Original Article

The Influence of Methyl Jasmonate on Growth and Caffeic Acid Derivative Contents of *In vitro* Shoot and Roots in Echinaceae (*Echinacea Purpurea*)

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ABSTRACT

This study was carried out to determine the effects of methyl jasmonate (MeJA) applications on growth and accumulation of caffeic acid derivatives (CADs) in shoots and roots grown *in vitro*. For this aim, 28 days old plants obtained from seeds were cultured in ½ Murashige and Skoog media containing different concentrations of MeJA (0, 10, 50 and 100 μM) and plants were harvested in three times at 15 days intervals. After harvest, growth parameters and CADs in shoot and roots were determined, separately. All growth parameters decreased in line with the elevating level of MeJA applications. But MeJA applications increased the CADs both shoots and roots compared to the controls. The highest cichoric acid, the main CAD of Echinacea, was found in shoots and roots applied with 100 μM MeJA and harvested in 45 days after application.

Keywords: *Echinacea Purpurea*, Methyl Jasmonate, Caffeic Acid Derives, Cichoric Acid

INTRODUCTION

Echinacea purpurea is an herbaceous perennial species that has gained international popularity because of its nutraceutical and medicinal properties.[1] Besides alkamides, polysaccharides and glycoproteins, *E. purpurea* mainly contains caffeic acid derivatives (CADs).[2] Synthesis of CADs requires expensive, difficult and time-consuming procedures and it is well known that the natural combinations of these compounds are more effective than the artificially obtained compounds.[3] *In vitro* techniques combined elicitor treatments may be utilized effectively to improve the secondary metabolites production in plants. Methyl jasmonate (MeJA), a jasmonic acid derivative, is one of the most effective and frequently used elicitor in secondary metabolite production.[4] MeJA plays a key role in co-ordination of plant defense gene expression. It is a signalling and regulatory molecule influencing enzymes of the biosynthetic pathway responsible for secondary metabolite synthesis.[5]

As the first study on the effects of MeJA in Echinacea, it was aimed to determine the effects of MeJA on growth and production of CADs in *in vitro* shoots and roots in this study.

MATERIAL AND METHODS

Plant Materials

In this study, 28 days old plants obtained from seeds in *in vitro* conditions were used as plant materials. After washing 70% ethanol for 30 seconds, seeds were surface-sterilized with 15% (v/v) sodium hypochlorite containing 0.1% Tween 20 for 15 min, then rinsed three times in sterilized water. After sterilization, seeds were cultured on ½ MS medium supplemented with 3% sucrose and 0.6% agar at 25°C with a photoperiod of 16:8 h light: darkness.

Methods

28 days after sowing, plants were subculture in ½ MS medium containing 3% sucrose, 0.6% agar and different concentrations of

DOI: 10.5530/ijper.51.3s.77

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MeJA (0, 10, 50 and 100 µM) and plants cultured at same conditions. Plants were harvested in three times at 15 days intervals. Experiments were performed in triplicate and 12 plants were used for each replication. The harvested plants were divided into shoot and root. Then roots were washed with distilled water to remove the media residues and soaked with tissue paper to remove the surface water. Then shoot and root lengths were determined as cm. The fresh weights of shoots and roots were recorded as g, separately. After dried at 50°C to a constant weight, dry weights of shoots and roots were expressed as mg. Extractions and the quantifications of CADs (cichoric acid, caftaric acid, chlorogenic acid, echinacoside, caffeic acid and *o*-coumaric acid) by HPLC were performed by the method of Liu *et al*.[6]

Data were subjected to analysis of variance with mean separation by Tukey's multiple range test. Differences were considered statistically significant at the $p \leq 0.05$ levels.

RESULTS AND DISCUSSION

MeJA applications significantly affected both growth as well as CAD contents of *in vitro* shoots and roots in Echinacea. All growth parameters including shoot and root lengths and fresh and dry shoot and root weights decreased in line with the elevating level of MeJA (Table 1). The highest values were obtained from the control plants while MeJA especially in high concentrations caused a decline in all growth parameters. These results supported the knowledge that exogenous MeJA applications in *in vitro* conditions adversely affect the growth of cultures in different plants.[7,8] However, an increase in harvest time led to rise in all parameters. The highest values were obtained from the shoots and roots after 45 days.

Application of MeJA to *in vitro* plants resulted in substantial increases in the production of CADs. All MeJA applications significantly increased the amounts of CADs compared to the control (Table 2). The highest contents of cichoric acid, the most abundant CAD of Echinacea, were obtained from the shoots and roots treated with 100 µM MeJA and harvested at 45 day as 35.51 mg/g and 54.87 mg/g, respectively. Caftaric acid contents were also changed depending on the MeJA applications. The maximum caftaric acid contents were found in shoots and roots applicated with 100 µM MeJA after 45 days as in cichoric acid contents. 50 µM MeJA and harvest on 45th day combination was found as an optimum application to get maximum chlorogenic acid both shoots and roots. The highest amount of echinacoside in shoots was obtained with 50 µM MeJA after 30 days as 0.25 mg/g while 100 µM MeJA and 45th day were found as an optimum MeJA concentration and harvest time for roots. Caffeic acid and *o*-coumaric acid are intermediates in the biosynthesis steps of CADs such as cichoric acid, chlorogenic acid and caftaric acid.[9] Application of MeJA depending on its concentration resulted the increase of caffeic acid and *o*-coumaric acid contents while not being synthesized in control

Table 1: Effects of MEJA applications on the growth parameters of shoots and roots

	MeJA (µM)	Harvest time					MeJA (µM)	Harvest time			
		Day 15	Day 30	Day 45	Mean			Day 15	Day 30	Day 45	Mean
Shoot length (cm)	0	8.59 Ba*	10.61 Aa	10.85 Aa	10.02	Root length (cm)	0	5.35 Ba	7.35 Aa	7.85 Aa	6.85
	10	4.90 Cb	7.72 Bb	8.77 Ab	7.13		10	3.54 Cb	5.88 Ab	5.25 Bb	4.89
	50	3.78 Bc	6.06 Ac	6.14 Ac	5.33		50	3.20 Cb	4.28 Bc	5.23 Ab	4.24
	100	3.37 Bc	4.17 Ad	4.30 Ad	3.95		100	3.39 Bb	3.89 ABc	4.08 Ac	3.79
	Mean	5.16	7.14	7.52			Mean	3.87	5.35	5.60	
Fresh shoot weight (g)	0	0.37	0.69	0.74	0.60 a	Fresh root weight (g)	0	0.23 Ba	0.71 Aa	0.73 Aa	0.56
	10	0.20	0.40	0.48	0.36 b		10	0.16 Bb	0.58 Ab	0.55 Ab	0.52
	50	0.19	0.40	0.39	0.32 b		50	0.14 Bb	0.39 Ac	0.42 Ad	0.32
	100	0.18	0.27	0.27	0.39 b		100	0.09 Cb	0.33 Bd	0.49 Ac	0.30
	Mean	0.24 B	0.44 A	0.47 A			Mean	0.15	0.50	0.55	
Dry shoot weight (mg)	0	41.95 Ba	67.75ABa	156.85 Aa	88.85	Dry root weight (mg)	0	26.66	77.70	90.79	65.05 a
	10	37.34 Cab	55.18 Ba	85.37Aab	59.30		10	20.64	73.40	75.65	56.56 ab
	50	33.61 Cab	51.74 Ba	73.99 Ab	53.11		50	18.23	57.92	75.27	50.48 b
	100	30.81 Ab	49.49 Aa	54.93 Ab	45.07		100	13.64	54.59	75.62	47.95 b
	Mean	35.93	56.04	92.78			Mean	19.80 C	65.90 B	79.33 A	

*The lower cases show the difference between treatments at each harvest time, the capital letters show the difference between harvest times in each treatment.

Table 2: Effects of MEJA applications on the CAD contents of shoots and roots

	MeJA (µM)	Shoots				Roots			
		Day 15	Day 30	Day 45	Mean	Day 15	Day 30	Day 45	Mean
Cichoric acid (mg/g)	0	5.97 Cb*	13.21 Bc	18.39 Ad	12.52	7.66 Cc	13.88 Bd	28.92 Ac	16.82
	10	12.48 Cc	20.41 Bb	23.60 Ac	18.83	30.10 Cb	40.15 Bc	44.51 Ab	38.25
	50	17.33 Cb	21.05 Bb	25.34 Ab	21.24	32.97 Cb	44.08 Bb	48.53 Ab	41.86
	100	18.60 Ca	31.07 Ba	35.51 Aa	28.39	40.57 Ca	49.71 Ba	54.87 Aa	48.38
	Mean	13.59	21.43	25.71		27.82	36.95	44.21	
Caftaric acid (mg/g)	0	2.41 Cc	2.88 Bc	3.58 Ad	2.96	1.04 Bd	2.97 Ac	3.13 Ac	2.38
	10	3.59 Cb	4.46 Bb	5.48 Ac	4.51	3.15 Cc	6.08 Bb	7.05 Ab	5.43
	50	4.43 Ba	4.49 Bb	6.16 Ab	5.03	3.83 Cb	6.45 Bb	7.33 Ab	5.87
	100	4.48 Ca	6.13 Ba	7.28 Aa	5.96	6.14 Ca	7.55 Ba	8.85 Aa	7.51
	Mean	3.73	4.49	5.62		3.54	5.76	6.59	
Chlorogenic acid (mg/g)	0	0.14 Cc	0.19 Bd	0.38 Ad	0.24	1.60 Bd	1.64 Bb	2.34 Ac	1.86
	10	0.42 Ca	0.63 Bb	0.71 Ab	0.59	2.39 Bc	2.70 Ab	2.74 Ab	2.61
	50	0.47 Ca	0.72 Ba	0.87 Aa	0.68	3.06 Bb	3.19 Ba	3.49 Aa	3.25
	100	0.26 Bb	0.44 Ac	0.48 Ac	0.39	2.71 Bb	3.01 Aa	2.74 Bb	2.82
	Mean	0.32	0.50	0.61		2.44	2.64	2.83	
Echinacoside (mg/g)	0	0.12 Ac	0.12 Ad	0.09 Ab	0.11	0.02 Cc	0.03 Bc	0.04 Ac	0.03
	10	0.14 Ab	0.14 Ac	0.13 Aa	0.14	0.05 Cb	0.06 Bb	0.07 Ab	0.06
	50	0.19 Ba	0.25 Aa	0.11 Cb	0.18	0.05 Bb	0.07 Aa	0.07 Ab	0.06
	100	0.18 Aa	0.17 Ab	0.01 Bc	0.12	0.06 Ca	0.07 Ba	0.08 Aa	0.07
	Mean	0.16	0.17	0.09		0.04	0.06	0.06	
Caffeic acid (µg/g)	0	0.00	0.00	0.07	0.02 d	14.20 Bb	17.32 Ac	15.52 ABc	15.68
	10	0.00	4.17	1.90	2.03 c	14.62 Bb	22.32 Ab	20.70 Ab	19.21
	50	2.13	18.00	12.10	10.74 a	21.31 Ca	29.90 Aa	25.53 Ba	25.58
	100	2.43	11.90	3.17	5.83 b	12.00 Bb	20.23Abc	13.92 Bd	15.38
	Mean	1.14 C	8.52 A	4.32 B		15.53	22.44	18.92	
o-Coumaric acid (mg/g)	0	0.00	0.00	0.00	0.00 c	0.29 Cd	0.54 Ad	0.33 Bb	0.38
	10	0.08	0.25	0.10	0.14 a	0.55 Bc	0.62 Ac	0.35 Cb	0.51
	50	0.01	0.20	0.08	0.10 b	0.61 Bb	0.78 Ab	0.55 Ca	0.65
	100	0.01	0.19	0.08	0.09 b	0.77 Ba	0.84 Aa	0.59 Ca	0.73
	Mean	0.02 C	0.16 A	0.07 B		0.56	0.69	0.46	

*The lower cases show the difference between treatments at each harvest time, the capital letters show the difference between harvest times in each treatment.

shoots and roots. The highest caffeic acid contents were found in shoots and roots treated with 50 µM MeJA and harvested on 30th day as 18.00 µg/g and 29.90 µg/g, respectively. Maximum *o*-coumaric acid in shoots (0.25 mg/g) was measured from the shoots raised under 10 µM MeJA while 100 µM MeJA was the most optimum concentration for roots in order to get the maximum *o*-coumaric acid level (0.84 mg/g). On the other hand, 30th day was found as an optimum harvest time both shoots and roots for *o*-coumaric acid. Another interesting result is that all caffeic acid derivatives except echinacoside were found in higher concentrations in roots than shoots. Similarly Bauer[10] reported that the biochemical constituents of Echinacea roots are often greater than in its shoots.

In this study MeJA was used to enhance the accumulation of CADs in shoots and roots of Echinacea. It is well known that MeJA plays a key role in the plant defence response through altering the gene expression.[11] MeJA is a signalling and regulatory molecule influencing enzymes of the biosynthetic pathway responsible for secondary metabolite accumulation.[12] In the present study, MeJA applications significantly increased the CADs. The induction effect of MeJA on phenolic production was in agreement with reported data which revealed that MeJA strongly increased phenolics in

different plants.[13-15] According to Chen and Chen[16], stress signalling molecules increase the synthesis of secondary metabolites by reducing the synthesis of the primer metabolites. These results suggest that the effects of MeJA on secondary metabolite production seem to be due to the activation of related metabolism.

CONCLUSION

All growth parameters of both shoot and roots decreased in line with the elevating level of MeJA treatments while MeJA treatments increased the CADs in both shoots and roots applied with 100 µM MeJA and harvested in 45 days after application. To conclude, MeJA may be promising compound for use in the production of secondary metabolites in Echinacea plants.

ACKNOWLEDGEMENT

The authors are thankful to TUBITAK (Scientific and Technolog-ical Research Council of Turkey) for the financial support for thisresearch Project (Project number: TOVAG 113O144).

CONFLICT OF INTEREST

The authors declare No conflict of Interest

ABBREVIATION USED

CAD: Caffeic Acid Derivative; CADs: Caffeic Acid Derivatives; HPLC: High Performance Liquid Chromatography; MeJA:Methyl Jasmonate; MS: Murashige and Skoog

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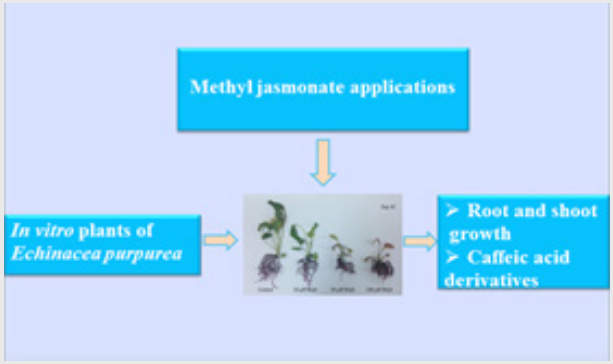
PICTORIAL ABSTRACT



SUMMARY

- All growth parameters of both shoot and roots decreased in line with the elevating level of MeJA applications in Echinacea.
- MeJA applications increased the caffeic acid derivatives in both shoots and roots compared to the controls.
- The highest cichoric acid, the main caffeic acid derivative found in Echineceae, was found in shoots and roots applied with 100 µM MeJA and harvested in 45 days after application.

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Cite this article: Demirci T, Özmen S, Yilmaz EG, Asci ÖA, Baydar NG. The Influence of Methyl Jasmonate on Growth and Caffeic Acid Derivative Contents of *In vitro* Shoot and Roots in Echinaceae (*Echinacea Purpurea*). Indian J of Pharmaceutical Education and Research. 2017;51(3)Suppl:S513-17.