

A PRELIMINARY STUDY OF FLORAL DEVELOPMENT AND BREEDING SYSTEM OF *Orthosiphon aristatus* (BLUME) MIQ

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ABSTRACT. *Orthosiphon aristatus* (Blume) Miq. belongs to the family Lamiaceae. There are two varieties, white corolla (OAV-1) and purple corolla (OAV-2) varieties. An observation on inflorescence and flower development of *O. aristatus* was conducted alongside with the study on its breeding system. Inflorescence of OAV-1 and OAV-2 varieties started to develop approximately two and a half months after transplanting the rooted cuttings. The initiation of inflorescence until the senescence took about 75 days. Flower buds started to appear on the inflorescence approximately after 17 days of the commencement of inflorescence development. The development from flower bud until flower senescence took around 50 days. The artificial pollination tests, however, suggested that *O. aristatus* is predominantly a self-pollinated species.

KEYWORD. *Orthosiphon aristatus*, floral biology, artificial pollination

INTRODUCTION

Orthosiphon aristatus (Blume) Miq. is a perennial herb under the family Lamiaceae, and it is widely distributed in Southeast Asia and Australia. *O. aristatus* has several synonyms include *O. stamineus* Benth., *O. longiflorum* Ham., *O. spiralis* Merr., and *O. grandiflorus* Bold. Various vernacular names were given to *O. aristatus* such as Misai Kucing (Malaysia), Cat's whiskers, Java Tea (Europe), Mao xu cao (China) and Kabling gubat (Philippines) (Malaysia Herbal Monograph Committee, 2009). The prominent floral feature of this herb is the extension of pistil and stamens beyond the corolla that gives the "cat's whiskers" visual (Adnyana *et al.*, 2013).

O. aristatus is widely known for its medicinal value and it has been vastly used in traditional remedies to treat diseases such as jaundice, hypertension, diabetes, rheumatoid, oedema, and gall-stone (Adnyana *et al.*, 2013). It also has potent antioxidant and anti-inflammation properties (Ameer *et al.*, 2012). In addition, this herb has the synergistic bio-

enhancing capacity for tamoxifen against breast cancer (Ahamed Basheer & Abdul Majid, 2010). The demand for *O. aristatus*-related products increases with the changing lifestyle of modern generation that prioritizes a healthy lifestyle. The products are usually marketed in the form of herbal tea or in capsules as a supplementary diet

There are two varieties of *O. aristatus* in Malaysia based on its floral and calyx colour, which are *O. aristatus* white variety (OAV-1) and *O. aristatus* purple variety (OAV-2) (Chan and Loo, 2006). Between these two varieties, there are morphological variations (Chan and Loo, 2006) and phytochemical variations (Lee, 2004) that may be utilised to breed new varieties with improved qualities and yield. Unfortunately, the information on the floral biology of this species, which are crucial in performing traditional breeding, is scarce. A brief description of the flower development was done by Almatar *et al.*, (2013). This study reports the inflorescence and flower development of *O. aristatus* together with its breeding system.

MATERIAL AND METHODS

Plant Materials

Stem cuttings of OAV-1 and OAV-2 from different locality were rooted on sand bed in the greenhouse at Universiti Malaysia Sarawak. After one month, each rooted cutting was transplanted into plastic polyethylene bag (30 x 30 cm) containing 3: 2: 1 soil medium (topsoil: peat soil: sand). At least 10 plants for each variety with almost similar inflorescence growth stage were used to observe the inflorescence and flower development. A similar number of plants were used for each artificial pollination test.

Inflorescence and Flower Development

The development of the inflorescence and individual flowers for OAV-1 and OAV-2 was described to estimate the lifespan of an inflorescence and flower. At the same time, the stage of anthesis was recorded.

For the inflorescence development, 30 earliest inflorescence primordials observable by the naked eye for each variety were tagged as 'Inflorescence Day 1' (I-Day 1) and their development was observed and described.

Using the same 30 selected inflorescences, their first flower buds observable by the naked eye were tagged as 'Flower Day 1' (F-Day 1) and their flower development was observed until the flowers bore seeds and senesce. From F-Day 1 until senescence, 10 tagged flower buds/flowers were carefully collected successively from different growth stages. The flower buds/flowers were then carefully dissected and observed under a microscope. The dehiscence of anther was attentively observed and images were taken. The information of the dehiscence of anther and anthesis are used to guide the artificial pollination tests.

Artificial Pollination Tests

After selecting the flower buds for artificial pollination, other flower buds or flowers near the vicinity were removed. Emasculation was performed, if needed, before the dehiscence of the anther. After artificial pollination, the flower buds were bagged using a transparent plastic bag with fine pores. All the artificial pollination tests were considered successful if seeds developed.

Three types of artificial pollination tests were conducted, i.e cross-pollination test, self-pollination test and apomixes test involving both OAV-1 and OAV-2.

The self-pollination tests were conducted by (1) pollinating the stigma of a selected flower with pollen grains from the same flower buds (selfing type A) and (2) pollinating the stigma with pollen grains from a different flower of the same inflorescence or plant (selfing type B). For selfing type A, the selected flower buds were bagged and let to pollinate naturally whilst for the selfing type B, the flower buds were emasculated and artificially pollinated.

The cross-pollination tests were performed by first emasculating all the selected flower buds. For crossing type A, the emasculated flower buds were artificially pollinated using the pollen grains from flowers of different plants of the same variety. In crossing type B, the emasculated flower buds of one variety were pollinated using pollens from flowers of another variety.

The apomixes test was performed by emasculating the selected flower buds of both varieties and bagged afterwards without artificial pollination.

RESULT AND DISCUSSION

Inflorescence Development

The inflorescence of OAV-1 and OAV-2 commenced development approximately two and a half months after transplanting the rooted cuttings. An inflorescence primordial of the two varieties can develop either at the node of the main stem supported by peduncle or at the terminal of a lateral branch or branchlet (Figure 1). At the node of the main stem, an inflorescence primordial was formed between two pairs of bracts. The bracts had obovate shape, arcuate venation with an entire margin. The inflorescence primordial formed at the terminal or lateral branch or branchlet was supported by peduncle without bracts but was raised directly above two young leaves (Figure 1).

On I-Day 1, the inflorescence primordial was pyramidal-like with a smooth hairy surface in yellowish-green colour (Figure 2). Five days later, the terminal part of the inflorescence primordial became creamy in colour. Many layers of early stage bracts were already formed, overlapping each other (Figure 3).

About two weeks later, the development of bracts could be clearly observed with whorled arrangement. The bracts shape ranged from cordate-like at the younger layer (top), to deltoid-like at older layer (bottom) (Figure 4). Flower buds also started to emerge (Figure 5). The inflorescence was developed into an opposed cyme arranged in terminal racemes. Six flower buds were formed at each layer.

On I-Day 39, the corolla fully emerged from the calyx (Figure 6). The closed corolla was larger in size to accommodate the growth of stamens and pistil inside. Besides that, tints of purple were found at the centre of the corolla in both varieties that manifested the dark purple pigment of stigma and anthers inside the corolla. For I-Day 42, the flowers bloomed into bilabiate corolla and both varieties could be easily distinguished by the accumulation of purple pigment on the flowers of OAV-2 (Figure 7). The long stamens and pistils, with estimated lengths of 4.5-5.0 cm and 5.5-6.0 cm respectively, stretched out of the corolla resembling cat's whiskers.

The flowers on each layer of inflorescence bloomed sequentially one layer after another as the observation continued until Day 55. This type of inflorescence is classified as raceme. Each flower lasted for approximately one week before it abscised. As it was, when the flower buds on the middle layer of the inflorescence started to bloom, the corolla of the first few layers of flowers had already started to abscise. After the abscission of the corolla, the base of the calyx started to bloat, indicating the development of ovaries, i.e. the fertilization has occurred (Figure 8). Simultaneously, the colour of the calyx also started to fade from green to light green. Around Day 75, the calyx becomes crispy, shrivelled and the tip of the calyx showed a tint of purple colour (Figure 9). In the calyx, the ovaries had developed into the nutlets which are black in colour. The next round of inflorescence primordial continued to develop at the top of the old inflorescence or the inflorescence straight away wilted.

Flower Development

The observation of flower development started when the flower bud first appeared on the lower layer of bracts on the I-Day17 inflorescence (labelled as the F-Day 1 in this section). The early stage flower bud had a heart shape. The calyx had a whitish pubescent surface (Figure 10). The corolla became visible around 10 days after F-Day 1 stage. The size of corolla slowly increased to accommodate the growth of stamen and pistil within (Figure 11- Figure 13). The surface of the corolla was translucent and purple tint appeared at the centre due to the visible dark purple stigma and anthers inside the corolla.

Flower bud at F-Day 25, the corolla elongated to 9.5 mm long for OAV-1 and 9.0 mm for OAV-2. The calyx for both varieties was *c.* (*c.* meaning around) 5 mm in length. On the corolla, the purple pigment had increased for OAV-2 but not for OAV-1, making it easy to distinguish between the two varieties. The length of stamen of OAV-1 was measured *c.* 35 mm and 32 mm for OAV-2. The length of pistil was *c.* 24 mm for OAV-1 and 22 mm for OAV-2. The stamen and pistil entangled with each other with the anthers and stigma

positioned at different locus inside the enclosed corolla cavity. The stigma was clavate with two lobes for both varieties and was dark purple in colour. The pollen sacs of didynamous anthers started to dehisce and spherical pollen grains were released. The pollen grains were purple in colour. No pollen grains were observed on the stigma (Figure 14). The ovary of both varieties sized *c.*1 mm in length and was separated into four oblong-ovoid sections in light green colour.

The F-Day 27 flower samples had their corolla fully opened (anthesis occurred), showing bilabiate flower form. The flower was tubular with a pubescent surface. The upper lip had entire margin consisted of four lobes. The lower lip had one lobe. The stamen was *c.*4.3 mm in length for OAV-1 and *c.*3.6 mm for OAV-2, whilst the pistil was *c.*3.2 mm in length for OAV-1 and *c.*2.7 mm for OAV-2. The dehisced pollen sacs released a large number of pollen grains. The pollen grains were spherical with six furrow-like colpus and netted muri (Chan and Loo, 2006). Meanwhile, the stigma was curved upwards. The colour of the stigma turned into darker purple (Figure 15).

From stage F-Day 29 to F-Day 30, the surface of stigma started to wrinkle. The size of the ovary of both varieties remained the same as previous stage observed (Figure 16). The pollen sacs shrivelled and turned brownish with no pollen grains spotted. The flowers started to abscise around Day 31.

Observation of F-Day 38 showed the ovaries increases in size in both varieties i.e., fertilization occurred. The length of ovaries was *c.*1.5 mm for OAV-1 and *c.*1.4 mm for OAV-2. The ovaries in both varieties changed colour from light green to white which seemed spongy (Figure 17). The calyx of both varieties for F-Day 46 had wilted as their surface changed colour from green to light brown. Meanwhile, the nutlets in both varieties turned colour from white to brown and had reached *c.*2.2 mm long and *c.*2 mm wide in both varieties. The nutlets were sticky but became harder in tactile impression (Figure 18). Around Day 52, the four nutlets could be easily separated and each brown nutlet was in an ellipse shape.

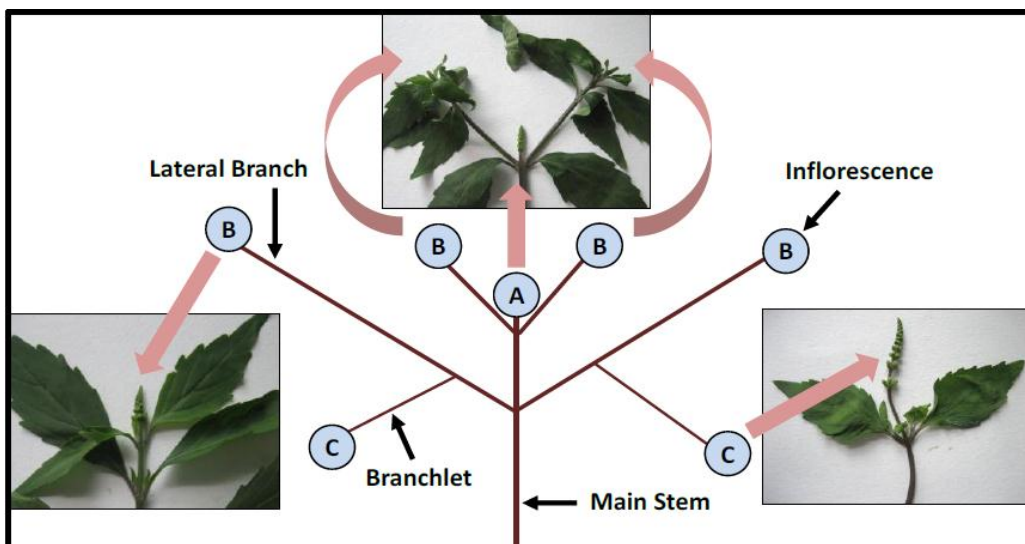


Figure 1: Inflorescence of *O. aristatus* which can develop on (A) the node of the main stem, (B) top of lateral branch, or (C) terminal of a branchlet.



Figure 2: Development of inflorescence primordial of (a) OAV-1 and (b) OAV-2 at day 1 with light yellowish green hairy surface and light yellowish-white at the top surface (Bar= 2.5 mm).



Figure 3: Development of inflorescence primordial of (a) OAV-1 and (b) OAV-2 at day 5. The terminal part of inflorescence primordial became cream in colour. Layers of early stage bracts were formed overlapping each other. The bracts on upper layer were more developed than those on the lower layer (Bar= 2.5 mm).



Figure 4: Development of inflorescence primordials of (a) OAV-1 and (b) OAV-2 at day 14. There were two bracts per layer opposite to each other with whorled arrangement. Bract shape ranges from cordate at the younger layer (top) to deltoid-like at older layer (bottom) (Bar= 2.5 mm).



Figure 5: The development of inflorescence primordials of (a) OAV-1 and (b) OAV-2 at day 18. The flower buds began to appear on the lower layer of bracts (Bar= 3 mm).

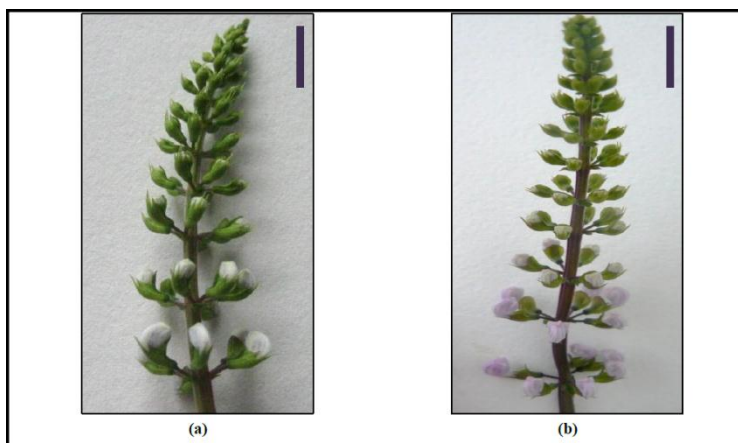


Figure 6: Development of inflorescence of (a) OAV-1 and (b) OAV-2 at day 39. The corolla emerged out of calyx while continued to enlarge in size from lower layer to the upper layer (Bar 10 mm).

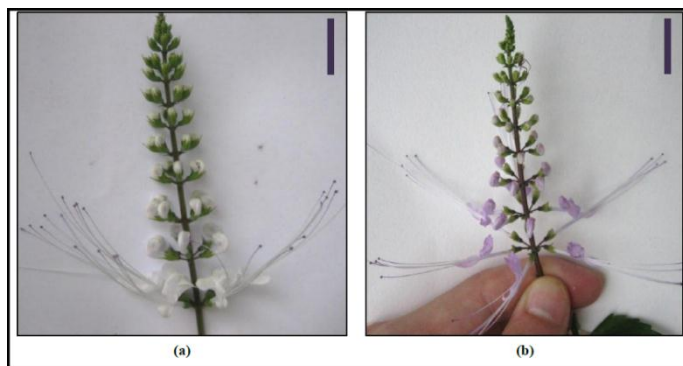


Figure 7: Development of inflorescence of (a) OAV-1 and (b) OAV-2 at day 42. The flowers at the lowest layer started to bloom. The flowers of both varieties showed long stamens and pistils protruding outwards resembling cat's whiskers (Bar= 13 mm).

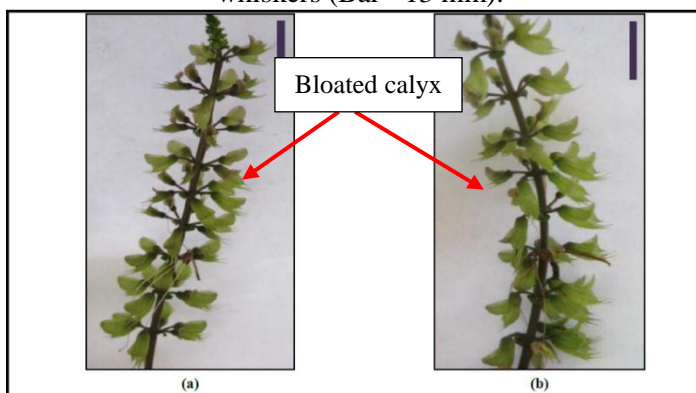


Figure 8: Development of inflorescence of (a) OAV-1 and (b) OAV-2 after abscission of all corolla at day 56. The base of calyx started to bloat which indicates the swelling of ovaries i.e. fertilization had occurred. Simultaneously, the colour of calyx faded from green to light green (Bar= 14 mm).

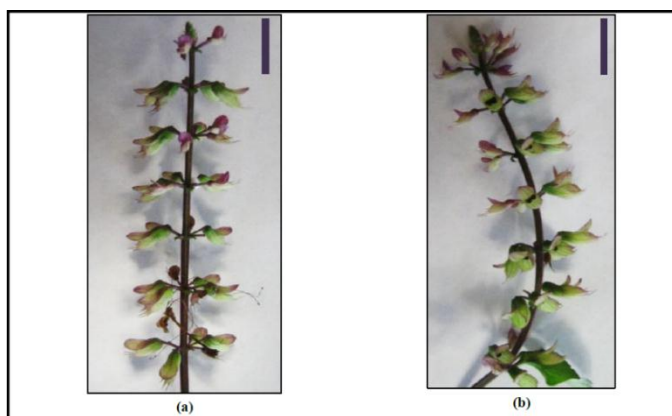


Figure 9: Development of inflorescence of (a) OAV-1 and (b) OAV-2 at day 75. The calyx became crispy, shrivelled and the tip of calyx showed a tint of purple colour. In the calyx, the ovaries had developed into black nutlets (Bar= 17 mm).



Figure 10: Flower bud of (a) OAV-1 and (b) OAV-2 at day 1. The bud was initially observed to have pump shape wrapped with whitish pubescent surface of the calyx by inseparable operculum tooth (Bar= 0.8 mm).

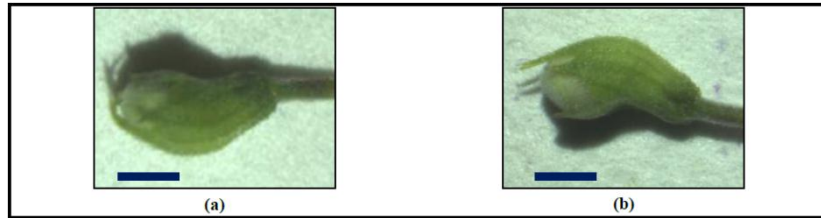


Figure 12: Flower bud of (a) OAV-1 and (b) OAV-2 at day 20. The enclosed corolla started to emerge from the calyx (Bar= 2 mm).

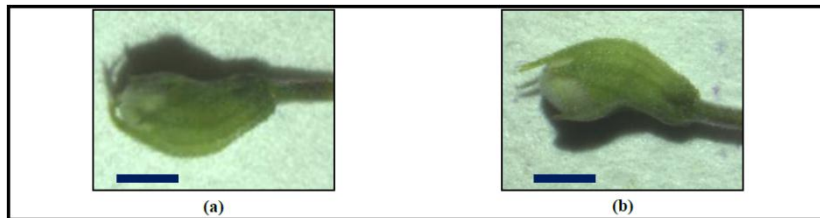


Figure 13: Flower bud of (a) OAV-1 and (b) OAV-2 at day 20. The enclosed corolla started to emerge from the calyx (Bar= 2 mm).

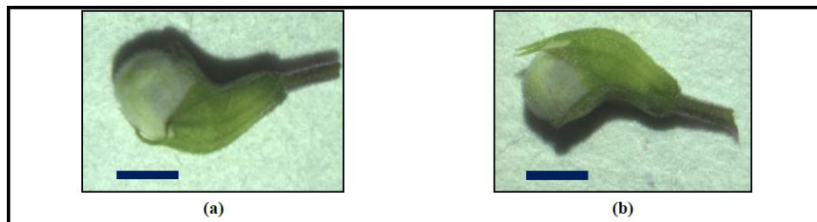
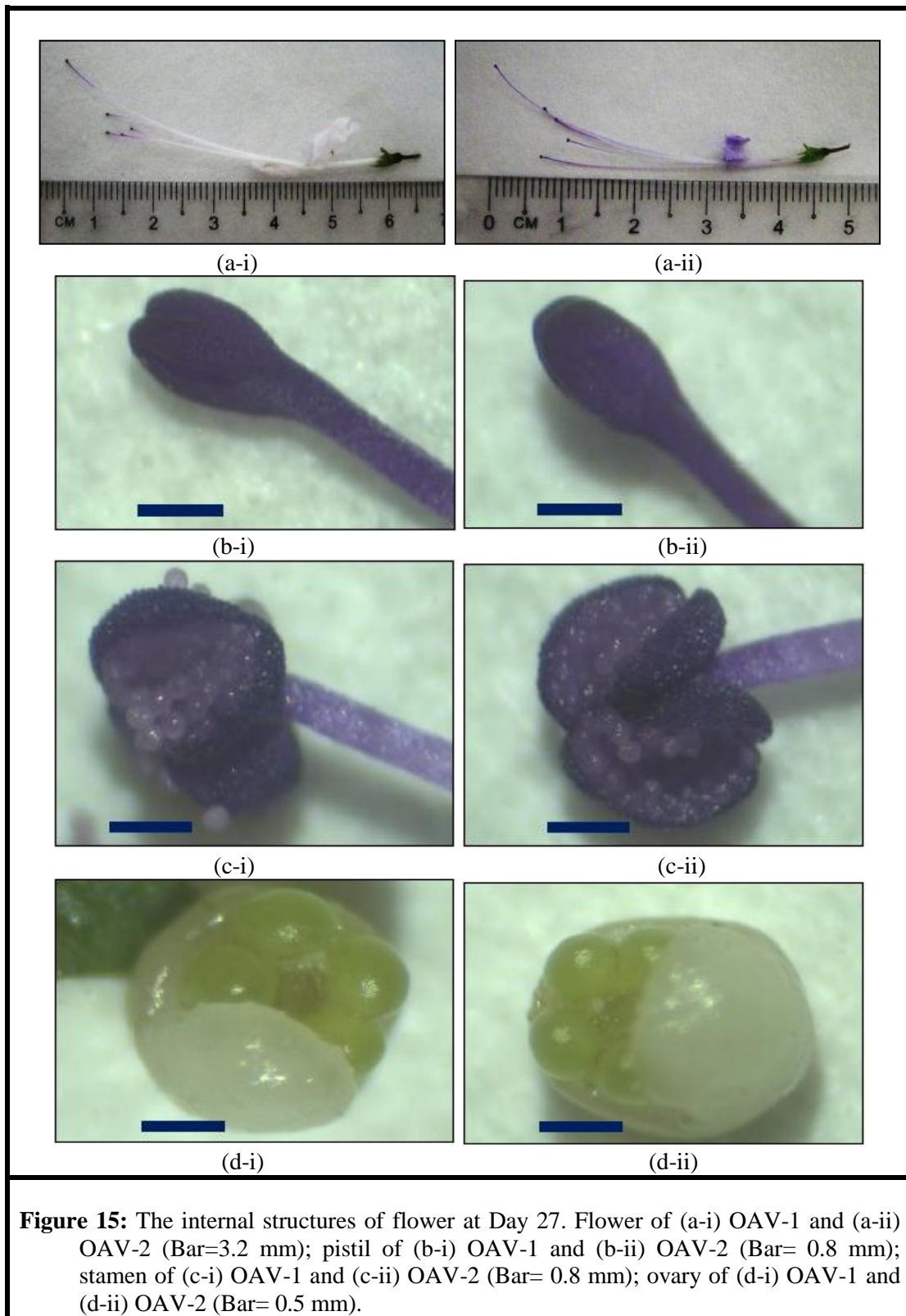
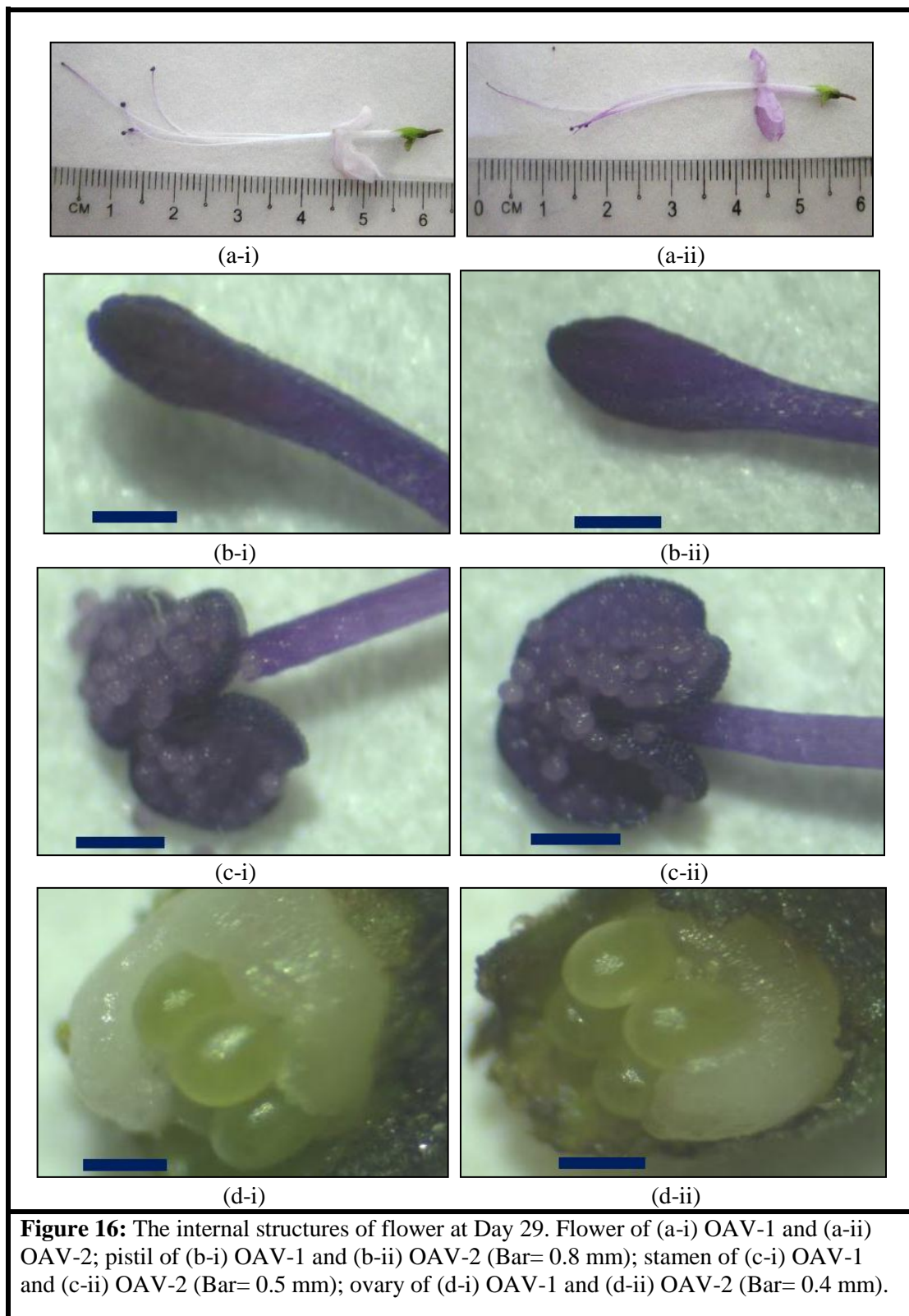
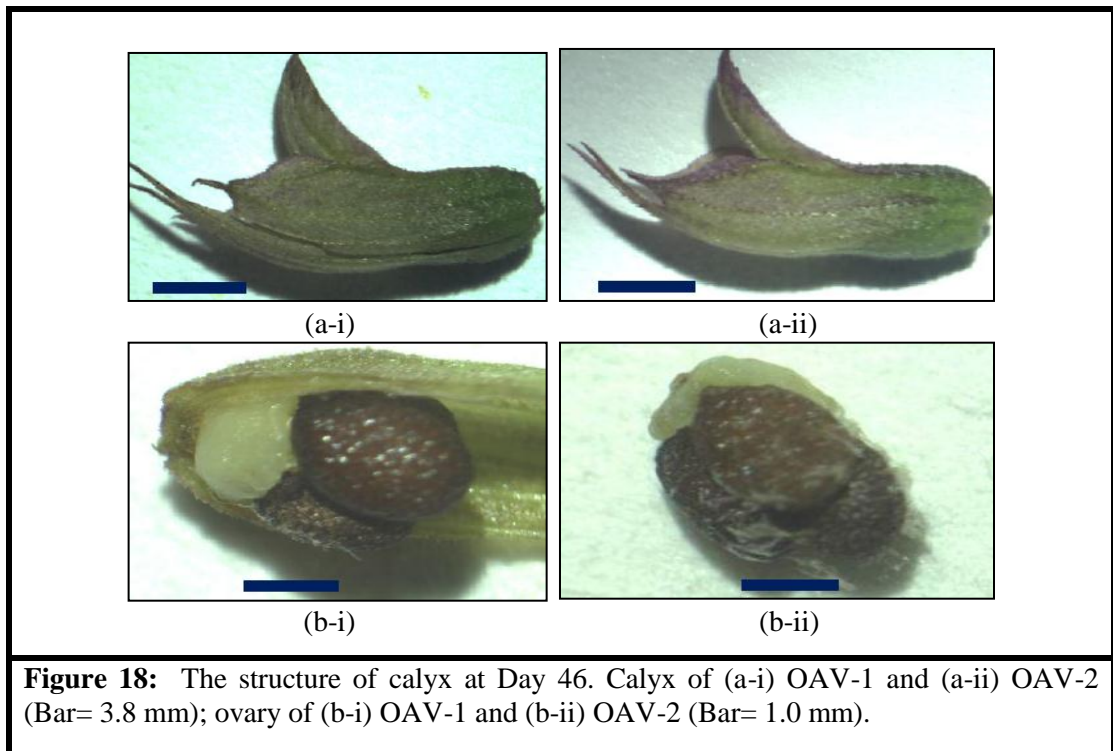
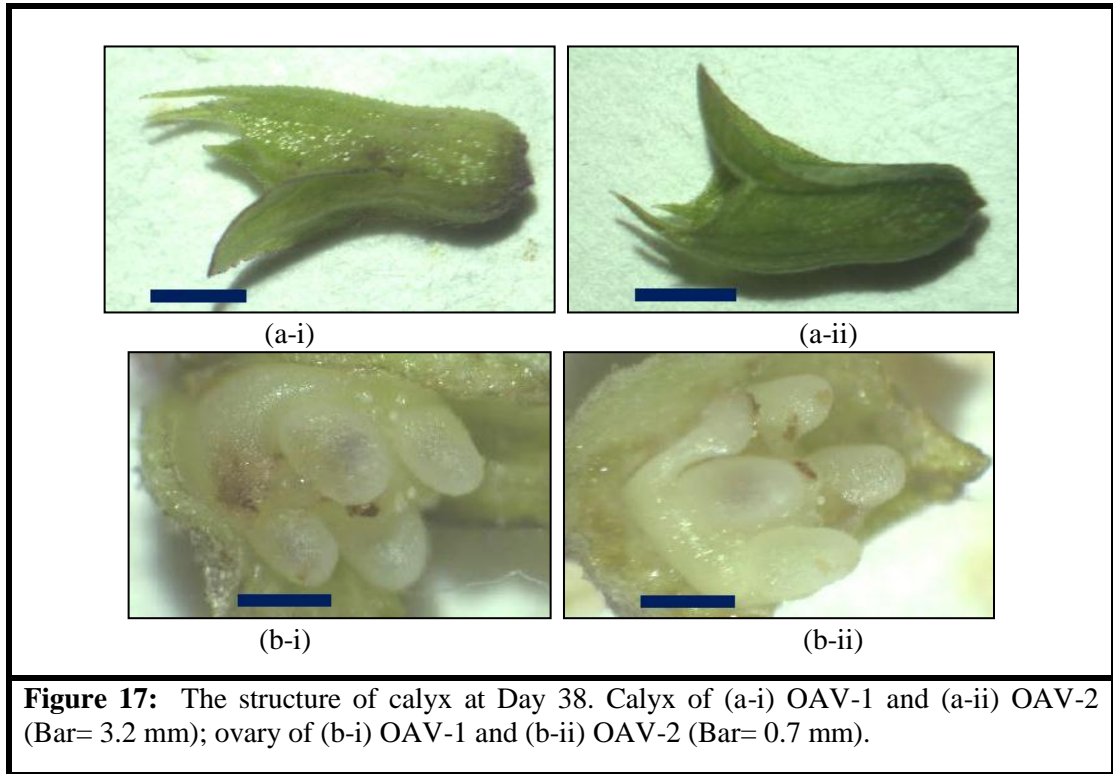


Figure 14: Flower bud of (a) OAV-1 and (b) OAV-2 at day 23. The enclosed corolla slowly increase in size to accommodate the growth of stamen and pistil inside (Bar= 3 mm).







Artificial Pollination Tests

Artificial self-pollination, cross-pollination and apomixes tests were conducted. The artificial self-pollination tests had an average of 95% success. For artificial cross-pollination tests, 28% in average was recorded. Seed set was not observed for apomixes test. The results of artificial pollination tests are summarized in Table 1.

Table 1: The success of seed set for artificial pollination tests on OAV-1 (white) and OAV-2 (purple).

Artificial Pollination Test	OAV-1 (White)					OAV-2 (Purple)				
	Selfing	Selfing	Crossing	Crossing	Apomixis	Selfing A	Selfing	Crossing	Crossing	Apomixis
	A	B	A	B		B	A	B		
Number of flowers tested	50	70	70	60	25	15	55	30	30	25
Number of flowers successfully set seed	43	63	20	21	0	15	50	8	10	0
Percentage of success to set seed (%)	86.00	90.00	28.57	35.00	0.00	100	91.00	26.67	33.33	0.00

Breeding System of *Orthosiphon aristatus*

The opened flower of *O. aristatus* had protruding pistil and stamens, with stigma extended away from the anther. This species was reported to be pollinated by small butterflies which use the lower lip as a landing platform. The upper lip restricted the movement of pollinator. As a result, the pollen grains will be deposited on the pollinator body parts such as head, thorax and leg bases. The pollen grains would then landed on the stigma, either of the same flower or another flower of the same inflorescence or different inflorescence (van der Pijl, 1972).

The pollinating agent suggested by van der Pijl (1972) for *O. aristatus* is only true for OAV-2, the purple-flowered variety. Butterflies are known in general to visit flowers with bright colours, such as red, blue and purple (Hirota *et al.*, 2012), mild scented and with tubular shape (Abrol, 2011; Hirota *et al.*, 2012). For OAV-1, the white-flowered variety of *O. aristatus*, the small moth is the possible pollinator. The moth is known to visit a white-coloured flower in general (Abrol, 2011; Hirota *et al.*, 2012). The breeding system is, however, unknown.

The current study suggests that pollinator may not be involved in the pollination of this species. *O. aristatus* is predominantly a self-pollinated species and can be cross-pollinated. This suggestion is based on the high success rate of artificial self-pollination tests (approx. 95%) and low success rate of artificial cross-pollination tests (approx. 28%). Further study should be done to determine the viability of the seeds obtained from the artificial pollination tests.

It is possible that *O. aristatus* is self-pollinated through chasmogamic self-pollination which is preanthesis self-pollination (Frankel and Galun, 2012). The flower development observation of *O. aristatus* recorded that some pollen grains were spotted on anthers in flower buds of F-Day 24. There were, however, no pollen grains found on the stigma of flower buds at F-Day 24 and F-Day 25. The flower buds open up between the developmental stage of F-Day 25 (close) and F-Day 27 (open) (Figure 14 and Figure 15). At this window period, pollen grains are possibly brushed onto the stigma when the tangled pistil and stamens are unrolling and protruding out from the corolla. The mechanism chasmogamic self-pollination where shedding of pollen grains in concurrent with anthesis is also reported for sesame – *Sesamum indicum* (Abrol, 2011; Weiss, 1971), a predominantly self-pollinated species. Sesame can also be cross-pollinated naturally (Pathirana, 1994). For *O. aristatus*, the occurrence of natural cross-pollination is unknown, but this study indicates that it can be artificially cross-pollinated.

CONCLUSION

To summarize, the development of inflorescence for *O. aristatus* lasted up to 75 days. The anthers dehisced before anthesis, which was around 25 days after the first appearance or development of the flower bud. The breeding system of *O. aristatus* was considered predominantly self-pollinated and can be artificially cross-pollinated.

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