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Zinc Efficiency is Not Related to Bicarbonate Tolerance in Iranian Rice Cultivars

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Abstract: In this study this hypothesis was evaluated using 6 Iranian rice cultivars with different Zn efficiency as ranked in a solution culture study. Results showed that, bicarbonate (10 mM) strongly reduced shoot and root growth and particularly root length of all studied genotypes in the exception of cv. Mianeh. The root growth particularly root length of this cultivar was even stimulated up to 70% for example in Zn deficient plants. The inhibitory or stimulatory effect of high pH was not prominent as bicarbonate, which could be the result of the effect of bicarbonate independent from accompanying pH. Zinc and Fe content of shoot and root reduced in response to bicarbonate and high pH and the reduction was similar in genotypes with different Zn efficiency and bicarbonate tolerance. It was concluded that, because of different chemical factors of soil on which these genotypes are cultivated, the Zn efficiency trait is not necessarily associated with bicarbonate tolerance in all studied rice genotypes. In soils with low bicarbonate content such as rice fields in north of Iran, bicarbonate response was not selected together with Zn deficiency tolerance in frequently cultivated genotypes. In contrast, cv. Mianeh, being cultivated in calcareous soils of west and northwest of Iran, shows a clear correlation between these two traits.

Key words: Rice genotypes, Zn deficiency, Zn efficiency, bicarbonate

INTRODUCTION

Zn deficiency is one of the most important micronutrient disorders of rice. Zn deficiency in wetland rice occurs on soils with pH greater than 7.0 and soils with low level of Zn and high amounts of bicarbonate. The presence of bicarbonate in soils is commonly accompanied by decreased nutrient availability and consequently their uptake and transport in plants (Forno *et al*., 1975a; Dogar and Hai, 1980). Consequently, bicarbonate is considered a reliable means of classifying cultivars according to susceptibility to Fe (Coulombe *et al*., 1984) and Zn (Forno *et al*., 1975a; Yang *et al*., 1994a) deficiency.

In rice plants, there are reports suggesting that bicarbonate may inhibit root absorption of Zn (Dogar and Hai, 1980) and immobilize Zn in the root with subsequent inhibited translocation to the shoot (Forno *et al*., 1975b). Yang *et al*. (1993) showed that external bicarbonate decreased concentrations of Zn and Fe in the shoot of Zn-inefficient but not Zn-efficient genotypes grown on calcareous soils.

Previous studies using IR and Chinese genotypes on the physiological mechanisms of Zn efficiency have shown that, Zn deficiency in lowland rice is related to the presence of high concentrations of bicarbonate ions in the soil solution. Thus genotypic differences in susceptibility to Zn deficiency might be also arise due to differences in growth inhibition by bicarbonate (Forno, 1975a; Yang *et al*., 1994a).

Using two IR rice genotypes differing in Zn efficiency, it was shown that, bicarbonate in the nutrient solution caused significant impairment of root growth in Zn-inefficient rice genotype and a stimulation of that in Zn-efficient genotype (Yang *et al*., 1994a). Further study using seven IR and one Chinese genotype showed that, there is a significant correlation between Zn deficiency and bicarbonate tolerance in tested rice genotypes (Hajiboland *et al*., 2003). However, experiment with wheat and rye genotypes showed that this correlation is not a common feature of Zn efficiency trait in cereals and is restricted to (studied) rice cultivars.

The aim of the present study was to investigate the effect of bicarbonate on Iranian rice cultivars and its dependence to their Zn efficiency trait, therefore to test the general validity of the relationship between bicarbonate tolerance and Zn efficiency of the rice cultivars other than IR and Chinese genotypes.

Firstly, a hydroponic screening experiment using 6 cultivars commonly cultivated in north (all tested cultivars in the exception of cv. Mianeh) or west and northwest of Iran (cv. Mianeh), was conducted in order to

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determine the Zn-efficient and Zn-inefficient genotypes. Thereafter, the selected genotypes were used to study the effect of bicarbonate on shoot and root growth as well as Zn amounts in plant. In order to separate the effect of bicarbonate ion from the accompanying high pH, the high pH treatment using HEPES, was also included in the experimental design.

MATERIALS AND METHODS

Plants culture and treatments: For screening experiment, six genotypes of rice (*Oryza sativa* L.) were used including Mianeh, Amol, Shafagh, Tarom Hashemi, Fajr and Onda. Seeds were provided by Iranian Rice Research Institute, Guilan Province, Iran.

Seeds were germinated in darkness on filter paper. Germinated seeds were then transferred on a nylon net and pre-cultured for 2 days on 0.02 mM $CaSO_4$, 3 days on 25% and 3 days on 50% nutrient solution. Thirteen-days-old rice seedlings were transferred into 2.5 L plastic pots with 5 bundles consisting of 3 plants per bundle. Plants were grown under incandescent-fluorescent light at about 400 μmol $m^{-2}s^{-1}$, with 18/6 h light/dark period, 25/18°C day/night temperature and a relative humidity of 75/85%. The nutrient solutions were not aerated and were replaced every 5 days.

In a preliminary experiment with 16 Iranian rice cultivars using chelator-buffered technique, we have determined the cultivars Mianeh, Amol and Shafagh as Zn-efficient and cultivars T. Hashemi, Fajr and Onda as Zn-inefficient. In order to test the evaluation of the results of the preliminary experiment and reproduce the data, an experiment was conducted using conventional nutrient solution to screen the genotypes in this system. Plants were grown in nutrient solution (Yoshida *et al.*, 1972) with the following composition (mM): NH_4NO_3 1.50, $CaCl_2$ 1.00, $MgSO_4$ 1.60, K_2SO_4 1.00, KH_2PO_4 0.30 and (μM): H_3BO_3 2.0, $MnSO_4$ 5.0, $CuSO_4$ 0.2 and $(NH_4)_6Mo_7O_{24}$ 0.05. Iron was supplied as FeEDTA at 0.1 mM. The Zn level of 0 μM were supplied using nutrient solution made by double-distilled water without Zn addition. The concentration of Zn at 0.1 and 0.5 μM was supplied by addition of $ZnSO_4$ at the defined concentrations to the nutrient solution. The pH of nutrient solution was 6.0 and adjusted every day.

Application of high pH and bicarbonate at low and adequate Zn supply: To study the effect of bicarbonate on shoot and root growth, as well as the effect of Zn nutritional status of plants on the bicarbonate-induced changes of growth, an experiment was conducted with bicarbonate and three levels of Zn supply. To separate the effect of bicarbonate from high pH, plants were treated with 10 mM bicarbonate as well as high pH (8.0). The pH was adjusted to 6.0 using 10 mM KOH-MES (2-[N-morpholino]-ethansulfonate) as a control and to 8.0 using 10 mM KOH-HEPES (N-2-hydroxyethylpiperazin-N-2-ethansulfonate) as the high pH treatment. Bicarbonate was supplied as 10 mM $NaHCO_3$ (pH~8.0). Prior to the bicarbonate and high pH treatments, plants were pre-cultured for 3 days in 50% nutrient solution.

Harvest: Plants were harvested 21 days after starting of the treatments in the screening experiment and 14 days in the experiment with bicarbonate treatment. Before harvest, chlorophyll content of leaves was measured using a chlorophyll meter (Minolta, SPAD 502). Then, shoots and roots were separated, roots were rinsed for one min with distilled water, pre-dried on filter paper and the fresh weights were determined. Oven-dried samples at 70°C for 48 h, were used for determination of dry weights. The roots of second group were used for the measurement of root length according to Tennant (1975) after determining fresh weight.

For determination of Zn and Fe, oven-dried samples were ashed in a muffle furnace at 500°C for 8 h and then digested in 1:3 HNO_3. The digested samples were dried on a heating plate and subsequently ashed at 500°C for another 3 h. Samples were resuspended in 2 mL 10% HCl and made up to volume by double-distilled water. Zinc and Fe concentration was determined by atomic absorption spectrophotometry (Shimadzu, AA 6500).

All experiments were conducted using 3 or 4 independent replications. The statistical analyses were performed using Sigma stat (3.02).

RESULTS

According to the results of screening experiment, the cultivars Mianeh, Amol and Shafagh showing significantly lower reduction of shoot and root growth, could be ranked as Zn-efficient genotypes (Table 1). The other three cultivars, T. Hashemi, Fajr and Onda with up to 69% (T. Hashemi) and 54% (Onda) reduction of shoot and root growth respectively, could be defined as Zn-inefficient genotypes. Root length was affected more than root weight by low Zn supply, for example, in cv. T. Hashemi this parameter was reduced up to 70%, while root biomass decreased only 36%. The roots at all Zn deficient treatments were obviously thicker than control plants (data not shown). Any stimulation of root length was observed either at sever (Zn = 0 μM) or moderated (Zn = 0.1 μM) Zn deficiency. As expected, the chlorophyll content (relative values) responded to Zn supply more

Table 1: Shoot and root dry weight and root length of various Iranian rice cultivars grown in nutrient solution with low and sufficient Zn supply

	Zn = 0 (μM)	Zn = 0.1 (μM)	Zn = 0.5 (μM)
Shoot DW (mg plant^{-1})			
Mianeh	96.2±8.2^{a}	108.4±4.5^{a}	100.4±4.5^{a}
Amol	84.4±6.1^{a}	93.2±9.5^{a}	100.2±9.1^{a}
Shafagh	69.3±6.9^{a}	67.3±3.4^{a}	79.4±4.5^{a}
T. Hashemi	37.4±6.8^{c}	48.7±1.2^{b}	92.3±4.4^{a}
Fajr	53.9±4.1^{c}	72.4±5.3^{b}	100.2±6.8^{a}
Onda	36.2±1.4^{c}	59.1±3.7^{b}	66.4±4.9^{a}
Root DW (mg plant^{-1})			
Mianeh	16.6±1.2^{a}	21.2±1.3^{a}	17.7±1.2^{a}
Amol	17.7±1.3^{a}	19.8±1.9^{a}	17.9±1.9^{a}
Shafagh	13.4±1.4^{a}	15.1±1.6^{a}	13.3±1.7^{a}
T. Hashemi	6.1±1.0^{b}	7.4±1.2^{ab}	9.6±1.1^{a}
Fajr	12.2±1.5^{b}	13.6±1.4^{ab}	16.4±2.2^{a}
Onda	6.3±1.7^{b}	9.0±1.8^{ab}	13.7±1.9^{a}
Root length (cm plant^{-1})			
Mianeh	212.0±45^{a}	295.0±35^{a}	317.0±77^{a}
Amol	210.0±27^{b}	259.0±14^{a}	282.0±22^{a}
Shafagh	285.0±8^{b}	325.0±21^{a}	342.0±18^{a}
T. Hashemi	92.0±9^{c}	323.0±13^{b}	311.0±81^{a}
Fajr	112.0±17^{c}	252.0±21^{b}	348.0±12^{a}
Onda	179.0±4^{c}	266.0±18^{b}	358.0±47^{a}

Data in each row followed by the same letter(s) are not significantly different ($p<0.05$)

prominently in Zn-inefficient than in Zn-efficient genotypes (Fig. 1).

In response to bicarbonate, the most reduction in chlorophyll amounts was observed in three Zn-inefficient genotypes T. Hashemi, Fajr and Onda. In contrast, in Mianeh and Shafagh, any significant effect of bicarbonate and high pH was observed on the chlorophyll content of leaves (Fig. 1). A dramatic inhibition of shoot and root growth was observed in response to both bicarbonate and high pH (Fig. 2). However, genotypic differences were also observed, cv. Mianeh was the most tolerant species to bicarbonate and high pH particularly under low Zn supply. Cultivar T. Hashemi was also relatively tolerant to high pH, in respect to shoot and root growth. The most sensitive genotype to bicarbonate was cv. Fajr with up to 80% reduction of shoot and root weight at 10 mM bicarbonate (Fig. 2).

Root length responded to bicarbonate and high pH similar with the dry mass of plants (Table 2). Generally, the inhibitory effect of bicarbonate on root length in Zn deficient plants was lower than that in Zn sufficient ones.

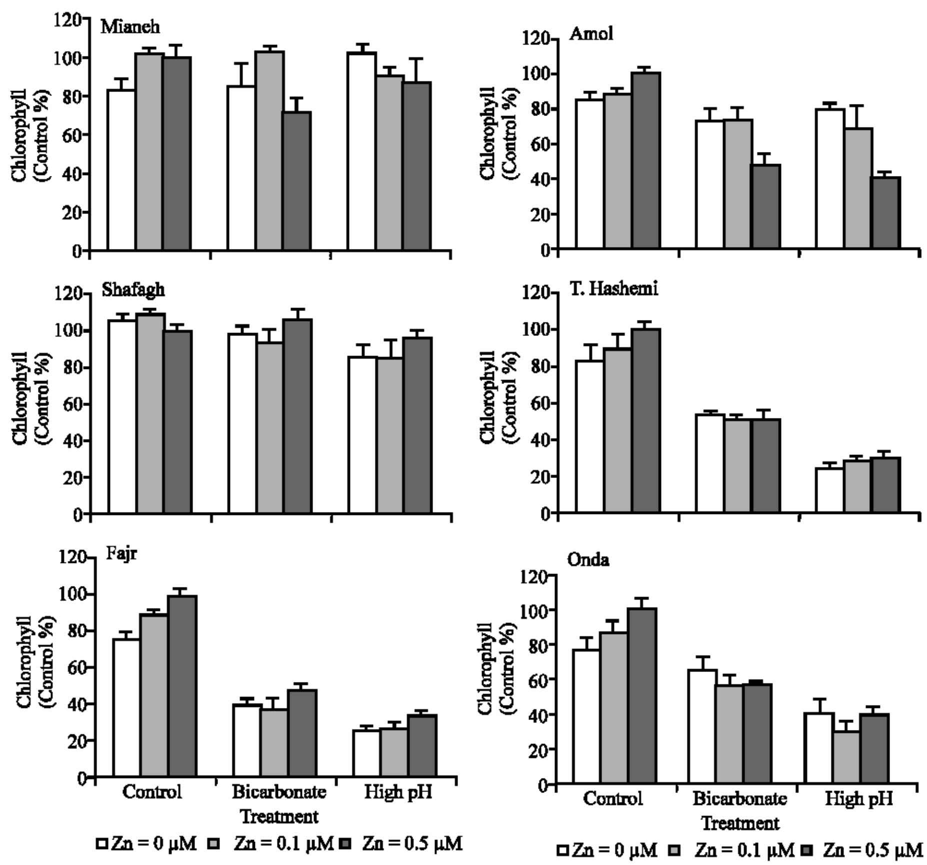

Fig. 1: Effect of bicarbonate and high pH (pH = 8.0) on shoot and root dry weight (% over control) of six Iranian rice cultivars grown in nutrient solution with different Zn levels

Table 2: The effect of bicarbonate (10 mM, pH = 8.0) and high pH (10 mM HEPES, pH = 8.0) on root length (cm plant^{-1}) of six Iranian rice cultivars grown in nutrient solution with low (0 and 0.1 μM) or adequate (0.5 μM) Zn supply

	Control	Bicarbonate	HEPES
Zn = 0 (μM)			
Mianeh	761±124^{b}	1292±123^{a}	876±137^{b}
Amol	480±74^{b}	98±22^{c}	117±22^{c}
Shafagh	496±48^{b}	86±9^{c}	58±5^{c}
T. Hashemi	230±46^{b}	60±9^{d}	80±16^{d}
Fajr	308±20^{b}	72±25^{c}	102±14^{c}
Onda	326±47^{b}	64±9^{c}	96±22^{c}
Zn = 0.1 μM			
Mianeh	854±23^{a}	662±18^{a}	809±141^{a}
Amol	592±103^{ab}	88±13^{c}	72±18^{c}
Shafagh	591±74^{ab}	40±6^{d}	64±6^{c}
T. Hashemi	251±15^{b}	63±10^{d}	127±16^{c}
Fajr	321±54^{b}	66±8^{c}	105±20^{c}
Onda	344±19^{b}	98±19^{c}	85±16^{c}
Zn=0.5 μM			
Mianeh	940±68^{a}	634±148^{a}	708±122^{a}
Amol	688±36^{a}	47±7^{c}	111±23^{c}
Shafagh	626±65^{a}	30±2^{d}	56±5^{c}
T. Hashemi	378±24^{a}	90±11^{d}	128±22^{c}
Fajr	628±181^{a}	77±14^{c}	114±13^{c}
Onda	557±33^{a}	62±5^{c}	72±11^{c}

Data in each row followed by the same letter(s) are not significantly different ($p<0.05$)

Similarly, in cv. Mianeh the stimulation of root length was only prominent in Zn deficient plants and the presence of bicarbonate did not affect root length of Zn sufficient plants.

Zinc and Fe content of shoot and root was strongly reduced in plants treated with bicarbonate or high pH (Table 3). Additionally, the extent of reduction was similar in four analyzed cultivars. However, the reduction of root Zn content in response to bicarbonate and high pH was not significant in two Zn-efficient cultivars Mianeh and Shafagh. The reducing effect of bicarbonate was not differed from that of high pH in the exception of that in T. Hashemi and Onda at Zn = 0.1 and 0.5 μM.

However, Zn and Fe concentrations responded differently because of a large negative growth response of plants to bicarbonate and high pH. Bicarbonate induced enhancement in Zn concentration of shoot and root, particularly in cultivars with high bicarbonate susceptibility such as Onda. However, the Zn concentration of shoot was not affected significantly in Mianeh neither by bicarbonate nor high pH at any of Zn treatments (Table 4).

Table 3: Zinc and Fe content (μg plant^{-1}) of shoot and root in four Iranian rice cultivars grown in nutrient solution with low and sufficient Zn supply at the bicarbonate (10 mM, pH = 8.0) or high pH (HEPES, pH = 8.0) treatments

	Shoot			Root		
Zn content	Control	Bicarbonate	HEPES	Control	Bicarbonate	HEPES
Zn = 0 (μM)						
Mianeh	3.25±0.57^{a}	2.26±0.48^{b}	2.47±0.25^{ab}	2.38±0.48^{a}	1.50±0.23^{a}	1.52±0.28^{a}
Shafagh	4.97±0.38^{a}	2.56±0.23^{b}	2.55±0.19^{b}	1.90±0.29^{a}	1.17±0.64^{a}	1.12±0.07^{a}
T. Hashemi	2.99±0.58^{a}	1.88±0.21^{b}	2.18±0.12^{b}	1.83±0.15^{a}	0.95±0.13^{b}	1.04±0.19^{b}
Onda	3.63±0.46^{a}	1.98±0.12^{b}	1.97±0.19^{b}	2.27±0.71^{a}	1.09±0.43^{b}	1.16±0.05^{b}
Zn = 0.1 (μM)						
Mianeh	4.65±0.79^{a}	2.53±0.48^{b}	2.79±0.50^{b}	2.94±0.44^{a}	1.46±0.23^{b}	1.54±0.27^{b}
Shafagh	6.61±0.24^{a}	2.58±0.09^{b}	2.85±0.18^{b}	2.42±0.21^{a}	1.49±0.13^{b}	1.51±0.02^{b}
T. Hashemi	3.59±0.48^{a}	1.91±0.15^{c}	2.98±0.18^{b}	2.52±0.18^{a}	1.59±0.15^{b}	1.85±0.13^{b}
Onda	5.37±0.23^{a}	2.70±0.19^{c}	3.22±0.29^{b}	3.35±0.37^{a}	1.47±0.32^{b}	1.12±0.13^{b}
Zn = 0.5 (μM)						
Mianeh	5.45±0.55^{a}	2.65±0.72^{b}	2.78±0.97^{b}	3.11±0.77^{a}	1.42±0.23^{b}	1.84±0.45^{b}
Shafagh	7.70±0.25^{a}	3.46±0.78^{b}	3.27±0.06^{b}	2.88±0.23^{a}	1.97±0.29^{b}	1.93±0.01^{b}
T. Hashemi	4.49±0.67^{a}	2.23±0.17^{b}	2.86±0.13^{b}	2.73±0.48^{a}	1.96±0.17^{b}	2.09±0.27^{ab}
Onda	7.89±0.31^{a}	3.81±0.19^{c}	4.27±0.11^{b}	3.86±0.07^{a}	2.12±0.14^{b}	2.91±0.79^{b}
	Shoot			Root		
Fe content	Control	Bicarbonate	HEPES	Control	Bicarbonate	HEPES
Zn = 0 (μM)						
Mianeh	9.48±1.48^{a}	2.75±0.48^{b}	3.62±1.21^{b}	21.98±1.28^{a}	10.26±1.09^{b}	10.63±1.64^{b}
Shafagh	11.06±1.09^{a}	2.35±0.14^{b}	2.63±0.11^{b}	28.63±1.43^{a}	14.25±3.48^{b}	14.28±4.53^{b}
T. Hashemi	10.21±1.78^{a}	2.29±0.65^{b}	1.88±0.26^{b}	21.31±2.24^{a}	10.39±1.57^{b}	10.30±1.31^{b}
Onda	14.86±2.38^{a}	3.68±0.50^{b}	2.77±0.15^{b}	39.48±3.43^{a}	17.72±1.87^{b}	17.91±4.28^{b}
Zn = 0.1 (μM)						
Mianeh	10.69±0.97^{a}	3.79±0.78^{b}	3.76±0.64^{b}	21.35±1.38^{a}	10.30±1.19^{b}	10.39±1.50^{b}
Shafagh	11.12±0.70^{a}	2.71±0.34^{b}	2.52±0.16^{b}	25.92±2.23^{a}	22.33±2.78^{a}	22.61±2.28^{a}
T. Hashemi	11.35±1.12^{a}	2.63±0.56^{b}	2.03±0.28^{b}	21.26±3.28^{a}	10.19±1.12^{b}	10.51±1.76^{b}
Onda	13.68±0.99^{a}	1.83±0.17^{b}	2.56±0.44^{b}	47.28±2.09^{a}	11.58±2.78^{c}	21.06±4.39^{b}
Zn = 0.5 (μM)						
Mianeh	10.40±0.69^{a}	2.75±0.5^{b}	3.25±1.42^{b}	18.53±1.24^{a}	10.39±1.38^{b}	10.50±1.21^{b}
Shafagh	9.35±1.59^{a}	2.32±0.07^{b}	2.56±0.23^{b}	24.12±1.7^{a}	10.49±1.68^{b}	14.48±2.97^{b}
T. Hashemi	10.77±1.28^{a}	2.49±0.59^{b}	2.89±0.51^{b}	17.96±1.28^{a}	10.73±1.08^{b}	10.40±1.12^{b}
Onda	12.52±1.08^{a}	3.18±0.25^{b}	2.64±0.41^{b}	11.93±3.49^{a}	12.94±3.62^{a}	10.66±3.34^{a}

Data in each row within each plant part followed by the same letter(s) are not significantly different ($p<0.05$)

Table 4: Zinc and Fe concentration ($\mu g\ g^{-1}$ DW) of shoot and root in four Iranian rice cultivars grown in nutrient solution with low and sufficient Zn supply at the bicarbonate (10 mM, pH = 8.0) or high pH (HEPES, pH=8.0) treatments

	Shoot			Root		
Zn concentration	Control	Bicarbonate	HEPES	Control	Bicarbonate	HEPES
Zn = 0 (µM)						
Mianeh	33.8±5.9^{a}	33.0±7.0^{a}	27.3±2.8^{a}	143.4±28.9^{a}	76.6±11.2^{b}	64.9±11.9^{b}
Shafagh	71.7±5.5^{b}	103.9±9.3^{a}	104.0±7.8^{a}	141.8±21.6^{b}	164.8±8.4^{ab}	189.8±11.9^{a}
T. Hashemi	79.9±15.5^{b}	126.2±14.1^{a}	91.6±5.0^{b}	300.0±24.6^{a}	255.4±34.9^{a}	172.5±31.5^{b}
Onda	100.3±12.7^{b}	252.2±15.3^{a}	124.1±11.9^{b}	360.3±11.1^{a}	637.4±23.4^{b}	334.3±14.4^{a}
Zn = 0.1 (µM)						
Mianeh	42.9±7.3^{a}	60.7±11.5^{a}	50.7±9.1^{a}	138.7±20.7^{a}	108.9±17.2^{ab}	100.6±17.6^{b}
Shafagh	98.2±3.6^{c}	258.5±9.0^{a}	172.9±10.9^{b}	160.3±13.9^{c}	534.1±46.6^{a}	287.6±38.1^{b}
T. Hashemi	73.7±9.9^{c}	121.5±9.5^{a}	92.9±5.6^{b}	340.5±24.3^{b}	548.3±51.7^{a}	342.6±31.5^{b}
Onda	90.9±3.9^{c}	262.4±18.5^{a}	171.3±15.4^{b}	372.2±41.1^{b}	668.2±145.4^{a}	320.0±14.4^{b}
Zn = 0.5 (µM)						
Mianeh	54.3±5.5^{a}	95.8±26.1^{a}	72.9±25.5^{a}	175.7±43.5^{b}	208.8±33.8^{b}	296.8±72.6^{a}
Shafagh	96.9±3.2^{b}	143.1±32.3^{a}	117.5±2.2^{ab}	216.5±17.3^{c}	486.4±71.6^{a}	349.6±18.5^{b}
T. Hashemi	48.7±7.3^{b}	96.9±7.4^{a}	57.0±2.6^{b}	284.4±50.0^{c}	816.8±70.8^{a}	401.9±51.9^{b}
Onda	118.8±4.7^{b}	183.8±9.2^{a}	95.4±2.5^{c}	281.8±15.1^{b}	495.3±32.7^{a}	303.1±82.3^{b}
	Shoot			Root		
Fe concentration	Control	Bicarbonate	HEPES	Control	Bicarbonate	HEPES
Zn = 0 (µM)						
Mianeh	98.5±15.4^{a}	40.2±7.0^{b}	39.9±13.4^{b}	1324±77^{a}	524±56^{b}	454±70^{b}
Shafagh	158.7±15.7^{a}	93.4±5.7^{b}	106.0±4.5^{b}	2134±107^{a}	1468±490^{a}	2407±768^{a}
T. Hashemi	272.7±47.6^{a}	153.7±43.6^{b}	78.9±10.9^{c}	3693±367^{a}	2793±422^{b}	1708±217^{c}
Onda	408.8±65.7^{a}	458.6±63.7^{a}	170.1±9.4^{b}	625±544^{c}	10351±1094^{a}	5158±1233^{b}
Zn = 0.1 (µM)						
Mianeh	97.8±8.9^{a}	90.9±18.7^{ab}	68.3±11.6^{b}	1007±65^{a}	769±89^{b}	679±98^{b}
Shafagh	164.9±1.0^{b}	270.5±34.1^{a}	151.7±9.7^{b}	1715±148^{c}	7993±996^{a}	4305±434^{b}
T. Hashemi	233.1±2.3^{a}	167.3±35.6^{b}	63.3±8.7^{c}	2873±443^{a}	3514±386^{a}	1949±326^{b}
Onda	230.1±1.5^{a}	174.9±16.5^{b}	132.9±23.4^{c}	5244±232^{a}	5227±1264^{a}	6017±1254^{a}
Zn = 0.5 (µM)						
Mianeh	103.6±6.9^{a}	99.4±18.1^{a}	85.3±37.3^{a}	1047±70^{b}	1528±203^{a}	1693±195^{a}
Shafagh	117.1±20.0^{a}	95.1±2.9^{ab}	89.8±8.3^{b}	1812±128^{b}	2568±415^{ab}	2609±538^{a}
T. Hashemi	115.9±13.9^{a}	108.2±25.6^{a}	57.6±10.2^{b}	1871±133^{b}	4471±450^{a}	2000±215^{b}
Onda	188.3±16.3^{a}	149.5±12.1^{b}	58.1±9.2^{c}	869±255^{b}	3014±846^{a}	1104±348^{b}

Data in each row within each plant part followed by the same letter(s) are not significantly different ($p<0.05$)

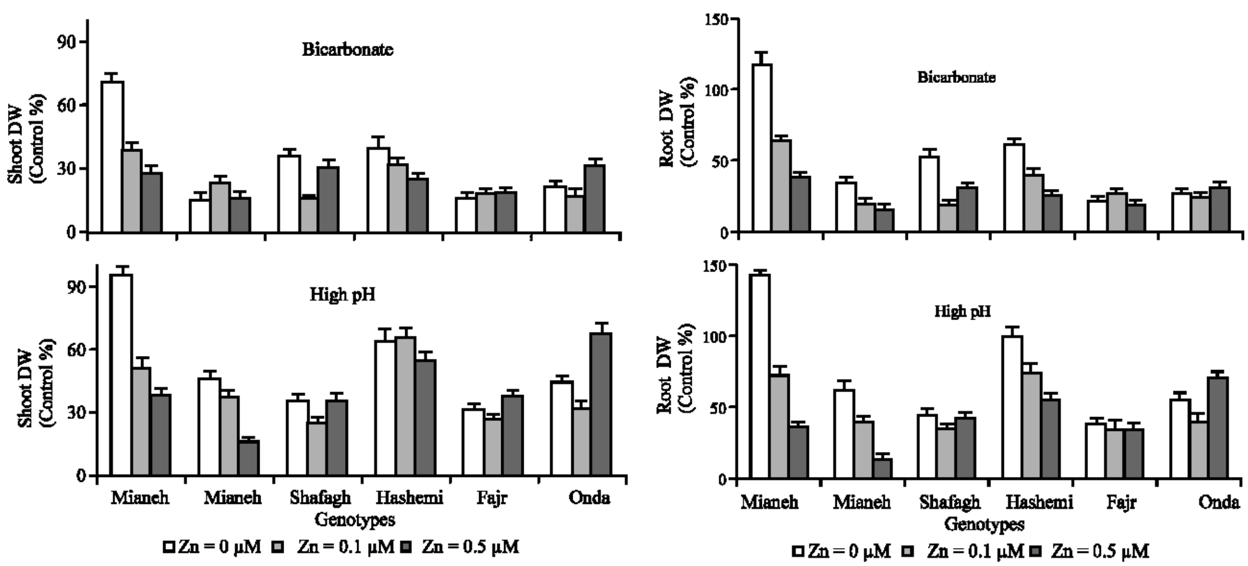

Fig. 2: Effect of bicarbonate and high pH (pH = 8.0) on chlorophyll content (% over control) of six Iranian rice cultivars grown in nutrient solution with different Zn levels

Bicarbonate and high pH induced changes in Fe concentrations of shoot and root in tendency or significant. In contrast to Zn concentration, Fe concentration of shoot was reduced at all Zn treatments and cultivars by bicarbonate and high pH. In contrast, high pH particularly bicarbonate cause a substantial accumulation of Fe in roots at all Zn treatments and in all analyzed cultivars with the exception of Mianeh at low Zn supplies (Table 4).

DISCUSSION

The visual Zn-deficiency symptoms as well as responses of fresh and dry matter production to low Zn supply demonstrated that Mianeh, Amol and Shafagh are the genotypes most resistant and T. Hashemi, Fajr and Onda are the genotypes most susceptible to Zn deficiency. The results of the field experiment were in accordance with the nutrient solution study (Hajiboland and Salehi, unpublished data).

In contrast to the results obtained for wheat (Rengel and Graham, 1995; Cakmak *et al*., 1996) in all of studied genotypes enhancement of root weight or root length was not observed in response to low Zn supply. Similar results were obtained in experiment with IR and Chinese rice genotypes (Hajiboland, 2000).

Growth results of tested genotypes demonstrated that, there is a high susceptibility to bicarbonate in Iranian rice cultivars. For example in Amol and Fajr, at sufficient Zn supply, biomass production was inhibited up to 84 and 81% in shoot and 91 and 80% in roots, respectively. Study of the effect of similar concentrations of bicarbonate (10 mM) on shoot and root growth of IR rice genotypes showed that in the most susceptible genotype (IR26), the shoot and root dry weight decreased only 19% and 45% compared to control (Hajiboland *et al*., 2003). The similar range of tolerance was recorded in different genotypes of wheat and rye originated from the Turkey (Hajiboland *et al*., 2003). It could be concluded that, the bicarbonate sensitivity in Iranian rice cultivars (in the exception of cv. Mianeh, see below) is much higher compared with IR or Chinese genotypes.

The highest tolerance to bicarbonate was observed in the Mianeh particularly at low Zn supply. In this cultivar and treatment, reduction of shoot growth was only 29% than the control plants. In the Mianeh, the root growth was rather slightly (root dry weight, 18%) or even significantly (root length, 70%) increased in response to bicarbonate (Table 2). Interestingly, the root length improvement was observed only in Zn deficient plants. The plants supplied with moderate or sufficient Zn, did not respond positively to bicarbonate in terms of root length. In the experiment with IR and Chinese genotypes it was also shown that, root length response to bicarbonate is strongly dependent to the level of Zn in the medium. In Zn-efficient genotypes root length was rather inhibited in response to bicarbonate when plants supplied with Zn higher than the recommended amount for rice. The opposite was observed in Zn-inefficient genotypes, in which at this Zn level, a stimulatory effect of bicarbonate was also appeared in this genotype. It was concluded that, the inhibitory effect of bicarbonate on root and shoot growth in Zn-inefficient rice genotypes is due to reduction of Zn supply to plant (Hajiboland *et al*., 2003).

Stimulated root growth was evaluated as an important adaptive mechanism of plants grown on calcareous soils (Hajiboland *et al*., 2003). It is known that the Zn bioavailability is limited by low Zn mobility in the soil solution and thus by low spatial availability. Accordingly, root growth and surface area are important parameters influencing bioavailability of Zn (Marschner, 1993). Therefore, in this work a significant increase of root length in cv. Mianeh, could be an important mechanism for acquisition of Zn in calcareous-soils grown plants.

Generally, the inhibitory effect of high pH on growth parameters was lower than that of bicarbonate. For example, the rate of reduction for Fajr at the high pH treatments were only 62 and 66% for shoot and root growth, which are significantly lower than the inhibition rate under the bicarbonate effect (80%). This is in agreement with the results obtained for IR rice genotypes as well as wheat and rye cultivars (Yang *et al*., 2003). These authors suggested that, because of a prominent effect of bicarbonate on shoot and root growth than that of high pH, there is most likely an effect of bicarbonate *per se* than the accompanying high pH (Hajiboland *et al*., 2003). In soil grown plants, there is strongly negative correlation between soil pH and rice yield when no fertilizer is added (Marschner, 1993). On the other hand, in the pH range of 6.5 to 8.0, the content of DTPA extractable Zn only slightly declines, but the drastic decrease in Zn uptake was attributed to elevated levels of bicarbonate (Marschner, 1993).

In addition of a high susceptibility to bicarbonate, these responses were not related to the Zn efficiency trait of cultivars. The most sensitive cultivars to bicarbonate (e.g., Amol and Fajr) were classified in the screening experiment as two contrasting cultivars concerning the Zn efficiency trait. However, the relatively high tolerance of the Mianeh, a Zn-efficient cultivar could be described independent from its Zn efficiency trait.

A close relationship between bicarbonate tolerance and Zn efficiency trait of IR rice genotypes, was attributed to the simultaneous development of these two trait during natural and artificial selection of genotypes which have been cultivated on calcareous soils with low Zn availability (Hajiboland *et al*., 2003). However, the lack of such relationship in Iranian rice cultivars, could be explained similarly by the soil characteristics of cultivation and breeding sites. The soils of rice fields in north Iran are classified as soils with relatively low pH (6.0-6.5) having low bicarbonate content. Therefore, the bicarbonate tolerance trait was not improved and selected during the breeding of these cultivars. In contrast, the genotype Mianeh, is cultivated in west and northwest Iran, on soils classified as calcareous soils (pH = 8.0-8.5). Therefore, a relatively high tolerance of this cultivar to bicarbonate is also due to its cultivation sites. In this cultivar, a stimulation effect of bicarbonate on root length up to 70%, was similar with the results obtained for the Zn-efficient IR rice cultivars (Hajiboland *et al*., 2003). The effect of bicarbonate on root morphology (Hutchinson, 1967; Geisler, 1963) and supply of carbon to plants leading to a growth improvement of roots via dark fixation (Bialczyk and Lechowski, 1992; Bialczyk *et al*., 1994) was reported.

In contrast to growth results, the chlorophyll content of cultivars in response to bicarbonate and high pH was correlated to their Zn efficiency trait. The cause of the discrepancy between chlorophyll and growth responses to bicarbonate in relation to Zn efficiency trait of cultivars is not known.

Mechanisms of growth inhibition of root by bicarbonate and high pH were studied by some workers. Many authors have explained the root inhibition caused by bicarbonate treatments as being the result of excess accumulation of organic acids, particularly malate, which is an important product of dark fixation (Lee and Woolhouse, 1969a,b; Yang *et al*., 1994a; Yang *et al*., 2003). This could be also the cause of root growth inhibition in presenr study.

However, the strong reduction of shoot growth could be only described as disturbance of nutrients uptake in plants induced by bicarbonate and high pH. As it was shown also in Table 3, the Zn and Fe content of plants were strongly reduced in bicarbonate and high pH treated plants. The inhibitory effect of bicarbonate on uptake of nutrients including Zn, Fe and Cu in soil grown plants (Kausar *et al*., 1976; Rahmatullah *et al*., 1976; Rashid *et al*., 1976; Yang *et al*., 1994b) as well as in nutrient solution cultures (Hale and Wallace, 1960; Yang *et al*., 1994a; Hajiboland, 2000) was well documented. In this work, the reduction of Zn content in cultivar Mianeh in response to bicarbonate in Zn deficient plants was lower (30%) than other cultivars (up to 48% in Shafagh) and consequently any change in Zn concentration of shoot was observed in the former cultivar. However, for study of a possible differential effect of bicarbonate on Zn uptake and transport in cultivars with different bicarbonate tolerance, it is necessary to carry out a short-term experiment with isotopes and without bicarbonate induced-differential root growth throughout the experiment. In such a study with two contrasting IR genotypes, it was shown that, inhibition of ^{65}Zn uptake was higher in bicarbonate susceptible than tolerant genotype in response to 10 mM bicarbonate (Hajiboland, 2000).

However, the Zn concentration in the shoot and root was not reduced but rather increased compared with control plants (Table 4). It means that, the growth inhibition was higher than the reduction of uptake, leading to an accumulation of Zn in plants. In contrast, because of a much strong reduction of Fe uptake in bicarbonate treated plants, the Fe concentrations of shoot was reduced though a strong reduction of shoot biomass. However, the Fe concentration of root was increased substantially in the exception of Mianeh at low or moderate Zn supplies. A high root Fe concentration could be not only the result of root growth inhibition but also the result of the formation of iron plaques (Otte *et al*., 1989) on root surfaces that most likely could not be removed during the washing of roots with distilled water.

Growth reduction of particularly shoot under bicarbonate treatment could be the result of inactivation of Zn, Fe and other micronutrients within plants. Bicarbonate causes a precipitation of Fe in leaf apoplasm and lead to Fe deficiency though being a normal total Fe concentration in leaves (Nikolic *et al*., 1998). Therefore, available Fe fraction instead of total Fe was proposed as indicator of Fe nutritional status (Zhang *et al*., 1995). For Zn, there are also some reports on the reduction of bioavailable Zn within plants under the treatments such as phosphate (Cakmak and Marschner, 1987) and bicarbonate (Hajiboland, 2000). Therefore, it could be assumed that, bicarbonate and high pH reduce the bioavailable fraction of Zn and Fe (and likely other micronutrients) and subsequently cause the growth reduction.

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