

Effects of Exogen Lipoic Acid on the Mineral Compositions of Maize

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Abstract

This study, which does not aim to research the possible modulating role of exogenous lipoic acid supplementation on the inorganic composition of the root, coleoptile and endosperm of germinated maize, is the first one in the literature. Maize seeds were germinated with lipoic acid at concentrations of 10, 15, 25 and 30 $\mu\text{mol L}^{-1}$ for 4 days as defined in the material method section for the study. Macro elements (magnesium (Mg), phosphorus (P), potassium (K) and calcium (Ca)), the concentrations of essential and nonessential micro elements (boron (B), manganese (Mn), iron (Fe), nickel (Ni), copper(Cu), zinc (Zn), beryllium (Be), aluminum (Al), selenium (Se) and molybdenum (Mo)) were analyzed using ICP-MS technique in root, coleoptile and endosperm of maize plant. When roots and coleoptiles treated with all lipoic acid applications compared with control groups, an important decrease was determined in endosperms (except for Mn) while a significant increase was recorded in Mg, P, K, Ca, B, Mn, Fe, Ni, Cu, Zn, Be, Se, Mo and Al contents (except for Al in coleoptile groups). When taking account of the maximum changes in the concentrations of the analyzed elements, the best results were obtained in the application of 25 $\mu\text{mol L}^{-1}$ lipoic acid. It can be said that the application of lipoic acid significantly affects the inorganic composition of the plant by increasing the inorganic element contents in maize through its transport from the endosperms to the roots and coleoptiles when all the results are considered together.

Keywords: Lipoic Acid, ICP-MS, Inorganic Element Contents, Maize, Germination.

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1. Introduction

Lipoic acid (LA; 6,8-thiolic acid or 1,2-dithiolane-3-pentanoic acid) is a sulphur-containing 8-carbon disulphide molecule which is in charge with energy production and metabolism, with important functions in pyruvate and glycine metabolism and it also plays a role as a cofactor of some enzymes (pyruvate dehydrogenase complex and glycine decarboxylase complex, and α -ketoglutarate dehydrogenase) (Gueguen et al., 2000; Packer and Cadenas, 2010). There are two forms of lipoic acid, LA and DHLA, and these forms can be easily transformed into one another by oxidation-reduction reactions (Karaca, 2008).

In addition to its role in energy metabolism mentioned above, in both LA and DHLA in-vivo and in-vitro conditions, it chelates metal ions by reducing the oxidized forms of vitamin C, glutathione and tocopherol molecules, detoxify free radicals, repairing oxidized proteins, as well as exhibit a unique antioxidant effect that can inhibit lipid peroxidation (Gorcek and Erdal, 2015; Sears, 2013; Turk et al., 2018). Due to the fact that the LA molecule has a dithiol ring, both oxidized and reduced forms of LA show strong redox activity, this situation makes it unique compared to other antioxidants (Navari-Izzo et al., 2002;Gorcek and Erdal, 2015; Yildiz et al., 2015; Turk et al., 2018). In addition, the only antioxidant property of LA and DHLA forms of lipoic acid, which can be easily soluble in both aqueous and lipid environments, facilitates its effect on both the cytoplasm and the cell membrane. Although numerous studies have been conducted on the effects of LA on the treatment of various metabolic pathways and diseases in animals, similar studies on the presence, effect, and function of LA in plants are still in its infancy. In the first studies, the biosynthetic pathway of LA was investigated in the plastid and mitochondria of Arabidopsis thaliana (Yasuno and Wada, 2002), changes in the endogenous content of LA and DHLA in wheat seedlings grown under copper stress and basil seeds exposed to salts stress

were examined (Sgherri et al., 2002; Tarchoune et al., 2013). Another study noted that LA also had a significant effect on proteins associated with basic metabolic processes such as photosynthesis, energy metabolism, signal conduction, and stress defense (Yildiz et al., 2015). In other studies, in addition to the antioxidant properties of LA, it has also been proven to have the ability to chelate metal ions (Sgherri et al., 2002). Turk et al. (2018) investigated the antioxidant effects of lipoic acid in wheat seedlings germinated under lead stress. In addition to the effects of lipoic acid on the antioxidant system, the effect of LA on essential elements in wheat seedlings exposed to salt stress was determined in another study (Gorcek and Erdal, 2015). In a thesis study conducted with germinated maize seeds in recent years, LA's relationship with energy metabolism along with its antioxidant effect has been tried to be explained (Karayel, 2019). When the current literature is examined, the number of studies carried out, especially in the germination phase, which is the first and most important cycle of plants, is quite limited. However, there is no research in the current literature indicating the effect of lipoic acid on the content of inorganic elements of germinated seeds.

Nutrients are one of the important factors in the survival of plants like sufficient water, light, temperature, and air (Turk, 2019). They participate in many metabolic processes such as structural, electrochemical, and catalytic roles in biological organisms like plants. The lack of one or more of these essential elements can extremely affect the life cycle of plants negatively. Plants need 18 basic elements to maintain their growth and development properly. These basic elements are divided into two classes, including macro elements and microelements according to the amount needed by plants. While microelements (boron (B), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), nickel (Ni), and chlorine (Cl)) are vital for plants in very small quantities, excess amounts of them can cause toxic effects on plants. Plants need macroelements (magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), and sulfur (S)) at higher rates than microelements (Dumlupinar et al., 2007; Genisel et al., 2015; Turk, 2019). Minerals play vital roles in increasing plant tolerance, playing a signaling role and ensuring the stability of the cell membrane

(Dumlupinar et al., 2007; Erdal and Dumlupinar, 2011). In addition, minerals take part in synthesis reactions such as photosynthesis and protein synthesis, and also function vitally as the building blocks of DNA, RNA, ATP, NADP, proteins, vitamins and enzymes. (Dumlupinar et al., 2007; Erdal and Dumlupinar, 2011).

When it is considered the significant effects of minerals and LA separately on plant metabolism as a whole in the present study, it was tried to clarify how maize had an effect on macro and microelement concentrations in root, coleoptile, and endosperm during the germination phase of exogenous LA application for the first time.

2. Materials and Methods

Experiment condition, Lipoic Acid applications, and growth analyses

In the present study, maize seed (*Zea mays* cv. Hido) was used. Before planting, the seeds were quickly washed with 96% alcohol and subjected to surface sterilization in 5% sodium hypochlorite for approximately 10 minutes. After the sterilization process, the seeds were thoroughly washed with distilled water. While the control group was shaken in pure water, the application groups were continuously shaken in LA solutions (10, 15, 25 and 30 $\mu\text{M L}^{-1}$ lipoic acid) for 6 hours in room conditions. The solutions were prepared by dissolving L-(±)- α -Lipoic acid (Sigma Aldrich, $\geq 99.0\%$ (titration) powder) in high purity water. After the swelling period, 13 ml of their own solution was added for each group and the 10-swollen maize seeds were placed on Petri dishes containing two layers of filter paper. The seeds were germinated at 25 °C for 4 days in dark conditions. The germinating seeds were harvested on day 4 the root and the cotyledon lengths of maize seedlings were measured by using the centimetric scale.

ICP-MS analysis and sample preparation procedure

Maize roots, coleoptiles and endosperms were oven-dried at 70 °C for 72 hours. The dried samples were then grounded with liquid nitrogen for microwave digestion procedure. Milestone Ethos up Microwave system was used for the digestion process. The specific procedure for maize was chosen and all samples were weighed in Teflon containers to a maximum of 0.5 g.

Next, nine milliliters of HNO₃ (65%) and one milliliter of H₂O₂ (30%) were added into the samples. Then all containers were sealed and placed in the microwave system and conducted digestion process at 210 °C for 35 minutes. After degradation, all samples were transferred to sterile vials and their volume was made up with 40 ml of ultrapure water. The samples were filtered through a 0.45 µm filter and diluted in 2% HNO₃ solution and loaded into the instrument. Concentrations of B, Mg, Al, P, K, Ca, Mn, Fe, Ni, Cu, Zn, Be, Se, and Mo were determined in inductively coupled plasma mass spectrophotometry (ICP / MS; Agilent 7800, Japan) (Turk, 2019). All standards and reagents were obtained from Merck and Agilent.

Before analysis, the quartz, glass materials and cone parts in the whole system were thoroughly cleaned with 5% HNO₃ solution. Before the analysis, the instrument was calibrated with Tune solution. Different seven calibration points were determined for elements at 0, 10, 25, 50, 100, 250 and 500 ppb concentrations. All of the standards were prepared in %2 HNO₃ solution.

Statistical analysis

The study was carried out with 3 independent samples and 3 parallel readings. The analysis of variance (ANOVA) was used to compare significant differences between samples. Statistical analyzes were performed using SPSS 20.0. Statistical significance was defined as $p < 0.05$ (Duncan's multiple range method).

3. Results and Discussion

Plants need essential elements to maintain their productivity and water-mineral balance alongside growth and development (Turk et al., 2018). Microelements are very important for plants although they are needed in very little quantity compared to macroelements. The quantitative decrease in the content of any microelements inhibits or delays the growth and development of the plant, no matter how small the amount needed. In the current study, we found that LA applications (especially 25 µM LA) significantly increased the concentrations of essential microelements (B, Mn, Fe, Ni, Cu and Zn) at the root and coleoptile of maize seeds. Compared to the

control, it was observed that the concentration of Mn increased in endosperms while a decrease happened in the concentration of other microelements. It is known that Mn, Cu, Fe, Zn, and Ni activate many enzymes as cofactors. Zn catalyzes enzyme-substrate binding, participates in nucleic acid metabolism and protein synthesis, and also it may be necessary for chlorophyll synthesis in some plants (Erdal and Dumlupinar, 2011), while Fe is an essential element for chlorophyll production, protein biosynthesis, and starch content (Pazurkiewicz-Kocot et al., 2008). Cu plays a role as a redox cofactor in the basic processes of cellular metabolism such as respiration and photosynthesis, furthermore, like Ni, also participates in hormone signaling, lignin biosynthesis, and oxidative stress (Broadley et al., 2012; Vatansever et al., 2017). Moreover, while Mn and Ni act as cofactors in the activation of defense enzymes including SOD and CAT (Marschner, 1995; Wei Yang et al., 2008; Fabiano et al., 2015; Shahzad et al., 2018). B has a key role in cell division, elongation, and photosynthesis (Ahmad and Prasad, 2011).

There are many studies in the literature showing that microelements affect growth, plant yield, and macroelement balance. For example; B can affect the absorption of N, P, and K, and while a lack of B can change the optimal balance of these three macroelements (Turk et al., 2016) a high concentration of Mn accumulation can prevent the reception and displacement of other essential elements such as Ca, Mg, Fe, and P due to their similarities in ionic radii (Marschner, 2012; Millaleo et al., 2013). In the current study, significant increases were found in B, Cu, Zn, Fe, Mn, and Ni concentrations of 107.07%, 68.71%, 159.80, 102.02%, 78.78%, and 157.33 respectively, compared with control groups in roots treated with 25 µM LA (Table 1a-1b). In coleoptiles, it was determined that the contents B, Cu, Zn, Fe, Mn, and Ni increased significantly in 25 µM LA groups according to control, 56.49%, 74.57%, 95.92%, 266.76%, 66.11%, and 8772, 27% respectively (Table 1a-1b). Unlike root and coleoptile results, 96.63%, 73.69%, 55.27%, 32.69%, and 76.82% reductions were found in B, Cu, Zn, Fe and Ni concentrations respectively, compared to control in endosperms treated with 25 µM LA (Table 1a-1b). Unlike other elements, an increase of Mn content of 77.46% was recorded in endosperms

applied 25 μM LA compared to the control (Table 1a).

It can be expressed that these increases, which occur by transporting from endosperms to the root and coleoptile, contribute to plant growth with cell elongation. These results are similar to increases in root and coleoptile lengths of LA application groups (Figure 1). In the 25 μM LA application, an increase of 30.44% was recorded in root length and 28.21% in coleoptile length compared to control groups (Figure 1). The decrease in Cu content in endosperms can be said to have a positive effect on plant growth, taking into account root and coleoptile length values, as well as being effective in all cellular metabolic processes by moving from here to the root and coleoptiles. The increase of mineral Zn in roots and coleoptiles and the decrease in endosperms support that Zn is transported from endosperms to roots and coleoptiles as in the Cu element. The increase in the determined mineral Fe is very remarkable in terms of the inclusion of various enzymes, proteins and chlorophyll, which are as important for the growth and development of the plant as well as for its survival. This increase is very important in terms of cofactoring the enzymes that play a vital role in the plant, positively affecting lipid and carbohydrate metabolism and triggering plant growth. Besides, it is possible to mention that increases in Fe, Ca, Mg and P contents with Mn (to be mentioned later) are not high in the groups that applied LA and this accumulation is at the demanded level for the plant, since extreme Mn accumulation can prevent the removal and displacement of essential elements such as Ca, Mg, Fe, and P because of similarities in ionic radii (Marschner, 2012; Millaleo et al., 2013).

When the vital importance of the Ni element mentioned above is taken into account, the increase in Ni content is quite important for germinated maize. The increase in this element in all LA groups can contribute to plant growth by positively affecting many vital processes from photosynthesis to stress, endurance and defense, from nitrogen metabolism to plant growth. In this study, it can be stated that essential microelements are transported from endosperms to roots and coleoptiles, and LA contributes to supporting plant growth by increasing microelements content, especially in roots and coleoptiles. It can be said that lipoic acid can achieve this in line with the faster growth of plants by

affecting the intake, distribution, and relocation of inorganic elements

Non-essential elements have also important roles in plant metabolism. Beryllium (Be) is not a major nutrient for higher plants but a low amount of Be can stimulate the growth of certain plant species and also activate certain enzymes (Sajwan et al., 2003). It has also been reported that it is possible to interfere with plant nutrition in line with the inhibition of certain enzymes of Be as well as an antagonism pathway between Be and Ca, Mg, or phosphate (Williams and Le Riche, 1968). In our study, while the Be content decreased by 59.44% compared to the control in endosperms treated with 25 μM LA, it increased by 70.56% in the root and 82.65% in coleoptile (Table 2). Selenium (Se), one of the trace elements, has a toxic effect on plants at high concentrations whereas at low concentrations it has positive effects on plants (Calabrese and Baldwin, 2003). Se is similar to sulfur which is a chemically important plant macronutrient (Cruz-Jimenez et al., 2005). A high rate of Se content suppresses protein synthesis, nucleic acid synthesis, growth and organogenesis (Terry et al., 2000). Se, with affecting the distribution of metals, sometimes also increases the excretion of toxic elements due to its antioxidant properties (Pazurkiewicz-Kocot et al., 2003, 2008). In this study, while an increase in Se content in the root by 38.17% and 163.67% in coleoptile in 25 μM LA application compared with the control, a rate of 40.21% reduction was recorded in endosperm tissues (Table 2). Se accumulation in the root and coleoptile of the plant with LA application may stem from the antioxidant properties of this mineral and its capacity to protect the plant against possible oxidative damage. Aluminum (Al) is the third most abundant element in the earth's crust. In this research, Al content decreased in coleoptile and endosperms but increased in the roots. For increased Al in the roots, we can say that it can stimulate nutrient absorption by promoting root growth in plants. A high rate of Al concentration causes rapid inhibition of plant root growth under normal conditions (Horst et al., 1992; Ryan et al., 1995) damaging the root system, restricting nutrient and water intake (Kochian et al., 2004). In this study, it was determined that in the 25 μM LA application, while Al content in coleoptile and endosperm tissues decreased by 59.17% and 61.43% respectively compared to the control, an increase of 11.30% in the

root (Table 2). The increase in the root length and Al content (Figure 1) shows that Al concentration is not toxic to germinating maize seeds. Because the primary purpose of Al toxicity is the root tips in the distal part of the transition zone between the elongation area and cell division, and in the main binding regions where the root meristem is localized in the cell walls (Sun et al., 2020). In some studies with the tea plant, the increase in root growth and rooting in the presence of Al has a quality that supports our finding (Fung et al., 2008; Mukhopadhyay et al., 2012; Sun et al., 2020). Molybdenum (Mo) is a necessary micronutrient for the growth of many living organisms (Sauer and Frebort, 2003; Kaiser et al., 2005). The main physiological function of molybdenum in higher plants is that an essential component of mononuclear Mo enzymes, which play a vital role in many metabolic processes such as C-, N- and S-cycles in plants (Stallmeyer et al., 1999; Mendel and Haënsch, 2002). In the current study, it was determined that the Mo content decreased by 63.60% in endosperm compared to the control in 25µM LA application but increased by 214.90% and 283.03% in root and coleoptile respectively (Table 2). It can be stated that it has a positive effect on plant growth and development by participating in the structure of enzymes affecting important metabolic processes thanks to the transportation of Mo content from endosperms to roots and cotyledons.

Potassium (K) which is known as the macro element and has essential importance is a cation abundant in plants and it has vital roles in a wide range of metabolic pathways, including the transport of sugar and nutrients, photosynthesis, protein synthesis, enzyme activation, product quality, osmotic potential and regulation of stoma movement (Erdal and Dumlupinar, 2011; Genisel et al., 2012; Turk and Erdal, 2015; Turk, 2019). In a study, it was noted that LA application provided a significant increase in the K content of wheat seedlings grown under normal and salty conditions compared to the control group (Gorcek and Erdal, 2015). In this study, again, it was also noted that lipoic acid had a significant effect on photosynthetic activity by improving the leaf surface area, chlorophyll content and Rubisco activity. In the current study, with 25µM LA application compared to the control, the K content increased significantly by 69.95% in the root and 56.71% in coleoptile while the

endosperm decreased by 31.20% (Table 3). It can be said that increased K content may cause an increase in growth parameters by stimulating biosynthesis reactions and the transportation of related products. Another important macro element, phosphorus (P), has many important roles in plant metabolism (Turk et al., 2016). Since P takes part in the structure of NADP and ATP, it plays very important roles in metabolic events such as photosynthesis, respiration and fatty acid biosynthesis (Dumlupinar et al., 2007; Erdal and Dumlupinar, 2011). P is also a component of DNA and RNA (Taiz and Zeiger, 2003). In the current study, it was recorded a decrease of 25,09% in P content in endosperms applied 25µM LA compared to the control while a 72.09% increase in roots and 61.68% in coleoptiles (Table 3). It can be said that the P content which is vitally important during germination and necessary for energy metabolism can have an influence upon energy and other metabolic pathways, moving from the endosperms of LA applied groups to their roots and cotyledons. In a thesis study on wheat seedlings exposed to salt stress, significant increases in P content with the LA application were observed compared to the control group (Gorcek, 2013). In another thesis study with maize, significant increases were recorded in the gene activities of the enzymes (citrate synthase, cytochrome oxidase, pyruvate dehydrogenase, ATP synthase) which participate in the energy metabolism in LA-applied roots and coleoptiles compared to the control (Karayel, 2019). Based on these results, it can be said that LA application can stimulate mitochondrial respiration and other pathways by increasing the P content at the roots and coleoptiles. Similar to P, endosperms treated with 25 µM LA showed a decrease in calcium (Ca) and magnesium (Mg) contents by 63.07% and 17.23% respectively. Increases of 145.44% and 68.54% were recorded in the root for Ca and Mg, and 30.13% and 65.40% for Ca and Mg in coleoptile respectively (Table 3). Ca is a component of the cell wall and ensures root growth even though the effects of elements on plant metabolism are different from each other. Ca also acts as a secondary messenger against both environmental and hormonal stimuli in addition to its use in the synthesis of middle lamella in newly divided cells in the plant (Sanders et al., 1999). Ca also affects many metabolic processes by binding with the calmodulin present in the cytosol. While Ca

is essential for plant cell membranes to maintain normal function, Mg participates in many physiological and biochemical reactions involved in plant growth and development (Turk and Erdal, 2015; Turk, 2019). Mg, which participates in the structure of chlorophyll, has an important effect on chloroplast enzymes (Mengel et al., 2001). It was recorded that in a study with wheat seedlings grown under normal and salty conditions, LA application increases Ca and Mg and also ensures important increases in total chlorophyll content (Gorcek and Erdal, 2015). In the current study, the decrease in the minerals Ca and Mg in the endosperms and the increase in the roots and coleoptiles indicate that they are transported in these elements as in others. Increases in Ca content support the increase in root and coleoptile length (Figure 1). Considering this situation, it can be expressed that Ca is transported to be used in many processes such as root and coleoptile formation, cell permeability, intracellular communication and osmotic potential. The increase in Mg content in root and coleoptile may suggest that it is carried for use specifically in DNA and RNA synthesis and respiration, as well as it can act as a cofactor for chlorophyll formation and chloroplast enzymes.

4. Conclusion

Considering all these results, LA applications stimulated nonessential element contents as well as the positive changes it created in basic macro and microelement concentrations, and the best results were obtained in the application of 25 μ M LA. It can be stated that the positive effect of lipoic acid on macro and microelements regulates plant metabolism by affecting biochemical processes, thus contributing to the growth and development of the plant. Lipoic acid indicated this effect by transporting macro and microelements from endosperm to root, especially to coleoptiles. This finding will provide a very important contribution in order to illuminate the other metabolic processes lipoic acid connected in addition to understanding its mechanism on plant metabolism at the macro and microelement level.

5. Acknowledgement

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Declaration of competing interest

The author declares no conflict of interest.

CRedit authorship contribution statement

Hulya Turk: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization.

Table 1a. Effects of lipoic acid applications on essential microelement concentrations in 4-day-old maize endosperm, root and coleoptiles

Essential Micro Elements (mg. kg ⁻¹)									
	Boron			Copper			Zinc		
	E	R	C	E	R	C	E	R	C
C	33.87 ^a	21.60 ^e	26.12 ^e	0.74 ^b	6.96 ^e	4.45 ^e	65.13 ^a	65.27 ^e	64.63 ^c
10 LA	17.55 ^b	25.36 ^d	31.40 ^c	0.59 ^c	7.19 ^d	5.14 ^d	53.67 ^b	139.88 ^c	79.11 ^c
15 LA	7.75 ^c	34.09 ^b	35.33 ^b	0.27 ^d	7.41 ^c	5.98 ^b	37.80 ^c	148.05 ^b	108.36 ^{ab}
25 LA	1.48 ^e	44.72 ^a	40.87 ^a	0.19 ^e	11.74 ^a	7.76 ^a	29.14 ^d	169.56 ^a	126.63 ^a
30 LA	6.83 ^d	28.10 ^c	30.24 ^d	1.13 ^a	8.29 ^b	5.72 ^c	56.90 ^b	130.08 ^d	84.68 ^{bc}

Different letters in the same group indicate statistically significant differences (p<0.05)

(C: Control, 10 LA: 10 µmol L⁻¹ Lipoic acid, 15 LA: 15 µmol L⁻¹ Lipoic acid, 25 LA: 25 µmol L⁻¹ Lipoic acid, 30 LA: 30 µmol L⁻¹ Lipoic acid and E: Endosperm, R: Root and C: Coleoptile)

Table 1b. Effects of lipoic acid applications on essential microelement concentrations in 4-day-old maize endosperm, root and coleoptiles

Essential Micro Elements (mg. kg ⁻¹)									
	Iron			Manganese			Nickel		
	E	R	C	E	R	C	E	R	C
C	39.52 ^a	54.38 ^e	55.57 ^e	8.46 ^d	8.75 ^e	13.72 ^d	0.51 ^a	1.71 ^d	0.13 ^e
10 LA	35.01 ^b	68.75 ^d	73.19 ^c	9.71 ^c	9.50 ^d	15.48 ^c	0.47 ^b	1.85 ^c	1.95 ^c
15 LA	30.04 ^d	75.79 ^c	85.40 ^b	12.00 ^b	10.36 ^c	18.44 ^b	0.43 ^c	2.81 ^b	2.99 ^b
25 LA	26.60 ^e	109.85 ^a	205.47 ^a	15.02 ^a	15.65 ^a	22.80 ^a	0.12 ^d	4.40 ^a	11.50 ^a
30 LA	34.50 ^c	77.84 ^b	61.71 ^d	9.86 ^c	11.02 ^b	12.67 ^e	0.42 ^c	1.26 ^e	0.90 ^d

Different letters in the same group indicate statistically significant differences (p<0.05)

(C: Control, 10 LA: 10 µmol L⁻¹ Lipoic acid, 15 LA: 15 µmol L⁻¹ Lipoic acid, 25 LA: 25 µmol L⁻¹ Lipoic acid, 30 LA: 30 µmol L⁻¹ Lipoic acid and E: Endosperm, R: Root and C: Coleoptile)

Table 2. Effects of lipoic acid applications on non-essential microelement concentrations in 4-day-old maize endosperm, root and coleoptiles

Non-Essential Micro Elements (mg. kg ⁻¹)												
	Beryllium			Aluminum			Selenium			Molybdenum		
	E	R	C	E	R	C	E	R	C	E	R	C
C	0.11 ^a	0.06 ^c	0.07 ^{cd}	14.28 ^a	7.85 ^d	9.13 ^a	0.54 ^a	0.72 ^e	0.51 ^e	2.02 ^a	0.81 ^e	0.83 ^e
10 LA	0.07 ^c	0.08 ^{ab}	0.08 ^c	12.74 ^b	12.52 ^c	5.02 ^c	0.47 ^b	0.83 ^d	0.58 ^d	1.34 ^b	1.09 ^d	1.33 ^d
15 LA	0.06 ^c	0.09 ^a	0.11 ^b	9.92 ^c	14.53 ^b	4.23 ^d	0.39 ^c	0.85 ^c	0.89 ^c	0.99 ^d	1.27 ^c	1.75 ^b
25 LA	0.04 ^d	0.10 ^a	0.13 ^a	5.83 ^e	17.15 ^a	3.52 ^e	0.32 ^d	1.00 ^a	1.35 ^a	0.73 ^e	2.57 ^a	3.18 ^a
30 LA	0.08 ^b	0.06 ^{bc}	0.07 ^d	9.43 ^d	16.79 ^a	6.38 ^b	0.43 ^b	0.90 ^b	0.99 ^b	1.17 ^c	1.38 ^b	1.50 ^c

Different letters in the same group indicate statistically significant differences (p<0.05)

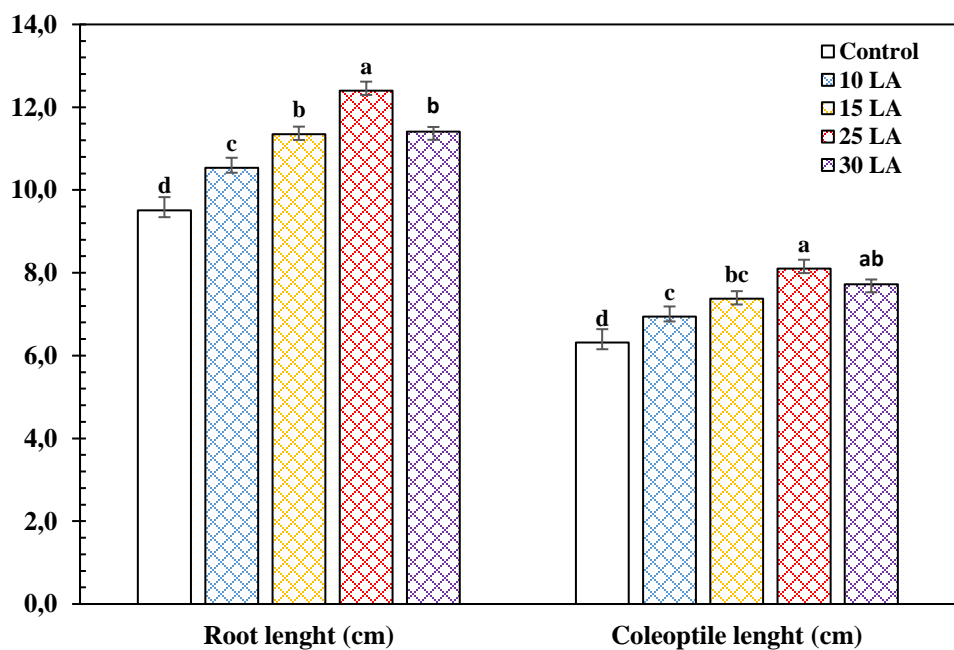
(C: Control, 10 LA: 10 µmol L⁻¹ Lipoic acid, 15 LA: 15 µmol L⁻¹ Lipoic acid, 25 LA: 25 µmol L⁻¹ Lipoic acid, 30 LA: 30 µmol L⁻¹ Lipoic acid and E: Endosperm, R: Root and C: Coleoptile)

Table 3. Effects of lipoic acid applications on macro element concentrations in 4-day-old maize endosperm, root and coleoptiles

Macro Elements (mg. kg ⁻¹)						
	Magnesium			Phosphorus		
	E	R	C	E	R	C
C	1775.38 ^a	1299.32 ^e	1672.38 ^e	4233.60 ^a	6927.87 ^e	8076.71 ^e
10 LA	1657.67 ^b	1419.22 ^d	1772.53 ^d	3856.51 ^b	7198.39 ^d	8716.00 ^d
15 LA	1594.22 ^c	1469.41 ^c	1807.46 ^c	3635.23 ^c	7567.66 ^c	9504.55 ^c
25 LA	1469.46 ^e	2191.29 ^a	2766.10 ^a	3171.29 ^e	11921.94 ^a	13074.92 ^a
30 LA	1503.12 ^d	1623.00 ^b	1853.46 ^b	3364.39 ^d	8219.89 ^b	10195.38 ^b
	Potassium			Calcium		
	E	R	C	E	R	C
C	1869.88 ^a	17164.46 ^e	18121.44 ^e	84.53 ^a	133.54 ^e	73.82 ^e
10 LA	1626.48 ^b	19474.60 ^d	19183.46 ^d	54.48 ^b	147.99 ^d	78.22 ^d
15 LA	1444.30 ^d	20906.10 ^c	22367.04 ^c	50.64 ^c	259.40 ^b	81.02 ^b
25 LA	1286.47 ^e	29170.74 ^a	28398.61 ^a	31.22 ^e	331.78 ^a	96.06 ^a
30 LA	1601.20 ^c	21301.02 ^b	25112.39 ^b	37.02 ^d	199.38 ^c	79.82 ^c

Different letters in the same group indicate statistically significant differences ($p < 0.05$)

(C: Control, 10 LA: 10 $\mu\text{mol L}^{-1}$ Lipoic acid, 15 LA: 15 $\mu\text{mol L}^{-1}$ Lipoic acid, 25 LA: 25 $\mu\text{mol L}^{-1}$ Lipoic acid, 30 LA: 30 $\mu\text{mol L}^{-1}$ Lipoic acid and E: Endosperm, R: Root and C: Coleoptile)

Figure 1. Effects of exogenous lipoic acid supplementations on root and coleoptile lengths in 4-day-old maize seeds

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