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ORIGINAL ARTICLE

Seed characteristics and germination properties of four *Garcinia* (Clusiaceae) fruit species

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Abstract – Introduction. Seeds from *Garcinia* species are recalcitrant, which do not survive dessication and cold temperature. This poses a significant challenge for their long-term preservation, and hence more research is needed to understand the biology of such seeds. **Materials and methods.** Seeds from three under-investigated *Garcinia* species, namely *G. atroviridis*, *G. hombroniana* and *G. prainiana*, as well as the economically important mangosteen (*G. mangostana*), were studied using histochemical tests and microscopy techniques as well as moisture content and germination tests. **Results and discussion.** The seeds of these species varied in shape and size. *G. hombroniana* seeds were the largest, while those of *G. prainiana* were the smallest. Furthermore, during a period of desiccation, larger seeds (*G. hambroniana* and *G. mangostana*) retained their moisture content longer than the smaller seeds (*G. atroviridis* and *G. prainiana*). Consequently, species with larger seeds seemed to maintain a higher germination percentage compared with the species with smaller seeds. Moreover, *G. prainiana* seeds had the thickest testa, which perhaps underlay their significantly delayed germination time. Large amounts of lipid and calcium oxalate were also observed in all four *Garcinia* species, which may have a role in food storage and herbivore protection, respectively. During germination, all species had radicles and shoots emerged from two opposite ends, conforming to the *Garcinia*-type germination. The procambial tissues also increased in length during germination towards the ends where the radicle and plumule emerged. **Conclusion.** This study successfully characterised the seeds from four different *Garcinia* species, some of which are not yet reported elsewhere.

Keywords: Malaysia / *Garcinia* spp. / seed germination / seed structure

1 Introduction

There are approximately 200 *Garcinia* L. (Clusiaceae) species grown around the tropical areas [1] and among these, mangosteen (*G. mangostana*) fruit is the most economically important because of its potential uses in the healthcare industry. The fruit contains xanthone, a secondary metabolite with high antioxidant [2], antitumorigenic [3] and antimalarial activity [4].

Plants such as *Garcinia* are propagated through seeds. Seeds serve as a means of reproduction and an important element to ensure the survival of any flowering plant species. Hence, seed banking may serve as a way to preserve genetic diversity, particularly for endangered plant species [5]. However, unlike orthodox seeds such as melon seeds, recalcitrant seeds such as from mangosteen (*Garcinia mangostana* L.) [6] may lose viability upon storage in freezers [5]. Therefore, germplasm conservation for recalcitrant seeds is still a huge challenge, and more research is needed to understand the biology and anatomy of these seeds to develop better preservation strategies in the future.

A few earlier studies have documented some characteristics of recalcitrant seeds, other than their desiccation and freezing sensitivity features [7]. For example, Hamilton *et al.* [8] reported that out of 71 Eastern Australian rainforest species studied, a recalcitrant seed can be identified by its larger size, higher seed moisture content and less investment in the seed coat. On the other hand, the desiccation tolerance in orthodox seeds can be acquired through accumulation of starch, lipid and insoluble proteins, which provide a buffering capacity as well as resistance against cell collapse during the dehydration process [9]. Therefore, these characteristics can be studied using histochemical tests to further justify their nature, either orthodox or recalcitrant, for successful seed preservation.

Several *Garcinia* seeds have been considered as recalcitrant [6, 10, 11] and possess some interesting structural

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and germination characteristics. For example, *G. mangostana* seeds do not have a well-differentiated plumule and radical [12]. A few studies also documented that *Garcinia* seeds have much larger hypocotyls and small cotyledons [13, 14]. Moreover, several seeds from *Garcinia* species, for instance *G. mangostana* [15, 16], *G. indica*, *G. cambogia*, *G. xanthochymus* [17] and *G. gummi-gutta* [18], can be cut into segments and a shoot and root will develop from each segment. Recently, research on *Garcinia* seeds including germination properties has been extended by Di Stefano *et al.* [19] (*G. intermedia*), Abdullah *et al.* [20] (*G. mangostana*) and Asomaning *et al.* [21] (*G. kola*). However, seeds from a few other *Garcinia* species bearing edible fruits such as *G. atroviridis* Griff., *G. prainiana* King. and *G. hombroniana* Pierre have not been studied so far, and comparison with the commercially important *G. mangostana* will be vital to investigate their morphology, anatomy, water content and chemical composition as well as germination characteristics for seed storage consideration.

2 Materials and methods

2.1 Seed origin

Seeds of *G. mangostana* were obtained from Karak, Pahang; *G. atroviridis* and *G. prainiana* from Kampung Gosong, Machang, Kelantan, and *G. hombroniana* from Rimba Ilmu, Universiti Malaya. Seeds were cleaned and rinsed with water. Seed size, weight and moisture content were determined from 50 seeds randomly selected for each species.

2.2 Histochemical and morphological investigations

Sections of seeds were stained with Sudan III solution to detect lipids, IKI reagent to detect starch, 0.1% toluidine in ethanol-xylene 2:1 v/v to detect protein, ferric chloride to detect tannin, acidified phloroglucinol to detect lignin or dilute hydrochloric acid to detect calcium oxalate. The surface structure of the seeds was observed under a Scanning Electron Microscope (Phillips XL 30, Netherlands).

2.3 Measurement of the testa

The thickness of the testa was measured using a Quantimet 570 Image Analyser (Leica Cambridge, provided by Delta Advantech (M) Sdn. Bhd.) connected to a compound microscope. Measurements of the testa were made at different parts of the seed chosen at random and a total of 102 measurements were made for each species.

2.4 Moisture content and germination tests

Seeds were desiccated non-aseptically in open Petri dishes (27 ± 2 °C) at different times. After each desiccation period, moisture content was determined and a germination test was carried out using the in-sand method [22]. For the measurement of moisture content, three replicates of 10 seeds per replicate were dried at 103 °C for 16 h. Meanwhile, three replicates of 15 seeds per replicate were used for the germination test. Germination was based on seeds which produced morphologically normal seedlings after three weeks of germination at 28−30 °C under 12-h light/12-h dark with light photon flux density of 26−30 μmol $m^{-2}s^{-1}$. The experiment was repeated three times.

2.5 Germination

Seeds (100−200 per species) were germinated in sand to observe full germination, which included the time taken for emergence of the radicle, plumule and adventitious root and the occurrence of polyembryony. Histological changes in the seeds during germination were also analysed. Every three or six days from the start of germination until radicle and plumule emergence, ten were fixed in FAA solution, embedded in paraffin and sectioned longitudinally from the plumule to the radicle end on a rotatory microtome, then stained and mounted following [23].

The mean germination time (MGT) was calculated according to Ellis *et al.* [24] equation:

$$MGT = \frac{\sum n \cdot D}{\sum n}$$

where n is the number of seeds germinated on day D, and D is counted from the first day of germination.

3 Results

3.1 Seed characteristics and moisture content

The seed size and fresh moisture contents of the four *Garcinia* species are shown in *table I*. Seeds from the four *Garcinia* species can be categorised into two groups, the species with larger (*G. hombroniana* and *G. mangostana*) or smaller seeds (*G. atroviridis* and *G. prainiana*). In terms of length, *G. hombroniana* seeds were the largest (2.5 cm), while those of *G. prainiana* were the smallest (1.1 cm). However, based on weight, *G. atroviridis* had the lightest seeds (0.3 g). *G. mangostana* seeds were flat and variable in shape, while those of *G. hombroniana* and *G. atroviridis* were kidney-shaped and flattened (*figure 1*). *G. prainiana* seeds, on the other hand, were round with a smoother seed coat.

The moisture content of fresh seeds ranged from 35−61%. Larger seeds (*G. hambroniana* and *G. mangostana*) contained a higher moisture content (> 50%) compared with the smaller seeds (*table I*).

The moisture content of seeds after desiccation was also determined and the germination percentages are shown in *figure 2*. At harvest, the moisture content of the *Garcinia* seeds

Table I. Mean seed size, weight and moisture content of *Garcinia* species. Data values are means ± standard deviation (n = 102). FW: fresh weight, CV: coefficient of variation.

Species	Seed length (in cm)	Seed width (in cm)	Seed weight (in g)	Moisture content (in % FW)	CV (in % FW)
G. hombroniana	2.5 ± 0.05	1.4 ± 0.04	1.0438 ± 0.0314	51.52	21.29
G. mangostana	1.9 ± 0.03	1.3 ± 0.03	0.8420 ± 0.0072	61.12	20.29
G. atroviridis	1.3 ± 0.03	0.6 ± 0.01	0.3328 ± 0.0032	34.92	6.73
G. prainiana	1.1 ± 0.02	0.9 ± 0.01	0.4642 ± 0.0077	41.77	11.76

Figure 1. Seeds of *Garcinia* species: (A) *G. hombroniana*, (B) *G. mangostana*, (C) *G. atroviridis* and (D) *G. prainiana.* Bar: 1 cm.

studied ranged from 38−53% (*figure 2A*). During desiccation, the germination percentage decreased rapidly (*figure 2B*) in line with the decreasing moisture content, particularly below the 30% moisture level. However, *G. hambroniana* and *G. mangostana* retained moisture between 30−40% longer (at 72 h) with 85% germination percentage, compared with *G. atroviridis* and *G. prainiana*, which retained moisture content between 9−14% on desiccation with 20% germinability at 72 h.

3.2 Histochemical test

The histochemical tests showed that the seeds of all species contained a large amount of lipid and calcium oxalate (*table II*, *figure 3*). Tannin was observed only in *G. mangostana* and *G. hombroniana*, whereas *G. mangostana* was the only species with abundant starch grains. Other minerals such as proteins and lignin were present in very small quantities in the seeds of all four species (*table II*).

3.3 Procambial and testa thickness

The procambial strand in the seeds of all species studied was observed to be in the form of a complete sphere/ellipse. For *G. mangostana* and *G. hombroniana*, the procambial strand was broadly elliptical (*figure 4A, 4B*), while in *G. atroviridis* and *G. prainiana* it was more elongated and narrow (*figure 4C, 4D*).

The testae in all species were observed to be thin and membranous, consisting of parenchyma cells. In *G. prainiana*, the innermost layer of the testa was made of thick-walled macrosclereids. The thickness of the testa also varied among the species (*table III*, *figure 5A−5D*), being thickest in *G. prainiana* (401.57 ± 0.76 μm), and thinnest in *G. atroviridis*

Table II. Seed composition of several *Garcinia* species (+ + + : large amount; + : small amount; − : negative).

Structural compounds	*G. hombroniana*	*G. mangostana*	*G. atroviridis*	*G. prainiana*
Lipid	+ + +	+ + +	+ + +	+ + +
Starch	+	+ + +	+	+
Protein	+	+	+	+
Tannin	+	+	−	−
Lignin	+	+	+	+
Calcium oxalate	+ + +	+ + +	+ + +	+ + +

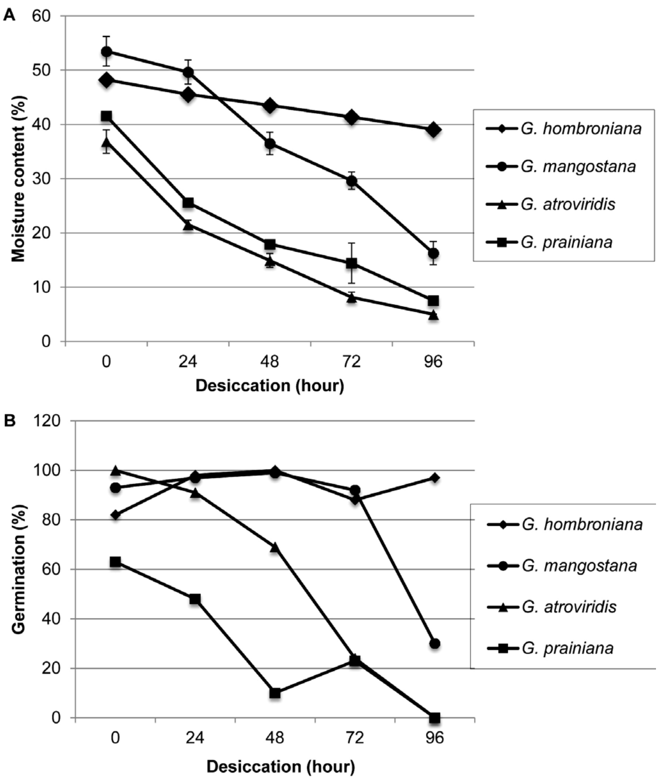

Figure 2. Effect of desiccation on moisture content (A) and germination percentage (B) on seeds of four *Garcinia* species. Data values are means ± standard deviations (*n* = three replicates of 10−15 seeds per replicate).

and *G. hombroniana* (approximately 146 µm). Meanwhile, the thickness of the testa for *G. mangostana* was intermediate (231.39 ± 2.01 µm). Furthermore, the seed coat surface was smooth for the four species, but they varied in cell arrangement (*table III*, *figure 5E, 5F*).

Before germination, the procambium consisted of two to three layers of cells. During germination, the procambium increased in length towards the two ends of the seed where the radicle and plumule emerge (*figure 6A*). For the polyembryonic seeds, each embryo was observed to have its own procambium (*figure 6B*). A xylem was first observed between the sixth and ninth days of germination for *Garcinia* species that germinated in two to three weeks. For *G. prainiana*, the xylem appeared between the 18th and 21st days of germination.

3.4 Germination

The seed germination of *G. mangostana, G. hombroniana* and *G. atroviridis* conformed to the *Garcinia* type [25, 26], where the radicle emerged from one end followed by the plumule from the opposite end of the seed (*figure 6C*), as also observed in earlier studies by Malik *et al.* [26]. The procambial tissues elongated and developed into primordium tissues. An adventitious root developed soon after at the base of the young shoot, after which the first radicle died (*figure 6D*). This is the root that functions for further growth of the seedling. However, for *G. prainiana*, there was no adventitious root formation and the first root remained functional. For *G. hombroniana* and *G. atroviridis*, radicle emergence was observed on the third day of sowing and the plumule was observed on the sixth day (*table IV*). Radicle and plumule emergence for *G. mangostana* occurred on the sixth and ninth day, respectively. *G. prainiana* seeds took the longest to germinate, 24 days for radicle emergence and 30 days for the plumule to appear (*table IV*). Adventitious roots emerged on the fifteenth, eighteenth and twelfth day for *G. atroviridis, G. mangostana* and *G. hombroniana*, respectively. Polyembryony was observed in *G. hombroniana* (*figure 6E*) and *G. mangostana. G. atroviridis* showed the occurrence of multiple shoots and these shoots had a common root system.

4 Discussion

4.1 Seed size and testa structure determine the recalcitrance and germination percentage of *Garcinia* seeds

In this study, seeds from four *Garcinia* species were investigated; *G. hombroniana*, *G. mangostana*, *G. atroviridis* and *G. prainiana*. These species showed various ranges of seed size and moisture content (*table I*, *figure 1*), which may have influenced the recalcitrance and germination percentage of respective seeds (*figure 2*). *G. hambroniana*, for example, had the largest seeds with a relatively high moisture content compared with the other three species (*table I*). Perhaps due to this large seed size, *G. hambroniana* retained moisture (approximately 20−60% moisture content) within the 96-h desiccation period, while the moisture content of the other small-seeded species, particularly *G. atroviridis* and *G. prainiana*, dropped drastically (*figure 2A*). This drop in moisture content affected the germination percentage of both species such that

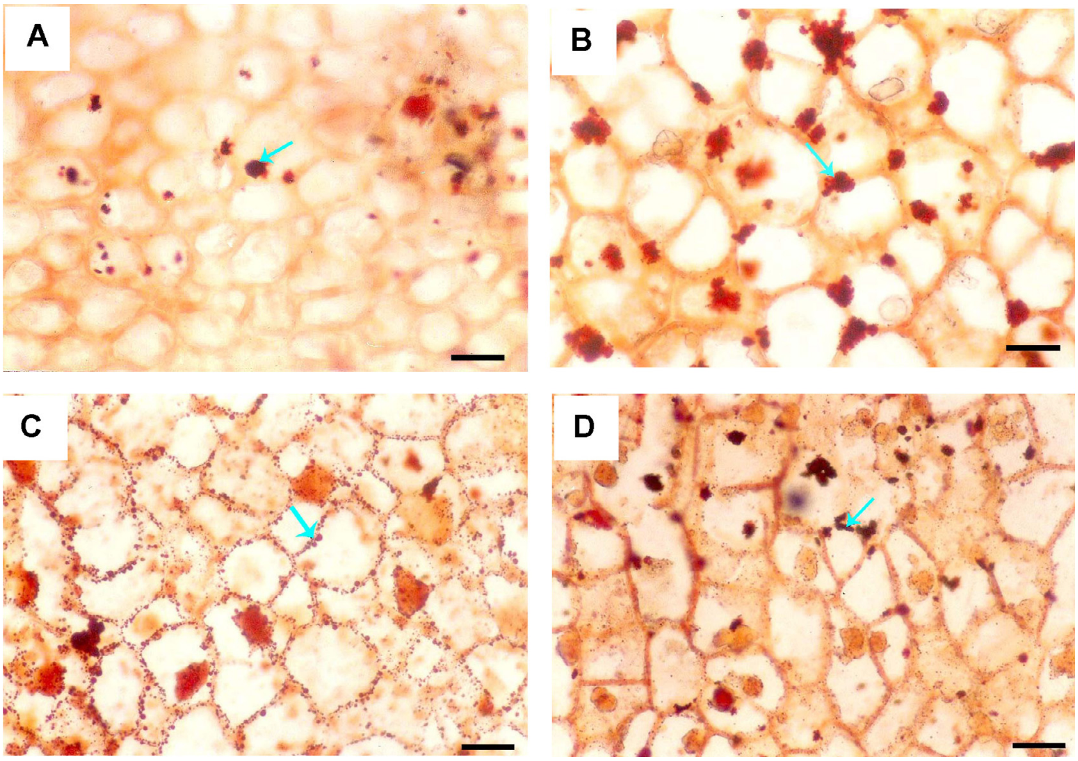

Figure 3. Histochemical localisation of lipids indicated by arrows in cells of seeds of the *Garcinia* spp. (A) *G. hombroniana*, (B) *G. mangostana*, (C) *G. atroviridis* and (D) *G. prainiana.* Bar: 50 µm.

at 96 h, seeds lost germinability (*figure 2B*). Meanwhile, *G. mangostana* also had a high moisture content comparable with that of *G. hambroniana* during harvest (*figure 2A*), but the moisture level was consistently reduced upon desiccation and when the moisture content dropped more than 30% after 72 h, the germination percentage fell rapidly (*figure 2B*). Normah *et al.* [6] also reported similar observations and concluded that *G. mangostana* seeds were highly recalcitrant. Furthermore, earlier studies documented that seeds from other *Garcinia* species were also intolerant to desiccation and low moisture content including *G. indica*, *G. cambogia*, *G. xanthochymus* [17, 26], *G. cowa* [27] and *G. kola* (Heckel) [28]. This suggests that seeds from most *Garcinia* species are highly recalcitrant, as drying negatively impacts their viability [5, 9].

Another factor that influences germination is the testa (or seed coat) thickness. An investigation by Liu *et al.* [27] showed that *G. cowa* seed dormancy was mainly contributed by the thick testa structure. Removal of this structure expedited the mean germination time of *G. cowa* from 120 days to just 13 days [27]. In this study, *G. prainiana* seeds germinated the slowest, approximately 47 days mean germination time compared with just 22−25 days for the other three species (*table IV*). The testa thickness of *G. prainiana* seeds was approximately 401.57 µm, about two times thicker than the other species (*table III*). Interestingly, the testa of the dormant *G. cowa* seeds can reach up to 1.1 mm [27], suggesting the importance of this structure during seed germination. Furthermore, the testa of *G. prainiana* seeds contains a hydrophobic suberin layer (*figure 5*) which may act as a mechanical barrier to both air movement and water uptake. Consequently, this layer may significantly hinder the imbibition process as well as possibly preventing radicle protrusion from the *G. prainiana* seeds. Further experimentation is needed to verify this hypothesis, including the removal of the testa structure from the seeds to examine their germination properties, as previously done by Liu *et al*. [27].

4.2 Significance of seed composition and structure in *Garcinia* species

Seeds can be composed of different stored reserves and minerals. All four *Garcinia* seeds studied contained large amounts of lipid (*table II*, *figure 3*), as documented in several other *Garcinia* species before [29–31]. This suggests that lipid may well be the main food reserve in *Garcinia* seeds for germination and possibly not starch, as shown in this study, particularly for *G. hombroniana*, *G. atroviridis* and *G. prainiana* seeds (*table II*). Interestingly, lipid content in the recalcitrant seeds of fluted pumpkins (*Telfairia occidentalis*) increased upon desiccation while the starch content decreased [32]. This further implies that recalcitrant seeds might utilise starch in lipid biosynthesis, as lipids are preferred as storage reserves.

Furthermore, besides lipid, calcium oxalate was also abundant in all four studied *Garcinia* seeds (*table II*). This mineral is important for protection against herbivores and the

Table III. Testa thickness and surface structure of the seed coat of *Garcinia* species. Data values are means ± standard deviation (n = 102).

Characters	*G. hombroniana*	*G. mangostana*	*G. atroviridis*	*G. prainiana*
Testa thickness (µ)	145.89 ± 0.90	231.39 ± 2.01	147.35 ± 1.24	401.57 ± 0.76
Seed coat surface	Smooth	Smooth	Smooth	Smooth
Cell arrangement	Scales with sclerotesta	Scales with sclerotesta	Non-uniform thickness	Reticulate

Table IV. Time^{y} taken for the emergence of the radicle, adventitious root formation, plumule emergence, mean germination time (MGT) and occurrence of polyembryony.

Recorded parameters	*G. hombroniana*	*G. mangostana*	*G. atroviridis*	*G. prainiana*
Time^{y} to emergence (1−2 mm) of radicle	3	6	3	24
Time^{y} to emergence (1−2 mm) of plumule	6	9	6	30
Time^{y} to adventitious root formation	12	18	15	–^{z}
Mean germination time^{y}	22.2	24	25	47
Type of germination	Garcinia-type	Garcinia-type	Garcinia-type	Semi-Garcinia
Germination (in %)	95.6	75.6	57.3	48.9
Occurrence of polyembryony (%) (adventitious shoots)	3.49	11.76	9.30 (2-3)	0 (0)

^{y} In days, ^{z} no root observed.

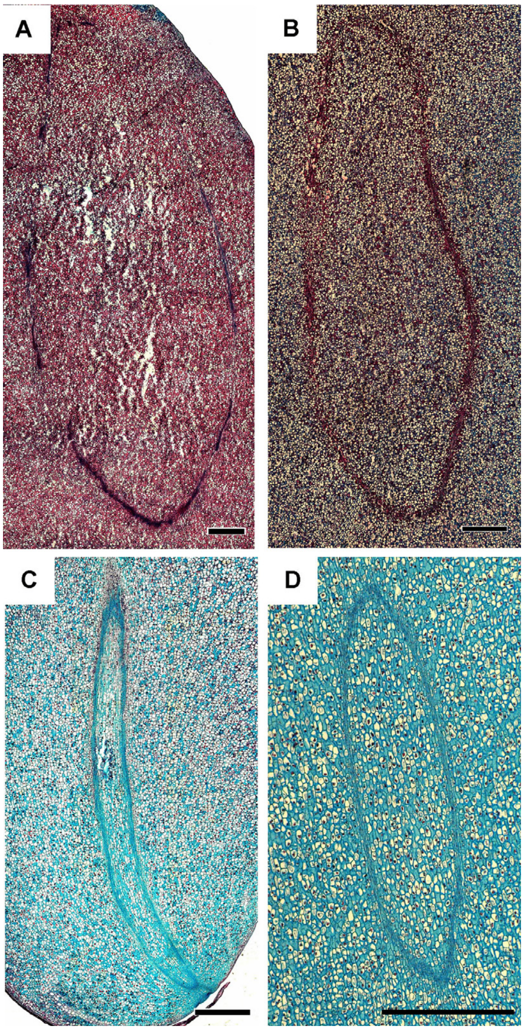

Figure 4. Longitudinal sections of *Garcinia* seeds showing the procambial ring. Elliptical type observed in (A) *G. hombroniana* and (B) *G. mangostana.* Elongated and narrow procambial in (C) *G. atroviridis* and (D) *G. prainiana*. Bar: 1,000 µm.

regulation of the calcium level, as well as heavy metal detoxification in plants [33]. Meanwhile, tannin was observed only in *G. mangostana* and *G. hombroniana* (*table II*) and might contribute to their lower initial water loss (*figure 2A*), possibly due to the buffering capacity of this phenolic compound [34].

In terms of seed structure, *G. mangostana, G. hombroniana, G. atroviridis* and *G. prainiana* did not have differentiated embryos, no trace of a radicle and cotyledons as well as what can be interpreted as tuberculous hypocotyls, as suggested earlier by Sprecher [12]. Berjak *et al.* [35] suggested that seeds as such might be best described as having a dual function, that of embryo cells in the usual sense, and also as reserve storage tissues. In this study, the plumule and radicle were observed to emerge from the two opposite ends of the seeds regardless of species (*figure 6A*), conforming to the *Garcinia*-type germination [25]. In addition, the procambium cell layers in the germinating seeds increased in length towards the two opposite ends of the seed where the plumule and radicle each emerged. The increase in the procambium length implies that the procambium cells divided rapidly and developed into vascular tissues for water and nutrient transports during the early stages of germination. From the seed transverse sections, it was observed that each shoot has its own procambium, indicating that each procambium can differentiate into an individual plumule and radicle (*figure 6C*).

5 Conclusion

The present study revealed the characteristics of seeds from four *Garcinia* species. The information obtained will be beneficial for further understanding of desiccation intolerance (a characteristic of recalcitrant seeds) and the germination behavior of these species. Further investigation of morphogenesis, seed biology and evolutionary ecology as well as the impact of maturation drying would generate more knowledge on the unique characteristics of these *Garcinia* seeds.

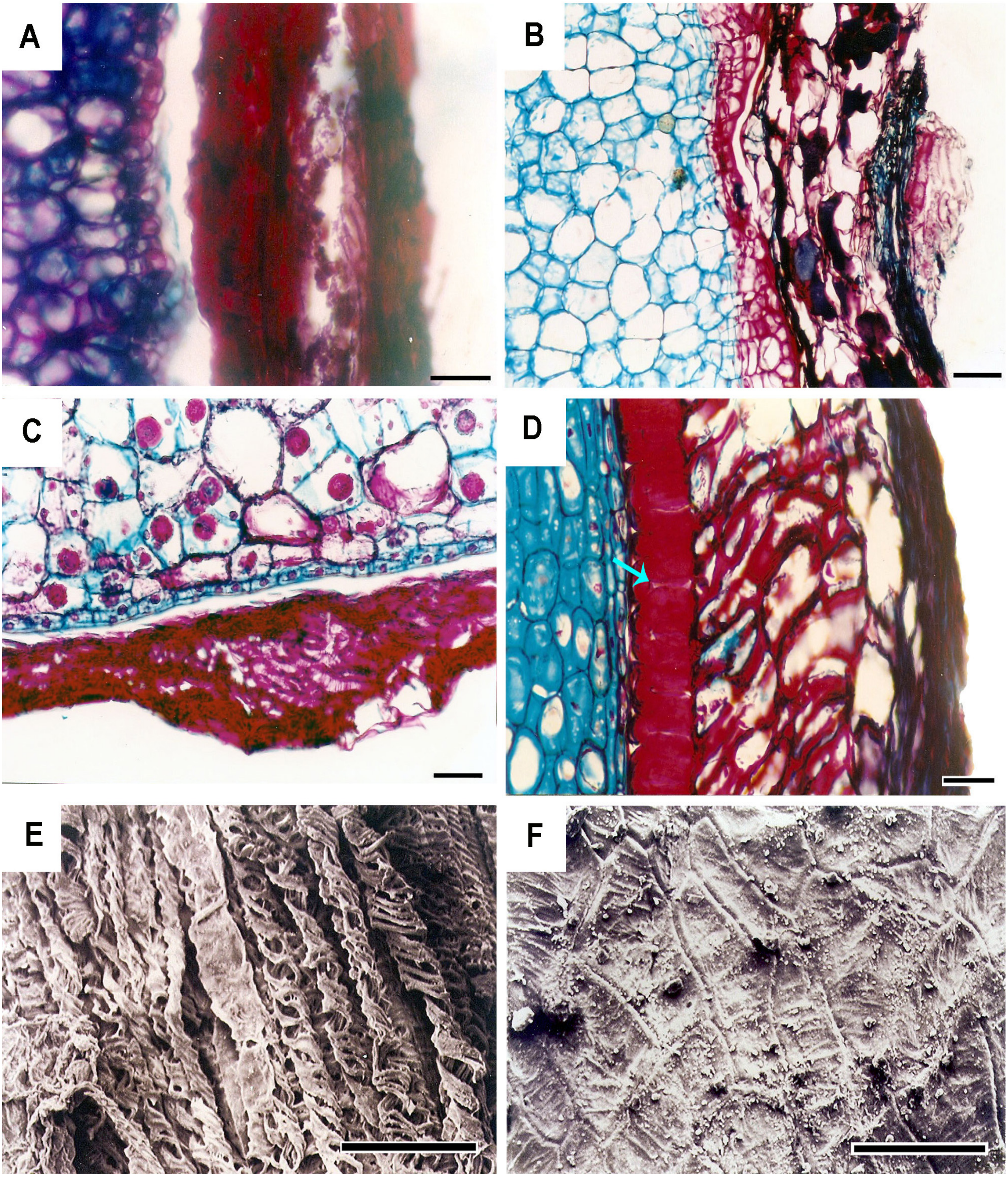

Figure 5. (A−D). Longitudinal sections showing the testa of *Garcinia* seeds. (A) *G. hombroniana*, (B) *G. mangostana*, (C) *G. atroviridis* and (D) *G. prainiana* (arrows show the suberin layers). Bar: 50 μm. (E, F) Scanning electron micrographs of the seed surface showing the cell arrangement in different *Garcinia* seeds. Scale with sclerotesta type in (E) *G. hombroniana* and reticulate in (F) *G. prainiana.* Bar: 50 μm.

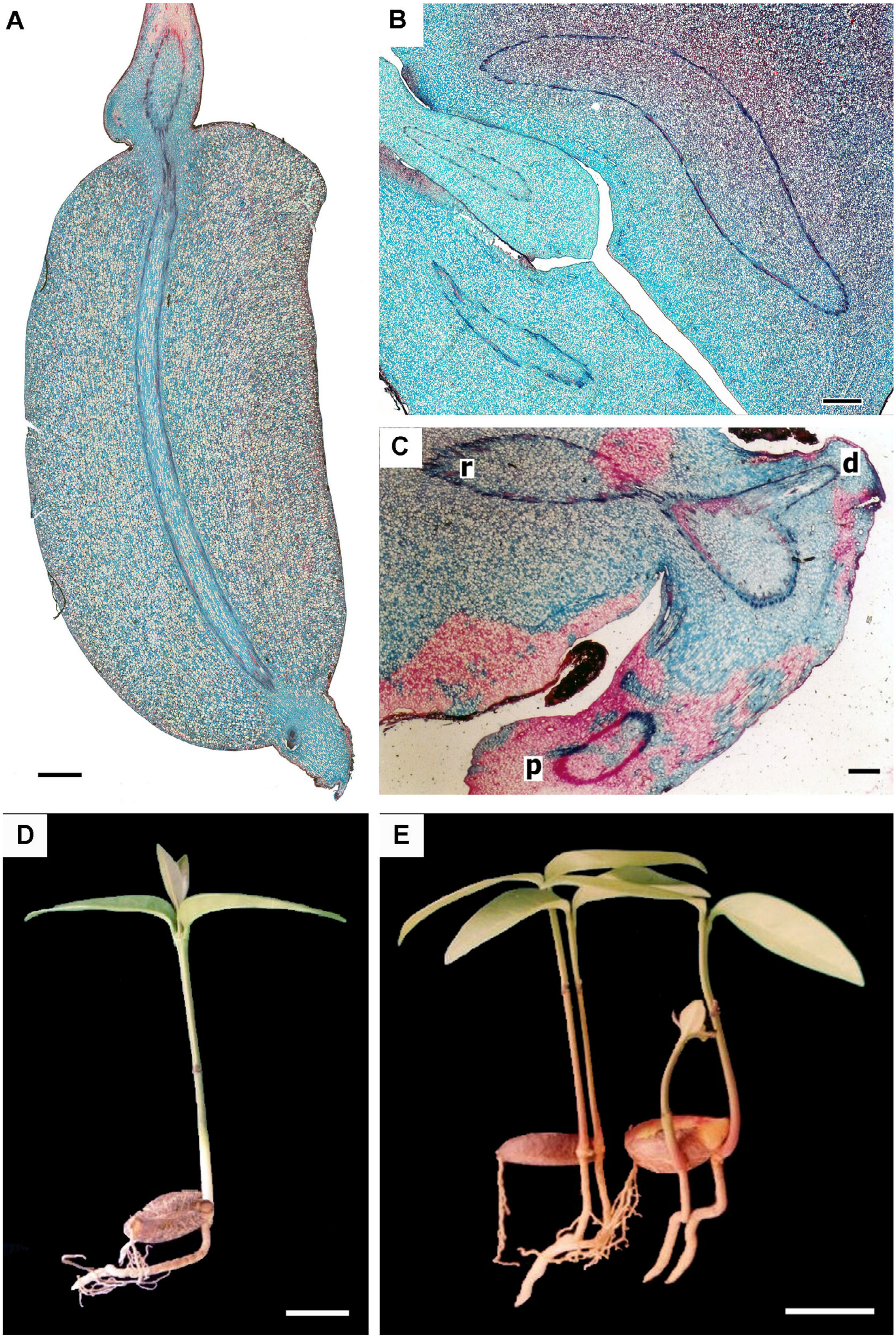

Figure 6. Procambium structure during germination and polyembryony. (A) Elongated procambium towards the two ends of the seed from where the radicle and plumule emerge. (B) Polyembryonic seed showing several embryos each with a separate procambium. (C) The presence of adventitious roots at the basal part of the shoot. (p: shoot, d: adventitious roots and r: the radicle development). (D) Single seedling from a seed of *G. hombroniana*. (E) Polyembryony characteristic of *G. hombroniana*, with two seedlings produced from a single seed. Bar: A, B: 1,000 μm, C: 50 μm, D, E: 1 cm.

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