# IMPROVEMENT OF HYDROPONIC CULTURE MEDIUM BY ADDING CALCIUM-ZEOLITE

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## Abstract

**In order to add the buffering effect of calcium (Ca) ions to hydroponic solution, Ca-zeolite was introduced to the solution. Tomato plants (*Lycopersicon esculentum* Mill.) were grown in hydroponic solution containing excess amounts of copper ions. Even though excess copper ions inhibited the growth of tomato plants, when Ca-zeolite was added to the hydroponic solution, the recovery of growth was observed. Thus, it was demonstrated that Ca-zeolite could be used as a buffering agent when harmful ions are dissolved in the hydroponic solution. In order to test more practical applications under hydroponic conditions, the concentration of the hydroponic solution was increased until blossom-end rot was induced in tomato fruits. When Ca-zeolite was added to the solution, blossom-end rot could be reduced drastically. It was shown that Ca-zeolite added to hydroponic solution could be used to avoid the onset of physiological disorders associated with salt stress.**

## Key index words

**Blossom-end rot, Calcium, Copper, Hydroponic culture, Tomato, Zeolite, Zinc.**

## 1. Introduction

**Hydroponic culture is currently the most popular cultivation method in automated systems in plant factories and greenhouses, and the effect of variations in ion concentrations of hydroponic solution upon plant growth is a concern which was chosen as the focus of this study. Nutrient solution composed of a variety of ions influenced absorption and water status of plants completely differently from a solution composed of single ions under salt stress conditions (Nonami et al., 1992). When zeolite is added to a solution, zeolite has cation exchange capacity, and the solution increases buffering capacity against changes in pH and cation concentrations (Fukuyama et al., 1994). Because zeolite has cation exchange capacity, if zeolite is conjugated with divalent elements, zeolite will have a large divalent ion exchange capacity. Calcium-zeolite was used in the present study, because Ca is known to be essential for preventing the blossom-end rot in tomato plants (Evans and Troxler, 1953; Maynard et al., 1957; Van Goor, 1968; Ward, 1973; Wiersum, 1966).**

**Although micro-elements such as copper (Cu) and zinc (Zn) are essential for plant nutrition, if concentrations of Cu and Zn are too high, plant growth will be inhibited. Because Cu, Zn and Ca ions are divalent ions, Ca-zeolite may have a large ion exchange capacity with Cu and Zn ions in solution. We tested whether the addition of Ca-zeolite was effective for preventing Cu and Zn overdose in hydroponic culture.**

## 2. Materials and Methods

### 2.1. Seedling preparation

**Tomato plants (*Lycopersicon esculentum* Mill. cv. Hausu-Momotaro) (Takii Seed Co. Ltd., Kyoto, Japan) were used in the present study. Seeds were sown in vermiculite under greenhouse conditions. From the 23rd day after germination, seedlings were grown hydroponically in solution having electric conductivity (EC) of 0.1 S/m diluted from Hoagland's solution. All culture solutions were made with pure water and chemicals of high quality.**

### 2.2. Copper ion treatment

**In order to induce growth inhibition, copper ions were added to the culture solution in amounts of 80 μM. After adding 80 μM of copper ion, 5.0 g/L of Ca-zeolite was added to the culture solution to avoid growth inhibition due to copper toxicity. Relative growth rates of leaves were determined by measuring the leaf area of tomato plants. Immediately after determination of relative growth rates of leaves, water potentials of leaves were measured with isopiestic psychrometers (Boyer and Knipling, 1965).**

### 2.3. Induction of blossom-end rot in tomato fruits

**Blossom-end rot in tomato fruits was induced by changing compositions of the hydroponic solution from Hoagland's solution to MS salt medium (Murashige and Skoog, 1962), and also, the concentration of the solution was elevated gradually from 0.1 S/m to 0.6 S/m of EC. In order to avoid the occurrence of blossom-end rot, the Ca-zeolite was added to the hydroponic solution at concentrations of 0.5 g/L, 3 g/L, 10 g/L, 20 g/L or 40 g/L. Eight tomato plants were grown in each treatment plot.**

### 2.4. Measurements of ion concentrations

**Plant tissue was excised, and immediately after excision, the tissue was set between plastic plates fixed on a vise. Cell sap was extracted from the tissue by squeezing with the vise, and filtered through an ashless paper filter. Concentrations of ions soluble in the hydroponic solution and plant tissues were analyzed by using an atomic absorption and flame emission spectrophotometer (AA-880 mark II, Nippon Jarrel-Ash Co., Ltd., Uji, Japan).**

## 3. Results

When the concentration of copper ions was increased to 80 µM in the hydroponic solution, growth of tomato plants was inhibited. When 4.0 g/L of Ca of zeolite was added to the copper-added hydroponic solution, the growth rates recovered to the same level as the control plants. Thus, Ca-zeolite could be used to avoid growth inhibition due to copper toxicity.

While the growth inhibition due to the copper toxicity was occurring, the relation between the water status of the growing region and relative growth rates of tomato leaves was measured by using the isopiestic psychrometer. When the growth was inhibited by adding copper ions to the hydroponic solution, the water potential of the young leaves became -0.8 MPa (Fig. 1). When the relative growth rate of the copper-treated plants was $1.3 \times 10^{-6}$ $S^{-1}$, the water potential decreased further to -1.2 MPa (Cu (80 µM) in Fig. 1).

When the copper-treated plants were grown in the hydroponic solution containing 4.0 g/L of Ca-zeolite, the growth recovered and the water potential of the growing region rose to the same level as that of the control plants (Fig. 1). This indicates that the copper toxicity contributed to the decrease in water potential in the growing region while the growth of the plants was retarded by the addition of copper ions.

When concentrations of the hydroponic solution were increased, tomato plants tended to have fruits having blossom-end rot. When Ca-zeolite was added to the hydroponic solution, the symptoms of the blossom-end rot in fruits decreased (Fig. 2). When the concentration of Ca-zeolite in the hydroponic solution was adjusted to higher than 20 g/L, blossom-end rot was prevented (Fig. 2).

The addition of Ca-zeolite to the hydroponic solution increased the concentration of Ca ions in the hydroponic solution (Fig. 3A). Concentrations of Ca ions in root and stem tissues were significantly lower than Ca concentration of the hydroponic solution (Fig. 3A). Although the addition of Ca-zeolite to the hydroponic solution increased the concentration of Ca ions in the stem tissue, the concentration of Ca ions in tomato fruits was not increased significantly (Fig. 3B). The concentration of Ca ions in tomato fruits was always higher at the side closer to the calyx (Fruit (Up) in Fig. 3B) than the side closer to the tip of tomato fruits (Fruit (Low) in Fig. 3B), indicating that there exists a Ca concentration gradient in tomato fruits.

The addition of Ca-zeolite to the hydroponic solution decreased the concentrations of Zn ions in the hydroponic solution, root tissues and stem tissues (Fig. 4A). Further, the decrease in concentration of Zn ions occurred in fruits, and the concentration of Zn ions at the side closer to the calyx (Fruit (Up) in Fig. 4B) became lower than that at the side closer to the tip of tomato fruits (Fruit (Low) in Fig. 4B) when the concentration of Ca-zeolite became higher than 20 g/L. Because the decrease in Zn ion concentrations coincided with the decrease in blossom-end rot occurrence rates by the Ca-zeolite treatments (Figs.

**2 and 4), the Zn ion concentrations may be directly related with the metabolic disorder related with blossom-end rot in tomato fruits.**

## 4. Discussion

**Growth inhibition was induced by adding copper ions to the hydroponic solution. Although copper ions are essential for plant nutrition (Rains, 1976), when the concentration exceeds more than the adequate level, plant growth may be inhibited as shown here. Even though a growth-inhibiting concentration of copper ions was added to the hydroponic solution, when an adequate amount of Ca-zeolite was added to the solution, growth recovery was observed.**

**Ca-zeolite had a buffering capacity with cations in the solution, and thus, copper ions could be exchanged with Ca bounded with zeolite. If so, copper ions could be absorbed by zeolite, and instead of copper ions, Ca ions could be released from zeolite in the solution. Therefore, the toxicity of copper could be avoided as shown in Fig. 1 in the present study.**

**As essential micro-element nutrients, plants require a larger amount of zinc than copper (Rains, 1976), and thus, fertilizer contains significantly more zinc than copper. When higher concentrations of fertilizers were given to plants without modifications of concentrations of micro-elements, high concentrations of the micro-elements might have prohibited physiological metabolisms in plants. In the present study, concentrations of Zn ions were more than necessary for Zn requirement for tomato plants, and thus, blossom-end rot in tomato fruits might be directly caused by zinc toxicity. When Ca-zeolite was added to the hydroponic solution, the concentrations of Zn ions in the hydroponic solution as well as root tissues, stem tissues and fruit tissues were reduced significantly (Fig. 4). Because the addition of Ca-zeolite decreased the occurrence rate of blossom-end rot in fruits drastically (Fig. 2), it is most likely that blossom-end rot in tomato fruits in the present study could be induced by an overdose of zinc ions in the hydroponic solution.**

**When zeolite is added to a solution, the solution increases its capacity for pH buffering (Fukuyama et al., 1994). By using this characteristic of zeolite, a new tissue culture medium was developed by mixing zeolite together with tissue culture medium solution (Fukuyama et al., 1994). The buffering capacity of pH in the medium increased significantly against both acidification and alkalization. When embryos taken out from *Phaseolus vulgaris* seeds were grown in media containing zeolite, the length of plantlets formed from the embryos became larger and the dry weight of the plantlets became heavier than plantlets grown in media containing no buffering agents (Fukuyama et al., 1994). A similar principle should be applicable to the hydroponic culture, and thus, Ca-zeolite can be used as a buffering agent of pH and nutrient for growing plants hydroponically as shown in the present study.**

Cell enlargement can be inhibited by inadequate water uptake in the growing tissues. When plants are growing, water potential gradient between the water source and elongating cells exist in the growing tissue (Nonami, 1993; Nonami and Boyer, 1990a; Nonami and Boyer, 1993). Such a water potential gradient associated with growth is called the growth-induced water potential (Nonami, 1993; Nonami and Boyer, 1990a; Nonami and Boyer, 1993). The size of the growth-induced water potential can be obtained from a difference in water potential between the water source and the growing tissue (Nonami and Boyer, 1990a; Nonami and Boyer, 1993). Relationship between the growth-induced water potential and growth rates can be expressed in the following equation;

$$G = L(\Psi_o - \Psi_w)$$

where G, L, $\Psi o$ and $\Psi w$ indicate relative growth rate, hydraulic conductance, water potential of the water source and water potential of the elongating region (Nonami and Boyer, 1990a; Nonami and Boyer, 1993). The ($\Psi o$ - $\Psi w$) is the growth-induced water potential. The hydraulic conductance is related to the water flow path into the enlarging cells and indicates water permeability of the cell membrane (Nonami and Boyer, 1990a; Nonami and Boyer, 1990b).

When copper ions were added to the hydroponic solution, the growth of tomato plants was inhibited, and the slope of water potential against the relative growth rate became steeper than that of the control plants (Fig. 1). Because the slope of water potential against the relative growth rate is corresponding to the hydraulic conductance, an increase in the slope indicates that water permeability of the cell membrane decreased in the growing tissue. Therefore, it can be said that copper toxicity contributed to the decrease in the water permeability in cell membranes in the growing tissue.

When Ca-zeolite was added to the solution containing copper ions, the growth of tomato plants recovered, and simultaneously, the water potential of the elongating region increased to the level equivalent to the control plants (Fig. 1). This indicates that the addition of Ca-zeolite to the hydroponic solution recovered the membrane permeability of cells in the growing tissue, resulting in the increase in water potential of the growing tissue. These results indicate that copper toxicity causes the physiological disorders in cell membranes by decreasing hydraulic conductance of the water flow path into the enlarging cells.

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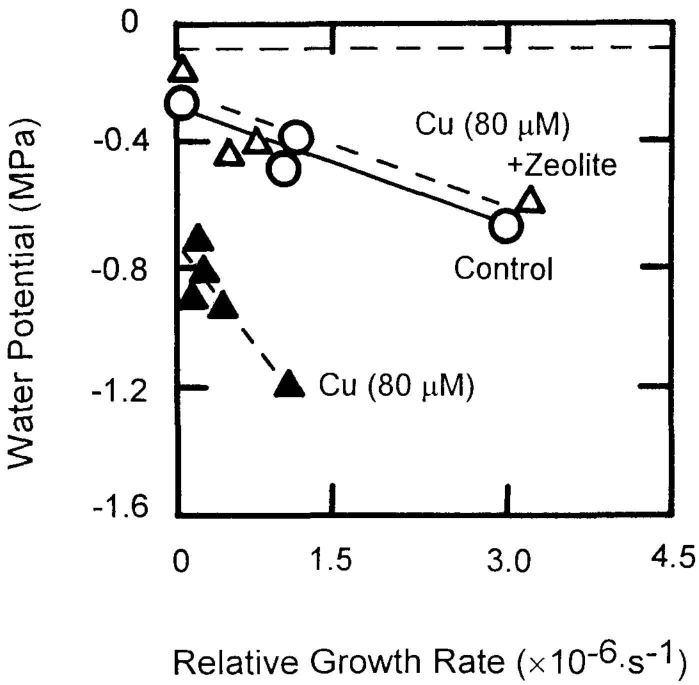

Fig. 1. Relationship between water potentials of the expanding leaves and relative growth rates of leaves of tomato plants grown in the hydroponic solution containing 80 μM of Cu ions (▲), without additional Cu ions (Control; ○) and with 5.0 g/L of Ca-zeolite + 80 μM of Cu ions (△).

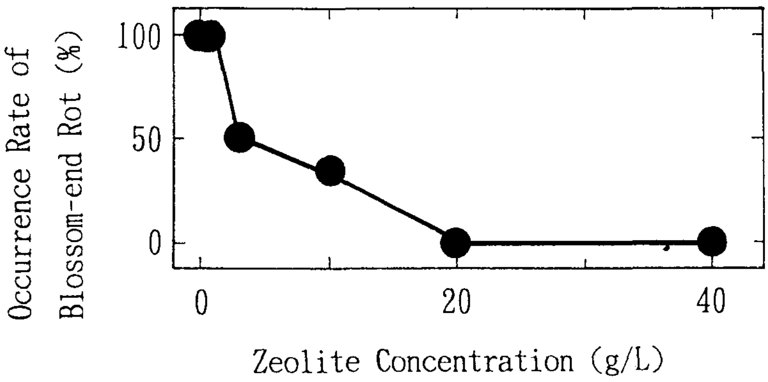

Fig. 2. Occurrence rates of the blossom-end rot in tomato fruits of plants which were grown in hydroponic solution added with various concentrations of Ca-zeolite.

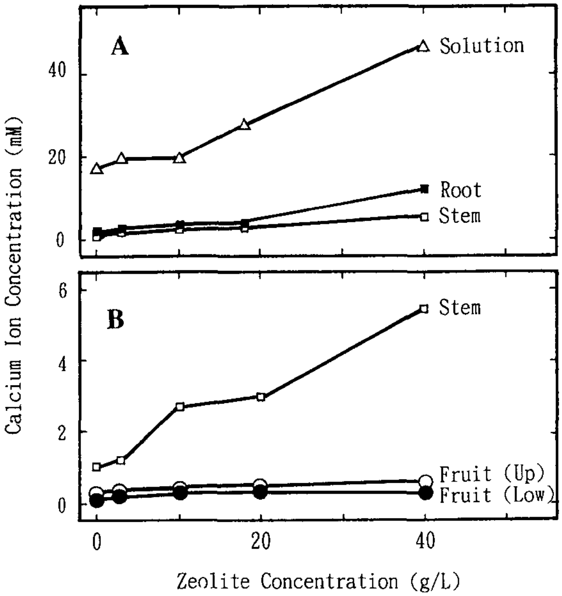

**Fig. 3. Calcium ion concentrations the hydroponic solution (A), roots (A), stems (A, B), and the upper part of fruits (B) and the lower part of fruits (B) of plants cultivated with addition of various concentrations of Ca-zeolite.**

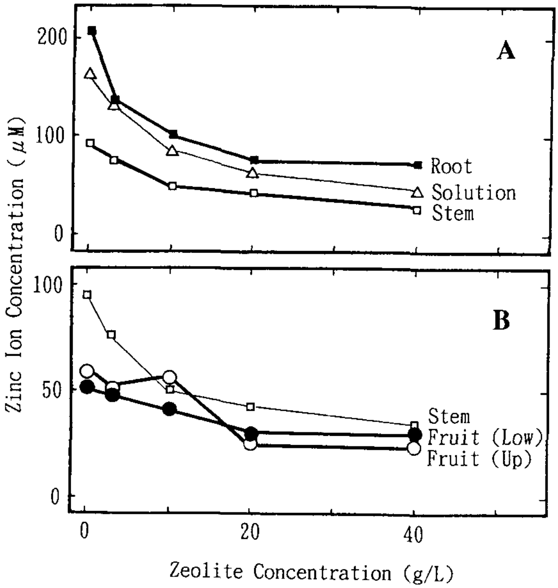

**Fig. 4. Zinc ion concentrations the hydroponic solution (A), roots (A), stems (A, B), and the upper part of fruits (B) and the lower part of fruits (B) of plants cultivated with addition of various concentrations of Ca-zeolite.**