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Enlargement of Tubers from Flower Blooming to Seeds Ripening in Konjac Plant (Amorphophallus muelleri Blume)

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ABSTRACT

The cultivation of konjac plants (Amorphophallus muelleri Blume) is mainly initiated by the accumulation of glucomannan in their tuber. Many studies of konjac plants are limited until konjac tubers are suitable for harvest, not extended to the flowering stage. This study focused on tubers' development in the flower bud formation phase until seed development. The results of this study show that only one flower grows from each tuber. It takes 40+2 days for the konjac flower to bloom fully but 3+1 days for the flower to wither. The weight, diameter, and thickness of the konjac tubers used as planting material were 0.342±0.014 kg, 8.23±0.26 cm, and 5.75±0.17 cm, respectively, and significantly grew to 2.70±0.156 kg, 19.96±0.560 cm, and 12.73±0.335 cm, respectively at the time of harvest. Konjac plants promote thick lateral roots at the base of the petiole and fibrous roots on the tuber skin. During the enlargement of the tubers, the thickness and diameter ratio did not change. The cross-sectional shape of petioles and spadix is slightly oval. The number of seeds per plant correlated with the female flower's length, diameter, and cylindrical area. Finally, tubers decompose, and a new one or more starts to grow.

INTRODUCTION

Some Amorphophallus species grow wild in tropical forests, yet some have been intensively cultivated, especially after it was discovered that the konjac plant contains a significant concentration of glucomannan. Glucomannan could easily and quickly be isolated from fresh konjac tubers, requiring no further purification.

Konjac plants (Amorphophallus muelleri Blume) can be cultivated using reproductive seeds, bulbils, or tubers. It takes 3-4 years from seed to blossom (Liu et al., 2023). Konjac plants only produce one single flower. The konjac flower consists of spathe and spadix. Hundreds to thousands of small fruits were produced at the lower segment of the

spadix. Each fruit produces a seed (Shenglin et al., 2020). Nevertheless, farmers rarely use seeds as planting material in konjac cultivation since it takes extra years for the tubers to be suitable for harvesting.

Bulbils are formed at the armpits of compound leaves of the mother plant. They are clones of the parent plant; consequently, each bulbil has identical genetic material. Bulbils are commonly used in the first year of planting for early shoot emergence and faster growth. Shu et al. (2024) argued that bulbil was an important asexual reproductive structure of konjac plants. Bulbils have played a significant role in the reproduction of konjac plants by serving as propagation materials, energy reserves, and

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survival mechanisms in adverse environmental conditions.

Although all three types of planting material can be used in the first year, only tubers are used in subsequent years. Tubers are expected to continue to grow until they are suitable for harvesting and are the size of 2-3 kg. To produce seeds, the tubers continue to be replanted for the following year(s) until the tubers bloom. If the konjac tubers are continuously replanted for 3-4 growing seasons, they will likely bloom and produce reproductive seeds (Shenglin et al., 2020).

Most of the research on the cultivation of konjac plants starts from planting seeds, bulbils, or tubers as planting materials, the response of konjac plants to certain treatments, including media mixtures (Xu et al., 2024), artificial lighting for enhancing seed germination (Zahra et al., 2023), breaking dormancy (Shu et al., 2024), and shade intensity (Nurshanti et al., 2023) until the tubers are harvested. Tubers are the most important organ because they contain glucomannan. Tubers are the most important organ because they contain glucomannan (Nurlela et al., 2022; Satiti et al., 2025). Harvesting tubers is carried out before flowering. This research focuses on morphological changes during the development of flowers and seeds of koniac plants. It is generally believed that koniac tubers will soon be graded after the flowers bloom and true seeds age. Still, the reality is that no one has scientifically proven how and how long the decomposition process takes place.

MATERIALS AND METHODS

The planting material used was konjac tubers that had undergone three dry-rainy growing cycles for three years. Planting material originated from South Sumatra, Indonesia. The tubers were replanted at the beginning of the rainy season in October 2023 and harvested after the flowers dried. Seeds were fully developed at the beginning of the dry season in April 2024. This research activity was carried out in Jakabaring, Palembang, Indonesia.

The average weight, diameter, and thickness of tubers used as planting material were 342.01 g, 8.23 cm, and 5.75 cm, respectively. The 24 whole tubers were planted on loosened soil at 50 cm spacing. Planting started when there was a tuber within the stock that had already shown the presence of emerging buds. The bud emergence

in konjac plants is relatively diverse. Based on the results of our earlier studies, the bud emergence was faster and more uniform if true seeds were used (Nurshanti et al., 2023), but it will take 3-4 years to wait until the konjac plant blooms. Not all (4.17%) tubers produced flowers. Instead, some of them continue to produce leaves. In this study, only flowers produced were used.

Measurements of the konjac flower's growth rate were carried out starting from the base of the petiole to the highest tip of the spathe. Since the occurrence of the bud emergence greatly varies, the measurement point of flower development was recorded based on the bud emergence event, not based on the calendar dates. Therefore, all measurement points were fixed to condition when new shoots are visible or less than 5 mm above ground level.

It should be known that when konjac plants produce flowers, the organs that appear above the ground surface are only one floret consisting of petioles grow directly from tuber (which is sometimes referred to as pseudo stem) and one flower consisting of a spathe shaped like a saucer (which is a deformed corolla) and a spadix consisting of three segments. The lowest segment connected to the petiole is a base for hundreds to thousands of small female flowers to develop. Secondly, the middle segment is where the male flowers produce pollen. Thirdly, the uppermost segment of the appendix produces pheromones to attract the presence of pollinating insects.

Measured traits included time from bud emergence to flower fully bloom; the flower height; petiole length and diameter; spathe diameter; middle section of petiole and spadix; cylindrical area and length of female flower segment; number of true seed/flower; diameter, thickness, and fresh weights of tuber as planting material and at harvest; ratio of thickness/weight; and tuber fresh weight at harvest. The water content within sliced konjac tubers could not dry in the oven because the konjac tubers became sticky and formed a big chunk of jelly.

Some traits that affect the development of other traits or are suspected to be interrelated were carried out using regression or correlation tests. Regression testing was performed to determine whether the updates or changes had caused new defects in the existing functions. Correlation analysis was used to discover if there was a relationship

between the two datasets. Both tests were performed using Microsoft Excel for Windows (developed by Microsoft, Redmond, Washington, US).

RESULTS AND DISCUSSION

Growth and Development of Konjac Flower

The development of konjac flowers begins with the initiation of buds appearing from underneath the soil surface. At the beginning of emergence, it is difficult to distinguish between leaf buds and flower buds. After the spathe opens, it can be determined that what grows is a leaf or flower. It takes about 40±2 days from the emergence of flower buds until they reach full bloom (Fig. 1). After that, the flower only takes 4±1 days to wither and dry. In this study, the time of bud emergence varies greatly; still, it is not yet known whether it is caused by the influence of agrocilmatology conditions or due to the diversity of tuber used. The growth and development of konjac flowers follow a quadratic curve, with slow growth at the beginning and accelerated when the petiole begins to lengthen.

Konjac plants, when flowering, produce only flowers without leaves. The flower bud appears from the planted tuber. A short bract fully protects the bud from penetrating the soil layer. Then comes

out the slightly elongated bract from inside the short bract. After that, from inside the bract emerges the tip of the spathe. The next process is the rapid elongation of the petiole. Therefore, the upper part of the petiole is clearly visible. This process pushes the flower up while it is still tightly wrapped by the spathe. While the petiole lengthens, the bract begins to dry and shrink in size so that the entire length of the petiole can be seen; meanwhile, the spadix begins to appear through the opening of the spathe gap. After the spathe opens fully (full bloom), the three parts of the spadix become visible, where the appendix protrudes up and out of the spathe. The middle and bottom segments of the spadix stay within the spathe i.e., the male and female flower segment.

Chua et al. (2013) suggested the six evelopmental stages of konjac plant, starting from (1) dormancy, (2) leaf bud emergence, (3) leaf bud elongation, (4) leaflet emergence, (5) leaf expansion, and ending with (6) shoot senescence. However, these stages were relevant for conditions before the konjac plant entered the reproductive cycle. Konjac plant is unique; it enters the reproductive stage after 3 to 4 planting cycles. It grows only to produce flowers and reproductive seeds during the reproductive stage. There is no leaf developed.

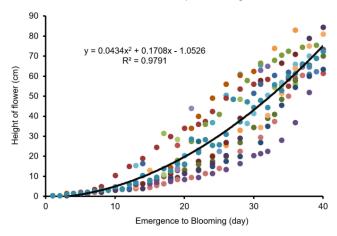


Fig. 1. Growth of konjac flower started with bud emergence and ended as the flower had fully bloomed

This process takes about 40 days from the flower bud emerging out of the soil surface until the flower reac solution. Handayani et al. (2020) reported that the flowering process included six phases, which altogether needed 22 to 36 days, namely the developments of inflorescence bud, cataphyll, spathe and spadix, appendix, fully bloomed inflorescence, and flower anthesis. The difference in time needed from the emergence of flower buds to fully open flowers between the two studies can be caused by differences in the starting point of measurement, and the first phase of flower bud growth occurred very slowly. In the research, measurements began when the flower bud cracks the soil surface at ± 5 mm.

Parts of Konjac Flower and Their Function

The flower bud was covered by spathe. The spathe was positioned between the upper end of the petiole and the base of the spadix, so it rose as the petiole lengthened; meanwhile, the bract stayed at the base of the petiole which then withered and dried out earlier. During anthesis, the flower consists of petiole, spathe, and spadix, respectively, from proximal to distal. The saucer-shaped spathe acts as a spadix protector. Furthermore, the spadix consists of a female flower segment at the base, a male flower segment at the middle section, and an appendix at the tip, respectively, from proximal to distal (Fig. 2). Shortly after 3-4 days of full bloom, the flower started to wilt and dry.

As planting material, konjac tubers, bulbils, and true seeds produce only one petiole with an oval-shaped cross-section, long and sturdy enough to support a single large flower. The saucer-shaped spathe is an outside protector of the male and female flower segments inside of the spathe. The spathe looks like a petal, but it is actually a modified leaf (Song et al. 2024). In the flowering phase, konjac plants have absolutely no leaves. Green petiole bark with white patches and the reddishgreen outer surface of the spathe are the parts of the konjac flower that chlorophyll.

Henry et al. (2020) emphasized that other parts of the plant, such as petiole and spathe, should also be able to conduct photosynthesis. Some plants that conduct C3 photosynthesis in the leaves have been reported to use C4 photosynthesis in petioles and flowers. Although, the chlorophyll contents and chloroplast numbers of the petioles were lower than those in leaves. The total photosynthetic rates of the petioles were equivalent to 6~8% of those at the leaf blade (Sun et al. 2021). The ability of konjac plants to carry out photosynthesis through petioles and spathe is the answer to the increasing size of konjac tubers during the flowering and seed ripening processes. Ripening konjac seeds takes about 5-6 months if they are under the shade. In the case of Pinellia ternata tubers, Xue et al. (2021) found that shading improved tuber quality.

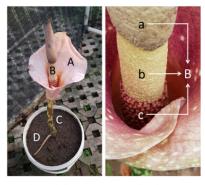


Fig. 2. The fully blooming konjac flower (left) and inner parts of the flower consist of spathe (A), spadix (B), petiole (C), and bract (D). The spadix divides into appendix (a), male flower segment (b), and female flower segment (c)

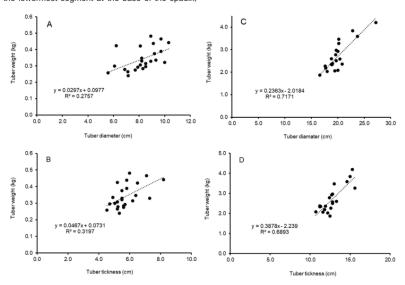
Metabolic heat is produced in floral organs during the anthesis with primary functions being to increase scent volatilization for pollinator attraction (Claudel et al. 2023). The comprehensive integrated analysis provided insight into spathe coloration regulation. The microRNAs may function as a negative regulator in anthocyanin accumulation (Lin et al. 2022). Konjac tubers, bulbils, and true seeds. as planting material, produce only one petiole with an oval-shape cross section, long, and sturdy to support a single large flower (Wunnenberg et al., 2021). Moreover, spathe can also protect flowers from herbivores by displaying warning signals, camouflage conspicuous reproductive organs, or providing physical barriers or toxic chemicals (Song et al. 2024).

The actual flowers of konjac plant are very tiny and found in spirals on the spadix. The spadix consists of three segments. Female flowers are on the lowermost segment at the base of the spadix;

male flowers are on the middle segment above the female flower segment; and the segment at the end of the spadix is the appendix. The number of female flowers may vary between a few hundred to more than a thousand seeds.

Tuber Growth During the Period of Flowering and Seed Ripening

The konjac tuber's weight, diameter, and thickness used as planting material were 0.342±0.014 kg, 8.23±0.26 cm, and 5.75±0.17 cm, respectively. Meanwhile, the weight, diameter, and thickness of the konjac tubers at the time of harvest were 2.70±0.156 kg, 19.96±0.560 cm, and 12.73±0.335 cm, respectively. Konjac tubers grew continuously after planting, as shown by the increase of tuber fresh weight, diameter, and thickness during the development of konjac flowers and beyond. Further enlarging during the long period of the seed maturity process (Fig. 3).



 $\textbf{Fig. 3.} \ \, \textbf{The diameter, thickness, and weight of the tubers increase significantly as shown by the difference in tuber weight at the time of planting (A and B) and harvesting (C and D)$

A source-to-sink transport to the konjac tuber during the vegetative phase occurred after the leaf blade enlarged and increased after the leaf reached its maximum size (Chua et al. 2013). Nevertheless, in this study, the same pattern occurred after the petiole was fully elongated and the flower had bloomed. Furthermore, the increase in tuber fresh weight, diameter, and thickness went beyond flowering. It continues to increase during the seed maturity period. Furthermore, the increase of tuber fresh weight, diameter, and thickness went beyond flowering, it continues to increase during seed maturity period (Zhao et al., 2024).

Storage root and tuber formation are initiated in an underground precursor organ. Hence, they were modified stems originating from the plant's main stem. The development of a tuber undergoes different phases: (a) stolon induction and initiation, (b) stolon elongation and branching, (c) cessation of the longitudinal stolon growth, (d) tuber induction by swelling of the stolon subapical region, and (e) tuber growth and enlargement (Zierer et al. 2021). In konjac cultivation, a small tuber is used as planting material; therefore, the growth continues the enlarging process.

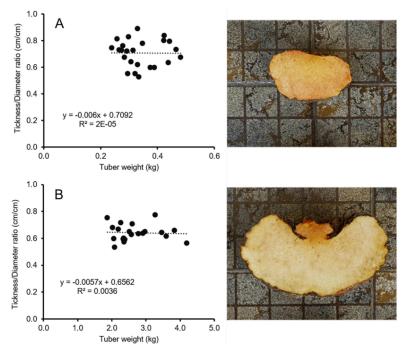


Fig. 4. The average ratio of thickness/diameter in konjac tubers was similar regardless of widely different tuber sizes

The ratio of thickness/diameter (T/D ratio) of the tuber did not change significantly as the flower approached full bloom. As konjac tubers grew, the average thickness/diameter ratio decreased insignificantly from 0.709 at planting to 0.656 at harvest. Parallel to the process of flower formation, the shape of konjac tubers also curved upwards to form a bowl-shaped tuber. Petiole was positioned at the center of the bowl. The base of the petiole became the main site for lateral roots to grow (Fig. 4).

Changes in the ratio of thickness/diameter (T/D ratio) can be used to proxy changes in the shape of the konjac tuber. In this study, as the konjac tubers enlarged until the seeds were ripe and ready to be harvested, the average thickness/diameter ratio decreased from 0.709 at planting to 0.656 which indicated that when the tuber increases in size, the tuber shape becomes 7.48 % slimmer.

There is no significant correlation between the weight of small tubers used as planting material and the weight of harvested larger tubers (Fig. 5). This finding shows that in the cultivation of konjac, there was no need to argue on the variability of tuber sizes used as planting materials because the size of the harvested tubers was more influenced by 6 or more months of exposure to microenvironmental conditions during the process of tuber growth and development.

Konjac plants can also be propagated using micro-tubers which can be used for commercial use in the field (Zhang et al. 2024). The difference in the weight of the mini tuber used as planting material does not directly determine the yield of harvested tubers. Tuber enlargement was more influenced by the conditions during the six months of exposure to the microenvironment on each koniac plant. The growth and development of konjac plants were reportedly influenced by shade intensity (Nurshanti et al. 2023), media mixtures used (Xu et al. 2024), various treatment applications for breaking dormancy (Shu et al. 2024), and artificial lighting for enhancing seed germination (Zahra et al. 2023). The correlation between the weight of tubers at planting and harvest is useful to determine the optimal weight or size of tubers to be used as planting material in the cultivation of konjac plants.

The cross-sectional shape of the petioles and female flower segment at the spadix base was not perfectly round but rather oval, as the red line (oval) did not fully follow or overlap with the blue line (round) (Fig. 6). However, the oval shape was only diverged by 6.78 percent and 4.75 percent of the perfectly rounded shape for the petiole and spadix base, respectively. The cross-section of petioles and spadix base reached the perfectly round shape only if the blue and red circle lines overlapped perfectly.

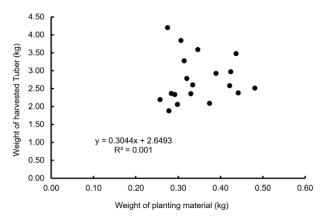


Fig. 5. There was a weak relationship between the tuber size used as planting materials and the harvested tubers after 6 months of cultivation

The nearly perfect circular shape of both petiole and spadix causes the flowers to stand firmly during the seed ripening or under strong wind (Shiba et al. 2023). The cross-sectional shape of the petiole and spadix showed that these two organ parts were not fully round in konjac plants. However, the elliptical shapes were only diverged by 6.78 percent and 4.75 percent of the perfectly rounded shape for petiole and spadix, respectively. This study proved that the petiole and the base of the spadix, where the fruit and seed of the konjac plant attached, remained unaffected during the fruit ripening process which lasted up to six months.

Konjac plants with longer petioles do not automatically have a longer female flower segment. The R^2 within the oval shape (circled with a broken line) was 0.7013 without the outliers; however, it was very low at 0.162 if the outliers were included. On the other hand, the length of the female flower segment was strongly correlated with the cylindrical area where the hundred to thousand yet tiny konjac fruits grew (Fig. 7). A spathe protected a spadix by constructing a floral chamber.

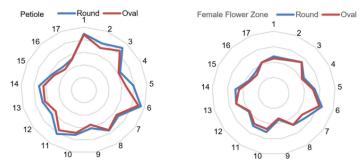


Fig. 6. Long (blue) and short (red) side diameters are measured at the middle section of the petiole and female flower segment at the spadix base in konjac plants

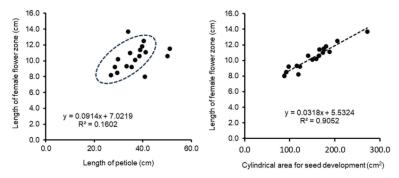


Fig. 7. The length of the petiole did correlate to the length of most of the female flower segment, but some outliers were also found

Although 76.5 percent of konjac plants with long petioles tend to form longer female flower cylinders, there are 23.5 percent that behave very differently, namely having long petioles but producing short female flower cylinders. Currently, it cannot be concluded whether this phenomenon is caused by genetic variation or due to microenvironmental conditions experienced by the plant. Meanwhile, petiole length is closely related to the total area of the cylindrical display of female flow.

Konjac tuber remains enlarging during the flower development process as long as the petiole and female flower segments are still active, continuing until the fruits are mature (left column). Tubers gradually decompose if the next generation plant(s) has started to grow (middle column). A single-parent tuber can grow into several new plants. The decomposition or decay of the parent tubers continues as the next generation grows bigger (right column) (Fig. 8).

Konjac plants have two types of roots: lateral and fibrous (Fig. 9). Lateral roots grow at the base of the petiole. Lateral roots have a larger diameter than the diameter of fibrous roots. The function of lateral roots seems to strengthen the above-ground organs. Fibrous roots grow sporadically on all parts of the tuber skin. The function of fibrous roots is to absorb water and nutrients needed by konjac plants.

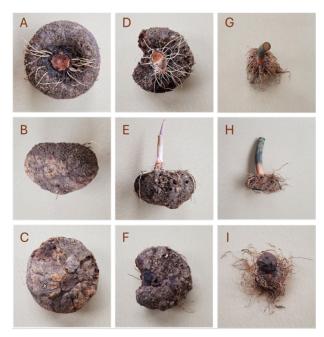


Fig. 8. Konjac tubers continue to grow during the flowering process and the formation of true seeds (A, B, C) but begin to decompose as buds begin to appear on the soil surface (D, E, F), and the next-generation plants develop into mature plants (G, H, I). Photos on the top row (A, E, G) are the bird's eye view; the middle row (B, E, H) is the side view, and the bottom row (C, F, I) is the upward view



Fig. 9. Lateral roots appear earlier at the base of the petiole (A) and then are followed by the fibrous roots (B) which sporadically spread and randomly appear on the surface of the konjac tuber

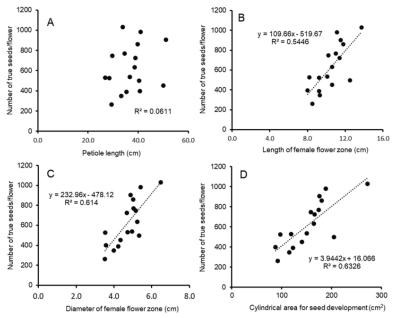


Fig. 10. Only traits directly related to the female flower segment are correlated with true seed count

Different morphologies of roots and their functions are also found in several types of plants, for example, in bamboo (Wang et al. 2023), corn (Protto et al. 2024; Rishmawi et al. 2023), and many other plants. In addition to the strong petiole and lower section of the spadix, the tick lateral roots that grow from the base of the petiole and extend laterally help strengthen the upright petiole of konjac fruit bunches until ripe for securing seeds harvest. Meanwhile, the thin but highly branching fibrous roots absorb nutrients and water needed to increase the size of tubers. The function of fibrous roots is more for the absorption of water and nutrients needed by konjac plants (Xu et al., 2023).

Petiole length did not correlate with number of true seeds per individual konjac plant; however, length, diameter, and cylindrical area for seed development did correlate with number of true seeds per plant (Fig. 10). It is easy to understand since the last three variables directly correlate with the number of seeds per plant. The length, diameter, and area of the female flower segment are parts of the cylindrical segment of female flowers; meanwhile, petiole length is not a part of the cylindrical segment of female flowers.

CONCLUSION

It took 38-42 days from bud emergence to full bloom. Flowers begin to wither 3-4 days after anthesis and six months to produce mature seeds. The petiole function is to support the upright flower crown and produce chlorophylls for synthesizing carbohydrates, which then accumulate in tubers. Spathe serves as a spadix protector and attracts pollinating insects. Spadix consists of an appendix as a source of pheromone in the upper segment, a pool of pollens at the middle segment, and a pool of female flowers. During the six-month process of seed ripening, konjac tubers continue to grow. Tubers begin to decompose when the next generation plant(s) starts to grow from the mother tuber.

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