

Physiological Characterization of Strawberry Cultivars with Differential Susceptibility to Iron Deficiency

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Abstract: Chlorosis resulted by iron (Fe) deficiency is a common problem for many crops in calcareous soils, which results in a decrease in growth and yield. Strawberries (*Fragaria ananassa* Duch.) exhibited a wide genotypic variation in their tolerance to Fe deficiency. Selection and development of Fe-efficient strawberry cultivars is a beneficial approach for their growth in Fe deficient calcareous soils. In this study, we screened 33 strawberry cultivars in terms of their response to iron chlorosis by means of bicarbonate application. The genotypes were grown in six liter pots with sand:soil mixture in the green house, evaluated in terms of size and TSS (Total Soluble Solids) contents of fruits and chlorophyll, total and active Fe contents and by visual chlorosis symptoms (or rank scores) on the leaves. Based on these results, another experiment was done using two iron-efficient ('Tango' and 'Spadeka'), two iron-inefficient ('Camarosa' and 'Dorit') and one moderately efficient ('Osmanli') cultivars grown in hydroponics. The selected cultivars were tested for their differences in uptake and total concentration of Fe in the leaves under sufficient and deficient concentrations of Fe. The results agreed with the first study in the soil mixture, which further support the methodology of screening.

Key words: Bicarbonate · chlorophyll · Fe deficiency · genotypic variation · hydroponics · strawberry

INTRODUCTION

Chlorosis caused by iron (Fe) deficiency is a common micronutrient problem and occurs mostly in calcareous soils, particularly in arid and semi-arid regions [1-4]. In the Mediterranean region, Fe chlorosis is one of the major abiotic stresses affecting fruit trees and other crops. The treatments include soil iron amendments, foliar sprays and solid implants in tree trunks and branches. Strawberries can be grown in a wide range of soil types, from light to heavy textured soils. Yet, highest yields are obtained when plants are grown in deep fertile soil, with high organic matter, good drainage and with pH of 6.0-6.5 [5]. On calcareous soils with high pH, however, Fe deficiency appears to be a major constraint for production of strawberries [6].

In soils with very high pH, high level of CaCO₃, low levels of organic matter and soil moisture and some other soil factors are predominantly responsible for low availability of Fe to plants [7]. Fe easily forms barely soluble compounds (i.e., oxides, hydroxides) that

are not available to plants [8]. Solubilization/mobilization of these compounds by plant roots is essential for Fe adequate nutrition of plants in calcareous soils [9]. It was well documented that plant species and genotypes of given species differ greatly in their sensitivity to Fe deficiency in calcareous soils [10, 11]. Many studies were conducted on Fe nutrition of strawberries over the past 50 years to determine the most effective and economical methods of correcting Fe deficiency chlorosis [4, 12-14]. Yet, no study could suggest an efficient screening method of cultivars regarding their Fe efficiency.

In Turkey, strawberries are mostly grown on calcareous soils of the Mediterranean and Aegean coastal regions. Therefore, strawberry production is seriously affected by CaCO₃ induced Fe-deficiency [14]. Eyuboglu [15], reported that roughly 59% of Turkish soils contain more than 5% CaCO₃ and over 63% of the soils have a pH higher than 7.5. In these regions plants are usually grown under drip irrigation systems. Farmers routinely supply Fe with the irrigation water in form of synthetic chelates to correct for Fe deficiencies, which increase the production

expenses. Thus, selection and improvement of strawberry genotypes for higher tolerance to Fe deficiency is a most realistic approach and more economical and practical solution to dissolve this problem. Since plant species and also the varieties show different response to Fe nutrition and plant Fe concentrations may differ even if they are grown in the same growing conditions. So, plants develop some adaptation mechanisms to Fe deficiency. In this paper, we present results of 33 strawberry genotypes screening for variation in resistance to Fe deficiency chlorosis induced by application of HCO_3^- to create artificial stress conditions for fixation or FeCO_3 precipitation. In addition, further characterization of Fe deficiency were done with pre-selected efficient, moderate and in-efficient strawberry cultivars using hydroponic culture system.

MATERIALS AND METHODS

Two series of experiments were carried out in this study during 2 years. In the first experiment, sixteen strawberry varieties and seventeen hybrids were tested; the genotypes (with named attached) and hybrids (with number) are listed in Table 1. Six (6) Plants of each genotype were transplanted to six liter pots with sand: soil (scale: 1:1) mixture in the greenhouse and at the beginning stage some physical and chemical characteristics of soil mixture (1:1 soil:sand) were detected and given in Table 2. Then, 200 ppm $\text{Ca}(\text{NO}_3)_2$ (as a source of N), 100 ppm triple super phosphate (as a source of P) were applied. Six plants were used for each genotype: three were amended with Fe and the other three were not. Fe was applied as 30 ppm Fe-chelate (EDDHA, Sequestrine 138, Ciba-Geigy). All plants were irrigated with 10 mM NaHCO_3 during November-June growing period. The genotypes were compared as regards to their level of Fe chlorosis by visual ranking, chlorophyll, total Fe and active Fe contents in their leaves and fruit size (scores:3,5,7,9) and total soluble solid contents (%). The fruit size of genotypes was compared according to the strawberry descriptor while the latter were determined by a hand type refractometer.

In the second experiment, two Fe-efficient (Tango and Spadeka), two Fe-inefficient (Camarosa and Dorit) and one moderately Fe-efficient (Osmanli) cultivars, selected based on the results of the first experiment. The plants were grown under greenhouse conditions for 120 days (until the early flowering stage), in hydroponics, with the following nutrients: 2 mM $\text{Ca}(\text{NO}_3)_2$; 1 mM MgSO_4 ; 0.2 mM KH_2PO_4 and 6.2 mg l^{-1} H_3BO_3 ; 0.25 mg l^{-1} $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 0.5 mg l^{-1} $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 0.25 mg l^{-1}

Table 1: Parents name of the strawberry cultivar candidates

Hybrid No	Parents
475/3	OsmanlıXVista/Pajaro/Blerubi
475/4	OsmanlıXVista/Pajaro/Belrubi
477/1	OsmanlıXCruz
477/2	OsmanlıXCruz
477/3	OsmanlıXCruz
488/1	OsmanlıXAiko
489/A	OsmanlıXVista/Pajaro/Belrubi
496/2	OsmanlıXCruz
496/6	OsmanlıXCruz
502/B	OsmanlıXTufts
504/7	OsmanlıXVista/Pajaro/Belrubi
613	OsmanlıXChandler
614	OsmanlıXChandler
625	Salvi-15XES 1044
647	504/7XDorit
691	504/7XDouglas
692	504/7XDouglas

Table 2: Some physical and chemical characteristics of soil mixture (1:1 soil:sand)

P_2O_5 (kg da^{-1})	8.32
K (ppm)	400.00
Zn (ppm)	1.50
Fe (ppm)	3.70
Mn (ppm)	8.60
Cu (ppm)	0.60
B (ppm)	0.20
pH	7.25
Salt (%)	0.25
Sand (%)	84.54
Silt (%)	4.16
Clay (%)	11.30

$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$; 0.55 mg l^{-1} $\text{ZnSO}_4 \cdot 3\text{H}_2\text{O}$ and 0.076 mM Fe DTPA in 5.5 liter containers. Ten plants were grown of each cultivar: five of them were amended with 50 ppm Fe-EDDHA while the other five were not. The five strawberry cultivars were tested for chlorophyll and total Fe contents of their leaves.

Determination of chlorophyll, active and total Fe contents and chlorosis symptoms: Chlorosis was scored visually according to a scale from 1 (severe chlorosis and necrosis) to 5 (intense green color). Chlorophyll contents of the leaves were determined by spectrophotometry after extraction of fresh leaf tissues with acetone [16]. In the first experiment, the concentration of total Fe were measured by in dry-ash samples at 550°C, dissolved in

3.3% HNO₃; active Fe was determined in dried leaf samples shaken in 1 N HCl for 2 h at 15 cycle/minute. In the second experiment, total Fe was analyzed by Flame photometry using the digestion method of Walinga *et al.* [17].

Experimental design and statistical analysis: The experiment were designed Completely Randomized Design with three replicates.

Analysis of variance and correlations were done using MSTAT and COSTAT statistical softwares; differences among the treatments were analyzed by the Tukey Multiple Comparison test. Tukey's procedure (HSD, p = 0.05) was used to test for significant differences among the blackberry genotypes.

RESULTS

Characterization of the 33 strawberry genotypes for Fe stress: The response of the tested strawberry genotypes to Fe deficiency stress were ranking, graded on 1-5 scale (Table 1). Chandler, Selva, Camarosa, Giresun, Vista, Dorit, 502/B, 504/7 and 613 number genotypes were found as the most inefficient (Score 1). The veins of their leaves became yellowish and necrosis formation was observed in the leaves. In addition, the leaves of the inefficient genotypes were smaller than the efficient ones. Muir, Oso Grande, Douglas CVs and genotype numbers 475/3, 477/1, 477/3, 488/1 having the 2 score and found to be efficient.

Based on the severity of leaf symptoms, CV Osmanlı and hybrid numbers 477/2, 475/4, 489/A, 496/2, 614, 625, 647 and 691 were shown to be moderate efficiency and having the 3 score. Nyoho, Senga Sengana, Early Glow cultivars and 496/6 and 692 hybrid numbers were found to be efficient and having scaled 4. Tango and Spadeka were classified as the most efficient genotypes having the highest score [5].

The results of fruit size and TSS content of experimental genotypes were given in Fig. 1 and 2. As shown in Fig. 1 the fruit size of experimental genotypes were found to be bigger under Fe applied conditions except Tango and Spadeka Cvs. The biggest fruit size was obtained from Cv Camarosa whereas Cv Osmanlı was the smallest one. However, the fruit size was highly affected negatively in Cv Camarosa compare to the other genotypes under non Fe treatment condition. As seen Fig. 2 the highest TSS content was obtained from the hybrids. Among the Cvs the highest TSS content was obtained from Osmanlı and Tango. In another fertilization study, Paydaş *et al.* [18] reported that the TSS content of Cvs Dorit, Oso Grande, Douglas and Chandler respectively as 11.39, 14.34, 10.92 and 8.98% under non fertilized conditions and in the present study we also detected parallel results.

Chlorophyll a, b and total chlorophyll contents of leaves of Fe treated and non-Fe treated strawberry genotypes were highly variable (Table 3). Fe treatment affected positively chlorophyll a contents of strawberry

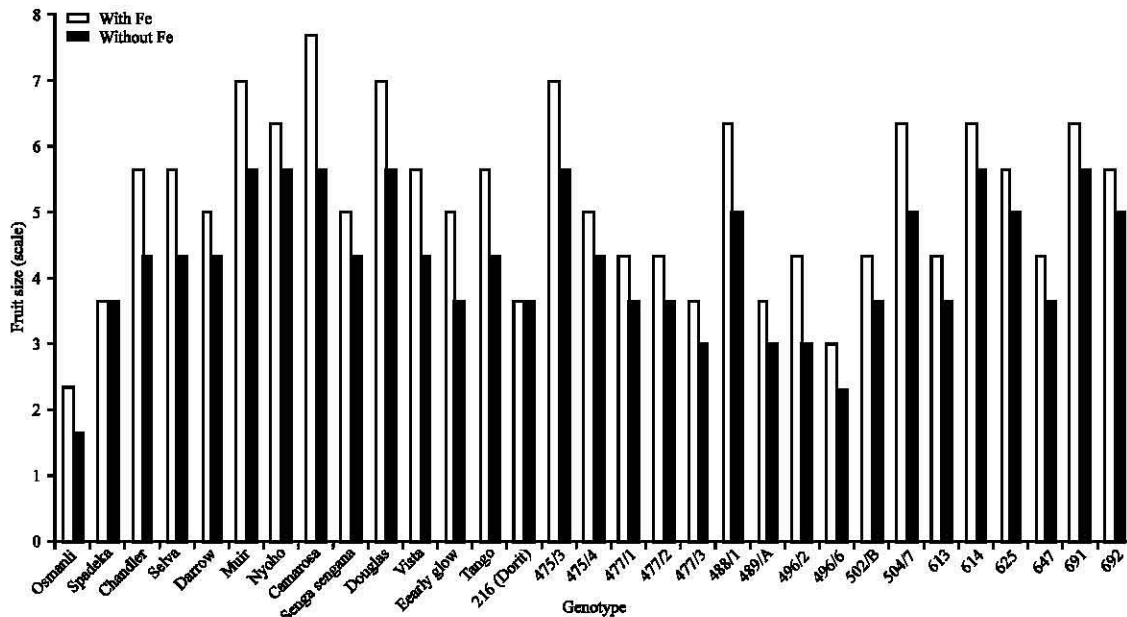


Fig. 1: The effect of Fe emended on fruit size of experimental genotypes

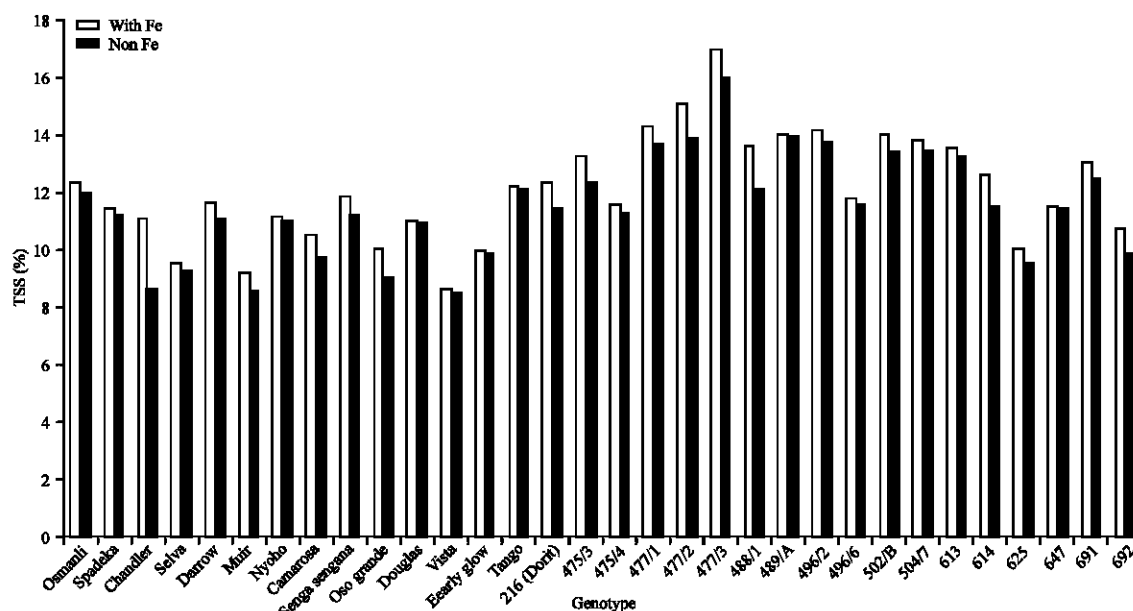


Fig. 2: The effects of Fe-amended on TSS contents (%) of experimental genotypes

genotypes. In average, chlorophyll a contents of the Fe-treated genotypes were higher (0.89 mg g^{-1}) than those of non-treated ones (0.27 mg g^{-1}). At genotypic level, CVs Tango and Spadeka showed the highest chlorophyll a contents in both treatments, whereas the highest difference between treatments was observed in CV Giresun and hybrid numbers 488/1, 504/7613, 692.

Chlorophyll b contents of Fe treated and non-treated strawberry genotypes are also presented in Table 3. Fe amendments positively affected chlorophyll b contents of all strawberry genotypes. On the average, chlorophyll b content was higher (0.30 mg g^{-1}) than the non-treated ones (0.09 mg g^{-1}). Spadeka and Tango had the highest chlorophyll b contents among the un-amended genotypes, whereas the highest difference between Fe treatments was obtained in Darrow, Selva, Muir, Camarosa and 475/3, 488/1, 496/2, 692 hybrids.

Fe treatment positively affected total chlorophyll contents of strawberry genotypes. Thus, total chlorophyll content of the genotypes was found to be higher (1.36 mg g^{-1}) than non-treated ones (0.45 mg g^{-1}). At genotypic level, however, Tango and Spadeka CVs had similar total chlorophyll contents in both treatments whereas the highest difference between treatments was obtained from CVs Darrow, Selva, Muir, Giresun and hybrid numbers 504/7, 488/1, 613, 692. So, these results show that these genotypes can be separate from one another in terms of Fe-efficient and inefficient.

Total Fe contents of the Fe treated and non treated genotypes were significantly affected by bicarbonate

applications (Table 3). Among the Fe treated genotypes, the highest total Fe was obtained from CVs Osmanli, Chandler, Early Glow and hybrid numbers 477/2, 496/6 and 692 whereas the lowest total Fe was obtained from CVs Nyoho, Muir, Giresun, Douglas and hybrid numbers 489/A and 647. The least variation between Fe treated and non Fe-treated plants was observed in CV Tango (107 and 93 ppm, respectively). In general, active Fe contents in strawberry leaves appeared to be smaller than their total Fe contents. Active Fe contents of the genotypes were affected by Fe treatment as well. Average active Fe content of Fe treated strawberry genotypes was found 68 ppm, whereas non-Fe treated ones had 41 ppm. The effect of Fe treatment was the least in Vista, 477/3, 489/A genotypes, whereas Fe treatment increased highly the active Fe contents of hybrid numbers 477/1, 614, 692.

Further characterization of selected strawberry genotypes:

The response of 33 strawberry genotypes to Fe supplement revealed that Tango, Spadeka, Nyoho, Senga Sengana, Darrow, Early Glow and hybrid numbers 496/6 and 692 certain strawberry genotypes seemed to be Fe efficient, whereas others are Fe inefficient. To study and to understand the characteristics that make some genotypes more efficient than others, we focused on five common cultivars for further characterization, which exhibited in the first phase significant differences in their response to Fe CVs Camarosa, Osmanli, Dorit, Spadeka and Tango.

Table 3: Chlorophyll and Fe contents of 33 strawberry genotypes with Fe-amended and non amended (mg g⁻¹ fresh wt; ppm of dry wt in the extract)

Genotypes	Chlorophyll a (mg g ⁻¹)		Chlorophyll b (mg g ⁻¹)		Total Chlorophyll (mg g ⁻¹)		Total Fe (ppm)		Active Fe (ppm)	
	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe
Osmanli	1.13	0.59	0.38	0.16	1.73	0.84	230	168	92	63
Spadeka	1.38	0.75	0.62	0.47	2.33	1.61	107	81	68	33
Chandler	0.80	0.18	0.22	0.11	1.13	0.35	194	139	78	55
Selva	0.52	0.17	0.57	0.06	1.52	0.25	118	73	44	35
Darrow	1.26	0.23	0.43	0.03	1.96	0.29	121	89	60	27
Muir	1.07	0.23	0.35	0.05	1.54	0.31	75	62	62	44
Nyoho	0.97	0.34	0.32	0.07	1.48	0.46	70	59	43	24
Camarosa	0.89	0.24	0.25	0.04	1.25	0.30	142	84	86	39
Senga Sengana	1.12	0.25	0.29	0.07	1.58	0.69	109	68	55	34
Giresun	1.21	0.18	0.00	0.08	1.76	0.23	87	49	67	38
Oso Grande	0.67	0.21	0.21	0.06	0.98	0.63	151	101	74	30
Douglas	0.97	0.45	0.30	0.13	1.42	0.65	84	69	50	33
Vista	1.04	0.32	0.31	0.08	1.55	0.39	153	114	44	37
Early Glow	0.99	0.22	0.36	0.08	1.60	0.34	168	142	48	35
Tango	0.81	0.70	0.77	0.53	1.80	1.36	107	93	50	32
216 (Dorit)	1.05	0.27	0.19	0.05	1.28	0.65	144	101	54	40
475/3	0.66	0.18	0.28	0.05	1.15	0.26	158	108	67	44
475/4	0.87	0.43	0.26	0.10	1.27	0.59	131	108	56	43
477/1	0.91	0.23	0.26	0.05	1.33	0.59	119	76	64	26
477/2	0.83	0.40	0.25	0.15	1.20	0.61	222	114	108	63
477/3	0.82	0.29	0.22	0.09	1.15	0.42	167	102	57	43
488/1	0.74	0.06	0.20	0.02	1.06	0.15	99	47	52	28
489/A	0.94	0.26	0.25	0.06	1.32	0.36	80	67	63	51
496/2	0.60	0.16	0.25	0.02	0.93	0.21	167	101	76	42
496/6	0.81	0.30	0.24	0.09	1.16	0.43	260	158	155	103
502/B	0.82	0.24	0.23	0.05	1.17	0.32	136	98	59	35
504/7	0.60	0.09	0.17	0.02	0.85	0.12	151	82	51	34
613	0.93	0.08	0.24	0.16	1.29	0.11	158	136	76	55
614	0.88	0.19	0.41	0.05	1.42	0.31	146	79	71	29
625	0.78	0.18	0.21	0.03	1.12	0.41	158	113	49	33
647	0.85	0.25	0.18	0.05	1.16	0.33	93	47	55	30
691	0.64	0.19	0.15	0.04	0.91	0.23	124	62	85	43
692	0.97	0.14	0.29	0.02	1.44	0.12	272	256	136	59
Average	0.89	0.27	0.30	0.09	1.36	0.45	143	98	68	41

Table 4: Chlorophyll and Fe contents of pre-selected genotypes with Fe-amended and non-amended (mg/g fresh wt ; ppm of dry wt in the extract)

Genotypes	Chlorophyll a (mg g ⁻¹)		Chlorophyll b (mg g ⁻¹)		Total Chlorophyll (mg g ⁻¹)		Total Fe (ppm)	
	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe	+Fe	-Fe
Tango	0.99	0.72	0.21	0.02	1.30	1.03	342	222
Spadeka	0.94	0.77	0.19	0.02	1.24	1.07	302	221
Dorit	0.68	0.23	0.07	0.00	0.82	0.29	298	225
Camarosa	0.59	0.26	0.11	0.00	0.77	0.34	272	208
Osmanli	0.87	0.61	0.31	0.03	1.29	0.91	260	176
Average	0.81	0.52	0.18	0.01	1.09	0.73	295	169

Similar to the results of the first experiment; the veins of Camarosa, Dorit and Osmanli leaves respectively became yellowish under Fe starved conditions and necrosis formation observed in the leaves. However, Tango and Spadeka did not show the chlorosis symptoms at all, as in the first experiment.

In Cv Spadeka, the leaves of Fe treated plants had 0.94 mg g^{-1} chlorophyll a, whereas non Fe-treated plants had 0.77 mg g^{-1} chlorophyll a. Chlorophyll b contents of Fe treated and non-treated strawberry genotypes are presented in Table 4. Fe treatment affected positively chlorophyll b contents of strawberry genotypes. Average chlorophyll b content of the Fe-treated genotypes was higher (0.18 mg g^{-1}) than non-treated ones (0.01 mg g^{-1}). At genotypic level, Osmanli, Spadeka and Tango genotypes had the highest chlorophyll b content in non Fe treatments as well, whereas the highest difference between Fe treatment was obtained from Camarosa and Dorit (Table 4). Total chlorophyll content of Fe treated and non-Fe treated strawberry genotypes of the 2nd trial are presented in Table 4. Fe treatment affected positively total chlorophyll contents of strawberry genotypes. Total chlorophyll content of the Fe-treated genotypes was higher (1.09 mg g^{-1}) than non-treated ones (0.73 mg g^{-1}). At genotypic level, Tango and Spadeka genotypes had similar total chlorophyll contents in both treatments, whereas the highest difference between treatments was obtained from Camarosa and Dorit.

Total Fe contents of the Fe treated and non Fe treated genotypes were significantly affected by bicarbonate applications. Among the Fe treated genotypes, the highest total Fe was obtained from CVs Tango (342 mg g^{-1}) and Spadeka (302 mg g^{-1}) genotypes, whereas the lowest total Fe was obtained from Osmanli (260 ppm), Dorit (298 ppm) and Camarosa (272 ppm) CVs, respectively. The least variation between Fe treated and non Fe-treated plants was observed in Cv Dorit (272 ppm and 208 ppm, respectively).

DISCUSSION

The results of the present study indicate the occurrence of a large genotypic variation among the strawberry genotypes in their sensitivity/tolerance to Fe deficiency. Genotypes greatly differed in their chlorophyll and Fe levels under sufficient vs. Fe deficient conditions. The results indicated that the HCO_3 method is useful in screening genotypes for their tolerance to lime-induced Fe chlorosis in strawberries. Such a test was used in other plant species grown in alkaline soils, as in citrus

rootstocks [19], in grapevine [20] and peach and their hybrids growing in alkaline soils [21-23] Bindra *et al.* [24], proposed another test to screen peach rootstocks based on the capacity of the plants to absorb and translocate radioactive Fe. However, the use of bicarbonate test is easier, quicker and cheaper than the other screening methods. The results of this study showed that the bicarbonate test is a reliable method for screening studies.

Under Fe supply, in the first experiment, total Fe was changed between 70 ppm (Nyoho) to 272 ppm (692) while under Fe deficiency it was changed between 47 ppm (647) to 256 ppm (692). Similarly, Pritts and Hendley, [25] reported that Fe deficiency started 40 ppm (dry wt) below, sufficient stage can be changed from 60 ppm to 250 ppm (dry wt). Excess of 350 ppm of Fe were measured in leaves (dry wt). The authors suggested that Fe deficiencies could be detected by conducting a leaf analysis where the plants exhibit symptoms. In the present study, in hydroponics results show that total Fe uptake was higher than under soil conditions.

Nelson and Jolley [26], reported a large variation for tolerance to Fe deficiency among the strawberry genotypes. Iron deficiency chlorosis can greatly reduce yields in strawberries grown in high pH calcareous soils. In the present study, size and TSS contents of fruits affected Fe amendment depends on the genotypes. Zaiter *et al.* [13], reported that Fe-EDDHA increased the yield in 33%. However, genotypes responded differently to the amendment: Fe-EDDHA the yield increase was 13% for "Motto"; 30% for "Chandler"; 56% for "Douglas". Fe was found to be a major constraint for production of many crops including strawberry (*Fragaria X ananassa*), when it is grown on high pH calcareous soils of Lebanon [6]. Consequently, Fe application is required in the growing of Fe-inefficient strawberry cultivars particularly as chelates (like Fe EDDHA) that can improve the economic yield. However, this will be costly for the growers. Alternatively, selection and/or breeding of Fe-efficient cultivars are realistic approaches to remedy this problem for strawberry cultivation in high pH calcareous soils. In hydroponic culture, the results show that total Fe uptake was higher than under soil conditions. In the present study, we demonstrated that screening by bicarbonate induced Fe chlorosis is a practical and economical testing tool. The CVs "Spadeka" and "Tango" were found to be Fe-efficient. These two genotypes can decrease the rhizosphere pH and, as a result, they can take up Fe more effectively. From this point of view, we aimed to use these cultivars to develop by breeding having Fe-efficient, in high yielded and high quality characteristics. These

results will provide very important information for breeders to understand the genetic mechanism of Fe efficiency in strawberry and will help identification of molecular markers link to Fe-efficiency in strawberry for further studies.

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