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Research Article

Proliferation of porang (*Amorphophallus muelleri* Blume) from bulbils and leaf cutting treated by NAA and BA

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ABSTRACT

Javanese konjac or iles-iles (Amorphophallus muelleri Blume, Araceae) is a tuber crop native to Indonesia as a source of carbohydrat, also contains a lot of glucomannan, which has high economic value as a raw material in industry. The study aimed to develop the propagation method of A. muelleri from bulbils and leaf cuttings through the plant growth regulator (PGR) application of α-naphthaleneacetic acid (NAA) and benziladenin (BA). The research was conducted from August 2020 to December 2021 at IPB University, Bogor. The study consisted of three experiments based on the type of propagation material. Each experiment used a randomized complete block design with three factors, i.e., NAA concentrations (0, 2, and 4 mg L^{-1}), BA concentrations (0, 5, 10, and 15 mg L^{-1}), and propagation materials (Experiment 1: small, medium, large bulbils; Experiment 2: immature and mature peak leaflet cuttings; Experiment 3: immature and mature base leaflet cuttings). The results showed that there was an interaction of three factors in the bulbils and PGR application, which had a significant effect on the percentage of axillary shoot proliferation and growth. In Experiment 2, PGR application was unable to regenerate peak leaflet cuttings, whereas in Experiment 3 PGR encouraged base leaflet cuttings to form shoots, roots, and bulbils, especially at concentrations of 2 mg L^{-1} NAA+15 mg L^{-1} BA and 4 mg L^{-1} NAA+15 mg L^{-1} BA.

Keywords: α-naphthaleneacetic acid, benziladenin, iles-iles, Javanese konjac, plant growth regulators

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INTRODUCTION

Porang or Javanese konjac or *iles-iles* (*Amorphophallus muelleri* Blume, Araceae) is a tuber crop native to Indonesia that contains a lot of glucomannan (Sugiyama & Santosa, 2008; Nurlela et al., 2021). Glucomannan is widely used as raw materials in food, beverages, biotechnology, chemical, textile, and paper industries (Behera & Ray, 2016; Supriati, 2016; Wardani et al., 2021). The wide use of glucomannan stimulates the extensive planting area of *A. muelleri* in Indonesia.

The Indonesian government encourages farmers to cultivate *A. muelleri*, especially in forest margins and open land (Dewi et al., 2021). For example, tuber production in East Java continues to increase, and in 2017 it was recorded at 4,840 tonnes (Dishut, 2018). Many *A. muelleri* fields implement agroforestry systems, with average productivity of 2-4 tonnes per hectare (Afifah et al., 2014; Santosa, 2014). The low tuber productivity is affected by limited genetic improvement due to low genetic diversity, long life cycle,

dormancy, and apomicts (Sugiyama & Santosa, 2008; Poerba et al., 2016; Yulianto et al., 2016; Tajuddin et al., 2020). Therefore, a breakthrough is needed to increase productivity through improved cultivation, for example by providing quality seeds.

Conventionally, propagation of *A. muelleri* uses tubers, bulbils, and seeds (Sugiyama & Santosa, 2008; Hidayah et al., 2018; Ibrahim, 2019). Azizi & Kurniawan (2021) recommended bulbils as planting material because their morphology resembles tubers. Nevertheless, such propagation takes a long time because there is a dormancy period of 1-5 months (Sumarwoto, 2005; Sugiyama & Santosa, 2008). Ibrahim (2019) recommended using tissue culture for rapid propagation. However, in vitro propagation requires experts, the process is complex and the materials used are relatively more expensive, making it difficult for farmers to implement. Therefore, a mass propagation method that is more affordable and applicable to farmers is needed.

Santosa & Wirnas (2009) carried out rapid propagation using tuber skin, but the success rate of nurseries is still far from expected. Prayoga et al. (2022) used a plant growth regulator (PGR) available in the market to increase the shoot growth of bulbils; he revealed 5 mL L^{-1} of PGR application accelerated the number of growing points.

According to Rademacher (2015), PGR is a chemical compound in the form of a hormone that can control plant growth and development. PGR application to increase the growth of *A. muelleri* leaf cuttings was evaluated by Sumarwoto (2008), who found that indole-3-butyric acid (IBA), α-naphthaleneacetic acid (NAA), and indole-3-acetic acid (IAA) at concentrations of 0, 500, 1,000 and 1,500 ppm increase the growth rate of leaf cuttings by 34%. Previously, Aryadi (2004) manipulated leaf cuttings to produce bulbil-sized tubers using Rootone-F but it was unsuccessful in producing shoots. Thus, information related to the influence of the type of planting material and its potential for PGR is still not well consolidated. The research aimed to develop a method for propagating quality seeds from bulbils and leaf cuttings through the application of NAA and benziladenin (BA).

MATERIALS AND METHODS

The research was conducted from August 2020 to December 2021 at the Tissue Culture Laboratory and the Microtechnic Laboratory, Department of Agronomy and Horticulture, Bogor Agricultural University, IPB Dramaga, Bogor, Indonesia. The seedling was maintained in the plastic shade house. Plant material was obtained from a collection belonging to the Department of Agronomy and Horticulture.

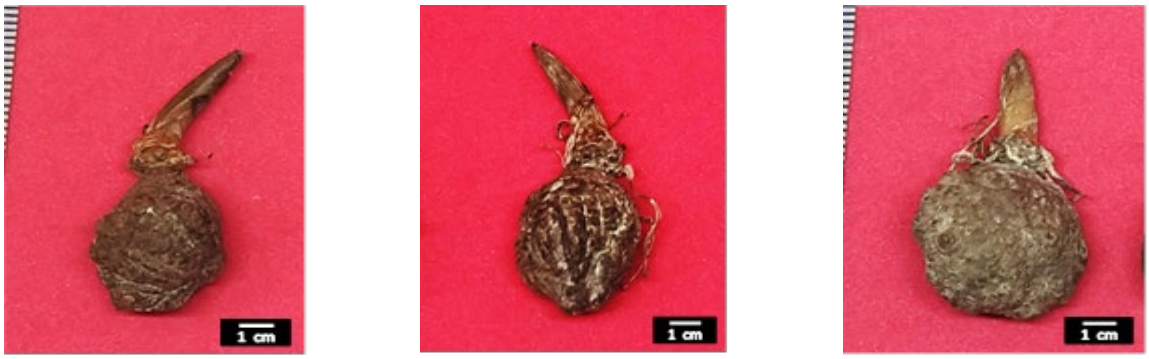
The study consisted of three experiments. Each experiment used a randomized complete block design (RCBD). The three experiments were carried out in parallel activities.

Experiment I

The first factor was the BA concentration with 4 levels (0, 5, 10, and 15 mg L^{-1}), and the second factor was NAA concentration with 3 levels (0, 2, and 4 mg L^{-1}). The third factor in the first experiment was the bulbil as planting material which was harvested about 5 months earlier (Figure 1). Bulbils were grouped based on diameter including small (1.3-1.5 cm), medium (1.6-1.7 cm), and large (1.8-2 cm). Block was made based on the size of the bulbil. Each experimental unit consisted of 3 bulbils so there were 108 units of observation.

The planting media was a mixture of soil and husk charcoal (2:1, v/v) and then sterilized by autoclaving for 20 minutes. The media was kept in a tray (36 cm x 28 cm x 11.5 cm) with the top covered with plastic wrapping. Solutions combination of BA and NAA were prepared in 500 mL.

PGR was applied by soaking the bulbil for 1 hour. After drying, the bulbils were planted with a spacing of about 2 cm x 2 cm in a tray. After planting, the top of the tray is covered by plastic wrap. The time of planting was noted as 0 weeks after planting (WAP). At 2 WAP, bulbils or plants were transferred into polybags (5 kg) and observed until 9 WAP.



(A) (B) (C)

Figure 1. Propagules used in experiment. (A) Small bulbil, (B) Medium bulbil, (C) Large bulbil.

Observations included: increase in shoot height, plant height, time of leaf and shoot emergence, new tuber diameter, number of shoots, number of plants experiencing proliferation (%), number of bulbils forming shoots (%), number of bulbils producing plants (%) and organogenesis. Histological analysis was performed on the apical and axillary shoots formed in each treatment using Mendoza et al. (1993). Samples were hydrated using a series of alcohol grades and immersed in a mixture of 100% alcohol and resin (1:1, v/v; Hisstoresin, Leica, Heidelberg, Germany) for 4 hours and stored in pure historesin for 24 hours. The resin was polymerized by the addition of a compactor and the samples were divided into 8-µm sections using a rotatory microtome. Samples were stained for 2 minutes with 0.05% toluidine blue and 0.01% fuchsin acid and covered with a cover glass. The samples were observed under a microscope (Olympus CX23) with 400X.

Experiment II

The second experiment used RCBD with 3 factors. The first and second factors were the same as Experiment I, while the third factor used cuttings of leaves collected from plants aged 3 years; see Sugiyama & Santosa (2008) for criteria. The peak leaflets were taken from different leaf ages (immature and mature leaves). The immature leaf was still lance-shaped about 2 weeks after emergence, while the mature leaf aged > 3 months after emergence. The treatment was repeated 10 times.

The experimental procedure was the same as Experiment I. The peak leaflets were cut into triangular-shaped (±5 cm long) and then the cutting part was dipped into the PGR solution according to concentration for ±5 seconds.

Soaked leaves were planted in a tray with a spacing of 2 cm x 2 cm then the top of the tray was covered by plastic wrap. The planting media was the same as in Experiment I. The observed variables were the percentage of live cuttings, the percentage of rooted cuttings, the percentage of sprouting cuttings, the percentage of cuttings forming bulbils, and the percentage of plants that experienced proliferation.

Experiment III

The design of Experimental III was the same as Experiment I. The first and the second factors were the same as in Experiment I, the difference was the third factor. The third factor was base leaflet cuttings which were grouped based on leaf age (immature and mature leaves). The criteria for immature and mature leaves were the same as in Experiment II. The base leaflets were 1-2 cm in diameter, and the cutting was about 10 cm long. The treatment was repeated 5 times. Each experimental unit consisted of a single cutting material. The growing media and observation variables were the same as in Experiment I.

Data analysis

Data from the three experiments were processed using Microsoft Excel, and subjected to the F test using SAS version 9.4. For any significant effect, data then was evaluated using the Duncan Multiple Range Test (DMRT) with a level of 5%. Histology data were processed using the ImageJ application.

RESULTS AND DISCUSSION

Shoot from bulbils

ANOVA showed that the treatment of bulbil size, NAA, and BA did not significantly interact with the weekly plant height (data not shown). Bulbil responded to BA application significantly on plant height at 2, 4, and 5 WAP (Figure 2A). The control plants tended to be taller than the other BA treatments even though the statistics were not significantly different (Figure 2A). The application of exogenous PGR together with endogenous, functioned to control vegetative growth according to Paulo & Dias (2019). This effect is thought to have affected *A. muelleri* height in this experiment, although according to Mangena (2020), BA functions to stimulate protein synthesis and cell division thereby increasing shoot initiation.

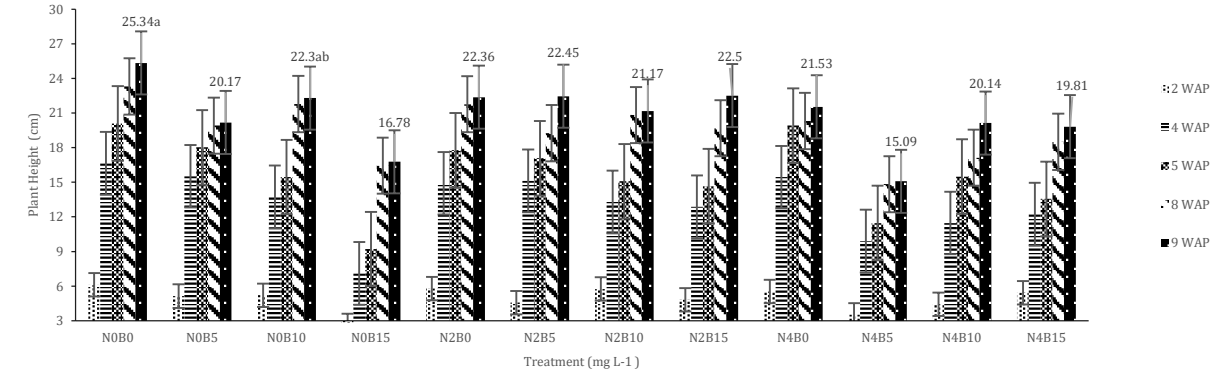
In the early growth of *A. muelleri*, Sugiyama & Santosa (2008) stated that food reserves in seed tubers or bulbils were used for growth until established and then relied on assimilation. Susanto et al. (2014) also stated that food reserves in tubers play an important role in the initial growth process of *Amorphophallus*.

The morphology of *A. muelleri* bulbils has similar characteristics to corms but they are smaller in size and emerge on leaf branches (Nugrahaeni et al., 2021). On the surface of the bulbil skin, many dormant buds form small protrusions on the entire bulbil surface (Santosa & Sugiyama, 2007; Sugiyama & Santosa, 2008). The shoots that emerged from the dormant buds in this study were referred to as axillary buds.

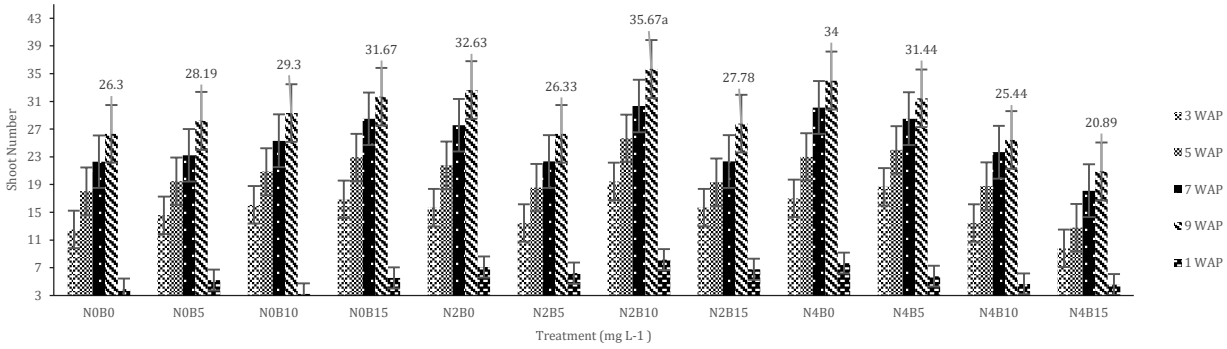
Based on ANOVA, it was shown that diameter treatment, single NAA, single BA, and the interaction of the three factors had no significant effect on the number of shoots and the time of shoot formation each week. The interaction of NAA × BA showed a significant effect on the number of shoots and the time of shoot formation (Figure 1). The treatment of 2 mg L^{-1} NAA with 10 mg L^{-1} BA produced the highest average number of shoots, i.e., 8.2 shoots (Figure 2B) with the fastest shoot formation time, i.e., 1.0 WAP (Table 2) although not significantly different for all treatments.

Figure 3 is a visualization of the buds emerging from the bulbil. On each bulbil, regardless of size, one main bud (main apical bud) with the largest size appeared among the other buds. Just below the main apical bud was a new tuber (called daughter corm). The apical bud is considered to have a large apical dominance (Sugiyama & Santosa, 2008), which suppresses the growth of other axillary buds. However, the existing axillary shoots also enlarged, although not as large as the main shoot (Figure 3B). Mulyani & Ismail (2015) stated that exogenous PGR could activate endogenous auxin performance and increase cytokinin levels in plants thereby stimulating the emergence of new shoots.

ANOVA showed that NAA treatment and interaction of NAA×BA×tuber size had no significant effect on shoot height (data not shown). Single BA treatment and bulbil size had a significant effect on the increasing shoot length. Treatment of 0 mg and 10 mg L^{-1} BA produced the best shoot length gain for all bulbil sizes but they were not significantly different from the other treatments (Table 1). The increase in shoot length in the BA treatment and without BA could be influenced by several factors such as the plant growth phase, PGR type, concentration, and method of PGR application (Saefas et al., 2017). It is speculated that the bulbil has passed the slow growth phase and underwent the exponential growth phase (Mata, 2021). In addition, the increase in shoot height is less than optimum as a result of the large number of shoots that form. Bozsó & Barna (2021) stated that BA plays a role in activating cell division so that it stimulates the formation of shoots.

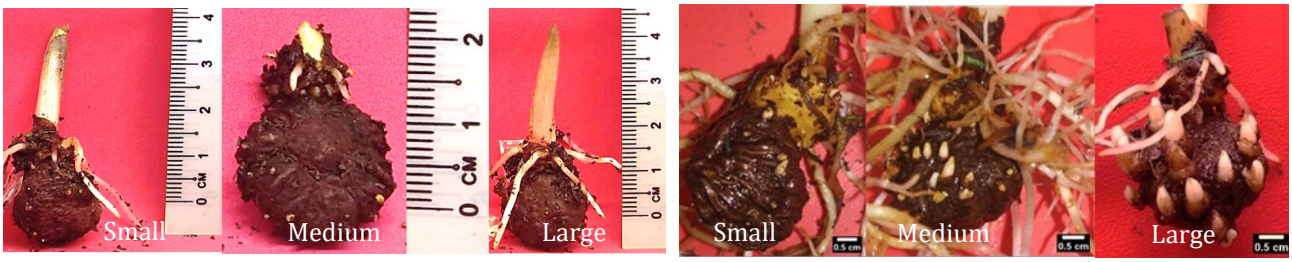


(A)



(B)

Figure 2. Plant height and bud number of *A. muelleri* growing from bulbils on different NAA and BA concentrations. (A) Plant height, (B) Bud number; N0-0 mg L^{-1} NAA, N2-2 mg L^{-1} NAA, N4-4 mg L^{-1} NAA; B0-0 mg L^{-1} BA, B5-5 mg L^{-1} BA, B10-10 mg L^{-1} BA, and B15-15 mg L^{-1} BA; WAP-weeks after planting.



(A) (B)

Figure 3. Buds growth of different-sized bulbils of *A. muelleri* treated with 4 mg NAA L^{-1} and 15 mg BA L^{-1}. (A) bud growth at 2 weeks after planting; (B) bud growth at 9 weeks after planting.

Table 1. Bud length, daughter corm diameter, and apical bud proliferation of *A. muelleri* from different propagule sizes and BA concentration at 4 weeks after planting.

Treatment		Bud length	Diameter of	Apical bud
Bulbil size	BA (mg L^{-1})	(cm)	daughter corm (cm)	proliferation (%)
Small	0	1.0±0.3ab	1.4±0.1a	11±0.2b
	5	0.6±0.2b	1.4±0.0ab	0.0 a±0.0c
	10	1.0±0.7ab	1.2±0.2b	33±0.6b
	15	0.6±0.5b	1.1±0.1b	67±0.6a
Medium	0	1.2±0.4a	1.4±0.1a	11±0.2b
	5	0.6±0.4b	1.3±0.1ab	11±0.2b
	10	1.0±0.5ab	1.2±0.2b	22±0.2b
	15	0.8±0.3ab	1.3±0.1ab	89±0.2a
Large	0	1.3±0.4a	1.5±0.1a	0.0±0.0c
	5	0.6±0.5b	1.2±0.3b	0.0±0.0c
	10	0.8±0.2ab	1.3±0.2ab	56±0.5a
	15	0.5±0.4b	1.2±0.1b	100±0.0a
F test		*	*	*
CV (%)		9.9	25.9[t]	14.2[t]

Note: Values in the same column and treatment followed by the same letter are not significantly different at DMRT α = 5%; *= significant effect; [t]Data transformation using $(n+0.5)^{1/2}$; Mean±SD

ANOVA showed that the NAA treatment and the interaction between NAA×BA× bulbil size had no significant effect on new tuber diameter. Likewise, the treatment of bulbil size and NAA×BA interaction had no significant effect on the time of leaf formation (data not shown).

Bud of bulbil grew and formed leaves. The fastest leaf emergence was obtained from control plants without BA irrespective of NAA concentrations (Table 2). The fastest time for leaves to appear was 3.4 WAP in the control without BA followed by the application of 5 mg L^{-1} of BA which was 3.8 WAP although not significantly different from the other treatments. The leaves developed from the apical bud as the main growing point. When the apical bud develops and produces leaves, generally the axillary buds/lateral buds around the apical bud experience dormancy. Axillary buds can grow and develop into buds if there is interference with the apical bud (Xue et al., 2020).

Plants of bulbil origin produced daughter corm in all PGR treatments with relatively the same size (Table 1). The finding indicates that plants from bulbils had the natural ability to produce new daughter corm, and it took ± 3 weeks to develop a fully expanded leaf. Figure 2A shows that the daughter corm had formed even though in the bud stage.

ANOVA showed that NAA × BA × bulbil size interaction was not significant on the percentage of bulbils forming plants, new tubers, apical shoots, and axillary shoots (data not shown). Table 2 shows that the percentage of plants producing bulbils and daughter corm was 89% or more in all NAA and BA treatments. The percentage of plants producing bulbils was significantly lower in the 10 mg BA L^{-1} treatment in the presence of NAA. The effect of BA rebound was similar to control without NAA and BA by 15 mg BA L^{-1} application.

The percentage of plants producing apical buds reached 100% in control plants without BA treatment (Table 2). In bulbils treated with 2 mg NAA L^{-1}, an increase in BA concentration affected the percentage of plants producing apical shoots. However, at 4 mg NAA L^{-1}, only 5 mg BA L^{-1} treatment significantly suppressed apical shoot growth. The difference in the response of NAA and BA application on the apical bud is likely correlated with a balance of auxin and cytokinin as responsible hormones for cell division. The situation of high auxin (low cytokinin) and high cytokinin (low auxin) in the tissue encourages the formation of shoots (Su et al., 2011; Yulizar et al., 2014).

Table 2. Time to shoot and leaf emergences, percentage plant produced apical bud, new tuber, bulbils, and axillary buds of *A. muelleri* from different NAA and BA concentrations.

Treatment		Shoot emergence (week) [z]	Leaf emergence (week) [z]	Plant produced apical bud (%) [y]	Plant produced axillary bud (%) [y]	Plant produced new tuber (%) [y]	Plant produced bulbils (%) [y]
NAA (mg L^{-1})	BA (mg L^{-1})						
0	0	1.7±0.8a	3.4±0.4b	100±0.0a	100±0.0a	100±0.0a	100±0.0a
	5	1.0±0.1b	3.8±0.5ab	100±0.0a	100±0.0a	89±0.1b	100±0.0a
	10	1.2±0.6a	4.1±0.4a	100±0.0a	100±0.0a	89±0.1b	100±0.0a
	15	1.2±0.6a	4.1±1.4a	33±0.6b	100±0.0a	100±0.0a	100±0.0a
2	0	1.1±0.4b	3.6±0.4ab	100±0.0a	100±0.0a	100±0.0a	100±0.0a
	5	1.0±0.1b	4.2±0.2a	67±0.6a	100±0.0a	100±0.0a	100±0.0a
	10	1.0±0.1b	4.0±0.6a	67±0.6a	100±0.0a	100±0.0a	89±0.1b
	15	1.0±0.1b	3.7±0.0ab	67±0.6a	67±0.6a	100±0.0a	100±0.0a
4	0	1.6±0.7a	3.4±0.2ab	100±0.0a	100±0.0a	100±0.0a	100±0.0a
	5	1.1±0.4b	4.3±1.0a	33±0.6b	67±0.6a	100±0.0a	89±0.1b
	10	1.9±0.9a	3.9±0.8ab	100±0.0a	100±0.0a	100±0.0a	89±0.1b
	15	1.0±0.1b	4.1±0.5a	100±0.0a	100±0.0a	100±0.0a	100±0.0a
F test		*	*	*	*	*	*
CV (%)		23.9	25.9[t]	22.6	15.8[t]	5.8	8.3

Note: Values in the same column and treatment followed by the same letter are not significantly different at DMRT α = 5%; *= significant effect; [z] At 4 weeks after planting, [y] At 9 weeks after planting; [t] data transformation using $(n+0.5)^{1/2}$; Mean±SD

In general, the percentage of plants that had auxiliary buds was not affected by NAA and BA applications (Table 2). The finding is different from the statement of Sugiyama & Santosa (2008) where apical dominance is high at the time apical shoots grow and suppress the axillary buds action. The presence of active axillary buds in control plants without NAA and BA treatment requires further research. There were likely variations in apical dominance strength between bulbils.

The percentage of apical shoot proliferation (Table 1) and axillary shoot proliferation (Table 3) showed that the concentration of 4 mg NAA L^{-1} with 15 mg BA L^{-1} resulted in 100% bud proliferation in large bulbils although not significantly different from other treatments. Shoot proliferation is a process of multiplying shoots in an explant or certain plant parts (Baidowi & Wiendi, 2017). Kebrom (2017) noted that to stimulate the formation of axillary shoots more markedly, apical shoot pruning is advised.

Table 3 shows that axillary bud proliferation was erratic. High shoot proliferation predominantly was influenced by concentrations of BA than NAA. At 10 and 15 mg BA L^{-1} shoot proliferation was higher and more evenly distributed at various concentrations of NAA than at lower BA concentrations. Ardian et al. (2012) concluded that the application of BA 0.2 mg L^{-1} and NAA 0.1 mg L^{-1} stimulates high proliferation for cassava in vitro.

The percentage growth of axillary bud reached 100% at concentrations of 4 mg L^{-1} NAA +15 mg L^{-1} BA in medium and large bulbils (Table 3). Axillary shoots were considered able to form leaves (Figure 4C). Rapid growth in large bulbils in the present research is in line with previous studies where bulbil size determines the initial growth rate of plants (Soedarjo et al., 2020). The arrow in Figure 4A indicates the locations where the next leaf will appears. Growth of axillary bud, especially in the form of leaf shoots, can appear more than once on a bulbil because of the suspected weak apical dominance (Figure 4B). Sumarwoto (2005) pointed out that large bulbils mean larger energy to form roots and faster shoot growth.

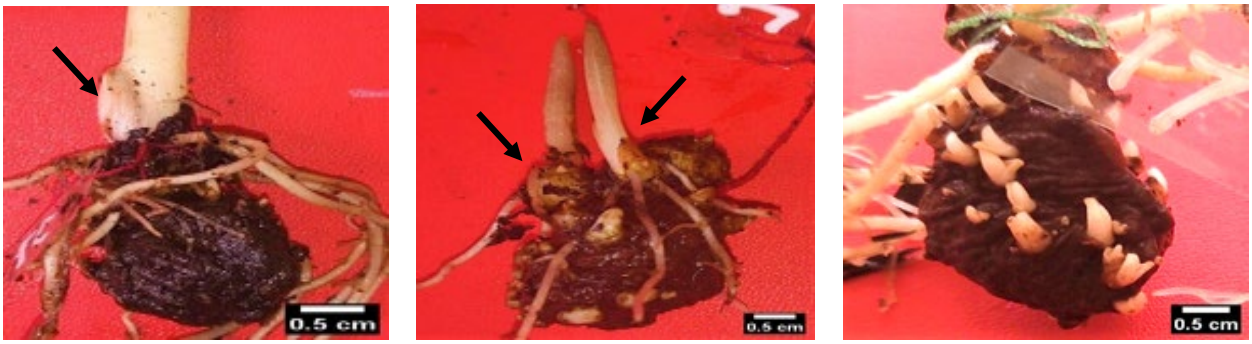
Figure 4C shows the number of active axillary shoots that potentially developed into propagules. The axillary shoots can be used as propagation material by detaching shoots from the bulbil's surface. *A. muelleri* has a basic chromosome number of X = 13 which is triploid (2n=3x=39) and apomictic (Sugiyama & Santosa, 2008; Lontoh et al., 2019; Zhao et al., 2021). The apomictic causes the genetic properties of the offspring to be the same as the parent (Hand & Koltunow, 2014). In addition, it is suspected that the selection of the right planting material as a source of seeds also influences the success of producing propagules for *A. muelleri*. Present research findings were in line with other researchs where medium and large bulbils have higher viability (Dewi et al., 2015).

Histological observations on the apical and axillary shoots of bulbil-origin plants showed the presence of shoot apical meristem (SAM) and leaf primordia (LP) (Figure 5). SAM is at a growing point where there are cells that will regenerate into leaves (LP) and stems (Xue et al., 2020). The presence of SAM and LP can be used as an indicator to determine shoot formation.

Table 3. Growth of axillary bud and proliferation of *A. muelleri* from different bulbil sizes treated with different NAA and BA concentrations

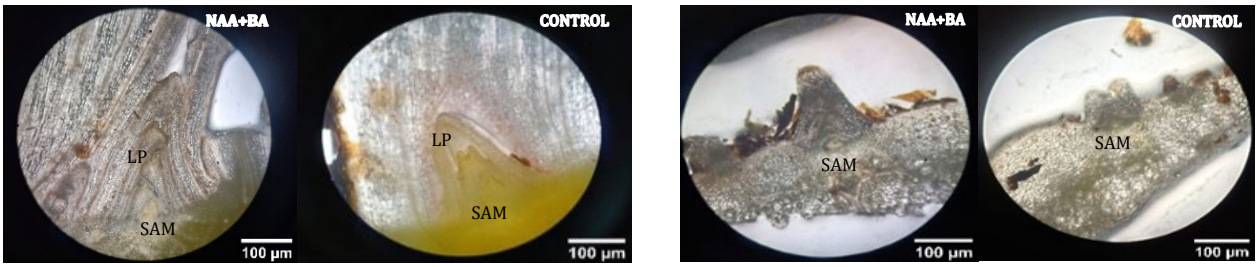
Treatment		BA (mg L^{-1})			
Bulbil size	NAA (mg L^{-1})	0	5	10	15
		Growth of Axillary bud (%)			
Small	0	0.0±0.0a	0.0±0.0a	33±0.6b	67±0.6a
	2	33±0.6a	0.0±0.0a	33±0.6b	67±0.6a
	4	0.0±0.0a	33±0.6a	67±0.6a	100±0.0a
Medium	0	0.0±0.0a	0.0±0.0a	33±0.6b	67±0.6a
	2	0.0±0.0a	0.0±0.0a	33±0.6b	33±0.6b
	4	0.0±0.0a	33±0.6a	67±0.6a	100±0.0a
Large	0	0.0±0.0a	0.0±0.0a	33±0.6b	67±0.6a
	2	33±0.6a	33±0.6a	33±0.6b	100±0.0a
	4	0.0±0.0a	33±0.6a	67±0.6a	100±0.0a
F test			*		
CV (%)			15,9[t]		
		Axillary bud proliferation (%)			
Small	0	0.0±0.0b	0.0±0.0a	33±0.6b	67±0.6a
	2	67±0.6a	0.0±0.0a	0.0±0.0b	33±0.6b
	4	67±0.6a	0.0±0.0a	0.0±0.0b	0.0±0.0b
Medium	0	33±0.6b	0.0±0.0a	33±0.6b	67±0.6a
	2	0.0±0.0b	33±0.6a	67±0.6a	100±0.0a
	4	0.0±0.0b	0.0±0.0a	33±0.6b	100±0.0a
Large	0	0.0±0.0b	0.0±0.0a	33±0.6b	67±0.6a
	2	33±0.6b	0.0±0.0a	0.0±0.0b	67±0.6a
	4	0.0±0.0b	0.0±0.0a	67±0.6a	100±0.0a
F test			*		
CV (%)			25.8[t]		

Note: Values in the same column and treatment followed by the same letter are not significantly different at DMRT test α = 5%; *= significant effect; [t] Data transformation using (n+0.5)1/2; Mean±SD



(A) (B) (C)

Figure 4. Proliferation of different buds of *A. muelleri* treated with 4 mg NAA L^{-1} and 15 mg BA L^{-1}. (A) Proliferation of apical bud at 5 weeks after planting, (B) Proliferation of axillary bud at 5 weeks after planting, (C) Growth of axillary bud at 5 weeks after planting.



(A) (B)

Figure 5. Histological evaluation of different buds of *A. muelleri* treated NAA+BA (4 mg NAA L^{-1} + 15 mg BA L^{-1}) and control. (A) Apical bud at 10 weeks after planting, (B) Axillary bud at 10 weeks after planting

Proliferation of peak leaflets cutting

Based on the results of ANOVA, it was shown that the interaction of NAA, BA, and leaf age did not significantly affect the survival percentage of peak leaflet cuttings. The same thing happened to the interaction of two factors, namely the interaction of BA × NAA, BA × leaf age, and the PGR single factor which had no significant effect on the survival percentage of peak leaflet cuttings (data not shown). Interaction of leaf age NAA significantly existed to the survival percentage of peak leaflet cuttings.

Treatment of NAA 2 mg L^{-1} on mature peak leaflet cuttings showed an average survival rate of 85% at 3 to 5 WAP reaching an average of 35% (Table 4) although not significantly different from other treatments. Immature leaf cuttings experienced necrosis (yellowing) more quickly. Leaflets with severe necrosis usually rot thereafter. The cause of the leaf-cutting death is still unclear. Excess moisture in soil media probably causes leaflets to rot.

During the experiment, peak leaflet cuttings soaked in PGR had a low survival rate of cuttings, which was 35%, which was the highest for mature peak leaflet cuttings treated with 2 mg L^{-1} NAA (Table 4). The cause of this high mortality is still unknown. According to Aisoi (2021), the success of vegetative propagation is marked by the occurrence of regeneration to form shoots and roots on cuttings. The cause of the low survival rate of peak leaflet cuttings might relate to the absence of the main leaf veins in the leaf cuttings. The absence of leaf veins might affect the effectiveness of both assimilate and water translocation. In addition, the absence of roots that form on peak leaflet cuttings disrupts the food supply and consequently, the cuttings die. As a result, some leaf-cutting exhibited rolling and brownish leaves.

Table 4. The survival rate of *A.muelleri* peak leaflet cuttings from different leaf ages and NAA treatment.

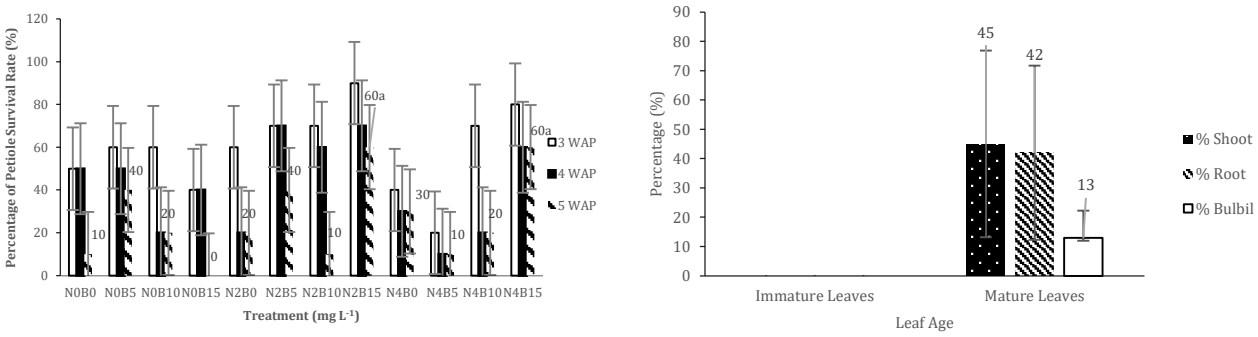
Leaf age	NAA (mg L^{-1})	The survival rate of leaf-cutting (%)		
		3 WAP	4 WAP	5 WAP
Immature leaf	0	75±0.1a	10±0.1b	10±0.1
	2	70±0.1ab	10±0.1b	10±0.1
	4	10±0.1b	10±0.1b	10±0.1
Mature leaf	0	65±0.2ab	40±0.3ab	10±0.1
	2	85±0.2a	35±0.1ab	35±0.1
	4	75±0.1a	60±0.3a	20±0.3
F test		*	*	ns
CV (%)		24.9	32.6^{t}	29.5^{t}

Note: Values in the same column and treatment followed by the same letter are not significantly different at DMRT test α = 5%; ns= non-significant, *= significant effect; ^{t} data transformation using $(n+0.5)^{1/2}$; Mean±SD

Proliferation from base leaflet

ANOVA analysis showed that the treatment of petiole age, NAA, BA, and the interaction of petiole age × NAA × BA had no significant effect on the survival percentage of base leaflet cuttings (data not shown). PGR treatment had a significant effect on the survival percentage of base leaflet cuttings (Figure 6). The success rate of cutting material reached an average of 60% at 5 WAP for the two combinations of NAA with BA treatments, namely 2 mg L^{-1} NAA + 15 mg L^{-1} BA, and 4 mg L^{-1} NAA + 15 mg L^{-1} BA (Figure 6A). At 9 WAP, all base leaflet cuttings experienced death which was indicated by the color of leaves that were dry or rotten. The death is likely due to the plant entering the dormant period.

In contrast to bulbil origin, base leaflet cuttings did not produce shoots but produced daughter corm immediately. The daughter corm was located at the basal of the base leaflet cuttings. According to Sumarwoto (2008) corm experiences dormancy for 5-6 months after formation from petiole cutting, and the application of PGR in the form of IAA and IBA stimulates germination in more than 70% of cuttings.



(A) (B)

Figure 6. Survival rate of propagule from petiole of *A. muelleri* from different NAA and BA concentrations, and shoot; root, and bulbil production of propagule from different leaf ages; (A) Survival rate of petiole after NAA and BA treatments, (B) Percentage of petiole propagule from different leaf age to produce shoot, roots, and bulbils; WAP-weeks after treatment; Treatment combination see Figure 1.

The low percentage of base leaflet cuttings proliferated in the present study as compared to the finding by Sumarwoto (2008) is likely influenced by two things, i.e., the type of PGR used and the growing environment, especially the media used. Apriani & Suhartanto (2015) state that planting media affects the percentage of live cuttings. The good planting media to support cutting is indicated by a high ability to retain moisture, good aeration and drainage, low salinity, and free from pests and diseases (Febriani et al., 2015). Sumarwoto & Maryana (2011) state that soil media has a light to medium texture, loose, fertile, and high organic matter content is very suitable for growing *A. muelleri*.Treatment concentrations of NAA, BA, and leaf age as well as their interactions had no significant effect on the percentage of cuttings forming bulbils, shoots, roots, and the number of bulbils produced from base leaflet cuttings (data not shown). Base leaflet cuttings from mature leaves produced higher shoots, roots, and bulbils than petiole cuttings from immature leaves (Figure 6B). There is a possibility that the mature leaves already contained enough assimilate stock, contrary to young leaves which lack reserves leading to unable to produce shoots and the tubers continued dormant. Unfortunately, nutritional content such as sugar or carbohydrates was not evaluated in the present study. In pineapple, Etriadi et al. (2019) stated that the application of cytokinin in the form of BAP did not increase the germination of leaf cuttings compared to controls.

From three experiments, three findings are concluded. First, the proliferation rate is strongly influenced by the planting material, where the bulbil regardless of its size had the highest proliferation compared to peak leaflet cuttings and base leaflet cuttings. The larger the size of the bulbil due to the higher number of dormant buds has greater the potential

for proliferation. In general, one main apical bud will appear on each bulbil which suppresses the growth of other shoots, although more than one apical bud was also obtained in this study (Figure 4B and 4C).

Second, the age of the planting material for both peak and base leaflet cuttings affects the proliferation rate. The older the planting material tends to be more capable of proliferating than the younger ones. In cases of immature from peak and base leaflet cuttings, most of the plants become dormant, or even cuttings die for various reasons. Rotten or dry were common signs for the unsuccess which may be caused by infectious diseases or by unknown physiological causes. Hormonal imbalance and adequacy of nutrient reserves in immature leaves could be the low success in immature leaves. Sumarwoto (2008) proved that the application of IAA and IBA increases the growth of leaf cuttings with a higher proliferation rate than in the present study. However, Sumarwoto (2008) did not explain the leaf age.

Third, the prospect of NAA and BA to increase the proliferation of *A. muelleri* seedlings is challenging. Application of high BA (15 mg L^{-1}) followed by 2 or 4 mg L^{-1} NAA gave the highest proliferation rate as compared to the control, especially in bulbils and base leaflet cuttings. In base leaflet cuttings, the effect of NAA with BA application was inconsistent, further study is needed. It is possible that the lack of a large leaf vein in the material used for base leaflet cuttings also affected the consistency of the results.

This research implies that bulbils can be used to obtain large quantities of *A. muelleri* seedlings. Morphologically, bulbils are located in the axils of *A. muelleri* leaflets (Sugiyama & Santosa, 2008). PGR application could stimulate bulbil formation, even though they are small or very small. Application of the right PGR concentration might stimulate the growth of vegetative organs such as shoots and roots as well as stimulate endogenous PGR activity as stated by Azmi & Handriatni (2019). In addition, the high-quality planting media to support the growth of cuttings determined the success of cuttings propagation according to Anam (2019) and Sari et al. (2019), besides providing proper propagules by plant origin, plant age, environmental conditions, and PGR.

CONCLUSIONS

The interaction of NAA, BA, and bulbil size was significant on the percentage of proliferation and growth of axillary bud where the concentration of 4 mg L^{-1} NAA with 15 mg L^{-1} BA produced the highest value indicating practical implication for seedling production. No interaction existed between NAA, BA, with leaf age for both cuttings of peak and base leaflets on bud proliferation. However, mature base leaflet cuttings supplemented by 2 mg L^{-1} NAA + 15 mg L^{-1} BA and 4 mg L^{-1} NAA + 15 mg L^{-1} BA could produce substantial buds and produce plants with bulbils, shoots, and roots. Therefore, the preference for rapid and mass propagation was bulbils > petiole > leaflets.

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