## Effect of bicarbonate and root zone temperature on uptake of Zn, Fe, Mn and Cu by different rice cultivars (*Oryza sativa* L.) grown in calcareous soil

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#### Abstract

Pot experiments were conducted with a calcareous soil (Inceptisol) to elucidate the effects of bicarbonate (0 and 20 mM) and root zone temperature (15° and 25°C) on the uptake of Zn, Fe, Mn and Cu by "Zn-efficient" and "Zn-inefficient" rice cultivars. Bicarbonate decreased concentrations and total uptake of Zn in shoots of "Zn-inefficient" cultivars, especially of IR 26 at 25°C, but not in Zn-efficient cultivars. Bicarbonate decreased concentrations and uptake of Fe in shoots of Zn inefficient cultivars, particularly in IR 26. Concentrations and total uptake of Mn were lower in bicarbonate treatment in the Zn-inefficient cultivars at 15°C, and in all cultivars. Compared to the 25°C root zone temperature, the concentrations and total uptake of both Zn and Cu in shoots at 15°C were lower in Zn-inefficient than in the Zn-efficient cultivars. The results indicate that Zn-efficiency in rice is causally related to high tolerance of plant to elavated bicarbonate concentrations in soil solution.

#### Introduction

Bicarbonate has been regarded as a major causal factor for Zn deficiency in rice grown on calcareous soil (Forno et al., 1975; Yoshida and Tanaka, 1969). Bicarbonate might inhibit root absorption of Zn (Dogar and Hai, 1980), immobilize Zn in roots and inhibit translocation to shoots. Zn deficiency is more common and more severe in early-season rice because of cool weather during transplanting in early Spring. Severe inhibition of Zn uptake into rice shoots occured at sub-optimal temperature (Mikkelson and Brandon, 1969). Low root zone temperature (RZT) decreased both uptake rate and translocation (Engels et al., 1992; Engels and Marschner, 1992), and concentrations of macronutrients in shoots of cool-sensitive plants like maize (Engels and Marschner, 1990). However, little information is available about interactions of RZT and bicarbonate to induce Zn deficiency in rice. Differences of rice cultivars in Zn efficiency could be mainly associated with their differences in sensitivity to bicarbonate (Forno et al., 1975). However, enhanced Fe uptake seemed to be responsible for the cultivar differences in susceptibility to Zn deficiency (Chaudhry et al., 1977). The objectives of this study were to determine effects of bicarbonate and root zone temperature (RZT) on uptake of Zn, Fe, Mn and Cu in "Zn-efficient" and "Zninefficient" rice (*Oryza sativa* L.) cultivars grown on calcareous soil.

#### Materials and methods

Rice cultivars used in these studies were IR 26 and IR 8192-31-2 obtained from the International Rice Research Institute (IRRI) and Che 64-7 and Shanyou 10 obtained from China. IR 8292-31-2 and Shanyou 10 were considered to be "Znefficient" and IR 26 and Che 64-7 to be "Zninefficient" (growth differences at low soil Zn) (H.U. Neue, 1989, personal communication; Yang et al., 1991, unpublished data).

Plants were grown in soil pot experiments in a growth chamber with (20 mM) and without bicarbonate (as NaHCO<sub>3</sub>) at RZT of 15° and 25°C. A calcareous soil (Inceptisol), obtained from a Zn deficient rice field in Zhejiang province, China was air-dried and sieved to pass a 2 mm screen. Initial chemical properties of the soil were: pH 7.77 (1 soil : 2.5 water); in mg kg<sup>-1</sup> soil; total N 3.1 available P (0.5 M NaHCO<sub>3</sub> extractable) 53.1 and available K (1 N NH<sub>4</sub>Ac extractable) 28.5 and Mn 40.0, Fe 30.0, Cu 1.5, and Zn 0.7 (0.005 M DTPA extractable). The basal fertilizers were 63.3 mg N as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 18.8 mg P as  $Ca(H_2PO_4)_2$  and 69.0 mg K as KCl per pot of 450 g soil. 20 germinated seeds were planted to each pot and plants were grown 6 days before bicarbonate and RZT treatments were administered. Bicarbonate was added to each pot in solution. For the low RZT treatment, the root part of the plants were placed in a water bath in which the temperature gradually decreased and was maintained at 15°C by coils through which cooled water was circulated, while the shoot parts of the plants were cultured at the air temperature 25°/20°C (light/dark). For the optimal RZT treatment, both root and shoot parts of the plants were cultured at the air temperature (25°/20°C light/dark). The experiments were conducted in controlled environmental conditions with light/dark regime of 15/9 h, a photon flux density of about 400  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> and a relative humidity of 80 %/85 % day /night.

Plants were grown in the treatments for 14 days (20-day-old). Shoots were cut 2 cm from the soil surface, weighed, rinsed with distilled water, dried at 65°C and again weighed. Roots were washed from soil, dried and weighed. The shoot samples were ground to pass a 0.5 mm (40-mesh) sieve for analysis. Samples weighed and dry-ashed (550°C 6h), residues were digested with 1:3(v/v) HNO<sub>3</sub> twice and dissolved in 1:30(v/v) HCl, concentrations of Zn, Fe, Mn and Cu were analyzed by Atomic Absorption Spectroscopy (AAS). Water soluble Fe and Zn were extracted by 1.0 mM Mes (2-(N-morpholino) ethanesulfonic acid) buffer (pH

6.0)(Cakmak and Marschner, 1987). Fe and Zn concentration in the extractions were analyzed by AAS.

#### Results

### Effects of bicarbonate and root zone temperature on growth

Plants after 7 days treatment with bicarbonate at different RZT showed distinct differences in growth. The RZT of 15°C decreased shoot growth of all cultivars, particularly of IR 26 and Che 64-7. The older leaves of these two cultivars developed red-brown spots and new leaves almost completely ceased growing. Chlorosis was observed in the middle parts of some new leaves of IR 26 at 25°C RZT, and symptoms were more severe with bicarbonate treatment. In contrast, no symptoms were observed in IR 8192-31-2 and Shanyou 10. After 14 days treatment, shoot dry weights of all cultivars at 25°C RZT were twice as high as at 15°C RZT (Fig. 1). Bicarbonate treatments had no effects on shoot dry weights of all cultivars at 15°C RZT, but shoot dry weights of IR 26 and Che 64-7 were decreased when grown with bicarbonate at 25°C RZT (Fig. 1). Root dry weight of all cultivars was reduced by 15°C RZT (Fig. 1). At 15°C RZT, root dry weight was not significantly decreased by bicarbonate but at 25°C RZT it was significantly decreased in IR 26 and Che 64-7 but not in IR 8192-31-2 (Fig. 1). Thus, bicarbonate decreased root and shoot growth of Zn-inefficient rice cultivars at optimal RZT, but not that of Znefficient cultivars.

#### Effects of bicarbonate and root zone temperature on shoot concentrations of Zn, Fe, Mn, and Cu

Zinc concentrations in shoots of all cultivars were significantly lower at 15°C than at 25°C RZT (Fig.2). At 15°C RZT, Zn concentration in the shoot of IR 8192-31-2 was about 20 mg kg<sup>-1</sup> dry wt., and about ~15 mg kg<sup>-1</sup> dry wt. in IR 26 and Che 64-7. Bicarbonate had only small effects on Zn concentration. However, bicarbonate significantly reduced Zn concentrations of the shoots at 25°C RZT in IR 26 and Che 64-7,



Fig. 1. Effects of root zone tempearture and bicarbonate on root and shoot dry matter yields of different rice cultivars. Plants were 20-day old and treatments lasted for 14 days. All data are means of 3 replications. The vertical bars depict least significant difference (LSD, P < 0.05) among treatments.

but not in IR 8192-31-2, implying that the Zn-inefficient cultivars were more sensitive to bicarbonate. The Cu concentration in the shoots of the cultivars were decreased at RZT 15°C, but not by bicarbonate. At 15°C RZT, shoot Cu concentration in IR 26 was only about half that of IR 8192-31-2 (Fig. 2). Zninefficient cultivars were more sensitive to sub-optical RZT than Zn-efficient cultivars with respect to Zn and Cu accumulation in shoots. Sub-optimal RZT had no effects on Fe and Mn concentrations in shoots of all cultivars, whereas bicarbonate treatment considerably decreased Fe concentrations in IR 26 and Che 64-7, but had little effect in IR 8192-31-2 (Fig. 3). Bicarbonate decreased shoot Mn concentrations at both temperatures in IR 26 and Che 64-7, but had much less effect in IR 8192-31-2 and Shanyou 10. Bicarbonate depressed Fe and Mn accumulation in shoots of rice cultivars susceptible to Zn deficiency.

# *Effects of bicarbonate and root zone temperature on concentrations of water soluble Fe and Zn*

Water soluble Zn concentrations in shoots of all cultivars were much lower at 15°C



Fig. 2. Effects of bicarbonate and root zone temperature on shoot concentrations of Zn and Cu of different rice cultivars. All data are means of 3 replications, vertical bars depict LSD (P < 0.05) among treatments.

RZT than at 25°C RZT (Fig. 4). Bicarbonate did not decrease the concentrations of water soluble Zn and Fe in the shoots. In shoots of IR 8192-31-2 (Zn-efficient), water soluble Zn cocentration was actually increased at both temperatures. Water-soluble Fe concentrations in shoots of IR 8192-31-2 doubled with bicarbonate treatment at both temperatures. The water-



Fig. 3. Effects of bicarbonate and root zone temperature on shoot concentrations of Fe and Mn of different rice cultivars. All data are means of 3 replications, vertical bars depict LSD (P < 0.05) among treatments.

soluble Fe concentration in shoots of the other three cultivars were also significantly increased by bicarbonate (Fig. 4).

#### Discussion

Bicarbonate has been regarded as a causal factor of "lime-induced Fe chlorosis". It inhibits root reducing capacity, Fe absorption and translocation to leaves, and Fe immobilization in the plant. In lowland rice, bicarbonate may be the major factor in Zn deficiency (Forno et al., 1975; Dogar and Hai, 1980). Our results showed that at 25°C RZT, bicarbonate inhibited the uptake of not only Zn but also of Fe and Mn in the Zn-inefficient rice cultivars (Table 2). Bicarbonate was reported to inhibit the uptake of not only Zn, but also of Fe, K, N, P, and Mg in rice (Dogar and Hai, 1980). Bicarbonate also decreased K concentrations in shoots, particularly in Zn-inefficient cultivars (data not shown). These results imply that bicarbonate appears to inhibit nutrient uptake by impairing root activity.

In paddy soils under flooding conditions, concentrations of  $Fe^{2+}$  and  $Mn^{2+}$  are high in the soil solution (Rahmatullah et al., 1976). Conventionally, Zn deficiency in lowland rice has



Fig. 4. Effects of bicarbonate and root zone temperature on shoot concentrations of water soluble Fe and Zn of different rice cultivars. All data are means of 3 replications, vertical bars depict LSD (P < 0.05) among treatments.

been considered to be associated with high concentrations of Fe and Mn in both soils and plant (Giordano et al., 1974; Rahmatullah et al., 1976; Chaudhry et al., 1977). However, bicarbonate concentration increased, whereas Zn concentration in soil solution decreased during flooding (Giordano et al., 1974; Forno et al., 1975) Therefore, inhibition of bicarbonate on uptake of nutrients could easily cause Zn deficiency in lowland rice due to low availability of Zn in flooded soils. Shoot growth was little affected by bicarbonate in all rice cultivars (Fig. 1). Root growth in Zn-inefficient cultivars was significantly reduced by bicarbonate, especially at 25°C RZT (Fig. 1). Thus, sensitivity of root growth in the Zn-inefficient rice cultivars because of bicarbonate may be an earliest effect of bicarbonate ion to induce Zn deficiency in rice.

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