BIOCHEMICAL STUDIES OF MICROELEMENTS IN GREEN PLANTS VI. EFFECTS OF MICROELEMENT DEFICIENCIES ON THE STATUS OF PHOSPHORUS FRACTIONS IN BARLEY PLANTS

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VI. EFFECTS OF MICROELEMENT DEFICIENCIES ON THE STATUS OF PHOSPHORUS FRACTIONS IN BARLEY PLANTS

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If a plant is deficient in any one of the essential microelements, some characteristic changes of its chemical status with the emergence of visual symptoms should occur.

Concentration of phosphorus in the plant tissues deficient in microelements appears to differ more or less from that in the normal one. For instance, it was shown in the previous paper (1) that total phosphorus increased in the barley leaves deficient in iron. Steinberg *et al.* (2) have reported on the increase of phosphorus content of tobacco plants deficient in microelements. However, the status of phosphorus fractions in such plant tissues seems to have been studied only by Reed (3), who found histochemically that the leaves and stems of the zinc deficient tomato accumulated inorganic phosphate highly.

At present, it is well known that various phosphorus compounds play important roles in the crucial metabolic processes of the plant such as respiratory process, photosynthesis, protein and nucleic acid metabolism. Accordingly, it is of interest to examine the effects of microelements deficiencies on the fractional distribution of phosphorus in plant tissues as a research on the role of microelements in plants.

The present work deals with the changes of inorganic, cold acid soluble organic, lipid-, ribonucleic acid (RNA)-, deoxyribonucleic acid (DNA)-, and acid insoluble residual phosphorus fraction within the leaf-tissues of barley plants which were grown on the cultural solutions eliminated in microelements and that revealed the characteristic deficiency symptoms.

Materials and Methods

Deficient culture of barley plants. Two week old barley seedlings (var. Shokimugi) brought up on glass distilled water were grown on the cultural solutions from which iron, manganese, zinc, copper, and molybdenum were individually eliminated. The methods of culture and the composition of the cultural solution were the same as those described before (4). After the culture for ten weeks, when the deficiency symptoms of the microelements became apparent, the aerial parts, which consisted almost entirely of leaf-tissues *viz.* leaf blades and sheaths at this stage of growth, were taken from the plants and the necrotic portion, if present, was removed from them. Then they were analysed for the phosphorus fractions.

Fractionation of phosphorus. For the fractionation of phosphorus, the under mentioned procedures were used refering to the methods originally proposed by Juni *et al.* (5) and modified by Klein (6), and by Ogur *et al.* (7). The digestion of the supernatant or the filtrate and the final residue obtained through the procedure was made by heating them with hydrogen peroxide and sulfric acid and the determination was made according to Allen's method (8).

(*1*) *Inorganic phosphorus.* About 3 g of fresh leaf-tissues were macerated with an appropriate volume of 10 percent trichloroacetic acid solution and the macerate was kept below 4°C for two hours, then centrifuged at 3000 rpm. for ten minutes by a refrigerated centrifuge at 1°C. The residue was again suspended in 5 ml of trichloroacetic acid solution then centrifuged. This washing was repeated three times, and subsequently each supernatant liquid was combined and immediately the phosphorus content was determined as inorganic phosphorus.

(*2*) *Cold acid soluble organic phosphorus.* Another aliquot of the extract obtained above was digested and the total phosphorus content was determined. The difference of this value and the inorganic phosphorus corresponds to cold acid soluble organic phosphorus.

(*3*) *Lipid-phosphorus.* The solid residue obtained above was suspended in 5 ml of ethanol-ether mixture in 3:1. The suspension was gently boiled on a water bath for three minutes then filtered through a glass filter. This procedure was repeated until the residue became colorless. All filtrates were mixed together and analyzed for lipid phosphorus after digestion.

(*4*) *RNA-phosphorus.* The residue from the above procedure was suspended in 5 ml of 1 N perchloric acid solution at 4°C for 18 hours and centrifuged. The residue was treated twice in the same manner and the supernatant was combined together then its phosphorus content was determined as RNA-phosphorus.

(*5*) *DNA-phosphorus.* The residue obtained above was suspended in 5 ml of 0.5 N perchloric acid solution and kept at 70°C for 20 minutes, then centrifuged. This extracting procedure was repeated again. The phosphorus content of the combined supernatant was determined as DNA-phosphorus.

(6) *Acid insoluble residual phosphorus.* The final residue was digested and the phosphorus content determined.

Results and Discussion

Growth of plants. The length and the dry weight of the aerial parts and roots per plant are given in Table 1. In general, the rate of growth was low, because the culture in this experiment was done in the coldest season, ten weeks from the beginning of January, in the green house which was warmed only to the degree to prevent freezing of the cultural solution at night.

Table 1. Status of growth of barley plants deficient in microelements after ten weeks culture.

Treatment	Aerial parts		Roots	
	Length	Dry weight per plant	Length	Dry weight per plant
	cm	mg	cm	mg
Full nutrients	9.6	125	8.5	55
-Fe	8.8	57	13.8	33
-Mn	6.4	59	8.7	36
-Zn	6.0	75	7.3	35
-Cu	8.5	76	7.6	37
-Mo	8.3	108	4.8	34

Dry matter of the aerial parts was low in zinc and copper deficient plants and much lower in iron and manganese deficient ones. In these plants, the characteristic symptoms of the deficiency were clearly observed. With molybdenum deficient plant, no clear symptom appeared and the retardation of growth in the aerial parts was slight. However, its roots were very short and became necrotic. This may be regarded as a sequence of molybdenum deficiency on the barley plants (4).

Nitrogen contents. Total and protein nitrogen were determined on the leaf-tissues used for the fractionation of phosphorus. The data obtained are shown in Table 2.

Table 2. Total and protein nitrogen in leaf-tissues of barley plants deficient in microelements. (% dry weight)

Treatment	Total-N	Protein-N
Full nutrients	4.68	3.02
-Fe	6.04	3.18
-Mn	5.90	3.28
-Zn	5.48	2.28
-Cu	5.44	3.34
-Mo	3.78	2.17

Total nitrogen showed an increase in all deficient plants except the molybdenum deficient one, in which it decreased as compared with that in the normal plant. The level of protein nitrogen was a little higher in copper, manganese, and iron deficient plants than in the normal plant, but it was remarkably low in zinc and molybdenum deficient plants. In general, all the deficient plants, especially the zinc deficient one in the extreme, showed a low ratio of protein nitrogen relative to total nitrogen.

Phosphorus fractions. Table 3 and Fig. 1 show the data from the fractionation of phosphorus. The sum of phosphorus in each fraction and the amount of total-phosphorus determined directly on the dry matter of the leaf-tissues showed good agreement within the error of 5 percent.

Table 3. Concentration of phosphorus fractions in leaf-tissues of barley plants deficient in microelements.

(mg P/g dry weight)

Fraction of P / Treatment	Inorganic	Cold acid soluble organic	Lipid	RNA	DNA	Acid insoluble	Total
Full nutrients	0.99	1.18	0.89	0.94	0.25	0.28	4.53
-Fe	2.48	1.17	1.09	1.25	0.53	0.37	6.89
-Mn	2.06	0.91	0.94	0.91	0.43	0.38	5.63
-Zn	3.24	0.76	0.71	0.50	0.18	0.18	5.57
-Cu	1.38	0.83	1.08	0.86	0.32	0.30	4.77
-Mo	0.95	0.58	0.82	0.66	0.14	0.19	3.34

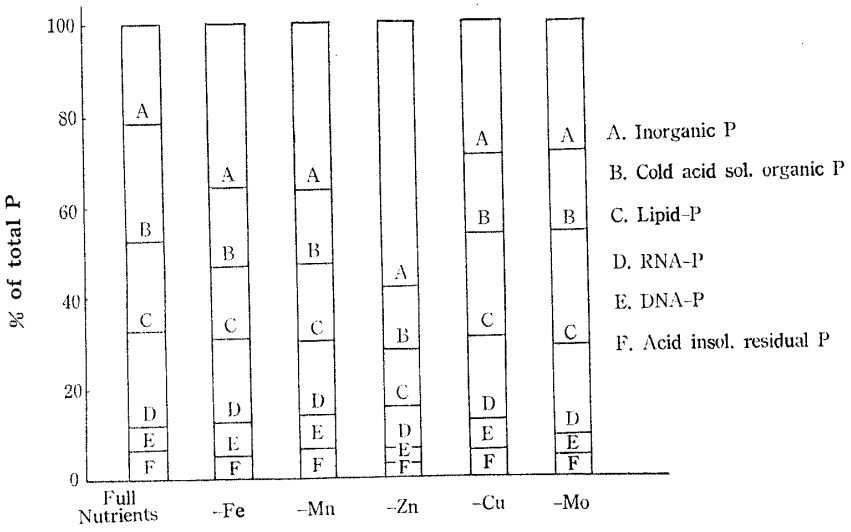

Fig. 1. Percentage distribution of phosphorus fractions in leaf-tissues of barley plants deficient in microelements.

(*1*) *Inorganic phosphorus.* Except for the molybdenum deficient plant, the concentration of inorganic phosphorus in the deficient plants was higher than that of the normal one. Especially, the zinc deficient plant gave an extremely high value and more than one half of its total phosphorus was found in this

fraction. This result was consistent with the description of Reed (3). The ratio of inorganic to total phosphorus was far higher in all deficient plants, even in the molybdenum deficient one, than that in the normal one. Therefore, it may be concluded that the efficiency for the assimilation of the uptaken phosphorus diminishes in the microelement deficient plants.

(*2*) *Cold acid soluble organic phosphorus.* Concerning this fraction, all deficient plants, especially the zinc and molybdenum deficient ones, showed low values. This fraction is considered to be consisted mainly of phosphate esters of soluble organic components in plants such as intermediates of carbohydrate and protein metaboliam and nucleotides which are co-factors of enzymic reactions or units of nucleic acids, so that the phosphorus in this fraction may be regarded as that incorporating into metabolic processes and inorganic phosphorus as its pool. Since all deficient plants showed considerably low ratio of this fraction to inorganic phosphorus, it could be supposed that the incorporating efficiency of inorganic phosphorus into the metabolic process dropped in them.

(*3*) *Lipid-phosphorus.* The components peculiar to green plant-tissues of this fraction may be the phospholipids present in the lamellae of the chloroplasts (9). Consequently, it is noteworthy that iron, manganese, and copper deficient plants had comparatively high lipid-phosphorus though they were all more or less chlorotic. On the other hand, the content of lipid-phosphorus was low in the zinc and molybdenum deficient plants.

(*4*) *RNA-phosphorus.* RNA is now accepted to participate to the biosynthesis of protoplasmic protein or enzymes (10) (11). Concentration of this phosphorus was very low in the zinc and molybdenum deficient plants, in which the level of protein nitrogen was also low, while it was appreciably high in the iron deficient plant which had relatively high protein nitrogen. These data suggest the presence of correlation between the level of RNA and protein in the plant tissues deficient in microelements. However, as RNA-phosphorus was relatively low in the copper deficient plant which had the highest protein nitrogen and a similar trend was shown by the manganese deficient plant, it seems that the correlation mentioned above does not hold always.

It was reported earlier (12) that under the condition of zinc deficiency free amino acids are accumulated in the leaf-tissues at a high concentration whereas the level of protein is low. For such reason, it is considered that the synthesis of some particular amino acid which is essential to the biosynthesis of protein is blocked by the shortage of zinc. But, from the above results, it may also be probable that the protein synthesis from amino acids is blocked due to the depression of RNA formation in zinc deficient plant tissues. Holden (13) reported that zinc inhibited the ribonuclease activity *in vitro* and Brachet (14) found that the exogenous ribonuclease inhibited the elongation, the incorporation of amino acids into protein, and RNA metabolism in onion root tips. But the functional

mechanism of endogenous ribonuclease relating to the RNA synthesis *in vivo* remains yet unkown.

(5) *DNA-phosphorus.* It is said that DNA exists solely in the nucleus of the cell and its content is constant within a narrow limit for the nuclei of different somatic tissues of a given species of organisms (15). DNA-phosphorus was high in iron, manganese, and copper deficient plants whereas low in zinc and molybdenum deficient ones. Thus, if the constancy of DNA content of the nuclei holds in these cases, the cell number that constitutes a unit weight of dry matter may be larger in the former plants and smaller in the latter than in the normal plant. Moreover, the DNA metabolism is assumed to be disturbed in zinc and molybdenum deficient plants. These assumptions should be examined further by cytochemical observations.

(6) *Acid insoluble residual phosphorus.* This fraction may be regarded as phosphorus mainly bound with protein, though it seems that the evidence on the occurence of the phosphoprotein has not yet been obtained for the green plants. The variation of this fraction among the deficient plants appears to be parallel to that of protein nitrogen.

(7) *Total phosphorus.* Total phosphorus increased more or less in iron, manganese, zinc, and copper deficient plants, though it decreased in the molybdenum deficient one. Generally speaking, when a plant is deficient in an essential element, it tends to absorb other elements at relatively high concentrations, so that the low value of total phosphorus seems to be a notable effect of molybdenum deficiency. In this connection, Stout *et al.* (16) reported that the absorption of molybdenum by plants was enhanced in the presence of high phosphate in the nutrient medium. The inter-relationship of phosphorus and molybdenum appears to be an interesting problem on the nutrient absorption by plants. It may also be pointed out that this feature is found in total nitrogen, which was low only in molybdenum deficient plant.

To sum up the results described above, the changes of the status of phosphorus fractions in leaf-tissues by deficiencies of microelements were found to be not only uniform but also characteristic to each element seemingly corresponding to the characteristic deficiency symptoms.

Summary

The changes of the status of phosphorus fractions were examined on the leaf-tissues of barley plants deficient in iron, manganese, zinc, copper, and molybdenum respectively. The results obtained on the dry weight basis are:

1) Concentration of inorganic phosphorus was higher in iron, manganese, copper, and extremely zinc deficient plants whereas that of cold acid soluble organic phosphorus was lower in all deficient plants than in the normal one.

2) Lipid-phosphorus increased in iron, manganese, and copper deficient plants

and decreased in the zinc and molybdenum deficient ones.

3) RNA-phosphorus of zinc and molybdenum deficient plants was very low corresponding to the low protein nitrogen in them. DNA-phosphorus was also markedly low in these plants, whereas it was high in iron, manganese, and copper deficient ones.

4) Total phosphorus was low only in the molybdenum deficient plant, though it was high in all other deficient plants. Shortage of molybdenum appears to restrain the uptake of phosphorus.

5) Commonly in all the deficient plants examined, the ratio of inorganic phosphorus to cold acid soluble or total organic phosphorus was much higher than that in the normal one, so that the efficiency to metabolize the uptaken phosphorus was suggested to be depressed in them.

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