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Variation in floral morphology of agamosporous (*Amorphophallus muelleri* Blume) in natural and gibberellin induced flowering

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Abstract

Morphological variation in triploid apomicts of *Amorphophallus muelleri* has been postulated as low. Here, we present for the first time macroscopic variation in floral morphology from natural and gibberellin treatments. Agamosporous (*A. muelleri*) exhibited morphological variations on 35 out of 45 floral characters. Organ variation was apparent on peduncle, spathe, stigma, carpel, and appendix; that could relate to different genetic background. It seems that gibberellin application at initial stage of flowering increased the existing morphological variation; causing phenotypic plasticity in three characters, *i.e.*, sheath length, disposition of second spathe and degree of limb apex shape. Present study implies that floral characters can be used as key to determine true-to-type of *A. muelleri*. Further extensive research is needed to clarify the variation in apomict of *A. muelleri* using approaches such as metabolomics and hormonal dynamics during flowering.

Key words: *Amorphophallus muelleri*, apomictic, Araceae, clonal variation, floral morphology, flower diversity, gibberellin, glucomannan, iles-iles, morphological variation, natural flowering, phenotypic plasticity

Introduction

Amorphophallus muelleri Blume (synonym: *A. blumei* Scott, *A. oncophyllus* Prain and *A. burmanicus* Hook. f.) (Jansen *et al*., 1996) belongs to Araceae family and it is an emerging commercial tuber crop. In the tropical country like Indonesia, *A. muelleri* (locally known as *iles-iles*) grows in agroforestry systems because it adapts to shading (Santosa *et al*., 2003; 2006a). The corm contains 47-55 % glucomannan on dry matter basis (Sumarwoto, 2005), and many industries use it to produce low calorie foods (Liu *et al*., 1998).

The plant traditionally propagates through aerial bulbils (Sumarwoto, 2005), and buds of corm skin (Santosa and Wirnas, 2009). A leaf is able to produce 2-65 aerial bulbils in every growing season depending on plant age (Sumarwoto, 2005; Harijati and Mastuti, 2014), and corm skin contains 1-12 lateral buds in every centimeter square (Sugiyama and Santosa, 2008). Flowering occur for the first time at fourth year from planting and biennial subsequently, and the flowers produce mature seeds within about a year (Santosa *et al*., 2016a). Thus, the plant exhibits dominant clonal propagation rather than seeds.

Developing new varieties with low oxalic content, high tuberization rate, short dormancy, and resistant to disease requires genetic variation in *A. muelleri*. However, *A. muelleri* produces apomictic triploid seeds (x=13) that set without pollination (Jansen *et al*., 1996; Dani, 2008). In the agamosporous population, Hand and Koltunow (2014) stated that sibling is genetically identical to the maternal. Many studies have shown that *A. muelleri* has low variation in molecular variability (Poerba and Martanti, 2008; Mekkerdchoo *et al*., 2011; Wahyudi *et al*., 2013; Nikmah *et al*., 2016), and leaf morphology (Harijati and Mastuti, 2014; Santosa *et al*., 2016b).

On the other hand, genetic variation in apomicts have been identified in many plants species such as dandelions (Van der Hulst *et al*., 2003), *Erigeron* (Noyes and Rieseberg, 2000), mangosteen (Mansyah *et al*., 2010; Matra *et al*., 2016), and *Ranunculus sp*. (Paun *et al*., 2006). Since visible morphological identity is important in the breeding program especially on the field selection step and crop identification, therefore, we evaluated floral characters in *A. muelleri* as a simple marker for genotyping in the field.

Considering phenotypic plasticity (Schlichting, 1986) that may occur in *A. muelleri* due to hormonal levels (Santosa *et al*., 2006b) or resource allocation (Sumarwoto, 2005; Santosa *et al*., 2016a, c); inflorescences from natural and gibberellin (GA_3) treatments were compared. Objective of present study was to access morphological variations and possibile presence of phenotypic plasticity in floral organ of *A. muelleri*. The results define the potential of floral characters for developing new variety; the possibility of phenotypic plasticity and its consequenses on apomicts status in *A. muelleri* is discussed.

Materials and methods

Two simultaneous experiments, *i.e.*, natural and GA_3 induced flowerings, were conducted at Leuwikopo Farm of Bogor Agricultural University, Bogor, Indonesia (260 m above sea level) from May, 2015-Nov, 2016. The experiments used original Latosolic soil supplemented with lime ($CaCO_3$) applied @ one

ton per ha. Soil had 65 % clay (sand:silt:clay= 13:22:65 %), pH 5.6, high amount of total N (2.8 g kg^{-1} by Kjeldahl method), low amount of phosphorus (34.0 mg kg^{-1} of Bray I) and moderate amount of available potassium (174.8 mg kg^{-1}). Air temperature during experiment was 23-32 ºC (26.9 ºC on average) with RH 62-96 %. Average monthly rainfall during the experiment was 293 mm, except rainfall 0-100 mm occurred within Aug-Oct, 2015.

Planting material and cultivation method: Three-year-old dormant corms were harvested from farmer field in May, 2015. Previously, the plants were maintained under 11-year-old teak agroforest in Bogor with light intensity ranging from 35 to 45 %. The three-year-old corms was originated from bulbils that had undergone three growing cycles. In every growing season, the plants received N:P:K fertilizers (15:15:15) 200 kg ha^{-1}. After harvest, the corms were stored in the room (26-28 °C, RH 79 -82 %) until the buds were swollen (Fig. 1).

Corms with flower buds were planted on 3rd Aug, 2015 and maintained until seed maturity on 30th Nov, 2016. The corms were arranged in equidistance 50 x 50 x 50 cm under artificial shading net with light intensity reduced up to 65 %. Corm weight and diameter at planting was 1977±325 g and 10±3 cm, respectively. The flower bud was 2.5±1.0 cm in length at planting. As a control, 16 corms of two-year-old were planted and expected to produce leaves.

A two-gram carbofuran (Furadan 3G ®) per corm was applied at planting to protect from termite. No supplemental NPK fertilizers were applied, except goat manure @ 10 tons ha^{-1} applied one month before planting. The manure (pH 7.8) contained 1.13 % of total N, 0.07 % of phosphorus and 0.28 % of potassium. Overhead irrigation using sprinklers was applied if rainfall for two consecutive days was less than 3 mm.

Natural flowering: A total of 287 inflorescences were observed. Morphology of inflorescence were observed at anthesis (Fig. 1C-D). Twenty-two out of 70 floral characters described by Sedayu *et al*. (2010) were adopted with slight modification. Additional 23 morphological characters were formulated, a total of 45 descriptors were used (Table 1). Time of anthesis was determined when spathe was fully expanded and /or flower started to emit putrid odor.

In addition, a set of 800 corms aged three-year-old was planted in July 2016 in order to observe impact of physical damage to flower organ. Five hundred and fifty corms were planted upside-down and 200 corms were planted as in common culture practice. Twenty young flowers from common culture practice were scratched vertically using sterile knife.

Gibberellin flowering: The 3-year-old corms with small bud (± 5 mm) were treated with gibberellin (GA_3) at levels of 0.5, 1.0, 1.5

Fig. 1. Seed corm and inflorescence growth in *A. muelleri*. A: Corms at budding. B: Nitidulidae insects visit female flowers. C: Typical *A. muelleri* inflorescence at anthesis: D: Young spadix 10 days after emergence covered by sheaths (arrow): fm-female zone. E: Leaf co-exist during fruiting: arrow indicates berry set. F: immature flower with green stigma and root (arrow). G: Worm attacks appendix. H: Inflorescence emerges after leaf growth in GA_3 treated corms. I: Node-like on peduncle. Scale bar 1 cm.

and 2.0 g L^{-1}, on 10 July, 2015. GA_3 solution (Bioscience, USA) about 2.5 to 3.0 mL was sprayed onto bud and corm. Control corm was sprayed with distilled water. On 3rd August, 2015 corms with flower bud were selected and planted. A set of 16 corms was planted for each GA_3 level in a randomized complete block design with four replicates. In total, 256 from GA_3 and 65 control flowers were evaluated as in natural flowering.

Statistical analysis: Statistical evaluation was conducted using ANOVA, and significance of mean was analyzed further with LSD at 5 % level. A chi-square (χ^2) test at 5 % level was performed to test between natural and GA_3 flowerings.

Results

Inflorescence growth: Thirty-six out of 45 descriptors exhibited variation (Table 1). *A. muelleri* had no variation for nine descriptors, *i.e.*, (1) stamen color predominantly yellow, (2) inflorescence with adjacent female to male zone, (3) disposition of stamen congested or slightly distant, (4) male zone aligned/ fused into a spiral/lax spiral, (5) transition zone between male and appendix was contiguous, (6) all inflorescence had appendix, (7) no substantial elongation on peduncle during fruiting, (8) berry red/orange at maturation, and (9) all berry with smooth surface.

Inflorescence grew similarly from natural and GA_3 induced flowering. GA_3 flowering tended to emerge later than natural flowering, but statistically similar. In general, GA_3 flower had similar characteristic to natural flower, except sheath (cataphyll) length, disposition of double spathe and spathe limb apex shape.

Inflorescence was solitary. However, 19 inflorescences had leaf co-exist (Table 1; Fig. 1E). The leaf emerged at 2-4 months after anthesis from the main bud and /or axillary buds on corm skin. All leaves stood for about 3-4 months; and withered at the same time (on March-Apir, 2016) with the leaves from control plants without the flower. Unexpectedly, 11 out of 19 inflorescences co-existing with leaf were dead before they produced any mature berry. The remaining inflorescence with leaf co-exist produced higher number of seedless berry than those of solitary ones (57 % *vs*. 2 %, $P<0.000$). Extensive root formation at petiole base was common during leaf growth (Santosa *et al*., 2016a), and the roots competed for space with peduncle especially if the leaf emerged from the main bud. As a result, the peduncle strangled and died. It seems that leaf co-exist is a kind of plant mechanism to maintain vegetative growth by aborting the inflorescence.

The size of the spadix, flower organ and its ratio was not affected by the GA_3 application, except appendix length (Table 2). Spadix varied in length from 11-38 cm, with average 25 cm in natural flowering. According to Santosa *et al*. (2016a) spadix size is determined by the corm size.

Peduncle length varied from 2-45 cm, irrespective of GA_3 treatments (Table 2). The peduncle had light, dark green or blackish color with various shape of spots and creamy white or light green stripes. However, *A. muelleri* peduncle had no specific color pattern.

Spathe was shorter or equaling to peduncle length on 48.8 % accessions while 51.2 % had spathe longer than peduncle (Table 1). Inflorescence sheath was commonly short (79.4 %), but 14.6 % inflorescence from natural flowering had sheaths longer than the peduncle. In GA_3 treatments > 1 g L^{-1}, the final sheath size were significantly longer than those of natural flowering (Table 2). It is likely that sheath size is sensitive to level of GA_3; higher GA_3 concentration application promoted inflorescence to produce longer sheath.

Roots emerged from the base of a peduncle (Fig. 1F). However, root was absent in 19.8 % of inflorescence (Table 1). Variation in rooting ability among inflorescence has been reported by Santosa *et al*. (2016a, c). The flower produced seed although it grew without roots. It is unlikely that rooting ability is determined by cultural practice, all plants were regularly irrigated and planted properly.

Spathe number and variation: Spathe expressed morphological variation (Fig. 2). Spathe varied in number, withering time, color, spots, morphology, and opening (Table 1). Three spadices of each natural and GA_3 flowerings had double spathes (Figs. 2A-B), separated from typical single spathe (Fig. 1C). All spathes withered at the same time about one week after anthesis. In the double spathes, the remnant of the second spathe formed a node-like with a small bud on the peduncle (Fig. 1I). Across accessions, however, there was variation in spathe withering time. Three spadices of natural and one of the GA_3 flowerings remained green for up to 3.5 months after anthesis (Fig. 2C).

Young spathe commonly enclosed spadix straightly (Fig. 2D), but it twisted in 16 inflorescences (Table 1, Fig. 2E). Mature spathe open at anthesis with its limb wrinkled outward (Fig. 1C). In natural flowering, however, the limb of 30 accessions (10.5 %) remained smooth or enclosed until the spathe withered (Fig. 2F-H). In GA_3 flowering, many smooth limbs associated with translucent spathe, unlike in the natural ones.

Spathe color at about a week before anthesis was light to dark brown-purplish/pinkish (Table 1). However, 12 accessions (7.7 %) had with light-dark green spathe with dark brown stripes along limb and limb apex (Table 1) and 16 accessions had black spots/ stripes at spathe base near constriction part (Fig. 2I). Dark green spathe had two forms, *i.e.*, thin and thick spathes. Both forms existed in both natural and GA_3 flowerings. The gibberellin levels unlikely induced dark green spathe production, because one of each dark green spathe was found at GA_3 level of 1.5 and 2.0 g L^{-1}. Interestingly, all dark green with thick spathes withered later than the other typical spathes (Fig. 2C).

Limb apex of spathe was obtuse, retuse, emarginate and accuminate (Table 1, Figs. 2J-N). Obtuse/retuse and emarginated apex commonly had a single (Fig. 2J) or double apex (Fig. 2K). Acuminate limb apex with single (Fig. 2L), double (Fig. 2M) and triple tips (Fig. 2N) arose in 11, 5 and 1 accessions of natural flowering, respectively. In acuminate apex of GA_3 flowering, each one accession had the limb apex seized five times longer and 20 times longer than the typical. Moreover, in GA_3 flowering, the limb apex of many acuminate spathe elongated showing a star-like structure (Fig. 2B).

Staminate and pistillate flowers, and transision zone: Reproductive organs exhibited variation in *A. muelleri* (Fig. 3, Table 1). The flower was unisexual with male disposed above female zone with clear border (Fig. 1D; 3A). Nevertheless, 14 accessions (4.9 %) from natural flowering exhibited a transition

Table 1. Floral morphology of *A. muelleri* from natural flowering and percentage of the particular characters obtained from flowering population in 2015

No	Flower part	Morpholocical description	Percentage description (%)			
			1	2	3	4
	Female flowers					
1	Stigma (lobe number)	1 one-lobed; 2 mixed –lobed type	11.5	88.5	-	-
2	Stigma (lobe color)	1 yellow; 2 white pinkish; 3 white-greenish	97.2	1.7	1.0	-
3	Carpel (color)	1 cream white; 2 pink; 3 pale red/purple; 4 bright dark red	1.4	1.0	97.3	0.3
4	Number of pistillate flower	1 below average; 2 average; 3 above average	58.2	8.4	33.4	-
5	Ovary: disposition	1 congested; 2 slightly distant; 3 loosely arranged	1.7	96.2	2.1	-
6	Ovary: arrangement	1 aligned/fused into vertical ridges; 2 aligned/fused into a lax spiral; 3 (sub) verticillate/dense spiral	-	2.4	97.6	-
	Male flowers					
7	Number of stamen	1 below average; 2 average; 3 above average	48.1	12.2	39.7	-
8	Color of stamen	1 yellow; 2 other	100.0	-	-	-
9	Pollen	1 extruded; 2 non extruded	3.8	96.2	-	-
	Spadix					
10	Leaf coexist	1 absent; 2 present	93.4	6.6	-	-
11	Length relative spadix to spathe	1 distinctly shorter than spathe; 2 ± equalling spathe; 3 longer than spathe.	3.5	10.8	85.7	-
12	Female zone: length rel. to male zone	1 much shorter than male zone (less than 0.2×); 2 shorter than male zone (0.2 - 0.9×); 3 ± equalling male zone; 4 longer than male zone (1.1×and more).	0.3	85.7	7.3	7.0
13	Female to male zone	1 adjacent (contiguous); 2 separated by sterile zone.	100.0	-	-	-
14	Zone between male and female zone	1 with staminodes or pistillodes; 2 with staminodes and pistillodes	95.1	4.9	-	-
15	Male zone: disposition stamens	1 congested or slightly distant; 2 loosely arranged	100.0	-	-	-
16	Male zone: disposition stamens:	1 aligned/fused into a lax spiral; 2 dense spiral.	100.0	-	-	-
17	Male zone: transition to appendix	1 contiguous; 2 naked zone, distinct from appendix (“stipe”).	100.0	-	-	-
	Appendix					
18	Presence	1 present; 2 absent.	100.0	-	-	-
19	Apex shape	1 conical; 2 cubical; 3 flattened; 4 irregular	74.6	3.1	19.9	1.7
20	Appendix: susceptibility to worm	1 non susceptible; 2 susceptible	97.6	2.4	-	-
21	Roughness	1 smooth; 2 slightly rough/very rough	86.4	13.6	-	-
22	Color at emergence	1 creamy white/pinkish; 2 Pinkish-purplish white; 3 brownish; 4 yellowish	16.4	72.1	10.1	1.4
23*	Appendix: length rel. to male ± fem. zone	1 shorter; 2 ± equal; 3 longer.	18.8	23.7	57.5	-
24	Appendix: diameter (base) rel. to male zone	1 less than in male zone; 2 ± equal or slightly broader; 3 exceeding.	-	0.3	99.7	-
25	Appendix: general shape (lateral view)	1 ovoid (1:1 – ca. 1.5:1); 2 shortly conical (ca. 2:1); 3 elongate (2.5:1 or more).	0.7	0.7	98.6	-
	Spathe					
26	Number of spathe	1 one; 2 two	97.9	2.1	-	-
27	Spathe color	1 brown/brown purplish/brown pinkish; 2 light green/dark green; 3 dark red/reddish-purplish/pink; 4 others	62.4	8.7	28.9	-
28	Spathe: overall color intensity/-type inside	1 pale (whitish, greenish, pinkish, brownish); 2 dark (purple, blackish-grey).	19.2	80.8	-	-
29	Black spot on spathe	1 absent; 2 present	92.3	7.7	-	-
30	Shape young spathe	1 straight; 2 fold/twisted	71.4	28.6	-	-
31	Overall spathe appearance	1 thick/dark; 2 translucent	94.4	5.2	-	-
32	Transition base-limb	1 gradual, not or shallowly constricted; 2 distinctly constricted.	2.4	97.6	-	-
33	Spathe base: surface within	1 smooth; 2 sculptured.	0.3	99.7	-	-
34	Limb: apex	1 acuminate; 2 obtuse/retuse; 3 emarginate	5.2	41.1	54.0	-
35	Limb: margin shape	1 straight; 2 undulate; 3 plicate/strongly folded.	4.2	3.5	92.3	-
36	Spathe at anthesis	1 opening, separating from spadix; 2 clasping the spadix.	89.5	10.5	-	-
	Peduncle					
37	Presence of node	1 absent; 2 present	97.9	2.1	-	-
38	Length	1 below average; 2 average; 3 above average	44.3	4.2	51.6	-
39	Length rel. to spathe	1 shorter than or equalling spathe; 2 longer than spathe.	48.8	51.2	-	-
40	Last sheath rel. to peduncle	1 shorter (0.2 - 0.9×); 2 ± equaling peduncle; 3 longer (1.1- 1.9×); 4 much longer (≥2.0×).	79.4	5.9	12.5	2.1
41	During fruiting	1 elongating; 2 no substantial growth.	-	100.0	-	-
	Berries					
42	Anthesis time	1 early; 2 average; 3 late	46.0	11.5	42.5	-
43	Berries mature: color	1 red/orange; 2 other.	100.0	-	-	-
44	Berries mature: surface	1 smooth; 2 ridged/verrucate.	100.0	-	-	-
45	Rooting ability	1 rooted; 2 without root	80.1	19.9	-	-

* Variation of GA_3 iduced flowering is larger than natural flowering if abnormal appendix is excluded from calculation; ‘-‘ no accession fit with criteria.

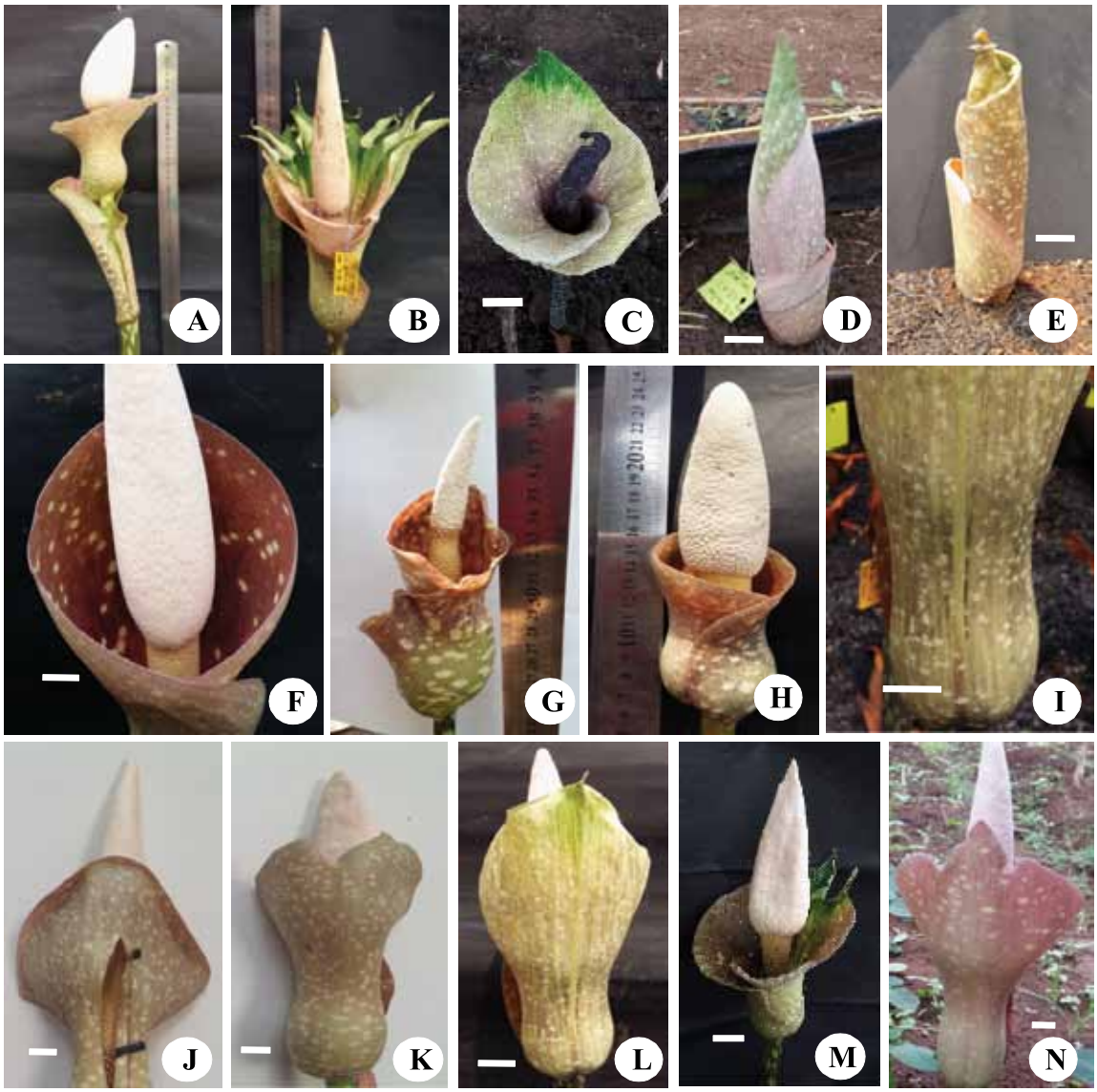

Fig. 2. Spathe variation in *Amorphophallus muelleri*. A: Double spathe of natural flowering. B: Double spathe from GA_3 flowering; first and second spathe close. C: Spathe remains green at 3 months after anthesis. D: Spathe encloses smoothly. E: Spathe encloses twisted. F: Straight limb. G: Rough appendix with wrinkled limb. H: Rough appendix with straight limb. I: Black spot. J: Obtuse/retuse limb apex. K: Emarginated apex. L. Acuminate apex. M: Acuminate-emarginated fork-shaped apex. N: Obtuse fork-shaped apex. Scale bar 2 cm.

zone with some bisexual flowers, *i.e.*, one carpel with four stamens (Fig. 3C). Staminate flowers mostly organized compactly, but it clustered in a four-stamen in four natural inflorescences (Fig. 3A).

Staminate flower rarely released pollen (Table 1). Anthers of some inflorescences released 3-10 pollen at anthesis and after spathe dry, *i.e.*, 11 and 5 inflorescences, respectively (Fig. 3D). However, most pollen was empty (sterile) based on microscopic observation. According to Gusmalawati *et al*. (2013), *A. muelleri* has sticky dimorphic pollen, *i.e.*, transparent (3.5-6.8 µm in diameter) and dark brown (7.0-10.0 µm).

Pistillate flower exhibited variation in carpel and stigma color (Table 1). Stigma lobe was mostly yellow at anthesis, but 1.7 and 1.0 % of inflorescences exhibited white-pinkish and white-greenish lobes, respectively. Lobe periphery was dark red-purplish (Fig. 3F), but five of natural and one of gibberellin induced flowering exhibited the yellow (Fig. 3G). Some inflorescences had large stigma lobes (Fig. 3H) departed from common small lobes. Lobe number varied within and among inflorescences; it ranged 1-7 with two lobes as the most dominant followed by three lobes (Fig. 3H-J). Stigma lobe number has been used as an important trait on mangosteen determination (Mansyah *et al*., 2010). In *A. muelleri*, however, it is unlikely specific because 88.5 % inflorescence contained different lobe number (Table 1). For example, on inflorescence with dominant stigma had two-lobes, other stigma with different lobe number such as single, triple or more lobes were observed. In such case, stigma with three lobes ranged 0-32.8 %.

Color and disposition of carpel varied among accessions (Table 1), and it was independent of gibberellin application. Carpel color was mostly pale red-purplish (Fig. 3I) followed by light pink (Fig. 3J) and creamy white at proportion 97.3, 1.4 and 1.0 %, respectively. Style and ovary color varied among inflorescences composed of permutation of white, pink and red; resulted in nine combinations of carpel color. In a rare case, inflorescence had carpel of bright red. The carpel disposition varied, *i.e.*, slightly

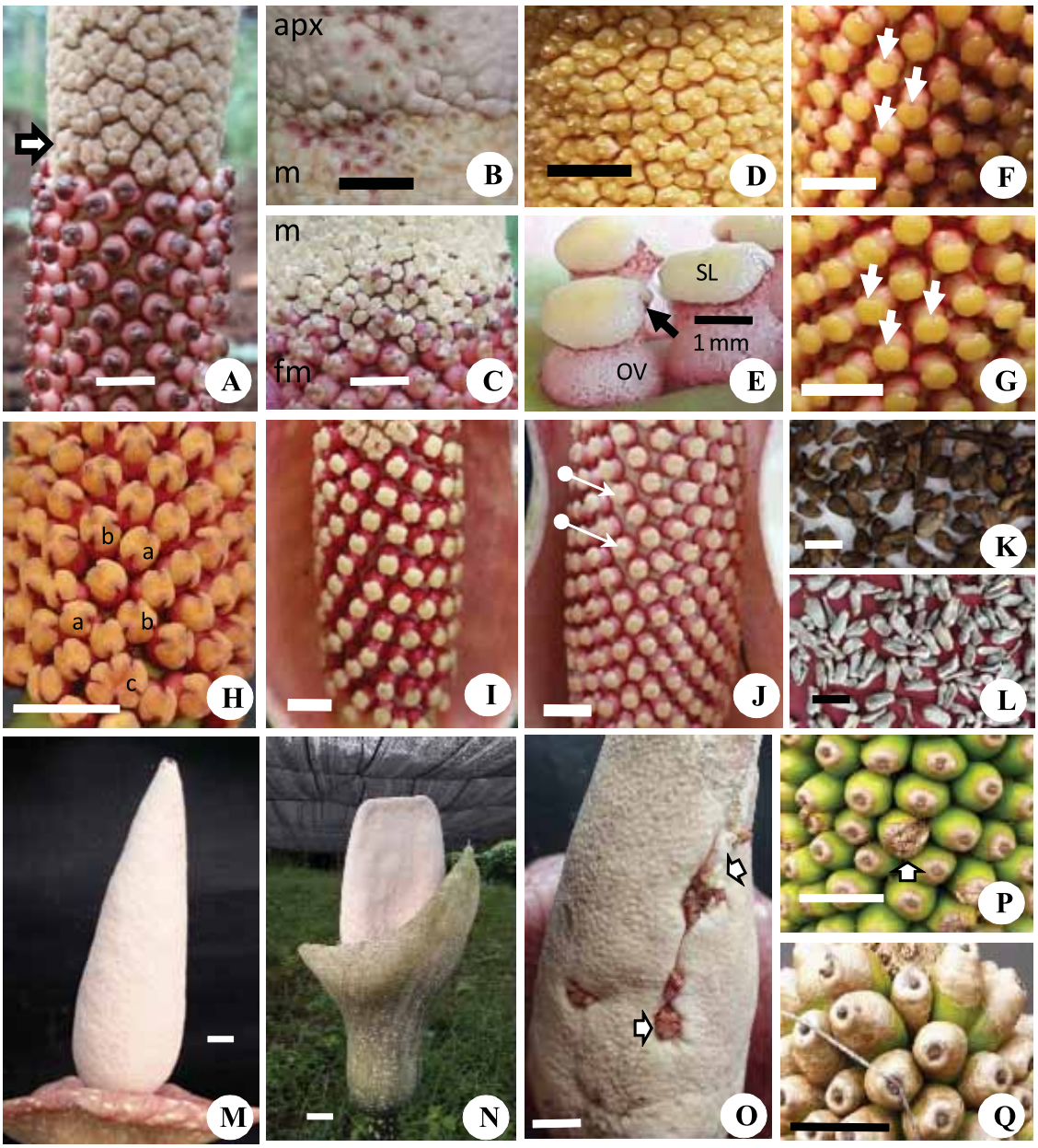

Fig 3. Reproductive organs of *Amorphophallus muelleri*. A: A male flower with four stamen (arrow). B: Transition zones between appendix (apx) and male zone (m). C: Bisexual flowers at transition part between male (m) and female zones (fm). D: Staminate flowers release pollen. E: Pistillate flowers (OV-ovary, SL- stigma lobe, arrow-lobe periphery). F: Stigma lobe with dark red-purple edge (arrows). G: Stigma lobe with yellow periphery (arrows). H: Stigma with predominant large lobes of two (a) three (b) and five (c). I: Carpel with dark red ovary and style. J: Carpel with pink-creamy ovary and white style: arrows show three lobes among two lobes. K: Seeds shape bell from one-seeded berry. L: Seeds shape half-bell from two-seeded berry. M: Conical appendix. N: Appendix with flat apex. O: Male flowers develop on appendix (upper arrow) and female flower (lower arrow). P: Berry has small cap: 6 months after anthesis: some cracked-berry (arrow). Q: Berry with large silvery cap. Scale bar 1 cm.

distant, loosely and congested arrangement at proportion 96.2, 2.1 and 1.7 %, respectively. The carpel arrangement was mostly dense spiral clockwise or anticlockwise.

Female zone elongated slightly during berry development, *e.g.*, 3-8 cm, depending on a number of berries. It elongated markedly in an inflorescence with a larger number of berry. Peduncle elongation in *A. muelleri* is insignificant as compare to other *Amorphophallus* species (Sugiyama and Santosa, 2008; Sedayu *et al*., 2010).

Appendix variation: Appendix was sterile; it exhibited variation in color, shape, and roughness (Table 1). At emergence, an appendix of natural flowering was pinkish/purplish white (72.1 %), followed by pinkish creamy-white (16.4 %), brownish white (10.1 %), and yellow (1.4 %). The appendix changed into pale at anthesis, irrespective the color at emergence. We observed one appendix in GA_3 treatment had light red-purplish colour at anthesis, but none in the natural flowering. In general, there was no marked appendix variation at anthesis.

Appendix shape was conical (Fig. 3M), flat-conical, and flat-cubical (Table 1, Fig. 3N). In natural and GA_3 flowerings, 1.7 and 2.7 % appendix-shaped irregular, respectively. From the lateral view, appendix shape was elongate-conical, followed by ovoid and shortly conical. Mostly appendix was smooth (Table 1), but 13.6 % of natural (Fig. 2G-H) and 10.3 % of GA_3 flowerings

Table 2. Inflorescence characteristic of *A. muelleri* at anthesis from natural and GA_3 flowerings obtained from flowering in 2015

Characters	Natural flowering[Y]		GA_3- flowering[Z]		Significance
	Mean±SD	Range	Mean±SD	Range	
Time to anthesis (days)[X]	89±15	49–125	95±14	60–124	ns
First sheath length (cm)	2±2	0–7	2±2	1–9	ns
Second sheath length (cm)	5±2	2–14	6±3	2–15	ns
Third sheath length (cm)	8±4	2–24	11±4	4–25	*
Fourth sheath length (cm)[W]	14±5	3–35	17±5	8–38	*
Peduncle length (cm)	18±6	2–44	21±5	4–46	ns
Peduncle base diameter (cm)	1.8±0.4	1.0–2.8	1.8±0.3	1.2–2.7	ns
Spadix length (cm)	25±5	11–38	27±4	15–39	ns
Spathe length (cm)	19±3	11–30	21±3	14–32	ns
Female zone length (cm)	5±1	2–9	6±1	4–10	ns
Number of pistillate flower	206±69	90–425	205±72	89–433	ns
Male zone length (cm)	6±1	3–9	7±1	4–10	ns
Number of stamen[W]	1635±560	744–2810	1622±567	698–2890	ns
Apx. length (cm)	13±3	2–21	15±3	7–25	*
Ratio peduncle to spathe	0.98±0.28	0.14–1.73	1.03±0.25	0.29–1.70	ns
Ratio peduncle to spadix	0.73±0.21	0.11–1.56	0.77±0.18	0.25–1.55	ns
Ratio spadix to spathe	1.35±0.16	0.91–2.01	1.35±0.18	0.93–2.00	ns
Ratio female to male zone	0.85±0.16	0.29–1.68	0.84±0.13	0.57–1.23	ns
Ratio female zone to Apx.	0.43±0.18	0.19–2.75	0.40±0.10	0.29–0.99	ns
Ratio male zone to Apx.	0.51±0.23	0.29–4.00	0.49±0.10	0.29–1.20	ns
Ratio Apx. to male+female	1.12±0.20	0.15–1.96	1.15±0.18	0.46–1.96	ns
Ratio Sheath to peduncle	0.82±0.33	0.29–2.56	0.84±0.36	0.01–2.56	ns

[X] From planting; [Y] n=287; [Z] n=256 (pooled of 500, 1000, 1500 and 2000 ppm GA_3); [W] final sheath; * Statistically different by Chi-test at level of confidence 5 %; ns-non significant; Apx-appendix.

produced the rough appendix. The appendix was commonly longer than reproductive organ (female plus male zones), but 18.8 % accessions had a shorter appendix. All GA_3 flowering had appendix about 1.5 times longer relative to the length of reproductive zones, unless the inflorescence with deformed appendix. However, due to a large number of deformed appendix in GA_3 flowering, there was no significant difference in the ratio of appendix to reproductive zones between natural and the GA_3 induced flowering (Table 2).

Seeded-berry and seed size: Berry, irrespective of natural and GA_3 flowerings, matured at 9-11 months after anthesis as indicated by bright dark red colour. At harvest, some berries were seedless and distributed randomly on pistillate flowers. Seedless berry ranged from 3-37 % (average 12 %) in inflorescence, irrespective of natural and GA_3 flowerings.

A berry contained 1-5 seeds. One- or two-seeded berries were common in an inflorescence followed by three- and four-seeded. Within three- and four-seeded berry, it was common to find a large seed alongwith other small seeds. On average, seed size ranged from 1-10 mm in diameter and 7-14 mm in length. Average seed weight tended to decrease by increasing number of seed in a berry. A 100 seeds of single-seeded berry weighed 22.0 g, while it was 12.2 g and 11.1 g in double- and three-seeded berry, respectively.

The mature seed was gray or dark green, and shape of seed depended on its number within berry. Seed from single-seeded berry commonly shaped bell or round (Fig. 3K), while double-seeded shaped half bell (Fig. 3L). Some seeds shaped irregular such as twisted, twisted-tubular and hooked, especially from those of three-or more-seeded berries.

Discussion

Phenotypic plasticity: From 36 polymorphic characters, 35 were independent of GA_3 treatment (Table 1). GA_3 applications affected three characters, *i.e.*, sheath size, disposition of the second spathe, and shape of spathe limb apex. The third and final sheaths elongated in the presence of GA_3 (Table 2). In inflorescence with double spathes, the position of first and the second spathe separated 2-4 cm in GA_3 (Fig. 2B), while 16-17 cm apart in natural flowerings (Fig. 2A). The shape of limb apex of the first or the second spathe was mostly emarginate-acuminate with three or more apex, but GA_3 treatment extended the apex forming star-like structure (Fig. 2B). Thus, minor phenotypic plasticity could be present in *A. muelleri* inflorescence especially after GA_3 application.

We treated young appendix by scratching to induce damage. Surprisingly, it recovered within a week and the appendix slightly deformed at anthesis. In subsequent flowering in Nov., 2016 when the corms were planted upside down, one out of more than 500 inflorescences produced appendix with additional form like male and female flowers (Fig. 3O). It is probable that such formation is an excess of recovery growth after mechanical damage by the action of soil impedance. Thus, it seems that mechanical damage is another inducer of phenotypic plasticity of the appendix.

At immature stage or about 5-6 months after anthesis, berries of 16 % inflorescence cracked (Fig. 3P). The rate of cracked-berry in an inflorescence was 2-16 independent of berry number in an inflorescence and GA_3 applications. Commonly, berries matured from upper zone downward. However, the cracked-berry matured earlier irrespective of the position on pistillate zone. The crack is unlikely due to lack of calcium or water fluctuation because all

plants received uniform liming and watering.

Most inflorescences produced berries with cap. The cap had different color with berry, especially at immature stage. The cap color was gray, orange, or shiny-silver that faded after the berry got mature. Some inflorescences had large cap (Fig. 3Q), different from the common small one (Fig. 3P). Across all inflorescences, the largest cap was half of the length of a berry. It needs further study whether the cap could be used as additional descriptor for *A. muelleri* identification.

In present experiment, one GA_3 treated corm produced inflorescence after leaf emergence (Fig. 1H), different to co-existing leaf in natural flowering (Fig. 1E). The inflorescence was small and failed to produce any seed. We suspected that the flower emerged from particular corm originally aged less than 3-year-old. According to Santosa *et al*. (2006b), in small corms, GA_3 applications induce leaf growth in *Amorphophallus* followed by inflorescence with deforms flower organs. Therefore, hormonal dynamics during flowering of *A. muelleri* will be an interesting study in the near future.

Genetic variation: Genetic properties could attribute variation in floral morphology of *A. muelleri*. Nine out of 45 characters were monomorphic in the inflorescence of *A. muelleri*. One among 36 polymorphic characters, *i.e.*, appendix length relative to reproductive organs, was excluded because it was affected by GA_3 treatment. Variation in limb apex was observed in both natural as well as GA_3 flowerings, but star-like shape was exclusive to GA_3 treatment. Thus, the shape of limb apex was likely stable characters, but extended shape of limb apex could be plastic. By using 35 applicable markers, 118 (41.1 %) from 287 inflorescences of natural flowering exhibited morphological variation. Briefly, 17, 7, 4, 1 and 5 markers generated variation up to 10 %, 11-20 %, 21-30 %, 31-40 % and larger than 41 %, respectively in the flowering population.

Genetic variations in *A. muelleri* could relate to mutation, gene insertion and gene translocation (Poerba and Martanti, 2008; Mekkerdchoo *et al*., 2011). According to Van der Hulst *et al*. (2003), in absolutely triploid apomicts, accumulation of mutation creates genotypic differentiation. Progenitor of the present study was obtained seeds from East Java province in 2000, similar study site of Santosa *et al*. (2003). The foundation population was established in Bogor through bulbils and seeds for about 14 years. Thus, somaclonal variation may be responsible in the present variation. In *A. rivieri*, somaclonal variation rate from corm organogenesis is 12.2 % (Hu *et al*., 2011). However, the clonal variation in *A. muelleri* has neven been evaluated.

Nikmah *et al*. (2016) noted that there is *LEAFY* gene variation in *A. muelleri* from Grobogan population. Interestingly, genetic variation based on chloroplast DNA is more marked (Mekkerdchoo *et al*., 2011; Wahyudi *et al*., 2013) than genomic DNA (Poerba and Martanti 2008; Nikmah *et al*., 2016). Santosa *et al*. (2014) found leaf chimera after seeds of *A. muelleri* were exposed to gamma ray at the level of 50 Gy. It is speculated that the plants produce different siblings or both sexual and asexual co-occur, as stated by Van der Hulst *et al*. (2003) on general apomict cases. Evaluation of mutation rate in *A. muelleri* in the near future would be interesting.

In conclusion, floral morphological variation was apparent in apomict *A. muelleri*. The variation in descriptors included peduncle length, spathe shape, translucent, spots and color, appendix shape and roughness, stigma lobe, and carpel color. The flower phenotype especially length of sheaths, disposition of double-spathe, and degree of spathe limb apex shape were plastic by the exogenous GA_3 application.

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