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Phenotypic leaf character of katokkon chili pepper (*Capsicum chinense* Jacq.) result of polyploidization with colchicine

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Phenotypic leaf character of katokkon chili pepper (*Capsicum chinense* Jacq.) result of polyploidization with colchicine

Novitasari[1], R Sjahril[2,3], I R Saleh[3], F Haring[3], K Mantja[3], A Tambung[3], and F Noviany[2]

[1] Master student, Faculty of Agriculture, Universitas Hasanuddin, Jl. Perintis Kemerdekaan,km. 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia.
[2] Laboratory of Plant Bioscience and Reproduction Biotechnology, Department of Agronomy,Faculty of Agriculture, Universitas Hasanuddin, Jl. Perintis Kemerdekaan, km. 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia.
[3] Department of Agronomy, Faculty of Agriculture, Universitas Hasanuddin, Jl. Perintis Kemerdekaan, km. 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia.

Email: rinaldi.sjahril@gmail.com

Abstract. The formation of a new variety of katokkon chili through mutation breeding using colchicine was conducted in this study. This study aimsto determine the effect of the phenotypic leaf characters of katokkon chili pepper resulting from polyploidization with colchicine. The study was conducted at the Malino Horticulture Seed Garden Installation, Tinggimoncong District, Gowa Regency, South Sulawesi at an altitude of 1047 m above sea level. This study used samples of katokkonchili pepper plants that had been induced by colchicine and were analyzed using flow cytometry. The results showed that treatment with 0.10% colchicine concentration and48 hours of soaking time and 0.20% colchicine concentration and 24 and 48 hours of soaking time that is tetraploid detected plant affected on darker leaf color and irregular branching shape compared to wild-type. At the same time, tetraploid plants leaf length, width and thickness did not show significant differences with diploid and mixoploid plants.

1. Introduction

Katokkon chili pepper (*Capsium chinense Jacq.*) is a location-specific superior variety that is only widely cultivated in highland areas such as Tana Toraja and its surroundings [1], when planted at different altitudes or land, there will be a risk of decreased growth and productivity. Efforts that can bemade to overcome this are with plant breeding. Plant breeding is an activity to change the genetic composition of plants that aims to get plants with better properties [2]. There are several breeding methods, one of which is mutation breeding by polyploidization.

Polyploidy in plants will affect plant morphology, such as increased fruit size [3], larger leaf size, wider [4] thicker [5] and darker leaf color [6] compared to diploid plants. The most common type of mutagen used in plants is colchicine because of its efficiency and reliability in inducing polyploid plants [7]. Colchicine ($C_{22}H_{25}O_6N$) is a natural alkaloid extracted from the entire plant *Colchicum autumnale* L. [8]. Colchicine will prevent the formation of spindle threads during mitotic division which will cause chromatids to be difficult to separate resulting in chromosome multiplication which can be cause plant become polyploidy plants at the right concentration and time [9].The use of colchicine in each plant will give a different response depending on the concentration and duration of soaking [10].

Research results have been published on colchicine induction in red chili [11], bird's eye chili [12] and katokkon chili [13] but little information is available regarding the effect of colchicine induction on the phenotypic leaf character of katokkon chili pepper, which is polyploid. Based on this, this research was conducted to determine the response phenotypic leaf character of katokkon chili pepper by colchicine treatment.

2. Materials and method

The research was conducted at the experimental station of the Malino Horticultural Seed Garden Installation, Tinggimoncong District, Gowa Regency, South Sulawesi at an altitude of 1047 m above sea level (S: 5º14'39", E: 119º51'26"). The research was conducted from October 2021 – July 2022.

The research was carried out using a two-factor factorial design, with a randomized block design (RBD) as the environmental design. The first factor was the concentration of colchicine, which consisted of 4 levels, namely: 0.00%, 0.05%, 0.10%, and 0.20%. The second factor is the immersion time, which consists of 4 levels, namely: 6, 12, 24, and 48 hours.

The seeds used in this study seed were obtained from fruit which is the second harvest and has a pattern purple color as a characteristic of the local Toraja katokkon in its immature fruit. Seeds are removed and then selected from empty seeds and placed in a 25% Bayclin solution for 3-5 minutes and rinsed with distilled water. Rinsed using distilled water and then soaked with Dithane fungicide solution for 1-2 minutes and rinsed again with distilled water three times. The seeds were air dried for 2 days. The dried seeds were then arranged on a petri dish that has been filled with wet opaque paper. The moisture of the opaque paper was maintained by adding enough water. Seeds that have sprouted (radicle approximately 3-5 mm) were then treated with colchicine by soaked as immersion time treatments. After treatment, the sprouts were rinsed three times with distilled water and then transferred to the growing media in seedling trays (36 holes) for 1.5 months, then flow cytometry analysis was carried out before being transferred to the bed. Flow cytometry observations were made on 10 plant samples while leaf morphology observations were made on 5 leaf samples for each treatment and replicate. Observation of leaf color using a color chart (Royal Horticulture Society color chart sixth edition, 2015; 2019 reprint). Quantitative observation data were analyzed for variance using Microsoft Excel 2013. The quantitative characters observed included leaf length, width, and thickness,, while the qualitative characters observed were leaf color and crown shape.

3. Results and discussion

The observations on the characters of the length, width and thickness of the leaves of the katokkonchili plant, it showed that the treatment with colchicine concentration and soaking time had no significant effect. The average length, width and thickness of the leaves of the katokkon chili plant can be seen in Table 1.

Table 1 shows that the most extended leaf length was found at 0.00% colchicine concentration and 12 hours of soaking time (k0w2) (10.52 cm), while the shortest leaf length was seen at 0.20% colchicine concentration and 24 hours of soaking time (k3w3) (7.46cm). Then the widest leaf width wasseen at 0.00% colchicine concentration and 48 hours of soaking time (k0w4) (16.46 cm), while the narrowest leaf width was found at 0.20% colchicine concentration and 24 hours of soaking time (k3w3)(4.13 cm). Furthermore, the thickest leaf was seen at 0.00% colchicine concentration and 48 hours soaking time (k0w4) (2.14 mm), while the thinnest leaf was seen at 0.05% colchicine concentration and 24 hours soaking time (k1w4) (1.63 mm). This shows that soaking has not been able to affect the leaf length, leaf width, and leaf thickness of katokkon chili plants. The variation in plants morphological characters is due to random mutagens influence [14], resulting in various surfaces [15]. Polyploid plants generally have more significant vegetative parts, so they are more vigor than diploid ones [16]. However, this effect is not universal because several polyploids are similar to or weaker thantheir diploid parents.

Table 1. The average length, width and thickness of the katokkon chili pepper leaf after poliploidization

Treatment	Parameter		
	Leaf length (cm)	Leaf width (cm)	Leaf thickness (mm)
k0w1	8,86	5,48	1,93
k0w2	10,52	5,58	1,78
k0w3	9,23	4,67	1,95
k0w4	10,10	**6,46**	**2,14**
k1w1	10,04	4,84	2,07
k1w2	10,14	5,05	1,86
k1w3	9,39	4,82	1,86
k1w4	**10,55**	5,80	1,63
k2w1	9,21	4,85	1,80
k2w2	9,84	5,27	1,79
k2w3	8,64	4,55	1,68
k2w4	8,49	4,77	1,76
k3w1	9,27	4,93	1,89
k3w2	10,27	5,14	2,13
k3w3	7,46	4,13	1,73
k3w4	9,04	4,85	1,98
Average	9,44	5,07	1,87
Variant	0,71	0,31	0,02
Std	0,84	0,55	0,15
Significance	ns	ns	ns

Note: k0 (colchicine 0.00%), k1 (colchicine 0.05%), k2 (colchicine 0.10%), k3 (colchicine 0.20%), w1 (6 hours)w2 (12 hours), w3 (24 hours), w4 (48 hours). ns = not significant at the BNT 5% test level. Bolded numbers indicate the treatment that has the highest value on each parameter.

Based on the results of flow cytometry analysis, the treatment of 0.10% colchicine concentration and 48hours of soaking time and 0.20% of colchicine concentration and 24 and 48 hours of soaking time resulted in plants that were detected as mixoploid and tetraploid (data not shown). The growth of plants induced by colchicine, when viewed from the character of the leaf color and the shape of the branches observed visually, differed from the plants that were observed (wild-type). Images of plants detected as diploid, mixoploid and tetraploid can be seen in Figure 1.

Figure 1 shows that plants detected as diploid (A) have moderate olive green B 137 leaf color, mixoploid plants (B) have dark green A 135 and tetraploid plants (C) have dark green A 136 leaf color. Leaf colors of plantsdetected as tetraploid have different colors darker than diploid plants. This indicates that tetraploid plants have more chlorophyll content than diploid plants. Polyploidization in plants will cause the size of plant cells to become larger so that the size of the stomata becomes large, thus the chloroplasts in the guard cells become more numerous and cause darker leaf green color [17] and higher chlorophyll content [18].

The shape of the canopy branching on the katokkon chili pepper shown in Figure 1 shows that diploid plants (A) have regular shapes and are densely branched, different from tetraploid plants (C) which have irregular shapes and sparse branches. This difference is the effect of colchicine induction which causes abnormal plant conditions [19]. The plants appear stunt ed, with fewer leaves and fewer leaf nodes than wild-type [20].

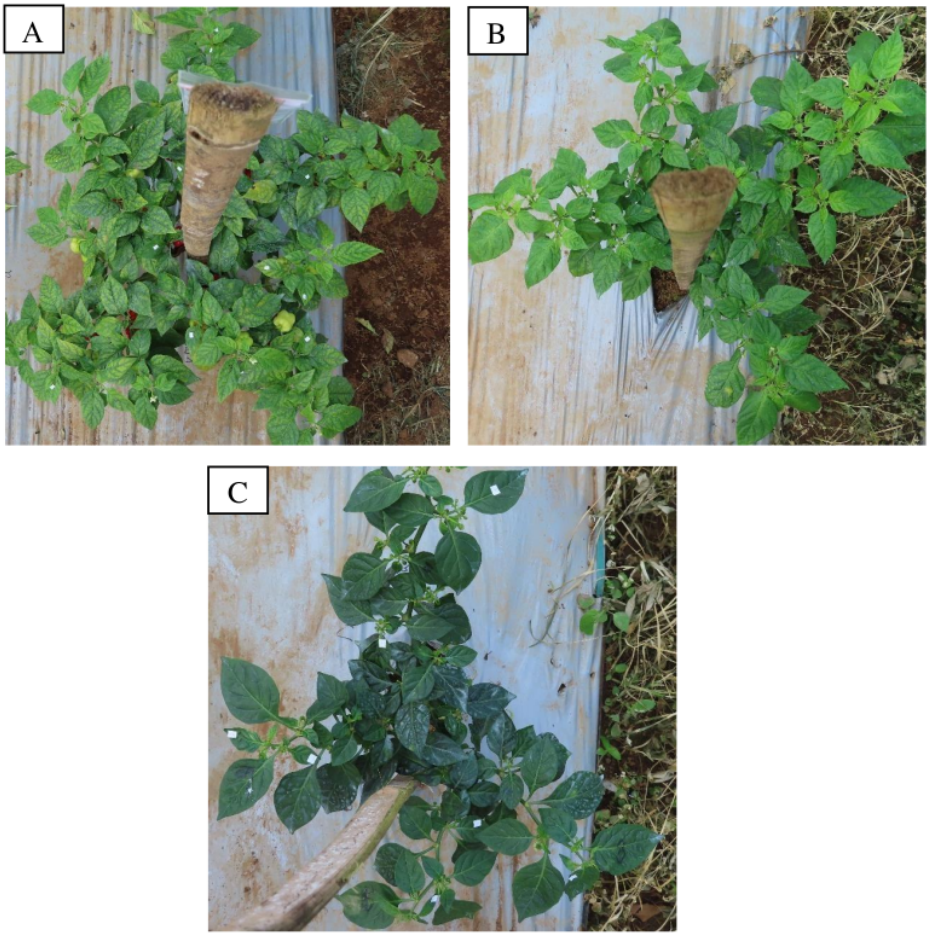

Figure 1. Differences in leaf color and crown design of katokkon chili pepper plants induced bycolchicine (A) diploid (wild-type), (B) mixoploid and (C) Tetraploid

4. Conclusion

Treatment with 0.10% colchicine concentration and 48 hours of soaking time and 0.20% of colchicine concentration and 24 and 48 hours of soaking time affected the phenotypic leaf character of the katokkon chili pepper, which could be seen from the dark green leaf color (Dark green A 136) and the sparse form of branching, while the length, width, and thickness of the leaves showed no significant difference.

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