BIODIVERSITAS
Volume 20, Number 2, February 2019
Pages: 328-337

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200204

Morphological characters, flowering and seed germination of the Indonesian medicinal plant *Orthosiphon aristatus*

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Manuscript received: 17 April 2018. Revision accepted: 12 January 2019.

Abstract. *Febjislami S, Kurniawati A, Melati M, Wahyu Y. 2019. Morphological characters, flowering and seed germination of the Indonesian medicinal plant* Orthosiphon aristatus. *Biodiversitas 20: 328-337. Orthosiphon aristatus* (Blume) Miq is a popular medicinal plant in Southeast Asia. The morphological variation of *O. aristatus* is narrow and information on flowering and seed germination is limited. This study aimed to determine the morphological characters, flowering and seed germination of *O. aristatus*. The study was conducted on 19 accessions (*ex situ* collections) of *O. aristatus* from West, Central and East Java. It was found that the differences in morphological and flowering characters were mainly based on shape and color. The dominant stem color is strong yellowish green mixed with deep purplish pink in different proportions. The dominant leaf shape was medium elliptic. *O. aristatus* flower has three kinds of colors: purple, intermediate and white (the most common color). *O. aristatus* has heterostyled flower with a long-styled morph. The stigma has two shapes: open and close (the dominant shape). The open and close stigma was found in Tuban accession, the open stigma was found in Pamekasan accession. *O. aristatus* accessions have a narrow diversity of 84% similarity rate. *O. aristatus* started flowering at 4-5 weeks after planting and blooming 2-3 weeks later. *O. aristatus* seeds begin to germinate on the fourth day and take about eight days to grow from seedling to become complete sprouts. Count I ranged from day 5-6 and count II on days 10-11. Tuban accession has the highest germination rate of 72.97%. The results of this research can be considered as new information, regarding information about the diversity of morphological, flowering characters and the ability of *O. aristatus* seed to germinate.

Keywords: Cat's whiskers, flowering, germination, morphology, *Orthosiphon aristatus*

INTRODUCTION

Cat's whisker (*Orthosiphon aristatus* (Blume) Miq, or *O. stamineus* Benth.) is one of the most widely used traditional medicinal plants in Southeast Asia. The plant is found mainly throughout Southern China, South Asia, Southeast Asia and Tropical Australia. The leaf of *O. aristatus* has been introduced to Europe and Japan as a healthy tea, that usually known as "Java Tea" (Ameer *et al.* 2012). The planting area of *O. aristatus* in Indonesia is mostly found in West Java (Bogor and Sukabumi), East and West Sumatra, Aceh, and North Sulawesi (Syukur 2008; Sembiring et al. 2012).

Orthosiphon aristatus propagation is generally done through vegetative propagation with cuttings (Thijssen 1989). Vegetative propagation produces a plant that is genetically the same as the mother plant, so *O. aristatus* propagated by using cuttings has narrow genetic diversity (Febjislami 2017). For the purpose of plant breeding, the genetical variation might be increased by combining with other genetical sources of *O. aristatus* collected from various agro-ecosystem conditions. Agro-ecosystem conditions affect the genetic potential of medicinal plants, thus affecting the diversity, quantity, and quality of medicinal plant material (Wahid 1998; Rahardjo and Rosita 2003). There were two varieties of *O. stamineus* in Malaysia, the purple and white varieties that could be differentiated merely based on the color of corolla and calyx and the leaf characteristics (Keng and Siong 2006).

Orthosiphon aristatus also produces seed that can be used for generative propagation. *O. aristatus* easily propagated using seeds, but the seed germination rate rapidly declining (Febjislami 2017). Information on flowering of *O. aristatus* is limited. Generally, research was done to study the bioactive content of *O. aristatus* and its potentiality as medicine (Tezuka *et al.* 2000; Mohamed *et al.* 2012). There have been several studies based on the development stage of flower, germination of *O. aristatus* pollen and pollination on cat's whiskers flower (Almatar et al. 2013; Mohamad 2015), but there is no any report yet on plant propagation study by using seed and on seed germination rate of *O. aristatus*. The information about the propagation of a cat's whiskers seeds and its germination rate is also required to increase the diversity of *O. aristatus* through conventional breeding.

Therefore this study aimed to determine morphological character, flowering and seed germination potential for propagation of cat whiskers using seeds from several accessions plant from West Java (Lembang), Central Java (Bojong, Banyumas 1, Banyumas 2, Banyumas 3, Banyumas 4) and East Java (Kebomas, Pamekasan, Poncokusumo, Jenu, Semanding, Tuban, Patram, Sumber Baru, Sumbersari, Randuagung, Pronojiwo, Wonoasih, Kraksaan).

MATERIALS AND METHODS

Study area

The experiment was conducted at IPB Experimental Station, at Sawah Baru Dramaga Campus, Bogor. Two subsequent experiments were conducted. The first experiment was morphological study located at 6°33'44.8" South and 106°44'04.2" East and carried out from March to September 2015. The second experiment was the flowering study located at 6°33'43.1" South and 106°44'03.6" East and carried out from March to July 2016. The seed viability test was conducted in Biology and Seed Biophysics Laboratory, Department of Agronomy and Horticulture, IPB Dramaga Campus, Bogor during March to April 2017.

Procedures

Morphological Characterization and Flowering Study

Eighteen accessions of *O. aristatus* collected from Central Java, East Java and one accession from Manoko Lembang Experimental Garden (West Java) were used as plant materials. The characterization of plant morphology referred to descriptor of *Salvia officinalis* L. (ECPGR 2011) and *Ocimum basilicum* L. (UPOV 2016) that have been modified (Table 1).

Plants were grown under plastic house at 27.6^0-41.8^0C in 25 x 30 cm polybag containing clay loam soil mixed with cow manure (1:1 soil: cow manure media ratio).

Flowering stages were observed at the initiation of flower blooming until seed formation. One flower raceme was observed for every plant sample. The variables to observe were days of flower formation, flower growth, flowering time, number of flowers per raceme, petal development, seed formation, female organ (pistil) position against male organ (stamen) and pollination type.

Viability test

Seeds originated from three randomly selected accessions harvested at different dates and numbers (Table 2), stencil paper and sterile distilled water along with Petri dish (9 cm in diameter), seed germinator IPB 73-2A, tweezers and hand sprayer, microscopes, cameras and other tools that support research were required to check the viability of the seeds. Only brown colour seeds were harvested at the stage when petals can be taken easily or fall off when touched. After harvesting, seeds were air dried and then stored in airtight plastic.

Top of paper method (TP) were used to check the seed viability which have been modified by Purbojati and Suwarno (2006). Three pieces of stencil paper (cut to fit with Petri dish size) was arranged inside each Petri dish and moistened with sterile distilled water. Seeds were arranged on stencil paper and spaced apart inside the Petri dish. Petri dishes were then closed and labeled before inserted into IPB 73-2A seed germinator. The seeds observed over the next 24 hours. The parameters observed were germination rate (GR), calculated by using the following formula (ISTA 2014):

$$GR\ (\%) = \frac{t1 + t2}{tb} \times 100\%$$

Where:
t1 = normal seed germinated on count I.
t2 = normal seed germinated on count II.
tb = Sum of seed sowed.

Table 1. *Orthosiphon aristatus* morphological characters

No.	Character	Explanation
1	Plant height	[3] short [5] medium [7] tall
2	Anthocyanin coloration in stem	[1] absent or very weak [3] weak [5] medium [7] strong [9] very strong
3	Leaf shape	[1] broad ovate [2] medium ovate [3] medium elliptic [4] narrow elliptic
4	Leaf length	[1] very short [3] short [5] medium [7] long
5	Leaf width	[1] very narrow [3] narrow [5] medium [7] broad
6	Anthocyanin coloration intensity on leaf	[1] absent or very weak [3] weak [5] medium [7] strong [9] very strong [99] other (remarks)
7	Anthocyanin coloration distribution on leaf	[1] mainly along veins [2] on basal part [3] on basal and central part [4] trough out [99] other (remarks)
8	Leaf glossiness	[3] weak [5] medium [7] strong
9	Leaf serration of margin	[1] absent or very weak [3] weak [5] medium [7] strong [9] very strong
10	Leaf undulation of margin	[1] absent or very weak [3] weak [5] medium [7] strong
11	Petiole length	[1] short [2] medium [3] long
12	Flowering stem length	[3] short [5] medium [7] long
13	Corolla color	[1] white [2] pink [3] light violet [4] dark violet
14	Style color	[1] white [2] light violet [3] dark violet
15	Fully grown leaf color	[1] pale green [3] greyish green [5] green [7] dark green
16	Calyx color	[1] Light green [2] green [3] dark green [4] purple [5] violet [99] other (remarks)
17	Filament color	[1] white [2] light violet [3] dark violet

Table 2. Harvest time and number of seeds for seed viability test

Accession	Harvest time (WAP)	Seeds number	Seed total	Sow time (DAH)
Semanding	45,9 and 49,4	6 and 17	23	35 and 10
Tuban	49,4	62	62	10 and 29
Wonoasih	49,4 and 50,6	28 and 14	42	10 and 2

Note: Tuban accession for each replication one and two used 25 and 12 seeds, 25 other seeds used different treatments so that the data were not used; WAP = Weeks after planting; DAH = Days after harvest.

Data analysis

During seed viability study, randomized Complete Block Design with one factor (3 accessions namely Semanding, Tuban and Wonoasih) repeated 2 times were used during the experiment so that there were six experimental units. The flowering study used Randomized Complete Block Design with one factor (19 accessions), five times replication with one sample plant in each experimental unit, so there were 95 plant samples.

The quantitative data were analyzed using Ms. Excel software. Similarity analysis was performed on qualitative data using Minitab software and presented as a dendrogram for cluster analysis.

RESULTS AND DISCUSSION

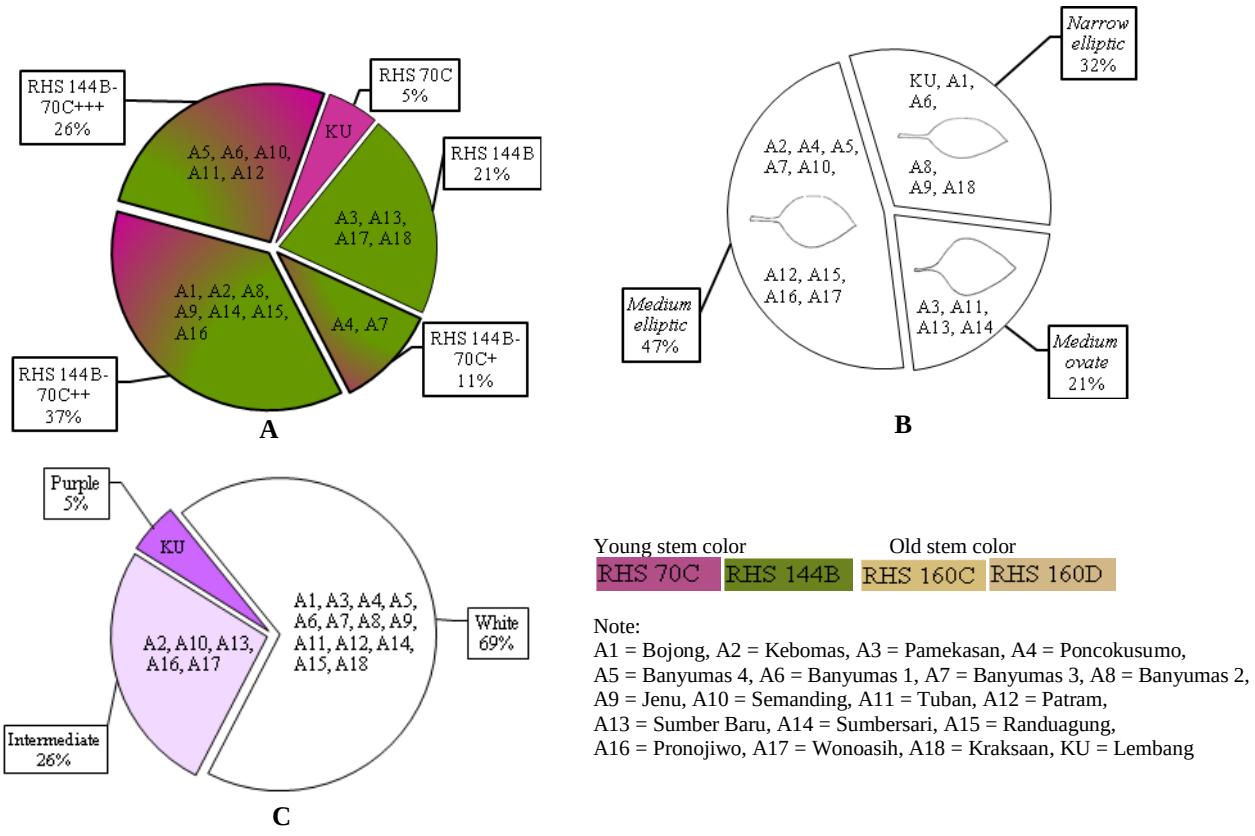
Morphological Characterization

All *O. aristatus* accession having quadrangular stem, trichomes on the stems and veins. Approximately 75% of accessions have young stems with the color consist of dominant color mix of strong yellowish green (RHS 144B) and deep purplish pink (RHS 70C) with different proportions. Only Lembang accession has deep purplish pink color stems. The color of old stems of all accessions is almost same, that ranges between green yellow pale (RHS 160C) and pale yellow color (RHS 160D) (Figure 1a).

Three shapes of leaf were found, namely medium ovate, medium elliptic and narrowly elliptic. Medium elliptic leaf shape is present in 47% of observed accessions (Figure 1b). The young and old leaves color almost same. Young leaves are light green or yellowish green and old leaves are green to dark green in colour.

Flower color of *O. aristatus* accession can be grouped into three colors: white, intermediate and purple (Figure 1c). White was the dominant flower color found in 69% of *O. aristatus* accession. Some accessions have intermediate colored flowers, between white and purple (Figure 2a). The intermediate colored flowers look pale or white purple with a purple tinge when not blooming yet. When the flowers are blooming they look white from a distance, but if with closer observation, it can be seen that there is a purple tinge at the edge of the corolla (Figure 2b).

The style and filament of the purple flower have light purple color differs from the intermediate and white flower that has style and filament with a gradation color from white at the base and becoming purple at the end of stigma and anther (Figure 3a). Calyx color is green and generally almost the same for those three flowers. Each flower has corolla with five dents. There are two different types of stigma shape, *i.e.*, close-shape and open-shape; close-shape stigma was dominantly found in tested samples. Except for the two accessions, Tuban accession has both types of stigma (close-shape and open-shape) while Pamekasan accession is dominated by open-shape stigma. The anther has purple color with two anther lobes on one stamen. The pollen color of all accessions is pale purple (Figure 3b).



Note:
A1 = Bojong, A2 = Kebomas, A3 = Pamekasan, A4 = Poncokusumo, A5 = Banyumas 4, A6 = Banyumas 1, A7 = Banyumas 3, A8 = Banyumas 2, A9 = Jenu, A10 = Semanding, A11 = Tuban, A12 = Patram, A13 = Sumber Baru, A14 = Sumbersari, A15 = Randuagung, A16 = Pronojiwo, A17 = Wonoasih, A18 = Kraksaan, KU = Lembang

Figure 1. Diversity of young stem color (A), leaf shape (B) corolla color (C) cat's whiskers accession. Purple color: + = weak, ++ = medium, +++ = strong. (Source: stem color: Azalea Society of America 2007; leaf shape: *descriptor Ocimum basilicum* L, UPOV 2016).

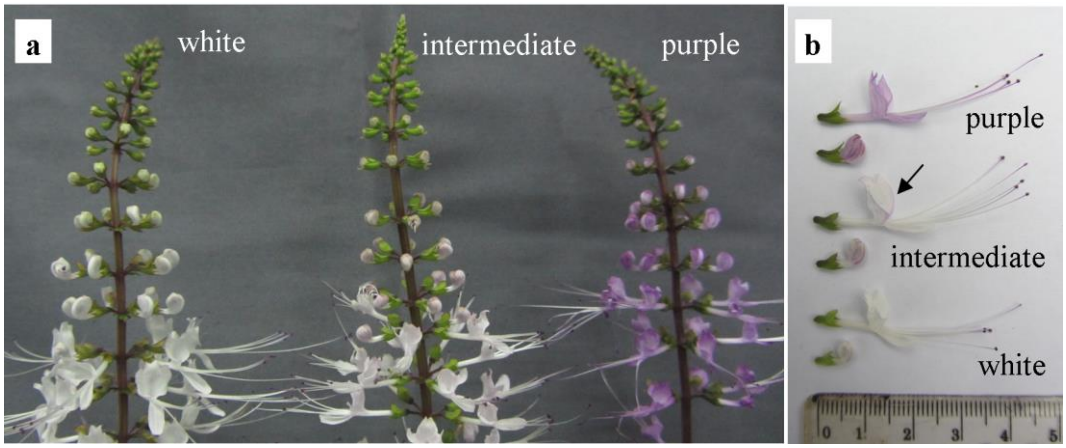


Figure 2. *Orthosiphon aristatus* flower. Arrow refers to purple tinge

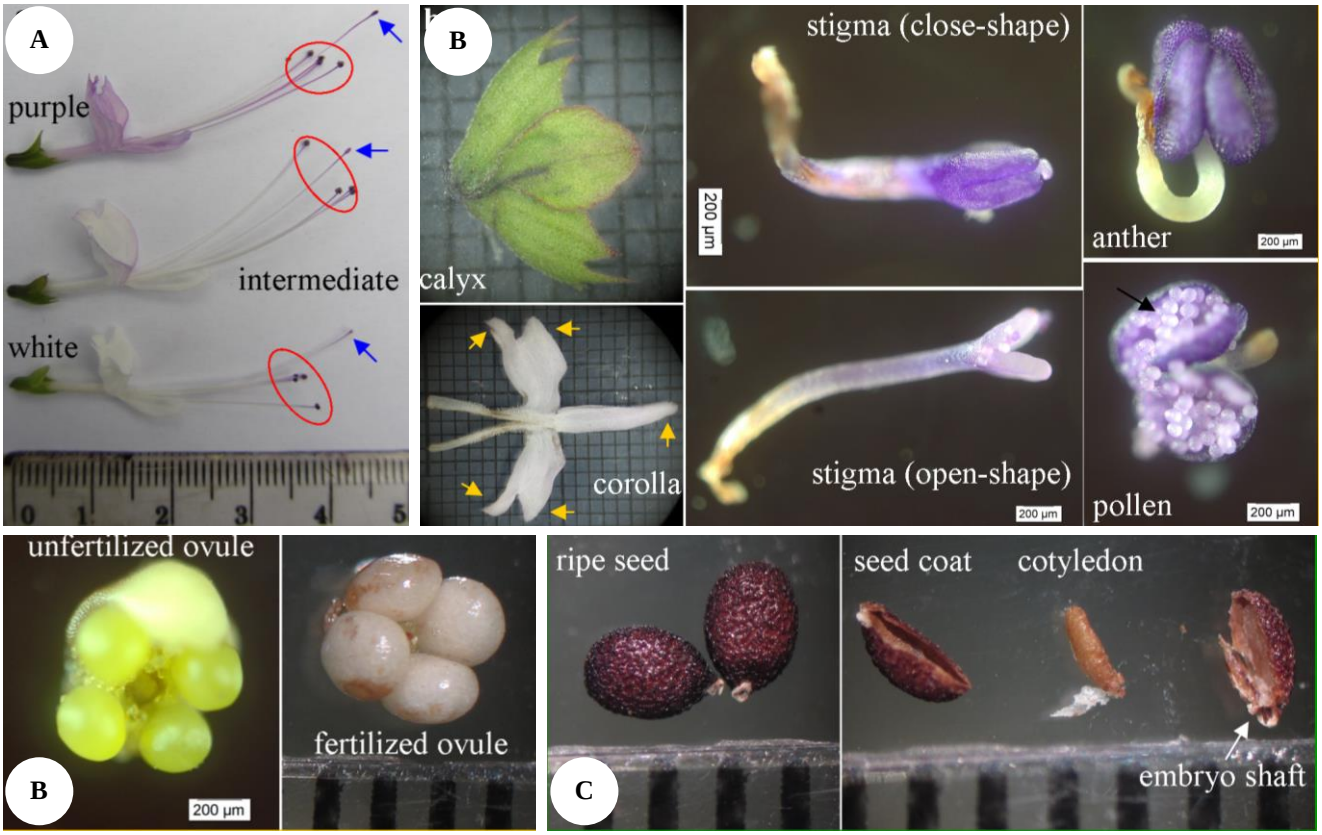


Figure 3. Morphological of *Orthosiphon aristatus* flower (A), parts of the flower (B), and ripe seeds with parts (C).The blue arrow shows the pistil; The red circle shows the stamens; The black arrow shows the pollen; The white arrow shows embryo shaft; Box length and distance between lines = 1 mm

The seed shapes of all accessions are oval with a slight taper at the ends attached to the base of the petals. The color of young (unfertilized and fertilized ovule) seed is pale green and white (Figure 3b) while the color of ripe seeds is reddish brown to dark brown (Figure 3c). All accessions have the same amount of corolla, pistil, stamen, and ovule. The amount of corolla per-flower is one corolla, one pistil, four stamens and four ovules in one flower.

Cluster analysis

The resulted dendrogram obtained from cluster analysis of *O. aristatus* accessions show narrow diversity (Figure 4). There are four clusters at 85% similarity level. Characters that make Bojong, Pamekasan and Kraksaan accessions grouped on the first cluster and also become distinguishing characters with another cluster were anthocyanin coloration intensity and distribution on the leaf; corolla, style, calyx and filament color. The second

cluster consists of 12 accessions that grouped because of anthocyanin coloration distribution on the leaf; style and filament color. Sumber Baru and Wonoasih accession were grouped on the third cluster have similarities on all characters except on leaf shape, leaf width and leaf undulation of margin. The fourth cluster has anthocyanin coloration on the stem, leaf width, leaf serration, undulation of margin, calyx, style and filament that different with another and brings on Lembang accession separated from another accession on another cluster (Table 3). This result showed that tested accessions have narrow diversity because of highest similarity percentage *i.e.*, 85%. Suratman et al. (2000) reported that *I. aquatica* and *I. reptans* has similarity index of 81%, that means *I. aquatica* and *I. reptans* has a highest genetic relationship and big uniformity of morphology character or small diversity of character.

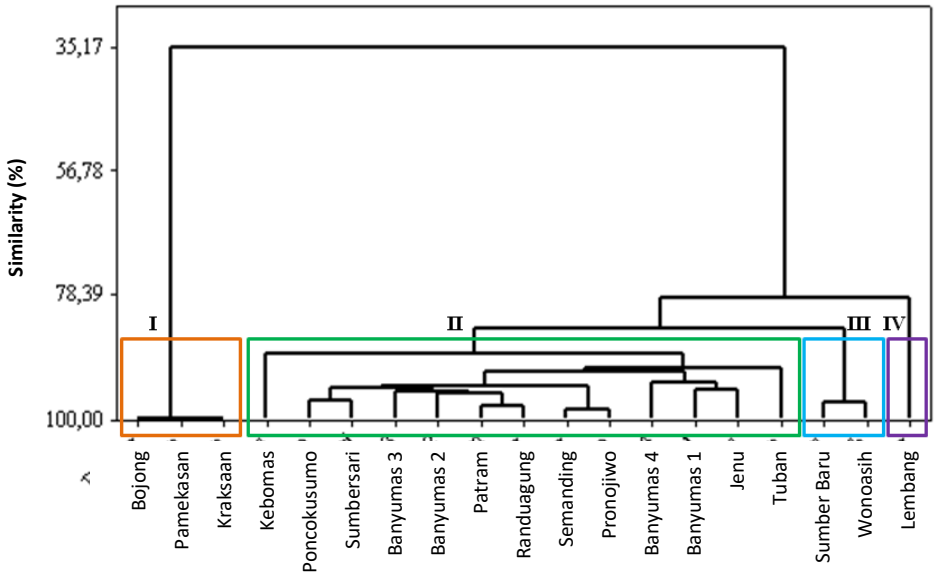


Figure 4. Dendrogram of 19 *Orthosiphon aristatus* accession

Table 3. Differentiation of *Orthosiphon aristatus* morphological characters

Character	Accession																		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Plant height	5	3	5	3	5	5	5	5	7	5	5	5	5	7	5	5	7	7	3
Anthocyanin coloration on stem	5	1	1	3	3	5	3	5	7	5	7	5	7	7	5	7	1	1	9
Leaf shape	4	2	4	3	3	2	3	4	3	3	3	3	3	4	4	2	2	3	4
Leaf length	7	3	5	5	7	7	5	7	7	7	7	7	5	5	7	5	5	5	5
Leaf width	3	5	3	5	5	5	5	3	5	5	5	5	5	3	3	5	5	3	1
Anthocyanin coloration intensity on leaf	99	99	99	1	3	3	1	1	1	1	1	1	1	1	1	5	3	3	5
Anthocyanin coloration distribution on leaf	99	99	99	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Leaf glossiness	3	5	5	3	5	5	5	7	7	5	7	7	3	5	3	5	3	3	7
Leaf serration of margin	7	5	5	5	5	7	5	5	7	7	7	5	7	7	7	7	5	5	9
Leaf undulation of margin	1	3	3	1	1	1	3	3	3	3	1	1	3	1	3	3	1	3	5
Petiole length	3	1	3	2	2	3	2	3	3	3	1	1	1	2	1	3	3	3	3
Flowering stem length	3	3	7	3	5	7	5	5	7	7	7	7	5	5	5	7	7	7	7
Corolla color	1	1	1	3	1	1	1	1	1	1	3	3	1	1	1	1	3	3	4
Style color	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
Fully grown leaf color	7	5	5	5	7	7	7	7	7	5	7	7	5	7	7	7	7	7	7
Calyx color	1	1	1	2	1	1	1	1	1	1	2	2	3	1	1	1	2	2	3
Filament color	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2

Note: A = Bojong, B = Pamekasan, C = Kraksaan, D = Kebomas, E = Poncokusumo, F = Sumbersari, G = Banyumas 3, H = Banyumas 2, I = Patram, J = Randuagung, K = Semanding, L = Pronojiwo, M = Banyumas 4, N = Banyumas 1, O = Jenu, P = Tuban, Q = Sumber Baru, R = Wonoasih, S = Lembang

Flowering study

Flowering and Bloom Time

The initiation of flowering ranges from 29 to 38 days or about 4-5 weeks after planting. Seven accessions began flowering at 29-31 days, and they could be classified as fast-flowering plants, and 12 accessions started flowering at 36-38 days (could be grouped into slow flowering plants) (Table 4). Flower blooming ranges from 11-18 days or 2-3 weeks since the initiation of the flower. The flower blooms gradually from the bottom to the top of the flower raceme (Figure 5a). It takes 2 days for flower buds to blooms. When the flowers are not blooming yet, the pistils and stamens are arranged in a circle because style and filament have long measurement (Figure 5b). The style of *O. aristatus* flower has a higher position than the filament. The anther lobes dehisce a few moments after flower blooming, seen by anther condition that not dehisces yet when the flower still in bud (Figure 5c)

Flower and seeds development

Development of flowers and seeds of *O. aristatus* show that flower buds and new blooming flower have green sepal and light green ovule (Figure 5d). After the pollination and fertilization, sepal base part begins to enlarge due to ovule development (Figure 5e). The fertilized ovule will enlarge in size and change color to white. Unfertilized ovule will not develop so that from four ovules there are only 1-3 ovules that develop into seeds. During seeds maturity process, the color changed into reddish brown (Figure 5f). Seeds that have reached physiological maturity are marked with yellowish green sepal color, and the color of the seeds becomes brown (Figure 5g). A flower that blooms asynchronously produced seed for generative propagation resources that can't be harvest at the same time. The seed must harvest asynchronously according to the period of physiology maturity.

Based on observation at the research site, not all of *O. aristatus* flower successful forming seed flower drooping which occurs due to improper pollination. Bloomed *O. aristatus* flower showed some pollen stick to the surface of stigma (Figure 3b) clearly observed under the microscope. This condition confirmed that *O. aristatus* seed formed by the pollination and fertilization process. This is supported by research by Mohamad (2015) that showed pollen of *O. aristatus* can normally germinated, and artificial pollination on *O. aristatus* flower was successful till seed forming.

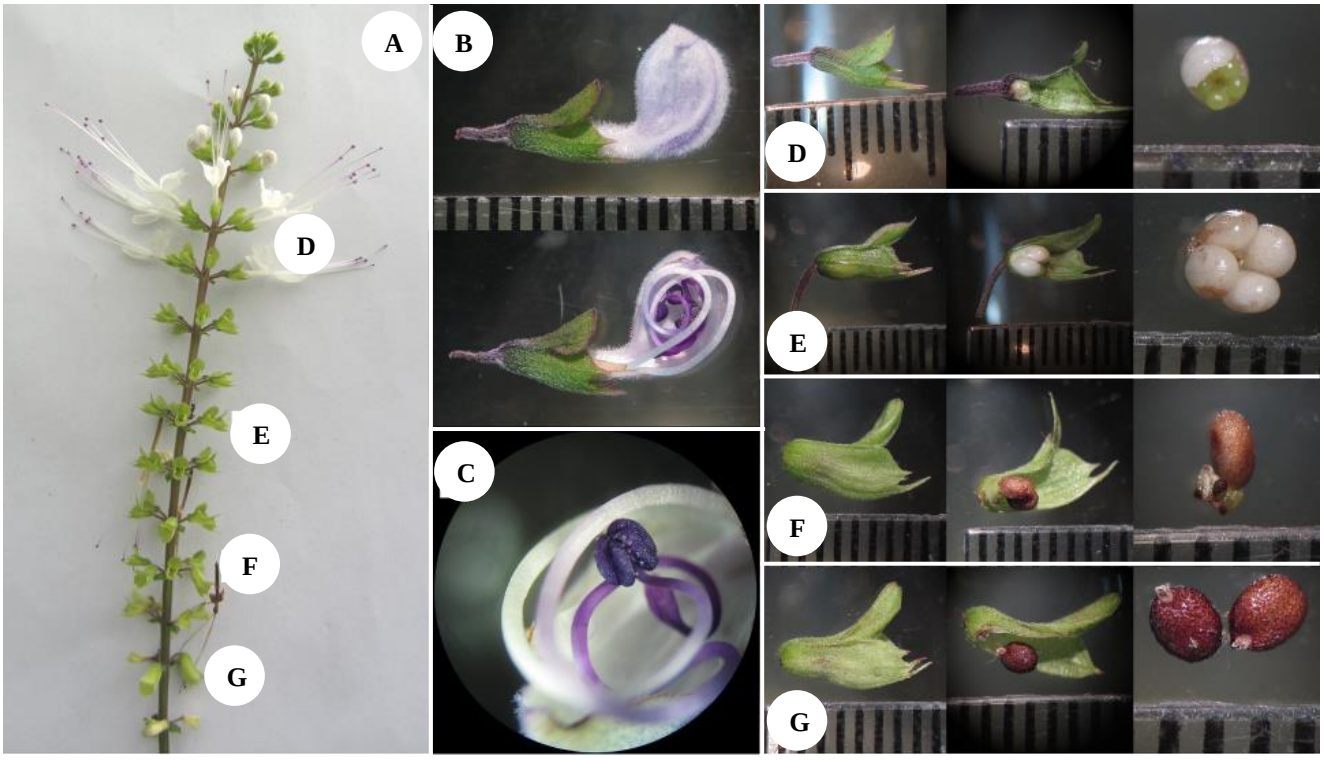


Figure 5. *Orthosiphon aristatus* flowers when blooming (A), still in buds (B), anther condition in bud flower (C), seeds development in bud and new blooming flower (D), after pollination and fertilization (E), during seeds maturity process (F), and reached physiological maturity (G)

Table 4. Flowering time 19 *O. aristatus* accessions

Flowering time (days)	Accessions
29-34 (fast)	Lembang, Bojong, Banyumas 3, Kebomas, Pamekasan, Sumber Baru, Randuagung
35-38 (slow)	Banyumas 4, Banyumas 1, Banyumas 2, Poncokusumo, Jenu, Tuban, Patram, Sumbersari, Pronojiwo, Semanding, Wonoasih, Kraksaan

Seeds viability

Germination testing

O. aristatus seed needs 8 days to germinate. *O. aristatus* seed that still pithy when tested the viability showed development covered under mucilage (some with thick and some with a thin layer) produced by the seed coat that cover the seeds when exposed to water. Seed coat on embryo shaft cracked and radical (root) appeared which develops into root with fine roots. Changes of color happened on the part near the cotyledons (become green), and the cotyledons begin to lift until the cotyledons removed from the seed coat but the position is still closed. Seeds have germinated perfectly to form a complete sprout that can be distinguished between the primary roots, stems, and cotyledon (Figure 6a-h).

Germination time and rate

The seeds started to germinate after 2-6 days, with the average of 4 days after sowing. The earliest seeds to germinate are from Tuban accession (Table 5). showed from count I earlier (day-5) than other accessions. Count II of Semanding and Tuban accessions (day-10 and day-11) fastest than Wonoasih accessions (day-18). This condition showed seed of Semanding and Tuban accession has germination pattern fastest than Wonoasih accession. This difference is possibly due to differences in the physiological condition of seeds during harvesting. The differences in harvesting time were influenced by not simultaneously time of flower blooming in a single bunch of flowers. The average germination rates of the three accessions from the lowest to the highest were Wonoasih (57.14%), Semanding (62.75%) and Tuban (72.87%). Tuban accession seed has the highest germination rate than other two accessions (Figure 7) so it potentially used as propagation *O. aristatus* plant using seed.

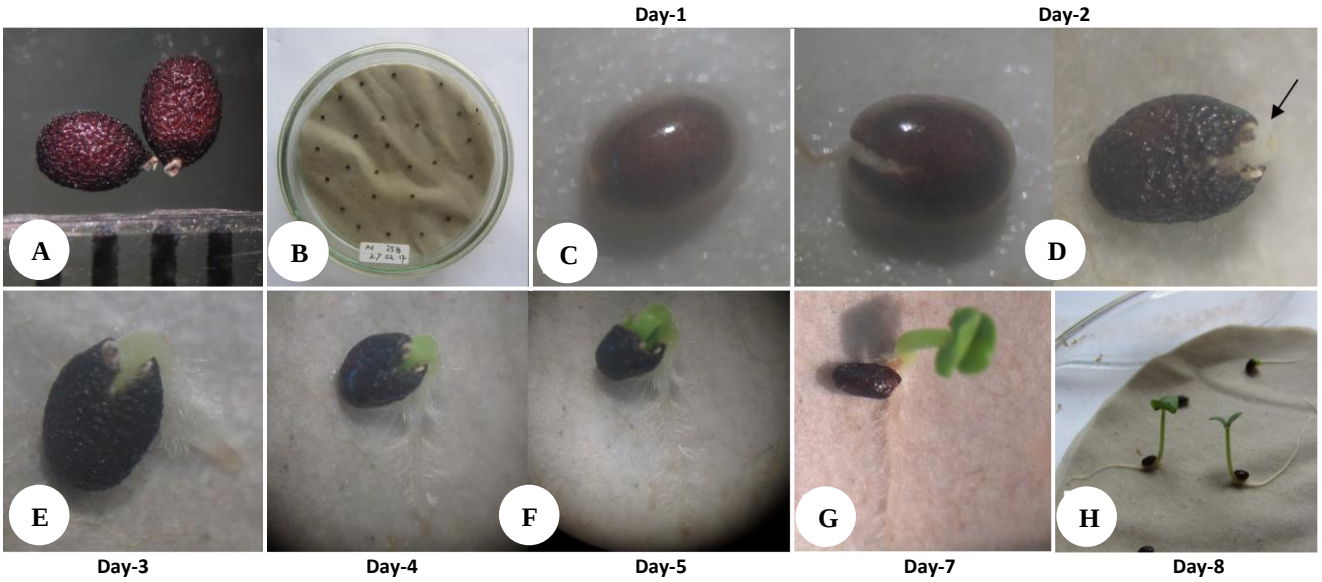


Figure 6. *Orthosiphon aristatus* seed germination. Dried and pithy sample seed (A), seed viability test using on paper method test (B), seed covered under mucilage (C), seed coat cracked and the appearance of radical root (D), development of radical root into a root with fine roots (E-F), the cotyledons released from the seed coat (G), and complete sprout of *O. aristatus* seed (H). The black arrow shows the radical (root) emergence

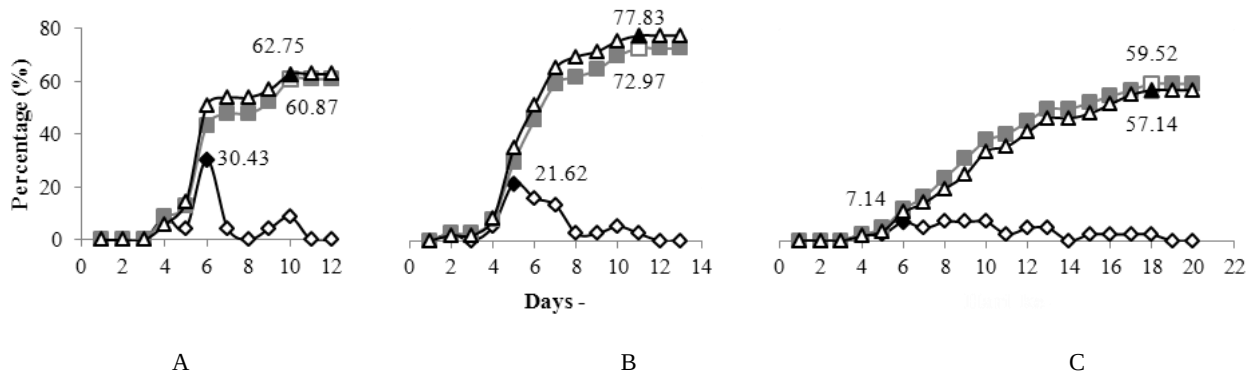


Figure 7. Count I (◊), count II (■) and germination percentage rate (Δ) Semanding (A), Tuban (B) and Wonoasih (C) accession seed

Table 5. Time and number of seeds germinated

Parameter / Replication	Semanding accession		Σ	Tuban accession		Σ	Wonoasih accession		Σ
	1	2		1	2		1	2	
Germination time (days to-)	5	4	-	2	4	-	4	6	-
Number of seeds sowed	6	17	**23**	25	12	**37**	14	28	**42**
Number of seed germinated	4	10	**14**	16	11	**28**	7	18	**25**
	(66.67%)	(58.82%)	**(62.75%)**	(64.00%)	(91.67%)	**(72.97%)**	(50.00%)	(64.29%)	**(57.14%)**

Discussion

In the present study three colors of petal on *O. aristatus* accessions namely purple, intermediate and white were found. The characteristics of white color flowers are not different from those of white flower varieties according to Keng and Siong (2006), but purple color flower is different. The floral characters in purple varieties have a light purple tint on the edge of white petal lobe. Unlike the purple flowers in this study, the color of petals is entirely light purple. The description of purple flower characters is more suitable for an intermediate flower that found in 5 (26%) accessions.

Elliptic is the dominant leaf shape found in 19 accessions. Other leaf shapes *O. aristatus* are ovate and rhomboid (Kite shape) (Keng and Siong 2006) and lanceolate (narrowly ovate) (Almatar et al. 2013). The ovate shape leaves are always present among leaves of this plant. The leaf color of 19 accessions was not different from that of Almatar et al. (2013) that was a green color. The current experiment showed that purple varieties had green color but in contrast to the study of Keng and Siong (2006), there were yellow spots on top and bottom surfaces of the leaves.

The differences among 19 accessions in this research were found in flower color, leaf shape and anthocyanins content and distribution on leaf and stem. Venkateshappa and Sreenath (2013) reported that some Lamiaceae species (*Orthosiphon rubicundus* Benth., *Ocimum basilicum* Linn., *Leucas cephalotes* Spreng., and *Coleus forskohlii* Briq) show some differences and similarities in stem and leaf morphology, stem and leaf anatomy and also in flower color. So, these findings supported the current experiment that the diversity of *O. aristatus* group of the Lamiaceae family depends on stem, leaf and flower characters.

There are two possible types of pollination in this plant, namely self and cross-pollination. Self-pollination might be more dominant based on the asynchronous blooming of flowers from base to top of flower bunch. However, cross-pollination might occur based on the position of stigma and anther, where the position of stigma is higher than anther. Cross-pollination might also occur because anther dehisces after flower bloom. Appropriate with Palupi (2013) statement that flower with heteromorphic structure (pistil longer than stamen or stamen longer than pistil) was one of mechanism that used by the plant to prevent self-pollination and boosted cross-pollination.

Different length of style and filament makes the position of stigma is higher than anther; this is known as hetero-style. Hetero-style is found in 24 families of flowering plants. Hetero-styled species with two forms of flowers called distylous. Flower shape with long pistil and short stamens called a long-styled or pin shape while flower shape with short pistil and long stamen called a short-styled or thrum shape. Most all hetero-styled taxa are self-incompatibility (Ganders 1979). Self-incompatibility is a condition when pollen tube development blocked so can't reach ovules and fertilization failed to happen (Palupi 2013). However, controlled pollination experiments on *Salvia brandegeei*, belongs to Lamiaceae family, showed that there are hetero-style taxa without self-incompatibility. Self-pollination has occurred, and the percentage of seeds is not different from intra-morph pollination (pollen from flowers of the same shape) and inter-morph (pollen from different flower forms) (Barret et al. 2000).

Plants with hetero-style flowers generally required the help of insects for the pollination process. Ferrero et al. (2009) reported that long-proboscis fly and butterfly have a role as a pollinator of *Plumbago auriculata* (Plumbaginaceae) that has a short and long-morph flower. Celep et al. (2014) also found from field investigation revealed that 19 insect species pollinated *Salvia virgata* and four pollinated *Salvia verticillata* (Lamiaceae), including 16 bee species from seven genera of the Apidae and three fly species from three genera of the Nemestrinidae and Tabanidae. Cat whiskers for flowering study planted in a plastic house with a closed net condition around it. This condition causes the usual insects that have a role in pollination of Lamiaceae plants that were butterflies and bees could not help pollination. This affects a number of seeds formed (seed set) so that the seeds that are successfully harvested in this study were in a few amounts.

Other causes that affect the least amount of seeds that could be harvested are pest attacks during the flower development process. The pest identified are grasshopper which eats flowers, caterpillar stem causing shoots wither and *Cochlochila bullita* (Peng et al. 2013) attack is a type of mites that suck fluid on young leaves in the shoots that cause buds to dry and die (Smith-Pardo 2013). This pest attack disrupts the development of flowers and inhibits the growth of the cat's whiskers flowers on the top of the plant.

The current experiment showed that seed color for the viability study is brown which indicated that seeds were harvested at mature stage as also reported by Sales et al. (2011) where bush mint seeds (*Hyptis marrubioides* EPL belongs to Lamiaceae family), were harvested at more mature stage (dark brown color) presented the greatest germination values both at the beginning and throughout storage and maintaining 70% germination after 18 months.

Seed at this stage has better structure and containing larger amounts of food reserves.

Although the harvested seeds were in the mature physiological phase with brown color, not all seed were pithy. During the seed development, there was pest attack of brown marmorated stink bug (*Halyomorpha halys*) (Jacob 2013) that sucks seed fluid. The risk of being attacked by pest and disease is high because seeds are not covered by fruit flesh and open petals structure and uncover seeds after pollination makes the seeds directly being exposed to outside environment.

The current study showed that some seeds are not covered or covered with thin layer mucilage. Tuban accession has some seeds with thin mucilage layer and Semanding accession has some seeds that have thick mucilage layer. According to Hasan and Al Thobaithi (2014), most of the species from Lamiaceae family produce mucilage when germinated. Furthermore, they said that mucilage-produced species have two type of exocarp; namely mucilaginous and non-mucilaginous. The mucilaginous latter cells are much larger, radially elongated, thick-walled, the cavity filled with pigment in the center, while the non-mucilaginous cells are often narrow as in *Lavandula citriodora*.

It can be concluded that cat's whiskers plant or *Orthosiphon aristatus* have a narrow diversity but character differences are still visible on the color of the stems, leaf shape, and flower color. The dominant color of the stem is a mixture of strong yellowish green and deep purplish pink. Leaf shape is dominated by elliptic medium and the dominant flower color is white. The *O. aristatus* started flowering at 4-5 weeks after planting then blooming 2-3 weeks later. *O. aristatus* seeds formed from pollination and fertilization process so it can be potentially used for generative propagation and for conventional breeding. *O. aristatus* has a hetero-styled flower with a long-styled morph. There are two types of stigma formed *i.e.*, close and open-shape. The mature seeds when ripe is brown color started germinating about 4 days after planting and it takes about 8 days to develop into a complete sprout. Count I ranged from day 5-6 and count II from days 10-11. The highest germination rate of *O. aristatus* (Tuban accession) reached 72.97% and it is showed seed of *O. aristatus* potentially used as generative propagation.

ACKNOWLEDGEMENTS

This research partly supported by Graduate Education Scholarship Grant (BPPDN) from Directorate General of Resources for Science, Technology and Higher Education, Ministry of Research, Technology, and Higher Education, Republic of Indonesia, awarded to the first author. Part of the research costs was also supported by Dr. Ani Kurniawati, from cross-faculty/center research funds, BOPTN 2013. Authors would like to thank Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, West Java, Indonesia for supporting facilities.

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