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Determination of Zn Efficiency in Rice (*Oryza sativa* L.) Genotypes with Use of Response to Bicarbonate

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Abstract: Diversity of root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice genotypes under bicarbonate treatment can be an ecological strategy for plants grown on calcareous soils with low Zn availability. In this study, the effects of bicarbonate on root and shoot fresh and dry weight, root length, number of roots per plant and the accumulation of malate and citrate on four genotypes of rice (*Oryza sativa* L.) were examined. Two genotypes, Pokkali (Zn-efficient) and IR26 (Zn-inefficient), from IRRI were used as known cultivars for Zn efficiency and two genotypes from Iran (Shafagh and Domsiah) were examined for Zn efficiency. The results indicated that the one of Iranian cultivars, Shafagh, was a Zn-efficient and Domsiah (local variety) was Zn-inefficient. Results showed that bicarbonate significantly decreased the growth of roots in the Zn-inefficient genotypes but induced their growth in the Zn-efficient genotypes particularly after 8 days of treatment. The accumulation of malate and citrate were greater in Zn-inefficient genotypes, as compared with the Zn-efficient genotypes. We conclude that root characteristics and the accumulation of organic acids can be used in rice genotypes for screening Zn efficiency under bicarbonate treatment.

Key words: Bicarbonate, Zn efficiency, root growth, organic acids, rice

INTRODUCTION

Zinc (Zn) deficiency was first identified as a field problem in rice in 1966 (Nene, 1966). It is now wildly recognized as one of the most widespread soil constraints in rice production, with as much as 50% of all lowland rice soils prone to Zn deficiency (Sillanpää, 1990; White and Zasoski, 1999; Cakmak *et al.*, 1998). Similar to Fe deficiency, most of the Zn deficiency occurred in calcareous soils (Forno *et al.*, 1975a; Yoshida and Tanaka, 1969; Yang *et al.*, 1993; Yang *et al.*, 1994a). Calcareous soils, generally with a pH>7.4 have relatively low available zinc concentration because the solubility of zinc decreases with increasing pH. Very often the total zinc content of calcareous soils is similar to those in soils of the other types, or even higher, but the availability is low (Alloway, 2004). In calcareous soils, after the flush in microbial activity that follows submergence, bicarbonate is the predominantation, which mainly decreases Zn transport from root to shoot and to a lesser extent, Zn uptake by roots (Singh *et al.*, 2005). 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). The inhibitory effect of bicarbonate on root growth appeared to be related to high accumulation and compartmentation of organic acids in root cells (Lee and woolhouse, 1971; Yang *et al.*, 1994b; Yang *et al.*, 2003). The findings showed that the inhibitory effect of bicarbonate on root growth of Zn-inefficient rice genotypes was closely associated with high accumulation of organic acids, malate and citrate in particular, in roots (Yang *et al.*, 1994b, 2003; Hajiboland *et al.*, 2005). In the present study, the effect of bicarbonate concentrations in nutrient solutions were examined on root and shoot growth and the accumulation of organic acids in roots for determination of Zn efficiency in rice genotypes.

MATERIALS AND METHODS

Plant culture: Four genotypes of rice plants (*Oryza sativa* L.) were selected for this study. Two cultivars were provided by IRRI (personal communication). One being Zn-efficient (Pokkali) and the other Zn-inefficient (IR26) which were used as control. The others two genotypes from Iran, Shafagh (as a cultivar) and Domsiah (as a local variety) were unknown for Zn-efficiency. Rice seedlings were grown in nutrient solution with the following composition (Yoshida *et al.*, 1972) (mM): NH_4NO_3 1.43, $CaCl_2$ 1.00, $MgSO_4$ 1.64, $K_2SO_4$1.32, NaH_2PO_4 0.32 and (µM): $MnCl_2$ 9.5, $FeCl_3$ 35.6, $ZnSO_4$ 0.15, $CuSO_4$ 0.15, $(NH_4)_6Mo_7O_{24}$ 0.075 and H_3BO_3 1.9. Bicarbonate was

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supplied at four concentrations: 0, 5, 10 and 20 mM as sodium salt. Germinated rice seeds were placed on nylon net and precultured for 5 days in a solution of 0.02 mM $CaSO_4$. Five-day-old rice seedlings of similar size were selected and transplanted to 3 L plastic containers, each container having eight clusters and three plants per cluster. Prior to the bicarbonate treatment, plants were precultured for 7 days in 0.5 strength nutrient solutions. The pH of the nutrient solution during preculture was 6.0 and 8.0 during bicarbonate treatment. Plants were grown under controlled environmental conditions with temperature regimes of 25/18°C, 14/10 h light/dark, a relative humidity of 75/85% and at a photon flux density of about 400 μmol $m^{-2}s^{-1}$.

Measurements: Plants were harvested at 0, 2, 4 and 8 days after bicarbonate treatments. Shoot fresh and dry weights were recorded. Roots from each replication were divided into three parts after determination of fresh weights. One part was used for the determination of dry weight, the second for root number and length (Tannat, 1975) and the third part was used for the determination of organic acids.

Determination of organic acids: Determination of malate and citrate were carried out after extraction from homogenized samples with 5% (v/v) H_3PO_4 [1 mL per 200 (mg FW^{-1})]. The extracts were centrifuged at 10000 g for 10 min and after suitable dilution with 18 mM K_2HPO_4 (pH = 2.1), were analyzed by RP-HPLC with UV detection at 215 nm (Neumann *et al*., 1999).

Data analysis: All data were subjected to analysis through ANOVA and means were separated using the least significant difference (LSD, $p<0.05$). All the data are means of four replications±SE.

RESULTS

Effect of bicarbonate on dry matter of roots and shoots: Bicarbonate at 5-20 mM significantly reduced the root dry weight of IR26 and Domsiah after 2 days of treatment (Fig. 1). After 4 days of treatment with the concentrations of 5-20 mM bicarbonate, the root dry weight of the Zn-inefficient cultivar (IR26) and Domsiah were decreased by 21.7 and 25.1%, respectively and after 8 days of treatment, they were decreased by about 28 and 31%, respectively (Fig. 1). The inhibitory effect of bicarbonate on root dry weight in Zn-inefficient cultivar was noticeable even at 5 mM bicarbonate in nutrient solution. However, the root dry weight of the Zn-efficient cultivar (Pokkali) and Shafagh had minimum fluctuation with the concentrations of 5, 10 and 20 mM bicarbonate up to day 4 and 8 treatment (Fig. 1). Thus, the root growth of the Zn-efficient cultivars quickly adapted to the bicarbonate concentrations. Both fresh and dry weights of the shoots of IR26 (Zn-inefficient) and Domsiah were significantly reduced by added bicarbonate (5-20 mM) up to 4 and 8 days of treatments (Table 1). Shoot fresh and dry weights of Pokkali (Zn-efficient) and Shafagh after 8 days increased 3-6% at the most, when plants were grown at bicarbonate concentrations of 5 and 10 mM, but decreased 13-16% at the most when grown at 20 mM bicarbonate. Thus, different effects of bicarbonate on root growth of the four cultivars (Fig. 1), in principle, were also similar on shoot growth.

Effect of bicarbonate on root length and number per plant: In IR26 (Zn-inefficient), even 5 mM bicarbonate severely depressed root length and number of roots per plant as early as 2 days treatment (Fig. 2 and 3). The longer the treatment, the more prominent the inhibitory effects of bicarbonate were, irrespective of the bicarbonate concentration. But root length of Domsiah was not affected by bicarbonate treatment after 2 days, whereas, it decreased greatly after 4 and then 8 days treatment. Also, root number alteration per plant of Domsiah was similar to IR26 cultivar. In general, increasing of bicarbonate concentrations from 5 to 20 mM decreased the root number and length of Zn-inefficient rice cultivars, distinctly. In contrast, neither root length nor number of roots per plant of Pokkali (Zn-efficient) and Shafagh were significantly affected by bicarbonate.

Effect of bicarbonate on accumulation of organic acids: Malate concentration in root of all cultivars was considerably increased with bicarbonate treatment (Fig. 4). In comparison with the control, malate concentration increased by 4-6 times in roots of IR26 and 3-6 times in roots of Domsiah grown at 5-10 mM bicarbonate after 8 days and it increased as much as 11 and 8 times when the plants were grown at 20 mM bicarbonate, respectively. However, malate concentration in the roots of Pokkali and Shafagh increased less, at the most 1-2 times at 5-10 mM and 3-4 times at the 20 mM bicarbonate compared with the control (Fig. 4).

Similarly, citrate concentration in roots of all cultivars increased when plants were grown under bicarbonate treatment (data not shown). After 2 days of treatment the citrate concentration in roots of IR26 and Domsiah increased 3 and 4 times at 5 mM, 11 and 13 times at 10 mM

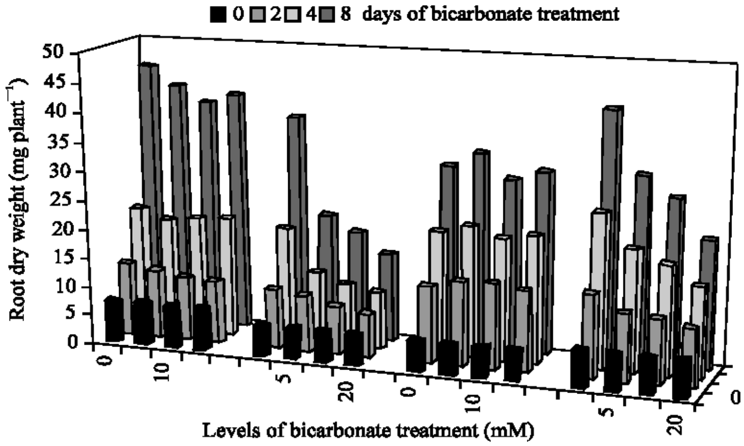

Fig. 1: Effect of bicarbonate on root dry weight of the Zn-inefficient and Zn-efficient rice genotypes. From left to right: Pokkali, IR26, Shafagh and Domsiah

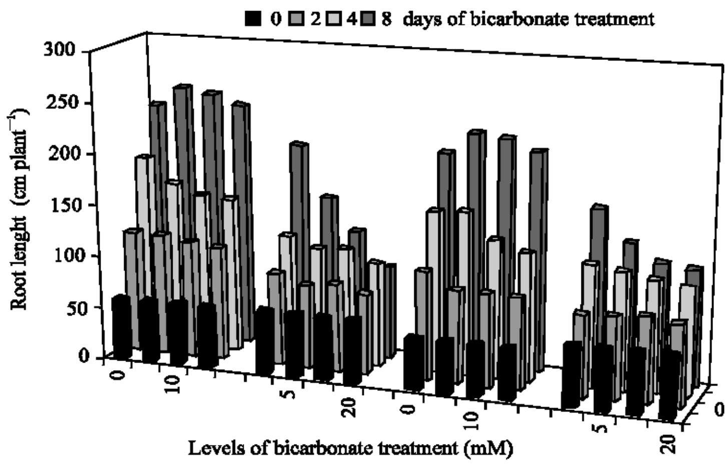

Fig. 2: Effect of bicarbonate on root length of the Zn efficient and Zn-inefficient genotypes. Genotypes from left to right: Pokkali, IR26, Shafagh and Domsiah

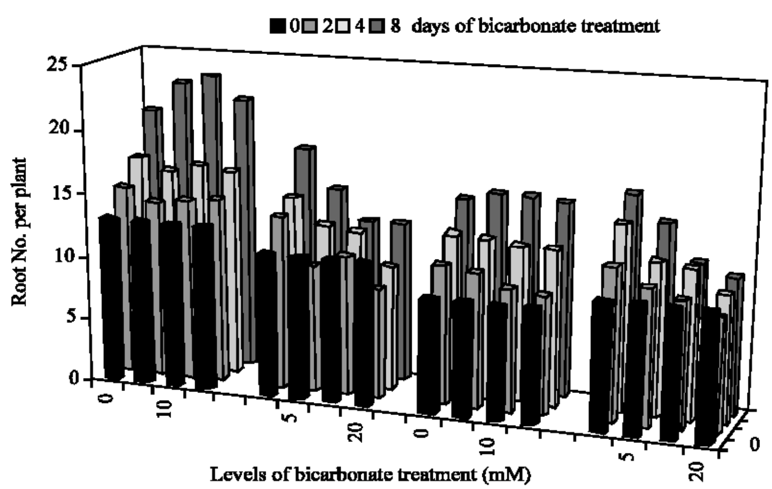

Fig. 3: Effect of bicarbonate on root umber per plant of the Zn-inefficient and Zn-efficient rice genotypes. Genotypes from left to right: Pokkali, IR26, Shafagh and Domsiah

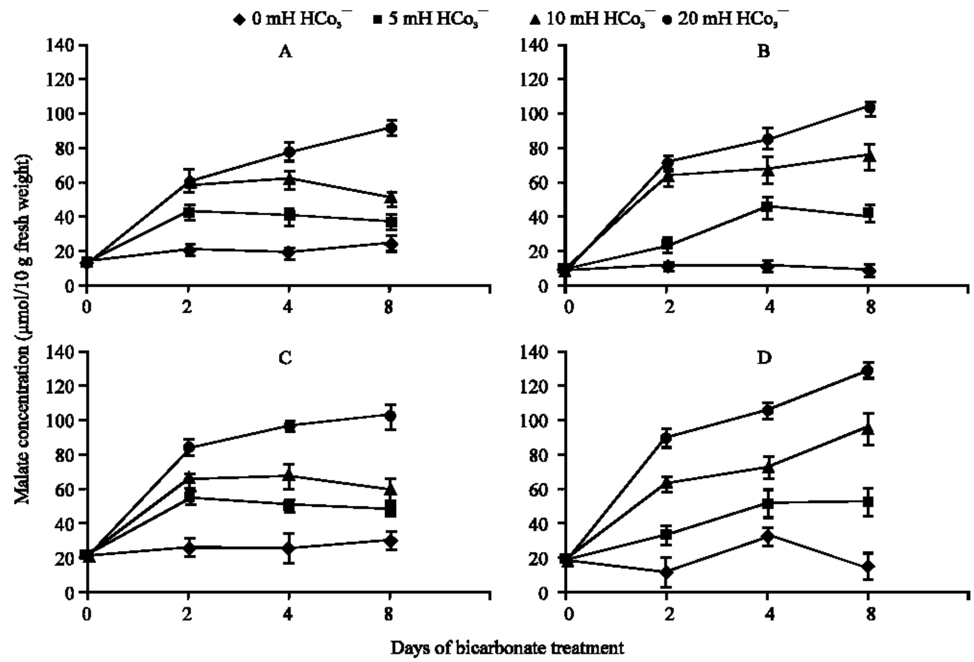

Fig. 4: Effect of bicarbonate on the concentrations of malate in the roots of Zn-efficient: A(Pokkali), C(Shafagh) and Zn-inefficient: B(IR26), D(Domsiah) rice genotypes. (Values represent the mean±SE of four replications)

and 15 and 18 times at 20 mM bicarbonate, respectively. Whereas, citrate concentration in roots of Pokkali and Shafagh grown at 5-20 mM bicarbonate were about 40-45 and 43-48% higher than in case of the control at day 2 and were little affected at day 4 of treatment.

DISCUSSION

Treatment of rice seedlings with different concentrations of bicarbonate inhibited fresh and dry weight of root (Fig. 1) and shoot (Table 1), root length (Fig. 2) and individual root number per plant (Fig. 3) in Zn-inefficient cultivars and these results conform to previous reports (Yang *et al.*, 1994b; Hajiboland *et al.*, 2005). Increases of bicarbonate concentrations from 5 to 20 mM, inhibited the root and shoot growth of Zn-inefficient rice cultivars and conversely improved the root elongation for Zn-efficient cultivars (Fig. 1-3). Present results as compared with previous works (Yang *et al.*, 1994b; Hajiboland *et al.*, 2003, 2005) indicates that root accumulation of organic acids can be used for the screening of Zn-efficient and Zn-inefficient genotypes. On the basis of this finding, obtained results of this research showed that the Iranian cultivar (Shafagh) was a Zn-efficient genotype and Domsiah was a Zn-inefficient local variety. Zinc deficiency in rice plant occurs mainly at early stages in calcareous soils, where Zn availability is low due to adsorption by calcium carbonate (Xie and Jian, 1994). Bicarbonate tolerance and Zn efficiency have been developed in rice simultaneously during natural or artificial selection for plant growth on alkaline and calcareous soils with low Zn availability. Dong *et al.* (1995) suggested that the difference in root morphology among genotypes is more likely to be a property of the genotype. Zinc uptake by higher plants appears to be mostly controlled by the transport of zinc across the plasma membrane, which is largely metabolism-dependent and genetically controlled. Zn-efficient genotypes may be able to maintain structural and functional stability of their root-cell plasma membranes better than Zn-inefficient genotypes under Zn deficiency (Rengel and Graham, 1995). In rice, under flooded conditions, Zn availability decreased because of the reduction in Zn solubility as pH increases. High bicarbonate concentration is one of the causes of Zn deficiency, which due to reducing conditions in calcareous soils with high organic matter content or because of large concentrations of bicarbonate in irrigation water. Bicarbonate has been regarded as a major factor for inducing Zn deficiency in lowland rice. The length of the total root system increased in response to bicarbonate in the Zn-efficient genotypes but decreased in the Zn-inefficient genotypes. Although the mechanism involved in these morphological changes is not known, these changes could be the main cause of bicarbonate-related Zn efficiency responses of various rice genotypes. Hajiboland *et al.* (2003) suggested that

Table 1: Effect of bicarbonate on shoot fresh and dry weight of the Zn-inefficient and Zn-efficient rice genotypes

Genotypes	$NaHCO_3$ (mM)	4 days ($mg\ plant^{-1}$) FW	DW	8 days ($mg\ plant^{-1}$) FW	DW
Pokkali	0	602	83.2	1328	162.1
	5	614	89.1	1332	168.3
	10	610	86.2	1331	165.4
	20	522	69.1	1118	140.1
IR26	0	332	48.1	592	71.3
	5	201	30.2	318	43.1
	10	267	34.1	378	54.2
	20	186	23.1	298	41.3
Shafagh	0	285	37.1	516	63.2
	5	302	41.2	525	67.3
	10	315	42.5	532	64.1
	20	273	35.1	487	60.2
Domsiah	0	311	40.9	542	67.1
	5	292	38.6	418	58.1
	10	218	29.1	325	43.5
	20	202	25.8	318	42.1
$LSD_{5\%}$		71.56	11.94	130.54	28.99

the impairment of root growth is due to bicarbonate in inducing Zn deficiency in lowland rice on calcareous soils. Root accumulation of organic acids in rice plants grown in bicarbonate containing nutrient solution exhibits genotypic differences (Yang *et al*., 1994b). Zinc-inefficient genotypes accumulated much more organic acids than Zn-efficient genotypes in response to bicarbonate treatment, which was associated with strongly inhibited root growth. In the Zn-inefficient rice genotype the increased accumulation of malate and citrate in roots by bicarbonate appeared to result from their less exudation to the rhizosphore and transport up to shoots. Different accumulation of organic acids in the roots of the four genotypes which caused different growth responses to bicarbonate may be the result of differences in the dark fixation of bicarbonate i.e., PEP activity, exudation of organic acids into the medium or transport to above ground parts of the plant. Another possibility could be the differential ability of the four genotypes in the use of bicarbonate-derived organic acids as respiratory metabolites in the TCA cycle. Lee and Woolhouse (1971) showed that the respiratory malate pool was more completely compartmented from other malate pools in the cells of the calcifuges, whereas a progressive mixing of the malate pool occurred in the calcicoles.

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