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Original Research Article

Floral Biology of Ginger (*Zingiber officinale* Rosc.)

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Abstract

Floral biology of large white ginger (*Zingiber officinale* Rosc.), belonging to the family Zingiberaceae, was studied by direct observation method, which started from the initiation up to anthesis stage. The structure of pollen and anthers were observed by Scanning Electron Microscopy. The viability of pollen was tested by pollen growth media. The presence of pollinators were also observed. The experiment indicated that ginger started to flower from 4 to 7 months after planting (MAP) depending on the environment. Environmental conditions such as temperature influenced flowering. Normal condition of temperature and humidity may cause flowering period longer. The time is required for anthesis in Bogor was found to be longer than in Cicurug. The period of flower initiation until wilt was 70-80 days, even the period of flower bloom or anthesis until wilt was occurring only a few hours (12-18 h). The morphology of flower shows that the position of the anthers is lower than stigma, indicating that the pollination in ginger flower is open type. No pollen tube is formed. The receptivity of stigma was occurring at the time when secretion was produced and the stigma tip was transparent (2.5 h after anthesis). No pollinator to visit when the flower is blooming.

Keywords

Anthesis
Flowering biology
Pollination
Stigma receptivity
Zingiber officinale Rosc.

Introduction

Large white ginger (*Zingiber officinale* Rosc.var.officinale) is a medicinal herb belongs to the family Zingiberaceae, in which the rhizome is utilized for spice or raw material of traditional medicines. Members of this family are perennials that frequently have sympodial (forked) or horn-like fleshy rhizomes (underground stems). Most species in the Zingiberaceae produce flowers, which develop on separate shoots arising from rhizomes. Ginger flowers are covered by bract (leaflike structures), from each bract emerges one complete flower (Purseglove et al., 1981). Peter et al. (2007) reported some times there were two bisexual flowers. Bracts are spirally arranged, and the flower clusters are spiral and cone-like called as spike. The Zingiberaceae flower resembles an orchid because of its labellum (two or three fused stamens) joined with a pair of petal-like sterile stamens. Nectar is present in the slender flower

tubes. The brightly coloured flowers may bloom for only a few hours and are thought to be pollinated by insects. This was in accordance with Endress (1994) who stated that Zingiberaceae is a large family of animal-pollinated tropical monocotyledons. Major pollinators include bees, hawkmoths and birds (Ippolito and Armstrong, 1993). Members of the Zingiberaceae family also display a broad range of pollination and breeding systems (Sakai et al., 1999).

The flowers of large white ginger are not of particular concern because propagation is commonly undertaken by using rhizomes and its flower is not parts of ornamental flower. On the other hand, big white ginger rarely produced flowers under normal condition, and even if the flowers are produced, they rarely produce seeds. Flowers usually open during day time and fall on the next day. Ramachandran (1992) reported that ratio between fertile pollen and germinated pollen grains depended on the quantity of pollen grains germinated on stigma or whether self incompatibility operates. Rahman (1998) reported that fertility of red ginger pollen (*Zingiber officinale* var. *Rubrum*) highly vary from 6 to 45% which was sufficient for fertilization. According to Peter et al. (2007), the failure fruit and seeds formation may be caused by a number of factors such as failure of pollination-due to limited pollinators. It is therefore possible that the absence of pollinators may cause ginger seldom produce seeds.

Biology of ginger flowers have not been studied comprehensively especially on the flower, seed formation and factors affecting it. Understanding on the floral biology is important for conventional breeding purposes. The objectives of this study was to observe phenology and flower development of large white ginger (*Zingiber officinale* Rosc.). It is hoped that this study will enlight upon why ginger rarely produce flowers and set seeds.

Materials and methods

Research site and plant material

The research was undertaken from November 2009 until June 2010 in two Experimental Gardens of the Indonesian Spice and Medicinal Crops Research Institute. The first location was in an open field in Cicurug, Sukabumi (500 m asl), the second location was in a glass house of Cimanggu garden (250 m asl), Bogor. In each site, gingers were planted in polybags. Observation on flowers was conducted on 40 plants per each location, started from flowers initiation until the flowers fall off.

Observation on flower phenology

Morphology of flower was monitored once a week started from a few months following flower development until the plants undergone senescence. Characters observed in flower phenology were number of flower/spike, length flowering time per spike and per plant. Monitoring also made on the changes of individual flower from initiation until the flowers wilted and fall off, also included colour of corolla. Bracts were monitored since flowers bud until anthesis.

Biology of pollen and stigma

Pollen was observed from randomly the newly blooming in the field. Pollen viability was observed by binocular microscope. The structure of pollen and anthers were observed by scanning electron microscopy (SEM) in the laboratory of Zoology, LIPI Cibinong.

The viability of pollen was observed by pollen staining and germination. Pollen staining was observed by acetocarmine staining 0.75% and aniline blue staining 0.2 %, while pollen germination was observed by Brewbaker and Kwack (100 ppm H_3BO_4, 300 ppm $Ca(NO_3)_24H_2O$, 200 ppm $MgSO_47H_2O$, 100 ppmKNO_3 in 1000 ml aquabidest) and PGM (10% sucrosa, 0.005% H_3BO_3, 10 m$MCaCl_2$, 0.005 mMKH_2PO_4, 4% PEG 6000. Pollen was taken every 15 min, until 5 times as soon as possible after the flowers bloom, Pollen viability was evaluated after 24 hours incubation. Pollen germination was determined by direct microscopic observation (at 40× magnification) in three view points per slide, and four replicates (slides) were used for each medium. A pollen was classified germinated when pollen tube length was equal or exceed the pollen grain diameter, while it was classified a normal when the pollen has become 70% stained dark blue with aniline blue or dark red with acetocarmine.

Pollen structure and stigma were observed by scanning electron microscope. The pollen samples were collected from fresh flowers and then prepared for

scanning microscopy with the usual fixation and dehydration procedures. Data were observed for pollen were include shape, texture and pore surface of pollen, while for the stigma observed were included: surface and texture of stigma. Observations of stigma were started the beginning of bloom until to full bloom. Fluid volume on the stigma was measured by a micro pipette. Fluid was taken as soon as possible after the flowers bloom. Besides that, the presence of pollinators was also observed.

Results and discussion

Flowering Period

Flower formation in Sukabumi was earlier than in Bogor. The flowers initiation occurred 4 months and lasted until 7 months after planting, it took about 3 months in Sukabumi. While in Bogor, flower initiation occurred at the fifth months and lasted only one month after. The peak flowering period in both locations was 5 months after planting (Table 1). Individual flower did not open at the same time. It took one by one to complete the whole flowers opened in a spike.

Initiation of flowers occurred earlier in Sukabumi than Bogor, may be influenced by altitude, sunshine, temperature and relative humidity. Adaniya et al. (1989) indicated that ginger rarely produce flowers, but when ginger was planted in a glass house equipped with heater at autumn produced flowers. Gracie et al. (2004) reported that development of generative shoots in *Zingiber myoga* was influenced by light intensity and temperature. At Sukabumi the ginger planted in a open field, while in Bogor was in the glass house. Ginger planted in Sukabumi received higher light intensity than in Bogor.

In terms of temperature not much different between the two locations. Average daily temperature in Bogor was 20-26°C (7.00 AM), 28-40°C (12 PM) and 23-26°C (16.00 PM). In Sukabumi, the temperature was 21-23°C (7.00 AM), 29-34°C (12.00 PM) and 21-23°C (16.00 PM). Stable temperature and humidity during the experiment may last the flowering period longer.

Table 1. Flowering period at Bogor and Sukabumi.

Locations	Spike emergence (%)			
	Age (Months After Planting)			
	4	5	6	7
Sukabumi	14.91	56.45	23.79	4.83
Bogor	0	83.09	16.90	0

Table 2. Time of anthesis, temperature and relative humidity at the time of anthesis.

Location	Time of anthesis	Temperature (°C)	RH (%)	Time taken for anthesis B1-B4 (min.)*	Climatic condition
Sukabumi	14.05-15.15 16.15-16.30	28-33 27	60-69 80	15-25 25-35	Bright day - Cloudy Rainy
Bogor	13.15- 16.00 16.55-17.30	22-29 20-21	80-88 88-89	20-30 60-90	Bright day - Cloudy Rainy

* Started from opening of corolla until corolla fully open (anthesis); B1 when calyx started to open flower; B4 when flowers fully open.

Time of anthesis

Time of anthesis at the two locations were similar ocurred in the afternoon around 13.00 – 17.30 (Table 2). Flowers anthesis occurred earlier in the day during sunny bright day, or later in the afternoon in the rainy day. Temperature and relative humidity influence time of anthesis. Higher temperature and relative humidity accelerated flowers to open. The time taken for flowers to fully open initiated by opening of the corolla until the corolla fully open was faster in Sukabumi than in Bogor, even faster during sunny bright day and slightly longer in rainy days. The environmental condition of an open field in Sukabumi, in which the lands received full sunshine, and higher temperature may be the reason why diffences occurred.

Environmental conditions such as humidity, light intensity and temperature influence flowering.

Changes in those factors will change plants respons to flowering (Darjanto and Satifah 1990). Lower temperature and higher relative humidity, made time of flowers open tend to be longer. Sri Rahayu et al. (2007) reported that in *Hoya lacunosa*, anthesis was influenced by temperature, relative humidity and light intensity. In bulbs producing plants, Khodorova and Boitel-Conti (2013) indicated that among several naturally occurring environmental factors, such as temperature, humidity and light intensity, temperature is considered to play a predominant role in controlling proper growth and flowering. Apparently, ginger faced similar situation, that temperature was dominant in determining flowering. As in other families of the Zingiberales, flowers of all species of observed gingers last for less than 1 day (Endress, 1994; Larsen et al., 1998).

Phenology and flower development

During plant growth and development, rhizome will form vegetative and/or generative shoots. Ginger flowers may developed directly from rhizome or from vegetative shoots. When flowers arised from rhizome, generative shoots usually developed indicated by rounded form, green colour with lighter green at its tip.

When the flowers almost open, a slit was develop around the tip (apex) trigerring emergence of flower. Vegetative shoots also developed from rhizome. The shape of vegetative shoot is mostly slender and fine and its tip was acute (Fig. 1 A and 1 B). In other occassion, generative shoots may emerged after vegetative shoots developed and elongated to form a pseudo stem from 3-5 leaves and then at its middle stem emerged generative shoots (Fig. 1 C).

At initial development of shoots which arised from rhizome, no peducle was observed. Along with its growth, green bracts gradually developed and made the shoots elongated and bigger to form spike. In generative shoot growth, shoots changed its shape from slender to become rounded and form spike. Spike made of a number bracts, and from its bract emerged corolla, indicating the spike formation was complete and mature. Flower emerged from each spike, one flower per bract up to 3 flowers, but flowers do not open every day. One to two flowers were formed in each bract (Fig. 2A). This also occurred in other in Zingiberaceae family, such as in genera Alpinia (*A. purpurata* "Kusuma" and *A. purpurata* "Bethari") (Oktaviani, 2009). This result was in contrast with Larsen et al. (1998) which stated that in Zingiberaceae, each bract only contained on flower. In general, flower anthesis started from the bottom part then spirally move upward. But in other occassion, the opposite was observed, especially when more than one flower was formed in one bract, and the second flower always open much later, when the flowers at the spice tip already opened.

Environmental conditions affected flower development. All flowers in the bract were complete flowers and will open when the time comes. However, heavy rain may hampered flowers to open and to much rain will make flowers rotten. This occurred in Sukabumi, when rainy season was longer than usual. Temperature also influence flower development and anthesis. High temperature during flowering period cause spice to stop its growth, and flowers becomed yellowish, dry and fail to open. This mostly found in ginger grown in the glass house in Bogor (Fig. 2 B). Fully developed spike retain its growth and colour, but some flowers may dry and rotten (Fig. 2 C).

Fig. 1: A) Vegetative and generative shoots; B) Generative shoot developed from rhizome; C) Generative shoot emerged from fully developed shoot.

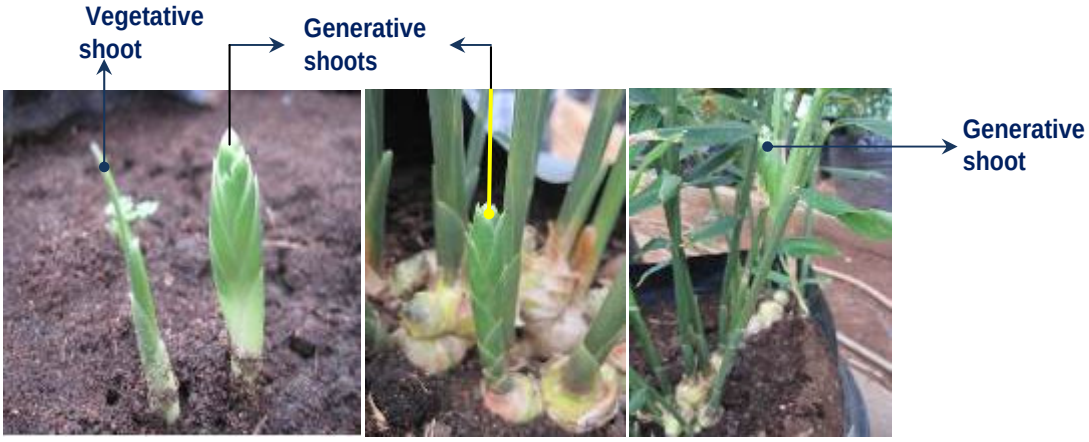


Fig. 2: A) Bract with two flowers; B) Dried spike and flowers; C) Dried flowers before anthesis.

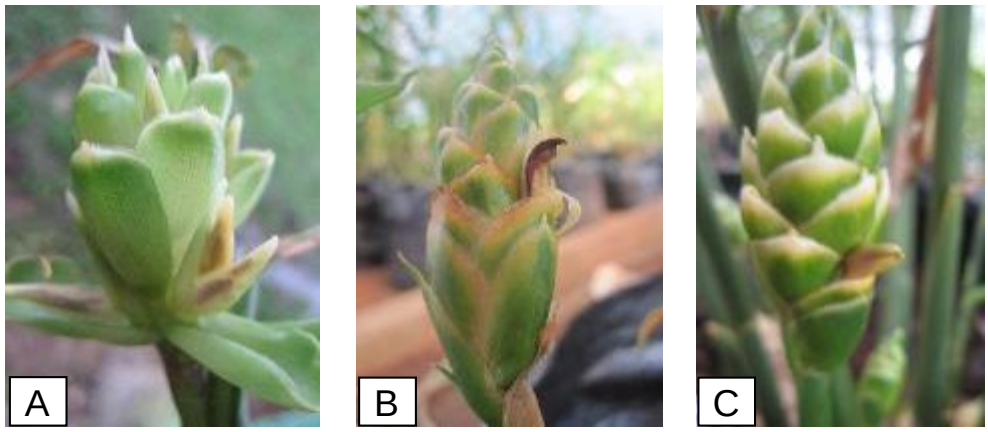


Table 3. Phenology of large white ginger (*Zingiber officinale* Rosc.).

Flowering stage		Time	Size	Note
Spike development period				
S0	Generative shoot identified	0 DASE	1-1.5 cm	Spike slender, greenish white, a slit was formed at the rounded tip
S1	Shoot tip enlarge and elongated	5-7 DASE	2 cm	Lapisan-lapisan braktea semakin jelas
S2	Spike can be distinguished from peduncles	10 DASE	4 cm	Colour turn to dark green, peduncle size vary
S3	Spike enlarged, difer from peduncles	14-18 DASE	7-20 cm	Bract emerged, flower developed inside
S4	Flower tip emerged from spike	18-20 DASE		Maximum size of bract
S5	Flower enlarged	23-25 DASE	6-8 mm	
S6	Flower completely developed and emerged from a bract	27-35 DASE	10-15 mm	Calyx turn to yellowish
S7	Bract turn reddish	50-60 DASE		Occurred after the last flower in a spike open
S8	Bract drying	70-80 DASE		Started from the spike tip, turning brown
Flower bloomy until wilt periode(12-18 h)				
B0	Fully flower	0		
B1	Corolla opening	±72 MAFF	Corolla color looks real from the outside .	
B2	Sepal begin to open Pistils begin to appear Mahkota masih membulat	±74 MAFF	Divided sepals 3: 1 large sepals, 2 small sepals. Yellow sepal color, corolla/petal began to appear. The position of the pistil between the large sepal and large petal	
B3	Clearly visible pistil	±76 MAFF	Sepals are separated	
B4	Open corolla	±87 MAFF	Divided petals 3: 1 large petals, 2 small petals.	
B5	Anthera broke	±93 MAFF	Anthera position at the base of the pistil like sticking	
B6 B7 B8	Pistils start to curve Anthers as touch the petal Flower wilt	±112 MAFF ±232 MAFF ±12-18 HAFF	Clear liquid in the anthers and receptive suspected Flowers wilt the next day	
Ket: DASE:day after spike emerged; MAFF: minute after full flowering; HAFF: hour after full flowering				

Flower phenology

The time taken from flower to develop which arised from rhizome was 70-80 days. The flowering period was divided into spice development and flower anthesis until wilting (Table 3, Fig. 3 and Fig. 4). Generative shoots arised from the terminal part of vegetative shoots were rather difficult to observe. This generative shoot developed after the spike enlarged, and covered by bract.

Flower bloomy until wilt period

Flowers that will bloom characterized by calix color fade. Calix seemed to thin out so it looks a tinge of red color of the corolla, Calix slowly opened so that the corolla visible (Figure 4.B2), Calix full opening and divided into 3 (1 big calix opening upwards and 2 small calix opening down) (Figure 4.B4), corolla opening filled with a receptive stigma, characterized by curved labellum and the anthers to the stigma end looks wet and shiny (Fig. 4 B7).

Ginger flowers have 3 calixs. 1 calix is larger than the others and light yellow transparent, so that when the flowers begin to bloom will look tinge of red which is the color of the labellum (corolla) are protected by calyx. Corolla dark red and mottled yellow, the base of the corolla is yellow. The longer the process of expansion of flowers the color red more real as if calyx thinning. When the flowers bloom, pistil stalk shaped curved edges touching the labellum.

Flowers to smell the fragrant scent like ginger, it indicates the anthers in a receptive state. Pistil stalk in the form of very fine threads and protected white thin layer of dark red at the edges (the curved part). The coating also protects the theca so the pistil stalk and theca as if fused, the position is beneath the theca and the sticky pollen. The position of the stigma and ginger theca is almost the same as the ornamental ginger Alpinia. Oktaviani (2009) states that the characterization of the genus of Zingiberaceae, *Alpinia* at various cultivars tested showed that flower only lasts one day later withered, and generally at the time of blooming flowers pistil position higher than the stamens. The position of theca is one of the factors that cause has not been found in plant seeds ginger. Bermawie et al. (1997) studied that the flowers of ginger full opened in afternoon and fall of the next day.

Pollination biology

During the study, there are no pollinating insects as vectors of ginger, because ginger flowers blooming in the afternoon until late afternoon (01.00 - 05.00 pm). Rarely, the pollinators (insect) found floating in nature. Not known vectors such as pollinating insects or other animals so that no seed formation ginger. Most of the insect pollinator activity from morning until late afternoon, as long as the plants are still producing attractant that usually occur during the day. The insects are active at 01.00 - 05.00 pm does not help the formation of cashew fruit (not insect pollinators) (Bhattacharya, 2004).

The other Zingiberaceae found the presence of insects as pollinators factor. There are some insects that are found in the "Bornean Gingers" are Halictid, Amegilla and birds (Spiderhunter) which acts as a vector of pollinators (Sakai et al., 1999). *Apis cerana cerana* as the main pollinator vector *Alpinia blepharocalix* and other insects that *Xylocopa* spp. while on *Alpinia kwangsiensis* found a bird (*Arachnothera longirostra*) as pollinators vector (Ling 2003). Shruti et al. (2009) many insects carry pollen at 09.00, followed by 12.00 and 15.00 hours slightly, presumably because of differences in high humidity and low temperatures differ by several Zingiberaceae which serves as an ornamental plant "Ornamental Ginger" and although often found in the seeds of wild life and not cultivated.

Pollen viability

Estimate of pollen viability by aniline blue staining showed pollen will stain dark blue indicates that the pollen viable and stained a deep red when using acetocarmine staining, the resulted in 31- 57%. Interaction between staining and germination time (Table 4), both used coloring can be used to estimate pollen viability of ginger. Using aniline blue has the hightest viability on 45^{th} minutes after anthesis. Acetocarmin staining showed that the lowest viability at 15^{th} minutes after anthesis and the peak at 60^{th} minute after anthesis, after that pollen viability decreases. Presumably after anthesis 60^{th} minutes, the viability of the pollen will be decrease. The anilin blue staining showed that pollen viability will be decreased 45^{th} minute after anthesis. Warid (2009) aniline blue staining is a dye that can infer pollen germination with PGM because it has a positive correlation of 0.624 to the family Euphorbiaceae, Solanaceae, Poaceae and

Myrtaceae. Adaniya and Shoda (1998) stained with an orcein acetic acid solution and counted ; the fertile pollen were 0.2 to 21.1%. Fertile pollen grains are those that had good stainability without vacuolization. Adaniya and Shirai (2001) used acetocarmin for pollen germination ginger Sanshu and obtained 40% viability. The media that used is not capable of germination of pollen. The absence of pollen tubes that form despite being observed until 72 h. The medium used for germination is Pollen Growth Media (PGM) is a commonly used medium for pollen germination consisting of compounds that is more complete than the media used for germination of pollen. The absence of pollen tube because the media used is not suitable, or environmental conditions that do not support the pollen to germinate.

Fig. 3: Spike development period of large white ginger (*Zingiber officinale* Rosc.).

S0) Generative shoot identified, **S1)** Shoot tip enlarge and elongated, **S2)** Spike can be distinguished from peduncles, **S3)** Spike enlarged, difer from peduncles, **S4)** Flower tip emerged from spike, **S5)** Flower enlarged, **S6)** Pistil starts to curve , **S7)** Bract turn reddish, **S8)** Bract drying.

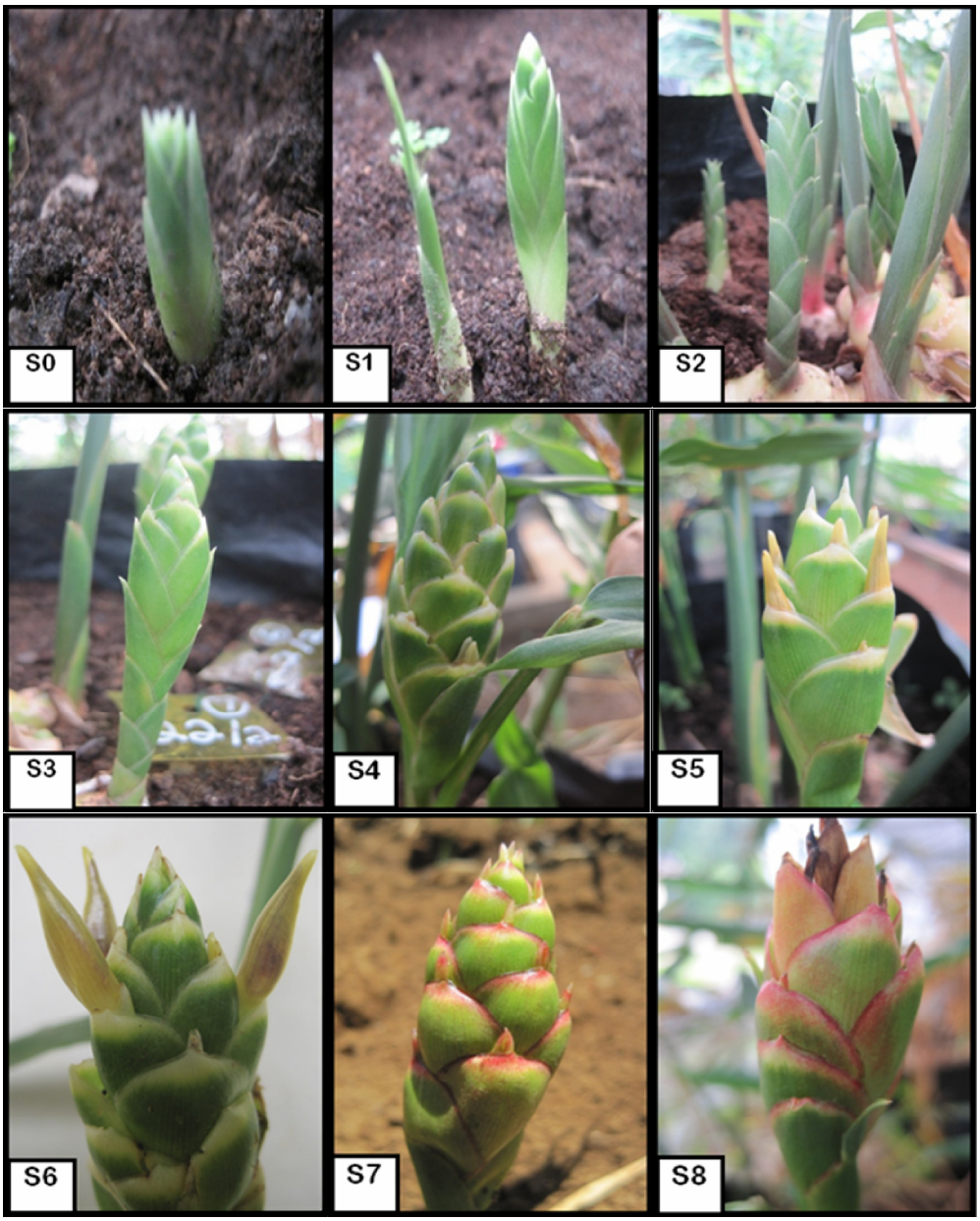
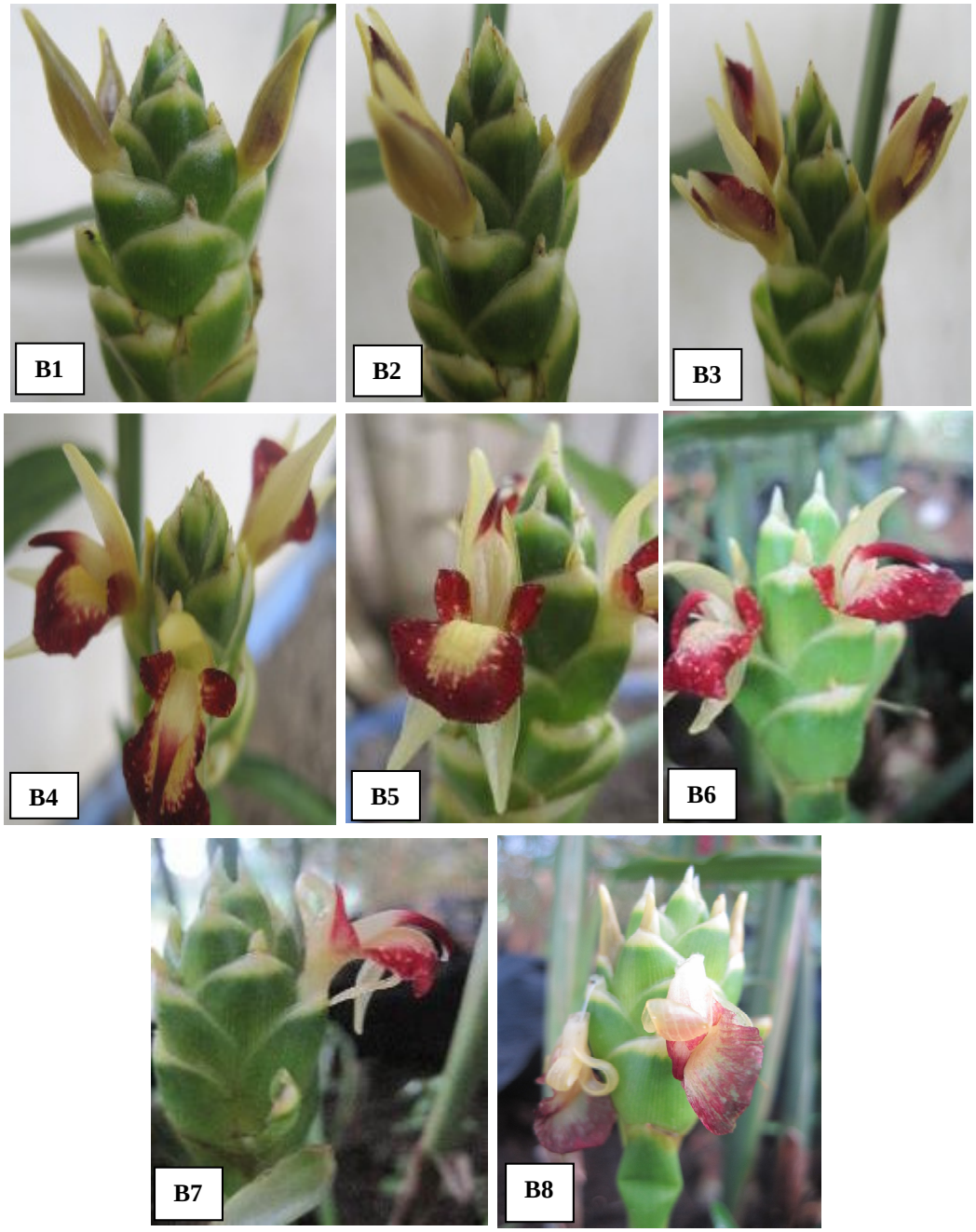


Fig. 4: Flower bloomy until wilt period of large white ginger (*Zingiber officinale* Rosc.).
B0)Flower opening, B1) Sepal begin to open, B2) Devided sepal 3, B4) Fully open corolla, B5) Anthera broke, B6) Pistil starts to curve, B7) Anthera as touch petals, B8) Flower wilt



Pollen morphology

Size of large white ginger pollen has 59,1±8.52 µm x 58,25±5.74 µm, which a round shape pollen on one side (Fig. 5A) and the other side is concave. No ornament and no porous (unporate), pollen surface texture forming regular patterns like nets and pollen will change shape after being separated from the parent plant due to dehydration. Observations by light microscopy using staining or not, it can be seen that the outer part of the pollen (exine) is without any bulges or other form of spines. Exine is apart like nets (reticulate), cell walls are thick and separate from the inside parts. The absence of pores on pollen and thickness cell wall may affect the emergence of pollen tubes.

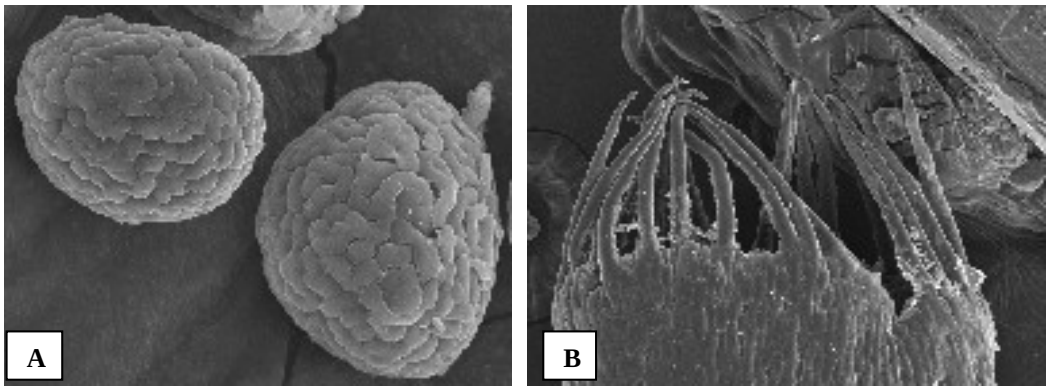
Table 4. The interaction between the staining with the time period of pollen to estimate pollen viability (% germination).

Staining	Time period of pollen (minutes)				
	15	30	45	60	75
Aniline blue	42.41 cd	42.00 cd	57.18 a	51.41 ab	39.47 d
Acetocarmin	31.47 e	47.92 cb	50.18 b	53.16 ab	51.0 ab
Same alphabet in values between columns are not significant at $p<0.05$.					

Polar axis length is almost equal to the equatorial diameter, it is indicated that the pollen form is almost round or spherical. Size of large white ginger pollen is relatively large compared to other Zingiberaceae pollen, as well as when compared to other families such as pollen (Euphorbiaceae, Solanaceae, Poaceae and Myrtaceae) but, smaller than pollen Malvaceae (*Hibiscus*). Saensouk et al. (2009) stated that the diameter of the equatorial plane pollen Zingiberaceae family, genus *Cornukaempferia*: (*C. aurantiflora*, *C. longipetiolata* and *C. larsenii*) respectively, are: 47.65, 42.47 and 43.75 µm.

Pollen has an ornament in the form of long spines with 4.28 ± 0.35 µm Zingiberaceae genus *Alpinia*, pollen diameter is 50 ± 4.15 µm to 102.50 ± 5.95 µm. Erdtman (1972) stated that some species in the family Solanaceae have not pores while the others have pores, it indicates that the species, in the same family does not always have the same morphology.

Fig. 5: A) Pollen and B) stigma of large white ginger (*Zingiber officinale* Rosc.).



Biology of stigma

The width of stigma were 708 ± 4µm, while the length of glands (hairs of stigma) is 312±5µm (Figure 5B). The stigma surface was smooth (did not wrinkled or wavy). Stigma was observed when flower was fully opened until 17.00. Volume secretion of the stigma surface was difficult to be measured by micro pipette. The secretion of fluid on the stigma surface was very little so, it was only observed by visually.

Secretion on the stigma was identified when the flowers full opened, the stigma touched the labellum. The peak secretion occured when the liquid were found at tip of stigma. It was happed in short time, because generally, ginger flowers bloom from 13.00 – 17.00. Observations finished at 17.00, because at that time already dark and hard to see visually. The next day the ginger flowers wilted and fall off. The flower blooming, secretion of glands at the base flower, presence of papilla, fragrant flower indicated that the stigma was receptive. The matured stigma had transparant secretion that containing substances are required for pollen germination (Darjanto and Satifah, 1990).

Conclusions

The results suggest that the floral period of large white ginger starts 4-7th months and it is affected by environment. Normal temperature and relative humidity, may cause the period of ginger flowering is longer. Plants flowering are very affected by climate conditions, especially air temperature. The period of time required to bloom in Bogor was longer than in Cicurug. The time required since flower initiation

(primordial) stage until flower wilt is 70-80 days, while periods of flower bloom until wilt is only 12-18 h. Flower morphology indicates that anthera position was lower than stigma. The period of blooming was just a few hours, and pistil was receptive at ±2.5 h after bloom, which was indicated by presence of maximum secretion. No pollinator to visit when the flower is blooming.

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