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EFFECT OF LOW SOLUTION pH ON GROWTH, APPEARANCE AND MINERAL COMPOSITION OF PLANT ROOTS

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In general, nitrate brings about satisfactory growth in many species of plants in solution culture, and seems to be superior to ammonium for plant growth. Many workers have studied the effect of the nitrogen source on the growth of plant roots. Reports have been made on : the stunted growth of ammonium-fed roots[3,8,9,10,12,21)], the appearance of the roots of the ammonium-fed plants[1,6,8,9,10,11)], the acceleration of root growth by nitrate[7,12)], and the inhibition of root growth by nitrate at high concentrations[2,5)].

As described above, the relationship between nitrogen sources and appearance or growth of roots is complicated. We previously showed that the root growth was stunted by the lowering of solution pH[15,16,17,18,19,20)]. Plant growth was accelerated in tops and roots even in the ammonium-fed plants, when the pH was not allowed to lower by the constant pH culture or the rush uptake of ammonium was prevented by the use of the nitrogen-restricted culture. In these experiments, however, the reason for the development of the characteristic root appearances in the conventional solution culture with ammonium could not be explained.

In the present investigation, culture experiments, by feeding the plants with ammonium and with nitrate, were conducted under different pH conditions, in order to clarify the effect of the nitrogen form and low pH on the growth and appearance of plant roots.

MATERIALS AND METHODS

Test plants used in this experiment were barley (*Hordeum vulgare* L. ; cv. Akashinriki), corn (*Zea mays* L. ; cv. Nagano 1), bean (*Phaseolus vulgaris* L. ; cv. Masterpiece), cucumber (*Cucumis sativus* L.; cv. Chihai), tomato (*Lycopersicon esculentum* Mill. ; cv. Beiju) and lettuce (*Lactuca sativa* L. ; cv. New York 515).

Table 1 shows the season, duration and temperature conditions of culture experiments. Room temperature was measured by a recording thermometer with thermister sensor at a height of one meter, but one in parentheses in the table was measured by a bimetallic recording

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TABLE 1. Season, duration and temperature condition of culture experiments

Plants	Days*1 in nursery	Experimental period		Mean temperature at 9:00(°C)	
		Initial and final days	Duration (days)	Room*2	Soln.*3
Constant low-pH culture					
Bean	7	Sep. 25 – Oct. 13	18	(28)	—
Repeatedly adjusted low-pH culture					
Barley	11	Jan. 8 – Feb. 10	33	18	17(14)
Corn	10	Nov. 20 – Dec. 16	26	20	18
Bean*4	7	Sep. 20 – Oct. 8	18	27	—
Cucumber*4	7	Sep. 14 – Oct. 13	29	27	—
Tomato	12	Oct. 26 – Dec. 3	38	20	18
Lettuce	9	Feb. 10 – Mar. 22	40	20	20(16)

*1 Days from seeding to transplanting.
*2 ( ) shows the temperature measured by a recording thermometer of bimetal type.
*3 ( ) shows the temperature in the pot for the conventional culture.
*4 Co-planted species.

thermometer. Solution temperature was measured by a thermister sensor dipping into the nutrient solution in the apparatus for the constant pH solution culture or in the pot for the conventional solution culture.

In the preliminary experiment, bean plants were cultured in the nitrate type nutrient solution under a low and constant pH condition (pH 3.3–3.5), using the apparatus for the constant pH culture[13,14]. This culture method is hereafter referred to as "the constant low pH culture". At the same time, bean plants were grown by the conventional culture using the ammonium or the nitrate type nutrient solution.

In the main experiments, barley, corn, bean, cucumber, tomato and lettuce were grown using the apparatus for the constant pH culture with the nitrate type nutrient solution. The solution pH in the apparatus was adjusted manually once or twice a day with dilute solution of hydrochloric acid or sodium hydroxide, so as to follow the pH change of the ammonium type nutrient solution in the case of the conventional culture which was performed simultaneously. This method is referred to as "the repeatedly adjusted low-pH culture" hereafter. For this culture, an apparatus for constant pH culture was neccessary to prevent the pH rise of the nitrate type nutrient solution.

The change of pH in the case of the conventional culture, and the simulated pH changes in the repeatedly adjusted low-pH culture with the nitrate type nutrient solution are shown in Fig. 1. The pH of the ammonium type nutrient solution in the case of the conventional culture changed in a saw-tooth wave pattern; the pH fell gradually with time

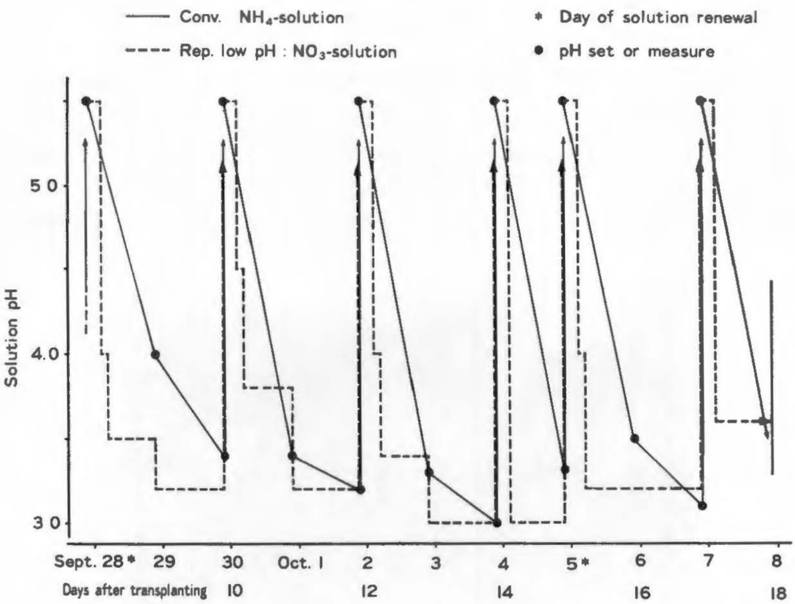


FIG. 1. Changes of pH of the ammonium type nutrient solution in the conventional culture (solid line) and its simulation by repeatedly adjusted low-pH culture (broken line).

and rose rapidly at manual pH adjustment. The pH of the nitrate type nutrient solution in the apparatus changed in a square wave pattern, because the pH in the apparatus was adjusted at intervals to the pH changes seen in the ammonium type nutrient solution.

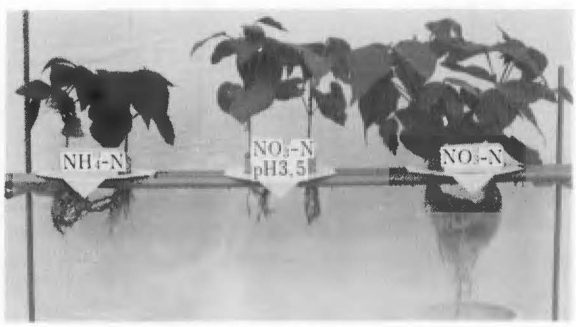
The fresh nutrient solution contained 5 mM of nitrogen, 4 mM of potassium and chloride, 2 mM of calcium, and 1 mM of phosphate, magnesium and sulfate. The sodium concentration (2 mM or less) varied depending on the kind of nutrient solution. Nitrogen compounds used for the ammonium type and the nitrate type nutrient solutions were ammonium sulfate and a mixture of potassium nitrate and sodium nitrate (4 : 1), respectively. The composition of micronutrients was 1.0 ppm of iron, 0.5 ppm of boron and manganese, 0.05 ppm of zinc, 0.02 ppm of copper and 0.01 ppm of molybdenum.

Nutrient solution was aerated continuously and renewed once a week. Iron (1 ppm) was added every other day to all cultures. In the case of the conventional culture, the pH of the nutrient solution was adjusted manually every other day, after the addition of iron. Most of the plant species, except bean and cucumber, were planted in separate apparatus. Planting density was six plants per pot for barley, and two plants per pot for the other species of plants as shown in Plates 1 and 2. Details of the methods of culture experiments and mineral analysis were described previously[15,17,19,20].

RESULTS

1. *The Effect of Constant Low-pH Culture on the Root Appearance*

Bean plants were cultured by the constant low-pH culture with nitrate and by the conventional culture with the nitrate or the ammoinum type nutrient solution. The appearance of roots is shown in Plate 1. Roots grown by the constant low-pH culture with nitrate showed thick



(A) (B) (C)

PLATE 1. Growth and appearance of bean roots in the constant low-pH culture.

(A) Conventional culture with ammonium (set pH 5.5).
(B) Constant low-pH culture with nitrate (pH 3.3 to 3.5).
(C) Conventional culture with nitrate (set pH 5.5).

and stunted appearance. However, root branching was few as compared with that of the plants grown by the conventional culture with ammonium, *i. e.* the so-called "stubby root system"[10]. In addition, in the constant low-pH culture, the root tips were necrotic.

2. *Effect of the Nitrogen Source and Repeatedly Adjusted Low-pH Culture on Growth and Root Appearance*

To examine the difference in root appearance of the plants grown by the conventional culture and the constant low-pH culture, the repeatedly low-pH culture was adopted. In this culture, the pH change in the ammonium type nutrient solution of the conventional culture was simulated with the nitrate type nutrient solution as shown in Fig. 1.

Fig. 2 shows the plant growth in the conventional cultures with the ammonium type and the nitrate type nutrient solutions, and that in the repeatedly adjusted low-pH culture with the nitrate type nutrient solution. All plants, except corn, showed stunted growth with the conventional culture with ammonium, and with the repeatedly adjusted low-pH culture with nitrate. Root growth seems to be more affected

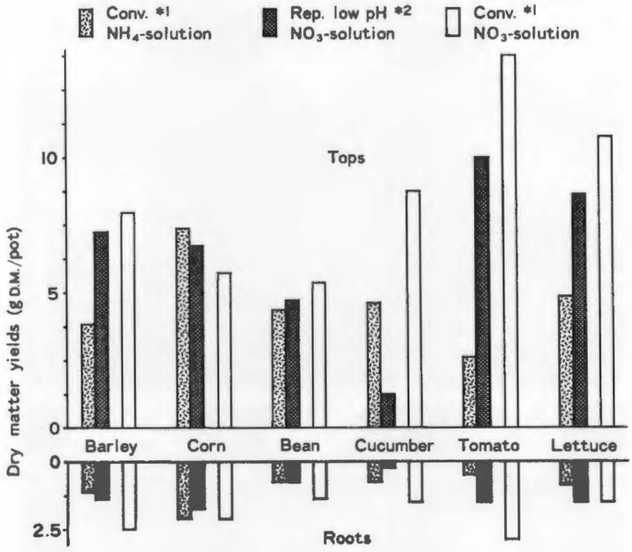


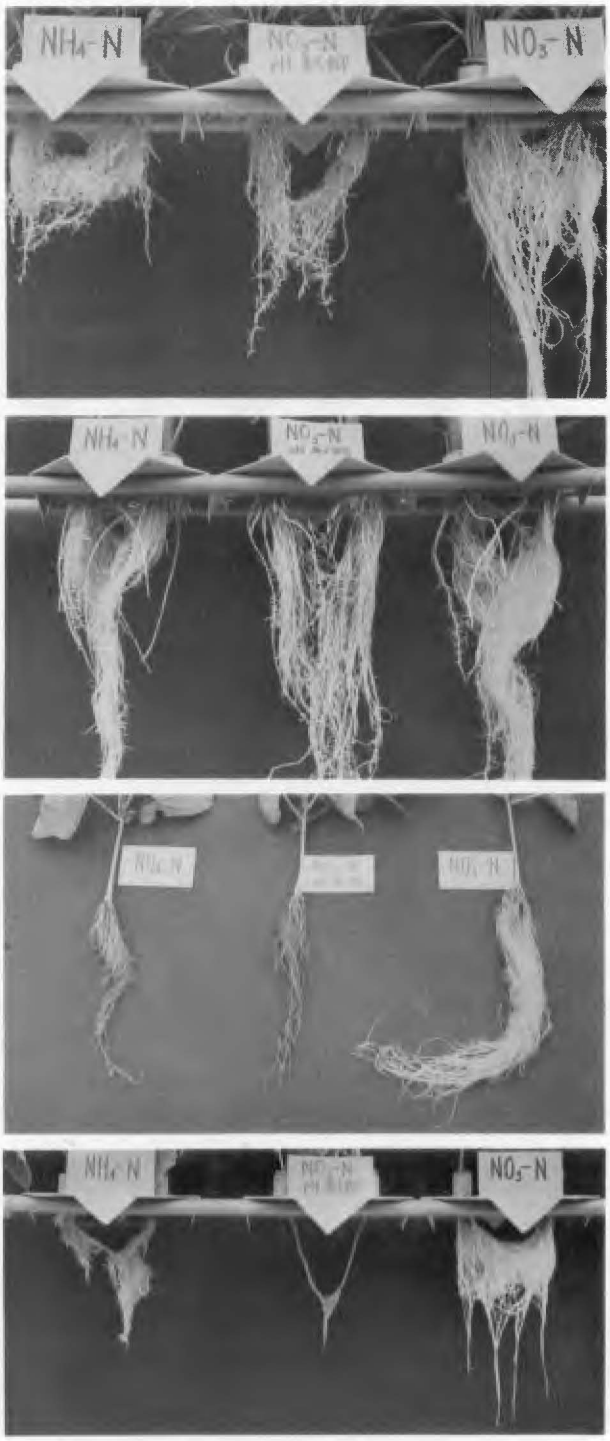
FIG. 2. Effect of low solution pH on plant growth.
*1 Conventional culture.
*2 Repeatedly adjusted low-pH culture.

by low pH or ammonium ion than top growth. Growth of corn plants were similar among the three different cultures.

Plate 2 shows the appearance of roots. Even in the nitrate type nutrient solution, the plants, except corn and cucumber, developed the thick, stunted and heavily branched roots with the repeatedly adjusted low-pH culture. This was similar to the root appearance of the so-called stubby root system. Except corn, the stubby root system was found in all the roots grown by the conventional culture with ammonium. In cucumber plants, the roots grown by the repeatedly adjusted low-pH culture with nitrate were more stunted than those grown by the conventional culture with ammonium.

3. *Effect of the Nitrogen Source and Low pH on Mineral Composition of Plant Roots*

The mineral contents of the plants grown in the conventional culture with nitrate or ammonium and by the repeatedly adjusted low-pH culture with nitrate are illustrated in Figs. 3 to 9. Except for potassium and calcium, only the mineral contents in the roots are shown, because the effects of the nitrogen source and low pH on the composition of these minerals were more clear in the roots than in the tops.



(A)

(B)

(C)

(D)

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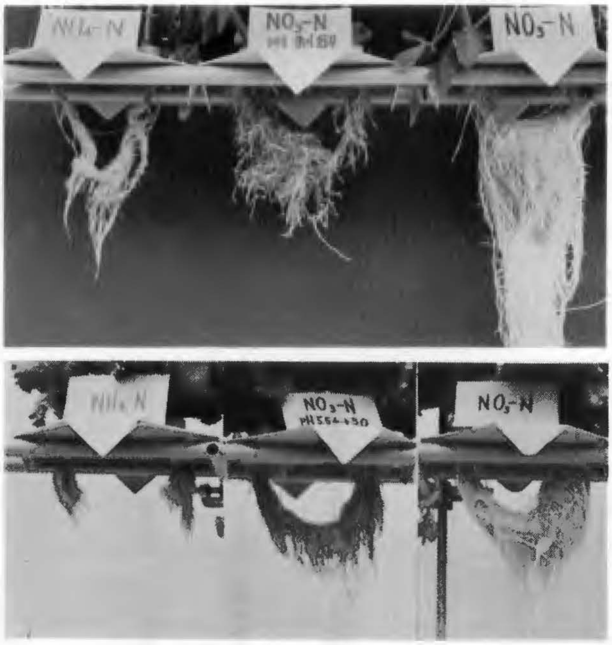
(E)

(F)



(A) Barley
(B) Corn
(C) Bean
(D) Cucumber
(E) Tomato
(F) Lettuce

Left: Conventional culture with ammonium (set pH 5.5).
Center: Repeatedly adjusted low-pH culture with nitrate (Solution pH was simulated to the conventional culture with ammonium).
Right: Conventional culture with nitrate (set pH 5.5).

PLATE 2. A–F Effect of repeatedly adjusted low-pH culture on growth and appearance of plant roots.

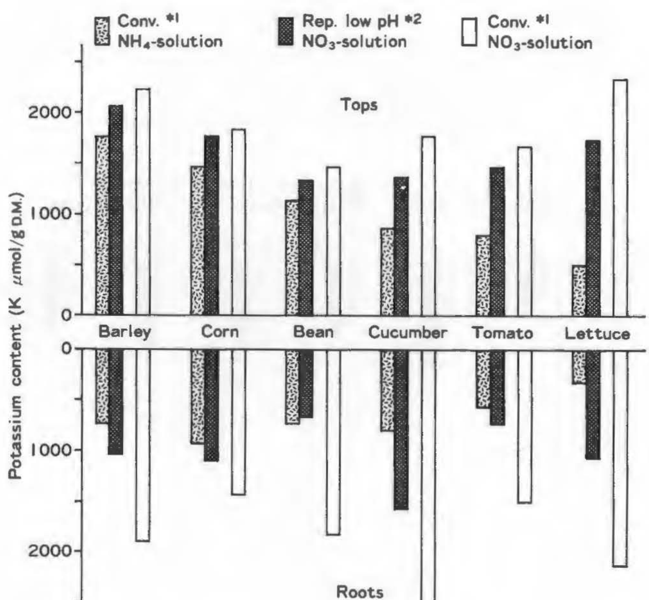


FIG. 3. Effect of low soltuion pH on potassium content of plant roots.

*1 Conventional culture.

*2 Repeatedly adjusted low-pH culture.

As shown in Fig. 3, the potassium contents of the roots decreased in the repeatedly adjusted low-pH culture with nitrate and in the conventional culture with ammonium, while the roots grown by the conventional culture with nitrate contained abundant potassium.

Fig. 4 shows the calcium content. In this figure, the scale for the roots is expanded. The contents decreased strikingly in the plants

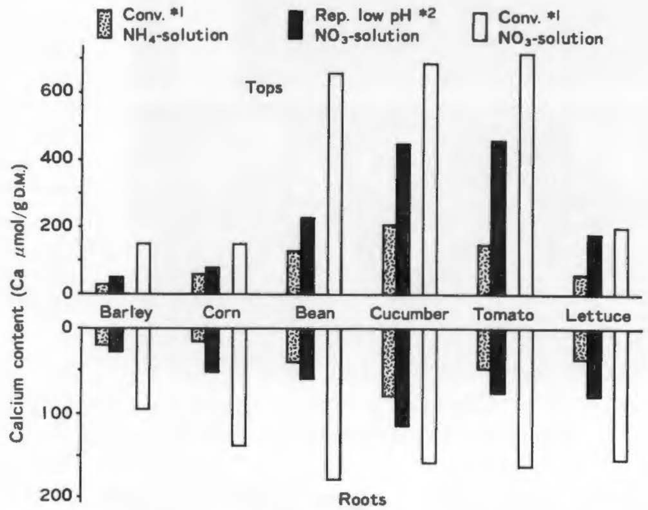


FIG. 4. Effect of low solution pH on calcium content of plants.
*1 Conventional culture.
*2 Repeatedly adjusted low-pH culture.

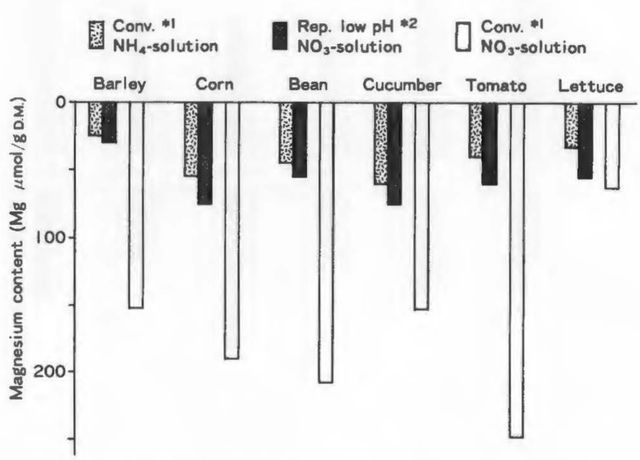


FIG. 5. Effect of low solution pH on magnesium content of plant roots.
*1 Conventional culture.
*2 Repeatedly adjusted low-pH culture.

grown by the repeatedly adjusted low-pH culture, even though the plants were fed with nitrate. As compared with the conventional culture with ammonium, the plants grown by the repeatedly adjusted low-pH culture with nitrate contained slightly more calcium. Magnesium contents of plant roots are illustrated in Fig. 5. The pattern in this figure is similar to those of calcium and the potassium contents in the roots (Figs. 3 and 4).

Manganese, zinc and iron were not determined in lettuce plants. Fg. 6 shows the manganese content of roots. It is similar to the other

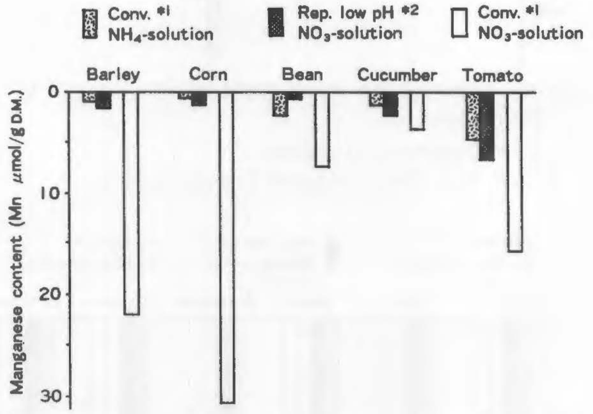


FIG. 6. Effect of low solution pH on manganese content of plant roots.

*1 Conventional culture.
*2 Repeatedly adjusted low-pH culture.

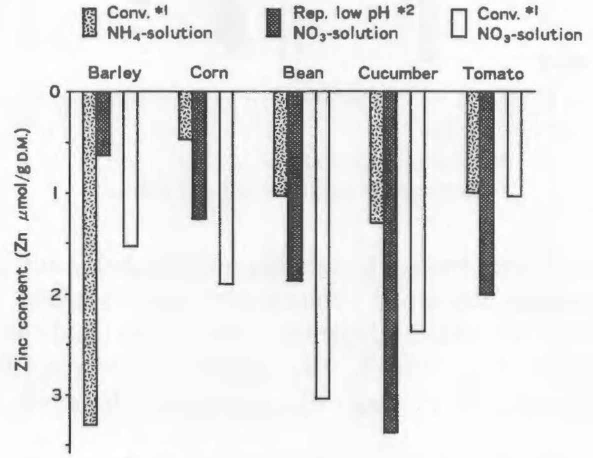


FIG. 7. Effect of low solution pH on zinc content of plant roots.

*1 Conventional culture.
*2 Repeatedly adjusted low-pH culture.

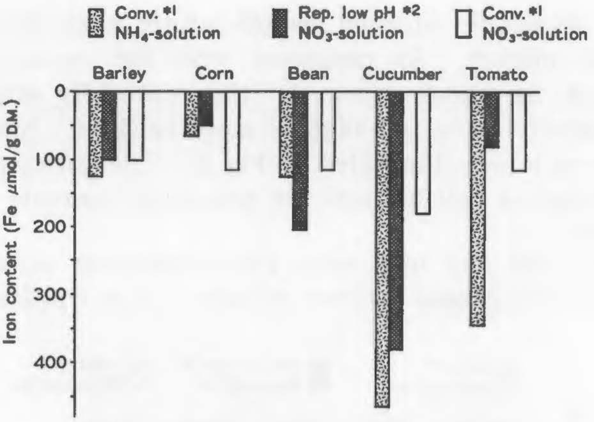


FIG. 8. Effect of low solution pH on iron content of plant roots.
*1 Conventional culture.
*2 Repeatedly adjusted low-pH culture.

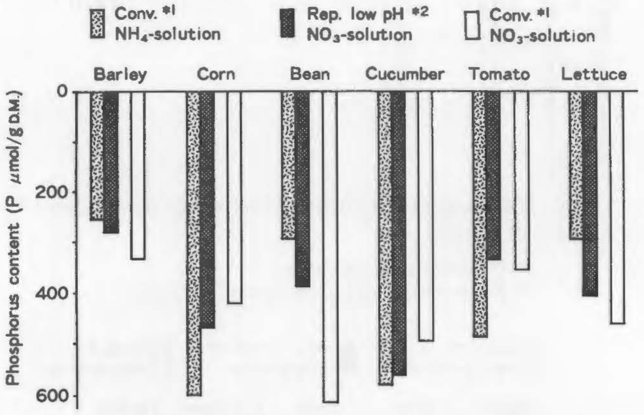


FIG. 9. Effect of low solution pH on phosphorus content of plant roots.
*1 Conventional culture.
*2 Repeatedly adjusted low-pH culture.

cation contents in the roots, though the nitrate-fed roots grown by the conventional culture contained remarkably large amount of manganese as compared with the other elements. Zinc, iron and phosphorus contents are shown in Figs. 7 to 9. No characteristic relation between pH treatments and contents of these elements was observed.

DISCUSSION

These experiments were conducted to clarify the possible conditions at which the characteristic root appearance, *i. e.* the so-called stubby root system, is developed in their conventional culture with ammonium[10].

A pH of lower than 3.6 was firstly postulated as a possible cause for the development of the stubby root system, because the thick and stunted roots were found frequently in the solution culture with a pH kept constant at 3.6 in either ammonium or nitrate nitrogen source[15,17]. However, the roots showed thick and stunted appearance with poor branching, only in the constant low-pH culture (Plate 1); the appearance was different between the roots grown by the conventional culture with ammonium and those grown by the constant low-pH culture with nitrate.

Then, the repeatedly adjusted low-pH culture was adopted for the nitrate-fed plants to simulate the pH change of the conventional culture with ammonium. As a result, except for corn and cucumber, growth and appearance of roots of plants grown by the repeatedly adjusted low-pH culture with nitrate were similar to those of the plants grown by the conventional culture with ammonium (Plate 2).

Therefore, ammonium ion does not seem to be essential for the development of the thick, stunted and heavily branched roots. The development of stubby roots is thought to be related to pH condition. At least in barley, bean, tomato and lettuce plants, thick and stunted roots might be caused by a low pH, and heavily branched roots was attributed to the repeatedly changed pH.

The process for developing heavily branched roots in the repeatedly adjusted low-pH culture is thought to be as follows: (1) when the solution pH is lowered, the growing points of the root tips are injured; (2) the growing points injured by the low pH do not recover during the suitable pH period following the manual pH adjustment; (3) then, the primordia of the lateral roots begin to grow; (4) the repetition of the above steps may result in heavily branched roots.

In corn plants, Becking has reported the thick and heavily branched roots as ammonium-poisoned roots[1]. In the present experiments, however, the appearances of the corn roots in the three different pH treatments were similar as shown in Plate 2 (B). This confliction may be caused by different environmental or cultural conditions between our and his experiments; it seems that the drop of the pH of the ammonium type nutrient solution was insufficient to develop the stubby root system of corn under the condition of the present experiments.

In cucumber plants, the roots grown by the repeatedly adjusted low-pH culture were more stunted than those grown by the conventional culture with ammonium. This may be ascribed to the low tolerance to acidity of cucumber plants, because, cucumber was co-planted with bean plants which may be more resistant to acidity than cucumber for the substantial period of the culture experiments (Table 1), and the pH changes in the ammonium type nutrient solution, in which bean plants were grown by the conventional culture, was used as the standard pH for both plants in the apparatus for the repeatedly adjusted low-pH culture. To prevent such an influence, the effect of the repeatedly adjusted low-pH culture was examined with single-planting in the other species of plants.

The thick, stunted, necrotic and sparcely branched roots which are similar to bean roots grown by the constant low-pH culture, have been reported for ammonium-fed plants[9]. This may be caused by a long exposure of plant roots to a low pH without adjusting the solution pH. Accordingly, when the pH of nutrient solution was adjusted exactly at intervals of two or three days, roots showed a stubby appearance in ammonium-fed culture as described by Kirkby *et al.*[10]

The repeatedly adjusted low-pH culture with nitrate resulted in low cation contents of roots, like the ammonium-fed roots in the conventional culture (Figs. 3 to 6). This shows clearly that the uptake of cations is severely depressed by a high concentration of hydrogen ion in the nutrient solution, in analogy with the depression by ammonium ion.

The difference in potassium content among the plants cultured by the three different methods was small as compared with that in calcium, magnesium and manganese contents (Figs. 3 to 6). This may be due to small repressive effects of hydrogen and ammonium ions on the uptake of potassium as compared with the other cations[15,16,17,18,19,20].

In the repeatedly adjusted low-pH culture, corn and cucumber plants contained less calcium as was the case for the other plants, though the roots did not clearly show the stubby root system. Accordingly, cation contents were independent of root appearance: stubby or non-stubby roots (Plate 2 and Figs. 2 to 7).

Manganese content of the roots of the plants grown by the conventional culture with nitrate was remarkably large compared with that of the plants grown by the repeatedly adjusted low-pH culture with nitrate or the conventional culture with ammonium (Fig. 6). This means that manganese uptake is inhibited drastically by hydrogen ions as has already been indicated[15,17]. It is also considered that an abundance of manganese in the roots grown by the conventional culture with nitrate results partially from its precipitation on root surface[15,17], because of a great difference in the manganese contents between tops and roots, though the content in tops was not shown here.

Zinc and iron contents in roots were difficult to characterize in the three different culture methods (Figs. 7 and 8). The complicated results concerning zinc contents may be due to the small uptake of zinc in the low pH range[4,15,17,22], the partial stimulation of uptake by ammonium feeding[15,17], and possible precipitation of zinc compounds on root surface[15,17].

Poorly characterized results concerning iron may be attributed to the depression of uptake in low pH range and smaller uptake in high pH range[15,17], together with possible precipitation of iron on root surface.

Phosphorus contents of the plants grown by the three different culture methods could not be characterized, and no specific difference was found in any plant species or any three culture methods (Fig. 9). This may be attributed to the abundance of root phosphorus due to the partial precipitation on the root surface[15,17] and to the acceleration of phosphorus uptake by ammonium or low pH[15,17,19,20].

SUMMARY

In general, most plants except rice grow vigorously on nitrate, than on ammonium in solution culture. But, the previous papers showed that the growth of plants in the ammonium type nutrient solution was accelerated to the level of nitrate-fed plants, when the plants were grown by the constant pH or the nitrogen-restricted culture.

On the other hand, there are several reports on the stunted roots, or the so-called "stubby root system" in the ammonium type nutrient solution. The stubby root system is characterized by thick, stunted and heavily branched root appearance. The present investigation was undertaken to study the relationship between solution pH and the stubby root system. Barley, corn, bean, cucumber, tomato and lettuce were used as test plants. The results were as follows.

1) Even in the nitrate type nutrient solution, thick and stunted roots appeared, when the solution pH was maintained at low level, though the root branches were sparse.

2) The stubby root system also appeared in the nitrate type nutrient solution, when the solution pH was frequently lowered by the apparatus for the constant pH culture, simulating the pH changes of the ammonium type nutrient solution in the conventional culture. The appearance and the growth of the nitrate-fed stubby roots resembled those of the stubby roots, which appeared usually in the ammonium type nutrient solution in the conventional culture in which the solution pH was adjusted every other day.

3) From the results described above, it was clear that the thick and stunted roots resulted from the low pH condition, and that the heavily branched appearance was attributed to the repeatedly adjusted low-pH culture, the solution pH being shifted according to the pH

changes in the conventional culture with ammonium. Therefore, it was concluded that those characteristic appearances depended mainly on the effect of hydrogen ions.

4) The mineral composition of the stubby roots, developed in the ammonium type nutrient solution, was characterized by a low content of cations such as calcium, magnesium, manganese and potassium. The cation content of the nitrate-fed stubby roots grown in the repeatedly adjusted low-pH culture was similar to that of the ammonium-fed roots in the conventional culture.

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