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Alteration in Root Exudates Level During Fe-Deficiency in two Cucumber Cultivars

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ABSTRACT

A water culture experiment was conducted with two-cucumber cultivars (*Cucumis sativus* L.) Cv. named Dotto (referred as cultivar I) and Beit alpha (referred as cultivar II) to investigate the differences between both cultivars in their capabilities to release root exudates (phenolic compounds, soluble sugars, organic acids and free amino acids) as a response mechanism to Fe-deficiency. Cucumber plants were grown in nutrient solution with the presence or absence of iron Fe-EDTA at pH 6.0 (un-buffered) or buffered by with addition of $CaCO_3$ and MES-NaOH (pH 7.2) in order to increase iron stress condition. The results indicated that either cultivar I or cultivar II exhibited high differences in the release of root exudates. In cultivar I the value of phenolic compounds under (-Fe) was 201.3 µg/g root dry weight (d.w.)/4 h compared with 50.3 µg/g root d.w./4 h in cultivar II respectively at 12 days old plant. Under Fe stress conditions with different treatments, the amount of soluble sugars released from the roots of cultivar I ware relatively higher compared to cultivar II. The presence of $CaCO_3$ and MES-buffer resulted in a remarkable increase in soluble sugars in cultivar I compared with cultivar II. In addition the total amount of organic acids released under stress conditions was 26.47 mg/g root d.w./4 h and 5.25 mg/g root d.w./4 h in cultivar I and cultivar II respectively. Oxalic acid represented more than 10 folds in cultivar I increase compared with cultivar II under Fe-stress. Also the total amount of free amino acids released from the roots of cultivar I increased by 94% compared to cultivar II under Fe deficiency. The behavior of cucumber cultivars grown under iron stress showed a high difference in their response to cope Fe-deficiency.

Key Words: Cucumber; Iron deficiency; Root exudates; Organic acid

INTRODUCTION

Plant genotypes differ widely in their response to micronutrient deficiency. The combined effects of soil properties, and plant characteristics may control availability of nutrients in the rhizosphere area. Plant genotypes which are able to grow better and yield more without developing symptoms of deficiency under nutrient stress are termed efficient genotype (Olsen *et al.*, 1981). Root exudates are considered as one of the main root products, which are well known to influence nutrient solubility and uptake when plants imposed for stress. Mono and dicots plant species released much more organic acids when grown under Fe-deficiency as demonstrated by Landsberg (1981). The accumulation of organic acids (citric & malice acids) may be involved in solubilization of iron from the soil. The phenolic compounds enhance the availability of iron to the roots (Julian *et al.*, 1983). In addition, El-Glala and Amberger (1988) revealed that amino acids act as a chelator for iron in maize plant. The objective of this study was to study the level root exudates as a rapid and simple laboratory method for assorts the response of two cucumber cultivars to Fe-deficiency stress.

MATERIALS AND METHODS

Two cucumber cultivars (*Cucumis sativus* L.) Cvs. named Dotto (referred as cultivar I) and Beit alpha (referred as cultivar II) were grown as previously described by Mohamed *et al.* (1998). Seeds were germinated on wet filter paper in the dark at 20°C for 4 days. Individual seedlings were transferred into 2.5 L plastic vessels (15 plant/pot) containing an aerated nutrient solution. Seedlings were grown up to 14 days under control conditions. Cucumber plants were grown in different hydroponics solutions with or without the addition of 80 µM Fe-EDTA. The nutrient solution was changed every 3 – 4 days and the pH adjusted to 6.2 with NaOH or buffered to pH 7.2 with 10 mM MES-NaOH. Alternatively, 1 gL^{-1} $CaCO_3$ was added. The experiment was conductive under the control environmental condition, as follows, light/ dark periods of 16/8 h at 24/20°C, relative humidity to 65-75% and a photosynthetic photon flux of 250 µ $mol/m^2/s$ at plant light.

Collection of root exudates. Intact plants were used for collection of root exudates after 4, 8 and 12 days. Intact plants were removed from the nutrient solution and after two hours of the onset of the light period, the roots were washed two times for one minute in deionized water. Then, plants were placed in 200 mL aerated deionized water for

four hours. The root exudates solution was filtered stored at - 20ºC until used and the root were cut, dried and weighted.

Analyses of root exudates. Root exudates solution was analyzed for: total phenolics, total soluble sugars, organic acids and free amino acids composition.

Phenolic compounds. Phenolic compounds content in plant root exudates were determined colorimetrically according to Lam and Street (1977).

Total soluble sugars. Total soluble sugars were determined in root exudates by the method of Dubois *et al.* (1956).

Organic acids. The method of Phillips and Jennings (1976) was used for organic acids determination (measured only at day 12) using gas chromatography ATI Unicom Series 610. UK model.

Free amino acids: Free amino acids were determined (measured only at day 12 under control condition) using Amino Acid Analyzer, model Eppendorf LC 3000 (physiological column) as reported by El-gala and Amberger (1988) using known amino acid standard.

RESULTS AND DISCUSSION

Release of phenolic compounds. Data presented in Table I showed that the release of phenolic compounds from the root of two cucumber cultivars reached the maximum value at 12 days old under all treatments. For example the value was greatly high when cultivar I grown under iron stress (-Fe), the value was 201.3, 122.1, 131.9 µg/g root d.w./4 h. Under control, $CaCO_3$ and MES buffer treatments respectively. But were 50.3, 44.2, 52.2 µg/g roots d.w./4 h. for cultivar II at the same age. This mean that Fe stress promoted the release of phenolic compounds in order to increase the availability of Fe uptake by chelation or reduction of iron and this release more evidence in cultivar I more than in cultivar II.

Also, the addition of $CaCO_3$ and MES buffer to the nutrient solution (to create more stress conditions) caused marked differences in the release of phenolic compounds from the two tested cultivars. The release of phenolic compounds from roots of cultivar II was greatly lower than of cultivar I when grown under iron deficiency combined with presence of $CaCO_3$ the value was 122.1 and 44.2, respectively at days 12.

This means that, cultivar I might able to overcome the iron deficiency by releasing more root exudates in the growth medium. In this respect Hether *et al.* (1984) indicated that, several plant species such as (tomato, soybean, sunflower & barley) showed different ability in response to iron stress by releasing some reductants (phenolic compounds). The high amount of phenolic compounds released from roots of efficient soybean cultivar keep Fe^{2+} in the reduced form, thereby increasing its mobility and availability for transport, while Fe inefficient cultivar released low amount of phenolic compounds (Brown & Ambler, 1973). These phenolic compounds not only act as reductants (by oxidizing the phenolic OH groups) for reduction of Fe^{3+} to Fe^{2+} but also acts as stable Fe^{3+} chelated (Römheld & Marschner, 1981; Barrett-Lennard *et al.*, 1983). In addition, the results of the present work are in a good agreement with the results obtained by Olsen *et al.* (1981) who studied Fe^{3+} reducing substances (e.g. caffeic acid & its derivatives) released from roots of Fe-stressed of two tomato efficient and inefficient genotypes. The authors showed that the content of phenolic compounds was approximately twice high in roots of Fe-efficient when compared with Fe inefficient genotypes.

Table I. Release of phenolic compounds from the roots of cucumber plants (cultivars I & II) grown in the presence (+Fe) and absence of iron (-Fe)

Plant age (days)	Treatments	Phenolic compounds (µg/g root d. w/4 hrs)					
		Cv I		Cv II	Cv I		Cv II
		+Fe	%*	+Fe	-Fe	%*	-Fe
4	Control	65.12	209	31.2	86.9	220	40.9
	$CaCO_3$	70.91	224	31.71	91.1	234	39.0
	Buffer	69.20	214	32.30	93.9	224	42.0
8	Control	67.19	209	32.20	120.1	244	49.2
	$CaCO_3$	72.10	207	34.92	112.9	263	42.9
	Buffer	76.91	257	29.91	114.2	244	46.9
12	Control	79.90	234	34.20	201.3	400	50.3
	$CaCO_3$	73.29	209	35.10	122.1	276	44.2
	Buffer	80.2	216	37.2	131.9	253	52.2

* Relative to cultivar II; Cv= Cultivar

Table II. Release of soluble sugars from the roots of cucumber plants (cultivars I & II) grown in the presence (+Fe) and absence of iron (-Fe).

Plant age (Days)	Treatments	Soluble sugars (µg/g root d. w/4 hrs.)					
		Cv I		Cv II	Cv I		Cv II
		+Fe	%*	+Fe	-Fe	%*	-Fe
4	Control	30.2	125	24.2	41.9	110	38.1
	$CaCO_3$	27.1	93	29.2	46.0	101	45.5
	Buffer	35.2	109	32.2	60.0	127	47.3
8	Control	85.9	106	80.9	120.1	109	110.1
	$CaCO_3$	115.2	144	79.9	134.0	134	100.1
	Buffer	99.8	126	79.1	135.0	135	99.9
12	Control	102.0	114	89.2	145.1	105	137.9
	$CaCO_3$	120.4	119	100.9	148.2	102	145.0
	Buffer	112.1	105	107.0	131.2	110	119.8

* Relative to cultivar II; Cv= Cultivar

Release of soluble sugars. It is quite obvious from the results in Table II that, cultivar I and cultivar II exhibited high differences in the release of soluble sugars. Generally when Fe withdraws from the growth media (-Fe) the amount of soluble sugars released from roots of cultivar I was relatively higher when compared to cultivar II. At 12 days old, under (-Fe) the amount of soluble sugars released from the roots of cultivar I was 145.1 µg/g root d.w./4 h. Meanwhile it was 137.9 µg/g root d.w./4 h. in case of cultivar II (nearly 10% increase). Data presented in Table II also showed that under Fe deficiency with addition of $CaCO_3$ or MES buffer, the amount of soluble sugars released from the roots of cultivar I and II was increased when compared to control treatment.

Table III. Major organic acids in root exudates of two cucumber cultivars grown in the absence (-Fe) and presence (+Fe) of iron at 12 days old plant

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Organic acids	Organic acids (mg/g root d.w. 4 h^{-1})											
	- Fe						+Fe					
	Cultivar (I)			Cultivar (II)			Cultivar (I)			Cultivar (II)		
	Control	$CaCO_3$	Buffer	Control	$CaCO_3$	Buffer	Control	$CaCO_3$	Buffer	Control	$CaCO_3$	Buffer
Oxalic acid	15.00	5.85	-	1.32	1.62	1.64	2.48	2.00	0.48	0.02	0.03	004
Succinic acis	7.17	-	2.60	-	-	-	-	-	2.50	2.79	-	-
Fumaric acid	1.50	9.64	2.56	-	-	-	-	-	-	1.89	3.22	-
Malic acid	-	-	-	3.93	0.46	0.69	2.90	-	-	-	-	2.48
Lactic acid	6.76	9.90	-	-	-	-	-	-	3.30	-	-	-
Citric acid	2.05	2.70	4.46	-	-	-	-	-	-	-	-	0.69
Total	26.47	28.09	9.62	5.25	2.08	2.33	5.38	2	6.28	4.70	3.25	3.21

Table IV. Composition of free amino acids in the root exudates of two cucumber cultivars grown in the absence (-Fe) or the presence (+Fe) of iron at 12 days

Free amino acids		µg/g root d.w. 4 h^{-1}			
		- Fe		+Fe	
		Cv I	Cv II	Cv I	Cv II
1	Glycine	11.73	10.21	9.40	9.71
2	Alanine	83.11	74.76	50.00	63.07
3	Valine	0.00	30.00	28.19	0.00
4	Leucine	30.94	30.54	33.18	26.60
5	Isoleucine	15.31	14.26	13.54	10.41
6	Phenylalanine	20.33	17.54	20.44	18.38
7	Tyrosine	4.59	8.31	7.24	6.54
8	Tryptophane	4.85	0.00	3.10	0.00
9	Aspartic acid	64.58	0.00	42.31	40.41
10	Glutamic acid	61.17	26.66	64.27	95.15
11	Lysine	17.36	19.39	8.45	31.33
12	Arginine	48.62	27.33	44.19	20.28
13	Histidine	13.27	7.92	9.27	5.91
14	3-CH3 Histidine	0.30	0.00	0.37	0.83
15	1-CH3 Histidine	2.51	0.00	2.00	0.00
16	Cystine	48.12	0.00	0.00	37.02
17	Methionine	3.44	3.13	5.00	2.31
18	Thronine	29.65	0.00	24.00	0.00
19	Serine	58.30	0.00	44.26	17.36
20	Phosphoserine	4.41	2.75	4.11	4.54
21	Proline	46.02	19.31	54.00	13.01
Total		568.63	292.11	467.32	402.86

These results are in agreement with the finding of Hale and Moore (1979) who reported that different species of plant exuded varying amounts and types of soluble sugars by their roots. This exudation led to alter the availability of the nutrient in rhizosphere area and the exudation rate was affected by the physiological age and the metabolic state of the whole plants. The authors added that one-gram dry weight of root could exude 4.5 – 6.5 mg of reducing sugars under controlled condition.

Release of organic acids. Results showed that the maximum release of organic acids from roots of cucumber plants was obtained at 12 days old (Table II). There was a great difference in the total organic acids released from the root cells of cultivar I grown with or without iron. The content of total organic acids were 5.38 mg/g root d.w./4 h and 26.47 mg/g root d.w./4 h in iron sufficient and iron deficient plant respectively (more than 390% increase). Whereas in cultivar II data showed no high differences in the concentration of total organic acids released from the roots of Fe- sufficient and Fe deficient plants respectively (4.7 & 5.25 mg/g root d.w./4 h). Marked differences were also observed in the type of organic acids, for example, oxalic acid was found to be the major organic acid released from the roots of cultivar I under (-Fe) treatment the value was 15.0 mg/g root d.w/4 h) and was 1.32 mg/g root d.w/4 h in cultivar II (more than 10 folds increase). Whereas fumaric acid was the lowest acid released in cultivar I and was absent in cultivar II. The same trend was obtained in case of $CaCO_3$ or MES buffer treatments under stress condition (Table III), the amount of total organic acids was increased and was higher in cultivar I than in cultivar II under iron stress condition.

The data of Table (III) showed that the release of organic acids by of cultivar I was much higher compared to cultivar II when grown under iron stress. This confirmed the finding of Clark *et al.* (1973) who found that the organic acids (mainly citrate), which released from roots of two maize genotypes were highly different in both genotypes when grown under Fe-stress an Fe-inefficient genotype showed lower exudation compared to Fe efficient genotype. Furthermore, Olsen and Brown (1980) pointed out that, Fe-efficient genotype exhibited high response to release reductants to the root media compared to Fe- inefficient genotype and this reductants may be acts as a source of proton release and to reduce Fe^{3+} to Fe^{2+}, so the Fe^{+2} become more available to plant In this concern, Pich *et al.* (1991) found a high citrate concentration in roots of Fe-efficient genotype compared to Fe-inefficient genotype. They suggested that regulation of citrate accumulation attributed to the genes, which is responsible for Fe-efficiency.

Under condition of iron stress combined with $CaCO_3$ or high pH (buffer), organic acids exudation was affected considerably (Table III). These results are in consistence with the observation of Rhoads and Wallace (1960) who found that bean plants grown under iron deficiency with the presence of $CaCO_3$ increased the amount of organic acids especially oxalate. This can be explained as; roots grown in

$CaCO_3$ may increase the CO_2 fixation by phospho enol pyrovate carboxylase (PEPs) enzyme, which led to increase the amount of organic acids synthesis.

Release of free amino acids. The composition of free amino acids released from the roots cells of both cultivars under iron stress was determined (Table IV) and the results showed that, amino acids was markedly varied in both cultivars. It was being 568.6 and 292.11 µg/g root d.w./4 h under iron stress in cultivar I and cultivar II respectively (more than 94% increase). Similar pattern was observed under Fe-sufficient treatment. Also the results in Table (IV) revealed that there was a distinct difference in the amount and type of amino acids released from the roots of cultivar I and II under Fe-deficiency. The difference magnitude observed in the detected amino acids in the both cultivars was; aspartic, glutamic, arginine, histidine, cystine, threonine, serine, and proline. The concentration of amino acids released from the roots of stressed plant depends presumably on either the concentration of amino acids in the root tissue or the permeability of membranes (Graham *et al.*, 1981; Kraffczyk *et al.*, 1984). Whereas Uren and Reisenauer (1988) suggested that some plant species have adaptive mechanism when grown under nutrient stress to increase the availability of mineral nutrients in the rhizosphere, such as the release of amino acids from plant roots. The results of increasing amino acids under Fe-stress were partially in agreement with those reported by El-gala and Amberger (1988) and Veliksar *et al.* (1997). They concluded that, the high amount of amino acids released from roots of Fe deficient plant than in Fe- sufficient plant may be due to the accumulation of amino acids in deficient leaves as a result of less structure of protein synthesis. From the results obtained it might be concluded that cultivar I and cultivar II of cucumber plant showed a high difference in their capabilities to release different compounds as a response mechanism to Fe-deficiency stress. These capabilities were greatly higher in cultivar I compared to cultivar II. This means that when cultivar I grown under iron stress it was able to initiate some metabolic process such as root exudates to increase Fe availability. This mechanism might be useful for studying the response of cultivars efficiency to Fe- deficiency stress.

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