Self and Crossability of Six Thailand Native Dancing Lady Gingers (*Globba* spp.)

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ABSTRACT

The nine species of *Globba* which had commercial potential characteristics were collected and used in this research. The determined chromosome numbers of the nine species were ranged from 20 to 32. The six of collected species were used for pollination. The results of self pollination of the six species represented the percentage of fruit set from 8.69 to 37.04%. The normal seeds could be obtained from their fruits except for self pollinated of *G. rosea*. Reciprocal crosses of *G.* sp. 1 × *G.* sp. 2 and *G. xantholeuca* × *G.* aff. *reflexa* represented high crossability. *G. rosea* showed high crossibility when using as pollen parantal plant in *G.* sp. 2 cross combination. *G. schomburgkii* revealed no crossability as a seed parental plant. The normal seed could be obtained only in reciprocal cross between *G. xantholeuca* × *G.* aff. *reflexa* as an intersectional cross combination. The crossability polygons revealed that using the lower chromosome number species as seed parental plants tend to obtain higher percentage of fruit setting for intersectional crosses. Furthermore, the results could clarify self and crossabilities and pollen quality which are really useful for *Globba* breeding program.

Keywords: Breeding program, chromosome number, *Globba*, pollen

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INTRODUCTION

Globba L. is a tropical rhizomatous geophyte and belongs to Zingiberaceae. Currently this genus has approximately 100 available species and it was distributed broadly around South East Asia, It is especially found in Thailand, Myanmar, Indonesia, Malaysia and Laos. (William *et al*., 2004; Larsen and Larsen, 2006). Almost all of *Globba* species are known as “Dancing lady ginger” because of their flowers which look similar to dancing lady or flying swan. Cytological studies of this genus revealed that they had wide range of chromosome numbers $2n = 2x = 22, 24, 28, 32, 44, 48, 56, 64, 80$ and 96 ($x = 8, 11, 12, 16, 17$ and 24) (Sharma and Bhattacharyya, 1959; Bisson *et al*., 1968; Ramachandran, 1969; Mahanty, 1970; Larsen, 1972; Lim, 1972a; 1972b; Mehra and Sachdeva, 1979; Beltran and Kiews, 1984; Newman, 1988; Eksomtramage *et al*., 2001; Takano, 2001; Eksomtramage *et al*., 2002; Saensouk and Chantaranothai, 2003; Bumrungthai *et al*., 2004; Khamtang *et al*., 2014).

Thailand exports some *Globba* species as commercial ornamental bulbs for supporting world ornamental plant markets. *G. winitii* C.H. Wright, *G. rosea* Gagnep., *G. sherwoodiana* W.J. Kress and V. Gowda and *G. schomburgkii* Hook.f. These were the well-known dancing lady gingers species as cut-flower and pot plants. Recently, *Globba* hybrids for commercial uses in Thailand are derived from interspecific hybridization and clonal selection (Jompuk *et al*., 2010; The Office of Agricultural Research and Extension Maejo University, 2014). Some of the obtained interspecific hybrids had to use an embryo rescue technique for overcoming post-fertilization barriers (Nontasawatsri and Suksathan, 2014). However, there were no an upright inflorescence type hybrid. The beneficial traits of upright inflorescence type are sturdiness of peduncle, erect and thick stalk, abundance of cluster with larger notable staminode and convenience for flower packaging management. The study of cross-pollinating ability was necessary for improvement of an upright inflorescence with permanent colorful bract hybrids.

In this research, the nine species of dancing lady gingers were collected and used for reciprocal crosses. *G. xantholeuca* Craib. has white flowers set into erect panicle inflorescence with tiny impermanent bract and bracteole. *G. adhaerens* Gagnep. shows primary bract, spatutlate, cuspidate apex like a wing as a prominent feature. *G.* aff. *reflexa* Craib has a splendid staminode with small greenish permanent bract and arching inflorescence. *G. rosea* Gagnep. presents the colorful bracts and rosette form of inflorescence stalk. *G. schomburgkii* shows up golden flowers and huge rosette inflorescence. *G. sherwoodiana* represents white spiral imbricated bracts on the raceme. *G. siamensis* (Hemsl.) Hemsl. has maroon permanent bracts, bracteole and capsule on the raceme with bamboo-like foliage. *G.* sp. 1 shows yellow flowers covered by ivory sizeable ovate bract everlastingly with vigorous erect leafy shoots and *G.* sp. 2 has a large-scale of pinkish bract and gorgeous yellowish flowers attached to cluster with long and sturdy peduncle, abaxial surface of leaves have a reddish tone distinctly. Therefore, this research aimed to determine self and cross pollinating ability among *Globba* species which had possibility or potential to produce new commercial hybrids.

MATERIALS AND METHODS

Chromosome Numbers

Nine species of dancing ladies gingers as follows: *G. xantholeuca*, *G. adhaerens*, *G.* aff. *reflexa*, *G. rosea*, *G. schomburgkii*, *G. sherwoodiana*, *G. siamensis*, *G.* sp. 1 and *G.* sp. 2 were collected from local forests of Thailand. The sample plants were grown in 8 inch diameter pots under 50% shading of a greenhouse in Bangkok. The root tips were collected at approximately 8:00 am and they were pretreated with 8-hydroxyquinoline for 4 h at 12°C and rinsed with tap water for 3 times. They were soaked with Canoy's fluid for an hour at room temperature after fixation and soaked with 95% and 70% ethanol for 4 min respectively. The sample root tips were hydrolyzed by 1 N hydrochloric acid for 8 min at 60°C and soaked in distilled water. Root meristem tissues of *Globba* were dyed with aceto-orcein except for the root tips of *G. schomburgkii* which were dyed with acetocarmine. The squashed slide was incubated with acetic acid overnight at room temperature. The clarifiable cells in metaphase stage were observed under light microscope and chromosomes were counted. Observed and counted 20 cells of each species except for the *G.* aff. *reflexa* which only recorded 5 tangible cells.

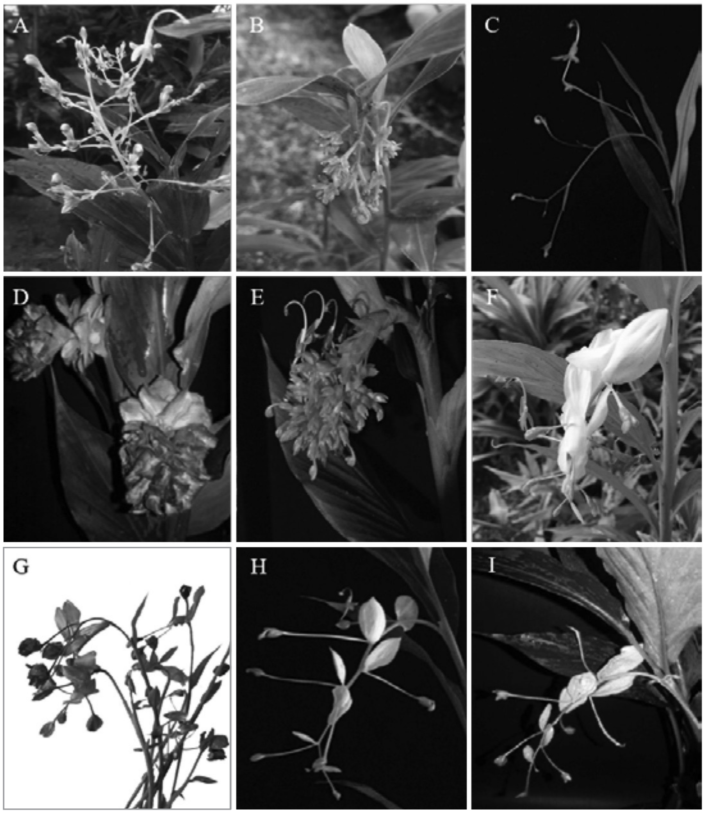

Figure 1 *Globba* spp.; (A) *G. xantholeuca*, (B) *G. adhaerens*, (C) *G.* aff. *reflexa*, (D) *G. rosea*, (E) *G. schomburgkii*, (F) *G. sherwoodiana*, (G) *G. siamensis*, (H) *G.* sp. 1, (I) *G.* sp. 2

Self and Cross Pollinations

The six native species of *Globba* : *G. xantholeuca*, *G.* aff. *reflexa*, *G. rosea*, *G. schomburgkii*, *G.* sp. 1 and *G.* sp. 2 were selected and used for pollinating study. Because of the other three species could not produce normal inflorescences under climate condition of Bangkok. The plants were grown in 8-inch diameter pots under 50% shading of the open greenhouse until flowered. The average of first flower blooming time of all sample species were taking about 14 to 68 days after planted. About 40 to 45 flowers of each species were used and hand self-pollinated. In case of the panicle and arching raceme inflorescences, *G. xantholeuca* and *G.* aff. *reflexa*, approximately 1 to 2 flowers of cluster, only selected 1 to 4 clusters of lower part of inflorescence, these flowers were chosen to pollinate. Because of some species in Sect. *Nudae* included *G. xantholeuca* presenting an andromonoecious behavior (Sangvirotjanapat *et al*., 2017). There were only few clusters (1 to 4 and/or 5 clusters) of the lower part of inflorescences consist of hermaphrodite flowers. In case of interspecific cross pollination, the flowers were emasculated before making hand pollination (Nontasawatsri and Suksathan, 2014). Reciprocal cross among 6 species were conducted. The percentage of fruit set, seed number per fruit, pollen viability and pollen germination were observed. Pollen viability was determined by Acetocarmine and pollen germination was tested by the hanging-drop method with a specific liquid medium (Sakhanokho and Rajasekaran, 2010). The pollen germination was observed from 5 to 180 min after cultured. An estimate of cross ability was determined by comparing the percentage of the fruit set and the number of seeds obtained.

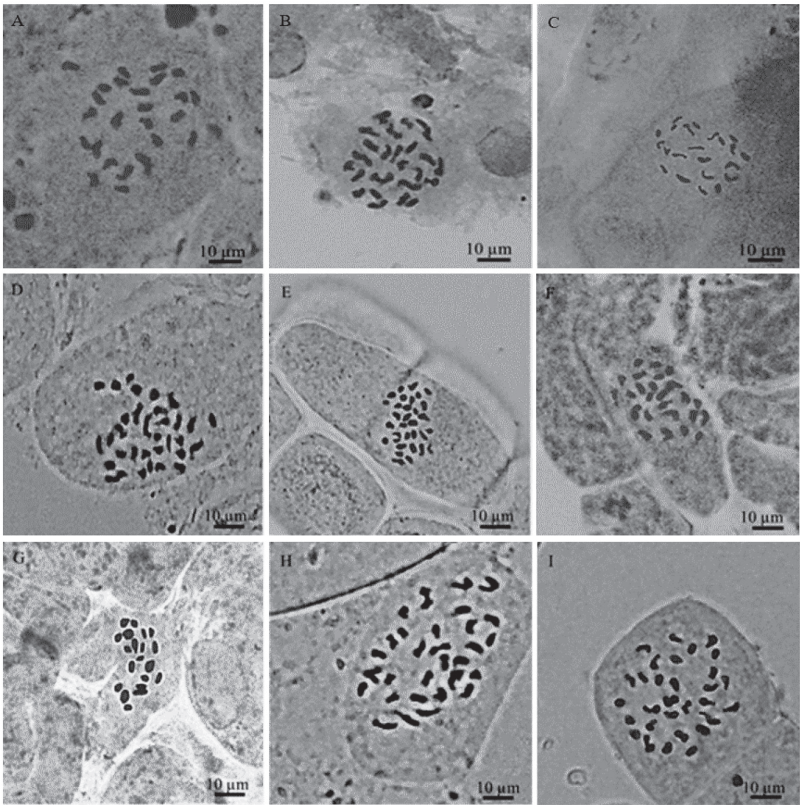

Figure 2 Chromosome of *Globba* spp. (1000X); (A) *G. xantholeuca* (2*n* = 2*x* = 26), (B) *G. adhaerens* (2*n* = 2*x* = 24), (C) *G.* aff. *reflexa* (2*n* = 2*x* = 22), (D) *G. rosea* (2*n* = 2*x* = 32), (E) *G. schomburgkii* (2*n* = 2*x* = 32), (F) *G. sherwoodiana* (2*n* = 2*x* = 26), (G) *G. siamensis* (2*n* = 2*x* = 20), (H) *G.* sp. 1 (2*n* = 2*x* = 30), (I) *G.* sp. 2 (2*n* = 2*x* = 30)

RESULTS AND DISCUSSION

Chromosome Numbers

A chromosome number of *G. xantholeuca* was determined as 2*n* = 2*x* = 26 (Table 1 and Figure 2A). This species was not reported in another publication and not recorded in the database of the Index to Plant Chromosome Numbers (IPCN). Nowadays it was listed in Sect. *Nudae* subsect. *Mediocalcaratae*. There were some previous chromosome number reports of other species in this section, Lim (1972) reported that *G. albiflora* Ridl. had 2*n* = 2*x* = 32. *The other* closely related species, *G. leucantha* Miq., was transferred to Sect. *Ceratanthera* and also had chromosome number 2*n* = 2*x* = 32 and *G. pendula* Roxb. had 32 and 48 sets of chromosome numbers. Newman (1988) reported chromosome numbers of *G. brachyanthera* K. Schum. and *G. fasciata* Ridl. as 2*n* = 2*x* = 32, which was similar to the report of Eksomtramage *et al.* (2001). Takano (2001) represented chromosome numbers of nine taxa of *Globba* i.e. *G. campsophylla* K. Schum., *G. franciscii* Ridley, *G. leucantha* var. *bicolor* Holttum and var. *flavidula* Holttum, *G. maculata* Blum. *G. peniculata* Roxb. *G. pendula* Roxb. and *G. platystachya* Baker. The chromosome numbers were determined as 2*n* = 2*x* = 32, except for the chromosome numbers of *G. leucantha* var.

bicolor, *G. peniculata* and *G. maculata* were 2*n* = 2*x* = 48. For Sect. *Globba* species were determined as widespread range of chromosome numbers as shown in Table 1. *G. adhaerens* had 2*n* = 2*x* = 24 (Figure 2B), *G.* aff. *reflexa* had 2*n* = 2*x* = 22 (Figure 2C), *G. rosea* had 2*n* = 2*x* = 32 (Figure 2D), *G. sherwoodiana* had 2*n* = 2*x* = 26 (Figure 2F), *G. siamensis* had 2*n* = 2*x* = 20 (Figure 2G), *G.* sp. 1 had 2*n* = 2*x* = 30 (Figure 2H) and *G.* sp. 2 had 2*n* = 2*x* = 30 (Figure 2I). These eight specimens were the first investigated chromosome number. In addition, *G.* aff. *reflexa* was gathered in Chiang Mai province which was same distribution area of *G. reflexa* (2*n* = 2*x* = 32) as a report of Larsen (1972). Furthermore, *G.* aff. *reflexa* in this study and *G. reflexa* (Larsen, 1972), which originated in the same province, were not identified to the same species because of their different chromosome numbers. *G. schomburgkii* had chromosome number 2*n* = 2*x* = 32 (Figure 2E), which was not similar to reports of Bisson *et al.* (1968) who found the chromosome number 2*n* = 2*x* = 48. Larsen (1972) reported that 2*n* = 2*x* = 48 and 64 could be a natural octaploid*y plant*, and also, the specimen was gathered in the north of Thailand. However, this result was similar to report of Khamtang *et al.* (2014) who clarified the chromosome number of a sample collected from Chaiyaphum province. Although Sect. *Globba* had wide ranges of somatic chromosome numbers, This section had been reported that 2*n* = 2*x* = 24, 32, 44, 48, 56, 64, 80 and 96 by former researches (Bisson *et al.*, 1968; Mahanty, 1970; Larsen, 1972; Lim, 1972a; 1972b; Beltran and Kiews, 1984; Newman, 1988; Eksomtramage *et al.*, 2001; Takano, 2001; Eksomtramage *et al.*, 2002; Saensouk and Chantaranothai, 2003; Bumrungthai *et al.*, 2004; Khamtang *et al.*, 2014). The presumptive causations of somatic chromosome number variation of *Globba* is the wide range of climatic distribution and the overlapping of species distribution (Mahanty, 1970). Including the different ecological regions that could induce a speciation as neoendemics (Larsen, 1972).

Table 1 Chromosome numbers of nine *Globba* species and previous somatic chromosome number reports

Species	Locations	Somatic chromosome numbers (2*n*)	Voucher number	Previous published reports	
				(2*n*)	Authors
G. xantholeuca Craib.	Prachinburi	26*	BK38925	-	-
G. adhaerens Gagnep.	Chaiyaphum	24*	BKF187625	-	-
G. aff. *reflexa* Craib.	Chiang Mai	22*	BKF166262	-	-
G. rosea Gagnep.	Prachinburi	32*	BKF133408	-	-
G. schomburgkii Hook. f.	Bangkok	32	BKF47121	48	Bisson *et al.* (1968); Larsen (1972)
				64	Larsen (1972)
				32	Khamtang *et al.* (2014)
G. sherwoodiana W.J. Kress and V. Gowda	Kanchanaburi	26*	BKF128904	-	-
G. siamensis Hemsl.	Nongkhai	20*	BKF158588	-	-
G. sp. 1	Prachinburi	30*	BKF194729	-	-
G. sp. 2	Prachinburi	30*	BK068534	-	-

Note: * First report and has not been recorded in database of IPCN

Self and Cross Pollinations

Percentage of pollen viability ranged from 44.33 ± 4.16 to 92.33 ± 2.52%. Percentage of pollen germination ranged from 21 to 82% (Table 2). *G. xantholeuca* had the highest percentage of normal fruits. Almost all of the obtained fruits had high average normal seed number per fruit about 23.14 ± 10.37 seeds. *G.* aff. *reflexa*. had percentage of fruit set about 37.04%. The average of normal seed number per fruit were only 3.00 ± 1.41 seeds. *G. rosea* showed the lowest percentage of fruit set and no obtained seed, while *G. schomburgkii*, *G.* sp. 1 and *G.* sp. 2 had a percentage of normal fruits consisting of more than 50% and had an average normal seed number of about 4 to 6 seeds per fruit (Table 2). A low percentage of fruit set may cause by the thickness of pollen wall that could delay pollen tube germination on stigma. *G. xantholeuca*, *G. rosea* and *G. schomburgkii* had thick exine layers of pollen might be retarded or inhibited tube germination (Figure 3A, 3C and 3D). Exine has the main function to protect, acclimate and to be a gametophytic food source of its own inside. Even if it landed in a stressful environment to germination (Heslop-Harrison, 1976; Tanaka *et al*., 2004; Sakhanokho and Rajasekaran, 2010).

Table 2 Percentage of fruit set and seed number of self pollination of six *Globba* species

Species	Percentage of pollen viability (%)	Percentage of pollen germination (%)	Percentage of fruit set (%)	Percentage of normal fruit (%)	Average seed number per fruit	Average normal seed number per fruit
G. xantholeuca	67.33 ± 2.52	30	25.00	70.00	24.86 ± 8.90	23.14 ± 10.37
G. aff. *reflexa*	44.33 ± 4.16	24	37.04	60.00	4.33 ± 1.21	3.00 ± 1.41
G. rosea	70.33 ± 6.66	21	8.69	0.00	-	-
G. schomburgkii	88.66 ± 4.51	32	17.86	60.00	5.92 ± 1.00	6.33 ± 1.53
G. sp. 1	91.66 ± 5.03	76	33.33	55.55	5.00 ± 1.58	4.40 ± 1.52
G. sp. 2	92.33 ± 2.52	82	27.90	50.00	6.83 ± 1.94	5.33 ± 1.21

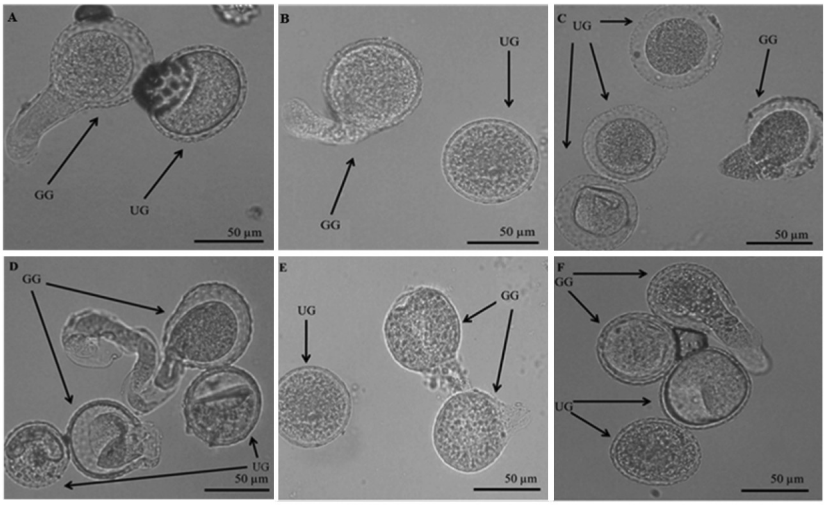

Figure 3 Germinated pollen grain (GG) and ungerminated pollen grain (UG) of *G. xantholeuca* (A), *G.* aff. *reflexa* (B), *G. rosea* (C), *G. schomburgkii* (D), *G.* sp. 1 (E) and *G.* sp. 2 (F)

The results of reciprocal cross pollination among six *Globba* species were shown in Table 3. There were only 10 cross combinations that could set fruits e.g. *G. xantholeuca* × *G.* aff. *reflexa*, *G.* aff. *reflexa* × *G. xantholeuca*, *G. xantholeuca* × *G.* sp. 1, *G. xantholeuca* × *G.* sp. 2, *G.* sp. 1 × *G. schomburgkii*, *G.* sp. 1 × *G.* sp. 2, *G.* sp. 2 × *G. xantholeuca*, *G.* sp. 2 × *G. rosea*, *G.* sp. 2 × *G. schomburgkii* and *G.* sp. 2 × *G.* sp. 1. The percentage of fruit set ranged from 5.26 to 16.67%. Almost all of the fruits developed and produced seeds. The obtained seeds from 10 interspecific crosses, there were seeds from 5 cross combinations of *G. xantholeuca* × *G.* aff. *reflexa*, *G.* aff. *reflexa* × *G. xantholeuca*, *G.* sp. 1 × *G.* sp. 2, *G.* sp. 2 × *G. rosea* and *G.* sp. 2 × *G.* sp. 1 and could be normally developed and germinated. In case of using *G. rosea* and *G. schomburgkii* as seed parental plants, they could not set fruit for any pollen parental plants in this research.

Table 3 Percentage of fruit set and seed number of reciprocal cross pollination among six *Globba* species

Cross combinations	Percentage of fruit set (%)	Percentage of normal fruit (%)	Average seed number per fruit	Average normal seed number per fruit
G. xantholeuca × *G.* aff. *reflexa*	13.33	100	7.00 ± 2.83*	7.00 ± 2.83*
G. xantholeuca × *G. rosea*	0.00	-	-	-
G. xantholeuca × *G. schomburgkii*	0.00	-	-	-
G. xantholeuca × *G.* sp. 1	12.50	100	2.00 ± 1.41	0 ± 0.00

Table 3 Continue

Cross combinations	Percentage of fruit set (%)	Percentage of normal fruit (%)	Average seed number per fruit	Average normal seed number per fruit
G. xantholeuca × *G.* sp. 2	5.55	100	8.00 ± 0.00	0 ± 0.00
G. aff. *reflexa* × *G. xantholeuca*	12.00	100	2.50 ± 0.71	1.00 ± 0.00
G. aff. *reflexa* × *G. rosea*	0.00	-	-	-
G. aff. *reflexa* × *G. schomburgkii*	0.00	-	-	-
G. aff. *reflexa* × *G.* sp. 1	0.00	-	-	-
G. aff. *reflexa* × *G.* sp. 2	0.00	-	-	-
G. rosea × *G.* aff. *reflexa*	0.00	-	-	-
G. rosea × *G. xantholeuca*	0.00	-	-	-
G. rosea × *G. schomburgkii*	0.00	-	-	-
G. rosea × *G.* sp. 1	0.00	-	-	-
G. rosea × *G.* sp. 2	0.00	-	-	-
G. schomburgkii × *G.* aff. *reflexa*	0.00	-	-	-
G. schomburgkii × *G. xantholeuca*	0.00	-	-	-
G. schomburgkii × *G. rosea*	0.00	-	-	-
G. schomburgkii × *G.* sp. 1	0.00	-	-	-
G. schomburgkii × *G.* sp. 2	0.00	-	-	-
G. sp. 1 × *G.* aff. *reflexa*	0.00	-	-	-
G. sp. 1 × *G. xantholeuca*	0.00	-	-	-
G. sp. 1 × *G. Rosea*	0.00	-	-	-
G. sp. 1 × *G. schomburgkii*	5.88	100	2.00 ± 0.00	0 ± 0.00
G. sp. 1 × *G.* sp. 2	16.67	100	3.33 ± 0.58	2.33 ± 0.58
G. sp. 2 × *G.* aff. *reflexa*	0.00	-	-	-
G. sp. 2 × *G. xantholeuca*	5.26	100	2.00 ± 0.00	0 ± 0.00
G. sp. 2 × *G. rosea*	9.10	100	1.50 ± 0.71	0.50 ± 0.71
G. sp. 2 × *G. schomburgkii*	5.88	100	1.00 ± 0.00	0 ± 0.00
G. sp. 2 × *G.* sp. 1	14.29	100	2.66 ± 0.58	2.00 ± 1.00

Polygon of crossability among the six *Globba* species was constructed based on percentage of fruit set and normal seed production (Figure 4). Reciprocal crosses between *G.* sp. 1 and *G.* sp. 2 could obtain fruits and normal seeds. They had similar chromosome numbers $2n = 2x = 30$ and represented the highest percentage of fruit set in the crossability polygon. The other reciprocal cross combination that could produce normal fruits and seeds was the combination of *G.* aff. *reflexa* and *G. xantholeuca*. They were separated in another section and had different chromosome numbers. In terms of chromosome numbers, they had very closed amount. The percentage of fruit set as intersectional cross was higher than 11%. Reciprocal crosses of *G. rosea* could obtain normal seeds when using *G.* sp. 2 as seed parental plants. It had no fertility with other four species for reciprocal cross combinations. *G. schomburgkii* showed no crossability when acted as seed parental plant for any reciprocal cross combinations. Nontasawatsri and Suksathan (2014) also reported about low crossability of reciprocal crosses between *G. rosea* and *G. schomburgkii*. When they used *G. rosea* as a seed parental plant, there was no fruit setting. Meanwhile *G. schomburgkii* was used as well and it had a low fruit setting percentage with few seeds inside. The two species had been classified as a close genetic relationship (William *et al*., 2004) and had a low percentage of fruit set when self pollinated, therefore self-incompatibility may be the barrier of these reciprocal cross combinations.

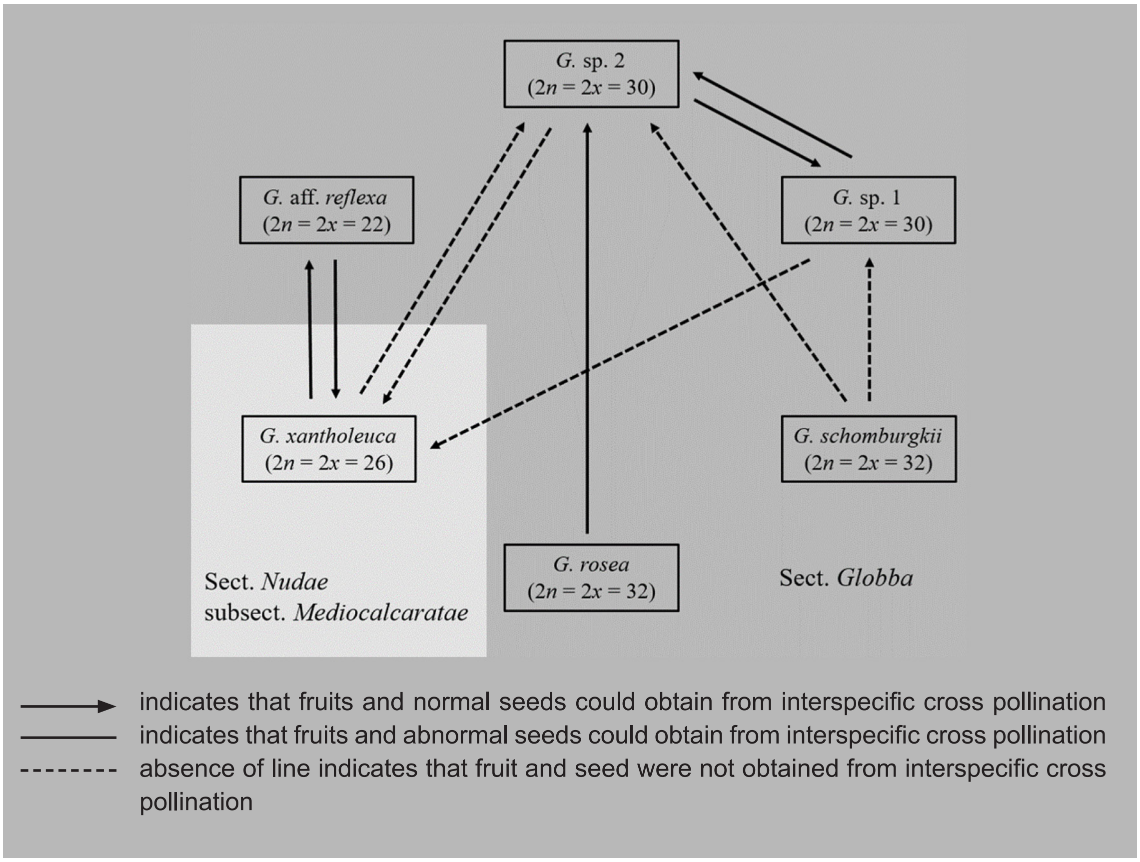

Figure 4 Crossability polygon of six *Globba* specie male and female crossing direction

For intersectional cross combinations, the fruit set occurred only reciprocal cross combintaions of *G. xantholeuca* × *G.* aff. *reflexa* and *G. xantholeuca* × *G.* sp. 2 including one direct cross of *G. xantholeuca* × *G.* sp. 1. Then merely, *G. xantholeuca* × *G.* aff. *reflexa* reciprocal cross combination had normal seeds. In this study, using lower chromosome number plants as seed parental plant seem to increase possibility to obtain normal seeds from infrasectional cross combinations, similar to crossability between *Cucumis sativas* and *C. melo* (Chen and Adelberg, 2000). The immature embryos were not successfully developed due to embryo abortion and endosperm degeneration. The other intersectional cross combinations of *Globba* which were the abnormal seeds obtained from these interspecific crosses may be caused from abnormal development of endosperm and/or embryo from different chromosome number parental plants (Cooper and Brink, 1940). These post-zygotic barriers could be to promote the hybrid zygote which is deadly in the early of development stage. The incompatibility between the endosperm and the embryo can be restored by embryo rescue technique certainly (Van Tuyl and De Jeu, 1997; Chanchula *et al*., 2013; Nontaswatsri and Suksathan, 2014).

CONCLUSION

The chromosome numbers of nine *Globba* species were determined. Some of them were not to be reported in previous publications and recorded in the database of the Index to Plant Chromosome Numbers (IPCN). The percentage of pollen viability ranged from 44.33 ± 4.16 to 92.33 ± 2.52%. The percentage of pollen germination ranged from 21 to 82%. The percentage of fruit set after self pollination of all species were lower than 50%, particularly a bulbil producer species (*G. rosea* and *G. schomburgkii*). Additionally, *G. xantholeuca*, *G. rosea* and *G. schomburgkii* had thick exine layers of pollen which retarded or inhibited tube germination. There were only 10 cross combinations that could set fruits but only five cross combinations could obtain fruits and seeds. In the case of using *G. rosea* and *G. schomburgkii* as seed parental plants, they could not set fruit for any pollen parental plants. According to the polygon of crossability among the six *Globba* species, reciprocal crosses between *G.* sp. 1 and *G.* sp. 2 could obtain fruits and normal seeds and showed the highest percentage of fruit set, moreover these two species might be a same species because of high crossabilities and same amount of chromosome numbers even if some leave characteristic is different. The other reciprocal cross combination that could produce normal fruit and seed was combination of *G. xantholeuca* and *G.* aff. *reflexa*, which is an intersectional cross. Reciprocal crosses of *G. rosea* could obtain normal seeds when using only *G.* sp. 2 as seed parental plants. *G. schomburgkii* as seed parental plant, showed no crossability with other pollen parental species. Therefore, using *G*. sp. 1 and *G*. sp. 2 as pollen plants showed high potential to produce an ideal putative hybrid which have an upright inflorescence with large-scale permanent colorful bract and attractive staminode.

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