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EFFECT OF NITROGEN SOURCE ON GROWTH AND MINERAL UPTAKE IN PLANTS UNDER NITROGEN-RESTRICTED CULTURE CONDITION*

Masumi MORITSUGU and Toshio KAWASAKI

In the previous papers[12,13], the effects of ammonium and nitrate nitrogen on the growth and mineral uptake of higher plants were examined by the constant pH and conventional culture methods. The pH of the nutrient solution was kept constant at 5.5 ± 0.1 in the former method, and was adjusted manually to 5.5 every other day in the latter method.

The growth of such plants as tomato, cabbage, chinese cabbage, spinach and radish (group 1) fed with ammonium was stunted even in the constant pH solution culture. On the other hand, plants such as barley, corn, sorghum, cucumber, lettuce and carrot (group 2) grew vigorously on ammonium in the constant pH culture. Rice plants grew rapidly with ammonium even in the conventional solution culture. The group 1 plants seemed to be sensitive to both ammonium and hydrogen ions; the group 2 plants seemed to be sensitive to hydrogen ion, and less sensitive to ammonium ion. The rice plants did not seem to be sensitive to either ion[12,13].

However, it is supposed that the growth of ammonium sensitive plants may be accelerated when the rate of uptake of ammonium ion is maintained at lower than that of the assimilation of ammonium in plants. To examine this possibility, a new culture method, which can facilitate a continuous supply of a small but sufficient amount of ammonium or nitrate ions, was designed. In this method the plants were grown in a nitrogen (ammonium or nitrate)-restricted nutrient solution. This new method was used to examine the growth and mineral uptake of higher plants grown on either ammonium or nitrate.

MATERIALS AND METHODS

Plants used in the present investigation were: cucumber (*Cucumis sativus* L.; cv. Chihai), tomato (*Lycopersicon esculentum* Mill.; cv. Beiju), cabbage (*Brassica oleracea* L.; cv. Natsu-maki nigatsu-dori and Aki-maki-wase), chinese cabbage (*Brassica pekinensis* Rupr.; cv. Harumaki goku-wase and Kashin), spinach (*Spinacia oleracea* L.; cv. Ujoh)

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and radish (*Raphanus sativus* L.; cv. Kouhaku-hatsuka and Mino-wase).

As described previously[12,13], these plants are sensitive to ammonium and hydrogen ions, except cucumber which is insensitive to ammonium ion but sensitive to low pH. In the present investigation, culture experiments were conducted employing the device for the nitrogen-restricted culture at solution pH 5.5.

Fig. 1 shows the block diagram of the apparatus consisting of two systems used for the constant pH culture. One of them was used to

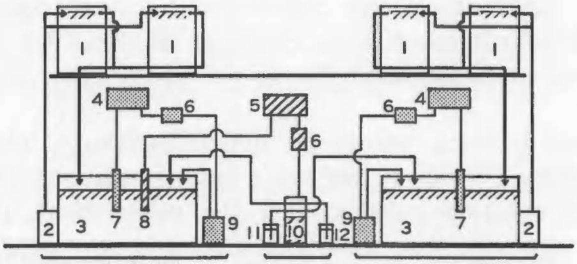


FIG. 1. Block diagram of the device for the nitrogen-restricted culture method.

1. Culture pots (a/5000, quartet respectively).
2. Magnet pump for circulation of nutrient solution.
3. Reservoir for nutrient solution.
4. Industrial pH indicating controller (for pH regulation).
5. Industrial pH indicating controller (for NO_3^- regulation).
6. Dual action timer.
7. Electrodes (pH, reference and temperature compensation).
8. Electrodes (NO_3^- and reference).
9. Acid or alkali injection pump.
10. Nitrogen injection pump (Dual channel).
11. Stocked ammonium solution.
12. Stocked nitrate solution.

regulate the concentration of nitrate ions and the other to feed ammonium. The lowered nitrate concentration detected by the nitrate electrode was compensated by the supply of nitrate ion to the system for the nitrate-restricted solution culture, through one channel of the peristaltic injection pump with dual channel. At the same time, the other channel of the pump was used to feed ammonium ion to the system for the ammonium-restricted solution culture. The same amount of nitrogen can be supplied to the apparatuses for the ammonium- and the nitrate-restricted cultures, when the stock solutions of both nitrogen sources were of the same normality.

The apparatus continued to supply nitrogen sufficiently to maintain vigorous growth even after the plants had grown to be fairly large, and to prevent the ammonium injury resulting from the rush uptake of ammonium.

Table 1 shows the temperature and nitrogen concentrations during culture experiments. The nitrogen concentration of the nitrogen-restricted nutrient solution was kept constant at low levels as shown in Table 1 (expressed as nitrate concentration). The accuracy of pH control in this culture method was the same as that in the constant pH culture[8,9,10,11].

Table 2 shows the composition of the nutrient solution used for the nitrogen-restricted culture. Plants were also grown by the conventional culture with nitrate for reference. As can be seen in Plate 1, two

TABLE 1. Season, duration, temperature and nitrate concentration of the experiment

Plants	Days*1 in nersery	Experimental period		Mean temperature (°C) at 9:00			N conc.*2 (mM)
		Initial and final days	Duration (days)	Room	Solution		Set/Final
					N-rest.	Conv.	
Cucumber	8	Oct. 10–Dec. 9	60	21	20	17	0.25/ —
Tomato	8	Jun. 21–Aug. 2	42	30	27	25	0.20/0.19–0.02
Cabbage	19	May 14–Jun. 13	30	28	22	22	0.10/ —
Chinese cabbage	12	Mar. 5–Apr. 7	33	21	20	17	0.05/0.14–0.03
Spinach	9	Jan. 27–Mar. 3	35	18	20	19	0.05/ —
Radish	12	Mar. 5–Mar. 31	26	21	20	17	0.05/ —

*1 Days from seeding to transplanting.
*2 Set or final shows the concentrations of nitrate and ammonium ions in the freshly prepared nutrient solution and nitrate concentration in the nutrient solution used for a week, respectively. —: not measured.

TABLE 2. Composition of nutrient solution for the nitrogen restricted solution culture

Macronutrients		Micronutrients	
Nitrogen*		Fe(citrate)	1.0 ppm
KH_2PO_4	1.0 mM	B(H_3BO_3)	0.5
K_2SO_4	1.5	Mn($MnCl_2$)	0.5
$CaCl_2$	2.0	Zn($ZnSO_4$)	0.05
$MgSO_4$	1.0	Cu($CuSO_4$)	0.02
		Mo(Na_2MoO_4)	0.01

* Nitrogen concentration was adjusted to designated concentration shown in TABLE 1 with stock solutions of $NaNO_3$ or $(NH_4)_2SO_4$.

plants were planted in each pot, but for spinach plants, the density was increased to 4 plants per pot, because the cultivar used in this investigation was relatively small. Mono-culture system was undertaken in the present experiments, because the mixed-culture of plants having different grades of ammonium sensitivity was thought to be difficult. Details of culture and analytical methods were described previously[8,9,10,11,12,13.].

RESULTS

1. *Effect of the Nitrogen Source on Plant Growth under Nitrogen-restricted Culture Condition*

Fig. 2 shows the growth of plants fed with low concentrations of nitrogen as compared with those fed with nitrate by the conventional culture. Cucumber plants and all the ammonium sensitive plants examined, *i. e.* tomato, cabbage, chinese cabbage, spinach and radish, grew

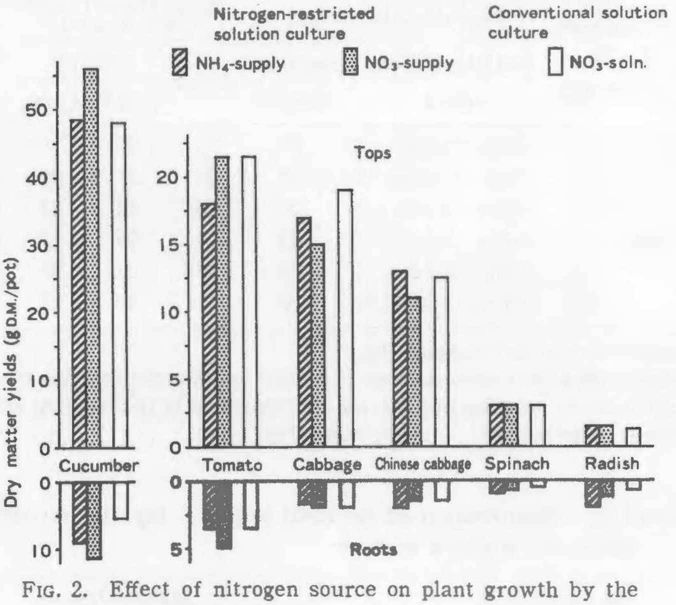


FIG. 2. Effect of nitrogen source on plant growth by the nitrogen-restricted culture method.

vigorously by the ammonium-restricted culture method like the plants grown by the nitrate-restricted culture method or those grown by the conventional culture with nitrate.

Plate 1 shows the growth of chinese cabbage plants under several culture conditions and the growth of tomato, spinach and radish in both nitrogen-restricted cultures and conventional culture with nitrate. The

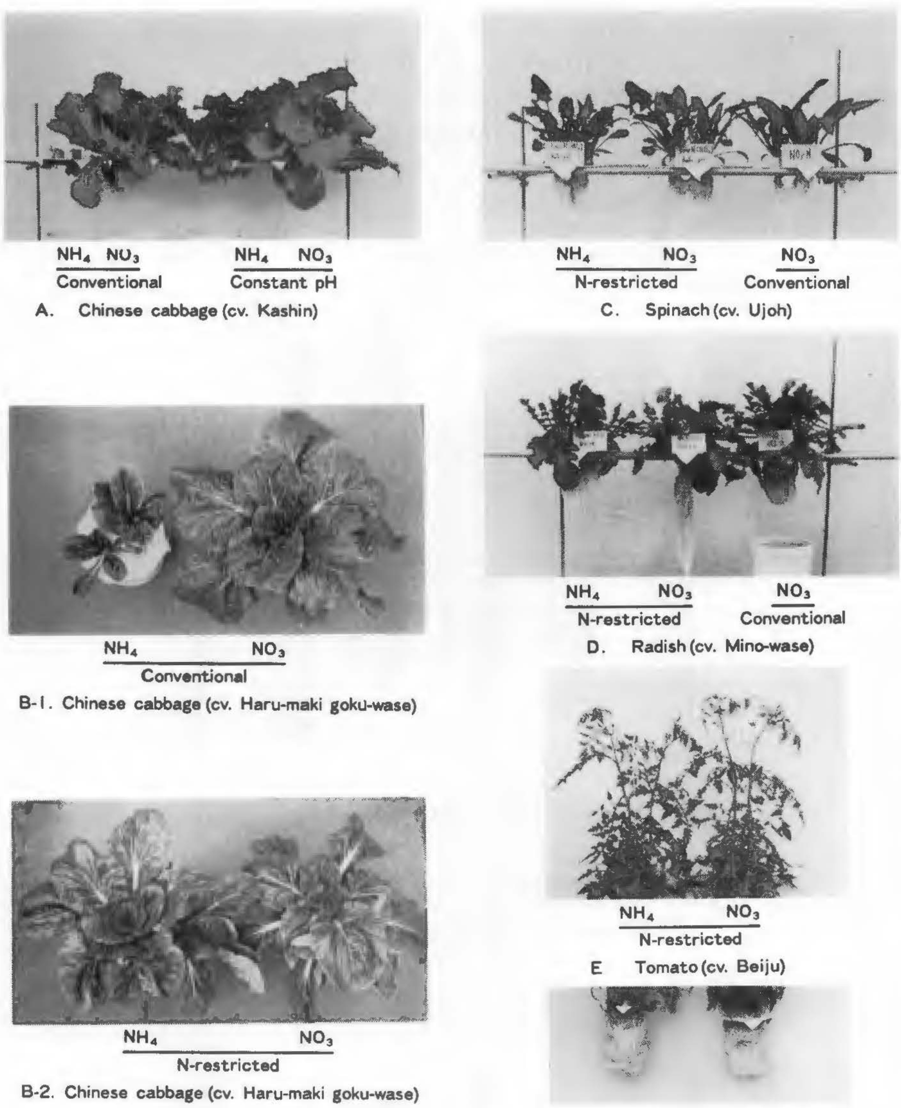


PLATE 1. Effect of nitrogen source and culture method on growth of ammonium sensitive plants.

growth of ammonium sensitive plants, for example chinese cabbage, in the conventional and the constant pH cultures was stunted by ammonium feeding as shown in Plate 1 and in the previous papers[12,13].

2. *Effect of the Nitrogen Source on Mineral Uptake in Plants under Nitrogen-restricted Culture Condition*

The effects of low concentrations of ammonium and nitrate ions on mineral uptake by the ammonium sensitive plants were examined. Fig. 3

shows the potassium content of the plants grown by the ammonium- or the nitrate-restricted culture or by the conventional culture with nitrate. Potassium content did not seem to be affected by a low concentration of ammonium or nitrate ; plants grown by the ammonium-restricted and

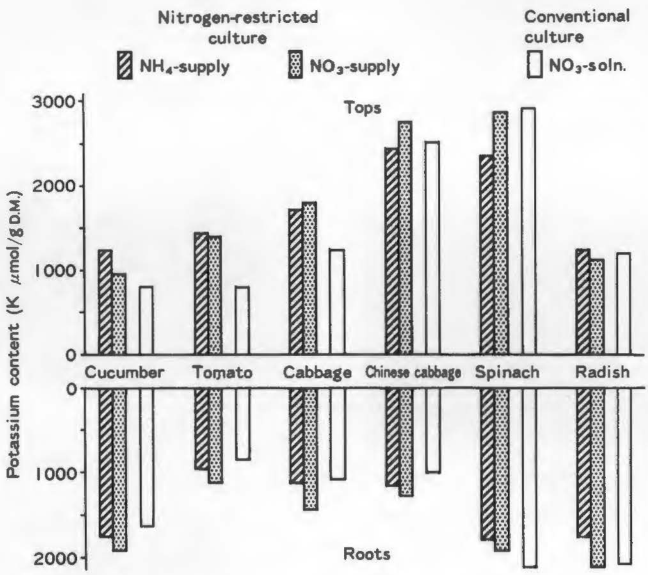


FIG. 3. Effect of nitrogen source on potassium content of plants by the nitrogen-restricted culture.

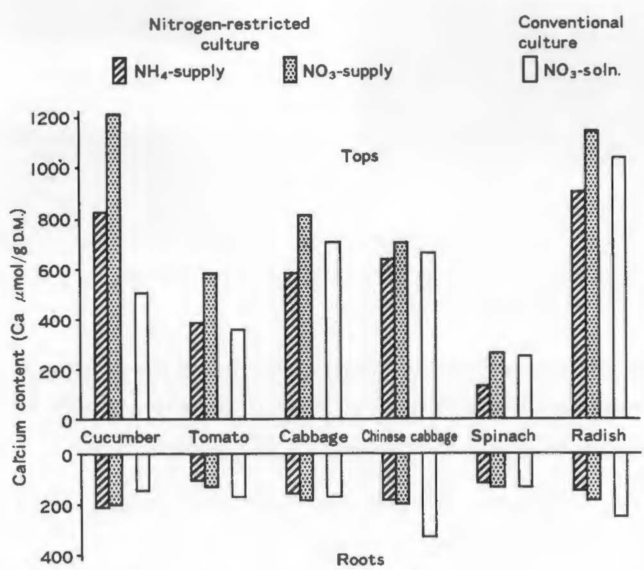


FIG. 4. Effect of nitrogen source on calcium content of plants by the nitrogen-restricted culture.

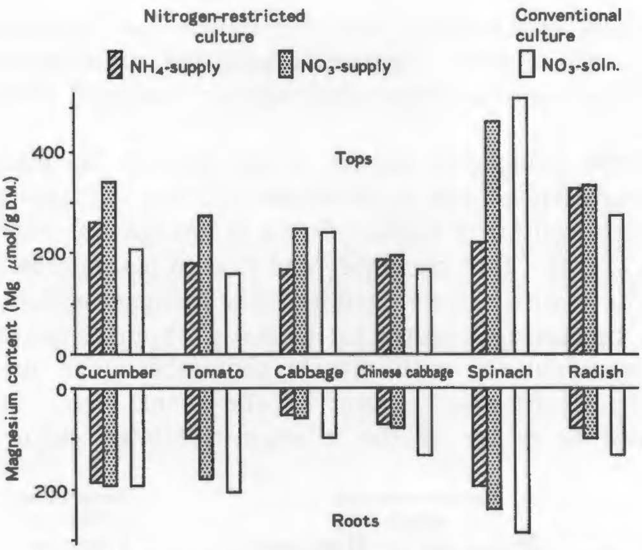


FIG. 5. Effect of nitrogen source on magnesium content of plants by the nitrogen-restricted culture.

the nitrate-restricted cultures showed similar potassium contents. But, cucumber, tomato and cabbage plants grown by the conventional culture with nitrate contained somewhat less potassium than those grown by either of the nitrogen-restricted cultures.

As shown in Figs. 4 and 5, the plants grown by the ammonium-restricted culture had less calcium and magnesium than in the plants grown by the nitrate-restricted cultures. On the other hand, cucumber and tomato plants grown by the conventional culture with nitrate con-

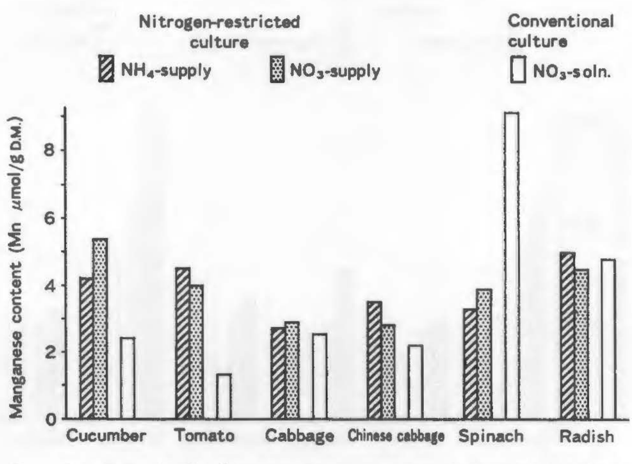


FIG. 6. Effect of nitrogen source on manganese content of plant tops by the nitrogen-restricted culture.

tained somewhat less calcium and magnesium than those grown by the nitrate-restricted culture. Spinach plants, fed with nitrate, contained more magnesium and somewhat less calcium than the other plants examined here.

Fig. 6 shows manganese content of the tops of the plants grown by both nitrogen-restricted and conventional cultures. Plants grown by the former methods contained similar levels of manganese independently of the nitrogen source. But, cucumber and tomato plants grown by the conventional culture with nitrate contained less manganese than those grown by either of the nitrogen-restricted cultures. Spinach plants grown by the conventional culture with nitrate contained much manganese.

Fig. 7 shows the zinc content of the plant tops. Plants, except spinach, grown by either of the nitrogen-restricted cultures contained

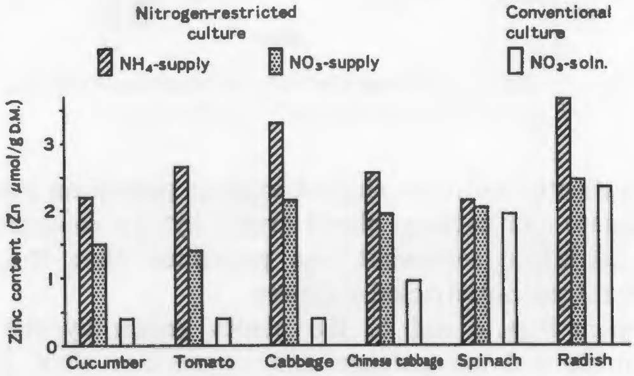


FIG. 7. Effect of nitrogen source on zinc content of plant tops by the nitrogen-restricted culture.

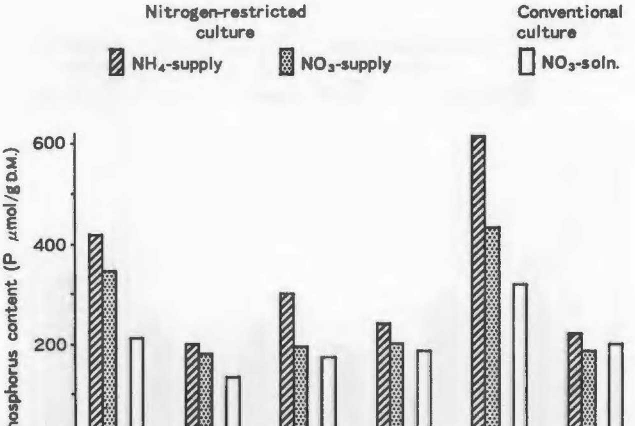


FIG. 8. Effect of nitrogen source on phosphorus content of plant tops by the nitrogen-restricted culture.

more zinc than those grown by the conventional culture with nitrate. In comparison with the plants grown by the nitrate-restricted culture, the plants grown by the ammonium-restricted culture generally showed higher zinc contents.

Fig. 8 shows the phosphorus content of the plant tops. In general, the ammonium-fed plants contain more phosphorus than the nitrate-

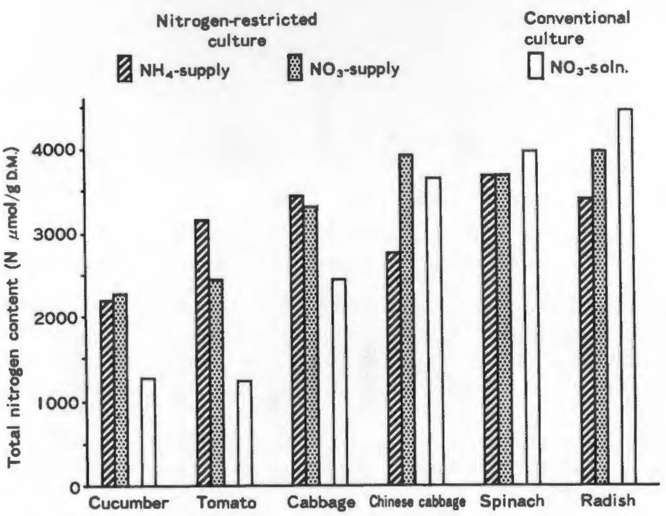


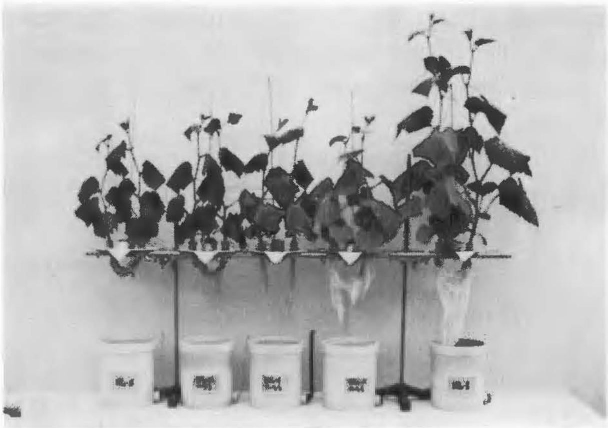
FIG. 9. Effect of nitrogen source on total nitrogen content of plant tops by the nitrogen-restricted culture.

fed plants. Fig. 9 shows the total nitrogen content of the plant tops. Cucumber, tomato and cabbage plants grown by the nitrogen-restricted cultures contained more nitrogen than those grown by the conventional culture with nitrate.

DISCUSSION

Studies have been made on the effect of low concentrations of ammonium and nitrate on plant growth[6,14]. An experiment using turnip plants which might be sensitive to ammonium and hydrogen ions showed a preference for nitrate ion at all nitrogen levels tested[6]. In another experiment, oat plants, which might be somewhat resistant to both ammonium and hydrogen ions, preferred ammonium ions at low nitrogen concentrations[14].

The growth of plants is generally hindered by nitrogen deficiency, when the level of nitrogen supplied is low. For example, Plate 2 shows cucumber plants grown by the conventional culture, in which a small amount of nitrogen was supplied a few times a week. As shown in



(1.) (2) (3) (4) (5)

PLATE 2. Effect of reduced and divided supply of ammonium on growth of cucumber plants.

(1) Conventional solution culture feeding with the ammonium type nutrient solution (5 millimoles/ℓ of ammonium ions was given once a week).
(2) Ammonium supply (1 millimole/ℓ of ammonium was given manually at the rate of 3 times/week. Total amounts were equivalent to 3.0 millimoles /ℓ/week).
(3) Ammonium supply (0.5 millimoles/ℓ of ammonium was given manually at the rate of 6 times/week. Total amounts were equivalent to 3.0 millimoles/ℓ/week).
(4) Ammonium supply (0.25 millimoles/ℓ of ammonium was given manually at the rate of 6 times/week. Total amounts were equivalent to 1.5 millimoles/ℓ/week).
(5) Conventional solution culture feeding with the nitrate type nutrient solution (5 millimoles/ℓ of nitrate ions was given once a week).

this plate, the application of nitrogen (ammonium) in divided doses had no effect on plant growth except for a small acceleration of root growth in treatment-4, the lowest level of ammonium was supplied most frequently. The plants grown by this treatment had yellowish leaves, a sign of nitrogen deficiency, while the leaves of the plants grown by the other treatments (1 to 3) were dark green.

Therefore, it is important to increase the supply of ammonium, according to plant growth, to obtain vigorous growth on ammonium. Thus, by the ammonium-restricted culture, the ammonium-sensitive plants may be grown vigorously on ammonium, without producing nitrogen deficiency.

However, the device for the nitrogen-restricted culture has its weakness. The concentration of nitrogen can not be adjusted so accurately, as shown in Table 1. This may be caused mainly by the following: meter precision, temperature change, and aging of the electrode.

The error of the meter is ± 0.1 pH unit as described previously[8,9,10,11]. If the pH units are converted to molar concentration, the variation at 0.1 mM is equivalent to 0.079 to 0.126 mM. This range of molar variation seems somewhat wide in this low concentration range.

The temperature error in nitrate regulation can not be compensated by the temperature compensation circuit in the pH measurement unit, because the electric potential of the nitrate anion is reverse of that of hydrogen cation. Then, the temperature error is directly related to the reduction of the accuracy of nitrate regulation.

If the factors not associated with temperature are eliminated, and the rise of temperature is assumed as 10°C (*e. g.* 15°C to 25°C), the nitrate concentration at 25°C (298°K), equivalent to measurable voltage at 15°C (288°K), is calculated from Nernst's equation as follows.

$$\mathrm{Ea} - 2.3\,\frac{288\mathrm{R}}{\mathrm{F}}\log\,[\mathrm{NO_3^-}(288)] = \mathrm{Ea} - 2.3\,\frac{298\mathrm{R}}{\mathrm{F}}\log\,[\mathrm{NO_3^-}(298)]$$

$$\log\,[\mathrm{NO_3^-}(298)] = \frac{288}{298}\log\,[\mathrm{NO_3^-}(288)]$$

$$[\mathrm{NO_3^-}(298)] = [\mathrm{NO_3^-}(288)]^{\frac{288}{298}}$$

The last line shows that the nitrate concentration at 25°C can be expressed by $\frac{288}{298}$ powers of those at 15°C. This means that the more the concentration decreases, the more the effect of exponent increases. For example, the error, which arises from 10°C elevation, increases from 0 to 38 percent, when the concentration decreases from 1.0 M to 0.1 mM.

The effect of electrode aging on the error was large. The lifetime of a commercial nitrate electrode was about three months. In later period of the lifetime of the electrode, the accuracy of nitrate regulation must be decreased gradually.

Thus, the difference between the set and the final concentrations (Table 1) might be caused by a combination of the above errors. However, the apparatus in the present investigation was practically satisfactory to examine the effect of low levels of ammonium and nitrate on plant growth, because the plants less resistant to ammonium ion, such as tomato, cabbage, chinese cabbage, spinach and radish[12,13], could grow rapidly on ammonium by the nitrogen-restricted culture, like the plants grown by the nitrate-restricted culture and the conventional culture with nitrate (Plate 1 and Fig. 2).

Judging from the results described above, the stunted growth of such plants induced by ammonium feeding in the constant pH culture is obviously related to ammonium toxicity as described previously[12,13]. It can be concluded that the stunted growth of such plants is recovered by the prevention of rush uptake of ammonium ion by the ammonium-restricted culture.

The uptake of calcium and magnesium was considerably small (Figs. 3 and 4), even at the low concentration of ammonium (0.05 to 0.25 mM). These results were similar to those reported by other workers[1,2,3,4,7,15,16]. Phosphate uptake seems to be accelerated by ammonium ion or depressed by nitrate ion even at such a low concentration (Fig. 8) as indicated in other papers[1,16].

On the other hand, such a low concentration of ammonium does not affect the uptake of potassium or manganese (Figs. 2 and 6). Zinc uptake seems to be accelerated by a low concentration of ammonium in all plants in the present experiments (Fig. 7). Depression of zinc uptake by ammonium[4] was not found, at least, in the present experiments.

Rayer *et al.*[15] have concluded that the uptake of phosphate by soybean plants was accelerated by 17.8 μM to 3.57 mM of ammonium, the uptake of potassium was depressed by 500 μM or more of ammonium, and the uptake of calcium or magnesium was depressed by 357 μM or more of ammonium. In the present experiments, the concentration of ammonium was indirectly controlled. However, if the concentration of ammonium in practice does not exceed that of nitrate, the present results of potassium and phosphorus contents seem to be similar to the results they obtained[15]. On the other hand, the present results on calcium and magnesium are different from their results[15]. It is thought that the confliction between our and their results might be caused by the difference of the method of ion control; they adjusted the concentrations of ammonium and hydrogen ions by the daily renewal of nutrient solution, and we regulated automatically both nitrogen concentration and pH in the present experiments.

Spinach plants, fed with nitrate, contained abundant magnesium as compared with the other plants, and the level of their magnesium content was higher than that of calcium (Figs. 4 and 5). This tendency seems to be strong in nitrate predominant cultures, because Hara *et al.*[5] have also observed abundant megnesium in spinach plants under field condition or in their nitrate type nutrient solution, in addition to our previous results obtained by the constant pH culture with nitrate[12,13].

The uptake of ammonium and nitrate was similar in both nitrogen-restricted cultures. This may be attributed to the equivalent supply of both forms of nitrogen to either nitrogen-restricted culture. In cucumber, tomato and cabbage plants, the contents of total nitrogen were higher in both nitrogen-restricted cultures than in the conventional culture. This means that the plants were sometimes starved in nitrogen by the conventional culture, while the supply of nitrogen was kept constant in both nitrogen-restricted cultures even after the plants grew to be fairly large. Consequently, this tendency was clear in big plants or in the case of long term experiments (Table 1, Figs. 2 and 9).

In addition, such tendency was also found in the case of the other nutrients, *i.e.* potassium, calcium, magnesium, manganese and zinc (Figs. 3 to 7). This might be induced by abundant supply of nutrients in the nitrogen-restricted culture compared to the conventional culture, because the apparatus for the former method had a reservoir of nutrient solution (Fig. 1).

SUMMARY

An apparatus for nitrogen-restricted culture method was developed so that ammonium or nitrate ion could be supplied continuously at a low and constant concentration (*e.g.* 0.1 mM). In addition to the constant pH condition, the supply of nitrogen was automatically increased with plant growth by this method. In the preceding paper, tomato, cabbage, chinese cabbage, spinach and radish were characterized by a high sensitivity to ammonium and hydrogen ions. In the present experiments, those plants were grown by the nitrogen-restricted culture (feeding either ammonium or nitrate), and by the conventional culture with nitrate. Then, plant growth and mineral uptake were compared between the ammonium-fed and the nitrate-fed plants. The results were as follows.

1) Even in the plants characterized by a high sensitivity to ammonium ion, ammonium poisoning was not found in the ammonium-restricted culture. In this case, the ammonium-fed plants grew rapidly, like the nitrate-fed plants by the nitrogen-restricted or the conventional culture.

2) Even at a low concentration of ammonium or nitrate, the effect of the nitrogen source on the uptake of macro-nutrients differed between the ammonium- and nitrate-feeding with the exception of potassium. In general, the cation contents in the ammonium-fed plants were lower than those of the nitrate-fed plants, being opposite to the case of phosphorus.

3) In the case of the nitrogen-restricted culture, the effect of the nitrogen source on manganese contents was similar in both nitrogen feeding conditions as was the case for potassium.

4) Studies using the plants characterized by a high sensitivity to ammonium ion showed that the content of zinc in the ammonium-fed plants was higher than in the nitrate-fed plants.

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