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In vitro screening of octoploid *Fragaria chiloensis* and *Fragaria virginiana* genotypes against iron deficiency

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Abstract: Iron (Fe) deficiency is one of the common problems in strawberry (*Fragaria* × *ananassa* Duch.) growing regions. In vitro screening enables researchers to evaluate genotype performance under several concentrations of Fe in a relatively fast and economical way. Therefore, we evaluated the responses of several genotypes belonging to octoploid *Fragaria chiloensis* (L.) Mill. and *Fragaria virginiana* Mill. (the progenitors of cultivated strawberry) against Fe treatments under in vitro conditions. In the first experiment, HM 1, Pigeon Point, CFRA1267 (*F. chiloensis* subsp. *pacifica*), and CA1541 (*F. chiloensis* subsp. *chiloensis*) were tested with various Fe concentrations (0%, 10%, 15%, 25%, 50%, 65%, 80%, and 100%, where 100% = 27.8 mg L^{-1} $FeSO_4.7H_2O$). Different Fe concentration treatments resulted in differences in plant height, fresh and dry weight, as well as macro- and microelement concentrations among the tested genotypes. In the second experiment, 16 octoploid *Fragaria* genotypes were tested with 4 Fe concentration treatments (0%, 10%, 50%, and 100%, where 100% = 27.8 mg L^{-1} $FeSO_4.7H_2O$). Iron concentration and genotype interactions were significant for all studied characteristics except sulfur (S) and manganese (Mn) concentrations, and the 16 genotypes responded differentially to varying Fe treatments. The results of these experiments indicated that *F. chiloensis* and *F. virginiana* genotypes exhibited considerable variation under different Fe treatments in vitro, and the more resistant genotypes could be utilized to develop new strawberry cultivars with tolerance to low Fe concentrations.

Key words: Breeding, genetic resources, iron, strawberry, supercore, tissue culture

1. Introduction

Cultivated strawberry, *Fragaria* × *ananassa*, is the natural hybrid of *F. chiloensis* and *F. virginiana* and is thought to be only about 300 years old (Hancock, 1999). Earlier researchers have reported that the cultivated strawberry has very narrow genetic diversity when compared with its progenitor species (Dale and Sjulin, 1990; Hancock, 2006; Horvath et al., 2011). Since the progenitor species can easily be crossed with the cultivated strawberry, several attempts have been made to utilize the wild species to expand the genetic base of cultivated strawberry. An example of successful utilization of the wild species includes the introgression of day-neutrality from *F. virginiana* subsp. *glauca* into *F.* × *ananassa* (Ahmadi et al., 1990; Serce and Hancock, 2005; Shaw and Famula, 2005; Luby et al., 2008). Indeed, there have been attempts to create a synthetic cultivated strawberry through hybridization after selection of elite *F. chiloensis* and *F. virginiana* clones for the development of superior strawberry cultivars with high genetic diversity (Luby et al., 2008; Hancock et al., 2010; Stegmeir et al., 2010).

In the last 3 decades various studies have been conducted on the progenitor species of cultivated strawberry. First, several collections have been undertaken in North and South America (Luby et al., 1991, 2008; Hancock et al., 2010; Stegmeir et al., 2010). More than 2000 clones were sampled and sent to the National Clonal Germplasm Center at Corvallis, Oregon, USA (Hummer, 1991; Yao et al., 2012). A world collection with more than 380 clones was developed and evaluated for both horticultural and taxonomical traits (Hancock et al., 2003, 2004). A supercore collection consisting of 38 clones was created and evaluated by several researchers. For example, the horticultural performance of these genotypes was tested at 5 locations in the USA (Hancock et al., 2001a, 2001b). CO_2 assimilation rates under cool and hot temperatures were determined by Serce et al. (2002). Serce and Hancock (2005) also studied the flowering patterns of these clones

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under different environmental conditions. Several other studies tested the reactions of these clones against spider mites (Serce and Hancock, 2002), fungal diseases (Schilder et al., 2003), and anthracnose (Smith et al., 2003).

Iron deficiency is a common nutritional problem in plants and soils. There are many reports describing yield reductions caused by Fe deficiency in field crops (Hansen et al., 2006) and in fruits (Rombola and Tagliavini, 2006) and the detrimental effects of Fe-chlorosis on both fruit yield and quality (*Álvarez-Fernández* et al., 2006). The differences in sensitivity to Fe deficiency among plant species have been reported by several researchers (Vose, 1982; Awad et al., 1994; Tagliavini and Rombola, 2001; Erdal et al., 2004; *Álvarez-Fernández* et al., 2011; Pestana et al., 2012; Zuo and Zhang, 2011), and the strawberry is one of the most sensitive species to Fe deficiency (Vose, 1982; Zaiter et al., 1993; *Álvarez-Fernández* et al., 2006; Kafkas et al., 2007; Pestana et al., 2011, 2012).

Iron deficiency is one of the most common problems in strawberry growing areas, as the cultivated strawberry (*F.* × *ananassa*) is very sensitive to Fe deficiency. In vitro screening enables researchers to evaluate genotype performances under several treatments in a relatively fast and economical way. Herein, we use these methods to determine responses to Fe deficiency among a diverse sample of octoploid *Fragaria* genotypes from the supercore collection. The *Fragaria* genotypes more resistant to Fe deficiency could be utilized to develop new strawberry cultivars having tolerance to low Fe concentrations.

2. Materials and methods

2.1. Experiment 1

In this first experiment 4 genotypes, HM 1, Pigeon Point, CFRA1267, and CA 1541 were selected from the supercore collection (Table 1). These genotypes were selected because they exhibited differential responses to

Table 1. *Fragaria* genotypes, their names, PI numbers, and sampling sites used in the experiments.

No.	Name	Other name(s)	PI no.	Sampling site
		F. chiloensis subsp. *pacifica*		
1	RCP 37	CFRA 34	551445	California
2	BSP 14	CFRA 48	551459	Oregon
3	Pigeon Point	CFRA 357; CA 1367	551728	California
4	Auke Lake	CFRA 368	551735	Alaska
5	HM 1	CFRA 1691	612489	Oregon
6	CFRA 1267		612488	British Columbia
7	Scotts Creek	CFRA 1692	612490	California
		F. chiloensis f. *chiloensis*		
8	Darrow 72	CFRA 24	236579	Chile
9	CA 1541		551736	Peru
10	2 BRA 1A	CFRA 1075	612316	Chile
		F. chiloensis f. *patagonica*		
11	2 TAP 4B	CFRA 1092	612317	Chile
		F. virginiana subsp. *glauca*		
12	Cascade	CFRA 110	551527	Oregon
13	BT 3	CFRA 1693; CA 1226; BH3	612491	Utah
14	BH 2	CFRA 1696; LH 5-1	612494	South Dakota
15	RH 43	CFRA 1698; N8688	612496	Alaska
16	LH 30-4	CFRA 1703	612501	Montana
		F. virginiana subsp. *virginiana*		
17	Eagle 14	CFRA 1694	612492	Ontario
18	JP 95-1	CFRA 1435	612570	Florida

Fe treatments when they were grown under greenhouse conditions in a previous trial. The stolons were harvested from genotypes grown in unheated greenhouse conditions in 2.5-L pots. These plant materials were sterilized with 70% ethyl alcohol for 3 min and were treated with 20% NaClO + 1–2 drops of Tween-20 for 10 min. They were then washed with sterile water to remove the sterilants.

The shoots from sterilized plant materials were trimmed to 0.5–1.0 mm sections containing the meristem and placed in Murashige and Skoog (MS) (1962) tissue culture media containing the standard macro- and microelements and plant growth regulators [benzyl adenine (BA) at a concentration of 1 mg L^{-1}]. The materials were subcultured every 4 weeks during micropropagation. The previously developed protocol by Aka-Kaçar and Çetiner (1995) was used to root the shoots.

After 6 weeks of rooting, the plantlets were transferred to multiplication media. The propagation media was prepared in the same way as the shoot development media, according to the protocol described by Aka-Kaçar and Çetiner (1995). Plantlets were kept under a light intensity of 1500–2000 lx for 16 h at 25 ± 1 °C.

The maximum Fe concentration of the MS media at the propagation, subculturing, and rooting stages was set at 27.8 mg L^{-1} $FeSO_4.7H_2O$ (100%). Other sets of media were produced at different concentrations of Fe (0%, 5%, 15%, 25%, 50%, 65%, 80%, and 100%). The cultured plants were harvested according to their root number, root length, and plant height.

At the end of this experiment, the root number, root length, and plant height were measured. Data on the fresh and dry weights of the plants and soil plant analysis development (SPAD) unit values indicating chlorosis levels of leaves and chlorophyll concentrations were also recorded for these tissue cultured plants.

The SPAD values were recorded using a chlorophyll meter (Minolta SPAD 502). The chlorophyll concentrations were measured using young leaves having a standard position and size according to methods described by Arnon (1949). In short, 100–200 mg of the young leaf tissue was homogenized on 80% acetone and filtered. Then the total chlorophyll contents were measured at 652 nm and expressed in milligrams per gram fresh weight. Analysis of the elements in plant samples was performed according to Jones et al. (1991) and Cakmak et al. (2010). For this aim, the samples were dried at 70 °C for 48 h. The dried samples were used to determine the macro- and micronutrient concentrations. Plant tissues (0.25 g) were mixed with 2 mL of H_2O, 2 mL of H_2O_2 (30%), and 4 mL of HNO_3 and were subjected to the wet digestion method by microwave (Milestone 1200 Mega) and filtered through blue band filter paper (Çakmak et al., 2010). The filtered extract was completed to 25 mL with distilled water, and micro- and macronutrients of the resultant extract were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Jobin, Yvon–Paris). The values were controlled by standard reference material (SRM 1547 Peach Leaves, National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.2. Experiment 2

The second experiment was conducted under conditions similar to those of Experiment 1. However, only the traits that showed significant correlation with Fe concentrations in Experiment 1 were analyzed. Sixteen genotypes from the supercore collection were used as plant material in Experiment 2 (Table 1). Fewer Fe treatments were used in this experiment; 27.8 mg L^{-1} $FeSO_4.7H_2O$ was considered the adequate Fe concentration and was assigned the value of 100%. Three different levels of Fe concentration (0%, 10%, and 50%) were used to determine the reactions of the genotypes.

Analyses of variance were conducted utilizing a factorial design through the GLM procedure of computer software program SAS (SAS, 2005). The mean tables were computed using the TABULATE procedure of SAS, while correlation coefficients and their significance among the different studied variables were calculated using the CORRELATION procedure of SAS.

3. Results

3.1. Experiment 1

The appearance of 4 *Fragaria* genotypes grown under standard tissue culture conditions with varying Fe concentrations is presented in the Figure. The Fe treatments had a significant effect on all the variables tested except copper (Cu) concentrations (Table 2). This indicated that the desired screening environment was created in the experiment. The 4 genotypes also significantly differed for all experimental variables except SPAD values and phosphorus (P) concentrations. The Fe concentration treatments (T) and the genotype (G) interactions were significant for all the studied plant characteristics as well as macro- and micronutrient concentrations. This indicated that the 4 genotypes tested in this experiment reacted differentially to the different Fe treatments.

In general, the mean values of the tested traits increased linearly with increasing levels of Fe concentration; however, the rate of change declined after the 50% Fe concentration. For example, average plant heights across Fe concentration treatments were 1.40, 2.56, 4.13, 4.12, 4.37, 4.82, 3.39, and 4.81 cm. These results suggested that Fe concentrations from 15% to 50% were adequate to distinguish the differential responses of variant genotypes. Thus, the 0%, 10%, 50%, and 100% concentrations were utilized in further screening studies.

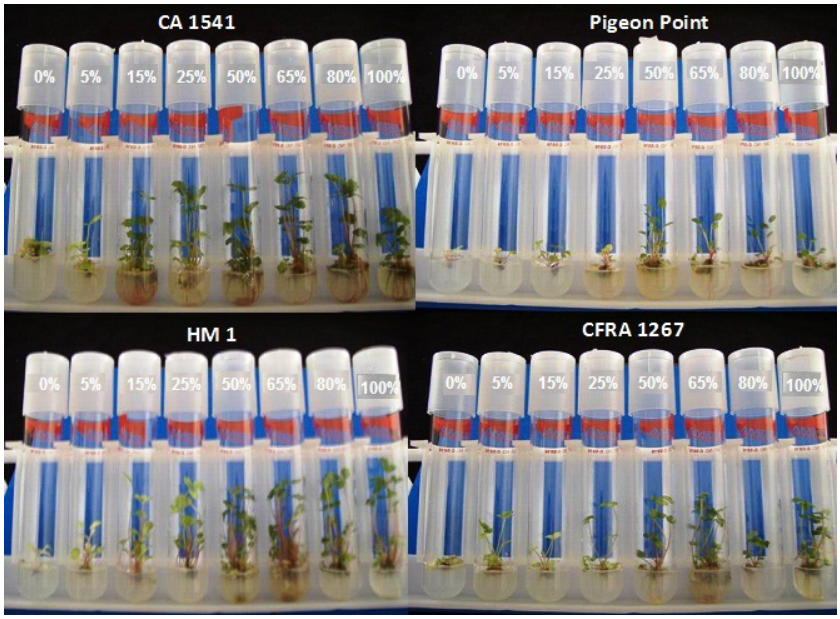

Figure. The appearance of 4 *Fragaria* genotypes grown under standard tissue culture with varying Fe concentrations (0%, 5%, 15%, 25%, 50%, 65%, 80%, and 100%).

Chlorophyll concentration was significantly correlated with SPAD values (Table 3). Chlorophyll concentrations rapidly increased from 0% to 50% Fe concentrations, but were stable after the 50% Fe concentration level. Although similar trends were observed for all SPAD values, there were considerable deviations in the degree of response among genotypes. These results indicated that the ultimate responses of *Fragaria* genotypes to varying Fe concentrations should not be evaluated only by chlorophyll concentrations and SPAD values; several other traits should be considered as well.

The mean values for T × G are presented in Table 3. There were significant T × G interactions for all variables. The root number varied between 5.1 (CFRA 1267; 65%) and 28.7 (HM 1; 50%). CFRA 1267 also showed the longest root length (10.7 cm), while HM 1 had the tallest plant (7.3 cm). The SPAD values varied greatly. CFRA 1267 had the lowest values (3.4) at 0% Fe concentration, whereas HM 1 at 50% had the highest value (44.1). HM 1 also had the highest dry matter content (49.7 mg plant^{-1}). The plant macro- and micronutrient contents showed significant T × G interactions. Overall, there was a trend in which higher Fe concentration treatments had higher Fe content for all genotypes; however, the magnitude of change varied across genotypes. HM 1 had the lowest Fe concentration at 5%, whereas CA 1541 exhibited the highest Fe concentration at 100%. CA 1541 had the lowest zinc (Zn), Cu, and P and highest Mn concentrations at 15% Fe. HM 1 had the highest Cu (15.8 mg kg^{-1}) and P (0.70%) for all Fe treatments.

The correlation coefficients and their significance at 5% for all variables tested in Experiment 1 are given in Table 4. Many variables were significantly correlated. Fe concentrations were significantly and positively correlated with SPAD, chlorophyll content, and dry weight; however, it was not significantly associated with root number, root length, or plant height. The Fe concentration was also significantly correlated with all other element concentrations either positively (Mn) or negatively (Zn, Cu, and P).

3.2. Experiment 2

In Experiment 2, the ANOVA analysis indicated that only a few variables were significantly associated with variation in Fe concentration [Fe, Zn, and boron (B) concentrations; Table 2]. However, the genotypes were significantly different for all the variables tested. T × G interactions were also statistically significantly different for dry weight content, and Fe, P, Cu, Zn, and B concentrations. This indicated that the genotypes responded differently to the various Fe concentration treatments.

The dry matter content varied between 0.8 g (LH 30-4) and 4.1 g (HM 1) mg plant^{-1} (Table 5). Fresh weights ranged from 0.06 g plant^{-1} for RCP 37 to 1.25 g plant^{-1} for BH 2 (Table 5). Overall, the highest fresh weights were observed at 50% Fe concentrations. LH 30-4 had the shortest plant height (6.7 mm), while 2 TAB 4B had the tallest plant height (76.8 mm). Plant height was significantly associated with level of Fe concentration.

HM 1 had the longest plants regardless of Fe concentration. In general, the genotypes had similar Fe

Table 2. Mean squares and significances of several variables for 4 *Fragaria* genotypes grown under standard tissue culture with varying Fe concentrations (0%, 5%, 15%, 25%, 50%, 65%, 80%, and 100%) (Experiment 1) and variables for 4 *Fragaria* genotypes grown under standard tissue culture with varying Fe concentrations (0%, 10%, 50%, and 100%) (Experiment 2).

Experiment 1

Source	df	Root number	Root length (cm)	Plant height (cm)	SPAD	Chlorophyll content	Dry weight (mg plant^{-1})	Concentration				
								Fe	Zn	Mn	Cu	P
Treatment (T)	7	197*	27.3*	31.9*	1891*	5.83*	0.006*	1238*	0.16*	96766*	5249	55.7*
Replication /T	15	60*	8.5	1.5	16	0.11	0.001	83	0.01	793	2560*	4.9*
Genotype (G)	3	1265*	112.7*	184.0*	248	4.12*	0.007*	3033*	0.38*	92253*	122869*	46.8
T × G	21	104*	19.0*	7.6*	93*	0.82*	0.009*	352*	0.03*	10703*	5506*	15.9*
Error	39	40	8.5	1.5	19	0.35	0.001	97	0.01	560	1418	2.2

Experiment 2

Source	df	Dry weight	Concentration						
			Fe	P	S	Cu	Mn	Zn	B
Treatment (T)	3	0.012	288,886*	0.001	0.001	1.53	1431	23,606*	132.7*
Replication /T	36	0.001*	813*	0.001	0.001	0.10	119	306	6.7
Genotype (G)	15	0.018*	24,184*	0.043*	0.026*	40.88*	7516*	17,283*	163.1*
T × G	45	0.001*	15,261*	0.001*	0.001	1.70*	521	1848*	35.1*
Error	522	0.001	388	0.001	0.001	0.25	352	279	14.3

Table 3. Mean values of several variables for 4 *Fragaria* genotypes grown under standard tissue culture with varying Fe concentrations (0%, 5%, 15%, 25%, 50%, 65%, and 80%). Element concentrations are presented in mg kg^{-1} except P which is presented in %.

Genotype	Fe treatment (%)	Root number	Root length (cm)	Plant height (cm)	SPAD	Chlorophyll content	Dry weight (mg $plant^{-1}$)	Concentration				
								Fe	Zn	Mn	Cu	P
	0	10.6	2.4	3.2	5.1	0.59	23.4	26.8	240	218	12.1	0.55
	5	22.2	3.6	7.0	7.0	0.99	13.2	23.7	158	329	15.8	0.70
	15	16.8	3.1	6.3	22.2	1.56	44.2	26.4	78	305	6.3	0.47
HM 1	25	22.4	3.7	5.3	30.8	2.05	30.7	33.4	74	270	8.0	0.58
	50	28.7	3.4	7.2	44.1	3.48	51.9	56.8	65	329	6.3	0.28
	65	18.2	2.1	5.7	39.8	2.76	53.5	50.4	57	386	5.3	0.33
	80	24.2	3.0	7.3	40.3	3.06	27.2	25.9	70	261	6.9	0.49
	100	---	---	2.8	40.9	3.17	49.7	49.3	59	381	3.5	0.36
	0	7.0	1.0	1.1	6.6	1.47	5.4	30.9	615	214	14.6	0.67
	5	10.2	2.2	1.9	8.4	0.98	5.9	81.2	311	171	7.2	0.56
	15	5.5	1.8	2.2	16.1	1.31	12.0	47.1	212	168	3.6	0.52
Pigeon Point	25	18.2	2.4	2.4	21.9	2.15	5.5	47.7	109	145	5.6	0.69
	50	6.0	2.1	1.6	40.6	3.00	20.6	52.6	120	154	3.2	0.47
	65	8.8	1.3	1.9	37.7	1.79	29.1	85.8	125	177	1.5	0.41
	80	6.3	2.3	2.0	40.7	3.59	11.1	---	234	154	4.6	0.44
	100	---	---	1.0	38.1	1.90	20.6	---	171	165	5.8	0.46
	0	6.4	3.7	2.9	3.4	1.04	14.5	35.1	537	184	8.6	0.50
	5	6.3	2.4	1.7	31.6	1.86	15.7	29.7	51	296	5.6	0.69
	15	13.0	2.5	3.0	5.9	0.74	11.6	28.8	232	212	9.9	0.68
CFRA 1267	25	14.9	4.6	4.3	31.6	3.19	12.1	37.5	81	274	11.9	0.58
	50	12.2	6.1	4.3	43.1	3.76	28.4	56.5	86	205	8.1	0.50
	65	5.1	1.8	2.8	39.1	3.41	27.7	57.5	51	250	8.0	0.41
	80	11.4	10.7	5.1	38.5	3.05	10.9	33.8	67	217	7.8	0.52
	100	---	---	1.1	41.1	4.15	9.5	79.1	44	238	5.5	0.53
	0	8.6	3.6	3.1	6.3	0.18	17.4	50.2	350	290	5.6	0.37
	5	14.8	5.9	5.8	20.3	2.38	28.5	37.5	100	317	7.8	0.44
	15	9.1	4.2	4.5	37.3	3.68	36.9	53.8	48	378	3.8	0.32
CA 1541	25	12.6	5.7	5.5	36.4	2.81	28.7	47.9	45	295	4.3	0.42
	50	13.3	8.4	5.9	46.2	4.41	40.5	67.2	48	352	3.8	0.27
	65	10.3	6.6	5.4	45.4	3.91	35.6	70.7	46	340	4.3	0.29
	80	13.0	4.9	5.0	41.3	3.57	29.9	71.2	47	300	5.4	0.21
	100	---	---	0.9	44.0	3.92	25.8	100.1	54	309	4.0	0.31

Table 4. Correlation coefficients and significances of several variables for 4 *Fragaria* genotypes grown under standard tissue culture with varying Fe concentrations (0%, 5%, 15%, 25%, 50%, 65%, 80%, and 100%). Significant correlation coefficients are presented in bold.

Variable	Root length	Plant height	SPAD	Chlorophyll content	Dry weight	Concentration				
						Fe	Zn	Mn	Cu	P
Root number	0.12	**0.78**	0.15	0.12	**0.42**	–0.23	**–0.38**	**0.41**	0.21	–0.02
Root length		**0.51**	0.37	**0.47**	0.17	0.06	–0.37	0.34	–0.07	–0.32
Plant height			0.15	0.18	**0.54**	–0.35	**–0.39**	**0.59**	0.16	–0.26
SPAD				**0.90**	**0.51**	**0.48**	**–0.75**	0.29	**–0.60**	**–0.59**
Chlorophyll content					**0.40**	**0.48**	**–0.64**	0.34	**–0.42**	**–0.55**
Dry weight						**0.14**	**–0.51**	**0.72**	**–0.39**	**–0.71**
Fe							–0.25	0.02	**–0.59**	**–0.57**
Zn								**–0.45**	**0.45**	**0.35**
Mn									**–0.07**	**–0.49**
Cu										**0.61**

concentrations at 0% Fe but had significantly different values at 50% and 100% Fe. The greatest Fe concentrations at 100% Fe treatment were observed in LH 30-4, RH 43, Eagle 14, and BH2 genotypes belonging to *F. virginiana*. We should also note that these genotypes had the lowest dry weights as well.

Surprisingly, plant Fe concentration decreased in BT 3 as the Fe concentration in the media increased. Most genotypes showed higher Fe levels as Fe concentrations in the treatments were raised. LH 30-4 had the highest Fe concentrations among genotypes at all Fe treatment levels. LH 30-4 had the highest P concentration while Pigeon Point had the lowest. LH 30-4 had the highest S concentration of any genotypes at 0%, 25%, and 50% Fe concentrations. RCP 37 had the highest overall Cu concentration. The differences between RCP 37 and other genotypes were quite dramatic, especially at the 0%, 25%, and 100% Fe treatments. 2 BRA 1A had the highest Mn concentration among all genotypes. The highest Mn concentration was recovered at the 0% Fe treatment and gradually decreased; a similar trend was observed in most of the other genotypes. Similar to the results obtained in P and S concentrations, LH 30-4 had the highest Zn concentrations at all Fe treatments; the concentration decreased as Fe concentration increased, although most other genotypes did not exhibit this trend. Boron (B) concentrations were similar among genotypes, except for 2 BRA 1A which had significantly higher concentrations at 0% and 25% Fe but average concentrations at 50% and 100% Fe.

4. Discussion

We tested the responses of a diverse array of *Fragaria* genotypes to different Fe concentrations during micropropagation. In Experiment 1 we studied the response of 4 genotypes to 8 different Fe concentrations to identify variables for further study in a larger trial with more genotypes. We found that the most important variables were plant dry weights and element concentrations. Similar strategies have been utilized by other researchers (Dolcet-Sanjuan et al., 1992; *Álvarez-Fernández* et al., 2003, 2011).

Many other researchers have employed other laboratory techniques such as growth chambers and tissue culture to conduct their screening studies (Stephens et al., 1990; Muleo et al., 1995; Tangolar et al., 2008; Yilmaz et al., 2008) when field studies are difficult. The reactions of *Pyrus* and *Cydonia* to low levels of Fe have been tested both under field conditions and via tissue culture. Similar responses were obtained from the genotypes under these varying environments indicating that tissue culture could be used for screening purposes (Dolcet Sanjuan et al., 1992).

After determining an effective screening system in Experiment 1, a large number of *Fragaria* genotypes were screened at 4 different Fe concentrations. The results indicated that the genotype exhibited a great deal of variation for almost all the variables tested. The significant interaction between genotype and Fe concentration indicated that the pattern responses of the genotypes were variable as well.

We found that some strawberry genotypes, such as 2 BRA 1A, were quite tolerant to even the lowest Fe

Table 5. Mean values of several variables for 4 *Fragaria* genotypes grown under standard tissue culture with varying Fe concentrations (0%, 10%, 50%, and 100%). The concentrations are presented in mg kg^{-1} except P which is in %.

Genotype	Fe treatment (%)	Dry weight (mg $plant^{-1}$)	Concentration						
			Fe	P	S	Cu	Mn	Zn	B
2 BRA 1A	0	1.4	60	0.28	0.26	6.1	314	253	56.3
	10	2.3	121	0.28	0.31	5.7	249	199	71.9
	50	1.1	151	0.27	0.19	5.2	236	158	45.9
	100	2.7	321	0.29	0.23	4.9	195	135	45.9
BH 2	0	2.9	37	0.45	0.27	2.0	130	230	42.0
	10	2.7	64	0.42	0.32	2.0	118	182	38.8
	50	3.1	140	0.38	0.28	1.6	110	138	34.4
	100	2.9	271	0.37	0.26	1.3	112	138	36.5
LH 30-4	0	0.9	119	0.63	0.36	6.1	129	411	50.5
	10	1.0	157	0.59	0.37	6.3	116	334	44.3
	50	1.0	314	0.50	0.35	4.4	101	260	39.1
	100	0.8	574	0.53	0.35	5.9	111	251	43.7
Darrow 72	0	3.1	21	0.37	0.27	1.8	134	182	36.6
	10	2.7	57	0.32	0.16	3.1	146	172	41.7
	50	2.6	132	0.31	0.25	2.8	124	136	36.5
	100	3.0	192	0.32	0.22	2.4	132	123	39.8
HM 1	0	4.0	26	0.28	0.20	1.9	147	136	46.6
	10	4.1	58	0.33	0.21	2.3	152	141	49.3
	50	4.1	119	0.29	0.19	2.1	153	123	50.0
	100	3.9	160	0.27	0.19	2.7	157	115	51.9
Cascade	0	2.1	32	0.37	0.20	4.3	118	306	48.9
	10	2.3	64	0.35	0.20	3.1	123	246	45.0
	50	2.7	161	0.31	0.18	2.4	100	150	41.3
	100	2.7	266	0.31	0.22	2.6	112	144	40.6
Scotts Creek	0	3.3	69	0.32	0.23	2.0	120	183	36.9
	10	3.7	68	0.33	0.24	1.2	135	136	42.8
	50	3.6	143	0.34	0.22	1.2	127	128	41.0
	100	3.4	215	0.32	0.25	1.5	135	124	43.3
2 TAP 4B	0	2.8	24	0.32	0.25	1.6	159	210	42.5
	10	3.0	41	0.33	0.26	1.2	128	154	38.4
	50	3.8	109	0.30	0.25	1.3	131	129	39.3
	100	3.3	171	0.29	0.27	1.2	137	117	38.7

Table 5. (Continued).

Genotype	Fe treatment (%)	Dry weight (mg plant^{-1})	Concentration						
			Fe	P	S	Cu	Mn	Zn	B
RH 43	0	1.5	56	0.36	0.33	2.8	147	231	46.6
	10	1.6	78	0.31	0.28	2.2	135	176	44.2
	50	1.5	165	0.30	0.28	1.8	131	144	41.4
	100	1.6	495	0.35	0.31	2.2	138	167	46.2
Pigeon Point	0	2.8	40	0.27	0.21	3.5	144	111	44.9
	10	2.5	50	0.26	0.24	3.3	127	105	40.6
	50	3.0	116	0.22	0.19	3.4	143	97	43.0
	100	2.6	198	0.26	0.20	3.6	140	101	41.9
BT 3	0	2.7	330	0.38	0.31	4.4	132	152	41.7
	10	2.6	210	0.37	0.32	3.6	157	159	41.1
	50	2.4	65	0.34	0.34	4.2	157	165	41.4
	100	2.5	38	0.43	0.33	3.2	135	201	37.9
Auke Lake	0	2.7	26	0.40	0.30	3.9	147	228	43.6
	10	2.7	58	0.35	0.27	2.4	166	161	42.0
	50	2.8	140	0.35	0.29	1.8	125	147	34.9
	100	2.6	266	0.34	0.27	4.1	154	148	41.0
BSP 14	0	2.6	56	0.30	0.25	2.6	139	139	42.5
	10	2.5	123	0.34	0.23	5.2	121	189	41.6
	50	2.7	255	0.36	0.25	4.7	117	181	38.8
	100	2.0	366	0.32	0.22	6.7	126	153	44.4
Eagle 14	0	1.4	48	0.34	0.32	6.8	192	221	47.3
	10	1.5	83	0.30	0.32	5.8	179	169	42.9
	50	1.2	265	0.29	0.32	8.5	211	154	46.6
	100	1.3	471	0.32	0.36	6.4	175	147	42.7
RCP 37	0	1.3	60	0.37	0.24	10.0	147	223	45.3
	10	1.8	116	0.39	0.25	13.1	118	218	40.6
	50	1.2	293	0.36	0.23	8.7	114	173	39.2
	100	1.2	316	0.43	0.27	10.2	149	211	47.3
JP 95-1	0	2.9	44	0.37	0.32	2.1	180	210	45.3
	10	2.5	66	0.37	0.31	2.7	178	174	48.4
	50	3.2	115	0.33	0.31	1.8	139	130	38.0
	100	2.7	307	0.29	0.28	2.4	171	126	39.6

concentrations. Determining how these genotypes cope with low levels of Fe will be of great importance and may lead to a better understanding of internal Fe usage efficiency. The special issues that could be addressed include Fe compartmentation at a cell level, the level of active Fe in the metabolic pool during low Fe conditions, and the presence of other mechanisms responsible for Fe efficiency.

The results of the experiment indicated that *F. chiloensis* and *F. virginiana* genotypes exhibited considerable variation in response to different Fe concentrations during micropropagation, and this variation can likely be utilized to develop new strawberry cultivars with tolerance to low Fe concentrations. In vitro Fe deficiency tests could be used as an early diagnostic method to screen strawberry cultivars for response to Fe or to identify Fe-tolerant strawberry genotypes for genetic improvement.

The results obtained in this study open new windows on the selection and use of strawberry genotypes tolerant to conditions in which Fe chlorosis could become a problem. However, it is desirable to confirm the behavior of the most tolerant genotypes under field and/or greenhouse conditions.

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