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Plant nutrition status, yield and quality of satsuma mandarin (*Citrus unshiu* Marc.) under soil application of Fe-EDDHA and combination with zinc and manganese in calcareous soil

Ying Zhang, Cheng-Xiao Hu*, Qi-Ling Tan, Cang-Song Zheng, Hui-Ping Gui, Wei-Nan Zeng, Xue-Cheng Sun, Xiao-Hu Zhao

College of Resources and Environment/Micro-element Research Center, Huazhong Agricultural University, Wuhan, Hubei Province 430070, PR China

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ABSTRACT

The purpose of this study was to investigate citrus tissue's response to varying applications of Fe-EDDHA, zinc (Zn) and manganese (Mn) combinations and the resulting effects on citrus fruit yield and quality in calcareous soil. A field experiment was conducted for three years with satsuma mandarin trees planted in calcareous soil. Four treatments were conducted: (i) control; (ii) soil Fe application; (iii) soil Fe + Zn application; and (iv) soil Fe + Zn + Mn application. Soil available Fe and Zn concentrations were raised through the application of Fe-EDDHA and Zn, whereas the soil available Mn concentration was not increased through the application of Mn. Applying Fe-EDDHA raised the leaf active Fe content significantly without influencing the leaf and fruit Fe concentrations. The Zn concentrations in leaf and fruit decreased when Fe-EDDHA was applied exclusively, but increased when Fe + Zn and Fe + Zn + Mn were applied in the third year. There was no significant difference in leaf and fruit Mn concentrations among all treatments between 2010 and 2012. Soil Fe, Fe + Zn and Fe + Zn + Mn application increased fruit number by 23.5%, 26.7% and 24.9%, respectively, and soil Fe + Zn application increased the yield in 2012. Fruit TSS, TA and Vc content were higher through the application of Fe + Zn and Fe + Zn + Mn in 2012. The results revealed that Fe and Zn play different roles that affect fruit yield and quality in calcareous soil. The citrus yield was mainly affected by Fe, whereas fruit quality was improved by Zn. The application of Fe-EDDHA alone could induce Zn deficiency, so the co-application of Fe-EDDHA and Zn fertilizer was useful for obtaining higher yield and quality in satsuma mandarin in calcareous soil.

1. Introduction

Iron (Fe) deficiency is frequently found in citrus growing on calcareous soils in semiarid climates, as the high pH and HCO_3^- not only reduce soil available Fe concentrations, but also affect the mobility of Fe in citrus trees' roots, raise intracellular pH and decrease the physiological activity of Fe (Mengel, 1994). High soil pH can also reduce the availability of the micronutrients manganese (Mn) and zinc (Zn) in soil (El-Fouly et al., 2001).

Fe, Zn and Mn are essential microelements of higher plants (Marschner, 2012). Fe catalyzes the production of chlorophyll and is involved in some respiratory and photosynthetic enzyme systems. Fe deficiency inhibits vegetative growth and the formation of flower buds, affecting the number of buds and fruit sets in citrus on calcareous soils (Zekri and Obreza, 2003). Zn plays an important role in starch metabolism, acts as a cofactor for many enzymes and affects photosynthesis, nucleic acid metabolism and protein biosynthesis (Alloway, 2004). Zn deficiency can inhibit the growth of fruit trees by impeding photosynthesis, carbon metabolism and respiration, which reduces the yield and quality of fruit (Srivastava and Singh, 2005, 2009). Mn is an activator of several enzymes and plays roles in photosynthesis and in the formation of chlorophyll (Zekri and Obreza, 2009).

The chelate Fe-ethylenediamine-di(o-hydroxy-phenylacetic acid) (Fe-EDDHA) has been recognized as one of the most effective Fe sources for correcting Fe deficiency in calcareous soils (Mortvedt, 1991). The soil application or foliar spraying of

* Corresponding author at: Huazhong Agricultural University, No. 1, Shizishan Street, Hongshan District, Wuhan, Hubei Province 430070, PR China. Tel.: +86 27 87282043; fax: +86 27 87395111.

E-mail addresses: yingzh@webmail.hzau.edu.cn (Y. Zhang), hucx@mail.hzau.edu.cn (C.-X. Hu), qitan@mail.hzau.edu.cn (Q.-L. Tan), zhengcangsong@163.com (C.-S. Zheng), huiping.828@163.com (H.-P. Gui), zwnzpp@163.com (W.-N. Zeng), sxccn@mail.hzau.edu.cn (X.-C. Sun), xhzhao@mail.hzau.edu.cn (X.-H. Zhao).

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Fe-EDDHA resulted in a rapid increase in leaf chlorophyll and, in the same season that the treatment took place, both fruit size and tree yield increased observably in kiwifruit (Loupassaki et al., 1997) and strawberry (Zaiter et al., 1993). Moreover, some studies have reported that Zn application benefitted vegetative growth, yield and fruit quality in alkaline soils (Swietlik, 2001; Núñez-Moreno et al., 2009; Srivastava and Singh, 2009). Savvas et al. (2009) proved that tomatoes planted in a nutrient solution with standard or high Mn content exhibited better vegetative growth and fruit yield than those planted in a low Mn nutrient solution. However, most of the research has focused on the lack of a single element to carry on the fertilization treatments. Given that there is a synergistic effect or antagonism for microelement absorption in citrus trees, a large number of individual elements may affect the absorption of other elements. Therefore, single microelement fertilizer applications cannot comprehensively improve crops' nutrient status in calcareous soil.

China's Hubei Province, in the Yiling district of Yichang, is a predominant region for *Citrus reticulata* production. A large area of satsuma orange orchards comprises calcareous soil with low Fe, Zn and Mn availability. Thus, the aim of this study was to determine the effects of various treatments on satsuma mandarin grown in calcareous soil with low Fe, Zn and Mn availability over a three-year period. Fe-EDDHA was applied alone, with Zn and also with Zn and Mn to examine the effects on Fe, Zn and Mn absorption, fruit yield and quality.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was conducted from 2010 to 2012 in a commercial orchard in the Yiling district of Yichang city, Hubei Province, China (111°26′ N, 30°46′ E; 149 m above sea level). The citrus cultivars were 34-year-old satsuma mandarin trees (*Citrus unshiu* Marc.) grafted onto trifoliate orange [*Poncirus trifoliata* (L.) *Raf.*], which were planted in a calcareous soil (calcareous purple soil). Some of the physicochemical properties of the soil from the surface horizon (0–20 cm) are given in Table 1. The citrus trees were planted with a spacing of 4 × 2.5 m and trained as small hemisphere canopies. Each year, N, P and K fertilizers were applied as urea (900 kg ha^{-1}), monoammonium phosphate (450 kg ha^{-1}) and potassium sulfate (750 kg ha^{-1}), respectively, which were applied two times at the spring tip germination (with proportion: 60%, 60% and 40%, respectively) and the rapid fruit growth period (with proportion: 40%, 40% and 60%, respectively) using the furrow application method. All of the necessary cultural practices and plant protection measures were followed uniformly for all treatments during the entire period of experimentation.

Table 1
Some physicochemical properties of the soil.

pH	7.64
$CaCO_3$ (%)	14.2
Organic matter (g/kg)	19.71
Avail-N (mg kg^{-1})	84.19
Avail-P (mg kg^{-1})	39.89
Avail-K (mg kg^{-1})	212.66
Avail-Ca (mg kg^{-1})	2777.3
Avail-Mg (mg kg^{-1})	271.4
Avail-Fe (mg kg^{-1})	6.63
Avail-Mn (mg kg^{-1})	3.43
Avail-Cu (mg kg^{-1})	0.90
Avail-Zn (mg kg^{-1})	0.92

2.2. Experimental design and sampling

The experiment comprised four treatments replicated four times, with four mandarin trees of uniform growth chosen each time. The Fe, Zn and Mn in the forms of Fe(III)-*o*,*o*-EDDHA (Fe(III)-EDDHA, Fe = 6%), zinc sulfate ($ZnSO_4·7H_2O$) and manganese sulfate ($MnSO_4$) were applied at the spring tip germination period in the following treatments: (i) control; (ii) soil Fe application (60 g Fe-EDDHA per tree); (iii) soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4·7H_2O$ per tree); and (iv) soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4·7H_2O$ per tree + 30 g $MnSO_4$). The specific applying method is: dissolve each kind of microelement fertilizer separately, and use the root-irrigation method to irrigate soil circles surrounding orange trees, sequentially, clean barriers on the top soil before applying in order to make the fertilizers rapidly permeate the soil. The soil was fertilized with Fe, Zn and Mn annually at the spring tip germination period throughout the 2010–2012 study period, and the soil samples were collected annually from the surface horizon (0–20 cm) before Fe, Zn and Mn application. Leaf samples located at the second or third position from the mature branch tip were sampled, respectively, at six growth periods: spring tip germination (S1), full bloom (S2), young fruit (S3), fruit development (S4), the beginning of veraison (S5) and mature fruit (S6). Fruit samples were collected at the S3, S4, S5 and S6 periods.

2.3. Measurements and observations

Soil samples were air-dried, triturated with a wooden roller to pass through a 1 mm sieve and stored at room temperature after homogenization. The experimental parameters were measured according to the soil physicochemical analysis handbook (Bao, 2000). Soil organic matter was measured using the chromic acid and titration procedure. Soil pH was determined potentiometrically in 1:2.5 soil/distilled water suspensions after shaking. The alkaline hydrolysis diffusion method was used to determine the soil-available N. Available P was determined using Olsen's method. Available K was extracted with 1 mol L^{-1} NH_4OAC and determined using a flame photometer (FP6410, INESA, China). Exchangeable Ca and Mg were extracted with 1 mol L^{-1} CH_3COONH_4 and available Fe, Mn, Cu and Zn were extracted with a DTPA extracting agent (5 mmol L^{-1} DTPA, 0.01 mol L^{-1} $CaCl_2$ and 0.1 mol L^{-1} TEA). Exchangeable Ca, Mg and available Fe, Mn, Cu and Zn were determined using an atomic absorption spectrophotometer (Z 2000, HITACHI, Japan). Soil B was extracted using hot water and the concentration of B in the solution was determined using the colorimetric curcumin method (Bao, 2000).

Freshly collected leaves and fruits were washed in consecutive volumes of deionised water. Peel and pulp were carefully excised from the fruits and all the two fruit parts were mixed respectively as peel and pulp samples. Leaves and fruit sections were inactivated by heating at 105 °C for 40 min, then dried at 70 °C in a forced air oven until they maintained a constant weight. Leaf and fruit samples were digested with $HNO_3/HClO_4$ (v/v, 4:1) and an atomic absorption spectrophotometer (Z 2000, HITACHI, Japan) was used to measure Fe, Zn and Mn concentrations. The Fe, Zn and Mn concentrations in leaves and fruits were expressed in relation to dry weight (DW). Leaf active Fe was extracted with 1 mol L^{-1} HCl (Pierson and Clark, 1984) and determined using an atomic absorption spectrophotometer (Z 2000, HITACHI, Japan). Leaf active Fe was expressed in relation to fresh weight (FW).

In the S6 period, yield per tree was recorded by weighing and counting the total number of fruits per tree, and the mean fruit weight was calculated by weighing fruits on a digital electronic balance. The fruit quality parameters were assessed as total soluble solids (TSS), titratable acidity (TA), TSS:TA and ascorbic acid (Vc) content. TSS was measured from the fruit homogenate juice using an Abbe refractometer in the juice squeezed from the fruit homogenate. The TA of the juice was determined by titrating against 0.1 mol L^{-1} sodium hydroxide (NaOH) and expressed as percent citric acid (%). The TSS:TA ratio was calculated for each sample by dividing the TSS by the corresponding TA value. The Vc content of the juice was determined following the method described by Ruck (1963), and expressed as mg per 100 g.

2.4. Statistical analysis

Unless otherwise noted, the results are given as means ± standard error (SE). Data were analyzed using analysis of variance (ANOVA) and the differences between means determined by the least significant difference (LSD) test at $p < 0.05$. The SPSS PASW Statistics 18.0 analytical software package was used for all statistical analyses.

3. Results

3.1. Soil available Fe, Zn and Mn concentrations of the citrus orchard

The experimental citrus orchard soil pH was 7.64, slightly alkaline. The soil available Fe, Zn and Mn concentrations in the first year were generally low (6.6, 0.9 and 3.4 mg kg^{-1}, respectively) (Table 1). The soil-available Fe and Zn concentrations were all raised significantly through the application of Fe-EDDHA and Zn in 2011 and 2012, respectively, but there was no significant difference in the soil Mn concentration among all treatments from 2010 to 2012 (Table 2).

3.2. Fe, active Fe, Zn and Mn concentrations in citrus leaves and fruit

3.2.1. Fe, active Fe, Zn and Mn concentrations in citrus leaves

The seasonal dynamics of leaf Fe concentration exhibited no difference among treatments. Leaf Fe concentration was the highest at the full bloom period. Leaf active Fe concentration decreased during the young fruit period and thereafter increased notably, reaching a peak at mature fruit period (Fig. 1). There were no significant difference in leaf Fe concentration among all treatments between 2010 and 2012, but the leaf active Fe concentration in 2011 and 2012 was increased by applying Fe-EDDHA (Fig. 1).

The seasonal dynamics of leaf Zn concentration showed that initially increased and then decreased during the young fruit period, then thereafter increased, reaching a peak at the beginning of the veraison period in 2010 and at the fruit-development period in 2011 and 2012, at which point it decreased (Fig. 2a). In 2011 and 2012, the leaf Zn concentration was lower in the Fe alone treatment than that in the other treatments. In 2012, the leaf Zn concentration was higher in the Fe + Zn and Fe + Zn + Mn treatments than that in the control and Fe alone treatments (Fig. 2a). The leaf Mn concentration did not vary in the early stages, but increased notably at the beginning of the veraison and fruit mature stage. There were no significant differences in leaf Mn concentrations among all of the treatments between 2010 and 2012 (Fig. 2b).

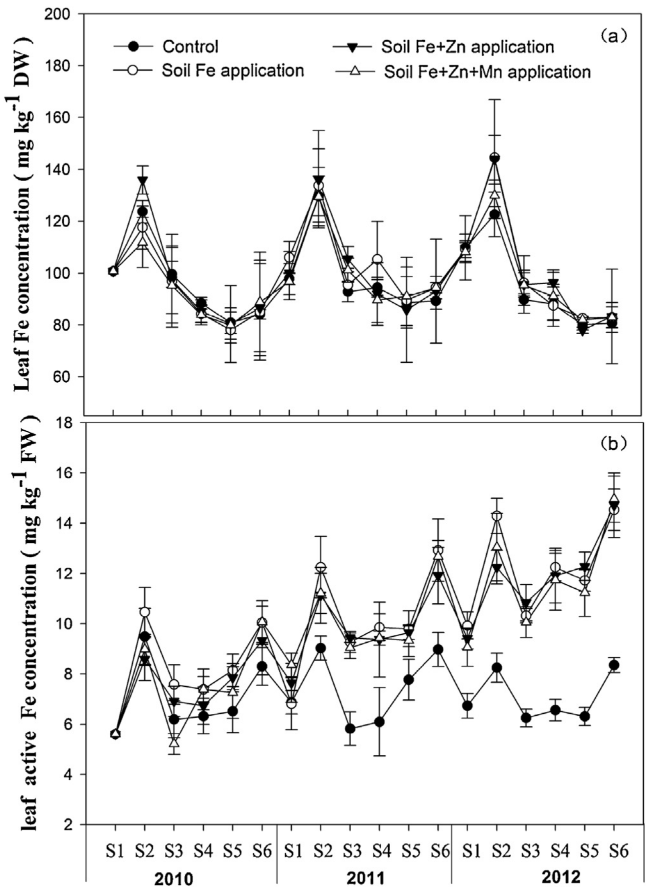

Fig. 1. Dynamics of leaf Fe (a) and active Fe (b) concentrations in satsuma mandarin trees grafted on trifoliate orange with different treatments. Control; soil Fe application (60 g Fe-EDDHA per tree); soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4·7H_2O$ per tree); soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4·7H_2O$ per tree + 30 g $MnSO_4$). Growth periods: spring tip germination (S1), full bloom (S2), young fruit (S3), fruit development (S4), beginning of veraison (S5) and mature fruit (S6). Values represent means ± SE ($n = 4$).

3.2.2. Fe, Zn and Mn concentrations in citrus fruit

For all of the treatments, the Fe and Mn concentrations in fruit peels initially increased and then decreased throughout the fruit growth periods. However, the Zn concentration in fruit peel initially increased, reaching a peak at the beginning of the veraison period, at which point it decreased. Seasonal changes of the Fe, Zn and Mn concentrations in fruit pulp presented similar decreasing trends.

Table 2
Effect of Fe-EDDHA, Zn and Mn applications on soil available Fe, Zn and Mn concentration in citrus orchard soil.

Treatment[a]	Soil Fe concentration (mg kg^{-1})		Soil Zn concentration (mg kg^{-1})		Soil Mn concentration (mg kg^{-1})	
	2011	2012	2011	2012	2011	2012
Control	6.90 ± 0.49 c	6.28 ± 0.74 b	0.99 ± 0.09 b	0.91 ± 0.11 b	3.56 ± 0.36 a	3.90 ± 1.00 a
Soil Fe application	9.55 ± 0.74 b	16.04 ± 1.41 a	1.01 ± 0.12 b	0.93 ± 0.12 b	3.45 ± 0.49 a	3.31 ± 0.26 a
Soil Fe + Zn application	12.70 ± 0.62 a	15.28 ± 0.86 a	1.71 ± 0.10 a	1.80 ± 0.06 a	3.81 ± 0.43 a	3.78 ± 0.47 a
Soil Fe + Zn + Mn application	11.67 ± 1.14 ab	15.40 ± 1.25 a	1.65 ± 0.07 a	1.67 ± 0.11 a	3.64 ± 0.48 a	4.13 ± 0.58 a

Values are means of four replicates. Values represent the means ± SE ($n = 4$). Within a column, the different letter means significant difference ($p < 0.05$).
[a] Control; soil Fe application (60 g Fe-EDDHA per tree); soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4·7H_2O$ per tree); Soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4·7H_2O$ per tree + 30 g $MnSO_4$).

Table 3
Effect of application of Fe-EDDHA, Zn and Mn on number of fruit and mean fruit weight of citrus.

Treatment[a]	Number of fruit per tree			Mean fruit weight (g)		
	2010	2011	2012	2010	2011	2012
Control	230.3 ± 40.7 a	99.5 ± 6.5 a	204.2 ± 17.2 b	142.7 ± 2.3 a	148.8 ± 7.8 a	142.9 ± 6.8 a
Soil Fe application	218.1 ± 15.3 a	124.8 ± 11.8 a	252.2 ± 9.5 a	142.6 ± 7.6 a	142.6 ± 6.9 a	137.1 ± 2.5 a
Soil Fe + Zn application	228.3 ± 21.1 a	117.8 ± 6.4 a	258.8 ± 16.1 a	148.1 ± 3.8 a	156.6 ± 6.7 a	149.3 ± 10.4 a
Soil Fe + Zn + Mn application	243.0 ± 20.4 a	123.3 ± 15.4 a	255.1 ± 15.0 a	144.1 ± 3.9 a	150.9 ± 4.8 a	152.4 ± 6.3 a

Values are means of four replicates. Values represent means ± SE ($n = 4$). Within a column, the different letter means significant difference ($p < 0.05$).

[a] Control; soil Fe application (60 g Fe-EDDHA per tree); soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4 \cdot 7H_2O$ per tree); Soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4 \cdot 7H_2O$ per tree + 30 g $MnSO_4$).

There were no significant differences in peel and pulp Fe concentrations among all of the treatments from 2010 to 2012 (Fig. 3). In 2012, the Zn concentration in peel and pulp were lower in the Fe alone treatment than that in the other treatments, the Zn concentration in peel was higher in the Fe + Zn and Fe + Zn + Mn treatments than that in the control and Fe alone treatments at the beginning of the veraison and fruit mature period (Fig. 4). There were no significant differences in fruit peel and pulp Mn concentrations among all of the treatments from 2010 to 2012 (Fig. 5).

3.3. Citrus fruit yield

In 2012, soil Fe application, soil Fe + Zn application and soil Fe + Zn + Mn application increased fruit number by 23.5%, 26.7% and 24.9% compared to the control, respectively. However, there were no significant differences in fruit weight among all of the treatments (Table 3). Fruit yield was increased significantly by the application of Fe + Zn in 2012 (Table 4). In addition, the number of fruit per tree ($R^2 = 0.2630$, $p = 0.0422$) and the mean fruit weight ($R^2 = 0.3949$, $p = 0.0091$) were positively correlated with leaf active Fe concentrations and leaf Zn concentrations at the beginning of the veraison period, respectively, which was considered to be the most suitable nutrient diagnosis period for citrus (Fig. 7).

3.4. Citrus fruit quality

The soil application of Fe-EDDHA alone did not influence TSS over the three-year study period. Applying Fe + Zn raised TSS

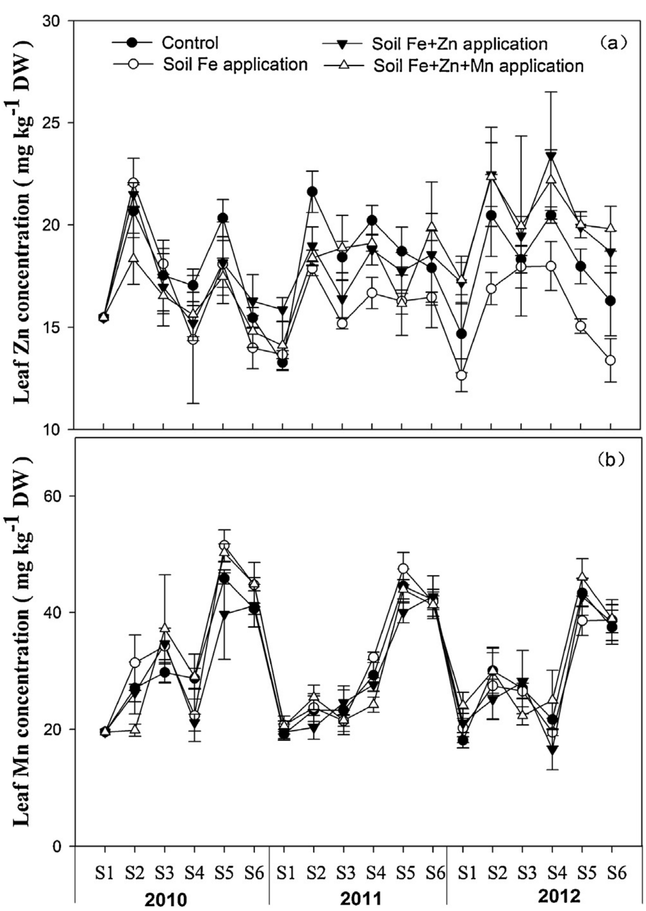

Fig. 2. Dynamics of leaf Zn (a) and Mn (b) concentrations of satsuma mandarin trees grafted on trifoliate orange with different treatments. Control; soil Fe application (60 g Fe-EDDHA per tree); soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4 \cdot 7H_2O$ per tree); soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4 \cdot 7H_2O$ per tree + 30 g $MnSO_4$). Growth periods: spring tip germination (S1), full bloom (S2), young fruit (S3), fruit development (S4), beginning of veraison (S5) and mature fruit (S6). Values represent means ± SE ($n = 4$).

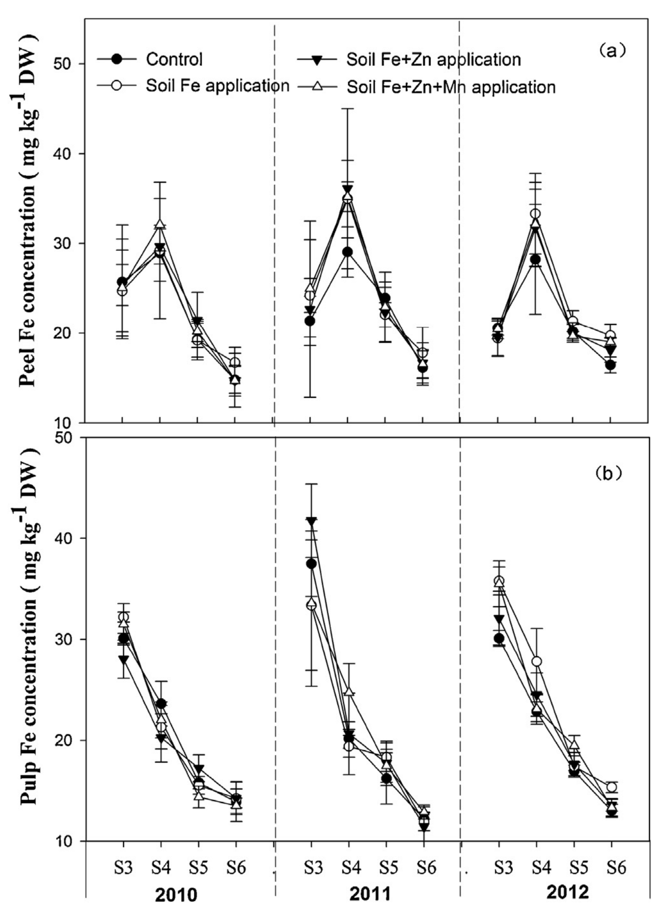

Fig. 3. Dynamics of Fe concentration in peel (a) and pulp (b) of satsuma mandarin trees grafted on trifoliate orange with different treatments. Control; soil Fe application (60 g Fe-EDDHA per tree); soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4 \cdot 7H_2O$ per tree); soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4 \cdot 7H_2O$ per tree + 30 g $MnSO_4$). Growth periods: young fruit (S3), fruit development (S4), beginning of veraison (S5) and mature fruit (S6). Values represent means ± SE ($n = 4$).

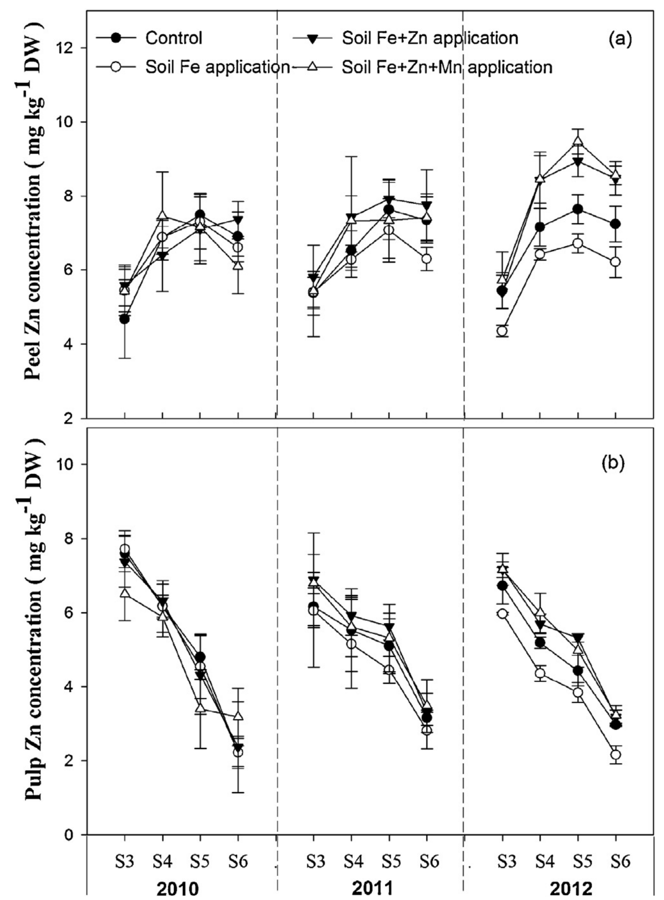

Fig. 4. Dynamics of Zn concentration in peel (a) and pulp (b) of satsuma mandarin trees grafted on trifoliate orange with different treatments. Control; soil Fe application (60 g Fe-EDDHA per tree); soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4{\cdot}7H_2O$ per tree); soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4{\cdot}7H_2O$ per tree + 30 g $MnSO_4$). Growth periods: young fruit (S3), fruit development (S4), beginning of veraison (S5) and mature fruit (S6). Values represent means ± SE ($n = 4$).

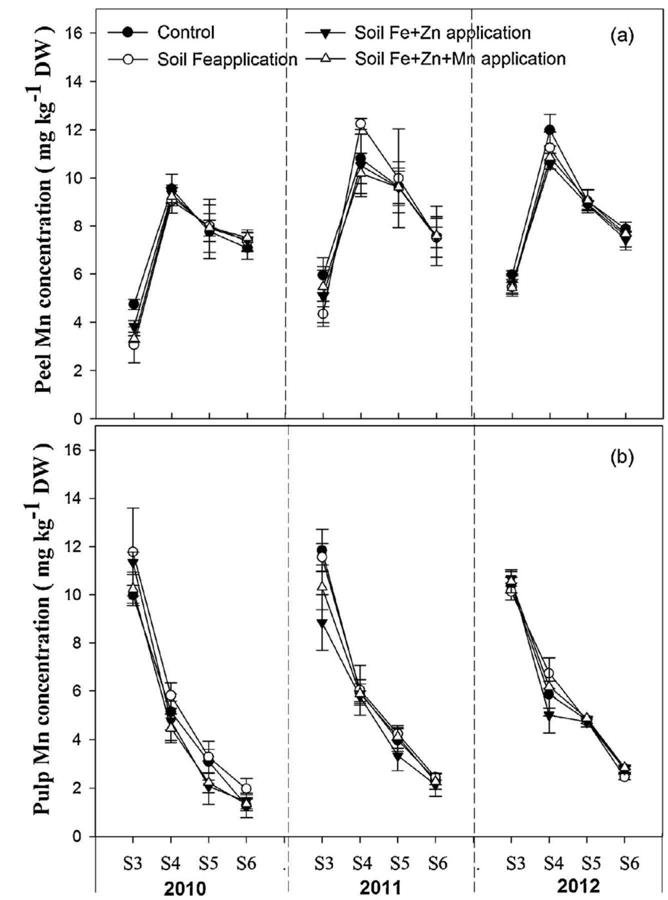

Fig. 5. Dynamics of Mn concentration in peel (a) and pulp (b) of satsuma mandarin trees grafted on trifoliate orange with different treatments. Control; soil Fe application (60 g Fe-EDDHA per tree); soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4{\cdot}7H_2O$ per tree); soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4{\cdot}7H_2O$ per tree + 30 g $MnSO_4$). Growth periods: young fruit (S3), fruit development (S4), beginning of veraison (S5) and mature fruit (S6). Values represent means ± SE ($n = 4$).

significantly from 2011 to 2012 while applying Fe + Zn + Mn increased TSS significantly from 2010 to 2011 (Fig. 6a). There were no significant differences in the TA content among all of the treatments in 2010. However, the TA content was higher in the Fe + Zn + Mn treatment than that in the Fe alone treatment in 2011, and the TA content was higher in the Fe + Zn + Mn treatment than that in the control and Fe alone treatments in 2012 (Fig. 6b). There were no significant differences in the TSS:TA from 2010 to 2011. However, the TSS:TA was raised significantly by applying Fe-EDDHA alone in 2012 (Fig. 6c). The soil application of Fe-EDDHA alone did not influence the Vc content over the three-year study period, but the combined application of Fe-EDDHA and Zn increased Vc content significantly in 2012 (Fig. 6d). Moreover, TSS ($R^2 = 0.2954$, $p = 0.0296$), TA ($R^2 = 0.6069$, $p = 0.0004$) and Vc ($R^2 = 0.2810$, $p = 0.0347$) were positively correlated with leaf Zn concentrations at the most suitable nutrient diagnosis period for citrus, TSS:TA ($R^2 = 0.2785$, $p = 0.0356$) was negatively correlated with leaf Zn concentrations (Fig. 8).

Table 4
Effect of application of Fe-EDDHA, Zn and Mn on yield of citrus.

Treatment[a]	Yield (kg tree^{-1})		
	2010	2011	2012
Control	32.6 ± 5.5 a	14.8 ± 1.2 a	30.5 ± 3.1 b
Soil Fe application	31.4 ± 5.5 a	17.9 ± 2.3 a	36.1 ± 2.8 ab
Soil Fe + Zn application	33.6 ± 2.3 a	18.3 ± 0.7 a	40.8 ± 3.5 a
Soil Fe + Zn + Mn application	35.3 ± 5.2 a	18.6 ± 2.4 a	39. 5 ± 2.0 ab

Values are means of four replicates. Values represent means ± SE ($n = 4$). Within a column, the different letter means significant difference ($p < 0.05$).

[a] Control; soil Fe application (60 g Fe-EDDHA per tree); soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4{\cdot}7H_2O$ per tree); soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4{\cdot}7H_2O$ per tree + 30 g $MnSO_4$).

4. Discussion

4.1. The efficiency of Fe in leaf and the inhibiting for Zn absorption

Although the application of Fe-EDDHA could not influence leaf Fe concentration, leaf active Fe concentration in 2011 and 2012 was increased by applying Fe-EDDHA (Fig. 1). Leaf Fe concentration is not correlated with the degree of chlorosis expressed as chlorophyll content when trees are grown in calcareous soils had been confirmed previous reports (Mengel et al., 1994; Pestana et al., 2001b). This may be because the Fe was immobilized somewhere in the leaf in an unavailable form (Morales et al., 1998). Although why Fe accumulate in leaves and fail to alleviate the Fe deficiency is not well understood, most researches consider that iron is transported through the xylem as Fe^{3+}-citrate to reach the leaf apoplast, and the high pH of leaf apoplast induced by soil HCO_3^- could immobilize Fe, reduce the Fe to transport to mesophyll cytoplasm, and decrease the efficiency of Fe in the leaves (Mengel, 1994). Shi et al. (1993) suggested that the leaf active Fe content decreased with the

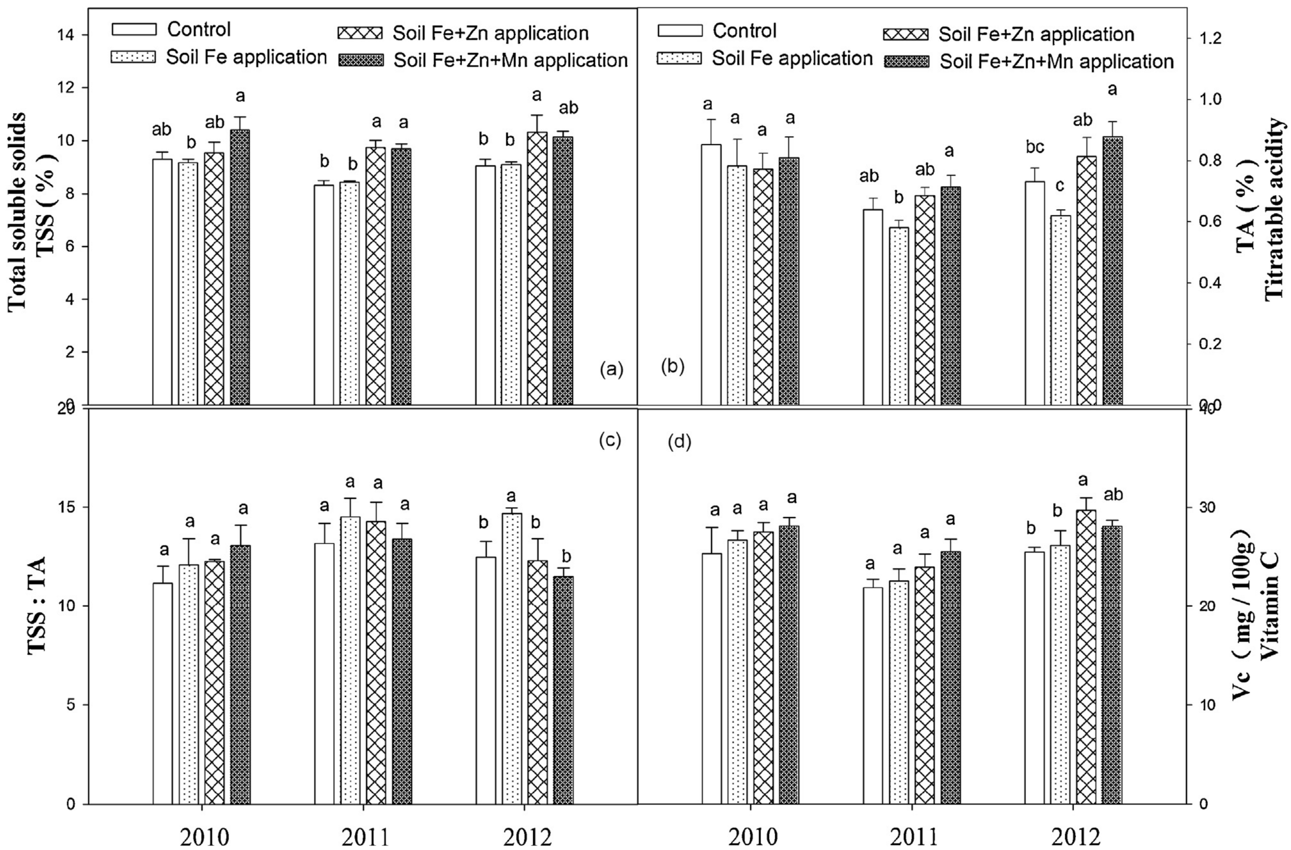

Fig. 6. Effect of application of Fe-EDDHA, Zn and Mn on total soluble solids (TSS) (A), titratable acidity (TA) (B), TSS:TA ratio (C) and Vc contents (D) of satsuma mandarin trees grafted on trifoliate orange with different treatments. Control; soil Fe application (60 g Fe-EDDHA per tree); soil Fe + Zn application (60 g Fe-EDDHA + 40 g $ZnSO_4{\cdot}7H_2O$ per tree); soil Fe + Zn + Mn application (60 g Fe-EDDHA + 40 g $ZnSO_4{\cdot}7H_2O$ per tree + 30 g $MnSO_4$). Values represent means + SE (n = 4). In the bars, the different letter means significant difference ($p < 0.05$), and the same letter means not significantly different ($p > 0.05$).

increasing of HCO_3^- level in nutrient solution. It can be concluded that the proportion of active Fe in the total leaf Fe indicated the efficiency of Fe in the leaves. Our data also showed that the leaf active Fe provides a better diagnosis of Fe nutritional status compared with the total Fe content (Fig. 1), it consistent with the results of Smith and Cheng (2006).

In our study, the soil application of Fe-EDDHA alone decreased leaf and fruit Zn concentrations significantly in 2012, but there was no significant influence on leaf Mn concentration (Figs. 2 and 4). This may be because the transporters of Fe and Zn ions usually compete for the same site, and Zn may be diminished under Fe-sufficient compared with Fe-deficient conditions (Alam et al., 2001; von Wirén et al., 1996). Zn deficiency in leaves could become the new limitation in Fe deficient citrus trees after Fe-EDDHA application to alleviate citrus Fe deficiency (Huang et al., 2012). However, applying Fe-EDDHA in combination with Zn fertilizer

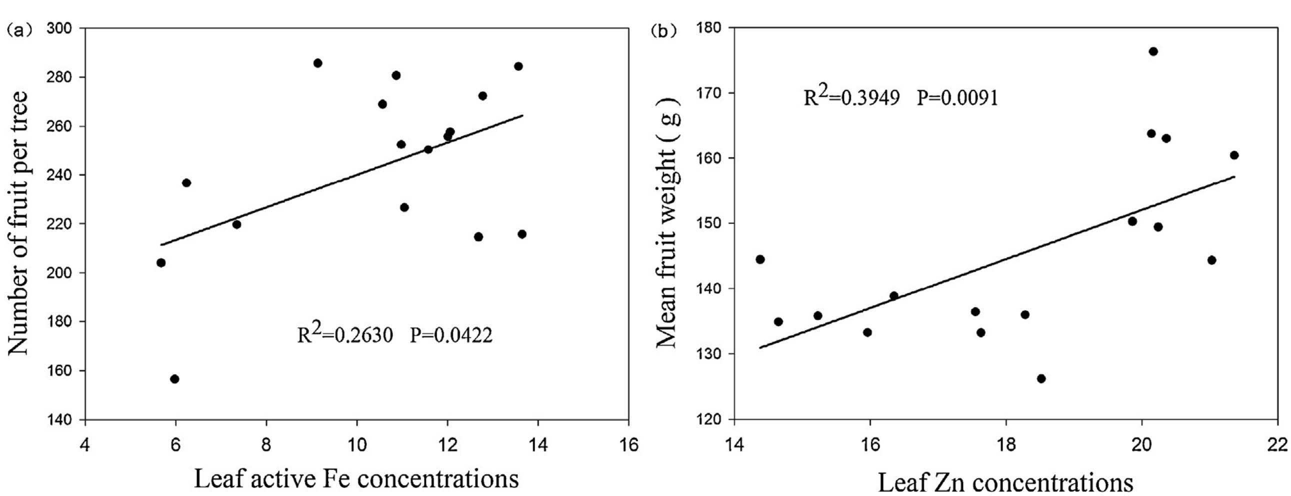

Fig. 7. Effect of leaf active Fe and leaf Zn concentrations at the beginning of veraison period on number of fruit per tree (a) and mean fruit weight (b) of satsuma mandarin trees grafted on trifoliate orange in 2012. (The leaf nutrient in spring tips at the beginning of the veraison period was considered to be the most suitable nutrient diagnosis period for citrus.) (n = 16).

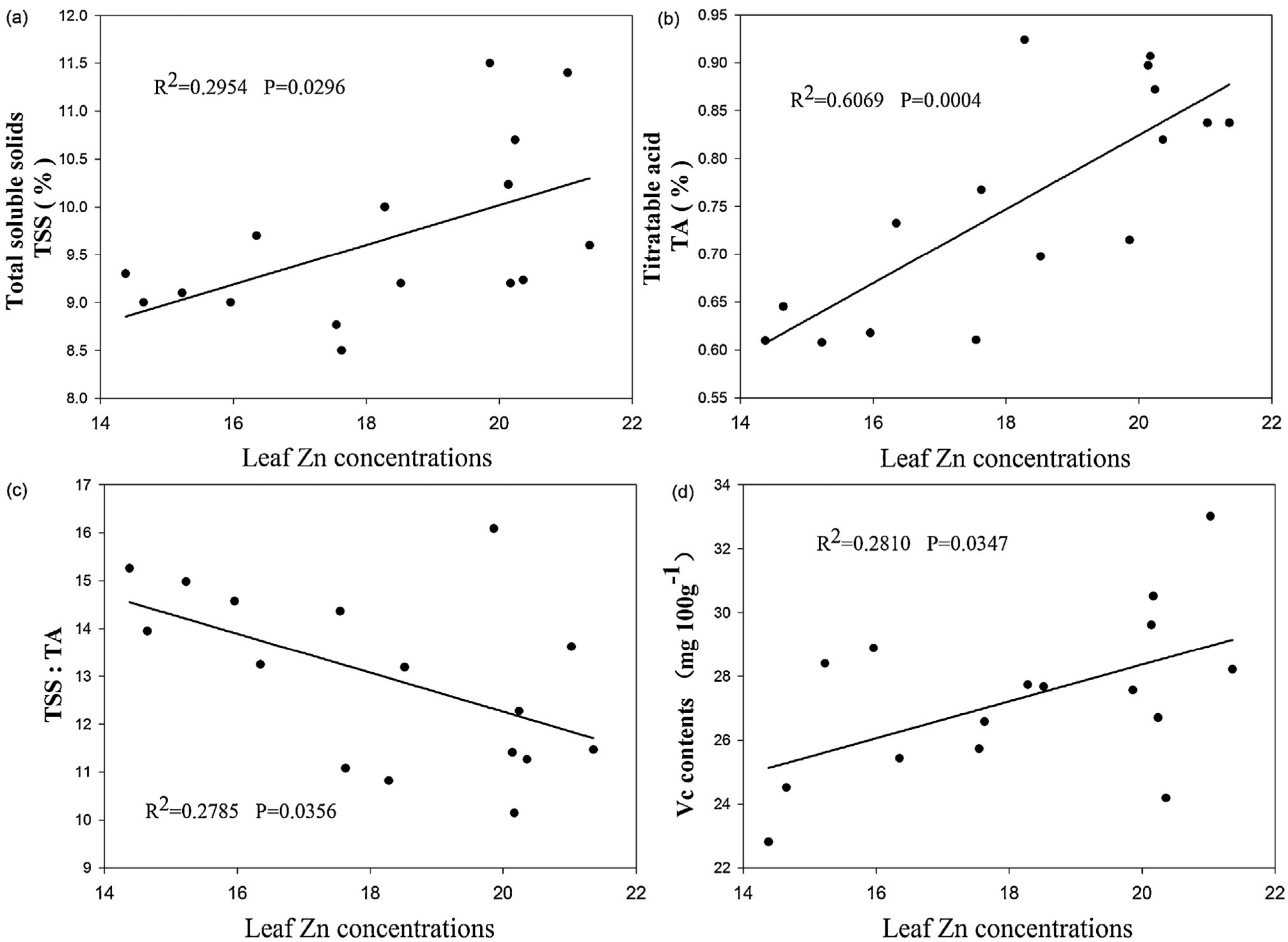

Fig. 8. Effect of leaf Zn concentrations at the beginning of veraison period on total soluble solids (TSS) (a), titratable acidity (TA) (b), TSS:TA (c) and Vc contents (d) of satsuma mandarin trees grafted on trifoliate orange in 2012. (The leaf nutrient in spring tips at the beginning of the veraison period was considered to be the most suitable nutrient diagnosis period for citrus.) ($n = 16$).

could offset the Fe–Zn antagonism and improve Zn absorption (Figs. 2 and 4). Therefore, we should attach importance to Zn supplementation when applying Fe-EDDHA to correct Fe deficiency. The co-application Fe-EDDHA with Zn fertilizer proved the best fertilization measure in calcareous soil.

4.2. Fe and Zn play different roles in yield improvement

The citrus trees that received Fe-EDDHA applications had a higher fruit number than the control trees in 2012 (Table 4) and the fruit number ($R^2 = 0.2630$, $p = 0.0422$) was positively correlated with leaf active Fe concentrations in 2012 (Fig. 7a). This can be ascribed to the fact that Fe deficiency decreases the amount of chlorophyll-containing thylakoid membranes and the electron transport capacity of leaves, severely restricting tree vigor and the growth of leaves and decreasing the number of fruits per tree and fruit size (Àlvarez-Fernàndez et al., 2006; Morales et al., 1998). Similar studies have reported that the soil or foliar application of Fe-EDDHA increased yield per plant, mostly by increasing the fruit numbers in kiwifruit (Loupassaki et al., 1997) and strawberry (Zaiter et al., 1993). Although applying Zn did not significantly influence fruit weight, fruit weight ($R^2 = 0.3949$, $p = 0.0091$) was positively correlated with leaf Zn concentrations (Table 4 and Fig. 7b). Zn was required for the synthesis of tryptophan, and as a precursor to the synthesis of indoleacetic acid, which is involved in the fruit growth and development process (Swietlik, 1999). It could be concluded that Fe and Zn play different roles in fruit yield. The increase in yield after the application of Fe-EDDHA was due to augmentation of the fruit number while Zn improved yield by increasing the mean fruit weight. The treatment that applied a combination of Fe-EDDHA and Zn had the highest yield in 2012 (Table 4).

4.3. Increase in citrus quality was mainly related to Zn application

Applying Zn significantly increased fruit TSS, TA and Vc content (Fig. 6), which were positively correlated with leaf Zn concentrations in 2012 (Fig. 8). This can be ascribed to Zn played an active role in the photosynthesis, nucleic acid and starch metabolism, and promoted activities of various enzymes in these biochemical reactions (Alloway, 2004). These results are in line with the work of Khan et al. (2012), who reported that trees treated with Zn fertilizer at fruit set stage exhibited higher TSS and TA in citrus. Numerous studies have also reported that Zn application can improve quality parameters in peach (Sidhu et al., 2012), pomegranate (Hasani et al., 2012) and mango (Bhowmick et al., 2012). Although applying Zn did not influence TSS:TA significantly, TSS:TA ($R^2 = 0.2785$, $p = 0.0356$) was negatively correlated with leaf Zn concentrations in 2012 (Figs. 5c and 8c). In this case, it is possible that although Zn application could simultaneously increase the TSS and TA, Zn might have had a greater influence on the fruit acidity of citrus.

Although Fe-EDDHA alone did not influence the TSS and Vc content, Fe-EDDHA application decreased TA and increased TSS:TA, significantly (Fig. 6). These results are in line with the work of Pestana et al. (2001a), who reported foliar sprays Fe fertilizer decreased TA content. However, research on the effect of Fe deficiency on the organoleptic quality in citrus has been scant, perhaps because Fe-EDDHA application inhibits Zn absorption, resulting in the TA decrease. It could be concluded that Zn was a more important factor than Fe in the synthesis of quality parameters.

4.4. Seasonal changes of Fe, Zn and Mn in citrus leaves

The seasonal dynamics of leaf active Fe, total Zn and Mn concentrations exhibited difference. Fe and Zn are considered to have intermediate phloem mobility, leaf could transport them to other plant tissues through phloem (Marschner, 2012; Shi et al., 2011; Zhang et al., 1995). Leaf active Fe concentration showed the lower levels at the period of fruit rapid enlargement (S2–S4), and leaf Zn concentration showed the decreasing trend at the young fruit (S2–S3) and fruit veraison (S5–S6) period (Figs. 1b and 2a). Although the processes that differentially modulate phloem transport of Fe and Zn into fruit/fruit parts are not clearly understood, it was possibly related to the different requirement for fruit growth and quality parameters accumulation, the significant increase in the fruit number by application of Fe-EDDHA and the better yield and quality improvement by combination application with Zn could prove the conclusion from another perspective. Mn is a phloem-immobile micronutrient (Marschner, 2012), which probably explains the relative increasing trend of leaf Mn concentration in the whole growth period (Fig. 2b).

5. Conclusions

The present study revealed that Fe and Zn played different roles in fruit yield in calcareous soil, with the former affecting the citrus fruit number and the latter influencing the mean fruit weight. Moreover, Zn was a more important factor than Fe in citrus fruit quality. The application of Fe-EDDHA alone could induce zinc deficiency, thus the co-application of Fe-EDDHA and Zn fertilizer was useful for obtaining higher marketable yield and quality in satsuma mandarin, with a continuous application of at least three years in calcareous soil.

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