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Flowering and Reproductive Biology of *Zingiber spectabile*

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ABSTRACT

The species of Zingiberales are sources of globally important spices and ornamental plants, and have long been used in Asian traditional medicine, cuisine and as herbs. Some species have high ornamental value due to their attractive foliage or flowers, including *Zingiber spectabile* Griff. Hybridization has been the major source of genetic variation in flower and ornamental breeding and understanding the flowering season and peaks of flowering is important for flower growers. Stigma receptivity, or the effective pollination period, is one of the important factors determining successful fertilization and has been rarely studied in Zingiberaceae. The objectives of this study were to examine the *Z. spectabile* reproductive biology, to investigate stigma receptivity under several flowering developmental stages, and their reproductive success. The inflorescence development of *Z. spectabile* from the start of the bracts opening to fully open bracts took 13-17 weeks. The ideal time for artificial pollination was between 11:00-13:00 hours, and the anthers dehisced prior to stigma receptivity. Our study demonstrates that *Z. spectabile* is self-compatible and cross-pollination does not increase fruit set and seed set.

Keywords: Flower structure, ornamental traits, pollen viability, stigma receptivity

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INTRODUCTION

The ginger family (Zingiberaceae) has over 1500 species, mostly native to Asia and the Pacific (Leong-Skornickova & Gallick, 2010). Members of Zingiberales are sources of globally important spices and ornamental plants, and have long been used in Asian traditional medicine, herbs and culinary. Some species have high ornamental values due to their attractive foliage or flowers,

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including *Zingiber spectabile* Griff, Alpinia, and Costus that had been commonly used as cut flower. Other Zingiberaceae species such as Globbas, Calatheas, Curcuma and Tapeinochilos are just beginning to be available in the market in Indonesia.

Zingiber spectabile is also known as beehive gingers; its local names include *bihip* (Indonesia), and *tepus tanah* (Malay). Another species of beehive ginger that has been cultivated is *Zingiber olivaceum*. *Zingiber spectabile* is usually 2.5-3 m tall with lots of basal cones, whereas *Z. olivaceum* is 1.5-2 m tall with smaller basal and terminal cones. *Zingiber spectabile* inflorescences have yellow to brown bracts with yellow spots on their true flowers; the inflorescences are popular as cut flowers due to the variable bract colours (Loges et al., 2011) and long-lasting shelf life (Chee & Hoo, 2010).

Zingiber spectabile is a short-day species; it requires at least nine weeks of consecutive short days in order to initiate and develop flowers (Criley, 2011). The inflorescence is terminal and can measure up to 30 cm in height. The bracts vary in colour from white, yellow, orange to red, often darkening as the bracts mature. Zingiberaceae flowers are usually zygomorphic, bisexual with a single functional stamen, and five sterile stamens are transformed into labellum and staminodes (Leong-Skornickova & Gallick, 2010). Flexistyly, a form of heterodichogamy, has been reported to be widespread in Zingiberaceae (Li et al., 2001, Takano et al., 2005); flexistyly can have two floral morphs, i.e. anaflexistylous (protogynous) and cataflexistylous (protandrous) morphs according to the direction of stigma movement and time of pollen release during anthesis (Takano et al., 2005).

Zingiber spectabile foliage ranges from 2-3 m in height. The leaves have been traditionally used to treat various ailments, and their rhizomes are used as a germicide, stimulant and in the treatments of cough and asthma (Sadhu et al., 2007). Sivasothy et al. (2012) evaluated anti-bacterial activities of oils extracted from *Z. spectabile* leaves and rhizomes. Eighty compounds were isolated and identified, and some demonstrated activities against Escherichia and Salmonella. Sivasothy et al. (2013) reported that curcuminoids from the rhizome of *Z. spectabile* had preservative properties with higher antioxidant and antibacterial activities.

Despite their huge economic, cultural and ornamental importance, studies on Zingiberaceae are limited, particularly on *Z. spectabile* flowering biology and hybridization. *Zingiber spectabile* is available in very limited variants, and no information is available on the self -incompatibility of this species. Self-incompatibility has been linked to the reduction in the fitness of the progenies, mainly due to the increased expression of deleterious or lethal genes (Olmstead, 1989). Hybridization has been the major source of genetic variation in flower and ornamental breeding, and half of the flowering plants show self -incompatibility (Gibs, 2014). Therefore, understanding pollination, self-incompatibility, and seed

setting is valuable for breeders in creating new variants. Stigma receptivity refers to the ability of the stigma to support germination of viable and compatible pollens (Yi et al., 2006). Stigma receptivity determines an effective pollination period, i.e. the longevity of the ovule minus the time lag between pollination and fertilization (Dafni & Maues, 1998); it is one of the important factors determining successful fertilization. Knowledge on self-incompatibility provides information for plant breeders to plan breeding programs (Wickramasinghe et al., 2010). The objectives of this study were to examine *Z. spectabile* reproductive biology, to investigate stigma receptivity under several flowering developmental stages, and their reproductive success. The results of this study will allow a better understanding of the flowering of this species, particularly its flowering seasons and peaks of flowering, which is important information for the commercialization of this species, as well as to provide strategies to optimize pollination and increase fruit set.

MATERIALS AND METHODS

The experiments were conducted at the Ornamental Plant Research Station of Indonesian Ornamental Crops Research Institute (IOCRI), at Segunung, Cianjur, West Java, Indonesia (6.7°S, 107.0°W GPS) between January to September 2010. The research station is located on the highland of ± 1100 m above sea level. The plants were grown in a net house which transmitted ± 55% of natural light intensity. The relative humidity inside the net house ranged from 70-90%, with average day/night temperatures of 24-26°C/18-20°C.

Three-year-old plants of the *Z. spectabile* collection of IOCRI were used for this study. The plants were grown on 18 plots of 1 m x 8 m, using a mixture of soil: manures: rice husk media with ratio 1:1:1 (v/v/v). The plants began flowering 8 months after planting and continuously produced flowers. The inflorescence of *Z. spectabile* has 1-3 flowers open at a time acropetally, thus every floret in one inflorescence exhibits a range of different developmental stages.

Description of the Flower Stage, Inflorescence Structure and Floral Biology

The growth stages of *Z. spectabile* was based on the principal growth stage described in Biologische Bundesanstalt, Bundessortenamt and Chemical industry (BBCH) scale (Meier, 1997) and growth stage of *Etlingera elatior* determined by Choon et al. (2016). Morphological changes of the shoot apices were observed daily to study the onset of floral initiation. Ten (10) flower buds were tagged to record the duration of flower opening, temporal and spatial separation of pollen shedding and stigma receptivity. The stigma was considered as receptive when extended papillae and exudates were visible on the stigmatic surface. Morphological characteristics such as length and diameter of the bracts, length and diameter of the stem, bract colour, and percentage of open bracts were measured weekly starting at bud stage, i.e. 10% of bract opening, to

full opening, i.e. >80% of bract opening, or when no more florets developed. Petal and labellum length and width, petal colour, length of the pistil and pollen, number of ovules, and number of pollens per anther were measured at anthesis on ten flowers from randomly selected plants. Bract and petal colours were determined using the Royal Standard Colour Chart as reference. All data were recorded between 8:00 and 16:00 hours daily between March-August 2010. The flower structure and number of ovules per ovary were observed from five flowers from different plants under the dissecting microscope (Nikon SMZ1000) at 40x magnification.

Pollen Viability

Five (5) nearly open flowers were collected hourly between 08:00-14:00 hours from the inflorescences of randomly selected plants, and the pollens were collected to study pollen germination. Each inflorescence had 1-3 opened flowers on the same day. Flowers were immediately put in a cool box after collection and brought to the laboratory for further study. The anthers of five randomly picked open flowers were transferred to a single concave microscope slide containing Brewbaker and Kwack (1963) medium for germination study. The Brewbaker and Kwack medium was prepared by dissolving 100 mg of H_3BO_3, 300 mg of Ca $(NO_3)_2.4H_2O$, 200 mg of $MgSO4.7H_2O$ and 100 mg of KNO_3 in 100 mL of double-distilled water as stock solution. The pollen germinating medium was prepared by adding 90 mL of distilled water to 10 mL stock solution. The pollens were incubated in the medium for one hour prior to germination count under the light microscope (Olympus BX51) at 100x magnification. Pollen viability, i.e. those having the pollen tube no less than the pollen diameter, was calculated as percentage of germinated pollen.

The number of pollens of five flowers was counted using a hemacytometer. Pollen from the two-lobes anther was extracted, mixed with 1 ml distilled water, stirred thoroughly and counted on the hemacytometer.

Time and Duration of Stigma Receptivity

Stigma receptivity was evaluated by monitoring the secretion on the stigmatic surface and style extension. Stigma receptivity was also recorded based on fruit set and seed set from artificial pollinations conducted hourly between 08:00 to 14:00 hours on five flowers and replicated three times.

The time of stigma receptivity was determined based on the time of anthesis, position of the stigma and style, maximum stigma secretion, and the time when fruit set, and seed set were highest following hand pollination.

Fifteen flowers were emasculated early on the day of anthesis but before anther dehiscence prior to a hand pollination which was conducted hourly between 08:00-14:00. Pollination was conducted using a tweezer with pollens from flowers of different plants. The stigma was covered with a

plastic covering until the following day to avoid pollination by insect pollinators. Fruit set was calculated as the percentage of pollinated flowers that developed into fruits, and the seed set was calculated as the percentage of ovules within an ovary that developed into viable seeds.

Mating System

To study the mating system, fifteen flowers each were self and cross hand pollinated and another fifteen flowers were left for open pollination as control. For self pollination, flowers were emasculated early on the day of anthesis and then pollinated with the pollen of the same flower, whereas for cross pollination, flowers were pollinated with the pollens from flowers of different plants. For open pollination, flowers were left to natural (insect) pollination. Pollination was conducted between 08:00 to 12:00 hours to avoid the high temperature during midday. The percentage of fruit set, and seed set were calculated from different pollination types and reproductive success was calculated as described by Wiens et al. (1987).

RESULTS

Description of the Flower Stage and Inflorescence Structure

The flowering of *Z. spectabile* in the study location began between January and February (during the rainy season) and the seeds matured during June or July. The spikes grew directly from the rhizome. The flower structure was visible when a tiny spike with fully differentiated bractea developed from the terminal growth apex of the shoot, as comparable to stage 30 of *E. elatior* (Choon et al., 2016) of the principal growth stage 3 or peduncle elongation (Meier, 1997). It took 7 days from the first visible bud to spike emergence of about 20 mm above ground. The emerged spikes took about 3 weeks to fully develop; this stage is comparable to stage 39 of *E. elatior* (Choon et al., 2016), and another 2 weeks to reach bracts opening (a total of about 6 weeks), or comparable to stage 50 of *E. elatior* (Choon et al., 2016) of the principal growth stage 5 or inflorescence emergence according to Meier (1997) (Figure 1A - stage 1). The spike consisted of a stem and inflorescence that comprised of bracts. The flowers were born from the ovate bracts.

Based on its value as cut flower, the morphological development of the *Z. spectabile* inflorescence was classified into 5 stages as follows: 1) bracts showed about 10% opening (3.97 ± 2.07cm long) and were pale yellow in colour (Figure 1A); 2) bracts were elongated (7.07±2.47 cm long) and their colour changed to yellow; 20-30 bracts were open (Figure 1B); 3) flowers emerged from the bracts (30-40 bracts), starting from the lowest position along the inflorescence (Figure 1C); 4) bracts' colour changed to reddish and inflorescence continued to grow until reaching its maximum size (13.47±2.06 cm long) (Figure 1D); 5) bracts' colour changed to bright red indicating the end of flowering in the inflorescence. Flowers rarely emerged from the distal part of the inflorescence (Figure 1E). The morphological characteristics of the inflorescence development in *Z. spectabile* are described in Table 1.

(A) (B) (C) (D) (E)

Figure 1. Five stages of inflorescence development of *Zingiber spectabile*. (A) Stage 1: inflorescence with pale yellow bracts at 6 weeks after spike emergence. (B) Stage 2: inflorescence with yellow bracts at 2-3 weeks after stage 1 (20-30 bracts). (C) Stage 3: inflorescence with reddish bracts, beginning of flower emergence, 4 weeks after stage 2. (D) Stage 4: inflorescence with pale red bracts, flower emergence reached the distal part of the inflorescence, 4-5 weeks after stage 3. (E) Stage 5: inflorescence with bright red bracts, the end of flower emergence, 3-5 weeks after stage 4. Red arrows show opened flowers on each bract

Table 1
Inflorescence development of Zingiber spectabile

Stage	Characteristics	Time	Inflorescence size* (mm)		Notes
			Length	Diameter	
1	Bracts started opening	6 weeks after spike emergence	39.7±20.7	36.6±18.5	Bract colour was pale yellow
2	More opening of bracts	2-3 weeks after stage 1	70.7±24.7	62.0±9.8	Bract colour changed to yellow
3	Bracts fully opened	4 weeks after stage 2	101.9±21.2	70.9±5.7	Around March; first flower opened
4	Bracts' colour changed to reddish	4-5 weeks after stage 3	134.7±20.6	74.9±4.1	Opened flowers up to the middle of the bracts
5	Bracts' colour changed to bright red	3-5 weeks after stage 4	134.7±20.6	75.2±3.9	No more flowers opened

*Values indicates averages ± S.E. (n =15)

Stage 1 of the inflorescence development in this study occurred at the end of January to early February and lasted ± 2-3 weeks before the inflorescence reached stage 2. Stage 3 occurred ± 4 weeks after stage 2 (when the bracts were fully open) by the end of March. The whole development of the inflorescence from stage 1 to stage 5 lasted about 13-17 weeks. The development of the spike from emergence to bract opening took ± 6 weeks, so the actual inflorescence development took about 19-23 weeks. This study was conducted in a location with high elevation in the mountain area of West Java, Indonesia. Flowering phenology changes with elevation gradients. Plants at lower elevation and warmer temperatures typically flower earlier than plants of the same species growing at higher elevation and cooler temperatures (Ziello et al., 2009). Therefore if *Z. spectabile* is to be grown in a lower elevation, this species is likely to flower

earlier. The longer growing period at higher elevation produces larger inflorescences and more marketable cut flowers.

The *Z. spectabile* inflorescence is suitable as cut flower at all stages of its development. Stages 3, 4 and 5, however, display the most attractive bract colour and good- sized flowers for floral arrangement. The selection of inflorescence sizes depends on its purpose of use in a floral arrangement. Thus, the main production of *Z. spectabile* as cut flower lasts between May-July. However, a limited number of inflorescences is available almost all the time provided the plants are maintained carefully. Lessa et al. (2015) reported that the postharvest longevity of *Z. spectabile* in different vase solutions was about 9 days, and that tap water was sufficient to maintain the quality and longevity of *Z. spectabile* inflorescences. Understanding the time of flowering, flower development and flowering period is useful for commercial purposes.

Flower Structure

Zingiber spectabile flowers comprise three petals, one purple labellum with yellow spots, a pistil and one fertile stamen. The *Z. spectabile* flower development began with the appearance of flower buds enclosed by transparent sepals from the bracts around 08:00-09:00 hours (Figure 2A). The sepals splitted open around 10:00 hours (Figure 2B). Hence, the petals splayed while the style was still curving, followed by the appearance of a curved style around 11:00 hours (Figure 2C). At around 12:00 hours the flower was fully blooming, indicated by straightened styles (Figure 2D). The stamen consists of a short undeveloped filament and an anther; the anther consists of two lobes and is positioned at the lower part of the style (Figure 2E). The style is long with the hairy stigma at the tip (Figure 2F). The whole structure of the pistil, i.e. ovary, style and stigma, is longer than the stamens (Table 2) so the stigma is located at a higher position than the anthers at anthesis.

Anthesis occurred at 11:00 hours and the opened flowers began to wilt by 16:00 hours of the same day, so the flowers only last for one day. Every day, one to three flowers open in an inflorescence during the flowering period until all the flowers of the inflorescence have opened. The ovary comprises 3 locules and bears 43.8±10.7 ovules (Table 2). The average number of ovules in an ovary determines the potential number of seeds in a fruit (capsule),

Table 2
Floral characteristics of Zingiber spectabile

Floral parts	Measurement (average±S.E.)
Flower length (cm)	6.80±0.35
Labellum length (cm)	3.17±0.24
Labellum width (cm)	1.36±0.10
Corolla length (cm)	3.23±0.15
Corolla width (cm)	0.72±0.05
Corolla colour	yellow group 10C – 14D*
Pistil length (cm)	5.84±0.25
Anther length (cm)	1.16±0.23
Ovules number in an ovary	43.8±10.7
Pollen number in an anther	143,360±94,979

*Colour classification was based on Royal Standard Colour Chart

although the number of pollens in the anther (143,360±94,979) was considerably higher than the number of ovules. The high number of pollens produced by a flower could serve as pollen stock for other flowers.

Anther dehiscence started around 09:00 hours before flower blooming, during which time the secretion on the stigmatic surface was still absent. However, pollen germination was low, indicating that some pollen was still at maturation stage when the anther had dehisced. The pollen matured 3 hrs after anther dehiscence as it reached highest germination (Table 3). Flower opening (anthesis) started to occur at 11:00 hours at which time secretion started to appear on the stigma and the style was still curving. Full bloom occurred around 12:00 hours, accompanied by the straightening of the style, and higher secretion appeared on the stigmatic surface that lasted until 13:00 hours. The high secretion coincided with high pollen germination (Table 3), however, spatially the stigma was at the farthest position from the anther, and so voluntary self pollination was unfeasible. The temporal separation of anther dehiscence and stigma receptivity is common in Zingiberaceae

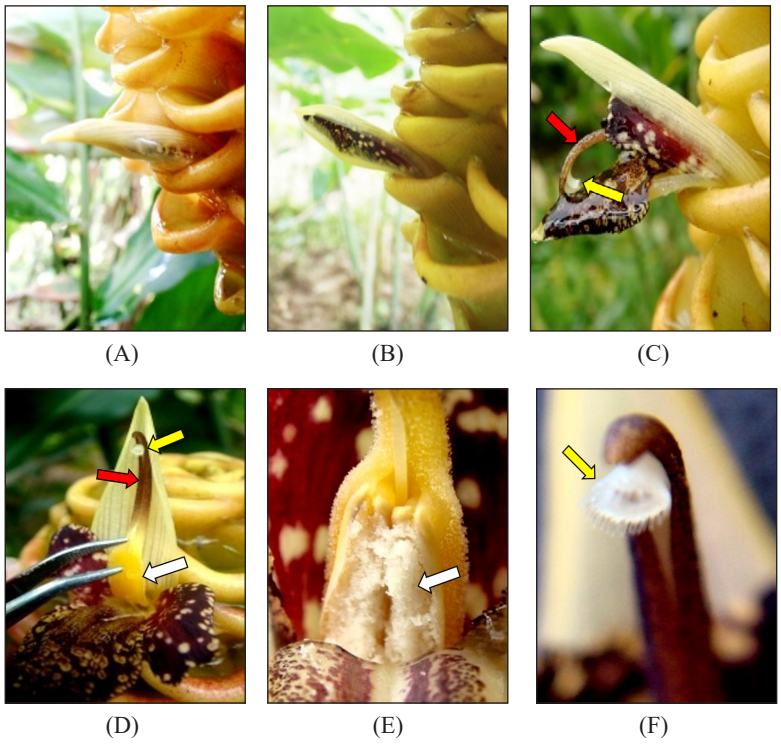


Figure 2. Stages of *Zingiber spectabile* flower development: (A) Flower bud enclosed by yellowish sepals appears from the bract, 08:00-09:00 hours. (B) The sepals split open showing the petals, 10:00 hours. (C) Flower starts to bloom, petals splay, and the style is still curving (red arrow) with the stigma still folded (yellow arrow), 11:00 hours. (D) Fully blooming flower, the style has straightened (red arrow), the stigma is exposed (yellow arrow) and the anther is at the lower part of the style (white arrow), 12:00 hours. (E) The dehisced anther (white arrow). (F) The stigma with stigmatic hairs (yellow arrow) at the tip of the style

although the overlapping between the two events varies among species. *Curcumorpha longiflora* anthers dehisce one day before the stigma becomes receptive (a two-day flower) (Gao et al., 2004). In *Curcuma aeruginosa* the difference is a matter of several hours (Aswani & Sabu, 2017) whereas it is only around 20 minutes in *Zingiber officinale* (Melati et al., 2015). In this case the flower of *Z. spectabile* cannot be categorized as flexistyly because the flower's function as protogynous or protandrous was determined temporally and was not according to the direction of stigma movement. The period of high pollen germination and high stigmatic secretion suggests that pollination is best performed between 12:00-13:00 hrs. Information on the time and duration of stigma receptivity is crucial for successful breeding or artificial pollination as it determines the success of pollination, i.e. higher fruit set and seed set (Dafni & Maues, 1998).

Based on the flower development hand cross pollination was carried out from 08:00 (before anther dehiscence) to 14:00 hours (secretion from the stigmatic surface had started diminishing). Hand cross pollination showed that the fruit set, and seed set did not follow the pattern of pollen germination and stigmatic secretion (Table 4). Fruit set (60-93.3%) and seed set (45.7-72.4%) were not significantly different during the day of anthesis. Although the anther dehisced in the morning, pollen germination was low, and the stigma was somehow unexposed for pollination, the fruit set, and seed set were equally high as with any other time of the day. It is possible that the maturing pollen that arrived onto the unreceptive stigma synchronized so that the recognition process between the pollen and stigmatic surfaces occurred at the prime time and resulted in fertilization, hence yielding fruit set and seed set. It was surprising that the higher pollen germination and the receptive stigma (high secretion) at 12:00 hours (Table 3) did not result in a higher fruit set and seed set than the earlier time of the day when the pollen germination was low, and the stigma was not receptive yet. The higher temperature during the daytime (24-26^{o}C) might be harmful to the pollen and reduce its viability rapidly, thus, lowering the fruit

Table 3
Morphological changes in anther and stigma during anthesis of Zingiber spectabile

Time	Flower stage	Anther	Pollen germination (%) *	Style	Stigmatic surface
08.00	Flower bud	Intact	20.95^{b}	curving	No secretion
09.00	Flower bud	Dehiscence started	14.60^{b}	curving	No secretion
10.00	Flower bud	Dehiscence	34.15^{b}	curving	Secretion noticeable
11.00	Start to bloom	Dehiscence	36.58^{b}	curving	More secretion
12.00	Fully blooming	Dehiscence	62.67^{a}	straightening	High secretion
13.00	Fully blooming	Start withering	32.46^{b}	straightened	High secretion
14.00	Fully blooming	Withered	35.88^{b}	straightened	Less secretion

*Numbers followed by the same letter are not significantly different based on DMRT at 0.05

Table 4
Time of pollination, fruit set, seed set and reproductive success of Zingiber spectabile

No	Time of pollination	Fruit set (%)	Number of seeds per fruit (seed set %) *	Reproductive success (%) **
1	08.00	14 (93.3)	29.5 (67.4)	62.9
2	09.00	14 (93.3)	26.9 (61.4)	57.3
3	10.00	13 (86.7)	31.7 (72.4)	62.8
4	11.00	11 (73.3)	23.5 (53.7)	39.4
5	12.00	14 (93.3)	27.5 (62.8)	58.6
6	13.00	9 (60.0)	20.0 (45.7)	27.4
7	14.00	14 (93.3)	24.6 (56.2)	52.4

*Seed set was calculated based on number of ovules per ovary (43.8) (Table 2) on 15 pollinated flowers
** Reproductive success was calculated according to the method described by Wiens et al. (1987)

set and seed set. The data indicated that despite dichogamy, pollination success was comparable throughout the day of anthesis. So, it was concluded that the optimum time for hand pollination was between 11:00-13:00 hours. Technically, hand pollination is much easier when the flower has started to bloom as the stigma is more exposed and accessible. Pollination after midday tends to produce fewer seeds per fruit which could be due to the deterioration of the entire flower making it unfit for pollination and fertilization.

The pre-emergence reproductive success indicates the proportion of the ovules that developed into viable seeds. Our study demonstrated that the reproductive success following cross pollination was relatively high, ranging between 27.4 – 62.9% (Table 4), which meant 274-629 out of 1000 ovules produced by a *Z. spectabile* could potentially develop into viable seeds.

The number of ovules in an ovary averaged 43.8 (Table 2), but the number of seeds per fruit ranged from 20.0-31.7 (45.6-72.4%), indicating that not all ovules could develop into viable seeds following cross pollination. Our observation also revealed that not all bracts could produce viable flowers. Most of the blooming flowers were those positioned at the two third midsection of the inflorescence. IOCRI reported that the average number of bracts per inflorescence of *Z. spectabile* was 159 (IOCRI/balithi.litbang.pertanian.go.id/leaflet-download-08-zingiber.pdf). Hence the potential for seed yield is relatively high and gives breeders considerable opportunity to accomplish hybridization.

Fruit set from self pollination was not significantly different from that of cross pollination, ranging from 74.3-80% (Table 5), whereas open pollination did not set any fruit. Similarly, the seed set from self pollination was similar to that from cross pollination, ranging from 15-36 seeds per fruit (34.2-82.2%). These data imply that *Z. spectabile* is self-compatible. This finding is rather unexpected. So, we reckon that the temporal and spatial separation of the reproductive organs (stigma is positioned higher than the anther and anthers dehisce

Table 5
Zingiber spectabile *fruit set and seed set following different types of pollination*

Types of pollination	Flower	Fruit set (%)	Number of seeds per locus			Number seeds per fruit
			Side locule	Middle locule	Site locule	
Self	1	100	13±1.7	7.3±2.5	11.7±2.5	32±5.6
	2	100	11.3±3.1	8.3±3.8	10.3±1.5	30±7.9
	3	33.3	14±0	8 ±0	14±0	36
	4	66.7	7±9.9	4±5.7	5±7.1	16±22.6
	5	100	10.3±2.3	8±3.6	10.7±1.2	29±4.6
	Average	80.0				
Cross	1	66.7	5±7	3.7±4.7	6.3±7.1	15±18.7
	2	66.7	11±1.4	3.5±5.0	9	23.5±3.5
	3	100	12.3±4.5	5	15±3.5	32.3±7.6
	4	33.3	11	7	6	24
	5	100	11.7±4.2	8±4.4	10.7±3.5	30.3±11.4
	Average	73.3				
Open	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0

before the stigma is receptive), is the mechanism of the species to prevent self pollination. Gao et al. (2004) reported that self-compatibility was also found in *Curcumorpha longiflora* (Zingiberaceae) as indicated by the similar rate of germinated pollen and pollen tube length following self and cross pollination.

The consequence of self-compatibility is that emasculation will have to be done for hybridization which will make the crossing tasks more tedious. However, emasculation might not be necessary in *Z. spectabile* because the anther is embedded at the proximal end of the pistil (near the ovary), whereas the stigma is at the distal end, and so voluntary self pollination is not feasible. Therefore, restriction of insect visits is necessary to avoid unwanted hybridization when emasculation is not implemented.

The diameter of the ovaries of the self and cross-pollinated flowers increased significantly and reached its maximum size during the first four weeks after pollination/ WAP (Figure 3), and was larger than that of open pollination, merely due to the number of seeds developed within (Table 5). The ovary diameter did not show more growth until the fruits and seeds matured, indicating that the seeds were morphologically mature at four weeks after pollination when the embryo had been formed. The accumulation of storage reserve in the seeds likely occurred between 4-10 WAP until the seeds were physiologically mature.

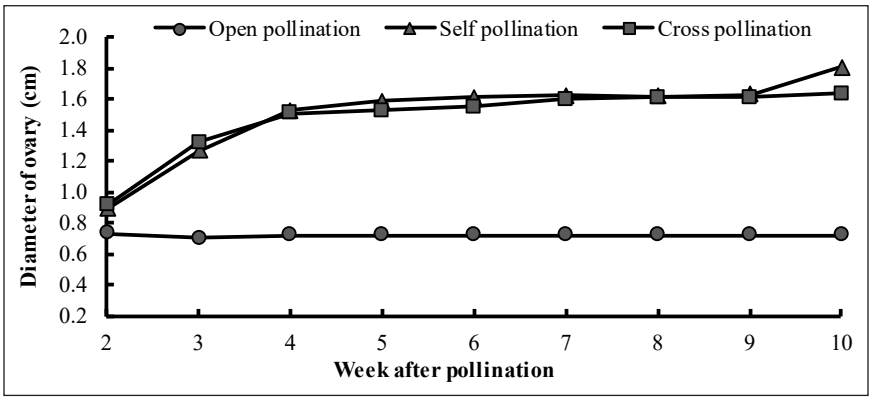


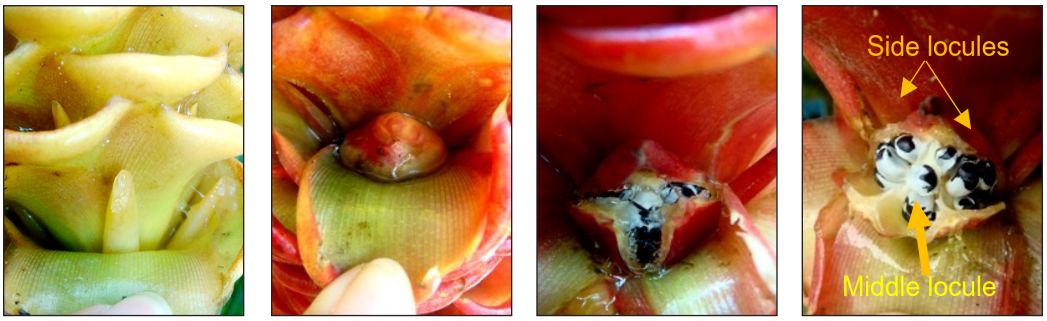
Figure 3. Development of *Zingiber spectabile* ovary diameter following pollination

Open pollination failing to set fruits and seeds was possibly due to the absence of suitable pollinating insects in the area of study. The effective time for insect pollinators to forage the flowers was from morning till early afternoon (08:00-14:00 hours), during which time the insects collected pollens from the dehisced anther and deposited them onto the stigma. Based on our study, very little nectar was available when the flower was fully blooming at 12.:00 hours. So, the primary attractant for the pollinating insect is the colourful petals. This narrows the pollination window considerably. Moreover, the embedded anther at the proximal end of the pistil makes it difficult for the insect to collect the pollens; the delicate structure and position of the stigma make it difficult to be in contact with the insect pollinators.

Phenological flowering stages can have inter-annual variability and large spatial differences (Koch et al., 2009). Variabilities exist between individual plants, and are affected by biotic and abiotic environment, particularly by temperature and precipitation (Koch et al., 2009).

The ovary comprises three locules, and each locule bears several ovules. In most cases the percentage of seed set in the outer/edge locules was higher than that of the inner/middle ones (Table 5). This could be simply because the outer locules are more spacious than the inner locules, allowing the seeds to grow fully. The success of a pollination can be evaluated at 1 WAP, when the ovary appears to be fresh (Figure 4A). The ovary enlarges rapidly within 3 WAP (Figure 4B) and continues to enlarge until it cracks open around 9 WAP (Figure 4C) showing the developing seeds inside. At this stage the seeds are intact in each locule. At 10 WAP the crack gets wider as the seeds continue to develop and become loosely intact (Figure 4D). Harvesting the seeds at this stage is much easier.

Understanding phenology could greatly enhance growers' ability to plan management practices in relation to the events occurring within the plants. Information about the time of flowering, fruit set, and the relationship between these events will allow better planning management of cultural practices at optimum times. The fact that hand



(A) (B) (C) (D)

Figure 4. Development of *Zingiber spectabile* ovary following cross pollination: (A) an ovary after pollination (1 WAP); (B) enlarged ovary (3 WAP); (C) ovary starts to crack (9 WAP), showing the intact seeds; (D) ovary cracks open showing the loosely mature seeds (10 WAP). WAP=week after pollination

pollination successfully set fruits and viable seeds (data not included), the hybridization of *Z. spectabile* will be feasible and more flowers variations can be expected.

CONCLUSION

The inflorescence development of *Zingiber spectabile* from the start of the bracts opening to fully open bracts take 13-17 weeks. The ideal time for artificial pollination is between 11:00-13:00 hours. Anthers dehisce prior to stigma receptivity. *Zingiber spectabile* is self- compatible and cross pollination does not increase fruit set and seed set.

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