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SHORT COMMUNICATION

VARIABLE TOLERANCE OF TWO GENOTYPES OF RICE TO EXCESS COPPER

B.K. DUBE, RAJEEV GOPAL, PRATIMA SINHA AND C. CHATTERJEE*

Department of Botany, Lucknow University, Lucknow-226 007, India

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To assess comparative copper tolerance and its inhibitory effect on metabolism of rice (*Oryza sativa* L.) two genotypes viz. Pusa Basmati and Pusa Sharbati were grown in refined sand in complete nutrient solution for 36 days after sowing. On 37th day plants were divided into 3 lots. One lot was allowed to grow with complete nutrient solution to serve as control (0.001 mM Cu) while the other two lots were supplied with excess copper (as copper sulphate) at 0.1 and 0.2 mM, respectively. After 9 days of metal supply (d 46), at 0.2 mM Cu, growth of rice was depressed and young leaves developed marginal interveinal chlorosis. Later the effects intensified and irregular brown necrotic spots developed on the affected leaves. With increase in age the affected leaves were completely bleached, The symptoms were delayed by seven days in plants at 0.1 mM Cu. Excess copper reduced the biomass, concentration of chlorophylls a, b, total and active iron and activities of catalase, acid phosphatase and polyphenol oxidase and increased the activity of peroxidase in leaves. In both the genotypes, the accumulation of Cu was higher in roots, than leaves, more so in Pusa Sharbati than Pusa Basmati. Rice genotype Pusa Sharbati appears to be more sensitive to Cu toxicity.

***Key words* : Copper, metabolism, phytotoxic lesions, rice.**

Copper is extesively used in agriculture in the form of fertilizers, growth promoters, bactericides(Adriano, 1986). This in turn creates copper toxicity in soils. Normal growth of plants requires 5-20 ppm of Cu (Marschner 1995) and less than 4 ppm is deficient, more than 20 ppm is considered to be toxic. Reports suggest that deficiency of Cu affects multitude of physiological processes in plants such as carbohydrate metabolism, cell wall metabolism, water relations, seed production and disease resistance (Bussler 1981).

Cu accumulates through roots in plants, even in such cases where roots have been damaged by toxicity (Jiang *et al*. 2000). The toxicity effects of excess Cu by different plant species are usually accompanied by 100 – 300 ppm Cu. The toxic effects of excess Cu in various plants are reduction in growth, poorly developed and discolored root system, leaf chlorosis, stunted plants with reduced branches and reduction in economic yield due to male sterility. The yellowing of young leaves in excess Cu is often similar to that of iron chlorosis (Hewitt 1983). In this paper efforts have been made to differentiate the effect of excess Cu on rice genotypes on the basis of changes in metabolism and growth by growing the plants in refined sand.

Rice (*Oryza sativa* L.) genotype Pusa Basmati and Pusa Sharbati were grown in refined sand in a glass house under controlled conditions (Agarwala and Chatterjee 1996). Plants were grown in polyethylene containers (5L capacity) having a central drainage hole, which was covered with an inverted watch glass whose rim was lined with glass wool. The composition of the complete nutrient solution was: 4 mM KNO_3; 4 mM $Ca(NO_3)_2$; 2 mM $MgSO_4$; 1.5 mM NaH_2PO_4; 100µM Fe-EDTA; 10µM $MnSO_4$; 30µM H_3BO_3; 1µM $CuSO_4$;

* Corresponding author

1µM $ZnSO_4$; 0.2µM Na_2MoO_4; 0.1µM $NiSO_4$; 0.1µM $CoSO_4$ and 0.1 mM NaCl. The plants were maintained in complete nutrient solution for 36 days after sowing. On 37th day plants were divided into three lots. One lot was allowed to grow with complete nutrient solution to serve as control. The remaining two lots were supplied with excess copper (as copper sulphate) at 0.1 and 0.2 mM, respectively. In each treatment there were four replicates and in each pot two plants were maintained. The nutrient solution was supplied daily, except on Sundays when the sand was flushed with distilled water to remove accumulated salts and deleterious substances from the rooting medium.

In addition to the periodical record of visible toxicity symptoms, both genotypes were harvested and sampled at d 70 (33 days after Cu supply) for biomass and concentration of Cu and Fe in different parts. Thoroughly washed plant material were dried in an electric oven at 70^0 C for 48h. After wet digestion in HNO_3: $HClO_4$ (10:1) , the concentration of Cu and Fe were estimated in clear digests by using Atomic Absorption Spectrophotometer.

All metabolic parameters were measured in fully expanded young leaves. The concentration of chlorophylls (a and b) at day 46 (9 days after Cu supply) and activities

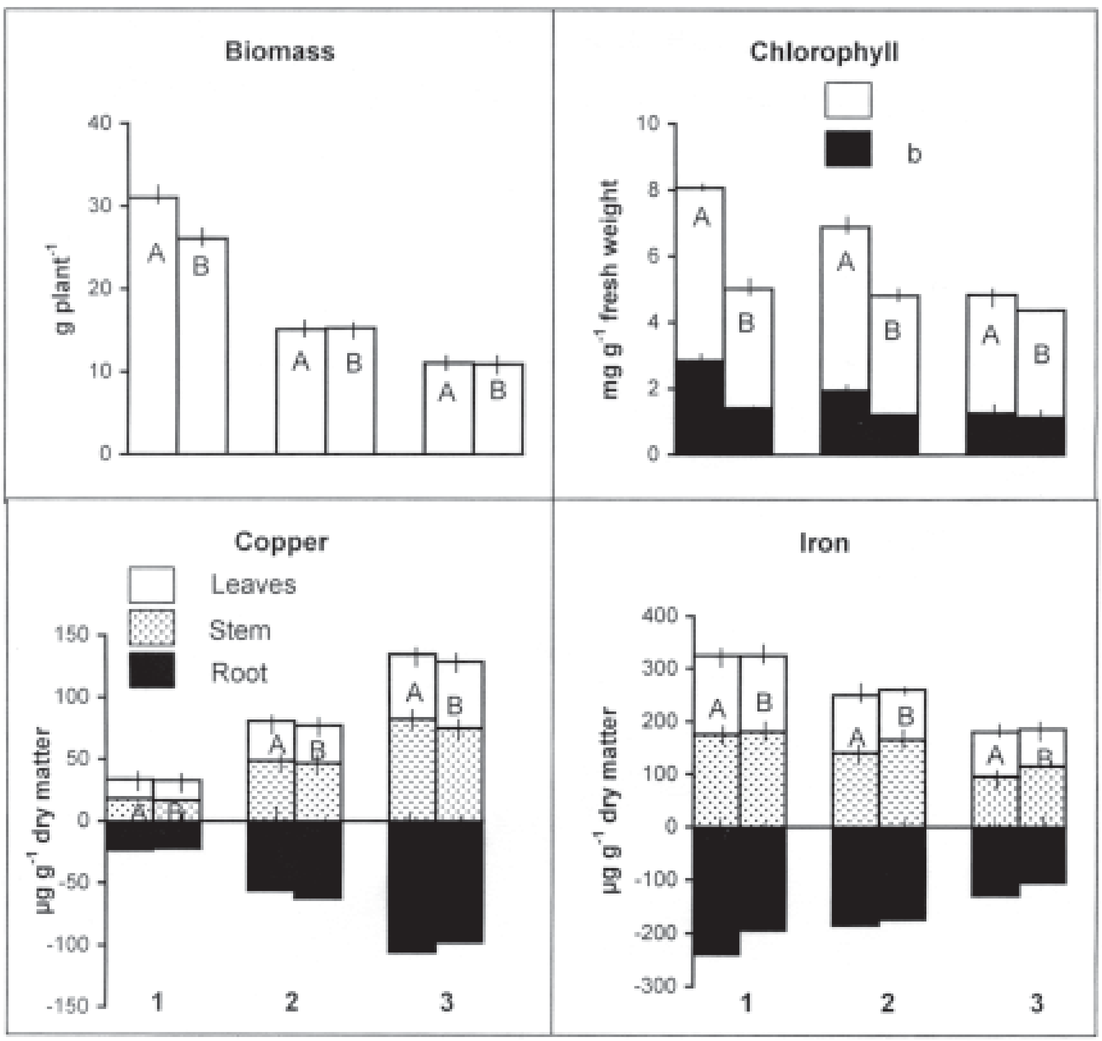

Fig. 1. Effect of Cu on biomass, chlorophyll, copper and iron concentration in various parts of rice genotypes. Vertical lines denote ± SE. A = Pusa Basmati, B = Pusa Sharbati, 1 = 0.001 (control), 2 = 0.1 and 3 = 0.2 mM copper

Table 1. Effect of excess Cu on height, active iron and specific activity of some enzymes in rice genotypes (±SE).

Days after metal supply	Pusa Basmati mM Cu 0.001	0.1	0.2	Pusa Sharbati mM Cu 0.001	0.1	0.2
			Height (cm)			
33	61±0.82	43±0.76	28±0.18	54±0.61	44±0.32	32±0.24
			Active Fe (μg g^{-1} fresh weight) With hydroxylamine			
28	120±1.34	102±1.68	70±1.02	116±1.18	99±1.32	73±1.12
			Without hydroxylamine			
	70±0.42	64±0.72	58±0.32	64±0.63	63±0.58	54±0.28
			Peroxidase (μmol p- phenyldiamine oxidised min^{-1} mg^{-1} protein)			
12	0.216±0.005	0.22±0.001	0.252±0.005	0.204±0.001	0.216±0.002	0.24±0.005
			Catalase (μmol H_2O_2 decomposed min^{-1} mg^{-1} protein)			
	349±12.0	334±7.2	305±1.5	340±8.0	330±6.2	275±0.9
			Acid phosphatase (μg Pi liberated min^{-1} mg^{-1} protein)			
	11.5±0.005	9.24±0.03	8.64±0.01	11.5±0.01	8.10±0.02	7.77±0.01
			Polyphenol oxidase (μmol DOPA oxidised min^{-1} mg^{-1} protein)			
	0.054±0.001	0.024±0.0	0.021±0.0	0.024±0.001	0.003±0.001	0.009±0.0

of peroxidase, catalase, polyphenoloxidase and acid phosphatase at day 49 (12 days after Cu supply) were assayed as per the methods describe earlier by Nautiyal *et al*. (1999). Active iron was also determined (Mehrotra and Jain 1992) in fresh leaves at day 65 (28 days after Cu supply). For expressing specific activity of enzyme, soluble protein in crude leaf extracts was determined (Bradford 1976). All determinations were carried out in triplicate and data were tested for significance (± SE) (Sukhatme and Amble 1985).

In rice genotypes visible symptoms of excess copper were discernible 9 days after treatment in Pusa Sharbati and at 14 days after treatment in Pusa Basmati. Excess copper depressed the growth of rice after 15days of Cu supply more at 0.2mM. The visible symptoms of excess copper are somewhat superficially similar to that of iron deficiency (Van Assche and Clijsters 1990, Lanaras *et al*. 1993). At day 46 (9 days after Cu supply) the young leaves exhibited interveinal chlorosis towards the apex and it gradually spread downwards. Irregular brown necrotic spots appeared near the margins of the affected chlorotic leaves. After few days, the affected leaves of genotype Pusa Sharbati at excess Cu (0.2 mM) completely bleached followed by necrosis initiating from the base. In these plants, the growth of root system was also reduced (Foy *et. al*. 1978). At this stage owing to excess Cu , growth as well as tillering of plants was highly restricted. These symptoms were more severe in Pusa Sharbati than in Pusa Basmati at 0.2 mM Cu supply. The effects of copper toxicity were less severe at 0.1 mM copper supply in both the genotypes. The growth of root system was also restricted as has been observed in taro (Hill *et al*. 2000) and sunflower (Jiang *et al*. 2000), where it has been suggested that a large copper supply usually restricts root length, lead to macronutrient depletion in the restricted rooting zone, consequently inhibiting plant growth.

The decrease in biomass of rice genotypes (Fig. 1) might be due to poor protein synthesis (Marschner 1995) or due to restriction in photosynthesis at excess Cu as a consequence of alterations in the structure of chlorophyll (Hill *et al*. 2000) in excess Cu treatments. The decrease in biomass of rice in excess copper might also be due to disturbed nitrogen and carbohydrate metabolism in such conditions. This was also supported by higher accumulation of copper in various plant parts including that of roots. These results are somewhat similar to that in sugarcane (Agarwala *et al*. 1993) and tomato (Liao *et al*. 2000) suggesting immobility of copper from lower to upper parts. The increase in copper concentration in excess copper treated rice genotypes (Fig. 1) is simultaneously accompanied by a decrease in the uptake and translocation of iron, the magnitude of change in nutrient concentration was almost same in both the genotypes. Copper toxicity effects in rice genotypes closely resembled iron deficiency, which might be due to displacement of iron by copper (Van Assche and Clijsters 1990, Lanaras *et al*. 1993). The less availability of iron due to excess Cu is also responsible for low chlorophyll a and b concentration (Fig. 1) as iron is required for the synthesis of chlorophyll and this in turn reduces the net photosynthetic rate resulting in low biomass of excess copper treated rice (Hill *et al*. 2000). Compared to total, the decrease in active iron as a result of excess copper might suggest that requirement of iron for utilization in different metabolic processes is not facilitated properly under excess copper conditions and as a consequence of unavailability of adequate iron at different molecular levels, iron deficiency type effects appeared on plants at excess copper.

In rice genotypes, the low activity of catalase (Table 1) one of the anti- oxidative enzymes in leaves of rice genotypes treated with excess copper. It should be due to complete or partial replacement of Fe from active sites or might be due to hindrances in incorporation of Fe in the protein moiety of enzymes and are similar to that reported for barley (Agarwala *et al*. 1977) and oat (Luna *et al*. 1994). The peroxidase activity was stimulated (Table 1) at Cu concentrations in rice genotypes. This enzyme plays a protective role by scavenging O_2 (superoxide radical) and H_2O_2 and minimizing catalytic iron in the system (Stohs and Bagchi 1995, Foyer *et. al*. 1997).

The decrease in acid phosphatase activity (Table 1) in excess copper condition in rice genotypes might be due to low inorganic phosphorus pool creating disturbances in incorporation of phosphorus in different biomolecules (Tyler 1976, Agarwala *et al*. 1985). The significant decrease in polyphenol oxidase (Table 1) in leaves of excess Cu treated rice genotypes is similar to that reported on some other crops (Marschner 1995, Nautiyal *et al*. 1999).

From the observed results in rice genotypes it appears that excess Cu damaged the metabolism in addition to appearance of characteristic and specific visible symptoms of high copper. These effects were more pronounced in genotype Pusa Sharbati than Pusa Basmati rice.

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