THE UTILIZATION OF AMMONIUM SALTS, NITRATE SALTS, AND THE SYNTHESIS OF AMINO ACIDS IN THE RICE SEEDLING

by

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INTRODUCTION

The most important inorganic salts of nitrogen which can be absorbed and utilized by plants are ammonia and nitrates. These two play a role in various degrees of efficiency in the growth of plants.

Thers are many kinds of plants which prefer ammonia salts to nitrates as their nitrogenous nutrients. Rice plant is one of them, and may be accordingly classified as an "ammophile plant". On the other hand, some plants will grow more vigorously in a nitrate medium; and they are grouped as the "nitrophiles" such as jute, buck-wheat and potato etc.

The question of the relative efficiency of ammonium and nitrate as sources of nitrogen for green plants is one which has often been investigated since the days of Hutchinson, Miller (1911, cited in Clark and Shive 1934), and Prianischnikov (1926). They have reviewed the early experiments which proved that plants could absorb and assiminate either ammonium or nitrate nitrogen.

Physiological studies of nitrogen substances on the nutrition of the rice plants were generally conducted by means of water or sand cultures. Under these cultures, the reaction change of the solution depends in great degree upon its chemical composition, especially upon the kind of salts used as nitrogen source.

On the nutritive value of the nitrogen source for the rice plant many authors have made studies with ammonium sulfate and calcium nitrate under various culture conditions with special reference to the hydrogen-ion concentration. In most cases they came to the conclusion that the former was usually superior to the latter.

Willis and Carrero (1923) pointed out that nitrates were less suitable than ammonium salts for the fertilization of young rice plants. Clark and Shive (1934) studied the absorption of ammonium and nitrate nitrogen by using tomato plants from culture solutions with various hydrogen-ion concentrations. They found that the plants grew heavily upon the NH_4-N during the early stages of growth, and less heavily upon the NO_3-N, but that during the later stages of development this condition was reversed. They also found that the rates of absorption of NH_4-N were higher from solutions of high pH than from solutions of low pH whereas the rates of absorption of NO_3-N were higher from solutions of low pH than from solutions of high pH.

Yamayuchi (1935) and Bonner (1946) dealt mainly with the effect of nitrogen sources on the growth of the rice-plant. They confirmed the fact that the rice seedling utilizes ammonium better than nitrates as a source of nitrogen. Prianishnikov (1951) has also demonstrated that plants treated with ammonium showed better development than those with nitrates.

Although different theories have been proposed to explain why nitrates are not so suitable as the nitrogen source for the rice plant, the problem has not yet been definitely solved.

As to the kinds of free amino acids in the plant body, they bear a close relationship to the absorbed inorganic nitrogen sources. These amino acids may also be variable in the growing stages. And the free amides usually influence them indirectly. So, to explain the nitrogen assimilation process in the plant body, the free amino acids and amides are some of the key points. Some workers who dealt with theoretical studies have noticed the state of transformation and composition of free amino acids in the plant at the different stages of growth. For instance, Izawa and Natake (1954) worked on wheat plant, Yemm (1954) on barley seedling, Saio K., and Kimura J. (1957) on young rice seedling and Akai S. (1957) on leaves of the rice plant.

In this paper, the writer repeats a culture experiment to study the utilization of nitrogen substances by rice seedlings by supplying ammonium and nitrate as nitrogen sources in connection with hydrogen-ion concentration.

Furthermore, the free amino acids and amides in the plant are detected by the paper chromatographic method, which is used for the preparatory experiment of quantitative analysis. This experiment is thus an attempt to find out the different kinds of the free amino acids and amides in the rice seedling under the different nutrient conditions.

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MATERIALS AND METHODS

The Rice seeds (Taichung No. 65) used in this study were supplied by the Taiwan Agricultural Research Institute.

Rice seeds were treated with 0.1% granosan solution for 20 minutes and washed four times with dist. water before being used. They were then soaked for 12 hrs. in tap water, and finally put in Petri-dishes at room temperature for 80 hrs. for germination.

When the primary buds of the seedling were about 0.4 cm long, selected seedlings were divided into two series of cultures. One series was supplied with ammonium sulfate (A) in a nutrient solution at pH 6.0, 6.5, 7.0, 7.5, and 8.0, the other with calcium nitrate (B) at pH 4.0, 4.5, 5.0, 5.5, and 6.0. The initial pH was obtained by suitable addition of H_2SO_4 and KOH.

The chemical composition of the culture solutions was in accordance with that used by Kasugai, the formula of the solutions is as follows;

Table 1. Chemical Composition of Nutrient Solutions

Salts	Nutrient Solutions	
	A	B
$(NH_4)_2SO_4$	0.132 gm	—
$Ca(NO_3)_2 \cdot 4H_2O$	—	0.236 gm
KCl	0.172	0.172
KH_2PO_4	0.122	0.122
$FeSO_4$	Trace	Trace
$MgSO_4 \cdot 7H_2O$	0.074	0.074
$CaCl_2$	0.111	—
Dist•Water	1,000 ml	1,000 ml

Fifty seedlings were placed on gauze, which tightly covered one end of the vessel. Eight vessels were placed in a glass pot. The space between the vessel and pot was filled with culture solution up to the level of the vessel. These pots were kept in the green-house. The solution lost by evaporation and transpiration was replaced by fresh nutrient solution. The cultures were renewed every three days at 6 pm; and their pH value changes were determined by the used of a pH-meter just after the solutions were renewed.

Samples of the seedlings were removed from the culture solutions at the end of each 3-day culture period, and dried in an oven at 70°C until they were really dry, and then their weights were taken.

The nitrogen absorbed by the seedlings from these culture solutions was determined. One was by the Lumetron Colorimeter to detect the content of the nitrate nitrogen and the other was by the Micro-kjeldahl to detect the content of the ammonium nitrogen. Every three days the length of the final roots and shoots of all the seedlings were recorded until the end of the 15 th day.

As for the next procedure of this experiment, the largest seedlings from the different nitrogen sources (one with ammonium sulfate, at pH 8.0, the other calcium nitrate, at pH 4.0) were selected for analytical study by paper chromatography.

The samples were examined with two-dimensional paper chromatography on sheets of Tu Yo No. 2 filter paper with butanol-acetic acid-water in the first dimension and phenol-water in the second dimension. These methods follow the patterns used by Izawa and Natake (1954).

RESULTS

1. Changes in hydrogen-ion concentration of the culture solutions.

Table 2. Changes in hydrogen-ion concentration when Ammonium was used, as a nitrogen source of culture solution.

Final values at the end of each 3-day period from initial value of pH 6.0, 6.5, 7.0, 7.5 and 8.0. Solutions were renewed every 3-day, immediately after determination of final pH values.

Initial pH values	Final pH values				
0 day	3rd day	6th day	9th day	12th day	15th day
6.0	4.5	4.6	4.4	4.5	4.4
6.5	4.3	4.4	4.6	4.4	4.6
7.0	5.4	5.2	4.4	4.8	5.1
7.5	6.2	6.0	4.7	4.9	5.8
8.0	6.3	6.5	5.2	4.8	5.1

Table 3. Changes in hydrogen-ion concentration when Nitrate was used, as a nitrogen source of culture solution.

Final values at the end of each 3-day period from intial values of pH 4.0, 4.5, 5.0, 5.5 and 6.0. Solutions were renewed every 3-day, immediately after determination of final pH values.

Initial pH values	Final pH values				
0 day	3rd day	6th day	9th day	12th day	15th day
4.0	5.3	5.8	5.2	5.8	6.0
4.5	5.7	6.1	6.2	6.0	6.4
5.0	6.7	7.0	6.6	7.0	6.8
5.5	6.85	7.0	6.9	7.2	7.1
6.0	6.9	7.3	7.0	7.0	7.2

From Tables 2 and 3 it was shown that the solutions containing ammonium nitrogen increased their acidity under the influence of the plant while those containing nitrate nitrogen decreased their acidity (Yamaguchi, 1935). These results bear a striking resemblance to those of Weissman (1950) with wheat seedlings.

It was shown that the reaction of the culture solution is markedly influenced by preferential absorption of cation of NH_4-salts or the anion of NO_3-salts.

This suggests that the reaction rates of absorption of NO_3 ions and of NH_4 ions will determine the reaction changes. That differential absorption of salt cations and anions might alter the hydrogen-ion concentration of the solution has been shown by many investigators.

2. Growing state of the seedlings.

Table 4. Length of the rice seedlings at different growing periods in solution with Ammonium as the nitrogen source and varying the initial pH.

Sept. 10–25 1957 Temp. 23–42.6°C

Initial pH	Seedlings	Length (cm)					Leaf color	Growing state
		3rd day	6th day	9th day	12th day	15th day		
6.0	Shoot Root	1.59 2.65	8.61 6.06	10.42 6.12	14.97 6.30	16.75 6.40	A part of leaves with light green color	Good
6.5	Shoot Root	1.55 2.47	9.60 6.18	11.14 6.22	16.07 6.50	17.65 6.80	Leaf green	Good
7.0	Shoot Root	1.67 3.50	9.71 6.55	11.46 6.77	16.70 6.77	17.75 7.30	Leaf green	Good
7.5	Shoot Root	1.66 2.88	8.93 7.20	14.10 7.37	17.40 7.77	19.60 7.95	Leaf green	Very good
8.0	Shoot Root	1.67 2.75	9.76 7.75	14.27 7.80	19.54 8.40	24.05 8.65	Leaf green	Pretty good

Table 5. Length of the rice seedlings at different growing periods in solution with Nitrate as the nitrogen source and varying the initial pH.

Initial pH	Seedlings	Length (cm)					Leaf color	Growing state
		3rd day	6th day	9th day	12th day	15th day		
4.0	Shoot Root	1.67 2.78	10.43 9.02	13.60 9.80	18.10 10.23	20.0 10.41	Leaf light green in color (rarely)	Good
4.5	Shoot Root	1.46 2.36	7.68 9.88	9.20 9.97	11.80 10.03	13.30 10.23	Leaf yellow green in color (mostly)	Poor
5.0	Shoot Root	1.37 1.67	7.25 8.21	9.47 8.40	14.46 8.93	15.10 9.45	Leaf yellow color (mostly)	Poor
5.5	Shoot Root	1.55 2.27	7.48 8.36	9.12 8.45	12.67 8.76	13.85 8.93	Yellow in color	Very poor
6.0	Shoot Root	1.50 2.93	6.61 7.93	8.27 8.02	12.67 8.67	13.25 8.80	All turn to yellow color	Very poor

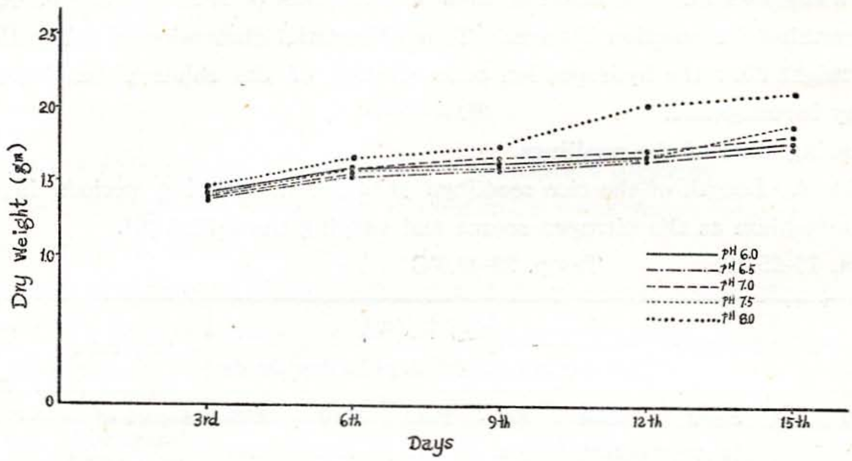

Fig. 1. Dry weight (gm) of the rice seedlings (100 individual seddlings) at the different growing periods in solution with ammonium as nitrogen source and varying initial pH.

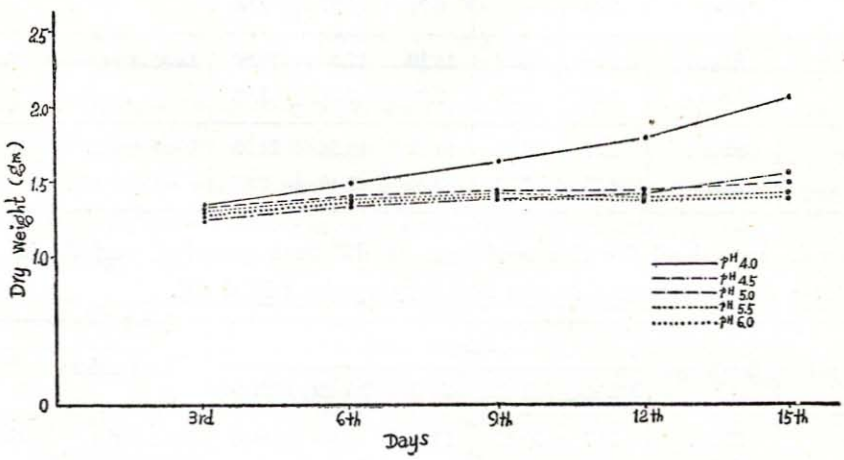

Fig. 2. Dry weight (gm) of the rice seedlings (100 individual seedlings) at the different growing periods in solution with nitrate as nitrogen source and varying initial pH.

Tables 4 and 5 showed that the seedlings grown with ammonium nitrogen showed more luxuriant growth than those with nitrate nitrogen. The leaves of the seedlings cultivated in the nitrate solution ranged from light green to yellow in color. Furthermore, these seedlings were comparatively smaller (except at pH 4.0) than those of the other groups of seedlings grown in ammonium solutions (Refer to Fig. 7).

Tables 4 and 5 indicated also that the seedlings grew fast on the 6 th day. In the ammonium source the speed of the growth on the 6 th day was 5½ or 6½ times more than that on the 3 rd day, and seedlings grew continuously after the 6 th day. In the nitrate source, the seedlings (when the pH was 4.5 to 6.0) grew 4 times faster than that on the 3 rd day but their growth was severely inhibited after the 6 th day (except at pH 4.0).

At pH 8.0 seedlings supplied with ammonium nitrogen grew better than those in the same culture solutions with other pH values. The growth of the seedlings at pH 4.0 and the culture receiving nitrate nitrogen made a greater volume of growth than those receiving the same culture solution in different pH values.

From Figs. 1 and 2 we also recognized that the dry weight of both the ammonium seedlings and the nitrate seedlings increased evidently on the 6 th day. After that day the dry weight of the ammonium seedlings went on increasing (especially at pH 8.0), while that of the nitrate seedlings increased only at pH 4.0; and no evident increase was observed when the solution were in a range of pH 4.5–6.0. This might be explained by the fact that pH 4.5–6.0 were prohibitive to their growth.

These results again indicated the advantage of ammonium nitrogen at a high pH value and nitrate nitrogen at a low pH value. It is recognized that the length of shoot is markedly influenced by the hydrogen-ion concentration. With nitrate as the source of nitrogen, the length of shoot was sharply reduced at pH 4.5, 5.0, 5.5 and 6.0. On the other hand, the length of shoot was less affected with ammonium as the nitrogen source (Refer to Fig. 7).

3. Analysis of the seedlings.

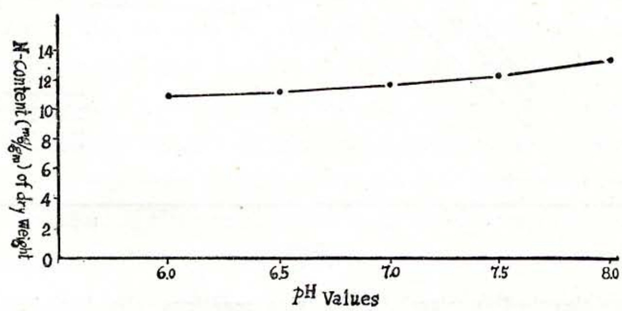

Fig. 3. Nitrogen content* (mg/gm dry weight) in the rice seedlings grown 15 days in solutions with ammonium as nitrogen source and varying initial pH. Each figure is an average of three determinations.

* Samples were analyzed to test the nitrogen content of the seedlings on the fifteenth day.

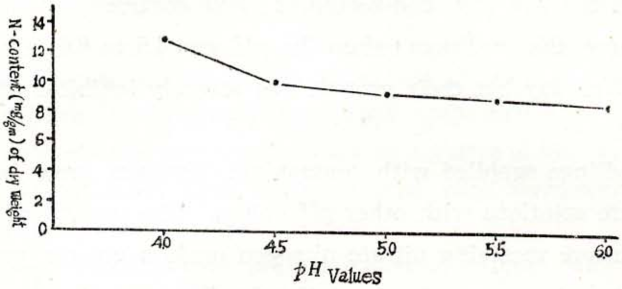

Fig. 4. Nitrogen content* (mg/gm dry weight) in the rice seedlings grown 15 days in solutions with nitrate as nitrogen source and varying initial pH. Each figure is an average of three determinations.

* Samples were analyzed to test the nitrogen content of the seedlings on the fifteenth day.

From Figs. 3 and 4, it might be seen that with ammonium as nitrogen source the nitrogen content increased as the pH value increased. In the presence of nitrate as nitrogen source, it decreased as the pH value advanced. Data indicated that the effect of the hydrogen-ion concentration on the rate of nitrate assimilation was greater than that of the ammonium seedling.

4. Nitrogen absorbed by the rice seedling from culture solutions.

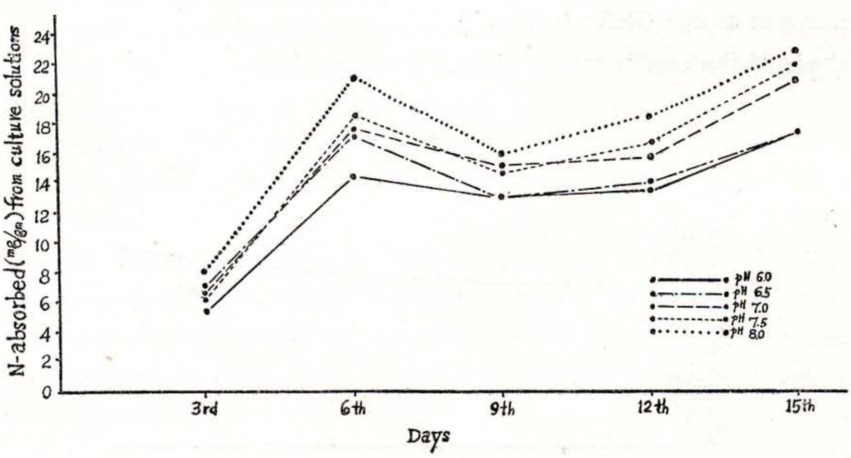

Fig. 5. Nitrogen absorbed (mg/gm) by the rice seedlings (100 individual seedlings) grown 15 days in solution with Ammonium as the nitrogen source and varying the initial pH.

As shown in Figs. 5 and 6, it can be seen that as NH_4-N was applied to the seedlings, the absorption markedly increased as pH value increased, but under NO_3-N conditions the absorption apparently was restricted except at pH 4.0. The pH of the solution was probably the limiting factor for absorption.

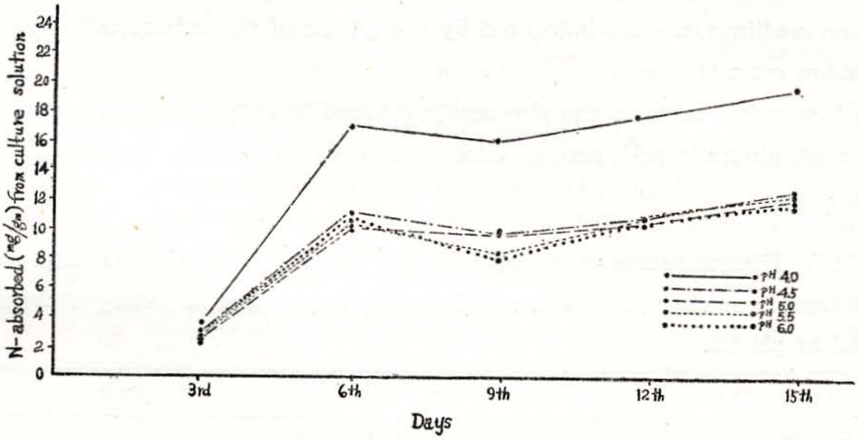

Fig. 6. Nitrogen absorbed (mg/gm) by the rice seedlings (100 individual seedlings) grown 15 days in solution with Nitrate as the nitrogen source and varying the initial pH.

It has been previously shown that the hydrogen-ion concentration has less influence on nitrogen absorption when ammonium was supplied to the seedlings, than vice versa.

The observations show that the rice seedlings absorbed ammonium salts from culture solution more rapidly at high pH value whereas they absorbed nitrate more rapidly at a lower pH and this has been reported by many workers.

5. Free amino acids in the rice seedlings.

The distribution of alcohol-soluble amino acids on the chromatogram are shown in Fig. 8 and the spots were identified in Fig. 8 too.

The amino acids could be determined by the comparison of their relative color densities with visual observation as shown in Tables 7 and 8. Rf. values of amino acids and its color developed by ninhydrin is distinctly shown in Table 6.

Table 6. Rf values of Amino acids and its color developed by ninhydrin.

Solvents / Amino acids	Butanol Acetic acid Water (4:1:5) Ascending	Phenol Water (4:1) Ascending	Colour reaction
Aspartic acid	0.12±0.02	0.14±0.02	Blue colour
Glutamic acid	0.19±0.01	0.26±0.01	Violet
Serine	0.11±0.01	0.35±0.01	Violet
Asparagine	0.09±0.01	0.43±0.02	Orange brown
Glutamine	0.12±0.01	0.61±0.01	Deep violet
Alanine	0.24±0.02	0.62±0.02	Violet
Methionine	0.42±0.02	0.82±0.01	Violet
Valine	0.50±0.02	0.81±0.02	Violet
Leucine	0.69±0.02	0.88±0.02	Violet

From the results shown in Tables 7 and 8, it is clear that the free amino acids in the rice seedlings are not influenced by the nature of the culture solutions and its hydrogen-ion concentrations.

The free amino acids in the rice seedling found by paper chromatography were aspartic acid, glutamic acid, serine, alanine, methionine, valine, leucine, asparagine, and glutamine.

Table 7. Determination of amino acids by visual observation of rice seedlings at the different growing periods in solution with ammonium as nitrogen source and the initial at pH 8.0.

Amino acids	Shoot						Root					
	3rd day	6th day	9th day	12th day	15th day Noon	15th day Night	3rd day	6th day	9th day	12th day	15th day Noon	15th day Night
Aspartic acid	++	++	++	+++	++	+++	++	++	+	+	+	+
Glutamic acid	++	++	++	+++	++	++	+	++	++	+	+	+
Serine	+	+	+	++	++	++	++	++	++	++	+++	+++
Alanine	+	+	+	++	++	++	++	+	+	+	+	+
Methionine	+	++	++	+	++	+	++++	+++	++	++	++	+
Valine	+	+	+	+	+	+	+	+	+	+	+	+
Leucine	+	+	+	+	+	+	+	+	+	+	+	+
Asparagine	+++	++++	++++	++++	++++	++++	+	++	+++	+++	+++	+++
Glutamine	++	++	++	++	+++	+++	++	++	++	++	++	++

Table 8. Determination of amino acids by visual observation of rice seedlings at the different growing periods in solution with nitrate as nitrogen source and the initial at pH 4.0.

Amino acids	Shoot						Root					
	3rd day	6th day	9th day	12th day	15th day Noon	15th day Night	3th day	6th day	9th day	12th day	15th day Noon	15th day Night
Aspartic acid	++	++	+++	++	++	++	+++	++	++	+	+	++
Glutamic acid	++	++	+++	++	++	++	++	++	++	+	+	++
Serine	++	++	++	++	++	++	+	++	++	++	++	++
Alanine	+	+	+	++	++	+	++	+	+	+	+	+
Methionine	++	++	++	++	+	+	+++	+++	++	+	+	+
Valine	+	+	+	+	+	+	+	+	+	+	+	+
Leucine	+	+	+	+	+	+	+	+	+	+	+	+
Asparagine	+++	+++	+++	+++	++++	+++	+	++	+++	+++	+++	++
Glutamine	++	++	++	++	++	++	++	++	++	++	++	++

From Tables 7 and 8 the results can be divided into two parts: one, when the shoot was used as the sample, in this experiment, it is evident that a considerably greater amount of asparagine was found than that of glutamine throughout its whole seedling stage. And a much larger amount of asparagine was found in the ammonium than in the nitrate seedlings. Asparagine and glutamine were present in largest amounts: valine and leucine were the least; while aspartic acid and glutamic acid were in between them. The other part of this experiment concerns the root. It indicated that there was much methionine in the seedling. It reached its highest point when the seedlings were in their early growing stages, and declined gradually when the seedlings came to their later stages.

DISCUSSION

In general, the differences between the ammonium seedlings and the nitrate seedlings were caused by the nature of the nitrogen sources and the hydrogen-ion concentration in the culture solutions.

From Table 3, it can be seen that when the nitrogen was supplied in the form of nitrate, the pH value always rose toward the neutral point, regardless of the initial hydrogen-ion concentration. This rise in pH value might be attributed to the presence of NO_3 radical which was derived from the hydrolysis of $Ca\ (NO_3)_2$. This was neutralized by H^+ either as ion pairs or as molecules. In the development of chemical theory there seems to be no need for assuming that any significant hydrolysis of $Ca\ (NO_3)_2$ takes place in accordance with the following hypothetical reactions:

$$
\begin{array}{l}
Ca\ (NO_3)_2 \rightleftarrows Ca^{++} + 2\ NO_3^- \\
2\ H_2O \rightleftarrows 2\ OH^- + 2\ H^+ \\
\qquad\qquad\quad \downarrow\uparrow \qquad\quad \downarrow\uparrow \\
\qquad\quad Ca\ (OH)_2 \quad 2\ HNO_3
\end{array}
$$

But if the hydrolysis of $Ca\ (NO_3)_2$ does occur, the solution should contain more HNO_3 owing to an increase in the ionic product $[H^+] \times [NO_3^-]$. So it is suggested that the rise in pH values as shown in Table 3 might be due to the fact that some of the NO_3 ions had been neutralized by H ions.

The reactions shown in Tables 2 and 3 indicated that some of the NH_4 ions had been absorbed by the seedlings in company with the OH ions, the latter of which came from the solution and that some of the NO_3 ions were neutralized by H ions.

According to the generally accepted view, $(NH_4)_2\ SO_4$, when it is present in the culture solution, reacts with the ions of water in the following fashion:

$$
\begin{array}{l}
(NH_4)_2\ SO_4 \rightleftarrows 2\ NH_4^+ + SO_4^= \\
2\ H_2O \rightleftarrows 2\ OH^- + \quad 2\ H^+ \\
\qquad\qquad\quad \downarrow\uparrow \qquad\qquad \downarrow\uparrow \\
\qquad\quad 2\ NH_4OH \quad H_2SO_4 \\
\qquad\qquad\quad \downarrow\uparrow \\
\qquad\quad 2\ NH_3 + 2\ H_2O
\end{array}
$$

The fall in the pH values of culture solutions might be explained by assuming that NH_3 or NH_4OH was absorbed more rapidly than H_2SO_4 or $(NH_4)_2$ SO_4.

This was in accordance with the idea previously suggested by Trelease and Trelease (1935) that the absorption of ammonium ions was accompanied by the removal of hydroxyl ions of the solution.

Experiments conducted with $(NH_4)_2$ SO_4 as the only source of nitrogen showed that the NH_4 ions were absorbed at a much higher rate than any other ions; the pH value of the solution decreased in direct relation to the NH_4 absorption.

As mentioned above, a plant would absorb nitrate ions more readily from an acid medium than from an alkaline one because it could easily secure hydrogen ions that must accompany the nitrate into the plant.

Similarly an alkaline medium would easily supply the hydroxyl ions that would accompany the ammonium ions into the plant. This was in keeping with the aforementioned findings of Weissman (1951).

It was suggested that the entrance of nitrate might depend upon the ionic product $(H^+)\times(NO_3^-)$ within the cell and in the external solution (Osterhout, 1936).

The principles outlined above might furnish an explanation for the reaction changes shown in Tables 2 and 3.

The growing state of the seedlings were given in Tables 4 and 5. A study of the data suggests that differences in the growing states might be explained most satisfactorily if it is assumed (1) that the growth of the seedlings is directly related to the rate of absorption of nitrogen (in the NH_4 or the NO_3 form); (2) that nitrogen content tended to increase with increasing pH value of the NH_4 solution; and (3) that nitrogen content increased with decreasing pH value of NO_3 solution.

These assumptions were supported by the findings of a number of studies reported in the literature.

For example, Bonner (1946). found that rice, at the seedling stage utilized ammonium rather than nitrate as a source of nitrogen which was confirmed in this experiment, Tiedjens and Bobbins (1931, cited in Maximov, 1935) found that tomato plants absorbed and assimilated ammonium salts most efficiently at an initial pH of 7 or 8.

The data of Fig. 2 showed that with an initial pH value of 4.0, the dry weight exhibited a general tendency to increase with an increase of seedling growth (Refer to Table 5).

The most vigorous growth was secured in the solution that had the lowest pH value of NO_3. It might be supposed that NO_3 absorption predominates at the generally low pH value of the solution and that the most rapid absorption of nitrogen and hence the greatest growth, occurs in solutions having the lowest hydrogen-ion concentration (Refer to Fig. 6 and Tabled 5).

We found (Fig. 1) that with an initial pH value of 8.0, the dry weight tended to increase in proportion to its pH value. The greatest growth was obtained in the solution having the highest pH value (Refer to Table 4). From these studies we can understand that the absorption of nitrogen in the NH_4 form is very important and the growth of the seedlings was mainly determined by the supply of NH_4 in the solution.

From Table 4 it is seen that good growth occurs in all the seedlings where ammonia is supplied as the source of nitrogen.

The result of the previous experiment shows that the plants growing in the nitrate culture were inferior to those in the culture with ammonium sulfate, and this unfavorable effect of nitrate might be probably due to the accumulation of nitrites which were produced by its decomposition. This was suggested by Willis and Carrero (1923) that nitrates were less suitable than ammonium salts for the fertilization of young rice plant might be based on the influence on the plants of the residues which generally have been ascribed to the toxic effects of the nitrites derived from the nitrates by reduction. It was a manifestation of chlorosis caused by the action of the basic residues of the nitrate salts used as nutrients.

The change of hydrogen-ion concentration of the culture solution causes the precipitation of iron; and available iron for the plant growth was much diminished, from which chlorosis on the leaves resulted.

According to the analysis of the seedlings, it also showed that NH_4-N in the culture was favorable for the growth of the rice plant in its early stage and the NO_3-N was only favorable to the seedling when the pH value was 4.0.

As shown in Figs. 5 and 6 the amount of nitrogen absorbed by the seedlings increased much on the 6 th days. On that day the amount absorbed by ammonium seedlings was 3 times more than that on the 3 rd day and that absorbed by the nitrate seedling was 4 times more than that on the 3 rd day. This meant that the seedling grew faster on the 6th day and absorbed more nitrogen from the culture solution.

On the 9 th day the amount of nitrogen absorbed was far less than on the 6 th day. But the absorption rate increased again after the 9 th day. However the nitrate seedlings in the solution within a range from pH 4.5 to 6.0 did not absorb nearly as much nitrogen. This condition is in agreement with the growing condition of the seedlings.

This experiment showed evidently that the utilization of NH_4-N and the suitable pH range for the seedling were better and wider than that of NO_3-N. This might be supported by the experiment of Bonner and Weissman. (1946; 1950).

In Tables 7 and 8 of this experiment, we can see that the free amino acids found in the shoot of rice seedling were similar to those in the root.

Free amino acids and amides present on chromatograms were aspartic acid, glutamic acid, serine, alanine, methionine, valine, leucine, asparagine and glutamine.

The results in Tables 7 and 8 of this experiment disclose the fact that the nitrogen sources had no effect on the synthesis of amino acids in the seedlings, although they could affect their growth.

In regard to the metabolism of the amino acid in the plant body the most significant fact is known as transamination. This important biological reaction consists of the bodily transfer of an amino group from one compound to a second.

According to the fact that these three amino acids (i. e. aspartic acid, glutamic acid and alanine) must be in free state in the plant body, we might estimate that the nitrogen metabolism process was similar to the assimilation process of nitrogen in the plant body.

These amino acids, among which glutamic acid and aspartic acid were found in the richest amount, indicated that they were metabolites of great importance in the plant.

It was reported that preparations of pea roots could aminate α-keto-isocaproic acid at the expense of glutamic acid with the formation of leucine. The reaction between glutamic acid and α-keto-isovaleric acid to form the amino acid valine is also known in the plant.

It is clear in any case that the transamination for reactions other than the glutamic-aspartic and glutamic-alanine systems are present in plant tissues even only in low concentration.

Serine was also one of the important amino acids in the plant, yet whether it has something to do with the transamination or is rélated to other parts of amino acid metabolism is unknown. However, the fact that it must be in free state in the plant body was one of the key points to explain the amino acid metabolism in the plant body.

Among amides, asparagine and glutamine were seen in the two N-sources. Whether these two amides are present or not depends upon the nutritive condition of the plant, and they are thus different from the amino acids mentioned above. In other words, the less the nitrogen was supplied, the less the amount of asparagine was formed.

It disappears if the supply of nitrogen is discontinued. On the other hand, glutamine did not show the same behavior as that of the asparagine (Izawa and Natake, 1954).

The asparagine was found in a greater amount than glutamine in all seedling stages. (Refer to Tables 7 and 8). It was also found that in the seedlings, the asparagine contained more NH_4-N than NO_3-N. There was no doubt that it was available for the rice seedlings in the early stages with ammonium as a nitrogen salt.

Mothes, (1940, cited in Mayer and Anderson, 1952) found that if the plant was kept in darkness to prohibit the formation of energy substances, the formation of glutamine was also somewhat prohibited. On the other hand, there would be a large amount of asparagine.

Prianishnikov stated (1951) that in the same organ of the plant the ratio between the two amides might change depending upon the nature of the nutrition used. Glutamine was found favorable whereas asparagine was accumulated when there was a high expenditure of energy material. In the latter case, glutamine might serve as a source for the formation of asparagine. (This occurs only when the carbohydrates show deficiency).

By this way, if free asparagine could be one of the indicators of plant nitrogen nutrition, glutamine could be that of plant carbohydrate nutrition too.

Schylze (1897) showed definitely that under normal conditions asparagine was produced from proteins and not from inorganic nitrogen sources. But through further studies largely with the seedling of legumes Schulze supplied evidence in support of the hypothesis that asparagine might be formed readily from ammonia and organic acid.

He also found that during early stages of germination and growth of the seedlings, cotyledons contained only primary products of protein hydrolysis, the amino acids (leucine, tyrosine etc.). During later development of the plant, amino acids disappeared from the cotyledons and asparagine was accumulated in large amounts in the shoot. Therefore asparagine did not seem to be produced directly from proteins but resulted from the breaking down of amino acids.

From Tables 7 and 8 we might find that methionine was accumulated in the roots during the earlier stage of growth. Later on, it dwindled gradually and left only a trace in the later stage of growth.

Although we have not found the metabolism process of methionine in detail, yet it is not difficult to visualize some of its important reactions.

Besides, it was found that a very small amount of valine and leucine was present at any stage. Their function in amino acid metabolism in the plant has not yet been worked out.

That the amount of different amino acids in the shoot was much more than that in the root was shown in the paper chromatography. So this concept, that the amino acids in the rice seedlings were synthesized in the leaf from which they were transferred to the root part, was probably true.

In conclusion, the assimilation process of nitrogen is very complex. It is influenced by many factors. Evidences for such a view from this study was insufficient to make any final conclusion. However, the problems discussed above may be of some use in further study.

SUMMARY

1. Rice seedlings were grown in culture solutions with initial pH values adjusted to 4.0, 4.5, 5.0, 5.5, and 6.0 with nitrate and 6.0, 6.5, 7.0, 7.5, and 8.0 with ammonium nitrogen sources.

2. Records were made during a culture period of 15 days.

3. It was found that with low pH values, the pH values of the solutions increased rapidly and approached, in extreme cases, to a pH value of 7.2. With high pH value, the pH values decreased rapidly tending to a limited value of 4.3.

4. Excellent growth of the seedlings was found in culture solutions with a pH value of 8.0 and of 4.0

5. The dry weight and the nitrogen content of the ammonium seedlings were much higher than those of the nitrate seedlings. The amount of nitrogen absorbed from the culture solutions with ammonium was greater than that from the nitrate solutions.

6. It is concluded that the rice seedling during its early develoment grew better with ammonium salts than with nitrates.

7. Free amino acids and amides in the rice seedlings were detected by paper chromatography. These amino acids and amides were aspartic acid, glumatic acid, alanine, serine, methionine, valine, leucine, asparagine and glutamine. These two amides were larger in amount, valine and leucine were the least; while aspartic acid and glutamic acid were between them.

8. It was not observed that the free amino acids synthesis of the rice seedlings could be influenced by the nature of the nitrogen sources and its hydrogen-ion concentrations.

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Fig. 7. Rice seedlings of the experiment in solution cultures at 15 days with a nutrient solution containing calcium nitrate at initial pH 4.0, 4.5 5.0, 5.5, and 6.0 (upper) and ammonium sulfate at initial pH 6.0, 6.5, 7.0, 7.5, and 8.0 (lower).

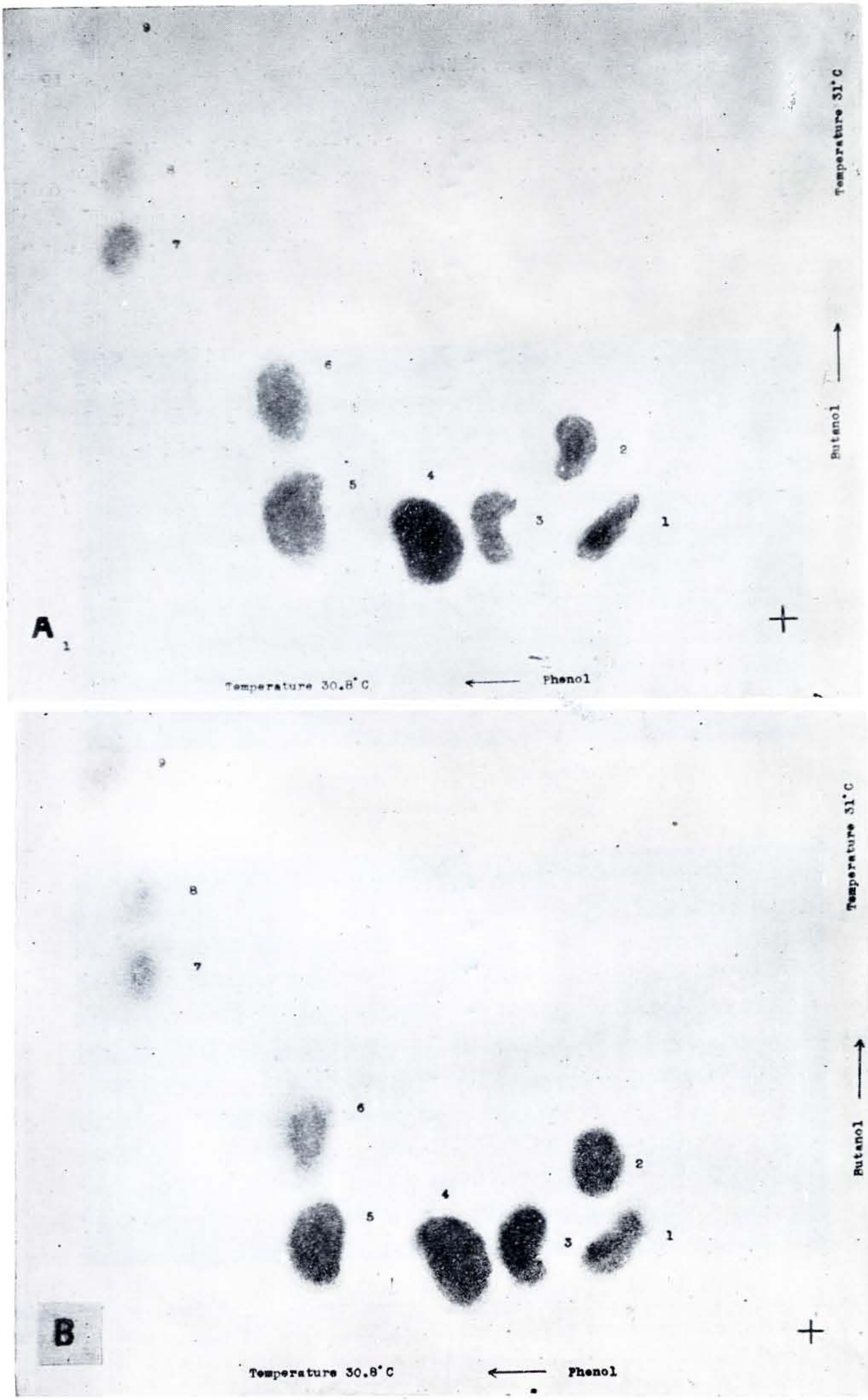

Fig. 8. Amino acids and amides in the rice seedling. Two-dimensional Paper Chromatogram.

Legend: 1, Aspartic acid; 2, Glutamic acid; 3, Serine;
4, Asparagine; 5, Glutamine; 6, Alanine;
7, Methionine; 8, Valine; 9, Leucine.

A. A rice seedling grown in ammonium sulfate at inital pH 8.0.

B. A rice seedling grown in calcium nitrate at initial pH 4.0.

The shoots were removed from the seedlings at the end of the 15 th day.