Morphological and anatomical characters of diploid and tetraploid taro (*Colocasia esculenta* L. Schott) cv. Bentul grown in lathhouse

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**Abstract.** A number of tetraploid taros Bentul has been produced by *in vitro* polyploidization with oryzalin treatment to achieve optimal production and to overcome pests, diseases and drought stress. Planlets achieved from polyploidization had high survival rate on acclimatization process. Characterization was conducted for diploid and tetraploid Taro plants grown in Lathhouse. The aim of morphology and anatomy characterization of tetraploid taro was to compare those with diploid one. The results showed that the morphological characters of seven clones of tetraploid taro Bentul presented 56% similarity with the diploid and divided into three different subgroups with 63% similarity. The thickness of leaves tissue was not significantly different. Stomata size of tetraploid taro was larger than diploids but the stomata density was lower. Thickness of petiole epidermis of diploid taro was significantly different with tetraploid 75.2.1. Thickness of root epidermis of diploid taro was significantly different with tetraploid 7.5.12.2; 30.4.6; 60.2.6 and 75.2.3. Diameter of root stele of taro Bentul was not significantly different between diploid and tetraploid clones.

**1. Introduction**

Food diversification is one of the government programs to reach food security in Indonesia. The diversity of biological resources in the group of cereals, tubers and fruits plants can be used as a potential alternative food source. One of the tuber plant species that is being developed as an alternative food is taro [1]. Taro is useful for alternative food source because containing nutritional value in the tubers, especially as a non-rice calorie food source. Taro tuber contains protein, carbohydrates, fat, crude fibre, phosphorus, calcium, iron, thiamine, riboflavin, niacin and vitamin C [2]. Proteins contained in taro tubers (1.9%) are higher than that in cassava (0.8%) and sweet potatoes (1.8%), although the carbohydrate content (23.78%) is smaller than that in cassava (37.87%) and sweet potatoes (27.97%) [3]. Talas Bentul is one of the most cultivated taro cultivars in Bogor (West Jawa, Indonesia) because of its relatively high productivity, tasty and having fluffy tuber taste [4], but Bentul taro is not particularly resistant to...
leaf pests attack and is susceptible to tuberous rot [1]. The first paragraph after a heading is not indented.

In order to achieve optimal production and overcome pest attacks and abiotic environmental stresses, genetic improvement efforts have been carried out through plant tissue culture application. Induction of polyploidy can produce superior characters in plants, including thickening of leaves and stems in the Spathiphyllum wallisii [5]. This character in polyploid plants serves as the plant’s main defense against disease attacks from the environment. Thicker leaf tissue (especially the epidermis) causes the penetration of the disease can be inhibited, so that the plant can survive longer from disease attacks before eradication on pests and diseases is carried out [6]. Induction of polyploidy in onion plant affected the plant organs becoming more resistant to leaf pests and tuberous disease attack because the phenotype expression of polyploid plants is superior to their diploid parents [7]. Induction of polyploidy on taro cv Bentul is important to increase crop yields, so that tubers produced from taro polyploid was larger than diploid taro [8].

Stomata density from polyploid plants also changes. Banana plants (Musa spp.) with higher levels of ploidy have larger stomata size but lower in the density [9]. Stomata have an important role in transpiration and defence against drought. Decreasing stomata density will affect plant defences against drought stress. Low stomatal density will reduce the evaporation process of water in plants because it reduced number of leaf pores [10]. Morphological and anatomical changes can be recorded as plant characteristics [11]. This character is also important to determine the potential of plants as a source of food, medicinal raw materials, furniture raw materials, utilization of landscapes to conservation [12] [13] [14] [15] [16]. This study was aimed to observe morphological and anatomical characteristics of tetraploid taro cv. Bentul and to compare that with diploid control genotype.

2. Materials and Methods
2.1. Materials
The plant materials used were 7 clones of taro tetraploid plants namely: tetraploid 7,5.10.3 (4 individuals), tetraploid 7.5.12.2 (7 individuals), tetraploid 30.4.6 (9 individuals), tetraploid 60.2.6 (3 individuals), tetraploid 75.2.1 (5 individuals), tetraploid 75.2.3 (10 individuals), tetraploid 75.8.1 (10 individuals) as a result of the oryzalin induction treatment carried out by Wulansari et al [8] and 1 clone of diploid taro clone. All plant materials were from collection of Laboratory of Plant Cell and Tissue Culture at Research Center for Biotechnology, LIPI at Cibinong, West Java, Indonesia. All plants grow in Lathhouse and are ± 3-4 months old. The chemical materials used include sodium hypochlorite (NaClO) 5.25%.

2.2. Methods
2.2.1. Acclimatization from in vitro culture. Acclimatization was carried out on rooting plantlets. Plantlets were removed from the culture tubs, washed using tap water. Plantlets were planted in acclimatization media consisted of autoclaved soil, coco peat and rice husk charcoal mixtures with a ratio of 1: 1:1, then they were covered by a plastic bag and placed in the greenhouse. The percentage of plantlet life was recorded after the fourth week.

2.2.2. Morphological Characterization of Taro cv Bentul
2.2.2.1. Habitus Characterization. Habitus characterization observed were plant span, plant height, number of stolons and suckers based on taro descriptor [17]. This quantitative observation is repeated three times per individual per clone.
2.2.2.2. Leaves, Petiole and Root Morphological Characterization. Leaf morphology observed were leaf base shape, predominant position (shape) of leaf lamina surface, leaf blade margin, leaf blade color, leaf blade margin color, leaf lamina appendages, leaf lamina length/width ratio,
petiole junction pattern, petiole junction color, sap color of leaf blade tip, leaf main vein color, vein pattern, petiole/lamina length ratio [17]. Morphology of petiole investigated were petiole color, petiole stripe, petiole basal-ring color, cross-section of lower part of petiole, ratio of sheath length/total petiole length, leaf sheath color [17]. Root morphology observed were root color and uniformity of root color [17]. Quantitative observation of leaf morphology done with three replicates of each individual per clone. Qualitative observations for leaf color followed the color codes based on the color chart according to the Royal Horticultural Society (RHS) in 2015.

2.2.2.3. Grouping analysis of taro diploid and tetraploid cv Bentul according to morphological character. Similarities of plant habitus, leaves, petiole and root morphological characterization between taro diploid and tetraploid clones were presented as dendogram, results of grouping analysis were done by PAST software.

2.2.3. Anatomical Characterization of Taro cv Bentul

2.2.3.1. Leaf stomata. Non-permanent slide of epidermis leaves was made according to Sass method [18]. Five leaves from 5 individual taro plant of each clone were taken, then slashed the opposite part of the leaf blade as sample. To observe the stomata in the adaxial part, epidermal cells of the abaxial section are peeled off and do the opposite if we want to observe the other side of leaves. The leaf blade was cut for 1 x 1 cm then sliced using a razor blade which has been moistened with sodium hypochlorite (NaClO) to obtain the thinnest layer. The sample was then soaked in 5.25% sodium hypochlorite solution for 3-5 min followed by rinsing them with distilled water.

Samples were put on the glass object and covered with a glass cover, then observed under an inverted microscope (Leica DMIL LED). Microscopic observation for leaf paradermal section was carried out in five fields of view with five replicates of each clone. Data were analyzed by one-way analysis of variance (ANOVA), followed by the Duncan Multiple Range Test (DMRT) with 95% probability. The parameters observed in the leaves of the paradermal section were type, size, and stomatal density. Stomata was measured by measuring wide and length of guard cells. Stomatal density was calculated according to Willmer (stomatal density = number of stomata/field view width in mm²).

2.2.3.2. Transversal section of leaf, petiole and root. Leaf-pieces size approximately 1x1 cm were taken from mid position, manually sectioned transversely with a razor blade. Samples were then placed on a glass object and observed under a microscope (Leica DMIL LED). The parameters observed were thickness of upper and lower epidermal tissue, thickness of palisade tissue, thickness of spongy tissue, and thickness of leaves.

The upper third part of petiole and the mid part of the main root was cut about 2 cm long then manually sectioned transversely as thin as possible using a razor blade under a stereo microscope. Samples were then placed on a glass object and observed under a microscope (Leica DMIL LED). Observation of microscopic slides of petiole and roots were carried out in two fields of view with two replicates of plants for each clone. The parameters observed for transverse petiole section were thickness of epidermal tissue, thickness of epidermal tissue and stele diameter of roots. Anatomical characterization data were analyzed using one-way analysis of variance (ANOVA), followed by the Duncan Multiple Range Test (DMRT) test at alpha=5%.

3. Results and Discussion

3.1 Acclimatization.

Tetraploid and diploid plants had a high survival rate on acclimatization processes, but not for tetraploid clone 7.5.12.2 (Table 1). After acclimatization, plantlets were moved and maintained in the lathhouse for morphological and anatomical characterization.
Table 1. Survival rate of diploid and tetraploid taro cv. Bentul planlets after acllimatization

| Clone | Ploidy | Planlet Number | Number of not survive Planlets | Number of survive planlets | Survival Rate (%) |
|-------|--------|----------------|-------------------------------|----------------------------|-------------------|
| Bentul | Diploid | 6              | 0                             | 6                          | 100               |
| 7.5.10.3 | Tetraploid | 6              | 0                             | 6                          | 100               |
| 7.5.12.2 | Tetraploid | 8              | 1                             | 7                          | 87.5              |
| 30.4.6 | Tetraploid | 6              | 0                             | 6                          | 100               |
| 60.2.6 | Tetraploid | 3              | 0                             | 3                          | 100               |
| 75.2.1 | Tetraploid | 5              | 0                             | 5                          | 100               |
| 75.2.3 | Tetraploid | 5              | 0                             | 5                          | 100               |
| 75.8.1 | Tetraploid | 17             | 0                             | 17                         | 100               |

3.2. Morphological Characterization

3.2.1. Habitus Characterization. Taro cv Bentul diploid and tetraploid grown in Lathhouse have herbaceous habitus with stem no true woody tissue (Figure 1). Morphological characters of taro cv Bentul diploid and tetraploid after 3-4 months grown in lathouse are presented in Table 2. The plant span was commonly narrow, but tetraploid 75.2.1 was wide. This clone had medium height, with no stolon and sucker. Four out of seven clones of tetraploid taro had neither stolon nor sucker.

![Figure 1](image_url) Morphology of taro cv Bentul diploid (A) and tetraploid (B) 3-4 months grown in Lathhouse

Table 2. Morphological characters of taro cv Bentul diploid and tetraploid after 3-4 months grown in Lathhouse

| Plant Habit | Clones of Taro cv Bentul |
|-------------|--------------------------|
| Plant span  | Diploid      | Tetraploid 7.5.10.3 narrow (<50 cm) | Tetraploid 7.5.12.2 narrow (<50 cm) | Tetraploid 30.4.6 narrow (<50 cm) | Tetraploid 60.2.6 narrow (<50 cm) | Tetraploid 75.2.1 narrow (<50 cm) | Tetraploid 75.2.3 narrow-medium (50-100 cm) | Tetraploid 75.8.1 narrow-wide (0->100 cm) |
| Plant height| Dwarf-medium (0-100 cm) | Dwarf (<50 cm) | Dwarf (<50 cm) | Medium (50-100 cm) | Dwarf-medium (0-100 cm) | Dwarf-medium (0-100 cm) | Dwarf-medium (0-100 cm) | Medium (50-100 cm) |
| Number of stolon and sucker | 1-3 | None | 0-1 | None | None | 2-4 | None | 1-4 |
Different growth rate and morphological characters were also found in diploid and tetraploid in vitro shoots of taro at 4 weeks old [8]. Changes in height and span of plants due to chemical mutagen induction were also seen in Rejang bananas after oryzalin induction [20]. The difference in shoot growth in plants having higher levels of ploidy is due to the reorganization and restructuring of the genome of polyploid plants resulting in changing patterns of gene expression [21]. Changes in the pattern of gene expression may also due to inter-genomic translocations. Anti-mitotic mutagen which inhibits spindle thread formation causes chromosomes to double without cell division. The number of chromosomes is doubled, so the chances for inter-genomic translocation are greater. These chromosome translocation events resulted in increased in plant heterozygosity [22].

3.2.2. Leaves, Petiole and Root Morphological Characterization. Morphological character of leaf blade in all taro clones were peltate leaf base shape, cup shape predominant position (shape) of leaf lamina surface, absent of leaf appendages, 1:1 leaf lamina length/width ratio, medium petiole junction pattern, Y-shaped vein pattern (Figure 2). Differences in morphological characters are shown in Table 3. Only tetraploid 60.2.6 had the same leaf blade margin undulate similar to diploid plants. Other tetraploid clones had leaf blade margin of more undulate. All leaf blade color was green but with different intensity. The only different on the leaf blade margin color was found on tetraploid 75.2.1 Green-Grey. Other clones including diploid had leaf blade margin color green. Leaf main vein color of all diploid and tetraploids was the same, all were green. Leaf blade color changes due to the induction of chemical mutagens also occur on Garden Pansy (Viola wittrockiana) ornamental plant [23], and leaf blade margin shape of Lychnis[24].

![Figure2](image-url) Morphology of taro leaf blade cv Bentul (a) diploid; (b) tetraploid

Table 3. Different leaf blade characteristics of taro cv Bentul diploid and tetraploid after 3-4 months grown in lathhouse

| Characters        | Diploid | Tetraploid 7,5.10.3 | Tetraploid 7,5.12.2 | Tetraploid 30.4.6 | Tetraploid 60.2.6 | Tetraploid 75.2.1 | Tetraploid 75.2.3 | Tetraploid 75.8.1 |
|-------------------|---------|---------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Leaf blade margin | Undulate| More Undulate Green | More Undulate Green | More Undulate Green | More Undulate Green | More Undulate Green | More Undulate Green | More Undulate Green |
| Leaf Blade Color   | GG137A  | GG139A              | GG139A              | GG136A            | GG137-GG139       | GG138A            | GG139A            | GG139A            |
| Leaf Blade Margin  | GG      | GG                  | GG N138C            | GG 138A           | Green-Grey        | Green 138A        | Green GG 138A     | Green GG N138C    |
The same morphological character of diploid and tetraploid taro petiole cv Bentul in all clones was 1:1 for sheath length:total petiole length. Cross-section of lower part of petiole was closed. All petiole sheath edge color was purple. Different characteristics of diploid and tetraploid taro petiole was defined by different color as shown in Table 4 and Figure 3. Color of petiole was yellow green except for tetraploid 60.2.6 which was green. Color of petiole sheath varied between diploid and tetraploid clones which were yellow green, purple and green. Petiole basal-ring color was different. Root morphological characteristics for all clones was uniform, all clones had brown roots. Mutagen oryzalin did not changed the root morphology.

Table 4. Different petiole characteristics of taro cv Bentul diploid and tetraploid after 3-4 months grown in Lathhouse

| Character                  | Diploid | Tetraploid | Tetraploid | Tetraploid | Tetraploid | Tetraploid | Tetraploid | Tetraploid |
|----------------------------|---------|------------|------------|------------|------------|------------|------------|------------|
| Color of petiole           | Yellow  | Yellow     | Yellow     | Yellow     | Green      | Yellow     | Yellow     | Yellow     |
|                           | green   | green      | green      | green      | N137A-C    | green      | green      | N137A-C    |
|                           | YGG     | YGG 145 A- | YGG 145 A- | YGG 145 A- | YGG 145 A- | YGG 145 A- | YGG 145 A- | YGG 145 A- |
|                           | C       | C          | C          | C          | C          | C          | C          | C          |
|                           | Purple   | Purple     | Purple     | Purple     | Purple     | Purple     | Purple     | Purple     |
|                           | PG N77  | PG N77     | PG N77     | PG N77     | PG N77     | PG N77     | PG N77     | PG N77     |
|                           | A       | A          | A          | A          | A          | A          | A          | A          |
| Color of petiole sheath    | Yellow  | Purple     | Green      | Green      | Purple     | Purple     | Purple     | Purple     |
|                           | green   | PG N77 A   | GG N137C   | GG N137C   | PG N77 A   | GG N137C   | GG N137C   | GG N137C   |
|                           | YGG     | 145 A-     | 147 B      | 147 B      | 147 B      | 147 B      | 147 B      | 147 B      |
|                           | A-C     |            |            |            |            |            |            |            |
| Petiole basal ring color   | Purple  | Purple     | Green      | Green      | Purple     | Purple     | Purple     | Purple     |
|                           | brown   | brown      |            |            | brown      | brown      | brown      | brown      |

Figure 3. Morphology of taro petiole cv Bentul (a) diploid; (b) tetraploid
3.2.3. **Grouping analysis of taro Bentul diploid and tetraploid morphological character.** Parameters used in the grouping analysis of taro diploid and tetraploid plants are based on morphological characters consisting of habitus, leaves, petiole, and root morphological characterization. Grouping analysis of morphological characters based on 1999 IPGRI descriptors is shown in a dendogram (Figure 4). This analysis was used to classify the similarity and differences of morphological characters between diploid and tetraploid taro clones. Binary values (0) was used to define the same characteristics and (1) for different morphological characteristics of tetraploid clone compared to diploid one, then processed using the correlation-paired group equation in the PAST application.

The results of grouping analysis showed that there are two main groups. The first group was divided into three subgroups with the similarity of 63%. The first subgroup was tetraploid 7.5.10.3, tetraploid 7.5.12.2 and tetraploid 75.8.1 taro with 80% similarity. The second subgroup was tetraploid 75.2.1 and tetraploid 30.4.6 with similarity of 85%. The third subgroup is tetraploid 60.2.6 and tetraploid 75.2.3 with a similarity of 80%. The second group consisted only of diploid taro cv Bentul with 56% similarity to seven other tetraploid clones. Morphological characters of tetraploid taro were different from diploid includes fewer or no number of stolon and suckers, leaf blade margin undulate or sinuate, color of top third petiole was green until purple. Tetraploid clone 60.2.6 and 75.2.3 are very close to diploid ones, while tetraploid taro clone 7.5.10.3, 7.5.12.2 and 75.8.1 are taro with the lowest similarity to the diploid ones.

![Figure 4](image-url) Clustering of taro cv Bentul diploid and tetraploid clones based on morphological characteristics (A= clone 7.5.10.3, B= clone 7.5.12.2, C= clone75.8.1, D= clone75.2.1, E=clone30.4.6, F= clone60.2.6, G= clone 75.2.3)

The advantages of polyploidization is that more vigorous plants are formed, suppressed recessive characteristics and increase the ability to reproduce asexually. However, polyploidy induction on taro cv Bentul reduces the ability of plants to form stolons and sucker. The disadvantages of polyploidization are the change in cell structure and its regulation, cells are difficult to carry out meiosis and mitosis and the instability of epigenetic plants [25].

3.2.4. **Anatomical Characterization of Taro cv Bentul.**

3.2.4.1. **Leaf stomata.** The outermost layer of taro leaf blade is the epidermis. Taro leaf epidermis cells, both in diploid and tetraploids have 5-6-sided polygonal shapes, notched straight cell walls with irregular arrangement on the adaxial side of leaves (Figure 5). The epidermis serves to protect the underneath tissue. The arrangement of leaf epidermal cells has a very important function as a link between mesophyll and the external environment. The epidermis in
leaves plays a function in the process of transpiration, respiration, and leaf protection from pest attacks [26].

Stomata on taro cv Bentul leaves are found on the adaxial and abaxial sides of the leaves. Irregular epidermal cells on the adaxial and abaxial side of the leaves make the stomata looks overlapping between the surrounding epidermal cells. Taro cv Bentul leaves stomata are surrounded by 4-6 neighboring cells that are as large and indistinguishable from surrounding epidermal cells (Figure 5), so that leaves stomata of taro cv Bentul diploid and tetraploid are included in the anomocytic type [27][28]. The stomata size is calculated based on the length and width of the guard cells. All clones of tetraploid taro cv Bentul have stomata length and width are significantly different from diploid plants of the adaxial and abaxial part (Table 5). Increasing the set of chromosomes in cells causes an increase in stomata size, as occurs in the addition of Secale cereale plant chromosome sets [29]. Increasing stomata size and decreasing stomatal density of abaxial and adaxial part of leaves also occur in Olea europaea L [30].

Figure 5. Stomata with 4-6 guard cells (anomocytic type) of taro cv Bentul (a) diploid, (b) tetraploid. Ep=Epidermis, Sp=guard cell. Bar=50 µm

Table 5. Stomata size and density of taro cv Bentul tetraploid

| Clone         | Stomata size (µm) | Stomatal density (mm⁻²) |
|---------------|-------------------|-------------------------|
|               | Adaxial size      | Abaxial size            |                             |
|               | Length | Width | Length | Width | Adaxial | Abaxial |
| Diploid       | 25,87a  | 17,64a | 26,77a  | 18,08a | 40,80c  | 57,31c  |
| Tetraploid 7.5.10.3 | 32,77bc | 23,85bc | 33,81bcd | 24,00bc | 16,72ab | 31,33a  |
| Tetraploid 7.5.12.2 | 32,60bc | 24,41bc | 32,89bcd | 23,19b  | 27,59b  | 50,94bc |
| Tetraploid 30.4.6 | 30,92b  | 23,92bc | 31,75bc | 23,24b  | 23,11b  | 40,80bc |
| Tetraploid 60.2.6 | 31,25bc | 22,25b  | 35,75cde | 25,75bc | 20,25b  | 29,25c  |
| Tetraploid 75.2.1 | 31,46bc | 24,81c  | 39,03f  | 26,32bc | 27,12bc | 26,89g  |
| Tetraploid 75.2.3 | 24,80a  | 18,38a  | 29,85ab  | 23,32b  | 8,25a   | 26,18a  |
| Tetraploid 75.8.1 | 35,03c  | 25,06c  | 36,85de  | 27,47c  | 25,47b  | 34,43ab |

Note: Numbers followed by the same letters in the same column show no significant difference (duncan test, P <0.05).

3.2.4.2. Transversal section of leaf, petiole and root. Leaf of taro cv Bentul consisted of the upper epidermal layer, palisade, sponges and lower epidermis (Figure 6). The epidermal, palisade and spongy mesophyll tissue of taro diploid leaves has not significantly different thickness from all tetraploid taro clones (Table 6). Thickness of epidermal tissue helps plants in the process of self-defense against pathogens [31]. Thicker palisade tissue tends to increase the ability of leaves to carry out photosynthesis [32] The thickened spongy mesophyll due to
polyploidization decreases the diffusion ability of CO$_2$ in cells. Romero-Aranda et al. showed that autotetraploid Citrus plants decrease in the ability of CO$_2$ assimilation due to a decrease in the area of spongy mesophyll in internal air space [33].

![Figure 6. Structure of transversely section of leaf taro cv Bentul (a) diploid, (b) tetraploid (Ue=Upper epidermis; Psd=palisade; Spg=Spongy mesophyll; Le=Lower epidermis)](image)

Table 6. Thickness of leaf blade tissue of taro cv Bentul diploid and tetraploids

| Clone        | Thickness of leaf blades tissue (µm) | Leaf Blade |
|--------------|-------------------------------------|------------|
|              | Upper Epidermis | Palisade | Spongy Mesophyll | Lower Epidermis |          |
| Diploid      | 7.67$^{ab}$      | 30.67$^{abc}$ | 49.80$^{abc}$ | 7.76$^{a}$ | 94.60$^{abc}$ |
| Tetraploid 7.5.10.3 | 7.78$^{ab}$ | 27.69$^{a}$ | 59.75$^{c}$ | 8.95$^{a}$ | 103.20$^{c}$ |
| Tetraploid 7.5.12.2 | 8.38$^{ab}$ | 29.62$^{a}$ | 48.26$^{abc}$ | 8.76$^{a}$ | 94.80$^{abc}$ |
| Tetraploid 30.4.6 | 6.68$^{a}$ | 32.02$^{abc}$ | 30.82$^{a}$ | 7.23$^{a}$ | 76.20$^{a}$ |
| Tetraploid 60.2.6 | 6.80$^{a}$ | 35.80$^{bc}$ | 37.80$^{ab}$ | 7.80$^{a}$ | 88.00$^{abc}$ |
| Tetraploid 75.2.1 | 8.31$^{ab}$ | 37.47$^{c}$ | 58.38$^{c}$ | 6.60$^{a}$ | 110.40$^{c}$ |
| Tetraploid 75.2.3 | 7.22$^{a}$ | 26.60$^{a}$ | 36.35$^{ab}$ | 7.84$^{a}$ | 77.40$^{ab}$ |
| Tetraploid 75.8.1 | 9.48$^{b}$ | 32.58$^{abc}$ | 51.81$^{bc}$ | 7.48$^{a}$ | 101.00$^{bc}$ |

Note: Numbers followed by the same letters in the same column show no significant difference (duncan test, P <0.05).

Taro cv Bentul is a monocotyledonous plant that has complete leaf, with blade, petiole and sheath. Petiole has a watery texture (herbaceous) and is rather soft. The tissue that composes petiole includes epidermal, cortex (parenchymal tissue) and stele (vascular system) (Figure 7). Petiole has a single layer of epidermal cells. Petiole stele as vascular system consist of two groups. The first group is neatly arranged underneath epidermal layer. The second group is arranged scattered in the deeper part of cortex. The type of arrangement of vascular system in taro petioles is collateral, composed of adjacent xylem and phloem, surrounded by sclerenchyma [34]. The diploid taro petiole epidermal tissue has a thickness of 7.78 µm, not significantly different from almost all taro tetraploid except 75.2.1 clone (Table 7).
Figure 7. Petiol transversal section of taro cv Bentul (a) diploid; b) tetraploid. Ep= epidermis; Kor=Cortex, Ste=Stele. Bar= 50 µm

Table 7. Thickness of epidermis cells of taro petiol cv Bentul diploid and tetraploids

| Clone                | Thick of petiole epidermis (µm) |
|----------------------|---------------------------------|
| Diploid              | 7.78<sup>a</sup>               |
| Tetraploid 7.5.10.3  | 7.56<sup>a</sup>               |
| Tetraploid 7.5.12.2  | 7.32<sup>a</sup>               |
| Tetraploid 30.4.6    | 7.60<sup>a</sup>               |
| Tetraploid 60.2.6    | 8.17<sup>ab</sup>              |
| Tetraploid 75.2.1    | 10.13<sup>b</sup>              |
| Tetraploid 75.2.3    | 8.70<sup>ab</sup>              |
| Tetraploid 75.8.1    | 8.53<sup>ab</sup>              |

Note: Numbers followed by the same letters in the same column show no significant difference (duncan test, P <0.05).

Roots of taro cv Bentul are composed of epidermis, cortex and stele (Figure 8). Diploid root epidermal thickness is 128.77 µm, not significantly different from 7.5.10.3 and 75.2.1 clones. Tetraploid 7.5.12.2 has the thickest epidermal root tissue which is 163.69 µm. The diameter of taro root stele between diploid clones and seven tetraploid clones did not show any significant differences (Table 8). Phloem and xylem at the root of taro cv Bentul form a vascular system that are mutually integrated with the polyarch arrangement, i.e the root vascular system has a large number of phloem bundles surrounding the xylem [34][35].

Figure 8. Root transversal section of taro cv Bentul (a) diploid; b) tetraploids. Ep= epidermis; Kor=cortex; Ste=Stele. Bar=50 µm
Table 8. Thickness of epidermis cell and diameter stele of taro root cv Bentul diploid and tetraploids

| Clone          | Thickness of Root Epidermis cell (µm) | Root Stele Diameter (µm) |
|----------------|--------------------------------------|--------------------------|
| Diploid        | 128.77\textsuperscript{b}            | 1234.75\textsuperscript{a} |
| Tetraploid 7.5.10.3 | 123.46\textsuperscript{b}          | 1186.00\textsuperscript{a} |
| Tetraploid 7.5.12.2 | 163.69\textsuperscript{a}          | 1423.00\textsuperscript{a} |
| Tetraploid 30.4.6 | 76.32\textsuperscript{a}            | 1087.75\textsuperscript{a} |
| Tetraploid 60.2.6 | 86.75\textsuperscript{a}            | 1153.00\textsuperscript{a} |
| Tetraploid 75.2.1 | 101.09\textsuperscript{ab}          | 1089.00\textsuperscript{a} |
| Tetraploid 75.2.3 | 85.50\textsuperscript{a}            | 1258.25\textsuperscript{a} |
| Tetraploid 75.8.1 | 123.82\textsuperscript{ab}          | 1372.75\textsuperscript{a} |

Note: Numbers followed by the same letters in the same column show no significant difference (duncan test, P <0.05).

4. Conclusions

Induction of polyploidy using oryzalin caused changes in anatomical and morphological characteristics of taro cv Bentul. Tetraploid taro has a greener leaf blade color, the leaf blade margin is undulate or sinuate, and the petiole color is more dominant purple than the diploid taro clone. The results of the grouping analysis on morphological characters, it can be seen that seven clones of taro cv Bentul tetraploid characteristics have 56% similarity coefficient with diploid one. Seven tetraploid clones were divided into 3 different subgroups.

The thickness of the tissue leaves of the diploid and tetraploid Bentul is not significantly different. Stomata size in leaf epidermis of seven tetraploid clones is larger than diploid ones. The density of the leaf stomata of the seven taro cv Bentul tetraploid clones tended to be lower than diploid ones. Thickness of root epidermis tissue taro cv Bentul diploid is significantly different from tetraploid taro 75.2.1 Thickness of root of taro cv Bentul diploid epidermal tissue is significantly different from tetraploid taro 7.5.12.2, 30.4.6, 60.2.6 and 75.2.3. Diametre of diploid taro root stele tends not to differ significantly from tetraploid taro.

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6. References

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