Physiological and biochemical adjustment of iron chlorosis affected low-chill peach cultivars supplied with different iron sources

Binayak Chakraborty · Pramod Narayan Singh ·
Alok Shukla · Daya Shankar Mishra

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Abstract A pot experiment was carried out to investigate the effect of iron supplementation on physiological and biochemical status of the low-chill peach cultivars (Saharanpur Prabhat, Shan-e-Punjab and Pratap) suffered from iron chlorosis in artificially created calcareous soil. Three most commonly used iron sources viz. Fe-sulphate (1.0 % and 0.5 %), Fe-citrate (1.0 % and 0.5 %) and FeEDTA (0.1 % and 0.2 %) were sprayed on the 4th and 5th leaves from the apex of the twig. And after 1 week of spraying, observation on various physiological and biochemical parameters in leaves were recorded. Improvement in plant physiological parameters like chlorophyll content index (CCI), photosynthetic rate (P_n), stomatal conductance (g_s) and intercellular CO_2 conc. (C_i) were recorded best with the application of 1.0 % Fe-sulphate both in treated and untreated upper leaves. The maximum recovery in biochemical parameters such as total leaf chlorophyll content, superoxide dismutase (SOD) and peroxidase (POD) activity was also noted with the application of 1.0 % Fe-sulphate. However, application of 1.0 % Fe-sulphate and 0.5 % Fe-sulphate had similar effect for most of the parameters under study. The ability of iron sources to induce physiological and biochemical responses in iron deficient low-chill peach plants were in the following order Fe-sulphate>Fe-citrate>FeEDTA. Differential responses in plant physiological and biochemical parameters were also exhibited by the low-chill peach

cultivars with regard to supplementation of various iron sources. Among the low-chill peach cultivars, Saharanpur Prabhat responded best with the application of iron sources followed by Shan-e-Punjab and Pratap.

Keywords Iron · Iron chlorosis · Chlorophyll · Photosynthesis · Low-chill peach

Introduction

Peach [*Prunus persica* (L.) Batsch] is an important stone fruit of the temperate region of the world. The red colour of skin and high nutritional value of the fruits attract the consumers in markets. Plant breeding methods have made it possible to develop a number of outstanding low chilling requirement cultivars suitable for growing in subtropical/submountainous parts of the world. However, iron chlorosis is the widespread nutritional disorder associated with cultivation of this crop in calcareous and alkaline soils. The most prevalent cause of Fe deficiency in these regions is the presence of high level of bicarbonate ion in calcareous soil. High level of bicarbonate ions in the soil affects the metabolic process in roots and leaves, decreases the soil and plant iron availability (Mengel 1995), leading to the condition known as lime-induced iron chlorosis (Pestana et al. 2005). In peach, lime-induced iron deficiency results in delayed ripening and impaired the fruit quality (Sanz et al. 1997).

Iron is the component of iron-sulfur (Fe-S) proteins and also non-heme iron proteins which are required during photosynthesis, respiration and N_2 fixation (Taiz and Zeiger 1991). It is also reported to be regulated the chlorophyll biosynthesis (Marschner 1995). About 80 % leaf iron is localized in chloroplast and 60 % of iron is involved in

B. Chakraborty · P. N. Singh (✉) · D. S. Mishra
Department of Horticulture, College of Agriculture,
G.B. Pant University of Agriculture and Technology,
Udham Singh Nagar,
Pantnagar, Uttarakhand PIN: 263 145, India
e-mail: pnsinghraghuvanshi@gmail.com

A. Shukla
Department of Biotechnology, Bhimtal Campus,
Kumaun University,
Bhimtal, Uttarakhand PIN: 263136, India

regular functioning of electron transport chain (Terry and Abadía 1986). Thus under iron deficiency, the level of chlorophyll fluorescence (Morales et al. 1991) and photosynthetic activity of the plants are decreased markedly (Iturbe-Ormaeche et al. 1995). Enzymatic activities of certain antioxidant enzymes like superoxide dismutase (SOD: EC 1.15.1.1) and peroxidase (POD: EC 1.11.1.7) are altered under such conditions. Superoxide dismutase is the major scavenger of superoxide (O_2^-) in plant system and its enzymatic reaction leads to formation of H_2O_2 and O_2 (Fridovich 1995). The hydrogen peroxide (H_2O_2) is decomposed in the presence of peroxidase (Minotti and Aust 1987). Hence, under iron deficient condition normal physiological and biochemical processes in the plant system are severely hampered.

Such condition can be restored with the application of iron, otherwise productivity and fruit quality are drastically affected (Sanz et al. 1997). Several management practices have been standardized to counteract this problem (Pestana et al. 2003). However, foliar application of iron sources offers the most efficient method to provide iron than soil application or trunk injections when plants are grown on calcareous or alkaline soils (Crane et al. 2007). A number of iron sources like inorganic and organic iron salts or synthetic iron compounds are available in the market at affordable price. These chemicals are being used to correct iron deficiency without having the knowledge about technical details like doses, timing and frequencies. All these technical aspects of foliar fertilization have a great influence on the internal physiological and biochemical mechanisms of plants. Therefore, without understanding the physiology and biochemistry of iron supplied plants, a successful management practice would not be prescribed. Keeping the above facts under consideration, an attempt has been made to examine the internal physiological and biochemical process associated with foliar application of different iron sources in low-chill peaches grown under iron deficient condition.

Materials and methods

Plant material and growing conditions

One year old plants of three low-chill peach cultivars viz. Saharanpur Prabhat, Shan-e-Punjab and Pratap were used for the study. All the low-chill cultivars are widely grown in the subtropics of North Western part of India and showed difference in tolerance level under calcareous soil. The plants were grown in nursery during previous year and transferred in the 6 kg capacity pots filled with sandy loam soil having $30\text{ g CaCO}_3\text{ kg}^{-1}$ soil. The plants were grown in a green house for 2 months to develop uniform iron

deficiency symptoms. For proper growth and establishment 30 ppm nitrogen as urea, 10 ppm phosphorus as KH_2PO_4 , 22 ppm potassium as KH_2PO_4 and KCl were supplied. Among the micro nutrients, 2.5 ppm Zn as $ZnSO_4 \cdot 7H_2O$ was applied in soil. Deionized water was used for watering to the plants every alternate day.

Treatment composition and method of application

After 40–45 days of growing, upon the appearance of interveinal chlorosis in the younger leaves, three different iron sources i.e., Fe-sulphate (1 % and 0.5 %), Fe-citrate (1 % and 0.5 %) and FeEDTA (0.1 % and 0.2 %) were applied as foliar application to abaxial side of the 4th and 5th leaves from the apex of the twigs in each plants with the help of a paint brush. The iron chlorosis affected low-chill plants of each cultivar were treated as control and the whole experiment was replicated thrice. Seven days after application of different iron sources the observations on physiological and biochemical parameters were recorded.

Chlorophyll content index, photosynthetic parameters and chlorophyll fluorescence

The chlorophyll content index determined with the help of SPAD meter (Opti Science, CMM-200, USA). The net photosynthetic rate (P_n), stomatal conductance (g_s), internal CO_2 concentration (C_i) were measured using the Li-6400 (Li-COR, Lincoln, USA). During day when the observations were taken, the temperature and photon flux density inside the green house were varied from 25 to 20 °C and $750\text{--}800\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ at plant level, respectively.

The chlorophyll fluorescence parameters (F_v/F_m , F_o/F_m ; F_o is the initial fluorescence, F_m the maximum fluorescence, $F_v = F_m - F_o$ the variable fluorescence) were recorded after a dark adaptation time for 30 min with a portable chlorophyll fluorometer (Handy Plant Efficiency Analyzer, Hansatech, UK). All the physiological parameters were taken in quadruplicate both for the treated leaves and untreated upper leaves separately between 11.00 A.M. and 1.00 P.M.

Leaf chlorophyll content

Concentration of total chlorophyll was analyzed following the method given by Arnon (1949). 0.5 g of leaf material was collected from the plants and grinded in 10 ml of 80 % acetone. After filtration final volume was made up to 50 ml. The 5 ml of this solution was measured using a UV-visible spectrophotometer (Thermospectronic Biomate 5) at wavelengths 663 nm and 645 nm. The Chl. a, Chl. b and total chlorophyll content of the leaves were calculated according to the following equations:

$$\text{Chl. a} = \frac{[(12.7 \times A_{663}) - (2.63 \times A_{645})]}{\text{Wt. of the sample (g)} \times 1000} \times \text{Final volume of the solution (ml)}$$

$$\text{Chl. b} = \frac{[(22.9 \times A_{645}) - (4.48 \times A_{663})]}{\text{Wt. of the sample (g)} \times 1000} \times \text{Final volume of the solution (ml)}$$

$$\text{Total Chlorophyll} = \frac{[(20.2 \times A_{645}) + (8.02 \times A_{663})]}{\text{Wt. of the sample (g)} \times 1000} \times \text{Final volume of the solution (ml)}$$

Superoxide dismutase (SOD) and peroxidase (POD) activity

Total SOD activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977). The reaction mixture (1.5 ml) contained 50 mM phosphate buffer (pH=7.8), 0.1 μ M EDTA, 13 mM methionine, 75 μ M NBT, 2 μ M riboflavin and 50 μ l enzyme extract. Riboflavin was added last and tubes were shaken followed by illuminated with two 20 W fluorescent tubes. The reaction was allowed to proceed for 15 min after which the lights were switched off and the tubes were covered with a black cloth. Absorbance of the reaction mixture was read at 560 nm. One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50 % inhibition of the NBT photoreduction rate and the results expressed as U g⁻¹ of fresh weight.

Peroxidase activity of the leaves was determined at 30 °C by a direct spectrophotometric method (Hammerschmidt and Kuc 1982). The reaction mixture consisted of 0.5 ml leaf extract, 1.5 ml of 0.05 M pyrogallol and 0.5 ml of 1 % hydrogen peroxide. The reaction was incubated in a water bath, and absorbance at 420 nm was recorded at 30 s intervals for 2 min. The enzyme activity was expressed as change in the absorbance of the reaction mixture min⁻¹ g⁻¹ of fresh weight.

Physiologically active iron (Fe²⁺) conc. of the leaves

Physiologically active iron (Fe²⁺) conc. expressed as mg kg⁻¹ DW of the leaves, was determined according to method described by Katyal and Sharma (1980). One gram fresh leaves were collected from each plant 7 days after foliar application of different iron sources. Harvested leaves were washed thoroughly with tap water followed by 1 N HCl and deionised water. After air drying, the leaves were chopped with a Ni coated scissor and soaked in 10 ml 1.5 % (w/v) 1–10 o-phenanthroline (pH 3.0) for 16 h. The filtrate orange colour solution was collected and transmittance recorded at 510 nm in a spectrophotometer. Another set of 1 g fresh leaves was harvested from each plant and placed in hot air oven for calculating the conc. on dry weight basis.

Total iron conc. of the leaves

Total iron conc. was analyzed following the A.O.A.C. (1990) standard procedure. The freshly harvested leaves

were dried at 72 °C. One gram dried leaves were crushed and digested with tri-acid mixture consisting of HNO₃, H₂SO₄ and HClO₄ (10:1:4 v/v). Atomic absorption spectrophotometer (AAS) was used to determine the total iron concentration. The total iron content of the leaves expressed as mg kg⁻¹ DW.

Statistical analyses

The whole experiment was laid out in two factorial completely randomized design with three replications during 2009 growing season and re-measured with same treatment and cultivar combinations in next growing season (i.e. during 2010). The data of both the years were pooled and then subjected to analyze for the variance. The least significant differences (LSDs) were calculated to compare the significant effects at $p \leq 0.05$ (Snedecor and Cochran 1967).

Results

Physiological parameters

Chlorophyll content index (CCI) and photosynthetic rate (P_n) after foliar application

In comparison to control, iron supplementation with different iron sources brought significant changes in chlorophyll content index (CCI) and photosynthetic rate in the low-chill peach cultivars (Fig. 1 (A_{TL}, A_{UUL} and B_{TL}, B_{UUL})). The maximum chlorophyll content index (CCI) and photosynthetic rate in treated as well as in untreated upper leaves were recorded in low-chill peach cultivar Saharanpur Prabhat when 1.0 % Fe-sulphate was used as iron source. Regardless of cultivars, application of 1.0 % Fe-sulphate caused maximum increase in chlorophyll content index and photosynthetic rate. However, there were no detectable differences observed between application of 0.5 % Fe-sulphate and 1.0 % Fe-sulphate for both the parameters in treated as well as untreated upper leaves. Among the low-chill peach cultivars, the treated and untreated upper leaves of Saharanpur Prabhat responded best with the application of different iron sources for improvement of chlorophyll content index as well as photosynthetic rate. These parameters were recorded minimum in low-chill peach cultivar Pratap.

Fig. 1 Recovery of plant physiological parameters (chlorophyll content index (A_{TL} , A_{UUL}), photosynthetic rate (B_{TL} , B_{UUL}), stomatal conductance (C_{TL} , C_{UUL}), intercellular CO_2 conc. (D_{TL} , D_{UUL}), chlorophyll fluorescence parameters: F_v/F_m (E_{TL} , E_{UUL}) and F_o/F_m (F_{TL} , F_{UUL})) in the leaves of low-chill peach cultivars 7 days after foliar application of different iron sources. T_1 =control, T_2 =0.1 % FeEDTA, T_3 =0.2 % FeEDTA, T_4 =0.5 % Fe-sulphate, T_5 =1.0 % Fe-sulphate, T_6 =0.5 % Fe-citrate and T_7 =1.0 % Fe-citrate. TL=Treated leaves and UUL=Untreated upper leaves. The vertical bars denote standard error ($n=3$)

Stomatal conductance and intercellular CO₂ conc. after foliar application

Stomatal conductance and intercellular CO₂ conc. were significantly affected with application of different iron sources over control (Fig. 1 (C_{TL}, C_{UUL} and D_{TL}, D_{UUL})). The treated and untreated upper leaves of Saharanpur Prabhat exhibited maximum stomatal conductance when treated with 1.0 % Fe-sulphate. No significant increase in intercellular CO₂ conc. of treated and untreated upper leaves was recorded among the low-chill peach cultivars with the application of 1.0 % Fe-sulphate. Both stomatal conductance and intercellular CO₂ conc. of the low-chill peach cultivars did not vary significantly when different conc. of Fe-sulphate was used. However, regardless of cultivars, the overall improvement in stomatal conductance and intercellular CO₂ conc. of treated and untreated upper leaves were recorded with the application of 1.0 % Fe-sulphate. The low-chill peach cultivar Saharanpur Prabhat recorded maximum stomatal conductance and intercellular CO₂ conc. in treated and untreated upper leaves after application of different iron sources while Pratap showed minimum value for these parameters.

Chlorophyll fluorescence parameters after foliar application

As shown in Fig. 1 (E_{TL}, E_{UUL} and F_{TL} and F_{UUL}) the chlorophyll fluorescence parameters in treated and untreated upper leaves of all the low-chill peach cultivars underwent a significant increase with the application of different iron sources. The maximum increase in F_v/F_m and F_o/F_m values of the treated and untreated upper leaves were gained with the application of 1.0 % Fe-sulphate in the low-chill peach cultivar Saharanpur Prabhat. Application of 0.5 % Fe-sulphate and 1.0 % Fe-sulphate had similar effect on overall improvement of chlorophyll fluorescence parameters among

the low-chill peach cultivars. As like photosynthetic parameters the chlorophyll fluorescence parameters were also recorded maximum in Saharanpur Prabhat and minimum found in Pratap.

Biochemical parameters

Total chlorophyll content and Chl. a: Chl. b after foliar application

Significant increase in total chlorophyll content was noted after application of different iron sources in all the low-chill peach cultivars over control (Table 1). The maximum increase in total chlorophyll content recorded with the application of 1.0 % Fe-sulphate in the leaves of Saharanpur Prabhat. However, no significant change in total chlorophyll content was detected with the application of 1.0 % Fe-sulphate and 0.5 % Fe-sulphate in the leaves of low-chill peach cultivars. The leaves of Saharanpur Prabhat possessed maximum total chlorophyll content while least improvement in chlorophyll content was occurred in Pratap. The minimum Chl. a : Chl. b was recorded in the leaves of Saharanpur Prabhat treated with 0.5 % Fe-sulphate (Table 1). The Chl. a : Chl. b of the leaves was maximum when 0.1 % Fe-EDTA as used as iron source. Leaves of low-chill peach cultivar Pratap exhibited maximum Chl. a : Chl. b while this ratio was recorded minimum in Saharanpur Prabhat.

Superoxide dismutase activity (SOD) and peroxidase activity (POD) after foliar application

The enzymatic activities of the leaves were restored significantly with the application of different iron sources (Table 2). Regardless of low-chill peach cultivars, significant increase in SOD activity was gained with the application of 1.0 % Fe-sulphate. The low-chill peach cultivar Saharanpur Prabhat and Shan-e-Punjab exhibited similar improvement in SOD

Table 1 Recovery of total chlorophyll content and Chl. a: Chl. b in the leaves of low-chill peach cultivars 7 days after foliar application of different iron sources

Treatments	Total chlorophyll (mg g ⁻¹ FW)			Chl. a: Chl. b		
	Saharanpur Prabhat	Shan-e-Punjab	Pratap	Saharanpur Prabhat	Shan-e-Punjab	Pratap
Control	1.15±0.03 ^c	1.09±0.12 ^c	0.84±0.03 ^d	3.07±0.01 ^c	3.95±0.22 ^a	2.43±0.06 ^d
0.1 % FeEDTA	1.30±0.02 ^b	1.18±0.02 ^c	1.03±0.02 ^c	3.34±0.02 ^b	4.28±0.33 ^a	3.41±0.33 ^b
0.2 % FeEDTA	1.38±0.05 ^b	1.31±0.05 ^b	1.20±0.12 ^b	3.40±0.33 ^b	3.33±0.01 ^c	3.38±0.03 ^b
0.5 % Fe-sulphate	1.80±0.01 ^a	1.66±0.02 ^a	1.52±0.33 ^a	2.42±0.67 ^d	2.92±0.02 ^c	3.14±0.04 ^c
1.0 % Fe-sulphate	1.82±0.22 ^a	1.66±0.11 ^a	1.53±0.24 ^a	2.49±0.01 ^d	2.95±0.02 ^c	3.12±0.06 ^c
0.5 % Fe-citrate	1.50±0.33 ^b	1.39±0.34 ^b	1.20±0.04 ^b	2.88±0.02 ^c	3.16±0.06 ^c	3.55±0.04 ^b
1.0 % Fe-citrate	1.70±0.15 ^a	1.51±0.03 ^b	1.36±0.12 ^b	2.32±0.15 ^d	3.29±0.04 ^c	3.57±0.01 ^b

For each measurement corresponding mean±standard error ($n=3$) followed by the same letter are not significant at $P\leq 0.05$

Table 2 Recovery of superoxide dismutase (SOD) and peroxidase (POD) activity in the leaves of low-chill peach cultivars 7 days after foliar application of different iron sources

Treatments	Superoxide dismutase (SOD) (U g ⁻¹ FW)			Peroxidase (POD) (ΔE_{420} min ⁻¹ g ⁻¹ FW)		
	Saharanpur Prabhat	Shan-e-Punjab	Pratap	Saharanpur Prabhat	Shan-e-Punjab	Pratap
Control	1.02±0.01 ^d	1.10±0.33 ^d	1.03±0.02 ^d	0.70±0.02 ^c	0.67±0.67 ^c	0.53±0.11 ^c
0.1 % FeEDTA	1.15±0.03 ^c	1.17±0.02 ^c	1.14±0.06 ^c	0.95±0.03 ^c	0.92±0.67 ^c	0.83±0.15 ^c
0.2 % FeEDTA	1.22±0.06 ^c	1.22±0.01 ^c	1.15±0.02 ^c	1.18±0.15 ^c	1.10±0.33 ^c	1.07±0.03 ^c
0.5 % Fe-sulphate	1.45±0.02 ^a	1.40±0.03 ^a	1.34±0.01 ^a	1.65±0.06 ^a	1.42±0.15 ^b	1.44±0.03 ^b
1.0 % Fe-sulphate	1.53±0.02 ^a	1.53±0.16 ^a	1.40±0.33 ^a	1.87±0.11 ^a	1.75±0.02 ^a	1.84±0.02 ^a
0.5 % Fe-citrate	1.16±0.11 ^c	1.26±0.02 ^b	1.24±0.15 ^b	1.51±0.33 ^a	1.25±0.02 ^c	1.23±0.33 ^c
1.0 % Fe-citrate	1.38±0.15 ^b	1.40±0.04 ^b	1.33±0.12 ^b	1.47±0.67 ^b	1.36±0.04 ^b	1.42±0.67 ^b

For each measurement corresponding mean±standard error ($n=3$) followed by the same letter are not significant at $P\leq 0.05$

activity when 1.0 % Fe-sulphate was used as iron source while this treatment brought maximum recovery in POD activity in the leaves of both Saharanpur Prabhat and Pratap. The overall improvement in SOD and POD activity upon foliar application of different iron sources were noted maximum in the leaves of low-chill peach cultivar Shan-e-Punjab and Sharanpur Prabhat, respectively. The SOD and POD activity were least activated in the leaves of low-chill peach cultivar Pratap.

Plant nutritional parameters

Physiologically active iron (Fe²⁺) and total iron conc. of the leaves after foliar application

Significant enrichment of both leaf physiologically active iron (Fe²⁺) and total iron conc. were achieved with the application of different iron sources (Table 3). Application of 1.0 % Fe-sulphate brought more than two fold increase in the leaf physiologically active iron (Fe²⁺) conc. over the control plants in all the low-chill peach cultivars. However, no significant effect of leaf physiologically active iron (Fe²⁺) conc. was found with the application 1.0 % Fe-sulphate and

0.5 % Fe-sulphate. Similar effect was also noted for leaf total iron conc. after foliar application. FeEDTA was found to be least effective to increase the leaf mineral content of the peach cultivars. Irrespective of different iron sources used, both physiologically active iron (Fe²⁺) and total iron conc. were recorded maximum in the leaves of low-chill peach cultivar Saharanpur Prabhat followed by Shan-e-Punjab and Pratap.

Discussion

Under iron deficiency loss of chlorophyll in the younger leaves is the common visible symptom. However, it is hypothesized that different factors are responsible for the decrease in the chlorophyll content under such conditions. Iron has a role in thylakoid lipid composition. Beside this, iron is directly or indirectly involved in the production of chlorophyll and deficiency of iron irreversibly damages chlorophyll synthesis (Jacobson and Oertli 1956). The common precursor for chlorophyll and heme synthesis is δ -aminolevulinic acid (ALA) and the rate of ALA formation

Table 3 Recovery of nutritional status (physiologically active iron and total iron content) in the leaves of low-chill peach cultivars 7 days after foliar fertilization of different iron sources

Treatments	Physiologically active iron/Fe ²⁺ (mg kg ⁻¹ DW)			Total iron (mg kg ⁻¹ DW)		
	Saharanpur Prabhat	Shan-e-Punjab	Pratap	Saharanpur Prabhat	Shan-e-Punjab	Pratap
Control	10.45±0.67 ^d	8.95±0.03 ^d	8.25±0.06 ^d	40.00±0.03 ^c	38.25±0.16 ^d	35.35±0.02 ^d
0.1 % FeEDTA	12.24±0.33 ^c	10.46±0.22 ^d	10.25±0.25 ^d	56.24±0.22 ^b	41.77±0.03 ^d	37.70±0.01 ^d
0.2 % FeEDTA	15.67±0.15 ^b	14.20±0.25 ^c	12.33±0.67 ^c	65.08±0.15 ^b	50.25±0.03 ^c	45.69±0.67 ^c
0.5 % Fe-sulphate	22.05±0.22 ^a	20.00±0.67 ^a	19.67±0.04 ^a	76.96±0.33 ^a	65.38±0.06 ^a	62.25±0.15 ^b
1.0 % Fe-sulphate	23.95±0.03 ^a	21.33±0.06 ^a	20.25±0.02 ^a	87.14±0.06 ^a	78.29±0.33 ^a	71.19±0.03 ^a
0.5 % Fe-citrate	17.50±0.12 ^b	15.97±0.20 ^b	14.95±0.33 ^b	65.45±0.02 ^b	55.25±0.06 ^b	42.14±0.24 ^b
1.0 % Fe-citrate	18.25±0.02 ^a	17.06±0.33 ^b	16.04±0.13 ^b	84.74±0.01 ^a	70.00±0.22 ^b	64.67±0.25 ^b

For each measurement corresponding mean±standard error ($n=3$) followed by the same letter are not significant at $P\leq 0.05$

is controlled by iron (Miller et al. 1995). In this experiment, application of iron sources significantly increased the SPAD meter readings (i.e. CCI) as well as the total chlorophyll content of the leaves. The chlorophyll content index is reported to be positively correlated with leaf chlorophyll content (Netto et al. 2005). Higher amount of iron penetration might occur in the leaf cells with 1.0 % Fe-sulphate than other sources and also could took part in the recovery of thylakoid membrane as well as regulated the genes involved in chlorophyll biosynthesis processes (Abadía 1992). Similar results were also found in grapefruit applied with 0.3 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ + 0.1 % L-77 (surfactant) as reported by Horesh and Levy (1981). Foliar application of Fe-sulphate was reported to increase the chlorophyll content of the leaves in kiwi (Rambolá et al. 2000) and citrus (Pestana et al. 2001).

Physiological activity of the peach cultivars was improved with supplementation of iron in Fe-sulphate treated plants as compared to other two iron sources. This might be attributed to more amount of iron used in photosynthetic electron transport chain and also the enrichment of iron in chloroplast was more when Fe-sulphate used as iron source. The spraying solution prepared with Fe-sulphate had iron in ferrous (Fe^{2+}) form whereas, Fe-citrate and FeEDTA containing solutions the form of iron was ferric (Fe^{3+}). In ferrous (Fe^{2+}) form, the uptake of iron was believed to be more, since reduction step was not required during entry into the cells. While plasmamembrane bound enzyme ferric chelate reductase (FCR) reduced the ferric form of iron into ferrous before entry into the cells (Brüggemann et al. 1993). Further, activity of this enzyme was reported to be controlled by light, pH and several other factors (González-Vallejo et al. 2000; Larbi et al. 2001). These type of findings indicated that higher amount of iron penetration occurred with Fe-sulphate than other sources (Table 3). The higher stomatal conductance might also be responsible for the same. Molassiotis et al. (2006) also recorded less stomatal conductance when they exposed peach rootstocks to iron deprived solution than iron containing solution. Improvement in chlorophyll fluorescence parameters were also observed with the application of 1.0 % Fe-sulphate. Iron deficiency decreases not only absorbance, but also increases the relative amount of light dissipated thermally by the photosystem II antenna (Morales et al. 1991). Similar to photosynthetic rate, the chlorophyll fluorescence parameters were not significantly affected by the application of 1.0 % Fe-sulphate and 0.5 % Fe-sulphate. This clearly indicated that a large fraction of energy went to drive the carbon assimilation in the plants treated with 1.0 % Fe-sulphate and 0.5 % Fe-sulphate. Thus, plants treated with 1.0 % Fe-sulphate and 0.5 % Fe-sulphate, showed an increase in photosynthetic rate in a non-significant manner. Overall, the improvement of all the physiological activities in the treated leaves was recorded higher as

compared to untreated upper leaves. This clearly established that iron is an immobile element within the plant system (Brown et al. 1958).

An increased antioxidant enzyme activity was recorded with the application of different iron sources. The maximum increase in SOD activity was recorded with the application of 1.0 % Fe-sulphate. This might be due to more amount of iron absorption through foliar application enhancing the superoxide dismutase activity (SOD) to scavenge the reactive oxygen species and provide better growing environment after iron supplementation. Further, it was reported that the oxidative stress was negatively correlated with iron availability (Mohamed and Aly 2004). Plants treated with 1.0 % Fe-sulphate had shown significant increase in peroxidase activity of the leaves as compared to control during both the years. Agarwala and Mehrotra (1977) also recorded increased peroxidase activity with the application of iron as FeEDTA in bael (*Agle marmelos*) and bougainvillea (*Bougainvillea spectabilis*). However, no information is available on the comparison of enhanced peroxidase activity in the low-chill peaches when applied with different iron sources.

Leaf mineral status (both physiologically active iron/ Fe^{2+} and total iron) was improved with the application of different iron sources. In our experiment, application of Fe-sulphate found to be the best iron source for foliar application. This might be attributed to more penetration capacity of this iron source across the leaf epidermis possibly due to lesser molecular weight than other two iron sources. The compounds having low molecular weight were reported to be suitable for foliar fertilization (Schönherr 2001). Further, no reduction of iron was required during entry into the cells by the enzyme ferric chelate reductase (FCR) when Fe-sulphate was used (Brüggemann et al. 1993). While during spraying of other two sources, iron was reduced at the plasmamembrane of the leaf cells. In Fe-citrate, iron is bind with an organic acid i.e. citric acid. Citric acid acts as a carrier of iron within the plant system during translocation (Tiffin 1965). This might be the plausible cause of intermediate effect of Fe-citrate among the three different iron sources. Larger molecular weight and FCR regulated reduction step restricted the leaf iron enrichment from FeEDTA source in low-chill peach cultivars. Absorption capacity of nutrient from inorganic sources by the leaves was reported to be higher than chelated form of nutrients (Abadía et al. 2002). Significant increase in total and ferrous iron content of the leaves had also been found with foliar application of ferrous sulphate + H_2SO_4 than Fe-EDDHA in avocado (Crane et al. 2007).

Among the low-chill peach cultivars, Saharanpur Prabhat responded best with iron supplementation in both the leaves than the other low-chill cultivars of peach. This might be ascribed to the higher absorption capacity of the foliar

applied nutrient by this cultivar. Furthermore, the utilization of foliar applied iron within the plant system to induce physiological and biochemical responses could also be higher. These findings indicated that this cultivar has a better genetic make up to respond upon iron application than other low-chill peach cultivars.

Conclusion

Application of 0.5 % Fe-sulphate proved to be the cost effective way for management of iron chlorosis in low-chill peaches. A genotype dependent plant physiological and biochemical responses were observed upon foliar application of different iron sources in the peach cultivars. The low-chill peach cultivar Saharanpur Prabhat found to be suitable for growing in calcareous soil. However, a strong recommendation to grow this cultivar in calcareous soil requires further study in the other aspects of science.

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