Yield improvement in zucchini under salt stress: determining micronutrient balance

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Abstract

Zucchini plants (Cucurbita pepo L. var. Moschata) grown in pots under greenhouse conditions were supplied with differing amounts of NaCl (0, 20, 40, and 80 mM). The foliar concentrations of Cl, Fe, Mn and Zn increased in response to rising NaCl levels. The Cu concentrations in leaves were highest in the 0 mM NaCl treatment. In fruits, concentrations of Cl, total Fe and extractable Mn increased with higher levels of NaCl. Fruit yield was increased only by NaCl at 80 mM. Length and fresh weight of fruit tended to increase with increasing NaCl rate, and fruit dry weight proved greatest at 80 mM. The activities of catalase and ascorbic acid oxidase assayed in fruits showed an inverse relationship with the salt concentrations applied. In this context, it is useful to determine the effect of NaCl on the micronutrient concentration, given that the improvement of crop watered with saline water enhances the micronutrient profile. Thus, at 80 mM NaCl treatment improved the micronutrient levels in the shoot, and significantly increased the micronutrient density in the edible part of this crop. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Micronutrients; Ascorbic acid oxidase; Catalase; Peroxidase; Ribonuclease

1. Introduction

In many irrigated areas of the world, farmers are forced to use saline water to irrigate their crops due to inadequate supplies of fresh water. The use of saline water can increase the salt concentration in the soil and thereby stunt plant growth and reduce yields (Akilan et al., 1997). Some irrigation waters contain high...
amounts of NaCl, which contributes to specific ion effects of Cl\(^-\), Na\(^+\), or both, and to antagonistic effects on nutrient elements (Al-Rawahy et al., 1992). Nutrient imbalances may result from the effect of salinity on nutrient availability, uptake, and partitioning within the plant or may be caused by physiological inactivation of a given nutrient, raising the internal requirement of the plant for that essential element (Vil\lora et al., 1997).

Salinity stress has stimulatory as well as inhibitory effects on the uptake of some micronutrients by crop plants. The uptake of Fe, Mn, Zn, and Cu generally increases in crop plants under salinity stress (Alam, 1994). The detrimental effects of NaCl stress on the nutrition of bean plants are reflected in higher concentrations of Cl and Mn in roots and Cl, Fe, and Mn in leaves and of Cl and Fe in fruits (Carbonell-Barrachina et al., 1998).

Biochemical and enzymatic methods involving marker enzymes offer an approach to assess the mineral-nutrition status of plants. These methods are based on the fact that the activity of certain enzymes in deficient tissue is lower or higher (depending on the nutrient) than in normal tissue (Marschner, 1995). For example, the activities of catalase and peroxidase can be used as indicators of the nutritional status of Fe in plants and are also useful in tracing Fe and Mn deficiencies (Valenzuela et al., 1995). Meanwhile, ascorbate oxidase decreases in copper-deficient plants and is a sensitive indicator of the nutritional status of Cu in plants, in close agreement with the leaf Cu levels of that plant (Delhaize et al., 1986). Kessler (1961) measured the activity of the ribonuclease to determine the Zn deficiency in different plant species, and Dwivedi and Takkar (1974) suggested that incipient Zn deficiency in young corn plants could be determined on the basis of ribonuclease activity, which was found to be much higher in zinc-deficient plants than in plants with normal Zn levels (Romero, 1995).

The above relationships were established in leaves, while in fruits, these relationships remain to be defined. In the present study, we tested the response of micronutrients and enzymatic activities to NaCl in the irrigation water, in order to achieve more accurate estimations of the nutritional status of zucchini and thus improvements in the greenhouse cultivation of this crop.

2. Materials and methods

2.1. Crop conditions and plant sampling

Plants of zucchini (Cucurbita pepo L. var. Moschata) were grown under the following controlled greenhouse conditions in Almeria (southern Spain) in 1993 and 1994: 25–30°C, relative humidity 60–80%, 400 \(\mu\)mol/m\(^2\)/s (PAR) for 14 h per day. The soil characteristics were: loamy sand (37.3% sand, 48.6% silt, and 10.1% clay); 26.82% CaCO\(_3\)-equivalent; 14.35% CaCO\(_3\)-active; 442.1 mmol/kg
The greenhouse was divided into 12 plots, providing four NaCl treatments: 0, 20, 40, and 80 mM, designated as $T_0$ (control), $T_1$, $T_2$ and $T_3$, respectively. Each treatment was replicated three times, with two plants per replication, in a randomized complete block design. The plants were grown on artificial soil described above in containers 60 cm × 45 cm × 40 cm and were given NaCl at 8 weeks old by adding NaCl to the culture solution. The concentration was built up over 16 weeks by progressive increments in NaCl until totalling the quantities noted above for the NaCl treatments. The following micronutrients were also applied: 1.5 mg iron/l as FeEDDHA; 0.6 mg manganese/l, 0.3 mg zinc/l, 0.03 mg copper/l and 0.02 mg molybdenum/l in the form of sulphates; 0.3 mg boron/l as H$_3$BO$_3$; together with nitrogen as NH$_4$NO$_3$ (1.5 g/l), phosphorus as H$_3$PO$_4$ (1.5 g/l) and potassium as K$_2$SO$_4$ (1.7 g/l). A computerized fertigation system was used. The irrigation water had a pH of 7.8 and electrical conductivity of 3.80 dS/m, with 0.54 g/l of chloride and 0.38 g/l of sodium. The total quantity of water applied was 130 l per container divided over 21 waterings for the entire cycle.

From the beginning of the fruiting period (120 days old), to the end of the biological cycle (180 days old), giving a total of 4 samplings, at 15-day intervals, starting with plants of 135 days old. The readings expressed in the tables are the means of the three replicates per treatment with four samplings. Leaf samples were taken only from plants with fully expanded leaves of the same size, picked from about one-third of the plant height below the plant apex. Three undamaged fruits of commercially acceptable size were taken from each plant. In the laboratory, the samples were rinsed three times in distilled water after desinfecting with 1% non-ionic detergent, then dried on filter paper and separated into two equal samples, one for fresh- and the other for dry-matter assay.

2.2. Assay of total micronutrients

The plant material (leaves and fruits) was air-dried at 70°C for 24 h, and 0.2–0.3 g of the dried ground matter was subjected to wet digestion with concentrated sulphuric acid (Jones, 1991) in order to determine total Fe, Mn, Zn and Cu levels, using atomic absorption spectrophotometry (Hocking and Pate, 1977).

2.3. Assay of extractable micronutrients

Dry matter (0.15 g) was extracted with 10 ml 1 M HCl for 30 min and then filtered. The concentrations of Fe, Mn, Zn and Cu extracted from leaves and fruits (Sánchez et al., 1994) were measured using atomic absorption spectrophotometry (Hocking and Pate, 1977). Chloride was extracted in water (Cataldo et al., 1975) and measured following the method used by Koltoff and Kuroda (1951).
2.4. Assay of enzyme activities

Modifying the method of Bar-Akiva et al. (1969), for the ascorbic acid oxidase assay, we homogenized 2 g fruit fresh weight (FFW) in McIlvaine buffer pH 5.6. After centrifugation at 3600 g for 15 min, the homogenate was filtered and 2.45 ml homogenization buffer and 0.5 ml of 1.4 µg/ml ascorbic acid were added to 0.05 ml of the extract. Each sample was prepared simultaneously with a blank in which reaction substrate (ascorbic acid) was replaced by homogenization buffer. Both reaction mixtures were incubated for 30 min at 30°C, kept for 15 min at 20°C and then measured at 265 nm in a spectrophotometer. Results were expressed as µg oxidized ascorbic acid per gram fresh weight per hour.

For the determination of peroxidase activity, 0.3 g FFW were homogenized in 5 ml of 50 mmol/l phosphate buffer pH 7.0. After centrifugation, 0.1 ml of the extract was placed into tubes, followed by 0.5 ml benedine solution, 0.5 ml ascorbic acid, and 0.5 ml 2% H2O2 (v/v, Perhydrol Merck®). The time taken to cover 0.4 absorbency units to 420 nm was recorded. The results were expressed as µmol oxidized ascorbic acid per gram FFW/h (Bar-Akiva, 1984).

For the catalase assay (Marsh et al., 1963), 1 ml from the extract for the peroxidase assay was added to 5 ml 1.5% NaBO3 (w/v). This reaction mixture was incubated for 5 min at 37°C after which the reaction was stopped by the addition of 5 ml 2N H2SO4. The acidified reaction mixture was titrated against 0.05N KMnO4 to determine the quantity of H2O2 consumed by the enzyme. The activity was expressed as meq of NaBO3 destroyed per gram FFW per hour.

The ribonuclease activity (Kessler, 1961) was assayed by using 1 g ground FFW in 10 ml of 0.2 mol/l citrate buffer pH 6.5. The homogenate was centrifuged for 15 min at 17 000 g and 5 ml of substrate (0.1% RNA in citrate buffer 0.025 mol/l pH 6.8) were added to 5 ml of extract. The reaction was incubated 60 min at 35°C and stopped afterwards by submergence in a bath at 0–2°C. Once the temperature of the reaction mixture was lowered, 10 ml of acetic acid:absolute ethanol (1:3) were added. The resulting mixture was left 150 min at room temperature (20°C) and then centrifuged at 12 000 g for 15 min. Next 0.5 ml of the last extract were diluted with 3 ml of distilled water and the absorbance was measured at 260 nm. Results were expressed in Wilson’s units per gram FFW.

2.5. Statistical analysis

The experimental results were submitted to an analysis of variance (ANOVA) and means were compared using LSD test (p≤0.05) and differences were represented by lowercase letters in the tables. Simple correlation analyses were performed to indicate possible relationships between treatments and the parameters analysed.
3. Results and discussion

Some of the experimental methods were intended to repeat as closely as possible techniques used by farmers in southern Spain. Local agricultural irrigation depletes the local aquifers, causing marine-water intrusion into groundwater which is also polluted by leaching from excess fertilizers applied to the crops in the area. For these reasons the irrigation water in our experiment had high levels of Na and Cl ions, as well as a high pH and electrical conductivity. These characteristics are usually deleterious to crop growth and yield. The T₀ (control) treatment was irrigated only with this water and would therefore have given different readings both for micronutrients and for enzyme activities if irrigated with fresh water.

The foliar concentrations of total and extractable Fe and Zn, as well as total Mn and Cl, increased significantly in response to rising levels of NaCl supplied, reaching the highest values at 80 mM NaCl (T₃; Table 1). The 20 mM treatment (T₁) had the highest levels of extractable Mn. The total and extractable Cu concentration in leaves decreased significantly as the amount of NaCl applied increased (Table 1).

The very high foliar Cl (Table 1) caused no visible symptoms of toxicity, in agreement with the view that this species is moderately salt tolerant, according to data on yield, salinity-tolerance threshold ECₑ (dS/m⁻¹) and slope (%) per dS/m⁻¹) reported by François and Maas (1994). In fact, genotypic differences in chloride tolerance are closely related to salt-tolerance mechanisms (Marschner, 1995), and the toxicity and tolerance to excess chloride varies among plant species. In fruit trees, beans and cotton, more than 3.5 mg Cl⁻/g leaf dry weight

Table 1
Leaf micronutrient concentrations (µg/g dry wt.) at different NaCl levels

<table>
<thead>
<tr>
<th>NaCl treatment (mM)</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀ (0)</td>
<td>201c</td>
<td>52c</td>
<td>120c</td>
<td>61b</td>
<td>50c</td>
</tr>
<tr>
<td>T₁ (20)</td>
<td>220bc</td>
<td>61bc</td>
<td>131b</td>
<td>77a</td>
<td>70b</td>
</tr>
<tr>
<td>T₂ (40)</td>
<td>239ab</td>
<td>78ab</td>
<td>140a</td>
<td>74ab</td>
<td>77a</td>
</tr>
<tr>
<td>T₃ (80)</td>
<td>256a</td>
<td>91a</td>
<td>146a</td>
<td>60b</td>
<td>82a</td>
</tr>
</tbody>
</table>

Significance: *** *** *** *** * *** *** *** ***

rᵇ 0.656 0.580 0.727 −0.045 0.652 0.701 −0120 −706 0.662

r²ᵇ 0.430** 0.337* 0.529*** 0.200** 0.425** 0.491*** 0.014** 0.499*** 0.438**

ᵃ The values within columns which differ significantly at the 5% level are indicated by different lowercase letters.

ᵇ The r and r² values and significance of r² (*p<0.05; **p<0.01; ***p<0.001) represents the correlation between NaCl levels and the micronutrient readings of all 48 data from the replicates of 4, not of the 4 values of the table.
have proved to be toxic. By comparison, barley, spinach, lettuce, and sugar beet can tolerate between 20 and 30 mg Cl⁻/g leaf dry weight without harmful effects.

These micronutrient concentrations in leaves are higher than values reported for zucchini grown in greenhouses in the absence of NaCl reported by Mills and Jones (1996). These authors found that new leaves of mature zucchini (Cucurbita pepo var. melopepo) plants with average concentrations of total Fe of 112, Mn 82, Cu 13 and Zn 92 (µg/g) were in the normal to high but not toxic range (Jones, 1991).

Fruit yield per plant as well as fruit length and FFW increased significantly with the NaCl treatments (Table 2). In the fruits (Table 3), the concentration of extractable Mn and Cl increased significantly with the NaCl dosages applied while the concentrations of total Mn and total and extractable Fe, Cu and Zn followed no clear linear pattern in response to the salt treatments. In no case did

### Table 2
Crop yield in zucchini at different NaCl levels

<table>
<thead>
<tr>
<th>NaCl treatment (mM)</th>
<th>Yield per plant (Kg)</th>
<th>Length (cm)</th>
<th>Fresh wt. per fruit (g)</th>
<th>Dry wt. per fruit (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀ (0)</td>
<td>1.04b</td>
<td>13.4b</td>
<td>75.9c</td>
<td>4.4a</td>
</tr>
<tr>
<td>T₁ (20)</td>
<td>0.91c</td>
<td>13.5b</td>
<td>82.7b</td>
<td>4.9a</td>
</tr>
<tr>
<td>T₂ (40)</td>
<td>1.02b</td>
<td>13.8b</td>
<td>80.1b</td>
<td>4.4a</td>
</tr>
<tr>
<td>T₃ (80)</td>
<td>1.45a</td>
<td>14.5a</td>
<td>96.6a</td>
<td>5.4a</td>
</tr>
<tr>
<td>Significance</td>
<td>**</td>
<td>*</td>
<td>**</td>
<td>ns</td>
</tr>
</tbody>
</table>

*The values within columns which differ significantly at the 5% level are indicated by different lowercase letters (Extracted from Villora et al., 1999).*

### Table 3
Fruit micronutrient concentrations (µg/g dry wt.) at different NaCl levels

<table>
<thead>
<tr>
<th>NaCl treatment (mM)</th>
<th>Fe Total</th>
<th>Extraitable</th>
<th>Mn Total</th>
<th>Extractable</th>
<th>Zn Total</th>
<th>Extractable</th>
<th>Cu Total</th>
<th>Extractable</th>
<th>Cl Total</th>
<th>Extractable</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀ (0)</td>
<td>188b</td>
<td>98b</td>
<td>78a</td>
<td>18b</td>
<td>87ab</td>
<td>48a</td>
<td>27a</td>
<td>20a</td>
<td>2200c</td>
<td></td>
</tr>
<tr>
<td>T₁ (20)</td>
<td>203ab</td>
<td>113a</td>
<td>76a</td>
<td>18b</td>
<td>79b</td>
<td>41b</td>
<td>29a</td>
<td>18a</td>
<td>3810b</td>
<td></td>
</tr>
<tr>
<td>T₂ (40)</td>
<td>209ab</td>
<td>98b</td>
<td>76a</td>
<td>19b</td>
<td>85ab</td>
<td>44ab</td>
<td>27a</td>
<td>20a</td>
<td>4800ab</td>
<td></td>
</tr>
<tr>
<td>T₃ (80)</td>
<td>214a</td>
<td>109ab</td>
<td>85a</td>
<td>22a</td>
<td>91a</td>
<td>48a</td>
<td>29a</td>
<td>22a</td>
<td>5440a</td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>*</td>
<td>*</td>
<td>ns</td>
<td>***</td>
<td>**</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>rᵇ</td>
<td>0.240</td>
<td>0.075</td>
<td>0.051</td>
<td>0.551</td>
<td>0.036</td>
<td>0.022</td>
<td>0.114</td>
<td>0.130</td>
<td>0.658</td>
<td></td>
</tr>
<tr>
<td>r²ᵇ</td>
<td>0.057ns</td>
<td>0.006ns</td>
<td>0.003ns</td>
<td>0.304***</td>
<td>0.001ns</td>
<td>0.001ns</td>
<td>0.013ns</td>
<td>0.017ns</td>
<td>0.433***</td>
<td></td>
</tr>
</tbody>
</table>

*The values within columns which differ significantly at the 5% level are indicated by different lowercase letters.

ᵇ The r and r² values and significance of r² ("p≤0.05; "p≤0.01; "p≤0.001) represents the correlation between NaCl levels and the micronutrient readings of all 48 data from the replicates of 4 samplings, not of the values of the table.
these elements reach toxic levels. Our experimental results for zucchini contrast with findings for tomato, squash and green beans, in which the level of each micronutrient reportedly fluctuates with salinity, perhaps due in part not only to the salt treatments but also largely to the type of crop and the cultivar used in each experiment (Grattan and Grieve, 1994).

Catalase and peroxidase activities in leaves have been demonstrated to be valuable indicators of the nutritional status of Fe and Mn in plants (Lavon and Goldschmidt, 1999). In addition, these enzymatic activities are frequently used when studying stress responses (Evers et al., 1997). Similarly, ribonuclease activity is a good indicator for Zn (Dwivedi and Takkar, 1974). By contrast, a close positive correlation between Cu and ascorbic acid oxidase activity was found in leaves of different crops (Delhaize et al., 1986). Thus, these diagnostic methods are useful to solve particular problems of nutritional disorders and to supplement total and fractionated elemental analyses rather than to replace established techniques (Marschner, 1995).

Catalase and ascorbic acid oxidase activities in fruits declined significantly with increased NaCl concentrations, whereas ribonuclease activity remained unchanged as the NaCl dosage rose (Table 4). In contrast to other experiments in which peroxidase activity in Atriplex was found to intensify with the saline dosage (Wang et al., 1997), there was no significant correlation in zucchini.

Table 4
Enzyme activities in zucchini fruits, at different NaCl levels

| NaCl treatment (mM) | Enzyme activity |  
|---------------------|-----------------|---|---|---|---|
|                     | Catalase⁴ | Peroxidase⁵ | Ribonuclease⁶ | Ascorbic acid oxidase⁶ |
| T₀ (0)              | 62.6a       | 4.6b        | 2.0a        | 645.9a            |
| T₁ (20)             | 58.9a       | 8.6a        | 2.3a        | 259.8b            |
| T₂ (40)             | 54.4a       | 7.4ab       | 2.4a        | 108.3c            |
| T₃ (80)             | 30.9b       | 6.5ab       | 2.5a        | 90.5c             |
| Significance        | ***         | *           | ns          | *                 |
| r⁷b                 | −0.497      | 0.185       | 0.039       | −0.283            |
| r²⁷b                | 0.247***    | 0.034⁸ns    | 0.002⁸ns    | 0.080*            |

a The values within columns which differ significantly at the 5% level are indicated by different lowercase letters.

b The r and r² values and significance of r² (⁹ns p>0.05; * p<0.05; ** p≤0.01; *** p≤0.001) represents the correlation between NaCl levels and measurements of enzyme activities of all 48 data from the replicates of 4 samplings, not of the 4 values of the table.

⁴ meq NaBO₃/g FW/h.

⁵ μmol oxidized ascorbic acid/g FW/h.

⁶ Wilson units/g FW/h.

⁷ μg oxidized ascorbic acid/g FW/h.
In conclusion, in this case, saline irrigation significantly boosted crop yield and improved fruit quality (Villora et al., 1999). This might result from the greater Cl requirements in zucchini than for other horticultural crops, which generally display toxicity responses of stunted growth and depressed yield under such conditions.

In the present study, we established correlations between micronutrients and enzymatic activities. The total Fe \((r=-0.311^*)\) as well as the extractable Mn \((r=-0.274^*)\) and extractable Fe \((r=-0.249^*)\) concentrations in fruits, presented significant inverse correlations with catalase activity. We also found a significant correlation between total Mn concentration \((r=-0.327^*)\) and peroxidase activity. Those enzymes may therefore also help to indicate the nutritional status of Fe and Mn in fruit under saline conditions, more accurately than can chemical analysis alone, as also found by Sánchez et al. (1994) in melon. Similarly, ascorbic acid oxidase activity is considered a good indicator of the Cu status (Delhaize et al., 1986), but in this experiment the correlation between extractable Cu and the ascorbic acid oxidase activity was negative \((r=-0.301^*)\). It should be noted that enzymatic methods employed to establish the nutritional status of plants in this experiment did not by themselves prove sufficiently informative. Nevertheless, it is useful to ascertain the effect of NaCl on the micronutrient concentration, given that the crops watered with saline water had enhanced yields and the micronutrient profiles — a result important for human nutrition. Thus, in practical terms, the T₃ treatment improved yield and fruit quality (Villora et al., 1999), as well as the micronutrient levels in the shoot, particularly with regard to Fe and Zn in the fruits, thereby significantly increasing the concentrations of micronutrient in the edible part of this crop.

References


