

Influence of *Pseudomonas fluorescens* and ferrous sulphate on biochemical parameters of peach leaves

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Abstract

Foliar fertilization and microbe supplemented iron bioavailability can fulfill the iron demand of plants. Plants of low-chill peach cv. Pant Peach-1 were selected to find out the effect of *Pseudomonas fluorescens* PBAP-27 inoculation along with foliar spray of ferrous sulphate. The experiment consisted of eight treatments viz. T₁: control (water spray), T₂: *P. fluorescens* PBAP-27, T₃: FeSO₄·7H₂O (0.5 %) spray once, T₄: FeSO₄·7H₂O (0.5 %) spray twice, T₅: FeSO₄·7H₂O (0.5 %) spray thrice, T₆: *P. fluorescens* PBAP-27+FeSO₄·7H₂O (0.5 %) spray once, T₇: *P. fluorescens* PBAP-27+FeSO₄·7H₂O (0.5 %) spray twice, T₈: *P. fluorescens* PBAP-27+FeSO₄·7H₂O (0.5 %) spray thrice. Leaf area, chlorophyll content, active iron, total iron, ferric iron and iron: manganese ratios of leaves were studied. Maximum leaf area was recorded with *P. fluorescens* PBAP-27+FeSO₄·7H₂O (0.5 %) by spraying twice. Whereas, chlorophyll content, active iron, total iron, ferric iron and iron: manganese ratio were recorded higher in *P. fluorescens* PBAP-27+FeSO₄·7H₂O (0.5 %) sprayed thrice. Findings indicate that the three foliar spray of ferrous sulphate (0.5 %) along with inoculation of *P. fluorescens* strain can be used as useful strategy for iron fertilization for improving physiological parameters (leaf area and chlorophyll content) and leaves iron status (active iron, total iron, ferric iron) in low chill peach.

Key words: Ferrous sulphate, *Pseudomonas fluorescens*, Chlorophyll content, Iron content

Introduction

Peach [*Prunus persica* (L.) Batsch] is a temperate fruit crop belongs to family rosaceae, genus *Prunus*, and subgenus *Amygdalus*. In India, peach is one of the major temperate fruit crops, and cultivated in 18.10 thousand ha area with the production of 93.52 thousand tons (Anonymous, 2015). Iron, a multifunctional element, necessary for different physiochemical process, is classified as an essential micronutrient for the growth of plants. It is involved in several physiological activities of plants as a component of several enzymes and proteins that involved in ETC (electron transport chain), photosynthesis and respiration (Taiz and Zeiger, 1991; Lucena and Hernandez-Apaolaza, 2017). Due to iron limiting condition of the soil, the poor functioning of enzymes and proteins in plant system results in less yield of fruits.

The soil pH and bicarbonate concentration influence iron availability in the soil. With the increase in both, the iron becomes limited for uptake by the roots of the plants especially that depends on ferric reductases activity (Lucena *et al.*, 2007 and Jeong and Connolly, 2009). Peach is most affected by iron chlorosis (Alvarez-Fernandez *et al.*, 2005; Tsipouridis *et al.*, 2006). The effect is still worse with the plants growing in calcareous and alkaline soils (Tagliavini and Marangoni, 2002). Foliar fertilization with deferent iron sources is a common strategy to combat the problem. Though, the variable responses of exogenously applied iron are recorded.

Iron deprivation in soil through the microbes has been reported by Kloepper *et al.* (1980). According to findings, plant growth is promoted due to the iron chelating effect of siderophore produced by PGPR (plant growth promoting rhizobacteria). Soil and foliar management practices could be assisted with the microbe supplemented iron bioavailability. Microbial siderophore helps in iron transport has been demonstrated by Crowley *et al.* (1988) and Sah *et al.* (2017). *Chryseobacterium sp.* C138 from the rhizosphere of *Oryza sativa* and *Pseudomonas fluorescens* N21.4 from the rhizosphere of *Nicotiana glauca* are two siderophore-producing bacterial strains. Treatment of iron-starved tomato plants in hydroponics culture by siderophore of both bacteria with or without bacterial cell significantly increased chlorophyll and iron content over the positive controls; confirm the effectiveness of siderophore in providing iron nutrition to tomato plants (Radzki *et al.*, 2013). Bona *et al.* (2017) also reported that plant growth-promoting pseudomonads improve yield and quality of tomato.

The research on iron nutrition in peach has not given an effective solution, up to date. It is, therefore, necessary to evaluate the response of foliar iron (FeSO₄·7H₂O) spray and microbial (*P. fluorescens*) inoculation on peach. The present investigation was undertaken to study the physiological parameters and leaf iron status of low chill peach plants grown in calcareous pot media as influenced by foliar iron (FeSO₄·7H₂O) spray and microbial (*P. fluorescens* PBAP-27) inoculation.

Materials and methods

The experiment was conducted in the nursery at Horticulture Research Centre, Patharchatta of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) from December to June (2014-15 and 2015-16).

Experimental materials: One-year-old plants of low-chill peach cultivar Pant Peach-1 was selected for the experiment. Plants were prepared by tongue grafting on peach rootstock. All grafted plants were kept under uniform cultural practices in the nursery. The experiment was performed in pot culture. Artificially prepared calcareous soil media was used to fill pots for the experiment.

Preparation of calcareous soil: Soil between the depths of 10-25 cm was collected from the peach orchard located at Horticulture Research Station. CaCO_3 @ 3 percent was mixed with collected soil and dried in shade for a week. After complete drying, 5 kg soil was weighted out and put in each pot. The flooding of each pot was done with water and left as such until drying. Plants show iron stress when grown in calcareous soil due to a lower concentration of available iron (Loeppert, 1986).

Planting and aftercare: Planting was done after filling the pot with calcareous soil. Grafted plants of Pant Peach-1 were planted in each pot during the dormant season (December). Just after planting, plants were gently watered. Following planting, slight pruning was performed to maintain the uniform height of plants. Nitrogen, phosphorus and potassium were supplied in the pots for proper growth and establishment of plants. Among micronutrients, only zinc was applied @ 2.5 ppm as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. Nutrients were applied in solution form and then mixed it with the soil (Chakraborty *et al.*, 2014).

Experimental design: There were eight treatments replicated four times in Completely Randomized Design having one plant per pot per treatment.

Application of *Pseudomonas*: Powder form of *Pseudomonas* was collected from Bio Control Lab, Department of Plant Pathology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India. 10 g of the inoculants powder was mixed per liter of water and the solution poured into each pot for treatment in evening followed by mixing in the soil. Treatment was applied twice, first after planting and second after a month interval.

Application of Fe source: Ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was used as iron source to the plants. It was applied at the concentration of

0.5 percent prepared by dissolving 5 g of the chemical per litre of water. Fifteen days interval was given between each spray. First spraying was done in March.

Results and discussion

Leaf area: The data pertaining to the effect of *P. fluorescens* inoculation and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ treatments on leaf area clearly indicated that all the treatments had a significant effect on leaf area over control. Maximum leaf area 8.48 cm^2 was recorded under treatment T_7 [*P. fluorescens* PBAP-27+ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) sprayed twice] which was statistically *at par* with treatment T_8 [*P. fluorescens* PBAP-27+ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) sprayed thrice] whereas the minimum leaf area 6.39 cm^2 was recorded under control. Plant morphology is very much altered with iron deficiency (Briat, 2007; Jimenez *et al.*, 2019). Thereby, exogenous application of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ may increase peach leaf area.

Chlorophyll content: The mean of chlorophyll content is presented in Table 1. The maximum chlorophyll 'a' content 1.12 mg g^{-1} FW was recorded under treatment T_8 [*P. fluorescens* PBAP-27+ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) sprayed thrice], which was statistically *at par* with T_7 [*P. fluorescens* PBAP-27+ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) spray twice] *i.e.* 1.04 mg g^{-1} FW. The minimum chlorophyll 'a' content (0.80 mg g^{-1} FW) was recorded under T_1 (control). The maximum chlorophyll 'b' content (0.36 mg g^{-1} FW) was recorded under treatment T_8 [*P. fluorescens* PBAP-27+ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) sprayed thrice]. The minimum chlorophyll 'b' content (0.23 mg g^{-1} FW) was recorded under control *i.e.*, T_1 . The maximum total chlorophyll content 1.50 mg g^{-1} FW was recorded under treatment T_8 [*P. fluorescens* PBAP-27+ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) sprayed thrice]. The minimum total chlorophyll content (1.03 mg g^{-1} FW) was recorded under control (T_1).

It is evident from the data (Table 1) that chlorophyll content of peach leaves increased with the inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate because of the fact that Fe deficiency alters both chloroplast structure and photosynthetic rate in higher plants (Eberhard *et al.*, 2008). Sainy *et al.* (2017) also reported that *P. fluorescens* improve total chlorophyll content in *Zinnia elegans*. Increase in leaf chlorophyll content by exogenous application of ferrous sulphate has been also reported by Chakraborti *et al.* (2012) in peach.

Iron content: Data presented in Table 2 showed that the active iron content in leaves was significantly influenced by the inoculation of *P. fluorescens* and foliar spray of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The maximum active iron content of leaves (25.54 mg kg^{-1} DW) was determined

Table 1. Effect of the inoculation of *P. fluorescens* and foliar spray of ferrous sulphate on leaf area and chlorophyll content of peach leaves.

Treatment	Leaf area (cm^2)	Chlorophyll 'a' (mg g^{-1} FW)	Chlorophyll 'b' (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)
T_1 : Control	6.39	0.80	0.23	1.03
T_2 : <i>P. fluorescens</i> PBAP-27	6.82	0.83	0.28	1.10
T_3 : $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) spray once	6.90	0.81	0.27	1.08
T_4 : $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) spray twice	7.00	0.89	0.31	1.19
T_5 : $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) spray thrice	8.39	0.99	0.33	1.29
T_6 : <i>P. fluorescens</i> PBAP-27+ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) spray once	7.13	0.94	0.31	1.26
T_7 : <i>P. fluorescens</i> (PBAP-27)+ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) spray twice	8.48	1.04	0.35	1.37
T_8 : <i>P. fluorescens</i> (PBAP-27)+ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 %) spray thrice	8.46	1.12	0.36	1.50
S.Em	0.23	0.02	0.01	0.02
CD at 5%	0.69	0.08	0.03	0.08

Table 2. Effect of the inoculation of *P. fluorescens* and foliar spray of ferrous sulphate on iron content of peach leaves

Treatment	Active iron (mg kg ⁻¹ DW)	Total iron (mg kg ⁻¹ DW)	Ferric form iron (mg kg ⁻¹ DW)	Iron: manganese ratio
T ₁ : Control	10.57	44.79	34.22	1.23
T ₂ : <i>P. fluorescens</i> PBAP-27	17.14	57.33	40.20	1.69
T ₃ : FeSO ₄ .7H ₂ O (0.5 %) spray once	14.42	53.16	38.74	1.55
T ₄ : FeSO ₄ .7H ₂ O (0.5 %) spray twice	18.80	62.43	43.64	1.97
T ₅ : FeSO ₄ .7H ₂ O (0.5 %) spray thrice	22.97	73.47	50.50	2.45
T ₆ : <i>P. fluorescens</i> PBAP-27+FeSO ₄ .7H ₂ O (0.5 %) spray once	19.83	66.35	46.52	2.13
T ₇ : <i>P. fluorescens</i> (PBAP-27)+FeSO ₄ .7H ₂ O (0.5 %) spray twice	24.68	76.54	51.86	2.78
T ₈ : <i>P. fluorescens</i> (PBAP-27)+FeSO ₄ .7H ₂ O (0.5 %) spray thrice	25.54	81.06	55.52	3.22
S.Em	0.47	1.66	1.74	0.08
CD at 5%	1.38	4.85	5.09	0.25

under treatment T₈ [*P. fluorescens* PBAP-27+FeSO₄.7H₂O (0.5 %) sprayed thrice] which was statistically *at par* with treatment T₇ [*P. fluorescens* PBAP-27+FeSO₄.7H₂O (0.5 %) sprayed twice] *i.e.*, 24.68 mg kg⁻¹ DW. The minimum active iron content of leaves (10.57 mg kg⁻¹ DW) was recorded with control (T₁). It is evident from the data that treatment T₈ [*P. fluorescens* PBAP-27+FeSO₄.7H₂O (0.5 %) sprayed thrice] resulted maximum total iron (81.06 mg kg⁻¹ DW) which was statistically *at par* with treatment T₇ [*P. fluorescens* PBAP-27+FeSO₄.7H₂O (0.5 %) sprayed twice] *i.e.* 76.54 mg kg⁻¹ DW. The minimum content of total iron (44.79 mg kg⁻¹ DW) was recorded under T₁ (control). Data regarding ferric iron content of leaves revealed that treatments had a significant effect on total iron content in leaves. The maximum ferric iron content of leaves (55.52 mg kg⁻¹ DW) was recorded with the treatment T₈ [*P. fluorescens* PBAP-27+FeSO₄.7H₂O (0.5 %) spray thrice], which was statistically *at par* with treatment T₇ [*P. fluorescens* PBAP-27+FeSO₄.7H₂O (0.5 %) spray twice]. Whereas, the minimum ferric iron content of leaves (34.22 mg kg⁻¹ DW) was recorded under T₁ (control). The role of microbial siderophores on the iron acquisition by roots has been demonstrated by Crowley *et al.* (1991). Exogenous application of different iron sources significantly improved both physiologically active iron (Fe²⁺) and total iron concentration of leaves was also reported by Chakraborty *et al.* (2012) in peach.

Data regarding iron: manganese ratio of leaves revealed significant differences among the treatments. The highest ratio of iron: manganese in leaves (3.22) was recorded with treatment T₈ [*P. fluorescens* PBAP-27+FeSO₄.7H₂O (0.5 %) sprayed thrice], whereas, the minimum ratio of iron: manganese in leaves (1.23) was recorded under T₁ (control). It is evident from the data that Fe:Mn ratio differ significantly among the treatments direct competition between manganese and iron for a position in the heme nucleus of the enzyme (Twyman, 1951; Sideris and Young, 1949). Hence, the exogenous application of iron sulphate might have altered Fe:Mn ratio of peach leaves.

Physiological parameters and leaf iron status have a direct influence on fruit yield and quality. Based on the finding, it can be concluded that inoculation of *P. fluorescens* and foliar spray of ferrous sulphate was effective in increasing the chlorophyll content and iron status of leaves indicating that fruit yield and quality in peach can be increased with the inoculation of *P. fluorescens* and foliar spray of ferrous sulphate, under low iron availability conditions in soil.

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