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The Survival Rate and Starch Histochemical Assay of Various Stem Cutting Conditions of Mentega 2 Cassava Genotype at Initial Growth Stage

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A B S T R A C T

Background: : This study aimed to evaluate the growth ability of cassava mini stem cuttings with different node number and a variety of stem cutting shapes and their correlation with starch content in the stems at initial growth stages. **Methods.** In this study, the viability of cassava stem cuttings was identified in two type experiments i.e. mini-stem cuttings consisting 1 and 2 nodes and shape variation of single node mini-stem cutting. Parameters observed were shoots emergence period, number of sprouting cuttings, shoots number of individual stem cuttings, shoots height and number of leaves. In addition, starch histochemical test was also carried out on stems of young shoots and initial stem cuttings using Lugol's solution. **Results.** Both cassava stem cuttings consisting of 1 and 2 buds indicated the same survival rate of 100%. 1 bud stem cuttings with different shapes showed different survival rate, i.e. 60-80% for semicircular and fully circular cuttings and 30-40% for box shape cuttings. The difference in survival rate with different stem size is probably related to the availability of the amount of starch to support shoots growth. Observations at week 3 after planting generally showed that the stem cuttings with 2 buds were higher than those of stem with 1 bud. **Conclusion:** There were differences in the scores on the starch content test qualitatively with Lugol staining, in various parts of the plant originating from 1 bud and 2 bud cuttings which may indicate a breakdown of starch during shoot development.

Kemapuan Tumbuh dan Uji Histokimia Pati Berbagai Kondisi Stek Ubi Kayu Genotip Mentega 2 Pada Fase Awal Pertumbuhan Tanaman

Kata kunci:
Mata tunas
Pati
Stek mini
Ubi kayu

A B S T R A K

Background: Penelitian ini bertujuan untuk mengevaluasi kemampuan tumbuh stek mini ubi kayu dengan perbedaan jumlah mata tunas dan variasi bentuk stek serta korelasi dengan kandungan pati di batang saat fase awal pertumbuhan. **Metode:** Penelitian ini diamati daya tumbuh stek ubi kayu yang terdiri dari dua rangkaian percobaan, meliputi percobaan pertama yang menggunakan bahan tanam stek berukuran mini dengan 1 mata tunas (1 Mt) dan 2 mata tunas (2 Mt), serta percobaan kedua yang menggunakan variasi bentuk pada stek 1 Mt. Parameter yang diamati adalah waktu muncul tunas, jumlah stek bertunas, jumlah tunas pada setiap stek, tinggi tunas dan jumlah daun. Selain itu dilakukan pula uji histokimia pati pada batang stek dan batang dari tunas yang tumbuh menggunakan larutan Lugol. **Hasil:** Perlakuan bahan tanam menggunakan 1 Mt dan 2 Mt memiliki persentase kemampuan tumbuh yang sama yaitu 100%. Namun, perlakuan variasi bentuk stek dari 1 Mt memiliki kemampuan tumbuh yang berbeda, yaitu 60-80% untuk stek berbentuk setengah dan satu lingkaran serta 30-40% untuk stek berbentuk kotak. Pengamatan pada minggu ke-3 setelah tanam, kemampuan tumbuh tunas pada stek 2 Mt lebih tinggi dibandingkan stek 1 Mt.. **Kesimpulan:** Perbedaan kandungan pati pada berbagai bagian batang tanaman yang berasal dari stek 1 Mt dan 2 Mt diduga karena adanya penguraian pati selama perkembangan tunas.

Introduction

Cassava (Manihot esculenta Crantz) is an excellent source of carbohydrates for tapioca starch, bioethanol, environmentally-friendly plastics and various other products. In cultivation, cassava has wide adaptability both to soil nutrients and water availability. Some essential aspects for increasing the productivity of cassava are superior varieties, quality seeds, planting time arrangements, population and spacing, fertilisation, and harvesting. The very high need for cassava requires the provision of large amounts of seeds to support the productivity of the cassava agro-industry. Slow propagation is one of the obstacles in cassava production (Neves et al., 2020).

The use of quality cassava seeds is one of the determining factors and essential in increasing production. Cassava is generally cultivated using stem cuttings from plants aged 8-12 months with an optimum length of 20-25 cm cuttings with at least ten buds (Sundari, 2010). The significant need for cassava seedlings takes efforts to optimise the seeds, including the use of shorter cuttings and fewer buds. Cassava stems contain food reserves in the form of carbohydrates, water and others for metabolic growth. The foodstuffs will decrease over time because they are used for growth, both during the nursery and after being transferred to the field (Effendi, 2002). Cassava cuttings are recommended when the soil is loose and moist (Roja, 2009). During the planting season, it is necessary to provide a massive number of seedlings so that in conditions of limited seed supply, mini cuttings (5-6 cm long, with 3-4 buds) can be used with yields that are no different from ordinary cuttings. The initial phase of cassava growth consists of the adventitious phase of roots and shoots (5-15 days after planting /DAP) and leaf and root phases (18-40 DAP) (Saleh et al., 2016).

Several studies regarding the use of cassava cuttings made use of shoot cuttings with different book sections (Neves et al., 2020) and regular cuttings with different lengths, namely 10, 15, 20, 25, and 30 cm (Remison et al., 2015). Experiments using mini-cassava cuttings with different treatment of the number of books (1, 2 and 3 books) and treatment using or without naphthalene acetic acid showed the percentage of sprouted cuttings was 95-100% (Ardian, 2012; NurulNahar & Tan, 2012). Effendi (2002) states that cuttings with three buds can save 75-80% of seedlings with the average yield of crops not significantly different from conventional or conventional cultivation. Prasitsarn et al., (2018) also reported the use of stem cuttings with a length of 15 cm and 30 cm which were removed or removed by half the number of buds to see their effect on yield.

The vegetative life cycle of a plant can be described as a continuous process of obtaining, transferring, and storing the energy needed for growth, reproduction, and self-protection against abiotic and biotic environmental stresses. Carbohydrates (sugar) play a role in most long-distance energy transfer and long-term energy storage in plants (Zwieniecki et al., 2015; Tixier et al., 2019). Cassava stalks contain starch up to 30% of the dry weight of the biomass. The grain size and size distribution of the cassava stem starch and its size vary according to genotype, location of growth, and position along the stem (Ismail et al., 2016; Wei et al., 2018). A good understanding of the interaction between metabolic processes and plant development is needed so that the linkage mechanism between source and sink can optimise production (Fernie et al., 2020).

Several researchers have researched the effect of cutting length and the number of shoots of cassava both in Indonesia and abroad, but not many reports have been found regarding the role and metabolism of starch in stems, especially in the early stages of plant growth. In this study, the observation of growth ability and the starch histochemical test was carried out on cassava cuttings of genotype two butter with 1 and 2 buds and variations in the shape of the cuttings in 1 bud. Genotype Butter 2 is a local and prevalent genotype rich in beta content of beta carotene, protein Zn and Fe (Hartati et al., 2012). The study aimed to evaluate the growing ability of small cassava cuttings with differences in the number of buds and variations in the shape of the cuttings and their correlation with starch content in the stems during the early stages of growth.

Methods

Plant material

The plant material used was eight-month-old genotype butter two cassava stem cuttings raised in the cassava collection garden, Germplasm Farm of the Biotechnology Research Center, Indonesian Institute of Sciences (LIPI).

Research designs

Experimental design

The research was conducted in August - November 2019, which consisted of two sets of experiments. The first experiment used a different treatment of the number of buds on the planting material for cuttings consisting of 1 (1 Mt) and 2 shoots (2 Mt) (Figure 1a). The second experiment used a variation of the shape of the cuttings with 1 bud consisted of four levels, namely (1) one circle, (2) half circle, (3) box 1 cm x 1 cm x 1 cm with the base of the petiole, and (4) squares 1 cm x 1 cm x 1 cm without the base of the crate (Figure 1b). Each experimental set consisted of 3 replications with 30 cuttings planted for each replication. The cuttings are grown using compost media on a plastic tray horizontally. Every day the plants are watered by spraying water around the cuttings.

Shoots were observed visually from the beginning of planting or 1 to 21 DAP. Microscopic observations of shoot growth were carried out at 1, 3 and 7 DAP using a Leica EZ4HD microscope with a magnification of 12.5X. Variables observed included shoot emergence time, number of sprouting cuttings, number of shoots per cuttings, shoot height and number of leaves.

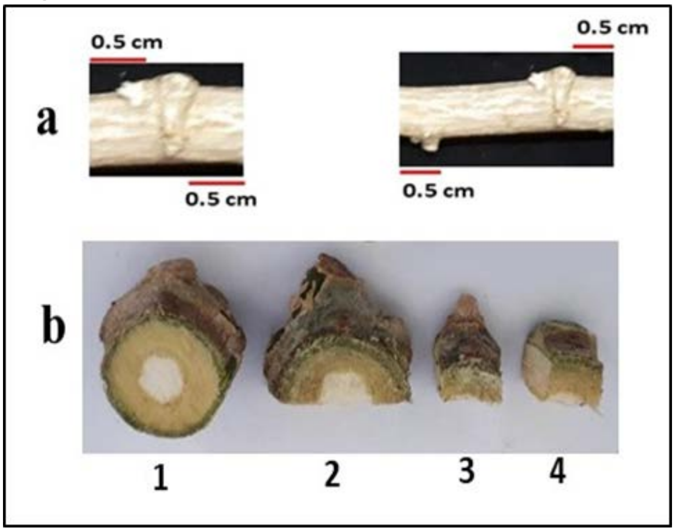

Figure 1. Plant material for growth ability test and starch histochemical test. a). cuttings with 1 bud (left) and 2 buds; b). 1 bud cuttings with various shapes in the form of a circle (1), a semicircle (2), a box 1 x 1 cm with and without the base of the crate (3 and 4).

Qualitative test of starch on cuttings and shoots

To determine the role of starch in cassava cuttings on shoot growth, a qualitative test of starch was carried out using staining with Lugol's reagent on the growing shoots and cassava cuttings used as seeds. Through the qualitative test of starch on several parts of the stem, it is hoped that the pattern of starch decomposition that occurs in the initial phase of plant growth, which is 8 weeks after planting, is expected. Starch identification was carried out on cuttings and shoots of cassava seedlings of genotype Butter 2 in the first experiment, namely 1 Mt and 2 Mt cuttings qualitatively using the Lugol test. The plant parts used in the shoot stem consist of four parts, namely the top, middle, bottom, and base of the stem, while the stem cuttings consist of five parts, namely the end, middle, end near the root, stem tissue around the roots and roots. Identification was carried out when the plants were 8 weeks after planting (WAP) (Figure 2). The starch histochemical test was carried out as many as 3 samples for each part of the plant. Plant samples were cut crosswise and dripped with Lugol's solution (2:1), were observed visually and then scored to determine the intensity of the colour resulting from the reaction of starch on the surface of stem tissue with Lugol's solution (Hartati et al., 2015). The determination of the starch colouring intensity score is categorised based on the level of colour intensity, namely the symbols +, ++, +++, ++++. Doubling the + symbol indicates an increase in the intensity of the colour.

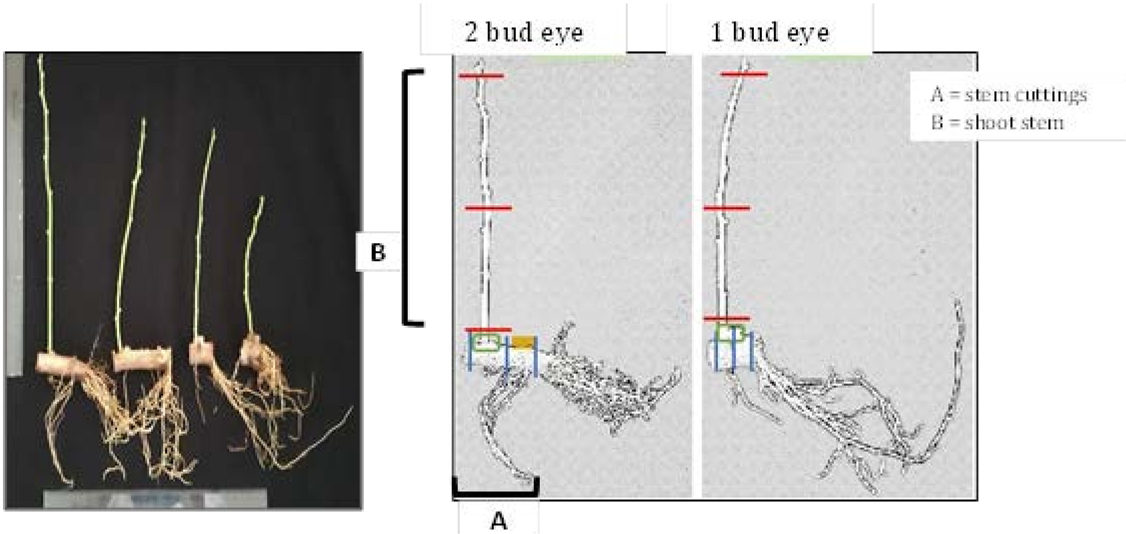

Figure 2. The position of plant sampling on shoots and cuttings for starch histochemical test using Lugol's stain.

Results

The ability to grow cassava cuttings based on the difference in the number of buds

All cassava cuttings in the first experiment, namely 1 Mt and 2 Mt of treatment, could grow with the same growth ability percentage of 100%. The 2 Mt treatment showed two responses to shoot growth, namely 85.72% experienced shoot growth from only one source of the bud, and 14.28% experienced shoot growth from both sources with shoot growth patterns consisting of dominant (higher) shoots and shoots that were smaller (Table 1).

Visually, the observation 3 weeks after planting (WAP) in the first experiment showed that the 2 Mt treatment had higher shoots, which reached up to 17 cm when compared to the average height of the cuttings in 1 Mt treatment, especially on cuttings that only responded to the growth of 1 fruit shoots. While the shoot height in the 2 Mt treatment which responded to the growth of 2 shoots, had a lower height growth. The 2 Mt treatment also produced more leaf number growth than the 1 Mt treatment. Root growth of the two treatments of 1 Mt and 2 Mt also showed a difference. The 2 Mt treatment had more roots because which is one end of the cuttings (Figure 3).

there were two root growth points, namely at the tip and middle of the cuttings, while in the 1 Mt treatment, the average root growth was only from the root growth point,

Table 1. The ability of shoot growth on mini cassava cuttings with variations in the number of buds and variations in the shape of 1 bud at the age of 3 weeks after planting

Number of shoots (MT)			Percentage of cuttings growth (%)	Day of shoot emergence	Range of Shoot Height (cm)	Range of Number of Leaves
1 Mt			100	3	7 – 15	4- 6
2 Mt			100	3		
	1 shoot				7 – 17	4 – 6
	2 shoots	shoot 1 (large)			7 - 11	4 – 6
		shoots 2 (small)			4 - 7	3 – 5

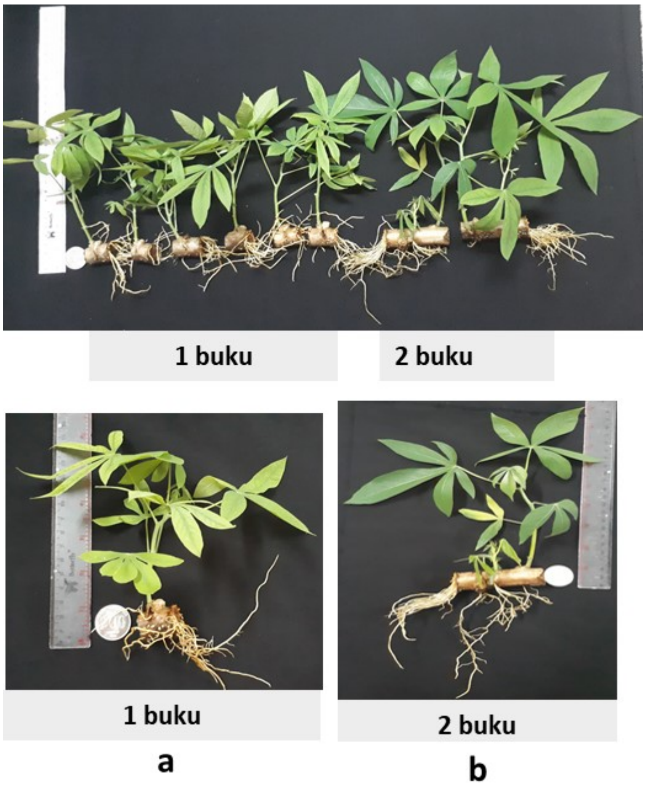

Figure 3. Growth of shoots and roots of plants in the first experiment with two treatments, a) 1 bud (1 Mt), and b). 2 buds (2 Mt) at the age of 3 WAP

The ability to grow cassava cuttings based on variations in cutting shape

In the second experiment, the treatment of shape variations in cuttings with 1 bud, there was a difference in shoot growth. The treatment of 1 circle and ½ circle shoots each could grow 76.67 ± 5.77% and 66.67 ± 11.55%, while the growth ability in the treatment of box-shaped cuttings measuring 1 cm x 1 cm x 1 cm with and without containers was 36.67 ± 5.77% and 33.33 ± 5.77%. The variation in the shape of the cuttings in one bud affected the difference in the size of the cuttings. The smaller the size of the cuttings indicates the lower ability of shoot growth. Variation of cuttings resulted in different growth responses to growth percentage, shoot emergence time, shoot height and number of leaves. The smaller the size of the cuttings, the smaller and lower all the parameters measured and observed were seen (Table 2).

In the second experiment, the variation of cuttings showed slow shoot growth, indicated by a short shoot height that only reached a range of 2.5 to 3 cm on the highest shoots, and fewer leaves were only able to form 4 to 5 leaves on the highest shoots. The results of microscopic observations regarding the emergence of shoots in the second experiment were variations in the shape of the buds with differences in the size of the buds. It was seen that at 1 DAP, there had not been any changes for all cuttings. New shoots were observed to appear at 3 DAP on circular and ½ circle cuttings, while on cuttings with a 1 x 1 cm box shape with the base of the petiole and 1 x 1 cm box without a crate, new shoots were observed to appear at 7 DAP (Table 2). The microscopic and visual observation of shoot growth in the fourth treatment in the second experiment showed differences in shoot growth at different cuttings. Apart from shoot height and number of leaves, the number of roots also showed a difference. The larger the size of the cuttings, the greater the number of roots that grow (Figure 4).

Table 2. The ability of shoot growth on mini cassava cuttings with variations in the shape of 1 bud at the age of 3 weeks after planting

Shape of cuttings	Percentage of cutting growth (%)	Day of shoot emergence	Range of Shoot Height (cm)	Range
1 circle	76.67 ± 5.77	3	2.5 – 3	4 – 5
½ circle	66.67 ± 11.55	3	1.5 – 2.5	3 – 4
1 x 1 cm box with the base of the petiole	36.67 ± 5.77	7	1.5 – 1	1
1 x 1 cm box without petiole	33.33 ± 5.77	7	0.3 – 0.5	1

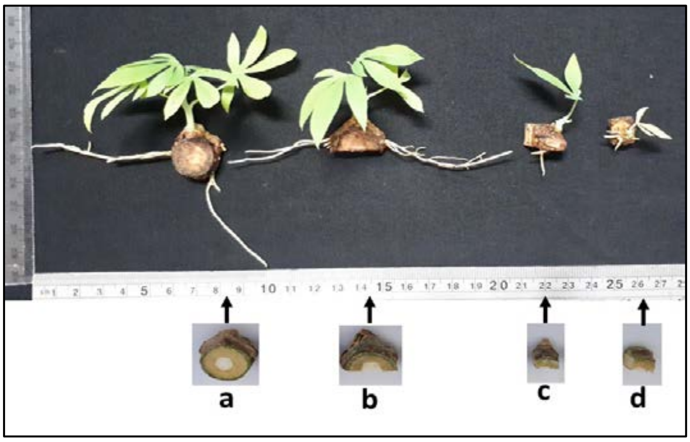

Figure 4. The growth of shoots and roots in the experimental treatment of variations in the shape of cuttings of 1 bud. a). one circle, b). semicircle, c). 1 x 1 cm box with the base of the crate, d). 1 x 1 cm box without the base of the crate.

Qualitative test profile of starch content in cuttings and shoots

The difference in the number of buds used as planting material showed different responses to shoot formation and growth. In addition, the number of shoots also caused differences in Lugol's absorption profile as indicated by variations in the intensity score of blue in the cuttings and shoots of cassava genotype Butter 2 at the age of 8 WAP (Table 2). The change indicates the difference in absorption of Lugol's solution by the plant tissue, namely the cuttings used as seedlings and the growing shoots that have young plant stem tissue in the surface colour of the stem slices to blue (Figures 5a and 5b). In very young shoot stem tissue (age 8 WAP), different Lugol absorption score patterns were obtained, both at 1 Mt and 2 Mt. In the 2 Mt treatment, it was seen that the highest score was found in the middle shoot stem, while the lowest score was found at the top and base of the stem. In the 1 Mt treatment, the highest score of bud stem Lugol uptake was at the bottom, while the lowest score was seen at the base of the stem (Table 2).

The results of the scoring pattern on the stem cuttings, 1 Mt and 2 Mt treatments, showed the same scoring pattern for all parts of the cuttings, namely the tip, middle, end near the root, stem tissue around the root and at the root. The highest uptake (score) was found in the stem tissue of cuttings around the root and the lowest was seen in the stem tissue at the end. Based on the study results, it was found that the root tissue did not show any Lugol uptake (Figure 5c).

Table 3. The score of the quality test score for Lugol's starch uptake on cuttings and shoots of the first experiment with two treatments of the number of buds aged 8 WAP. -: does not absorb lugol; +, ++. +++, ++++: absorbs lugol with different intensities.

Treatment	Plant parts		Lugol test score
1 Mt	Shoot stem	top	++
		middle	++
		down	+++
		base of stem	+
	Stem cuttings	end	+
		middle	++
		the end near the root	++
		stem tissue around the roots	++++
		root	-
2 Mt	Shoot stem	top	+
		middle	+++
		down	++
		base of stem	+

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Stem cuttings	end	+
	middle	++
	the end near the root	++
	stem tissue around the roots	++++
	root	-

Note: -: does not absorb lugol; +, ++. +++, ++++: absorbs lugol with different intensities.

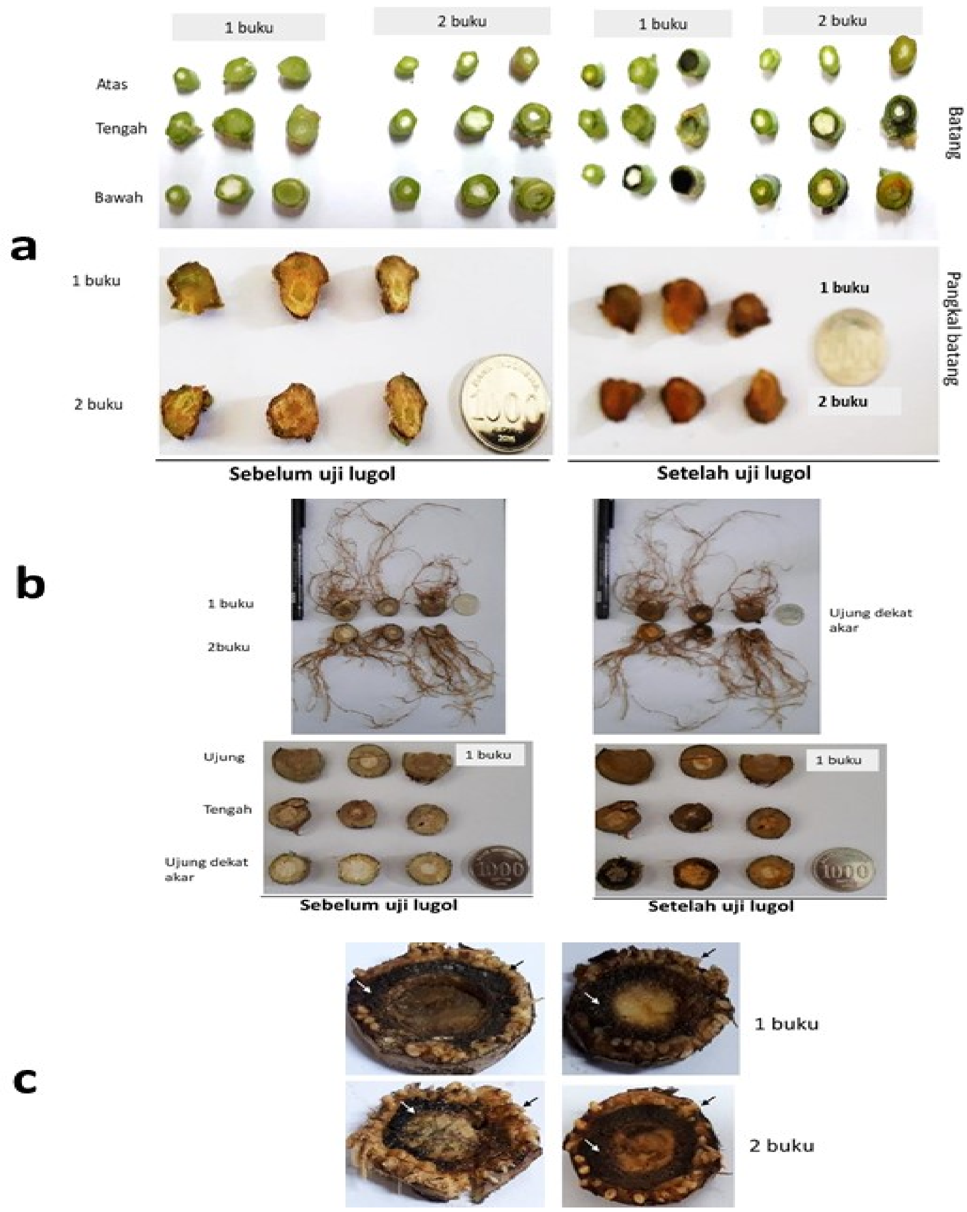

Figure 5. The results of the lugol uptake test of several parts of the stems and roots of cassava at 1 Mt and 2 Mt of treatment at 8 weeks after planting (MST). a. shoot stems, b. stem cuttings, c. stem network near the root (white arrow) and root (black arrow).

Discussion

The difference in the number of shots did not affect the ability to grow mini cassava cuttings.

The results showed that all (100%) cassava stem cuttings could sprout either from the planting material using 1 bud or 2 buds. In another research report, the use of cassava cuttings with 1,2 and 3 buds can also grow with a high percentage at the nursery level without growth regulators, reaching 81 - 100% (Effendi, 2002) and with naphthalene acetic acid treatment 500, 100, 2000 ppm growth reaches 95-100% (Ardian, 2012). Thus, the number of buds with different lengths of cuttings did not affect the growth of shoots.

In cuttings with two nodes, most of the buds that develop into buds are only one. However, some other plants showed that the two buds could develop into shoots even though the growth rates of the two shoots were different. This is probably due to the dominance of one of the shoots. The yield of cassava from mini cuttings needs to be observed in the subsequent growth conditions after being transferred to the field and also the yield. Effendi, (2002) reported that cuttings from 1, 2 and 3 buds showed no significant difference in yield. The research results show the opposite result by Bridgemohan & Onell (2014) which stated that the dry weight of cassava from cuttings with 3 buds was higher than cuttings with 1 and 2 buds.

Differences in cuttings shape affect the ability to grow mini cassava cuttings.

The growth pattern of cassava consists of vegetative growth (phasic development) and assimilate production (Ekanayake et al., 1998). In the vegetative growth phase, which begins with the cuttings forming shoots and leaf formation followed by root formation. The initiation phase or the initial phase of cassava growth occurs during the first 6 weeks, in which there is a growth of axillary shoots and adventitious roots, and there is a decrease in biomass weight on cuttings and constant total plant weight. Roots emerge from the tip of the cuttings and root thickening occurs (Lowe et al., 2011). In the first experiment, using treatment of 1 bud and 2 buds, and the second experiment with the treatment of variations in the shape of the buds, in the first treatment, 1 bud cuttings with intact stem cuttings (1 circle) and ½ circle, when the shoots appeared the same, namely on the third day.

One-eye cuttings with a smaller size need a longer shoot emergence time of up to 7 days. This is presumably due to differences in the availability of food reserves in the cuttings related to the size of the cuttings. Although cassava cuttings can grow to a petite size, further observations need to be made by considering the minimal number of roots formed which will correlate with yield in the field. However, the results of this study open up opportunities for cassava seed propagation to be combined with tissue culture propagation using one bud explant with a petite cuttings size which can facilitate explant handling in addition to increasing the availability of seeds.

Differences in the distribution of starch in the cuttings and cassava shoot from mini cuttings.

Colour change due to absorption of Lugol solution in stem tissue occurs because starch binds to iodine. The colour change to blue occurs when the glucose polymer present in starch is greater than 20 monomers such as amylose. Differences in starch content probably cause the difference in Lugol uptake intensity. A positive result in the Lugol test is the formation of dark blue to black colours after the addition of Lugol's reagent. Lugol's solution stains starch due to the interaction of iodine with the circular structure of polysaccharides (Sutamihardja et al., 2018).

Food reserves in the form of sugar and complex carbohydrates such as starch are significant for shoot growth. In Rosa hybrida, sucrose plays an important role in axillary shoot growth (Barbier et al., 2015). The decomposition of starch is known to play an essential role in providing carbon sources in the early stages of sprouting growth of several types of plants such as barley (Andriotis et al., 2016), Arabidopsis thaliana (Hedhly et al., 2016), six types of wild grassland (*Chloris virgata, Kochia scoparia, Lespedeza hedysaroides, Astragalus adsurgens, Leonurus artemisia, Dracocephalum moldavica*) (Zhao et al., 2018). However, the role of sugars and complex carbohydrates in metabolism associated with the initial phase of shoot growth has not been widely reported, including cassava. In seeds, the presence of starch has an essential role in germination. Seed germination is the process by which the embryos found in the seeds develop into plumules and radicles. The seeds absorb water and swell the dormant tissue and initiate cell division. Radicles emerge from micropillars, which move downward and into the ground. These radicles are then transformed into roots which provide a supply of water and nutrients for the plant throughout its life. Enzymes such as amylase, protease, and lipase are responsible for dissolving spare foodstuffs in the form of starch, protein and lipids simultaneously in seeds and providing energy and others (Joshi, 2018).

The role of starch in cassava stem cuttings for shoot growth has not been widely studied. However, the mechanism of starch metabolism in some plants, such as the endosperm of germinated rice seeds (Oryza sativa L.) (caryopsis) has been well studied. During rice seed germination and seedling growth, the starch in the endosperm is hydrolysed into glucose, which is taken up by the epithelial cells of the scutella, converted into sucrose, and transported via vessel bonds to other embryonic tissues, such as buds and growing roots (Liu et al., 2010). In addition to seed germination, carbohydrates also play a role in the formation of vegetative shoots from the root tip of *Catasetum fimbriatum* root morphogenesis, where carbohydrate content in the tissue decreases progressively during plant development (Vaz et al., 1998).

The difference in the Lugol test scores on shoot stems (young stem tissue) and cuttings indicates a qualitative change in starch content during the growth and development of cassava shoots, especially in the early stages of growth. According to Fernie et al., (2020) the decrease or rate of transport of sucrose and other assimilation to the phloem root of tubers occurs simply. Photosynthate partitioning in cassava is a process of long-distance transport of sugar from the leaves to the storage roots through the phloem vascular system, sucrose which is synthesized in the leaves is then transported and loaded into the phloem in a simplistic or apoplastic manner by sugar transporters (Yan et al., 2019). In the root tissue, qualitatively, there was no visible starch accumulation at 8 weeks after planting. In research on the location and time of starch formation through the Lugol test on cassava varieties IAC 576-70, it is known that starch in the roots occurs 190 days after planting (Figueiredo et al., 2013).

This research needs to be further confirmed, such as quantitative tests and distribution of starch molecules in tissues, dissolved sugar tests resulting from the breakdown of starch into sugars and related molecular aspects. Although the results of this study are still in the form of preliminary results regarding the role of starch on the growth of cassava shoots, the information obtained can be used for further development to understand the importance of the economic value of starch content not only in tubers but also to determine the importance of starch in the stems to support seed quality.

Conclusion

Cassava cuttings consisting of 1 and 2 buds can grow with the same percentage of shoot growth. Experiments on the variation of cuttings with one bud with different shapes and sizes grew well with different survival rates. In the qualitative test of starch content with Lugol's staining on several parts of the stems, namely cuttings and cassava shoots in treatment 1 and 2 buds, there was a difference in the staining score which indicated the role of starch in shoot growth in the early stages of growth and the accumulation of starch from the beginning in the very young stem. However, further research is needed regarding the relationship between the availability of starch in stem cuttings with the growth and development of cassava, especially in the early stages of plant growth.

Declaration statement

The authors reported no potential conflict of interest.

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