Asian Journal of Advances in Agricultural Research

Volume 20, Issue 2, Page 7-17, 2022; Article no.AJAAR.94151
ISSN: 2456-8864

The Effect of Colchicine on Profiles and Contents of Withanolides of Ciplukan (*Physalis angulata*) *In vitro*

Retno Mastuti [a*], Aminatun Munawarti [a] and Mufidah Afiyanti [a]

[a] *Biology Study Program, Faculty of Mathematics and Natural Sciences, Brawijaya University, Jl. Veteran, Malang 65145, Indonesia.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJAAR/2022/v20i2392

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/94151

Original Research Article

Received: 01/10/2022
Accepted: 03/12/2022
Published: 12/12/2022

ABSTRACT

This study aimed to determine the effect of in vitro polyploid induction using colchicine on the content of withanolides in *Physalis angulata* shoots. Polyploidy induction used two types of tissue, namely seeds from in vivo ripe fruit and in vitro cotyledonary nodes. Each tissue type was soaked in 0.1% colchicine for 0, 24, 48 and 72 hours. Seeds that had been soaked in colchicine were germinated in vitro and the cotyledonary nodes of seedling were used as explants for shoot induction. Meanwhile, the cotyledonary nodes that had been soaked in colchicine were immediately cultured on shoot induction medium. Media for shoot induction was MS + BAP 2 mg/L + IAA 0.05 mg/L. The shoots derived from multiplication were used for the analysis of withanolides content using the HPLC method. The results showed that a total of 38 withanolides compounds were detected in the retention time 21.6 to 36.8 minutes. Soaked the seeds in colchicine solution was more effective in increasing the withanolides content than the cotyledonary node tissues. All exposure time in colchicine only affected the intensity but had no effect on the profile of the withanolides.

**Corresponding author: Email: mastuti7@ub.ac.id;*

Keywords: Colchicine; HPLC; in vitro; Physalis angulata; withanolide.

1. INTRODUCTION

Physalis spp. is an annual herb belonging to the Solanaceae family. This plant is often found growing wild as a weed in moist and fertile land. Members of the Physalis genus can live in the lowlands to plains with an altitude of about 1,500 m above sea level. In Indonesia, ciplukan plants which are members of the Physalis genus are widely found in several areas such as Java (Sundanese: cecenet or cecendet; Seram: lapinonat), Madura (nyurnyuran or yor-yoran), Bali (Angket, Kepok-kepokan, Keceplokan), Minahasa (Leletokan) and Lombok (Sasak: Dedes).

Ciplukan (*P. angulata*) in addition to producing sweet fruit is also known as a medicinal plant. Physalis genus have several species use to treat dermatitis, hepatitis, asthma, cancer, kidney and liver disorders, bacterial infections, and has immunomodulatory antipyretic properties [1-3]. The medicinal properties are mainly due to the presence of compounds of withanolide group. Some withanolides and four acetylated derivatives showed potent cytotoxicity against normal fetal fibroblast (MRC-5) cells, human head and melanoma (B16F10 and SKMEL-28), neck squamous, and cell carcinoma (JMAR and MDA-1986) [4,5] Withanolides are mainly synthesized by member of Solanaceae family, including Physalis. These compounds are a group of polyoxygenated steroids based on the ergostan skeleton and primarily in the aglycone form. There are many withanolides have been isolated from Physalis [6]. Genus Physalis also synthesize physalin, the δ-lactone/lactol type, which have different modified skeletons [5,7].

Efforts to improve the physiological and biochemical characters of medicinal plants lead to an increase in the quantity and quality of secondary metabolites with medicinal properties [8]. Improvement of this character can be done in in vitro system with artificial polyploidy induction [9]. Polyploidy is a condition in which a cell or organism has more than one pair of chromosomes (diploid). An increase in the number of chromosomes and additional genomic interactions and genetic changes often results in polyploid plants that have superior characters compared to their diploid plants [10,11]. This makes polyploidization a credible approach for crop improvement [12].

Medicinal plants with duplicates of the entire chromosome set often have more distinctive characteristics such as a modified phytochemical profile and a higher content of the desired pharmaceutical molecule [13]. Therefore, the induction of chromosome doubling in medicinal plants have significant economic consequences [14]. Polyploidy induction has been reported to be beneficial for increasing the medicinal value of various medicinal plants, including the tetraploid plant *Sophora tonkinensis* [15], *Trollius chinensis* Bunge [16], *Zingiber officinale* Roscoe [17], and *Bletilla striata* (Thunb.) Reichb.f. [18].

In vitro regeneration systems provide facilities for manipulating ploidy in breeding and developing new plants [19]. In vitro ploidy manipulation is usually induced using antimitotic agents such as colchicine [20-22], oryzalin [23,24] and trifluralin [25,26]. The success of polyploidy induction is influenced not only by the duration of exposure and the concentration of antimitotic agents, but also by the type of tissue, and the interaction with basal media and plant growth regulators. In vitro conditions that are specific to each individual at the taxa, genera, species, or cultivar level, often have specific needs to maximize polyploid induction. Effect of colchicine has been studied on plant growth and floral behavior in cape gooseberry (*P. peruviana* L.) (Kumar et al., 2019). Polyploidization using nodal segments of *P. alkekengi* var. Franchettii showed that MS medium supplemented with 0.08% colchicine for 24 h resulted tetraploides plant [27]. However, until now no one has reported the effect of colchicine on the profile and content of withanolides especially in Physalis plants. Therefore, this study aimed to observe the effect of colchicine treatment under in vitro conditions on the content of withanolides in in vitro regenerated *P. angulata* shoots.

2. MATERIALS AND METHODS

2.1 Explant Preparation

Seeds were obtained from the ripe fruit of the *P. angulata* plant which grows wild in the corn fields of the South Malang area. After the seeds were dried, they were then sterilized in a 20% commercial bleach solution for 15 minutes and rinsed with sterile distilled water for 5 minutes three times. The sterilized seeds were ready to be germinated and used for colchicine treatment.

2.2 Colchicine Application in In Vitro Systems

Colchicine application was carried out on two different types of tissue, namely 1) seeds that will germinate to produce seedling as explant sources and 2) cotyledonary node explants derived from in vitro seedling that were ready to be cultured on shoot induction medium.

2.2.1 Soaking the seeds in a colchicine solution

Seeds that have been surface sterilized were soaked in 0.1% colchicine solution for 24, 48 and 72 hours. Seeds without soaking in colchicine solution were used as control. In vitro seed germination was undertaken in a water medium solidified with agar without adding nutrients and plant growth regulators (PGR). Each treatment in colchicine was repeated five times (culture bottles). Cotyledon nodes from two weeks old seedling of control and colchicine treatment were subcultured into shoot induction medium (MS + BAP 2 mg/L + IAA 0.05 mg/L). Two weeks later the regenerated shoots clumps were separated and subcultured into the same medium for shoot multiplication. The multiplicated shoots were ready to be analyzed for withanolides profile and content using the HPLC method.

2.2.2 Soaking cotyledon node explants in colchicine solution

The process of seed germination to produce seedling was the same as described in the colchicine treatment of seeds. Cotyledonary nodes of seedling were excised from 2 weeks old seedling and then soaked in 0.1% colchicine. The exposure time and number of repetitions in colchicine treatment were the same as in the seeds treatment. Subsequently, shoot induction and identification of withanolides were carried out the same as in the seed soaking treatment.

2.3 Analysis of Withanolide Content by HPLC Method

Analysis of withanolide profiles begins with phytochemical screening and identification of withanolides using LC-MS [28].

2.3.1 Preparation of in vitro shoot

Shoot culture which were prepared for HPLC analysis consisted of seven types of samples, namely control shoots, shoots derived from soaking seeds for 24, 48 and 72 hours and shoots derived from soaking the cotyledon nodes for 24, 48 and 72 hours. In vitro shoots of 0.1 g were ground with mortar and pestle, then 10 mL of methanol p.a was added, stirred until homogeneous and allowed to stand for 30 minutes. The solution was sonicated with a sonicator (Laboratory Ultrasonic Homogenizer sino sonics) for 45 minutes at room temperature, filtered using a vacuum filter (Pyrex vacuum filter) and then concentrated using a rotary evaporator. The concentrated extract was redissolved with 4 mL of methanol, then homogenized with a sonicator for 45 minutes at room temperature. The solution was concentrated using a rotary evaporator. The concentrated extract was redissolved with 10 mL of methanol, then filtered to obtain the sample solution filtrate.

2.3.2 Measuring withanolide levels by HPLC

HPLC analysis refers to [29] with modifications and carried out with the SPD M20-Photo Diode Array Detector. Samples were analyzed at 35°C in Shim-pack VP ODS 5 Column DSm, 150 x 4.6 mm. The mobile phase using the isocratic method uses acetonitrile and water in a ratio of 60:40. The sample solution filtrate and the mobile phase (eluent) were filtered with a Polytetrafluoroethylene (PTFE) membrane and degassing was carried out. The sample solution was filtered again with a cellulose nitrate membrane. The standard of withanolides was compound library based on the result of LC-MS analysis (Shimadzu). The analysis was carried out at a wavelength of 215 nm with a flow rate of 1 mL/min for 50 minutes. The withanolides content was calculated based on the standard curve obtained from the internal standard through the LC-MS/HPLC (Shimadzu) program using Labsolution software. The results of running HPLC for each sample were in the form of a chromatogram, retention time (minutes) for each peak, and the name of withanolides type for each peak and the levels of withanolides per fesh weight (FW).

2.4 Data Analysis

The levels of withanolides were tabulated based on the type of tissue (seeds and cotyledonary nodes) and the exposure time. The percentage of withanolides based on their concentration and the average increase or decrease in withanolides levels was calculated by MS Excel 365. Changes

in the levels of withanolides in shoots in vitro without and with colchicine treatment were calculated by the following formula:

$$Xn_i = \left(\frac{bn_i - a_i}{a_i}\right) \times 100\%$$

Notes: a= levels of withanolides in in vitro shoots control (without soaking in 0.1% colchicine solution); b = concentration of withanolides in in vitro shoots soaked in 0.1% colchicine, n = exposure time 24, 48 and 72 hours, X = change in concentration of withanolides, i = type of withanolides 1, 2, …, n.

3. RESULTS AND DISCUSSION

3.1 Withanolide Intensity in *In Vitro P. angulata* Shoot Derived from Seeds Treated with Colchicine

All seeds soaked in 0.1% colchicine at various exposure times were able to regenerate shoots. Chromatographic analysis using the HPLC method identified 38 peaks of withanolides on shoot derived from seeds which were soaked or not soaked in 0.1% colchicine (Fig. 1). The retention time to elicit withanolides was from 21,6 to 36,8 minutes. Shoots derived from seeds soaked in 0.1% colchicine at all exposure times showed higher intensity of absorbances (milli Absorbance-unit) (mAU) rather than control. The seeds that are not soaked in colchicine regenerated shoots which had the lowest intensity of absorbance (Fig. 1A). Meanwhile, seeds soaked in colchicine for 24 hours (Fig. 1B) and 48 hours (Fig. 1C) produced shoots that had higher intensity of absorbance. However, the intensity of absorbance decreased when the soaking seeds was prolonged up to 72 hours (Fig. 1D). Shoots derived from the seeds after soaking in 0.1% colchicine for 48 h produced the highest intensity of absorbance compared to the control and other exposure time.

3.2 Withanolide Intensity in *In vitro P. angulata* Shoot Derived from Cotyledonary Nodes Treated with Colchicine

Cotyledonary node explants that soaked in 0.1% colchicine also showed capability of shoot regeneration. HPLC analysis identified the similar number and types of withanolides with the same retention time. However, they showed a different trend of intensity of absorbance (mAU). Shoots derived from soaking cotyledonary nodes for 24 hours (Fig. 2B) produced the highest intensity of absorbance of withanolides compounds compared to control (Fig. 2A) and soaking the cotyledonary nodes for 48 h (Fig. 2C) and 72 h (Fig. 2D). In addition, the cotyledonary nodes soaked for 72 hours even had lower intensity of absorbance (mAU) of withanolides compounds than the control without soaking in colchicine.

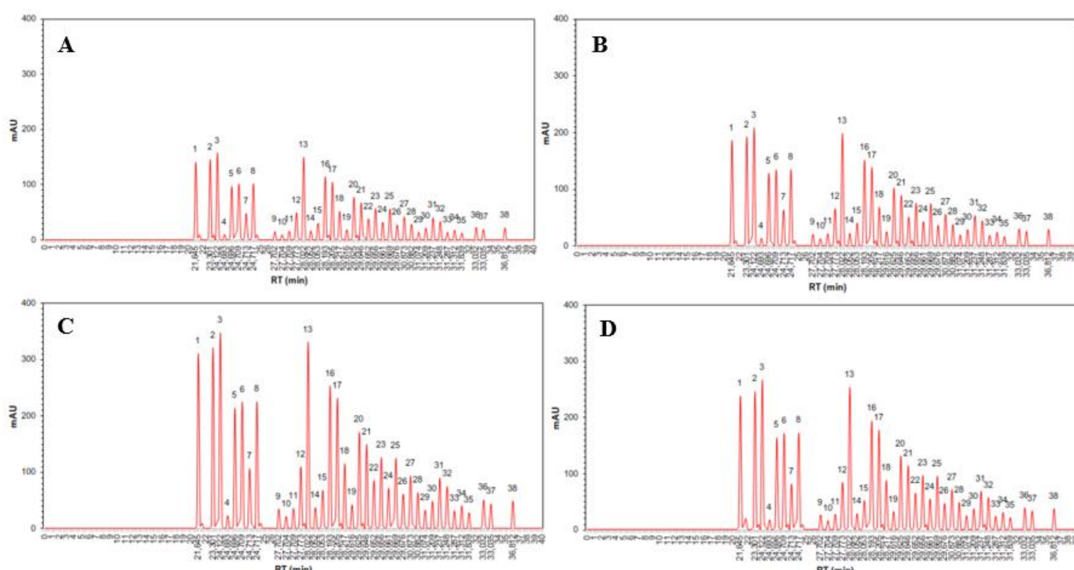


Fig. 1. Chromatograms profile of withanolides on Physalis in vitro shoots derived from soaking the seeds in 0.1% colchicine solution using the HPLC method. A. without soaking (control), B. soaking for 24 h, C. for 48 h, D. for 72 h

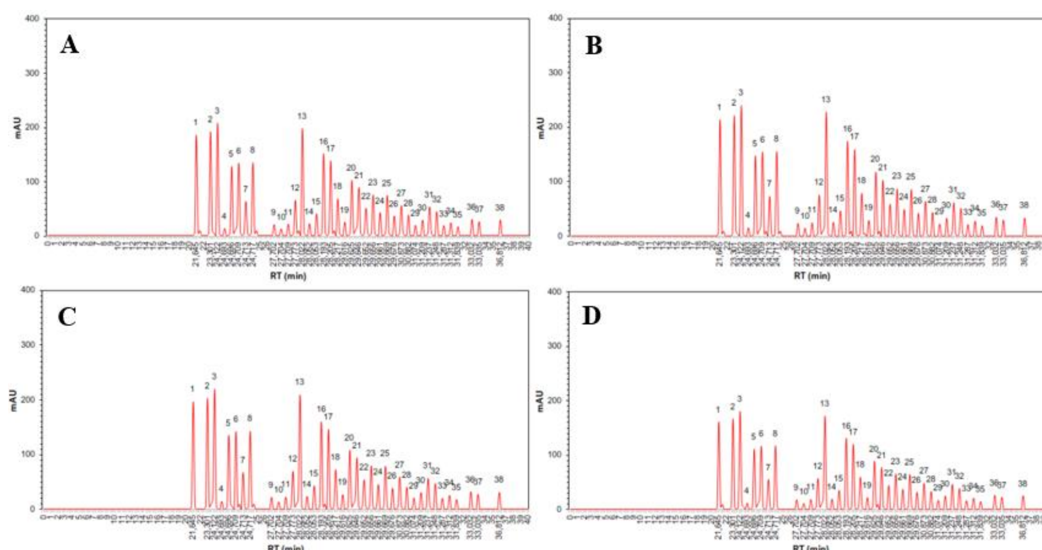


Fig. 2. Chromatograms profile of withanolides on Physalis in vitro shoots derived from soaking cotyledonary node explants in 0.1% colchicine solution using the HPLC method. A. without soaking (control), B. soaking for 24 h, C. for 48 h, D. for 72 h

Table 1. Diversity of withanolides isolated from *P. angulata* shoots in vitro

No	Group	Number of types	Type
1	hydroxywithanolide	3	18-hydroxywithanolide D 28-hydroxywithanolide 4B-hidroksiwithanolid E
2	dihydrowithanolide	2	2,3- dihydrowithanolide E** dihydrowithanolide E
3	phyperunolide	2	C, D
4	physagulin	4	D, L, M, N
5	physalin	9	A, B, D, E, F, G, H, I, J
6	Physanolide	1	A
7	withaferin	1	A
8	withangulatin	9	A, B, C, D, E, F, G, H, I
9	withanolide	4	A, B, D, E
10	Withanone	1	Withanone
11	withaphysalin	1	A*
12	withaphysanolide	1	A
Total		38	

These results indicated that soaking of seeds and cotyledon nodes in 0.1% colchicine for several exposure times had no effect on the profile of the synthesized whitanolides. The number and type of withanolides detected in control and treatment shoots did not differ. However, the different of exposure time affected the intensity of the absorbance of the withanolides.

3.3 Colchicine Effect on Withanolides Profile of *in vitro* Shoot of *P. angulata*

All types of 38 peaks compounds isolated from *P. angulata* shoots in vitro can be classified into 12 groups (Table 1). The diversity of withanolides showed that physalin and withangulatin compounds have the most types, as many as 9. Meanwhile, the only one type of withanolides were phyasnolid, withaferin, withanone and withaphysalin.

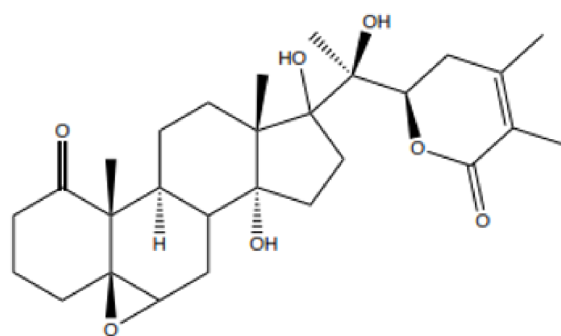
The contents of withanolides in the shoots of *P. angulata* in vitro without colchicine treatment ranged from 6.64 - 242.90 ug/g which the lowest and highest concentration was 2,3 dihydrowithanolide E and withaphysalin A, respectively (Table 2). Soaking seeds and cotyledonary nodes in colchicine generally regenerated shoots in vitro with increasing

Table 2. Effect of colchicine treatment on withanolides content

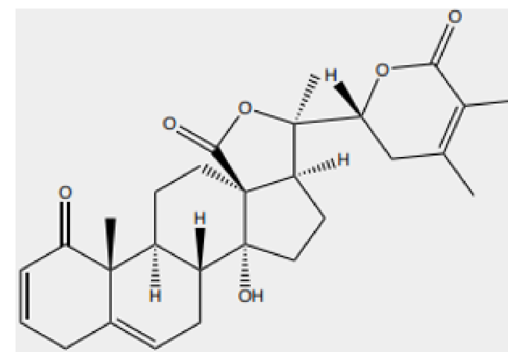
Peak No	Retention time (min)	Whitanolide compounds	Control (ug/g DW)	Increasing of withanolide content in *P. angulata* shoots in vitro (µg/g DW)					
				Soaking seeds in 0.1% colchicine solution (hours)[1)]			Soaking cotyledonary node explants in 0.1% colchicine solution (hours)[2)]		
				24	48	72	24	48	72
1	21.645	withaphysanolide A	216.45	16.15	91.42	47.53	36.00	21.93	-2.84
2	23.301	withanolide B	223.40	16.12	91.25	47.45	35.94	21.89	-2.84
3	24.122	withaphysalin A*	242.90	16.10	91.07	47.36	35.87	21.85	-2.84
4	24.693	withanone	7.89	31.56	188.45	97.75	71.10	44.40	-4.27
5	24.696	withanolide A	145.67	16.45	93.28	48.50	36.67	22.36	-2.87
6	24.709	withaferin A	154.14	16.36	92.72	48.21	36.47	22.23	-2.86
7	24.713	withanolide D	68.39	17.44	99.52	51.73	38.93	23.81	-2.96
8	24.717	withangulatin F	154.97	16.38	92.88	48.29	36.53	22.27	-2.86
9	27.702	18-hydroxywithanolide D	15.98	24.10	141.47	73.44	54.10	33.52	-3.58
10	27.704	2.3-dihydrowithanolide E**	6.64	35.71	214.58	111.27	80.55	50.45	-4.66
11	27.709	withanolide E	17.08	23.23	136.00	70.60	52.12	32.25	-3.50
12	27.773	dihydrowithanolide E	71.00	17.40	99.27	51.60	38.84	23.75	-2.96
13	28.022	physanolide A	231.83	16.13	91.26	47.46	35.94	21.90	-2.84
14	28.052	28-hydroxywithanolide	17.68	23.32	136.53	70.88	52.31	32.38	-3.51
15	28.053	4β-hydroxywithanolide E	40.04	18.82	108.24	56.24	42.08	25.83	-3.09
16	28.193	physalin B	174.73	16.33	92.52	48.11	36.40	22.19	-2.86
17	28.205	withangulatin B	158.57	16.39	92.89	48.30	36.53	22.27	-2.86
18	28.217	withangulatin E	74.83	17.29	98.61	51.26	38.60	23.60	-2.95
19	29.616	phyperunolide D	21.41	21.78	126.84	65.87	48.81	30.13	-3.36
20	29.625	physalin A	115.78	16.67	94.66	49.22	37.17	22.68	-2.89
21	29.646	withangulatin I	99.14	16.88	96.02	49.92	37.66	23.00	-2.91
22	29.652	physalin F	52.93	18.08	103.54	53.81	40.38	24.74	-3.02
23	29.656	physalin G	82.18	17.25	98.31	51.10	38.49	23.53	-2.94
24	29.661	physalin J	42.80	18.75	107.80	56.01	41.92	25.72	-3.08
25	29.669	withangulatin A	81.13	17.17	97.82	50.85	38.31	23.41	-2.93
26	29.676	physagulin M	35.52	19.33	111.46	57.91	43.25	26.57	-3.14
27	30.873	withangulatin C	58.79	17.79	101.76	52.89	39.74	24.33	-2.99
28	30.882	withangulatin H	37.52	19.02	109.47	56.88	42.53	26.11	-3.11

Peak No	Retention time (min)	Whitanolide compounds	Control (ug/g DW)	Increasing of withanolide content in *P. angulata* shoots in vitro (µg/g DW)					
				Soaking seeds in 0.1% colchicine solution (hours)[1)]			Soaking cotyledonary node explants in 0.1% colchicine solution (hours)[2)]		
				24	**48**	**72**	**24**	**48**	**72**
29	31.074	withangulatin G	14.55	24.80	145.86	75.71	55.69	34.54	-3.64
30	31.209	phyperunolide C	26.17	20.73	120.25	62.45	46.43	28.61	-3.27
31	31.237	physalin I	56.51	17.86	102.16	53.10	39.88	24.42	-3.00
32	31.248	physagulin N	44.92	18.61	106.87	55.53	41.59	25.51	-3.07
33	31.287	physalin D	14.04	24.80	145.90	75.73	55.71	34.55	-3.64
34	31.312	physalin E	20.40	22.14	129.12	67.04	49.63	30.66	-3.40
35	31.839	withangulatin D	11.27	27.53	163.06	84.61	61.91	38.52	-3.90
36	33.032	physagulin L	28.06	20.06	116.01	60.26	44.89	27.63	-3.20
37	33.035	physalin H	22.48	21.47	124.88	64.85	48.10	29.68	-3.33
38	36.812	physagulin D	26.57	20.60	119.45	62.04	46.14	28.42	-3.25

*Notes: *withanolides compounds with the highest content; ** withanolides with the lowest content*



2.3-dihydrowithanolide E



Withaphysalin A

Fig. 3. The types of withanolides with the highest (left) and the lowest (right) content

withanolides content. However, the pattern of increasing in the two of them is different. Soaking the seeds for 24 hours increased the content of withanolides the least. Soaking for 48 hours can produce compounds withanolides about five times more. However, the addition of exposure time up to 72 hours increased withanolides only three times more than soaking for 24 hours. This shows that soaking seeds in 0.1% colchicine solution for 48 hours is the optimal exposure time to produce the highest content of withanolides.

On the other hand, the highest increasing in the content of withanolides occurred when the cotyledonary nodes were soaked for 24 hours. The longer the soaking, the less withanolides levels increased. Even at 72 hours of soaking, the content of withanolides were less than the control, which was indicated by a negative value. This indicates that soaking the cotyledonary node for a long-time result in impaired the withanolides synthesis..

The results show that colchicine treatment in different tissues affected the levels of withanolide content. Colchicine treatment in seed tissue was able to provide higher changes in withanolide content than cotyledonary node tissue (Table 2). Among the 38 withanolides detected by HPLC 2.3-dihydrowithanolide E was synthesized in the highest amount, while withaphysalin A (Fig. 3) was synthesized in the least amount. This condition consistently occurred in both control and treatment shoots.

In this study, seed germination and shoot regeneration were not inhibited by colchicine treatment. According to [30], colchicine treatment of seeds is the most effective method to produce tetraploids. Colchicine treatment of seeds had little effect on germination [31]. Smaller seeds absorb more colchicine solution in a shorter time (surface area to volume ratio). However, colchicine treatment of 0.05 – 0.1% on soybean seeds reduced the germination rate [32]. According to [33], plant tissues or organs have different sensitivity to antimitotic agents. The difference in the effect of colchicine on different types of explants is due to the survival rate of the explants influenced by the permeability of the explant tissue and the ability to transport antimitotic agents [34].

Variations in exposure time also affected the content of withanolides synthesized in shoots from soaking seeds and cotyledonary nodes. In vitro shoots from seeds that were soaked for 48 hours produced the highest levels of withanolides, which ranged from 16.31 – 250.16 ug/g DW. The increasing of exposure time up to 72 h decreased the withanolides content (9.45 – 143.98 ug/g DW) although it was still higher than the withanolides content in shoots from cotyledonary nodes that were soaked for 24 h (4.43 – 68.5 ug/g DW).

Ploidy manipulation using chemical compounds has been widely carried out in vitro. The use of colchicine to induce polyploidy can increase the potential for secondary metabolite production. It has been widely used to produce important compounds in medicinal plants [35]. It was also explained that the induction of polyploidy can affect the physiological and biochemical processes of plants and affect the biosynthetic pathways of primary and secondary metabolites. This change is due to a reduction in the ratio of membrane to the amount of chromatin which leads to an increase in the contact between the chromatin material and the nuclear membrane and an increase in gene activity for each cell. The volume and proliferation rate of tetraploid versus diploid cells were 2 and 1.5, respectively. This is very beneficial in cellular production processes associated with cell surface metabolic activities.

Modification of ploidy is a strategy to increase metabolite production in plants because it can change the quantitative and qualitative patterns of secondary metabolite [36]. Medicinal plants undergoing chromosomal duplication often have a more distinctive phytochemical profile with a higher content of the desired pharmaceutical molecule. Therefore, the induction of medicinal plant chromosome doubling can have significant economic consequences [14].

The increase in the range of colchicine concentrations in the initial seed treatment was not always accompanied by an increase in the content of secondary compounds. This trend was also observed in anthocyanin content and glycyrrhizic acid in callus tissue produced from Licorice seeds [35]. The effect of chromosomal doubling is not always identical for all genes but depends on the genotype and species although it affects genomic activity [37]. This is because colchicine not only affects cell division but spreads through cells, disrupting cellular mechanisms and causing toxicity at high concentrations. Colchicine has an impact on the viscosity of the cytoplasm so that cells cannot function normally [38].

4. CONCLUSION

This study showed that increase the withanolide content in *P. angulata* was successfully provided by in vitro colchicine treatment. However, the content of withanolides was also influenced by the type of tissue and the duration of soaking in the colchicine solution. These results indicate that the in vitro system has the potential to be used to support the availability of herbal medicinal ingredients.

ACKNOWLEDGEMENT

Financial support for this study was provided by the Non-Tax State Revenue Fund (PNBP) Universitas Brawijaya in accordance with the Budget Implementation List (DIPA) Universitas Brawijaya 2021. The authors wish to thank Mufidatur Rosyidah, S.Si., M.Si for in vitro work. Thanks are also due to M. Ariesandy for HLPC analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mirzaee F, Hosseini AS, Askian R, Azadbakht M. Therapeutic activities and phytochemistry of physalis species based on traditional and modern medicine. Research Journal of Pharmacognosy. 2019;6(4):79–96.
   DOI:https://doi.org/DOI:http//doi.org/10.22127/rjp.2019.93529
2. Abdel-Fattah W, El-Bassyouni G. Medicinal functions of physalis fruits for biomedical applications. Austin Journal of Pharmacology and Therapeutics. 2021;9(2):1135.
   Available:www.austinpublishinggroup.com
3. Park HA, Kwon OK, Ryu HW, Min JH, Park MW, Park MH, Paik JH, Choi S, Paryanto I, Yuniato P, Oh SR, Ahn KS, Lee JW. Physalis peruviana L. Inhibits ovalbumin-induced airway inflammation by attenuating the activation of NF-?B and inflammatory molecules. International Journal of Molecular Medicine. 2019;43(4): 1830–1838.
   DOI:https://doi.org/10.3892/ijmm.2019.4110
4. Zhang H, Samadi AK, Gallagher RJ, Araya JJ, Tong X, Day VW, Cohen MS, Kindscher K, Gollapudi R, Timmermann BN. Cytotoxic withanolide constituents of *Physalis longifolia*. J. Nat. Prod. 2011; 74(12):2532–2544.
   DOI:https://doi.org/https://doi.org/10.1021/np200635r
5. Meng Q, Fan J, Liu Z, Xiwen L, Fangbo Z, Yanlin Z, Yi S, Li L, Xia L, Hua Erbing. Cytotoxic withanolides from the whole herb. Molecules. 2019;24(8):1–9.
   DOI:https://doi.org/10.3390/molecules24081608
6. Huang M, He JX, Hu HX, Zhang K, Wang XN, Zhao BB, Lou HX, Ren DM, Shen T. Withanolides from the genus Physalis: a review on their phytochemical and pharmacological aspects. Journal of Pharmacy and Pharmacology. 2020;72(5): 649–669.
   DOI:https://doi.org/10.1111/jphp.13209
7. Sun CP, Qiu CY, Zhao F, Kang N, Chen LX, Qiu F. Physalins V-IX, 16,24-cyclo-13,14-seco withanolides from Physalis angulata and their antiproliferative and anti-inflammatory activities. Scientific Reports. 2017;7(1):1–10.
   DOI:https://doi.org/10.1038/s41598-017-03849-9
8. Ghasemi M, Aelaei M, Akbari-Soltankohi F, Chenar HM, Rad EB. Studies on polyploidy induction for improvement of quality traits in ornamental and medicinal plants. Central Asian Journal of Plant Science Innovation. 2021;2(2):76–90.
   DOI:https://doi.org/10.22034/CAJPSI.2021.02.03
9. Eng WH, Ho WS, Ling KH. In vitro induction and identification of polyploid Neolamarckia cadamba plants by colchicine treatment. PeerJ. 2021;9:1–26.
   DOI:https://doi.org/10.7717/peerj.12399
10. Chen Y, Xu H, He T, Gao R, Guo G, Lu R, Chen Z, Liu C. Comparative analysis of morphology, photosynthetic physiology, and transcriptome between diploid and tetraploid barley derived from microspore culture. Frontiers in Plant Science. 2021;12(February).
    DOI:https://doi.org/10.3389/fpls.2021.626916
11. Cao Q, Zhang X, Gao X, Wang L, Jia G. Effects of ploidy level on the cellular, photochemical and photosynthetic characteristics in Lilium FO hybrids. Plant Physiology and Biochemistry. 2018; 133(October):50–56.

DOI:https://doi.org/10.1016/j.plaphy.2018.10.027

12. Corneillie S, Storme N. De, Rebecca Van Acker JUF, Bruyne M. De, Rycke R. De, Geelen D, Willats WGT, Vanholme B, Boerjan W. Polyploidy affects plant growth and alters cell wall composition. Plant Physiology. 2019;179:74–87. DOI:https://doi.org/doi.org/10.1104/pp.18.00967
13. Gantait S, Mukherjee E. Induced autopolyploidy—a promising approach for enhanced biosynthesis of plant secondary metabolites: an insight. Journal of Genetic Engineering and Biotechnology. 2021;19(4). DOI:https://doi.org/https://doi.org/10.1186/s43141-020-00109-8
14. Niazian M, Nalousi AM. Artificial polyploidy induction for improvement of ornamental and medicinal plants. Plant Cell, Tissue and Organ Culture. 2020;142:447–469. DOI:https://doi.org/10.1007/s11240-020-01888-1
15. Wei KH, Xu JP, Li LX, Cai JY, Miao JH, Li MH. In vitro induction and generation of tetraploid plants of sophora tonkinensis Gapnep. Pharmacogn Mag. 2018;14(54): 149–154. Available:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5909307/#idm140412024341360title
16. Zhang Q, Zhang F, Li B, Zhang L, Shi H. Production of tetraploid plants of trollius chinensis bunge induced by colchicine. Czech J. Genet. Plant Breed. 2016; 52(1):34–38.
17. Prasath D, Nair RR, Babu PA. Effect of colchicine induced tetraploids of ginger (Zingiber officinale Roscoe) on cytology, rhizome morphology, and essential oil content. Journal of Applied Research on Medicinal and Aromatic Plants. 2022; 31(August):100422. DOI:https://doi.org/10.1016/j.jarmap.2022.100422
18. Li M, Ding B, Huang W, Pan J, Ding Z, Jiang F. Induction and characterization of tetraploids from seeds of *Bletilla striata* (Thunb.) Reichb.f. BioMed Research International; 2018. DOI:https://doi.org/10.1155/2018/3246398
19. Touchell DH, Palmer IE, Ranney TG. *In vitro* ploidy manipulation for crop improvement. Front. Plant Sci. 2020;11:1–11. DOI:https://doi.org/doi.org/10.3389/fpls.2020.00722
20. Jeloudar NI, Chamani E, Shokouhian AA, Zakaria RA. Induction and identification of polyploidy by colchicine treatment in *Lilium regale*. Cytologia. 2019;84(3):271–276. DOI:https://doi.org/10.1508/cytologia.84.271
21. Nagat E, Kamla B, Khatab H. Phenotypic and molecular characterization of polyploidy Vicia faba induced by colchicine. GSC Biological and Pharmaceutical Sciences. 2020;11(3):235–243. DOI:https://doi.org/10.30574/gscbps.2020.11.3.0184
22. Bhuvaneswari G, Thirugnanasampandan R, Gogulramnath M. Effect of colchicine induced tetraploidy on morphology, cytology, essential oil composition, gene expression and antioxidant activity of *Citrus limon* (L.) Osbeck. Physiology and Molecular Biology of Plants. 2020;26(2): 271–279. DOI:https://doi.org/10.1007/s12298-019-00718-9
23. Kanchanapoom K, Koarapatchaikul K. In vitro induction of tetraploid plants from callus cultures of diploid bananas (*Musa acuminata*, AA group) “Kluai Leb Mu Nang” and “Kluai Sa.” Euphytica. 2012; 183(1):111–117. DOI:https://doi.org/10.1007/s10681-011-0516-9
24. Deans LE, Palmer IE, Touchell DH, Ranney TG. *In vitro* induction and characterization of polyploid hydrangea macrophylla and *H. serrata*. Hort Science. 2021;56(6):709–715. DOI:https://doi.org/10.21273/HORTSCI15783-21
25. Cheng ZH, Zhou XJ, Khan MA, Su L, Meng HW. In vitro induction of tetraploid garlic with trifluralin. Genetics and Molecular Research : GMR. 2012;11(3): 2620–2628. DOI:https://doi.org/10.4238/2012.July.10.13
26. Alavi J, Maroufi A, Mirzaghaderi G. Trifluralin-mediated polyploidization of fenugreek (*Trigonella feonum-graecum* L.) using in vitro embryo culture. Acta Physiologiae Plantarum. 2022; 44(10):1–11. DOI:https://doi.org/10.1007/s11738-022-03436-0

27. Santos GC, Cardoso FP, Martins AD, Rodrigues FA, Pasqual M, Ossani PC, Luz JMQ, Rezende RALS, Soares JDR. Polyploidy induction in Physalis alkekengi. Bioscience Journal. 2020;36(3):827–835.
DOI:https://doi.org/10.14393/BJ-v36n3a2020-47729
28. Mastuti R, Batoro J, Waluyo B. Pengaruh elisitor kitosan terhadap kandungan withanolid tunas in vitro aksesi tanaman *Physalis angulata* dari Pulau Madura. Jurnal Tumbuhan Obat Indonesia. 2021; 14(1):1–14.
DOI:https://doi.org/10.22435/jtoi.v14i1.4301
29. Cao C, Kindscher K, Gallagher RJ, Zhang H, Timmermann BN. Analysis of major withanolides in *Physalis longifolia* nutt. by HPLC-PDA. Journal of Chromatographic Science. 2015; 53(7):1044–1047.
DOI:https://doi.org/10.1093/chromsci/bmu162
30. Manzoor A, Ahmad T, Bashir MA, Hafiz IA, Silvestri C. Studies on colchicine induced chromosome doubling for enhancement of quality traits in ornamental plants. In Plants. MDPI AG. 2019;8(7).
DOI:https://doi.org/10.3390/plants8070194
31. Kobayashi N, Yamashita S, Ohta K, Hosoki T. Morphological characteristics and their inheritance in colchicine-induced Salvia polyploids. Journal of the Japanese Society for Horticultural Science. 2008; 77(2):186–191.
DOI:https://doi.org/10.2503/jjshs1.77.186
32. Mangena P. In vivo and in vitro application of colchicine on germination and shoot proliferation in soybean [*Glycine max* (L.) Merr.]. Asian Journal of Crop Science. 2020;12(1):34–42.
DOI:https://doi.org/10.3923/ajcs.2020.34.42
33. Sattler MC, Carvalho CR, Clarindo RW. The polyploidy and its key role in plant breeding. Planta. 2016;243:281–296.
DOI:https://doi.org/https://doi.org/10.1007/s00425-015-2450-x
34. Allum JF, Bringloe DH, Roberts AV. Chromosome doubling in a Rosa rugosa Thunb. hybrid by exposure of in vitro nodes to oryzalin: the effects of node length, oryzalin concentration and exposure time. Plant Cell Reports. 2007; 26(11):1977–1984.
DOI:https://doi.org/10.1007/s00299-007-0411-y
35. Bernard F, Moghbel N, Hassannejad S. Treatment of licorice seeds with colchicine: changes in seedling DNA levels and anthocyanin and glycyrrhizic acid contents of derived callus cultures. Natural Product Communications. 2012;7(11):1457–1460.
36. Madani H, Escrich A, Hosseini B, Sanchez-Muñoz R, Khojasteh A, Palazon J. Effect of polyploidy induction on natural metabolite production in medicinal plants. Biomolecules. 2021;11(6).
DOI:https://doi.org/10.3390/biom11060899
37. Lavania UC. Genomic and ploidy manipulation for enhanced production of phyto-pharmaceuticals. Plant Genetic Resources. 2005;3(2):170–177.
DOI:https://doi.org/https://doi.org/10.1079/PGR200576
38. Suliman HH, Asander HS. Influence of colchicine treatment on morphological, physiological and anatomical *Cercis siliquastrum* L. Seedlings growth (The effect of colchicine treatment on the growth, morphological, physiological, and anatomical characteristics of purple seedlings).Journal of Plant Production. 2019;10(8):721–730.
DOI:https://doi.org/10.21608/jpp.2019.58165

© 2022 Mastuti et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/94151