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Influence of foliar-applied zinc in the form of mineral and complexed with amino acids on yield and nutritional quality of onion under field conditions

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ABSTRACT

The aim of this field experiment was to investigate the efficiency of foliar-applied zinc (Zn) in the form of mineral ($ZnSO_4$) and complexed with lysine (Lys), methionine (Met), or threonine (Thr) as a strategy to improve yield and nutritional quality of two onion cultivars (*Allium cepa* L. cvs. Behbahan and Perimavera). Different Zn sources and free amino acids (i.e., Lys, Met, and Thr) were applied once at the early rapid vegetative growth stage and once 45 d later at concentration of 0.5%. A control treatment (free of Zn and amino acids) was also used. Plant response to Zn foliar application varied depending on the cultivar and Zn sources. In 'Behbahan', foliar application of Zn from all sources significantly increased bulb yield while in 'Primavera', using $ZnSO_4$ was ineffective. In both cultivars, the highest bulb yield was obtained by Zn-Lys complex. All Zn sources significantly increased Zn concentration in bulb, although the Zn-Lys complex was the most effective one. Foliar application of Zn-Lys complex resulted in a significant reduction of bulb nitrate accumulation while other Zn fertilizer sources had no significant effect on bulb nitrate content. In both cultivars, application of lysine either free or complexed with Zn significantly increased the bulb total soluble solids (TSS) compared with control while $ZnSO_4$ had no significant effect on bulb TSS. In 'Perimavera', all Zn sources increased bulb pyruvic acid concentration while in 'Behbahan', only Zn-Lys and Zn-Met complexes were effective in increasing bulb pyruvic acid concentration. According to the results, Zn-Lys complex can effectively be used as foliar spray for improving yield and quality of onion in Zn-deficient calcareous soils.

1. Introduction

Zinc (Zn) is an essential micronutrient that its deficiency, not only limits plant growth and yield, but also reduces nutritional quality of food crops in many agricultural lands worldwide (Kutman et al., 2011). Alkaline pH, high content of calcium carbonate, low levels of organic matter and excess application of phosphate fertilizers are main reasons for Zn deficiency in calcareous soils of arid and semi-arid regions (Marschner, 1995). Zinc plays roles in production of energy, synthesis of protein, and regulation of plant growth (Ishimaru et al., 2011). There are different approaches to correct Zn deficiency in plants. Among them, the easiest and most economic method is soil and foliar application of Zn fertilizer sources (Khoshgoftarmanesh et al., 2010). Due to low agronomic efficiency of soil applied-Zn, foliar application of Zn fertilizers is recommended as an alternative and effective strategy to correct Zn deficiency in plant (Almendros et al., 2015; Machado and Campos 2013; Khoshgoftarmanesh et al., 2010). Application of Zn as foliar spray can also be effective to achieve the aims of biofortification programs (Almendros et al., 2015; Khoshgoftarmanesh et al., 2010). Mineral sources, mainly $ZnSO_4$, and with a less degree, synthetic chelates of Zn (i.e., ZnEDTA and ZnDTPA) are commonly used for correcting Zn deficiency in soils and plants (Alloway, 2008). Mineral Zn sources may contain cadmium (Cd) and other toxic heavy metals as impurity (Khoshgoftarmanesh et al., 2010). On the other hand, synthetic chelates are expensive and their efficiency for foliar application is relatively low. Due to the large molecular size in comparison with the size of coticule and cell wall pores, it is supposed that the leaf uptake efficiency of Zn from synthetic Zn chelates is much less than $ZnSO_4$ (Marschner, 1995).

Recently, Zn-amino acid complexes have been synthesized as natural and effective sources of Zn for foliar spray applica-

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tion (Ghasemi et al., 2013). The efficiency of foliar application of these complexes in comparison with $ZnSO_4$ has been evaluated for wheat (Ghasemi et al., 2013) and lettuce (Mohammadi and Khoshgoftarmanesh, 2014). Amino acids as natural ligands are able to form relatively stable complexes with metals i.e., Zn via carboxylic groups (Ghasemi et al., 2013). In addition, amino acids have several beneficial effects on plants. Amino acids are structural units of proteins and play a role in biosynthesis of glutamine which is a precursor of nucleoids (Alcázar, et al., 2010), hormones and low molecular-weight nitrogen compounds (Lonnerdal, 2000). Enhanced protein content by application of amino acids has been reported in several plants. For example, foliar application of amino acids enhanced leaf yield and protein content of mulberry (*Morus alba* L. cv. S1) (Das et al., 2002). On the other side, translocation of amino acids is of great importance in plants. In contrast to reduced carbon that is translocated only by phloem, amino acids translocation occurs both in phloem and xylem. Therefore, re-translocation of amino acids helps in nitrogen recycling between roots and shoots and hasten translocation of immobile nutrient elements i.e., Zn in plant (Ortiz-Lopez et al., 2000). Re-translocation of Zn is of great importance for foliar application treatments particularly when Zn translocation to the roots or fruit is targeted.

Onion (*Allium cepa* L.) is the second most cultivated vegetable after tomato (Griffiths et al., 2002). This vegetable contains protein, calcium, vitamins, and ascorbic acid. Additionally, due to antimicrobial and anticarcinogenic properties and high antimutagenic capacity, onion plays an important role in human health. Onion is sensitive to Zn deficiency and significant reduction in yield and quality of this vegetable is expected under Zn deficiency conditions particularly in calcareous soil (Alloway, 2008; Ishimaru et al., 2011). The positive response of onion plant to soil application of Zn fertilizers has been reported (El-Nagerabi and Ahmed, 2003), but limited information is available on the efficiency of foliar application of Zn. Due to low agronomic efficiency of $ZnSO_4$ in some calcareous soils, foliar application is suggested as an alternative strategy to correct Zn deficiency. Therefore, in this field experiment, we tested the effectiveness of foliar applied Zn on yield and selected nutritional quality attributes (concentration of nitrate, Zn and pyruvic acid) of onion bulb. Additionally, the efficiency of $ZnSO_4$ was compared with synthesized complexes of Zn-amino acids.

2. Materials and methods

2.1. Synthesis and characterization of Zn-amino complexes

The complexes of Zn with lysine (Lys), methionine (Met) and threonine (Thr) were synthesized according to the procedure described by Ghasemi et al. (2013). Briefly, 2 mmol of each amino acid were dissolved in distilled water and mixed with 1 mmol $Zn(OAc)_2$ solution. Elemental analysis of the complexes was performed using a CHNS elemental analyzer (Perkin-Elmer 2400) and atomic absorption spectrometry (Model 3400, PerkinElmer, Wellesley, MA).

2.2. Experimental site and soil properties

A field study was conducted in growing season 2014–2015 in Agricultural Research Station at Behbahan (30, 36/N, 50, 14/E), Iran. The mean monthly maximum and minimum temperatures at the site were 43.16 °C (on June) and 4.33 °C (on December), respectively. The total annual precipitation during the growing season was 356.6 mm, all of which falling between November and May.

Soil cores (0–30 cm depth) were taken from the experimental location. The samples were air-dried, ground, sieved to less than 2-mm and used for chemical analysis. Soil pH was measured in 1:2.5 (w/v) soil:water suspension using a digital pH meter (Model 691, Metrohm AG Herisau Switzerland). Total nitrogen (TN) was determined using the Kjehldal method (Bremner and Mulvancy, 1982). Available phosphorus (P) was measured as described by Olsen and Sommers (1982). Soil Zn was extracted with diethylene triamine pentaacetic acid (DTPA) using the method of Lindsay and Norvell (1978) and analyzed by means of an atomic absorption spectrophotometer (Model 3030) from Perkin Elmer (Wellesley, MA). The soil of the experimental plots was classified as Aridic Calciustepts (US Soil Taxonomy). The experimental soil was calcareous (0.53 $g\,g^{-1}$ $CaCO_3$) with a pH of 7.7. Electrical Conductivity of the soil was 1.2 $dS\,m^{-1}$. Organic carbon and total N content of the soil was 7.6 and 1.0 $g\,kg^{-1}$, respectively. The available P and K were 6.8 and 237 $mg\,kg^{-1}$, respectively. The DTPA-extractable Zn in the experimental soil was 0.45 $mg\,kg^{-1}$. In comparison with the critical deficiency level for (1.0 $mg\,kg^{-1}$ dry soil) given by Mortvedt (1985), the soil was deficient in available Zn. Seeds of two onion cultivars (*Allisum Cepa* L. cvs. Behbahan and Perimavera) were grown in a nursery. The two cultivars are the most common onion cultivars grown in Khuzestan province, Iran. Two-leaf seedlings were transplanted in the experimental plots early October 2014. The size of each experimental plot was 12.5 m^2. The plants were planted in 6 rows with 4-m length and 50 cm spacing.

Before planting, soil in experimental plots was plowed by a chisel and nitrogen, phosphorus, and potassium fertilizers were applied at the recommended by Iranian Soil and Water Institute (Bybordi and Melekoti, 1999). Phosphorus was applied as triple super phosphate (150 kg P ha^{-1}), potassium in form of sulfate (200 kg K ha^{-1}) and nitrogen as urea at a rate of 90 kg N ha^{-1} at 3 times, one-third before planting, one-third 45 d after planting, and the rest at the early stage of bulb formation.

The crops were irrigated to keep soil moisture at approximately 70% field capacity, using basin irrigation method. Irrigation rates were based on evapotranspiration data collected at the local weather station of Behbahan. The irrigation water had an electrical conductivity (EC) of 1.8 $dS\,m^{-1}$.

Different Zn sources including $ZnSO_4$ and three synthesized Zn-amino acid complexes i.e., Zn-Lys, Zn-Met, and Zn-Thr were applied as foliar spray once at the fast growth stage and once 45 d later. Zinc concentration in all foliar-applied Zn solutions was 0.5% (w/v). To separate the effect of Zn and accompanying amino acids, blank treatments were used in which foliar application of free amino acids Lys, Met and Thr was performed with similar method mentioned for Zn treatments. In all treatments, the volume of applied solution was 1000 l ha^{-1}. Foliar application was performed early morning to inhibit possible leaf damage caused by salts on sunlight or high temperature. A control treatment free of Zn and amino acids was also used. According to the preliminary experiments, Lys, Met, and Thr form stable and soluble complexes with Zn.

The time of foliar application was selected by estimation of the fast growth stage of each onion cultivar. The fast growth stage of each onion cultivar was determined based on the growth degree days (GDD). The critical accumulative temperature for the fast growth of 'Perimaver' and 'Behbahan' was 1133.6 and 1103.2 GDD, respectively (Darabi, 2009). The GDD was calculated as following (Darabi, 2009):

$$GDD = \sum_{i=1}^{n} \left[\frac{T_{MAX} - T_{MIN}}{2} - T_b \right]$$

T_{MAX}, T_{MIN}, are maximum and minimum temperature, respectively; T_b is the base temperature (5.9 °C) and 'n' represents the number of days with temperatures higher than 5.9 °C.

Plants were harvested at the physiological maturity stage (early June 2015). At harvest, the neck of 50–80% of the plants were