The Influence of Hydrogen Ion Concentration on the Growth of the Seedlings of Some Cultivated Plants

(Preliminary report)

by

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In spite of the numerous works which have been done on the field of water and sand culture with regard to the influence of hydrogen ion concentration upon plant growth, nearly all the studies have been concerned with the initial reaction of the culture media. But the culture medium is not a fixed system. Recent researches show that the theory of unequal absorption of ions is not a mere pre-sumption. The reaction of a solution containing neutral salts may become acidic or alkaline as the cations or the anions are more or less unequally absorbed by the culture plants. Consequently in turn the growth of seedlings may be affected by this change of the reaction.

That this is the case is apparent from the results of the works of PANTANELLI (6), DAVISON AND WHERRY (2), PRIANISCHNIKOW (7) (8) (9) (10), and SALTER AND McILVAINE (13) in the culture of higher plants and those of NIKITINSKY (5), RITTER (11) and SAKAMURA (12) in the culture of fungi.

The writer has made some studies on the influence of hydrogen ion concentration upon the growth of seedlings with special respect to the change of reaction of the culture media. This report is but a preliminary one, the results in detail and the review of literature will be found in another paper.

METHODS

All the experiments were carried out in water culture. A modified KNOP solution was used as culture medium which $\text{Ca}(\text{NO}_3)_2$ was replaced by NaNO_3 and ammonium salt of some inorganic acids. The amount of nitrogen in ammonium salts and in sodium nitrate

was equivalent to that in the KNOP solution, disregarding the nitrogen in the nitrate radical in the case of ammonium nitrate. In order to prevent precipitation and the superfluous antagonistic action against hydrogen ions (3), calcium chloride was added in a small amount. One drop of a two per cent solution of iron salts, either in the form of ferric chloride, or ferric or ferrous sulphate was added to each 500 cc. of culture solution. MERCK's chemicals were used exclusively.

The distilled water used in the present work was prepared by treating it with MERCK's animal black or with KAHLBAUM's blood coal. All the vessels used were made of non-alkaline glass, some of them were of Jena glass. They were covered with black paper or placed in boxes to exclude sunlight.

The initial pH-value of the nutrient solutions was determined whenever they were prepared. The colorimetric method of CLARK AND LUBS (1) was employed in the determination of hydrogen ion concentration.

The germination of the seeds was carried out in wet saw dust, except in the case of wheat and paddy rice, where the seedlings were started in the moist chamber of the germination dishes. When the length of the shoots reached about 3—9 cc., they were transferred to the culture solutions. At the end of the experiments, the seedlings were dried at 100° C. in a dry oven.

All the experiments were carried out in triplicate, the average value was calculated from the experimental results.

EXPERIMENTAL RESULTS

Before the observation of the effect of reaction change due to the unequal absorption of ions by the seedlings, is made, it is necessary to trace out the daily change of pH-values of the nutrient solutions produced by them. For this purpose, experiments with seedlings of corn, paddy rice and buckwheat were conducted. Every experiment lasted eight days, during which period neither addition nor renewal of solutions was made. 5 cc. of the culture solution were taken with a pipette every day for determination of hydrogen ion concentration. As an example, the results with paddy rice will be shown in the following Table (Table I).

From Table I, it will be found that the hydrogen ion concentration changed considerably within a week or so. The reaction of the solution containing NH_4NO_3 , NH_4Cl and $(\text{NH}_4)_2\text{SO}_4$ became very

TABLE I

Paddy rice: 5 plants in each culture containing 270 cc. of nutrient solution. Culture duration; August 4—11, 1924.

N-source in the the solution	initial pH	Daily changes of pH-values						
		1	2	3	4	5	6	7
NaNO ₃	5.2	5.5	5.6	5.5	5.6	5.7	5.7	5.7
NH ₄ NO ₃	5.1	5.0	4.8	4.7	4.5	4.4	4.5	4.4
NH ₄ Cl	5.1	5.0	4.8	4.4	4.3	4.3	4.2	4.1
(NH ₄) ₂ SO ₄	5.1	5.0	4.8	4.4	4.2	4.2	4.3	4.2
(NH ₄) ₂ HPO ₄	6.9	6.8	6.8	6.7	6.7	6.7	6.7	6.7
NH ₄ HCO ₃	7.6	7.7	8.0	8.0	8.0	8.1	8.2	8.3

acidic, those which contained NaNO₃ inclined to alkaline, while the solutions with (NH₄)₂HPO₄ as nitrogenous source remained almost unchanged. The reaction of NH₄HCO₃-culture became very alkaline due to the escape of CO₂; but when the culture plants grew well in the solution, as in the case of corn and wheat, it usually changed, but very little. The results of experiments with corn and buckwheat were in agreement with these of paddy rice, except that in the case of corn, the reaction of the solution with NH₄NO₃ as nitrogenous source inclined to the neutral point instead of becoming acidic.

In these cases, the other conditions of the experiment were exactly the same except that sources of nitrogen were different among six kinds of nutrient solutions. Accordingly, the unequal absorption of the ions of the salt used as nitrogenous sources must account for the different degree of change of the reaction of nutrient solutions. Thus the direction of change of hydrogen ion concentration of the nutrient solutions depends largely upon the chemical composition of the solutions, especially the kinds of salts used as nitrogenous sources.

Having found out the fact and the tendency of the reaction change of the nutrient solutions, the writer undertook studies on the effect of this reaction change upon the growth of seedlings. Several experiments were made with corn, buckwheat, wheat, pea, broad bean and paddy rice. Among these, the results of experiments with wheat and paddy rice will be shown (Table II and Table III).

TABLE II

Wheat: 6 plants in each culture containing 270 cc. of nutrient solution.

No renewal of nutrient solution was made during the culture period. Sept. 13—Oct. 3, 1924.

N-source in the solution	Length (cm.)		Dry weight (g.)		pH	
	shoot	root	shoot	root	initial	final
NaNO ₃	55	11.7	0.124	0.027	5.2	6.7
NH ₄ NO ₃	47	6.7	0.109	0.022	5.1	4.2
NH ₄ Cl	33.3	6.7	0.089	0.018	5.1	3.0
(NH ₄) ₂ SO ₄	35.3	5.3	0.091	0.019	5.1	3.0
(NH ₄) ₂ HPO ₄	51	12.7	0.121	0.026	6.8	5.3
NH ₄ HCO ₃	45	9.7	0.108	0.022	7.6	8.1

TABLE III

Paddy rice: 6 plants in each culture containing 400 cc. of nutrient

solution. No renewal of nutrient solution was made during the culture period. June 24—July 13, 1925.

N-source in the solution	Length (cm.)		Dry weight (g.)		in pH	
	shoot	root	shoot	root	initial	final
NaNO ₃	10	13	0.0519	0.0738	5.2	6.0
NH ₄ NO ₃	25	12	0.3728	0.1160	5.1	3.1
NH ₄ Cl	18	8	0.1893	0.0805	5.1	2.8
(NH ₄) ₂ SO ₄	20	7	0.2177	0.0887	5.1	2.9
NH ₄ H ₂ PO ₄	15	14	0.1163	0.0718	5.6	4.1
NH ₄ HCO ₃	8	3	0.0365	0.0620	7.6	8.5

In general, the growth of seedlings became poorer and poorer in the solutions containing NH₄NO₃, NH₄Cl and (NH₄)₂SO₄, as the reaction of the solutions became more and more acidic. The solution containing NH₄HCO₃ also inhibited the plant growth, though the seedlings of wheat and corn could grow in it tolerably well.

(NH₄)₂HPO₄ and NH₄H₂PO₄ with the strong buffer action were suitable nitrogenous sources for most plants except rice plants and buckwheat. The slow inclination to alkalinity in the solution containing NaNO₃ produced favorable growth in most cases.

It should be pointed out that the growth features of paddy rice in these solutions were quite different from wheat and corn (Table III). The seedlings grew very excellently in NH_4NO_3 -, NH_4Cl - and $(\text{NH}_4)_2\text{SO}_4$ - culture whose acidity increased rapidly. Alkaline solutions or the solution whose reaction changed gradually from acidic to alkaline, was unsuitable for the growth of rice seedlings. The plants in NaNO_3 -culture; though they grew fairly well at first, became etiolated and ceased to grow at the middle of the experiment. These results agree with those of NAGAOKA (4), who found that paddy rice plants could not assimilate nitric nitrogen as well as they utilize ammoniacal nitrogen. Thus plants of different kinds are different in resistance to acidity and alkalinity.

Ammonia is usually said to be inferior to nitrate as the source of nitrogen for the growth of higher plants. The above results show clearly that this inferiority may to some extent be due to the unfavorable effect of increased acidity or alkalinity in the culture solution caused by unequal absorption of ions. If the increasing acidity of the nutrient solution could be avoided, ammonium salts will be quite as good sources of nitrogen as nitrate. As one of the means for prevention of the change of reaction in NH_4Cl - and $(\text{NH}_4)_2\text{SO}_4$ -cultures, the writer used a combination of two ammonium salts such as NH_4Cl and NH_4HCO_3 or NH_4Cl and $(\text{NH}_4)_2\text{HPO}_4$ in varied proportions as sources of nitrogen instead of single salts. Two experiments with buckwheat and wheat were carried out by this method. For example, in the case of wheat, the combinations of ammonium salts were made in the following proportions:

Culture No.	N-source			
	NaNO_3	$(\text{NH}_4)_2\text{SO}_4$	NH_4HCO_3	$(\text{NH}_4)_2\text{HPO}_4$
1	10	0	0	0
2	0	8	2	0
3	0	6	4	0
4	0	4	6	0
5	0	2	8	0
6	0	8	0	2

The results of this experiment are summarized in Table IV.

TABLE IV

Wheat: 15 plants in each culture containing 400 cc. of nutrient solution. Oct. 23 - Dec. 5, 1924.

Culture No.	Dry weight (g.)		pH		
	shoot	root	initial	Nov. 7	final
1	0.6180	0.1840	5.2	7.0	7.4
2	0.3215	0.1090	6.8	3.0	3.0
3	0.3620	0.1170	7.0	6.0	3.2
4	0.4560	0.1390	7.2	6.8	3.4
5	0.5000	0.1390	7.4	7.1	3.6
6	0.2460	0.1080	6.0	6.0	3.0

As will be seen from Table IV, the reaction of the culture solution containing the greatest amount of NH_4HCO_3 changed in the least degree. The dry weight of the seedlings is directly proportional to the amount of ammonium bicarbonate; the larger the amount of bicarbonate, the greater the dry weight. Here the beneficial effect of ammonium bicarbonate and ammonium phosphate is due to the buffer action. Then the question arises whether there are any salts of phosphoric acid other than ammonium phosphate which would act in the same way and whether the solution with the greatest buffer power is the best for plant growth. To answer these questions, a great number of experiments were carried out with the seedlings of wheat, corn, lupine, Azuki-bean and paddy rice. Here the influence of sodium phosphate was studied. M/10 solution of Na_2HPO_4 and NaH_2PO_4 were used throughout. It was found that in the solutions with NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$ as nitrogenous source, the increasing of acidity was retarded by adding sodium phosphates and this retardation resulted in better growth of plants. Solutions of stronger buffer power, however, are not always the best ones for plant growth; in this case the initial pH-values of the solutions play an important rôle in the growth. If the initial pH-values of the solution is not fit for growth, and its buffer action is strong, such is not the best culture medium. For example, the experiment with yellow lupine will be described here. The kinds of nitrogenous sources and varied proportions of Na_2HPO_4 and NaH_2PO_4 in the nutrient solutions were as follows:

Culture No.	N-source	Na ₂ HPO ₄	NaH ₂ PO ₄
		cc. in 1 litre of culture solution	
1	NaNO ₃	0	0
2	NH ₄ Cl	0	40
3	"	5	35
4	"	10	30
5	"	15	25
6	"	20	20
7	"	25	15
8	"	30	10

The results are given in Table V.

TABLE V

Yellow lupine: three seedlings grown in each culture containing 400 cc. of nutrient solution. Sept. 4 - 29, 1925.

Culture No.	Dry weight (g.)		pH	
	shoot	root	initial	final
1	0.4523	0.1007	5.2	4.2
2	0.3763	0.0757	5.6	4.0
3	0.3873	0.0833	6.0	5.0
4	0.4015	0.0940	6.4	5.4
5	0.3150	0.1047	6.6	5.8
6	0.2965	0.0790	6.8	6.4
7	0.3078	0.0790	7.0	6.6
8	0.2912	0.0761	7.1	6.8

Table V shows that No. 4 (for the shoot) and No. 5 (for the root) are most suitable for the growth of yellow lupine. In other words, lupine seedlings grow most favorably in the solutions of pH 6.3 or so with the moderate buffer action.

Besides the experiments described above, the antagonistic action of calcium ions against hydrogen ions was studied. In these experiments, M/10 solution of CaCl₂ was added in different amount to the solutions with NH₄Cl or (NH₄)₂SO₄ as nitrogenous source. It was found that in the presence of Ca-ions, wheat seedlings could grow tolerably well in such solutions whose acidity became pH 3.0 or 4.0, and which otherwise usually retard plant growth.

DISCUSSION

The above results show that cations of ammonium salts in the nutrient solution are always first absorbed by the tissue of young plants, leaving the solution very acidic. The phenomenon of increasing alkalinity in NaNO_3 -culture caused by growing seedlings might serve as a proof of the correctness of the theory of selective absorption. Whether this change of reaction in culture media is due to the selective power of plants or it is only a phenomenon of physico-chemical character, is a problem which requires further research. At any rate, ammonium salts are not bad nitrogenous sources for higher plants if unfavorable change of reaction in the culture solution containing them can be avoided.

From the results of this work, it is obvious that the influence of hydrogen ion concentration of the culture solution, which change during the course of culture, plays a great rôle in the growth of plants. Besides the injury of higher acidity and alkalinity to plant cells, the influence of hydrogen ion concentration on the process of absorption of nutrients is of fundamental importance. For the solution of the latter problem a series of experiments is now in progress. The results will be reported in the later papers.

SUMMARY

1. The hydrogen ion concentration of the nutrient solutions with NaNO_3 and ammonium salts of inorganic acids as nitrogenous sources is found to be under continual change as the culture plants grow.
2. The direction of this change depends to a great measure upon the chemical composition of the nutrient solutions especially the kinds of salts used as the source of nitrogen.
3. The great influence of the change of hydrogen ion concentration of the nutrient solutions upon several cultivated plants was investigated.
4. Individuality of plants in regard to the resistance to acidity is proved by this experimental method. Especially paddy rice is found to be different from corn and wheat in this point.
5. The change of hydrogen ion concentration of the nutrient solution — consequently its ill effect upon plant growth — may be avoided either by increasing the buffer power of the solution or by adding calcium ions to the solution.

The writer's thanks are due to Prof. T. SAKAMURA for his suggestion and guidance of this work.

January, 1927.

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現今本邦ニ産スト知レタ粘菌種ノ目録

南 方 熊 楠

K. MINAKATA: —A List of the Japanese Species of Mycetozoa

本誌第二六〇號ニ予ノ本邦産粘菌類目録ヲ出シタ時ハ七十四種、第三二一號ニ訂正目録ヲ出シタ時ハ一百八種デアツタ。然ルニ其後十三年間ニ辱知諸君ヤソノ又諸友ノ熱心ナル採集ニヨリ夥シク種ヤ變種ヲ本邦デ見及ブニ至ツタ。昨年十一月十日予ガ撰定シ置タ品種ヲ小畔君ガ整ヒ揃エテ 攝政宮殿下ニ献上シタ時ノ表啓ニ「現時帝國産粘菌ヲ點檢スルニ實ニ三十八屬一百九十三種ヲ算シ」ト有タガ、其後チ平沼大三郎君ガ今年夏秋ノ採集品四十六點ヲ贈ラレ、鏡檢シテ新種一ト本邦デ初メテノ種ニヲ見出タカラ、只今帝國ヨリ知ラレ居ル粘菌ノ種數ハ一百九十六デアル。件ノ表啓文ヲ本誌ヘ載ラレタニ、是等一百九十六種ノ名ヲ出サナイト物足ラヌ様ダカラ其目録ヲ拵ラエテ差上げ置ク、變種ノ名ヲモ悉ク書キ入レ、又予ガ新種ト判定シテ名ヲ付タ物共ノ圖録ヲモ掲ゲヤウト思フタレド、家内ニ故障有テ絲狀體ヤ孢子ノ大サヲ精細ニ觀察スルコトガナラズ、因テ其圖ト記載ハ追テ別ニ出ス事トシ、今ハタバ各種ノ名ヲ列スルニ止メオク。