Title
Zn and Mn o,p-EDDHA chelates for soybean nutrition in hydroponics in high pH conditions

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INTRODUCTION

The correction of metal micronutrient deficiencies is a problem still not fully solved in Agriculture. The low solubility of the iron, manganese and zinc oxides in the pH range of calcareous soils contributes, among other factors, to the low availability of these nutrients to plants. The Fe$^{3+}$ chelate of o,oEDDHA (ethylenediamine-N,N’bis(o-hydroxyphenylacetic) acid), a polyphenolic polyaminocarboxylic acid, and its analogues are the most efficient solution to correct the Fe deficiency (Norvell, 1991) with good results in hydroponic and soil conditions. The Mn deficiency is often treated with salts of Mn as Mn sulfate or as the Mn chelate of EDTA (Ethylenediaminetetraacetic acid) or analogous. Zinc sulphate has traditionally been the ‘‘reliable’’ source of Zn fertilizer but other sources of Zn are also available (Gangloff, 2006). New chelating agents such as o,pEDDHA (ethylenediamine-N(o-hydroxyphenylacetic)-N’(p-hydroxyphenylacetic)acid), a polyphenolic chelating agent with only five available donor groups or EDDS (SS-ethylenediaminedisuccinic), a biodegradable ligand with similar structure than EDTA, are been considered but there are not studies about the efficiency of the use of metal fertilizer mixes containing these chelating agents. The aim of this work was study the efficacy of the combined application of Fe, Mn, Zn and Cu chelates to correct the deficiencies in soybean in hydroponic solution in the presence of CaCO$_3$.

MATERIALS AND METHODS

Soybean (Glycine max L. cv Klaxon) seeds were germinated using a standard seed growing procedure in closed sterilised trays (García-Marco, 2006) and then seedlings were placed on containers with 10 L diluted (1/5) macronutrient solution (MS), diluted (1/20) micronutrient solution (mS) and 5.0 µM FeEDTA for 7 days. They grew for 4 days in a complete MS and 10.0 µM FeEDTA to induce the deficiency of Mn, Cu and Zn with a low Fe level. MS composition was 1.0mM Ca(NO$_3$)$_2$; 0.9 mM KNO$_3$; 0.3 mM MgSO$_4$ and 0.1 mM KH$_2$PO$_4$. mS composition was 1.0 µM MnSO$_4$; 0.5 µM CuSO$_4$; 0.5 µM ZnSO$_4$; 1.0 µM NiCl$_2$ and 0.1 µM CoSO$_4$. The pH of the solutions was adjusted to 8.2 with KOH 1M in the presence of HEPES 0.1 mM.

Then the stems of two individual seedlings were wrapped together with polyurethane foam and placed in 500 mL vessels preserved from the light by means of a black cover and with continuous aeration. At this point, treatments (simultaneous application of Fe, Mn, Cu and Zn chelates) were applied in the presence of the MS solution. The Fe was applied as o,oEDDHA/Fe$^{3+}$ in all the cases since it is known to be one of the best sources for the Fe nutrition (Lucena, 2005). The Mn, Zn and Cu were applied as o,pEDDHA, EDDS, EDTA, HEDTA or DTPA chelates, with the same chelating agent for the three metals in the same treatment and two additional treatments with Mn and Cu chelated by EDTA and Zn chelated by o,pEDDHA or EDDS. Three controls with no Mn, no Zn and no Mn and Zn with the remaining metal micronutrients chelated by EDTA were tested too. Moreover, 0.1g/l of CaCO$_3$ was added to the pots, in order to achieve calcareous soils conditions. The concentrations in the hydroponic solution were (mg/L) 1.00 Fe, 0.375 Mn, 0.250 Zn and 0.150 Cu. The chelate solutions were prepared as described by Alvarez-Fernandez et al. (2002). Three replicate pots per treatment were considered. The plants stayed for 7 days in these conditions. Chlorophyll index (SPAD 502, Minolta) was measured in all the leaves every two days. Samples were taken after 7 days of the treatment application. Plants were washed following the procedure described by Álvarez-Fernández et al. (2001), and fresh and dry weight of leaves, stems and roots determined separately. Then, micronutrient concentrations were determined in the plant organs after dry mineralization by atomic absorption spectrophotometry.
Data were statistically evaluated by analysis of variance (ANOVA) to assess the significance of the main factors. Means were also compared using Duncan’s test at $P < 0.05$ in order to find significant differences due to the treatments. Statistical analyses were done using SPSS 15.

**RESULTS AND DISCUSSION**

Micronutrient concentrations in the leaf after 7 days of treatment are presented in Table 1. The concentration of Fe in leaf was similar in all the treatments since the same iron chelate (o,oEDDHA/Fe$^{3+}$) was applied. The o,pEDDHA treatment presented the higher values of Mn concentration, significantly higher than EDTA and DTPA treatments, commonly used, and the MnCu-EDTA Zn-o,pEDDHA treatment. The rest of the treatments present lower Mn concentration but with no significantly differences with respect to o,pEDDHA. Similar tendency is observed for Zn concentration; the o,pEDDHA was the best ligand to provide Zn to plant. However the differences among treatments in Zn concentration are considerable higher. The treatments with single polyaminecarboxylic acids (EDDS, EDTA, HEDTA and DTPA) presented low values of Zn concentration compared to o,pEDDHA treatment (the polyphenolic compound). The treatments with o,pEDDHA and other ligand simultaneously were worse than the treatment with o,pEDDHA as the sole chelating agent. There were not satisfactory results about Cu nutrition in none treatment. The higher Cu values were for the treatments with deficiency in Mn and Zn followed by the EDTA.

Table 1. Leaf concentration of Fe, Mn, Zn and Cu ($\mu$g Metal / g.m.s.) and dry weight (g m.s./2 plants) in soybean plants after 7 days of treatment. Data ± SE.

<table>
<thead>
<tr>
<th></th>
<th>µg Metal / g.m.s.</th>
<th>g m.s./2 plants</th>
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<tbody>
<tr>
<td></td>
<td>Fe</td>
<td>Mn</td>
</tr>
<tr>
<td>o,pEDDHA</td>
<td>55.2 ± 1.9 b</td>
<td>73.3 ± 2.6 a</td>
</tr>
<tr>
<td>EDDS</td>
<td>63.2 ± 3.5 ab</td>
<td>66.6 ± 1.9 ab</td>
</tr>
<tr>
<td>EDTA</td>
<td>57.2 ± 5.2 b</td>
<td>60.8 ± 6.8 bc</td>
</tr>
<tr>
<td>HEDTA</td>
<td>58.2 ± 1.1 b</td>
<td>67.0 ± 1.5 ab</td>
</tr>
<tr>
<td>DTPA</td>
<td>69.1 ± 3.4 a</td>
<td>50.4 ± 0.3 c</td>
</tr>
<tr>
<td>MnCu-EDTA</td>
<td>61.6 ± 1.0 ab</td>
<td>66.8 ± 4.3 ab</td>
</tr>
<tr>
<td>Zn-o,pEDDHA</td>
<td>64.7 ± 3.0 ab</td>
<td>57.6 ± 6.2 bc</td>
</tr>
<tr>
<td></td>
<td>-Mn</td>
<td>63.1 ± 0.4 ab</td>
</tr>
<tr>
<td></td>
<td>-Zn</td>
<td>62.8 ± 3.6 ab</td>
</tr>
<tr>
<td></td>
<td>-Mn -Zn</td>
<td>68.1 ± 1.6 b</td>
</tr>
</tbody>
</table>

As expected, the control treatments showed lower SPAD index values (data not shown) than the other treatments. However the results obtained showed a tendency related to iron nutrition. The plants that presented a higher Fe content in leaf were the plants with the higher SPAD index independently of the Mn, Zn and Cu nutrition, with the exception of the control treatments. It seems that the SPAD index it is not adequate to evaluate the Mn and Zn nutrition in plant when the Fe nutrition is also a varying factor.
Plant dry weight at the end of the experiment (Table 1) showed that in the three treatments with Zn-o,pEDDHA, plants had higher values than with the other treatments. In addition, controls with no Mn were more affected than the control with no Zn. It seems that the Mn nutrition have a more relevant effect in the plant weight than the Zn nutrition. Plants treated with DTPA had the lowest values.

It is important to indicate that in general the best treatments are those with the chelates of lower stability, while the high stable chelates gives the worst results. This is the consequence of the competition between the plant and the chelating agent for the Zn$^{2+}$ and Mn$^{2+}$ as already studied by Halvorson and Lindsay (1977). Then, the results here presented are only valid for hydroponic like cultures.

In conclusion, the best treatment for the whole application of Mn and Zn was for the o,pEDDHA ligand that presents the higher levels for Mn and Zn in leaf especially for Zn and in the plant dry weight. It seems that the less stable polyphenolic chelates, like o,pEDDHA, are adequate for the nutrition of Mn and Zn in hydroponics due the low competence of this chelating agent with the plant for the metals.

ACKNOWLEDGEMENTS

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