Increase in lettuce (Lactuca sativa L.) production by foliar calcium application

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Keywords: Foliar fertilization, calcium oxide, calcium chloride, calcium chelate

ABSTRACT

Background: Calcium (Ca) deficiency in plants affects the growth of young leaves and triggers symptoms associated with cell disruption. The foliar application of Ca is recommended because it provides the nutrient directly to the organism of interest during critical growth stages. Foliar Ca application is controversial because such efficiency depends on the Ca source, applied dose and investigated culture. Objective: The present study aimed to assess whether foliar Ca application interferes with biomass accumulation and Ca concentration in the leaf. The treatments consisted of a factorial design and the foliar application of three Ca sources (calcium oxide, calcium chloride, calcium chelate) in four doses as well as a control group. Results: Most of the sources and dosages increased the number of leaves, and three treatments increased shoot biomass. Ca accumulation in the cell walls of certain treatments was observed using energy dispersive X-ray spectroscopy (SEM EDS) and histochemical tests with ruthenium red. The efficiency of foliar Ca application is dependent on the source and applied dosage, the Ca sources showed significant differences, with CaCl₂ at the recommended dosage showing the optimal cost benefits and efficiency compared with CaO and Ca chelate. Conclusions: Spraying increased the Ca can promote biofortification of plant foods by increasing Ca concentrations in the leaves concentration and accumulation of biomass because of Ca deposition in the cell wall as Ca pectate. Therefore, Ca spraying decrease post-harvest losses and improved durability and increase the time available for marketing the product.

INTRODUCTION

Calcium (Ca) is an essential macronutrient for plant growth, and it performs a number of functions within the plant cell according to its concentration and location. Ca is observed in the cell wall as a structural component, in the cytosol as a secondary messenger and in the vacuole as a counterion (Hepler, 2005; Hepler and Winship, 2010; White and Broadley, 2003).

Ca plays a role in photosynthesis, increased cell volume and division, cytoplasmic movements, cytoskeletal functions and plasma membrane stabilization (Hepler, 2005). In addition, Ca is an important intracellular messenger that operates in the plant defense system by inducing the synthesis of specific proteins and stress-regulating genes (Hepler, 2005; White and Broadley, 2003).

Ca is absorbed by the roots as a divalent cation and distributed through the xylem. However, translocation via phloem is low, and its transport relies on transpiration (Marschner, 1995). Ca is an important cell constituent and found at its highest concentration in the cell wall, mainly in the middle lamella, where it is associated with...
pectin as Ca pectate (Hepler and Winship, 2010). The absence of Ca in plant nutrition occurs in young leaves because of low transpiration rates at these sites, which disrupts the cells of young tissue (White and Broadley, 2003).

Foliar application is indicated to minimize Ca deficiency-related problems in short-cycle species. Foliar application provides direct nutrient to the plant during critical growth stages and obtains a fast response when the plant demand is high or when nutrient availability is limited because of soil conditions (Fageria et al., 2009; Fernández et al., 2013, Jusoh et al., 2015), as lettuce which is the main leafy vegetable consumed in Brazil towards healthy eating, lettuce has been gaining space in markets (Thiesen et al. 2016).

The present study aimed to evaluate whether Ca foliar application interferes with biomass accumulation and Ca concentration in the shoots of lettuce.

MATERIALS AND METHODS

Growth conditions:
The experiment was conducted in a greenhouse, and seeds of lettuce (Lactuca sativa L., Asteraceae) cv. Verônica were sown in polystyrene trays in April 2013. Two seedlings were transplanted 30 days after seed emergence to 2 L pots filled with Plantmax® substrate. The substrate had the following characteristics: dry matter = 73.35 g kg⁻¹; nitrogen (N) = 0.35 g kg⁻¹; phosphorus pentoxide (P₂O₅) = 0.57 g kg⁻¹; potassium oxide (K₂O) = 0.22 g kg⁻¹; magnesium oxide (MgO) = 2.76 g kg⁻¹; and calcium oxide (CaO) = 1.66 g kg⁻¹. Irrigation was performed to maintain field capacity at approximately 80%.

Experimental design:
The experiment followed a factorial design with an additional treatment (3 Ca sources x 4 dosages + 1 control with water application). The experiment was distributed in a randomized arrangement with four replicates.

Ca foliar applications:
Foliar applications began 15 days after transplantation (DAT) and were performed weekly. A manual sprayer with a flow of 400 L ha⁻¹ was used to apply the treatments.

The Ca sources included calcium chloride (CaCl₂), CaO and calcium chelate (Ca chelate). The CaCl₂ contained 140 g L⁻¹ Ca in its formulation; CaO contained 40 g L⁻¹ Ca, 0.3 g L⁻¹ boron (B), 3.1 g L⁻¹ zinc (Zn) and 6.9 g L⁻¹ N; and Ca chelate mixed mineral fertilizer contained 62 g L⁻¹ Ca and 6.2 g L⁻¹ B. The three Ca sources were applied in four dosages at 0.5, 1.0, 2.0 and 4.0 L ha⁻¹ of the product.

Plant analysis:
Samples at the apex of the fourth leaf node near the midrib of each plant were fixed in Karnovsky solution (Karnovsky, 1965) for leaf tissue analysis at 62 DAT.

The fixed samples were embedded in glycol methacrylate (Leica Historesin®, Leica Microsystem, Germany) and cross-sectioned with a rotary microtome (Olympus CUT 4055, Triangle Biomedical Sciences, Japan) for light microscopy. The sample sections were then stained with toluidine blue (O'Brien et al., 1965) to measure the leaf thickness, and a histochemical analysis was performed with ruthenium red to detect pectic substances (Johansen, 1940). Two fixed samples from each treatment were dehydrated in an ethanol series, subjected to critical-point drying with CO₂ and examined by energy dispersive X-ray spectroscopy (EDS) with a scanning electron microscope (TSM-6360 LV, Jeol, Japan) to quantify the chemical elements.

The aerial portion of the plants were sampled for morphological analyses. The number of leaves was determined, and the plants were placed in a greenhouse (65°C with air circulation) until reaching a constant weight to determine the shoot biomass.

Statistical analyses:
The data underwent a square root transformation, Kolmogorov-Smirnov normality test and analysis of variance (ANOVA). The treatments were compared with the control group using Dunnett’s test at 5% significance, and Tukey’s test (0.05) was used to compare treatments. ANOVA was selected as the linear model because the suitability of the polynomial equation could be biased with four doses.

Results:
The number of leaves, biomass and leaf blade thickness differed significantly among the treatments compared with that of the control group according to Dunnett's test (0.05) (Table 1). Foliar Ca applications led to a higher number of leaves compared with that of the control group with the exception of the treatment with 0.5 L ha⁻¹ Ca chelate. In addition, a higher accumulation of biomass was observed in treatments with 1.0 L ha⁻¹
and 2.0 L ha\(^{-1}\) CaCl\(_2\) and 4.0 L ha\(^{-1}\) CaO, with an increase in leaf thickness in the treatment with 0.5 L ha\(^{-1}\) CaO (Table 2).

The ANOVA performed between the products and dosages showed an interaction in relation to the number of leaves and biomass, whereas significant differences were not observed in the leaf blade thickness for Ca sources, dosages and interactions between these two variables (Table 1).

A greater number of leaves was observed at 1.0 and 2.0 L ha\(^{-1}\) CaCl\(_2\) and 1.0, 2.0 and 4.0 L ha\(^{-1}\) Ca chelate, whereas the leaf quantity was not affected by CaO (Table 2).

Biomass accumulation was higher with 0.5, 1.0 and 2.0 L ha\(^{-1}\) CaCl\(_2\) and lower with 4.0 L ha\(^{-1}\) CaCl\(_2\). Biomass accumulation was similar at 1.0 and 4.0 L ha\(^{-1}\), and the highest observed value was at the highest CaO dose. Biomass accumulation was higher at 0.5, 1.0 and 2.0 L ha\(^{-1}\) Ca chelate, and differences were not observed among these doses.

### Table 1: ANOVA of the number of leaves, biomass (g) and leaf thickness (mm) of lettuce obtained by factorial design in the treatments with three Ca sources applied in four doses as well as in the control treatment.

<table>
<thead>
<tr>
<th>Variation factor</th>
<th>p</th>
<th>Number of leaves</th>
<th>Biomass (g)</th>
<th>Leaf thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Products (P)</td>
<td>&lt;0.001</td>
<td>0.090</td>
<td>0.151</td>
<td></td>
</tr>
<tr>
<td>Doses (D)</td>
<td>0.004</td>
<td>0.017</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>P x D</td>
<td>0.002</td>
<td>0.007</td>
<td>&lt;0.050</td>
<td></td>
</tr>
<tr>
<td>Fatorial x Control</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.090</td>
<td></td>
</tr>
<tr>
<td>Variation coefficient</td>
<td>6.30</td>
<td>24.89</td>
<td>3.98</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Number of leaves, biomass (g) and leaf thickness (mm) of lettuce observed after the treatment that included foliar application of three Ca sources applied in four doses as well as in the control treatment, with the results subjected to Tukey’s (0.05) and Dunnett’s (0.05) tests.

<table>
<thead>
<tr>
<th>Doses</th>
<th>Products</th>
<th>CaCl(_2)</th>
<th>CaO</th>
<th>Quelato-Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of leaves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>12.67 a B *</td>
<td>12.95 a A *</td>
<td>8.58 b B</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>14.25 a AB *</td>
<td>12.29 ab A *</td>
<td>11.29 b A *</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>16.25 a A *</td>
<td>12.50 b A *</td>
<td>12.50 b A *</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>12.20 a B *</td>
<td>12.50 a A *</td>
<td>13.00 a A *</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biomass (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>3.09 a AB</td>
<td>2.36 a B</td>
<td>1.19 a B</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>4.59 a AB *</td>
<td>2.87 a AB</td>
<td>2.34 a AB</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>5.63 a A *</td>
<td>2.73 b B</td>
<td>3.55 ab AB</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>2.22 b B</td>
<td>5.43 a A *</td>
<td>3.89 ab A</td>
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</tr>
<tr>
<td>Control</td>
<td>1.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf thickness (mm)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>183.69</td>
<td>211.76 *</td>
<td>185.62</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>199.74</td>
<td>208.06</td>
<td>193.37</td>
<td></td>
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<tr>
<td>2.0</td>
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<td>196.57</td>
<td>186.97</td>
<td></td>
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<tr>
<td>4.0</td>
<td>175.73</td>
<td>181.49</td>
<td>185.23</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>170.96</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lower case letters compare means among the products. Uppercase letters compare means among doses. Means followed by the same letters do not differ by Tukey’s test (0.05); * Significant and higher than the control by Dunnett’s test (0.05).

The Ca source CaCl\(_2\) led to an increase of Ca in the leaves (mg Ca g\(^{-1}\) dry mass) until 2.0 L ha\(^{-1}\), and the Ca decreased at 4.0 L ha\(^{-1}\). The CaO and Ca-chelate applications led to a gradual increase in Ca concentration in mesophyll cells following an increase in applied dosage (Figure 1).

The ruthenium red test showed a strong reaction under light microscopy to the presence of pectic substances in the treatments with higher Ca concentrations in the leaf tissue, which were at 1.0 L ha\(^{-1}\) and 2.0 L ha\(^{-1}\) CaCl\(_2\) and 4.0 L ha\(^{-1}\) CaO (Figure 2).
Fig. 1: Number of leaves (A), biomass (B), leaf thickness (C) and Ca concentration in the leaf (D) in lettuce in the treatment with foliar Ca application of three Ca sources applied in four doses as well as in the control treatment.

Fig. 2: Cross sections of the leaf apex from the fourth lettuce node tested with ruthenium red for histochemical detection of pectin under different treatments: control (A), 1.0 L ha\(^{-1}\) CaCl\(_2\) (B), 2.0 L ha\(^{-1}\) CaCl\(_2\) (C) and 4.0 L ha\(^{-1}\) CaO (D); 50 µm.

Discussion:
This study showed that foliar Ca application is an efficient method of enhancing plant development because it promotes higher biomass accumulation, leaf number and Ca concentration in the leaf tissue. Leaves with thin cuticles and open stomata are desirable for more efficient and faster absorption of the applied solutions (Fageria et al., 2009). The applied Ca sources were absorbed because the lettuce leaf has a thin cuticle and stomata on both sides, thus facilitating product penetration and absorption inside the cells, which was also observed for sweet pepper (Weryszko-Chmielewska and Michalojć, 2009).
The control group showed a lower number of leaves compared with the treatments receiving foliar Ca application. Spraying led to an increase in cellular division rate and resulted in a higher number of leaves. Similarly, foliar Ca application in oregano was shown to reach the meristem and favored growth with an increase in the number of stems per plant (Dordas, 2009). Foliar Ca application interfered with mitotic processes by increasing Ca concentrations within the cells. Ca regulates the rate of chromosome movement during anaphase and stimulates microtubule depolymerization, directly affecting cell division (Zhang, 1992).

Foliar Ca application promoted higher biomass accumulation in the three treatments (Table 2). The Ca absorbed by the plant promoted increased Ca concentrations in the cytosol and accumulation in the cell wall as Ca pectate, which led to an increase in biomass. Ca is higher in the middle lamella and associated with structural rigidity of the cell wall. Such associations occur because acid residues are secreted as methyl esters during cell wall formation and de-esterified by pectin methylesterase, thus releasing a carboxyl group that binds to Ca and forms Ca pectate (Hepler, 2005). The mechanism by which biomass increases through Ca accumulation in the cell wall is clear because leaf thickness is not altered by Ca application, thus precluding the possibility of cell expansion, and treatments with higher Ca concentrations showed the highest biomass accumulation (Figure 1 and 2).

Treatments corresponding with the highest number of leaves did not show the same results for biomass accumulation. Plants directed the absorbed Ca towards cell division and cell wall synthesis in new cells, and significant accumulations of Ca in the middle lamella were only observed in treatments with 1.0 and 2.0 L ha⁻¹ CaCl₂ and 4.0 L ha⁻¹ CaO (Figures 1 and 2).

Different Ca sources provided varying results in crop development because of differences in product efficiency, which is related to the chemical properties of the sprayed ions, such as the formulation, concentration, pH and surfactants, which directly affect nutrient absorption rates (Wójcik, 2004; Fernández et al., 2013).

Maximum biomass production was reached at 2.0 L ha⁻¹ CaCl₂ (Table 2), and higher doses may be harmful to the plant (negative effect) because of the salinity caused by excessive chloride ions (Cl⁻) (Ruiz and Romero, 2001). Excess Cl⁻ causes stress and ion imbalances, which lead to reduced growth rates and small leaves (Bernstein, 1975). CaO showed variable results and achieved a higher biomass accumulation at 4.0 L ha⁻¹. The CaO product contained B, Zn and N in its formulation. B is responsible for structuring the cell wall by binding to pectic polysaccharides (Koshiba et al., 2009); Zn is important for overall plant metabolism, including protein synthesis, plasma membrane integrity maintenance and auxin regulation (Hafeez et al., 2013); and N is an essential component in proteins (Mokhele et al., 2012). Therefore, the set of nutrients administered to the plant promoted development at the tested dosage.

Ca chelate at 1.0 L ha⁻¹ showed maximum biomass accumulation efficiency, although this effect was not observed at higher doses. A gradient of Ca absorption through diffusion is observed when the product is applied until a maximum peak is reached (Fernández et al., 2013) where Ca concentration is higher at the leaf surface than in the mesophyll. The collection sites for Ca absorption were saturated, and an increase in penetration rate was not observed with increased product dose. (Stacey and Oosterhui, 2007) concluded that iron (Fe) and Zn chelate fertilizers are not indicated for foliar applications because they have low absorption rates, indicating that other fertilizer formulations with the same nutrients are absorbed more rapidly by the plants.

Foliar Ca applications had a direct effect on Ca concentration in the tissue (Figure 1) as reported by (Lopez-Lefebre et al., 2001; Singh et al., 2007; Dordas, 2009). All of the treatments showed Ca concentrations within the range recommended for normal lettuce growth from 0.1 to 5.0 mg g⁻¹ of dry matter (CHEMICAL AND SOIL FERTILITY COMMISSION (COMISSÃO DE QUÍMICA E FERTILIDADE DO SOLO) RS/SC, 2004).

In addition to acting on physiological processes, the absorbed Ca was accumulated as Ca pectate, which is a cell-wall stabilizing agent that inhibits the action of enzymes that degrade the cell wall and disintegrate the cell (Marschner, 1995; Glenn and Poovaiah, 1990). Therefore, Ca spraying may increase Ca concentrations in the cell wall and provide greater mechanical strength to the plant (Chéour et al., 1990; Singh, et al., 2007; Li et al., 2012) because of the increased total pectin content (Li et al., 2012) and delayed pectin dissolution (Glenn and Poovaiah, 1990).

Conclusions:
Calcium provided larger number of leaves and biomass, thus Ca spraying resulted in increased growth of lettuce, showing that calcium is absorbed and distributed by the plants. Besides that, spraying Ca can promote biofortification of plant foods by increasing Ca concentrations in the leaves tissue. In addition, higher cell Ca concentrations can result in a product with lower degradation rates and post-harvest loss, improved durability and longer marketing time.

The efficiency of foliar Ca application is dependent on the source and applied dosage, and CaCl₂ is the most recommended Ca source for lettuce at doses of 1.0 and 2.0 L ha⁻¹ because of its efficiency and cost-effectiveness. The Ca sources with CaO and Ca chelate have complex molecules that are patented products, and
they incur high costs to the grower. In addition, CaO and Ca chelate sources showed no significant differences from CaCl₂.

Suggests that further work be done at different times of the year, especially in the warm seasons and use of nutrient solutions without Ca in the soil to stimulate growth and the appearance of symptoms of Ca deficiency. For final suggestion for future works about the spraying Ca efficiency, it is suggested to conduct post-harvest tests to measure the efficiency in practice.

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