Effects of pH, calcium concentrations and sources of nitrogen on the growth and inorganic compositions of citrus seedlings in solution culture⁽¹⁾

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Summary

The experiment was performed to study the effects of pH, calcium concentrations and sources of nitrogen on the growth and inorganic compositions of natsudaïdai (*Citrus natsudaïdai* HAYATA) seedlings in solution culture under glass. Nitrogen was supplied with NaNO₃ or NH₄NO₃ in the concentration of 112 ppm, pH of solution was adjusted at 4, 5.5 and 7, and calcium concentrations supplied were low, medium and high, totalling 18 combination treatments.

The appearance of leaves of the seedlings at pH 5.5 in NaNO₃ series was normal, however, it was rather dark green in NH₄NO₃ series. At pH 4, leaves became somewhat dull green, and at pH 7 they tended to become chlorotic in both forms of nitrogen. Chlorosis was more remarkable in NaNO₃ series. Roots were white in pH 5.5, however, at pH 4 or pH 7 they turned light brown in NaNO₃ series. The elongation of roots at pH 4 was inferior to those at other pHs, and tips of laterals became stubby. In NH₄NO₃ series, roots generally became brown, and their elongation was less than that in NaNO₃ series. At pH 4, tips of laterals became stubby, and in advanced stage the cortex disintegrated and sloughed off. These symptoms were more remarkable in the treatment supplied with low Ca.

In general, seedlings supplied with NaNO₃ made

better growth than those supplied with NH₄NO₃. In both forms of nitrogen, growth of seedlings was the minimum at pH 4. In NaNO₃ series, growth of seedlings was the maximum at pH 5.5, and intermediate at pH 7. However, there was no clear difference of growth between pH 5.5 and pH 7 in NH₄NO₃ series except Ca low treatment. As for calcium concentration, seedlings supplied with medium Ca showed better growth at every pH in NaNO₃ series. On the other hand, no consistent difference of growth due to calcium concentration was observed in NH₄NO₃ series.

NaNO₃ plants had generally higher concentrations of K and Ca, and lower concentrations of N, P, and Mg as compared with NH₄NO₃ plants in their leaves. While, in their roots, NaNO₃ plants showed higher concentrations of P, Ca, and Mg, and lower concentrations of K than those of NH₄NO₃ plants. With the rise of pH level in the solution, concentrations of N, Ca, and Mg increased and K decreased in the leaves irrespective of nitrogen forms. In their roots also, concentrations of Ca, Mg, and P increased, and K decreased with the rise of pH. When Ca level in the solution was raised, only Ca concentration in the seedlings increased.

Many investigators have studied the influence of pH on the growth of citrus in soil^(1,9,10,19,20), or in solution culture^(5,7,11,14,15). In other plants, it is known that the effects of pH are modified by different sources of nitrogen⁽⁸⁾, or calcium concentrations^(2,3), however, the informations on citrus are rather scanty. This investigation was, therefore, conducted to study the effects of pH in connection with different sources of nitrogen and calcium concentrations on the growth and inorganic compositions of natsudaïdai (*Citrus natsudaïdai* HAYATA) seedlings in solution culture.

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Materials and Methods

Early in March, 1963, seeds of natsudaikai were sown in sand under glass house. From these materials, uniform and healthy seedlings were selected late in June. Twelve seedlings were transplanted in each flat, enamelled iron container covered with twelve-perforated wooden lid.

Treatments were the factorial combinations of two sources of nitrogen (NaNO_3 or NH_4NO_3), three levels of pH (4, 5.5, 7), and three concentrations of calcium (low, medium, high), totalling 18 treatments. Nutrients were supplied as follows per liter of solution, and tap water was used for dilution. Nutrient solution was supplied with 15 liters in each container.

2M NaNO_3 or M NH_4NO_3 4 ml, M KH_2PO_4 1 ml
M/4 K_2SO_4 4 ml, M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2 ml, 0.7 % Fe-EDTA 1 ml
Minor elements by HOAGLAND's formula 1 ml
M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0 ml (Ca low), 0.7 ml (medium), 2 ml (high)

The concentrations of calcium were about 20, 50, and 100 ppm in the Ca low, medium, and high treatments, respectively, adding about 20 ppm Ca in the tap water.

Adjustment to the respective pH of solutions were made daily by N/2 H_2SO_4 or NaOH, and the solutions were renewed weekly. Once between the solution renewals 0.7 % Fe-EDTA was added to each treatment to reduce chlorosis. Solutions were aerated with compressed air, 8 hours a day throughout the experimental period.

At the harvest middle in October, growth of seedlings was recorded and chemical analyses of leaves, stems, and roots were made after drying and grinding these materials. Total nitrogen was determined by GUNNING method, phosphorus by colorimetric method with vanadomolybdate, potassium by flame photometer, calcium and magnesium by EDTA titration method.

Results

Appearance of seedlings: In NaNO_3 series, the top growth of seedlings at pH 5.5 was greater than those at other pHs, and leaves were normal green. Seedling growth at pH 4 was poor, and leaves became somewhat dull green. The leaves at pH 7 turned to yellowish green, and in the upper leaves the contrast between green of veins and yellowish green of interveinal portions became clearer. Consequently upper leaves showed mottled appearance. Symptoms at pH 7 were rather conspicuous in high Ca treatment. In NH_4NO_3 series also, top growth at pH 5.5 was better and leaves were dark green. At pH 4 the leaves, especially upper leaves, became dull green or yellowish green, and at pH 7 also chlorotic. But in this case, there was no clear contrast between veins and interveinal portions such as in NaNO_3 series.

Roots were long and white at pH 5.5 in NaNO_3 series. At pH 4 and 7 roots turned to light brown, however, elongation of roots at pH 7 except high Ca was nearly equal to that at pH 5.5. On the other hand, elongation of roots at pH 4 was less than half of those at higher pHs tested, and tips of laterals became stubby. Especially, the cortex of roots supplied with low Ca was slimy.

Generally, roots of NH_4NO_3 series were inferior to NaNO_3 series in their linear growth, and turned brown. Particularly, at pH 4 the similar decrease of root length to NaNO_3 series was observed, and tips of laterals also were stubby. In advanced stage, the cortex of roots became slimy, disintegrated, and then sloughed off leaving their steles only. These symptoms were more remarkable in low Ca treatment.

Fig. 1. Effects of pH, calcium concentrations, and sources of nitrogen on the dry weight of citrus seedlings.

Growth of seedlings: The average dry weight per plant at harvest time was shown in Fig. 1. In NaNO₃ series, dry weight of the top (total of leaves and stem) was the minimum at pH 4 as a whole, the maximum at pH 5.5, and intermediate at pH 7. At all pHs tested,

dry weight increased from Ca low to medium, but decreased Ca medium to high. These tendencies were clearer at higher pH.

The similar trends to the tops were found also in the dry weight of roots, but the difference between pH 4 and pH 5.5 or 7 was greater than in the tops. As compared with the tops, the increase in the dry weight of roots from Ca low to medium was more at pH 4, and less at pH 5.5 or 7.

In NH_4NO_3 series, both dry weight of tops and roots were generally inferior to those in NaNO_3 series. As regards pH, dry weight at pH 4 was less than at higher pH, this tendency being clearer in the roots. Dry weight at pH 5.5 was slightly higher than at pH 7 in the top, however, in the root and total weight there was no clear difference between both pHs except low Ca treatment.

With regard to Ca levels, no consistent relationship was found throughout all pHs in NH_4NO_3 series. At pH 4, dry weight slightly increased from Ca low to medium, then decreased from Ca medium to high as shown in the top and total dry weight. The decrease was observed at pH 5.5 as Ca level increased, however, at pH 7 no consistent effect of Ca level was found on both top and root dry weight.

Though fresh weight was not shown, the tendency among treatments was similar to that of dry weight. For reference, height of seedlings, number and length of leaves, diameter of stem, and length of root in each treatment were shown in Table 1. These growth in the treatments also exhibited the same pattern as dry weight except slight discrepancy.

Table 1. Effects of pH, calcium concentrations, and sources of nitrogen on the growth of citrus seedlings.

pH	Ca conc.	Height of seedlings		Number of leaves		Length of leaves		Diameter of stem		Length of root	
		NaNO_3	NH_4NO_3	NaNO_3	NH_4NO_3	NaNO_3	NH_4NO_3	NaNO_3	NH_4NO_3	NaNO_3	NH_4NO_3
4	Low	13.4 cm	10.2	14.8	13.2	8.2 cm	7.5	3.0 mm	2.6	29.4 cm	23.8
	Med.	12.5	11.3	14.6	15.3	7.9	8.1	3.0	2.9	24.9	25.2
	High	12.4	11.7	14.5	13.6	6.9	7.6	2.9	2.7	28.7	24.8
5.5	Low	16.2	16.6	18.4	19.2	8.6	8.4	3.2	3.1	58.3	40.5
	Med.	20.5	13.4	21.7	16.3	8.5	6.7	3.3	2.9	67.2	33.6
	High	14.0	11.9	16.7	15.4	8.2	7.7	3.0	2.7	62.3	42.0
7	Low	14.7	12.0	18.3	16.2	7.1	6.9	2.9	2.8	63.5	30.0
	Med.	19.0	10.5	21.6	15.3	8.9	6.6	3.2	2.5	60.0	40.7
	High	10.5	11.6	12.5	15.3	6.1	7.0	2.7	2.6	41.6	42.6

Inorganic compositions: As shown in Figs. 2 and 3, N content (dry weight %) increased slightly in the leaves or root with rise of pH level in NaNO_3 series. In NH_4NO_3 series, N content of the leaves augmented with higher pH, while in the root it rather lowered at pH 7. In the leaves, NH_4NO_3 series had higher N content than NaNO_3 series, however, this tendency was not observed in the root. Ca level in the nutrient solution seemed not to have clear relationship with N content in both series.

Ca content in the leaves and root increased with rise of pH or Ca level of solution, and the degree of increment of Ca content in the leaves was greater than in the root. NaNO_3 series had higher Ca content than NH_4NO_3 series with few exceptions. Mg content of the leaves

As regards inorganic compositions in the stem, abbreviated to show in the Fig.3, the tendencies due to treatments were similar to those in the leaves. Respective total amounts of inorganic elements accumulated in the leaves or root, which were omitted to show, were almost proportional to the dry weights obtained in the respective treatments.

Fig. 3. Effects of pH, calcium concentrations, and sources of nitrogen on the inorganic compositions of citrus roots.

Discussion

As for nitrogen sources, growth of natsudaidai seedlings supplied with NaNO₃ was generally superior to that of seedlings supplied with NH₄NO₃. NH₄NO₃ seedlings had higher concentration of nitrogen including ammonium nitrogen in the leaves as compared with NaNO₃ seedlings. As it is accepted that ammonium nitrogen have injurious effects on the growth of citrus^(6,7), reduced growth in NH₄NO₃ treatment would be brought about by ammonium in spite of the presence of nitrate nitrogen.

In this experiment seedling growth was the minimum at pH 4 irrespective of nitrogen sources. In NaNO₃ series, seedling growth was the maximum at pH 5.5, and was lowered to some extent at pH 7, however, in NH₄NO₃ series, no clear difference was observed between pH 5.5 and 7 except Ca low treatment. In parallel with the reduced growth, the contents of nitrogen, calcium and magnesium at pH 4 were less than at higher pHs in both nitrogen sources. SMITH⁽¹⁶⁾ reported that growth at pH 4 was inferior to ones at pH 6, irrespective of nitrogen sources in the experiment with Pineapple sweet orange seedlings in aerated solution culture, and also found that seedlings at pH 4 had lower contents of calcium, magnesium and

potassium than those at pH 6. In pot culture with soil MARTIN *et al.*⁽¹¹⁾ found sweet orange seedlings made the maximum growth in the range of pH 5.7—6.5, and KOBAYASHI *et al.*⁽¹⁰⁾ showed Satsuma orange seedlings made the best growth in the range of pH 4.6—5.0 and pH 5.6—6.0. While, their growth was fairly reduced in the vicinity of pH 4, and was slightly lowered at pH 7 and over. It was also found that leaves of seedlings at lower pH had lower contents of cations such as calcium, magnesium and potassium.

Besides the decrease of nutrient absorption as affected by H ion, direct effect of H ion would be related to reduced growth at pH 4. RASMUSSEN *et al.*⁽¹⁴⁾ thought that H ion would be directly injurious to the roots of Pineapple orange seedlings at pH 4, because growth of seedlings at pH 4 was inferior to ones at pH 6 in the rain water culture without nutrients. They also observed brown, stubby, and swollen roots at pH 4 in contrast with white, smooth roots at pH 6 as were observed in our experiment. In addition to above causes, dissolution of aluminium and manganese, lowering of activity of nitrifying bacteria and deterioration of physical properties etc. seemed to be associated with reduced growth at lower pH in soil culture⁽¹¹⁾.

The lowering of growth at pH 7 as compared with ones at pH 5.5 in NaNO_3 series, seemed to be caused by deficiencies or inactivations of microelements such as iron or manganese in the tissues, as presumed by chlorotic or mottled appearance of leaves above-mentioned. However, further experiments accompanying analyses of these microelements will be necessary to corroborate this presumption. On the other hand, the solution maintained at pH 7 in NH_4NO_3 series tended to become lower pH. Accordingly, the reduction of deficiencies and of inactivations of microelements due to this lowering of pH appeared to be related with no clear difference of growth between pH 5.5 and 7.

As regards calcium, growth of seedlings increased with increasing supply from low to medium, however, decreased with higher supply in NaNO_3 series. In NH_4NO_3 series similar tendency was observed only at pH 4, and the decreasing and hardly no effect of calcium on the growth was found at pH 5.5 and 7, respectively. CHAPMAN *et al.*⁽⁴⁾ reported that adult trees of Washington navel orange had the maximum trunk circumference at Ca 200 ppm within the limits of Ca 15, 20, 50 and 200 ppm in the large scale solution culture supplied with nitrate as nitrogen source. In our experiment with younger seedlings of natsudaikai, calcium requirement for growth seemed to be lower than the above case. Furthermore, there was no such tendency that higher concentration of calcium was necessary for better growth at lower pH^(2,3).

The symptoms of calcium deficiency were not observed in the tops even at Ca low treatments because of the use of tap water in this experiment. But similar symptoms to those observed in the previous report⁽¹³⁾ appeared in the roots of seedlings of Ca low treatment at pH 4 in both nitrogen series. These seedlings had the minimum content of calcium and the poorest growth among all treatments.

Growth reduction at higher calcium concentration might be caused by chlorine. In this experiment tap water containing chlorine about 20 ppm was used for dilution of nutrients and calcium chloride was supplied as calcium source, so totalling about 160 ppm at Ca high treatment. HAAS⁽⁷⁾ presumed unfavorable effect of chlorine at the concentration of about 120 ppm

on the lemon cuttings in the soil culture. While, NAKAJIMA *et al.*⁽¹²⁾ reported that injury of chloride ion did not occur even at the concentration of 190 ppm on Satsuma orange seedlings in the nutrient spray culture to their roots. In our experiment also, NH_4NO_3 series did not show consistent responses to increasing supply of calcium chloride at respective pH, and chloride content in the seedlings was not analyzed. Accordingly, it would appear that further experiments are necessary to substantiate the effect of chlorine on the growth of natsudaikai seedlings.

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pH, カルシウム濃度ならびに窒素形態がカンキツの生育,
無機成分に及ぼす影響

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ナツダイダイ実生苗を水耕し、培養液の pH (4, 5.5, 7), カルシウム濃度 (低—20 ppm, 中—50 ppm, 高—100 ppm), ならびに窒素形態 (NaNO_3 , NH_4NO_3 , N としてともに 112 ppm) を変え、これらの組み合わせが生育、無機成分に及ぼす影響を調べた。

葉の外観は両窒素形態とも pH 5.5 では正常だが、pH 4 ではやや鈍緑色となり、pH 7 になると黄緑色がかかり、上葉にクロロシスを生じた。クロロシスは NaNO_3 区のほうが著しかった。 NaNO_3 区の根は pH 5.5 では白色だが、pH 4, 7 では淡褐変し、pH 4 では根長も短く、側根の先端がふくらんだ。 NH_4NO_3 区の根は一般に褐変し、伸長も NaNO_3 区より劣った。pH 4 では根長はとくに短く、側根の先端がふくらみ、さらに進むと皮層がほう壊脱落した。この症状は Ca 低濃度区で著しかった。

地上部、根の生育とも一般に NaNO_3 区のほうが NH_4NO_3 区よりまさった。両窒素形態とも pH 5.5 区で最も生育がよく、pH 7 区になるとやや劣り、pH 4 区で最も

劣った。ただし NH_4NO_3 区では pH 5.5 と pH 7 との差は Ca 低濃度区を除き NaNO_3 区ほどはつきりしなかった。カルシウム濃度については NaNO_3 区では各 pH とも Ca 中濃度区が最も生育がよかつたが、 NH_4NO_3 区では各 pH を通じての一貫した傾向はみられなかった。

無機成分濃度について、葉では一般に NaNO_3 区のほうが NH_4NO_3 区よりも K, Ca 濃度が高く、N, P, Mg 濃度が低かつた。一方、根では NaNO_3 区のほうが NH_4NO_3 区よりも P, Ca, Mg 濃度が高く、K 濃度は低く、N 濃度には一貫した傾向がみられなかった。pH を上げると葉では両窒素形態区とも N, Ca, Mg 濃度が増加し、K 濃度は減少したが、P 濃度には pH による差はみられなかった。根では pH を上げると両窒素形態とも P, Ca, Mg 濃度が増加し、K 濃度は減少したが、N 濃度は NaNO_3 区でのみやや増加した。Ca 施用濃度を上げてても両窒素形態とも Ca 濃度が増加するのみで他成分にははつきりした差はみられなかった。