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Induction of Root Browning by Chloramine in *Lactuca sativa* L. Grown in Hydroponics

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Summary

The cause of root browning (RB) in *Lactuca sativa* L. root, which often occurs in hydroponics when a nutrient solution is prepared with tap water, was investigated. The induction of RB occurred only when plants were cultured in the solutions containing both hypochlorous acid (HOCl) and ammonium ion (NH_4^+), but not in the solutions contained either HOCl or NH_4^+ alone. The addition of NaOCl (HOCl) followed by $NH_4H_2PO_4$ (NH_4^+) solution after a 7–10 day storage or $NH_4H_2PO_4$ followed by NaOCl solution induced no RB symptom. It was concluded that RB was induced by chloramine, a reaction product between HOCl and NH_4^+. Dechlorination of nutrient solutions was promoted in the presence of the iron ion in the solution under light conditions.

Key Words: chloramine, HOCl, *Lactuca sativa* L., NH_4^+, root browning.

Introduction

The occurrence of root browning (RB) in *Lactuca sativa* L. roots, an abnormal discoloration of the epidermis, has been frequently associated with hydroponic culture. This physiological disorder is often induced when a nutrient solution is renewed to prevent plant disease or to modify the balance of nutrient elements. Such RB develops within a few hours and causes the plants to wilt, leads to growth inhibition, or even death.

Krone and Weinard (1931), Zimmermann and Berg (1934), Buxton (1938), Bridgen (1986), and Frink and Bugbee (1987) reported that residual chlorine in the form of hypochlorous acid (HOCl) in tap water at near neutral pH induced RB and inhibited root and plant growth, but the critical concentrations of HOCl differed among the reports. It has been reported that NH_4^+ at 6 to 15 me·liter^{-1} in hydroponic culture causes RB (Maynard and Barker, 1969; Ikeda and Osawa, 1982; Findenegg, 1987) but not at less than 2 me·liter^{-1} generally present in nutrient solution.

In this paper, we examined why hydroponic culture causes the development of RB, and found that it occurred when chloramine, a reaction product between HOCl in tap water and NH_4^+, in the nutrient solution reaches a toxic level.

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Materials and Methods

Exp. 1. Identification of HOCl and NH_4^+ as causal elements for the occurrence of RB

Butter head lettuce (*Lactuca sativa* L.) cv. Okayama-saradana seeds, which were sown in commercial soil on July 3, 1999 and watered, were transferred to the half-strength of Enshi standard nutrient solution (HENS) at the 3-leaf stage. The concentrations of NO_3-N, NH_4-N, P, K, Ca and Mg as macro elements in the HENS were 8, 0.67, 2, 4, 4 and 2 me·liter^{-1}, respectively. Whereas Fe, B, Mn, Cu, Zn and Mo as micro elements were 3, 0.5, 0.5, 0.02, 0.05 and 0.01 mg·liter^{-1}, respectively. The pH of HENS, prepared with tap water in experimental field of Kyoto Prefectural University, was 6.2. Five seedlings were fixed on a styrofoam plate (100 × 400 × 20 mm) with a urethane cube (25 × 25 × 30 mm). Seven styrofoam plates were floated on 40 liters of the above mentioned nutrient solution, in a plastic container (440 × 740 × 240 mm). At the 12-leaf stage, 6 seedlings were fixed on another styrofoam plate (300 × 400 × 25 mm), and the plate was floated on 20 liters of each test solution in a plastic container (320 × 420 × 215 mm). Treatments were duplicated. The nutrient solution was aerated continuously with an air pump, and the pH of nutrient solutions was not regulated.

In Exp. 1.1, to determine which nutrient element causes RB, seedlings were transplanted to the HENS without one of following elements ; $Ca(NO_3)_2$, KNO_3, $MgSO_4$, $NH_4H_2PO_4$, NaFe-EDTA, and the other micro elements, prepared with tap water. In Exp. 1.2, plants were cultured in tap water with NaH_2PO_4 or $(NH_4)_2SO_4$ at concentrations equivalent to those in HENS. In Exp.

1.3, lettuce plants were cultured in HENS or $NH_4H_2PO_4$ solution, prepared with fresh tap water and stored for 10 days before use, or in two solutions, prepared with tap water previously stored for 10 days to test their stability. In Exp. 1.4, to test if RB occurs when both HOCl and NH_4^+ are in nutrient solutions, lettuce plants were cultured in deionized water containing either $(NH_4)_2SO_4$ or NaOCl, or both. The concentration of NaOCl was 0.5 mg Cl・liter^{-1}, equivalent to HOCl in tap water; the concentration of NH_4^+ was the same as in HENS. The concentration of HOCl was monitored with a colorimeter (SHIBATA) by adding *N, N*-Diethyl-*p*-phenylene-diamine (DPD) regent to the sample solution. The concentration of total chlorine was measured by adding potassium iodide to a water sample containing DPD. The concentration of chloramine was estimated as the difference between the concentration of total chlorine and that of HOCl.

In all experiments, the occurrence of RB and plant condition were evaluated two days after the start of the treatment.

Exp. 2. Occurrence of RB by alternate treatment with HOCl and NH_4^+

'Okayama-saradana' seedlings grown to the 12-leaf stage as in Exp.1 were transplanted to each test solution and cultured by the schedule shown in Table 4. Hypochlorous acid or NH_4^+ or both were added to tap water, dechlorinated by storing for 7 days, until the concentrations were equivalent to those in HENS in Exp.1. Plant numbers, replications, and management of each treatment were the same as Exp.1. Two days after the start of the treatment, the occurrence of RB and plant condition were evaluated.

Table 1. Occurrence of root browning (RB) in lettuce cultured in half-strength of Enshi standard nutrient solution (HENS)[z] prepared with fresh tap water, from which one of salts or all micro elements were excluded.

Solution	Brown symptom in root[y]
Tap water	−
Complete HENS	+
HENS without $Ca(NO_3)_2$	+
HENS without KNO_3	+
HENS without $MgSO_4$	+
HENS without $NH_4H_2PO_4$	−
HENS without NaFe-EDTA	+
HENS without Micro elements	+

[z] The concentration of NO_3-N, NH_4-N, P, K, Ca and Mg was 8, 0.67, 2, 4, 4 and 2 me・liter^{-1}, respectively, and that of Fe, B, Mn, Cu, Zn and Mo was 3, 0.5, 0.5, 0.02, 0.05 and 0.01 mg・liter^{-1}, respectively.

[y] +, root of all the plants in each replication turned brown ; −, root of all the plant in each replication not brown.

Exp. 3. Change in HOCl and chloramine concentrations in the nutrient solution with time

The changes in concentrations of HOCl and chloramine in various solutions with time under different light conditions were investigated by prerparing 60 liters of $NH_4H_2PO_4$ solution with tap water containing NaFe-EDTA or Na-EDTA, and storing them under natural light or in the dark. The concentrations of NH_4^+ as $NH_4H_2PO_4$ solution, Na-EDTA, and NaFe-EDTA were the same as those in HENS. The concentrations of HOCl and chloramine were measured daily as above.

Results and Discussion

In Exp. 1.1, RB occurred in all the plants cultured in the nutrient solution, containing $NH_4H_2PO_4$, but not in HENS without $NH_4H_2PO_4$ or in tap water (Table 1). In Exp. 1.2, RB was observed in all the plants that were cultured only in $(NH_4)_2SO_4$, but not in tap water or NaH_2PO_4 (Table 2). In both cases, lower leaves wilted 1 hr after transplanting, and RB was clearly observed 2 days after transplanting but only in the nutrient solution containing NH_4^+. Maynard and Barker (1969) reported that RB was induced by cultivation with the nutrient solution containing 15 me・liter^{-1} of NH_4^+ in bean, sweetcorn, cucumber and pea, whereas Ikeda and Osawa (1982) did not observe RB in the plants cultured in the solution containing 6 me・liter^{-1} of NH_4^+ and 6 me・liter^{-1} of NO_3^-. Furthermore, Findenegg (1987) reported that a 14- to 28-day treatment of 6 me・liter^{-1} of NH_4^+ at pH 4.0 induced RB in many plants but not at a neutral pH within a few days. In our experiments, NH_4^+ concentration in the nutrient solutions was only 0.67 me・liter^{-1} and pH was near neutral so that the disorder is not a case of simple NH_4^+ toxicity.

Krone and Weinard (1931) reported that irrigating with water containing 5 mg Cl・liter^{-1} suppressed plant height and fresh weight to 90 and 67% of the control, respectively, in seven different potted plants, including petunia. According to Buxton (1938), shoots and roots were injured by irrigation with chlorinated tap water. Water with more than 7.6 mg Cl・liter^{-1} inhibited growth and decreased flower number in zinnia plants (Bridgen, 1986). In Exp. 1.3, RB did not occur in either solution, which indicates that a compound that induces RB is present in tap water but it is decomposed during a

Table 2. Occurrence of RB in lettuce cultured in fresh tap water with either NH_4^+ or PO_4^{3-} added.

Solution	Brown symptom in root[z]
Tap water	−
$(NH_4)_2SO_4$	+
NaH_2PO_4	−

[z] See Table 1.

10-day storage. Neutral tap water contains residual chlorine as HOCl that decomposes with time (Kaneko, 1996), which indicates that HOCl might be involved in RB. In Exp. 1.4, RB did not occur in the solution containing only NaOCl (Table 3), but NaOCl in approximately pH neutral water hydrolyzes to HOCl. Frink and Bugbee (1987) found that the growth of the most susceptible species among eight different potted plants and four different vegetables was significantly decreased when they were irrigated with the water containing 2 mg $Cl \cdot liter^{-1}$ HOCl, but not with that containing 1 mg $Cl \cdot liter^{-1}$ HOCl. Zimmermann and Berg (1934) reported that less than 5 mg $Cl \cdot liter^{-1}$ HOCl had no effect on plant growth in most of the 13 species they examined. In our nutrient solution in this experiment HOCl was only equivalent to 0.5 mg $Cl \cdot liter^{-1}$, much less than that in the above reports, so that RB is not induced by HOCl.

In Exp. 1.4, intensive RB was observed in the solution containing both NaOCl and $(NH_4)_2SO_4$ (Table 3). The symptom and progress of RB were the same as in Exp. 1.1 and 1.2. This result strongly suggests that RB occurs when HOCl and NH_4^+ coexist in nutrient solution that react to form chloramine (Kaneko 1996). In Exp. 2, the presence of both NaOCl and $NH_4H_2PO_4$, induced RB and wilting in all plants (treatment 2 in Table 4 and Fig. 1). Plants, exposed to NaOCl, did not develop RB and wilt although the roots lost turgor (treatments 3, 5 and 6 in Table 4 and Fig. 2), which suggested that 0.5 mg $Cl \cdot liter^{-1}$, equivalence of HOCl, is slightly harmful to lettuce roots. It also suggests that the serious RB might be caused by chloramine, but not by HOCl or NH_4^+. Chloramine is known to change its form depending on the pH and chlorine: NH_4^+ ratio, e.g. mono-chloramine (NH_2Cl), di-chloramine ($NHCl_2$) and nitrogen trichloride (NCl_3). Since HENS is prepared with tap water in the experimental farm of Kyoto Prefectural University,

Table 3. Occurrence of RB in lettuce cultured in deionized water with containing NH_4^+ and/or HOCl added.

Solution	Brown symptom in root[z]
Deionized water	−
$(NH_4)SO_4$	−
NaOCl	−
$(NH_4)_2SO_4$+NaOCl	+

[z] See Table 1.

Table 4. Occurrence of RB in lettuce alternately treated with HOCl and/or NH_4^+.

Treatment	Brown symptom in root[z]
1. Dechlorinated tap water	−
2. NaOCl+$NH_4H_2PO_4$	+
3. NaOCl	−
4. $NH_4H_2PO_4$	−
5. 24-hr NaOCl → $NH_4H_2PO_4$	−
6. 24-hr $NH_4H_2PO_4$ → NaOCl	−

[z] See Table 1.

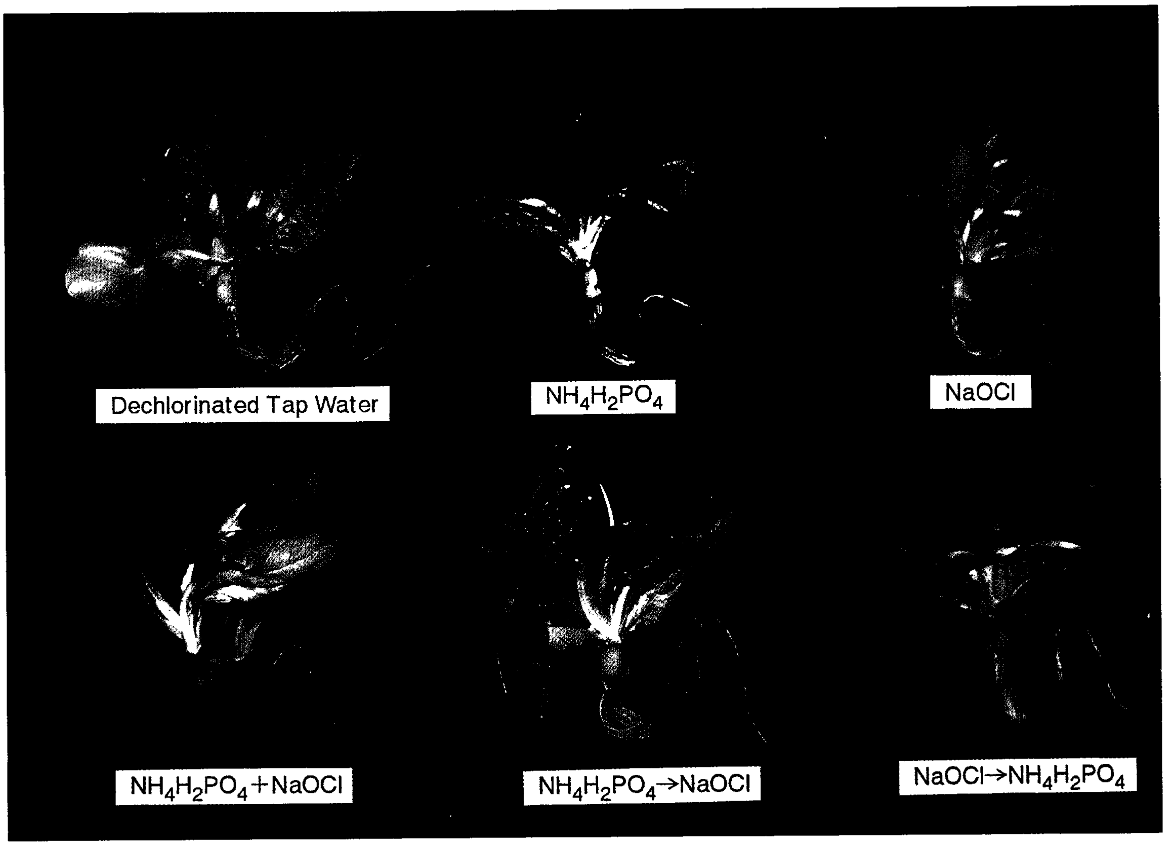

Fig. 1. Lettuce plants 4 days after alt..nate treatment with HOCl and/or NH_4^+ (Exp. 2).

pH is approximately neutral and chlorine: NH_4^+ ratio is approximately 1:24. Hence, the dominant chloramine in the present experiment is considered to be NH_2Cl (Kaneko, 1996).

Generally, in the water filtration plant, tap water is prepared by adding Cl_2, which form HOCl in neutral pH water, to suppress the propagation of harmful microorganisms. Iron and organic matter are known to promote the decomposition of HOCl (Yamada, 1987). Light, especially ultra violet light, breaks down NaFe-EDTA in the nutrient solution into iron ions and organic compounds. However, the equilibrium between HOCl and chloramine in the nutrient solution prepared with tap water is not known. In Exp. 3, the concentrations of HOCl and chloramine in the nutrient solution, containing many nutrient elements, change with time in the light and dark. At the start of the treatments, the concentration of total chlorine in all solution was 0.5 mg Cl · $liter^{-1}$ (Fig. 2) while that of chloramine in the nutrient solutions containing both HOCl and NH_4^+ was 0.3 mg Cl · $liter^{-1}$ at day 0. In the light, total chlorine in the solutions containing NaFe-EDTA decreased to zero

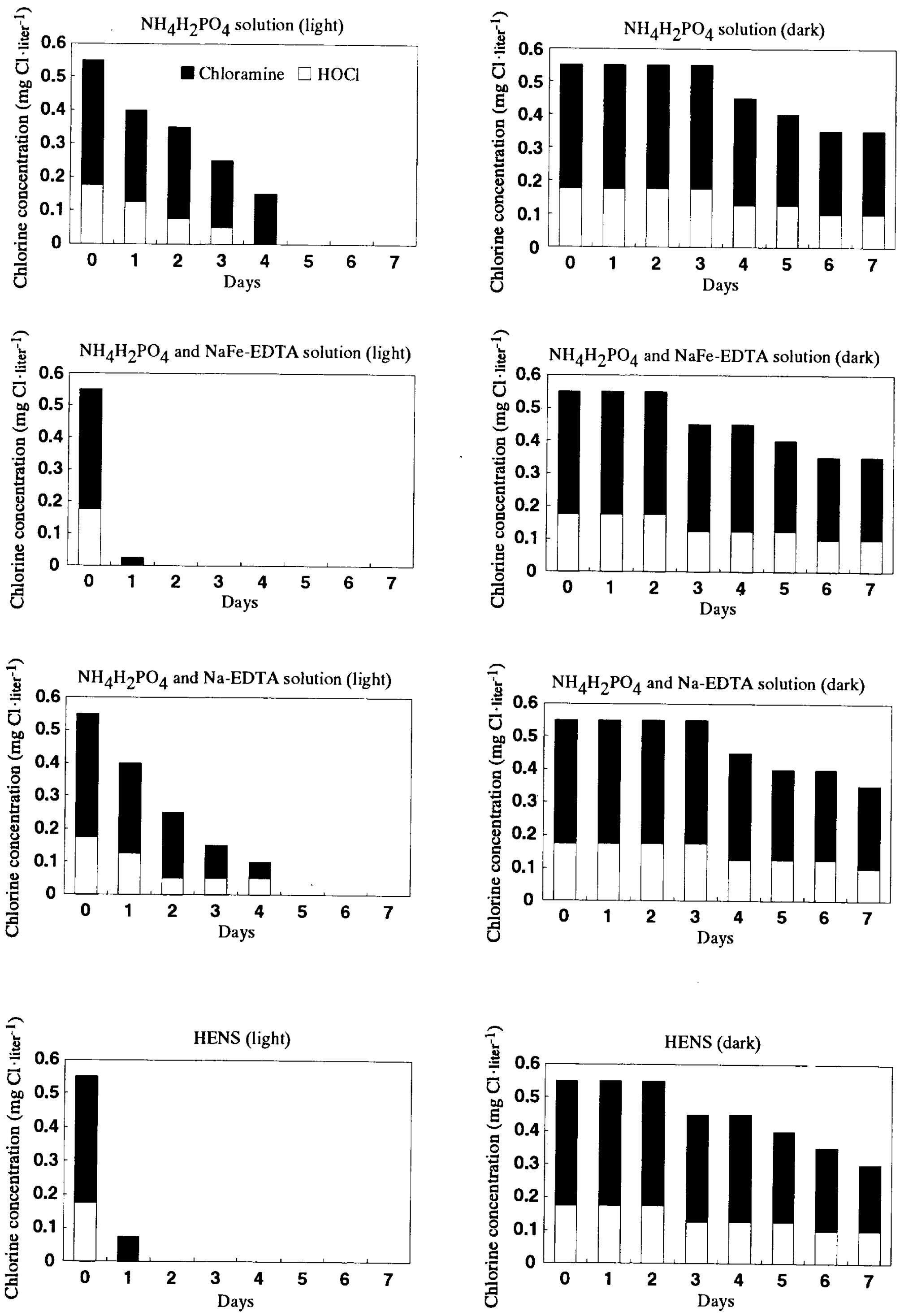

Fig. 2. Changes in the concentrations of chloramine and HOCl in $NH_4H_2PO_4$ solutions containing either NaFe-EDTA or Na-EDTA and in HENS prepared with tap water in the light and darkness. Almost same results were obtained in three independent experiments. Typical data are shown here.

on day 2 but it was day 5 in the absence of NaFe-EDTA. In the dark, disappearance of total chlorine was slow, the concentration remained higher than 0.3 mg Cl・$liter^{-1}$ even at day 7. These results show that the residual chlorine decreases faster in the light than in the dark, especially in the presence of NaFe-EDTA. Additional tests revealed that it was the iron ion rather than EDTA that caused the reduction of total chlorine in the light.

In this study, we concluded that chloramine induces the root injury in the lettuce plant. In Japan, 34% of hydroponic growers use tap water, 63% underground water and 6% rain water, according to the Research Data of National Institute of Vegetable, Ornamental and Tea No. 21 (1986). The use of chlorinated tap water prevents the spread of plant pathogens and pollution of underground water with nitrate and other nutrient elements. Tap water may contain more than 1 mg Cl・$liter^{-1}$, depending on the season and distance from the water filtration plant to the greenhouse. In addition, growers often treat the irrigation systems, seeds, and seedlings with HOCl to sterilize them; this results in the formation of chloramine and root injury. It is clear that chlorine compounds decompose in the light, especially in the presence of iron ion. But nutrient solutions are often kept in the dark so that chlorine compounds are not expected to decompose rapidly. Furthermore, iron ion forms iron hydroxide easily and cannot be absorbed by the plant. Thus, it may be necessary to dechlorinate the nutrient solution with a reducing agent such as sodium thiosulfate (Yamazaki, 1982), although the effect of reducing agents on plants is still unknown.

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水耕栽培におけるクロラミンにより発生するサラダナの根部褐変

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摘　要

水道水を用いて作成した培養液による水耕栽培で，しばしば発生する根部褐変の原因について調査した．サラダナの根部褐変は次亜塩素酸の形態で存在する水道水中の残留塩素とアンモニウムイオンが存在する培養液でのみ発生し，どちらか一方しか存在しない培養液では発生しなかった．また，次亜塩素酸あるいはアンモニウムイオンどちらかを含む培養液に交互に移植しても根部褐変は発生しなかった．従って，根部褐変は次亜塩素酸とアンモニウムイオンにより生成するクロラミンにより発生するものと考えられた．さらに培養液中の残留塩素濃度の低下は，光条件下で鉄イオンの存在により促進された．