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Research Article

Polyploidy Induction of Rutaceae through Bio-catharanthine Treatment

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Abstract

Indonesia has a tropical monsoon-type climate which suitable for agriculture and plantations. One of Indonesia's main centres of orange fruits (*Citrus* sp.) production and becoming the top ten with the largest production is in South Sulawesi. Farmers commonly cultivate siam oranges (*Citrus nobilis*). Lime (*Citrus aurantifolia*) has not yet become the main production, inversely proportional to the high demand in South Sulawesi. This study aims to determine the ability of bio-catharanthine as a polyploidy agent against the phenotype of the family Rutaceae cultivated in South Sulawesi. The research was carried out in Laboratory of Genetics and Molecular Biology, Department of Biology, Universitas Islam Negeri Alauddin Makassar using randomized complete block design consisted of two conditions (bio-catharanthine concentration including C1 = Bio-catharanthine 0.05%; C2 = Bio-catharanthine 0.075%; C3 = Bio-catharanthine 0.1% and immersion time including T1 = 3 h; T2 = 6 h). Phenotypic observations include plant height, the number of leaves and nodes, the length of roots, and the number per shoot. The study results showed that the bio-catharanthine immersion of 0.05-0.075% for 6 hours in Rutaceae significantly affected their phenotypic characters.

Keywords: Bio-catharanthine, *Citrus* sp., the phenotype of orange fruit; polyploidy agent

1. Introduction

As an agrarian country, Indonesia is inseparable from the horticultural commodity chain, which aims for the bright prospect, judging from its comparative and competitive advantages in the future recovery of the Indonesian economy (Nasikh et al., 2021; Arifin, 2013; Morgan, 2013). Fruits and vegetables are part of horticultural commodities containing nutritional value (Li et al., 2021; Mostafidi et al., 2020; Liu et al., 2012; Liu, 2013; Vincente et al., 2014). It cannot be synthesised in the human body and a few other species, also unavailable in different types of foods (Baldrick et al., 2011; Vincente et al., 2014; Baiano & Del Nobile, 2016). Fruits are commonly cultivated in Indonesia. Which suitable for tropical monsoon-type climate are members of the family Rutaceae, including siam oranges (*Citrus nobilis*), lime (*Citrus aurantifolia*), kaffir lime (*Citrus hystrix*), lemons (*Limonia acidissima*), grapefruit (*Citrus maxima*), bael (*Aegle marmelos*) and kingkit orange (*Triphasia trifoliata*). The supply and demand for orange fruits have continued to increase from 2015 to 2019 (PDSIP, 2015; Hanif, 2020). In 2019, Indonesia was able to produce 2.444.518 tons of Siam oranges (BPS, 2020). However, this production cannot stop the import value of orange fruits due to the increasing consumption trend since 2019.

The COVID-19 pandemic has increased public awareness of the importance of consuming fruits such as orange fruits.

One of Indonesia's leading centres of orange fruit production and becoming the top ten with the most significant show is in South Sulawesi (PDSIP, 2015). The 13 districts which are the centers of orange fruits production include Bantaeng, Selayar, Bulukumba, Enrekang, Bone, Soppeng, Wajo, Sinjai, Luwu, North Luwu, East Luwu, Gowa, and Sidrap (Sugiyatno, 2015). About 70-80% of the cultivar developed by farmers are still siam oranges. Siam orange is one of the mainstay commodities in North Luwu Regency (planted area of 77.000 ha). Pangkep Regency develops *Pamelo oranges* with four cultivars, including *Gula-Gula*, *Bencong*, *Putih*, and *Merah*. Bantaeng Regency was designated as the base area production of Keprok Batu 55 with a planted area of around 8 ha. *Keprok orange* of Selayar cultivar is developed in Selayar Regency with a planted area of more than 5.800 hectares (PDSIP, 2015; Tahir et al., 2018; BALITJESTRO, 2019).

For the management of *Citrus* cultivation in South Sulawesi, the local farmers still use hereditary methods such as seed propagation and other improper cultivation methods. As a result, many *Citrus* plants die if they are unable to adapt to the environment. The synergy of farmer groups with the government and research institutions in disseminating the innovation of *Citrus* cultivation has a positive impact on increasing fruit production and developing marketing distribution (Subekti et al., 2016; Rugayah et al., 2020). With the technology program to be adopted, the community will preserve the existence of orange varieties and increase the production of existing orange fruits. The expansive land and various cultivars can adapt to dry land, allowing it to be developed on a larger scale.

Polyploidy can increase genetic diversity, phenotypic plasticity, and heterozygosity. Hence it can contribute to the potential for adaptive polyploidy plants that can rapidly develop into new species with improved characters or be used in breeding programs (Lavania, 2013; Soltis et al., 2015; Bourke et al., 2018; Gallego-Tévar et al., 2018; Wei et al., 2019). This study aims to determine the ability of bio-catharanthine as a polyploidy agent against the phenotype of the family Rutaceae cultivated in South Sulawesi. This research describes the first work on polyploidy in some local *Citrus* sp. using bio-catharanthine. It is a market opportunity and an opportunity for the development of local orange fruit with a high diversity of genetic resources, thus supporting the availability and assembly of high-quality varieties.

2. Material and Method

The *Citrus* sp. sample preparation, including *Citrus aurantiifolia* and *Citrus maxima* cv. Gula-Gula (family Rutaceae) was carried out directly at the planting centre in Pangkep Regency, South Sulawesi. The samples are then taken to the Genetics and Molecular Biology Laboratory, Universitas Islam Negeri Alauddin Makassar. The seeds are removed and cleaned. Both *Citrus* sp. seeds were soaked with bio-catharanthine with various concentrations at room temperature, 20-26°C for 3-6 h. Then the germination process is

carried out using the Rockwool hydroponic system. The seeds sprout on the 5th-7th day after sowing.

Field experiments using the hydroponic system factorial (2 x 2) + 1 control arranged in a completely randomized block design, with a concentration bio catharanthine including C1 = bio-catharanthine 0.05%; C2 = bio-catharanthine; 0.075%; C3 = bio-catharanthine 0.1% and immersion time T1 = 3 h and T2 = 6 h (Aristya & Daryono, 2014; Muarifin & Daryono, 2015; Rosmaiti & Dani, 2015). Control was both of *Citrus* sp. without immersion in bio-catharanthine solution. The treatments were repeated three times, each treatment consisting of three plants.

Both *Citrus* sp. seeds were soaked in a solution of C1, C2, and C3 for 3-6 h. The solution is also sprayed on the shoots and young leaves every day for one week after planting. Furthermore, the solution is sprayed once a week. Phenotypic variables observed 12 weeks after planting were plant height, the number of leaves and nodes, the length of roots, and the number of roots per shoot. Phenotype data as quantitative data were analysed by ANOVA ($P<0.05$). In addition, the mean difference test between treatments was also carried out using Tukey's HSD in IBM SPSS ver. 25.0.

3. Results and Discussion

3.1. Results

In general, the results showed that the bio-catharanthine induced changes in the phenotype of both *Citrus* sp. including plant height, the number of leaves, the number of nodes, the length of roots, and the number of roots per shoot. This phenotype affects the changes in the chromosome number of both *Citrus* sp. T1 treatment did not show significant results, while T2 treatment is as follows in Table 1 and Table 2.

Table 1. The concentration variation of bio-catharanthine for six h on the phenotype growth of *Citrus aurantiifolia* at six weeks after planting.

Bio-catharanthine concentration (%)	Plant height	Number of leaves	Number of nodes	Length of roots	Number of roots per shoot
0	4.2a	3.8a	3.8a	1.3	4.5a
0.05	5.0b	4.9b	4.7b	1.0	3.0b
0.075	3.5c	2.4c	2.1c	1.0	2.8c
0.1	2.9c	1.5d	1.6c	1.0	1.0d

Note: Values followed by the same letter in the same column show results that are not significantly different based on the Duncan Multiple Range Test at α = 5%.

Table 1 shows the data on five phenotypes of *C. aurantifolia.* Two concentration variations (0.075% & 0.1%) resulted in a decrease in plant height, number of leaves, number of books, number of roots, and root length compared to the control.

Table 2. The concentration variation of bio-catharanthine for six h on the phenotype growth of *Citrus maxima* cv. Gula-Gula at six weeks after planting.

Bio-catharanthine concentration (%)	Plant height	Number of leaves	Number of nodes	Length of roots	Number of roots per shoot
0	6.7a	3.7a	2.6a	1.0	6.5a
0.05	7.1b	4.5b	3.6b	1.0	5.7b
0.075	6.3c	2.2c	2.1c	1.0	3.2c
0.1	3.9c	1.9c	1.5c	1.0	2.0d

Note: Values followed by the same letter in the same column show results that are not significantly different based on the Duncan Multiple Range Test at α = 5%.

Table 2 shows the data on five phenotypes of *C. maxima* cv. Gula-Gula. Only one concentration variations (0.05%) resulted in an increase in plant height, number of leaves, number of books, number of roots, and root length compared to the control.

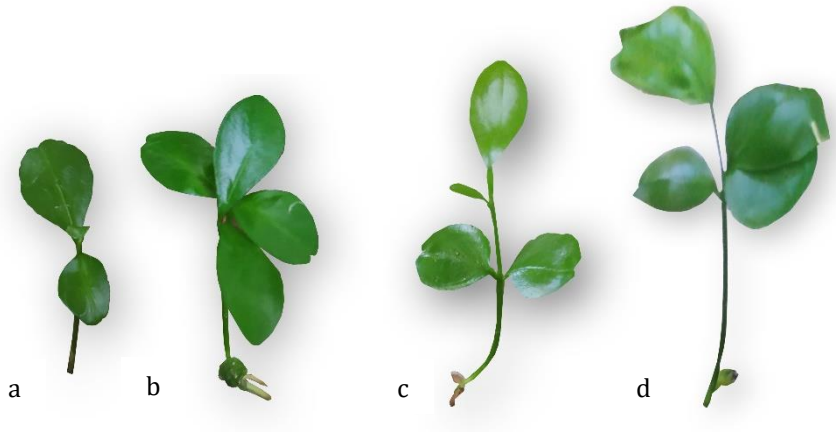

Figure 1. The phenotype of family Rutaceae induced by bio-catharanthine (a), *Citrus aurantiifolia* in control without the bio-catharanthine (b) *Citrus aurantiifolia* induced by 0.05% bio-catharanthine for 6 hours (c) *Citrus maxima* cv. Gula-Gula with no induction (d) *Citrus maxima* cv. Gula-Gula induced by 0.05% bio-catharanthine for 6 hours.

3.2. Discussion

Bio-catharanthine is an innovative product of a chromosome multiplying agent obtained from extracts of *Catharanthus roseus* developed by the Faculty of Biology, Universitas Gadjah Mada. *Catharanthus roseus* has several compounds such as vincristine, vindoline, vinblastine, and catharanthine used as antimitotic agents (Liu et al., 2014; Karimi & Raofie, 2019); it can multiply chromosomes (Begum, 2011; Moudi et al., 2013; Dall'Acqua, 2014; Alam et al., 2017). Its application produces polyploid plants with more extensive and robust roots, stems, leaves, flowers, fruit sizes, more resistance to pathogens and drought, and higher production.

The treatment of bio-catharanthine concentration had a significant effect on the increase in plant height, number of leaves, number of nodes, and number of roots per

shoot, but on the contrary, on the length of root both *Citrus aurantiifolia* and *Citrus maxima* cv. Gula-Gula at six weeks after planting (Figure 1).

Artificial polyploidy induction using bio-catharanthine provides growth variations in four phenotypes of *C. aurantiifolia* and *C. maxima* cv. Gula-Gula (Tables 1 & 2). The induction, which increased in plant size, was a concentration of 0.05% compared to the control. The increase in size did not apply to the other two concentrations. The results obtained from this study are in line with two previous studies that also used bio-catharanthine. Muarifin & Daryono (2015) showed an increase in size on the phenotype and ploidy of *Arachis hypogaea* L. 'Talam,' and Yuniasih & Maryani (2011), who examined Cultivar Melodi Gama-1 was polyploids with bio-catharanthine.

The plant organ size decreased after the bio-catharanthine concentration was added over 0.05% for six h (Tables 1 & 2). In contrast to previous studies by Aleza et al. (2009), a concentration of 0.1% colchicine for three h was obtained to induce the polymerisation of *Citrus clementina*. The increase in organ size is strongly affected by the amount of concentration applied and the appropriate length of immersion time. The lethality or survival percentage of mitotic agent-treated plants could inform each plant's optimum concentration and immersion duration (Eng & Ho, 2019). Sensitivity to mitotic agents could differ between plant species.

Bio-catharanthine containing vinca alkaloid compounds has a certain way of working on its effectiveness as an antimitotic and less toxic alternative to colchicine. Yulianti et al. (2015), showed *Citrus nobilis* cv. Simadu required 0.1% colchicine to produce twice as many chloroplasts as the control shoots. In our report, the bio-catharanthine in 0.05% was most effective for polyploidisation, lower than colchicine. We also found the obovate petiole wing shape on *Citrus maxima* cv. Gula-Gula considered mutations due to bio-catharanthine induction, some of which were not found in control. Yasin et al. (2017), also found the differences in the leaves character of *Citrus nobilis* cv. Pontianak as 0.15% colchicine induction results. The leaves showed a junction between the petiole and lamina.

The main mechanism of vinca alkaloid cytotoxicity is its interaction with tubulin and microtubule dysfunction (Moudi et al., 2013; Zhang et al., 2017). The presence of vinca alkaloids as tubulin modulators will inhibit polymerisation by binding to α/β tubulin protein. Thus the microtubule congregation interrupts, and microtubules fail to function normally (Sertel et al., 2011; Di Cesare et al., 2017; Quan et al., 2019). The formation of the division spindle will be inhibited. The doubling-chromosomes are not carried in the opposite direction; hence polyploid cells are formed. The disruptive effect of the vinca alkaloids of bio-catharanthine on microtubule dynamics, especially at the tip of the mitotic spindle, only occurs at the correct concentration capable of decreasing microtubule mass. Polyploidy in *Citrus aurantiifolia* and *Citrus maxima* cv Gula-Gula works effectively at a concentration of 0.05% bio-catharanthine induction.

Polyploidy made deliberately through the induction process assisted by bio-catharanthine will produce morphological characters of polyploid plants that are

different from their parents, such as larger cell size, thicker leaves, faster growth, and more vigorous, as well as more improved chemical composition. The critical improvement of this study was the assessment of efficient methods of utilising polyploids, improving the varieties, and increasing the genetic diversity of *Citrus* sp.

Conclusion

Bio-catharanthine immersion for 6 hours with the concentration of 0.05%, 0.075%, and 0.1% had a significant effect on the phenotypic properties of *Citrus aurantiifolia* and *Citrus maxima* cv. Gula-Gula (Rutaceae). A concentration of 0.05% indicates a larger phenotype character.

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