GROWTH AND COPPER UPTAKE OF RICE AND BARLEY PLANTS WITH VARYING COPPER SUPPLY

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By

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Introduction

By the pot culture with the soil of Kawatabi Farm of Tohoku University, barley plants suffered from copper deficiency so severely that they died back without heading. This soil is a volcanic ash soil acidic, rich in humus in the upper part of the profile, and short in nutrients including magnesium and copper. However, rice plants did not show any deficiency symptoms and could produce normal heads on the same soil (1).

The different susceptibility of plant species to copper deficiency, as shown between these plants, has been well known since early times, and some workers (2, 3) suggested that the cause of this difference relates with the dominancy of copper enzymes such as ascorbic acid oxidase in the cellular terminal oxidases. On the other hand, the authors (4) found that the decreasing effects of copper deficiency on cellular metabolic activities emerged primarily on the photosynthetic carbon dioxide assmilation but did not on the rate of respiratory oxygen uptake in both plant species. Consequently, it is supposed that the cause of the difference might relate with the requirement for copper in the photosynthetic processes.

In the first place, however, it was considered necessary to ascertain whether the cause of the difference depends upon the real nutritional requirement for copper or merely upon the power to absorb copper from the medium or upon the ability to redistribute and reuse the limited amount of absorbed copper from senescent parts to actively metabolizing parts within plant. Thus, rice and barley plants were grown on the soils of Kawatabi Farm and also in cultural solutions deficient or sufficient in copper to examine their growth and copper uptake.

Materials and Methods

Soil culture Rice (*Oriza sativa* var. Norin No. 16) and barley (*Hordeum vulgare* var. Shokimugi) were grown on 1/5000 a Wagner's pots each filled with 3kg of air dried soils which were taken separately from surface, subsurface, and sub-layer of Kawatabi Farm, Tohoku University. For rice 1.0 g of NH_4NO_3, 3.0 g of $Ca(H_2PO_4)_2 \cdot H_2O$, 0.4 g of K_2SO_4, and 0.1 g of $MgSO_4 \cdot 7H_2O$ were supplied per pot. For barley 1.8 g of NH_4NO_3, 5.0 g of $Ca(H_2PO_4)_2 \cdot H_2O$, 1.0 g of K_2SO_4, and 0.5 g of $MgSO_4 \cdot 7HO_2$ were supplied and moreover 7.5 g, 15 g, and 3 g of $CaCO_3$ were added respectively to surface, subsurface, and sub-soil in oder to neutralize the soil acidity. To one half the pots of respective soils 30mg of $CuSO_4 \cdot 5H_2O$ for rice and 60 mg for barley were supplemented.

These plants were grown in a green house always receiving deionized water which was made by ion exchange resin and had at least $10^6 \Omega/cm$ of resistance and less than 3 ppb of copper concentration. The soil condition of rice culture was not water-logged so as to make it comparable with that of barley culture. Rice plants were grown from 17th June to 17th October 1964 and barley from 16th December 1964 to 1st June 1965 and a group of the plants were taken as an intermediary harvest on 28th July for the former and on 3rd March for the latter. Rice plants were top-dressed with 1.0g of $(NH_4)_2SO_4$ and 0.2 g of K_2SO_4, on 12th August. The aerial portions harvested were oven-dried, weighed, and analysed for copper.

Water culture Since the amounts of nutrients involving copper supplied for barley differed from those for rice in the above soil culture, and in order to examine the problem at earlier stage of growth, these plants were grown respectively on the purified cultural solution of the same composition.

Ten-day old seedlings, which were brought up on glass distilled water, were transplanted on two-liter Pyrex beaker filled with a solution of the following composition: 1.2 mM $(NH_4)_2SO_4$, 0.6mM $NH_4H_2PO_4$, 1.5 mM KNO_3, 0.75 mM $Ca(NO_3)_2$, 1.0 mM $MgSO_4$, 1ppm Fe, 0.5ppm B and Mn, 0.05ppm Zn, 0.01 ppm Mo. The concentrations of copper were 0.00, 0.02, 0.5, and 2.5 ppm. The last two were involved as the treatment of high and excessive copper supply. The stock solution of macronutrient salts was purified by the copper sulfide co-precipitation method and glass distilled water was used for the cultural solution.

Barley seedlings were transplanted on 24th April and rice on 22th June 1965. They were harvested at two and three weeks after the transplantation and separated into aerial and root portion then oven-dried.

Copper determination The oven-dried materials were ground with a porcelain mortar and pestle and incinerated at 550°C then dissolved into 0.1N HCl. This ash solution was analaysed for copper by the colorimetric method with dithizone carbon tetrachloride solution (5).

Results and Discussion

Three separated soils from surface, subsurface, and sub-layer were used because, as will be reported in detail elsewhere, the 0.1N HCl extractable copper of the soils was found to differ with each other, *i.e.* 0.25ppm in surface, 0.09ppm in subsurface, and 0.13ppm in sub-soil. The growth (oven-dry weight per plant) on the soils is given in Table 1. It was observed that barley plants on subsurface soil without copper supply revealed a characteristic deficiency symptom of copper, namely unexpanded slim new leaves, by the time of the intermediary harvest, then died back before the last harvest time. As is shown in Table 1, they could not make heads at all and barley could produce only stunted ears on sub-soil but made almost normal growth and ear production on surface soil without copper supply. This result well agreed with the status of available copper in these soils as above mentioned. When copper was supplied, however, barley plants produced normal heads even on subsurface or sub-soil. On the contrary, rice plants showed normal growth and ear production regardless of the soil used and copper supply. Thus, it is evident that rice plants are resistant to copper deficiency whereas barley plants are susceptible to it.

Table 1. Relation between copper supply and growth of rice and barley plant on Kawatabi soil (oven dry weight in g/plant)

Plant	Copper supply	Soil	Intermediary harvest	Last harvest	
				Stems and leaves	Ears
Rice	−	Surface	0.91	10.31	5.31
		Subsurface	1.19	9.44	4.50
		Sub	1.31	9.52	4.29
	+	Surface	1.29	10.80	5.10
		Subsurface	1.38	9.63	5.24
		Sub	1.54	8.73	4.14
Barley	−	Surface	0.52	3.22	4.58
		Subsurface	0.36	1.88	0
		Sub	0.60	5.17	0.05
	+	Surface	0.56	4.22	4.92
		Subsurface	0.54	3.42	3.83
		Sub	0.68	5.58	3.63

The copper concentration in the whole shoot of the intermediary harvest is given in Table 2 and that in leaf-blades,-sheaths, and stems of the uppermost two nodes of the last harvest is in Table 3. The uppermost two nodes were analysed by the reason that copper deficiency appears most remarkable at the heading stage and the uppermost leaves have been known to be exclusively responsible

Table 2. Concentration of copper in the aerial portion of intermediary harvest of rice and barley

Copper supply	Soil	Rice	Barley
−	Surface	4.2 ppm	5.4 ppm
	Subsurface	2.8	3.0
	Sub	1.8	4.2
+	Surface	8.6	7.0
	Subsurface	7.0	8.5
	Sub	8.0	7.8

Table 3. Concentration of copper in leaf-blade, -sheath, and stem of the uppermost two nodes of rice and barley plant at the last harvest

Copper supply	Soil	Rice			Barley		
		Blade	Sheath	Stem	Blade	Sheath	Stem
−	Surface	4.6 ppm	2.1 ppm	2.4 ppm	5.6 ppm	5.4 ppm	1.9 ppm
	Subsurface	1.5	0.6	1.4	3.8	2.2	4.2
	Sub	1.8	3.2	1.2	2.1	1.6	1.9
+	Surface	4.2	2.4	3.7	6.3	3.6	3.9
	Subsurface	4.1	2.4	3.0	7.0	2.8	4.6
	Sub	5.6	4.9	4.3	11.0	4.2	5.0

for the development of ears.

Table 2 shows that the concentration of copper in the intermediary harvest of barley grown without copper supply was lower on sub-soil than on surface soil and on subsurface soil than on sub-soil. This result also is in accord with the status of available copper in the soils. The concentration of copper in rice plants on each soil without copper supply was rather lower than that in barley on the corresponding soil. In both plant species copper concentration was increased to almost the same level by copper supply. As is shown in Table 3, the results almost similar to the intermediary harvest were found with the last harvest especially with leaf-blades of the uppermost two nodes.

Connecting these results, it may be concluded that the resistance of rice to copper deficiency is not due to the ability to absorb copper from deficient media or to redistribute it from the senescent parts to the upper active parts being so efficient as the copper concentration in shoots or leaves reaches to a level in those of sufficient copper supply, but due to the nutritional requirement for copper being low.

The growth of plants obtained by the water culture experiment is shown in Fig. 1. From this result it was confirmed that barley is more susceptible to copper deficiency than rice even at a very early stage of growth and moreover that the

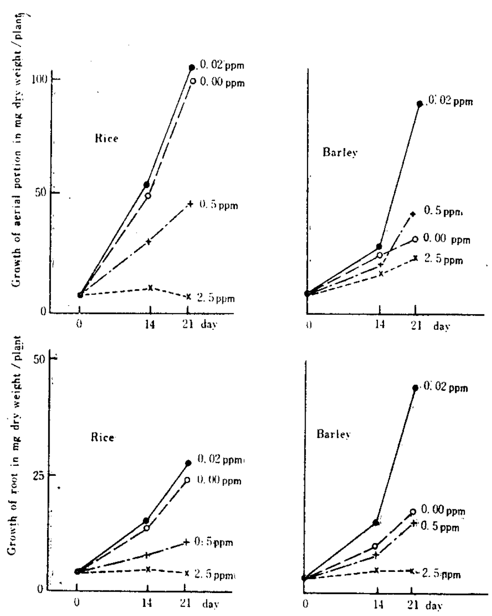

Fig. 1. Relation between growth and copper supply in rice and barley plants

Table 4. Concentration of copper in the aerial and root portion of rice and barley grown by water culture

Plant	Portion	Harvest	Copper supply in ppm		
			0.00	0.02	0.5
Rice	Aerial	1st	3.0 ppm	22.6 ppm	53.6 ppm
		2nd	2.5	13.8	43.3
	Root	1st	4.1	52.1	635
		2nd	5.3	23.9	713
Barley	Aerial	1st	3.3	8.5	64.0
		2nd	3.6	8.7	52.9
	Root	1st	5.8	16.1	195
		2nd	6.0	12.0	269

former is more resistant to excessive copper than the latter. The copper content of these plants is given in Table 4. The result obtained with copper deficient plants agreed with that from the soil culture experiment. Although the growth of plants receiving 0.00ppm copper was by far nearer the normal one in rice than in barley, the copper content was decreased equally in both plants. But copper content of rice roots supplied with 0.02ppm and 0.5ppm copper was much higher than that of barley roots. Total amount of copper taken up by plants on a two-litre beaker was calculated for the first harvest and is given in Table 5. Since the amount absorbed by rices receiving 0.02ppm copper was found to be 41.1γ, it may be estimated that they absorbed the supplied copper exhaustively for two weeks whereas barleys of the same treatment did not. Accordingly, copper content of the second harvest of the former should become about one half that of the first harvest. In general, the total amounts of copper was found to be greater in rice than in barley of the corresponding treatments. Perhaps, this could be seen from the soil culture experiment for the dry matter produced by rice was much greater than that by barley while copper content commonly differed only slightly between both plants of the same treatment. At any rate, the data in Tables 4 and 5 suggest that rice plants, especially its roots, tend to absorb copper more

Table 5. Amounts of copper taken up by 24 plants of rice and barley from a beaker at 2 weeks after transplantation

Copper supply	Rice	Barley
0.00 ppm	4.21 γ	3.56 γ
0.02	41.10	12.12
0.5	109.3	77.9

efficiently than barley and that the cause of the high susceptibility to excessive copper of rice might relate with this tendency. It should be interesting to know whether this tendency holds for other heavy metals than copper or not.

To sum up the results obtained by the soil and the water culture experiments, it may be concluded that rice plants are more resistant to copper deficiency and more susceptible to excessive copper than barley plants and that the former appear to have a tendency to absorb copper more efficiently than the latter, but that the copper concentration in shoots or leaves was equally lowered in both plants grown on the copper deficient media, so the minimal nutritional requirement for copper is probably lower in rice than in barley.

Summary

The relation between growth, copper uptake and status of copper in media was examined comparatively for rice and barley plants by soil and water culture methods.

It was confirmed that rice plants are more resistant to copper deficiency and more susceptible to excessive copper than barley plants. Though rice appears to absorb copper more efficiently than barley, the minimal nutritional requirement for copper within plant-tissues is probably lower in the former than in the latter judging from the equally lowered copper content of both plants grown on the deficient media.

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