BIOCHEMICAL STUDIES OF MICROELEMENTS IN GREEN PLANTS 1. DEFICIENCY SYMPTOMS OF MICROELEMENTS ON BARLEY PLANTS AND CHANGES OF INDOLACETIC ACID OXIDASE ACTIVITY

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1. DEFICIENCY SYMPTOMS OF MICROELEMENTS ON BARLEY PLANTS AND CHANGES OF INDOLACETIC ACID OXIDASE ACTIVITY

By

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Introduction

It is known that some characteristic pathological symptoms in green plants are due to the deficiency of microelements, and that those elements participate in biochemical reactions(1). Owing to the scanty knowledge on the functions of microelements in the metabolism of green plants, we made investigations thereon.

When plants are grown by a series of culture solutions in which each one of microelements is eliminated, characteristic symptoms due to the deficiency of the corresponding element will appear. Simultaneously, the plant growth is depressed accompanied with the development of the deficiency symptoms. This growth depression suggests the presence of some interaction between the microelements and the growth substance levels within the plants together with other relationships. In this concern Tsui (2) found that zinc participates the synthetic process of tryptophan which is now believed to be the precursor of indolacetic acid (IAA), the native plant auxin. Tang and Bonner(3) reported that the IAA oxidase, an enzyme which destroyes excessive IAA through oxidation to regulate the levels of IAA in plants, appeared to contain heavy metal, probably iron, as an essential component of its prosthetic group. On the other hand, Wagenknecht and Burris(4) reported that IAA oxidase might be a copper protein and activated by manganese in some cases. Thus the changes of IAA oxidase activity were examined on the tissues of barley plants deficient in each microelement.

Material and Methods

Cultivation of plants

Barley seeds were sawn on a gauze stretched on the surface of redistilled water filled in a pyrex glass tray. Each culture vessel consisted of a 2–litre pyrex glass beaker and its cover plate with six holes made of plaster and paraffined. Three two-week old seedlings were transplanted to each hole with the aid of cotton, and the vessel was filled with the basal culture solution of the following composition :

Salt	$(NH_4)_2SO_4$	$NH_4H_2PO_4$	KNO_3	$Ca(NO_3)_2$	$MgSO_4$
Molar concentration	0.00175	0.001	0.0025	0.001	0.001

In addition to this basal solution, 0.5 of Mn, 0.05 of Zn, 0.02 of Cu, 0.01 of Mo, 0.5 of B, and 1.0 per week of Fe, each in p.p.m., were supplied for the complete nutrition, and these were alternatively eliminated for the corresponding element dificient culture.

The stock solutions of the major nutrients had been purified cautiously through bubbling with hydrogen sulfide gas and filtration with filter papers to remove the minimum contaminations of heavy metals as precipitated sulfides.

In all cultures redistilled water was used, which was prepared from desalted water by distillation with a pyrex glass distiller.

Estimation of IAA oxidase activity

Tang and Bonner(3) devised the method of estimation of IAA oxidase activity by the colorimetric determinations of IAA. Wagenknecht and Burris (4) used the manometric method measuring the oxygen uptake in the IAA oxidation process. We used the colorimetric method since the manometric method was found to be somewhat unconvenient as discussed later. For the colorimetry of IAA Gordon and Weber(5) proposed a modification of the Tang and Bonner's reagents, the latter was used because this seemed to develop a more stable color. The conditions in the procedure for enzyme reaction were similar to those of Tang and Bonner.

Each 250 mg portion of fresh tissues were taken from plants which developed the respective microelemrent deficiency symptoms. These were ground with a mortar and pestle, then homogenized in 5 ml of cold phosphate buffer of pH 6.4 adding 5 ml of 100 mg/1 IAA solution. This mixture was made to 20 ml by the addition of water, hence the final concentration of IAA attained was 25 mg per litre, After incubation at 28°C for 3 hours, the solution was boiled a few minutes to stop the enzyme reaction, cooled and centrifuged, then the colorimetric determination was carried out on the samples of 2 ml supernatant solution.

Results

Deficiency symptom

In this investigation barley seeds were sawn on January 18, and the cultures of the seedlings started on the end of the same month. After four or five days from the beginning of the culture, the leaves of iron deficieny barley plants became yellow then gradually turned white yellow in the upper leaves, and the growth was soon ceased. However, their root remained white and healthy during a long period thereafter.

In the manganese deficient culture, the growth of shoots was checked within about ten days and the intervenal streaky chlorosis appeared. The youngest leaf could not expand its blade and became chlorotic thereafter necrosis appeared. By the culture eliminated from zinc, the growth of the shoots was also checked within ten days and the upward elongation of the young leaves was suppressed, although their leafblades expanded to some extent. However the shoots soon became stunted and necrosis developed. In this case, on other remarkable phenomenon was observed. The guttation was abnormally intensive from the beginning when the deficiency symptoms became severe, and so high a concentration of soluble organic matter containing nitrogen was excreted that white granules of that matter were left there after the evaporation of guttae. When copper was deficient, the color of the whole shoots changed slightly to yellowish light green and the young leaves could not expand their blades, then died off from their tips after about three weeks. By the shortage of molybdenum, the growth of the roots was abnormally depressed showing a necrotic appearance, but the aerial portion showed no chlorotic symptoms throughout the one-month experimental period and the number of tillers was rather numerous. In boron deficient culture, the plants showed no notable symptoms, although the growth was somewhat depressed. The growth indexes of each culture after one month of cultivation are given in Table 1.

Table 1. Growth indexes of each culture of barley plants grown for one month.

Sample	Length of shoot	Length of root	Number of tillers	Dry weight per plant	
				Shoot	Root
	cm	cm		mg	mg
Complete nutrition	12.3	6.3	3.3	202	66
Minus Fe	8.4	8.3	1	62	18
〃 Mn	8.7	7.7	2	92	38
〃 Zn	6.3	7.4	1	70	25
〃 Cu	7.9	6.4	2	62	17
〃 Mo	9.8	4.4	4	250	48
〃 B	9.8	7.4	3.4	168	50

IAA oxidase activity

The changes of IAA oxidase activity was determined colorimetrically on the

root tissues only, for the reason discussed later. The results obtained are shown in Table 2. With plants deficient in Cu and Mo the IAA oxidase activity in

Table 2. IAA oxidase activity of root tissue homogenate.

Sample	Amount of IAA destroyed	Per cent destruction of IAA	Index to the complete nutrition
	mg/l	%	
Complete nutrition	20	80	100
Minus Fe	20	80	100
〃 Mn	20	80	100
〃 Zn	20	80	100
〃 Cu	5	20	25
〃 Mo	2	8	10
〃 B	18	75	94

The initial concentration of IAA was 25 mg/l of the reaction mixture.

their roots was very small and only 20 and 8 per cent of that with complete plants, while the root tissues of plants grown in each nutrient solution, in which Fe, Mn, Zn, and B was eliminated alternatively, had almost the same activity of IAA oxidase as those in complete nutrition.

To see whether the deficient plants would restore the IAA oxidase activity in their roots, the individual eliminated element was added to the corresponding nutrient solution after the deficiency symptoms had developed. After five days the activity of each root tissue was measured. Through the supplement of the corresponding element, the copper dificient plants could recover the activity of IAA oxidase almost completely, while in the case of molybdenum the recovery was not indicated (Table 3). The addition of the other corresponding elements had essentially no effects.

Table 3. Restoration of IAA oxidase activity in the roots by the supplement of the corresponding element to the deficient plants.

Supplement	Amount of IAA Destroyed	Per cent destruction of IAA	Index to the complete nutrition
	mg/l	%	
Cu	19.0	76	95
Mo	1.5	6	8

The initial concentration of IAA was 25 mg/l of the reaction mixture.

Discussion

When barley seedlings were grown in solutions lacking one of the micro-elements, characteristic symptoms appeared as described above. However the growth responses of barley to each element seems to differ for the shoot and the

root. Generally in each plant deficient in Fe, Mn, Zn, and Cu, the dry weight of both its shoots and roots was far less than that of the control, and its shoot length was also shorter, although the length of the roots was rather longer. In molybdenum deficient plants, it is interesting that the depression of root elongation and its necrotic appearance was very notable, while the dry weight of the aerial portions and the number of tillers was large. The effect of boron elimination was not so obvious and this might be cause by the minute quantities of boron being dissolved into the nutrient solution from pyrex glass beaker used as the vessel, or it may be probable that the boron requirement of barley in this period of growth is very slight.

It has been generally accepted that the IAA oxidase is present in inactivated states within geen leaves(6). This was confirmed by us to be true with barley leaves by the colorimetric method, because leaf tissue homogenates showed no appreciable ability to oxidize IAA. However, when the oxygen uptake of homogenates added with IAA was measured manometrically in Warburg respirometer, the increased oxygen uptake was observed on the homogenates of green leaves as well as of root tissues. Since the oxygen uptake by plant tissue homogenates may be increased by the addition of IAA without oxidation of IAA, oxidation of IAA itself should be distinguished from its own stimulating effect on the respiratory process. It may be said that if plnat tissue homogenates or expressed saps were used themselves as the enzyme solution, the manometric method would be unsuitable for the estimation of IAA oxidase activity, because the reaction between IAA and such materials might be extremely complex. Therefore, to practice the manometric method, Tang and Bonner (3) prepared an enzyme solution through dialysis to remove the respiratory substrates. On the other hand, in the colorimetric determination the initial rate of the reaction was lowered with the increase of the addition of homogenate. This may be caused by the restraint of IAA oxidation due to the decrease of oxygen parital pressure induced by the increasse of other oxidative process. Hence, for a more accurate estimation of this enzyme activity by the colorimetric method, it seems to be necessary as in the manometric method to remove the restiratory substrates.

The extremely low levels of IAA oxidase activity in the roots of copper and molybdenum deficient plants (Table 2) indicate the close relation of these two elements to this enzyme. According to the results given in Table 3, it can be supposed that the IAA oxidase may be a copper protein and that the fundamental protein metabolism may be disturbed by molybdenum deficiency.

Summary

Barley plants were grown in a series of nutrient solutions in which each individual microelement was eliminated, and the deficiency symptoms and the

growth rate were examined.

The changes of IAA oxidase activity were estimated on the root tissues of these plants, and the results obtained are summarized as follows :

1) The IAA oxidase activities of each root tissues of iron, manganese, zinc, and boron deficient plants were almost the same as that of the control plants, although their growth had been depressed.

2) In the copper and molybdenum deficient plants the IAA oxidase activity of the roots was very weak, being only 20 and 8 per cent respectively compared with that in the complete nutrients.

3) By the addition of copper to the plants deficient in it, the IAA oxidase activity of their roots restored almost completely to the level of the control plants within five days. While in the case of molybdenum no restoration was recognized.

4) From these results it may be supposed that the IAA oxidase is a copper protein and that molybdenum plays an important role in the synthetic processes of this enzyme.

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