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Exogenously Treated Carnitine Upregulates the Contents of Macro and Micro-elements in the Leaves of Maize (*Zea Mays* Cv. Hido) Seedlings

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Abstract

Carnitine, a common compound in living organisms, is involved in a number of metabolic functions from lipid metabolism to defense metabolism. The objective of this study was, for the first time, to investigate the possible modulating role of exogenous carnitine application on inorganic composition of plants. 11-d-old maize plants were sprayed with 10, 25, and 50 µmol L^{-1} concentrations of carnitine. The seedlings were harvested at the end of 4th days after treatment of carnitine solutions and the concentrations of macro (magnesium (Mg), phosphorus (P), potassium (K), and calcium (Ca)) and trace microelements (boron (B), aluminum (Al), manganese (Mn), iron (Fe), cobalt (Co), copper(Cu), zinc (Zn), sodium (Na), selenium (Se), aluminum (Al), beryllium (Be), nickel (Ni), titanium (Ti), chrome (Cr), and barium (Ba)) of maize leaves were determined using ICP-MS spectroscopy technique. At all the concentrations treated, carnitine significantly augmented the concentrations of B, Mg, Al, P, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, and Se as compared with control plants; however, drastic decline was recorded at the concentrations of Na. On the other hand, the Ba concentrations were not significantly affected by carnitine applications. The maximum changes in the concentrations of elements studied were obtained at 25 µmol L^{-1} carnitine-treated seedlings. Taken together, the present results indicate that carnitine significantly affects inorganic composition of maize leaves by altering the concentrations of macro and microelements. These results will be used to better understand mode of action of carnitine on plant metabolism.

Keywords: Carnitine; ICP-MS; Maize; Macro Element; Trace Element

Introduction

Carnitine (3-hydroxy-4-N-N-N-trimethylaminobutyrate), which belongs to the group of quaternary ammonium compounds, plays a fundamental role in animal and fungal energy metabolism by esterifying fatty acids to acetate and thus mediates fatty acids to incorporate intra-mitochondrial respiration via beta-oxidation. Moreover, it is involved in different metabolic functions, such as modulation of intra-mitochondrial CoA/Acyl-CoA ratio and binding/removing acyl residues deriving from amino acid metabolism in animals, yeast, and bacteria. It is also a compound having osmoprotectant and antioxidant properties [1].

In 1951, the presence of carnitine in plants was first shown using Tenebrio worm bioassay in corn and wheat germs [2]. Since then, a number of studies have been published showing presence and amount of carnitine in different plant tissues. These studies revealed that carnitine is present in various plant tissues and their levels are approximately one thousand times less than animal tissues.

On the other hand, the determining presence of acylcarnitines and carnitine acyltransferase activities in plant tissues suggested that as in animals, in plants carnitine could play a role in lipid metabolism and mitochondrial and peroxisomal fatty acid β-oxidation process. In the following years, carnitine has been shown to play crucial role fatty acid trafficking and peroxisomal β-oxidation process in plants; however, its role on mitochondrial β-oxidation process in plants remains still controversy [3]. A recent study regarding effects of carnitine in plants is shedding new light on the relationship between fatty acid metabolism and mitochondrial respiration. The study reported that exogenous application of carnitine increased the transport of fatty acids into mitochondria and stimulated mitochondrial respiration in maize seedlings grown under normal and cold conditions [4].

Carnitine is also known to confer tolerance to plants against environmental stresses, such as salt stress, drought stress, and cold stress [1]. In these studies, the mitigating effect of carnitine against different stress factors was mainly attributed to its stimulating property on antioxidant system and modulatory role on abscisic acid pathway [5-7]. In our prior study, we found that when

applied exogenously, carnitine improved growth and cold tolerance of maize seedlings by modulating photosynthesis and nitrogen assimilation pathways as well as stimulation of antioxidant system [8]. However, it is evident from the literature that there is no research on the effect of carnitine on inorganic element content of plants.

The physio-biochemical and molecular alterations occurring in plants-treated with exogenous substances are intensively investigated; however, the alterations occurring in composition and concentration of inorganic elements are unknown in most cases. Plants need to essential nutrients as well as water, air, suitable temperature, and light in order to sustain their lives. Essential nutrients are divided into two groups according to their required quantity by plants. Micro or trace elements (boron (B), manganese (Mn), iron (Fe), copper(Cu) [9], zinc (Zn), nickel (Ni), and chlorine (Cl)) are those that are required by plants in only small amounts, and macro elements (magnesium (Mg), phosphorus (P), potassium (K), and calcium (Ca), sulphur (S)) which are demanded in relatively high levels [10-12]. In addition to essential elements, various elements (e.g. selenium (Se), aluminium(Al), beryllium (Be), cobalt (Co), chrome (Cr), titanium (Ti), barium (Ba), sodium (Na)) is also present in plant structure and involves in specific metabolic pathways [13-15]. Although microelements are present in plants at rates of only 10-3-10-5 times the amount of macro elements, they are nevertheless equally essential for normal plant growth. It is well-documented that there is a tight connection between inorganic elements and metabolic reactions. They play structural, electrochemical, and catalytic roles in a variety of metabolic processes [10,14]. Therefore, even small alterations in element content can directly affect plant metabolism.

Taking into account the vital importance of inorganic elements on plant metabolic processes and multifarious effects of carnitine on plant metabolism, it is the first time the present study aimed to determine whether exogenous carnitine application has an affect the concentrations of macro and microelements in maize leaves.

Materials and Methods

Experimental Conditions and Treatments

Maize seeds (Zea mays cv. Hido) were surface-sterilized with 96% ethanol for a short time and 5% sodium hypochlorite for 5 min and rinsed with running tap water and finally with few times distilled water. After sterilization, the maize seeds were germinated on moist filter paper containing pure water in a germination cabinet in dark at 25 °C for 2 d. Afterwards, the germinated uniform seedlings were selected and transferred to a hydroponic system containing Hoagland solution and were grown in day/night temperature of 25–27/20–22 °C using a 14/10 h light/dark cycle at 450 μmol quanta m^{-2} s^{-1} in a climate cabinet under relative humidity of 60/70% for 9 d more. Eleven-day-old maize seedlings were divided into four different groups (I. Control, II. 10 μmol L^{-1} carnitine, III. 25 μmol L-1 carnitine, and IV. 50 μmol L^{-1} carnitine). The solutions were prepared by dissolving L-Carnitine hydrochloride (Sigma Aldrich, ≥97.0% (HPLC)) in high pure water. While control group were sprayed with distilled water, the other groups were sprayed with different carnitine solutions. The treated plants were grown at the same conditions for more 4 days and harvested for further analyses.

Preparation of Plant Samples and Inorganic Element Analysis

The leaves of maize seedlings were oven-dried at 68 °C for 48 h. Then the dried samples were ground with liquid N_2 in a mortar for microwave digestion procedure. Milestone Ethos up digestion system was used to prepare the samples. The specific procedure for maize leaves was selected and 0.5 g samples were weighed into Teflon vessels. Then nine milliliters of HNO_3 (65%) and one milliliter H_2O_2 (30%) were added into samples. After that, all covered vessels were placed into the device and microwave digestion operation was performed for 35 min at 210 °C. After digestion, all samples were transferred into the sterile flask and completed with forty milliliters of ultra-pure water. All materials were cleaned with 5% HNO_3 before using for analysis.

Concentrations of B, Mg, Al, P, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Na, Ba and Be were determined with inductively coupled plasma mass spectrophotometry (ICP/MS; Agilent 7800, Japan). All standards and solutions were obtained from Merck.

Statistical Analysis

The experiment was randomly designed with three independent experiments. To compare significant differences between the samples was used the analysis of variance (ANOVA). Statistical significance was defined as $p < 0.05$ (Duncan's multiple range method). The statistical analyses were performed using SPSS 20.0. In addition, the results were evaluated by principal component analysis (PCA) to comprehensively explain the impact of carnitine applications on the concentrations of inorganic elements. For PCA, XLSAT 2019 software was used to examine the correlation between plant response and application groups.

Results and Discussion

Plants need to mineral elements, which are indispensable for plant growth, productivity, and water relations, in various amounts depending on developmental stage and environmental conditions [16]. We found that all the tested concentrations carnitine significantly enhanced the concentrations of macro elements (K, P, Ca, and Mg) in maize leaves in comparison to their controls (Table 1). The highest concentrations of macro elements were determined as 97630 mg. kg^{-1} for K, 20999 mg. kg^{-1} for P, 1084 mg. kg^{-1} for Ca, and 4796 mg. kg^{-1} for Mg, respectively, at 25 μmol L^{-1} carnitine-treated plants. Whereas, macro element contents of control plants were only 77859 mg. kg^{-1} for K, 16994 mg. kg^{-1} for P, 852 mg. kg^{-1} for Ca, and 3922 mg. kg^{-1} for Mg, respectively. The increase rates were 25, 24, 27, and 22% for K, P, Ca, and Mg, respectively, at 25 μmol L^{-1} carnitine-treated plants in comparison to their controls.

Macro elements (mg. kg^{-1})				
	Potassium	**Phosphorus**	**Calcium**	**Magnesium**
Control	77859^{c}	16994^{c}	852^{c}	3922^{c}
10 µmol L^{-1} carnitine	89598^{b}	18859^{b}	956^{b}	4343^{b}
25 µmol L^{-1} carnitine	97630^{a}	20999^{a}	1084^{a}	4796^{a}
50 µmol L^{-1} carnitine	88042^{b}	18695^{b}	982^{b}	4296^{b}
Different letters in the same group indicate statistically significant differences ($p<0.05$)				

Table 1: Effects of carnitine applications on macro element concentrations in 15-day-old maize leaves

K, the most abundant cation in plants, has a number of vital roles on metabolic pathways, including sugar and nutrient transportation, photosynthesis, protein synthesis, enzyme activation, and crop quality [13,14,17]. In addition, it has also a significant effect on the regulation of osmotic potential and stomatal movement. In our prior research, we found that carnitine application exhibited a significant effect on the photosynthetic activity by improving leaf surface area, stoma aperture, chlorophyll content, and activity and gene expression of Rubisco in maize seedlings grown under normal and cold conditions [8]. It is likely to say that carnitine applications stimulated biosynthesis reactions and transportation of related-products by increasing K concentration in leaves, and thus resulted in an increase in growth parameters, photosynthetic indices, and cold tolerance of maize plants. Moreover, cells have a Na/K balance. If this balance changes in the favor of Na, a K deficiency happens in plants. Na, not an essential element, is toxic to plants at higher concentrations [18,19]. We determined that Na concentration drastically decreased at carnitine-applied plants as compared to control plants (Table 3). The lowest content of Na was detected in 25 µmol L-1 carnitine-treated plants where the highest content of K was detected. This means that carnitine applications changed Na/K ratio in the favor of K and prevented excessive accumulation of Na in the leaves [15,20].

Another essential macro element, P plays many important roles in metabolism [21,22]. It is the structural unit of nucleic acids, sugar phosphates, ATP, and NADP, and is indispensable many of energy transfer processes [10,14]. As similar to increase in K content, carnitine-induced increase in P content may contribute to plant growth promotion by enhancing biosynthesis and energy reactions (Table 1). In our prior study, we found that carnitine application stimulated mitochondrial respiration as well as a number of metabolic processes in the leaves of maize seedlings grown under normal and cold conditions [4]. Similarly, carnitine applications resulted in marked elevations Ca and Mg contents in comparison to their controls (Table 1). It is well-documented that while Ca is required for maintaining normal function of plant membranes, Mg participates in many physiological and biochemical reactions related to plant growth and development [17,20]. In our prior study, we determined that carnitine application markedly decreased cold-induced elevations in levels of membrane damage indicators such as electrolyte leakage and lipid peroxidation [8]. Also, carnitine altered percentage of saturated and unsaturated fatty acids in membranes in favor of unsaturated fatty acids to protect cellular membranes from damage caused by cold stress. Thus, one the one hand, carnitine application protected the structure and integrity of membranes, on the other hand, reduced ROS level. It is highly likely that carnitine maintained low ROS level by decreasing the formation of ROS through the Ca-induced membrane stabilization process. Mg is known as a constituent of chlorophyll and has great impact on chloroplast enzymes. In our prior study, we found that carnitine application increased chlorophyll content and gene expression and activity of Rubisco [8]. These means that carnitine supports plant growth by exhibiting a synergistic effect on the contents of macro elements in the leaves.

On the other hand, carnitine applications gave rise to significant increases in the concentrations of essential and non-essential microelements when compared with their controls. The content of essential microelements (B, Mn, Fe, Ni, Cu, and Zn) is 13 to 38% higher for carnitine-treated plants than for the control (Table 2). These increases in microelement contents were consistent with elevation in the concentrations of macromolecules. The importance of microelements should not be neglected although they are needed in minor quantities. Any deficiency of an element, no matter how small the amount needed, it will retard plant growth and development. For most microelements the determination of their concentration in the leaf tissue gives a clear indication of their status in the plant. It is well-known that Mn, Cu, Fe, Zn and Ni activate a lot of enzymes as a co-factor. Besides, while Zn helps binding enzymes to substrates and participates in nucleic acid metabolism and protein synthesis, Fe is a necessary element for chlorophyll formation, protein biosynthesis, and starch content [14]. These data put forward that carnitine upregulated the inorganic element content and thus contributed to plant growth promotion. It is possible that it carried out this alteration by affecting uptake, distribution and replacement of inorganic elements, while they lead to faster growing of plants. As in stated above, Fe, Cu, Zn, and Mn act as a cofactor in the activation of oxidation-reduction enzymes including SOD and CAT. The increased concentration of these elements was compatible with the high activities of antioxidant enzymes [8]. Dumlupinar et al. [10] reported that plants have a distributing and replacing effect on inorganic element content in order to improve their hardiness against environmental factors. In literature, there are numerous studies showing that microelements increase growth and yield of plants and effect macro element balance. For example, B can affect the absorption of N, P, and K, and its deficiency can alter the optimal equilibrium of those three macro elements [21,22].

Essential microelements (mg. kg^{-1})						
	Boron	**Manganese**	**Iron**	**Nickel**	**Copper**	**Zinc**
Control	10.4^{d}	80.8^{c}	51.6^{c}	2.17^{b}	8.3^{c}	84.1^{c}
10 µmol L^{-1} carnitine	11.8^{c}	94.6^{b}	61.9^{b}	2.68^{a}	9.4^{b}	99.3^{b}
25 µmol L^{-1} carnitine	14.3^{a}	110.7^{a}	70.9^{a}	2.66^{a}	10.5^{a}	116.4^{a}
50 µmol L^{-1} carnitine	12.8^{b}	111.5^{a}	63.1^{b}	2.60^{a}	9.6^{b}	97.7^{b}
Different letters in the same group indicate statistically significant differences ($p<0.05$)						

Table 2: Effects of carnitine applications on essential microelement concentrations in 15-day-old maize leaves

Non-essential elements also have many important roles in plant metabolism. We determined that the amount of Be, Ti, Cr, Se, and Co (except Ba and Na) exhibited a marked elevation from 14 to 69% in carnitine-treated plants than for the control (Table 3). Carnitine- induced increase in Se content can contribute to improvement of nitrogen assimilation by increasing activity of nitrate reductase. Furthermore, Se, an important component of the antioxidant system, may protect plants from various forms of oxidative stress [23]. Increasing Al concentrations may stimulate root growth and constant nutrient absorption in plants [24]. Co, a transition element, is an essential component of several enzymes and co-enzymes involved in growth and metabolism of plants. While Cr and Se are toxic for plants at higher concentrations, the lower concentrations may stimulate plant growth and development [23,25]. Ti can increase crop performance by stimulating the activity of some enzymes, enhancing chlorophyll content and photosynthesis, promoting nutrient uptake, strengthening stress tolerance, and improving crop yield and quality [26]. In this study, the recorded increases in the contents of non-essential elements suggest that carnitine modulates plant metabolism by stimulating the concentrations of non-essential elements as well as essential macro- and microelements. These increases of non-essential element concentration were harmony with the increased plant growth (root and plant length, total protein content and dry weight), photosynthetic processes (leaf surface area, chlorophyll content, sucrose content, Rubisco activity and aperture of stomata) nitrogen metabolism (Nitrate reductase, Glutamine synthase and Glutamate synthase), stress tolerance and antioxidant system (Superoxide dismutase, Guaiacol peroxidase, Catalase, Ascorbate peroxide and Glutathione reductase) in maize seedlings grown under normal and cold conditions [8].

Essential microelements (mg. kg^{-1})								
	Beryllium	**Sodium**	**Aluminum**	**Titanium**	**Chrome**	**Selenium**	**Barium**	**Cobalt**
Control	0.06^{c}	120^{a}	46.3^{c}	1.69^{d}	1.32^{d}	0.17^{c}	0.16^{a}	0.25^{d}
10 µmol L^{-1} carnitine	0.09^{b}	106^{b}	54.6^{b}	1.92^{b}	1.54^{c}	0.21^{b}	0.17^{a}	0.37^{b}
25 µmol L^{-1} carnitine	0.11^{a}	89^{c}	63.9^{a}	2.12^{a}	1.87^{a}	0.26^{a}	0.16^{a}	0.45^{a}
50 µmol L^{-1} carnitine	0.09^{b}	103^{b}	56.2^{b}	1.89^{c}	1.70^{b}	0.22^{b}	0.16^{a}	0.32^{c}
Different letters in the same group indicate statistically significant differences ($p<0.05$)								

Table 3: Effects of carnitine applications on non-essential microelement concentrations in 15-day-old maize leaves

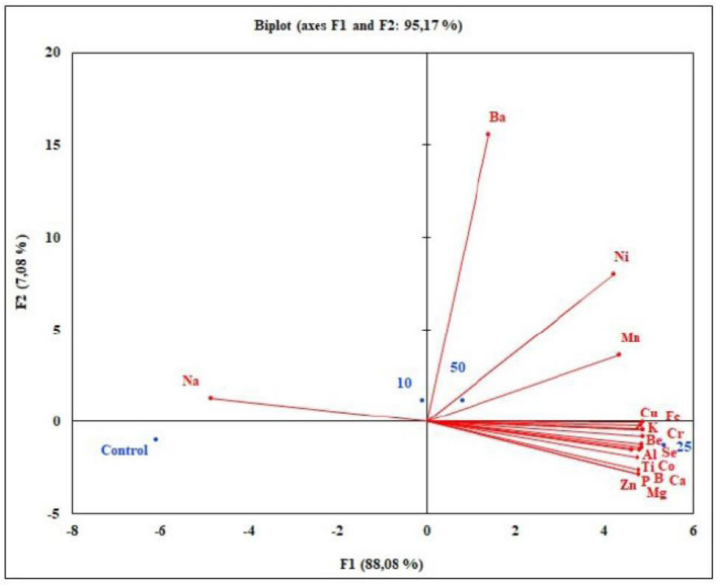

Figure 1: A biplot-based principal component analysis (PCA) with first two principal components for macro- and microelement concentrations of maize leaves treated with carnitine applications. (10, 10 µmol L^{-1} carnitine; 25, 25 µmol L^{-1} carnitine; 50, 50 µmol L^{-1} carnitine)

The effects of individual macro and microelements are discussed separately but important interactions happen between elements. Thus, in addition to increase in element contents, it is very important to maintain a balance between the concentrations of the different elements for an efficient plant growth. We also carried out principal component analysis (PCA) to see the correlation of plant response regarding the concentrations of macro and microelements to carnitine applications. The combined PCA accounts for 95.17% variation for all elements. All applications exhibited almost similar response with carnitine applications, thus, arranged in same axes in every PCA analysis. A significant correlation of macro- and microelements in response to carnitine applications was observed in integrated PCA, which was supported by both macro- and microelement plots individually (Figure 1). The most effective concentration of carnitine was observed as 25 μmol L^{-1}. As compared with the other elements, while Na exhibited a negative correlation, Ba was markedly separated. These findings were consistent with the changes in individual element concentration.

Conclusion

In conclusion, our data revealed that carnitine has a significant effect on the concentrations of inorganic elements in plants. This finding will provide an important contribution to the revealing of effect mechanism of carnitine on plant metabolism.

Author's Contribution

Dr. H. Turk designed the experimental framework and performed all the experiments, the statistical analysis of data, and the paper writing.

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