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The Natural Chelators in the Root Exudates of Barley and Their Ability to Dissolve Sparingly Soluble Source of Iron

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ROOTS of Fe-deficient grasses such as barley release phytosiderophores which greatly enhance mobilization and uptake of Fe. The present work was conducted in order to determine the total amounts of amino acids, organic acids and phytosiderophores in the root exudates of Fe-efficient barley plant (Giza 125), particularly in response to iron deficiency and to evaluate the ability of such organic compounds to dissolve sparingly soluble source of iron, *i.e.*, Fe^{3+} phosphate and Fe^{3+} hydroxide. Results show that total amounts of amino acids as well as organic acids are clearly higher in case of Fe-deficient barley root compared to those of Fe-sufficient ones; the relative increases are 775 and 170%, respectively. Similarly, phytosiderophores released from barley roots under iron deficiency reach about four fold that of sufficient condition. On the other hand, root exudates collected from Fe-deficient barley and shacked with Fe^{3+}phosphate Fe^{3+}hydroxide show that considerable amounts of Fe^{3+} are solubilized from such compounds. Increase in Fe^{3+}solubility represents about four fold that of control (only $CaSO_4$ solution).

Keywords: Iron-root exudates, Hytosiderophores, Amino acids, Organic acids *Hordeum vulgare* L.

In recent years, considerable progress has been made in identification of adaptive mechanisms which plant species and genotypes developed to overcome iron deficiency in soils, which have low availability of iron. It is known that plant roots exude organic compounds into the rhizosphere such as certain amino acids, organic acids, vitamins and other substances, which may affect nutrients flux into the root (Vancura, 1964 and Mengel &Kirkby, 1982).

In response to iron deficiency, roots of graminaceous species such as barley release so-called phytosiderophores, which are highly effective in solublizing, by chelation, the sparingly soluble inorganic Fe compounds (Takagi *et al.*, 1984). They stated that a natural chelator designated mugineic acid was isolated from the root washings of barley. However, Romheld & Marschner (1986) found that under the stress of Fe deficiency, some graminaceous plants secret phytosiderophores

such as avenic acid in oat, and 2-deoxy mugineic acid in wheat plant. Loeppert *et al.* (1994) added that the predominant phytosiderophores are compounds of the mugineic acid family. The phytosiderophores have a high affinity for Fe^{3+} and can facilitate the dissolution of soil Fe and increase the availability of Fe to plant roots.

The objective of this work is to determine the natural chelators, *i.e.*, amino acids, organic acids, and phytosiderophores contents of Fe-deficient and Fe-sutficient barley plant and to evaluate the ability of root exudates to dissolve the sparingly soluble source of iron.

Material and Methods

An experiment was carried out in a control chamber to study the effect of root exudates, particularly, phytosiderophores of barley plant on solublizing sparingly soluble sources of iron (Fe^{3+}phosphate or Fe^{3+}hydroxide). Pre-experiment was conducted to evaluate Fe-efficiency of five barley cultivars according to Brown & Jones (1976). Details of such experiment are found in Elhedek (2000). Dark plastic pots of 500 ml capacity, covered with polyvinyl chloride plate having ten holes were used as nutrient solution culture. The following nutrient solution of Cakmak *et al.* (1989) was used. The nutrient solution consists of 2, 0.88, 0.65, 0.25 and 0.10 mmol of Ca $(NO_3)_2$, K_2SO_4, $MgSO_4$, KH_2PO_4 and KCl, respectively and 10, 1, 0.1, 0.01 and 1 umol of H_3BO_3, MnSO4, CuSO4, $(NH_4)_6Mo_7O_{24}$ and $ZnSO_4.7H_2O$, respectively. The pH and EC of this solution were 6 and 0.8 dS/m, respectively.

Ten seedlings of barley, *Hordeum vulgare* L. cv. Giza 125, selected as the most Fe-efficient cultivar, was transferred to the experimental culture, which received 0 or 0.04 mmol/l Fe added as Fe EDTA. The plants were grown under controlled conditions (16/8hr day/night regime, relative humidity between 65-75% and light intensity 12K Lux). Aeration was adjusted, and then the nutrient solutions were changed every 3 days. Pots of Fe-deficient plants were replicated 11 times while those for Fe-sufficient were 8.

After 15 days from Fe treatments, root exudates were collected in 500 ml plastic pots containing 450ml of $CaSO_4$. $2H_2O$ (0.2 mM) of pH 6.85 as described elsewhere (Romheld & Marschner, 1986 and Elsharawy, 1989). Root exudates of 8 pots of Fe-deficient and 8 pots of non-deficient plants were collected from 2 to 6 hr after the beginning of the light period from each pot. The remained 3 pots of Fe deficient plants received 25 mg Fe/500 ml solution as $FePO_4$ or freshly precipitated $Fe(OH)_3$ and remained for 6 days until plants recovery.

Plants were washed, harvested and separated into roots and shoots, then dried at 70°C . Subsamples 0.5 g of each was digested by sulphuric acid and H_2O_2, and Fe concentration was determined. The pH of the exudate was immediately measured. The root exudates were filtered and concentrated to about 10 ml by

dryness under vacuum at 40^0C, then prepared for amino acids determination by the ninhydrin colorimetric method (Rosein, 1957). Determination of total organic acids was done according to Dragunova (1958).

The phytosiderophores concentration in the root exudates were determined indirectly by the method of Takagi (1976) as follow: One half ml of 0.5 M sodium acetate buffer solution, pH 5.6 was added to 10 ml of the sample solution of phytosiderophores and the pH was adjusted to 5.6 by adding a few drops of diluted NaOH or H_2SO_4 solution. Then, 2 ml of freshly prepared ferric hydroxide were added to the phytosiderophores sample solution and vigorously shaken at room temperature for 2 hr. Finally the suspension mixture was carefully filtered into flask containing 0.2 ml of 3N H_2SO_4. Iron chelated with phytosiderophores was determined colorimetrically using O-Phenanthroline.

To study the effect of root exudates collected from Fe-deficient plants on the solubility of relatively insoluble Fe compounds without interfering effect of plant roots through uptake of solubilized Fe and further effects due to root morphology such as absorption or partial precipitation of Fe in root apoplast, fifty mg Fe as $FePO_4$ or freshly precipitated Fe $(OH)_3$ were placed in 1 liter aerated plastic bottle together with 500 ml of the following solution: a) 0.2 mM $CaSO_4.2H_2O$ as control treatment, or b) collected root exudates from barley seedlings grown under Fe deficient condition. The plastic bottles were left standing for 24 hr., then shacked for 48 hr. using an over head shaker and filtered, then Fe was measured in the filtrate.

Results and Discussion

Dry weight, concentration and total Fe uptake of barley plants

Data in Table 1 show root and shoot dry weights values of barley plant grown for 15 days in water culture. Fe nutrition resulted in a significant increase in dry weights of shoots and roots compared to control. The relative increase in dry weight of barley shoot was 443% while increase in barley root dry weight was 302%. Values of Fe concentration in roots as well as shoots of barley plant are shown in Table 1. As expected barley roots contained higher Fe concentration than those of shoots. Addition of Fe resulted in higher Fe concentration as well as total uptake, which had a similar trend to those of Fe concentration. These results are in agreement with those reported by Fleming & Foy (1982) and Gangadhar *et al.* (1992).

TABLE 1. Root and shoot dry weight, Fe concentration and total uptake of barely plants grown in water culture as effected by Fe addition (means of 8 replicates ± S.D.).

Treatment	Root	Shoot
	Dry weight (g/pot)	
- Fe	0.51 ±0.07	0.88 ± 0.55
+ Fe	2.05 ±0.44	4.78 ± 0.20
	Fe Conentration (u`g/g)	
- Fe	53.8 ± 0.17	29.1 ± 0.37
+Fe	688 ± 0.72	245 ±0.94
	Fe Uptake (ug/10 plants)	
- Fe	27.4 ± 0.80	25.6 ± 0.15
+Fe	1410 ± 0.97	1173± 0.49

Amino acids, organic acids and phytosiderophores content and pH values of root exudates released by Fe-efficient barley .

Total amounts of amino acids and organic acids released into collection solution of Fe-deficient and non-deficient barley plants are graphically illustrated in Figure 1. In general, total amounts of organic acids released by plant roots are higher than those of amino acids and this is in agreement with the results of Kraffczyk *et al.* (1984) who found that amino acids and organic acids represent 2% and 33% of the corn root exudates, respectively.

Data also show that total amounts of amino acids are higher in case of Fe-deficient barley roots compared to those of Fe-sufficient ones; the relative increase in amino acids is 775%. Helal (1998) stated that the total amounts of amino acids of chlorotic barley plants (iron deficient) were higher by 17% than non-chlorotic (iron sufficient). Increasing amino acids in chlorotic plants was also reported by Elgala & Amberger (1988) who found that Fe-deficiency increased amounts of amino acids exudation by about 31.5% over those of non deficient plants. The increase in amino acids content in chlorotic than non-chlorotic barley may be due to the accumulation of amino acids in the chlorotic one as a result of less structure of protein materials and nucleus acid in plants.

Concerning the organic acids exudation, data show that iron deficiency caused such increase in the total amount of organic acids released by barley root reaching 170% relative to those of Fe-non deficient plants. Similarly, Landsberg (1981) reported that the amount of organic acids, particularly citrate and malate

in the root tissues of mono cots (barley, oats and millet) during iron stress reached to 330 % over those of Fe non-deficient plants. Increase of amino acids and organic acids exudation under Fe-deficiency could be explained as the result of an increase in root cell plasma membrane permeability and is not a specific root-response mechanism (Cakmak & Marschner, 1988).

As shown in Fig. 1 amounts of phytosiderophores released from Giza 125 barley roots under iron deficiency are clearly higher compared with those of sufficient conditions. Such increase reaches four-fold those of Fe-sufficient barley roots. Similar results about phytosiderophores release by barley were obtained by Romheld & Marschner (1990) and Wiren (1994) who found that both the rate of phytosiderophores production and the capacity of Fe^{3+} uptake were several fold enhanced under Fe-deficiency. Zhang *et al.* (1991) found that the rate of phytosiderophores release was about 35 times higher in the Fe-deficient as compared with the Fe-sufficient barley roots.

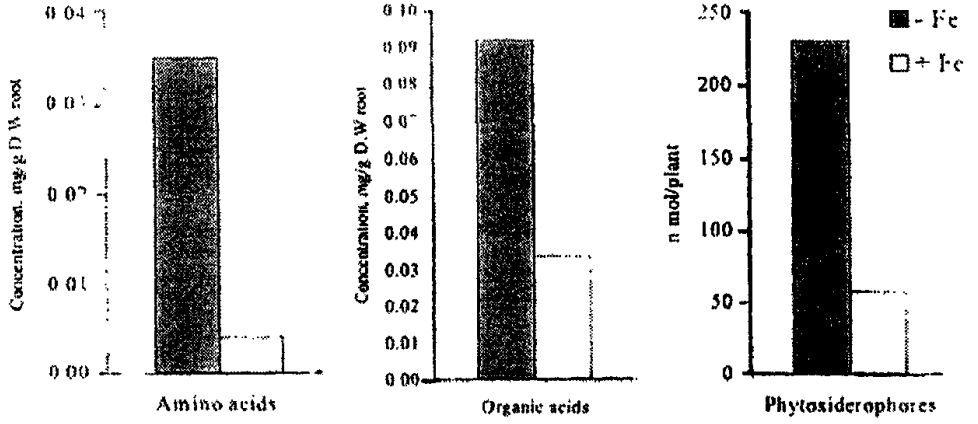

Fig.1. Amino acides, organic acids and phytosiderophores content of root exudates of Fe-deficient and non-deficient barley plants.

Up till now, two different types of root response to Fe deficiency (strategies) have been identified in the plant species. Marschner & Romheld (1995) reported that, in strategy I which occurs in dicotyledenous and monocotyledenous species, with the exception of the graminaceous species (grasses) and is characterized by enhanced net excretion of protons and in many instances, enhanced release of reductants/chelators (mainly phenolics). As for, strategy II which is confined to grasses and is characterized by two components, release of phytosiderophores and a high-affinity transport system in the plasma membrane of root cells for Fe^{3+}phytosiderophores. The release of phytosiderophores is strongly enhanced by iron deficiency and shows a distinct endogenous diurnal rhythm.

Concerning the pH values of the root exudates, the Fe-deficient barley plants show a marked increase in release of H^+ ions as indicated by the lower pH values, (5.56) comparing with the pH of the collecting solutions (6.85) or Fe-sufficient plants (6.68). In accordance, Brown & Jolley (1986) reported that Fe-efficient plant responds or adapts to Fe-stress by a) release of hydrogen ions and

(b) release of reducing compounds from their roots, (c) reduction of Fe^{3+} to Fe^{2+} by their roots, and (d) increases in organic acids. Each of these factors decreases the pH values.

Effect of root exudates on the solubility of sparingly soluble iron compounds

After root exudates collection, some replicates of the Fe-deficient plants were treated with either $FePO_4$ or $Fe(OH)_3$ containing 25 mg Fe/pot. Six days later from iron application, plants were recovered from chlorosis but not as green as the Fe sufficient ones. On the other hand, root exudates of another Fe-deficient plants were collected and shacked with the same rate of $FePO_4$ or $Fe(OH)_3$ (50 mg Fe/liter). Data given in Table 2 summarize the solubilized Fe compounds in presence or absence of root exudates .It is clear that amounts of solubilized Fe by barley exudates are clearly higher (about four-fold) that of control (only $CaSO_4$ solution). These results are in harmony with those obtained by Takagi (1976) and Takagi *et al.* (1984) who found that in the absence of interfering ions the collected phytosiderophores solution was able to solubilize hydrated Fe^{3+}. The effect of the relatively acidic pH could be also considered, that the solubility of Fe^{3+} is highly pH-dependent and it decreases 1000 fold for each unit increases in pH (Lindsay, 1979). Moreover, the role of amino acids in increasing nutrient availability to plant, particularly iron is well demonstrated. Amino acids may act as a chelator for iron, whereas Wallace (1962) reported that K- constant for Fe-cystine was 36 compared to 25 for Fe-EDTA. The relatively higher solubility products of Fe^{3+}phosphate compared to Fe^{3+}hydroxide as reported by Lindsay (1979) resulted increasing the soluble Fe in the medium of the former over the later one.

TABLE 2. Solubilization of Fe^{3+}phosphate and Fe^{3+}hydroxide as affected by root exudates collected from Fe-deficient barley (means of 3 replicates ± S.D.).

	Solubilized Fe mg / 500ml solution		Solubilized Fe corresponded to control			
			mg /500 ml root exudate		mg/g dry weight root	
Treat - ment	**Fe^{3+} phos.**	**Fe^{3+} hydroxide**	**Fe^{3+} phos.**	**Fe^{3+} hydroxide**	**Fe^{3+} phos.**	**Fe^{3+} hydroxide**
$CaSO_4.2H_2O$ (control)	0.15±0.09	0.10±0.08				
Barley root exudates	0.60±0.08	0.42±0.07	0.45±0.07	0.32±0.08	0.88	0.63

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المخلبيات الطبيعية فى إفرازات جذور الشعير ومقدرتها على إذابة مصادر الحديد شحيحة الذوبان

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تفرز جذور الشعير النامية فى بيئة ينقص بها الحديد مركب يسمي phytosiderophores والذى يشجع من حركة الحديد وامتصاصة بواسطة جذور النبات . وقد تم عمل هذا البحث لتقدير كميات الأحماض الأمينية والأحماض العضوية phytosiderophores, والتي توجد فى إفرازات جذور نبات الشعير (صنف جيزه ١٢٥) خاصة تحت ظروف نقص الحديد ، كذلك تقييم مقدرة تلك المركبات الطبيعية على إذابة مركبات الحديد شحيحة الذوبان مثل فوسفات الحديديك ، هيدروكسيد الحديديك .

أوضحت النتائج زيادة كميات الأحماض الأمينية والأحماض العضوية التي أفرزت تحت ظروف نقص الحديد مقارنة بحالة توفر الحديد وكانت قيم الزيادة ١٧٠،٧٧٥٪ على الترتيب ، وبالمثل ازدادت كميات الـ phytosiderophores تحت ظروف نقص الحديد لتصل إلى اربعة أضعاف قيمتها فى حالة توفر الحديد ومن جهة أخرى فقد تم تقدير كميات واضحة من الحديد فى مترشح المحلول الناتج من رج فوسفات الحديديك أو هيدروكسيد الحديديك مع إفرازات الجذور بقيم تصل إلى اربعة أضعاف تلك الناتجة من الكنترول (محلول يحتوي على كبريتات الكالسيوم فقط) .