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 bulbis, have slightly higher glucomannan content than tubers from side bulbis, 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 carbohydrate in *A. muelleri*[8] and stored in the tubers. Tubers of *A. muelleri* start to have economic value after at least three periods of growth. In Indonesia, *A. muelleri* is mostly planted in secondary forest under teak trees and is harvested for the first time about 3 years after planting and then each following year. Tuber size and yield are determined by various factors. One factor is the type of seeding material, including the tuber, bulbil, and seed. Initial tuber and bulbil size determine tuber size at harvesting. Larger bulbils produce heavier tubers than small bulbils.[9] After harvest, tubers are either directly chopped, dried, and stored or sent to the factory to be processed into flour. The time between tuber pooling until processing in the factory varies between several weeks and months. Dandago and Gungula[10] showed decreased levels of protein and starch and increased vitamin C and A levels in sweet potato stored for 5 months, while short-term storage (15 days) of taro tubers did not alter the quality of the tubers in terms of ash content, coarse fat content, crude protein content, and coarse fiber content.[11] During post-harvest, some farmers choose to chop *porang* vertically (adaxial to abaxial), resulting in a thickness of 0.2-1.0 cm. From a biological point of view, the actual size of the cells in a tuber varies, as mentioned in Takigami *et al.*'s research.[12] The author stated that cells in the periphery zone of *A. konjac* tubers were smaller than those in the center.[12] By staining *A. konjac* tuber tissues, Zhao *et al.*[13] recognized a translucent sac structure with a size of 0.25-0.70 mm, which is more than 5-10 times larger than ordinary cells. This sac structure is called the glucomannan idioblast,[14] and its distribution differs between the edge and the center of the tubers. Sumarwoto and Maryana[9] investigated different bulbs of different sizes as planting materials, *i.e.* small (1.5 g), medium (5 g), and large (10 g), but they were not specifying the origin of the bulbils. Bulbils harvested from 1-year-old *A. muelleri* plants differed in weight and size from those harvested from 2-year-old plants. Similarly, in the same age range, the size and weight of the bulbil from the *main stem* differed from that from the *branching stem*.

To date, there are no studies on the use of main bulbils (derived from the main stem) and side bulbils (derived from the branch). In this sense, it is interesting to see if bulbils taken from different parts of one mother plant (main or side) will produce the same tuber weight and the same glucomannan content. *A. muelleri's* exact harvest time is marked by shoot collapse.[15] In the field, the collapse of all shoots does not occur simultaneously. Suprisingly, the timing of harvest of *A. muelleri* has not been associated with glucomannan content. Harvested tubers, especially those that will be used as seeds, are often left on the terrace until the rainy season. Tubers with high economic value (age three years) can be left at the yard around house until sold. During this period, reserve substances, such as glucomannan, are likely to undergo changes. It is therefore crucial to investigate the impacts of storage time on glucomannan contents to take steps or strategies to maintain high glucomannan contents. Previous studies showed variation in idioblast glucomannan content, making it necessary to measure glucomannan in the edge (by peeling the edges of tubers of a certain thickness) and in the center of the tubers. Such studies could assist farmers in developing adequate processing methods.

Materials and Methods

All *Amorphophallus muelleri* Blume specimens were provided by a farmer from Oro-Oro Waru Village, Madiun District, East Java Province, Indonesia.

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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.

Glucomannan analysis

Glucomannan analysis was conducted according to Tatirat and Charoenrein,[16] with modifications. Briefly, 30 g of fresh tuber were finely sliced, 200 mL 0.3% $Al_2(SO_4)_3$ 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:

$$Glucomannan\ (\%) = \frac{glucomannan\ (dry\ weight)}{tuber\ sample\ (dry\ weight)} \times 100\% \quad (1)$$

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

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.

Bulbil seeding source	Bulbil weight (g)	Tuber yield (g)	Plant height
Main bulbil	25.44±3.67a	80.28±32.7a	60.86±4.45a
Side bulbil	7.88±1.15b	45.57±9.99b	47.43±4.24b

Different letters within a column indicate significant differences in the unpaired t test at =0.05.

Figure 1. Bulbil position: thick arrow shows a main bulbil; thin arrow marks a side bulbil.

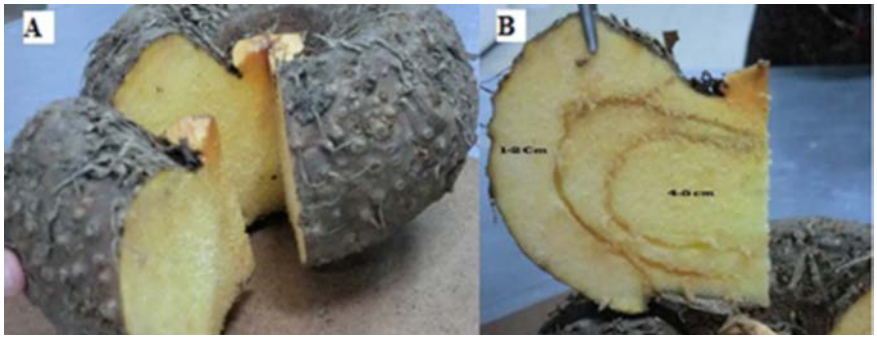

Figure 2. A) Pie cut of *A. muelleri* tuber; B). Mapping tuber parts considered as edge and center part.

(Figure 3). 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 bulbil or the side-bulbil. Heavier bulbil (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

Table 2. Glucomannan content reduction during storage of *A. muelleri* tubers.

Storage period (weeks)	Glucomannan content (%)	Reduction (%)
0	42.75	0
5	16.75	61
10	13.5	68
15	4	90

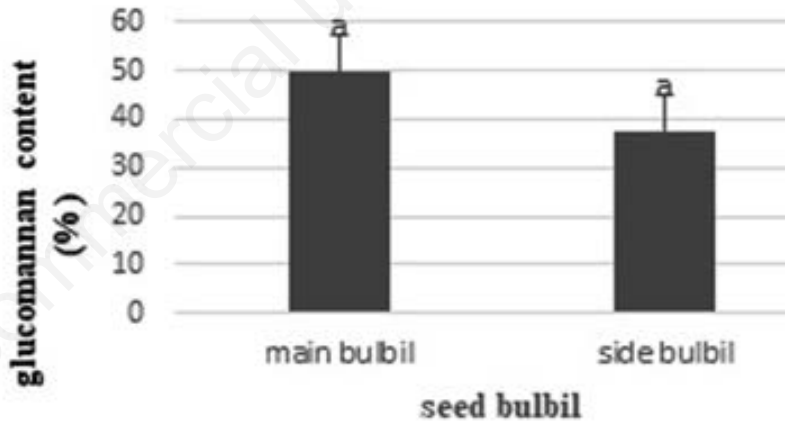

Figure 3. Glucomannan content of tubers derived from main and side bulbils. Note: Different letters indicate significant differences in the t test at α=0.05.

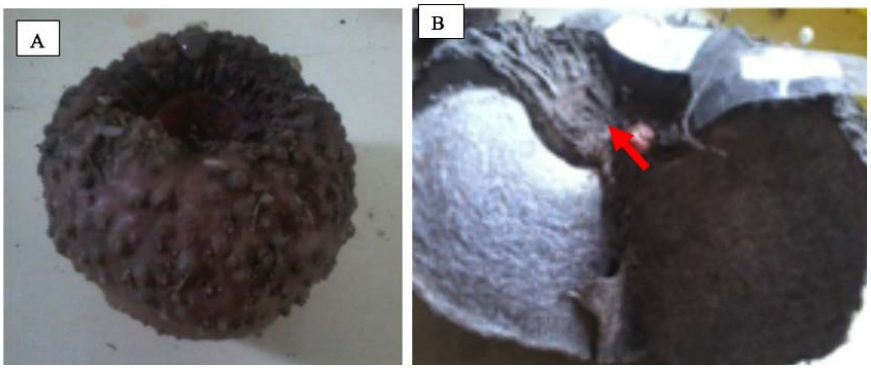

Figure 4. Tuber of A. *muelleri* at storage week zero (A), tuber of A. *muelleri* at storage week 10, beginning to show signs of germination (arrow).

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[19] mentions that during germination, major storage reserves are degraded to produce buds or other structures for germination. Fait *et al*.[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.[19] The products of respiration in the form of a C-skeleton and energy are used for embryonal growth.[18] Glucomannan as a food reserve[21,22] is used by *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, *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 *et al.*[14] indicated that the middle zone of the konjac tuber (belonging to the genus *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 *et al.*[14] and Takigami *et al*.,[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, *A. muelleri* tubers are chopped without being washed first, usually containing a layer of soil. To increase the quality of *A. muelleri* chips, the tubers should therefore be washed or peeled prior to processing.

Conclusions

Large-sized bulbils (main-bulbils) as seeded tubers produce higher tuber yields and higher glucomannan amounts than tubers from small bulbils (side bulbils). Tubers of *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.

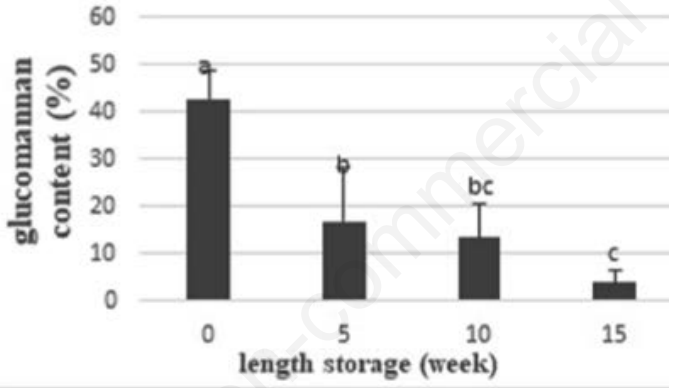

Figure 5. Glucomannan contents at four different storage times. Different letters indicate significant differences in Duncan's test.

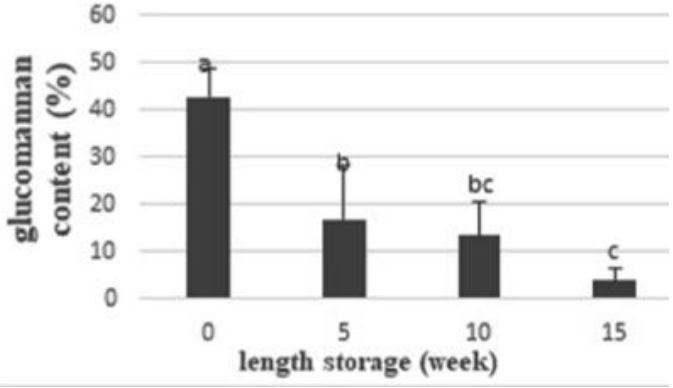

Figure 6. Glucomannan content of the edge and the center of the tubers. From edge and center part of tuber. Different letters indicate significant differences in the t test at α=0.05.

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