THE EFFECT ON BARLEY SEEDLINGS OF SOME INTERRELATIONS OF CATIONS AND ANIONS IN A THREE-SALT NUTRIENT SOLUTION

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(WITH TWO FIGURES)

Many investigations have been made of the nutrition of plants grown in sand and water culture, and more particularly of the results of the deficiency of one or more specified ions in an otherwise complete nutrient solution. In almost every case, the deficient element was replaced in the nutrient solution by another one.

Recently a method was described by HAMNER (**4**)[1] whereby the anions or the cations of a three-salt nutrient solution could be varied and yet have the rest of the nutrient solution almost constant without the addition of other elements than the original ones. Thus, calcium, magnesium, and potassium could be varied while the nitrates, sulphates, and phosphates remained nearly constant, and *vice versa*. By this method, involving the use of two triangle systems, the concentrations of only three ions, cations or anions as desired, are varied whereas in the ordinary triangles both the anions and cations are varied, thus resulting in the variations of all six ions at the same time (**7**).

This method was first used in studying growth responses of a legume, and since this type of plant often has a different physiological response from other plants, the following experiments were carried out with barley, a member of the grass family.

Barley seeds selected for uniformity were planted in quartz sand in glazed 4 × 8-inch self-draining crocks. Ten seeds were planted in each crock and three crocks were used for each point of the triangle. Plants were watered with distilled water until the first leaf was about 5 cm. tall, after which they were watered on alternate days with nutrient solution and were flushed out each week with distilled water.

All experiments were repeated twice later in the summer, so that a total of about four thousand plants was used. The investigations were begun in June and continued through September. Plants were grown under ordinary greenhouse conditions of light and humidity.

Two nutrient triangles were used. In the first triangle, three stock solutions, N, S, and P were prepared. Solution N consisted of 0.0045 molar KNO_3, 0.0045 molar $Mg(NO_3)_2$, and 0.006 molar $Ca(NO_3)_2$. Solution S consisted of 0.0045 molar K_2SO_4, 0.0045 molar $MgSO_4$, and 0.006

[1] Somewhat similar attacks upon this problem have been made by BECKENBACH, WADLEIGH, and SHIVE (**1**), and WADLEIGH (**8**).

molar $CaSO_4$. Solution P consisted of 0.006 molar $Ca(H_2PO_4)_2$, 0.0045 molar KH_2PO_4, and 0.0045 molar $Mg(H_2PO_4)_2$. The latter compound rather than $MgHPO_4$ was used because of its greater solubility and because its use allows the anions to be kept more nearly constant. The $CaSO_4$, instead of being added as such, was made by adding equivalent amounts of finely powdered $CaCO_3$ and H_2SO_4. This was done because of the slowness with which $CaSO_4$ dissolves. By these two modifications, it is possible to have all components go quickly into solution and to avoid the formation of a fine sludge in certain of the stock solutions which often occurs if the original method is followed.

By varying the amounts used of each of the solutions N, S, and P, the anions could be varied, while the cations remained practically constant.

In the second triangle there were three stock solutions: M, K, and C. Solution M consisted of 0.0045 molar $Mg(NO_3)_2$, 0.0045 molar $MgSO_4$, and 0.0045 molar $Mg(H_2PO_4)_2$. Solution K consisted of 0.0045 molar KNO_3, 0.0045 molar K_2SO_4, and 0.0045 molar KH_2PO_4. Solution C consisted of 0.006 molar $Ca(NO_3)_2$, 0.006 molar $Ca(H_2PO_4)_2$, and 0.006 molar $CaSO_4$, the latter being added by the same method as described above for the first triangle. By varying the amounts of each one of the nutrient solutions M, K, and C the cations can be varied at will while the anions remain practically constant.

In the first triangle, plants at one corner were watered only with solution N, at a second corner with S, and at the third only with P. Similarly, in the second triangle, the corner plants were watered only with solution M, or K, or C. All intermediate points of each triangle were watered with solutions made by mixing together various amounts of the three stock solutions, so that each point on the triangle varied by $\frac{1}{6}$ from every other point, thus making a total of 28 different nutrient solutions in each triangle (see figs. 1 and 2).

In addition to the plants in the triangle, a group of 50 was set aside as a control and was fed with a three-salt nutrient solution composed of 0.006 molar $Ca(NO_3)_2$, 0.0045 molar KH_2PO_4, and 0.0045 molar $MgSO_4$.

All plants were harvested when twenty days old. Heights of plants and dry weights of roots and tops are recorded in figures 1 and 2. Since the light was much poorer during August than during July, the plants grown later in the season did not synthesize as much dry matter and were somewhat slower to respond to the various treatments than were those grown earlier, but the same relative results were obtained.

Results and discussion

Triangle I. NO_3, SO_4, PO_4 varied; Mg, K, Ca constant

Initial deficiency symptoms in this triangle appeared within three or four days after the first application of nutrients. In that series along the side of the triangle in which there was a complete absence of NO_3, and varying amounts of SO_4 and PO_4, the leaf tips began to show a slight yellowing, followed shortly by a progressive death of the leaf from tip toward base. This necrosis occurred where the SO_4 was decreased to $\frac{2}{3}$ or less than $\frac{2}{3}$ of its maximum value, becoming more severe as the SO_4 decreased

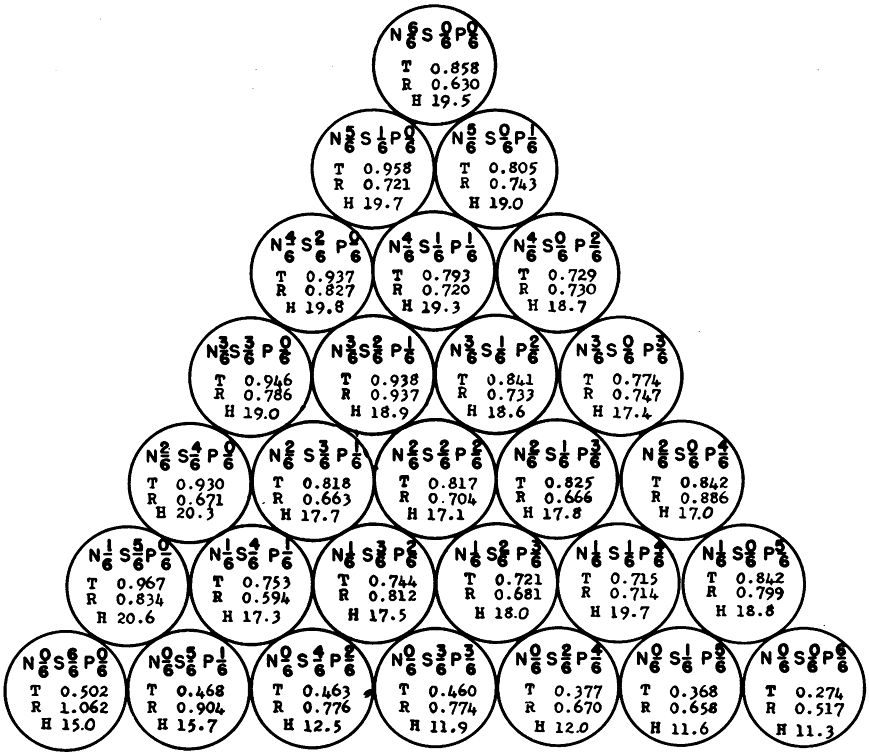

FIG. 1. Triangle I. Each point of the triangle was watered with the fractions of solutions N, S and P indicated in each circle. Solution N contains various nitrates; S, sulphates; and P, phosphates. T and R indicate dry weights in grams of tops and roots, respectively. H indicates average heights in cm. of plants in each treatment.

Data for the controls:

Tops	= 0.877 grams
Roots	= 0.764 grams
Height	= 18 cm.

and the PO_4 increased. The plants given the solution in which the PO_4 was increased to its maximum and the SO_4 omitted were almost entirely dead by the time of harvest. On the other hand, if the SO_4 was raised to $\frac{5}{6}$ of its maximum and the PO_4 reduced to $\frac{1}{6}$, the plants were only slightly stunted and a little yellowed because of the lack of NO_3. Whether this decrease in toxicity with the increasing amount of SO_4 is caused by the actual presence of larger amounts of SO_4 or merely by the decreased amount of PO_4 is not known, since as the SO_4 increases the PO_4 decreases according to the set-up of the triangle.

In that series watered with solutions containing no SO_4 but having varying relative amounts of NO_3 and PO_4, the same toxic symptoms appeared when PO_4 was relatively high in amount and the NO_3 low, although these plants were markedly better than those given the SO_4–PO_4 complex in the absence of NO_3. NO_3 was highly effective in preventing the toxic effect of excessive PO_4, even a small amount of NO_3 preventing the toxic effect of a large amount of PO_4.

That series of plants supplied with a solution lacking PO_4 and with varying relative amounts of NO_3 and SO_4 were uniformly good, the plants at the time of harvest being still too young for the PO_4 deficiency to have been manifested. All this points definitely to a pronounced PO_4 toxicity when NO_3 is absent, or present in only a low concentration. This has been previously shown by HAMNER (**4**) in his work on bean seedlings. MOORE (**6**) also found evidence of PO_4 toxicity in peanut seedlings.

The results of the treatments in the central regions of the triangle showed such slight variations as not to be significant, those with relatively low N being almost as vigorous as those with much N.

That solution containing only NO_3 and no SO_4 or PO_4 produced plants comparable to those receiving balanced solutions. This is probably because there were enough materials in the seeds to take care of the deficiencies in the solutions until the time of harvest. The solution containing only SO_4 as the cation produced plants which, although stunted and a little lighter green because of the lack of NO_3, were nevertheless in good condition. This was in marked contrast to those plants receiving PO_4 as the only cation. The latter were very poor. This again indicates the toxicity of PO_4 in the absence of NO_3.

In this triangle the roots were but slightly affected by the different nutritional treatments. Their size and weights were all remarkably similar, except for that series given the solution entirely lacking NO_3 and having varying amounts of SO_4 and PO_4. Here again, as in the case of the tops, there was a definite decrease in weight as the PO_4 increased and the SO_4 decreased.

TRIANGLE II. Mg, K, Ca VARIED; NO_3, SO_4, PO_4 CONSTANT

Here the first and the most severe symptoms to appear were those of K deficiency. In fact, these manifestations appeared in 2 to 3 days, even before the PO_4 toxicity or the NO_3 deficiency in triangle I. The first apparent reaction to K deficiency was a stunting of the plant, which was much more marked than the stunting brought about in a comparable period of time by lack of N. This stunting was followed rapidly by a yellowing and subsequent browning of the leaf starting at the tip and progressing towards the base. This occurred in the entire series of plants on that side of the triangle lacking K and having varying amounts of Ca and Mg. Here the plants were all uniformly very poor, there being apparently no Ca-Mg relationship affecting this die-back. Whether high in Ca and low in Mg or

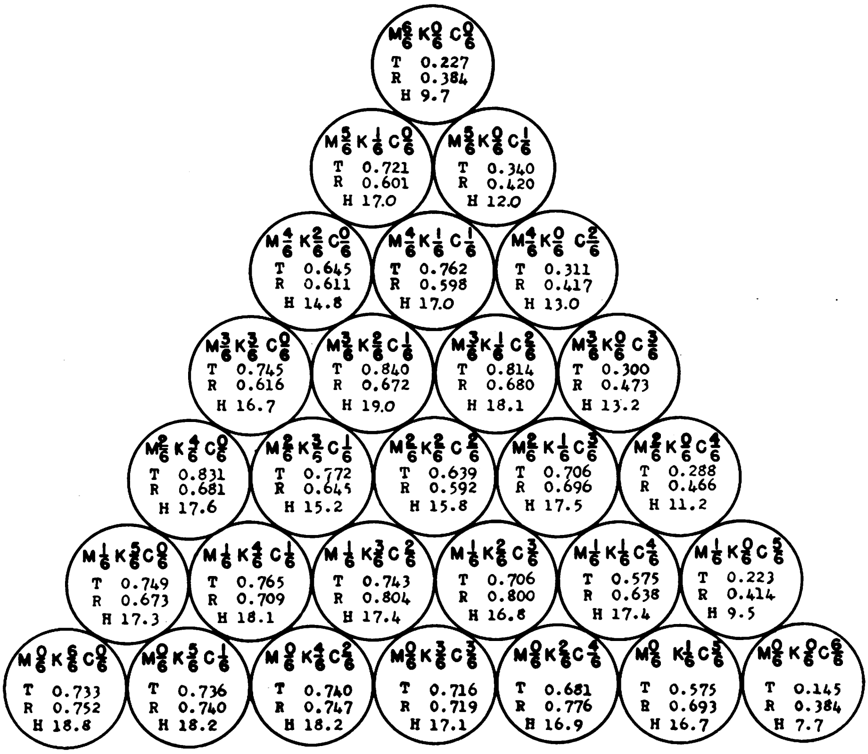

FIG. 2. Triangle II. Each point of the triangle was watered with the fractions of solutions M, K, and C indicated in each circle. Solution M contains Mg salts; K, potassium salts; C, calcium salts. T and R indicate dry weights in grams of tops and roots, respectively. H indicates average heights in cm. of plants in each treatment.

vice versa, the K deficiency symptoms appeared at about the same time and progressed at about the same rate. The reaction was so severe that in some cases more than $\frac{4}{5}$ of the plant tops were dead at the time of harvest. Those plants given the solution in which Mg was the only cation, however, reacted somewhat differently. Instead of yellowing they became a creamy white, and the tips shriveled more slowly than did the others, so that the basal parts remained white and without withering for a long time; finally only $\frac{1}{2}$-inch of the extreme basal parts of the leaves remained a healthy green color. This reaction is undoubtedly caused by the rôle which Mg plays in the formation of the chlorophyll molecule.

In the series on the side of the triangle given the solutions containing no Ca and varying amounts of Mg and K, the leaves were not seriously affected as they were in the series lacking K. Here where the amount of Mg was high as compared with K, the growing points were killed. This occurred in all cases where the Mg was more than $\frac{1}{2}$ of its possible maximum. Even where it was $\frac{1}{2}$, there were many individual cases with dead growing points. Where the Mg was decreased to $\frac{1}{3}$ its maximum value, however, and K increased to $\frac{2}{3}$, the growing points were usually unaffected; any further decrease in the Mg left the growing points healthy. Thus, in the absence of both Ca and Mg in the nutrient solution the growing points remained healthy. This points definitely to a toxic effect of Mg, rather than to a Ca deficiency in these seedlings. GAUCH (**3**) in his work on bean seedlings points out the extreme toxicity of Mg in the absence of Ca. It is not known whether variations in the concentration of K may affect this Mg toxicity manifested in the absence of Ca, since in accordance with the setup of this triangle as the K concentration increases, the Mg concentration is proportionately decreased. Therefore, this decrease of injury with the increasing K may be caused simply by the accompanying reduction in the amount of Mg.

The plants on the side of the triangle lacking Mg were fairly good. They were best at the end where the concentration of K was highest, and decreased in weight as the K decreased and the Ca increased.

Of the three cations present in the nutrient solution, the K was the most important one in maintaining the plants in a vigorous state. Deficiency of K was the first deficiency to appear and plants suffering from it were the poorest of all deficient plants at the time of harvest.

Since Ca and K, particularly the latter, are known to be associated with root growth, it might be expected that the roots would be markedly affected by the different nutritional treatments in this triangle. They were actually affected to such a degree that in some cases they were less than $\frac{1}{2}$ the normal size and weight. The most pronounced effect was in that series lacking K. Although in this series the amount of Ca varied inversely with the amount of Mg in the nutrient solutions, the roots were in all cases rather uniformly stunted. The roots, although dwarfed, were apparently healthy.

Considering all plants in both triangles, it may be said that in general those given the solutions in which the K, Ca, and Mg were kept constant and the NO_3, SO_4, and PO_4 were varied did better than the plants in the other triangle. The most vigorous plants, in either triangle, however, compared very favorably with those in the other. Certain combinations of nutrients in the triangles produced plants that are comparable with those given the three-salt nutrient solution. The former are the equals of the latter in every way. Data for the controls are found in the legend for figure 1.

GREGORY (**2**) based his mathematical approach to this problem upon the false philosophical assumption that the effects of the ions involved are additive and did not allow for interaction among them. He recognized this fault but used the assumption for the purpose of simplification. Nevertheless, several of his conclusions are in accord with experimental evidence presented in this paper, and it is still more interesting when one considers that his data came from JOHNSTON (**5**), who had designed his experiment for an entirely different purpose. The author disagrees with GREGORY in one important point. The latter finds Ca to be the most important cation and K the least while the present work indicates K to be more important than Ca.

In arriving at these conclusions for the work reported here, it must be remembered that the experiments were with seedlings and these results might be different if the plants were grown to maturity.

Summary

1. A study was made of the effect on barley seedlings of the interrelations of the cations and the anions present in a three-salt nutrient solution.

2. The plants of the triangle in which the anions were varied were as a whole better than plants of the triangle in which the cations varied.

3. The variations in the nutrient solutions had their greatest effect upon the top growth of the plants. The root growth was much more uniform except in the absence of K which caused a marked decrease in size and weight of roots.

4. In the absence of nitrates, or when a high phosphate-low nitrate relationship existed a marked phosphorous toxicity became apparent.

5. Potassium deficiency symptoms appeared extremely early, before signs of deficiency or toxicity of any other element. A severe die back of the leaves of these seedlings resulted.

6. A high concentration of Mg in conjunction with an absence of Ca in the nutrient solution resulted in the death of the terminal meristem, and caused some stunting and chlorosis.

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