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DEVELOPMENT OF APICAL SHOOTS AND ENDOGENOUS ABA CONCENTRATIONS IN PORANG TUBERS (*AMORPHOPHALLUS MUELLERI* BLUME) AFTER HARVEST

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ABSTRACT

This study aimed to analyze the development of apical shoots and the concentration of endogenous abscisic acid (ABA) in porang tubers after harvest. The development of apical shoots was prepared as a semi-permanent preparation which was observed using a microscope and ABA concentrations were analyzed using the High Performance Liquid Chromatogram (HPLC) method every two weeks. At 0 WAH, the meristem cells were differentiated and continued to grow until they reached 0.38±0.02 mm and formed shoot apical meristems (SAM) at 6 weeks after harvest (WAH) of 16.67%. At 12 WAH all tubers had formed SAM and shoots began to appear on the tuber surface. At the time when SAM had not yet formed, the concentration of endogenous ABA was high (433.07±39.26 ng/g), but when SAM was formed and the meristem height reached 12.52±4.90 mm the concentration decreased to 102.90±22.19 ng/g (20 WAH). The emergence of shoots and a decrease in the concentration of endogenous ABA at 12 WAH indicated that the dormancy period had ended. Based on this, the age of the best tubers is not more than 10 WAH. Over 10 WAH, tuber quality will decrease due to biochemical changes, so that the nutrient content, especially glucomannan, is reduced, therefore it is not ideal as industrial material.

Key words: endogenous ABA, dormancy, meristem, postharvest

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INTRODUCTION

Porang (*Amorphophallus muelleri* Blume) is a perennial herbaceous plant classified into the Araceae family (Wahyudi *et al*., 2013). Porang plant can be found in the Andaman Islands of India, Myanmar, Thailand and Indonesia (Java, Sumatera, Sulawesi, Bali, and Nusa Tenggara) (Kurniawan *et al.,* 2011; Yuzammi *et al*., 2017; Hafsah *et al*., 2018). This tuberous plant has four growing periods in its life cycle (Indriyani, 2011). Tubers that were used for industrial purposes were generally harvested from plants in the third growing period, because they have the highest concentration of glucomannan compared to tubers harvested from other plants in the growing period (Sumarwoto, 2005; Gusmalawati *et al.,* 2019). Glucomannan is an excellent polysaccharide for diet programs, lowers cholesterol, prevents heart disease, treats hypertension, and diabetes (Behera and Ray, 2016). In addition, glucomannan in the industrial sector can be used as raw material for textiles, paper, cosmetics, food, medicines, rubber, and others (Koswara, 2013). The many benefits of glucomannan cause porang tubers to have high economic value (Rokhmah and Supriadi, 2015). Proper post-harvest handling needs to be done, especially in determining the right age/time in tuber processing after harvest to get the best quality tubers as food and other industries (Nabubuya *et al.,* 2017). The dormancy period and subsequent emergence of shoots after the tubers are harvested can cause changes in the structure and metabolic activity, thus affecting the quality of tubers as raw material for industry, especially glucomannan concentrations (Sumarwoto, 2005; Chua *et al.,* 2013; Nurlela *et al.,* 2019).

Porang tubers have a dormancy period which is ended by the emergence of shoots, around 12-20 weeks after harvest (WAH) (Indriyani, 2011). Dormancy is a period in organisms when growth, development and physiological activities (respiration, enzyme activity, starch, and sugar) occur slowly because it is preferred to store food reserves (Cheema, 2010; Hamadina, 2011). Thus, in this dormancy period the tubers have the best nutritional content for food and industry (Craufurd *et al.,* 2001; Muthoni *et al.,* 2014). The end of the dormancy period is marked by the appearance of shoots on the tubers. The emergence of shoots on the tubers at the end of the dormancy period is one of the important factors affecting the deterioration of tuber quality as a result of the remobilization of stored compounds, especially carbohydrates, proteins, and water (Mani *et al.,* 2014).

The starch concentration in the *Ipomea batatas* tubers after harvest decreased as a result of the activity of the amylase enzyme in hydrolyzing the glycosidic bonds in starch to simple sugars (Nabubuya *et al.,* 2017). Glucomannan concentration in *A. konjac* is high during the dormancy period, and then decreases when shoots appear. This decrease is due to an increase in the activity of the enzymes β-mannanase, β-mannosidase, and β-glucosidase which play a role in degrading glucomannan into glucose and mannose. Increased glucose and mannose are used as a source of energy for shoot growth (Gille *et al.,* 2011; Chua *et al.,* 2013; Nabubuya *et al*., 2017). Changes in complex compounds such as starch and glucomannan into simple sugars in tubers after harvest are used as an energy source for shoot growth, resulting in a decrease in the quality of tubers as food (Gille *et al.,* 2011; Nabubuya *et al.,* 2017).

The dormancy period in tubers after harvest is influenced by several factors, namely: environment, physiological control mechanisms, and hormones (Martin *et al*., 2010; Sonnewald and Sonnewald, 2014). One of the hormones that plays an important role in initiating and maintaining dormancy in potato tubers is *abscisic acid* (ABA) (Aksenova *et al.,* 2012; Wróbel *et al.,* 2017). ABA concentrations are endogenous in potato tubers during a high dormancy period, and then decrease at the end of the dormancy period until shoots emerge (Destefano-Beltran *et al.,* 2006). Giving exogenous ABA to potato tubers after harvest prolongs the dormancy period and slows shoot growth (Suttle *et al.,* 2012). The concentration of endogenous ABA in potato tubers is closely related to the development of apical shoots. When the endogenous ABA concentration is high, meristem cells are not yet actively dividing. However, when the endogenous ABA concentration was low, meristem cells were actively dividing and differentiating to form apical shoots. Low concentrations of endogenous ABA in these tubers are a prerequisite for the induction of apical shoots (Sonnewald and Sonnewald, 2014). The apical shoots grow as tubers age after harvest (Viola *et al.,* 2007). In contrast to potato tubers, *Dioscorea* tubers do not have internal and external apical shoots. *Dioscorea* tubers have a layer of meristem cells just below the tuber surface in the shoot area (Craufurd *et al.,* 2001). The development of apical shoots in the *Dioscorea* tuber begins with the activation of meristem cells, the formation of *developing shoot apical meristems* (DAM), formation of *shoot apical meristems* (SAM), and then the emergence of apical shoots on the tuber surface. The stage from the activation of meristem cells to the emergence of apical shoots on the tuber surface takes about 16-20 WAH (Ile *et al.,* 2006). Changes in meristem activity might play an important role in the growth of apical shoots which were assumed to initiate the end of the dormancy period (Sonnewald and Sonnewald, 2014).

The development of apical shoots and the concentration of endogenous ABA after harvest in the tubers were factors that affect the dormancy period until shoots appear. Research on the development of apical shoots and endogenous ABA concentration in porang tubers after harvest has never been carried out. Therefore, the aim of this study was to analyze the relationship between apical shoot development and endogenous ABA concentrations in porang tubers from the dormancy period to shoot emergence. This information is very necessary to determine the right time for the utilization of good quality porang tubers as industrial raw materials.

MATERIALS AND METHODS

Tuber sample: This research was conducted from February to December 2018. The sample of porang tubers used in this study was obtained from the porang center in Rejosari Village, Bantur, Malang, and East Java, Indonesia. Tubers were harvested from plants in the third growing period when the plants have collapsed, their leaves and stems have dried up and released from the tubers. The first day of tuber harvesting was determined as tuber age 0 WAH (initial dormancy). As many as 66 tubers were used in intact condition (not rotten) and each weighing about 650-1850 g. Cleaned tubers were placed on bamboo racks at room temperature of 22-30°C and humidity of 49-69% in Merjosari, Lowokwaru, Malang City, East Java, Indonesia. The development of apical shoots, the percentage of tubers forming SAM, meristem height, and endogenous ABA concentration were observed and measured every 2 weeks up to 20 WAH (weeks 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20). This research was conducted using 6 replications.

Procedures

Analysis of apical shoot development: Analysis of shoot development on porang tubers at each age of observation was carried out by making semi-permanent preparations referring to the method of Chua *et al*. (2013) and Gusmalawati *et al*. (2013). The upper tubers (the area where the shoots appeared) were cut according to the size of the shoots, and then fixed with *formaldehyde acetic-acid alcohol* (FAA) for 24 h. Each sample piece was sliced longitudinally (in the direction of the tuber height) using a *clamp on hand microtome*. The results of the thin incisions were then stained with Toluidine Blue 1% for 1 minute and then rinsed with distilled water. The preparation was placed in a glass beaker, then given glycerin and covered with a cover glass. The anatomical structure of apical shoot development in porang tubers was observed microscopically with a CX-31 binocular microscope (tuber aged 0-12 WAH) and a stereo microscope (tuber aged 14-20 WAH) which refers to Ile *et al*. (2006) and Sonnewald and Sonnewald (2014). Meristems in the process of development (not yet dome-

like) were defined as *developing shoot apical meristems* (DAM), meristems that resemble domes (≥ 0.375 mm) were expressed as SAM, and SAM that has developed into organs was defined as *apical shoots* (AS). Meristem height was measured from the base to the tip of the meristem using a micrometer. Then, the percentage of tubers that make up SAM was calculated from the number of tubers that make up SAM divided by the total number of tuber samples multiplied by 100%. The observed preparations were then documented using a digital camera.

Analysis of endogenous ABA concentrations: Analysis of endogenous ABA concentrations in porang tubers at each observation age refers to the method of Wei *et al.* (2016). ABA standard stock solution was prepared by dissolving 5 mg of standard ABA (Sigma Aldrich) in 80% to 1000 mL methanol. From the stock solution, five types of solutions were made with concentrations (mg/L) 0, 0.1, 0.5, 1, 2.5, and 5. These solutions were used to determine the standard curve equations.

As much as 1 g of the tuber sample, pounded until smooth, then added with 20 mL of liquid nitrogen, and mashed until it becomes a powder. Furthermore, 15 mL of 80% methanol was added and placed in a closed brown glass bottle to be stored for 12 h at 4°C. Then the solution was filtered with a vacuum filter device. The filtrate obtained was collected (first filtrate), while the residue was redissolved with 15 mL 80% methanol and stored again in a closed brown glass bottle for 2 h at 4°C. Then the re-extracted solution was filtered again with a vacuum filter, and the filtrate (second filtrate) was obtained. The first and second filtrates were combined, and then 0.2 mL of concentrated HCl was added to create an acidic atmosphere. Furthermore, 5 ml of ethyl acetate solution was added, and then the solution was evaporated with a vacuum evaporator until a solution of 5 mL was obtained. The volume of the solution was then made into 10 ml with the addition of 80% methanol. The solution was ready to use for HPLC analysis.

Standard solution, sample solution, and mobile phase (eluent) were filtered with a 0.45 µm filter membrane and degassed before being injected into the HPLC device. Endogenous ABA analysis was performed using a Shimadzu HPLC device set on the CBM 20A system controller, a solvent delivering unit LC 20AT, 20 A CTO column oven, 20 A SPD UV-Vis Detector, a Shimadzu VP ODS column µm 150 x 4.6 mm at 25°C. Mobile phase using Acetonitrile (A); 0.1% v/v H_3PO_4 (B) by mobile phase method (Gradient method): 20% A, 80% B (0 min), 25% A, 75% B (5 min), 30% A, 70% B (8 min), 35% A, 65% B (15 min), and 45% A, 55% B (25 min) at a flow rate of 0.6 mL/min. The extract was injected as much as 10 µl into a 260 nm wavelength UV detector with a run time of 30 min. Endogenous ABA concentrations were analyzed using HPLC Software, namely Shimadzu LC Solution Ver 5.6.1. for windows.

Data analysis: The qualitative data on the development of apical shoots in the form of anatomical images were analyzed descriptively, while the quantitative data on the percentage of tubers forming SAM, meristem height, and endogenous ABA concentrations were analyzed by one-way ANOVA test followed by Tukey's test at $\alpha = 0.05$ to determine the age of the tubers after harvest which gave different effects. The relationship between endogenous ABA concentration with meristem height and the percentage of tubers forming SAM after harvest were analyzed using the Bivariate Correlation test. All quantitative data were analyzed using SPSS Statistics version 16.0 Software (SPSS Inc. Chicago, IL, USA).

RESULTS AND DISCUSSION

The structure of apical shoot development, height of meristem and percentage of tubers forming SAM: Based on microscopic observations, tubers aged 0-4 WAH were composed of parenchyma tissue, vascular bundles and meristems covered by several layers of small protective leaves (bractea). Meristem cells have been actively dividing which was indicated by the formation of DAM that was, meristem cells have developed almost to form SAM (Figure 1.ABC). Tubers aged 6-10 WAH have the same constituent tissue as tubers aged 0-4 WAH, but the meristem cells have been arranged into SAM which has a dome-like shape (Figure 1.DEF). At the age of 12 WAH SAM tubers had developed into larger size spadix so that the whole preparations could not be observed. However, the spadix showed a round protrusion which indicates in the development of female flower (gynoecium) (Figure 1.GH) and the apical shoots had appeared to the tuber surface because the outer layer of the bractea had been torn off. At the tubers aged 14-20 WAH, the size of the apical shoots was getting bigger and there were spadix, spathe, and bractea (Figure 1.IJKL). Tubers aged 16-20 can be seen clearly on the spadix, there was a round protrusion which was a gynoecium (Figure 1.JKL). However, in tubers aged 18-20 the WAH of the organs could not be observed as a whole because their size had also increased (Figure 1.KL).

Based on the ANOVA test, the age of porang tubers after harvest had a significant effect ($\alpha = 0.05$) on the meristem height and the percentage of tubers that formed SAM (Figure 1M). At the age of 0-4 WAH there were no tubers that form SAM. Even though until the age of 4 WAH there were no tubers that formed SAM, but at 0, 2, and 4 WAH the meristem cells had formed DAM which had a height of 0.21±0.01, 0.22±0.01, and 0.23±0.02 mm, respectively. Up to 12 WAH when all tubers (100%) had formed SAM, the meristem continued

to increase in height but did not differ significantly. At 16 WAH tubers, the height of the meristems began to increase significantly to reach 6.3±3.85 mm and at 18 WAH to 11.92±2.44 mm. At the age of 20 WAH tubers, the height of the meristems still increases, although not significantly, reaching 12.52±4.30 mm.

The slow growth of the apical shoots might be due to the tuber experiencing physical damage. In this study, the meristem height and the percentage of tubers that formed SAM increased with increasing age of the porang tubers after harvest. During the dormancy period the meristems continue to experience growth as indicated by the presence of high growth. This height increase resulted in the development of DAM, the emergence of SAM in all tubers at 12 WAH and was accompanied by the emergence of shoots on the tuber surface indicating the end of the dormancy period. The increase in the height of meristems and the percentage of tubers that form SAM in porang tubers after harvest may be influenced by physiological activities (hormones, carbohydrates, proteins, and enzymes) which affect shoot development. According to Suttle (1995), the shoot growth pattern of the tubers is influenced by the age after harvest. In *Dioscorea* tubers the longer the age after harvest, the higher the meristem, followed by the emergence of shoots on the tuber surface (Ile *et al*., 2006). Apart from the age of the tubers after harvest, genetic factors, and pigments (carotenoids) also influence shoot growth (Vranov´a *et al*., 2013; Sonnewald and Sonnewald, 2014).

The activity of meristem cells and the development of SAM in porang tubers aged 0-4 WAH was closely related to the formation of apical shoots, so it is assumed that meristem cells have begun to actively divide before the tubers were harvested. Previous research on porang seeds found that embryonic development can be observed 20 weeks after the flowers bloom. Embryo differentiation occurs before the plant collapsed, which was indicated by the presence of an apical meristem at the base of the seed. At 32 weeks after blooming, the plants collapse, and the size of the embryo increased. At the age of 36 weeks after the flowers bloom, there were leaf primordia and buds that have appeared on the surface of the seeds (Gusmalawati, 2013). So, it is assumed, the development of apical shoots in porang tubers has a similar pattern to embryonic development in porang seeds. Research by Gille *et al.* (2011) showed that *A. konjac* tubers harvested during the vegetative phase (17 weeks after planting and the plant has not collapsed) apical shoots had been formed. In the dormancy period, the potato tuber has dormant internal apical shoots. The apical shoots might be having formed simultaneously with tuber development in the vegetative phase (Sonnewald and Sonnewald, 2014). Based on this, it is assumed that the apical meristem development in porang tubers has occurred during the vegetative growth phase, so that when the tubers were harvested at the end of the vegetative phase the meristem cells have developed and were characterized by the presence of apical shoots on the tuber surface. However, this was different from the *Dioscorea* tuber which has two layers of meristem cells that have not been actively dividing at 0-4 WAH. The meristem cells become 10 layers vertically at 8 WAH, then develop to form DAM at 12 WAH, and then form SAM at 16 WAH. *Dioscorea* tubers aged 20 WAH contained leaf primordia accompanied by the appearance of apical shoots on the tuber surface (Ile *et al*., 2006). Each species has a different pattern of shoot development (Craufurd *et al.,* 2001).

The stages of SAM formation in porang and *Dioscorea* tubers have similarities, but the time required was different. The process of forming SAM in porang tubers was faster than in *Dioscorea* tubers. Leaf primordia on the *Dioscorea* tubers that appeared at the age of 20 WAH showed the appearance of apical shoots on the tuber surface, while the appearance of spadix on the 12 WAH porang tubers accompanied by cracking of the outer bractea layer indicated the appearance of flower buds on the tuber surface. This was closely related to the dormancy period of the porang and *Dioscorea* tubers. Sumarwoto (2005) states that the dormancy period in porang tubers occurs at 12-16 WAH and in *Dioscorea* tubers occurs at 16-20 WAH (Ile *et al.,* 2006). Gusmalawati (2013) stated that the porang tuber from the third growing period after harvest experienced generative growth. Tubers at the age of 12-16 WAH produce buds in the form of flower buds that emerge from the surface of the tubers, and then the buds were fully bloomed at 28-32 WAH. Porang flowers consist of the main parts: flower stalks (pedunculus), flower cobs (spadix) and large protective leaves (spathe), spadix consisting of: gynoecium, androecium, and appendix. Based on this research, the porang tubers after being harvested in the third growing period experienced generative growth to produce flowers, fruits, and seeds.

The emergence of shoots on the porang tubers was indicated by the cracking of the outer bractea layer which occurs at the age of 12 WAH. Sonnewald and Sonnewald (2014) stated that germination is a physiological stage to reactivate metabolic activity and the end of the dormancy period. Based on Hamadina's (2012) research, the germination of the *Dioscorea alata* tuber begins with the division of the meristem cells that were just below the tuber surface, which produces a large and undifferentiated cell mass. This mass of cells was immediately differentiated within it. The skin of the overlying tuber swells and subsequently cracks, exposing a shiny cell mass because of meristem activity, and then the shoot tip was differentiated. The place where the skin breaks and where the cells underneath is visible were called the germ areas. When the tip of the shoot was completely organized, it appears from the outside as a

bud and then elongates to produce shoots that become new plants. The growth of shoots on tubers is strongly influenced by physiological age (growth conditions, conditions after harvest, and age after harvest), but genetic factors are still very influential (Destefano-Beltran *et al.,* 2006; Peivastegan *et al.,* 2019). The emergence of shoots causes a decrease in the quality of potato tubers due to changes in stored compounds into energy used for shoot growth (Sonnewald and Sonnewald, 2014).

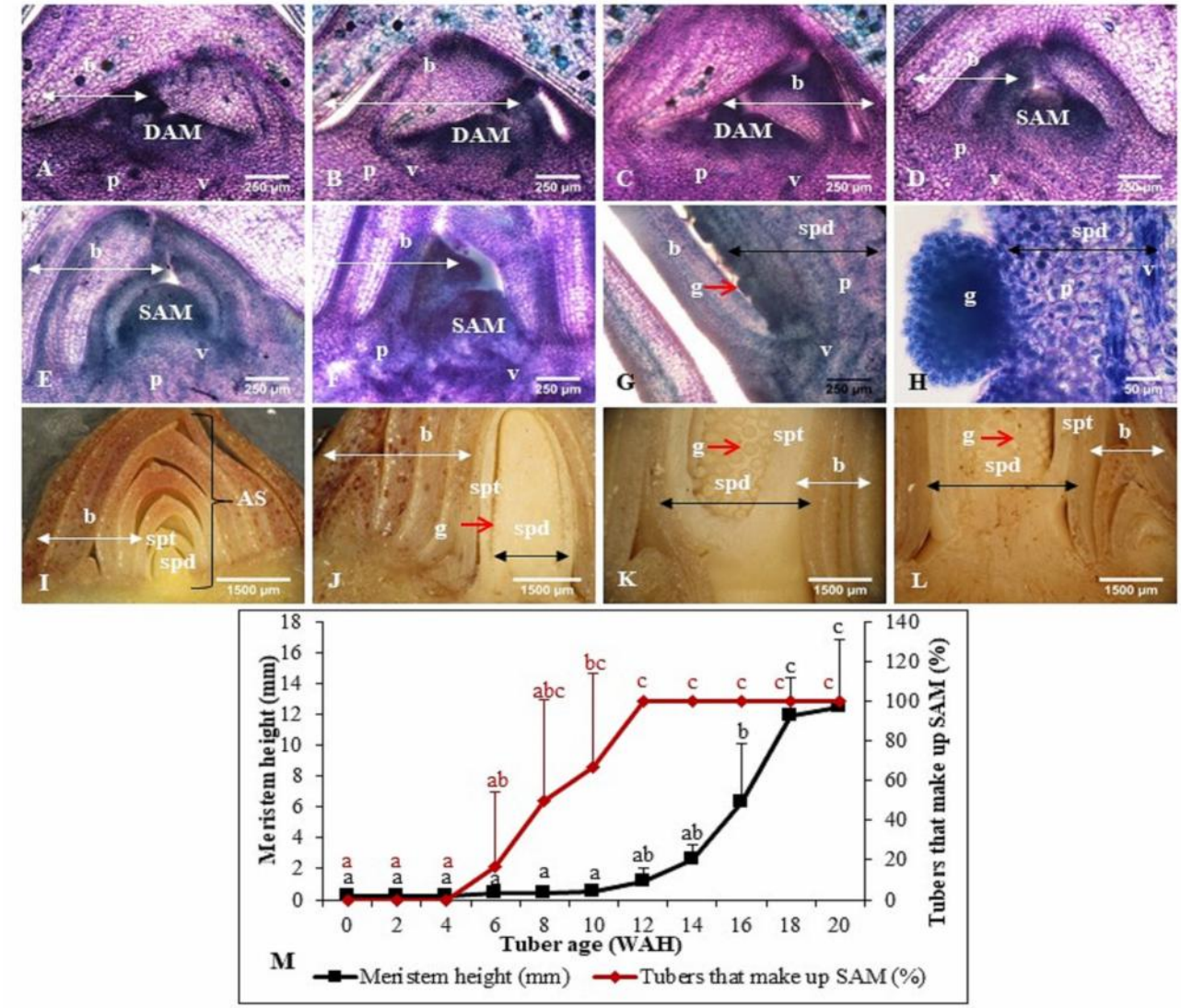

Figure 1. Development of apical shoots on porang tubers after harvest, A-L. apical shoot development structure based on longitudinal slices in the shoot area of the tubers: A. tubers aged 0 WAH, B. tubers aged 2 WAH, C. tubers aged 4 WAH, D. tubers aged 6 WAH, E. tubers aged 8 WAH, F. tubers age 10 WAH, GH. tubers aged 12 WAH, I. tubers aged 14 WAH, J. tubers aged 16 WAH, K. tubers aged 18 WAH, L. tubers aged 20 WAH; p: parenchyma, v: vascular bundle, DAM: developing shoot apical meristem, SAM: shoot apical meristem, spd: spadix, g: gynoecium, spt: spathe, b: bractea, AS: apical shoots, white arrow: bractea layer, red arrows: gynoecium, black arrows: spadix, A-G. with binocular microscope, H-K. with stereo microscope; M. meristem height and percentage of tubers forming SAM; The same letter notation for each variable shows no significant difference at the level α = 0.05, n = 6, bar sign = standard error (SE)

The dormancy period of the porang tubers in this study occurred up to 10 WAH, because during this period the meristem development occurred slowly, the tubers that formed SAM had not reached 100%, and the bractea in the outer layer had not been torn. However, at the age of 12 WAH all tubers (100%) had formed SAM although

the height increase of the meristem was still insignificant. The cracking of the bractea in the outer layer indicated that the dormancy period was over. Mani *et al*., (2014) states that the dormancy period in potato tubers shows slow morphological, physiological, and biochemical activities. This dormancy period was an attempt to store food reserves as a source of energy for growth. Previous studies revealed the dormancy period among species was different; *Dioscorea* has a dormancy period of 16-20 WAH (Hamadina, 2011), *A. campanulatus* 9-12 WAH (Ravi *et al.,* 2009), and 12-20 WAH in porang (Indriyani, 2011). The dormancy period in this study was the previous research (Sumarwoto, 2005), which occurs up to 10 WAH The dormancy period ends before the tubers reached age of 12 WAH, because at that time all the tubers have formed SAM followed by cracking of the bractea in the outer layer and the emergence of apical shoots on the tuber surface. The same phenomenon occurs when apical shoots appear on the surface of potato tubers, indicating that the dormancy period has ended. The dormancy period in tubers can be influenced by internal (hormone, genetic, and pigment) and external factors (temperature, humidity, light, harvest time, and age after harvest) (Suttle, 2007).

Endogenous ABA: HPLC analysis of early harvest porang tubers (0 WAH) showed the presence of endogenous ABA which was indicated by a peak on the chromatogram. Endogenous ABA peaks appeared at the retention time (RT) of 21.015 min with a peak height expressed in milli unit's absorbance (mUA) of 42 (Figure 2). The endogenous ABA that has been identified in this porang tuber may have a role in regulating and maintaining dormancy after harvest. Suttle (1995) stated that the endogenous ABA potato tubers were identified by HPLC analysis whose concentrations changed after harvest. The endogenous ABA in potato tubers plays a role in controlling dormancy and inhibiting shoot emergence.

Analysis of variance revealed that the age of tubers on porang after harvest had a significant effect (α= 0.05) on the endogenous ABA concentration. At the beginning of harvest, the endogenous ABA concentration was 295.32±40.62 ng/g, and continued to increase until the highest concentration reached 433.07±39.26 ng/g at 4 WAH. At 6 WAH the ABA concentration began to decrease to 368.24±31.69 ng/g and continued to decrease to 267.51±63.16 ng/g at 10 WAH, but the concentration was not significantly different from tubers aged 0 WAH. The endogenous ABA concentration continusly decreased to 207.90±37.30 ng/g at 12 WAH, and further decreased to 102.90±22.19 ng/g at 20 WAH (the concentration was significantly different from 0 WAH) (Figure 3). The longer the age of the tubers after harvest, the concentration of endogenous ABA decreased significantly. This is closely related to the dormancy period and the emergence of shoots on porang tubers after harvest. Based on the results of this study, high endogenous ABA concentrations in porang tubers after harvest at 2-6 WAH and decreased to 12 WAH played a role in maintaining dormancy. The concentration of endogenous ABA which decreased significantly at 14 WAH coincided with the process of increasing apical shoot height and the percentage of tubers that sprouted in porang tubers after harvest.

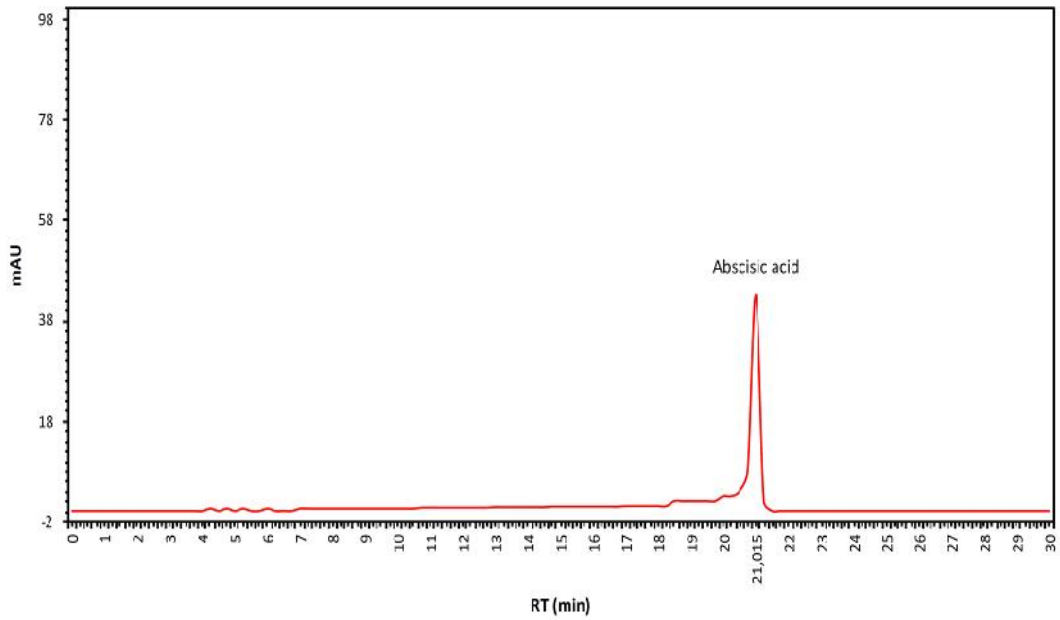

Figure 2. Chromatogram from HPLC analysis of porang tubers at early harvest (0 WAH), mUA: Milli Unit Absorbance, RT: retention time.

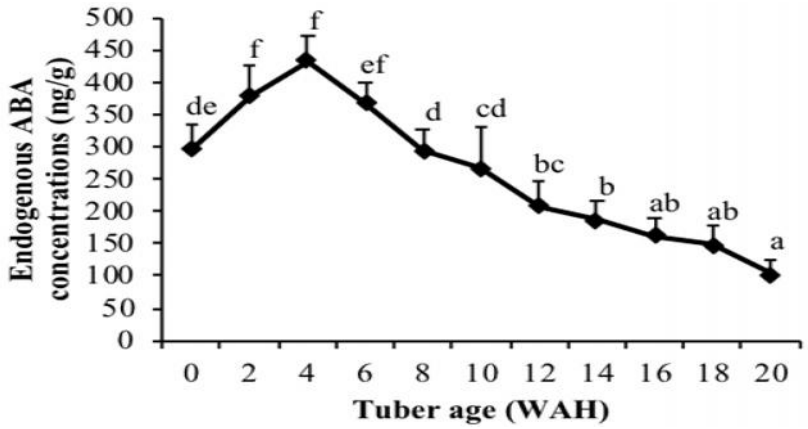

Figure 3. Endogenous ABA concentration in porang tubers after harvest WAH: week after harvest, the same letter notation shows no significant difference to Tukey's test at α = 0.05, bar sign indicates standard error (SE).

The change in the endogenous ABA concentration in porang tubers after harvesting the pattern was the same as that of potato tubers. Suttle (1995) stated that in potato tubers, the concentration of endogenous ABA was very high when harvested and then decreased with increasing age of tubers after harvest. Abscisic acid (ABA) has an important role in regulating dormancy. High endogenous ABA concentrations play a role in initiating, maintaining dormancy, and inhibiting shoot emergence, while low endogenous ABA concentrations play a role in initiating shoot emergence. The level of ABA content after harvest can determine the length of the dormancy period in an effort to store food reserves (Suttle *et al.,* 2012; Cheema, 2010). There was no information regarding the factors that affect changes in endogenous ABA concentrations in porang tubers, but external (temperature and humidity) and internal factors (pigments, genetics, and enzymes) that influence it in potato tubers (Flokova *et al.,* 2014) may also be factors that play a role in influencing the concentration of endogenous ABA in porang tubers.

The relationship between endogenous ABA concentrations and the percentage of tubers forming SAM and meristem height: The concentration of endogenous ABA in porang tubers after harvest showed a negative correlation with a significance value (α= 0.05) of -0.92 with the percentage of tubers forming SAM or a determination value of 83.76% (R= 0.8376) (Figure 4.A). This negative correlation shows that the lower the endogenous ABA concentration after harvest, the higher the percentage of tubers that form SAM, while the determination value shows that 83.76% of the variation in the percentage of tubers forming SAM was influenced by the concentration of endogenous ABA and 16.24% was influenced by other factors such as other hormones, sugar, and genetics. Research Ile *et al*. (2006) stated that the stages of SAM development occurred very slowly, the tubers that formed SAM occurred at 16 WAH tubers, and then at 20 WAH apical shoots appeared on the tuber surface. In addition, treatment with the gibberellin hormone caused the formation of SAM faster. In the potato tuber meristem, physiological stages affect the ABA concentration. In the dormancy period, the longitudinal incision of the apical bud area was detected with a higher ABA than in the shoot emergence period. This suggests that shoot development was affected by endogenous ABA in tubers after harvest (Sonnewald and Sonnewald, 2014).

The concentration of endogenous ABA in porang tubers after harvest showed a negative correlation with a significance value (α= 0.05) of -0.79 with shoot height or a determination value of 62.09% (R= 0.6209) (Figure 04.B). The negative correlation shows that the lower the endogenous ABA concentration, the higher the meristem height, while the determination value shows that 62.09% of the variation in meristem height was influenced by the endogenous ABA concentration after harvest and 37.91% was influenced by other factors such as tuber age after harvest. Meristem height in porang tubers increases with the age of tubers after harvest. Research Ile *et al.* (2006) stated that the development of apical shoots in *Dioscorea* tubers was influenced by the age of the tubers after harvest. At the beginning of harvest, the meristem cells consist of 2-4 layers with a height of 50 µm and then the tubers which form SAM with a height become 450 µm at 16 WAH. A decrease in endogenous ABA concentrations led to an increase in shoot height in potato tubers after harvest (Viola *et al.,* 2007). Endogenous ABA is required to initiate and maintain tuber dormancy (Suttle *et al.,* 2012). Endogenous ABA is the main hormone in initiating dormancy. Endogenous ABA concentrations are very high in the dormancy period and decrease after harvest (Destefano-Baltran *et al*., 2006). Apart from endogenous ABA, hormones that play a role in controlling dormancy

and germination are ethylene, gibberellin, cytokinins, and auxins (Tarkowska *et al.,* 2014; Dai *et al*., 2016; Asalfew, 2016). Reactive oxygen species (ROS), antioxidants, and nitric oxide (NO) also play a role in controlling dormancy and sprouting in potatoes (Essid *et al.,* 2014; Wang *et al.,* 2020). Other factors that affect dormancy and shoot emergence in tubers were environmental conditions, metabolic processes, and structural changes (Sonnewald and Sonnewald, 2014). Thus, in addition to the endogenous ABA concentration, it was suspected that these factors also affect the dormancy period until shoots appear on the porang tubers, so it needs to be studied.

The results of this study indicate that the structure of apical shoot development, the percentage of tubers forming SAM, the height of meristems, and the concentration of endogenous ABA in porang tubers after harvest can be used to determine the dormancy period. The dormancy period of porang tubers occurs for 10 WAH, due to the slow increase in the percentage of tubers that form SAM and the height of meristems and the decrease in endogenous ABA concentrations. Meanwhile, the dormancy period ends at 12 WAH tubers, which is marked by an increase in the percentage of tubers forming SAM, high meristem, and a rapid decrease in endogenous ABA concentration followed by cracking of the outer bractea layer which causes shoots to appear on the tuber surface. The development of apical shoots, the increase in the percentage of tubers that form SAM, the height of meristems, and the decrease in endogenous ABA concentrations were influenced by the age of the tubers after harvest and may be influenced by other factors such as environmental conditions, stage of development, hormones other than ABA, carbohydrates, and genetics. Thus, the factors that influence tuber changes after harvest that can have an impact on quality degradation need to be investigated further.

The conclusion in this study is that the increasing age of tubers after harvesting of porang tubers, the development of apical shoots, the percentage of tubers forming SAM, and the height of the meristems increased, while the endogenous ABA concentration decreased. The development of apical shoots, an increase in the percentage of tubers forming SAM, the height of meristems, and a decrease in endogenous ABA concentrations followed by the cracking of the outer bractea layer and marked by the appearance of shoots on the tuber surface at 12 WAH indicate that the dormancy period has ended. Based on this, tubers with an age of less than 12 WAH would be better if they were used as raw materials for the food, cosmetics, pharmaceutical, textile, and other industries.

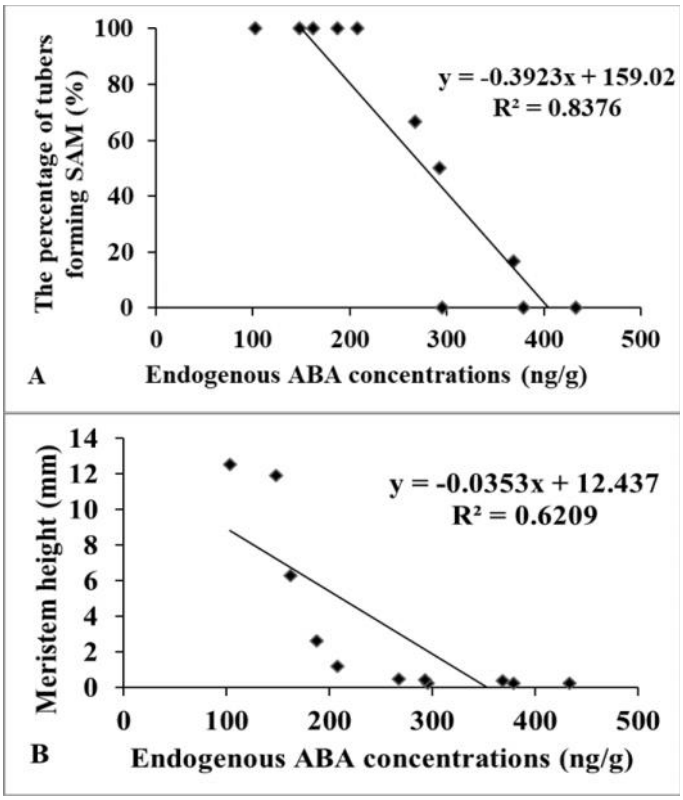

Figure 4. Correlation between endogenous ABA concentrations with meristem height and the percentage of tubers forming SAM, A. Correlation between endogenous ABA concentrations and the percentage of tubers forming SAM, B. Correlation between endogenous ABA concentrations and meristem height.

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