and mean germination time in days for the 5 different treatments. Duncan's post hoc test was used to compare multiple means. A P value < 0.05 was considered to indicate statistical significance.

Embryo length : seed length ratio (E:S ratio)

The embryo length : seed length ratio (E:S) was calculated for freshly collected naturally dispersed seeds and for seeds at the time of endocarp rupture. This helped determine embryo development. The *G. wynaadensis* embryo in fresh seeds is small and cannot be visualised and measured through a stereomicroscope. To measure the embryos of fresh seeds, scanning electron microscopy was performed. Mature seeds were cut into 2 halves, placed directly on brass stubs, coated with gold and observed using a Carl Zeiss Evo 18 scanning electron microscope. The embryo length of the seeds during dispersal was measured. The embryo length before the radicle emerged (at the time of endocarp split) was measured using a stereomicroscope. The % of embryo development before radicle emergence was calculated using the following formula:

% of embryo development = (average E:S ratio of seeds soon after endocarp rupture ÷ average E:S ratio of fresh seeds) × 100 (22).

Seed dormancy-breaking treatments

Soaking seeds in water

The seeds were soaked in distilled water for 24 h and then placed on germination paper in a KEMI KSG-2 seed germinator set to 80 % relative humidity and 30 ± 2 °C.

Stratification

The seeds were placed on germination paper and then incubated at various temperatures.

Cold stratification

The seeds were initially incubated at 5 °C and 10 °C for one week before being transferred to the germinator, where they were subjected to ideal conditions of 80% RH and 30 ± 2 °C. Three replicates, each containing 100 seeds were used.

Warm stratification

The seeds were initially incubated at 20 °C for one week and then transferred to a germinator set to ideal conditions of 80 % RH and 30 ± 2 °C. Three replicates, each containing 100 seeds, were utilized.

Effect of GA_3 on seed germination

GA_3 pre-treatment was conducted at various concentrations (0 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm) to assess the physiological dormancy of the seeds. All treated seeds were placed on germination paper and stored in a seed germinator at 30 ± 2 °C and 80 % RH (19).

Natural seed germination trials

The number of days for germination, germination % and the initiation of the first leaves were examined in studies on seedling growth under various growing conditions. Seedling development was monitored both in the JNTBGRI polyhouse and in natural environment.

Results

Seed morphology

Monocarps are 1-seeded. The seeds are ovoid, measuring 11.989 ± 0.954 mm long, and 6.470 ± 0.3767 mm wide, with an average weight of 0.3295 ± 0.0280 mm (Fig. 2A). The embryo size is small, the endosperm is copious, and the embryo must develop inside the seed before the radicle emerges. Therefore, the seeds of *G. wynaadensis* have underdeveloped embryos. The seed characteristics of *G. wynaadensis* are summarised in Table 1.

Seed purity

The seed purity of *G. wynaadensis* was 30.68 %. Approximately 70 % of the fruit were infested with larvae of fruit flies suspected to be in the genus *Euphranta*. The fruit remains intact at dispersal,with the endosperm and embryo

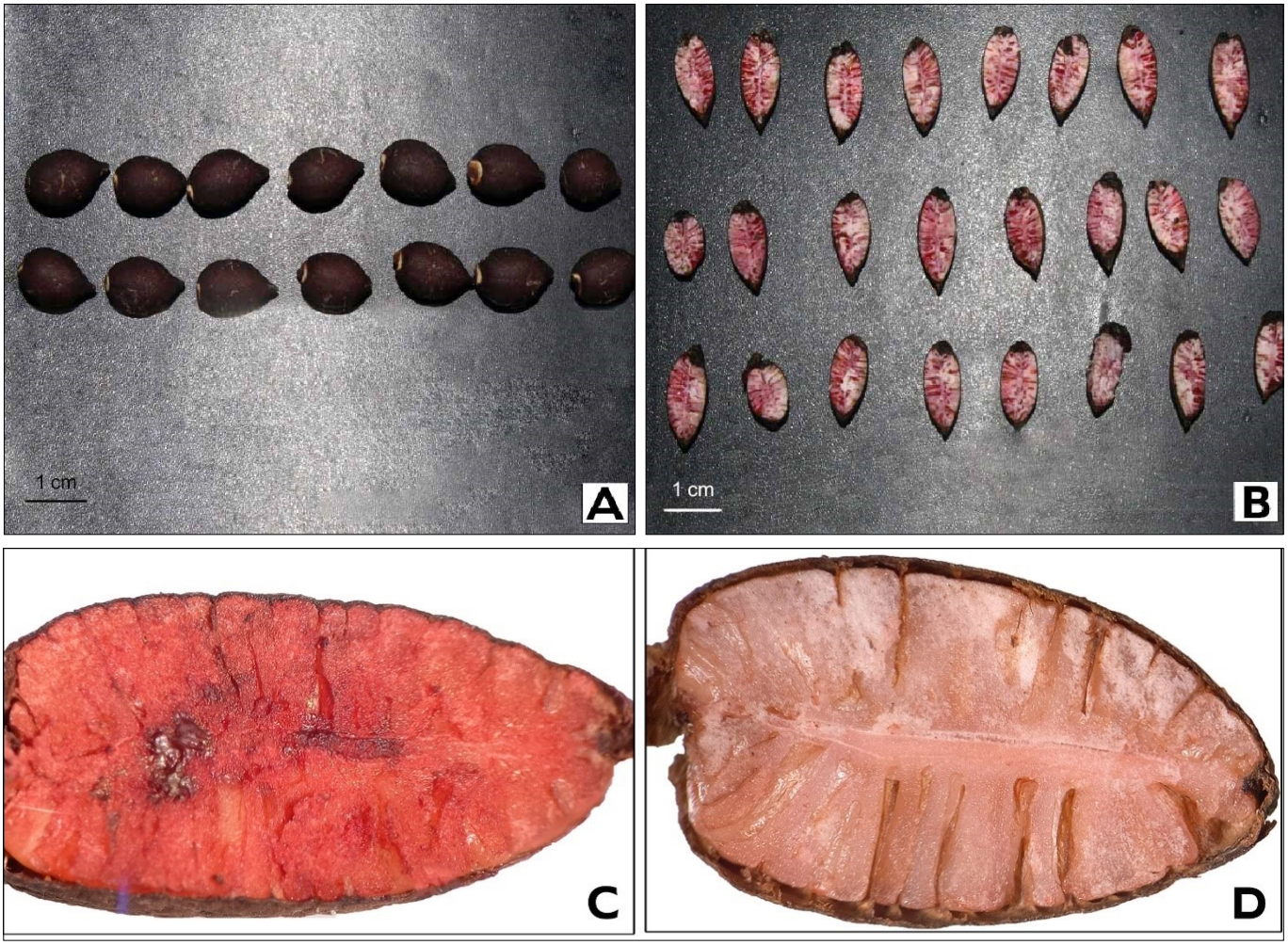


Fig. 2. Seed morphology and viability; **(A)** seeds of *G. wynaadensis,* (**B)** tetrazolium test showing partially stained endosperm, **(C)** fully stained endosperm, **(D**) unstained endosperm.

Table 1. Seed characteristics of *G. wynaadensis.*

Parameters	Measurements*
Seed Fresh weight(g)	0.3295±0.0280
Seed size(mm)	11.989±0.954 × 6.470±0.3767
Seed moisture content (% Dry weight basis)	28.41 ±2.5
Seed purity	30.68%
Endosperm	Ruminate
Embryo	Underdeveloped

* Values are presented as the means ± SDs of 100 samples.

being replaced by larvae of the fruit fly.

Seed imbibition test Genomic

Prior to initiating the seed germination studies, the seeds were checked for water imbibition. It was observed that there was an increase in seed weight every 3 h, with readings taken for up to 24 h (Fig. 3). An increase in seed weight after imbibition indicates that seeds of *G. wynaadensis* have permeable coats.

Seed moisture content

The seed moisture content of fresh seeds of *G. wynaaden-*

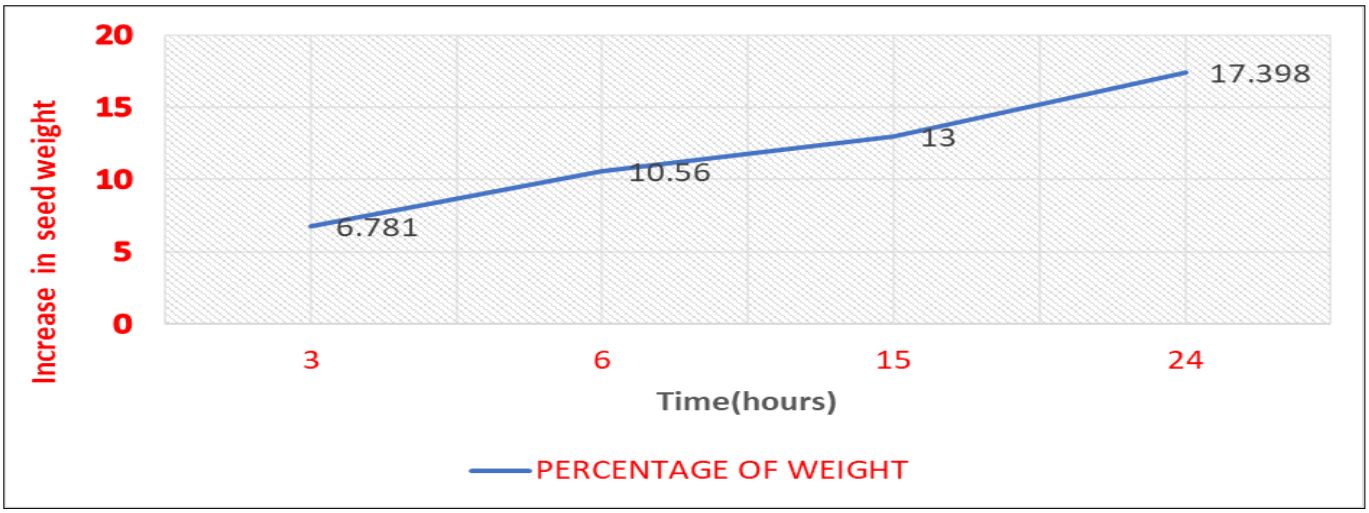


Fig. 3. Water imbibition curve for *G. wynaadensis* seeds.

sis was 28.41 ± 2.5% on a dry weight basis.

Seed viability

Through the tetrazolium test, fully stained, partially stained, and unstained seeds (Fig. 2D) were obtained and the results are summarised in Table 2. The principle behind this test is that active enzyme dehydrogenases in the living tissue of the seeds convert 2,3,5-triphenyl tetrazolium chloride to formazan, an insoluble red dye that stains the living tissues red. Fully stained (Fig. 2C) and partially stained (Fig. 2B) seeds were considered viable. Thus, after this tetrazolium test, 97.36±1.32% of the seeds were found to be viable.

Table 2. % of viable seeds according to the Tetrazolium test.

Tetrazolium test		
Staining Pattern	**Mean %***	**% of viable seeds**
Fully stained endosperm	7.894± 5.36	
Partially stained endosperm	89.47±7.52	97.36±1.32
Unstained endosperm	2.63± 1.11	

* Values are presented as the means ± SDs of 75 samples.

Seed germination

Seed germination trials were conducted using different methods: germination paper stored inside the germinator at 30 ± 2 °C and 80 % RH (germination trial 1), Petri dish with wet filter paper inside the germinator at 30 ± 2 °C and 80 % RH (germination trial 2), soil from the natural habitat at 30 ± 2 °C inside a polyhouse (germination trial 3), and soil from the JNTBGRI at 30 ± 2 °C inside a polyhouse (germination trial 4). The results are summarised in Table 3. Regarding germination % and mean germination time, germination trial 1 (using germination paper stored inside the germinator at 30 °C and 80 % RH) showed the most favourable response compared to the other trials, with a germination % of 73.40 ± 1.14 and a mean germination time of 41.27 ± 0.87 days. Further analysis revealed that germination took more than 30 days to occur. For seeds with underdeveloped embryos, radicle emergence typically happens within approximately 1 month or less, indicating morphological dormancy. Conversely, if radicle emergence is delayed and takes longer than 1 month, the seeds exhibit morphophysiological dormancy (MPD). Hence, seeds of *G. wynaadensis* demonstrates morphophysiological dormancy.

While the radicle emerges immediately after embryo growth, the epicotyl fails to emerge quickly after the radicle appears. Seeds with epicotyl dormancy do not exhibit growth of the upper portion (or shoot) of the plant immediately after radicle emergence. In case of *G. wynaadensis*, the epicotyl emerges only after days of warm stratification. Hence, seeds of *G. wynaadensis* exhibit epicotyl morphophysiological dormancy (eMPD).

Embryo growth

The embryo cannot be distinguished from the endosperm in seeds collected immediately after dispersal, even with a stereomicroscope. Therefore, scanning electron microscopy was performed to visualise the embryo. Scanning electron microscopic analysis of fresh seeds revealed a small embryo (Fig. 4 A-C), with the embryo-to-seed length ratio (E:S) in fresh seeds measured at 0.139 ± 0.04.

The E:S ratio of fresh *G. wynaadensis* seeds was 0.139 ± 0.04 and it increased to 0.5 ± 0.02 before radicle emergence (Fig 5 A-C).

The % of embryo development from the time of dispersal until radicle emergence in *G. wynaadensis* was calculated to be 359.71 %.

Seed dormancy-breaking treatments

Soaking seeds in water

Seeds of *G. wynaadensis* were soaked in water for 24 h, resulting in 80 % germination, with the mean germination time reduced to 18 days (Fig. 6B).

Stratification

Temperature requirements for embryo growth and radicle emergence : Cold stratification followed by warm stratification

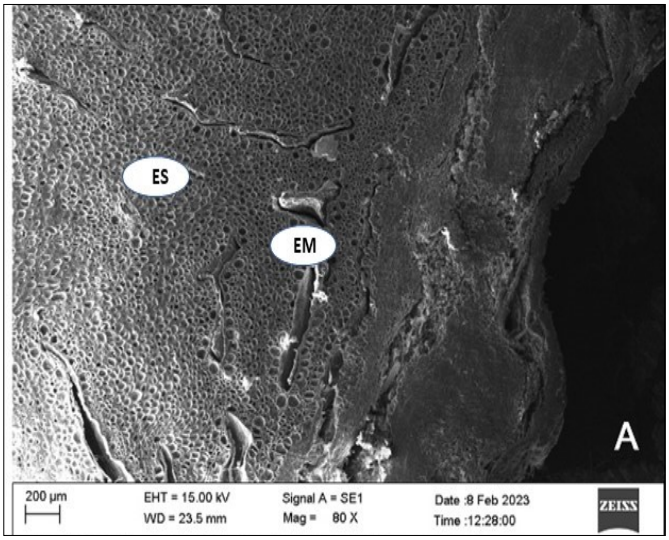
Cold stratification was conducted at 5 °C and 10 °C for 1 week, after which the samples were transferred to 30 °C. Following stratification at 5 °C for 1 week, the seed germination % was 60.00 ± 1.22 % and the mean germination time was reduced to 23.60 ± 0.89 days. Stratification at 10 °C for 1 week resulted in a germination % of 80.20 ± 1.10 % and the mean germination time decreased to 24.20 ± 0.84 days.

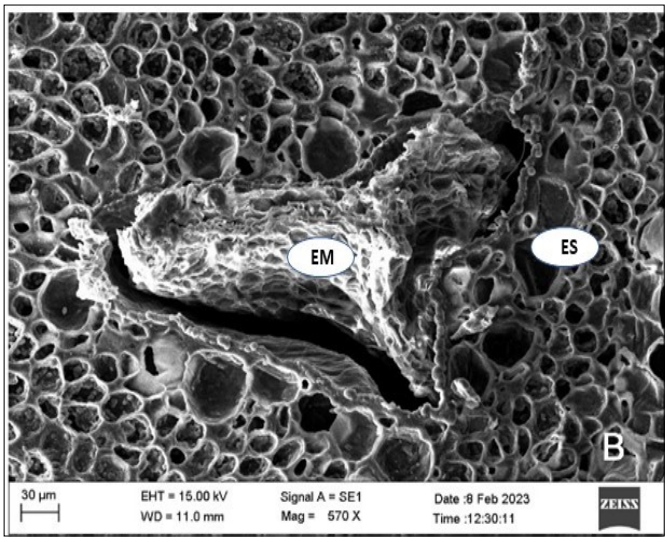
Warm stratification

Warm stratification was conducted at 20 °C for 1 week, following which the samples were transferred to 30 ± 2 °C. After stratification of *G. wynaadensis* seeds for 1 week, the germination % improved to 90.20 ± 1.10 % and the mean germination time decreased to 23.60 ± 0.89 days (Fig. 6C). The results are summarised in Fig. 7.

Table 3. Germination trials of *G. wynaadensis* seeds in different germination media

Treatment	Germination%	Emergence index	Germination speed	Germination energy	Mean germination value	Mean germination time
Trial 1 (on germination paper stored inside the germinator)	73.40 ± 1.14^{a}	1.980 ± 0.007^{a}	0.2702 ± 0.0071^{a}	0.3087 ± 0.0015^{c}	0.0168 ± 0.0004^{a}	41.27 ± 0.87^{d}
Trail 2 (on petridish with wet filter paper inside the germinator)	60.00 ± 1.22^{b}	1.364 ± 0.009^{b}	0.1763 ± 0.0015^{b}	0.2638 ± 0.0041^{d}	0.0052 ± 0.0003^{b}	52.00 ± 0.94^{c}
Trial 3 (soil from natural habitat)	46.67 ± 1.42^{c}	0.558 ± 0.006^{c}	0.1214 ± 0.0012^{c}	0.4288 ± 0.0146^{a}	0.0053 ± 0.0003^{b}	58.30 ± 0.51^{b}
Trail 4 (Soil from JNTBGRI inside polyhouse)	33.33 ± 0.69^{d}	0.565 ± 0.020^{c}	0.0815 ± 0.0008^{d}	0.3984 ± 0.0018^{b}	0.0025 ± 0.0005^{c}	61.48 ± 1.12^{a}
F Value	1125.969	16717.504	2445.641	499.023	1347.579	503.222
P Value	0.000	0.000	0.000	0.000	0.000	0.000





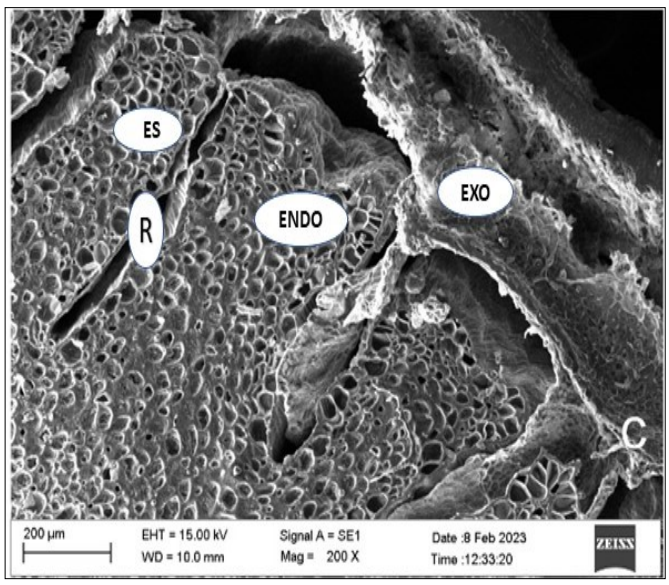


Fig. 4. Scanning electron microscopy image of seeds and embryos; **(A-C)** Scanning electron.microscopic view of the LS of seeds. [Abbreviations: ext - exotesta, ent - endotesta, emb - embryo, r - rumination, e - endosperm].

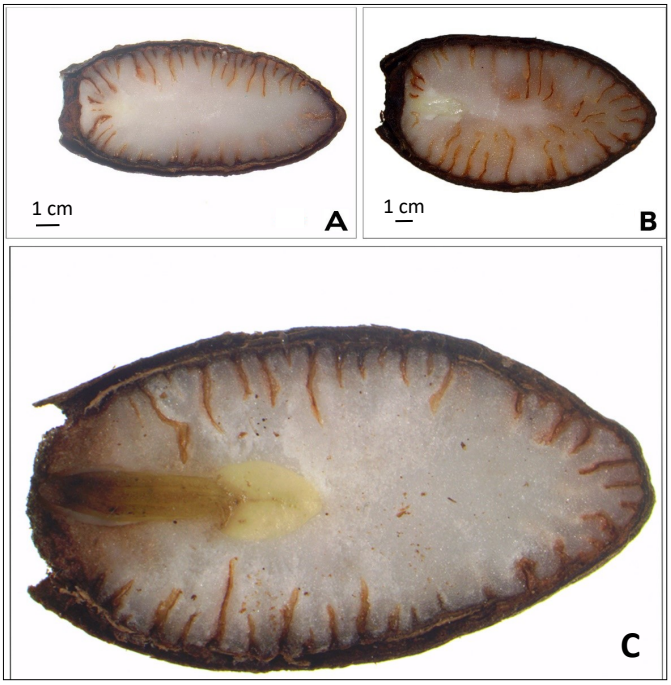


Fig. 5. Growth of an underdeveloped embryo in the seeds of *G. wynaadensis.* **(A)** stereomicroscopy image of fresh seeds**; (B)** stereomicroscopy image of seeds incubated at 30 °C for 5 weeks**; (C)** seeds incubated at 30 °C for 6 weeks.

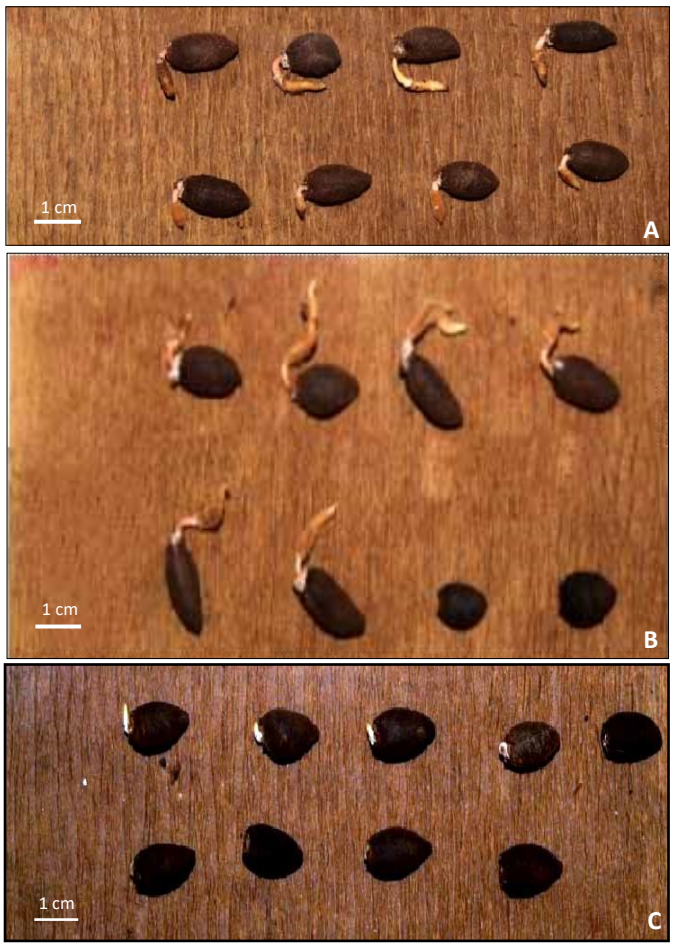


Fig. 6. Seed dormancy-breaking treatments. **(A)** 500 ppm GA3-treated seeds; **(B)** Seeds soaked in water for 24 h; **(C)** Seeds stratified at 20 °C.

The results of stratification revealed a statistically significant difference in the mean germination % among the 4 treatments (F = 617.987, P<0.05). Duncan's post hoc test for multiple comparisons revealed that the mean % of germination differed significantly among the 4 treatments. The highest mean germination % (90.20 ± 1.10%) was observed for seeds stratified at 20 °C, followed by those stratified at 10 °C (80.20 ± 1.10%). Regarding mean germination time, there was a significant difference among the 4 different treatments (F = 1498.334, P<0.05). Duncan's post hoc test for multiple comparisons revealed significant differences in mean germination time among the 4 treatments. The greatest mean germination time (41.27 ± 0.87

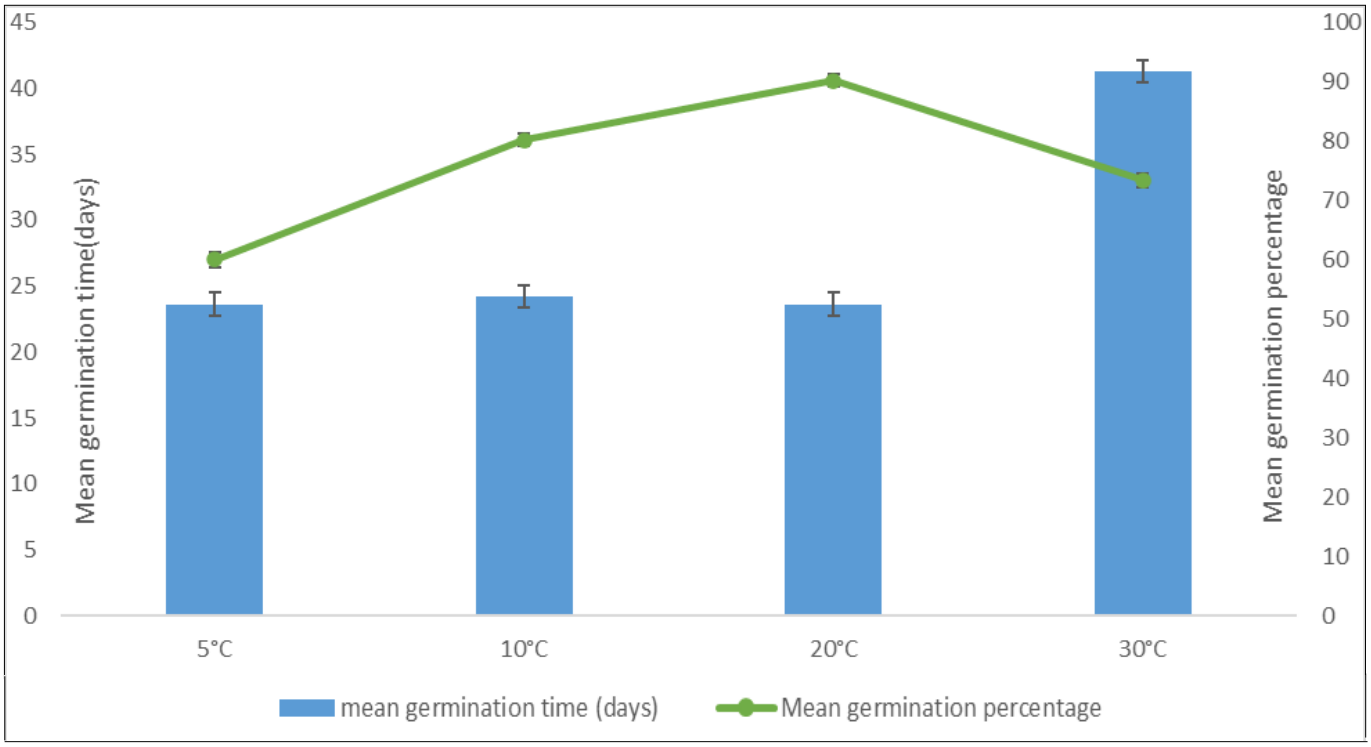


Fig. 7. Effect of stratification on the radicle emergence of *G. wynaadensis.*

days) was observed at 30 °C, followed by 24.20 ± 0.84 days at 10 °C. The lowest mean germination time (23.60 ± 0.89 days) was recorded at 5 °C and 20 °C.

of germinated seeds was observed in the 50, 100 and 250 ppm treatment groups. Notably, treatment with 500 ppm GA_3 for 24 h resulted in almost 99.60 ± 0.55 % seed germination. These results are summarised in Fig. 9. Statistical

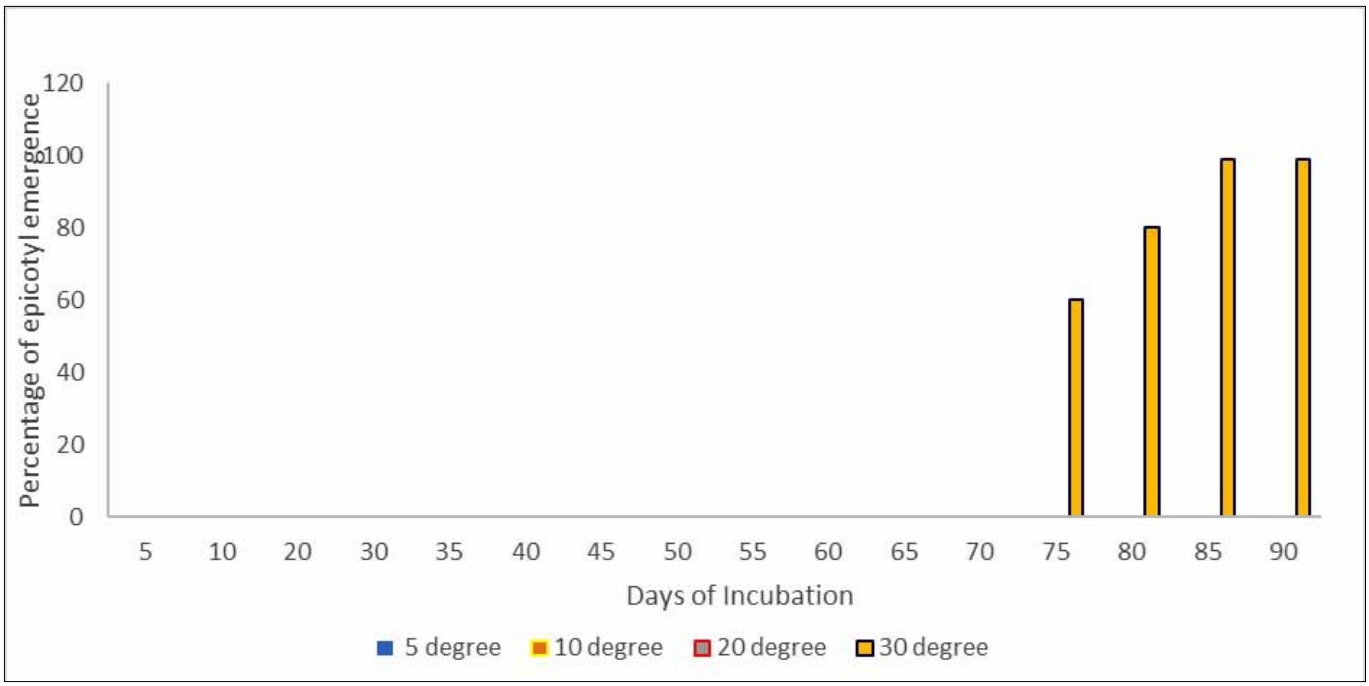


Fig. 8. Effect of temperature on epicotyl emergence.

Temperature requirements for epicotyl emergence

Epicotyls failed to emerge at temperature of 5 °C, 10 °C or 20 °C. However, in seeds stratified at 30 °C, 98 ± 2 % of the epicotyls emerged after 78–85 days (Fig. 8).

Effect of GA_3 on seed germination

Effect of GA_3 on radicle emergence

The control group (seeds soaked in water) exhibited 79.60 ± 0.55 % seed germination. A slight increase in the %

analysis revealed a significant difference in the mean germination % among the four different treatments ($F = 206.689$, $P<0.05$).

Duncan's post hoc test for multiple comparisons confirmed that the mean germination % significantly varied among the treatments. The highest mean germination % (99.60 ± 0.55 %) was observed for seeds treated with 500 ppm GA_3, followed by those treated with 250 ppm GA_3 (90.80 ± 1.64 %). Furthermore, the mean germination

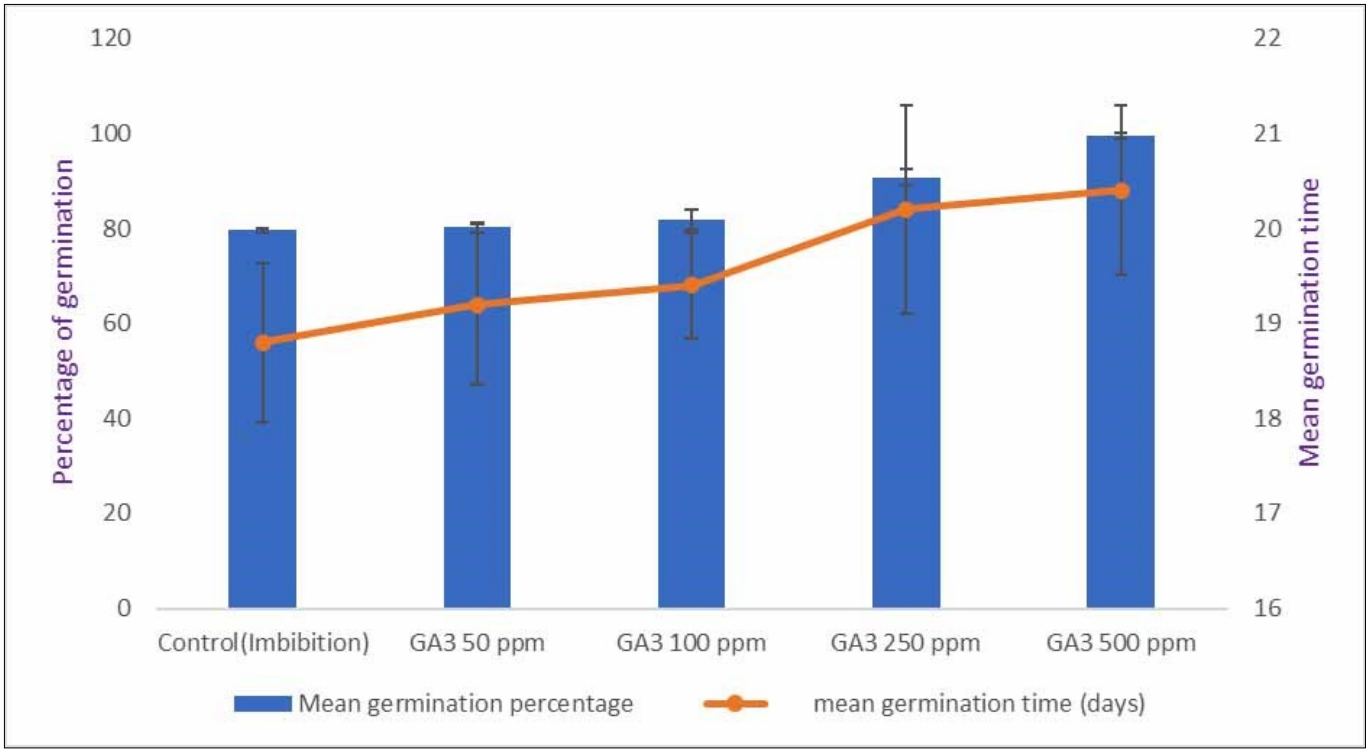


Fig. 9. Effect of GA_3 on radicle emergence.

time showed a significant difference among the treatments ($F = 3.108$, $P<0.05$). Duncan post hoc tests for multiple comparisons indicated significant differences in the mean germination times of the 4 treatments. The longest mean germination time (20.40 ± 0.89 days) was recorded for the 500 ppm GA_3-treated plants, followed by the 250 ppm GA_3-treated plants (20.20 ± 1.10 days). Conversely, the shortest mean germination time (18.80 ± 0.84 days) was observed during imbibition.

Effect of GA_3 on shoot emergence

Pretreatment (seeds before radicle emergence) and posttreatment (seeds after radicle emergence) of the seeds with various GA_3 concentrations were performed. The results are summarised in Fig. 10. Posttreatment with GA_3 did not significantly reduce the mean epicotyl emergence time. However, pretreatment with 500 ppm GA_3 reduced the mean germination time to 64 ± 1 day.

Seedling development

During seed germination, a radicle emerges from the mi-

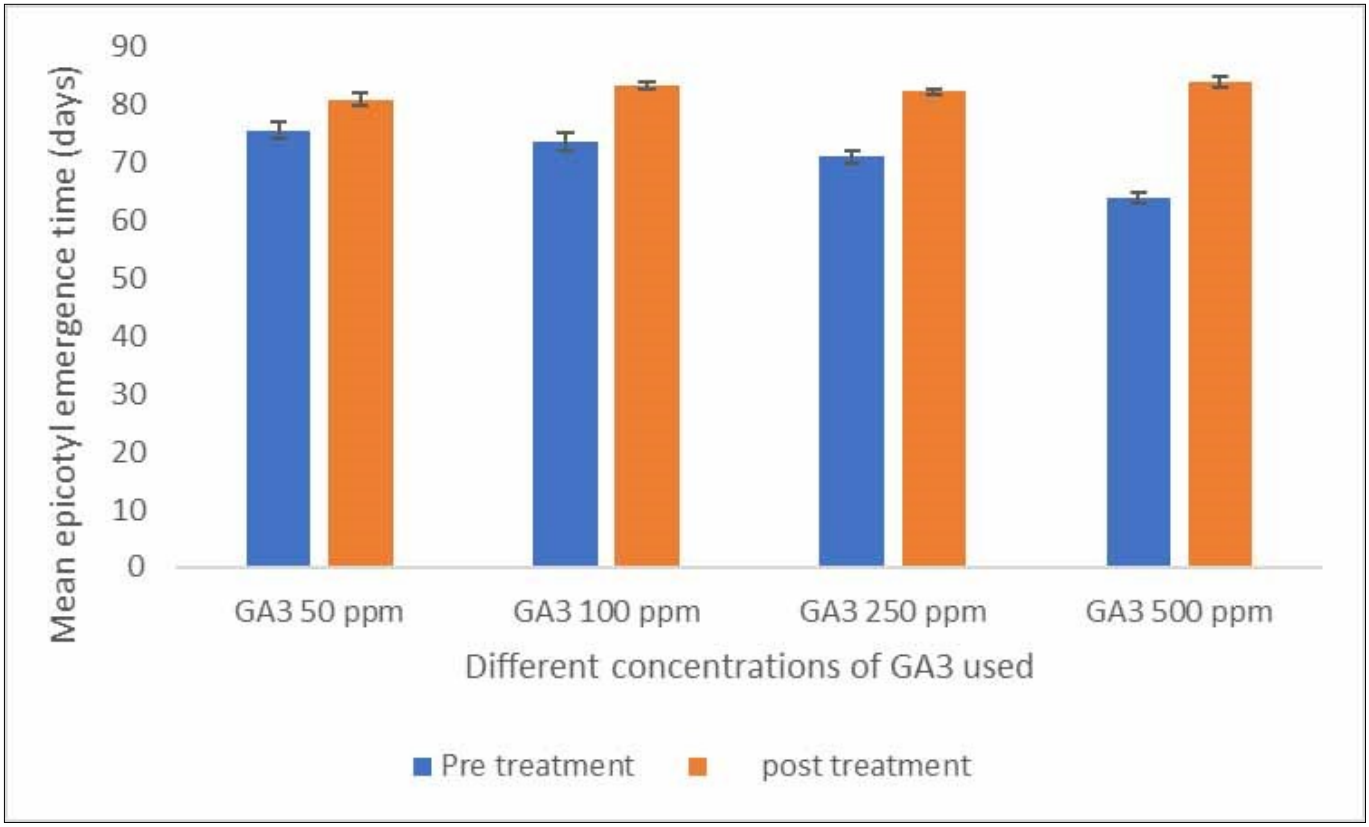


Fig. 10. Effect of GA_3 on shoot emergence.

cropylar end (Fig. 11 C). Before radicle protrusion, the testa and endosperm rupture (Fig. 11 A-B). Preceding the emergence of the hypocotyl from the seed coat, root enlargement and thickening occur (Fig. 10 D). Subsequently, the hypocotyl elongates and curves in a loop above the ground, carrying cotyledons enclosed in the testa above the soil (Fig. 12 A). The cotyledons of *G. wynaadensis* serve a haustorial function by absorbing nutrients from the en-

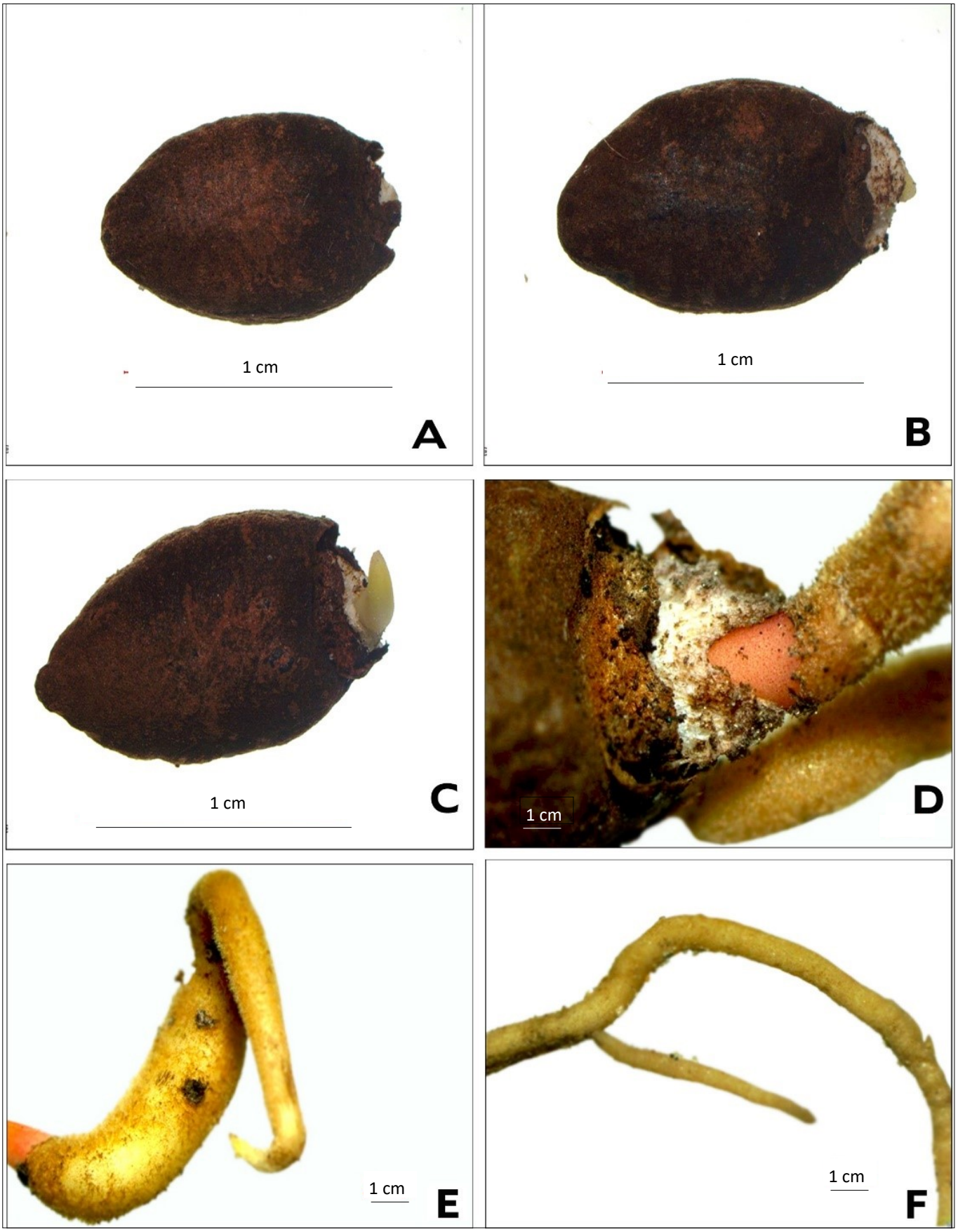


Fig. 11. Seed germination events visible during germination at 30 °C and 80% RH in the germination paper. **(A)** Rupture of the seed coat and endosperm rupture**, (B)** protrusion of the radicle**, (C)** elongation of the radicle**, (D)** formation of the hypocotyl, **(E**) further elongation of the radicle**,** and **(F)** secondary (lateral) root formation.

dosperm. The cotyledons of *G. wynaadensis* serve a haustorial function by absorbing nutrients from the endosperm. Remaining enclosed in the testa and attached to the apex of the hypocotyl, they inhibit the development of

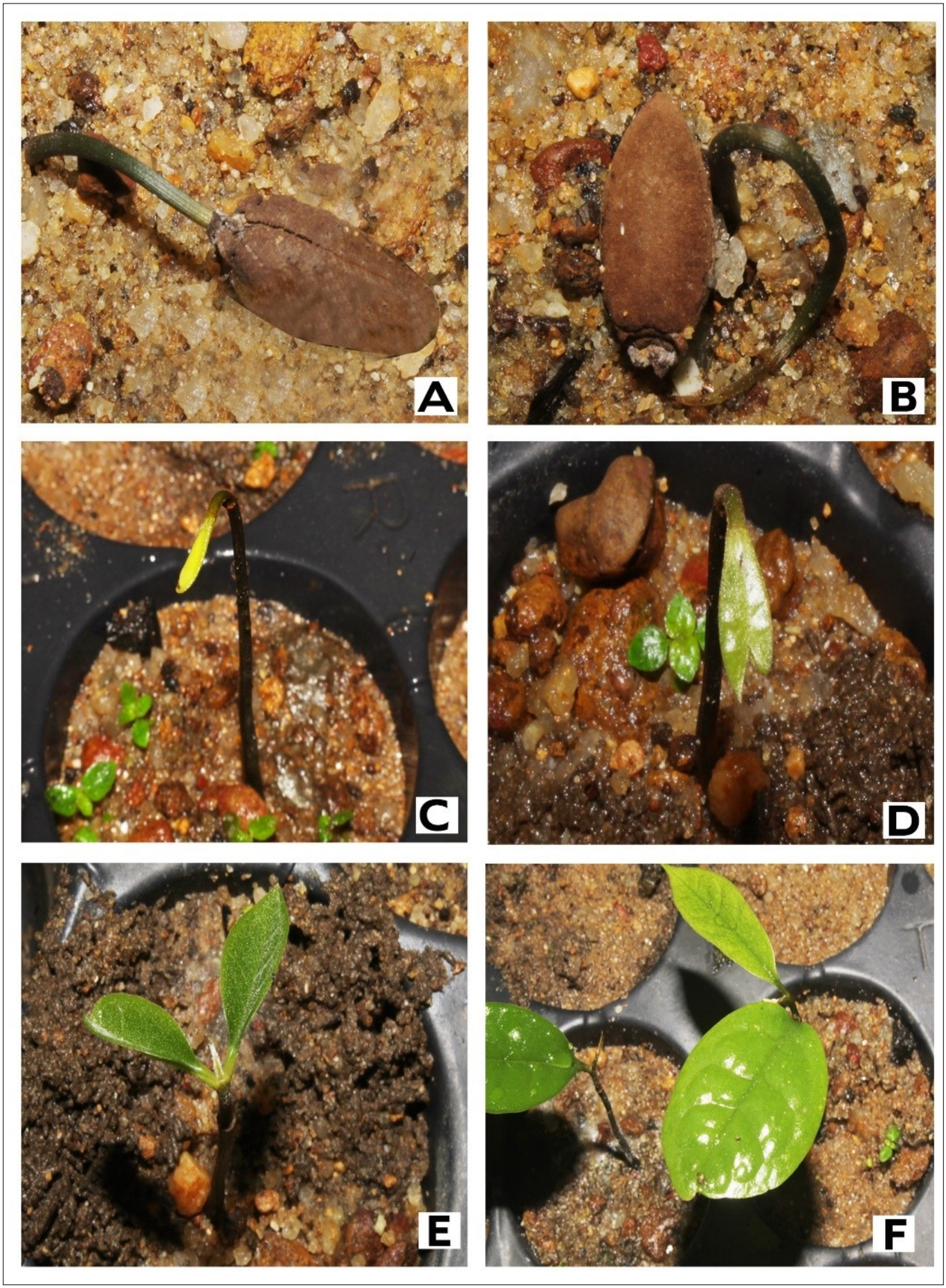


Fig. 12. Different stages of germination of *G. wynaadensis* seeds in the soil. **(A)** cotyledons enclosed in testa raised above the soil, **(B)** seedlings with cotyledons (excised) showing cataphylls, (**C-F)** development of the first true leaves.

the epicotyl and plumule (Fig. 12 A, Fig. 11 D). During this phase, the seedling enters a resting stage during which nutrients from the endosperm are transported into the hypocotyl, resulting in its development and increased strength (Fig. 10 D). Root thickening occurs, and lateral root emergences precedes epicotyl emergence (Fig 11 E-F). The cotyledons of *G. wynaadensis* remain non-emergent (Fig. 12 A-C) and eventually wither and detach along with the testa. Subsequently, the plumule develops into a shoot, and the initial leaves formed are cataphyll (Fig. 12 C -D). Due to the persistence of cotyledons within the seed coat and their position relative to the ground, *G. wynaadensis* rarely exhibits epigeal cryptocotyls during angiosperm seedling development.

Cataphyll forms true leaves after 16 ± 3.5 days, and the first formed leaves are opposite rather than alternate (Fig. 12 B-E). Initiation of the third apical leaf bud starts 25 ± 5 days after epicotyl emergence (Fig. 12 F). After one year of development, the seedlings had a mean height of 15.125 ± 2.175 cm and a diameter of 0.825 ± 0.125 cm.

Discussion

Seed morphology

The seeds of *G. wynaadensis* are ovoid, measuring 11.989 ± 0.954 mm long, and 6.470 ± 0.3767 mm wide, with an average weight of 0.3295 ± 0.0280 mm. When mature, they exhibits a dark brown coloration. In contrast, the elliptical-shaped seeds of *G. amuyon* have a diameter of approximately 5.5 mm and a length of approximately 7 mm, developing a brown or dark brown colour (23). The seeds of *G. wynaadensis* possess a ruminate endosperm. In Annonaceae, seeds are typically endospermous, with an oily endosperm that may occasionally be starchy and ruminate, characterized by an uneven, coarsely wrinkled texture. Additionally, a septate pith and resin canals are commonly present (24). Similar to *G. cardiopetalous* and *G. rhyncantherus*, *G. wynaadensis* bears 1 seed in each monocarp (25).

Seed moisture content

The fresh moisture content of the *G. wynaadensis* seeds was 28.41 ± 2.5 % (dry weight basis). Seed moisture content refers to the amount of water present in the seeds at the time of dispersal. Earlier reports have indicated that seeds of several species are dispersed at low moisture levels (known as orthodox seeds). However, recalcitrant seeds are dispersed at relatively high moisture levels (26). A study reported that *Goniothalamus amuyon* seeds have a fresh moisture content of 24.8 ± 0.4% (on a fresh weight basis) and exhibit intermediate storage behaviour (27). Therefore, the storage behaviour of *G. wynaadensis* warrants detailed study to determine whether the seeds are recalcitrant or orthodox.

Seed viability

Fresh *G. wynaadensis* seeds immersed in tetrazolium showed a viability of 97.36 ± 1.32%. According to one report, *Xylopia aromatica* (Annonaceae) seeds exhibiting morphophysiological dormancy can be effectively tested for viability by being imbibed at 30 °C for 48 h and then submerged in a 0.5 % tetrazolium solution for 24 h (26). Scolowski reported that the emergence of *Xylopia aromatica* seeds treated with promalin (6-benzyladenine + GA_4 + GA_7) was greater than the viability observed after tetrazolium treatment (28). The present study also supports these findings. The germination % of GA_3-treated seeds was 99.60 ± 0.55 %, while that of tetrazolium-treated seeds was 97.36 ± 1.32 %.

Seed germination

Germination trials conducted in different media at 30±2°C revealed that *G. wynaadensis* seeds took more than 1 month to germinate, indicating that the seeds were somewhat dormant. The water imbibition test showed that the seeds imbibed water; hence, the seed coat played no role in imparting seed dormancy. This finding implies that dormancy in *G. wynaadensis* is not physical.

The seeds have small embryos relative to the size of the endosperm. The embryos cannot be visualised using a stereomicroscope. In species with underdeveloped embryos, the embryo length to seed length ratio (E:S) at the time of seed dispersal varies. Previously reported species with E:S ratios include *Psychortia zeylanica* (E:S= 0.36 ± 0.04), *P. nigra* (E:S = 0.43 ± 0.0), *P. gardneri* (E:S= 0.44 ± 0.07), *Eremurus anisopterus* (E:S= 0.73 ± 0.01), *Gomphandra luzoniensis* (0.36 ± 0.07) and *Nothapodytes nimmoniana* (0.74 ± 0) (21, 27). Forbis reconstructed a phylogenetic tree based on the E:S ratio and found that the extant basal lineages (particularly within basal groups, including eumagnoliids, Illiciaceae, Schisandraceae, Austrobaileyaceae, Nymphaeaceae and Amborellaceae) have small embryos, reflecting the ancestral character of angiosperms. Amborella, the purported sister to all other angiosperms, has an extremely small embryo, with an E:S ratio of 0.097 and Nymphaeaceae, the next branch on the angiosperm tree, also has a small embryo with an E:S ratio of 0.036 (29). It was reported that fresh seeds of *G. amuyon* have an E:S ratio of 0.19 ± 0.03 (23), whereas *Goniothalamus wynaadensis* has an E:S ratio of 0.139 ± 0.04 at the time of dispersal. The embryo is small in relation to the copious endosperm, and scanning electron microscopic analysis is necessary to visualise and measure embryo length. Therefore, seed dormancy is a morphological factor. The cause of morphological dormancy is underdeveloped embryos (differentiated but not fully grown embryos). Within the seed, the immature embryo must lengthen 3 times before the radicle emerges. Before the radicle emerges, the embryo length:seed length ratios of *Psychortia* zeylanica, *P. nigra, P. gardneri* and *Goniothalamus amyuon* increase by 36 %, 40 %, 57 % and 300 % respectively (22, 23). The E:S ratio of *G. wynaadensis* increased to 359.71 %.

According to a report, seeds with differentiated but underdeveloped embryos are placed on a moist substrate and do not germinate for approximately 4 weeks; this type of seed exhibits morphophysiological dormancy (6). The germination of *G. wynaadensis* seeds took more than 4 weeks in different germination trials. This implies that seeds also have a physiological component of dormancy along with a morphological component. Hence, the seeds

of *G. wynaadensis* exhibit morphophysiological dormancy. Morphophysiological dormancy is common among basal angiosperms, particularly Magnoliids and Ranunculales, and is also found in some gymnosperms (6). Other species in Annonaceae, such as *Mitrephora maingayi*, *Monocarpia marginalis*, *Mezzettia leptopoda*, *Polyalthia cinnamomea*, *P. glaiica*, *P. jenkensii* and *P. sclerophylla*, have been reported to exhibit morphophysiological dormancy (6). According to one report, *Goniothalamus tortilipetalus* seeds exhibit delayed germination (8). It takes between 12 and 22 weeks for seeds to germinate, with 50 % of germination occurring in the first 16 weeks and 75 % occurring in the first 18 weeks. The germination % for seeds is 80% (8). This delayed dormancy in *Goniothalamus tortilipetalus* as morphophysiological dormancy (6). Additionally, the MPD of the seeds of the studied species is simple because embryo growth within the seeds occurs under warm conditions. Embryos in seeds containing complex levels of MPD require low temperatures (0–10 °C) to grow (6).

Three levels of physiological dormancy (PD) have been identified: nondeep, intermediate and deep (6). Reports revealed that GA_3 may or may not break intermediate PD and does not break deep PD, but it can alleviate nondeep PD (3). Our experiments revealed that 500 ppm GA_3 has a positive effect on the emergence of the radicle of this species and the emergence of the radicle in *G. wynaadensis* occurs at warm temperatures. Thus, the PD in *G. wynaadensis* is not deep.

Moreover, shoots from *G. wynaadensis* emerged 10 to 16 weeks after the emergence of the roots. Physiological dormancy and the requirement for warm temperatures to break this dormancy are the reasons for the delay in shoots or epicotyls emergence. *G. wynaadensis* has underdeveloped embryos and exhibits delay in both radicle and shoot emergence. This type of epicotyl dormancy is classified as epicotyl morphophysiological dormancy (e MPD). The dormancy of the seeds of these species can be explained as $C1_bB_b$ (radicle)–$C1_bB_b$ (epicotyl). $C1_b$ indicates that the root and epicotyl have a nondeep PD that is broken by warm stratification and B_b indicates that the embryo grows before radicle emergence and before epicotyl emergence under warm conditions. This is the first report of epicotyl eMPD in Annonaceae.

Reports are on numerous examples of eMPD in temperate species, but only a few tropical species exhibit this type of dormancy (6); for example, *Virola koschnyi* (Myristicaceae) and *Minquartia guianensis* (Olacaceae), *Psychortia zeylanica*, *P. nigra* and *P. gardneri* (Rubiaceae) also exhibit eMPD (22).

To explain the ecological significance of epicotyl dormancy, several theories have been proposed. Epicotyl dormancy may be an adaptation that allows seedlings of temperate deciduous forests to establish a well-developed root system during early spring cotyledon expansion (30). Physiological dormancy of the epicotyl found in recalcitrant tropical seeds may serve as a useful adaptation for plants to remain in the understorey until sufficient light is available (31).

Furthermore, reports revealed that in seeds with morphophysiological dormancy, the requirements for the resumption of embryo growth after dispersal vary and may represent adaptations to specific environments (32, 33).

It was proposed that epicotyl morphophysiological dormancy in the tropical species *Psychotria zeylanica* and *P. nigra* is synchronised with conditions favourable for seed development (22). According to this, these 2 *Psychotria* sp., radicle emergence occurs at the beginning of the rainy season, while plumule emergence occurs at the peak of the rainy season (22). Similar observations were made for the seed germination pattern of *G. wynaadensis*.

In Wayanad, 4 seasons are recognised :Winter (December–February), characterized by evening misting and minimal rainfall; Summer (March–May), partly dry but occasionally experiencing intense premonsoon events in the afternoon, along with lightning, strong winds and sporadic hail; Southwest Monsoon (June–September), the main rainy season with daily downpours; and Northeast Monsoon (October–November), a second rainy season with sporadic rain and high humidity (34). (34). Seeds of *G. wynaadensis* are dispersed in November during the northeast monsoon season. While the radicle emerges during this time, the epicotyl remains dormant inside the seed coat. Hypocotyl and root elongation occur from December to February when rainfall is minimal. In March, premonsoon showers prompt the emergence of the epicotyl. Therefore, epicotyl dormancy is an adaptation to reduce transpiration during the dry season and the development of a root system before the arrival of the southwest monsoon aids in its survival.

Effect of GA_3

Effect of GA_3 on radicle emergence

GA promoted the loss of dormancy in *Chaerophyllum tainturieri* seeds when they were incubated at a constant temperature of 20 °C for a 14 h daily photoperiod (6). This response to GA was expected, as GA overcomes dormancy in many seeds with nondeep PD. GA_3 replaced warm stratification and stimulated embryo growth in the seeds of *Fraxinus excelsior* (35), *Jeffersonia diphylla* (36) and *Panax ginseng* (37). However, *G. wynaadensis* exhibited the highest mean germination rate (99.60 ± 0.55) in seeds treated with 500 ppm GA_3 for 24 h and maintained at 30 °C. At 500 ppm GA_3, the mean germination time decreased to 20.20 ± 1.10 days.

Effect of GA_3 on shoot emergence

In *Paeonia ludlowii*, deep simple epicotyl morphophysiological dormancy is disrupted by 400 mg/L GA_3 (38). In *G. wynaadensis,* post-treatment with GA_3 after radicle emergence was ineffective in breaking epicotyl morphophysiological dormancy. However, pretreatment with 500 ppm GA_3 reduced the duration required to break eMPD.

Seedling development

During seed germination, a radicle protrudes from the micropylar end. Before radicle protrusion, rupture of the testa and endosperm occurs. Prior to the emergence of hypocotyl from the seed coat, root enlargement and thickening

takes place. The hypocotyl elongates and curves in a loop above the ground, carrying cotyledons enclosed in the testa above the soil. The cotyledons of *G. wynaadensis* serve haustorial functions and absorb nutrients from the endosperm. They remain within testa and attach to the top of the hypocotyl, blocking the development of the epicotyl and plumule. During this stage, the seedling enters a resting phase, during which nutrients from the endosperm are transferred into the hypocotyl, making it sturdy. As the cotyledons of *G. wynaadensis* remain non-emergent, they typically drop off along with the testa as they are consumed. Subsequently, the plumule develops into a shoot, and the first-formed leaves are cataphylls. Based on the persistence of cotyledons within the seed coat and their position above the ground, *G. wynaadensis* exhibits epigeal cryptocotyly, a rare phenomenon in angiosperm seedling development. The cotyledons of the plants, remaining permanently within the testa, are maintained above the soil. This type of seed germination is known as cryptocotylar epigeal germination (39).

However, unusual cases of cryptocotylar epigeal germination have been reported in seedlings of *Myristica*, *Jatropha multifida* (Euphorbiaceae), *Aegle marmelos* (Rutaceae), *Xylopia aromatica* and *Rollinia salicifolia* (Annonaceae) (39-40). This is the first report of epigeal cryptocotylar germination in the genus *Goniothalamus*. Several authors have documented a number of syndromes associated with cryptocotylar seedling establishment (39-41). Cryptocotylar species often have seedlings with first leaves that are cataphylls. For Malayan Forest cryptocotylar species, the seeds are larger and take longer to germinate than those of phanerocotylar species. In *G. wynaadensis*, seed germination is delayed, and the initial leaf cataphylls resembles those of Malayan forest cryptocotylar species. Reportedly, Australian cryptocotylar species exhibit common features such as non-endospermous seeds, a tree habitat and an alternate arrangement of first-formed leaves (39). In *G. wynaadensis*, the first-formed leaves are alternate and the species habit is a tree. Therefore, the cryptocotylar mode of seedling establishment syndromes shared by *G. wynaadensis* includes delayed seed germination, a tree habitat and the first leaves being cataphylls.

Depending on the function and position of the cotyledon, there are 5 seedling categories: 1) PEF, phanerocotylar epigeal with foliaceous cotyledons; 2) PER, phanerocotylar epigeal with reserve storage or absorption cotyledons; 3) PHR, phanerocotylar hypogeal with reserve storage or absorption cotyledons; 4) CHR, cryptocotylar hypogeal with reserve storage or absorption cotyledons; and 5) CER, cryptocotylar epigeal with reserve storage or absorption cotyledons (42). The *G. wynaadensis* seedling fits into the cryptocotylar epigeal with a reserve storage or absorption cotyledon (CER).

Conclusion

This study revealed that *G. wynaadensis* (Bedd.) Bedd. exhibits nondeep epicotyl morphophysiological dormancy, and different temperatures and GA_3 concentrations affect seed germination. Warm stratification and GA_3 treatment improved the germination % and reduced the germination time. However, epicotyl emergence did not ocuur at different temperatures. Post-GA_3 treatment was ineffective in breaking epicotyl morphophysiological dormancy in *G. wynaadensis*. Pretreatment with 500 ppm GA_3 reduced the duration required to break eMPD. Additionally, the study revealed that the species exhibited epigeal cryptocotylar germination, a rare condition in angiosperm seedling development.

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Authors' contributions

AJ and SAK conceived the idea and planned experiments. AJ performed the experiments under the supervision of SAK. All authors analysed the research data and drafted the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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