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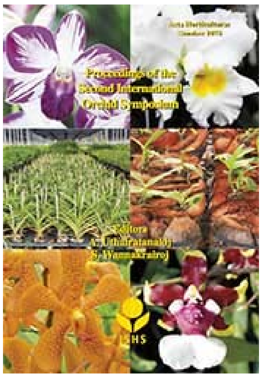

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Pollination Success among Standard Hybrids and Indonesian Species of *Phalaenopsis*

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Abstract

One of the constrains limitting the succes of *Phalaenopsis* breeding is incompatibility between the parents. The objective of this study was to evaluate pollination compatibilities among Indonesian *Phalaenopsis* species and the standard hybrid group. The species used were *Phal. violaceae* 'Sumatera', *Phal. cornu-cervi* 'Kalimantan', *Phal. amboinensis*, *Phal. tetraspis* and *Phal. modesta* and *Phal. amabilis*. The *Phalaenopsis* standard hybrids used were eight commercially available hybrid accessions in the Indonesian flower market. The pollination schemes evaluated include: self- and cross-pollination among standard hybrids (intra-hybrids) and reciprocal crosses among standard hybrids and the Indonesian species of *Phalaenopsis* (inter-hybrids). Prior to pollination, the following parental characters were noted: inflorescence length, flower number at anthesis, flower sizes, leaf stomatal size and stomatal density, respectively. After pollination, the following characters were recorded: pod set number, pod size, and seed set, respectively. Samples of mature pods of intra-hybrid crosses were harvested, the seeds were germinated aseptically, and the protocorm growth from the seeds were evaluated. Results of the evaluation indicated Indonesian *Phalaenopsis* species in general showed smaller inflorescence length, flower number at anthesis, and flower sizes than the standard hybrid accessions. The species also exhibited smaller stomatal cell size and higher stomatal cell densities than the hybrid ones. Standard *Phalaenopsis* hybrids commercially available in Indonesia might have different ploidy level than the Indonesian *Phalaenopsis* species. Such hypothesis was supported by the the contrasting morphological characters, stomatal cell sizes and stomatal density. Furthermore, it was supported by the failure to obtain either pod set or seed set in the majority of reciprocal cross pollination among *Phalaenopsis* standard hybrids and the species. Growth and multiplication of protocom derived from two intra-hybrid populations into protocorm like bodies (PLBs) was not affected by the medium composition but by the genetic background of the parents. Protocorm originated from cross pollination of White × Pink standard hybrid accessions gave higher multiplication rate than that of Pink × White standard hybrids.

INTRODUCTION

Phalaenopsis orchid is one of the most popular orchids in world floricultural market. In Indonesia, comsumers are interested in *Phalaenopsis* because of their flower beauty, long flower shelf life, and their affordable price. Usually, Indonesian orchid growers import *Phalaenopsis* planting material in the form of planlets, seedlings and mature flowering hybrid plants from Taiwan or Thailand.

With the existence of many *Phalaenopsis* species in Indonesia, it should be possible to develop Indonesian own breeding lines and planting materials. Therefore, breeding activities targetted to produce superior characters using local *Phalaenopsis* genetic materials need to be initiated in Indonesia. Although many crossing among

Phalaenopsis parental lines have routinely been done by orchid hobbyists in Indonesia, well documented data about the rate of success of polination among standard hybrids and Indonesian species of *Phalaenopsis* have not yet been available.

Tang and Chen (2007) stated that *Phalaenopsis* parental lines used for breeding purposes were classified into two groups, such as: either standard big flower group or novelty one. The standard big flower group includes the *Phalaenopsis* varieties with white, pink and stripes flower characters. On the other hand, the novelty group include those having small flower with special coloration or flowers with special fragrances.

Tang and Chen (2007) also stated that some *Phalaenopsis* orchid species were cross-incompatible, causing a number of constrains for their breeding and varietal improvement. Hsu et al. (2010) found that there might be breeding barrier in red *Phalaenopsis* orchids, associated with either the odd chromosome set or some other factors existed in the male or female parents, respectively. The objectives of this experiment were to evaluate pollination success rate among different pollination types and among *Phalaenopsis* standard hybrids and Indonesian species.

MATERIALS AND METHODS

The pollinations were conducted at Bogor, West Java, Indonesia during dry season 2012 (April to June 2012). Bogor is located approximately 250 m a.s.l.

The *Phalaenopsis* parental lines used in this research consisted of eight *Phalaenopsis* standard hybrids with white petal and sepal (six genotypes, i.e. AMP17, V3, four unknown commercial hybrids), pink petal and sepal (one genotype, i.e. KHM0421) and yellow petal and sepal (one genotype, unknown commercial hybrid). The hybrids were obtained from commercial traders who only classified them into standard and novelty hybrids based on the colour of the petals and the sepals. Six Indonesian *Phalaeopsis* species, including *Phal. amabilis*, *Phal. amboinensis*, *Phal. modesta*, *Phal. cornu-cervi*, *Phal. tetraspis*, and *Phal. violaceae*, were also used as parental lines in this experiment.

Self pollination was only carried out for the standar hybrid parental accessions. The cross pollinations, on the other hand, were conducted in the following schemes: (1) standard hybrid × standard hybrid (intra-hybrid), (2) standard hybrid × Indonesian species (standard hybrid was used as female parent), and (3) Indonesian species × standard hybrid crossed (Indonesian spesies was used as female parent). Scheme no. 2 and 3 were designated as the inter-hybrid.

All pollinations were conducted in the morning on flowers at 3-7 days after they were completely openned. Before pollination, the following characters were recorded among parental lines, such as: inflorescence length, flower number at anthesis, flower sizes, leaf stomatal size and stomatal density, respectively.

According to UPOV guidelines for *Phalaenopsis* description (2003), length of inflorescence is defined as the distance from the first flower to the end of spike; flower size is recorded as both flower length (the distance from the end of dorsal sepal to apical lobe) and flower width (the distance from the end of two petals). The stomatal size and density were recorded from the lower surface of the sample leaves.

After pollination, the following characters were recorded: pod set, pod size, and seed set, respectively. Once the pods were harvested, the seeds were isolated and counted. The harvested seeds were germinated non-symbiotically on a half strength of Murashige and Skoog (MS, basal nutrients and vitamines) medium (Murashige and Skoog, 1962), supplemented with sucrose (30 g/L) and coconut water (15% v/v).

The multiplication of protocorm into protocorm like bodies (PLBs) were evaluated on four medium compositions, such as: ½ strength MS medium; ½ strength MS medium suppemented with 15% of coconut water; commercial fertilizer medium (2 g/L NPK [25-5-20]); or commercial fertilizer medium (2 g/L NPK [25-5-20]) supplemented with 15% of coconut water. The MS medium consisted of MS basal nutrients and vitamines. All four media were supplemented with sucrose (30 g/L). The commercial fertilizer used was the Hyponex, with N:P:K compositions of 25:5:20 and without micro nutrients.

RESULTS AND DISCUSSIONS

The inflorescence length, flower number at anthesis, and flower size characters of the standard hybrids and Indonesian *Phalaenopsis* species used as parental lines in this experiment were presented in Table 1. The inflorescence length of the standard hybrid varied between accessions. In general, they showed longer inflorescence than the spesies ones. The average inflorescence length of the standard hybrids was 23.0 cm.

Standard hybrid accession of *Phalaenopsis* showed a ranged of 5-12 flowers per inflorescence. Among those, 7 to 10 flowers have opened completely almost at the same time and they were ready for self or cross polination treatments. The average number of flower per inflorescence for the standar hybrids was 7.5 (Table 1) and their average flower size were 7.7 cm (flower length) and 8.9 cm (flower width) (Table 1).

The Indonesian *Phalaenopsis* species, on the other hand, generally showed shorter inflorescence than those of standard hybrids. However, two of the *Phalaenopsis* species (*Phal. amabilis* and *Phal. tetraspis*) exhibited inflorescence length similar to that of standar hybrids. The number of flowers for Indonesian *Phalaenopsis* species were generally only 1-2 flowers per inflorescence (Table 1). However, the flower of *Phal. amabilis* could be as many as 9 flowers per inflorescence.

The flower size of two Indonesian *Phalaenopsis* species (*Phal. modesta* and *Phal. cornu-cervi*) in general were smaller and almost half size of the standar hybrid ones (Table 1). Although, flower size of *Phal. amboinensis*, *Phal. tetraspis*, *Phal. violaceae*, and *Phal. amabilis* were larger than the other two species, they were smaller than the standard hybrid ones. The flower sizes of *Phal. amabilis* were the closest to those of hybrid ones.

Results of stomatal cell observation among *Phalaenopsis* parental lines used in this experiment were presented in Table 2. The length of stomatal cells of *Phalaenopsis* standard hybrid samples ranged from 27.6-36.6 µm, while their width ranged from 25.9-32.5 µm (Table 2). The stomatal densities among the *Phalaenopsis* standard hybrids ranged from 11.8-13.6 stomatal cells per mm^2 (Table 2). The average stomatal cell length, width, and densities of the *Phalaenopsis* standard hybrids were 34.5 µm, 29.0 µm, and 13.1 cells per mm^2, respectively.

On the other hand, length of stomatal cells of Indonesian *Phalaenopsis* species ranged from 13.9-28.1 µm, while their width ranged from 12.6-22.1 µm (Table 2). The stomatal densities among the Indonesian *Phalaenopsis* species ranged from 18.7-50.9 stomatal cells per mm^2 (Table 2). The average stomatal cell length, width, and densities of the Indonesian *Phalaenopsis* spesies were 19.9 µm, 16.2 µm, and 32.3 cells per mm^2, respectively.

Based on data presented in Table 2, the size (length and width) of *Phalaenopsis* standard hybrids parental lines stomatal cells was higher than those of the Indonesia species. The ratio of the average stomatal size between standard hybrids vs. Indonesian species was 1.7 (stomatal length) and 1.8 (stomatal width). On the other hand, the ratio of the stomatal densities was 0.4. Such data pointed out that the Indonesian *Phalaenopsis* species and the standard hybrids might actualy have different ploidy levels. Since most of the Indonesian *Phalaenopsis* species are diploid, the standard hybrids used as parental lines in this experiment might actually be polyploid.

The effects of pollination types on pod set and pod size were shown in Table 3. The number of pollinations were conducted in ranged of 1-11 flowers. Among the tested pollination types, self pollination of the standard hybrids resulted in a total of 9 pods out of 11 pollinated flowers (81.8%). Self pollination of Pink flowerred standard hybrid resulted in 7 pods of 9 pollinated flowers (77.8%), while that of White flowerred standard hybrid resulted in 2 pods out of 2 pollinated flowers (100%) (Table 3).

Cross pollination among standard hybrid accessions (intra-hybrid) resulted in a total of 14 pods out of 16 pollinated flowers (87.5%). Cross polination between White × Pink standard hybrids resulted in 9 pods out of 11 pollinated flowers (81.8%), while the reciprocal cross resulted in 5 pods out of 5 pollinated flowers (100%) (Table 3). Cross pollination among standard hybrid × species and species × standard hybrid resulted in a total of 1 and 2 pods out of each 7 pollinated flowers, respectively (Table 3).

In the cross polination among standard hybrids and Indonesian species, only one pollinated flower for each combination, except for *Phal. violaceae* (3 pollinated flower) was conducted (total 7 cross polinated flowers). Among tested combinations, cross pollinated flowers among standard hybrids with Indonesian spesies in general were aborted. The only cross pollination resulted in pod formation was one between Pink standard hybrid × *Phal. amabilis*.

The reciprocal cross polination among Indonesian species × standard hybrids were conducted in a total of 7 flowers and only one flower for each combination of cross pollination. Cross pollination among Indonesian species × standard hybrids resulted in formation of a total 2 pods (28.6%). The combination of crossing among species x standard hybrids resulted in pods formation were *Phal. amabilis* × Yellow and *Phal. amabilis* × Pink standard hybrids, respectively.

Once the desired pods are obtained from pollinated flower, they would usually develop into normal pod sizes (Table 3). The pod sizes are affected by the female parent used in the pollination. Since all were using standard hybrids as female parent, the self pollination of standard hybrids, cross pollination of standard hybrid × standard hybrid, and cross pollination of standard hybrid × Indonesian spesies exhibited large pod sizes (Table 3). On the other hand, cross pollination of Indonesian spesies × standard hybrid used Indonesian species as female parent. As it was expected, the Indonesian species generally have small pod sizes; therefore, cross pollination among Indonesian spesies × standard hybrids also resulted in small pod sizes (Table 3).

The normal *Phalaenopsis* pods require approximately four months to fully develop and mature before they can be harvested. Data in Table 4 showed that only 77.7% pods (7 pods out of 9) developed into maturity and were harvested from self pollination of standard hybrids. Two pods could not develop into maturity and eventually were aborted. All pods (100%) from cross pollination of standard hybrids (intra-hybrids) reached maturity and harvested. Although resulted in 100% pods developed into maturity and harvested, reciprocal cross pollinations of standard hybrid and Indonesian species were drawn only from one and three evens, respectively. Therefore, the reciprocal cross pollinations of standard hybrid and Indonesian species need to be evaluated with larger pod set data.

In this experiment, pollinating more flowers for cross pollination between Indonesian species as female parent and standar hybrid as male was limited by the number of flowers per inflorescence except for *Phal. amabilis.* All the Indonesian species could only produce 1-2 flowers per inflorescence (Table 1). Therefore, to overcome such problem in the future evaluation, more clonal materials of the Indonesian *Phalaenopsis* species need to be prepared for parental lines. The validity of reciprocal cross polination success among Indonesian *Phalaenopsis* species and standard hybrid could then be determined more accurately by pollinating more flowers.

Since it is difficult to count the actual number of seeds within the harvested pods, they were only scored for the number of seeds per pods and grouped into three groups. The first group (no seed) indicated there was no normal seed developed in the harvested pod. The second group (few seeds) indicated normal seeds developed in the pods and the seed volume occupied less than half of the pod volume. The third group (lot of seeds) indicated normal seeds were produced and the seeds occupied more than half of the pod.

There was no seed set problem for the self pollinated and the cross pollinated standard hybrid accessions. Out of seven pods harvested from self pollinated standard hybrids, four pods (57.1%) contained normal seeds (Table 4). However, each of the harvested pod contained seeds of less than half of the volume of the pod and grouped as yielding few seeds (Table 4).

The cross pollination among standard hybrid (intra-hybrids) did not encounter pod set problems either. All cross polinated flowers developed into mature pods and were harvested (16 pods out of 16 pollinated flowers). All harvested pods derived from intra-hybrids crosses yielded normal seeds. The harvested pods also fully seeded and grouped as producing lot of seeds (Table 4).

Although the harvested pod from cross pollination of standard hybrid × Indonesian species also gave lot of seeds, but the data were from limited number of pod (Table 4). Therfore, more evaluations are neccessary to obtain more representative results than that generated in this experiment.

Non-symbiotic germination of the isolated seeds was carried out in vitro. Under this condition, the seed started to germinate and develop into protocorm at two weeks after planting. The multiplication of protocorm populations derived from two intra-hybrid crosses (cross pollination between White flowered standard hybrid accession × Pink flowered one and that between Pink flowered standard hybrid accession × White flowered one) to form protocorm like bodies (PLBs) were evaluated using four medium compositions.

The results of the experiment showed that there was no significant effect of medium compositions on PLBs multiplication (data not presented). The results also indicated genetic background might affect PLBs multiplication. Protocorm derived from cross pollination of White flowered standard hybrid × Pink flowered one exhibited higher PLBs multiplication than those derived from cross pollination of Pink × White flowered one. The PLBS multiplication data were presented in Figure 1.

Although in general all Indonesian *Phalaenopsis* spesies evaluated in this experiment exhibited inferior flower characters, they could be used to increase genetic diversity of the commercial cultivars. It might also be possible that the Indonesian *Phalaenopsis* species actually carry a number of useful characters for developing superior *Phalaenopsis* varieties in the future. Therefore, cross pollination among Indonesian *Phalaenopsis* species and commercial hybrids need to be initiated. Such activities would be beneficial for future local development of *Phalaenopsis* varieties in Indonesia. Moreover, since a number of *Phalaenopsis* species exist in Indonesia, Indonesian orchids community should have the most benefit from their utilization through their use in *Phalaenopsis* breeding activities. Unfortunately, such conditions have not been realized yet.

Based on the previous report, all of the *Phalaenopsis* species were reported as diploid (Lin et al., 2001). The common characters of the *Phalaenopsis* species include short inflorescence, low flower number per inflorescence, and small flower sizes. Some of the *Phalaenopsis* hybrids on the other hand, were reported as polyploid cultivars (Chen et al., 2011). The hybrids usually exhibited more superior characters such as: long inflorescence, large flower number per inflorescence, and large flower sizes. The standard hybrids and Indonesian *Phalaenopsis* species evaluated in this experiment fit to the general features described previously. Most of the Indonesian *Phalaenopsis* spesies exhibited short inflorescence, low number of flower per inflorescence, and small flower sizes. On the other hand, standard hybrids showed more superior characters.

The possibility of polyploid nature of the standard hybrids were indicated by the larger sizes of most of the described characters and also by the larger sizes of the leaf stomatal cells. Measuring stomatal cell size has been suggested as the general approach to identify the plant ploidy level (Dhooghe et al., 2011).

Previous studies have indicated that the stomatal cell size of polyploid species is larger than that of diploid one. Miguel and Leonhardt (2011) found that in general plants with 1.25 times of diploid stomatal cell size were suspected as polyploids. Omidbaigi et al. (2010) found that diploid dragonhead had stomatal and guard cell that was smaller in size than the tetraploids. In other case, Beaulieu et al. (2009) reported the existence of significant positive corelation among guard cell length and epidermal cell size with genome sizes (ploidy level).

Results of this observation indicated the stomatal cell size of Indonesian *Phalaenopsis* species were in general smaller than the standard hybrid ones. The average stomatal cell size ratio (length and width) between the standard hybrid and the Indonesian species were 1.8 and 1.7, respectively. Such finding supported the hypothesis that the standard hybrids commercially available in Indonesia might actually be polyploid.

Other indirect indicator of ploidy level is stomatal cell density. Beaulieu et al. (2009) reported that there was a significant negative correlation between ploidy level and stomatal density. Furthermore, the stomatal cell density was stated as a strong indirect predictor for ploidy level in angiosperms (Beaulieu et al., 2008). Chen et al. (2009) reported the existence of significant differences of stomatal density between diploid and tetraploid *Phalaenopsis*.

Results of this observation indicated the stomatal cell density of Indonesian *Phalaenopsis* species were in general higher than the standard hybrid ones. The average stomatal cell density ratio between the standard hybrid and the Indonesian species was 0.4, indicating that standard hybrid had lower stomatal cell density than that of species. Such finding further supported the hypothesis that the standard hybrids commercially available in Indonesia might actually be polyploid.

For orchids in general, polyploidy might be advantageous since such a high ploidy level results in larger morphological characters, such as longer inflorescence and larger flower size. Previous reports have indicated that the larger the ploidy level, the larger the cell size (Beaulieu et al., 2008, 2009). Ploidy level have also been reported to affect pollen size (Knight et al., 2010), although there was only a weak association. The orchid pollens are packed in a pollinia structure. However, comparison of the pollinia size among Indonesian species and the commercial hybrids has not been initiated.

Tang and Chen (2007) noted about the association between the parental line ploidy levels and the fertility and the pod set. In their study, tetraploid plant when used as male parents to pollinate diploid female parent resulted in no seed production. Furthermore, ploidy levels have also been reported to affect pollination success in *Phalaenopsis* (Hsu et al., 2010).

Results of this experiment showed that cross pollination among standard hybrid accessions with Indonesian *Phalaenopsis* species resulted in only one pod set out of six pollinated flowers. The five combinations of cross polination among standar hybrid × Indonesian species failed to develop any pod. The only cross pollination resulted in pod set was Pink standard hybrid × *Phal. amabilis*. A lot of viable seeds could be isolated from harvested mature pod.

Reciprocal cross pollination among Indonesian *Phalaenopsis* species with standard hybrid accessions resulted in three pod set out of eight pollinated flowers. The five combinations of cross polination among Indonesian species × standar hybrid failed to develop any pod. The one setting the pods were *Phal. amabilis* × Yellow, *Phal. amabilis* × Pink, and *Phal. equestris* × Pink standard hybrids. However, the harvested pod from these three combinations of cross pollination did not produce viable seed.

All of those data indicated that there might be pollination barriers among reciprocal crosses among standard hybrids and Indonesian *Phalaenopsis* species. These barries might partially be associated with differences in ploidy level among the standard hybrids and the species. Such cross pollination barriers, if they were true, might hamper efforts to introduce desirable genes from Indonesian *Phalaenopsis* species.

CONCLUSIONS

Standard *Phalaenopsis* hybrids commercially available in Indonesia might have different ploidy level from the Indonesian *Phalaenopsis* species. Such hypothesis was supported by the the contrasting morphological characters, stomatal cell sizes and stomatal density. Furthermore, it was supported by the failure to obtain either pod set or seed set in the majority of reciprocal cross pollination among *Phalaenopsis* standard hybrids and the species. Growth and multiplication of protocom derived from two intra-hybrid populations into protocorm like bodies (PLBs) was not affected by the medium composition but by the genetic background of the parents. Protocorm originated from cross pollination of White × Pink standard hybrid accessions gave higher multiplication rate than that of Pink × White standard hybrids.

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Tables

Table 1. The inflorescence length, flower number at anthesis, and flower size characters of the standard hybrids and Indonesian *Phalaenopsis* species used as parental lines.

Parental lines	Inflorescence length (cm)	Flower number at anthesis	Flower size (cm)	
			Length	Width
Phal. standard hybrids	23.0	7.5	7.7	8.9
Phalalaenopsis sp.:				
Phal. amboinensis	8.0	1	4.5	5.5
Phal. modesta	9.7	2	2.5	2.7
Phal. cornu-cervi	6.4	1	3.6	3.1
Phal. tetraspis	23.4	2	4.4	4.0
Phal. violaceae	5.7	1	5.7	4.6
Phal. amabilis	20.0	5	5.7	7.4

Table 2. Stomatal size and stomatal density characters among *Phalaenopsis* standard hybrids and Indonesian species.

Parental lines	Stomatal size		Stomatal cell density (cells/mm^2)
	Length (µm)	Width (µm)	
Phal. standard hybrids:			
White hybrid no. 1	36.6±5.6	32.5±8.0	13.6±7.8
White hybrid no. 2	36.5±3.6	30.9±1.3	11.8±2.9
White hybrid no. 3	35.2±4.9	26.6±1.9	13.5±2.9
Yellow hybrid	29.6±6.0	25.9±1.3	13.6±2.9
Average	34.5±5.0	29.0±3.1	13.1±4.1
Phal. species:			
Phal. cornu-cervi	28.1±2.8	22.1±5.7	50.9±2.0
Phal. tetraspis	17.7±3.8	13.9±2.9	27.2±1.5
Phal. modesta	13.9±0.8	12.6±0.6	18.7±0.6
Average	19.9±2.5	16.2±3.1	32.3±1.4
Hybrid vs. species ratio	1.7	1.8	0.4

Table 3. Effect of pollination types among standard hybrid accessions and Indonesian species of *Phalaenopsis* on pod sets and pod sizes.

Pollination types	Number of pod set			Pod size (cm)	
	Pollination	Frequency	%	Length	Diameter
Selfed standard hybrids:	11	9/11	82	12.1	1.0
White	9	7/9	78	11.5	1.0
Pink	2	2/2	100	11.3	0.9
Hybrid × hybrid crossed:	16	14/16	88	13.6	0.7
White × Pink	11	9/11	82	12.8	1.1
Pink × White	5	5/5	100	15.1	1.2
Hybrid × species crossed:	7	1/7	14	14.3	1.0
Yellow × *Phal. amboinensis*	1	0/1	0	0	0
Yellow × *Phal. modesta*	1	0/1	0	0	0
Pink × *Phal. amabilis*	1	1/1	100	14.3	1.0
White × *Phal. cornu-cervi*	1	0/1	0	0	0
White × *Phal. violaceae*	3	0/3	0	0	0
Species × hybrid crossed:	7	2/7	29	8.2	0.7
Phal. amboinensis × Yellow	1	0/1	0	0	0
Phal. amabilis × Yellow	1	1/1	100	8.0	0.7
Phal. tetraspis × Pink	1	0/1	0	0	0
Phal. amabilis × Pink	1	0/1	0	0	0
Phal. amabilis × Pink	1	1/1	100	8.3	0.6
Phal. violaceae × White	1	0/1	0	0	0
Phal. violaceae × White	1	0/1	0	0	0

Table 4. Effect of pollination types among standard hybrid accessions and Indonesian species of *Phalaenopsis* on harvested pods and seed sets.

Pollination type	Harvested pod		Seed set		
	Frequency	%	Frequency	%	Seeds/pod
Selfed hybrid	7/9	77.7	4/7	57.1	Few seeds
Hybrid × hybrid	16/16	100	16/16	100	Lots of seeds
Hybrid × species	1/1	100	1/1	100	Lots of seeds
Species × hybrid	3/3	100	0	0	No seed

Figures

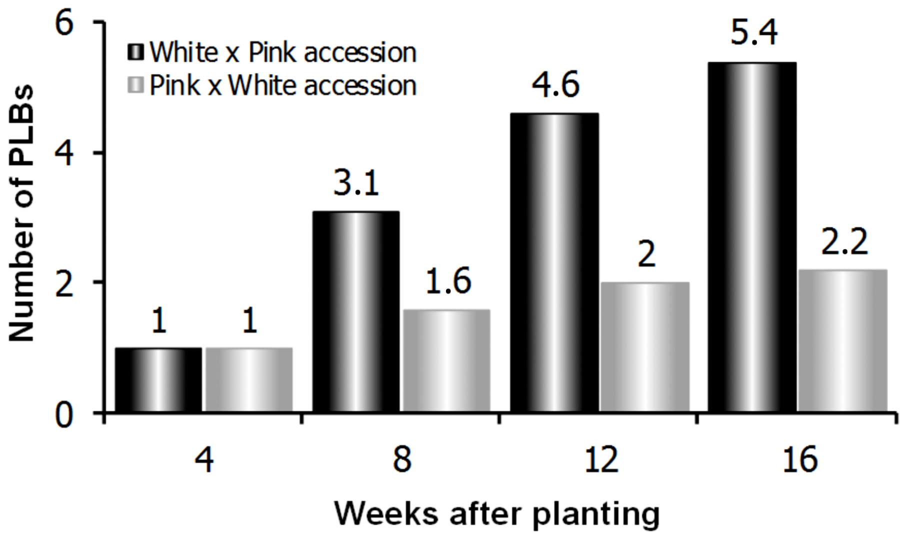

Fig. 1. Development of seeds from harvested pod of reciprocal intra-hybrid between White flowered standard hybrid accession and Pink flowered one into protocorm and multiplication of the protocorm-like bodies (PLBs).