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IDENTIFICATION OF THE COMPOSITION OF GROWTH HORMONES IN THE TUBER OF *DIOSCOREAALATA*

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ABSTRACT

The identification of the growth hormones in the *D. alata*'stuber was conducted to investigate the composition (mg/l) of auxin, gibberellin and cytokinin in the three sectional parts of the *D. alata*'stuber in order to understand the potential aspects of the other parts of the tuber to be used as the seed. The findings show that the head, middle and tail sections of the *D. alata*'stuber content at least one growth hormone with the concentration of averagely more than 2.5 mg/l. Auxin was found in all sections with a significant concentration of ~ 2.8 mg/l. The head section is likely to cause a rapid growth of shoots due to the presence of auxin and gibberellin with a considerable concentration (*c.a.* 2.9 mg/l). Additionally, the concentration of cytokinin (i.e. zeatin and kinetin) is very moderate (< 0.2 mg/l) indicating a predominant role of apical dominance in the *D. alata*'stuber.

INTRODUCTION

Discoreaalata is a kind of tubers potentially to support the food demands of Indonesian due to its nutrition composition which is likely to be comparable tothat of rice, the popular food resource in Indonesia. For instance, 100 gram of flour of *D. alata*consists of 101 kcal energy and 2.1 gram protein (Horton, 1988) which is comparable to that of the rice (100 gram of rice: 175 kcal energy, 4 gram protein, Adriyendi and Syahputra (2013)). Moreover, the diversity of nutrition in the 100 gram of flour of *D. alata* (i.e. 74% water, 0.2 gram fat, 1.0 mg ash, 20 mg Calcium, 69 mg phosphor, 600 mg Potassium, 0.1 mg thiamin, 0.04 mg riboflavin, 0.5 m niacinand 9 mg ascorbic acid) further supports the potential aspect of *D. alata*for being another primary food source for Indonesian(Horton, 1988). The tropical climate in Indonesian region, which is favorable for the growth of *D. alata*and enables an even distribution of the plant over the archipelago from the far west (Sumatera Island) until far east (Papua), additionally convinces the potential aspect of *D. alata*on the primary food source in the nation(Donohue and Denham, 2010; Lebot, 1999; Malapa *et al*., 2005).

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Despite the significantly potential aspects of *D. alata*, the multiplication ratio of the plant has not been optimal compared to the rice. For instance, the multiplication ratio of *D. alata*is only 0.2 which is far larger than that of potato(0.1) (Asea *et al*., 2010; Onwueme, 1978). The higher multiplication ratio of *D. alata*is probably caused by the conventional method in multiplying the plant by using whole tuber of *D. alata*with large weight (200-500 gram) or by using head tuber section (Fig. 1). However, this ratio can be reduced by alternatively considering the usage of small portion of tuber from the middle and tail sections of the tuber (Fig. 1); these sections can be stimulated to produce new shootsto be developed for new individuals(Onwueme, 1978). The usage of the small portion of the *D. alata*'stuber with light weight should consider the composition of the growth hormones inside the sectional tuber. The growth hormones play an important role to the growth and the development of a plant since the plant in stages of shoots, plant growth, the phase of flower, the phase of maturity in the plant and the post-harvest (Nickell, 1982). Another important thing to be considered is the distribution of the growth hormones due to the movement of the growth hormones following (basipetal) and inverting (acropetal) the direction of the earth gravity and thus, the accumulation of the growth hormones can occur at a particular location in the plant where the basipetal and acropetal movements meet.

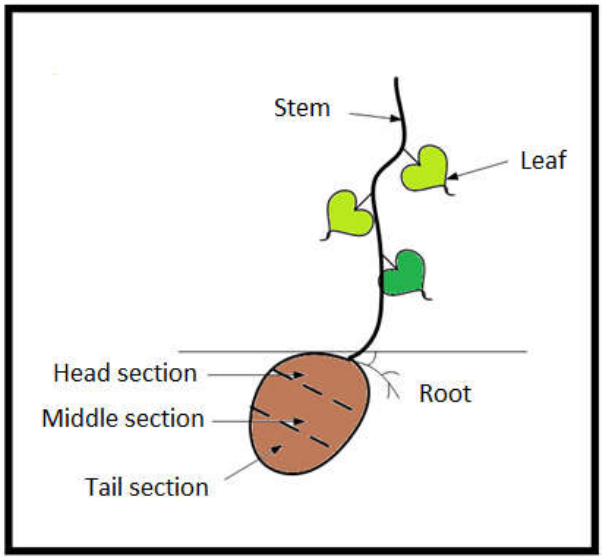

Figure 1.Theschematic diagram of tubers of *D. alata*'s

This indicates an uneven distribution of the growth hormones in the tuber and therefore, it is important to investigate the the distribution of the hormones e.g. auxin, gibberellin and cytokinin in the sections of the *D. alata*'stuber in order to determine the effectiveness of the small portion from the three sections (Fig. 1) of the *D. alata*'stuber to successfully produce new individuals. This present work can be considered as a preliminary study aiming to investigate the composition (in mg/liter) of auxin, gibberellin and cytokinin in the three sectional parts of the *D. alata*'stuber (Fig. 1) utilizing High Performance Liquid Chromatography (HPLC) 2487 combined with the UV-Vis detector following Linskens and Jackson (2012). The previous studies have investigated the composition of these growth hormones using the method of Linkensdan Jackson (1987) on seaweed with satisfied results (Sedayu *et al*., 2013).

MATERIALS AND METHODS

The source of the *D. alata*'stuber was from Saparua Island, Maluku Province of Eastern Indonesia with the diameter ranging between 15 and 18cm. the tubers then were divided following the sectional parts i.e. the head, middle and tail parts based on Fig. 1. The three sections of the *D. alata*'stuber were powdered using blender and the powder of the sections were extracted. The extracts of the *D. alata*'stuber were preserved using 2% formaldehyde liquid and were proceeded in advance in laboratory using the HPLC 2487 approach to identify the composition of auxin, gibberellin and cytokinin (i.e.zeatin and kinetin).

Preparation and analysis of Gibberellin

The 2 grams of extract of the *D. alata*'stuber were put into a separatory funnel. The extract was then titrated with using ethyl acetate 3×10 ml and then to be passed into silica resin. The result of elution was evaporated using vacuum evaporator with the temperature of 55 ^{o}C and then dried using freeze dryer. Next, the dried extract was dissolved into 10 ml methanol (65% methanol and 35% water). The solution was then filtered using milliphorepaper which were then used to be analyzed using HPLC waters 2487. A C18 silica was used for being the reversed-phase HPLC packing.

The gibberellin concentration was detected using UV-Vis detector with the wavelength of 254 nm.

Preparation and analysis of auxin and cytokinin

The 2 grams of extract of the *D. alata*'stuber were put into a separatory funnel to be extracted using 3×10 ml solution of methanol. The drying process of the extract used freeze dryer. The dried extract was dissolved into 30% solution of acetic acid with acetonitrile of 23 ml. Then, the solution was centrifuged with the rotate velocity of 4,000 rpm for 30 minutes. The centrifuged solution was then fltered using milliphore paper to be analyzed using HPLC for determining the concentration of cytokinin in the *D. alata*'stuber.

RESULTS AND DISCUSSION

Fig.2 shows the types of hormones detected using HPLC technique along with the application of the UV-Vis detector on the *D. alata*'stuber sections. It is interesting to notice that auxin was predominantly found in the three sections with the concentration of more than 2.5 mg/l. Moreover, it is also obvious that most of the growth hormones (auxin, gibberellin and cytokinin) were found to be mainly localized or concentrated in the head section of the*D. alata*'stuber. Furthermore, the cytokinin is very low in all sections (less than 0.4 mg/l).

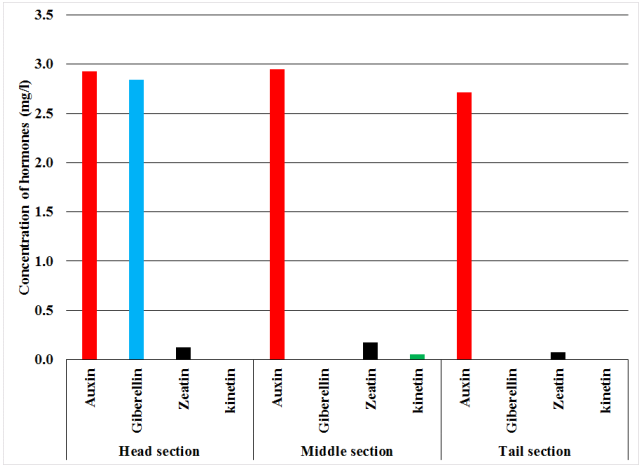

Figure 2. Distribution of growth hormones in the *D. alata*'stuber sections; the results were obtained from Indonesian Research Centre for Biotechnology and Bioindustry, Bogor

The presence of auxin in all sections of the *D. alata*'stuber indicates the potential aspects of the sections to produce new individuals e.g. the growth of shoots. Auxin has been reported by previous studies to play a significant role to directly influence the growth of shoots (Bandurski *et al*., 1995; McDavid *et al*., 1972; Normanly *et al*., 1995; Thimann, 1939).As a result, the usage of all sections in multiplying *D. alata* should need to be taken into account and is likely to reduce the larger value of the multiplication ratio of *D. alata* due to the usage of the whole tuber. The head section of the*D. alata*'stuberis more likely to produce rapid growth of shoots due to the considerable concentration of auxin alongside with the pronounced concentration of gibberellin.The roles of auxin and gibberellin in affecting growth of plant are specific. On one hand, auxin contributes to the plant growth via the extension of cell (Yang *et al*., 1996).

On the other hand, gibberellin stimulates the plant's growth via the increase of cell numbers along with the rise of cell length (Hu *et al*., 2008; Olszewski *et al*., 2002; Yang *et al*., 1996). In fact, the collaboration between both growth hormones with comparable concentrationwill provide more extent in length compared to the particular condition of either predominant gibberellin or auxin(Aloni, 1979). This indicates that the growth of new individuals from the middle and tail sections of the *D. alata*'stuber will be regulated by the extension cell due to the predominant concentration of auxin. In contrast, the growth of the new individuals form the head one will be governed by two ways i.e. the increase of cell numbers and the extension of cell due to the considerable concertationof auxin and gibberellin; this reflects a more rapid growth of new individual plants due to the usage of the head section compared to the other sections.

The evidence of auxin in the middle and tail sections of the *D. alata*'stuber can be alternative for the seed of *D. alata* despite the common usage of the head section. Most of Indonesian farmers tend to utilize the head section of the tuber due to the presence of shoots at the top of the head section which provides a confidence to produce new individuals. However, the growth of plant is significantly regulated by the growth hormones (Yang *et al*., 1996) and thus, the existence of auxin in the middle and tail sections of the *D. alata*'stuber reflects a potential aspect of the sections to be considered as the alternative seed. The insignificant concentration of cytokinin i.e. zeatin and kinetin is likely to affect the apical dominance in the *D. alata*'stuber. Some studies argue that the presence of cytokininprevent apical dominance (George *et al*., 2008; Heldt and Piechulla, 2004).This indicates that the apical dominance exists significantly in the *D. alata*'stuber.

Concluding Remarks

The identification of the growth hormones in the *D. alata*'stuber was conducted to investigate the composition (mg/l) of auxin, gibberellin and cytokinin in the three sectional parts of the *D. alata*'stuber in order to understand the potential aspects of the other parts of the tuber to be used as the seed. The findings show that the head, middle and tail sections of the *D. alata*'stuber content at least one growth hormone with the concentration of averagely more than 2.5 mg/l. Auxin was found in all sections with a significant concentration of ~ 2.8 mg/l. The head section is likely to cause a rapid growth of shoots due to the presence of auxin and gibberellin with a considerable concentration (*c.a.* 2.9 mg/l).Additionally, the concentration of cytokinin (i.e. zeatin and kinetin) is very moderate (< 0.2 mg/l) indicating a predominant role of apical dominance in the *D. alata*'stuber. It should be noticed that the results of this study only provides information on the composition of the growth hormones in the *D. alata*'stuber which is an outlook of the potential aspects of the sections of the tubers to be usage for the seed of *D. alata*. This information has not been verified yet with the studies in the field about the characteristics of the new individuals of *D. alata*(e.g. the growth rate, the morphological features) originally from the different tuber's sections. As a result, a useful focus of future work would beto directly measure the different characteristics of shoots and the growth rate of new individuals of *D. alata*from the head, middle and tail sections of the *D. alata*'stuber. This would reveal the roles of different composition of the growth hormones in the seeds in determining the characteristic of new individuals of *D. alata*.

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