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Mineral Uptake and Soluble Carbohydrates of Lettuce: Effects of Air Temperatures and Mineral Supply Levels

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INTRODUCTION

Both low and high temperatures affect plant growth and development at whole plant level, tissue and even cell level through a variety of metabolic changes. In this paper we investigated the extent of the inhibition of growth, macro–element uptake and soluble carbohydrate production, and the effect of extra–supply of minerals as a means of the recovery from the damage. Lettuce plants were grown four different growth temperatures (15/8, 22/15, 25/18 and 28/21°C), and extra–supply of minerals was composed of 1.5– and 2.0–fold stronger than the standard nutrition (1/2 strength of Hoagland’s solution). Low and high temperatures significantly adversely affected lettuce growth, mineral uptake and soluble carbohydrate production, and the extra–supply of minerals didn’t play any crucial roles to promote and recover them. Merely, P uptake was slightly increased by an extra–supply of nutrients in all temperature conditions. From this study, it was strongly suggested that further research should include the effect of extra–supply of minerals when plants are recovered from low or high temperature stress, and metabolic interaction such as mineral uptake and carbohydrates between shoot and root.

Key words: Lettuce, Mineral uptake, Carbohydrates, Temperature stress, Mineral supply

MATERIALS AND METHODS

Plant materials and growth conditions

This study was performed in an environment–controlled growth chamber, NAAS, RDA, South Korea in 2014. The uniformly growing seedlings of lettuce were transplanted into 1 L plastic box filled with pure sand soil, and then fed with 1/2 strength of Hoagland’s solution which is composed as follows; 5 mM Ca(NO₃)₂, 5 mM KNO₃, 2 mM MgSO₄, 0.5 mM KH₂PO₄, 1.5 mM Fe–EDTA, 1 mM NH₄NO₃, 2 μM H₂BO₃, 0.2 μM MnCl₂, 0.01 μM ZnSO₄, 0.01 μM CuSO₄ and 0.03 μM H₂MoO₄. The 1/2 strength of Hoagland’s solution, 100 mL per box, was lead to a considerable reduction in mineral uptake (Cumbus and Nye, 1984; Raju et al., 1990; Tindall et al., 1990).

Lettuce (Lactuca sativa L.) is the second largest leafy crops after Chinese cabbage, and the scale of greenhouses–based cultivation throughout South Korea was approximately 2,968 ha (MAFRA statistics, 2013). Furthermore, a year round cultivation of lettuce often causes the unexpected heat (high in summer season) and chilly (low in winter season) stresses, and thus results in deleterious effects on the growth and yield. Although the literature on plant responses to temperatures such as mineral uptake and carbohydrate production is abundant, the information about the effects of extra–supply of minerals there is only a very few. The objective of this study was to know the extent of a damage of growth, mineral uptake and carbohydrate production under low or high temperature conditions, and to know whether there is any effect of extra–supply of minerals to recover and promote those in lettuce plant.
supplied every day during the experiment. Plants were grown in four different temperature conditions, low (15/8°C, day/night), optimal (22/15°C), and high temperature (25/18 and 28/21°C). To investigate the effect of an extra-supply of minerals to mitigate the damage from temperature stress, lettuce plants were grown in three different nutrient conditions, standard (1/2 strength of Hoagland's solution), 1.5- and 2.0-fold stronger nutrient solutions. Lettuce plants were assigned with the completely randomized two factor factorial design (temperature and mineral supply) in an environment-controlled chamber and were taken to determine the contents of carbohydrates and mineral elements at 5, 10 and 15 days after treatment (DAT).

**Measurement of nutrients**

The oven-dried samples (0.3 g) which were at 80°C for 48 h were soaked in 5 mL of 368 mmol L⁻¹ salicylic acid in 84.7% sulfuric acid (H₂SO₄) for 24 h then digested in a digestion system, heated to 300°C for 3 h, followed by several drops of hydrogen peroxide (H₂O₂). The extracted solution was transferred to 100 mL volumetric flasks and then diluted to 100 mL with deionized water for mineral assays. The N concentration was colorimetrically determined using the automatic flow injection analyzer (BRAN LUBE, Germany). The P concentration was measured using the molybdate-blue colorimetric method (UV-2450, Shimadzu, Japan) and cation concentrations were determined with ICP-OES (INTEGRA XMP, GBC, Australia).

**Measurement of soluble carbohydrates**

Soluble sugar from dried shoots and roots was determined by the reaction of 1.0 mL of the alcoholic extract with 2.0 mL fresh 0.2% anthrone in sulfuric acid (w/v); the absorbance was read at 630 nm. After the extraction of the soluble fractions, the solid fraction was used for starch analysis. Starch was firstly extracted with 9.3 N (normal concentration) of perchloric acid and followed by 4.6 N. The extracts were combined and starch concentration was determined after reaction with the anthrone reagent. Glucose was used as the standard for soluble sugar.

**Statistical analysis**

This experiment was performed with the completely randomized two factor factorial design (temperature and nutrient level) with three repeats. The analysis of variance (ANOVA) was conducted to find effects of treatments. Least significant difference (LSD) was performed to determine the significance of the difference between the means of treatments. An α error value of 0.05 was chosen to indicate statistical significance. All statistical analysis was performed using version 9.01 of SAS (SAS Institute Inc, Cary, NC).

**RESULTS**

**Effects of temperature and extra-supplied nutrient on lettuce growth**

The shoot growth of lettuce (dry weight–based) from each temperature condition was shown in Fig. 1, and the growth rates between temperature conditions represented significant differences (p<0.01 and 0.001) at three time points. The growth was the highest at the optimal (22/15°C) and moderately high (25/18°C) temperature conditions during the whole experiment period whereas the low (15/8°C) and high (28/21°C) temperature conditions represented the marked reduction in the growth from Day 10. The effect of extra-supply of minerals (1.5 and 2.0-fold stronger) as a means of recovering the damage from temperature stresses was not observed (Fig. 2), and especially the extra-supply made the growth worse at high temperature condition.

![Fig. 1. Effect of temperature stresses on tomato plants grown under 1/2 strength Hoagland's nutrient solution (n=3). Star marks, *, ** and ***, mean significant difference at p<0.05, 0.01 and 0.001, respectively.](image)

![Fig. 2. Effect of an extra-supply of nutrient solution on lettuce growth grown under four different temperature regimes at 15 DAT (n=3). The concentration, × 1.0, means the 1/2 strength Hoagland's nutrient solution. The letters above vertical bars mean significant differences from LSD test.](image)
Effects of temperature and extra–supplied minerals on concentration and uptake of macro–elements

The concentrations of macro–nutrients in temperature–affected lettuce resulted in a tendency of the overall increase except N (67% compared to the optimal) and K (55%) concentration, which represented a marked reduction, at low temperature (Table 1). An increase in the concentration was obvious Ca (1.9 fold as high compared to the optimal) at low temperature and P (1.9 fold as high) at high temperature. By contrast, the uptake of macro–nutrients was greatly affected by temperature stresses (Table 2), and the uptake rates were ranged from 26 to 71% at the low temperature to optimal and from 48 to 66% in the high temperature. The uptake rate under moderately high temperature (25/18°C) resulted in slight decrease of N, P and K, and in great increase of Ca and Mg. The extra supply of minerals was non–effective to promote the nutrient uptake as lettuce was exposed extremely adverse temperature conditions although an effect was partially observed at 2.0–fold stronger mineral supply (Table 3). The supply of 1.5–fold stronger minerals led to the significant reduction in most elements which ranged from 18 to 56% at low temperature (15/8°C) to optimal temperature (22/15°C) and from 30 to 71% at high temperature (28/21°C). The supply of 2.0–fold stronger minerals promoted widely the nutrient uptake at low temperature (15/8°C) compared to control (× 1.0 of low temperature), whereas there was no effect at high temperature. Unlike other macro–elements, P uptake was slightly increased by an extra–supply of nutrients at all temperature conditions.

Effects of temperature and extra–supplied nutrients on soluble carbohydrates production

The contents (glucose equiv.) of soluble sugars and starch greatly differed with both temperature conditions and the period of temperature treatment (Fig. 3). The levels of soluble sugars and starch were the highest in optimal temperature at 5 and 10 DAT and in moderately high temperature at 15 DAT, and the relative rates of soluble sugars and starch in low and high temperatures represented 39 (21.1 ± 1.5 mg g⁻¹ DW), 37 (13.0 ± 1.1 mg g⁻¹ DW), 26 (13.9 ± 2.1 mg g⁻¹ DW) and 43 (15.4 ± 0.7 mg g⁻¹ DW) %, respectively, at 15 DAT. The extra supply of nutrients to enhance the accumulation of soluble sugars and starch didn’t show any effect in all temperature conditions (Fig. 4). The contents of soluble sugars and starch were the highest in moderately high temperature and followed by optimal, low and high temperatures. Moreover, the level of soluble sugars was significantly reduced by low temperature, and starch was markedly decreased in both low and high temperatures.

<table>
<thead>
<tr>
<th>Temperature (day/night, °C)</th>
<th>N</th>
<th>P₂O₅</th>
<th>K₂O</th>
<th>CaO</th>
<th>MgO</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/8</td>
<td>1.64 ~ 3.91(67)</td>
<td>0.38 ~ 0.49(123)</td>
<td>2.57 ~ 5.02(55)</td>
<td>1.21 ~ 3.04(190)</td>
<td>0.32 ~ 0.65(112)</td>
</tr>
<tr>
<td>22/15</td>
<td>3.32 ~ 4.00(100)</td>
<td>0.34 ~ 0.39(100)</td>
<td>6.08 ~ 6.66(100)</td>
<td>1.19 ~ 1.43(100)</td>
<td>0.39 ~ 0.51(100)</td>
</tr>
<tr>
<td>25/18</td>
<td>3.34 ~ 4.64(106)</td>
<td>0.30 ~ 0.50(110)</td>
<td>5.74 ~ 7.48(104)</td>
<td>1.23 ~ 2.34(138)</td>
<td>0.42 ~ 0.80(143)</td>
</tr>
<tr>
<td>28/21</td>
<td>4.92 ~ 5.10(137)</td>
<td>0.58 ~ 0.78(193)</td>
<td>6.87 ~ 7.67(115)</td>
<td>1.11 ~ 1.83(114)</td>
<td>0.43 ~ 0.62(117)</td>
</tr>
</tbody>
</table>

† Data indicate the range of the concentrations measured at three sampling points, 5, 10 and 15 DAT, and the data within the parenthesis represent a concentration index of macro–elements when the concentrations are defined as 100 in optimal temperature condition (22/15°C)

<table>
<thead>
<tr>
<th>Temperature (day/night, °C)</th>
<th>N</th>
<th>P₂O₅</th>
<th>K₂O</th>
<th>CaO</th>
<th>MgO</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/8</td>
<td>26.5 ± 9.0b</td>
<td>3.5 ± 0.5c</td>
<td>35.6 ± 7.6c</td>
<td>22.2 ± 6.3bc</td>
<td>5.5 ± 1.2c</td>
</tr>
<tr>
<td>22/15</td>
<td>87.2 ± 5.3a</td>
<td>7.7 ± 0.5a</td>
<td>137.6 ± 7.2a</td>
<td>31.1 ± 2.0b</td>
<td>11.0 ± 0.8b</td>
</tr>
<tr>
<td>25/18</td>
<td>73.3 ± 14.3a</td>
<td>6.5 ± 1.2a</td>
<td>126.3 ± 25.6a</td>
<td>50.7 ± 9.8a</td>
<td>17.1 ± 3.1a</td>
</tr>
<tr>
<td>28/21</td>
<td>42.5 ± 5.0b</td>
<td>5.1 ± 0.6b</td>
<td>65.3 ± 8.0b</td>
<td>15.1 ± 1.5c</td>
<td>5.3 ± 0.6c</td>
</tr>
</tbody>
</table>

† Data indicate the uptake of macro–elements of tomato shoots at three different temperature conditions at 15 days after treatment, and tomato plants were grown under the 1/2 strength Hoagland’s nutrient solution. The letters mean significant differences from LSD test (n=3)
Table 3. The effect of the extra-supplied nutrients on the uptake rates of macro-elements

<table>
<thead>
<tr>
<th>Temperature (day/night, °C)</th>
<th>Nutrient supply</th>
<th>N</th>
<th>P₂O₅</th>
<th>K₂O</th>
<th>CaO</th>
<th>MgO</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/8</td>
<td>× 1.0</td>
<td>30</td>
<td>46</td>
<td>26</td>
<td>71</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>× 1.5</td>
<td>19</td>
<td>50</td>
<td>18</td>
<td>56</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>× 2.0</td>
<td>31</td>
<td>71</td>
<td>28</td>
<td>108</td>
<td>58</td>
</tr>
<tr>
<td>22/15</td>
<td>× 1.0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>× 1.5</td>
<td>98</td>
<td>103</td>
<td>113</td>
<td>109</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>× 2.0</td>
<td>98</td>
<td>100</td>
<td>119</td>
<td>144</td>
<td>139</td>
</tr>
<tr>
<td>25/18</td>
<td>× 1.0</td>
<td>84</td>
<td>85</td>
<td>92</td>
<td>163</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>× 1.5</td>
<td>94</td>
<td>96</td>
<td>108</td>
<td>139</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>× 2.0</td>
<td>78</td>
<td>96</td>
<td>119</td>
<td>144</td>
<td>139</td>
</tr>
<tr>
<td>28/21</td>
<td>× 1.0</td>
<td>49</td>
<td>66</td>
<td>47</td>
<td>49</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>× 1.5</td>
<td>34</td>
<td>71</td>
<td>32</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>× 2.0</td>
<td>39</td>
<td>78</td>
<td>38</td>
<td>39</td>
<td>41</td>
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</tbody>
</table>

F-value

<table>
<thead>
<tr>
<th></th>
<th>Temperature</th>
<th>Nutrient</th>
<th>Temperature × Nutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>82.42***</td>
<td>26.98***</td>
<td>136.02***</td>
</tr>
<tr>
<td></td>
<td>47.13***</td>
<td>90.00***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.61</td>
<td>3.24</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>5.53*</td>
<td>7.29**</td>
<td></td>
</tr>
</tbody>
</table>

† The data indicate a percentage of macro-element uptake when the uptake is defined as 100 in optimal supply (1/2 strength Hoagland nutrient solution) at optimal temperature condition (22/15°C). The concentration, × 1.0, means the 1/2 strength Hoagland’s nutrient solution.

Fig. 3. Effect of temperature stresses on the accumulation of soluble sugars and starch in the leaves of lettuce plants grown under 1/2 strength Hoagland’s nutrient solution at 15 DAT (n=3). The letters above vertical bars mean significant differences from LSD test.

Fig. 4. Effect of an extra-supply of nutrient solution on the accumulation of soluble sugars and starch in the leaves of lettuce plants grown under three different temperature conditions at 15 DAT (n=3). The concentration, × 1.0, means the 1/2 strength Hoagland’s nutrient solution. The letters above vertical bars mean significant differences from LSD test.
DISCUSSION

Temperature stresses (low and high temperature) are the major environmental factors affecting plant growth and development, and also induce morphological, physiological and biochemical changes in plants. It is well known that crop growth rates are significantly reduced by temperature stresses as results of a decrease in metabolic processes and photosynthesis (Sharkova, 2001; Wise et al., 2004; Waraich et al., 2012), shoot and root growth inhibition, and leaf senescence (Vollenweider and Gunthardt-Goerg, 2005), and our result also represented only one-third in low or high temperature conditions compared to the optimal. An extra-supply of minerals to promote lettuce growth didn’t have any effect in low or high temperature stress (Fig. 2), the growth was not differed in the optimal or moderately high temperature conditions, and even high temperature led to a marked reduction in the growth under 1.5- and 2.0-fold stronger mineral conditions. Waraich et al. (2011) reported that N fertilization mitigated the adverse effects of temperature stresses which induced the reduction in photosynthesis and growth by photo-oxidative damage (Waraich et al., 2011). Increasing K supply also alleviated the damage of leaf and stem damage and decrease in crop yield (Grewal and Singh, 1980; Hakerlerler et al., 1997), however the effect mentioned above was not observed in our present work. The concentration and uptake of macro-elements was strongly different with temperature conditions. Certainly, high temperature led to substantial accumulation of macro-elements, while low temperature represented lower N and K concentrations or higher P, Ca and Mg concentrations. This suggests the existence of a controlling mechanism in plant, particularly root, which is mediated by physiological responses such as transpiration, mineral absorption, and leaf and root growth. Marked reduction in mineral concentration has been considered as a factor responsible for temperature stresses (Ali et al., 1998; Tindall et al., 1990; Engels and Marschner, 1996; Du and Tachibana, 1994). However, mineral concentrations except N and K at low temperature in lettuce shoots increased at both low and high temperatures, and it might be interpreted to be the mineral accumulation as the result of restricted utilization by growth cessation. Undoubtedly, a remarkable growth inhibition which was caused by low and high temperature stresses adversely affected macro-element uptake (Table 2), this means that mineral uptake greatly depends on ambient temperature conditions, similar to the observation by Ali et al. (1998) and Tindall et al. (1990). The extra-supply of minerals as a means of promoting mineral uptake wasn’t noticeable in all temperature conditions although two-fold stronger minerals affected a slight increase in macro-element uptake at low temperature stress (Table 3). Interestingly, an extra-supply of P led to the significant increase in P uptake as lettuce plant was suffered from temperature stresses. Low and high temperatures markedly reduced soluble sugars and starch levels in lettuce shoots (Fig. 3). More carbon losses due to increased respiration and the shortage of non-structural carbohydrate have long been considered as factors responsible for the growth inhibition under various temperature stresses (Youngner and Nudge, 1968; Canmore-Neumann and Kafkafi, 1983), consistent with our observation. Starch metabolism (diurnal fluctuation) is very sensitive to changes in the environment, and soluble sugars that accumulate in response to stress can function as osmolytes to maintain cell turgor against adverse environments, particularly temperature stresses (Madden et al., 1985; Todaka et al., 2000; Kaplan and Guy, 2004; Basu et al., 2007; Kempa et al., 2008). In this study, a decline of soluble sugars and starch levels is possibly considered as a result of restricted water and nutrient absorption and limited photosynthesis although further study is needed for the clarification of carbohydrate metabolism. The extra-supply of minerals didn’t also have any effect to enhance soluble sugars and starch levels, namely photosynthesis. In conclusion, temperature stresses caused significant reduction in crop growth, mineral uptake and carbohydrate production while macro-element concentrations in lettuce shoots seemed likely to be a temperature-dependent response. Moreover, extra-supply of minerals didn’t play crucial roles to promote an adverse damage caused by temperature stresses. Further research should include the effect of extra-supply of minerals when plants are recovered from low or high temperature stress, and metabolic interaction such as mineral uptake and carbohydrates between shoot and root.

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