RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
55 (1) 163-174, 2023
http://doi.org/10.54910/sabrao2023.55.1.16
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

ORYZALIN-INDUCED TARO (*COLOCASIA ESCULENTA* L.) TETRAPLOID AND DIPLOID ASSESSMENT FOR GROWTH AND AGRONOMIC TRAITS

T. HANDAYANI[1*], A.D. PRAWESTRI[1*], R.S. RAHAYU[2], and A. LEKSONOWATI[1]

[1]Research Center for Genetic Engineering, Research Organization of Life Science and Environment, National Research and Innovation Agency (BRIN), Cibinong Science and Technology Park, Bogor, Indonesia
[2]Research Center for Plant Conservation, Botanical Garden and Forestry, Research Organization of Life Science and Environment, National Research and Innovation Agency (BRIN), Bogor, Indonesia
*Corresponding authors' emails: trihandayani08@gmail.com, ad.prawestri@gmail.com
Email addresses of co-authors: resa.rahayu@gmail.com, aryanileksonowati@gmail.com

SUMMARY

Taro (*Colocasia esculenta* L. cv. Pontianak) is a popular and widely cultivated cultivar in the Indonesian farming community. In taro Pontianak, manipulating the ploidy level by chromosome doubling can enhance its genetic diversity. The latest study aimed to evaluate the oryzalin-induced polyploid taro cv. Pontianak compared with its diploid in terms of growth, anatomical, cytological, morphological, and agronomic traits in the greenhouse and the field. In vitro shoots of taro cv. Pontianak were soaked using a liquid medium supplemented with oryzalin with a concentration of 30 and 60 µM for one day. After five times of subcultures, flow cytometry analysis proceeded to determine their ploidy levels. The diploid (2n = 2x) and tetraploid (2n = 4x) plantlets were then acclimatized in the greenhouse for six to eight weeks and hardened for two months before planting in the field for 10 months. Squashing the root tip to ensure the ploidy levels of taro seedlings helped measure the chromosome numbers. Observing the growth, anatomical, and agronomic traits of diploid and tetraploid plants occurred during the hardening process until harvest time. The 30 µM oryzalin treatment produced tetraploid and mixoploid shoots; none from the 60 µM treatment. Oryzalin-induced tetraploid taro exhibited some morphological characteristics of tetraploid plants, such as, longer stomata size and low density. Tubers of tetraploid plants tended to have increased protein, ash, and water content compared with their diploid counterpart. However, tetraploid taro showed a slower growth rate both in the greenhouse and the field. Additionally, it gave lower yields than diploids in some agronomic characteristics, including delayed flowering time, lower plant fresh weight, and smaller tubers. This study provides information that oryzalin-induced tetraploid taro could exhibit increasing and decreasing nutritional-agronomic traits (as mentioned above) compared with its diploid.

Keywords: Taro (*Colocasia esculenta* L. cv. Pontianak), ploidy manipulation, genetic diversity, proximate analysis, field evaluation, growth traits

Key findings: Taro (*Colocasia esculenta* L. cv. Pontianak) ploidy level manipulation in vitro used 30 µM oryzalin. The oryzalin-induced tetraploid taro has varied agronomic traits and proximate contents compared with their diploid genotypes.

Communicating Editor: Prof. Naqib Ullah Khan

Manuscript received: September 23, 2022; Accepted: December 16, 2022.

Citation: Handayani T, Prawestri AD, Rahayu RS, Leksonowati A (2023). Oryzalin-induced taro (*Colocasia esculenta* L.) tetraploid and diploid assessment for growth and agronomic traits. *SABRAO J. Breed. Genet.* 55(1): 163-174. http://doi.org/10.54910/sabrao2023.55.1.16.

INTRODUCTION

Taro (*Colocasia esculenta* L.) is a polymorphic plant species that belongs to the family Araceae (Chaïr *et al.*, 2016). Taro is native to Southeast Asia and widely cultivated as a food crop in various tropical and subtropical countries, including China, India, and Africa (Rashmi *et al*., 2018). Although generative propagation can occur naturally, its monoculture and hetero-culture vegetative propagation also exist (Habibah and Astika, 2020). Most parts of taro plants are edible, including the tuberous portion, stolon, leaf blades, petioles, and flowers (Bammite *et al*., 2021). Taro tubers, which contain 70%–80% starch utilized as a staple food instead of rice, ranked ninth among food crops worldwide (Rashmi *et al*., 2018). In addition, taro contains anticancer compounds and immuno-modulators beneficial to health (Pereira *et al*., 2021).

In Indonesia, the genetic diversity of taro is relatively high, with the cultivation of at least 40 cultivars in various regions, i.e., Java, Kalimantan, and Sulawesi to Papua, Indonesia (Mulyaningsih *et al*., 2019). The taro cultivar Pontianak is a local genotype widely cultivated in West Kalimantan, and the tubers are white or purple-black. Although it also has a relatively high economic value in Malaysia and Japan, however, still unpopular in Indonesia (Kautsar, 2020; Nattasha, 2021). Based on the Indonesian Food Composition Data obtained from the Ministry of Health, Indonesia, a 100 g of fresh Pontianak taro tubers with an edible weight value of 83% have a nutritional food composition consisting of water (60 g), energy (163 Cal), protein (2.3 g), fat (0.5 g), carbohydrates (36.4 g), fiber (0.7 g), calcium (45 mg), phosphorus (80 mg), iron (1.7 mg), sodium (14 mg), potassium (323.1 mg), copper (0.3 mg), zinc (1 mg), thiamin (0.02 mg), and riboflavin (0.1 mg) (Ministry of Health, 2018).

The genus *Colocasia* consists of 20 types and has a basic number of chromosomes x = 14 (Wang *et al*., 2017) and two cytotypes, which are diploids with 28 chromosomes (2n = 2x = 28) and triploids with 42 chromosomes (2n = 3x = 42). Cytogenetic, morphological, and biochemical studies revealed that triploids are derivatives of autopolyploids spontaneously (Chaïr *et al*., 2016). Artificially, polyploidy induction use chromosome multiplier compounds, such as colchicine and oryzalin, and such ploidy manipulation aims to enhance genetic diversity and productivity (Wulansari *et al*., 2021). Increasing the genetic diversity of taro is required to assemble germplasm for tolerance and further development against biotic and abiotic stresses (Oktavianingsih *et al*., 2021). Past studies enunciated the use of oryzalin to induce polyploid on the taro cultivar Bentul (Wulansari *et al*., 2017) and the use of colchicine with Kaliurang cultivars in Indonesia (Ermayanti *et al*., 2018). Induction of polyploidy in a plant could affect growth and morphological, anatomical, phytochemical, and agronomic characteristics (Corneillie *et al*., 2019; Mo *et al*., 2020; Ridwan and Witjaksono, 2020; Wulandari *et al*., 2020).

Evaluating polyploid plant growth and its agronomic traits becomes imperative, especially for determining and selecting superior taro clones. In taro plants, the plant's size, fresh tuber weight, starch content, fiber and water in the tubers, and other post-harvest characteristics are the key indicators that need evaluation after the induction of polyploidy. Therefore, the latest study aimed to compare the oryzalin-induced tetraploid taro cv. Pontianak with its diploid in terms of growth, anatomical, cytological, morphological, and agronomic traits in the greenhouse and the field.

MATERIALS AND METHODS

Plant material

The taro cultivar Pontianak (*Colocasia esculenta* L.) served as the plant material. Initiating in vitro shoots from the tubers-derived buds underwent in vitro propagation five to six times subculture before being used for induction of tetraploid treatment. The study ensued during 2018–2021 at the Laboratorium of Plant and Tissue Culture and the experimental field, Cibinong Science and Technology Park, National Research and Innovation Agency, Bogor, Indonesia.

In vitro ploidy induction

Induction of tetraploid treatment began by soaking the shoots in a solution of oryzalin with a concentration of 30 and 60 µM for one day. The oryzalin induction used 20 shoot buds for each concentration treatment. A piece of shoot 0.8–1.0 cm, soaked in 30 mL of B_2 liquid medium, received an oryzalin supplement according to the concentration treatment. Shoots soaked with oryzalin solution in a 100 mL erlenmeyer were laid out on a rotary shaker at a speed of 100 rpm without direct lighting at a room temperature of 25±3°C.

After one day, the soaked shoots got rinsed with the same liquid medium without oryzalin and transferred to a semi-solid B_2 medium with five shoots/bottle. The composition of the B_2 medium was MS basal medium (macronutrient, micronutrient, and vitamins) (Murashige and Skoog, 1962), then added with 100 mg L^{-1} of Myo-inositol, 2 mg L^{-1} of BA (Benzyl-adenine), and 7 g L^{-1} agar. The medium was set at pH 5.7–5.8 by adding NaOH or HCl 1N before autoclaving.

The shoots obtained from the oryzalin treatment received a numbering of one to 20 at the time of subculture, with shoots growing from the multiplication being given different extra numbers based on the initial numbering. Shoots from oryzalin treatment gained subcultured four to five times into the same medium (B_2) every eight weeks. Observations of the effects of oryzalin treatment included counting the survival rates of the explants after oryzalin treatment and the number of regenerating shoots in subcultures 1, 3, and 5.

Ploidy analysis

After the fifth subculture, analysis of the growing shoots for their ploidy levels used flow cytometry. The analyzed ploidy levels consisted of in vitro leaf shoots treated with oryzalin randomly taken from each treatment, with the non-oryzalin treatment as a control. Placing pieces of in vitro taro leaves measuring 0.5–1.0 cm^2 in a petri dish and added 250 µL of CyStain Nuclei Extraction Buffer (Sysmex) got chopped with razor blades. The chopped leaves gained filtering with a CellTrics® sieve of 30 µm, with the filtrate inserted in a *cuvette* tube, supplemented by 800 mL of CyStain buffer solution (CyStain PI absolute, RNAse stock solution, and staining buffer; Sysmex) for analysis. The diploid control sample achieved calibration on channel 200 with the setting in the flow cytometry (Partec, Germany). Samples showing diploids will appear in peak histograms at channel 200, tetraploids at channel 400, and mixoploids will show peaks simultaneously on the two channels.

Chromosomes number

Calculating the number of chromosomes in diploid and tetraploid plants progressed. Counting the chromosome number in the taro cultivar Pontianak followed the method of Senavongse *et al*. (2018) with some modifications. The sample used the root tip of the taro cultivar Pontianak planted in a polybag. Collecting root tips (about 1 cm long) from healthy plants occurred at 07:30–08:00 am, then cleaned with tap water. Soaking the roots in a 0.8% 8-Hydroxyquinoline solution continued for 5 h at 4°C under dark conditions. Rinsing the roots with tap water for further fixation in Carnoy solution (acetic acid: ethanol, 1:3, v/v) proceeded for about 1 h at room temperature. Again, rinsing with tap water, the roots got stored in 70% alcohol at room temperature. In a dry bath, hydrolyzing the root tips in a 1 M HCl solution at 60°C for 5 min followed. The roots, rinsed with water, underwent soaking in a solution of lactophenol orcein (LPO) for 1 h for chromosomal staining. The root tip, cut to about 1 mm, was laid out on a glass slide, dripped with one drop of LPO, and then covered with a glass slide for further squashing. Observation of the sample ensued under a light microscope at a magnification of 40×.

Acclimatization and field cultivation

Diploid and tetraploid taro shoots propagated on the B_2 medium and subcultured to the B_0 medium (B_2 medium without hormone), induced rooting. The rooted plantlet received acclimatization on a mixture medium of soil, cocopeat, husk charcoal, and sand (composition 1:2:2:2, w/w/w/w) previously sterilized using a steamer. After acclimatization of the seedlings for approximately six to eight weeks, their transfer to polybags for hardening for two months transpired before planting into the field for agronomic characterization.

Planting the diploid (2n = 2x) and tetraploid (2n = 4x) seedlings proceeded in the field in a randomized complete block design (RCBD) with six replications to observe their growth and agronomic characteristics. Each replication (block) represented by a plant row (size 4.0 m × 0.6 m) contained eight plants, with a planting distance of 50 cm among the plants in the row. Plant fertilization used the chemical fertilizer NPK (16:16:16) at the rate of 60 g per plant, given two times (30 g first at the planting time and 30 g as the second dose at the three-month-old plant stage). Plant maintenance followed the recommended and standard cultivation technology for taro crops.

Anatomical and agronomic traits observation

Observing the growth traits of diploid and tetraploid plants during the greenhouse's hardening process continued after the

seedlings' transplanting into polybags. Recording of data covered the parameters viz., plant height, number of leaves, leaf length, and leaf width. Observations happened for four and eight weeks before the seedlings' planting in the field. Monitoring the above vegetative growth traits in the field also ensued for four, eight, and twelve weeks after planting. Before harvesting the tubers (after 10 months of planting), recording the data included plant height, number of leaves, number of new shoots, length and width of the leaf, leaf color (SPAD), length, and stomata density. The harvest parameters observed comprised the plant's total weight, the tuber's weight, and the diameter. In addition, the analyses of diploid and tetraploid taro tubers for the proximate content also took place.

Plant height measurement covered the stem base to the tip of the highest leaf, with the stem diameter covering the largest stem in each plant, about 5 cm above the soil surface. Calculation of the number of leaves included all fully opened shoots. Measurements of leaf length and width covered the broader leaf of each plant, measured at the center or symmetrical point of the leaf. Observations on the leaf color used the SPAD meter by measuring leaf color at three points on the broader leaf. Observations of the length and density of stomata used a simple method of applying acrylic nail polish before observing under a light microscope (Moore *et al*., 2008). Stomata density calculation through the view area of a light microscope at 40× magnification used the formula:

Stomata density = the number of stomata / the view area at 40× magnification

Where,

The view area = 0.196 mm^2

Evaluation of the plant's fresh weight consisted of measuring the entire plant except for the tubers. Separating the fresh tubers observed on taro plants from the canopy part of the plant occurred earlier. Measurement of the diameter of the tuber took place at the central part of the tuber. Analysis of the proximate content of tubers ensued at the Saraswanti Indo Genetech Laboratory (SIG), Bogor, West Java, Indonesia. Fresh tubers from diploid and tetraploid plants with three replicates for each ploidy gained analysis, with the data recorded on the parameters including protein, ash, carbohydrate, total energy, total fat, and water content.

Statistical analysis

The analysis of collected data resulted from comparing the obtained mean values at each stage of treatment using percentage data, total amount data, and the data presented in the form of average data ± standard deviation (St. Dev.) of each treatment. Analysis of the obtained data used a one-way analysis of variance. Employing Duncan's multiple range test ($DMRT_{0.05}$) assessed the significant difference among the various treatments.

RESULTS

Polyploidy manipulation

Results revealed that the oryzalin-treated and inoculated taro shoots showed survival rates of around 15%–30% (Table 1). After five times subcultures in the same medium, 34 shoots resulted from the 30 µM oryzalin treatment, with only 25 from the 60 µM oryzalin. The multiplication and regeneration rate of shoots obtained from 30 µM oryzalin treatment appeared higher than that of 60 µM. However, the shoots' survival rate at 30 µM oryzalin treatment rated only 15%.

The ploidy level analysis using flow cytometry of the oryzalin-induced in vitro shoots showed that in a total of 20 regenerated tested shoots, seven polyploids were obtained (four tetraploids and three mixoploids), with the remaining 13 as diploids (Table 2, Figure 1). Flow cytometry histogram showed that diploid shoots 2C DNA from non-treated oryzalin produces a peak at channel 200 determined as standard (Figure 1a). Tetraploid shoots with 4C DNA and mixoploids with 2C+4C DNA produced the histograms with peaks on channels 400 (Figure 1b) and 200+400 (Figure 1c). In the present research, the 30 µM oryzalin treatment developed the tetraploid and mixoploid shoots, with the 60 µM producing no polyploid shoots (Table 2). It indicated that the higher concentration of oryzalin inhibits the growth of the shoots. Likewise, the 30 µM oryzalin could induce polyploid through in vitro shoot buds of taro.

Also, confirming the ploidy level by counting the number of chromosomes used the squashing method. The results revealed that tetraploid plants resulting from polyploid induction have 48–56 chromosomes, double their diploid (24–28 chromosomes) (Figure 2). The number of chromosomes aligns with the flow cytometry analysis that tetraploids have double the genome size as their diploids.

Table 1. Survival rate and degree of multiplication of shoots in vitro on tetraploid induction treatment.

Oryzalin (µM)	Survival (%)	Number of shoots regeneration		
		Subculture 1	Subculture 3	Subculture 5
0	20 (100.0)	48	73	91
30	3 (15.0)	4	20	34
60	6 (30.0)	18	15	25

Table 2. Ploidy analysis on in vitro shoots resulted from oryzalin treatment using flow cytometry.

Oryzalin (µM)	Number of shoots	Peak (Mean-x)*		CV (%)*		Ploidy
0	4	238.55	± 56.78	5.31	± 1.14	Diploid (2x)
30	5	199.26	± 5.16	3.99	± 0.54	Diploid (2x)
	4	398.27	± 25.29	3.58	± 0.99	Tetraploid (4x)
	3	194.31	± 4.92	3.44	± 0.52	Mixoploid (2x+4x)
		393.36	± 7.55	3.27	± 0.58	
60	4	191.58	± 5.83	4.72	± 0.63	Diploid (2x)

Note: *Mean-x: the average value of the peak channel size; CV (%): coefficient of variation of peak values from the histogram of flow cytometry.

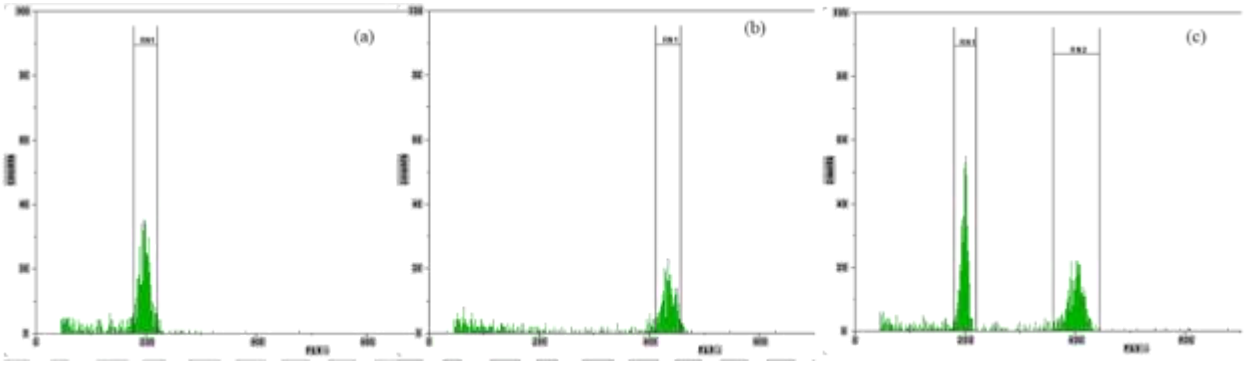

Figure 1. Histogram of flow cytometry on oryzalin-induced in vitro shoots of taro cultivar Pontianak, a) Diploid (2C peak at 200), b) Tetraploid (4C peak at 400), and c) Mixoploid (2C+4C peaks at 200 and 400).

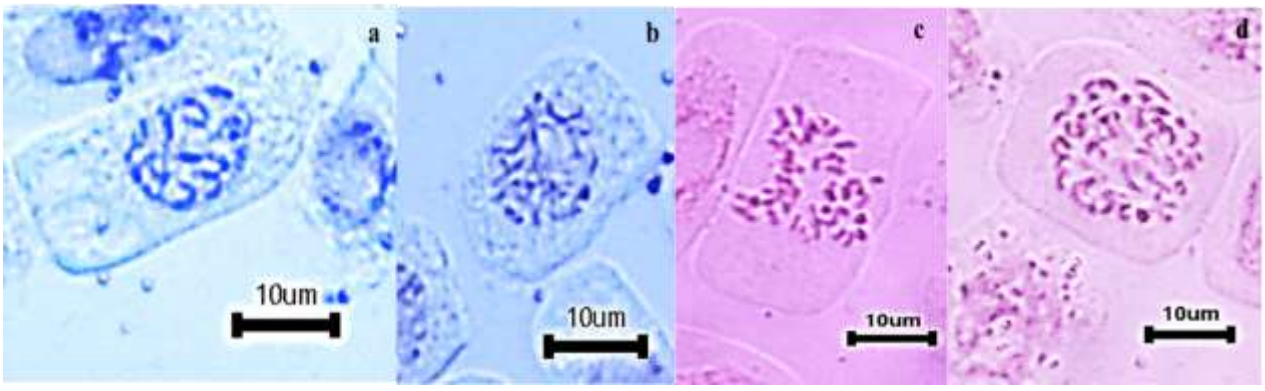

Figure 2. The number of chromosomes resulting from the taro cultivar Pontianak (a, b) diploid 2n = 2x = 24–28, and (c, d) tetraploid 2n = 4x = 48–56.

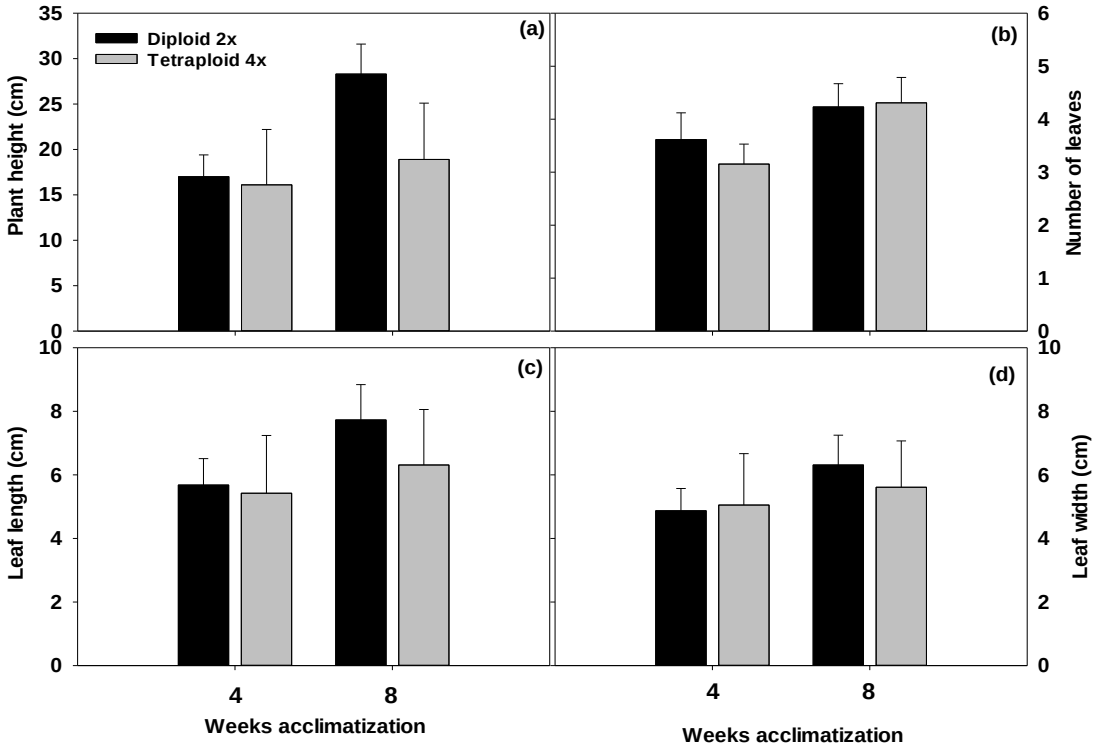

Figure 3. Growth of diploid and tetraploid seedlings of taro cultivar Pontianak in the greenhouse, a) Plant height, b) Number of leaves, c) Leaf length, and d) Leaf width.

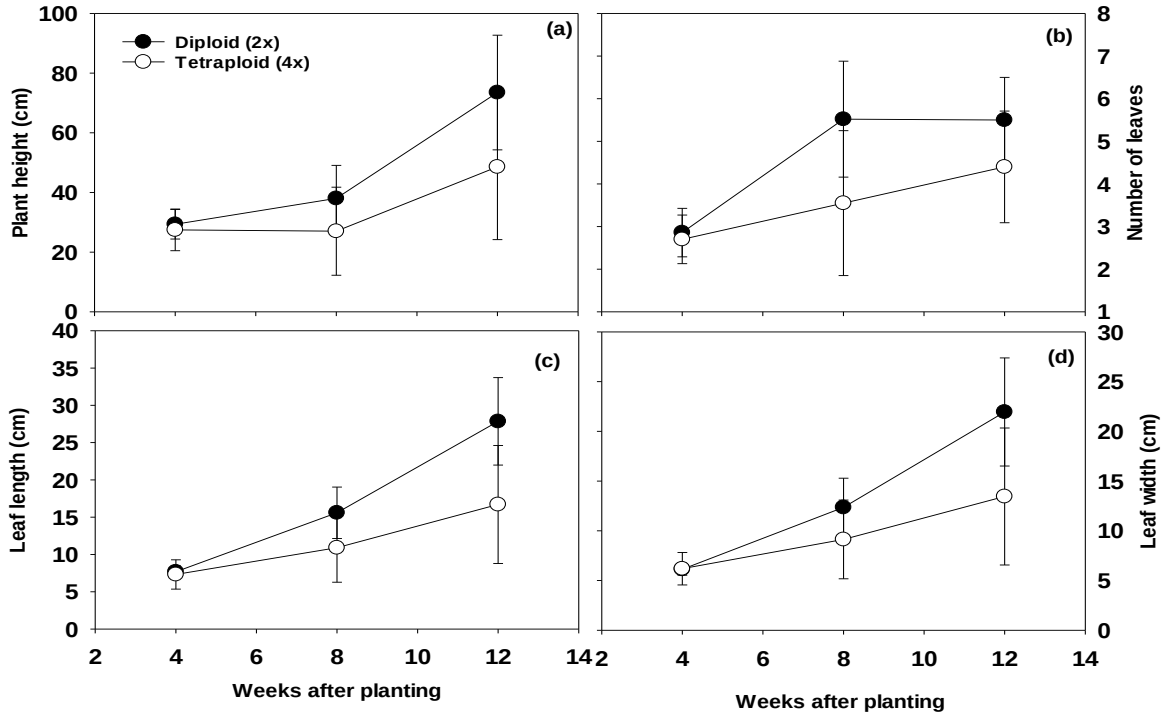

Figure 4. Vegetative growth of taro cultivar Pontianak diploid and tetraploid in the field, a) Plant height, b) Number of leaves, c) Leaf length, and d) Leaf width.

Vegetative and generative growth

The observations of seedling growth in polybags during the hardening in the greenhouse showed that diploid taro plants have faster growth than tetraploid, especially for plant height, leaf length, and width (Figure 3). In the parameters of plant height, number of leaves, leaf length, and width, observations gathered that the growth rate of diploid plants up to 12 weeks after planting (WAP) in the field was faster than that of tetraploids. The plant height of tetraploid taro at 12 WAP was 33.89% lower than that of diploid taro. Tetraploid taro plants also have fewer leaves and leaf sizes than diploid plants (Figure 4).

Until 10 months after planting and when the plants were in the generative phase, the average height of tetraploid plants scored 31.19% lower than that of diploids. Other plant growth parameters, such as, stem circumference, number of new shoots, number of flowers, and leaf size, also showed that tetraploid taro plants had a lower growth rate (Table 3). However, the tetraploid taro plants had a darker green color (SPAD), larger stomata size (longer stomata), and lower stomata density (Table 3). The slower growth rate of tetraploid plants has implications for the generative phase, so tetraploid plants could not produce flowers for up to 10 months of planting. Presumably, the tetraploid taro takes more time to reach the generative phase than the diploid. The higher vegetative growth rate also had implications for crop yields, where 10 months after planting, diploid taro plants had a fresh weight of 41.00% higher than the tetraploid plants. The weight and size of diploid taro tubers also showed a bigger size 10 months after planting (Table 3 and Figure 5).

Table 3. Anatomical and agronomic characteristics of taro cultivar Pontianak diploids and tetraploids at 10 months after planting.

Parameter	Diploid (2x)		Tetraploid (4x)	
Anatomical characteristics				
Leaf length (cm)	28.92	± 3.47 a	22.71	± 2.75 b
Leaf width (cm)	21.50	± 3.09 a	17.46	± 2.50 b
Leaf color (SPAD)	44.90	± 4.44 a	47.16	± 3.12 a
Stomata length (µm)	11.99	± 0.58 b	17.45	± 0.66 a
Stomata density (mm^2)	191.51	± 120.26 a	139.11	± 78.42 a
Agronomic characteristics				
Plant height (cm)	75.88	± 12.70 a	52.21	± 6.84 b
Number of leaves	2.88	± 0.38 a	2.17	± 0.30 b
Stem circumference (cm)	16.25	± 1.87 a	12.92	± 1.92 b
Number of new shoots	8.88	± 3.51 a	2.17	± 1.70 b
Number of flowers	0.88	± 0.52 a	0.00	± 0.00 b
Fresh weight of plants (g)	400.4	± 81.3 a	236.2	± 71.5 b
Fresh weight of tubers (g)	255.7	± 50.6 a	175.0	± 61.3 b
Diameter of tubers (cm)	6.646	± 0.507 a	5.522	± 0.874 b

Note: The different letters within the different parameters indicate a significant difference ($p \leq 0.05$) based on the DMRT test.

Figure 5. Morphological differences of diploid and tetraploid of the taro cultivar Pontianak leaf (A) and tubers (B) (Bar = 5 cm).

Table 4. Proximate content of the tubers obtained from diploid and tetraploid of taro cultivar Pontianak.

Proximate (per 100 g)	Ploidy level	
	Diploid (2X)	Tetraploid (4X)
Protein (%)	1.61 ± 0.55 a	2.03 ± 0.46 a
Ash (%)	1.31 ± 0.18 a	1.55 ± 0.11 a
Energy from fat (Kcal/100 g)	7.41 ± 0.36 a	2.40 ± 0.10 b
Total of fat (%)	0.82 ± 0.04 a	0.27 ± 0.01 b
Water content (%)	61.91 ± 2.27 b	66.67 ± 3.23 a
Total energy (Kcal/100 g)	151.24 ± 8.65 a	128.43 ± 13.03 b
Carbohydrates (%)	34.34 ± 2.05 a	29.48 ± 3.69 b
Crude fiber (%)	0.90 ± 0.07 a	0.69 ± 0.19 b
Dietary fiber (%)	3.33 ± 1.02 a	3.09 ± 0.73 a
Starch content (%)	29.08 ± 3.79 a	24.63 ± 4.35 a

Note: The different letters within the different parameters indicate a significant difference ($p \leq 0.05$) based on the DMRT test.

Proximate composition of the taro tubers

Based on the analysis of the proximate content, results showed that the nutritional content of the tetraploid Pontianak taro tubers increases the protein content by 20.06% but compensates for a carbohydrate decrease of 14.15% (Table 4). The ash content of tetraploid increased by 18.32% compared with the diploid control, showing that tetraploid tubers tended to have higher inorganic residue from the incineration of organic matter. The tubers from the tetraploid plants have a water content of 4.67% higher than their diploids, which can save more water in their cells. However, the tetraploid taro gave lower scores compared with the diploid for the parameters of total energy, total fat, energy from fat, carbohydrate, and crude fiber content (Table 4), with the total energy from fat and total fat content of tetraploid taro tubers is about 67% lower than diploid ones.

DISCUSSION

Employing polyploidy induction in various plants has used trifluralin, oryzalin, and colchicine compounds as inhibitors of the mitosis process and reduction division (Touchell *et al*., 2020). Genome duplication due to polyploidization treatment will result in several epigenetic changes and changes in gene expression. In plant breeding, scientists developed polyploidization to overcome the non-viability and infertility of interspecific hybrids, obtaining seedless polyploid cultivars and increasing resistance and tolerance to the biotic and abiotic factors (Eng and Ho, 2019). The employment of polyploidy induction mainly sought to increase genetic diversity and provide a source of crossbreeding related to genetic improvement at the chromosomal level, such as, banana plants (Poerba *et al*., 2019), shallot (Yun *et al*., 2021), and watermelon (Bae *et al*., 2020).

In the taro cultivar Bentul, a study reported the use of oryzalin compounds in research on polyploidy induction with concentrations ranging from 7.5 to 75 µM, showing the concentrations of 30 µM and 60 µM produced tetraploid and mixoploid shoots, reaching 50% of the total number of shoots. The higher the concentration of oryzalin given, the lower the shoot proliferation, petiole length, number of leaves, and number of shoots in vitro of taro cultivar Bentul plants (Wulansari *et al*., 2016).

In this study, despite the lower explant survival rate in the 30 µM oryzalin treatment compared with the 60 µM, only the 30 µM produced the tetraploid and mixoploid shoots. Allegedly during the subculture process, many shoots were unable to thrive and not survive, so up to the fifth subculture, shoots that grew on 60 µM oryzalin treatment were only diploid and had a lower multiplication rate. Sree-Ramulu *et al*. (1991) explained that the process of doubling chromosomes gained influence from the frequency of metaphase cells exposed to the antimitotic compound. Higher metaphase frequencies, especially with well-dispersed chromosomes after oryzalin treatment, resulted in a larger percentage of micronucleated cells, with single or groups of two or more metaphase chromosomes changed directly into micronuclei without centromere division and chromatid separation. Treatment with oryzalin at 30 µM might be more efficient in inducing higher micronucleated frequencies and increasing the efficiency of doubling chromosomes than treatment with oryzalin at

60 µM. Ascough *et al.* (2008) stated that the effectiveness of antimitotic compounds in vitro depends highly on the concentration applied, duration of treatment, type of explant, and penetration of the compound. Explant survival declined when treated with high concentrations and a long duration of colchicine and oryzalin, further affecting the rate of multiplication and reducing growth rate.

On confirmation of the number of chromosomes using the squashing method, the number of chromosomes in diploid plants ranged from 24–28, and the number of chromosomes in tetraploid plants ranged from 48–56. The range of chromosome numbers in taro plants varied, i.e., 2n = 2x = 22, 26, 28, 38, and 42, and the polyploid-induced chromosomes around 5%–15% have aneuploidy numbers lower or higher than the normal chromosomes (Ermayanti *et al*., 2019).

After acclimatization, the observation of shoot growth traits either in the greenhouse or in the field showed that the tetraploid taro plants had a slower growth rate than the diploid taro plants. The plant height, number of leaves, and size of the leaf of tetraploid taro plants also displayed smaller than the diploid ones. In several species of plants, it is known that tetraploid plants generally have larger plant sizes (plant height, leaves, and flowers), such as, moringa (Ridwan and Witjaksono, 2020), hydrangea (Deans *et al*., 2021), caladium (Zhang *et al*., 2020), and ginger (Zhou *et al*., 2020). However, in some species of plants, tetraploid plants have smaller leaf sizes, such as, watermelon plants (Bae *et al*., 2020), slower growth in eucalyptus plants (Fernando *et al*., 2019), and lower fresh and dry weight in rubber dandelion plants (*Taraxacum kok-saghyz*) (Luo *et al*., 2018).

Previous studies showed that the ploidy level is not directly proportional to the increase in cell size and biomass (Tsukaya, 2013). An optimum ploidy exists to obtain certain optimum characters in a plant, one of which is the dry weight of Arabidopsis (*Arabidopsis thaliana*) plants at a ploidy level of 4x (tetraploid) higher than 2x (diploid), 6x (hexaploid), and 8x (octoploid). Thus, one can conclude that 4x ploidy is the optimum ploidy for optimization of the dry weight of Arabidopsis plants (Corneillie *et al*., 2019). In addition, an increase in cell size does not necessarily lead to an increase in overall plant size; however, it usually becomes smaller due to the higher number of polyploid cell divisions (Trojak-Goluch *et al*., 2021). At each ploidy level, the growth rate of plants will be different, with a possibility that the triploid taro may have more optimal growth than tetraploids, such as, in banana plants (Poerba *et al*., 2017).

Stomata length and density parameters showed that tetraploid taro plants tended to have larger stomata sizes with lower stomatal density levels. The present study's results align with the studies on the taro cultivar Bentul (Wulandari *et al*., 2020). Stomata are one of the important characters affected by the chromosomal manipulation and polyploidization process. Changes in the size and density of stomata due to polyploidization affect the photosynthesis process of a plant. Harrison *et al.* (2020) stated that fewer but larger stomata are more beneficial to the plant. It shows that larger stomata sizeism with lower stomatal density level ascertains a favorable character for the plant. The leaves of tetraploid plants also gave an appearance of a dark green leaf color than those of diploid plants of the same age, probably due to the increased chloroplast number and enhanced chlorophyll content. The result indicated that larger stomata and high content in chloroplast characteristics can serve as effective parameters for preliminary screening of putative tetraploid, the same characteristic found in species *Echinacea purpurea* (Abdoli *et al*., 2013) and other tetraploid plants (He *et al.*, 2016; Bae *et al*., 2020; Yun *et al*., 2021; Bhattarai *et al*., 2021).

In this study, the tetraploid taro had a smaller leaf size, dark green leaf color, larger stomata, and lower density. Characters in tetraploid plants also reveal to be possessed in tetraploid teak plants (Ridwan *et al*., 2018). Characters found in tetraploid teak, one of which plays a role in the plant tolerance to drought, where tetraploid teak plants are more tolerant to drought than diploids. A report on the potential for drought tolerance in tetraploid taro by in vitro testing using PEG (polyethylene glycol) compounds stated the tetraploid taro cultivar Bentul plants had higher viability at 10%–15% PEG concentration than its diploids (Wulansari *et al*., 2021).

No flowers appeared in tetraploid plants for 10 months of observation in the field. Instead of the inability to produce flowers, researchers hypothesized that the tetraploids have not yet reached the reproductive phase. The previous study in chamomile (*Matricaria recutita* L.) showed that diploid plants bloomed, on average, significantly earlier than tetraploid ones, demonstrated by flowering time (FT) analysis. It may be due to the longer time necessary for replication of a doubled genome as a result of

polyploidization, which consequently may lead to the slowing of metabolism and growth rates in polyploids (Otto *et al*., 2017). Another study in radish reported that phytohormones, including ABA and IAA, are positively associated with delayed bolting and flowering in tetraploid through the quantification of endogenous phytohormone levels. Furthermore, based on qRT-PCR analysis, most of the genes positively related to flowering and bolting are expressed much lower in tetraploid plants compared with diploid (Pei *et al*., 2019).

Regarding the observations on the agronomic characteristics, the crop yields showed that the tetraploid plants had smaller tuber sizes than their diploids. The slower growth rate may have caused the tetraploid plant tubers after 10 months of planting may not reach the optimal age of harvesting. The analysis of proximate content found that tetraploid taro plants tended to have higher protein, ash, and water content than diploid taro, although the increase was not significant enough. Generally, tetraploid plants will have a higher protein content due to their larger genome size. One suspects that the cell size in tetraploid plants is also larger, which may be able to accommodate water and other metabolites. Reports of increased protein content in tetraploid plants also showed in ginger (*Zingiber officinale* Roscoe cv. 'Fengtou') (Zhou *et al*., 2020) and in moringa plants (Ridwan and Witjaksono, 2020).

The induction of polyploidy will affect a plant's physiological and biochemical processes and the biosynthetic pathway of primary and secondary metabolites. Increased ploidy levels are also associated with changes and rearrangements of genes after chromosome doubling. Changes in gene expression in the biosynthetic pathway of primary and secondary metabolites can alter the expression of key genes for various growth, morphological, and yield-related traits. Regulating genes on or off can also lead to increased production of specific compounds, as well as changes in the production patterns of certain compounds (Madani *et al*., 2021). Therefore, the pattern of change and enhancement of a metabolite compound in each plant induced by polyploidy may differ among the genotypes and cultivars of the different species. The anatomical-agronomic differences between diploid and tetraploid taro indirectly suggest that these tetraploids can be essential materials for one resource for enhancing genetic improvement related to biotic or abiotic tolerance and resistance. In addition, the increasing and decreasing value of some nutritional content from the tuber between tetraploid and diploid could emphasize their values as one source of functional food diet in the future.

CONCLUSIONS

In the taro cultivar Pontianak, the use of 30 µM oryzalin proved successful in the chromosomal manipulation and induced tetraploid levels in vitro. Tetraploid taro genotypes have a slower growth rate with lower plant height and leaf size, displaying dark green leaf color, larger stomata, and lower stomata density. The slower growth rate of tetraploid genotypes results in a smaller tetraploid tuber size than the diploids if harvested at the same age. Tetraploid taro tubers have a protein content, which tends to be higher but with a lower carbohydrate content. Compared to its diploid counterpart, oryzalin-induced tetraploid taro exhibits increasing and decreasing nutritional-agronomic traits.

ACKNOWLEDGMENTS

This research received partial financing from LIPI's DIPA activities in 2017–2019. The authors acknowledge K. Utami Nugraheni, Yuli, Hasrat E. Prayogi, and Omi for their technical assistance during the research. All the authors have an equal contribution to this work.

REFERENCES

Abdoli M, Moieni A, Badi HN (2013). Morphological, physiological, cytological, and phytochemical studies in diploid and colchicine-induced tetraploid plants of *Echinacea purpurea* L. *Acta Physiol. Plant.* 35(7): 2075-2083. http://doi.org/10.1007/s11738-013-1242-9.

Ascough GD, Van Staden J, Erwin JE (2008). Effectiveness of colchicine and oryzalin at inducing polyploidy in *Watsonia lepida* N.E. Brown. *HortSci.* 43(7): 2248-2251.

Bae SJ, Islam MM, Kim HY, Lim KB (2020). Induction of tetraploidy in watermelon with oryzalin treatments. *Hortic. Sci. Technol*. 38 (3): 385-393. https://doi.org/10.7235/HORT.20200037.

Bammite D, Matthews PJ, Dagnon DY, Agbogan A, Agre P, Akintayo TO, Odah K, Dansi A, Abberton M, Tozo K (2021). Genetic diversity in *Colocasia esculenta* and *Xanthosoma mafaffa* in Togo, West Africa. *Adv. in Hortic. Sci.* 35(3), 255-267. https://doi.org/10.36253/ahsc-9689.

Bhattarai K, Kareem A, Deng Z (2021). In vivo induction and characterization of polyploids in gerbera daisy. *Scien. Hortic.* 282(March):

110054. https://doi.org/10.1016/j.scienta.2021.110054.

Chaïr H, Traore RE, Duval MF, Rivallan R, Mukherjee A, Aboagye LM, Van Rensburg WJ, Andrianavalona V, De Pinheiro Carvalho MAA, Saborio F, Prana MS, Komolong B, Lawac F, Lebot V (2016). Genetic diversification and dispersal of taro (*Colocasia esculenta* L. Schott). *PLoS ONE* 11(6): 1-19. https://doi.org/10.1371/journal.pone.0157712.

Corneillie S, De Storme N, Van Acker R, Fangel JU, De Bruyne M, De Rycke R, Geelen D, Willats WGT, Vanholme B, Boerjan W (2019). Polyploidy affects plant growth and alters cell wall composition. *Plant Physiol.* 179(1): 74-87. https://doi.org/10.1104/pp.18.00967.

Deans LE, Palmer IE, Touchell DH, Ranney TG (2021). In vitro induction and characterization of polyploid *Hydrangea macrophylla* and *H. serrata*. *HortSci.* 56(6): 709-715. https://doi.org/10.21273/HORTSCI15783-21.

Eng WH, Ho WS (2019). Polyploidization using colchicine in horticultural plants: A review. *Scien. Hortic*. 246(2018): 604-617. https://doi.org/10.1016/j.scienta.2018.11.010.

Ermayanti TM, Ellfy RD, Wulansari A, Martin AF, Al Haflizh E (2019). Variasi jumlah kromosom talas bentul (*Colocasia esculenta* L. Schott) in vitro hasil perlakuan orizalin. *J. Biol. Indonesia* 15(1): 53-64. https://doi.org/10.47349/jbi/15012019/53.

Ermayanti TM, Wijayanta AN, Ratnadewi D (2018). Induksi poliploidi pada tanaman talas (*Colocasia esculenta* L. Schott) kultivar kaliurang dengan perlakuan kolkisin secara in vitro. *Biol. Indonesia* 14(1): 91-102. https://doi.org/10.14203/jbi.v14i1.3667

Fernando SC, Goodger JQD, Chew BL, Cohen TJ, Woodrow IE (2019). Induction and characterization of tetraploidy in *Eucalyptus polybractea* R.T. Baker. Ind. Crops Products 140: 111633. https://doi.org/10.1016/j.indcrop.2019.111633.

Habibah N, Astika IW (2020). Analisis sistem budi daya tanaman talas (*Colocasia esculenta* L.) di Kelurahan Bubulak, Bogor Barat, Jawa Barat. *J. Pusat Inovasi Masyarakat* 2(5): 771-781.

Harrison EL, Arce Cubas L, Gray JE, Hepworth C (2020). The influence of stomatal morphology and distribution on photosynthetic gas exchange. *Plant J.* 101(4): 768-779. https://doi.org/10.1111/tpj.14560.

He M, Gao W, Gao Y, Liu H, Yang X, Jiao H, Zhou Y (2016). Polyploidy induced by colchicine in *Dendranthema indicum* var. *aromaticum*, a scented chrysanthemum. *Eur. J. Hortic. Sci.* 81(4): 219-226. http://dx.doi.org/10.17660/eJHS.2016/81.4.5

Kautsar M (2020). 2 Jenis Talas Ini Populer di Jepang, Tapi Tidak di Indonesia. *Sari Agric*. https://pertanian.sariagri.id/56771/2-jenis-talas-ini-populer-di-jepang-tapi-tidak-di-indonesia.

Kementerian Kesehatan (Ministry of Health) (2018). Data Komposisi Pangan Indonesia. https://www.panganku.org/id-ID/view.

Luo Z, Iaffaldano BJ, Cornish K (2018). Colchicine-induced polyploidy has the potential to improve rubber yield in *Taraxacum kok-saghyz*. *Ind. Crops Prod*. 112(2017): 75-81. https://doi.org/10.1016/j.indcrop.2017.11.010.

Madani H, Escrich A, Hosseini B, Sanchez-Muñoz R, Khojasteh A, Palazon J (2021). Effect of polyploidy induction on natural metabolite production in medicinal plants. *Biomolecules* 11(6). https://doi.org/10.3390/biom11060899.

Mo L, Chen J, Lou X, Xu Q, Dong R, Tong Z, Huang H, Lin E (2020). Colchicine-induced polyploidy in *Rhododendron fortunei* Lindl. *Plants* 9(424): 1-13. https://doi.org/10.3390/ plants9040424.

Moore GW, Cleverly JR, Owens MK (2008). Nocturnal transpiration in riparian *Tamarix* thickets authenticated by sap flux, eddy covariance, and leaf gas exchange measurements. *Tree Physiol*. 28(4): 521-528. https://doi.org/10.1093/treephys/28.4.521.

Mulyaningsih ES, Anggraheni YGD, Sulistyowati Y (2019). Genetic diversity in taro (*Colocasia esculenta* L. Schott) germplasm procured from seven provinces of Indonesia. *SABRAO J. Breed. Genet.* 51(3): 295-310.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*. 15(3): 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Nattasha (2021). Aneka Ragam Talas, Petani Pilih Mana? Tabloid Sinar Tani. https://tabloidsinartani.com/detail/indeks/pangan/17555-Aneka-Ragam-Talas-Petani-Pilih-Mana.

Oktavianingsih L, Suharyanto E, Daryono BS, Purnomo (2021). Genetic diversity of taro (*Colocasia spp*.) from Kalimantan island, Borneo, Indonesia, based on RAPD markers. *SABRAO J. Breed. Genet.* 53(3): 447-458.

Otto LG, Mondal P, Brassac J, Preiss S, Degenhardt J, He S, Reif JC, Sharbel TF (2017). Use of genotyping-by-sequencing to determine the genetic structure in the medicinal plant chamomile, and to identify flowering time and alpha-bisabolol associated SNP-loci by genome-wide association mapping. *BMC Genomics* 18(1): 1-19.

Pei Y, Yao N, He L, Deng D, Li W, Zhang W (2019). Comparative study of the morphological, physiological, and molecular characteristics between diploid and tetraploid radish (*Raphunas sativus* L.). *Scien. Hortic.* 257(August): 108739. https://doi.org/10.1016/j.scienta.2019.108739.

Pereira PR, Mattos ÉB, Corrêa ACNTF, Vericimo MA, Paschoalin VMF (2021). Anticancer and immunomodulatory benefits of taro (*Colocasia esculenta*) corms, an underexploited tuber crop. *Int. J. Mol. Sci.* 22(1): 1-33. https://doi.org/10.3390/ijms22010265.

Poerba YS, Martanti D, Handayani T, Witjaksono (2019). Morphology and reproductive function of induced autotetraploid banana by chromosome doubling. *SABRAO J. Breed. Genet*. 51(2): 175-190.

Poerba YS, Handayani T, Witjaksono (2017). Characterization of tetraploid Pisang Rejang induced by oryzalin. *Berita Biol.* 16(1): 85-93. https://doi.org/10.14203/beritabiologi.v16i1.2210.

Rashmi D, Raghu N, Gopenath T, Palanisamy P, Bakthavatchalam P, Karthikeyan M, Gnanasekaran A, Basalingappa KM (2018). Taro (*Colocasia esculenta*): An overview. *J. Med. Plants Studies* 6(4): 156-161.

Ridwan, Handayani T, Riastiwi I, Witjaksono (2018). Tetraploid teak seedling was more tolerant to drought stress than its diploid seedling. *J. Penelitian Kehutanan Wallacea* 7(1): 1-11. https://doi.org/10.18330/jwallacea.2018.vol7iss1pp1-11.

Ridwan, Witjaksono (2020). Induction of autotetraploid moringa plant (*Moringa oleifera*) using oryzalin. *Biodiversitas* 21(9): 4086-4093. https://doi.org/10.13057/biodiv/d210920.

Senavongse R, Saensouk S, Saensouk P (2018). Comparative karyotype analysis in five strains of *Colocasia esculenta* L. Schott (Araceae) in Thailand. *Cytologia* 83(2): 169-173. https://doi.org/10.1508/cytologia.83.169.

Sree-Ramulu K, Verhoeven HA, Dijkhuis P (1991). Mitotic blocking, micronucleation, and chromosome doubling by oryzalin, amiprophos-methyl, and colchicine in potato. *Protoplasma* 160(2–3): 65-71. https://doi.org/10.1007/BF01539957

Touchell DH, Palmer IE, Ranney TG (2020). In vitro ploidy manipulation for crop improvement. *Front. in Plant Sci.* 11(June): 1-11. https://doi.org/10.3389/fpls.2020.00722

Trojak-Goluch A, Kawka-Lipińska M, Wielgusz K, Praczyk M (2021). Polyploidy in industrial crops: Applications and perspectives in plant breeding. *Agronomy* 11(12): 1-26. https://doi.org/10.3390/agronomy11122574.

Tsukaya H (2013). Does ploidy level directly control cell size? Counterevidence from Arabidopsis genetics. *PLoS ONE* 8(12): 1-7. https://doi.org/10.1371/journal.pone.0083729

Wang GY, Zhang XM, Qian M, Hu XY, Yang YP (2017). Chromosome number and genome size variation in Colocasia (Araceae) from China. *J. Plant Res.* 130(6): 989-997. https://doi.org/10.1007/s10265-017-0959-8.

Wulandari DR, Ningrum RK, Wulansari A, Ermayanti TM, Ratnadewi D (2020). Morphological and anatomical characters of diploid and tetraploid taro (*Colocasia esculenta* L. Schott) cv. Bentul grown in lathhouse. IOP Conference Series: *Earth Environ. Sci.* 439(1). https://doi.org/10.1088/1755-1315/439/1/012050.

Wulansari A, Marthin AF, Ermayanti TM (2016). Induksi tanaman poliploid talas (*Colocasia esculenta* L.) dengan perlakuan orizalin secara in vitro. *J. Biologi Indonesia* 12(2): 297-305. https://doi.org/10.14203/jbi.v12i2.2898

Wulansari A, Purwito A, Sukma D, Ermayanti TM (2021). Growth response of diploid and tetraploid taro (*Colocasia esculenta* [L.] Schott) shoot culture to drought stress using polyethylene glycol. IOP Conference Series: *Earth Environ. Sci*. 913(1). https://doi.org/10.1088/1755-1315/913/1/012016.

Wulansari A, Wulandari DR, Sari L, Ermayanti TM (2017). Pengaruh perlakuan sitokinin terhadap pertumbuhan in vitro talas diploid pontianak dan talas triploid bolang hitam. Prosiding Seminar Nasional 2017 Fakultas Pertanian UMJ, 138-146.

Yun RQA, Dinarti D, Husni A, Kosmiatin M (2021). Callus regeneration and polyploidy induction of *Allium cepa* L var. Bima Brebes using oryzalin. IOP Conference Series: *Earth Environ. Sci*. 948(1). https://doi.org/10.1088/1755-1315/948/1/012043.

Zhang YS, Chen JJ, Cao YM, Duan JX, Cai XD (2020). Induction of tetraploids in 'Red Flash' caladium using colchicine and oryzalin: Morphological, cytological, photosynthetic and chilling tolerance analysis. *Scien. Hortic.* 272(April), 109524. https://doi.org/10.1016/j.scienta.2020.109524.

Zhou J, Guo F, Fu J, Xiao Y, Wu J (2020). In vitro polyploid induction using colchicine for *Zingiber officinale* Roscoe cv. 'Fengtou' ginger. *Plant Cell, Tissue Organ Cult.* 142(1): 87-94. https://doi.org/10.1007/s11240-020-01842-1.