BIOCHEMICAL STUDIES OF MICROELEMENTS IN GREEN PLANTS V. EFFECTS OF MICROELEMENT DEFICIENCIES ON THE RATES OF PHOTOSYNTHESIS AND RESPIRATION OF RICE AND BARLEY PLANTS

著者	FUJIWARA Akio, TSUTSUMI Michio
journal or publication title	Tohoku journal of agricultural research
volume	13
number	4
page range	389-395
year	1963-02-09
URL	http://hdl.handle.net/10097/29419

BIOCHEMICAL STUDIES OF MICROELEMENTS IN GREEN PLANTS

V. EFFECTS OF MICROELEMENT DEFICIENCIES ON THE RATES OF PHOTOSYNTHESIS AND RESPIRATION OF RICE AND BARLEY PLANTS

By

Akio FUJIWARA and Michio TSUTSUMI

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received, September 25, 1962*)

Micro-nutrient-elements for plants, such as iron, copper, manganese, and zinc, are considered to participate in the respiration process in a broad sense of plants, because iron and copper have been found to be functional parts of particular oxidative enzymes present within the plant-tissues (1), and manganese and zinc have been shown to act as essential cofactors in the process of glycolysis and Krebs' cycle which have been found to anticipate and link to the terminal oxidative systems (2). Therefore, it may be expected that the deficiency of any one of these elements affects the rate of respiration of plant-tissues perhaps suppressively.

Nason *et al.* (3) have measured the endogenous oxygen uptake of the homogenates prepared from the leaf-tissues of the microelement deficient tomato plants, as well as the activities of several oxidative enzymes. However, they have not examined the respiration itself of the intact leaves. The status of the respiration of intact leaves should not be identical with that of such a endogenous oxygen uptake. Moreover, the effects of microelement deficiencies on the activities of oxidative enzymes have been studied by other workers (4) (5), but the information concerning those on the respiration of the leaf-tissue appeares to be scanty. Thus, the rates of respiration were measured on the rice and barley leaves deficient in microelements.

Previously (6), the rates of photosynthesis have been measured on the barley leaves deficient in microelements and found to be remarkably reduced. Thus, The rates of photosynthesis or the chlorophyll contents were also determined on the same materials as used in the respirometry, in an attempt to compare the changes in the rate of respiration with those in the rate of photosynthesis caused by the microelement deficiencies.

Materials and Methods

Deficient culture of plants: The one or two week old rice and barley seedlings were grown on the cultural solutions from which iron, manganese, zinc, and copper were eliminated individually as described before (7). Rice plants were grown for two weeks in summer and barley plants for six weeks in autumn. But exceptionally, the rice plants on the copper eliminated nutrient solution were grown for three weeks since the effects of deficiency on the growth did not appear within two weeks.

Techniques of measurements: Immediately after the plants were taken, the blades of the upper expanded leaves were cut into the slices of 2 cm in length. Fifteen slices were placed on 2 *ml* of water contained in the Warburg's vessels of about 15 *ml* in capacity, then the oxygen uptake was measured at 28°C for 2 hours according to the ordinary Warburg's manometric method.

The rate of respiration was determined as the volume of oxygen uptaken per mg dry weight of these materials per hour.

The chlorophyll content was determined spectrophotometrically on the extracts of the leaf-blades by 80 per cent acetone, and the rate of photosynthesis was measured by tracing the incorporation of $^{14}CO_2$ into the blades of upper expanded leaves. The incorporation of $^{14}CO_2$ was made as described previously (6).

Results

Growth of plants: Both rice and barley plants used in this experiment revealed not only the deficiency symptoms but also the retardation of growth within each growing period. The status of the growth can be seen in Table 1 and 2. Though the retardation of growth in the rice plant deficient in copper was comparatively small, the deficiency symptom that the expanding young leaf became yellow brown in color and withered was observed by the end of the three week cultural period.

Table 1. Status of growth of rice plants deficient in microelements.

Treatment	Length of shoot	Dry weight of shoot per plant	Dry weight/ Fresh weight
Full nutrients	30.0 cm	64.2 mg	26.4
−Fe	21.7	26.3	21.6
−Mn	18.0	19.1	21.8
−Zn	17.7	21.5	27.0
Full nutrients	31.4	126.0	31.8
−Cu	33.1	109.8	31.5

Table 2. Status of growth of barley plants deficient in microelements.

Treatment	Length of shoot	Dry weight of shoot per plant	Dry weight/ Fresh weight
Full nutrients	14.8 cm	134 mg	20.5
−Fe	14.1	96	18.0
−Mn	9.8	54	15.9
−Zn	8.5	49	22.9
−Cu	10.9	81	15.2

Status of leaf blades: The concentration of total- and protein-nitrogen and chlorophyll in dry matter of the leaf blades are shown in Table 3 and 4. Both species of plants showed the similar tendencies about the effects of microelement deficiencies on these chemical status.

Table 3. Total- and protein-nitrogen and chlorophyll contents in rice leaf blades deficient in microelements (% dry weight)

Treatment	Total-nitrogen	Protein-nitrogen	Total-chlorophyll	Chlorophyll a/b
Full nutrients	4.46	3.74	1.57	3.10
−Fe	5.45	3.86	0.86	3.99
−Mn	5.63	3.86	0.99	2.72
−Zn	6.59	2.84	1.07	2.84
Full nutrients	4.35	3.56	1.18	2.87
−Cu	5.00	3.76	0.92	3.54

Table 4. Total- and protein-nitrogen and chlorophyll contents in barley leaf blades deficient in microelements (% dry weight)

Treatment	Total-nitrogen	Protein-nitrogen	Total-chlorophyll	Chlorophyll a/b
Full nutrients	5.60	4.41	1.01	2.77
−Fe	6.33	4.17	0.40	3.42
−Mn	6.90	4.78	0.76	2.64
−Zn	6.06	2.68	0.54	2.95
−Cu	6.78	4.93	0.90	3.36

Rate of photosynthesis: The rate of photosynthesis was measured on rice plant only and the results obtained are given in Table 5, where the data are represented as the index number of the counts of photosynthetically incorporated ^{14}C per unit dry weight of leaf blade and that per unit weight of chlorophyll.

For the barley plants, the rate of photosynthesis had been measured already in the previous work (3) and the results obtained were generally consistent with those for the rice plants as shown Table 5. The results that the chlorophyll contents of leaf blades and the dry matter productions, an apparent resultant of photosynthesis, decreased (Table 2, 4) appear to be the evident indication for the reduction of the rate of photosynthesis of the barley plants used in this experiment.

Table 5. Rate of photosynthesis in rice leaf blades deficient in microelements.

Treatment	Index number on dry weight basis	Index number on chlorophyll basis
Full nutrients	100.0	100.0
−Fe	50.7	91.8
−Mn	35.8	57.0
−Zn	37.5	55.1
−Cu	61.6	78.5

Rate of respiration: Table 6 shows the rates of respiration of both species of plants represented as the oxygen uptake on the basis of dry weight and protein-nitrogen per hour. In general, the oxygen uptake of barley leaves was apparently higher than that of rice leaves. This tendency may be caused by the difference of the range of the temperatures between at the respiration measurement and at the cultures of rice plants and barley plants. Setting aside this problem, though it seems to be an interesting one, the effects of deficiencies of microelements examined on the rate of respiration showed features common between both species of plants.

Table 6. Rate of respiration of rice and barley leaf blades deficient in microelements

Treatment	O_2 uptaken by rice leaf blades per hour		Treatment	O_2 uptaken by barley leaf blades per hour	
	μl/mg dry weight	μl/mg protein-N		μl/mg dry weight	μl/mg protein-N
Full nutrients	2.58	69.0	Full nutrients	4.46	101.1
−Fe	2.68	69.3	−Fe	4.01	96.2
−Mn	4.35	112.7	−Mn	5.75	120.2
−Zn	2.87	101.0	−Zn	4.36	162.5
			−Cu	4.16	86.2
Full nutrients	2.58	72.5			
−Cu	2.37	63.1			

In spite of the evident retardation of growth, the decreases of the rate of photosynthesis and the chlorophyll content, the oxygen uptake on the dry weight basis and protein basis did not change significantly in iron deficient leaves. On the contrary, the remarkable increases of oxygen uptake were found in manganese deficient leaves on the dry weight basis and manganese and zinc deficient ones on the protein-nitrogen basis. In copper deficient leaves, though the oxygen uptake appeared to decrease slightly the decrease was not so evident as that of the rate of photosynthesis in rice plants or that of growth in barley plants. At any rate, according to these results, it may be concluded that the depressed changes due to the deficiencies in the microelements are brought about primarily to a great extent not on the rate of respiration but on the rate of photosynthesis.

Some inconsistent trends were found between the changes of the oxygen uptake by the barley leaf blades deficient in microelements revealed in this experiment and those by the tomato leaf homogenates described in the report of Nason *et al.* (3). For instance, in copper deficiency the oxygen uptake by the leaf blades changed only slightly whereas that by the leaf homogenate increased remarkably and in manganese deficiency *vice versa*. These inconsistencies may be due to the difference of species of the plants used, so this problem should be studied further on the same plant materials.

Discussion

Brown *et al.* (4, 5) have reported that the activities of catalase and peroxidase, the iron enzymes, and ascorbic acid oxidase, a copper enzyme, in plant tissues of several species are depressed due to the deficiencies of iron and copper respectively. However, it is still unknown whether these enzymes play any roles in the respiratory process of the plants (10). It is cytochrome oxidase that is accepted in general as the functioning terminal oxidase in the plant respiration (10), and recently Griffiths *et al.* (11) have found that cytochrome oxidase contains copper as well as iron as the functional constituent for its activity. Although the cytochrome oxidase examined by Griffiths *et al.* has been prepared from not the plant material but the beef heart and also the authors have not noticed any reports concerning the change of activity of cytochrome oxidase in plant tissues caused by the deficiencies of iron and copper, it may be supposed that the concentration of this enzyme within the tissues decreases due to the deficiency of constituent metal, namely either iron or probably copper. If it had happened, however, it might be difficult that the decrease of this enzyme reached to so far an extent as limiting the rate of respiration, because the oxygen uptake did almost not decrease by the shortage of neither iron nor copper whereas the rate of photosynthesis, for instance, decreased evidently in both cases.

Since in the zinc deficient leaves the concentration of protein-nitrogen decreased remarkably, it may also be supposed that the concentrations of the proteins of oxidative enzymes become low in parallel with it. However, the oxygen uptake was maintained at the same level as the normal one. Therefore, if the decrease of oxidative enzyme-protein occured, it might not exceed the limit from which the rate of respiration begins to be checked. Moreover, the rate of respiration on the basis of protein-nitrogen in the zinc deficient leaves was found to increase considerably. This increase may be attributed to the decrease of the chloroplast content as described previously (6), assuming that the proteins of respiratory enzymes do not decrease so largely as the chloroplast protein which decreases accompaning with chloroplasts themselves. Generally speaking, it appears that the cellular oxidative enzymes can hardly be lowered over the extent from which the rate of respiration begins to be limited.

The acceleration of the cell respiration is recognized to be caused for one thing when the oxidation of the respiratory substrate is not coupled with the phosphorylation that forms the energy rich phosphate compound (12). The respiration of some plant materials has been shown to be accelerated by the administration of an uncoupling agent such as dinitrophenol (8, 13, 14). On the other hand, Lindberg *et al.* (9) have found that manganese plays the role as a co-factor in the oxidative phosphorylation of the rat liver mitochondria. If this is assumed to be so in the plant cells, the increase of the oxygen uptake in manganese dificient leaves can reasonably be explained by regarding the manganese deficiency as an uncoupling action. The authors hope to study further on this question.

Summary

The rates of photosynthesis and respiration of rice and barley plants defiicient in iron, manganese, zinc, and copper were measured.

The changes of the rate of respiration due to the microelement deficiencies showed features common between both species of plants. The rate of respiration of leaves deficient in iron and copper changed only a little whereas that of manganese deficient leaves increased remarkably and that of zinc deficient leaves on the protein-nitrogen basis also increased, On the contrary the growth, the chlorophyll content, and the rate of photosynthesis were found to be low in general. It was infered, therefore, that the depressed changes due to the deficiencies of these elements are brought about primarily not on the respiratory process but on the process of photosynthesis.

References

1) Sumner, J.B. and G.F. Somers (1953). Chemistry and Methods of Enzymes, Academic Press, N.Y.
2) MacElroy, W.D. and A. Nason (1954). Annual Review of Plant Physiol., **5**, 1.
3) Nason, A., Oldewurtel, H. A. and L. M. Propst (1952). Arch. of Biochem. and Biophys., **38**, 1.
4) Brown, J.C. and S.B. Hendricks (1952). Plant Physiol., **27**, 651.
5) Brown, J.C. and R.A. Steinberg (1953). Plant Physiol., **28**, 488.
6) Fujiwara, A. and M. Tsutsumi (1962). Tohoku J. Agr. Res., **13**. 169.

7) Fujiwara, A. and M. Tsutsumi (1957). Ibid., **7**, 397.
8) Stenlid, G. (1949). Physiol. Plant., **2**, 61.
9) Lindberg, O, and L. Ernster (1954). Nature, **173**, 1038.
10) Goddard, D.R. and W.D. Bonner (1960). Plant Physiology, **IB**, (Ed. Steward F.C.), Academic Press, N.Y.
11) Griffiths, D.E. and D.C. Wharton(1961). J. Biol. Chem., **236**, 1857.
12) Krebs, H. (1959). Regulation on Cell Metabolism, (Ed. G. E. W. Wolstenholme) J. and A. Churchill, London.
13) Eliasson, L. and I. Mathiesen (1956). Physiol. Plant., **9**, 265.
14) MacDonald, I.R. and P.C. Dekock (1958). Ibid., **11**, 464.