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II. RELATIONS BETWEEN CONCENTRATIONS OF INORGANIC IONS IN THE MEDIUM AND GROWTH OF THE ISOLATED WHEAT ROOTS

By

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Introduction

It is well known that peculiar symptoms in the plant roots as well as in the shoots can develop under various concentrations of inorganic ions in the culture solution. In the water culture of the rice plant, for example, the root system generally develops in proportion to the concentration of nitrogen in the liquid medium, but, on the contrary, the deficiency of nitrogen in the medium produces long and slender roots. The same is true with retarding to the root elongation at a high level of phosphate supply. It is easily supposed that the reqirement of the roots alone are quite different from that of the plant as a whole. The possibility that the optimum concentration of inorganic ions for the root growth does not always agree with that for the shoot growth, should be also admitted. It is perhaps of interest to agronomists to research on the relation between inorganic ion concentrations in the medium and the root growth of the plant, which is considered without any connection with the plant itsself.

As regards the recent studies on the intact plant root, Bosemark (1) investigated the influences of NO_3 in the medium on the growth of the wheat roots, and suggested that the amount of natural auxin in the roots correspondingly rose with the increase of nitrogen supply. Burström (2) reported that calcium acts as a genuine cell elongation factor of the roots at some pH-level of the solution. Thus it has long been established that some inorganic ions have specific functions for intact plant roots. It may be able to assume that the studies on organic growth substances were over emphasized, and so those on inorganic ion in the tissue culture have been neglected. Literatures bearing on the exact relationship between the inorganic ions and the isolated root growth are scanty. In the case of systematic investigations on the inorganic ions in the root tissue culture, so far as we are aware there is White's study (3) on the major ions and Glasston's (4) on the minor elements.

In the previous paper (5), we reported on the environmental conditions for the isolated root growth of wheat and the rice plant; the isolated roots were cultured under various concentrations of inorganic ions in the medium. The investigation presented in this paper is an attempt to elucidate how the inorganic ions are related with the root growth.

Material and Methods

Plant material and sterilization of the seed: The sterilization of the plant seeds in the tissue culture is one of the most difficult techniques in securing the sterility and for keeping the active germination of the seeds. For such reasons the sterilizing methods were fully tested in this experiment.

The experimental material used in this study was the root of wheat (Norin No. 54). The selected seeds were pretreated with 70 per cent alcohol and sterilized with calcium hypochlorite solution, which was prepared by suspending 5 g calcium hypochlorite in one liter of water and filtrating the suspension. After the treatment with alcohol, the seeds were kept in a vacum flask until the sterilizer touched the seed surface thoroughly. The seeds were further soaked in a new hypochlorite solution for 6 or 8 hours and washed twice with sterilized deionized water. Then, they were transfered and kept in sterilized water for about the same period, the soakening being necessary to remove the poisonous effect of hypochlorite. The sterilizer did not inhibit the growth of the seedling, although the use of 0.1 per cent $HgCl_2$ solution in the previous experiment caused some injuries. Gautheret's method (6) without washing and Almestrand's (7) with washing only twice after the treatment with hypochlorite appreciably inhibited the growth of the seedling in the Petri dish. As far as we experienced, calcium hypochlorite was the best in both the effect of sterility and the poisonous action among the detergents, such $HgCl_2$ solution (8), (9), soap for sterilizing (10), usuplun, alcohol (11), (12) and formaldehyde solution (13) which have been employed by many investigators as the sterilizer of plant tissues. It was necessary to keep the seed grains in the dark during sterilizing with the hypochlorite solution. This fact may depend upon the lowered pH value of the solution caused by HCl derived from decomposition of HClO on the light, or lumi-decomposition. According to Eliasson (14), both sterilization and the poisonous action of the hypochlorite are controlled by the hydrogen ion concentration of the solution. If the pH is maintained at 12, the seeds can

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be kept in Eliasson's solution for more than 40 hours without injury. At pH 8, the same solution would kill them in less than one hour.

The amount of solution for a given number of seeds is also important because of its injurious effect. It is thought that the pH value of the solution is lowered by the carbon dioxide evolved from the seeds as by lumi-decompsition of HClO and as a consequence the proportion of HClO is strongly increased during the soaking. The amount of the hypochlorite solution was 100 ml per 100 grains, and this proportion hardly showed the poisonous effect without a decrease of the sterilizing action. If the number of grains were too much for amount of the solution, the seed germination would be inhibited, because free HClO of effective constituent begin to act severely on due to the increasing hydrogen ion concentration in the solution caused by the carbon dioxide on the respiration. By contrast, if the number of grains were reverse to the relation mentioned above, the sterilizing action would be unsatisfactory. For the same reason, the grains should be spread on a large tray-vessel containing the solution instead of piling inside of a bottle-like-container during the sterilization. We have used the Petri dish of 20 cm in diameter for this reason. If we pay attention to these points described above, the sterilization of the material will be relatively uniform.

Environmental condition of the root culture: Culture vessels used in this experiment were 100 ml Erlenmeyer flasks and the volume of the medium was 20 ml. Two or four isolated root tips were placed into each flask and cultured under the condition of darkness and at 23°C for ten days. During the culture, the solution in the flask was shaked by hand every other day to supply oxgen. Initial length of the root tips was 3 mm or 10 mm in NO_3-, PO_4- and K-series, 5 mm in Ca- and Mg-series, and 3 mm in Fe-series.

The medium compositions and its preparation: The major ion composition of the medium is shown in Table 1. In addition to these macro nutrients given in Table 1, 10 mg of glucose and 0.1 mg of thiamin, 0.5 mg of pyridoxine and 0.5 mg of nicotinic acid per liter were supplied. Most of these chemicals were obtained from Merck or Baker, and the water was deionized with the ion exchange resin. The employed ion concentration level is shown in Fig. 1, 2.

Table 1. Concentrations of ions in the culture solution.

ions	mg-ion	mg-equivalent	mg per liter	salts applied
NO_3	1.000	1.000	62.0	$Ca(NO_3)_2$, KNO_3
PO_4	0.300	0.150	38.1	KH_2PO_4
K	0.500	0.500	19.5	KNO_3, KH_2PO_4
Ca	0.600	0.300	24.0	$Ca(NO_3)_2$
Mg	0.200	0.100	4.86	$MgSO_4$
Fe	0.005	0.002	0.28	$Fe_2(SO_4)_3$

Besides those major ions, as minor ions, $MnCl_2 \cdot 4H_2O$, 1.00, $ZnSO_4 \cdot 7H_2O$, 0.02, HBO_3, 0.02, ammonium molybdate 0.02 mg per liter were added to the basal media at all times.

Besides the standard level, five modifications were tried; none, half, two and four times of standard level on NO_3-, PO_4- and K-series, and besides these modifications, the level eight times were tried in Mg-, Ca- and Fe- series. These ion preparations are summarized in Table 2. The other ion compositions of the basal medium except the tested ions were the same as that of the standard medium.

Table 2.

ions	salts added	salts replaced
NO_3	$NaNO_3$	KCl for KNO_3, $CaCl_2 \cdot 2H_2O$ for $Ca(NO_3)_2$
PO_4	NaH_2PO_4	KCl for KH_2PO_4
K	KCl	$NaNO_3$ for KNO_3, NaH_2PO_4 for KH_2PO_4
Ca	$CaCl_2 \cdot 2H_2O$	$NaNO_3$ for $Ca(NO_3)_2$
Mg	$MgCl_2$	
Fe	$Fe_2(SO_4)_3$	

As the hydrogen ion concentration of the medium always fell one or two units of pH by the autoclaving, the test media were prepared with NaOH of N/10 to adjust pH 5.0 to 5.4 after the autoclaving.

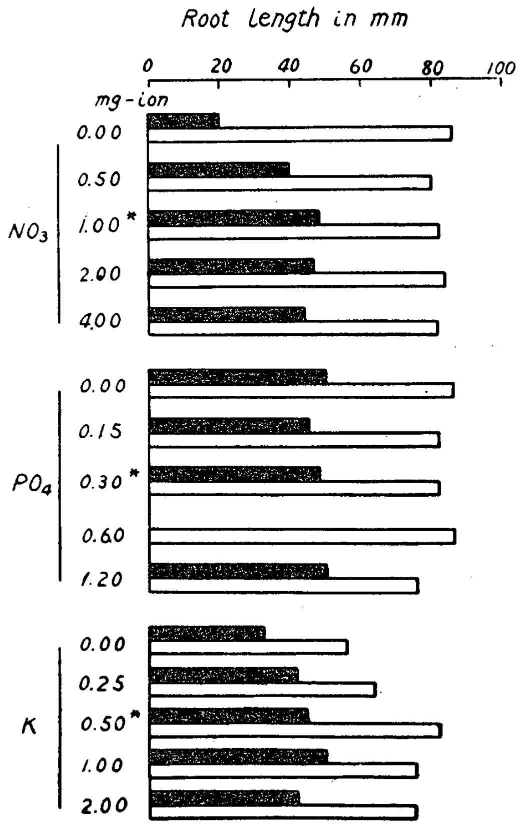

Fig. 1. Influence of NO_3-, PO_4- and K-ions on the growth of the isolated roots of wheat.
■ inoculated 3 mm root tips
□ inoculated 10 mm root tips
* standard medium

Results and Discussion

The effects of NO_3-, PO_4- and K-ions in shorter and longer root growth are shown in Fig. 1 and those of Ca-, Mg- and Fe-ions in Fig. 2.

NO_3 : When 10 mm long root tips were used, the longer roots with sufficient mature tissue begin to grow in the several media of the different NO_3-concentrations, the isolated roots in the standard medium of 1.0 mg-ion NO_3 per liter attained an average length of 82 mm after ten days. Omittion and the highest concentration of nitrogen in the media supported about 100 and 105 per cent of the growth obtained in the standard medium respectively. At any rate, omitting NO_3 or raising or lowering its concentration in the medium showed no influence on the growth within the concentration given here. When 3 mm root tips were used the shorter roots including insufficient mature tissue, were grown in a medium without nitrogen, the influence of its deficiency appeared in root length. The roots grown in the media without NO_3 were only 42 per cent of that of the standard medium. In 0.5 mg-ion level, there was recognized a slight tendency of nitrogen deficiency. Supplying 1.00 mg-ion NO_3 to the medium, at least, seemed to be necessary to obtain satisfactory growth. Concequently, the nitrogen requirement for its growth depends on the initial root length within a given culture term. The reason for this is that the mature tissue in the basal part of the isolated root relates to meristematic activity of root apex through nitrogen supply. Habarlandt (15) reported that the celluler division and differentiation of young meristematic plant cells may be directly influenced and controlled by the presence of mature, differentiated vascular tissues. Support of the view that the mature tissues are essential for the activity of meristematic tissues has come from numerous reports of failure to culture successfully excised root meristem lacking mature vascular tissues (16), (17), (18). The results that the omittion of NO_3 ions did not result in poorer growth in this experiment, may be due to the supply of organic nitrogen, amino acids and amides in the mature tissue, in the seed endosperm during the germination process.

PO_4 ; Omitting PO_4 or raising or lowering its concentration in the medium gave no significant effect on the growth in both cases of longer and shorter initial root lengths. In both case of 3 and 10 mm long root tips, omitting PO_4 gave a root length of about 105 per cent of that obtained in the standard medium. Thus, it, is clear that the root tips would further grow for a time without the addition of PO_4, as they were growing satisfactorily at the end of ten days. In all levels of concentrations of the medium, the addition of PO_4 gave no significant effect on the growth within the limits of the concentration levels given.

Burström (19), however, showed that phosphate relates chiefly to the cell multiplication, while nitrate has a close relation to the cell elongation. Street *et al* (20) have stated that phosphate intimately involves carbohydrate assimilation of the tomato root. Skoog *et al* (21) found phosphate ion is closely interrelated in the process of the control of organ formation in tobacco tissue together with adenine, carbohydrate and auxin. From these reports, it may be inferred that the disturbance of any metabolism in the living cell of the root although omitting PO_4 produces no deficient symptom on the root growth. We should also remember that the period of root culture was only ten days and the residual effects from the seed nutrient may possibly be involved in the isolated root growth.

K : The isolated root growth was influenced by omitting K in both cases.

When inoculated with 3 mm long root tips, the root grew to the length of 34.8 mm, and, when inoculated with 10 mm long root tips, the root length attained 55.6 mm after ten days. These root lengths are respectively equal to 70 and 68 per cent of that of the control root. No difference was found in the root growth between two or four times level and normal level, but in the half times level of K-ion (0.25 mg-ion per liter) deficiency of K-ion for the root growth appeared. Heller (22), studying the normal tissues of the carrot and Virginia creeper, concluded that the concentration of potassium should be increased about five fold of normal 10 mg-ions K per liter. At any rate, the presence of K-ion in the medium was necessary for the growth of the roots and its concentration range for the growth appeared to be very wide as in the case of NO_3- or PO_4-ion.

Ca: In this experiment to examine the effects by varying the concentration of Ca-ion, the culture of the isolated roots was carried out in the initial root length of 5 mm. The root length in the standard medium attained 52.8 mm after ten days and the root growth by omitting Ca attained only 31.8 mm, that is, 63 per cent of that of the standard medium. Omitting Ca retarded the growth of the main root and sprouted side roots apparently. In the medium of 0.2 mg-ion concentration, however, the growth was about 116 per cent of that obtained in the standard medium. This fact suggests that the less content of Ca-ion in the medium can produce satisfactory growth. Burström (2) showed that there is a maximal rate of root cell multiplication at 10^{-6} M of Ca-ion concentration or 0.04 mg Ca per liter. In the field, lime must be always supplied not only for the reclamation of acidic soils but for the plant roots to grow satisfactorily. The experimental result that Ca-ion gave the acceleration role to the root growth, therefore, is in accord with Burström's result and also of other investigators.

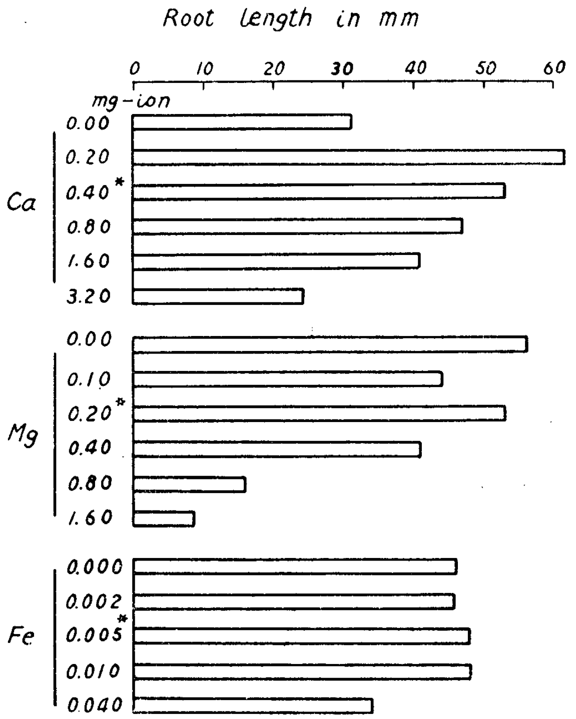

Fig. 2. Influence of Ca-, Mg- and Fe-ions on the growth of the isolated roots of wheat.
* standard medium

In 3.2 mg ion medium of the highest levels, the root growth was inhibited severely and the root length was only 63 per cent of the control root length.

The root growth in higher concentrations of Ca-ions, generally, showed a tendency to be inferior to that in lower concentrations of Ca-ions.

Mg: Omitting Mg resulted in producing 106 per cent of the growth obtained in the standard medium. Higher concentrations of 0.8 mg-ion and 1.6 mg-ion per liter of the medium progressively retarded the root growth, and the growth in the former attained only 31 per cent and in the later only 16 per cent of the growth of the standard medium. An excess of Mg leads to browning of the root tips of about 5 mm and the root cap was lost by culture, the meristem part swelled and its structures could not be discriminated.

Almestrand reported that barley and oat roots showed no effect in 0.1~100 mg Mg per liter. It is well known that Mg is an element consisting of cholrophyll, but the specificity of this element for the plant root growth is still obscure.

Relation between Ca and Mg: To find the relation between Ca- and Mg-ions in the root growth, the isolated roots were cultured under several combinations of these ions. The results are shown in Fig. 3.

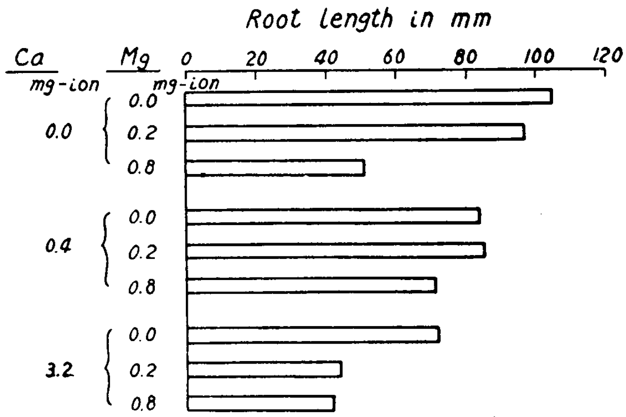

Fig. 3. Root growth in the culture media containing various concentrations of Ca- and Mg-ions. Inoculated 10mm root tips.

In proportion to Ca and Mg lower concentrations, the root growth generally was excellent and by omitting both Ca and Mg resulted in 120 per cent of the growth of the standard medium. By contrast, the raising of the concentrations of both ions inhibited root growth and non-Ca~0.2-mg-ion Mg series were only 50 per cent of the growth of the standard medium. In Mg excess series the roots browned, but by adding the proper quantity of Ca cured the Mg inhibition of growth. Thus, in the series containing a proper quantity of Ca, the growth recovered to 80 per cent of the control growth. This phenomenon is likely to be an antagonism which suppressed the injurious effects of Mg excess by supplying Ca. It is possible that the cell destruction is connected with the replace of Mg for Ca in the pectate of the cell wall. However, the same effect of Mg for the inhibition of Ca excess was not found apparently.

Fe: Omitting Fe or lowering or raising its concentration apparently gave no significant difference in the root growth. In the 0.04 mg-ion level of the highest concentration, the root growth was injured by its excess.

The requirement for iron in the experiment did not agree with other researches. Glasstone (4) showed that the supplying of Fe and Cu was necessary for the growth in the subculture of the side root of the tomato. White (3) reported also that this element accelerated the growth of wheat root tips. The fact that we were unable to find the necessity of Fe for the root growth, may be due not only to differences of plant sorts or variety, but also to the existence of the trace of Fe-ion that may be supposed to have been derived from salts and water or from the culture vessel.

Summary

1) The isolated wheat roots were grown in the sterilized nutrient solution. The sterilizing method of the seeds was described together with the influence of the inorganic ions on the root growth. As the sterilizer of the plant seeds, calcium hypochlorite solutions were superior to the other disinfectants in securing the sterility and keeping the activity of the seed germination.

2) To see the influences of inorganic ions on the growth of the root itself, the isolated wheat roots were cultured in the media containing different concentration levels of the inorganic ions for ten days and the results may be summarized as follows.

3) Omittion of NO_3-, PO_4- and Mg-ions respectively brought no notable effects on the growth along the main-axis. The fact that omitting these inorganic ions resulted in no poorer growth, may be due to the supply of the nutrients originated in the endosperm from the mature tissue during the germination process. When either Ca- or K-ion was omitted, the influence of its deficiency was clearly found in the growth. The same was true for NO_3, if the shorter root tips were inoculated into the culture solution. It seems possible, therefore, that mature tissues are essential for the actvity of meristematic tissues.

4) In case of increasing Ca- or Mg-ion concentration by four times, the roots failed to grow, though without a significant difference in the same concentration of NO_3-, PO_4- and K-ions respectively.

5) When Mg was added excessively, the tips of the roots appeared brown and their growth was inhibited severely. The development of abnormal symptoms by the presence of excess supply of Mg was cured by supplying the optimum levels of Ca-ion. This effect of Ca-ion suggested that it may be antagonistic to Mg-ion.

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