International Journal of Secondary Metabolite
2019, Vol. 6, No. 3, 241–250

https://dx.doi.org/10.21448/ijsm.521498

Published at http://www.ijate.net http://ijsm.ijate.net/en/ Research Article

Effects of Methyl Jasmonate and Putrescine on Tryptanthrin and Indirubin Production in *in vitro* Cultures of *Isatis demiriziana* Mısırdalı

Özgür Karakaş *1

[1] Department of Agricultural Biotechnology, Faculty of Agriculture, Sirnak University, 73300, Idil, Sirnak, Turkey

Abstract: Tryptantrin and indirubin are pharmacologically active compounds used in treatment of disseases such as cancer and Alzheimer's. In this study, we investigated the influences of different concentrations of methyl jasmonate (MeJa) and putrescine (Put) on tryptanthrin and indirubin production in leaf explants and development of *Isatis demiriziana* Mısırdalı grown in vitro. In all media treated with methyl jasmonate, tryptanthrin production in leaves of plantlets showed an increase. The highest increase in tryptanthrin production was observed in solid Murashige-Skoog (MS) medium containing 1.0 mM MeJa (154.026 ± 0.11 µg g-1), about 2.85-fold higher than the control (untreated plantlets) (40.017 ± 0.031 µg g-1). Production of tryptanthrin decreased about 2.56-fold in the leaves of plantlets treated with Put, when compared to control. The highest indirubin production was obtained in the leaves of plantlets grown in the MS medium containing 0.1 mM MeJa (11.274 ± 0.035 µg g-1) but treatments with Put didn't show any positive affect on the indirubin production. Analysis of tryptanthrin and indirubin were performed using high performance liquid chromatography (HPLC).

ARTICLE HISTORY

Received: February 05, 2019
Revised: May 29, 2019
Accepted: August 22, 2019

KEYWORDS

Tryptanthrin,
Indirubin,
Methyl jasmonate,
Putrescine,
Isatis demiriziana

1. INTRODUCTION

Isatis demiriziana Mısırdalı is a biennial member of *Isatis* genus and an endemic to South-Eastern Anatolia Region of Turkey [1]. *Isatis* is herbaceous plants belonging to the family Brassicaceae (Cruciferea). The roots and leaves of *Isatis* have anti-viral, anti-inflammatory and anti-tumor effects and the leaves of these plants are used as a source of indigo (blue dye) [2-5]. Recently, a number of active constitutients have been isolated from plants belonging to the *Isatis* genus mainly *Isatis tinctoria* and *Isatis indigotica* such as terpenoids, phenylpropanoids and alkaloids. The indole alkaloids in *Isatis* have significant biological activities. Among these compounds, many reports are available about indole alkaloids such as indirubin and tryptanthrin [6,7]. Tryptanthrin has a wide range of biological activities and this natural product is a yellow and basic alkaloid. Moreover, tryptanthrin has been suggested as both a component of many dyes and medicinal herbal treatments [8]. Tryptanthrin has anti-cancer [9,10], anti-inflamatory [11] and anti-bacterial effects [12,13]. Indirubin is a red

CONTACT: Özgür KARAKAŞ okarakas80@gmail.com Department of Agricultural Biotechnology, Faculty of Agriculture, Sirnak University, Idil, 73300, Sirnak, Turkey

ISSN-e: 2148-6905 /© IJSM 2019

coloured compound and isomer of indigo. In addition to the dye properties, due to its biological and pharmaceutical activities, indirubin has been used for the treatment of various diseases such as chronic myelocytic leukemia (CML) [14,15] and Alzheimer's [16].
Plant secondary metabolites are of a major importance as raw materials of drugs and dyes. In recent years, these products has gained great attention for their commercial importance and, many of these raw materials are produced from leaves and roots of the plants [17]. *In vitro* plant cultures have potential for large scale production of secondary metabolites. To increase the production of secondary metabolites via *in vitro* plant culture techniques have been used different ways. Among these ways, using of various biotic and abiotic elicitors has been effective to stimulate production of a series of important secondary metabolites [18-20]. Of these elicitors, Jasmonic acid (JA) and MeJa are important signalling agents in terms of promoting morphological and physiological changes in plants [21]. MeJa treatment can give rise to the accumulation of several classes of alkaloids [22], flavonoids and phenolics in some plants grown *in vitro* [23,24]. Polyamines (putrescine, spermine and spermidine) play an important physiological roles in plant growth and development [25]. Put is a precursor and stimulating agent for important alkaloids such as the pyrrolidine alkaloids (nicotine and nomicotine) in tobacco plants, pyrrolizidine alkaloids (retronecine) and some tropane alkaloids (hyoscine, meteloidine and hyoscyamine) possibly phenanthroindolizidines (tylophorine) [26,27].

To the best of our knowledge, this is the first report on the influences of MeJa and Put on tryptanthrin and indirubin production in *Isatis* species grown under *in vitro* conditions. In the current study, it was investigated the influences of different concentrations of MeJa and Put on tryptanth

2. MATERIAL and METHODS

2.1. Plant material and in vitro culture conditions

The mature seeds of *I. demiriziana* were collected in june 2014 from Diyarbakır-Ergani (1482 m above sea level), Turkey. Voucher specimens were deposited at the Herbarium of Dicle University, Faculty of Science (voucher no. DUF-6050). Specimens were identified by Prof. Dr. Ömer SAYA, from the same institution. Specimens were identified by. The seeds were washed thoroughly in tap water for 3 min and surface sterilized by dipping in a 70% ethanol solution for 60 sec, followed by immersion in a 6% sodium hypochlorite (NaOHCl) for 8 min, and then rinsed with sterile distilled water five times. After the sterilization stage, the seeds were placed on MS solid medium [28] supplemented with 0.6% agar and 3% sucrose prior to autoclaving at 1 atm, 121ºC for 20 min. The cultures were incubated under a photoperiod of 16 h light and 8 h darkness in a growth chamber at 25 ± 2 ºC. After 3 weeks initiation of cultures, plantlets produced from the germinated seeds were transferred to solid MS basal medium containing 0.6% agar and 3% sucrose for plant proliferation. Different concentrations of MeJa (0.05, 0.1, 0.25, 0.5 and 1.0 mM) and Put (0.5, 1.0 and 2.0 mM) were applied to three-week-old plantlets.

2.2. Reagents and chemicals

Tryptanthrin (≥98%), indirubin (≥98%), N,N-Dimethylformamide (DMF, ≥99.9%), MeJa (≥95%) and putrescine dihyrochloride (Put≥97%) were purchased from Sigma (St. Louis, Mo, USA). Methanol (≥99.9%) and acetonitrile (ACN, ≥99.9%) were purchased from Merck (Darmstadt, Germany) and trifluoroacetic acid (TFA, ≥99%) was purchased from Merck (Hohenbrunn, Germany).

2.3. Treatment and preparation of MeJa and Put

MeJa and Put were dissolved in methanol and then their stock solutions were prepared as 1 mg mL^{-1} and 5 mg mL^{-1} in methanol, respectively. 3-week-old plantlets were transferred to MS medium containing different concentrations of MeJa (0.05, 0.1, 0.25, 0.5 and 1.0 mM) and Put (0.5, 1.0 and 2.0 mM). The plantlets treated with MeJa and Put were harvested to determine influences of MeJa and Put on tryptanthrin and indirubin production in the leaves of *in vitro* grown plants after 12 days of treated with elicitors.

2.4. Calibration curves of tryptanthrin and indirubin

Standarts of tryptanthrin and indirubin were dissolved in DMF (1 mg mL^{-1}). The standard solutions of tryptanthrin and indirubin were prepared at seven different concentrations (0.05, 0.1, 0.25, 0.5, 1.0, 2.5; 5.0 µg mL^{-1}) by diluting their stock solutions with DMF. To generate calibration curves (Figure 1A-B), each standard solution was injected in triplicate. The concentrations of tryptanthrin and indirubin in the extracts were calculated using calibration curves of the compounds.

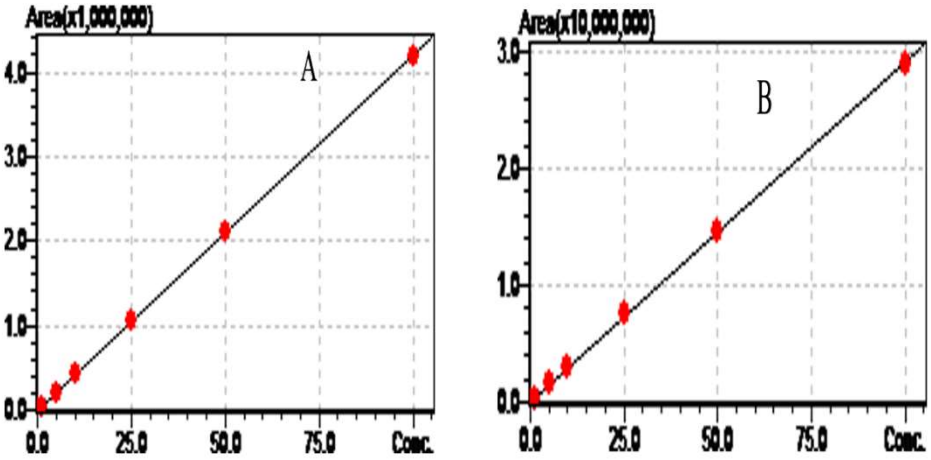

Figure 1. Calibration curves of tryptanthrin and indirubin. (A) Tryptanthrin; (B) Indirubin

2.5. Extraction of tryptanthrin and indirubin

It was used method developed by Liau et al., [29] for extraction. The leaves of plants were ground into powder using a laboratory blender. 0.2 mg of leaf sample was accurately weighed and extracted with 10 mL of methanol by ultrasonication (Jeiotech, US-05, Korea) for 5 min at 45 ^{0}C, in triplicate (10 mL x 3). Methanol in extracts was removed in vacuo (Labtech EV311, Evaporator), following metanolic extracts were combined. The final samples were dissolved in methanol of 5 mL and the final volume was adjusted to 40 mL with methanol. The solution was filtered through a 0.45 mm nylon filter membrane (Merck-Millipore® syringe filter) prior to analysis.

2.6. Analysis of tryptanthrin and indirubin using HPLC

Analysis was performed by using an HPLC system (Shimadzu Corporation, Japan) equipped with Inertsil ODS-3 C18 coloumn (5µm x 4.6 mm x 250 mm), LC-20AT pump, DGU 20A5R degaser, SIL 20A-HT autosampler and SPD M-20A PDA detector. The modified method of Zou et al. [4] was used to analysis of tryptanthrin and indirubin. The mobile phase consisted of water/acetonitrile 40/60 with 0.1% TFA for tryptanthrin and indirubin compounds. The mobile phase was filtered through a 0.45 mm filter and then degassed by ultrasonication. For both compounds, an isocratic elution profile was used, coloumn temperature was adjusted

to 30 ^{0}C and flow rate was 0.5 mL min^{-1}. Separation process was carried out at room temperature. Tryptanthrin and indirubin were detected at wavelengths of 305 and 275 nm, respectively. Retention times of reference compounds of tryptanthrin and indirubin were 14.9 and 13.4 min, respectively (Figure 2A-B). Injection volume was set as 20 µl. The correlation coefficients (*R*) of the standards were 0.9999 for tryptanthrin and 0.9997 for indirubin. Quantification of the two compounds were performed by comparing the retention time of the standards.

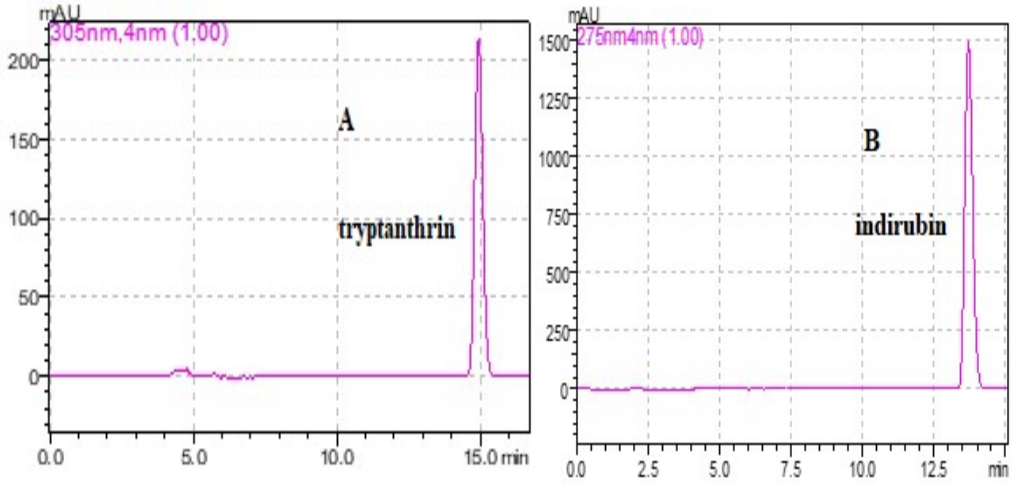

Figure 2. HPLC chromatograms of reference (standard) compounds. A. Tryptanthrin (100 ppm), B. Indirubin (100 ppm)

2.7. Statistical analysis

Statistical analyses were performed using SPSS Software Version 16.0 for Windows. Analysis of variance was performed using ANOVA. Duncan's multi-way range test was used for comparing of data. All data are expressed as the mean values ± standard deviation (SD). Differences were considered statistically significant at $p \leq 0.05$.

3. RESULTS and DISCUSSION

3.1. Influences of MeJa and Put on tryptanthrin and indirubin production

Table 1 shows the influences of MeJa and Put on tryptanthrin and indirubin production in the leaves of *I. demiriziana* grown *in vitro*. In the present study, I investigated the influences of MeJa and Put treatment on tryptanthrin and indirubin production in the leaf explants and development of *I. demiriziana* grown under *in vitro* conditions. Moreover, plantlets treated with MeJa and Put were compared to control plantlets (untreated plantlets). MeJa and Put treatment showed significant differences in production of tryptanthrin and indirubin. MeJa treatments were found to be more effective in production of tryptanthrin and indirubin. The retention times of tryptanthrin and indirubin compounds in the extracts were 14.9 and 13.4 min, respectively (Figure 3A-B, Figure 4A-B). The highest increase in tryptanthrin production was observed in the leaf explants of plantlets treated with 1.0 mM MeJa (154.026 ± 0.11 µg g^{-1}) and this value was about 2.85-fold higher than in the control samples (40.017 ± 0.031 µg g^{-1}). Tryptanthrin content of leaf explants in medium treated with 0.05 mM MeJa (43.922 ± 0.035 µg g^{-1}) was close to the *in vitro* control (40.017 ± 0.031 µg g^{-1}) but the amounts of tryptanthrin in MS solid media containing 0.5, 0.25 and 0.1 mM MeJa were 1.59, 1.06 and 0.97 times higher than that of the control, respectively.

All the MeJa treatments enhanced indirubin production, and the highest indirubin production was found in the leaf explants of plantlets treated with 0.1 mM MeJa (11.274 ±

0.035 µg g^{-1}) and this quantity was about 3.32-fold higher than in *in vitro* control plantlets (2.607 ± 0.027 µg g^{-1}). It was found that MeJa treatment enhanced about 6.5-fold vindoline (an indole alkaloid) production in shoot cultures *of Catharanthus roseus* compared to the control cultures [30]. The similar study showed that production of stemofoline alkaloids enhanced 1.42-fold in root extracts of *Stemona* sp. treated with 1.0 MeJa, when compared with control plants [31]. MeJa treatment induced the accumulation of dihydrosanguinarine alkaloid about 1.5-fold higher than in control [32]. Similarly, the another research was showed that adding of MeJa to cell cultures of *Catharanthus roseus* enhanced the production of ajmalicine alkaloid [33]. The results of our study were consistent with previous reports which indicated that MeJa induced the production of some important alkaloids in *in vitro* cultures.

As shown in Table 1, Put treatments did not show any affects on indirubin production compared to *in vitro* control plants and the differences between the quantities of indirubin in the leaf explants of plantlets treated with Put were statistically insignificant. Tryptanthrin production in leaf explants of plants treated with Put showed a decrease in all treatments but the highest decrease was observed in the medium treated with 0.5 mM Put (2.56-fold less than the control) compared to the control (Table 1). It was reported that Put treatment enhanced the content of capsaicin alkaloids in cell suspension cultures of *Capsicum frutescens* [34]. Similarly, it was found that Put treatment increased content of betalains in hairy root culture of *Beta vulgaris* [35]. However, Put adding to medium of transformed root cultures of *Datura stramonium* decreased the production of some tropane alkaloids [36]. As mentioned in the previous studies, Put treatment to the *in vitro* cultures revealed different results in the production of some important alkaloids. In the present study, we found that Put treatments significantly decreased the tryptanthrin production in *in vitro* shoot cultures of *I. demiriziana*. However, Put treatment did not affect on the indirubin production (Table 1).

Table 1. Influences of MeJa and Put on tryptanthrin and indirubin content (µg g-1) of leaf samples and survival percentage of *I. demiriziana* grown *in vitro*.

Treatment (mM)	Tryptanthrin content	Indirubin content	Survival percentage
In vitro control	40.017 ± 0.031f	2.607 ± 0.027e	100
0.05 MeJa	43.922 ± 0.035e	4.881 ± 0.093d	92.5
0.1 MeJa	78.947 ± 0.034d	11.274 ± 0.035b	87.5
0.25 MeJa	82.750 ± 0.032c	6.939 ± 0.019a	47.5
0.5 MeJa	103.751 ± 0.15b	4.047 ± 0.041c	20
1.0 MeJa	154.026 ± 0.11a	4.806 ± 0.026d	5
0.5 Put	11.226 ± 0.092g	2.481 ± 0.018e	100
1.0 Put	12.853 ± 0.010i	2.598 ± 0.025e	100
2.0 Put	15.572 ± 0.020h	2.657 ± 0.072e	100

Values expressed mean of three different experiments. A P-value of less than 0.05 were significant in terms of differences between data according to Anova multi-way Range Test. Meja; methyl jasmonate, Put; putrescine

3.2. Influences of MeJa and Put on appearance of plantlets

The treatment of different concentration of MeJa and Put had remarkable effects on development and survival percentage of plantlets in shoot cultures of *I. demiriziana*. The effect of elicitation on plant development was eveluated both morphologically and in terms of survival percentage.

As seen from the Figure 5A-B, plantlets in culture media supplemented with 0.05 mM MeJa and control medium were healthy and normal but in the remaining media had deformations in leaves and/or stems of plants in terms of morphological appearances (Figure 5C-F). Morphological appearances of plants treated with Put and grown in control medium

were normal and healthy at the end of 12 th day (Figure 5A). At the end of 12th day, significant differences were observed in both survival percentage and morphological appearance of plantlets grown in the media containing MeJa. Survival percentage of plantlets in medium containing 0.05 mM MeJa was 92.5%, in 0.1 mM MeJa was 87.5%. Plantlets grown in 0.25 and 0,5 mM MeJa were 47.5% and 20%, respectively and the majority of stems and leaves of plantlets grown in both media was observed drying. Among the media tested, the maximum loss was appeared in media containing 1.0 mM MeJa (5%) in terms of survival percentage of plants (Table 1). It was reported that the root biomass of *Panax ginseng* grown in suspension cultures treated with MeJa decreased 10% by day 9 [23] and MeJa treatment declined 20% on day 5 in hairy root culture of *Rehmannıa glutinosa* when compared with control cultures [37].

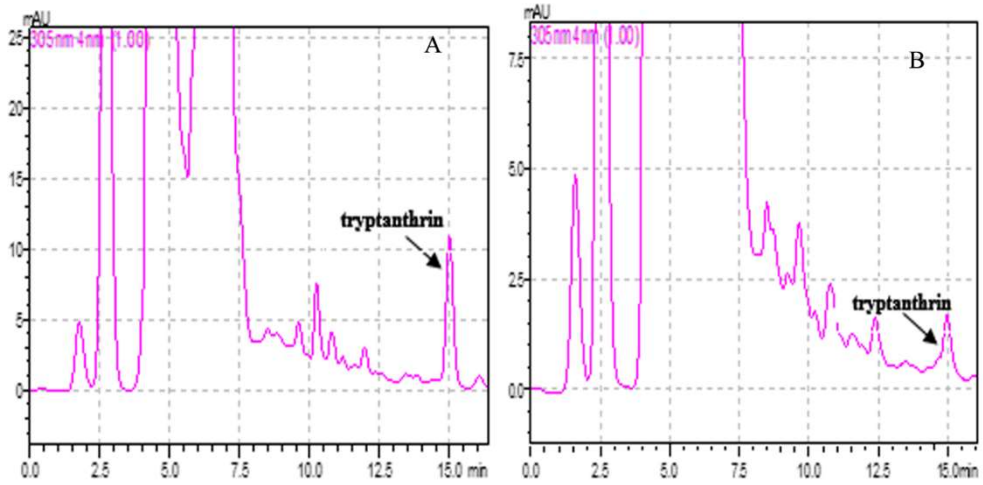

Figure 3. HPLC chromatogram of tryptanthrin. A. Treatment with 1.0 mM MeJa; B. Treatment with 2.0 mM Put. Peaks were enlarged for clarity

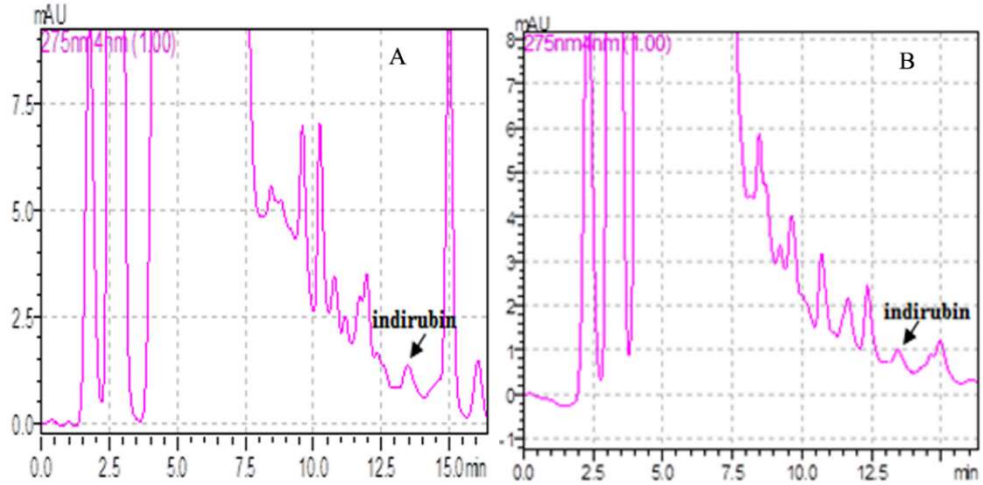

Figure 4. HPLC chromatogram of indirubin. A. Treatment with 1.0 mM MeJa; B. Treatment with 2.0 mM Put. Peaks were enlarged for clarity

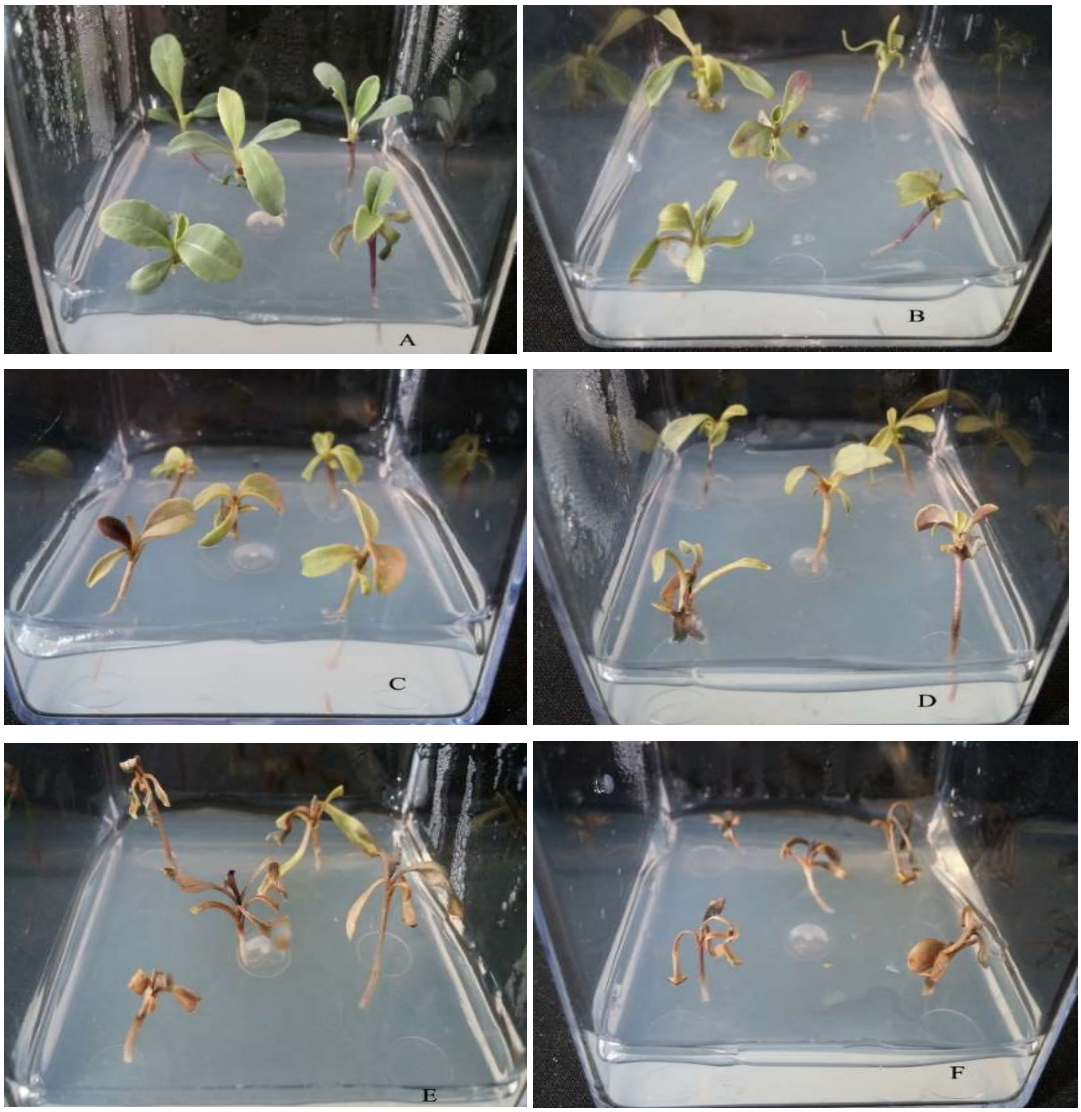

Figure 5. In vitro cultures of I. demiriziana treated with MeJa at different concentrations A. Control plantlets grown in vitro, B. 0.05 mM MeJa, C. 0.1 mM MeJa D. 0.25 mM MeJa, E. 0.5 mM MeJa, F. 1.0 mM MeJa

4. CONCLUSION

The present study demonstrated that the MeJa treatments significantly enhanced both tryptanthrin production (about 2.85 times more than that of control) and indirubin production (3.32 times more than that of control) in leaf explant of *I. demiriziana in vitro* grown. Put treatment reduced tryptanthrin production (1.57-2.56 times more less that of control) but it did not show any affects on indirubin production. Our study showed that adding of MeJa to *in vitro* culture media was an efficient way for the production of tryptanthrin and indirubin in *in vitro* conditions.

Acknowledgements

This work was financially supported by the Dicle University Research Project Council (grant number: 12-FF-141).

Orcid

Özgür KARAKAŞ https://orcid.org/0000-0003-3339-4811

5. REFERENCES

[1]. Mısırdalı, H. (1985). Taxonomic and cytological investigations on the species of Isatis L., grown in the Eastern and South Eastern Anatolia and over the regions of Eastern Mediterranean. (TUBITAK Project No: TBAG-535, Eskisehir, Turkey).

[2]. Maugard, T., Enaud, E., Choisy, P., Legoy, M.D. (2001). Identification of an indigo precursor from leaves of Isatis tinctoria (woad), *Phytochemistry. 58(6)*, 897-904. DOI: 10.1016/S0031-9422(01)00335-1.

[3]. Angelini, L.G., Tozzi, S., Nassi, N. (2007). Differences in leaf yield and indigo precursors production in woad (Isatis tinctoria L.) and Chinese woad (Isatis indigotica Fort.) genotypes. *Field Crops Research, 101 (3)*, 285–295. DOI: 10.1016/j.fcr.2006.12.004.

[4]. Zou, P., Koh, H.L. (2007). Determination of indican, isatin, indirubin and indigotin in Isatis indigotica by liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Communication in Mass Spectrometry*, *21(7)*, 1239-1246. DOI: 10.1002/rcm.2954.

[5]. Mohn, T., Suter, K., Hamburger, M. (2008). Seasonal changes and effect of harvest on glucosinolates in Isatis leaves. *Planta Medica, 74 (5)*, 582-587. DOI: 10.1055/s-2008-1074504.

[6]. Honda, G., Tosirisuk, V., Tabata, M. (1980). Isolation of an antidermatophytic, tryptanthrin, from indigo plants, Polygonum tinctorium and Isatis tinctoria. Planta Medica, 38 (3), 275-276. DOI: 10.1055/s-2008-1074877.

[7]. Mohn, T., Plitzko, I., Hamburger, M. (2009). A comprehensive metabolite profiling of Isatis tinctoria leaf extracts. *Phytochemistry 70 (7)*, 924-934. DOI: 10.1016/j.phytochem. 2009.04.019.

[8]. Tucker, A.M., Grundt, P. (2012). The chemistry of tryptanthrin and its derivatives. *Arkivoc, 2012 (1)*, 546-569. DOI: 10.3998/ark.5550190.0013.113.

[9]. Koya-Miyata, S., Kimoto, T., Micallef, M.J., Hino, K., Taniguchi, M., Ushio, S., Iwaki, K., Ikeda, M., Kurimoto, M. (2001). Prevention of azoxymethane-induced intestinal tumors by a crude ethyl acetate-extract and tryptanthrin extracted from Polygonum tinctorium Lour. *Anticancer Research, 21 (5)*, 3295-3300.

[10]. Yang, S., Li, X., Hu, F., Li, Y., Yang, Y., Yan, J., Kuang, C., Yang, Q. (2013). Discovery of tryptanthrin derivatives as potent inhibitors of indoleamine 2,3-dioxygenase with therapeutic activity in Lewis Lung Cancer (LLC) tumor-bearing mice. *Journal of Medicinal Chemistry, 56 (21)*, 8321-8331. DOI: 10.1021/jm401195n.

[11]. Ishihara, T., Kohno, K., Ushio, S., Iwaki, K., Ikeda, M., Kurimoto, M. (2000). Tryptanthrin inhibits nitric oxide and prostaglandin E2 synthesis by murine macrophages. *European Journal of Pharmacology, 407 (1-2)*,197-204. DOI: 10.1016/S0014-2999(00)00674-9.

[12]. Honda, G., Tabata, M., Tsuda, M. (1979). The antimicrobial specificity of tryptanthrin. *Planta Medica, 37(2)*, 172-174. DOI: 10.1055/s-0028-1097320.

[13]. Krivogorsky, B., Grundt, P., Yolken, R., Jones-Brando, L. (2008). Inhibition of Toxoplasma gondii by indirubin and tryptanthrin analogs. *Antimicrobial Agents Chemotherapy, 52(12)*, 4466-4469. DOI: 10.1128/AAC.00903-08.

[14]. Hoessel, R., Leclerc, S., Endicott, J.A., Nobel, M.E., Lawrie, A., Tunnah, P., Leost, M., Damiens, E., Marie, D., Marko, D., Niederberger, E., Tang, W., Eisenbrand, G., Meijer, L. (1999). Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases. *Nature Cell Biology, 1(1)*, 60-67. DOI: 10.1038/9035.

[15]. Nam, S., Scuto, A., Yang, F., Chen, W., Park, S., Hwa-Seung, Y., Konig, H., Bhatia, R., Cheng, X., Merz, K.H., Eisenbrand, G, Jove, R. (2012). Indirubin derivatives induce apoptosis of chronic myelogenous leukemia cells involving inhibition of Stat5 signaling. *Molecular oncology, 6(3)*, 276-283. DOI: 10.1016/j.molonc.2012.02.002.

[16]. Leclerc, S,. Garnier, M., Hoessel, R., Marko, D., Bibb, J.A., Snyder, G.L., Greengard, P., Biernat, J., Wu, Y.Z., Mandelkow, E.M., Eisenbrand, G., Meijer, L. (2001). Indirubins inhibit glycogen synthase kinase-3β and CDK5/P25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? *The Journal of Biological Chemistry, 276(1)*, 251-260. DOI: 10.1074/jbc.M002466200.

[17]. Hussain, M.S., Fareed, S., Ansari, S., Rahman, M.A., Ahmad, I.Z., Saeed, M. (2012). Current approaches toward production of secondary plant metabolites. *Journal of Pharmacy and Bioallied Sciences, 4(1)*, 10-20. DOI: 10.4103/0975-7406.92725.

[18]. Lambert, E., Faizal, A., Geelen, D. (2011). Modulation of triterpene saponin production: In vitro cultures, elicitation, and metabolic engineering. *Applied Biochemistry and Biotechnology, 164(2)*, 220-237. DOI: 10.1007/s12010-010-9129-3.

[19]. Sahu, R., Gangopadhyay, M., Dewanjee, S. (2013). Elicitor-induced rosmarinic acid accumulation and secondary metabolism enzyme activities in Solenostemon scutellarioides. *Acta Physiologiae Plantarum, 35(5)*, 1473-1481. DOI: 10.1007/s11738-012-1188-3.

[20]. Ramirez-Estrada, K., Vidal-Limon, H., Hidalgo, D., Moyano, E., Golenioswki, M.M., Cusidó, R., Palazon, J. (2016). Elicitation, an Effective Strategy for the Biotechnological Production of Bioactive High-Added Value Compounds in Plant Cell Factories. *Molecules, 21(2)*, 182. DOI: 10.3390/molecules21020182.

[21]. Largia, M.J.V., Pothiraj, G., Shilpha, J., Ramesh, M. (2015). Methyl jasmonate and salicylic acid synergism enhances bacoside A content in shoot cultures of Bacopa monnieri (L.). *Plant Cell, Tissue and Organ Culture, 122(1)*, 9-20. DOI: 10.1007/s11240-015-0745-z.

[22]. Zaker, A., Sykora, C., Gössnitzer, F., Abrishamchi, P., Asili, J., Mousavic, S.H., Wawroschd, C. (2015). Effects of some elicitors on tanshinone production in adventitious root cultures of Perovskia abrotanoides Karel. *Industrial Crops and Products, 67*, 97-102. DOI: 10.1016/j.indcrop.2015.01.015.

[23]. Ali, M.B., Hahn, E.J., Paek, K.Y., (2007). Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in Panax ginseng bioreactor root suspension cultures. *Molecules, 12(3)*, 607-621. DOI: 10.3390/12030607.

[24]. Horbowicz, M., Chrzanowski, G., Koczkodaj, D., Mitrus, J. (2011). The effects of methyl jasmonate vapors on content of phenolic compounds in seedlings of common buckwheat (Fagopyrum esculentum Moench). *Acta Societatis Botanicorum Poloniae, 80(1)*, 5-9. DOI: 10.5586/asbp.2011.001.

[25]. Ali, R.M. (2000). Role of putrescine in salt tolerance of Atropa belladonna plant. *Plant Science, 152(2)*, 173-179. DOI: 10.1038/srep14390.

[26]. Tiburcio, A., Kaur-Sawhney, R., Ingersoll, R.B., Galston, A.W. (1985). Correlation between polyamines and pyrrolidine alkaloids in developing tobacco callus. *Plant Physiology, 78(2)*, 323-326. DOI: 10.1104/pp.78.2.323.

[27]. Bais, H.P., Madhusudhan, R., Bhagyalakshmi, N., Rajasekaran, T., Ramesh, B.S., Ravishankar, G.A. (2000). Influence of polyamines on growth and formation of secondary metabolites in hairy root cultures of Beta vulgaris and Tagetes patula. *Acta Physiologiae Plantarum, 22(2)*, 151-158. DOI: 10.1007/s11738-000-0070-x.

[28]. Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia Plantarum, 15(3)*, 473-497. DOI: 0.1111/j.1399-3054.1962.tb08052.x.

[29]. Liau, B.C., Jong, T.T., Lee, M.R., Chen, S.S. (2007). LC-APCI-MS method for detection and analysis of tryptanthrin, indigo and indirubin in daqingye and banlangen. *Journal of*

Pharmaceutical and Biomedical Analysis, 43(1), 346 - 351. DOI: 10.1016/j.jpba.2006.06.029.

[30]. Vázquez-Flota, F., Hernández-Dominguez, E., De Lourdes, Miranda-Ham, M., Monforte-González, M. (2009). A differential response to chemical elicitors in Catharanthus roseus in vitro cultures. *Biotechnology Letters, 31(4)*, 591-595. DOI: 10.1007/s10529-008-9881-4.

[31] Chaichana, N., Dheeranupattana, S. (2012). Effects of methyl jasmonate and salicylic acid on alkaloid production from in vitro culture of Stemona sp. *International Journal of Bioscience, Biochemistry and Bioinformatics, 2(3)*, 146-150. DOI: 10.7763/IJBBB.2012.V2.89.

[32]. Cho, H., Son, S.Y., Rhee, H.S., Yoon, S.H., Lee-Parsons, C.W.T., Park, J.M. (2008). Synergistic effects of sequential treatment with methyl jasmonate, salicylic acid and yeast extract on benzophenanthridine alkaloid accumulation and protein expression in Eschscholtzia californica suspension cultures. *Journal of Biotechnology, 135(1)*, 117-122. DOI: 10.1016/j.jbiotec.2008.02.020.

[33]. Lee-Parsons, C.W.T., Ertürk, S., Tengtrakool, J. (2004). Enhancement of ajmalicine production in Catharanthus roseus cell cultures with methyl jasmonate is dependent on timing and dosage of elicitation. *Biotechnology Letters, 26(20)*, 1595-1599. DOI: 10.1023/B:BILE.0000045825.37395.94.

[34]. Sudha, G., Ravishankar, G.A. (2003). Putrescine facilitated enhancement of capsaicin production in cell suspension cultures of Capsicum frutescens. *Journal of Plant Physiology, 160(4)*, 339-346. DOI: 10.1078/0176-1617-0092.

[35]. Suresh, B., Thimmaraju, R., Bhagyalakshmi, N., Ravishankar, G.A. (2004). Polyamine and methyl jasmonate-influenced enhancement of betalaine production in hairy root cultures of Beta vulgaris grown in a bubble column reactor and studies on efflux of pigments. Process Biochemistry, 39(12), 2091-2096. DOI: 10.1016/j.procbio.2003.10.009.

[36]. Robins, R.J., Parr, A.J., Bent, E.G., Rhodes, M.J. (1991). Studies on the biosynthesis of tropane alkaloids in Datura stramonium L. transformed root cultures: 1. The kinetics of alkaloid production and the influence of feeding intermediate metabolites. *Planta, 183(2)*, 185-195. DOI: 10.1007/BF00197787.

[37] Piątczak, E., Kuźma, L., Wysokińska, H. (2016). The Influence of Methyl Jasmonate and Salicylic Acid on Secondary Metabolite Production in Rehmannia glutinosa libosch. Hairy Root Culture. *Acta Biologica Cracoviensia Series Botanica, 58(1)*, 57-65. DOI: 10.1515/abcsb-2016-0004.