Comparative Studies on the Water, Sand and Soil Cultures of Rice Plant, with Special Reference to the Nitrogen Source and Hydrogen Ion Concentration

By

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Physiological studies on the nutrition of the higher plants are generally conducted by means of sand and water cultures. It happens however that the conditions of these cultures differ from those of the soil or field culture in many respects, under which the natural growth of plants goes on. Therefore, as a matter of course, many differences are expected between the results obtained by these culture methods, and when the results from the two methods are applied to the soil culture, very careful comparative studies between them are previously needed, either in the scientific or practical meaning.

The paddy rice plant is a marsh plant ecologically speaking and the conditions under which it grows may be regarded to be very near to those of the sand culture, that is, conditions intermediate between the water and soil culture. In this respect the paddy rice plant seems to be a suitable material for a comparative investigation such as that mentioned above.

The reaction change of the culture solution depends in great degree upon its chemical composition, especially upon the kind of salt used as the nitrogen source. In this relation nitrogenous salts are often called physiologically acidic, alkaline or neutral. That the secondary change of the reaction plays an important rôle, directly or indirectly, upon the plant growth in the cases of the water and sand culture is a matter of course. In a previous paper (1929) the writer found that the hydrogen ion concentration of the culture solution should be taken into consideration for the explanation of the utilization of urea by corn seedlings and of its injurious action.

On the nutritive value of the nitrogen source for the rice plant many authors have made studies with ammonium sulphate and sodium nitrate under various culture conditions, with special connection with

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hydrogen ion concentration. In most cases they came to the conclusion, that the former is usually superior to the latter. Although many investigations have been undertaken on the problem why nitrate is not suitable as the N-source for the rice plant no conclusive explanation has yet been attained. That the hydrogen ion concentration is much concerned with this problem, can not be denied.

As stated above, it is very probable that the difference of plant growth according to the hydrogen ion concentration has considerable connection with the difference of culture methods as well as that of the nitrogen source. In the present paper the writer proposes to report the results of culture experiments with rice plants, applying these different culture methods and using ammonium sulphate, sodium nitrate and urea as nitrogen sources. This may show on one hand how important the water and sand cultures are for the physiological investigation and on the other hand how carefully the knowledge attained by these cultures must be applied to the soil or field culture, which has bearing upon soil science in many points.

Culture experiments were carried out in solution, in coarse sand and in clayey loam. The culture vessels used in the present work were porcelain cylinders. As culture plants mostly *Oryza sativa* (Bozu No. 6.), but sometimes *Zea Mays* (Sapporo Eight Lines), were used throughout the work. Seeds were supplied by the Agricultural Experimental Station, Hokkaido Government, for which the writer wishes to express here his many thanks.

A. WATER CULTURE

The growth of rice plants in relation to the nitrogen source

Urea and ammonium sulphate were used as nitrogen source owing to the following reasons:

1) The hydrolysis of urea depends greatly upon the conditions of the culture medium, especially its hydrogen ion concentration. Urea is an alkaline manure in the physiological sense.

2) Ammonium sulphate is widely used as a nitrogen source for the culture of rice plants, and this salt is physiologically acidic.

3) Urea and ammonium sulphate are available as nitrogen sources equally in the form of ammonia. Both of them cause the change of the reaction of the culture solution in opposite directions,

and a wide pH-range soon results from their use. By using nitrogen sources of these two kinds, studies on the relation between the resorption of ammonia and hydrogen ion concentration are possible.

As the original culture solution a modified KNOP's solution was prepared, using calcium chloride instead of calcium nitrate. The composition of this solution was as follows:

(A)

KCl	0.120 gm
$MgSO_4 \cdot 7H_2O$	0.250 gm
$CaCl_2 \cdot 2H_2O$	0.896 gm
$FeCl_3$(2%)	3 drops
Distilled water	1000 cc.

As the nitrogen source urea and ammonium sulphate were added in the following concentration to 1000 cc. of the culture solution A.

(B) nitrogen source

1	$CO(NH_2)_2$	0.366 gm
2	$(NH_4)_2SO_4$	0.805 gm
3	$CO(NH_2)_2$	0.185 gm
	$(NH_4)_2SO_4$	0.402 gm

Experiment I.

Six rice seedlings (shoot ca. 4 cm, and root 5 cm) in separate culture vessels, each containing 400 cc. of nutrient solution. March 3–March 16, 1931. Temp. 16°–35°C.

From the Table I, it will be seen that the pH-value of the culture solutions with ammonium sulphate or with both urea and ammonium sulphate combined changed from 3.4 to 6.8. Such reaction change of the culture solution depends greatly on the quantity ratio between urea and ammonium sulphate, and the method and time of the fertilization. The hydrogen ion concentration of the solution decreased, when there was urea in a larger amount than ammonium sulphate, but an inverse relation was recognized, if more ammonium sulphate was given than urea. Such a reaction change of the culture solution during the course of experiment certainly depends on the nature of the physiological alkalinity and acidity of urea and ammonium sulphate respectively. Under natural conditions and sometimes in the ordinary water culture urea is decomposed into ammonium and carbonic acid by the action of bacteria or with the aid of urease contained in the plant bodies, and ammonia thus formed

Table I.

N-source	Manuring	pH		Dry weight (gm)		
		Initial	Final	Shoot	Root	Total
Not given		4.5	4.6	0.066	0.200	0.266
$(NH_4)_2SO_4$	once	4.5	3.6	0.071	0.390	0.461
$CO(NH_2)_2$	once	4.5	6.8	0.060	0.290	0.350
$(NH_4)_2SO_4(\frac{1}{3})$ $CO(NH_2)_2(\frac{2}{3})$	once	4.5	6.5	0.065	0.390	0.455
$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$	once	4.5	6.1	0.065	0.390	0.455
$(NH_4)_2SO_4(\frac{2}{3})$ $CO(NH_2)_2(\frac{1}{3})$	once	4.5	4.7	0.111	0.730	0.841
$(NH_4)_2SO_4(\frac{3}{4})$ $CO(NH_2)_2(\frac{1}{4})$	once	4.5	5.3	0.088	0.560	0.648
$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$	* Urea once	4.5	3.6	0.069	0.390	0.459
$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$	* Urea divided twice	4.5	3.4	0.072	0.380	0.452
$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$	* Urea divided three times	4.5	3.5	0.073	0.390	0.463
$(NH_4)_2SO_4(\frac{1}{4})$ $CO(NH_2)_2(\frac{3}{4})$	* Urea once	4.5	3.8	0.074	0.510	0.584
$(NH_4)_2SO_4(\frac{1}{4})$ $CO(NH_2)_2(\frac{3}{4})$	* Urea divided twice	4.5	3.4	0.085	0.440	0.525
$(NH_4)_2SO_4(\frac{1}{4})$ $CO(NH_2)_2(\frac{3}{4})$	* Urea divided three times	4.5	3.7	0.065	0.360	0.425
$(NH_4)_2SO_4(\frac{1}{4})$ $CO(NH_2)\ (\frac{3}{4})$	* Urea divided four times	4.5	3.7	0.075	0.400	0.475

* Ammonium sulphate was given at the beginning of the experiment and subsequently urea.

plays a rôle in the reaction change of the culture solution. This reaction change is influenced by many factors such as the culture conditions, the initial pH-value, the buffer action of the culture solution, the amounts of urea and urease. For instance, the degree of the decrease of hydrogen ion concentration in the culture solution is not so great, when the pH-value is small (3.0–4.0) or when its buffer action is very strong at the beginning of the experiment.

A similar manner of the reaction change was confirmed by the writer (1931) even in the sterile water culture of *Zea Mays* which was proved to contain very little urease in its body. As found in that experiment, the reaction changed very slightly in the water culture in which ammonium sulphate at the beginning and subsequently urea was given as the source of nitrogen. In such a case, the increase of hydrogen ion concentration in the solution with ammonium sulphate renders the decomposition of urea very unsatisfactory, which was given subsequently. Urea may be, therefore, absorbed as itself, which causes no reaction change of the culture solution.

The development of rice plant fertilized with urea was poorer than in any of the cultures with other nitrogen sources, while that in the culture to which urea and ammonium sulphate combined were given, went on remarkably well. In a solution containing urea only as nitrogen source, the reaction of the solution becomes less acidic or more alkaline during the course of culture than it was at the beginning. This reaction change, in turn, often favourably affected the action of urease, consequently the amount of ammonia in the solution increased very rapidly. In such low hydrogen ion concentration, ammonia may be easily absorbed in the form of NH_4OH or of NH_3 by the root system of plants, and extremely much accumulated in the plant body, which may act harmfully on the growth of plants, especially on the root.[1]

An equally harmful effect is caused by the extreme resorption of urea as itself, which phenomenon was reported in the writer's previous paper (1929).

From the facts that the growth of rice plants in the culture solution with urea as nitrogen source occurred more poorly than in the control which contained no nitrogen source, it may be concluded that urea in a large amount does not serve as a favourable nitrogen source, but it acts rather injuriously on the growth of rice plant. On the contrary the hydrogen ion concentration of the $(NH_4)_2SO_4$ culture was much shifted and this reaction change, in turn, brought about a rather favourable condition for the growth of rice plant. These results show how important are factors related with hydrogen ion concentration in the development of rice plant in the water culture in the early growing stage.

1) MEVIUS (1928, a) and PIRSCHLE (1929).

The influence of buffer action of the nutrient solution containing urea on the growth of rice plant

As seen in the foregoing experiments, in the water culture with urea, the growth of rice plants was greatly influenced by the change of reaction of culture solution, which has much to do with its buffer action. As the nutrient solution contained many kinds of salts, the buffer action of the solution should be different according to kind, concentration and composition of these salts. LOO (1927, b) stated that the initial pH of the culture medium and the buffer capacity of the solution exert very great influence upon the plant growth, and that if the initial pH is not suited to the growth, the solutions with strong buffer action are rather injurious to the plants.

In the soil culture, also, the buffer action of the soil is very importantly related with the change of its reaction.[1] That the buffer action of the nutrient solution or soil is an equally important factor as the hydrogen ion concentration itself, on which the plant growth depends, must be taken into consideration in culture experiment. The following experiments were carried out in order to see the relation between the reaction change and plant growth in water culture, provided sufficiently with phosphate mixtures as buffer, and with urea as a nitrogen source. As culture solution a modified KNOP's solution was used, in which the total amount of nitrogen of urea and ammonium sulphate was equivalent to that in the ordinary KNOP's solution.

A definite amount of phosphate mixture was added to this solution. The concentration of sodium phosphate in the culture solution was M/100, which was adopted according to the results of the previous culture experiments with the rice plant.

Experiment II.

There rice seedlings in separate culture vessels, each containing 400 cc. of nutrient solution. March 18–March 31, 1931. Temp. 13°–32°C.

From Tables II and III, it will be seen that the shoot length of the cultures with various nitrogen sources can be arranged as follows: ammonium sulphate > calcium nitrate > urea.

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1) ARRHENIUS (1926), MEVIUS (1927), KAPPEN (1929).

Table II.

(Seedlings were cultivated preliminarily for 10 days in tap water.)

N-source	Phosphate mixture	pH		Shoot length (cm)
		Initial	Final	
$(NH_4)_2SO_4$	0	4.7	3.0	20.0
$CO(NH_2)_2$	0	4.7	6.4	16.0
$Ca(NO_3)_2$	0	4.6	5.7	18.0
$CO(NH_2)_2$	M/5 H_3PO_4 (0.32 c.c.) M/5 NaH_2PO_4 (19.68 c.c.)	3.8	4.4	16.0
$CO(NH_2)_2$	M/5 H_3PO_4 (0.04 c.c.) M/5 NaH_2PO_4 (19.96 c.c.)	4.4	5.0	15.0
$CO(NH_2)_2$	M/5 NaH_2PO_4 (20.00 c.c.)	4.6	5.1	18.0
$CO(NH_2)_2$	M/5 NaH_2PO_4 (19.84 c.c.) M/5 Na_2HPO_4 (0.06 c.c.)	4.8	5.0	17.0
$CO(NH_2)_2$	M/5 NaH_2PO_4 (19.70 c.c.) M/5 Na_2HPO_4 (0.30 c.c.)	5.2	5.4	17.0
$CO(NH_2)_2$	M/5 NaH_2PO_4 (19.20 c.c.) M/5 Na_2HPO_4 (0.80 c.c.)	5.5	5.5	16.0

Table III.

(Seedlings were cultivated preliminarily for 5 days in tap water.)

N-source	Phosphate mixture	pH		Shoot length (cm)
		Initial	Final	
$(NH_4)_2SO$	0	4.7	3.5	13.0
$CO(NH_2)_2$	0	4.7	6.3	7.0
$Ca(NO_3)_2$	0	4.6	5.5	10.0
$CO(NH_2)_2$	M/5 H_3PO_4 (0.32 c.c.) M/5 NaH_2PO_4 (19.68 c.c.)	3.8	4.8	7.0
$CO(NH_2)_2$	M/5 H PO_4 (0.04 c.c.) M/5 NaH_2PO_4 (19.96 c.c.)	4.4	4.6	9.0
$CO(NH_2)_2$	M/5 NaH_2PO_4 (20.00 c.c.)	4.6	5.0	7.0
$CO(NH_2)_2$	M/5 NaH_2PO_4 (19.84 c.c.) M/5 Na_2HPO_4 (0.06 c.c.)	4.8	5.3	9.0
$CO(NH_2)_2$	M/5 NaH_2PO_4 (19.70 c.c.) M/5 Na_2HPO_4 (0.30 c.c.)	5.2	5.5	5.0
$CO(NH_2)_2$	M/5 NaH_2PO_4 (19.70 c.c.) M/5 Na_2HPO_4 (0.80 c.c.)	5.5	5.5	5.0

The results of cultures with urea, however, varies according to the pH-value and to the amount of phosphate buffer mixture. Both of these factors should be equally taken into consideration, because the pH-values of various culture solutions in this experiment were not beyond the beneficial region for the growth of rice plant. LOO (1927,b) and KIMURA (1931) found that the excess of phosphate in the culture solution has some special ill effects on the rice seedlings. As above mentioned, urea is decomposed into ammonia in a comparatively short time under the natural culture condition, and thereby the reaction of the culture solution becomes less acidic or more alkaline than that at the beginning. This reaction change may cause ill effects on the growth of seedlings in turn. Therefore, the best growth of rice plant can be secured, if the beneficial hydrogen ion concentration of the culture solution is kept as nearly unchanged as possible by the aid of buffer material, which should be added in such amount that it does not influence the growth of the plant. This ill action in the urea culture was more remarkable in the early stage of development of seedlings than in the adult stage. NEUBAUER's method, which is applied to-day for the determination of the available amount of nutrients in soil, was applied also to the studies on the physiological change of the hydrogen ion concentration of fertilizers in the field by KAPPEN (1929), OSUGI and ENDO (1932). The writer's results mentioned above show that young seedlings are very sensitive to many cultural conditions. Therefore, it may be considered that NEUBAUER's method is available not only for the purpose in the original significance, but also can be applied to the investigation on the relation between the plant growth and soil and manure both together, in a relatively short time and precisely.

The influence of concentration of urea on the reaction change of culture solution and the plant growth

The result of the previous experiment shows that the plant growth in the urea culture is inferior to that in the cultures with ammonium sulphate or with both urea and ammonium sulphate together, and this unfavourable effect of urea may be probably due to the accumulation of ammonia, which is produced by its decomposition. If the concentration of urea in the culture solution is very high, a large amount of urea is decomposed into ammonia in a relatively short time, and a harmful effect appears hand in hand with the decrease of hydrogen ion concentration and the increase of ammonia in the molecular form. The following four experiments were performed in order to see, in what degree various amounts of urea, causing reaction change of culture solutions, differently influence the development of seedlings of *Zea Mays* in the early stage. The amounts of urea used for the cultures were the same as in the foregoing experiment.

Experiment III.

Six seedlings of *Zea Mays,* Sapporo Eight Lines (shoot ca. 1.5 cm and root 8 cm) were cultured in separate culture vessels, each containing 400 cc of nutrient solution. June 10–June 24, 1931. Temp. 9°–34°C.

Table IV.

(Seedlings with endosperm)

Urea in mol	pH		Dry weight (gm)			Growth	Remarks
	Initial	Final	Shoot	Root	Total		
0.05	4.8	6.9	0.971	0.171	1.142	++	
0.04	4.8	6.8	0.860	0.200	1.060	++	
0.03	4.8	6.4	0.969	0.186	1.155	+++	
0.02	4.8	6.5	0.640	0.142	0.782	+++	
0.01	4.8	3.6	0.845	0.219	1.064	+++	Anthocyan appeared in shoot
0.005	4.8	3.0	1.325	0.289	1.614	+++′	„

Table V.

(Endosperm was removed from seedling at the beginning of the experiment)

Urea in mol	pH		Dry weight (gm)			Growth	Remarks
	Initial	Final	Shoot	Root	Total		
0.05	4.8	7.3	0.235	0.056	0.291	+	Culture solution turbid a little
0.04	4.8	6.9	0.386	0.123	0.509	++	
0.03	4.8	7.1	0.215	0.310	0.509	+′	
0.02	4.8	8.4	0.278	0.060	0.338	+++	
0.01	4.8	7.0	0.475	0.136	0.611	++++	
0.005	4.8	6.3	0.656	0.176	0.832	++++++	

From the Tables IV and V, it will be seen that the growth of *Zea Mays* seedling in the culture solution containing 0.005 molar urea was the best, and the development of the plant was poorer in accordance with the increase of the concentration of urea in the culture solution, in both cases of the presence and absence of endosperm. This unfavourable effect of urea in a large amount was observed more clearly in the seedling from which the endosperm was removed at the beginning of the experiment. This phenomenon can be explained in the following manner: The rich content of carbohydrate in the endosperm of corn seedlings is used, combined with ammonia derived from urea, for the synthetic formation of protein, so that the plant growth occurred normally, escaping from injury caused by accumulation of urea or ammonia in the plant body.

The growth of seedlings cultured in a solution which contains 0.005 molar urea, occurred pretty well, and anthocyan appeared noticeably at the lower part of the shoot and at the base of the adventitious root. This anthocyan formation is probably caused by the accumulation of sugar, which is derived from the endosperm or newly produced by photosynthesis without being utilized for the synthesis of nitrogenous organic compounds, owing to the want of nitrogenous source, because the urea was given in a small amount. In this case, the reaction of culture solutions with larger amounts of urea became alkaline or less acidic, and this changed reaction of the solutions yielded ill effects on the seedlings in various manners. As seen in Table IV, the root development of seedlings of *Zea Mays* without endosperm, was very poor, especially in the solution which contained 0.02 molar urea, and the pH-value of which changed from 4.8 to 8.4. Such a poor growth of root may be regarded as the result of an enormous accumulation of ammonia in the plant body, which is accelerated by the decrease of hydrogen ion concentration in the solution. Such results indicate clearly that the concentration of urea is closely related with the reaction change of culture solution and with the growth of seedlings. The following experiments were attempted in order to prove more evidently the above mentioned relation between the concentration of urea and the plant growth.

Experiment IV.

Three seedlings of *Zea Mays* (shoot ca. 2 cm and root 8 cm) were cultured in separate culture vessels, each containing 400 cc. of nutrient solution. August 1–August 14, 1931. Temp. 19°–33°C.

The foregoing experiments show that the growth of seedlings in the urea culture became increasingly poorer in proportion to the concentration of urea in the culture solution, regardless of the existence or absence of endosperm. The reaction of the solutions changed in accordance with the variation of the concentration of urea, namely the more urea there was contained in the culture solution, the more remarkably its reaction became less acidic or more alkaline than that at the beginning. On the contrary, the less urea there was contained in the solution, the less the reaction of the solution changed. At the end of the present experiment the growth of seedlings in a range of pH-value 4.0–5.0 was very good. The results of two other experiments which were conducted in the same manner, were on the whole the same as the result of this experiment. The description of their data is therefore omitted.

The seedlings, from which endosperm was removed at the beginning of the experiment, were more harmfully affected by the high concentration of urea than the seedlings with endosperm. The removal of the endosperm results in a lack of sugar, which substance is necessary to transform the nitrogen source into more complex N-compounds. This causes extreme accumulation of ammonia in the plant body and acts harmfully.

KULTZCHER (1932) found that the unfavourable effects caused by the accumulation of ammonia in plant bodies which were cultured in the solution containing urea as a nitrogen source, are much decreased by the addition of glucose to the culture solution.[1] TANAKA (1931) has made studies on the growth of *Sisyrinchium* in a sterile culture using a solution containing urea as the nitrogen source. Also in his case the ill effects on the plant growth caused by a large amount of urea, could be eliminated by the addition of a small amount of glucose. This result agrees very well with that of the writer's experiments described above, where such ill effect was diminished by saving the endosperm.

Though it appears that theoretically intimate relations exist among the concentration of urea, reaction change of the culture solution and plant growth, they are somewhat complicated in practice, because the decomposition of urea into ammonia is influenced by many factors.

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1) As KULTZCHER used a relatively large amount of urea—about 0.166 mol —, it is probable that the plant growth is much influenced also by urea absorbed as itself.

Table VI.

(Endosperms were removed from seedlings at the beginning of the experiment.)

Urea in mol	Culture	pH		Dry weight (gm)	Growing state of plants				Appearance of plant at the time of harvest.
		Initial	Final		4/VIII	6/VIII	8/VIII	14/VIII	
0.1	a	4.9	6.5	0.001	Leaf margin became yellow	Tip of leaf withered. Root hair did not develop.	Leaf withered	Died	—
	b	4.9	6.7	0.001	,,	,,	,,	Died	—
	Control (No seedling)	4.9	6.4	—					
0.05	a	4.9	6.4	0.230	Leaf green	Leaf green	Leaf slightly gray	Poor growth	Leaf yellowish green +
	b	4.9	6.9	0.246	,,	,,	,,	,,	,, +
	Control	4.9	6.3	—					
0.02	a	4.9	6.1	0.515	Leaf green	Leaf green	Leaf green	Pretty good growth	++++
	b	4.9	6.1	0.445	,,	,,	Leaf green	,,	++++
	Control	4.9	6.8	—					
0.01	a	4.9	5.4	0.381	Leaf green	Leaf green	Leaf green	Good growth	+++′
	b	4.9	3.3	0.382	,,	,,	,,	,,	+++′
	Control	4.9	6.4	—					
0.004	a	4.9	5.4	0.454	Leaf green	Leaf green	Leaf green	Pretty good growth	++++′
	b	4.9	5.3	0.425	,,	,,	,,	,,	++++
	Control	4.9	6.4	—					
0.001	a	4.9	3.1	0.411	Leaf green	Leaf green	Leaf green	Good growth	Anthocyan appeared remarkably at the lower part of shoot. +++′
	b	4.9	3.3	0.419	,,	,,	,,	,,	,, +++′
	Control	4.9	5.5	—					

Table VI. (*Continued*)

Urea in mol	Culture	pH		Dry weight (gm)	Growing state of plants				Appearance of plant at the time of harvest.
		Initial	Final		4/VIII	6/VIII	8/VIII	14/VIII	
0.0005	a	4.8	3.2	0.487	Leaf green	Leaf green	Leaf green	Good growth	Anthocyan appeared at the lower part of shoot. +++
	b	4.8	3.2	0.373	,,	,,	,,	,,	,, +++
	Control	4.8	5.2	—					
0	a	4.7	3.3	0.380	Leaf green	Leaf green	Leaf green	Shoot short	Anthocyan appeared remarkably at the lower part of shoot. ++
	b	4.5	4.4	0.337	,,	,,	,,	,,	,, ++

Table VII.

(Endosperms were not removed from seedlings at the beginning of the experiment.)

Urea in mol	pH		Dry weight (gm)	Growing state of plants				Appearance of plant at the time of harvest
	Initial	Final		4/VIII	6/VIII	8/VIII	14/VIII	
0.1	4.9	7.5	0.324	Leaf green	Leaf gray	Died	—	—
0.05	4.9	7.0	0.675	Leaf green	Leaf green	Leaf gray	Died	+
0.02	4.9	5.7	0.637	Leaf green	Leaf green	Leaf green	Good growth	++++
0.01	4.9	5.0	0.708	Leaf green	Leaf green	Leaf green	Good growth	++++′
0.004	4.9	3.7	0.609	Leaf green	Leaf green	Leaf green	Good growth	++++
0.001	4.8	3.3	0.796	Leaf green	Leaf green	Leaf green	Very good growth	+++++
0.0005	4.8	3.3	0.657	Leaf green	Leaf green	Leaf green	Pretty good growth	Anthocyan appeared at the lower part of shoot. ++++
0	4.5	3.5	0.654	Leaf green	Leaf green	Leaf green	Short shoot	Anthocyan appeared remarkably at the lower part of shoot ++++

If the hydrolysis of urea begins, it is accelerated autocatalytically owing to the decrease of the hydrogen ion concentration. In such a case seedlings, especially young ones, are sometimes injured by the abundant resorption of urea. On the contrary, if urea is resorbed as itself before the decomposition into ammonia, the reaction of the solution has no chance to become less acidic or more alkaline, so that the plants are saved from the injury due to extreme accumulation of ammonia in their bodies. This is noticeable especially, if the plant contains no urease in its body; the plant growth went on very well. though the concentration of urea was moderately high. However, if the plant contains urease and its distribution and occurrence very according to the part and the stage of development, the above relations become more complex again. If the concentration of urea is so low that a remarkable decrease of hydrogen ion concentration does not result, the plant is protected from the ill effects of urea, but soon suffers from the want of nitrogen source. Such an unfavourable condition caused by the extreme high or low concentration of urea may be avoided, for instance by using the method of the continuous flow of nutrient soultion, of which the urea concentration is relatively low, as in PIRSCHLE's experiments with *Zea Mays.*

TANAKA (1931) reported that in the agar sterile culture of *Sisyrinchium,* using 0.005 molar urea in one litre of the culture solution, which amount of urea is nearly the same as that used in the writer's experiments, the plant growth occurred very well. The concentration 0.001–0.005 mol of urea was most favourable for the water culture of *Zea Mays.*

The above described experiments (Exp. I–IV) were carried out with the ordinary water culture, but it happens sometimes that a sterile culture is necessary, because urea can be easily attacked and decomposed by bacteria or fungi, before it is absorbed by the cultivated plant (YAMAGUCHI 1931, TANAKA 1931). The above conclusion was reached also on the basis of the results of the experiments with such sterile culture in the writer's foregoing paper, though for the purpose of the present investigation the ordinary water culture was enough.

B. SOIL CULTURE

Nitrogen source and growth of rice plant

As mentioned in the introduction to the present paper, it is very necessary to know how far the conclusions obtained from the water culture experiments can be applied to the soil culture. The clayey loam used for the following experiments was secured from the non-manured section of the experimental ground of the First Farm of our University.

Seedlings of *Oryza sativa* (Bozu No. 6) were cultured in soil in the green house for some days. After this preliminary culture the uniformly grown seedlings were planted in porcelain culture pots. The diameter of each pot was about 15.4 cm. and its depth was about 14.2 cm. Use was made of the afore-mentioned modified KNOP's culture solution, which contained urea or ammonium sulphate instead of culcium nitrate.[1] The amount of nutrient salts given per pot was as follows:

(A)	
KH_2PO_4	0.250 gm
$MgSO_4 \cdot 7H_2O$	0.250 gm
$CaCl_2 \cdot 2H_2O$	0.896 gm
$FeCl_3$	0.020 gm
(B) (nitrogen source)	
$(NH_4)_2SO_4$	0.805 gm
or $CO(NH_2)_2$	0.366 gm

The manuring occurred in the following 14 ways. Part B was given in every culture.

No. of culture	N-source	manuring
1	not given	only with part A.
2	$(NH_4)_2SO_4$	at the beginning
3	$CO(NH_2)_2$	at the beginning
4	$\begin{cases}(NH_4)_2SO_4(\frac{1}{3})\\ CO(NH_2)_2(\frac{2}{3})\end{cases}$	at the beginning
5	$\begin{cases}(NH_4)_2SO_4(\frac{1}{2})\\ CO(NH_2)_2(\frac{1}{2})\end{cases}$	at the beginning
6	$\begin{cases}(NH_4)_2SO_4(\frac{2}{3})\\ CO(NH_2)_2(\frac{1}{3})\end{cases}$	at the beginning
7	$\begin{cases}(NH_4)_2SO_4(\frac{3}{4})\\ CO(NH_2)_2(\frac{1}{4})\end{cases}$	at the beginning
8	$(NH_4)_2SO_4(\frac{1}{2})$	at the beginning
	$CO(NH_2)_2(\frac{1}{2})$	afterwards, once
9	$(NH_4)_2SO_4(\frac{1}{2})$	at the beginning
	$CO(NH_2)_2(\frac{1}{2})$	afterwards, divided two times
10	$(NH_4)_2SO_4(\frac{1}{2})$	at the beginning
	$CO(NH_2)_2(\frac{1}{2})$	afterwards, divided three times
11	$(NH_4)_2SO_4(\frac{1}{4})$	at the beginning
	$CO(NH_2)_2(\frac{3}{4})$	afterwards, once
12	$(NH_4)_2SO_4(\frac{1}{4})$	at the beginning
	$CO(NH_2)_2(\frac{3}{4})$	afterwards, divided two times
13	$(NH_4)_2SO_4(\frac{1}{4})$	at the beginning
	$CO(NH_2)_2(\frac{3}{4})$	afterwards, divided three times
14	$(NH_4)_2SO_4(\frac{1}{5})$	at the beginning
	$CO(NH_2)_2(\frac{4}{5})$	afterwards, divided two times

1) Calcium was given in the form of $CaCl_2$.

The pH-value of the soil solution in the pot was determined with its upper portion, which was taken with a glass tube, employing the colorimetric method of CLARK and LUBS (1926).

Experiment V.

Seven seedlings of *Oryza sativa* (shoot ca. 9 cm and root 7.5 cm) were planted in each pot. March 4–June 10, 1932:

Temp.	15°—33°C	(March)
	15°—33°C	(April)
	15°—30°C	(May)
	13°—34°C	(June)

1 2 3 4 5 6 7 8 9

1. $(NH_4)_2SO_4(\frac{1}{4})$ $CO(NH_2)_2(\frac{3}{4})$ urea subsequently, divided three times.
2. $(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$ urea subsequently, divided two times.
3. $(NH_4)_2SO_4(\frac{3}{4})$ $CO(NH_2)_2(\frac{1}{4})$ both together.
4. $(NH_4)_2SO_4(\frac{2}{3})$ $CO(NH_2)_2(\frac{1}{3})$ both together.
5. $(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$ both together.
6. $(NH_4)_2SO_4(\frac{1}{3})$ $CO(NH_2)_2(\frac{2}{3})$ both together.
7. $CO(NH_2)_2$ once.
8. $(NH_4)_2SO_4$ once.
9. N-source not given.

The growth of seedlings manured with ammonium sulphate only was the worst, that of the seedlings manured with urea was the best, and the combined application of both urea and ammonium sulphate showed the growth of a degree intermediate between them. The degree of growth of the last-mentioned culture was, however, variable according to the method of manuring and ratio of these two nitrogen sources.

The pH-value of soils supplied with ammonium sulphate was nearly 5.5–5.8, and the hydrogen ion concentration of the soil manured

Table VIII.

N-source and manuring	Culture	pH		Length of shoot (cm) 17/V	Appearance of plant growth	10/VI	
		Initial	Final			Length of shoot (cm)	Green weight (gm)
Not given	1–a	5.6	6.4	15.0	++	80.0	23.0
,,	1–b	5.4	6.6	20.0	++	62.0	19.0
	Average			17.5		71.0	21.0
$(NH_4)_2SO_4$, once	2–a	5.5	5.7	20.0	++	88.0	51.0
,, , ,,	2–b	5.4	5.8	20.0	++	92.5	57.0
	Average			20.0		96.0	54.0
$CO(NH_2)_2$, once	3–a	5.6	6.8	20.0	+++	96.0	56.0
,, , ,,	3–b	5.7	7.0	19.5	+++	98.0	60.0
	Average			19.7		97.0	58.0
$(NH_4)_2SO_4(\frac{1}{3})$ $CO(NH_2)_2(\frac{2}{3})$, together	4–a	5.4	6.5	20.0	++′	95.0	61.0
,, , ,,	4–b	5.6	6.8	21.0	++′	80.0	57.0
	Average			20.5		87.5	59.0
$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$, together	5–a	5.4	6.6	19.0	++′	88.0	47.0
,, , ,,	5–b	5.5	6.8	20.0	++′	73.0	56.0
	Average			19.5		80.5	51.5
$(NH_4)_2SO_4(\frac{2}{3})$ $CO(NH_2)_2(\frac{1}{3})$, together	6–a	5.4	5.8	15.0	++	88.0	52.0
,, , ,,	6–b	5.7	6.2	15.0	++	75.0	38.0
	Average			15.0		81.5	45.0
$(NH_4)_2SO_4(\frac{3}{4})$ $CO(NH_2)_2(\frac{1}{4})$, together	7–a	5.5	6.3	14.0	+′	65.0	26.0
,, , ,,	7–b	5.7	6.3	13.0	+′	Died	—
	Average			13.5		65.0	26.0
$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$, * (18/III)	8–a	5.5	7.0	16.0	++	90.0	53.0
,, , ,,	8–b	5.6	6.6	16.0	++	93.0	57.0
	Average			16.0		91.5	55.0
$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$, * (18/III, 30/III)	9–a	5.4	—	17.0	++	73.0	46.0
,, , ,,	9–b	5.4	6.4	18.0	++	80.0	50.0
	Average			17.5		76.5	48.0
$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$, * (18/III, 30/III, 16/IV)	10–a	5.5	6.9	16.0	++	96.0	70.0
,, , ,,	10–b	5.6	6.9	16.0	++	80.0	51.0
	Average			16.0		88.0	60.5

Table VIII. (*Continued*)

N-source and manuring	Culture	pH Initial	pH Final	Length of shoot (cm) 17/V	Appearance of plant growth	10/VI Length of shoot (cm)	10/VI Green weight (gm)
$(NH_4)_2SO_4(\frac{1}{4})$ * $CO(NH_2)_2(\frac{3}{4})$, (18/III)	11-a	5.5	—	16.0	+′	90.0	59.0
” , ”	11-b	5.7	6.6	12.0	++	80.0	56.0
	Average			13.5		85.0	57.5
$(NH_4)_2SO_4(\frac{1}{4})$ * $CO(NH_2)_2(\frac{3}{4})$, (18/III, 30/III)	12-a	5.3	7.0	14.0	++	90.0	53.0
” , ”	12-b	5.6	7.0	17.0	++′	80.0	55.0
	Average			15.5		85.0	54.0
$(NH_4)_2SO_4(\frac{1}{4})$ * $CO(NH_2)_2(\frac{3}{4})$, (18/III, 30/III, 16/IV)	13-a	5.7	6.8	15.0	++	80.0	48.0
” , ”	13-b	5.6	7.2	14.0	++	82.0	63.0
	Average			14.5		81.0	55.5
$(NH_4)_2SO_4(\frac{1}{5})$ * $CO(NH_2)_2(\frac{4}{5})$, (18/III, 30/III)	14-a	5.5	7.0	13.0	++	—	—
” , ”	14-b	5.6	6.8	17.0	++′	87.0	52.0
	Average			15.0		87.0	52.0

* Ammonium sulphate was given at the beginning of the experiment, and urea subsequently at intervals in two or three parts.

with urea became less acidic or more alkaline (pH 5.5–7.0). The reaction change of soils manured with both ammonium sulphate and urea combined varied according to the proportion of the two nitrogen sources. For instance, if the culture contained a larger amount of urea than of ammonium sulphate, the soil reaction became less acidic or more alkaline and in reverse ratios the reactions were the contrary.

The physiologically acidic and alkaline character of ammonium sulphate and urea respectively, appeared clearly to some degree, as in the case of the water culture. Especially the amount of urea remarkably influenced the reaction of the soil, because ammonia was autocatalytically produced from it. This phenomenon rendered the soil reaction continuously less acidic or more alkaline. Not only the amount of the nitrogen source, but also the number of times of its manuring and the proper nature of the soil influenced the reaction change of the soil.

The effect of the reaction change on the plant growth was not always uniform in the soil and water culture. The poisonous effect on the growth of plants caused by the high concentration of free ammonia, accompanied with high pH-value, was not recognized in the soil culture, while such happened often in the water culture.

C. SAND CULTURE

Nitrogen source and the growth of rice plant

The results of the foregoing experiments show that the growth of rice plant in the water culture with urea or ammonium sulphate as nitrogen source was very different from that in the soil culture with the same nitrogen source and the same pH-value of the medium. In order to ascertain the relation between the plant growth and the reaction change of a sand culture and to compare the results obtained from the soil, water and sand cultures, the following experiment was carried out.

The coarse sand used in this experiment was obtained from the River Toyohira in the suburbs of Sapporo. It was washed sufficiently with tap water before use. Rice seedlings (Bozu No. 6) were grown in the germinating vessel in the green house, and uniformly grown materials were planted in cylindrical culture pots. Both of the depth and diameter of the pots were about 14.4 cm. Each pot was quite filled with sand, and the same nutrient salts were added as in the soil culture. The amount of salts was, however, increased a little as in the following manner:

(A)

KH_2PO_2	0.500 gm
$MgSO_4 \cdot 7H_2O$	0.500 gm
$CaCl_2 \cdot 2H_2O$	2.660 gm
$FeCl_3$ (2%)	2 c.c.

(B)

a) $(NH_4)_2SO_4$	1.610 gm
b) $CO(NH_2)_2$	0.732 gm
c) $NaNO_3$	2.072 gm

Part A was given in every culture. The kinds of cultures and the methods of manuring were as follow:

No of cultures	N-source and method of manuring.	
1	Not given	
2	$(NH_4)_2SO_4$	once
3	$CO(NH_2)_2$	once
4	$NaNO_3$	once
5	$NaNO_3$ $CO(NH_2)_2$	at the same time[1]
6	$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$	at the same time
7	$(NH_4)_2SO_4(\frac{1}{2})$ .... $CO(NH_2)_2(\frac{1}{2})$	at the beginning afterwards once
8	$(NH_4)_2SO_4(\frac{1}{2})$ .... $CO(NH_2)_2(\frac{1}{2})$	at the beginning afterwards, divided three times
9	$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$	afterwards, divided three times
10	$NaNO_3(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$	at the same time
11	$NaNO_3(\frac{1}{2})$ .... $CO(NH_2)_2(\frac{1}{2})$	at the beginning afterwards
12	$NaNO_3(\frac{1}{2})$ .... $CO\ NH_2)_2(\frac{1}{2})$	at the beginning afterwards, divided two times
13	$NaNO_3(\frac{1}{2})$ .... $CO(NH_2)_2(\frac{1}{2})$	at the beginning afterwards, divided three times

The soil water was sucked from the rhizosphere of the rice plants using a pipette with some cotton on its terminal end, and its pH-value was determined by the colorimetric method of CLARK and LUBS.

Experiment VI.

Five rice seedlings (shoot 10 cm and root 7.5 cm) were planted in each pot. June 14–September 2, 1932:

Temp.:	13°—34°C	(June)
	14°—34°C	(July)
	19°—36°C	(August)
	15°—27°C	(September)

From Table IX it may be seen that the growth of rice plants in the sand culture manured with both urea and ammonium sulphate combined as the nitrogen source was the best, while growth in the culture manured with ammonium sulphate only was good next to the former. The growth of plants manured with urea or sodium nitrate either singly or combined was the poorest, and plants of these cultures almost all died in a comparatively early stage of the experiment.

1) pH of soil was regulated to 3.3 with HCl at the beginning of the experiment.

Table IX.

N-source and manuring	Culture	pH					Length of shoot (cm)			Length of root (cm) 2/IX	Remarks
		14/VI	20/VI	8/VII	2/VIII	30/VIII	4/VII	18/VII	2/IX		
Not given	1–a	6.3	6.5	6.7	6.9	7.4	26.0	42.0	55.0	12.0	Leaf chlorotic
,,	1–b	6.3	6.4	6.6	6.9	7.4	20.0	42.0	53.0	20.0	Leaf chlorotic
	Average						23.0	42.0	54.0	16.0	
$(NH_4)_2SO_4$	2–a	6.4	6.5	6.5	5.7	4.3	25.0	45.0	68.0	11.0	Good growth, flowered (14/VIII)
,,	2–b	6.4	6.1	4.4	4.3	4.3	27.0	43.0	57.0	11.0	,,
	Average						26.0	44.0	62.5	11.0	
$CO(NH_2)_2$	3–a	6.5	7.6	7.4	—	—	10.0	10.0	—	—	Poor growth, leaf gray, died (27/VI)
,,	3–b	6.6	7.6	7.5	—	—	10.0	10.0	—	—	,,
	Average						10.0	10.0	—	—	
$NaNO_3$	4–a	6.4	7.0	7.1	—	—	11.0	20.0	—	—	Very poor growth, leaf chlorotic, died (7/VII)
,,	4–b	6.5	6.9	7.0	—	—	11.0	10.0	—	—	,, , ,,
	Average						11.0	15.0	—	—	
$NaNO_3$(pH 3.3)	5–a	3.3	7.1	7.3	—	—	12.0	12.0	—	—	Leaf chlorotic, died (22/VII)
,,	5–b	3.3	7.1	7.0	—	—	11.0	11.0	—	—	,,
	Average						11.5	11.5	—	—	
$(NH_4)_2SO_4(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$, together	6–a	6.2	7.1	7.0	6.6	6.1	12.0	30.0	—	—	Poor growth
,, ,, ,,	6–b	6.3	7.1	7.0	6.5	5.9	11.0	22.0	60.0	11.0	,,
	Average						11.5	26.0	60.0	11.0	
$(NH_4)_2SO_4(\frac{1}{2})$ * $CO(NH_2)_2(\frac{1}{2})$, (19/VI)	7–a	6.4	6.3	6.3	6.7	6.4	24.0	48.0	64.0	13.0	Good growth
,, ,, ,,	7–b	6.4	6.3	5.6	6.7	6.4	23.0	48.0	67.0	12.0	,,
	Average						23.5	48.0	65.5	12.5	

Table IX. (*Continued*)

$(NH_4)_2SO_4(\frac{1}{2})$ * $CO(NH_2)_2(\frac{1}{2})$, (19/VI, 7/VII)	8–a	6.3	6.6	6.4	6.7	6.3	29.0	49.0	66.0	12.0	Very good growth, flowered (11/VIII)
,, , ,,	8–b	6.3	6.4	5.6	5.8	5.6	25.0	49.0	57.0	12.0	,,
	Average						27.5	49.0	61.5	12.0	
$(NH_4)_2SO_4(\frac{1}{2})$ * $CO(NH_2)_2(\frac{1}{2})$, (19/VI, 7/VII, 1/VIII)	9–a	6.3	6.8	6.3	6.8	6.1	23.0	50.0	47.0	10.0	Flowered (11/VIII)
,, , ,,	9–b	6.3	6.5	6.2	6.4	6.3	22.0	45.0	81.0	13.0	Flowered (13/VIII)
	Average						22.5	47.5	64.0	11.5	
$NaNO_3(\frac{1}{2})$ $CO(NH_2)_2(\frac{1}{2})$, together	10–a	6.4	7.3	7.3	—	—	12.0	12.0	—	—	Very poor growth, died
,, , ,,	10–b	6.4	7.3	7.2	—	—	11.0	11.0	—	—	,,
	Average						11.5	11.5	—	—	
$NaNO_3(\frac{1}{2})$ * $CO(NH_2)_2(\frac{1}{2})$, (19/VI)	11–a	6.4	7.1	7.3	—	—	13.0	20.0	—	—	Poor growth
,, , ,,	11–b	6.3	7.1	7.1	—	—	12.0	12.0	—	—	,,
	Average						12.5	16.0	—	—	
$NaNO_3(\frac{1}{2})$ * $CO(NH_2)_2(\frac{1}{2})$, (19/VI, 7/VII)	12–a	6.7	7.1	7.0	—	—	11.0	11.0	—	—	Poor growth, died
,, , ,,	12–b	6.7	7.0	6.8	—	—	16.0	28.0	—	—	,,
	Average						13.5	19.5	—	—	
$NaNO_3(\frac{1}{2})$ * $CO(NH_2)_2(\frac{1}{2})$, (19/VI, 7/VII, 1/VIII)	13–a	6.3	7.0	7.1	—	—	15.0	29.0	—	—	Poor growth, died
,, , ,,	13–b	6.3	6.9	7.0	—	—	15.0	16.0	—	—	,,
	Average						15.0	22.5	—	—	

* Ammonium sulphate or sodium nitrate was given at the beginning of the experiment, and urea subsequently, divided, two or three times.

The pH-value of the medium in the sand culture was about 7.0 as indicated in Table IX. When ammonium sulphate was applied, the reaction of the sand culture became more acidic (6.5–4.3), but if it was manured with urea or sodium nitrate the pH-value became 7.0 or higher. Generally the reaction of the sand culture manured with both urea and ammonia sulphate together changed dependently upon the manuring methods and the ratio of these two nitrogen sources. For instance, when the concentration of urea was very high, a large amount of ammonia was produced from it in a relatively short time, causing the increase of the pH-value (7.0 or larger). An extreme accumulation of ammonia caused in turn poor growth of rice plant. Moreover, in the culture of such high pH-values, the solubility of iron so remarkably decreased that it easily caused chlorosis of rice plants.

The pH-value of sand culture which was supplied with ammonium sulphate changed from 6.5 to 4.3, in which pH region the resorption of ammonia by seedlings occurred moderately and the solubility of iron and of phosphoric acid was not diminished. The growth of rice plant went on very well in this case.

General Discussion

From the comparative study on water, sand and soil cultures the following points may be noticed:

1. In soil, sand and water cultures, the reaction change of the culture medium depends largely on the kinds and amount of nitrogen source and the methods of manuring.

2. The best growth of rice seedlings in the water and sand cultures with urea, occurred at pH 6.0–4.3. If it happened that the reaction became less acidic or weak alkaline during the culture, the plant growth was very poor. In the soil culture the relation was very different. The growth of rice plant in the cultures supplied with ammonium sulphate was relatively poor, even if the pH-value of the medium was 6.0–4.5. On the other hand, the plant growth in the cultures supplied with urea only was very good in lower concentrations of hydrogen ion (for instance at pH 7.0). The same hydrogen ion concentration of the medium, therefore, does not always have the same effect on the growth of plants in the soil, sand and water cultures. The good plant growth which could be seen in the water and sand cultures, occurred rarely in the soil culture, even if the pH-value was the same in all the cultures.

DOBEBECK[1] found that the absorption of ammonia gas by quartz sand was increased proportionally to the fineness of its grain sizes. KÖNIG and HASENBÄUER (1921) found that the finer the soil particle was, the more ammonia was absorbed from the solution of ammonium chloride and that it was displaced by an equivalent amount of calcium. In general, the adsorption of ammonia by soil depends greatly on the composition and size of the soil particles, and especially humus plays a rôle in this case. As sandy soil contains very little humus and its granules are larger than those of clayey loam, its adsorption power for nutrient elements, including ammonia, remains far behind clayey loam. This is an important reason why the fertility of sandy soil is inferior to that of clayey loam. As KNOP (1871)[1] already stated, soil of sufficient fertility have a high adsorption power, and it is not unreasonable to say that the fertility of soil partially depends on this adsorption power. In respect to the adsorption of ammonia, clayey loam has the most strong power, sandy soil comes next, while in the water culture such power does not come into consideration. In the last case the absorption of ammonia by the plant body occurs freely, because there is no comparison between soil and plant in attraction towards ammonia. This occurs especially in the neutral or alkaline reaction and causes the accumulation of ammonia in plant body. The same thing can be taken into consideration to some extent in the case of the sand culture.

Extreme accumulation of ammonia poisons the plant as described by MEVIUS (1928, a), namely ammonia-poisoning. According to MEVIUS the absorption of ammonia by corn plants from the culture solution in the neutral or alkaline reaction is always greater than that on the acidic side, consequently a greater accumulation of ammonia takes place in the plant body, and in an extreme case this reacts injuriously upon the plant growth. Therefore the nutritive value of an ammonium salt for the growth of corn seedling varies according to the pH-value of the culture solution. MEVIUS and ENGEL (1929) found that such an accumulation of ammonia occurred in the root system, when seedlings of corn were grown in an alkaline nutrient solution. They stated that the nutrient values of ammonium salts and nitrates are the same at the pH-value between 5.3 and 5.6. PIRSCHLE (1929) confirmed MEVIUS' opinion, using the method of

1) Cited after BLANCK (1930).
2) Cited after RUSSEL (1932).

continuous flow of nutrient solution, which allows the keeping of a constant hydrogen ion concentration.

In the soil culture, however, the plant growth occurs somewhat differently, even if the same nitrogen source is given and the same hydrogen ion concentration is measured. For instance the growth of rice plant in the clayey loam soil, using urea as a nitrogen source, was very good at pH 7.0. Under such a culture condition the strong adsorption power of soils controlled the free resorption of ammonia by the root system of plants, though a large amount of ammonia was formed in the soil by the decomposition of urea. The soil acted as a reservoir of ammonia. Later MEVIUS (1928,b) stated that the soils which have a high adsorption power of ammonia, can counteract the poisoning caused by the accumulation of ammonia. From these considerations it may be said that the adsorption power of soil acts as the most important factor not only for the storage of nutrient elements, i.e. fertility of soils, but it plays a great rôle in the reaction change of a culture medium, in the resorption of ammonia by the root system and consequently in the plant growth.

In one series of sand culture, manured with sodium nitrate or both sodium nitrate and urea combined as the source of nitrogen, it was observed that the rice seedlings turned a more or less deep yellow and then died in every instance. These ill effects on the growth of rice plant were always seen in the cultures fertilized with nitrate. Many investigations on sodium nitrate as the nitrogen source for the rice plant have been carried on by many authors. In most cases it was concluded that the sodium nitrate is an unfavourable nitrogen source for the culture of paddy rice plants, especially, in the water culture.

NAGAOKA (1904), DAIKUHARA and IMASEKI (1907) found that the growth of paddy rice plants was very poor in the sand culture containing sodium nitrate, and they came to the conclusion that it is unsuitable for rice plant culture. As one of the factors, which are involved in the interference with the utilization of nitrates, they pointed out the formation of poisonous nitrite from nitrate by reduction.

KELLEY (1911), too, found that rice plant grew very poorly and the leaf colour changed to yellow when it was supplied with nitrates as a source of nitrogen, and that in one series of sand cultures, using sodium nitrate, the seedlings in every instance stood for some days, turned yellow and later died.

WILLIS and CARRERO (1923), LOO (1927, b, 1928), and METZGER and JANSSEN (1928) also found that the sodium nitrate is less suitable than ammonium salts as a fertilizer for young rice plants. The observation of FUKAKI (1929) showed that ammonium salts and nitrate are equally suitable for the growth of paddy rice plants in a water culture, if the pH-values of the culture solution are regulated within a certain moderate region. ESPINO and ESTIOKO (1931) found that nitrate nitrogen in a culture medium is essential for the normal growth and development of young rice plant about fifteen days old, but not for younger plants. WILLIS and CARRERO (1923) stated that the occurrence of chlorosis on the leaves of the rice plant cultured in the solution containing sodium nitrate as the nitrogen source, results from the decrease in the hydrogen ion concentration. This change of hydrogen ion concentration of the culture solution causes the precipitation of iron, and available iron for the plant growth is much diminished, from which chlorosis on the leaves results. However METZGER and JANSSEN (1928), using sodium nitrate as the nitrogen source, are of the opinion that chlorosis appears in the case of lack of available nitrogen, which is induced by various causes. From the above mentioned facts, it is very probable that the occurrence of chlorosis is closely connected with the decrease of the hydrogen ion concentration of the culture solution. The view of METZGER and JANSSEN that lack of available nitrogen is the only cause of chlorosis and that the change of hydrogen ion concentration does not play any rôle in this connection, seems to be unsoundly based.

SALINAS (1932) stated that the nutrient values of nitrate salts for the young rice plants are influenced greatly by the action of cations contained in the nitrate salts.

On the problem regarding the nutrient value of nitrate especially for the rice plant culture the writer carried out some experiments.[1] Neither nitrite nor even a trace of it could be detected in the plant body of rice or in the sterile culture solution of *Zea Mays*. In the ordinary water, sand and clayey loam cultures using nitrate as a nitrogen source, the formation of nitrite was very slight. Also some water cultures of paddy rice plants in solutions of various pH-values were carried out, using a larger amount of nitrate than that found in the clayey loam or sand cultures. In this case, no poisonous effect of

1) Not yet come to publication.

nitrite on the rice plant was observed, even when the reduction of nitrate into nitrite by micro-organismus might probably enough happen, but the plant growth was very good. From this result it may be said that the nitrite which may be produced from nitrate, did not act injuriously on the rice seedlings.

It was observed that the growth of rice plant occurred normally in the culture, of which the pH-value was estimated approximately 7.3, if the method of cultivation was suitable. But the growth of the rice plant in the sodium nitrate culture was very bad in the same hydrogen ion concentration, even if much care was taken for cultivation. Considering these facts, it may be said that the decrease of the hydrogen ion concentration caused by the physiologically alkaline nature of sodium nitrate can not be regarded as sole factor which causes the harmful effects on the plant growth, though its indirect action in connection with this phenomenon is very probable.

Concerning unfitness or the sometimes harmful character of sodium nitrate for rice plant culture further research is required. By the present writer any conclusive result could not be attained on this problem, though the opinion of SALINAS, that Na ions from nitrate act harmfully, seems to be very probable.

Summary

1. A comparative study on the water, sand and clayey loam cultures of *Oryza sativa* and *Zea Mays* were carried out using ammonium sulphate, sodium nitrate and urea as the source of nitrogen.

2. In the water and sand cultures, the favourable growth of paddy rice plants occurred on the acidic side, and poor growth near the neutral or on the alkaline side. In the clayey loam culture, on the contrary, poor growth occurred on the acidic side, the most favourable growth at near the neutral, and pretty favourable growth even in a weak alkaline reaction.

3. The difference of nutritive value of urea or ammonium sulphate in water, sand and clayey cultures greatly depends upon the difference of culture medium, especially, on the buffer action and adsorption power of the media.

4. The water and sand cultures, from which rather exact result can be expected, are necessary for the investigation of plant nutrient. But if their results should be applied practically to the field culture, the soil culture must be necessarily conducted before the application.

5. The use of young seedlings as experimental material may serve as a comparatively simple and adequate method for the determination of the nutrient value and of soil properties, because the young seedlings, in general, are most sensible to change in culture conditions.

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