

## Seed germination and seedling morphology of *Artabotrys hexapetalus*

TRI HANDAYANI\*

Center for Plant Conservation Botanic Gardens, Indonesian Institute of Sciences, Jl. Ir. H. Juanda No. 13 Bogor 16122, West Java, Indonesia. Tel./Fax. +92-251-8322187, \*email: irtri@yahoo.co.id

Manuscript received: 8 September 2016. Revision accepted: 16 December 2016.

**Abstract.** Handayani T. 2017. *Seed germination and seedling morphology of Artabotrys hexapetalus*. *Nusantara Bioscience* 9: 23-30. *Artabotrys hexapetalus* (L.f.) Bhandari (kenanga tanduk) is a fragrant, shrubby climber belonging to the Annonaceae family. In Indonesia, it is known as “kenanga tanduk”. It is a commercial ornamental plant due to its beautiful, aromatic flowers. This plant is also used in the perfume industry and in traditional medicine. It can be naturally propagated from seeds. However, it takes a long time to germinate. The research reported here aimed to determine the seed germination process, early seedling development, and seedling morphology of *A. hexapetalus* seeds planted under controlled conditions in Bogor Botanic Gardens. Ninety seeds were planted in a sand medium. The timing of germination, its characteristic pattern and the morphology of young emerged seedlings were observed and recorded. The seed characters were observed by using a microscope and camera. The results confirmed that *A. hexapetalus* seeds take a long time to germinate. Different periods of fruit maturation, small immature embryos, hard seed coat and hard endosperm texture are some of the factors that cause its seed to take a long time to germinate. The hard seed is due to the presence of exotesta, mesotesta and endotesta layers in the seed coat, as well as to the ruminant endosperm shaped like a wide plate and hard like glass (glass-like endosperm). The earliest seed germination occurred 159 days after sowing. The duration of seed germination from planting to last germinated seed took as long as 238 days. However, final seed viability reached 80%, with the speed of germination from sowing until the time of germination on a given day ranging from 0.01 to 0.08% per day. The type of seedling emergence is Phanerocotylar-Epigeal-Foliaceous (PEF). The seed germination process of *A. hexapetalus* from radicle appearance until the first open leaf can be divided into six stages; i.e. radicle growing phase, radicle and hypocotyl growing phase, hypocotyl emergent phase, cotyledon emergent phase, cotyledon opening phase and the first leaf opening phase. The pair of cotyledons look like true mature leaves and are firmly attached, not shedding easily.

**Keywords:** *Artabotrys hexapetalus*, germination stages, seed structure, seedling morphology

### INTRODUCTION

The Annonaceae family comprises 109 genera and 2.200 species (Couvreur et al. 2012; Shiva et al. 2015). The genus *Artabotrys* is among the relatively large genera of the family Annonaceae, consisting of at least 100 species (Fisher et al. 2002; Suleiman et al. 2014; Shiva et al. 2015), which are distributed mainly in tropical and subtropical regions of Africa and East Asia (Li and Gilbert 2011; Suleiman et al. 2014; Shiva et al. 2015). *Artabotrys hexapetalus* (L.f.) Bhandari, or “kenanga tanduk”, is one of its species that is widely known. This species grow in Sri Lanka, southern India, Bangladesh, Myanmar, China, Taiwan (Formosa), Peninsular Malaysia, Sumatra, Borneo, Java, and the Philippines (Fisher et al. 2002; Nurainas 2004; Li and Gilbert 2011).

Kenanga tanduk is easily recognizable from the flower stalks which are shaped like hooks (Fisher et al. 2002), and from its spoon-shaped petals, and clusters of berries (Nurainas 2004; Li and Gilbert 2011; Couvreur et al. 2012). It is a commercial, ornamental plant due to its beautiful and aromatic flowers (Fisher et al. 2002; Sowjanya et al. 2013). The plant is also used in the perfume industry (Nurainas 2004; Li and Gilbert 2011; Shiva et al. 2015), in traditional medicine (Li and Yu 1998; Li and Gilbert 2011), and as a repellent to ward off female

*Anopheline mosquitos* (Suleiman et al. 2014).

Seeds of Annonaceae have been of anatomical and morphological interest because of their unique structural characteristics: perichalazal growth, two or three integuments, fibrous mesotesta, ruminant endosperm, and micropylar plug, as well as their distinctive germination patterns (Svoma 1997, 1998). Annonaceae seeds are oval or oblong, 5-30 mm long, have ruminant endosperm, small embryo and thin cotyledons (Svoma 1997; Martinez-Maldonado et al. 2013; Shiva et al. 2015). Until recently, seed of Annonaceae were characterized as mesotestal only, however it is now known that this is not always the case. Svoma (1997) reported that seed of kenanga tanduk, besides having a mesotesta also has an endotesta, consequently the seed coat is very hard. The kenanga tanduk seed coat is composed of lignin fiber cells and stone cells, giving rise to the hard seed coat. The hard seed coat causes seed germination to be delayed significantly (Corner 1976). The seed coat exerts its germination-restrictive action mainly by being impermeable to water and/or oxygen or by its mechanical resistance to radicle protrusion (Debeaujon et al. 2000).

Seed is the main means for propagation of kenanga tanduk, but unfortunately, they take a long time to germinate (Handayani 2004). Seed germination is a complex physiological process beginning with water

imbibition and proceeding through until the emergence of the plumule above the growing medium (Bewley and Black 1994; Bewley 1997; Martinez-Maldonado et al. 2013). Under favorable conditions, the rapid growth of the embryo breaks the seed coat layers and presents the protrusion of the radicle. This emergence of the radicle is regarded as the completion of germination.

Seed germination depends on several factors. Santoso et al. (2012) reported seed germination is affected by two factors, i.e. internal and external factors. Vieira et al. (2010) refer to temperature, light, and soil humidity as the most important environmental factors controlling seed germination and seedling establishment. However, there are many factors that inhibit or prevent germination and different plant species vary in the range of factors that prohibit germination.

Following germination, the development of seedlings, their morphology and growth habit of the seedling are important characteristics to study. Some characters often used to study the morphology of seedlings of woody plant species are the emergence of seedlings (Zanne et al. 2005), the position and development of the cotyledons (Kitajima 1992; de Vogel 1980), and the function of the cotyledons (Kitajima 1992; Zanne et al. 2005). Cotyledons are the first leaves that appear on a plant, playing an important role in seedling development, especially in the early stages (Zheng et al. 2011). Based on the emergence of the seedling, cotyledons may remain inside the seed coat or emerge out of the seed coat (Kitajima 1992). The terms phanerocotylar and cryptocotylar refer to exposed and hidden cotyledons, respectively; if cotyledons emerge out of the seed coat they are called phanerocotylar, otherwise if the cotyledons remain inside the seed coat they are called cryptocotylar (Maia et al. 2005). Cotyledons may be lifted above the surface of the growing medium or else remain at or below the surface of the growing medium (Kitajima 1992; Zanne et al. 2005). Cotyledons raised above surface of the growing medium are called epigeal, whereas cotyledons which remain at or below ground level are called hypogeal (de Vogel 1980; Ibarra-Manriquez et al. 2001). Cotyledons can serve as a food-store and/or as photosynthetic organs (de Vogel 1980; Garwood 1995; Zanne et al. 2005; Ibarra-Manriquez et al. 2001). Cotyledons that serve photosynthesis are generally thin and resemble mature leaves, while cotyledons that serve as food reserves are thick and fleshy. By using these three characters, Kitajima (1992), Maia et al. (2005), and Zanne et al. (2005) classified types of seedlings into 3-5 groups, whereas de Vogel (1980) classified the types of seedlings into 16 groups.

The purpose of this current study was to identify the natural seed germination process, seed characters, seedling growth, and development stages, as well as the morphology of kenanga tanduk seedlings. Knowledge of morphological changes during germination can contribute to understanding the physiology and biochemistry processes during seed germination and seedling growth of plant species. Understanding these characteristics of seeds and seedlings can also contribute to a variety of other fields of science, such as plant identification, exploration, frugivory,

taxonomy, and ecophysiology.

## MATERIALS AND METHODS

This study was conducted from January 2015 to January 2016 at the Center for Plant Conservation Botanic Gardens (Bogor Botanical Gardens), Indonesian Institute of Science (LIPI), Bogor, West Java, Indonesia.

### Seed material

Seeds were collected from one mother plant growing in the Bogor Botanical Gardens' collection. Seeds were obtained from ripe fruit with yellow skins. The seeds were removed manually from the pulp and aryl, after which they were washed with water until completely free of pulp and then dried at room temperature for 24 hours. Seeds were then germinated in a sand media. Thirty seeds were sown in a batch, and this was replicated in three batches making 90 seeds in total. Observations of seed and seedling development were made every day until there were no more seeds germinating.

### Observation of natural germination

Parameters observed and recorded were days to first germination, the germination duration, the total germination percentage, the germination phases, the germination speed, and the seedling morphology. The earliest germination was recorded as the first day in which there was a seed visibly germinated; i.e. when the hypocotyl had appeared about 0.2-0.5 cm above growing medium surface (Martinez-Maldonado et al. 2013). The germination duration was calculated in days from the time of the first appearance of a germinated seed until the time of the last germinated seed. The germination percentage is the accumulated number of seeds that germinated successfully expressed as a percentage of the total number of seeds tested (Bewley 1997; Ranal et al. 2009; McNair et al. 2012). The speed of germination (expressed as % per day) from sowing until the time of germination on a given day was calculated from the percentage of tested seeds that germinated each day (Ranal et al. 2009), according to the formula  $R = (n/90) * 100 * (1/t)$  where n is the number of seeds that germinated on day t after sowing.

### Observation of seed characters

The seeds were removed from the pulp, then cut longitudinally. The sectioned seeds were observed using Olympus CX31 microscope with a magnification of  $4 \times 10 = 40x$ . Endosperm and seed coat were photographed using a Camera Canon Power Shot SX160IS.

### Observation of germination stages

Observation started from the emergence of the radicle until the first leaf opened. The stages of germination were photographed using the Camera Canon Power Shot SX160IS. Photographing began when the hypocotyl appeared at the growing medium surface and continued until 2-5 leaves had expanded. Seedling colors were identified using the RHS Color Chart. Observation of the

seedling types were based on the classification of Kitajima (1992), Maia et al. (2005), and Zanne et al. (2005) using three codes. Code 1 indicates the appearance of the cotyledons (i.e. phanerocotylar vs cryptocotylar); code 2 indicates the position of the cotyledons (i.e. epigeal vs hypogeal); and code 3 indicates the function of the cotyledons (assimilation vs food reserves).

### Observation of seedlings morphology

The morphology of seedlings were determined at the five expanded leaf stage. Observations followed the methods of Burger (1972), de Vogel (1980), and Malik et al. (2014). Seedlings were examined in terms of traits describing the type of seedling, the seedling roots, the hypocotyl, the cotyledons, the epicotyl, and the leaves. Determination of morphological characters was made by naked eye. The root length, petiole length of cotyledon, leaf petiole length, cotyledon blade length, cotyledon blade width, leaf blade length, and leaf blade width were measured with a ruler. Measurements of root and hypocotyl diameters were carried out using vernier calipers.

## RESULTS AND DISCUSSION

### The natural seed germination

Germination of seeds was not simultaneous but took place over a protracted period. The first seeds germinated 159 days after sowing, while the last to germinate did so on day 238 after sowing. Seed germination of kenanga tanduk can thus be classified as delayed, based on the criteria of Garwood (1995). He regards germination that takes longer than 16 weeks as delayed germination; but our kenanga tanduk seed required at least 23 weeks to germinate.

The delayed germination of kenanga tanduk seeds did not result in a low total germination percentage at the end of the observation period. In fact, the final germination percentage (80%) can be regarded as quite high. However, the drawn-out germination process meant that. Daily germination percentages were low, peaking at the highest percentage of 16.67 %, 202 days after sowing. It took place 43 days after the first appearance of a germinated seed. After that day, the germination percentage per day tapered off until the point 238 days after first sowing, the last day of a recorded germination (Figure 1). At the beginning of germination, the daily and cumulative germination percentage were low (3.33%). However, increasing numbers of seeds germinated in the subsequent days until at day 207 after sowing, the cumulative germination percentage of seeds reached 50%. This occurred 48 days after the first appearance of a germinated seed. The germination percentage on the last day of observed germination (day 238) was 80%. Thus, it can be concluded that seeds harvested from fruit of kenanga tanduk at the yellow skin stage were capable of a high germination percentage. The yellow skin of the fruit can be taken as a sign that the seeds it contains have reached physiological maturity. Seeds from physiologically ripe fruit result exhibit a good germination percentage (Bewley and Black

1994; Santoso et al. 2012), whereas it is possible that harvesting seed beyond this stage of fruit ripening will reduce seed germination percentage (Mayer and Mayber 1989).

The speeds of germination (expressed as % per day) from sowing until the time of germination on a given day were also low, ranging from 0.01% to 0.08% per day (Figure 2). The speeds of germination were closely related to the number of seeds that germinated on the days of observation. The low germination speeds of kenanga tanduk seed is due to the fact that seed germination was not synchronous and took place over a protracted time.

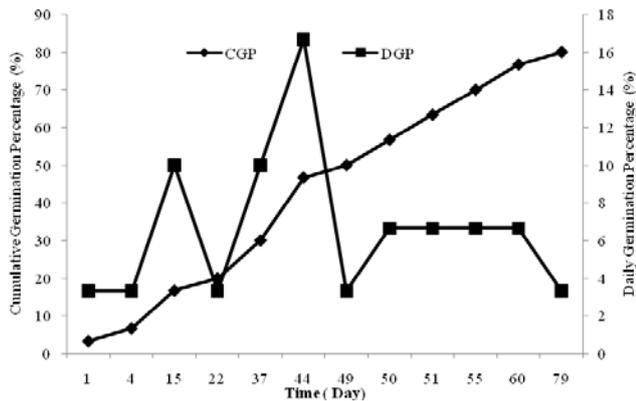
### Seed characters

Each fruit contains 1-2 seeds. Seeds are ovoid or oval shape, grooved on both sides, rather smooth in surface, the dorsal side is convex and the ventral side concave. The seed length is 1-2 cm and the width 1-1.5 cm, with diameter 1.5-2 cm, and color light brown to brown (Figure 3.A).

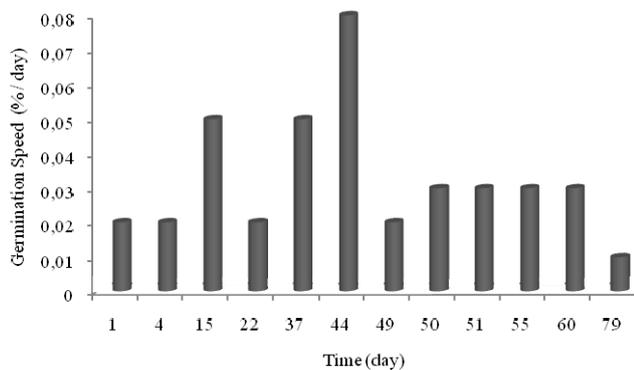
The time required for seeds to germinate is affected by differences in fruit maturation periods, and in seed characters. The results showed that although the seeds came from the same cluster of fruit, the germination times differed. Kenanga tanduk has bisexual flowers but anther dehiscence does not coincide with stigma receptivity. This causes irregularity in the time of fertilization, consequently physiological maturity is variable among its fruits. The hard seed coat, the small immature embryos, and the hard texture of the ruminant endosperm are factors causing the seeds to take such a long time to germinate.

Kenanga tanduk has a hard seed coat texture (Figure 3.B). In general, the seed coat of Annonaceae only consists of exotesta and endotesta layers (Corner 1976; Svoma 1998; Braga et al. 2010). However, for *Artabotrys hexapetalus*, anatomical observation shows that the seed coat consists of a mesotesta layer, in addition to the exotesta and endotesta layers (Figure 3.C). This is consistent with the report of Svoma (1997). The exotesta is formed by the outer epidermis, and is a single layer consisting of thin-walled cells which gradually fill with tannins. The mesotesta is a fibrous tissue layer, composed of thick cells and woody lignin (Figure 3.D). The endotesta is composed of several layers of stone cells. Around the micropyle of the endotesta layer is a thickened fibrous tissue that leaves only a small slit (Figure 4.A). Endotesta cells also form a cone-shaped plug (Figure 4.B) which serves to slow the transport of water and air into the endosperm (Corner 1976; Svoma 1997). The seed coat can inhibit seed germination by restricting / being impermeable to water and / or oxygen (Debeaujon et al. 2000; Oumar et al. 2012).

Anatomical observation showed that Annonaceae seeds have small immature embryos when the fruit are ripe (Figure 4.A), so it takes time for the maturation of embryos before sprouting (Svoma 1998; Martinez-Maldonado et al. 2013). Therefore, it still needs time for the embryo to develop. The time required for the maturation of embryos varies depending on each species. The maturation process of kenanga tanduk embryos takes about five months.



**Figure 1.** Daily and cumulative germination percentage. DGP: Daily germination percentage. CGP: Cumulative germination percentage



**Figure 2.** The speed of germination in kenanga tanduk seeds

The difficulty of water and air entering the seed is also influenced by the texture of the endosperm. According to Xue et al. (2012), Annonaceae has either a soft texture or a very hard endosperm. Kenanga tanduk seeds have glass-like ruminant endosperm which may slow down the imbibition process (Figure 4.C and 4.D). The endosperm is formed beneath the integuments, endotesta, and the inner layer of mesotesta fibers. Endosperm plays an important role in the supply of nutrients and water in seeds (Svoma 1998). Water and air are indispensable in the germination process of seeds (Meyer and Mayber 1989), stimulating the activity of germination enzymes (Kozłowski 1971). If imbibition is delayed, the enzyme-catalyzed respiration activity that plays a role in providing energy for germination is also delayed. According to de Vogel (1980), the germination process starts with the activity of the seed coat absorbing water. The seed coat swells and becomes more permeable to water, oxygen, and carbon dioxide. Swelling of the seed will stimulate further opening of the seed coat and the pushing out of the seed radicle.

### Growth and development stages of seedlings

Germination of kenanga tanduk seeds is indicated by the appearance of the white radicle emerging from a hole in

the seed apex. Appearance of the radicle is preceded by the opening of the operculum allowing the seed plug to part. Subsequently, the radicle appears out of that opening hole. From the time of emergence of the radicle up to the appearance of the first opened leaf, there are six stages, as presented in Figure 5.

#### Stage 1. Emergence of radicle

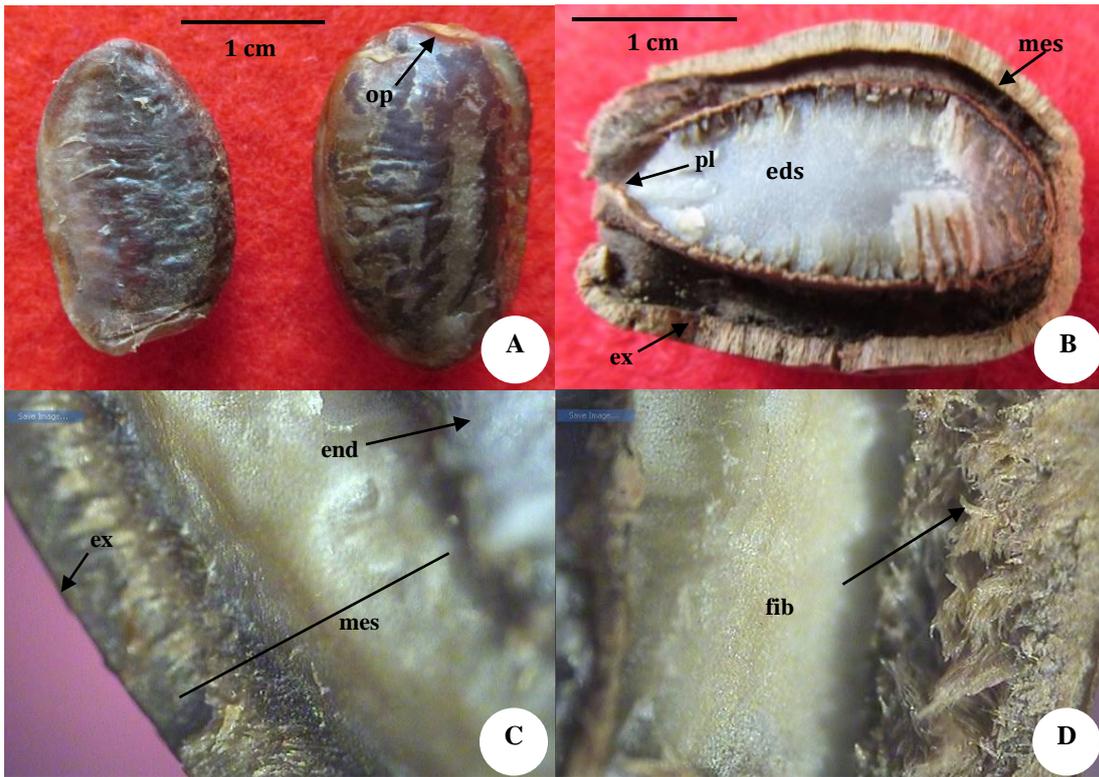
This stage is characterized by the emergence of a white yellowish-beige radicle from the small hole (micropyle) in the seed apex as a sign of early seedling growth of kenanga tanduk. The emergence of the radicle is preceded by the opening of the operculum (Figure 5.A). Enlargement of the radicle forces the seed coat around the micropyle to crack. The existence of such cracks facilitates the protrusion of the radicle. Further, the hypocotyl grows lengthwise. Elongation of the hypocotyl causes the radicle to protrude through the micropyle hole. According to Fisher (1992), seed germination occurs when the radicle emerges from the seed coat. In our experiment, this stage occurred 114 days after sowing.

#### Stage 2. Emergence of hypocotyl and roots

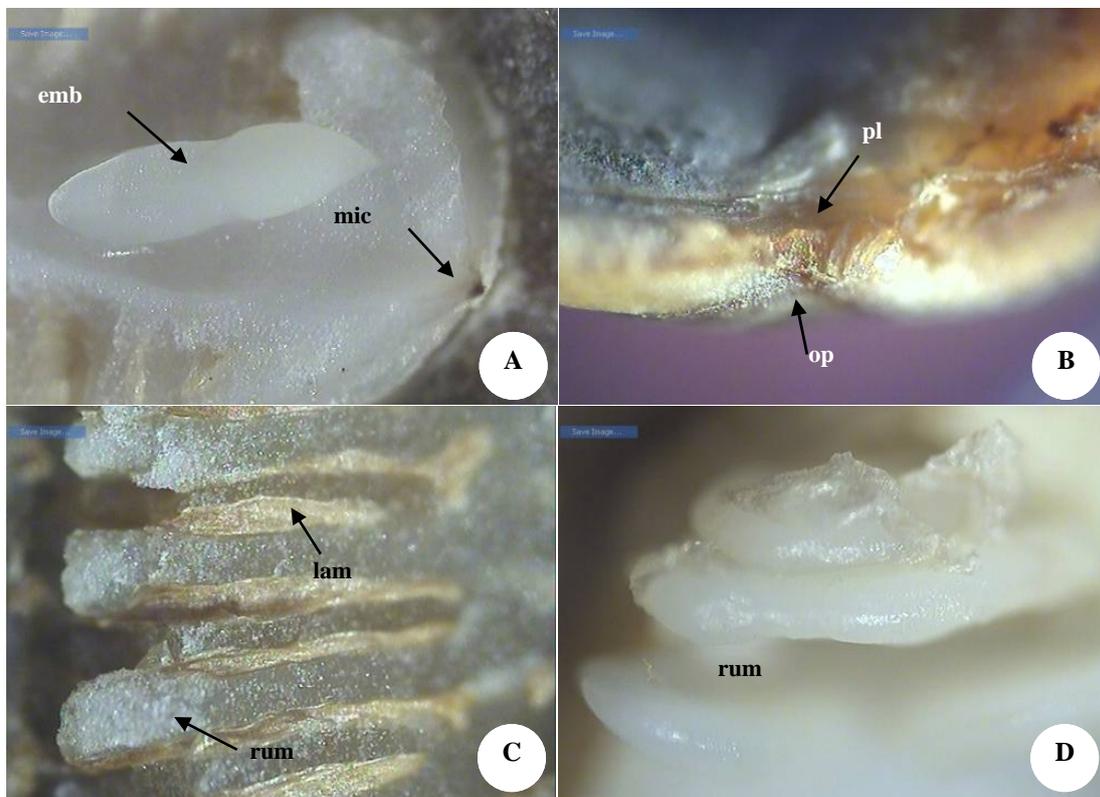
This stage is characterized by the emergence of radicle and hypocotyl. The radicle grows as a primary root. The primary root elongates and enlarges continuously. The color of the collet around the hypocotyl is white. The middle part is brownish yellow-beige. The color of the primary root tip is beige. During development, the primary root grows downward, the hypocotyl grows upward (Figure 5.B). The hypocotyl growth is bent like the letter "U" upside down. At this stage, secondary roots begin emerging around the base of the hypocotyl or collet. The roots grow downward continuously, while the hypocotyl grows upward approaching the growing medium surface. This stage occurred in our experiment approximately 121 days after sowing.

#### Stage 3. Emergence of hypocotyls above the growing medium surface

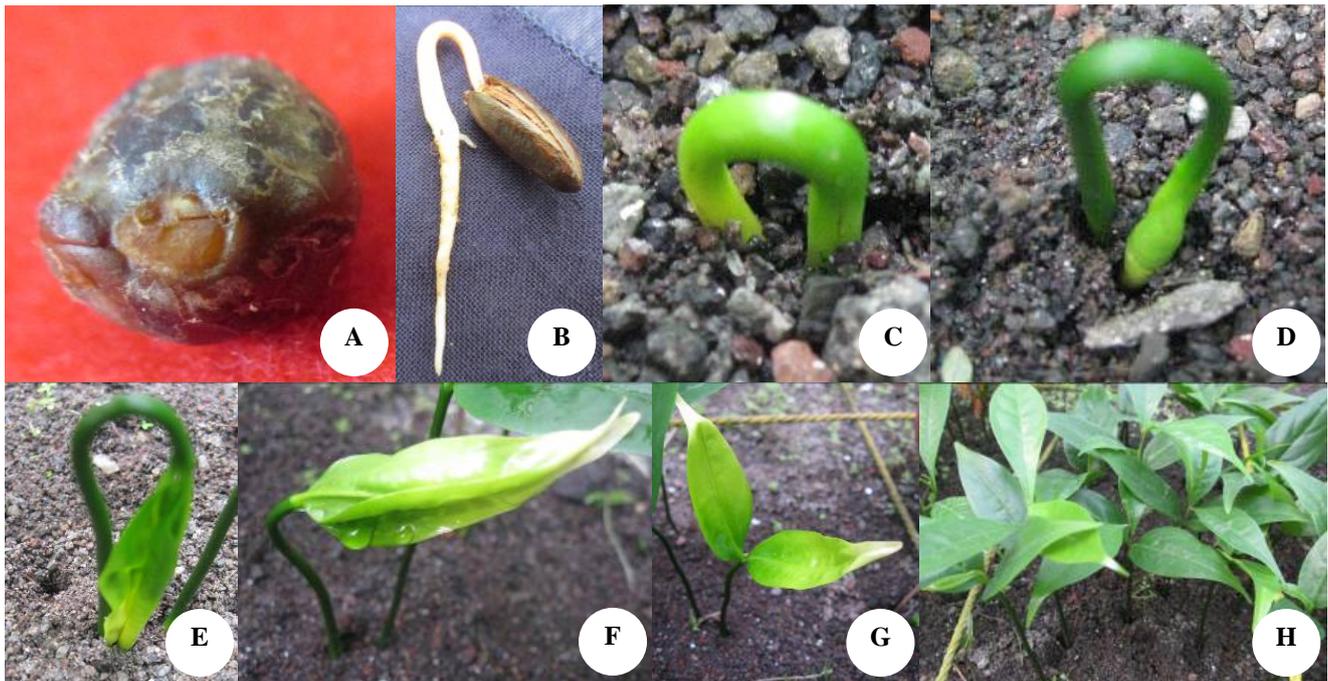
This stage is characterized by the emergence of the hypocotyl passing through the growing medium surface (Figure 5.C). The emergence of the hypocotyl is taken as the definitive indicator of seed germination (Martinez-Maldonado et al. 2013). The length of the hypocotyl is 0.3-0.5 cm. The color of the hypocotyl underground is light yellow (green 137), while above the ground it is yellowish-green (green 137 green 139 A or B). The crook of the hypocotyls extends upward so that the cotyledons appear above the growing medium surface. Cotyledons begin enlargement and elongation, resulting in the seed coat parting. The color of the unexposed cotyledons is yellow. The cotyledons are gradually drawn upward as a result of the lengthening hypocotyl. Initially, only one third of the cotyledons appear, then two thirds of them become visible (Figure 5.D). At this stage, the height of the seedling is 2.5-3.5 cm. In our experiment, this stage occurred 7-9 days after germination.



**Figure 3.** Seeds and seed coat kenanga tanduk. A-B using the camera, C-D using a microscope with a magnification of  $4 \times 10 = 40\times$ . A. The seed is intact, B. Longitudinal section, C. Seed coat layer, D. Mesotesta fibers. Note: op = operculum, mes = mesotesta, eds = endosperm, pl = plug, ex = exotesta, end = endotesta, fib = fibers



**Figure 4.** The embryo and endosperm of kenanga tanduk seeds at magnification  $4 \times 10 = 40\times$ . A. The small immature embryo, B. The anterior seed, C. Ruminant endosperm, D. Glass-like ruminant endosperm. Note: emb = embryo, mic = micropyle, pl = plug, op = operculum, lam = lamella, rum = ruminant endosperm



**Figure 5.** The germination stages of kenanga tanduk. A. First stage, B. Second stage, C-D. Third stage, E. Fourth stage, F-G. Fifth stage, and H. Sixth stage

#### Stage 4. Emergence of cotyledons

This stage is characterized by the emergence of cotyledons above the surface of the medium. The cotyledons are gradually drawn upward, caused by the lengthening of the hypocotyl. The cotyledons become more elongated, but the tip is still in the ground (Figure 5.E). At this stage, most of the cotyledons have separated from the seed coat. The color of cotyledons is greenish-yellow (yellow-green 150 D) later turning green (green 143 C). At this stage, the height of the seedlings is 4.5-5.5 cm. In our experiment, this stage took place 9-10 days after germination.

#### Stage 5. Opening of cotyledons.

This stage is characterized by the opening of the cotyledons. One to two days after the cotyledons have been lifted upward, the hypocotyl grows upright. In this case, the position of the cotyledons is either crooked or upright (Figure 5.F). After the cotyledons have grown upright, then 1-2 days later the cotyledon blades opened (Figure 5.G). The petioles of the cotyledons are 0.3-0.5 cm long. The cotyledon blades at this stage are 5-6.5 cm long and 2-2.5 cm wide. The color of the cotyledons is light green or greenish-yellow (yellow-green 144 C or 145 A yellow-green). The upper leaves are shiny. After opening the cotyledons, the plumule appears in the middle of the cotyledons. In our experiment, this stage happened 10-11 days after germination.

#### Stage 6. Opening of first leaves

This stage is characterized by the opening of the first leaf. The hypocotyl grows slowly. After the cotyledon blades open, the plumule grows steadily. The growing of

the plumule is stimulated by the elongation process of the epicotyl. The plumule grows larger and finally the first leaves open. The first leaves are green (green or green 137 C, 137 B). While the first leaves are opening, the second leaves appear, and then appear the next leaves (Figure 5.H). At this stage, tertiary rootlets begin to emerge along the secondary roots. Cotyledons still survive (not whole). The height of seedlings at this stage is 5.5-7.5 cm. This stage occurred one week after the cotyledons open fully.

#### Type of seed germination

The characterization of seedling type for kenanga tanduk, based on the 3 code classification of Kitajima (1992), Maia et al. (2005), and Zanne et al. (2005), is as follows:

*Code 1.* During the germination process of kenanga tanduk seeds, the enlargement of the cotyledons forces open the seed coat so that the cotyledons emerge out of the seed coat. This type of germination, exposing the two cotyledons outside the seed-coat, is called phanerocotylar.

*Code 2.* The cotyledons that have been separating from the seed coat are raised to emerge above the growing medium surface. Seedlings in which the cotyledons are lifted above the surface of the growing medium like this are classified as the epigeal type.

*Code 3.* In the early germinated seeds, the cotyledons are greenish yellow (yellow-green 150 D) later turning to green (green 143 C). The shape and arrangement of primary and secondary veins of the cotyledons resemble those of the mature leaves of the plant. These green cotyledons serve photosynthesis and assimilation, providing food for the new seedling's growth. Cotyledons which serve assimilation through photosynthesis are

generally thin, resembling mature leaves, and are eventually shed slowly in the same way as mature leaves.

Observation of cotyledon emergence, cotyledon position, and cotyledon function suggests that the seedling type of kenanga tanduk is PEF (Phanerokotilar-Epigeal-Foliaceous). Meanwhile, according to the classification of de Vogel (1980), kenanga tanduk seedlings are of the Macaranga type. Seedlings belonging this type have leaf-like cotyledons, expanding to be thin, and photosynthetic in function. The PEF type is the most common (more than 50%) seedling type in woody plant genera of the Malesian region. The *Blumeodendron* and *Horsfieldia* types in the classification system of de Vogel (1980) are also found in the Annonaceae family.

### Seedlings morphology

Seedling morphological characterization was carried out on seedlings at their five-leaf stage. The morphology was described following the standard terminology of Burger (1972) and de Vogel (1980). General characteristics of kenanga tanduk seedlings were as follows: *Type of seedlings*: phanerocotylar, epigeal, foliaceous (with assimilation function). *Roots*: the primary root is not enlarged; it is slender, not straight, light brown-dark brown, 12-14 cm long, and 0.1-0.3 cm diameter. Secondary roots are slender, located around the collet and along the primary root, 0.5-2.5 cm long, near the tip of the primary root becoming shorter, cream-brown. Tertiary roots are slender, dispersed along the secondary roots, up to 0.5 cm long, pale yellow-cream. *Hypocotyl*: tall, glabrous, dark green, 0.1-0.3 cm diameter. *Cotyledon*: emergence above the growing medium surface, a pair, distichous, shed slowly. Cotyledons thickened 0.3-0.5 cm long, dark green. Cotyledon blades elongated oval, 5-6.5 cm long and 2-2.5 cm wide, with base rounded, and tip acute, with the edges entire, green, glossy dark green on upper surface. The primary leaf veins above are sunken, below prominent. The number of secondary veins are 5-6 pairs, sunken above, prominent below. *Epicotyl*: upright, green. *Leaves*: single, alternate, petiole thickened, 0.3-0.4 cm long, glabrous, green. Leaf blades elongated oval, 3.5-9.5 cm long and 1.5-2.5 cm wide, with base rounded, tip acute-acuminate, edges entire, green, shiny upper surface. The upper primary leaf veins are sunken and glabrous, below prominent and glabrous. Secondary veins 4-9 pairs.

In summary, the present study showed that the seed germination of kenanga tanduk can be classified as delayed. The earliest seed germination occurred 159 days or 22 weeks after showing. The duration of seed germination from beginning to the end took as long as 238 days. The percentage germination reached 80% with the speeds of germination on a given day of germination ranging 0.01-0.08% per day. The seed germination process of *A. hexapetalus* from the time the radicle first appears until the time the first true leaf opens is divided into 6 phases. The type of cotyledon is epigeal. The type of seedling is Phanerocotylar-Epigeal-Foliaceous (PEF) or Macaranga type.

### ACKNOWLEDGEMENTS

The author thanks Dr. Graham Eagleton (Australian Volunteer International) for helpful discussion and comments on the manuscript's English. I am also thanks to Harto (Technician of the Bogor Botanic Gardens) for his helpful in collecting the seeds used.

### REFERENCES

- Bewley JD, Black M. 1994. Seeds: Physiology of development and germination. 2<sup>nd</sup> edition. Plenum Press, New York.
- Bewley JD. 1997. Seed germination and dormancy. *Plant Cell* 9: 1055-1066.
- Braga JF, Ferrira G, de Pinho SZ et al. 2010. Germination of *atmeya* (*Annona cherimola* Mill. x *A. squamosa* L.) CV. Gefner seeds subjected to treatments with plant growth regulators. *Intl J Sci Nat* 1 (2): 120-126.
- Burger HD. 1972. Seedlings of some tropical trees and shrubs mainly of South East Asia. Center for Agricultural Publishing and Documentation, Wageningen.
- Corner EJH. 1976. The seeds of dicotyledons 1 and 2. 1<sup>st</sup> ed. Cambridge University Press, Cambridge.
- Couvreux TP, Maas PJM, Meinke S et al. 2012. Keys to the genera of Annonaceae. *Bot J Linn Soc* 169: 74-83.
- de Vogel EE. 1980. Seedlings of Dicotyledons, Structure, Development, Types. Descriptions of 150 Woody Malesian Taxa. Centre for Agricultural Publishing and Documentation, Wageningen.
- Debeaujon I, Kloosterziel KML, Koornneef M. 2000. Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant Physiol* 122: 403-413.
- Fisher JB, Posluszy U, Lee DW. 2002. Shade promotes thorn development in a tropical liana *Artabotrys hexapetalus* (Annonaceae). *Intl J Plant Sci* 163 (2): 295-300.
- Handayani T. 2004. Perkecambahan benih sepuluh jenis anggota suku Annonaceae. *BioSMART* 6 (2): 109-112.
- Ibarra-Manriquez G, Ramos MM, Oyama K. 2001. Seedling functional types in a lowland rain forest in Mexico. *Am J Bot* 88 (10): 1801-1812.
- Kitajima K. 1992. Relationship between photosynthesis and thickness of cotyledons for tropical tree species. *Funct Ecol* 6: 582-599.
- Kozłowski TT. 1971. Growth and Development of Trees. Vol. I. Academic Press, New York.
- Li BT, Gilbert MG. 2011. *Artabotrys* R. Brown, Bot. Reg. 5: t. 423. 1820. *Fl China* 19: 701-703.
- Li T, Yu J. 1998. Studies on the chemical constituents of the leaves from *Artabotrys hexapetalus*. *Yao Xue Xue Bao* 33 (8): 591-596. [Chinese]
- Maia LA, Maia S, Pia P. 2005. Seedling morphology of non-pioneer trees in Central Amazonian varzea floodplain forest. *Ecotropia* 11: 1-8.
- Malik V, Seema A, Inam M. 2014. Seedling morphology of endangered *Eremostachys superba* Royle ex Benth. (Lamiaceae). *Intl J Pure Appl Biosci* 2 (5): 229-232.
- Martinez-Maldonado FE, Miranda-Lasprilla D, Magnitskiy S. 2013. Sugar apple (*Annona squamosa* L., Annonaceae) seed germination: morphological and anatomical changes. *Agron Colomb* 31 (2): 176-183.
- Mayer AM, Mayber AP. 1989. The germination of seeds. Pergamon Press, London.
- McNair J, Anusha S, Daniel F. 2012. How to analyse seed germination data using statistical time to event analysis: non-parametric and semi-parametric methods. *Seed Sci Res* 22 (2): 77-95.
- Nurainas. 2004. *Artabotrys* (Annonaceae) in Sumatra. *Floribunda* 2 (5): 117-128.
- Oumar BA, Maurice SAGNA, Mame Oureye SY. 2012. Germination capacity of Annonaceae seeds (*Annona muricata* L., *A. squamosa* L. and *A. senegalensis* Pers.) cultivated under axenic conditions. *Intl J Sci Adv Technol* 2 (6): 21-34.
- Ranal MA, Denise GDS, Wanessa RF et al. 2009. Calculating germination measurements and organizing spreadsheets. *Rev Bras Bot* 32 (4): 849-855.

- Santoso BB, Budianto A, Aryana IGPM. 2012. Seed viability of *Jatropha curcas* in different fruit maturity stages after storage. *Nusantara Biosci* 4 (3): 113-117.
- Schmidt, L. H. 2000. Guide to handling of tropical and subtropical forest seed. Danida Forest Seed Centre, Copenhagen.
- Shiva P, Sathiyaseelan R, Aron S et al. 2015. A review of the genus *Artabotrys* R. Br. (Annonaceae) from Andaman and Nicobar Islands, with a new record for India. *Indian J For* 38 (2): 159-164.
- Sowjanya KM, Swathi J, Narendra K, et al. 2013. Extraction and antimicrobial potential of secondary plant metabolites from *Artabotrys hexapetalus* (L.f.) Bhandari. *Intl J Res Ayurveda Pharm* 4 (5): 764-768.
- Suleiman RA, Quitino AM, Stephen SY. 2014. Chemical compositions and mosquito repellency of essential oils from *Artabotrys hexapetalus* and *Artabotrys rupestris*. *Intl J Biol Chem Sci* 8 (6): 2804-2812.
- Svoma E. 1997. Seed development and function in *Artabotrys hexapetalus* (Annonaceae). *Plant Syst Evol* 207: 205-223.
- Svoma E. 1998. Seed morphology and anatomy in some Annonaceae. *Plant Syst Evol* 209: 177-204.
- Vieira DCM, Socolowski F, Takaki M. 2010. Seed germination and seedling emergence of the invasive exotic species, *Clausena excavate*. *Braz J Biol* 70 (4): 1015-1020.
- Xue B, Su YCF, Thomas DC. 2012. Pruning the polyphyletic genus *Polyalthia* (Annonaceae) and resurrecting the genus *Monoon*. *Taxon* 61 (5): 1021-1039.
- Zanne AE, Chapman CA, Kitajima K. 2005. Evolutionary and ecological correlates of early seedling morphology in East African trees and shrubs. *Am J Bot* 92 (6): 972-978.
- Zheng W, Ping W, HongXiang Z et al. 2011. Photosynthetic characteristics of the cotyledon and first true leaf of castor (*Ricinus communis* L.). *Austr J Crop Sci* 5 (6): 702-708.