Effects of seeding material age, storage time, and tuber tissue zone on glucomannan content of Amorphophallus muelleri Blume

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Abstract
Among members of the genus Amorphophallus in Indonesia, Amorphophallus muelleri produces the highest amounts of glucomannan, which is a fiber carbohydrate that plays a significant role in controlling obesity and type 2 diabetes. Glucomannan in A. muelleri is stored in the tubers. Several internal and external factors affect the glucomannan content of the tubers. In this study, we only investigated the internal factors seeding material and tuber. The objectives were: i) to investigate the effect of the seeding material on tuber glucomannan levels; and ii) to assess the influence of the storage period and the tuber part on glucomannan contents. Glucomannan was extracted via centrifugation. The result showed that tubers, which yielded from the center bulbils, have slightly higher glucomannan content than tubers from side bulbils, even though insignificant. Our results indicate significant glucomannan losses at storage times of more than 3 months. Levels decreased by 90% after storage over 3.75 months since shoot collapse. Glucomannan levels of the central and the edge parts of the tubers did not differ significantly.

Introduction
Amorphophallus muelleri or porang is a plant native to Indonesia, and 23 species of the genus Amorphophallus occur in the country. Amorphophallus muelleri is well known as a significant source of glucomannan, which is used in the control of obesity and diabetes type 2,1 lowers total cholesterol, triglycerides, and LD cholesterol,2,3 improves weight loss,4,5 and helps to overcome constipation by decreasing the residence time of feces.6 As a hydrocolloid polysaccharide, it has a potential role in drug delivery.7 Glucomannan is the dominant carbohy-
Effect of main and side bulbil as seed planting material on tuber glucomannan content

The bulbil from the main petiole was called the main bulbil, while the bulbil from the rachis was called the side bulbil (Figure 1). Bulbs were taken from plants of a similar size. In total, 25 main bulbs (20.83-32.39 g) and side bulbs (5.87-10.58 g) were obtained. Four bulbs were selected, similar in weight and size, for planting and were planted in 40x40 cm polybags, using compost as media. Insect control was performed each day. When herbivorous insects were present (rarely observed), the insect was removed mechanically. Immediately after shoot collapse, the tubers were harvested, cleaned from soil, washed with tap water, air dried, and weighed. Glucomannan analysis was performed as soon as possible.

Effect of length of storage time on glucomannan content

The bulbs used for this study had an initial weight of 2.03-2.26 kg. Tubers were stored over a period of 15 weeks. Every 5 weeks, glucomannan contents were analyzed. To minimize the bias/variation factor of the tubers, at the beginning of the experiment, the tuber was divided by four pieces. The first piece was stored for 0 weeks, while pieces 2, 3, and 4 were stored for 5, 10, and 15 weeks, respectively. At week 0, glucomannan analysis was performed on four pieces from four different tubers; each piece represented a replicate. The analysis at weeks 5, 10, and 15 was the same as that in week 0, using four pieces. The tubers were stored on a laboratory table at room temperature, imitating storage after harvest.

Glucomannan analysis in the edge and center parts

The bulbs used for the study weighed 2.05-2.30 kg. To obtain the edge and center parts, the tubers were cut in pie-shaped manner (Figure 2A). Subsequently, 1-2-cm pieces were cut from the edge to the middle. To obtain a central cut, the proximal area was cut into 4-5-cm wide pieces in a distal direction (Figure 2B). Edge pieces were peeled and the other skin was removed. The thickness of the edge and the center parts depended on the tuber size. Distinguishing the edge and the central parts can be done by color gradation, as the edge has a slightly lighter color than the central. Both parts were subject to glucomannan analysis.

Statistical analysis

We used the unpaired t test and Duncan’s α test at 0.05 probability. The unpaired t test was used to analyze the glucomannan content to investigate the effects of main and side bulbils and of different tuber parts, with four and three replications, respectively. Duncan’s test, preceded by ANOVA, was used to determine the impact of the storage period on glucomannan content, using four replications. All tests were performed using SPSS 16 for Windows.

Results

Main bulbils produced significantly heavier tubers than side bulbils (P<0.05). Also, plants growing from the main bulbil had taller petioles than those obtained from side bulbils (Table 1). A trend was observed in tubers derived from main bulbils, which appeared to contain more glucomannan than those grown from side bulbils, although this difference was not significant.

Table 1. Tuber yield and plant height as a factor of seeding source.

<table>
<thead>
<tr>
<th>Bulbil seeding source</th>
<th>Bulbil weight (g)</th>
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<td>Main bulbil</td>
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Different letters within a column indicate significant differences in the unpaired t test at α=0.05.

Glucomannan analysis

Glucomannan analysis was conducted according to Tatirat and Charoenrein, with modifications. Briefly, 30 g of fresh tuber were finely sliced, 200 mL 0.3% Al2(SO4)3 were added, and the mixture was blended for 3 min. Subsequently, the suspension was heated at 55°C for 15 min in a water bath. During heating, the suspension was stirred with a glass rod. The suspension was then diluted to 600 mL and filtered through a chiffon cloth. The filtrate was centrifuged at 1500 rpm at 25°C for 30 min. The supernatant was collected and 95% isopropyl alcohol or 95% ethanol were added at a ratio of 1:1. The coagulated glucomannan was obtained by lifting with a glass rod and filtering through a Whatman filter; it was stored in 95% IPA to prevent discoloration. Inundation of glucomannan in 95% IPA was performed three times. Before drying at 45°C overnight, glucomannan was compacted between pieces of Whatman paper; glucomannan content was expressed as a percentage, using the following equation:

\[
\text{Glucomannan (\%) = \frac{\text{glucomannan (dry weight)}}{\text{tuber sample (dry weight)}} \times 100\% (1)}
\]

Fresh samples were converted to dry weight after correcting for moisture, which was determined by drying at 105°C for 2 h.

Glucomannan content in the edge and center parts

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During growth and development of A. muelleri, main bulbils appear earlier than side bulbils (Figure 1). As a consequence, main bulbils are generally larger than side bulbils, implying that the physiological age of the two kinds of bulbils is different, even though they have derived from the same mother plant. The data were obtained after seeded bulbils from both main and side sources were planted, producing plants of unequal stem height and tuber weight. The plants derived from main bulbils grow taller than those from side bulbils (Table 1).

Tubers of A. muelleri are rarely sold directly and are generally collected (Paidi, Personal communication). Once large quantities are collected, the tubers are transported to the factory. This means that the tubers are stored by the farmers prior to processing. At the beginning of the storage period, the tubers did not show any signs of sprouting (Figure 4A). However, at week 10, we observed the appearance of a coleoptile on the adaxial side of the tuber (Figure 4B). During storage, glucomannan contents decreased linearly (Figure 5). After more than 3 months, the decrease in glucomannan contents was significant, reaching up to 90% (Table 2).

When A. muelleri tubers are split, yellow flesh appears.8 Glucomannan sacs do not add color to the A. muelleri tubers, indicating that the yellow color is derived from carotene. The yellow color ranges from light yellow to dark yellow, indicating that the vacuole varies in size. Based on the different colors from the edge to the center of the tube, we assume that the glucomannan content differs throughout the tuber. Analysis showed that glucomannan levels were slightly higher in the center than in the edge, although this difference was not significant (Figure 6).

### Discussion

Our results were consistent with studies by Sukarman et al.17 on Temulawak, who stated that seed weight influenced yield. In addition to both height and weight measurements, we measured the glucomannan content of tubers yielded from either the main bulb or the side-bulbil. Heavier bulb (main bulbil) resulted in higher glucomannan yields than lighter bulbils (side bulbil), although this difference was not statistically significant. Similarly, our growth results are in agreement with those of Sumarwoto and Mariyana.9 However, these authors did not analyze glucomannan levels, but instead focused on the tuber diameter and thickness, yield, stem diameter, and plant height.

Given the physiological relationship between photosynthate source and sink in plants,18 we added canopy diameter as a parameter. Our results also showed that main bulbils produced more leaves and a wider canopy than side bulbils (data not shown). This means that plants derived from larger bulbils have higher photosynthesis rates, resulting in higher amounts of photosynthates, which are distributed to the sink area,18 including young leaves and tubers. Young leaves use photosynthesis for growth, whereas in tubers or storage organ, the photosynthate is stored as food reserves. Although tubers from main bulbils had higher glucomannan contents than those from side bulbils, the difference was not significant, indicating that seeds from the same mother plant or of the same age have the ability to produce similar yields. This raises the question whether the bulbil of a 4-year-old plant is as potent as the main bulbil of a 1-year-old plant. Chua et al.14 also compared the major bulb potential of old plants (4 years old) with the main bulbil of young main plants (age 1 year) in terms of...
glucomannan production and observed temporal glucomannan pockets, which were empty in young tissue, but full in mature tissue.

A sharp decline in glucomannan from week 10 to week 15 was related to the tubers entering the germination period. Evidence of the germination process was the emergence of germination signs in the form of a small, pink, dome-shaped structure. Bewley\textsuperscript{19} mentions that during germination, major storage reserves are degraded to produce buds or other structures for germination. Fait \textit{et al}.\textsuperscript{20} added that the germination process requires the reactivation of some metabolic processes. This requires balancing catabolic and anabolic activities to initiate physiological changes underlying emerging leaf buds, radicles, or plumules. During germination, there is an increase in respiratory activity.\textsuperscript{19} The products of respiration in the form of a C-skeleton and energy are used for embryonal growth.\textsuperscript{18} Glucomannan as a food reserve\textsuperscript{21,22} is used by \textit{A. muelleri} as a source of energy and a

source of carbon skeletons for growth and development. This study suggests that prolonged tuber storage results in a decrease in glucomannan contents.

In ancient times, \textit{A. muelleri} farmers chopped the tubers to a certain thickness and sun-dried (called porang chips by local farmers). These chips are sold at different prices. The habit of chopping the tubers has been conserved in Madiun and Nganjuk and has been widely adopted by farmers. Although Chua \textit{et al}.\textsuperscript{14} indicated that the middle zone of the konjac tuber (belonging to the genus \textit{Amorphophallus}) contains high amounts of glucomannan, farmers generally do not distinguish between the middle zone and the edge zone when slicing the tubers. Our results in are in agreement with findings from Chua \textit{et al}.\textsuperscript{14} and Takigami \textit{et al}.\textsuperscript{12} although the difference between the two zones is not statistically significant. From an economic point of view, the separation of middle and edge zones is laborious, but can be done with the help of machines. In the field, \textit{A. muelleri} tubers are chopped without being washed first, usually containing a layer of soil. To increase the quality of \textit{A. muelleri} chips, the tubers should therefore be washed or peeled prior to processing.

**Conclusions**

Large-sized bulbs (main-bulbs) as seeded tubers produce higher tuber yields and higher glucomannan amounts than tubers from small bulbs (side bulbs). Tubers of \textit{A. muelleri} harvested soon after shoot collapse produced optimum levels of glucomannan, while before shoot collapse or a few weeks after shoot collapse, glucomannan contents were lower. Storing the tubers at ambient temperatures will reduce glucomannan contents. Long-term storage (up to 15 weeks) in the open air greatly reduced glucomannan contents. In intact tubers, the central part contained higher glucomannan concentrations than the tuber edges. Therefore, in post-harvest processing, it is better to separate the edge and middle portions of the tubers.

**References**


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**Figure 5.** Glucomannan contents at four different storage times. Different letters indicate significant differences in Duncan’s test.

**Figure 6.** Glucomannan content of the edge and the center of the tubers. From edge and center part of tuber. Different letters indicate significant differences in the t test at $\alpha=0.05$. 

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