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Morphology, Structure and Potential of Bulbils Yield as a Planting Material of Onion in Tropical Region

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Abstract Onion is one of the essential horticultural commodities in Indonesia. The productivity of onion in 2019 is only 7.27 t/ha. Low productivity levels are a problem faced by farmers in Indonesia. One of the causes of low productivity is the low quality of planting material. The majority of onion cultivars can only be propagated vegetatively through bulbs and are known as sterile species. Therefore, other planting materials that have high production potential are needed. Bulbils are possible as planting material. Several onion varieties have a tendency to produce bulbils, one of which is the Tawangmangu Baru variety. Tawangmangu varieties of onion include the type of softneck that can form bulbils. This study aims to examine the morphological characteristics and structure of bulbils and to determine the yield potential of onion bulbils. Field experiments were carried out descriptively by planting onion bulbils. Each Tawangmangu Baru variety plant is capable of forming 1-5 bulbils. Bulbils that are formed have a small size with a single bulb shape. Bulbils and cloves have the same morphological and structural characteristics as the embryo. However, there is a difference in size between bulbils and clove bulbs, bulbils are smaller in length, size and weight. The longitudinal cross-section of the clove bulbs' bulbils is the first foliar leaf, leaf clove base storage. The cross-section of the bulbils and cloves consists of the carrier bundle, embryonic parenchyma, embryonic epidermis, bulb parenchyma. Growing bulbils require controlled environmental and climatic conditions. Bulbils can grow and produce bulbs with a productivity of 6 - 8 tons/ha. Bulbils have the potential to be developed as planting material.

Keywords Bulbils, Mulch, Structure of Bulbis, Tawangmangu Baru Variety

1. Introduction

Onion (*Allium sativum* L.) is an important horticultural commodity originating from Central Asia [1]. Today, onion is the largest plant among allium species grown worldwide. The level of consumption of onion in Indonesia in 2019 reached 1.77 kg/capita/year, amounting to 321,362 tons. However, Indonesia's onion production in 2019 is only 164,548 tons. The productivity of onion in Indonesia in 2019 was 7.23 t/ha and decreased by 7.78% compared to 2018 and a decrease of 20.46% compared to 2017 [2]. This causes the import of Indonesian onion to be quite high, reaching 226,213 tons. The problem of onion cultivation, in tropical regions such as Indonesia, is low productivity and high production costs. Based on the Ministry of Agriculture of the Republic of Indonesia (2018) that the productivity of onion in Indonesia in 2018 was 7.84 t/ha and decreased by 13.75% compared to 2017 (9.09 t/ha). Planting material is one of the causes of low productivity and high production costs. Farmers in Indonesia use cloves of consumption bulbs as planting material. This is because

the majority of onion cultivars can only be propagated vegetatively by planting bulbs and are known as sterile species [1, 3, 4].

Several varieties of onion have a tendency to produce bulbils [5]. Onion can form bulbils through inhibition of the development of the shoot apical meristem [6, 7]. Different ecotypes show great morphological diversity in size, color and shape of bulbs, leaves, flowers and bulbils development. [8]. There are two types of onion varieties, namely hardneck and softneck [9]. All hardneck onion forms bulbils, whereas softneck onion cannot form bulbils [10]. Tawangmangu Baru variety is a softneck variety capable of producing bulbils. Onion planting with bulbils has not been widely practiced. Bulbils are usually just a byproduct of the onion plant. Plants obtained from bulbils are more resistant to various diseases, including viral infections. Based on research results Dugan [11] that bulbils are less frequently infected by *Fusarium proliferatum* than bulbs. Besides that, Najda & Dyduch [12] reported that onion plants grown using bulbils contained total carbohydrates (18.82-21.52%), L-ascorbic acid (7.16-8.51%), chlorophyll (0.024-1.044 mg in 1 kg of fresh weight) and crude fiber (1.37-1.60%) which are classified as high. Onion bulbils have the potential to be used as planting material.

The tiny size of bulbils becomes an obstacle in using bulbils as planting material. Utilization as planting material in preliminary experiments, bulbils are not able to grow on land. Early growth is very resistant to environmental conditions, especially temperature and sunlight. This is due to the high temperatures and full sun all day in the tropics. These environmental conditions inhibit the initial growth of bulbils, causing bulbils to be unable to grow. [13] that temperature is the primary determinant in the success of horticultural product production, one of which is onion. The growth of seedlings from bulbils requires modification of the microclimate by shading and mulching treatments. Use of mulch as a ground cover to maintain soil moisture and temperature, reduce soil evaporation, control structure, control weeds and influence enteric bacteria in the soil [14]. Mulch affects soil hydrothermal, namely controlling the soil environment under mulch which in turn affects crop yields, productivity and efficiency of water use [15]. Based on the research results of Matyn [16] that mulch application has a positive effect on leaf number, plant fresh weight and fresh weight of onion bulbs at several different irrigation intervals. In addition, the results of research by Elshahawy [17] stated that the application of mulch can reduce sclerotial germination so that symptoms of white rot are reduced, which in turn increase the growth and yield of shallot and onion plant bulbs. In this study using black silver plastic mulch. Based on research results Hou [18] that black silver mulch can improve root growth, root dry matter, anthocyanin content, adenosine triphosphate (ATP), and starch content in potato plants in northern China. The novelty of this research is to examine the morphological characteristics and structure of bulbils and to utilize bulbils as planting material with microclimate modification with mulching and nutrient balance.

2. Materials and Methods

2.1. Analysis of the Morphology and Structure of Bulbils

The study was conducted from June to December 2017. Morphological and structural analysis of bulbils and onion cloves was carried out at the Biology Laboratory of Gadjah Mada University, Yogyakarta. The research stages included observation of morphology, the structure of bulbils and cloves of onion and the structure of bulbils and cloves of an embryo. Morphological observations include; diameter of bulbils using digital calipers (*Nankai, Japan*), the diameter of bulbs using digital calipers (*Nankai, Japan*), length of bulbils using a ruler (Kenko, Japan), length of clove bulbs using a ruler (*Kenko, Japan*) and weight of bulbils and cloves bulbs using analytical scales (*Radwag AS220 R2, Poland*).

Observation of the organ structure was carried out by direct observation using a binocular microscope (*Olympus, Japan*). The first stage in structural analysis is making preparations. The tools used in making preparations are glass objects, cover cups, measuring cups, small pipettes, large pipettes, preparation needles, bottles for chemicals, suction paper, microtomes, razors or razors, places for coloring, bottles balsam with a glass rod.

The process of making preparations is as follows:

Day 1:

(1) Fixation using a 70% alcohol solution
(2) Slicing by making cross or longitudinal slices using a Sliding microtome with a thickness of 20-30 µm. The slices are accommodated in a petridish which is given 70% alcohol.
(3) Staining using 1% safranin in 70% alcohol for 24 hours

Day 2:

Safranin is removed then replaced successively with:
(1) Alcohol 70% for 10 minutes
(2) Alcohol 80% for 10 minutes
(3) Alcohol 95% for 10 minutes
(4) Alcohol 100% for 10 minutes
(5) Alcohol 100% for 10 minutes
(6) Alcohol/xylol 3:1 for 10 minutes
(7) Alcohol/xylol 1:1 for 10 minutes
(8) Alcohol/xylol 1:3 for 10 minutes
(9) Xylol for 10 minutes
(10) Xylol for 10 minutes.

The slices are then arranged on a glass object covered with a cover glass by giving the Canadian Balsam first. The preparation was dried on a hot plate at 45oC until the

Canadian balsam was dry. Then the preparations are given names. Furthermore, it was observed with a binocular microscope. The results were analyzed descriptively by comparing the morphology and structure of bulbils and cloves.

2.2. Analysis of Potential Yield of Bulbils

Field research to assess the potential yield of bulbils was carried out in the experimental garden of Pancot Sub Village, Kalisoro Village, Tawangmangu District, Karanganyar Regency, Indonesia. Located at an altitude of 1300 masl with astronomical locations 1100 40 "- 1100 70" East Longitude and 70 28 "- 70 46" South Latitude. The research area was grumosol soil with a total N content of 0.09%; P2O5 96 mg/100g; K2O 11 mg/100g; Cation Exchange Capacity (CEC) 53.62 cmol; Base saturation 55.19%; pH 8.9; C-Organic 0.81%.

The research materials included onion bulbils, which are tillers from the Tawangmangu Baru onion variety, black silver mulch, UV plastic, paranet 50%. nitrogen fertilizers, phosphate, potassium, and cow manure with nutrient content as follows nitrogen 2.33%, P2O5 0.61%, K2O 1.58%, Calcium 1.04%, Magnesium 0.33%, Manganese 179 ppm and Zn 70.5 ppm. The field experiment used a descriptive study by planting air tubers on a 2 x 3 meter plot repeated 3 times. Planting was carried out at a spacing of 10 cm x 15 cm so that there were 360 plants per plot. Observation of the growth and yield of 3 plants per plot, so that the total sample was 9 plants and observations of yield per hectare with a sample of 1x 1m.

The observed variables observed in the analysis of potential yield of bulbils included: plant height, plant biomass, bulb weight per plant, bulb yield per m2, bulb diameter, number of cloves per bulb. The research data were analyzed using descriptive analysis by means of data tabulations and descriptions of growth and outcome variables. The observed data on growth and yield of bulbils (bulbil) planting material were then compared with descriptions of growth and yield of the parent variety, namely the Tawangmangu Baru variety based on the description from the Indonesian Ministry of Agriculture.

Table 1. Environmental conditions of soil temperature and humidity

Repetition	Soil Temperature (°C)			Soil Moisture (%)		
	Morning	Day	Night	Morning	Day	Night
1	10	31	24	34.5	46.5	40
2	10	29	26	42.5	54.5	32
3	10	30	22	23.6	56.5	31
4	7	29	25	24	45	32
5	7	31	22	46	57	40
6	9	30	24	43.5	30	40
Average	8.8	30.0	23.8	35.0	44.0	35.8

Environmental conditions such as temperature using a soil thermometer and soil moisture using a soil tester were observed every week at 6.00 WIT, noon at 12.00 WIB and 18.00 WIT (Table 1). Soil temperature in the morning, afternoon and evening in the treatment without mulch, namely 18.83; 30.00 and 23.83, while the soil temperature during the rice, afternoon and evening time in the mulch treatment was 11.83; 27.00 and 41.33.

3. Results

3.1. Morphological Characterization and Structure of Bulbil Compared to Clove Bulbs

The Tawangmangu Baru variety is a semi-genotype group that can form bolting, which then grows into bulbils (bulbils). Each Tawangmangu Baru variety plant can form 1-5 bulbs of bulbils (Figure 1). Bulbils in the Tawangmangu variety can only form on pseudostems (Figure 1). The morphological characteristics of bulbils and cloves have the same part (Figure 1). In onion, a bulb called a head consists of a smaller part and a clove. One bulb consists of several cloves. The difference in the morphological characteristics of bulbils and cloves lies in size, length, size and weight (Table 2). Size comparison of bulbils compared to cloves, namely: 1: 3 diameter; 1: 3 in length and 1: 5 in weight. The diameter and length of bulbils are only one-third the size of a clove. According to [19] there are three classes in the diameter of onion, namely: 3.2 -5.98 cm (small), 6.01 to 7.09 cm (medium) and 7.39 to 9.27 cm (large). Bulbils weigh less than an onion bulb, which is one-fifth of an onion bulb. [20] reported that the weight of 100 bulbils at 167 genotypes was lower than 10 g. This fact provides information that the food reserves contained in bulbils are very small. The structure of bulbils and cloves consists of paper skin, flesh and embryo [21], [22]. Comparison of the structure of bulbils and cloves is shown by transverse and longitudinal sections (Figure 1). The structure of bulbils and cloves each has the same arrangement of organs; what distinguishes between the two is the organ's size. Judging by the structure, bulbils have an embryo part that has the potential to grow into seeds.

Table 2. Size comparison of bulbils and cloves of onion in the Tawangmangu Baru variety

Variable	Bulbils from the Tawangmangu Baru variety	Tawangmangu Baru variety cloves
Clove diameter (cm)	0.53	0.96
Clove length (cm)	1.11	3.38
Clove weight (g)	0.23	1.09

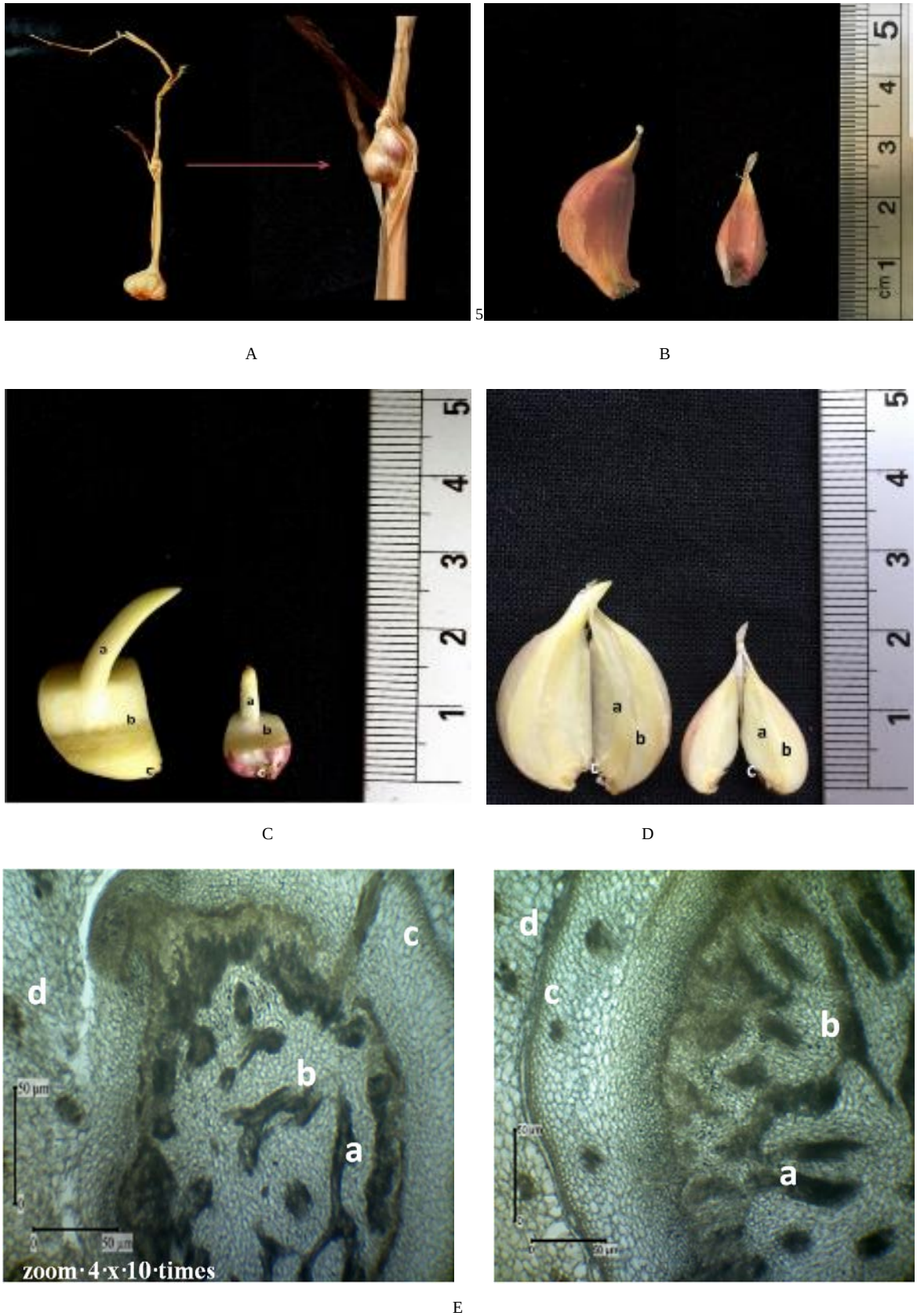

Figure 1. A: Tawangmangu Baru varieties of onion (a: bulb and clove, b: bulbils); B: The difference in size between cloves and bulbil (a: cloves, b: bulbil); C: Difference between clove and bulbil sprouts (a: first foliar leaf, b: storage leaf, c: clove base); D: Differences in longitudinal and bulbil sections (a: first foliar leaf, b: storage leaf, c: clove base); E: The cross-sectional differences of clove and bulbil embryos (a: embryonic bundle, b: embryonic parenchyma, c: embryonic epidermis, d: parenchyma, e: epidermis).

3.2. Growth and Bulb Yield Characteristics of Bulbil

Silver black plastic mulch can support the growth and yield of bulbils (Table 3). The average height of bulbils plants is 66.78 cm with 5 leaves. The yield of bulbs from bulbils planting material obtained ranges from 6 to 8 t/ha. The role of plastic mulch in maintaining temperature and humidity stability in the morning, afternoon and evening have an excellent effect on bulb growth and yield. The results showed that mulch could reduce soil temperature and increase soil moisture (Table 1). Based on research results by [23] mulch significantly increased the available water capacity by 18-35%, total porosity by 35-46% and soil moisture retention at low suction from 29-70%. The bulbils' size is smaller than the bulbs so that their growth requires stable temperature and humidity.

3.3. Plant Description of the Tawangmangu Baru Variety of Bulbils and Cloves

The Tawangmangu Baru variety of onion is the best onion variety in Indonesia. This variety was developed in the Tawangmangu area, Karanganyar, Central Java, Indonesia. This variety was relied on as a new variety selected in 1989 (Directorate General of Horticultural Seeds 1989). The Tawangmangu Baru variety has the advantages of large clove size, high productivity compared to other varieties grown in Indonesia, and a flat globe bulb shape with the cloves' color predominantly purplish white. The Tawangmangu Baru variety is a variety of the softneck group capable of forming bulbils (Figure 1). According to [24][25] that onion can form bulbils, which is a failure to form flowers caused by temperature factors. Bulbs produced from bulbils were not significantly different in size from bulbs produced from cloves (Table 5). According to [26] that onion grown from bulbils is the same size as the onion produced from the bulb. However according to [25] onion from bulbils produces fewer leaves than comparable varieties cultivated from bulbs. The comparison of plant descriptions from bulbils and cloves of origin shows many similarities (Table 2).

Table 3. Growth and yield of bulbils

Repetition	Plant heigh	Number of leaves	Bulb diameter (cm)	Number of cloves	Bulbs weight per crop (g)	Dry Bulbs Weight per crop (g)	Depreciation (%)	tuber weight per hectare (ton)
1	62	5	3.4	7	14.23	7.41	47	6027
2	60	4	3.6	9	15.08	7.91	47	6055
3	57	5	3	6	15.3	9.12	40	6020
4	58	5	3.3	6	15.49	9.23	44	6370
5	69	5	3.4	7	14.12	7.81	45	6860
6	64	5	3.7	10	14.87	8.42	43	7420
7	75	6	4	8	15.74	9.21	40	8050
8	77	5	3.8	9	16.32	9.04	44	7910
9	79	5	3.7	9	14.8	7.91	46	7840
Average	66.7778	5	3.54444	7.88889	15.1056	8.45111	44	6950.22

Table 4. Description of Tawangmangu Varieties based on the Ministry of Agriculture

No	Parameters	Descriptions
1	Origin	Tawangmangu, Karanganyar. Central Java, Indonesia
2	Harvest age	120 DAP
3	Leaf shape	Flat
4	Leaf color	Bluish green
5	Flowering ability	Not capable
6	Ability to form bulbils	Able
7	Plant habitus	Upright
8	Aroma	Strong

Table 5. Comparison of descriptions between Tawangmangu Baru (elder) varieties and plants made from bulbil planting material

No	Parameters	Variety of Tawangmangu baru (parent) *	Variety of Tawangmangu baru bulbil
1	Origin	Tawangmangu, Karanganyar. Central Java, Indonesia	Bulbil from Tawangmangu baru
2	Harvest age	120 – 140 DAP	120 DAP
3	Plant height	60 – 80 cm	57 – 79 cm
4	Leaf shape	Flat	Flat
5	Leaf color	Bluish green	Bluish green
6	Flowering ability	Not capable	Not capable
7	Ability to form bulbils	Able	Able
7	Plant habitus	Upright	Upright
8	Bulb diameter	4 – 5 cm	3 – 4 cm
9	Number of cloves	12 - 16	6 – 10
10	Aroma	Strong	Strong
11	Result	8 -12 t/Ha	6 – 8 t/ha
12	Depreciation	40 – 45%	40 - 47%

Note: DAP= Day After Planting, *): Data source Description of the variety of the Ministry of Agriculture of the Republic of Indonesia.

4. Discussion

Based on the onion genotype, there are three main morphological groups according to their ability to produce a scape (bolting), namely a genotype capable of forming a scape, a genotype that cannot form a scape and a semi bolting genotype [20]. The semi genotype group formed a scape in the pseudostem section. The Tawangmangu Baru variety is a semi-genotype group that can form bolting, which then grows into bulbils (bulbils). Each Tawangmangu Baru variety plant can form 1-5 bulbs of bulbils (Figure 1). Bulbils are spherical organs that are part of asexual reproduction in plants. Bulbils contain food reserves from the photosynthetic translocation of plants. The onion bulbils will then turn into segments known as cloves [27]. Bulbils that are formed have a small size with a single bulb shape. The morphological characteristics of bulbils and cloves have the same part (Figure 1). In onion, a bulb called a head consists of a smaller part and a clove. One bulb consists of several cloves. The bulb is slightly rounded and the clove is elongated in the shape of a crescent with a slightly concave lateral surface. The top of the clove is elongated and flat at the bottom [28].

Bulbils weigh less than an onion bulb, which is one-fifth of an onion bulb. [20] reported that the weight of 100 bulbils at 167 genotypes was lower than 10 g. This fact provides information that the food reserves contained in bulbils are very small. Food reserves are needed for early growth. Based on research results [29] that bulb size affects bolting, seed yield per bulb, bulb yield and time to bulb maturity. This is related to the amount of assimilation available in large bulbs [30], [31]. So that efforts are needed to support the growth of bublis. Silver black plastic mulch can support the growth of bulbils. This is because the mulch treatment can maintain soil moisture and temperature (Table 1). Plastic mulch can control the daily average temperature of 11–27 ^{o}C and average humidity of 41 - 61%. Based on research [32] that bulbils growth of *Poa alpina* (Poaceae) requires a lower atmospheric pressure and a lower temperature. Soil temperature affects bulb formation and bulb size [33]. Several studies have shown the effect of using plastic mulch in increasing the yield of onion bulbs [34]–[37]. Based on research results [38] that the growth of bulbils in lily plants is significantly influenced by temperature. Initial growth of bulbils requires a low temperature of 5-10^{o}C until the plants are eight weeks after planting, then after that, they are planted at a temperature of 27-30 ^{o}C until flowering. Besides, to support the growth and yield of bulbils, nutrients are needed. Research result by [38] that temperature and growth regulators affect growth, the dissolved carbohydrate content of bulbils, which will affect yield and flowering. Therefore growing bulbils as planting material requires more intensive treatment than usual.

The structure of bulbils and cloves each has the same arrangement of organs; what distinguishes between the two is the organ's size. Judging by the structure, bulbils have an embryo part that has the potential to grow into seeds. Onion bulb embryos can be developed from somatic embryogenesis [39]. The stages of onion somatic embryogenesis based on the scanning electron microscopy test (SEM) include the proembryo stage, the transition stage from proembryo to the globular stage, the globular stage, the mature embryo stage and the single cotyledon stage [40]. Studying the morphology and structure of bulbils can get information that bulbils have the potential to be developed as plant material.

Onion bulbils can be used as planting material and

produce good quality bulbs. The ability of the Tawangmangu variety onion bulbils has been investigated by [41] that the embryonic callus bulbils of onion can regenerate to form shoots and roots, based on this study results, bulbils have plant growth, such as plants grown from clove planting material. Bulbs produced from bulbils were not significantly different in size from bulbs produced from cloves (Table 5). According to [26] that onion grown from bulbils is the same size as the onion produced from the bulb. However according to [12] onion from bulbils produces fewer leaves than comparable varieties cultivated from bulbs. The comparison of plant descriptions from bulbils and cloves of origin shows many similarities (Table 4). Growth characters such as plant height, leaf shape, leaf color, flowering ability, ability to form bulbils and habitus, plants from bulbils and cloves of origin have the same characteristics. The variable yield of bulbils planting material is slightly lower than the planting material of cloves with a potential yield of 8 ton/ha. This is because the bulbils are smaller in size than clove bulbs. According to [42] that the size of the planting material affects yield. Based on the comparison of plant descriptions from bulbils and cloves planting material, it can be concluded that bulbils can be used as planting material.

5. Conclusions

Bulbils and onion clove bulbs have the same morphological and structural characteristics. The longitudinal cross-section of the clove bulbs' bulbils is the first foliar leaf, leaf clove base storage. The bulbils and cloves' cross-section consists of the carrier bundle, embryonic parenchyma, embryonic epidermis, and bulb parenchyma. Based on the morphological characteristics, there is a size difference between bulbils and cloves, namely bulbils, which have a smaller size. Silver black mulch can be used to support the growth and yield of bulbils. Bulbils can grow and produce bulbs with a productivity of 6 - 7.5 t/ha. Bulbils have the potential to be developed as planting material.

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