Effects of foliar spraying acetyl-coA on dry weight of leaves, chlorophyll a, and antioxidant enzymes of rosemary (*Rosmarinus officinalis* L.)

Mahshad Hosseini[1]*, Masoud Mashhadi Akbar Boojar[2], Babak Delkhosh[3], Pezhman Moradi[4]

1. Department of Horticultural Science, Islamic Azad University, Science and Research Branch, Tehran, Iran
2. Faculty of Biological science. Kharazmi University, Tehran, Iran
3. Department of Agronomy, Islamic Azad University, Science and Research Branch, Tehran, Iran
4. Department of Horticultural Science, Islamic Azad University, Saveh Branch, Saveh, Iran

Abstract

Acetyl coenzyme A is an important molecule in metabolism playing role in many biochemical reactions. Its main function is to convey the carbon atoms within the acetyl group to the citric acid cycle (Krebs cycle) to be oxidized for energy production. In order to find out if this chemical compound could affect physiological and morphological characteristics of rosemary plant, the present study was carried out in a factorial design including two factors (acetyl-co A and the time of treatment application) based on the completely randomized block design. Acetyl-coA was used in 6 different concentrations (0, 25, 50, 100, 200, and 400 mM) which were applied to plants 1, 2, and 3 times witha seven - day interval. The application of acetyl-coA 200 mM, had significantly improved the factors such as dry weight of leaves, chlorophyll a, and the activity of antioxidant enzymes catalase and glutathione peroxidase.

*Keywords:*acetyl-coA; rosemary; antioxidant enzymes activity; *Rosmarinus officinalis*

Hosseini, M., M. M. A. Boojar, B. Delkhosh and **P. Moradi**. 2014 'Effects of foliar spraying acetyl-coA on dry weight of leaves, chlorophyll a and antioxidant enzymes of rosemary (*Rosmarinus officinalis* L.)'. *Iranian Journal of Plant Physiology* 4 (2), 977-981.

Introduction

Acetyl-CoA is an intermediate common to a variety of metabolic processes that are distributed across at least five different subcellular compartments. In plastids, acetyl-CoA is the precursor for de novo fatty acid biosynthesis (Nikolau et al., 2003) and for the biosynthesis of glucosinylates (Falk et al., 2004). Mitochondrial acetyl-CoA is incorporated into the TCA cycle and is used for the generation of ATP and the synthesis of amino acid carbon skeletons.

*Corresponding author
E-mail address: mnhosseini66@yahoo.com
Received: October, 2013
Accepted: January, 2014

In microbodies, acetyl-CoA is generated during fatty acid b-oxidation. In the nucleus, acetyl-CoA is the substrate for the acetylation of proteins, such as histones and transcription factors, and regulates their function in maintaining or altering chromosome structure and/or gene transcription (Choi et al.,2003; Sun et al., 2003). In the cytosol, acetyl-CoA is required for the biosynthesis of a plethora of phytochemicals, many of which are important for plant growth, development, and responses to environmental cues (Schmid et al., 1990; Clouse, 2002; Souter et al., 2002). Furthermore, acetyl CoA is a vital primary metabolite involved in different aspects of

Table 1
Analysis of variance of the effect of foliar spraying time and acetyl-coA concentration on the studied characteristics of rosemary

Source of Deviation	df	Leaf Dry Weight (mg per shrub)	Chlorophyll a (mg/g.fw)	CAT (units/g.fw)	GPX(units/g.fw)
Block	3	0.21	0.50	3.14	1.97
Times of foliar spraying	2	0.27 ns	2.89*	24.38**	0.25 ns
Acetyl-coA concentration	5	3.10**	9.34**	30.45**	7.03**
Acetyl-coA concentration × time of foliar spraying	10	0.43 ns	1.64*	7.18**	1.55 ns
Error	38	0.30	0.78	0.94	0.97
Coefficient of variation	-	10.86	9.20	8.90	16.01

ns, × and ×× are no significant and significant effect at level of 5 percent and 1 percent, respectively.

metabolism like respiration and lipid metabolism. Three molecules of acetyl CoA is required for producing of mevalonate which is converted into isopentenyl pyrophosphate, precursor of variety of isopernoids and terpen derived compounds (Habibi et al., 2011). Thus acetyl CoA is implicated in metabolism of terpenoids and terpen-derived substances.

Rosemary is a valuable and important medicine plant. Plant material from rosemary (Rosmarinus officinalis L.) is of commercial interest for its essential oil content and its antioxidant compounds. During the past decade, antioxidant compounds from rosemary have received increasing interest for their use instead of synthetic antioxidant in food industry. Since, acetyl-coA as a chemical compound has not ever been reported to be applied on rosemary plant, this research was carried out to find out if it has any effect on this medicine plant.

Material and Methods

Plant and media preparation

Rosemary plants, Rosmarinus officinalis L., were obtained from commercial growers. Firstly, the plants were planted pots filled with 30% coco peat and 70% perlite and then placed in a greenhouse. This experiment was arranged in a factorial design based on randomized block design with 2 factors and 3 replications. Foliar spraying of acetyl-coA was applied as the first factor in 6 different concentrations (0, 25, 50,100, 200, and 400 mM) at 4 occasions (the second factor) when acetyl-coA was sprayed on plants 0, 1, 2 and 3 times with 7 days intervals.

Determination of physiological and morphological factors

The leaf dry weight was measured by a digital scale.

Determination of chlorophyll a

0.2 g leaf was gently mixed in 80% acetone for 2 minutes and then was centrifuged at 2500 round/ minute. and supernatant was extracted. The absorbance of each tube at A647 and A663 was read and recorded in the spectrophotometer (Porra et al., 1999). The amount of chlorophyll a was calculated by the following formulas:

Chl.a (mg/L) = (12.25×A663)-(2.79×A647)

Antioxidant enzymes

Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decomposition of H_2O_2 (e = 39.4 mM cm^{-1}) at 240 nm for 1 min (Abey 1984). Glutathione peroxidase (GPX, EC 1.11.17) activity was measured by monitoring the increase in absorbance due to tetraguaiacol formation (e = 26.6 mM cm^{-1}) at 470 nm (Tatiana et al. 1999).

Statistical Analyses

Data were analyzed using SPSS version 11 for windows (SPSS 2001). Analysis of variance (ANOVA) was used to test significance of the treatments on different growth parameters of the test species.

Table 2
Comparison of means for the effect of foliar spraying occasion on studied characteristics of rosemary

Times of foliar spraying	Leaf Dry Weight (mg per shrub)	Chlorophyll a (mg/g.fw)	CAT (units/g.fw)	GPX(units/g.fw)
1 time	5.02 a	10.05 b	10.19 c	6.67 a
2times	5.19 a	10.40 ab	11.79 b	6.88 a
3times	5.20 a	10.63 a	12.40 a	7.20 a

ns, × and ×× are no significant and significant effect at level of 5 percent and 1 percent, respectively.

Results

Leaf dry weight

Leaf dry weight was not significantly affected by increasing the number of spraying times. However, application of acetyl-CoA significantly increased the leaves dry weight and the highest leaf dry weight (5.78 g) was obtained by acetyl-CoA at 200 mM concentration compared to the control which had the lowest (4.50 g) leaves dry weight (Table 1).

Leaf chlorophyll a content

Leaf chlorophyll a content was influenced by increasing the number of sprayings times, so that the maximum content (10.63 mg/g.fw) was observed after three times spraying (Table2). The application of acetyl-CoA was significantly effective in the content of leaf chlorophyll a, by which the maximum content was obtained in the concentration of 200 mM (Table 3). Furthermore, it was noticed that the maximum chlorophyll a content was obtained by the concentration of 200 mM after three times sprayings (Table 2).

Catalase content

By increasing the number of spraying times, the amount of CAT was significantly increased so that, the highest level (12.40 g/fw) was recorded after three times application (Table2). Application of acetyl-CoA caused a significant increase in CAT as the highest amount (13.63 g/fw) was observed at 200 mM compared to the control (8.83 g/fw) which had the lowest CAT content (Table 3). As the amount of CAT increased with spraying times, the application of acetyl-CoA increased CAT level so that the highest amount (15.88 g/fw) was recorded at 100 mM concentration after three times spraying (Table 4).

Glutathione peroxidase (GPX) content

The amount of GPX was not significantly affected by increasing the numbers of spraying time, but it was found that the acetyl-CoA application could significantly influence the GPX content so the maximumGPX content (7.92 g/fw) was achieved by 200 mM compared to the other concentrations (Table3).

Table 3
Comparison of means for the effect of acetyl-coA concentrations on the studied characteristics of rosemary

Acetyl-coA Concentration	Leaf Dry Weight (mg per shrub))		Chlorophyll a (mg/g.fw)		CAT (units/g.fw)		GPX (units/g.fw)	
0	4.50	e	9.18	c	8.83	d	5.78	b
25	4.65	de	9.43	c	10.32	c	6.02	b
50	5.30	bc	10.56	b	12.16	b	7.26	a
100	5.57	ab	10.73	b	13.07	a	7.53	a
200	5.78	a	11.68	a	13.63	a	7.92	a
400	5.02	cd	10.59	b	10.91	c	7.00	a

ns, × and ×× are no significant and significant effect at level of 5 percent and 1 percent, respectively

Discussion

Application of acetyl-coA had enhancing effects on some morphological factors such as leaves dry weight. This compound also significantly enhanced catalase, GPX, and chlorophyll a. The highest amounts of these compounds were achieved at400, 200,200, 400, and 200 mM concentrations, respectively. Acetyl CoA takes part in different aspects of metabolism including carbohydrates, lipids and terpenoids. Therefore, it seems that physiological changes induced by the application of acetyl CoA resulted from increased terpenoids especially GA. In addition, acetyl CoA application, as a component of Krebs cycle, could change the status of ATP and reduce coenzymes in cells. GA-stimulated antioxidant enzymes result in decreased accumulation of active oxygen species and declined lipid peroxidation (Qing Zhu et al.,2011). Gibberellins could form sink via induction of especial physiological processes (Iqbal et al., 2011). Additionally, acetyl-CoA, provides organisms with the chemical flexibility to biosynthesize a plethora of natural products that constitute much of the structural and functional diversity in nature. This is particularly exemplified in the plant kingdom where acetyl-CoA metabolism via carboxylation, condensation, or acetylation reactions is used for the production of many different classes of metabolites. By distributing this metabolism among separate cellular and sub-cellular compartments, plants have the potential of simplifying the regulatory processes that control this complex network (Millerd and Bonner, 1954).

Table 4
Comparison of means for interaction between foliar spraying occasion and acetyl-coA concentration on characteristics of rosemary

Foliar Spraying Times	Acetyl-coA Concentration	Chlorophyll a (mg/g.fw)		CAT (g/fw)	
1 time	0	9.18	d	8.83	f
	25	9.08	d	8.70	f
	50	9.63	cd	9.25	fe
	100	10.38	bcd	12.05	bc
	200	11.20	abc	11.33	bcd
	400	10.83	abc	11.28	bcd
2times	0	9.18	d	8.83	f
	25	9.60	cd	9.87	def
	50	10.25	bcd	11.28	bcd
	100	11.10	abc	14.98	a
	200	11.68	ab	14.38	a
	400	10.60	abcd	10.93	bcd
3times	0	9.18	d	8.83	f
	25	9.63	cd	12.28	b
	50	10.95	abc	12.30	b
	100	11.55	ab	15.88	a
	200	12.15	a	14.60	a
	400	10.35	bcd	10.53	cde

Similar letters show no significant difference (P≤05)

References

Abey, H. 1984. 'Catalase in vitro'. *Methods Enzymol*, 105:121–126

Beauchamp, C. and **I. Fridovich**. 1971. 'Superoxide dismutase: improved assays and an applicable to acrylamide gels'. *Annal Biochem*. 44:278–287.

Choi, C.H., M. Hiromura and **A. Usheva**. 2003. 'Transcription factor IIB acetylates itself to regulate transcription'. *Nature*,424:965–969.

Clouse, S.D. 2002. 'Arabidopsis mutants reveal multiple roles for sterols in plant development'. *Plant Cell* 14: 1995–2000.

Falk, K. L., C. Vogel, S. Textor, S. Bartram, A. Hick, J. A. Pickett and **J. Gershenzon**. 2004. 'Glucosinolate biosynthesis: Demonstration and characterization of the condensing enzyme of the chain elongation cycle in *Eruca sativa*'. *Phytochemistry*, 65, 1073–1084.

Habibi A., G. Heidari, Y. Sohrabi, H. Badakhshan and **K. Mohammadi** .2011. 'Influence of bio, organic and chemical fertilizers on medicinal pumpkin traits'. *J Med Plants Res*. 5(23): 5590-5597.

Iqbal N., R. Nazar, M.I.R. Khan, A. Masood and **N. A. Khan**. 2011. 'Role of gibberellins in regulation of source-sink relations under optimal and limiting environmental conditions '. *Curr Sci*. 100 (7): 998-1007.

Millerd, A. and **J. Bonner**.1954. 'Acetate activation and acetoacetate formation in plant systems '. *Arch. Biochem. Biophys*. 49: 343–355.

Nikolau, B. J., J. B., Ohlrogge and **E. S. Wurtele**. 2003. 'Plant biotin containing carboxylases '. *Arch. Biochem. Biophys*. 414: 211–222.

Porra R. J., W. A. Thompson and P**. E. Kriedemann**. 1989. 'Determination of

accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy '. *Biochim Biophys Acta*; 975:384–94.

Qing Zhu L., L. Chao Han, Y. Xian Chang and **S. Qing Hua.** 2011. 'Gibberellin A3 pretreatment increased antioxidative capacity of cucumber radicals and hypocotyls under suboptimal temperature '.*Afr. J Agric Res.* 6(17): 4091-4098.

Schmid, J., P.W Doerner, S.D. Clouse, R.A., Dixon and **C.J. Lamb**.1990. 'Developmental and environmental regulation of a bean chalcone synthase promoter in transgenic tobacco '. *Plant Cell* 2: 619–631.

Souter, M., J. Topping, M. Pullen J Friml, K. Palme, R. Hackett, D. Grierson and **K. Lindsey**. 2002.Hydra mutants of Arabidopsis are defective in sterol profiles and auxin and ethylene signaling '. *Plant Cell* 14: 1017–1031.

Sun, J. M., V. A. Spencer, H.Y.Chen, , L. Li and **J.R. Davie**. 2003. 'Measurement of histone acetyltransferase and histone deacetylase activities and kinetics of histone acetylation '. *Methods* 31: 12–23.

Tatiana Z, K. Yamashita and **H. Matsumoto** . 1999. 'Iron deficiency induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber roots '. *Plant Cell Physiol* 40:273–280.

Zhang ZL. 1990. 'Guide to plant physiology experiments'. Beijing: Higher Education Press; China.