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Screening of Wild Strawberry Genotypes against Iron Deficiency under Greenhouse Conditions

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Abstract

The cultivated strawberry *Fragaria* × *ananassa* Duch. is the natural hybrid of *F. chiloensis* (L.) Mill. and *F. virginiana* Mill. The progenitor species have high genetic diversity compared with the cultivated genotypes; therefore, the use of wild relative in *F. chiloensis* breeding could provide a good for broadening the available genetic variations of cultivated species. In present study, 13 genotypes selected from strawberry super core collection were tested under Fe (-) and Fe (+) conditions for their response against Fe deficiency conditions in a growth medium (GM) (soil + sand + perlite), potentially able to simulate the actual GM in nature. SPAD-meter readings indicating chlorophyll levels of the leaf, shoot dry matter yield, Fe-efficiency rate, shoot total and active Fe concentrations were determined to evaluate the resistance levels of strawberry genotypes against Fe deficiency. Results of this study indicated that different response for strawberry subspecies and genotypes of the same subspecies grown in GM against Fe deficiency. Symptom for Fe deficiency of genotypes varied between 1-5, SPAD-meter readings 3, shoot dry matter yields and shoot Fe concentrations varied between 6.5-38, 1.02-6.06 g plant^{-1} and 41.8-233.1 mg kg^{-1} respectively. Iron-efficiencies of genotypes were found between 58–98%. Strawberry subspecies, *F. virginiana* spp. *glauca*, *F. chiloensis* ssp. *chiloensis* and *F. chiloensis* ssp. *pacifica* showed Fe-efficiency values of 93.8, 79.5 and 79.1% respectively. We concluded that shoot growth performance, Fe intake from GM, transfer of Fe from roots to shoots, shoot Fe-use efficiency, Fe deficiency symptom levels and SPAD-meter readings indicating chlorophyll levels were significant parameters to evaluate the resistance of strawberry genotypes against Fe deficiency. The most Fe-efficient genotypes belonging to *F. virginiana* spp. *glauca* could be used in breeding programs aiming at developing new strawberry genotypes suitable for growing under Fe deficient conditions.

Keywords: breeding, iron, genetic resources, strawberry, supercore, tissue culture

Introduction

Iron (Fe) deficiency is a common nutritional problem in human, plants and soils. El-Jendoubi (2012) reported that the incidence of Fe chlorosis is widespread in the Mediterranean basin (Abadía *et al*. 2004, Sanz *et al*. 1992) particularly it prevailed in Northern Greece (Tagliavini *et al*. 2000), Turkey (Tekin *et al*. 1998). Iron deficiency affects almost 1.6 million people worldwide and has significant impacts especially on pre-school children and pregnant women (McLeon *et al*., 2009; Cakmak *et al*., 2010; Karaköy *et al*. 2012). It is also is quite common problem both in Turkish soils (Eyupoglu *et al*., 1998; Cakmak *et al*., 1999) and people (Cavdar *et al*., 1983).

Iron deficiency in human and plants are directly related to available Fe concentrations of the soils (Fernández *et al*., 2008). Although total Fe concentrations of soils are usually at high levels (0.5-5.0%), however there are various soil and environmental factors hindering the plants to benefit from these high Fe levels (Tagliavini and Rombola, 2001). Iron and other micro-nutrient fertilizations to the soil and leaves are the primary measure to prevent Fe deficiency in plants. However, such types of activities are expensive, environmentally unfriendly and temporary solutions for Fe deficiency. Organic-based (chelated) fertilizers may provide an alternative source for soil fertilization but they are also expensive, lose their efficiencies in soils rapidly and increase Cu and Ni intakes (Palmer and Guerinot, 2009; Pestana *et al*., 2011).

The best alternative against Fe deficiency is to develop resistant genotypes to iron deficiency condition. Plants develop various mechanisms to increase solubility and intake and consequently not to be affected from the deficiency of abundant Fe which is unavailable to the plants (Marschner *et al*., 1986; Römheld and Marschner, 1986; Zuo and Zang, 2008).

Different groups of plant have developed various mechanisms against mineral deficiency such as entire monocotyledonous plants, except Gramineae, and dicotyledonous plants have special adaptation mechanisms called Strategy-I, while Gramineae develop an adaptation mechanism

called Strategy-II different from Strategy-I (Marschner *et al*., 1986; Römheld and Marschner, 1986).

Strawberry is a dicotyledonous plant and highly sensitive against Fe deficiency. Although strawberry cultivated lands and yields increase in Turkey in the last decades, however significant losses in both yields and quality are observed because of significantly low available Fe concentrations in the soils (Eyupoglu *et al*., 1998; Tamci, 1977). Therefore, there is need to screen and investigate strawberry genotypes resistant to Fe deficiency that could be used in strawberry breeding program to overcome such losses in yields and quality.

Despite the hydroponic studies carried out to determine the characteristics effective in resistance and sensitivity against Fe deficiency in different strawberry genotypes selected from super core collection representing the wide strawberry genetic resources; however, according to our best of knowledge, there is not any study studies reporting the response of the same genotypes in solid GM. Present study was carried out under greenhouse conditions to determine the deficiency symptom scores, SPAD meter readings indicating leaf chlorophyll levels, shoot dry matter yields, Fe-efficiency rates, shoot total and active Fe concentrations of genotypes.

Material and methods

Super core collection of the strawberry genotypes were created after morphological and molecular characterization of two progenitor species (*F. chiloensis* and F. *virginiana*) of cultivated species *Fragaria* × *ananassa*. Within the scope of present study, some strawberry genotypes were selected from the core collection in order to determine the sensitivity/resistance level against Fe deficiency-induced chlorosis in GM under greenhouse conditions.

Super core genotypes used in present study were previously tested for Fe-chlorosis. The whole collection is composed of 38 genotypes from *F. chiloensis* and *F. virginiana*. Shoot tips of genotypes were cultured into GM according to protocol specified in earlier studies carried out at laboratories of Department of Horticulture University of Çukurova (Aka Kacar and Çetiner, 1995). Cultured shoot tips were developed and became a full-shoot within six weeks after the culturing. These plantlets were then transferred to shoot development mediums (Aka and Çetiner, 1992). After we obtained the sufficient number of plantlets, they were transferred to rooting medium (Aka and Çetiner, 1992) and the rooted plantlets were then transferred to external conditions. These plantlets were used as the plant material in the present study.

Experiments were carried out at the Research and Experimental greenhouses of Department of Soil Science and Plant Nutrition, Faculty of Agricultural, University of Çukurova Adana, Turkey. The soils from Eskişehir-Sultanönü and different GM were added into the soil used as the GM in the present study, which was composed of GM. Physical and chemical characteristics of GM are given in Tab. 1.

Tab. 1. Chemical and physical characteristics of growth medium composed of Eskisehir-Sultanonu growth medium

Material	Fe	Zn	Cu	Mn	P	K	pH Salt $CaCO_3$
	(mg kg^{-1})						(mS) %
Growth medium	2.28	0.13	0.46	5.37	1.55	120	7.75 0.17 4.2

Genotypes used in experiments

Plant materials of current study consisted of the plants obtained by tissue culture and transferred into greenhouse conditions. Rooting processes of plant material were implemented at biotechnology laboratories of Department of Horticulture, Faculty of Agricultural University of Çukurova. Soil mixture composed of 1:1:1 (volume-based) soil + sand + perlite was used as the GM in greenhouse experiments for the adaptation of rooted plants at Research and Experimental Greenhouses of Department of Soil Science and Plant Nutrition, Faculty of Agricultural University of Çukurova. A total of 13 strawberry genotypes (RCP 37, Pigeon Point, CFRA 1267, HM 1, Scotts Creek, Darrow 72, CA-1541, 2BRA 1A, NAH, Cascade, LH 50-4, LH 30-4 and RH-43) were used tested in this study. Iron-deficiency symptoms and SPAD values were taken and plants were harvested around 4th month of growth.

Experimental Design and Implementations

Experiments were carried out in three replications with one plant in each replication and with Fe (Fe (+) containing 5 mg Fe kg^{-1} in the form of Fe-EDTA) and without Fe (Fe (-), 0 mg Fe kg^{-1}). Experimental pots were filled with 1.000 kg of above specified mixture (Tab. 1) and 200 mg N kg^{-1} (in the form of $(CaNO_3)2.4H_2O$), 100 mg P kg^{-1} (in the form of KH_2PO_4), 5 mg Zn kg^{-1} (in the form of $ZnSO_4.7H_2O$) and 50 mg S kg^{-1} gypsum ($CaSO_4.H_2O$) were applied in solutions to each pot as base fertilizers. Water needs of plant were supplied by distilled water until the harvest with moisture content around field capacity. Experiments were terminated by harvesting the plant shoots in 4th month.

Methods

Growth and development of strawberry genotypes were monitored and Fe deficiency symptoms were scored in a 5-point scale based on the severity of chlorothic patches over the leaves (as 1: very severe and 5: very slight or none) and severity was recorded as a SPAD value measured by a chlorophyll-meter (Minolta SPAD 502) indicating chlorophyll level of the plant.

Shoot Dry Matter Yield and Iron Efficiency Rate

Harvested shoots were dried at 70^{0}C for 48 hours and dry weights were measured (g $plant^{-1}$ or mg $plant^{-1}$). Iron-

efficiency rates of genotypes were determined by dividing plant weights of Fe (-) conditions to weights of Fe (+) conditions and expressed as percentage (%). This rate is commonly used in literature to indicate Fe-efficiency of species (Graham, 1984).

Total Iron Analysis

Dry shoots were grinded and digested in H_2O_2-HNO_3 acid mixture in a closed system wet digestion method by microwave (Milestone 1200 Mega) for half hour and resultant samples were filtered through blue-band filter paper for Fe analysis. Filtered extracts were then completed to 25 ml with distilled water and Fe concentrations of final extracts were measured in Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES, Jobin, Yvon-Paris).

Active Iron Analysis

100 mg Dry and grinded sample were taken and shaken in 10 ml 1 N HCl for 2 h in 120 rpm shaker and filtered through blue-band filter paper. Active Fe content of resultant extract was measured in Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES).

Analyses Performed over Growth Medium:

After performing the physical and chemical analyses in the GM for greenhouse experiments observation for various parameters were recorded according to methods described by previous researchers such as available P were determined in accordance with Olsen *et al*. (1954); available K according to Carson (1980); total salt contents by Wheatstone bridge method (US Salinity Laboratory Staff, 1954); pH by Jackson (1964); lime ($CaCO_3$) by Caglar (1949). Similarly Zn, Fe, Mn and Cu concentrations were determined according to standard procedure of DTPA-TEA extraction solution (Lindsay and Norvell, 1978).

Statistical Analysis

Analysis of variance was carried out according to randomized block design and LSD test was used to determine the differences (different groups) among means at 5% level. The means placed into the same group was indicated with the same letters in tables.

Results

Severity of Iron Deficiency Symptoms and Chlorophyll Levels (SPAD values) of Leaves

Sensitivity of 13 strawberry genotypes against Fe deficiency were tested with Fe (-) and Fe (+) treatments under greenhouse conditions. Iron deficiency symptoms among strawberry genotypes were observed as a homogeneous chlorosis over young leaves in earlier stage and necrotic patches in the stage (Fig. 1). Severity and occurrence time of Fe deficiency symptoms varied both among subspecies as well as genotypes of the same subspecies (Tab. 2). Under Fe (-) conditions, the most distinctive Fe deficiency symptom (2.0) was observed in *F. chiloensis* ssp. *pacifica* subspecies and it was followed by *F. chiloensis* ssp. *chiloensis* and *F. virginiana* spp. *glauca* with values of 2.6 and 4.8 respectively. Severity of Fe deficiency symptoms among genotypes of *F. chiloensis* ssp. *pacifica* subspecies varied between 1.0 - 3.5, while it changed between 1.5 - 4.5 among genotypes of *F. chiloensis* ssp. chiloensis and ranged from 1.0 to 5.0 among genotypes of *F. virginiana* spp. *glauca*. Except for Cascade and HM 1 genotypes, Fe deficiency generally decreased with Fe (+) treatment .

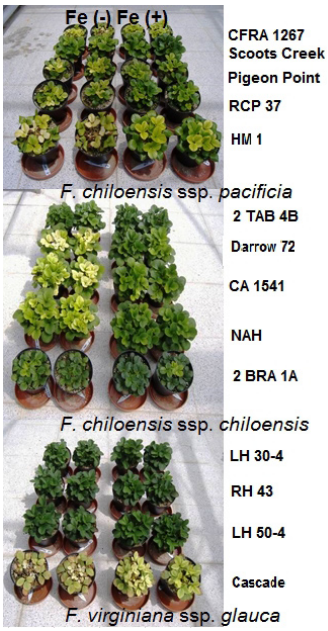

Fig. 1. A view of strawberry genotypes grown under greenhouse conditions with (Fe (+): 5 mg Fe kg^{-1}) and without (Fe (-): 0 mg Fe kg^{-1}) Fe-EDTA treatments in Fe (-) growth medium

Differences were also observed in SPAD-meter readings (indicating chlorophyll levels) of both subspecies and genotypes. SPAD-meter readings are given in. Under Fe (-), SPAD values of 4 genotypes (LH 50-4, LH 30-4, RH 43, 2BRA 1A) were above 30, one genotype (Scotts Creek) was between 20-30 (24.6) while the others were below 20. The genotype CFRA 1267 had the lowest SPAD value (6.5) under Fe (-) conditions. A positive correlation ($R2=0.58^{**}$) was observed between symptom levels and SPAD values of genotypes under Fe deficiency. Such a correlation indicated that empiric symptom rating could be used reliably to assess the sensitivity of genotypes against Fe deficiency.

Shoot Dry Matter Yield and Iron Efficiency

Iron deficiency decreased shoot dry matter yields of genotypes and such a decrease was prevented with Fe (+) treatment. Shoot dry matter yields of strawberry genotypes under Fe (-) conditions varied between 1.02-6.09 g

plant^{-1} and dry matter yields of subspecies varied between 2.20-4.54 g plant^{-1} (Tab. 2). These findings indicated 5.9 times difference between shoot dry matter production levels of genotypes. The average shoot dry matter yield of *F. chiloensis* ssp. *pacifica* subspecies with the most severe Fe deficiency symptoms was 2.20 g plant^{-1} under Fe (-) conditions, the average yield of *F. virginiana* ssp. *glauca* subspecies with the least severe symptoms was 2.86 g plant.

Average shoot dry matter yield of *F. chiloensis* ssp. *chiloensis* subspecies with Fe deficiency symptoms significantly higher than *F. virginiana* ssp. *glauca* and better than *F. chiloensis* ssp. *pacifica* with 4.54 g plant^{-1}. *F. chiloensis* ssp. *pacifica* (symptom level 2.0) and *F. virginiana* ssp. *glauca* (symptom level 4.8) with different symptom levels had closer but low shoot dry matter yields under Fe (-) conditions. However, the *F. chiloensis* ssp. *chiloensis* subspecies with a symptom level of 2.6 had about two times higher dry matter yield compared with other species under Fe (-) treatment.

Shoot dry matter yields increased with Fe (+) treatment supplied against Fe deficiency. Compared with control treatment, increases in shoot dry matter yields of *F. chiloensis* ssp. *pacifica*, *F. chiloensis* ssp. *chiloensis* and *F. virginiana* ssp. *glauca* subspecies were 26.3, 25.7 and 6.6% with Fe (+) treatment respectively. The lowest increase rate was observed in *F. virginiana* ssp. *glauca* subspecies and the other subspecies had more or less similar increase rates in dry mater yields. Similar to Fe (-) conditions, *F. chiloensis* ssp. *chiloensis* had the highest dry matter yield level (5.71 g bitki-1) under Fe (+) conditions.

Iron-efficiency rate is an important indicator of resistance against Fe deficiency. Complying with the shoot dry matter yields, *F. virginiana* ssp. *glauca* subspecies also had the highest Fe-efficiency level with 93.8%. The efficiency rates of *F. chiloensis* ssp. *pacifica*, and *F. chiloensis* ssp. *chiloensis* were found as 79.1 and 79.5% respectively. Iron-efficiency rates of entire strawberry genotypes in the present study varied between 58–99%.

The most distinctive genotypic differences with regard to Fe-efficiencies were observed in genotypes of *F. chiloensis* ssp. *pacifica* subspecies with efficiency rates varying between 58–95%. The genotypes of the same subspecies showed about 1.63 times difference for resistances against Fe deficiency. Such a diverse genetic variance in *F. chiloensis* ssp. *pacifica* subspecies indicated that this subspecies may contribute as significant genetic source in strawberry breeding.

Shoot Total and Active Iron Concentrations

Under Fe (-) conditions, Fe concentrations of 13 genotypes grown in GM under greenhouse conditions varied between 42-233 mg kg^{-1}. The highest (139.3 mg kg^{-1}) shoot Fe concentration under Fe (-) conditions was observed in *F. virginiana* ssp. *glauca* subspecies, followed by *F. chiloensis* ssp. *pacifica*, (72 mg kg^{-1}) and *F. chiloensis* ssp. *chiloensis* (64 mg kg^{-1}) subspecies (Tab. 3). Under Fe (-) condi-

Tab. 2. Symptom scores, SPAD values, shoot dry matter yields and Fe-efficiency rates of strawberry genotypes grown under greenhouse conditions with (Fe (+): 5 mg Fe kg^{-1}) and without (Fe (-): 0 mg Fe kg^{-1}) Fe-EDTA treatments in Fe-deficient growth medium

		Symptom scores		SPAD value						Shoot dry weight (g plant^{-1})						Fe-efficiency
	Genotypes	Fe0	Fe5	Fe0			Fe5			Fe0			Fe5			(%)
F. chiloensis ssp. *pacifica*	RCP 37	2.5	4.0	14.5	±	1.7cd	34.8	±	3.0a	1.98	±	0.50c	2.50	±	0.60bc	79
	Pigeon Point	2.0	3.5	12.3	±	3.5d	33.3	±	2.3a	1.94	±	0.35c	2.04	±	0.39c	95
	CFRA 1267	1.0	3.0	6.5	±	2.1e	14.7	±	1.2cd	1.64	±	0.46c	2.86	±	0.53abc	58
	HM 1	1.0	2.5	16.8	±	2.8c	23.4	±	2.8b	1.69	±	0.69c	2.56	±	1.14bc	66
	Scotts Creek	3.5	4.5	24.6	±	1.5b	35.1	±	2.4a	3.73	±	0.83ab	3.93	±	0.86a	95
	Mean	2.0	3.5	14.9			28.3			2.20			2.78			79.1
$LSD_{0.05}$						1.86						0.69				
F. chiloensis ssp. *chiloensis*	Darrow 72	1.5	4.5	11.1	±	2.8e	34.5	±	2.5b	4.88	±	1.47c	6.97	±	0.57ab	70
	CA-1541	2.0	4.5	9.8	±	1.7e	29.9	±	3.9c	5.22	±	1.66bc	6.62	±	0.91abc	79
	2 BRA 1A	4.5	5.0	34.8	±	2.4b	41.9	±	1.9a	1.98	±	0.69d	2.14	±	0.57d	93
	NAH	2.5	4.0	15.7	±	2.0d	33.7	±	3.4b	6.09	±	1.10abc	7.14	±	1.15a	85
	Mean	2.6	4.5	17.8			35.0			4.54			5.71			79.5
$LSD_{0.05}$						2.09						1.16				
F. virginiana ssp. *glauca*	Cascade	1.0	2.0	13.3	±	1.9d	15.9	±	2.1d	1.02	±	0.28c	1.51	±	0.25c	68
	LH 50-4	5.0	5.0	37.1	±	1.4c	44.4	±	1.6a	3.66	±	0.50ab	3.74	±	0.40ab	98
	LH 30-4	4.5	5.0	38.3	±	2.0c	40.7	±	2.1b	3.76	±	0.43ab	3.81	±	0.53a	99
	RH 43	5.0	5.0	37.7	±	1.4c	42.0	±	2.0b	3.00	±	0.53b	3.15	±	0.72ab	95
	Mean	4.8	4.2	31.6			35.7			2.86			3.05			93.8
LSD0.05						2.06						0.52				

tions, there was about 2.2 times differences between the highest and the lowest Fe concentration of the genotypes. Significant differences were observed between Fe concentrations not only between subspecies but also among the genotypes of the same subspecies under Fe (-) conditions. For instance, Pigeon Point genotype of *F. chiloensis* ssp. pacifica subspecies had shoot Fe concentration of 42 mg kg^{-1}whereas CFRA 1267 genotype of the same subspecies showed value of 131 mg kg^{-1} under Fe (-) conditions. There was about 3 times difference between the lowest and the highest values of the same subspecies. Such a finding indicated that Fe intake and transfer of genotypes may differ from each other.

It was interesting to note that CFRA 1267 genotype of *F. chiloensis* ssp. *pacifica* subspecies with high shoot Fe concentration under Fe (-) conditions had a low Fe-efficiency rate (58%), whereas the genotype Pigeon Point with low shoot Fe concentration had significantly high Fe-efficiency rate (95%). A similar case was also observed in genotypes of *F. virginiana* ssp. *glauca* subspecies. These findings indicated that genotypes may have different Fe-use efficiencies under Fe (-) conditions. Iron concentrations of genotypes did not have significant effects on Fe-efficiencies under both Fe (-) and Fe (+) conditions (respectively with R2=0.14 and R2=0.01). A similar relationship was also observed between active iron concentrations and Fe-efficiency values of the genotypes.

Discussion

In present study, Fe-efficiencies of 13 strawberry genotypes belonging to three different subspecies grown in GM under greenhouse conditions were tested under Fe (-) and Fe (+) conditions. Under Fe (-) conditions, chlorosis like patches were observed over young leaves and differences were observed in chlorosis of subspecies and even among the genotypes of the same subspecies (Tab. 2). On the other hand, chlorosis was relieved or totally eliminated under Fe (+) conditions. Such a relief or elimination was proved by Fe deficiency symptom scores and SPAD readings indicating chlorophyll levels. The genotypes with lower iron deficiency symptom scores generally had lower SPAD readings. Significant relationships between Fe nutrition and shoot chlorophyll levels, SPAD readings and Fe deficiency symptom scores were also reported in previous studies (Pestana *et al*., 2012; Álvarez-Fernández *et al*., 2011; Jelali *et. al*., 2010; Álvarez-Fernández *et al*., 2003). Similarly, Gulen and Eris (2003) reported a high regression coefficient between strawberry leaf chlorophyll content obtained and the SPAD readings in same leaves.

Under Fe (-) conditions, the genotypes with lower SPAD readings had generally lower Fe-efficiency values compared with genotypes with higher SPAD readings (Tab. 2). Results indicated that chlorosis may create significant problems in growth and yield of strawberries under Fe (-) conditions. Such a case was also pointed out in literature for different plants under Fe (-) conditions. For instance, Álvarez-Fernández *et al*. (2003) reported about 80% decrease in fruit yield with the decrease in chlorophyll level. Researchers also reported significant yield losses in peaches and pears with the decrease in SPAD values indicating a decrease in chlorophyll level.

Iron deficiency in pears caused about 36, 72 and 64% decreases in leaf chlorophyll concentration, number of fruit per tree and fresh fruit weight per tree respectively (Álvarez-Fernández *et al*., 2003). Similarly the decreases in leaf chlorophyll contents of peaches, pears and kiwi fruits grown over calcareous soils of Italy caused distinctive decreases in total yield (Rombolà *et al*., 1999).

High Fe-efficiency values of strawberry genotypes both under Fe (-) and Fe (+) conditions were mainly due to low shoot growth performance and low Fe-efficiency values of the genotypes particularly *F. chiloensis* ssp. *chiloensis* and due to high growth performance (Tab. 2). The relationships between growth performances and resistance against Fe deficiency were also pointed out for carob and lemon trees (Pestana *et al*., 2012). Significant decreases were observed in SPAD readings of lemon leaves under low or Fe (-) treated conditions. The difference between species was explained by low growth performance of carobs (Pestana *et al*. 2011) compared the citrus rootstocks and reported lower growth performances for Sour orange and indicated that such a low growth performance might be a significant mechanism in resistance against Fe deficiency.

In present study, Fe intakes from GM and transportation to shoots were found to be as significant factors in resistance against Fe deficiency. Shoot Fe concentrations of *F. virginiana* ssp. *glauca*, *F. chiloensis* ssp. *pacifica* and *F. chiloensis* ssp. *chiloensis* under Fe (-) conditions were 139.3, 72 and 64 mg kg^{-1} (Tab. 3) respectively. Iron-efficiency rates of the same subspecies were recorded as 93.8, 79.1 and 79.5% respectively (Tab. 2). High Fe-efficiency of *F. virginiana* ssp. *glauca* subspecies was mostly because of high Fe intake from GM and transportation to shoots. Rhizosphere pH reduction levels of genotypes or Fe intake capability through Fe-chelate reductase activity were indicated in literature as significant factors under Fe (-) conditions (Pestana *et al*., 2012; Jelali *et al*., 2010; Jolley *et al*., 1996).

It was observed in present study that high Fe concentration in leaves of strawberry genotypes does not always indicated resistance against Fe deficiency. For instance, CFRA 1267 genotype with high shoot Fe concentration (131 mg kg^{-1}) under Fe (-)conditions had a low Fe-efficiency rate (58%) and Pigeon Point genotype with low shoot Fe concentration (41.8 mg kg^{-1}) under the same conditions had a high Fe-efficiency rate (95%) (Tab. 2 and 3). These findings could be related to shoot Fe-use efficiencies of genotypes. It was reported that amount of Fe transported from roots to shoots of plants grown in calcareous soils, as observed in present study, might be low and consequently the species resistant against Fe deficiency may have higher

Tab. 3. Total and active Fe concentrations of strawberry genotypes grown under greenhouse conditions with (Fe (+): 5 mg Fe kg^{-1}) and without (Fe (-): 0 mg Fe kg^{-1}) Fe-EDTA treatments in Fe (-) growth medium

		Total Fe concentration (mg kg^{-1})						Active Fe concentration (mg kg^{-1})					
	Genotypes	Fe0			Fe5			Fe0			Fe5		
F. chiloensis ssp. *pacifica*	RCP 37	45.4	±	7.3 d	43.3	±	3.7 d	14.5	±	0.6 d	17.6	±	4.8 cd
	Pigeon Point	41.8	±	6.9 d	45.1	±	2.3 d	14.9	±	3.6 d	14.3	±	1.1 d
	CFRA 1267	131.1	±	9.8 b	81.5	±	15.2 c	20.5	±	1.8 abcd	22.2	±	2.2 abc
	HM 1	92.8	±	0.0 c	193.0	±	23.6 a	23.9	±	5.5 ab	24.7	±	5.3 a
	Scotts Creek	48.9	±	9.3 d	46.5	±	8.1 d	15.6	±	2.1 d	19.0	±	2.9 bcd
	Mean	72.0			81.9			17.9			19.6		
$LSD_{0.05}$				11.54						3.50			
F. chiloensis ssp. *chiloensis*	Darrow 72	69.5	±	14.8 a	60.5	±	9.7 a	17.5	±	0.6 a	21.1	±	1.1 a
	CA-1541	61.1	±	11.1 a	66.4	±	16.3 a	17.7	±	1.8 a	22.0	±	0.9 a
	2 BRA 1A	67.8	±	13.3 a	72.8	±	14.6 a	19.0	±	3.2 a	21.8	±	2.4 a
	NAH	59.3	±	7.2 a	67.1	±	12.7 a	17.5	±	1.9 a	20.7	±	1.6 a
	Mean	64.4			66.7			17.9			21.4		
$LSD_{0.05}$				ns						ns			
F. virginiana ssp. *glauca*	Cascade	233.1	±	5.4 a	113.2	±	18.8 cd	25.6	±	4.6 b	22.9	±	1.9 b
	LH 50-4	91.6	±	7.1 d	96.6	±	10.2 cd	37.0	±	2.0 a	39.2	±	5.4 a
	LH 30-4	128.9	±	24.8 ba	157.3	±	25.5 b	39.1	±	5.5 a	40.1	±	2.4 a
	RH 43	101.4	±	21.6 cd	122.9	±	38.5 bcd	37.1	±	2.4 a	37.5	±	3.2 a
	Mean	138.8			122.5			34.7			34.9		
$LSD_{0.05}$				22.67						3.79			

ns: Not significant

Different letters indicates significance at 5% level determined by LSD, Least Significant Difference method

Fe-use efficiencies (Jelali *et al*., 2010). Such a case may be explained by a term expressed in literature as "Fe-chlorosis paradox". Schmidt and Romheld (1997) reported that this phenomenon may be related to insufficient leaf growth due to Fe-chlorosis and consequent relative increase in leaf Fe concentration. The other element concentrations of green leaves and yellow leaves with chlorosis were compared, despite the similar Fe concentrations; it was observed that yellow leaves had higher nutrient concentrations than green leaves. This may be related to dilution due to growth of green leaves.

Results of present study revealed that shoot growth performance, Fe intake from GM, transfer of Fe from roots to shoots, shoot Fe-use efficiency, Fe deficiency symptom levels and SPAD readings indicating chlorophyll levels were significant characteristics to evaluate the resistance of strawberry genotypes against Fe deficiency conditions in GM.

Considering above mentioned parameters, it could be concluded that strawberry genotypes tested for Fe efficiency were *F. virginiana* ssp. *glauca* > *F. chiloensis* ssp. *pacifica* > *F. chiloensis* ssp. *chiloensis*. This result could be used by breeding programs aiming at developing Fe-efficient strawberry genotypes for sowing under the conditions having low levels of available Fe.

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