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EFFECT OF BICARBONATE AND HIGH PH ON ROOT GROWTH AND ACCUMULATION OF ORGANIC ACIDES IN TWO RICE CULTIVARS DIFFERING IN SUSCEPTIBILITY TO ZN DEFICENCY

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ABSTRACT

The objective of the present study was to evaluate the effect of bicarbonate and high pH treatments separately on shoot and root growth and accumulation of organic acids in the roots of two rice cultivars differing in susceptibility to Zn deficiency. The results indicated that shoot and root dry weight of both cultivars decreased by the treatments with bicarbonate and high pH and the decrease in shoot and root dry weight were greater when plants grown with bicarbonate than that with high pH. The inhibitory effect of bicarbonate end high pH on shoot and root dry weight of the Zn-efficient rice cultivar (S 101) was less than that of the Zn-inefficient rice cultivar (G172). Root length was significantly decreased by bicarbonate and high pH treatments for the Zn-inefficient rice cultivar (G172); whereas was considerably enhanced by bicarbonate and high pH treatments for the Zn-efficient rice cultivar(S101), and the enhancement of root length in the Zn-efficient rice cultivar (S101) was greater when plants grown with bicarbonate than that with high pH. Both bicarbonate and high pH treatments increased the concentrations of organic acids (malic, citric, fumaric and succinic) 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 low land 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

Zinc deficiency in plants is widespread throughout the world. It is estimated that more than 40% of the soils surveyed in 19 countries are zinc deficient (Graham *et al.*, 1992). In Egypt, zinc is one of the most limiting factors affecting rice production second only to nitrogen. Zinc deficiency of lowland rice occurs widely in almost neutral to alkaline soils, particularly calcareous soils (Sobhy *et al.*, 2002).

Bicarbonate and high pH combined with low Zn availability may be involved in the induction of Zn deficiency for the Zn-inefficient genotypes. The zinc-efficient genotypes are those that grow and yield normally when grown in soils with low Zn availability. The susceptibility of rice genotypes to Zn deficiency was found to be closely associated with high tolerance of plants to elevated bicarbonate concentrations (Yang *et al.*, 1994). Zinc deficiency in the field occurs generally 2–4 weeks after rice transplanting (Qin, 1988) or 4–6 weeks after submergence when bicarbonate concentration reaches as high as 40 mM (Forno, *et al.*, 1975).

Shoot growth of the Zn-inefficient rice cultivar was strongly inhibited by bicarbonate, whereas that of the Zn-efficient was not affected (Hajiboland et al., 2003 and Yang *et al.*, 2003). Little information is available, however, concerning the mechanisms how bicarbonate induces Zn deficiency. Dogar and Hai (1980) indicated that bicarbonate inhibited Zn absorption by rice roots, but Forno *et al*, (1975) reported that the primary effect of bicarbonate is to inhibit Zn translocation from roots to shoots.

One major factor may be the high accumulation of organic acids in root cells through stimulation of phosphoenolpyruvate -carboxylase in cytoplasm induced by bicarbonate or high pH. It has been reported that lime-induced chlorosis in some plants was related to high accumulation of organic acids (Yang *et al.*, 1994) Again; it is unknown whether bicarbonate has a specific effect on organic acid accumulation for Zn-inefficient rice genotypes, or whether high pH has the same effect. The objectives of this study were to examine the effects of high pH and bicarbonate separately on root growth and the accumulation of organic acids in Zn-efficient and Zn-inefficient rice genotypes.

MATERIALS AND METHODS

Primary experiment

Nine rice cultivars (Giza 171,172,176,177,178,181 and Sakha 101,102,103) were classified based on dry matter production (root plus shoot) at the low Zn level and Zn-use efficiency, Twenty-days-old seedlings of similar size from the cultivars were selected, sixteen seedlings transplanting together in each 3-liter container containing 2.5-liter from nutrient solution as described by yoshida, (1972), (mM): NH_4NO_3 1.43, $CaCl_2$ 1.00, $MgSO_4$ 1.64, K_2SO_4 1.32, KH_2PO_4 0.32 and (μM): $MnCl_2$ 9.5, $FeCl_3$ 35.6, $ZnSO_4$ 0.15, $CuSO_4$ 0.15, H_3BO_3 1.9, and $(NH_4)_6Mo_7O_{24}$ 0.075.while zinc were added at 3 levels of zinc as: (zero, 0.08 and 0.15 (μM). Nutrient solution was changed every 5 day and the pH adjusted daily for pH (5.5-6).

Thirty days after transplanting, the plants were harvested then separated into shoot and root, rinsed with distilled water and dry weight of shoot and root were recorded after drying at 70 oC in a forced-air oven, then ground and analyzed for zinc.

Main experiment

Two rice cultivars, one Zn-inefficient (Giza 172) and one Zn-efficient (Sakha 101), were selected for this study from the primary experiment. Seeds were germinated in Petri dishes over three layers of cotton saturated with distilled water. The dishes were then covered and left in the incubator for 48 h at temperature between 28-30 oC. The germinated rice seeds were raised on a nylon net for 5 days in a solution of 0.02 mM $CaSO_4$.

Prior to bicarbonate treatment, all rice seedlings were precultured for 7 days in the same composition of nutrient solution (Yoshida, 1972) as described in the primary experiment. Instead of $FeCl_3$, Fe was supplied as

FeEDDHA (Ethylene-bis-[2-(0-hydroxyphenyl) glycine]) at 0.01mM during this period.

Twelve-day-old seedlings of similar size were selected and transplanted to 3-L plastic containers containing the same composition of nutrient solution with 0.1 mM FeEDDHA. Each container had sixteen seedlings. Bicarbonate was supplied at three concentrations: 0, 10, and 20 mM as sodium bicarbonate. The pH of the nutrient solution was adjusted daily by NaOH or H_2SO_4 to pH 6 for the control and to pH 8 for the high pH and bicarbonate treatments. Each treatment was replicated 3 times. Plants were harvested after 0, 4, and 8 days of the start of bicarbonate treatments.

1-Root and shoot dry weight

Five plants from each replicate of the treatments were used for shoot and root dry weight measurement. Plants were rinsed with distilled water, separated to shoot and root and blotted between filter papers. Dry weight of shoot and root were recorded after drying at 70°C in a forced-air oven.

2-Root length

Another five plants of each replicate were used for root length measurement. Fresh root was used for root length measurement by using the method described by Newman, (1966).

Method of measuring root length:

The method is based on the assumption that if a root is laid within an area within which some straight lines lie at random, we should expect that the longer the root the more intersections it will make, on average, with the straight lines. Thus the number of intersections can be used to estimate the length of root by the following equation:

$$L = (\pi A N) / 2H$$

L = root length (cm) of the counted sample.
A = area of screen cm^2.
N = number of intersection.
H = length of hair line in the plain where the root are counted.

The N is calculated as (Sum of intersection / Sum field examined).
Root length per g of counted sample = L/C
Where C= dry weight of the counted sample.
Total length of the root = (L/C) × total dry weight of the root .

3-Organic Acids

The rest of the plants of each replicate were used for organic acid determination. Roots were rinsed with distilled water, blotted dry, weighed and stored at -20 °C for organic acid determination. 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, fumaric, and succinic acids were measured by Gas Chromatography (Mass Selective Detectors 5972 series). Each sample of the treatments with 3 replicates was analyzed twice.

RESULTS AND DISCUSSION

Classification of nine rice cultivars for zinc-use efficiency:

Nine rice cultivars were classified based on dry matter production (root plus shoot) at the low Zn level and Zn-use efficiency, Table (1) and Figure (1) illustrated these results.

Table (1) Zinc use efficiency (A-B/C-D) of several rice cultivars under different zinc levels.

Cultivars	A	B	C	D	Zn use Efficiency
1-Giza 172	7.40	4.75	162.41	86.23	34.79
3-Giza 176	6.07	4.77	168.42	104.67	20.39
5-Sakha 103	7.17	4.87	194.78	108.46	26.59
4-Giza 177	9.49	6.13	257.03	123.52	25.13
2-Giza 171	8.58	6.17	215.87	133.77	29.29
6-Giza 178	8.72	6.73	242.22	142.99	20.05
7-Sakha 102	9.42	7.39	260.22	158.08	19.83
8-Giza 181	8.36	7.59	227.46	150.59	9.97
9-Sakha 101	8.49	7.72	250.56	152.59	7.81

A= Dry matter yield of (root + shoot) across medium and high Zn level (g/pot).
B= Dry matter yield of (root + shoot) at low Zn level (g/pot).
C= Zn accumulation in (root + shoot) across medium and high Zn level (μg / pot).
D= Zn accumulation in (root + shoot) at low Zn level (μg / pot).

These rice cultivars were classified into four groups as follows:

1) Efficient and non responsive : In this group are cultivars which produced dry matter yield higher than the average of nine cultivars at the low Zn level, but Zn-use efficiency was lower than the average of nine cultivars. Cultivars G178, G 181, S101 and S102 fall into this category.
2) Non efficient and responsive : In this group are cultivars which produced dry matter yield less than the average of nine cultivars at the low Zn level, but Zn-use efficiency was higher than the average of nine cultivars. Cultivars G172, G 171, G177 and S103 fall into this category.
3) Non efficient and non responsive : In this group are cultivars which produced dry matter yield less than the average of nine cultivars at the low Zn level, but Zn-use efficiency was less than the average of nine cultivars. Cultivars G176 fall into this category.
4) Efficient and responsive : In this group are cultivars which produced dry matter yield higher than the average of nine cultivars at the low Zn level, but Zn-use efficiency was higher than the average of nine cultivars. Non cultivars fall into this category.

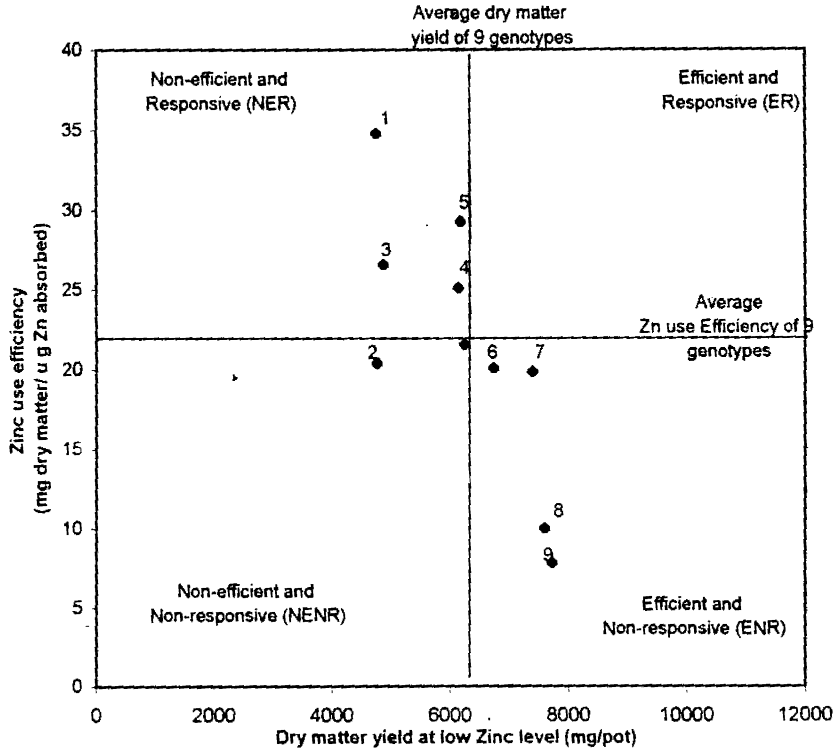

Fig.(1) Classification of several rice cultivars for Zinc-use efficiency

EFFECT OF BICARBONATE AND HIGH pH ON SHOOT AND ROOT DRY WEIGHT:-

Shoot and root dry weight of both the Zn-efficient cultivar (S101) and the Zn-inefficient cultivar (G172) were significantly decreased by the treatments with bicarbonate whereas pH treatments had no effect for 4-and 8-days compared with control (pH 6). The decrease in shoot and root dry weights were greater when grown with bicarbonate than with high pH (Figures 1-2). Similar results obtained by (Hajiboland *et al.*, 2003) and (Yang *et al.*, 2003).

The inhibitory effect of bicarbonate on shoot dry weight of the Zn-efficient cultivar (S101) was less prominent than that of the Zn-inefficient cultivar (G172). For instance, at day 8 of treatment, the decrease in shoot dry weight of the Zn-efficient cultivar was around 6 % for high pH treatment, 21% for 10 mM bicarbonate treatment and 29 % for 20 mM bicarbonate treatment, as compared with the control (pH 6), the corresponding values for the Zn-inefficient cultivar were 8, 46 and 51 %. This finding is in agreement with the results of (Hajiboland *et al.*, 2003) where they reported that the reduction in shoot growth due to bicarbonate treatment varied among the Zn-efficient and Zn-inefficient cultivars.

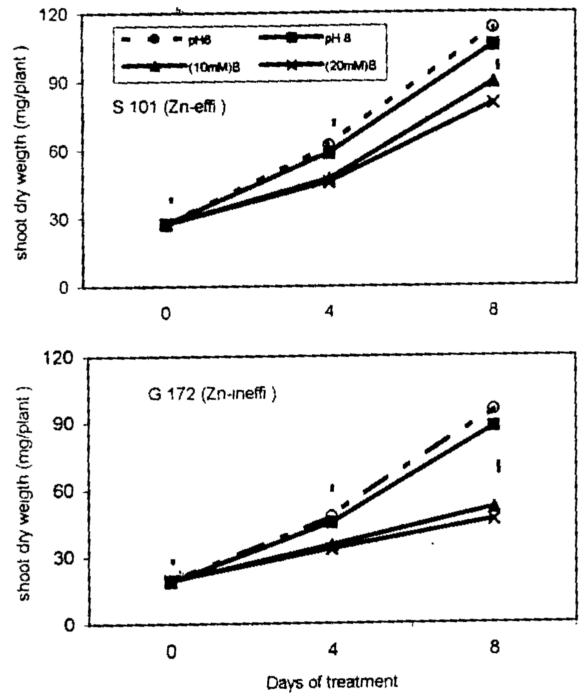

Fig (2) Effect of bicarbonate and high pH on shoot dry weight of the Zn-efficient (S101) and Zn-inefficient (G172) rice cultivars.

The bars depict the least significant difference (LSD, P< 0.01) for the corresponding group of the data.

The inhibiting effect of bicarbonate and high pH treatments on root dry weight of the Zn-efficient cultivar (S101) was less prominent than that of the Zn-inefficient cultivar (G172). For instance, at day 8 of treatment, the decrease in root dry weight of the Zn-efficient cultivar was around 5 % for the high pH treatment, 25% for 10 mM bicarbonate treatment and 27 % for 20 mM bicarbonate treatment, as compared with the control (pH 6), the corresponding values for the Zn-inefficient cultivar were 23, 60 and 69 %. Similar results obtained by (Yang *et al.*, 2003).

EFFECT OF BICARBONATE AND HIGH PH ON ROOT LENGTH:-

The root length of the Zn-efficient cultivar (S101) was considerably enhanced at day 4 and 8 when plants were grown with bicarbonate, but no obvious increase in root length was found when grown with high pH treatment (Fig. 3). On the contrary, the root length of the Zn-inefficient cultivar (G172) was obviously reduced at day 4 and dramatically reduced at day 8 of the bicarbonate or high pH treatments, and a greater reduction was noted for bicarbonate than for high pH at day 8 (Fig. 3).

The increases in root length for Zn-efficient cultivar were 54 % for 10 mM bicarbonate treatment and 64 % for 20 mM bicarbonate treatment at day

4, and reached to 74 % for 10 mM bicarbonate treatment and 83 % for 20 mM bicarbonate treatment at day 8. The decreases in the root length for Zn-inefficient cultivar were 13 % for 10 mM bicarbonate treatment and 42 % for 20 mM bicarbonate treatment at day 4, and 57 % for 10 mM bicarbonate treatment and 67 % for 20 mM bicarbonate treatment at day 8, compared with control (pH 6).

The results imply that bicarbonate might be the major responsible factor for enhancing root elongation in the Zn-efficient cultivar and inhibiting root elongation in the Zn-inefficient cultivar. The results of this study is in agreement with that of (Yang et al., 2003); they reported that the increases in root length was 75 % for Zn-efficient rice cultivar and the reduction in root length was 47 % for Zn-inefficient rice cultivar.

The strong enhancement of root elongation by bicarbonate in the Zn-efficient rice cultivar at the Zn-deficient and moderate Zn levels growth in the media could help plants develop a larger root surface area and enhance root capacity for absorbing Zn (Hajiboland et al., 2003).

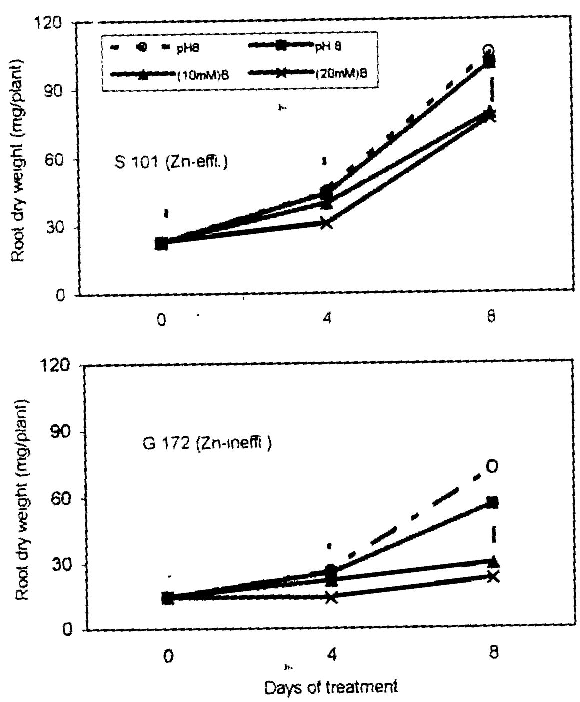

Fig (3) Effect of bicarbonate and high pH on root dry weight of the Zn-efficient (S101) and Zn-inefficient (G172) rice cultivars.

The bars depict the least significant difference (LSD, $P< 0.01$) for the corresponding group of the data.

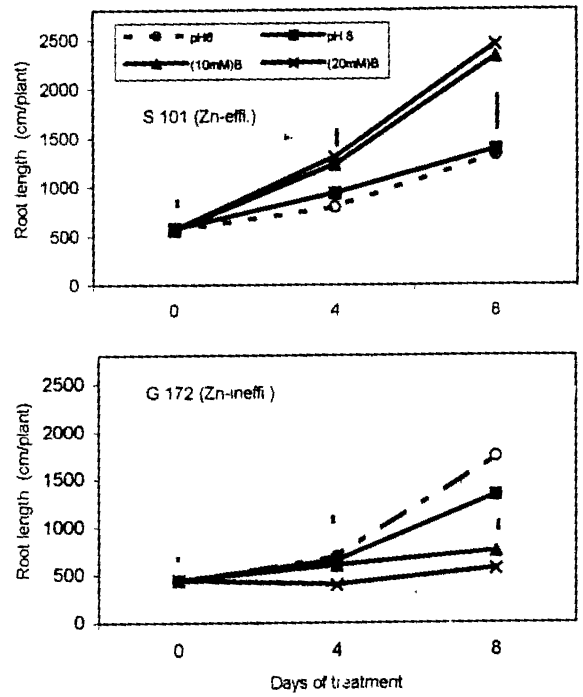

Fig (4) Effect of bicarbonate and high pH on root length of the Zn-efficient (S101) and Zn-inefficient (G172) rice cultivars.

The bars depict the least significant difference (LSD, P< 0.01) for the corresponding group of the data.

EFFECT OF BICARBONATE AND HIGH PH ON ACCUMULATION OF ORGANIC ACIDS IN ROOTS:-

The four major organic acids (i.e. malic, citric, fumaric and succinic acids) in rice roots are affected by bicarbonate and high pH treatments. In the roots of both Zn-efficient and Zn-inefficient cultivars malate was accumulated with the time.

At either day 4 or 8, malate concentration in the roots of the Zn-efficient cultivar was not affect by the high pH treatment, but increased ,on average, around three and five folds for 10, 20 mM bicarbonate treatments, respectively, compared with control (pH6) (Fig.4). Whereas, malate concentration in the root of the Zn-inefficient cultivar increased, on average, around three fold for high pH treatment and up to five and six folds for 10 and 20 mM bicarbonate treatment respectively, compared with control (pH 6) (Fig.4).

At day 4, malate concentration in the root of the Zn-efficient cultivar was, on average, 4 fold less than that of the Zn-inefficient cultivar. However, these differences were much less observed after 8 days of growth. These results were in agreement with those of Yang *et al.*, (1994).

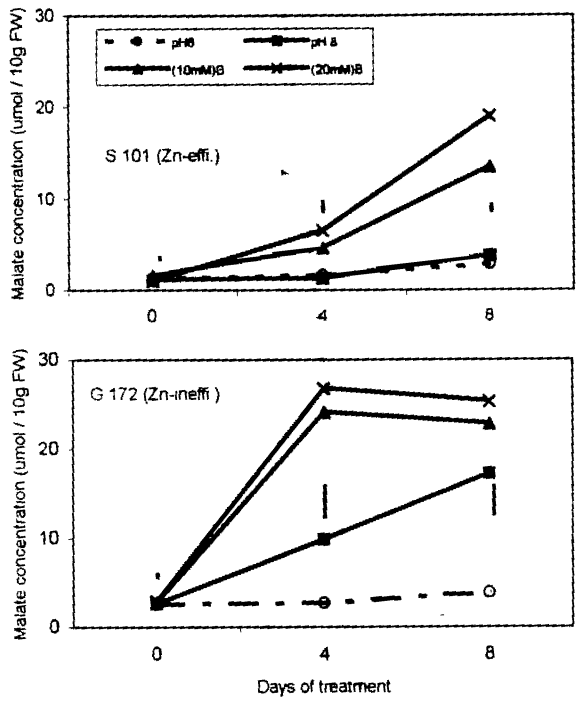

Fig (5) Effect of bicarbonate and high pH on Malate concentration in the roots of the Zn-efficient (S101) and Zn-inefficient (G172) rice cultivars.

The bars depict the least significant difference (LSD, P< 0.01) for the corresponding group of the data.

They reported that the synthesis of malate results from a marked activation of the cytoplasmic phosphoenolpyruvate carboxylase (dark fixation) induced by an increase in cytoplasmic bicarbonate and pH.

In general, malate concentration in the roots of the Zn-inefficient cultivar was, on average, around three fold greater than that of the Zn-efficient cultivar. These results are in agreement with (Yang et al., 1994, 2003) they reported that at 4 and 8 days of treatment, malate accumulation in the roots of the Zn-efficient rice cultivars was 2-3 folds less than that of the Zn-inefficient rice cultivars.

Large difference occurred in citrate concentration in the roots of Zn-inefficient and Zn-efficient treated with high pH and bicarbonate treatments (Fig.5). For the Zn-inefficient cultivar, citrate concentration in the roots reached its peak at day 4 for 20 mM bicarbonate and increased continually for high pH and bicarbonate treatments with the time.

Bicarbonate and high pH treatments had the most prominent influence on citrate concentration in the root of Zn-inefficient. After 4 and 8 days of treatment the citrate concentration in the roots of Zn-inefficient cultivar *increased*, on average, 7, 14 and 33 folds at high pH ,10 and 20 mM bicarbonate treatments, respectively, whereas, citrate concentration in roots of Zn-efficient was four fold at 20 mM bicarbonate treatment compared with control (pH 6). But no obvious difference in citrate concentration of the Zn-efficient cultivar was observed between high pH and 10 mM bicarbonate treatments compared with control (pH 6). Similar results obtained by Yang et al., (2003)

The other two organic acids which responsed to bicarbonate and high pH treatments in rice roots included succinate and fumarate. Fumarate takes the same trend of malate and citrate of Zn-inefficient cultivar. At 4 to 8 days, fumarate concentration in the roots of Zn-inefficient cultivar increased around 3 to 5 folds with high pH treatment and up to 6 to 7 and 7.7 to 8 folds with 10, 20 mM bicarbonate treatments, respectively. Whereas, fumarate concentration in roots of Zn-efficient reached its peak at day 4 for high pH and bicarbonate treatments and decreased (Fig.6).

In both cultivars (Zn-inefficient and Zn-efficient) bicarbonate treatments caused more fumarate accumulation than high pH treatment. Bicarbonate induced much more fumarate accumulation in the roots of the Zn-inefficient cultivar than that of the Zn-efficient cultivar at 4 and 8 days.

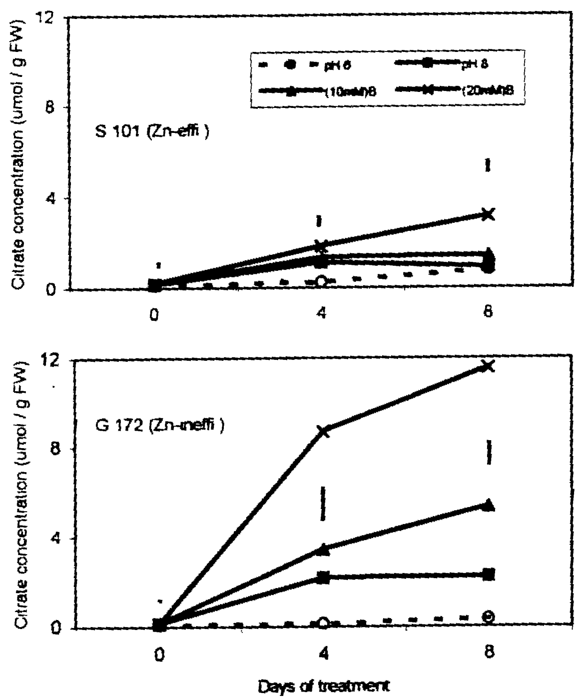

Fig (6) Effect of bicarbonate and high pH on citrate concentration in the roots of the Zn-efficient (S101) and Zn-inefficient (G172) rice cultivars

The bars depict the least significant difference (LSD, P< 0.01) for the corresponding group of the data.

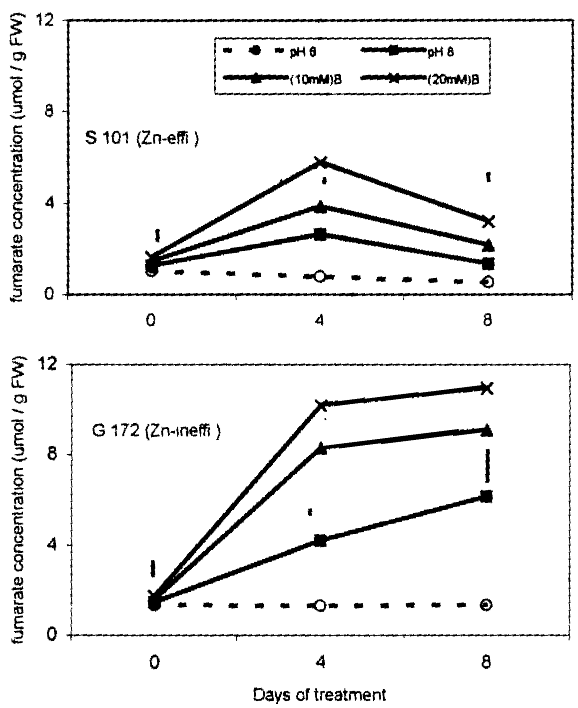

Fig (7) Effect of bicarbonate and high pH on fumarate concentration in the roots of the Zn-efficient (S101) and Zn-inefficient (G172) rice cultivars

The bars depict the least significant difference (LSD, P< 0.01) for the corresponding group of the data.

Succinate concentration was much lower than that other organic acids in the rice roots for Zn-inefficient and Zn-efficient. Succinate concentration in the roots of the Zn-inefficient cultivar increased by 60% and 80 % for high pH treatment, and up to 90 % and 96 % for 10 mM bicarbonate at day 4 and 8, respectively. In case of the 20 mM bicarbonate treatment succinate concentration reached a peak at day 4 (Fig.7), then leveled off. Succinate concentration in the roots of the Zn-efficient cultivar increased, on average, 30 % for high pH treatment , and up to 40 % and 110 % for 10 and 20 mM bicarbonate treatments, respectively, compared with control (pH 6).

No significant difference in succinate concentration of the Zn-efficient cultivar was observed between high pH and 10 mM bicarbonate treatments at 4 and 8 days, whereas, in the Zn-inefficent no significant difference in succinate concentration was observed between high pH and 10 mM bicarbonate treatment at day 8 only but there is a significant difference in day4.

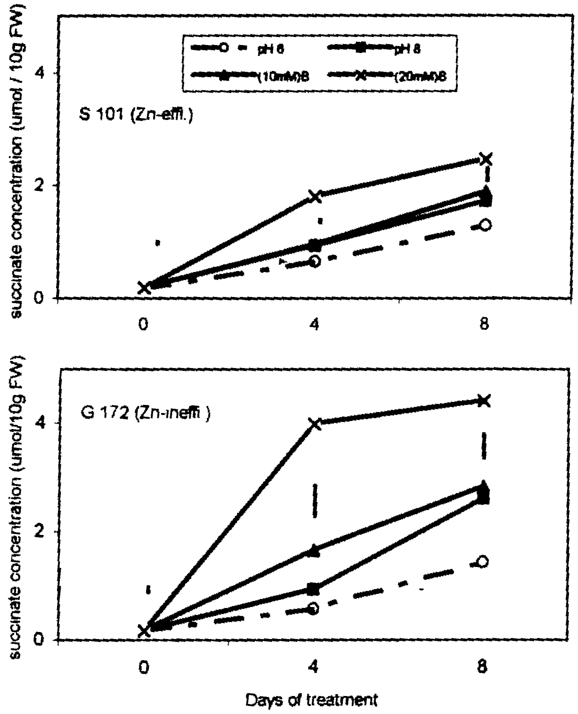

Fig (8) Effect of bicarbonate and high pH on succinate concentration in the roots of the Zn-efficient (S101) and Zn-inefficient (G172) rice cultivars.cultivars

The bars depict the least significant difference (LSD, P< 0.01) for the corresponding group of the data.

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تأثير البيكربونات ورقم الــ pH المرتفع على نمو الجذور وتراكم الأحماض العضوية فى صنفى أرز مختلفين فى حساسيتهما لنقص الزنك.

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تهدف الدراسة الحالية إلى تقييم اثر كلا من معاملات البيكربونات ورقم الــ pH العالى منفصلين على نمو المجموع الخضرى والجذرى وكذلك تراكم الأحماض العضوية فى جذور صنفين من أصناف الأرز مختلفين فى حساسيتهما لنقص الزنك. وقد دلت النتائج المتحصل عليها أن الوزن الجاف لكل من المجموع الخضرى والجذرى لكلا الصنفين قل نتيجة لتأثير كلا من معاملات البيكربونات ورقم الــ pH العالى وان النقص فى المجموع الخضرى والجذرى كان كبيرا فى حالة نمو النباتات فى معاملة البيكربونات عنه فى حالة نمو النباتات فى معاملة رقم الــ pH العالى. وان التأثير المثبط لكلا من معاملات البيكربونات والــ pH العالى على نمو المجموع الخضرى والجذرى كان قليلا فى صنف الأرز الكفؤ فى استخدام الزنك (سخا ١٠١) عن الصنف غير الكفؤ فى استخدام الزنك (جيزة ١٧٢) . أدت معاملات البيكربونات والــ pH العالى الى انخفاض معنوى فى الطول الكلى لجذور صنف الأرز غير الكفؤ فى استخدام الزنك(١٧٢)، بينما كان هناك تحسن ملحوظ فى الطول الكلى لجذور صنف الأرز الكفؤ فى استخدام الزنك (سخا ١٠١) كنتيجة لمعاملات البيكربونات ورقم الــ pH العالى وان هذا التحسن فى نمو جذور صنف الأرز الكفؤ فى استخدام الزنك (سخا ١٠١) كان كبيرا عند نمو النباتات فى معاملات البيكربونات عنه عند نموها فى معاملات رقم الــ pH العالى. أدت المعاملة بكل من البيكربونات ورقم الــ pH العالى إلى زيادة تركيزات الأحماض العضوية (الماليك والستريك والفيوماريك والسكسنيك) فى جذور كلا من صنفى الأرز ولكن بدرجة اكبر فى الصنف غير الكفؤ عن الصنف الكفؤ. وتشير النتائج إلى أن العجز فى نمو الجذور ربما يرجع إلى التأثير الأولى للبيكربونات وهو المسئول عن تشجيع حدوث نقص الزنك فى أراضى الأرز المغمورة وان التأثير المثبط للبيكربونات على نمو جذور الصنف غير الكفؤ ربما يكون كنتيجة للتراكم العالى وعدم التوزيع الكافى للأحماض العضوية فى خلايا الجذر.