Effect of bicarbonate on root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice cultivars (*Oryza sativa* L.)

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**Abstract**

Bicarbonate has been regarded as a major factor for inducing Zn deficiency in lowland rice, but the mechanisms responsible for this effect are not yet fully understood. The objective of the present study was to test whether early effects of bicarbonate (HCO₃⁻) are inhibition of root growth due to the accumulation of organic acids induced by HCO₃⁻. Solution culture experiments were conducted using two rice cultivars differing in susceptibility to Zn deficiency, and four bicarbonate concentrations (0, 5, 10, 20 mM). Bicarbonate (5-20 mM) strongly inhibited root growth of the Zn-inefficient cultivar within 4 days of treatments. In contrast, root growth of the Zn-efficient cultivar was slightly stimulated with bicarbonate at 5-10 mM and not affected at 20 mM. The inhibitory effect of bicarbonate on root growth in the Zn-inefficient cultivar was mainly that of impairment of new root initiation rather than suppression of elongation of individual roots. Bicarbonate (5-20 mM) increased the concentrations of malate, succinate and citrate in the roots of both cultivars, but to a greater extent for the Zn-inefficient than for the Zn-efficient cultivars. The results suggest that the impairment of root growth was likely to be the initial action of bicarbonate in inducing Zn deficiency in lowland rice, and the inhibitory effect of bicarbonate on root growth of the Zn-inefficient cultivar might result from high accumulation and an insufficient compartmentation of organic acids in the root cells.

**Introduction**

Bicarbonate (hydrogen carbonate) has been shown to be a direct or indirect cause of Fe-deficiency chlorosis in many plant species (Bertoni et al., 1992; Coulombe et al., 1984; McCray and Motocho, 1992; Mengel et al., 1984; Mengel and Geurtzen, 1986), and various mechanisms have been suggested to account for the relationships. These include inhibition of the reducing capacity of roots or Fe absorption and translocation to leaves (Bertoni et al., 1992; Fleming et al., 1984; Römheld et al., 1982; Romera et al., 1992; Venkatraj and Marschner, 1981); and immobilization Fe in leaves (Mengel and Mallissiosvas, 1981; Mengel and Bübl, 1983). Zinc deficiency in lowland rice is particularly associated with calcareous soils (Forno et al., 1975a; IRRI, 1971, 1972; Yoshida and Tanaka, 1969), and bicarbonate has been regarded as the major causal factor for zinc deficiency in rice (Forno et al., 1975b; Qin, 1988; Yang et al., 1993; Yoshida et al., 1971). Little information is available, however, concerning the mechanism by which bicarbonate induces Zn deficiency. The results of Dogar and Hai (1980) indicate that bicarbonate inhibits Zn absorption by rice roots, but Forno et al. (1975b) reported that the primary effect of bicarbonate is mainly inhibited Zn translocation from roots to shoots. Our previous studies showed that bicarbonate inhibited the uptake of not only Zn but also Fe, Mn, and K in the Zn-inefficient rice cultivars, but not in the Zn-efficient cultivars (Yang et al., 1993). We, thus, suggested that the earliest effect of bicarbonate might be to inhibit root growth of rice.

In the present study the effects of bicarbonate concentrations in nutrient solutions on root growth were
examined with relation to the accumulation of organic acids in roots of a Zn-efficient and a Zn-inefficient rice cultivar.

Materials and methods

Plant culture

Two rice cultivars one Zn-inefficient (IR 26) and one Zn-efficient (IR 8192-31-2), the seeds of which were provided by IRRI, were grown in nutrient solution (Yoshida, 1972) which is recommended for rice with the following composition (full strength) (mM): NH₄NO₃ 1.43, CaCl₂ 1.00, MgSO₄ 1.64, K₂SO₄ 1.32, NaH₂PO₄ 0.32, and (μM): MnCl₂ 9.5, FeCl₃ 35.6, ZnSO₄ 0.15, CuSO₄ 0.15, (NH₄)₆Mo₇O₂₄ 0.075 and H₃BO₃ 1.9. Bicarbonate was supplied at four concentrations: 0, 5, 10, 20 mM as sodium salt. Germinated rice seeds were placed on a nylon net and precultured for 5 days in a solution of 0.02 mM CaSO₄. Five-day-old rice seedlings of similar size were selected and transplanted to 3-L plastic containers, each container having eight clusters and four plants per cluster. Prior to bicarbonate treatment, plants were precultured for 7 days in the nutrient solution (see above); one quarter strength was used for the first two days and a half strength for the 3rd to 6th-day preculture, as well as during the bicarbonate treatments. The pH of the nutrient solution during preculture was 6.0, and 8.0 during bicarbonate treatments. To prevent plants from undergoing iron deficiency during bicarbonate treatment Fe was supplied as FeEDDHA (Ethylene-bis-[2-(o-hydroxyphenyl) glycine] at 0.01 M instead of FeCl₃. Plants were grown under controlled environmental conditions with temperature regimes of 25/18°C, 14h/10h light/dark, a relative humidity of 75%/85%, and at a photon flux density of about 400 μmol m⁻² s⁻¹. Plants were harvested at 0, 2, 4, and 8 days after bicarbonate treatments were started.

Measurement of root parameters

Six plants from each treatment were used at each harvest. Individual roots of each plant were carefully separated, placed on transparent film, and photocopied. From the photocopies of the roots, the number of individual roots per plant and root lengths per plant were measured using a length measuring machine (Digipl. 1335/Di). After photocopying, all roots were cut off, weighed, dried at 65°C and weighed again (root dry weight). Fresh and dry weights of the shoots were also obtained in a similar way.

Determination of organic acids

Roots from five other plants were cut off, rinsed with distilled water, dried with paper towel, weighed and immediately frozen in liquid nitrogen, and stored at -20°C for organic acid determination. The frozen roots were ground in a mortar with 70% v/v ethanol and acid-washed sand. The mixture was centrifuged at 4000 rpm for 10 min, and the pellet was extracted twice with boiling water. The supernatant from each of these extractions was transferred to a rotary evaporator and concentrated at 35°C under vacuum. The dried residues were dissolved in bi-distilled water and filtered through a membrane filter (0.2 μm). Concentrations of malic, citric, isocitric, tartaric and succinic acids were measured by ionchromatography (Dionex/SP). Each sample of the treatments with 4 replicates was analyzed twice. The eluant was 2.3 mM heptfluorobutyric acid (pH 2.6 adjusted with NaOH) at a flow rate of 0.3 mL min⁻¹ and the reagent solution was 5 mM tetrabutylammonium hydroxide at a flow rate of 10 mL min⁻¹.

Results

Root and shoot growth

Bicarbonate at 5-20 mM significantly reduced the root dry weight of IR 26 after 2 days of treatment (Fig. 1). After 4 days of treatment with the concentrations of 5-20 mM sodium bicarbonate the root dry weight of the Zn-inefficient cultivar (IR 26) was decreased by 30-45% and after 8 days of treatment by about 40-50% (Fig. 1). The inhibitory effects of bicarbonate on root dry weight in Zn-inefficient cultivar was dramatic even at 5 mM bicarbonate in the nutrient solution. However, the root dry weight of the Zn-efficient cultivar (IR 8192-32-2) slightly increased with the concentrations of 5 and 10 mM bicarbonate. At 20 mM bicarbonate there was a transient decrease in the rate of root dry weight production up to day 4 followed by a significant stimulation up to day 8 treatment. Thus, the root growth of the Zn-efficient cultivar quickly adapted to the bicarbonate concentrations.

Both fresh and dry weights of the shoots of IR 26 (Zn-inefficient) were dramatically reduced by added bicarbonate (5-20 mM) up to day 4 and day 8 of treat-