The effect of cutting the bulbil-porang (Amorphophallus muelleri) on its germination ability

N Harijati1*, and Ying D2

1Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia
2State Key Laboratory of Hybrid Rice, College of Life Sciences, Wuhan University, 430072 Wuhan, Hubei, PR China

* Correspondence : harijati@ub.ac.id

Abstract. Bulbil, an aerial tuber specifically found on porang, is one tool for multiplication of porang (Amorphophallus muelleri). The aim of this study was to determine the effect of bulbil cutting on the number of tubercles, the proportion of black and white tubercles, the potential for shoot production, the number and height of shoots produced from both cutting (2 and 4 cuts) which appeared in the abaxial or adaxial parts, and the direction of shoot growth. This study was designed using a completely randomized design, each unit of observation was four-replicated. The obtained data were analyzed using Anova, Tukey or independence T-test. The results showed the number of shoots from the whole bulbil, bulbil cut (two or four) differed significantly. The highest number of shoots obtained from bulbil cut was 4 which was 9 shoots. Those highest number of shoots was supported by the highest white tubercle. However, the potential for shoots to emerge from both whole and cut bulbils were almost the same, the length of the shoots did not differ significantly between them as well. The ratio between white and black tubercles seemed to affect the number of shoots, the high ratio produced more shoots. The tubercle ratio from intact bulbils, cut-2 bulbils and cut-4 bulbils tend to increase but not significantly different. The length of shoots that appear from the adaxial and abaxial parts was not different as well. All shoots from both the whole bulbil and cut bulbil grew upwards, none of which grows horizontally or towards the earth.

Keywords: white tubercle, bulbil, shoot, upward, abaxial, adaxial

1. Background
Glukomannan that sold in the market comes from the tuber Amorphophalus konjac and it is known as konjac glucomanan or KGM. KGM become the target of society because it was proven to overcome the ills of modern society. KGM can reduce blood sugar levels, cholesterol, triglycerides, and blood pressure and lowering body weight [1]. Amorphophalus konjac is a member of Amorphophallus which grows well in areas such as Japan, Korea and China. In Indonesia there is native Amorphophallus which is rich in glucomannan, Amorphophallus muelleri, common people call porang. Nowadays, porang is
trendsetter in trading as a tropical corm a source of glucomannan, so that many people open fields extensively to plant porang. The obstacle faced in extending the cultivation of porang is that the seeds are not widely available and the seeds price per Kg are expensive. There are three sources of porang seeds which include small tuber, bulbil and seeds. Among the three choices, bulbil is a wise choice based on price and ecological considerations. Bulbil porang can produce 1-4 [2]. Based on these facts, there is hope for multiplying seeds by cutting bulbil porang into 2 and 4. Bulbil porang has a unique morphological character, namely the presence of protuberances on the surface of the skin. The protuberances are known as tubercles. Some tubercles develop into shoots candidate. Tubercles which later develop into shoots have white color [3] and those that do not develop into shoot turn black color.

Bulbil is a tuber that is on the leaf surface or the surface of porang petiololus. Bulbil behaves like a corm because it is able to produce buds or shoots. In tubers, for example potatoes, the upper shoots (apical) grow more dominant than axillary shoots [4]. Potato axillary shoots grow rapidly when apical shoots are cut or removed [4]. The existence of these facts shows that in potato tubers apical dominance occurs. According to Muller and Leyser (2011) apical dominance occurs because the auxin from the apex shoots moves toward the basal which prevents the growth of axillary buds [5]. However, the morphology of the bulbil is different from potato. Bulbil porang does not show any notches that represent the node, also no structure that can be clearly identified as the shoot apex. Instead, basal parts can be identified based on the position of the parts attached to the parent plant. Often, the basal direction is called the proximal (abaxial) part and the opposite direction is the distal (adaxial) part. The purpose of this study was to determine the effect of cutting bulbil by horizontal way (to obtain two pieces) or horizontal then vertical (to obtain four pieces) on the number of tubercles, number of shoots, shoot length, and the direction of shoot grow.

2. EXPERIMENTAL DETAILS

Materials and preparation research

*A. muelleri* bulbil was obtained from Oro-Oro Waru sub-village in Sumberbendo village, Saradan sub-district, Madiun Regency, East Java Province, Indonesia. The bulbil weights were 4.09-4.72 grams. As a place to germinate, plastic jars with a diameter of 13.5 cm and a height of 6.5 cm were used. The germination plastic jar was washed, air dried and then rubbed out with 70% ethanol. Straw paper that will be used as germination media was cut to the same size as the base of the jar. The paper was sterilized using an autoclave at 1 atm for 15 minutes. Aquadest which will be used to moisturize germination media was autoclaved as well.

Cutting bulbil and Germination

Before cutting bulbil, bulbil got a sterilization treatment which includes soaking in 1.3% hypochlorite three times for 5 minute apart then washing with sterilized aquadest three times each of it 5 minute. The bulbil was divided into two or four parts. Intact bulbil was as control. The cut-bulbils or intact bulbils were placed in jars. To avoid contamination, after the jar was covered with its lid then the lid was sealed using sticky tape. Replication for each treatment was four. All jars were placed in the laboratory at room temperature.

Variable and analysis data

Independent variables were intact bulbil, bulbil cut into two parts (cut-2), bulbil cut into four parts (cut-4). While dependent variable included number of white tubercle, number of black tubercle, ratio of white to black tubercle, potency of shoot emerged (%) (= total shoot number/total number of white tubercle x 100%), shoot number, shoot height, shoot height from abaxial and adaxial site of cut-2 bulbil or cut-4
bulbil, direction shoot growth. The data was analysed using IBM SPSS ver. 20 for one-way anova or unpaired T-test.

3. Result and discussion

After the bulbil imbibed water, some tubercle turn to white color (then it is called as white tubercle) and the others toward black color (black tubercle) (Figure 1). White tubercle developed into shoot bud, while black tubercle did not develop. Total number of white tubercles were less than that of black tubercles. Tubers which were divided into four parts (cut-4) had the highest number of white- or black-tubercles (Figure 2 A, B) and significantly different with the other treatments (control and cut-2 bulbils). The number of shoots produced from three types of seeded bulbils were in accordance with the number of produced white tubercles and the ratio of white to black tubercles (Figure 5A). Cut-4 bulbils produced the highest number of both shoots (Figure 3B) and white tubercles (Figure 2A). However, the potency of shoot emerged was similar between three types of seeded bulbils (Figure 3A). It showed a trend of the more cut is done, the more potency of shoot emerged. These results indicated that cutting can reduce the dominance of some shoots to obtain a more nutrient supply over others shoots. Kebrom (2017) explains the rapid growth of the main shoot requiring more sugar so that the axillary buds cannot outgrow due to lack of sugar [6]. Base on figure 3A, it was clear that the potency to produce shoot was not affected by tuber size. It means that every part or slice with white tubercle, may develop a bud and a shoot. This fact was supported by Afifi et al. (2018) [7]. He proved that white tubercles contain apical meristem and all shoot systems developed from apical meristem [8]. Hence, there was a positive correlation between the number of white tubercles present on bulbil surface and number of shoot produced.

![Figure 1. White and black tubercles on intact bulbil. Bar showed 1 cm.](image-url)
Figure 2 Number tubercle which appear on intact, cut-2, and cut-4 bulbils. A) white tubercles; B) Black tubercles. Note: Letters on the same image showed no statistically significant difference in the Tukey α0.05

Figure 3. Shoot potency to emerge (A) and Shoot number (B) from intact-, cut2-, cut4-bulbil. Note: Letters on the same image showed no statistically significant difference in the Tukey α0.05

By cutting intact bulbil horizontally, it was expected that no auxin flowed from adaxial to basipetal part since the auxin source was in adaxial part. However, we found the bud come out from abaxial site with downward position (Figure 4A and 4B). It also was found that total shoot number from adaxial and abaxial part was similar (Figure 5B).
Figure 4. Germinated of cut-2 bulbils and cutting direction A). Germinated of both adaxial and abaxial parts. Abaxial part was marked by scar tissue. Bar showed 1 cm. B). Bulbil was cut horizontally and position both upward and downward.

Figure 5. The ratio of white to black tubercles (A), and total of all shoot number on adaxial and abaxial part (B). Note: same letters showed no statistically significant difference in the Tukey $\alpha=0.05$ (left) or the T-test $\alpha=0.05$ (right).

Figure 6. Shoot number on adaxial and abaxial part. A, shoot number from adaxial or abaxial part from cut-2 bulbils; B, shoot number from adaxial or abaxial part from cut-4 bulbils. Note: same letters showed no statistically significant difference in the T-test $\alpha=0.05$. 
Shoot number at both adaxial and abaxial parts was not significantly different. This was found at bulbils cut-2 and cut-4 as well (Figure 6 A and B). With some scar tissue at the base of bulbils, abaxial can be considered as morphology basal. It has been previously reported that auxin may migrate from apical meristem to morphology basal in consequence of inhibition of bud outgrowth [9]. In this experiment, perhaps no auxin migrated down or bud was not sensitive with the presence of auxin in abaxial so that bud outgrowth was occurred. By considering that cut-4 bulbils produced more shoot number than cut-2 bulbills (Figure 3B) and no significant different result of shoot number between adaxial and abaxial part (Figure 5B), we suggest to cut bulbils when preparing seeded material from bulbils. Our suggestion was also supported by the results of shoot height measurement. The result showed no difference shoot height measurement as well (Figure 7A). Shoot height is a reflection of shoot growth and development. To grow and develop requires a supply of nutrients [10]. From the 3 treatments, intact bulbil produced tallest shoots height but it was not significantly different with others. This result can be understood because intact bulbil has more food reserves than cut bulbil. The understanding relationship between shoot height with availability of food is little hard when it was applied in case of adaxial and abaxial shoots. Adaxial shoot height was taller than abaxial (Figure 7B.). It seems that the distribution of nutrients and hormones is uneven in bulbil. Therefore in subsequent studies it is necessary to measure hormonal and nutritional levels associated with budding (outgrowth of bud). Hormones, in particular, are likely related to apical dominance as well. According to Waldie and Leyse (2018) and Tan et al. (2019), auxin, cytokinin, and strigolactone play a significant role during the growth and growth of axillary buds [11].

Figure 7. Shoot height from intact and cut bulbils (A), and from both adaxial and abaxial parts. Note: same letters showed no statistically significant difference in the Tukey $\alpha$=0.05 (left hand side) or the T-test $\alpha$=0.05 (right hand side).
4. Conclusion
The amount of white tubercles in intact, cut-2 and cut-4 bulbils were lower than black tubercles. The highest ratio of white to black tubercles was given by cut-4 bulbils. The highest number of shoots was also given by cut-4 bulbils. However, the highest shoots were produced by intact bulbil. Potency to emerge shoot was not different between intact bulbils and cut bulbils. By separating the adaxial and abaxial parts of the bulbil, different results were obtained for the number of shoots and shoot height. Shoot number of adaxial tend to be lower than abaxial shoots, but shoot height of adaxial tends to be higher. To provide accurate answer why adaxial part produce less shoot than abaxial, it is necessary to measure the concentration of auxin, cytokinin, and strigolacton in abaxial and adaxial part around white or black tubercles in the next research.

5. ACKNOWLEDGMENT
A deep thanks to Head of Biology Department, Brawijaya University for his encouragement to provide research facilities. Also Lathifatuzzahro, she spent a lot of time to look after the bulbil and taking the data.

References
[1] Devaraj RD, Reddy CK, and Xu B 2019 Int J Biol Macromol 1(126) 273-281
[2] A’yun Q, Harijati N, Mastuti R 2019 Journal of Environmental Engineering & Sustainable Technology 6(1) 30-35
[3] Afifi MN, Harijati N, Mastuti R 2019 J.Exp. Life Sci. 9(1) 19-23
[4] Teper-Bammolker P, Buskila Y, Lopesco , Ben-Dor S, Saad I, Holdengreber V, Belaourov E, Zemach H, Ori N, Lers A, and Eshel D 2012 Plant Physiology 158 2053–206
[5] Muller D and Leyser O 2011 Ann Bot 107 1203–1212
[6] Kebrom TH 2017 Perspective Frontiers in Plants 1(8) 1-7
[7] Medförd J N 1992 The Plant Cell (4) 1029-1039
[8] Cline M 2000 Am. J. Bot. 87 182-190.
[9] Tan M, Li G, Chen X, Xing L, Ma J, Zhang D, Ge HJ, Han M, Sha G, and An N 2019 Frontiers in Plant Science 10 6-16
[10] Waldie T and Leyser O 2018 Plant Physiology 177 803–818
[11] Taiz L and Zeiger E 2006 Plant Physiology. 4th ed. Sinauer Associates: Sunderland