Journal of Plant Nutrition
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597277

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Online publication date: 07 December 2009

To cite this Article Neaman, Alexander and Espinoza, Gonzalo(2010) 'ADVANCES IN DIAGNOSIS OF IRON DEFICIENCY IN AVOCADO', Journal of Plant Nutrition, 33: 1, 38 — 45
To link to this Article: DOI: 10.1080/01904160903391065
URL: http://dx.doi.org/10.1080/01904160903391065

Journal of Plant Nutrition, 33:38–45, 2010

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904160903391065

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ADVANCES IN DIAGNOSIS OF IRON DEFICIENCY IN AVOCADO

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□ *Several methods for determination of extractable iron (Fe; or so-called "active Fe") have been proposed. In this study, three methods of Fe extraction were tested: 1.5% phenanthroline (pH 3) and 1 M hydrochloric acid (HCl) from fresh leaves, and 1 M HCl from oven-dry leaves. A six-year-old avocado orchard (cultivar 'Hass'), grown on a calcareous soil in the proximity of Cabildo (Valparaíso region, Chile), was selected for the study. Samples of mature (5–7 moths-old) leaves of different degree of chlorosis were collected. Total Fe concentrations in chlorotic leaves were similar or even greater than in green leaves. Regressions between the extractable Fe concentrations and the leaf SPAD-color were statistically significant for phenanthroline method, while non-significant for HCl methods. Thus, phenanthroline method was superior over others for diagnosis of Fe deficiency in avocado. Phenanthroline-extractable Fe concentration of 6 mg kg^{-1} was considered as a critical value for mature avocado leaves.*

Keywords: phenanthroline, Chile, iron chlorosis, calcareous soils, active iron, extractable iron

INTRODUCTION

Iron (Fe) chlorosis in crops grown on calcareous soils is a widespread problem in arid and semi-arid regions (Rashid and Ryan, 2004). It is well known that total leaf Fe does not often correlate well with Fe deficiency symptoms in plants (Katyal and Sharma, 1980; Nikolic and Kastori, 2000; Rao et al., 1987; Römheld, 2000; Zohlen and Tyler, 1997). This discrepancy is related to the localization and binding state of Fe in leaves: a proportion of Fe might be precipitated in the apoplasm of leaves and might not be physiologically available (Mengel and Geurtzen, 1988; Römheld, 2000). According to Marschner (2003), a fraction of Fe that undergoes reversible

Received 11 April 2008; accepted 20 December 2008.
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ferrous/ferric Fe oxidoreduction is of particular physiological importance. However, several other authors suggested that the fraction of Fe associated with chlorophyll synthesis is ferrous Fe (Katyal and Sharma, 1980; Lang and Reed, 1987).

Several techniques for extraction of so-called "active Fe" have been proposed and tested. The most common extractants are, perhaps, 1.5% phenanthroline (pH 3) and/or 1 M hydrochloric acid (HCl) solutions used for analysis of fresh or dry leaves (Basar, 2003; Katyal and Sharma, 1980; Köseoglu and Açikgöz, 1995; Lang and Reed, 1987; Mohammad et al., 1998; Rashid et al., 1997; Sönmez and Kaplan, 2004). However, there is no general agreement on which method is more suitable for diagnosis of Fe deficiency in crops and the exact composition of the operationally-defined "active Fe" is not yet sufficiently clear (Abadía, 1992; Nikolic and Kastori, 2000).

Despite of the fact that Fe chlorosis is a widespread problem in avocado trees grown on calcareous soils (Bar and Kafkafi, 1992; Decianzio, 1991), very little is known on effectiveness of different methods in diagnosis of Fe deficiency in avocado. Recently, Neaman and Aguirre (2007) compared three extraction methods from young (two months-old) avocado leaves of different degree of chlorosis: 1.5% phenanthroline (pH 3) and 1 M HCl extractions from fresh leaves, and 1 M HCl extraction from oven-dry leaves. The authors concluded that phenanthroline extraction was superior over other methods for diagnosis of Fe deficiency in young avocado leaves.

The choice of sampling of young leaves in the study of Neaman and Aguirre (2007) was due to clear visual symptoms of Fe deficiency in these leaves, while, in mature leaves, some apparent symptoms of magnesium deficiency made impossible the accurate visual diagnosis of Fe chlorosis. However, mature (five-seven months-old) leaves are commonly used for leaf analysis in avocado (Embleton and Jones, 1966; Lahav and Whiley, 2002). To this end, in the present study, we compared different methods for diagnosis of Fe deficiency in mature avocado leaves.

MATERIALS AND METHODS

A six-year-old avocado orchard (cultivar 'Hass') located in Cabildo (Valparaiso region, Chile) was selected for the study. Soil on the study site was calcareous, with pH in the range of 8.0–8.3. Trees with different degree of chlorosis were selected for the study. Samples of expanded mature (five-seven months-old) leaves from non-fruiting and non-flushing terminals of the spring shoot growth were collected during April, 2006. This type of sampling is common for leaf analysis in avocado (Rodríguez, 1992). Each sample was composed of about 20 leaves from the same tree. The total number of trees sampled was 46.

Leaf color was determined by a Minolta chlorophyll meter SPAD-502. Two measurements were performed for each leaf, giving about 40 total measurements. Variations in the measurement did not exceed, in general,

15% from the average value (Table 1). In the further discussion, the leaf color is expressed in SPAD units. The SPAD color, in turn, is known to correlate linearly and positively with the concentration of leaf chlorophyll (Pestana et al., 2004; Porro et al., 2001).

The leaf samples were stored overnight at 4°C prior to analysis. Then, leaves were washed in tap water, rinsed in deionized water, dried with paper towels, and ground in a Moulinex food processor for 30 seconds. A portion of each sample was oven dried at 70°C for 48 hours and then ground. Then, humidity factor of fresh leaves was determined.

The leaf concentrations of Fe, manganese (Mn), and zinc (Zn) were determined by atomic absorption spectrometry following the standard method of dry-ashing according to Sadzawka et al. (2007). These three elements were chosen because their deficiencies are very common in crops grown on calcareous soils (Marschner, 2003; Rashid and Ryan, 2004). In the further discussion, Fe determined by dry-ashing will be referred to as "total Fe", in contrast to "extractable Fe" determined by the following methods.

Solutions of 1.5% phenanthroline (pH 3) and 1 M HCl were used for extraction of Fe from fresh leaves and solution of 1 M HCl was used for extraction of Fe from oven-dry leaves. One gram of fresh or oven-dry leaves was places in a 250 mL Erlenmeyer flask and 10 mL of corresponding extracting solution were added. In the case of fresh leaves, the corresponding extracting solution was added immediately after grinding and weighing, in order to prevent possible oxidation of ferrous Fe. Then, the samples were left soaking overnight (for about 16 hours). Preliminary experiments demonstrated that increasing extraction time from 16 to 48 hours did not result in a considerable increase in extractable Fe. The extraction overnight (for about 16 hours) was chosen because it is convenient for running a routine laboratory analysis. At the end of the extraction, the samples were filtered through a Whatman filter paper and Fe concentrations in the filtrates were determined by atomic absorption spectrometry. The choice of atomic absorption spectrometry was due to the fact that pigments extracted by phenanthroline and HCl are known to interfere with colorimetric determinations (Takkar and Kaur, 1984).

All samples were analyzed in duplicate. The deviations in the values obtained from the duplicate runs were less than 10%. The average values are presented in the Table 1. All results are expressed on oven-dry basis.

RESULTS AND DISCUSSION

Manganese leaf concentrations were in the range of 50–250 mg kg^{-1} (Table 1). These Mn concentrations were considered as sufficient (Lahav and Whiley, 2002). In contrast, Zn leaf concentrations were in the range of 5–15 mg kg^{-1} (Table 1). Standards of avocado leaf analysis for Zn (Lahav

TABLE 1 Leaf color, concentrations of extractable Fe (by three methods) and concentrations of total Fe, Zn, Mn, and Mg in the leaves.

	Color, SPAD units			Extractable Fe, mg kg^{-1}			Total		
Sample	Average	STD	CV, %	1 M HCl, fresh leaves	1 M HCl, dry leaves	Phenanthroline, fresh leaves	Fe, mg kg^{-1}	Zn, mg kg^{-1}	Mn, mg kg^{-1}
A 17	53	2.8	5.2	31	22	10	51	7	223
A 36	46	3.3	7.1	30	16	6	44	5	145
A 45	48	4.6	9.7	30	17	8	47	7	160
B 07	52	2.9	5.6	22	16	9	50	14	188
B 20	55	2.6	4.6	23	20	13	48	10	159
B 32	48	2.4	5.1	25	17	6	55	11	120
C 20	52	3.0	5.8	36	16	8	44	7	178
C 21	56	3.0	5.3	33	26	8	60	8	234
C 37	20	3.2	16	23	17	3	57	6	108
C 42	41	4.0	9.6	24	13	5	42	7	131
D 08	54	4.0	7.5	20	18	8	38	6	92
D 11	48	3.7	7.7	27	19	8	49	6	100
D 18	53	3.3	6.3	24	15	6	44	7	161
D 36	49	1.9	3.9	31	19	6	47	8	211
D 43	53	3.9	7.5	25	15	10	50	13	180
D 44	48	2.7	5.7	23	16	11	45	10	258
D 45	36	5.9	16	27	17	5	45	9	170
D 46	51	4.3	8.6	27	20	6	42	6	151
D 48	48	3.3	6.8	22	19	6	35	6	45
E 17	29	3.8	13	16	16	2	43	5	111
H 18	29	3.9	14	21	13	2	47	5	201
C 17	27	2.5	9.2	15	14	3	49	9	227
C 21	54	2.1	3.9	19	14	4	37	8	199
D 30	32	4.0	13	18	12	4	35	9	115
D 42	21	2.2	10	33	14	4	71	14	175
D 43	50	2.0	4.0	16	12	5	33	9	72
E 11	46	2.4	5.3	19	13	6	53	6	75
E 17	19	1.6	8.3	18	10	3	38	8	80
E 20	24	3.3	14	29	10	4	42	7	83
E 30	35	2.1	6.0	23	11	5	60	9	127
E 33	35	3.5	9.9	27	13	4	68	10	180
E 34	21	2.1	10	25	11	3	57	10	157
E 38	52	2.5	4.7	21	13	5	54	10	143
F 09	48	1.6	3.3	19	14	4	54	8	113
F 10	52	2.4	4.6	22	14	4	47	8	103
F 11	45	2.4	5.4	19	15	5	49	8	98
F 15	52	4.9	9.4	24	16	5	59	9	165
F 27	51	1.8	3.6	20	15	6	55	9	128
G 23	50	2.6	5.3	20	13	6	67	8	202
G 41	54	2.5	4.6	23	18	13	56	8	101
G 44	25	3.4	14	23	13	4	63	6	72
G 46	49	6.0	12	28	14	5	42	8	111
G 47	45	2.4	5.3	20	13	7	50	6	99
G 48	51	2.6	5.1	30	13	5	45	7	142
H 07	53	2.9	5.4	17	15	5	40	7	165
H 21	55	2.6	4.7	51	22	10	63	9	168

STD = standard deviation and CV = coefficient of variation = STD * 100 / average. All analyses are expressed on oven-dry basis.

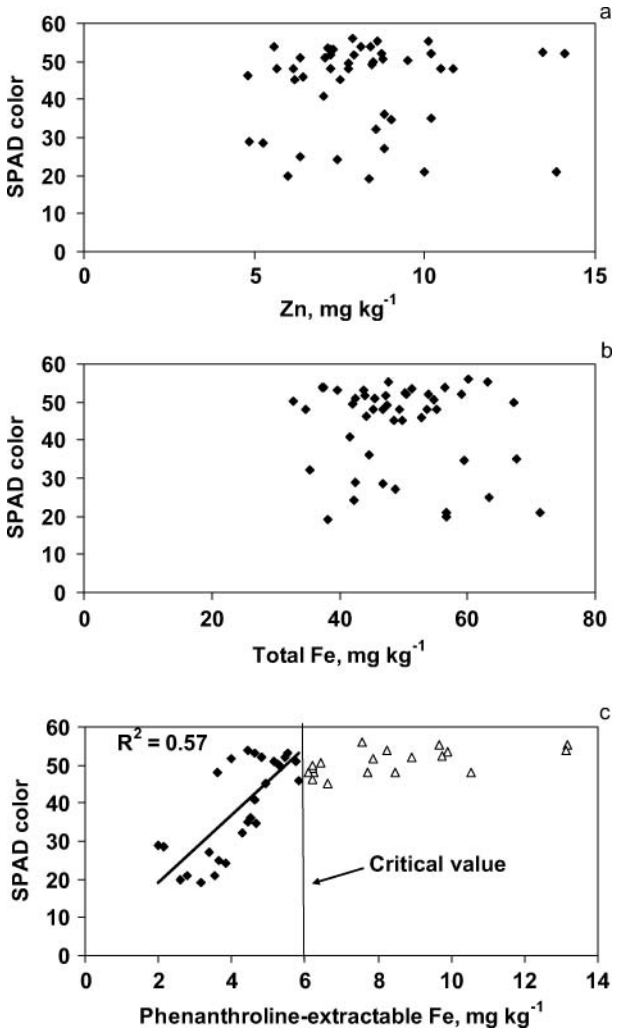

FIGURE 1 The relationship between leaf color and (a) Zn, (b) total Fe, and (c) phenanthroline-extractable Fe. Green and chlorotic leaves corresponded to SPAD color in the range of 45–60 and 20–30, respectively.

and Whiley, 2002) suggest that the range less than 10–20 mg kg^{-1} is deficient, while the range of 40–80 mg kg^{-1} is sufficient. However, we note that Zn was deficient (less than 10 mg kg^{-1}) in both green and chlorotic leaves (Figure 1a, Table 1) and regression between the leaf color and Zn concentrations was not significant (Table 2). This suggests that these deficient Zn concentrations (less than 10 mg kg^{-1}) did not produce any leaf color change.

Total Fe concentrations in chlorotic leaves were similar or even greater than in green leaves (Figure 1b, Table 1). Regression between the leaf color and total Fe concentrations was not significant (Table 2). Similarly, this

TABLE 2 Correlation coefficients between leaf color and leaf element concentrations determined by different methods. ns = not significant at 0.05 probability level

Element, method	R^2	Probability
Fe, phenanthroline (all samples)	0.44	0.001
Fe, phenanthroline (range of Fe < 6 mg kg^{-1})	0.57	0.001
Fe, 1 N HCl, fresh leaves	0.03	ns
Fe, 1 N HCl, oven-dry leaves	0.03	ns
Fe, total	0.02	ns
Zn, total	< 0.01	ns
Mn, total	0.04	ns

regression was not significant in the case of HCl-extraction methods for both fresh and oven-dry leaves. In contrast, regression between the leaf color and phenanthroline-extractable Fe was significant (Table 2). Values of SPAD color increased with phenanthroline-extractable Fe in the range of Fe < 6 mg kg^{-1} (Figure 1c). This suggests that Fe deficiency was the major nutritional constrain that determined the leaf color in the studied avocado orchard.

Leaf color was independent of phenanthroline-extractable Fe in the range greater than 6 mg kg^{-1} (Figure 1c). The regression coefficient (R^2) between the leaf color and phenanthroline-extractable Fe was 0.57 when the range of Fe up till 6 mg kg^{-1} was considered. Although this regression coefficient was rather small, it was highly significant ($P < 0.001$), while regressions were not significant in the case of HCl-extractable and total Fe (Table 2). This suggests that phenanthroline-extractable Fe was better parameter for diagnosis of Fe deficiency in comparison to HCl-extractable and total Fe. This observation is consistent with previous reports that total leaf Fe does not correlate well with Fe deficiency symptoms in plants and is not a valid index for Fe nutritional status of plants (Katyal and Sharma, 1980; Nikolic and Kastori, 2000; Rao et al., 1987; Römheld, 2000; Zohlen and Tyler, 1997).

The results of the present study suggest that the phenanthroline extraction is superior to the HCl extraction. Hydrochloric acid is expected to extract both ferrous and ferric Fe from the leaves and thus cannot be considered as a specific extractant for ferrous Fe. As the stability constant of Fe^{2+}-phenanthroline complex is considerably greater than that of Fe^{3+}-phenanthroline complex, Katyal and Sharma (1980) considered phenanthroline as Fe^{2+} specific extractant. The finding of the present study therefore support the hypothesis that the fraction of Fe associated with chlorophyll synthesis is ferrous Fe (Katyal and Sharma, 1980; Lang and Reed, 1987).

The phenanthroline-extractable Fe concentration of 6 mg kg^{-1} may be tentatively used as a critical value for "active Fe" in mature (five-seven

months-old) avocado leaves. However, further studies should be carried out to confirm this critical value.

CONCLUSION

Total Fe and HCl-extractable Fe were not suitable for diagnosis of Fe deficiency in mature (five-seven months-old) avocado leaves. Phenanthroline extraction applied on fresh leaves was superior over other methods for diagnosis of Fe deficiency in avocado. Phenanthroline-extractable Fe concentration of 6 mg kg^{-1} was considered as a critical value for mature avocado leaves.

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