ISSN 2085-2916 e-ISSN 2337-3652
Tersedia daring http://jai.ipb.ac.id

J. Agron. Indonesia, Agustus 2019, 47(2):171-179
DOI: https://dx.doi.org/10.24831/jai.v47i2.19445

Yield Evaluation of Selected Clones Apomictic Iles-Iles (*Amorphophallus muelleri* Blume) on Second Growing Period

Evaluasi Hasil Klon Terpilih Apomiktik Iles-Iles (Amorphophallus muelleri Blume) pada Masa Tumbuh Kedua

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Received 9 December 2018/Accepted 8 April 2019

ABSTRACT

Increasing corm production of iles-iles (Amorphophallus muelleri Blume) through genetic improvement is important for increasing farmers' income. However, the study on variety development is rarely reported. Here, yield evaluation of the second growing period was conducted at IPB Experimental Station Leuwikopo Farm, Bogor, Indonesia (-6.5647419, 106.7220331, 17.25z) from August 2017 to July 2018 in order to select candidate clone with high corm production. One-year-old corms of 21 F1 accessions were planted under 55% shading net with four replications. The parent population was set as a control. Results showed that accessions exhibited variation in corm size. The parent population produced corm ranged 622.3-908.3 g in weight (on average 764.2 g). The pooled accessions produced corm 180.7-1527.5 g in weight, corm diameter 71.0-145.8 mm, and corm height 46.6-87.6 mm. Nine accessions produced 5.91-99.88% higher average corms weight than the parent, i.e., BKB, BS, CF, CK, CR, DPG, DPP, SBM, and SHJ. Based on 30% as the basis of corm weight improvement, we concluded that five accessions, i.e., CK, CR, DPG, DPP, and SBM as prospective candidate clones. These accessions could be further evaluated in the third growing period to select best candidates of new variety of A. muelleri.

Keywords: Agamosporous, Araceae, breeding strategy, new variety, productivity

ABSTRAK

Peningkatan produksi umbi iles-iles (Amorphophallus muelleri Blume) melalui perbaikan genetik penting untuk meningkatkan pendapatan petani. Namun demikian, studi terkait hal tersebut masih jarang dilakukan. Uji daya hasil (UDH) tahun kedua dari klon terpilih dilakukan di Stasiun Percobaan IPB Kebun Leuwikopo Bogor, Indonesia (-6.5647419, 106.7220331, 17.25z) pada Agustus 2017-Juli 2018 dalam rangka merakit varietas baru yang memiliki produksi lebih tinggi. Umbi umur setahun dari 21 aksesi F1 ditanam di bawah paranet 55% dalam empat ulangan. Populasi tetua digunakan sebagai kontrol. Hasil menunjukkan adanya keragaman karakter umbi antar aksesi. Bobot umbi tetua berkisar 622.3-908.3 g (rata-rata 764.2 g). Bobot umbi aksesi yang diuji berkisar 180.7-1527.5 g, diameter umbi berkisar 71.0-145.8 mm, dan tinggi umbi berkisar 46.6-87.6 mm. Sembilan aksesi memiliki bobot umbi rata-rata di atas tetuanya yakni BKB, BS, CF, CK, CR, DPG, DPP, SBM, dan SHJ. Bobot umbi kesembilan aksesi tersebut meningkat antara 5.91-99.88% dari bobot rata-rata umbi tetuanya. Berdasarkan kriteria peningkatan bobot di atas 30%, diperoleh 5 genotipe yakni CK, CR, DPG, DPP, dan SBM yang potensial sebagai calon varietas baru. Aksesi-aksesi tersebut perlu diuji lanjut pada periode tanam ke-3 sebelum ditetapkan sebagai varietas unggul baru iles-iles.

Kata kunci: Apomiktik, Araceae, produktivitas, strategi pemuliaan, varietas baru

INTRODUCTION

Popularity of growing tuberous *iles-iles* (*Amorphophallus muelleri* Blume, Araceae) to sustain rural farmer income through agroforestry system is increasing in Indonesia (Afifah *et al*., 2014). The corm is harvested for glucomannan production, a kind of low digestive and neutral carbohydrate that applicable to be used in food, beverage, pharmacy and other strategic industries (Haryani and Hargono, 2008; Sugiyama and Santosa, 2008; Keithley *et al*., 2013; Hananto *et al*., 2015; Dai *et al*., 2016; Yang *et al*., 2017). From 100 g dried chips, about 20-31 g pure glucomannan is obtained depend on extraction method (Dwiyono, 2014; Widjanarko and Suwasito, 2014).

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The plant usually grows under intercropping of perennial trees due to shading requirement (Sugiyama and Santosa, 2008; Zhao *et al*., 2010; Afifah *et al*., 2014). The cost of glucomannan production from *A. muelleri* is considered lower than other *Amorphophallus* species that requires a large amount of pesticides, fertilizers and replanting (Sugiyama and Santosa, 2008). The plant has multiple propagation systems using aerial bulbils and seed, supports continuous growing for years under the intercropping without replanting requirement (Zhang *et al*., 2010).

Beside high advantage in the cultivation system and ecological impacts, there is still a fundamental problem on growing *A. muelleri*, namely low crop productivity. Low productivity resulted in lower competitiveness as compared to other shade-loving crops (Rachmawati, 2008; Pranamulya *et al*., 2013; Supriadi and Pranowo, 2015). Under current practice, average annual corm production in the agroforestry is 6-12 ton ha^{-1} (PPKKP, 2013), while Dwiyono (2014) reported 4-6 ton ha^{-1}. According to Rofik *et al*. (2017) fresh corm production ranges 1-4 ton ha^{-1} in east Java, depend on production system. Previously, Sugiyama and Santosa (2008) have estimated that production might reach 40 ton ha^{-1} annually. The low productivity is likely due to low corm size uniformity and long growing period. It is common that first harvest of the corm occurs at third year after planting from seeds or aerial bulbils (Zhang *et al*., 2010). Thus, genetic improvement with high corm uniformity, short growing period and high production is required. However, study on genetic improvement of *A. muelleri* is rarely reported.

Developing new variety in *A. muelleri* is rarely studied because it produces apomictic seed (agamosporous) that set without fertilization (Dani, 2008). It is known that apomict causes low genetic variation in many plants (Barcaccia and Albertini, 2013; Kandemir and Saygili, 2015). Nevertheless genetic variation in the *A. muelleri* has been noted (Poerba dan Martanti, 2008; Sedayu *et al*., 2010; Mekkerdchoo *et al*., 2011; Rosidiani *et al*., 2013; Nikmah *et al*., 2016; Santosa *et al*., 2018). Poerba and Martanti (2008) revealed that genetic diversity among populations ranges 0.02-0.36, and Nikmah *et al*. (2016) noted that using *LEAFY* markers *A. muelleri* population exhibits different haplotype according to its origin.

According to Acquaah (2012) variety development in apomictic crop is simpler than that of conventional breeding in non-apomictic crops; see Savidan *et al*. (2001) and Hand and Koltunow (2014) for reviews. Therefore, present experiment could be first practical experience in variety development on how to improve apomict *A. muelleri* for higher yield. Here, we used 21 populations of selected elite accessions in order to develop high yielding clone. History and steps on crop improvement strategy of *A. muelleri* is also discussed.

MATERIALS AND METHODS

Corm evaluation was conducted from August 2017-July 2018 at Leuwikopo Experimental Farm (245 m above sea level) of Bogor Agricultural University, Bogor, Indonesia (-6.5647419, 106.7220331, 17.25z).

Original propagule of *A. muelleri* was introduced to Bogor Agriculture University from Nganjuk District, East Java in early 2000s. Before determining the improvement method, crop life cycle was observed from separate experiment. The observation was made in 2006-2017 during populations establishment in both farmer fields (about one hectare), in Cikabayan (about 1000 m^2) and Leuwikopo Experimental Farm Bogor Agriculture University (about 3000 m^2). In brief, plant growth and development from seeds and bulbils were evaluated.

Twenty one accessions of one-year-old corms were evaluated in present experiment, i.e., BD, BKR, LSP, SBS, SC, SMM, STL, STS, SR, SB, SGH, SGBKK, BKB, BS, CF, CK, CR, DPG, DPP, SBM, and SHJ genotypes. Selected genotypes were originally from the farmer field that planted approximately 40,000 individuals (Figure 1). The parent population was maintained under agroforestry system under 15-year-old teak plantation in the farmer property near university farm in Bogor. Light intensity under the teak plantation was around 60-70% of full sunshine. Here, original population was determined as parent population (P0).

As clonal propagated-species, genotype improvement in *A. muelleri* followed Acquaah (2012). Establishment of genetic material for present experiment was from 2014-2017; the step was presented in Figure 2. About 500 corms were collected among those individuals that produced corms larger than 2 kg and noted as P1. The corms of P1 population was then planted in Leuwikopo (Y-2; 2014-2015) under artificial shading net 55% and supplemented with sprinkler irrigation; the P1 plants that exhibited superior growth, and high corm weight were tagged, grouped and noted as P2. In 2015-2016 (Y-1), P2 corms were enforced with gibberellin (1000-2000 ppm) to stimulate flowering and seed production. Selection was made in P2 population based on unique characters of inflorescence and infructescence. Unique characters included inflorescence with extra-large size, unusual color and shape of spate, cone, peduncle color,

Figure 1. Population of *Amorphophallus muelleri* under teak plantation in Bogor using agroforestry system. Land property belongs to Dr Fred Rumawas, and used for research by his permission

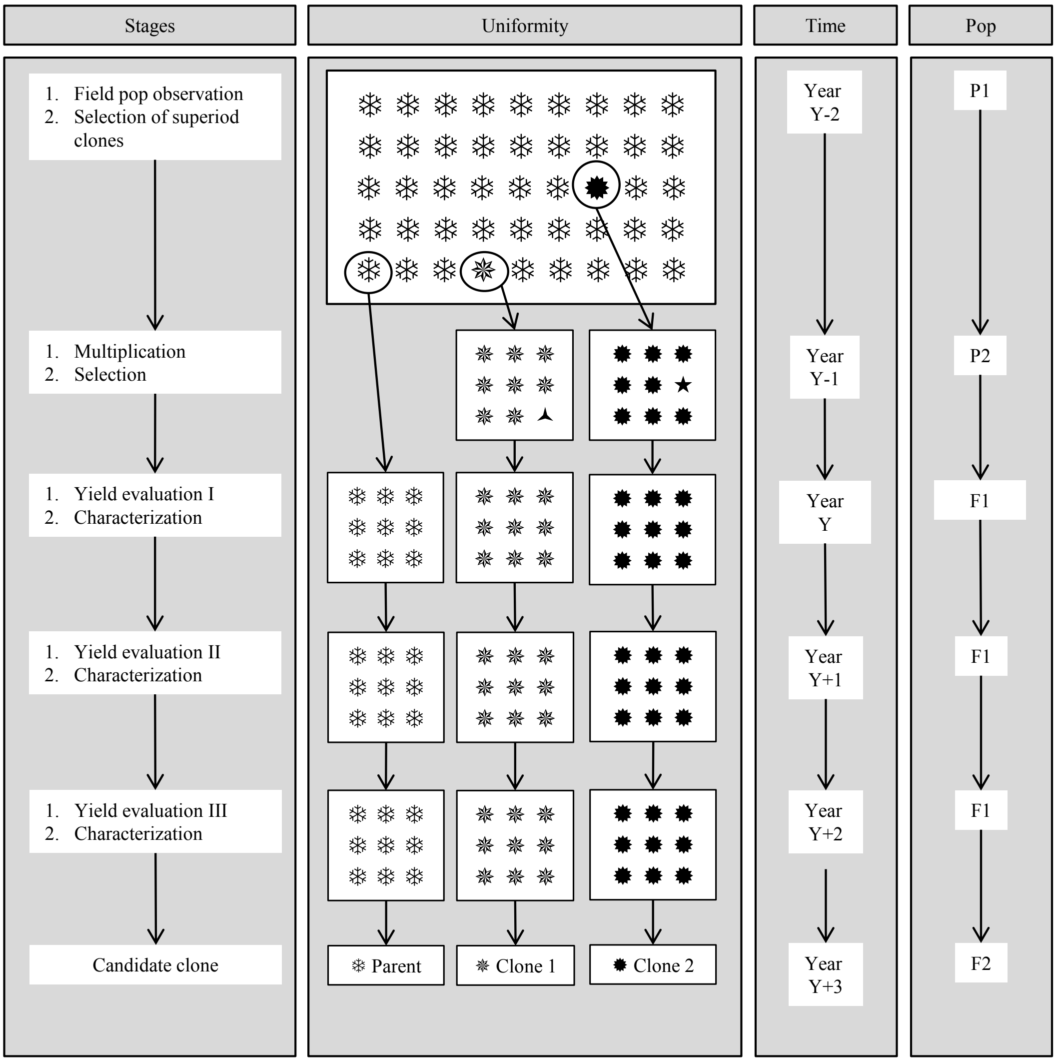

Figure 2. Five stages of new clone development on apomicts *A. muelleri* after modification from Acquaah (2012). Stage of present study is at Year Y+1. P-parent, F progeny

fruit shape, and other morphologies. From P2 population, we selected 21 accessions (F1) and harvested the seeds. Each progeny was from single corm. The seeds of each accession were planted for first yield evaluation in 2016-2017, and small corms were harvested at dry season of 2017. The harvested corm was noted as F1-1. The propagule of parent population evaluation was descended from bulbils.

In the evaluation of 21 accessions F1-1, each genotype was planted in four replications under 55% shading net; and arranged in randomized block design. Due to propagule limitation of each accessions, number of plant within replication differed, it ranged 20-87 plants.

Land was prepared using complete plowing method. Initially, the land was plowed using tractor and then harrowed twice. F1-1 seed corms (3-12 cm in diameter) were planted inside furrow seized 20 x 100 x 600 cm (Depth x Width x Length) using triangle planting distance of 50 x 50 x 50 cm.

At planting in August 2017, 1 kg m^{-1} of cow manure was applied inside the furrow and then the manure was covered with some mount of soil. At 10 weeks after planting (WAP), half of N:P:K fertilizers at rate of 100:60:80 ha^{-1} was applied. The other half of NPK fertilizers was applied at 20 WAP. Nitrogen was originally from compound NPK fertilizers (NPK Mutiara, 15% NPK). Additional nitrogen was obtained from urea (46% N), potassium from KCl (60% K_2O) and phosphorus from SP-36 (36% P_2O_5). Weeds were uprooted manually. Watering was provided from rainfall without any supplement irrigation.

Harvesting F1-2 corm was conducted at 35-40 WAP (May-July 2018) at the time plant entered dormancy as indicated by complete leaf senescence. Corm characters included fresh weight, diameter and height, corm shape and healthiness were evaluated. Marketable size was determined from the fresh corm weight ≥ 1,000 g, free from disease infection and shaped globose or depressed-globose.

Corm weight data of F1-2 was evaluated using ANOVA. Further evaluation was conducted using LSD at level of 5% when F test exhibited significant different. Here, we hypothesis that *A. muelleri* is obligate apomictic thus progeny is 100% maternal. In this context, estimates for selection advanced and expected genetic advance were based on broad sense heritability (h^2_{bs}), see Mulyadiana *et al*. (2019) and Syukur *et al*. (2015) with slight modification. Briefly, selection advanced (SA) $=\bar{x}F_c-\bar{x}F_p$; where $\bar{x}Fn$ = mean current population and Fc = mean parent population. Expected genetic advance (G) = $(i)(\sigma p)(h^2_{bs})$; where (i) = selection intensity, (σp)= standard deviation, (h^2_{bs}) = broad sense heritability. Broad sense heritability was estimated from equation: $(h^2_{bs}) = [(\sigma^2F_c-(\sigma^2F_p+\sigma^2F_c)/2]/\sigma^2F_c$; where σ^2F_c = variance of current population, σ^2F_p = variance of parent population. Selection proportion (SP) was estimated from average of two selection steps, i.e., (SP)={[(500/40000)+(21/500)] x 100%}/2={[0.0125+0.042] x 100%}/2=2.725% of the population; selection intensity (i) based on reference table was determined as 2.06.

RESULTS AND DISCUSSION

Crop Life Cycle

In the field growing period followed the climatic season. The seed, bulbils and seed corm actively produced leaf(s) during rainy season and went dormancy during dry season. The plant exhibited perennials life cycle. In the first three year, it predominantly produced leaf and then an inflorescence emerged in rainy season at fourth year (Figure 3). All inflorescences produced berries and seeds without any leaf, called as solitary inflorescence. The berries matured in subsequent dry season.

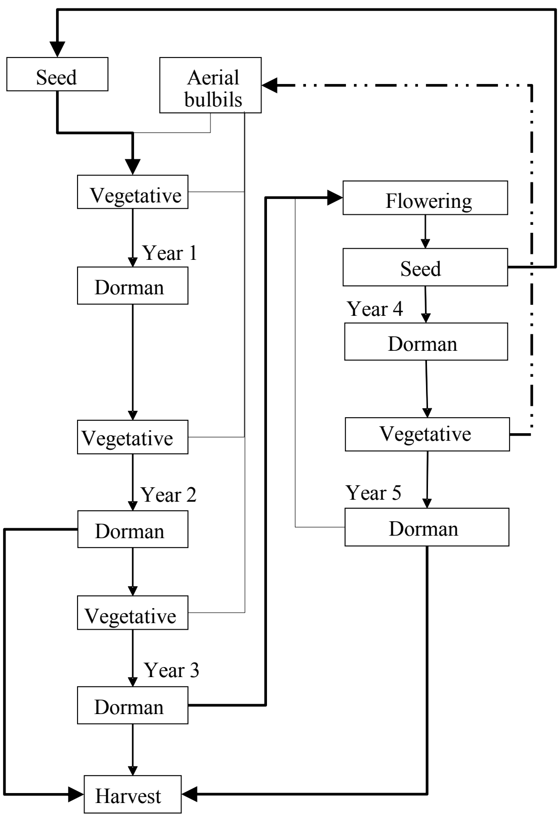

Figure 3. Life cycle of *A. muelleri* including seeds and aerial bulbils sources of an existing population

Corm after flowering continued to produce single large leaf in the next rainy season. After leaf dormancy, the corm produced another larger inflorescence than previous one. It meant that after first flowering, subsequent flowering time occurred at every two years (biannual cycle) as shown in Figure 3. This flowering cycle has been described (Santosa *et al*., 2016a). In our experiment, removing inflorescence stimulated vegetative growth; however, the corm produced another inflorescence in the next growing season.

The largest corm size was harvested after leaf senescence at the end of third year or at dormant state after vegetative (Figure 3). The harvest directly from flowering corm was not recommended, because the corm had abnormal shape. Also, according to Sugiyama and Santosa (2008), corms after flowering has low dry mass and glucomannan content. It is worthy to note that under Bogor climate, dry season commonly occurs May to August while rainy season occurs on September to April. Unlike vegetative growth that was completely suspended during dry season, the generative growth e.g. fruit maturation continued during dry season until fruiting complete at the end of the dry season. Nevertheless, in some years with extended rainy season (also meaning short dry season) such as in 2016 some plants with leaves postponed dormancy until May/June or produced leaves earlier in August than usually in September/October. Thus, it is important to consider climatic situation with distinct dry season to obtain optimum dry mass content in the *A. muelleri* production.

Yield Performance

Accessions of F1-2 population exhibited variation in corm weight, diameter and height (Table 1). The corm size variation was higher in the progeny than the parent population. Parent population produced corm 622.3-908.3 g (average 764.2 g), while average progeny population produced 11.0-2207.0 (average 760.0 g). Minimum corm weight across progeny accessions was 11.0-891.0 g and maximum 440.7-2207.0 g (average 180.7-1527.5 g). Minimum corm diameter was 23.0-117.9 mm and maximum 107.2-263.8 mm (average 71.0-145.8 mm). The minimum corm height ranged 21.6-77.5 mm and the maximum ranged 64.4-107.9 mm (average 46.6-87.6 mm).

Estimate values on selection advanced and expected genetic advanced were negative (Table 1). These negative values could be due to high incident of extreme small corm size in the some accession. After correction by omitting the extra small corms (< 50 g), selection advanced estimate for corm weight, corm diameter and corm height were 100.81 g, 11.08 mm and 4.98 mm, respectively. It indicated that all 21 accession collected need further selection.

From 21 accessions 12 accessions had average corm weight below its parent, i.e., BD, BKR, LSP, SBS, SC, SMM, STL, STS, SR, SB, SGH, and SGBKK; and 9 other accessions had above the parent, i.e., BKB, BS, CF, CK, CR, DPG, DPP, SBM, and SHJ. Across nine accessions, corm weight increased by about 5.91-99.88% from the parent. Based on 30% increasing rate in corm weight, five

Table 1. Genetic parameters of progeny population of *A. muelleri* from second growing period

Genetic parameters	Fresh corm weight (g)	Corm diameter (mm)	Corm height (mm)
Selection advanced (SA)	-110.320	-3.000	-0.770
Broad sense heritability (h^2_{bs})	-0.084	-0.013	-0.006
Expected genetic advanced (G)	-80.466	-0.882	-0.216

accessions, i.e., CK, CR, DPG, DPP, and SBM gained 99.88%, 48.60%, 46.38%, 34.90% and 31.02% higher from its parents, respectively (Table 2). Present finding showed that corm size in *A. muelleri* was likely affected by accession name; and it is not only determined by plant age as stated by Budiman and Arisoesilaningsih (2012). According to Poerba and Martanti (2008) *A. muelleri* in Saradan population exhibits high genetic diversity. The district name similar to the area of the original source of the propagule in present study; we speculate the presence of mutation in *A. muelleri* propagule as shown in present study.

The average minimum corm weight across accessions was mostly smaller than that of the parent, with exceptions DPG and CK genotypes were 43.18% and 22.45% larger than its parent, respectively (Table 2). The superiority of DPG and CK genotypes to the parent also was noted in diameter and height. Further selection based on corm weight is needed, because corm diameter and height according to Santosa *et al*. (2004) is affected by culture practice; deep planting causes corm elongation. Thus, corm weight seems as better estimator to distinguish *A. muelleri* accession. This case was observed in CR genotype, where average

Table 2. Relative percentage of corm weight, corm diameter, and height of twenty-one *A. muelleri* accessions to its parent from second growing period

Accession Code	Corm weight (%)[z]			Corm diameter (%)			Corm height (%)		
	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg
BD	-71.40bc	24.85bc	-16.65b	-45.23bc	14.92b	-4.23b	-21.52ab	24.01ab	3.12ab
BKB	-47.72bc	78.25ab	5.91ab	-12.73ab	26.11b	7.61ab	-2.79a	41.14a	17.04ab
BKR	-84.49c	-11.81bc	-43.04bc	-40.46b	0.42b	-14.70b	-34.90ab	6.51bc	-10.90bc
BS	-56.43bc	83.11ab	27.17ab	-15.36ab	34.41ab	15.33ab	-12.41a	38.86a	19.53ab
CF	-50.15bc	76.11ab	16.55ab	-13.51ab	24.44b	10.75ab	-12.97a	30.47ab	13.90ab
CK	22.45bc	142.98a	99.88a	2.40ab	38.54ab	26.80a	11.89a	29.70 ab	23.69ab
CR	-21.25b	101.23ab	48.60ab	2.85ab	30.91ab	21.62a	5.95ab	42.77a	27.55a
DPG	43.18a	51.05ab	46.38ab	13.83a	20.64b	18.74ab	25.99a	24.03ab	25.97a
DPP	-38.05b	74.94ab	34.90ab	-8.45ab	23.70b	15.56ab	1.94ab	24.13ab	17.00ab
SBM	-38.92b	99.30ab	31.02b	-13.86ab	29.56ab	12.39ab	-4.63a	35.54ab	17.62ab
LSP	-70.89bc	32.25b	-13.40b	-28.29ab	10.95b	-2.84b	-24.81ab	12.22b	-0.66b
SBS	-98.23c	-36.47c	-71.42c	-77.84c	-6.05b	-34.85c	-59.24b	-8.83c	-31.36c
SC	-68.10bc	21.09 bc	-25.11b	-31.28b	9.67b	-7.97b	-24.36ab	10.10b	-7.76b
SHJ	-40.46b	76.54ab	28.58ab	-9.06ab	17.80b	9.04ab	8.16a	23.18ab	18.81ab
SMM	-78.82bc	18.11bc	-24.15b	-38.94b	10.67b	-6.51b	-24.34ab	15.41b	-1.93b
STL	-75.37bc	40.34b	-8.70b	-30.52bc	13.22b	-1.89b	-20.39a	24.47ab	2.33b
STS	-97.48c	-51.48c	-76.35c	-72.90c	-15.27b	-38.22c	-64.83b	-14.86c	-32.24c
SR	-77.72bc	31.93b	-26.90b	-35.44b	15.22b	-6.91b	-26.69ab	20.01ab	-2.25b
SB	-83.61bc	92.47ab	-11.24b	-40.88b	108.50a	4.03ab	-41.87ab	41.87a	3.91b
SGH	-83.33bc	53.42ab	-13.38b	-33.88b	20.55b	1.26ab	-33.74ab	23.68ab	2.21b
SGBKK	-86.94bc	34.40b	-24.36b	-43.29b	20.55b	-3.12b	-52.44b	28.31ab	-6.12b
Parent[y]	622.30	908.30	764.20	103.60	126.50	115.00	61.50	75.60	68.70

Note: [z]Negative value indicates smaller than the parent. [y]Corm weight in gram, diameter and height of corm in mm. Values in a column followed by different alphabeth is significantly different after LSD test at 95% level of confident. Statistical analysis after data transformation using log(x+200) diameter and height of corm were larger but its corm weight was 21.25% smaller than the parent. Nevertheless, it needs further study because the shape of corm is commonly used to distinguish tuberous genotype like in *Dioscorea sp*. (Islam *et al*., 2011; Chinedu, 2017).

In term of production potential, assuming the planting density in the production field was 4 plant m^{-2}, five accessions had production potential about 40 ton ha^{-1} of freshtuber or larger, i.e., CK, CR, DPG, DPP and SBM (Figure 4). Dashed line is about 40 ton ha^{-1} based on potential yield proposed by Sugiyama and Santosa (2008). Interestingly, CK accession had potential yield 61.1 ton ha^{-1}, the highest among accessions.

Superiority of the parent across some tested genotypes is presumably due to differences on the initial propagule size at planting, as previously been observed by Sugiyama and Santosa (2008). In *A. paeoniifolius*, Santosa and Sugiyama (2007) noted that larger seed corms produced larger daughter corms. In present research, propagule of the parent was derived from bulbils (10-20 g) while progeny was from seed. The different propagule source could be another factor contributed to negative values of genetic properties of the population. In the future, it is important to evaluate similar propagule such as using aerial bulbils from both the parent and the progeny.

Marketable Size

Accessions exhibited different number on marketable sized-corm (Figure 5). The parent produced 32% corm as marketable size. Nine accessions, i.e., BKB, BS, CF, CK, CR, DPG, DPP, SBM and SHJ produced higher percentage of marketable corm weight than that of the parent; the highest percentage was from CK and CR accessions with percentage 66.67% and 66.98%, respectively. Judgment from such criteria, both CK and CR accessions could be further evaluated as the most prospective clones of *A. muelleri*.

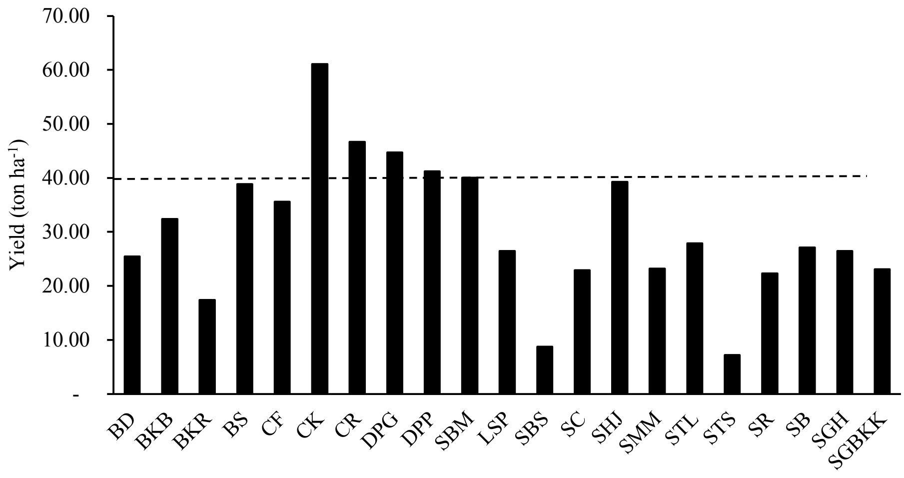

Figure 4. Potential yield of fresh corm production among *A. muelleri* accessions

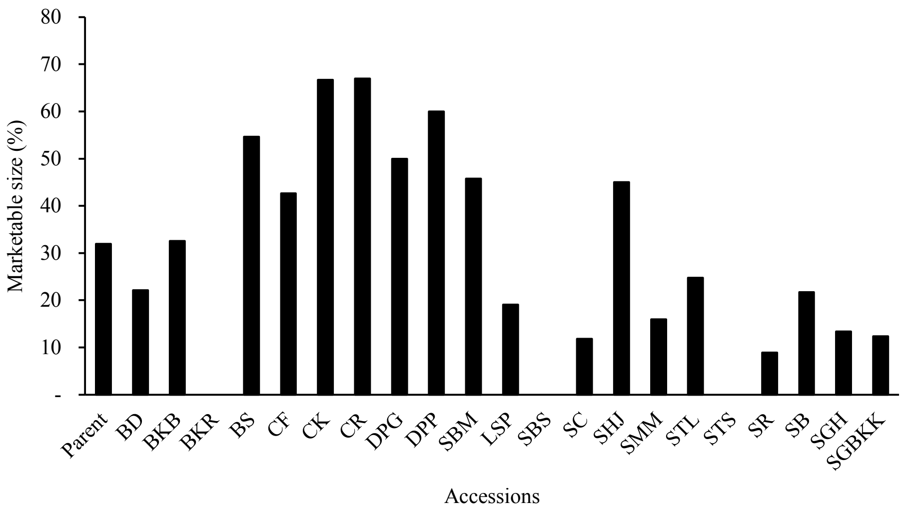

Figure 5. Percentage of marketable corms from 21 accessions of *A. muelleri* from second growing period evaluation. No value of BKR, SBS and STS accessions indicated those all accessions produced fresh corms smaller than 1,000 g

Indeed, Sugiyama and Santosa (2008) have noted that marketable size is 3-year-old corms with > 1500 g in weight. In *A. paeoniifolius* Santosa and Sugiyama (2007) concluded that seed corm size is known directly correlate with the corm weight at harvest, i.e., larger sized-seed corms absolutely produce larger sized-daughter corms at harvest. Here, we evaluated second growing period, and the corms will be used as seed corms for the third growing period. In farmer field, according to Sugiyama and Santosa (2008) harvests corm 500-600 g in weight is common from second growing period. In present experiment, corms weight was 1167-1527 g on average or 133-155% higher than that usually harvested by farmer. It means that early harvesting in *A. muelleri* is possible by obtaining fast growing clones such as using CK and CR accessions.

We noted that three accessions produced corms without marketable size in present experiment, i.e., BKR, SBS, and STS (Figure 6). The SBS and STS accessions mostly produced corms smaller than 250 g at rate 61 and 71 %, respectively, while BKR accessions mostly produced corm smaller than 500 g. The cause of light weight corm production in these three accessions need further study whether it was caused by genetic properties or by other factors. In *A. muelleri*, application of N and K fertilizers significantly increase corm weight (Sumarwoto and Widodo, 2008; Santosa *et al*., 2016b). According to Santosa *et al.* (2016b) application 100 kg N, 60 kg P_2O_5 and 80 kg K_2O for one hectare increases corm production by 63% than without fertilizer application.

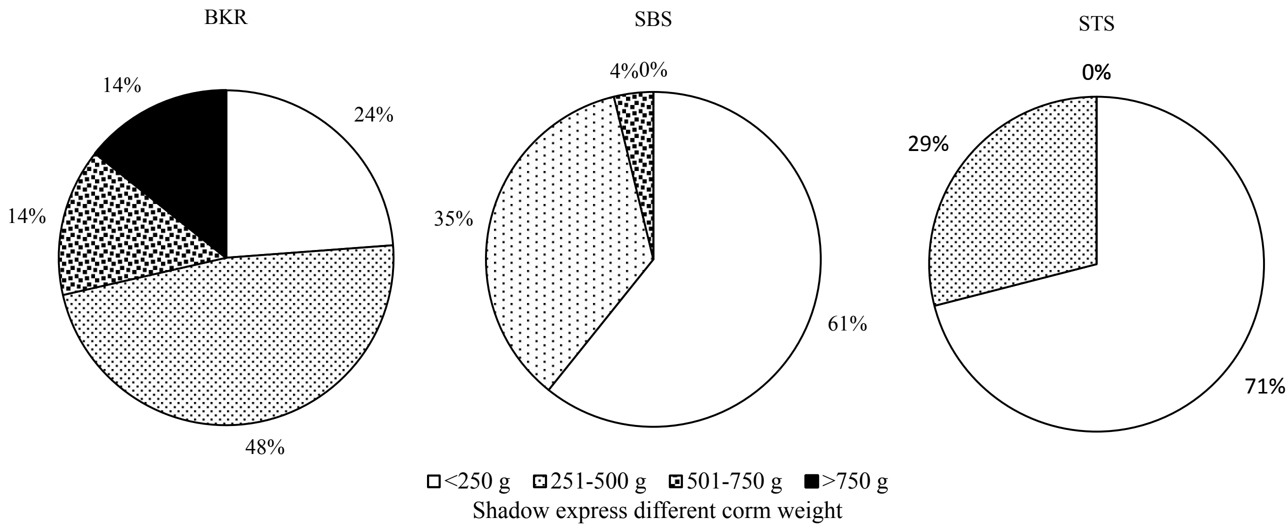

Figure 6. Corm weight distribution of BKR, SBS and STS accessions that failed to produce any marketable size

CONCLUSION

Nine out of 21 accessions were potential as new clonal of *A. muelleri* because they had average corm production 5.91-99.88% larger than the parent, i.e., BKB, BS, CF, CK, CR, DPG, DPP, SBM, and SHJ genotypes. Moreover, five accessions, i.e., CK, CR, DPG, DPP, and SBM gained 99.88%, 48.60%, 46.38%, 34.90% and 31.02% higher than its parent, respectively. Present study implies that improving production through clonal variation is prospective in *A. muelleri*. It needs further evaluation on genetic properties including heritability in order to understand the most valuable characters for selection in *A. muelleri* improvement.

ACKNOWLEDGEMENTS

We would like to thank Ministry of Research, Technology and Higher Education (Menristekdikti), The Republic of Indonesia for financial support by the Decentralized Strategic Applied Research Grant (PTUPT) for FY 2017-2018 to Dr Edi Santosa.

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