Scientia Horticulturae 186 (2015) 254–265

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Biofortification of zinc in onions (*Allium cepa* L.) and soil Zn status by the application of different organic Zn complexes

Patricia Almendros*, Ana Obrador, Demetrio Gonzalez, Jose M. Alvarez

Department of Chemistry and Agricultural Analysis, College of Agriculture, Technical University of Madrid (UPM), Ciudad Universitaria s.n., 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 8 October 2014
Received in revised form 3 February 2015
Accepted 16 February 2015
Available online 23 March 2015

Keywords:
Agronomic Zn biofortification
Zn concentration
Zn uptake
Transfer factor
% Utilization

ABSTRACT

A pot experiment was conducted to determine the influence of different natural and synthetic organic Zn complexes on the agronomic biofortification of onion (*Allium cepa* L.) in two types of soil: $Soil_{acid}$ (weakly acidic) and $Soil_{calc}$ (calcareous). Eight different organic Zn complexes were administered at two different Zn application rates. We determined the Zn distribution in different soil fractions and the Zn availability, pH and redox potential in soils with Nil-Zn and Zn treatments. The effectiveness of Zn sources in onion was assessed in terms of total Zn concentration, soluble Zn concentration, plant biomass and chlorophyll and carotenoid contents. Beneficial effects of Zn on onion response (DM plant biomass, total and soluble Zn concentration, utilization of applied Zn, etc.) were observed, with significant increment in the determined plant parameters, in comparison with the Nil-Zn treatment. The rates of increase varied among sources depending on the soil. Applications of Zn-aminolignosulfonate (Zn-AML) at the rate of 10 mg Zn kg^{-1} in $Soil_{acid}$ and of Zn-DTPA-HEDTA-EDTA at the rate of 10 mg Zn kg^{-1} in $Soil_{calc}$ produced the highest Zn and soluble Zn concentrations in plants. In general, the highest concentrations of Zn in labile forms and of potentially available Zn concentrations in both soils were associated with the application of the sources that contained Zn chelated by EDTA and/or DTPA. Zinc source and soil characteristics had a great influence on agronomic Zn biofortification in onions and therefore also on the quality and plant biomass of the crop. Applying Zn organic complexes to an onion crop improved not only productivity, but also Zn concentrations in onion.

1. Introduction

Onion (*Allium cepa* L.) is a high-value crop (Sullivan et al., 2001) and the second most important horticultural crop after tomato (Griffiths et al., 2002). It is widely used as a flavoring vegetable in different types of food. This crop is a source of vitamins and is valued for its antimicrobial and antioxidant activity, anticarcinogenic and antimutagenic properties and protection against cardiovascular disease (Corzo-Martinez et al., 2007; Stajner and Varga, 2003).

Zinc is essential in plant nutrition and is a fundamental component of various enzyme systems, in which it contributes to energy production, protein synthesis and growth regulation (Mortvedt, 2014; Ngole and Ekosse, 2009). A study conducted by the Food and Agriculture Organization (FAO) showed that Zn deficiency is the most common micronutrient deficiency and affects a wide range of soil types in many different agricultural areas (IZA, 2014).

Onion is a species which is particularly sensitive to Zn deficiency, with a relatively high responsiveness to Zn (Alloway, 2008; ILZRO, 1975; Mortvedt, 2014; Viets et al., 1954). Various authors have reported that applications of Zn significantly increase onion biomass, plant growth, Zn concentration and bulb quality (Gamelli, 2000; Maurya and Lal, 1975; Phor et al., 1995; Satbir et al., 1989).

Zinc deficiency is also a common nutritional problem in humans (Cakmak et al., 1999). Low levels of Zn in food crops are thought to be responsible for human Zn deficiency (Hotz and Brown, 2004). Zinc is an essential micronutrient for various functions in the

Abbreviations: CAR, carbonate bound; DM, dry matter; Eh, redox potential; EXC, exchangeable bound; FAAS, flame atomic absorption spectrophotometry; FeO_x, iron oxides bound; FM, fresh matter; Zn-HEDTA, [Zn-*N*-2-hydroxyethylethylenediaminetriacetate]; LMWOAs, low molecular weight organic acids; MES, [2-(*N*-morpholino)ethanesulfonic acid]; MnOx, Mn oxides bound; OM, organic matter bound; RES, residual; TEA, triethanolamine; TF, transfer factor; WS, water soluble bound; Zn-DTPA, Zn-diethylenetriaminepantaacetate; Zn-DTPA-HEDTA-EDTA, Zn-diethylenetriaminepentaacetate-*N*-2-hydroxyethylethylenediaminetriacetate-ethylenediaminetetraacetate; Zn-EDDHSA, Zn-ethylenediamine-*N*,*N*′-bis(2-hydroxyphenylacetate); Zn-EDTA, Zn-ethylenediaminetetraacetate.

* Corresponding author. Tel.: +34 913365650.
E-mail address: p.almendros@upm.es (P. Almendros).

http://dx.doi.org/10.1016/j.scienta.2015.02.023
0304-4238/

human body: it is required for the activity of over 200 enzymes which are involved in most major metabolic pathways and it is therefore necessary for a wide range of biochemical, immunological, and clinical functions. As a result, numerous body functions are affected by Zn deficiency (Cakmak, 2008). It is estimated that over 30% of the world's 6 billion people suffer from Zn deficiency (White et al., 2012).

Mineral malnutrition can be addressed by increasing the bioavailability of mineral elements in edible crops. Agronomic strategies to increase the concentrations of mineral elements in edible tissues generally rely on the application of mineral fertilizers and/or improvements in the solubilization and mobilization of mineral elements in the soil. Biofortification is a relatively new approach which aims to improve the nutritional status of the population by enhancing the micronutrient content of their staple plant foods, either through conventional breeding or genetic engineering. Agronomic biofortification through fertilization (its application to soils, seeds and/or leaves) helps to increase plant nutrient content, without changing the plant's genetic makeup (Storksdieck and Hurrell, 2009).

Zn concentration and Zn uptake are frequently used to evaluate the nutritional quality of plants (Machado et al., 2009; Machado and Campos, 2013; Basta et al., 2005; Luo et al., 2005; Mohammadi and Khoshgoftarmanesh, 2014). Other parameters can also be used to estimate the quality of plants, such as Zn soluble in water or in diluted acids or chelators (Alvarez, 2010; Mann and Takkar, 1983; Marschner, 1995) and chlorophyll content (Baglieri et al., 2014; Farghali, 1997; Pietrini et al., 2003; Ramirez et al., 2014). Others factors have also been used such as transfer factors (TF) to determine the level of micronutrients in plants and to estimate the quality of plants used as food (Chojnacka et al., 2005; Kabata-Pendias and Mukherjee, 2007; Vera-Tome et al., 2003).

The objective of this study was to determine the influence of different natural and synthetic organic Zn complexes on agronomic biofortification of onion crops grown in two types of soil under greenhouse conditions. The effectiveness of the Zn sources was assessed in terms of the Zn concentration, soluble Zn concentration, plant biomass, and chlorophyll and carotenoid contents. Zinc extraction procedures were applied to reveal the distribution of Zn in the fractions and its bioavailability in the different soils. The influence of pH and redox potential were also evaluated.

2. Materials and methods

2.1. Soil characterization

The two original soil surface horizons used in this study were obtained from two different regions of Spain. $Soil_{acid}$ was from Madrid (40°17′N, 4°03′W) and $Soil_{calcc}$ was from Guadalajara (40°39′N, 3°20′W). $Soil_{acid}$ was classified as a Typic Haploxeralf, and its main characteristics were as follows: Texture (USDA), sandy loam; clay, 100 g kg^{-1}; predominant clay, illite; pH [H_2O], 6.13; electrical conductivity, 0.037 dS m^{-1}; water-holding capacity, 6.6 g H_2O 100 g^{-1} soil; extractable P [0.025 M HCl, 0.03 M NH_4F] (Bray and Kurtz, 1945), 19.9 mg kg^{-1}; oxidizable OM [$K_2Cr_2O_7$] (Hesse, 1971), 5.00 g kg^{-1}; total N, 1.00 g kg^{-1}; cation exchange capacity, 4.72 cmolc kg^{-1}; Fe (active Fe_2O_3) [0.2 M $(NH_4)_2C_2O_4$, pH 3] (McKeague and Day, 1966), 141 mg kg^{-1}; total Zn, 9.97 mg kg^{-1}.

$Soil_{calc}$ was classified as a Typic Calcixerept, and its main characteristics were as follows: Texture (USDA), loamy sand; clay, 180 g kg^{-1}; predominant clay, smectite; pH, 8.13; electrical conductivity, 0.18 dS m^{-1}; water-holding capacity, 20.5 g H_2O 100 g^{-1} soil; extractable P [0.5 M $NaHCO_3$] (Olsen et al., 1954), 12.6 mg kg^{-1}; oxidizable OM [$K_2Cr_2O_7$], 12.9 g kg^{-1}; total N, 1.10 g kg^{-1}; cation exchange capacity, 23.5 cmolc kg^{-1}; Fe (active Fe_2O_3) [0.2 M $(NH_4)_2C_2O_4$, pH 3], 56 mg kg^{-1}; total $CaCO_3$, 134 g kg^{-1}; Free $CaCO_3$, 33.4 g kg^{-1}; total Zn, 44.25 mg kg^{-1}. The analytical procedures used were those described by Sparks (1996).

2.2. Greenhouse experiment

For the present study we used polyethylene containers (capacity, 15 L; internal diameter, 26.5 cm; height, 27.5 cm) containing 14 kg of air-dried soil. The nutritional status of the soil, in terms of N, P and K content, was assessed using the electroultrafiltration technique (Wiklicky and Nemeth, 1981). Additional N, P and K were applied at rates of: 50 mg N kg^{-1}, 50 mg P kg^{-1}, 146 mg K kg^{-1} and 50 mg S kg^{-1} [applied as $(NH_2)_2CO$, $Ca(H_2PO_4)_2$ and K_2SO_4].

To carry out this experiment, the different soils were treated with aqueous suspensions of eight different fertilizers, with different rates of Zn application (0, 5 and 10 mg kg^{-1}). The control treatment (Nil-Zn treatment) and Zn fertilizer treatments were replicated 3 times according to a randomized complete block design (total pot number: 102). Two days after Zn application the soils were manually mixed in the container.

The fertilizers used were: Zn-aminolignosulfonate (Zn-AML), Zn-EDDHSA (Zn-ethylenediamine-di-(2-hydroxy-5-sulfophenylacetate), Zn-EDTA (Zn-ethylenediaminetetraacetate), Zn-polyhydroxyphenylcarboxylate (Zn-PHP), Zn-HEDTA (Zn-*N*-2-hydroxyethyl-ethylenediaminetriacetate), Zn-EDTA-HEDTA, Zn-DTPA-HEDTA-EDTA (Zn-DTPA, Zn-diethylenetriaminepentaacetate) and Zn-EDDS (Zn-ethylenediamine disuccinate). These fertilizers are marketed by several different companies and have Zn concentrations (w/w) of 5.0, 3.6, 7.3, 3.0, 7.0, 6.0, 6.9 and 6.0%, respectively (Liñán, 2013).

Onion seeds (*A. cepa* L., cv. Grano) were kept in the two different soils in the growth room for 27 days. Thirty days after fertilizer application (the time required to equilibrate under these greenhouse conditions), three onion plants were grown in each container. The containers were kept in a greenhouse in which the temperature ranged from 9 °C (night) to 43 °C (day) and the relative air humidity ranged from 65% to 80% and was controlled using FICFOG spray nozzle technology. The containers were irrigated with water until soil field capacity was reached and this was then maintained. To evaluate evapotranspiration, the containers were weighed (balance A&D Instruments Ltd., UK, model FG-30 KBM) once a week and the volume of irrigation water required was estimated.

Seventy-five days after transplanting, the plants were collected, washed in deionized water, dried to a constant weight in an oven at 65 °C and then kept in sealed containers for analysis. When the plants were collected, the soil in each container was air-dried and manually mixed. A soil sample of approximately 200 g was taken from each container, sieved to <2 mm, and then stored for analysis in the laboratory.

2.3. Plant analysis

The total growing time of this crop was 75 days, from seedlings. At the end of the experiment, fresh second-to-last leaves were collected from the upper parts of the plants. The soluble Zn (MES) concentration in the fresh leaf was determined using 0.5 g of fresh leaf with 8 mL of 1 mM MES (2-(*N*-morpholino)ethanesulfonic acid) at pH 6 (ratio 1:32 w/v). The Zn concentration in the solution was determined by flame atomic absorption spectrophotometry (FAAS) (Perkin-Elmer model-AAnalyst 700).

The total Zn concentration in onion bulbs samples was determined by acid digestion, using 0.25 g of fresh ground samples and 10 mL of acid mixture [5 mL HNO_3 (65%), 2 mL HF (48%) and 3 mL