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PLANT PATHOLOGY

Influence of *Pseudomonas fluorescens* and Ferrous Sulphate on Iron Status of leaves and Fruit Quality of Low Chill Peach cv. Flordaprince

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ABSTRACT

An experiment was conducted to find out the effect of *Pseudomonas fluorescens* PBAP-27 along with foliar spray ferrous sulphate on low-chill peach plants suffering from iron deficiency. Leaf iron status viz. active iron, total iron and ferric iron and physico-chemical parameters of fruits viz. weight, volume, ascorbic acid, reducing sugar, non-reducing sugar and total sugar were recorded. The results obtained from the investigation showed significant increase in leaf iron status, fruit weight, fruit volume and ascorbic acid content in fruit with the inoculation of *Pseudomonas fluorescens* PBAP-27 as well as foliar spray of ferrous sulphate. The inoculation strain PBAP-27 of *Pseudomonas fluorescens* along with foliar spray of ferrous sulphate were noted as significant for increasing leaf iron status and ascorbic acid. Thus, inoculation of *Pseudomonas fluorescens* PBAP-27 and foliar spray of ferrous sulphate, can be used as strategy for iron fertilization for improving leaves iron status and fruit quality parameters namely , fruit weight, fruit volume and ascorbic acid in low chill peach.

Highlights

- Inoculation of *Pseudomonas fluorescens* along with foliar spray of ferrous sulphate improved iron status of leaves.
- Weight, volume and ascorbic acid content of fruits were improved by both treatments *i.e.* inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate.

Keywords: Peach, *Pseudomonas*, ferrous sulphate, fruit quality

Peach [*Prunus persica* (L.) Batsch], a stone fruit of temperate climate, belongs to family rosaceae, genus *Prunus* and subgenus amygdalus (Li 1984). Inside varietal wealth of peach, a distinct set of cultivars with low chilling requirement for bud burst and growth is called as low-chill peach. Farmers in subtropical areas of India prefer to grow low-chill peach because of its early arrival in market and premium prices. In India, peach is one of the major temperate fruit crops, and it is cultivated in 18.10 thousand ha area with the production of 93.52 thousand tons (Anonymous 2015). Peach suffered loss in yield and quality of fruit due to iron deficiency (Alvarez-Fernández *et al.* 2003). Iron, a multifunctional element, necessary for deferent physiochemical process, is classified as essential micronutrient for the growth of plants. It involved in several physiological activity 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).

Peach, a temperate climate fruit, is most affected by the problem of iron chlorosis. (Tsipouridis *et al*. 2002; Alvarez-Fernandez *et al*. 2005). Foliar fertilization with deferent iron sources is a common strategy to combat the problem. Though, the variable responses of exogenously applied iron are recorded. Treatment of iron-starved tomato plants in hydroponics culture by siderophore of both bacteria with or without bacterial cell significantly increased yield, chlorophyll and iron content over the positive controls with Hoagland solution, prove the effectiveness of siderophore in providing iron to plants of tomato (Radzki *et al*. 2013). An experiment conducted under green house condition on red bean showed significantly higher plant growth factors when inoculated with the strains of Flourescent *Pseudomonas* namely; 7NSK2, UTPF5 and UTPF 76 (Omidvari *et al*. 2010). Bona *et al.* (2017) also reported that plant growth-promoting pseudomonads improve yield, quality and nutritional value of tomato. It is with this background, present investigation was undertaken with the objectives to find out the efficacy of foliar spray of ferrous sulphate and *Pseudomonas fluorescens* PBAP-27 inoculation on iron status of foliage and fruit quality of *in vivo* peach plants.

MATERIALS AND METHODS

The present investigation was carried out at the Horticulture Research Center, Patharchatta and at the Laboratory of Department of Soil Science, G. B., Pant University of Agriculture and Technology, Pantnagar Uttarakhand during 2014-15 and 2015-16. The experimental site is situated at 29.5° North latitude and 79.3° of East longitude at an altitude of 243.84 meter above the mean sea level. Experiment conducted on seven year old trees of peach cv. Flordaprince, almost uniform in growth and vigour. Similar cultural operations were given during the course of the investigation. One tree selected as a unit of treatment in each replication. The experiment was laid out in split plot design with four replications, comprising two levels as main plot, *i.e.*, inoculation (I_1) of *Pseudomonas fluorescens* PBAP-27 and no inoculation (I_0) of *P. fluorescens* PBAP-27. Ten gram of the inoculant powder of *Pseudomonas fluorescens* was mixed with per liter of water. Two liter solution was poured to each tree basin for inoculation (I_1) in evening followed by mixing in soil. In sub plot, four levels of foliar spray (F) of ferrous sulphate (0.5%) *i.e.*, F_0 (no spray), F_1 (single spray), F_2 (two spray) and F_3 (three spray) were given. First spray of ferrous sulphate was given after fruit set and a fortnight interval was given between two sprays. The observations were recorded on leaves iron (active iron, total iron and ferric iron) and fruit quality (weight, volume, ascorbic acid, reducing sugar, non-reducing sugar and total sugar). Active iron concentration of leaves was determined according to the method described by Katyal and Sharma (1980). For analysis of total iron content in digested samples of leaves of peach plants, the samples were prepared according to the method A.O.A.C. standard procedure, and total iron content was analysed with the help of atomic absorption spectrophotometer (AAS). The ferric iron content was determined by subtracting the active iron content from the total iron content of the same plant and represented as mg kg^{-1} DW. Weight of ten fruits per replication was recorded by using physical balance. The average weight was obtained by dividing the total weight of fruits by the number of fruits. The volume of fruits was measured by water displacement method and average volume was worked out for a single fruit and expressed as ml. The ascorbic acid content in fresh fruit sample was estimated by reduction of 2, 6- dichlorophenol indophenol dye by ascorbic acid (AOAC 1980). Sugar (reducing, non-reducing and total sugar) was estimated by method of AOAC (1980). The results obtained during two years pooled and data thus obtained were subjected to statistical analysis as per methods outlined by Cochran and Cox (1966).

RESULTS AND DISCUSSION

Observation of data (Table 1) showed that physiologically active iron, total iron and ferric iron content in leaves were significantly influenced by the inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate. The interaction between inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate was significant for increasing in active iron content of leaves. It was evident from the data that the leaves of plants inoculated with *Pseudomonas fluorescence* (I_1) contained higher content of physiologically active iron (21.87 mg kg^{-1} DW), total iron (69.63 mg kg^{-1} DW) and ferric iron (47.70 mg kg^{-1} DW) in leaves

Table 1: Effect of the inoculation of *Pseudomonas fluorescens* PBAP-27 and foliar spray of ferrous sulphate on active iron, total iron and ferric iron content in peach leaves

Foliar spray of ferrous sulphate (F)	Inoculation (I)								
	Active iron (mg^{-1} kg DW)			Total iron (mg^{-1} kg DW)			Ferric iron (mg kg^{-1} DW)		
	I_0	I_1	Mean	I_0	I_1	Mean	I_0	I_1	Mean
F_0	9.90	15.00	12.45	49.36	53.66	51.51	39.46	38.66	39.06
F_1	11.85	22.78	17.31	54.28	71.03	62.66	42.44	48.25	45.34
F_2	17.68	24.03	20.86	60.08	73.93	67.00	42.40	49.90	46.15
F_3	20.13	25.66	22.89	66.55	79.90	73.22	46.42	53.99	50.21
Mean	14.89	21.87		57.57	69.63		42.68	47.70	
	S.Em.±	CD at 5%		S.Em.±	CD at 5%		S.Em.±	CD at 5%	
To compare two I mean	0.31	1.40		0.52	2.34		0.24	1.11	
To compare two F mean	0.34	1.01		0.92	2.74		0.48	1.42	
To compare two F mean at a given I	0.47	1.44		1.28	3.88		0.66	2.01	
To compare two I mean, either at a given F or at different F	0.51	1.84		1.22	4.03		0.62	2.04	

F_0: No iron spray (water spray), F_1: Foliar spray of ferrous sulphate (0.5%) once, F_2: Foliar spray of ferrous sulphate (0.5%) twice, F_3: Foliar spray of ferrous sulphate (0.5%) thrice, I_0: No inoculation, I_1 : Inoculation of P. fluorescens PBAP-27. S.Em.±: Standard error mean, CD: critical difference.

over the content of physiologically active iron, total iron and ferric iron with no inoculation of the *Pseudomonas fluorescens* (I_0) *i.e.,* 14.89 mg kg^{-1} DW, 57.57 mg kg^{-1} DW and 42.68 mg kg^{-1} DW, respectively. Three foliar spray of the ferrous sulphate (0.5%) resulted in maximum physiologically active iron content (22.89 mg kg^{-1} DW), total iron (73.22 mg kg^{-1} DW) and ferric iron (50.21 mg kg^{-1} DW) content of the leaves, whereas, the minimum physiologically active iron (12.45 mg kg^{-1} DW), total iron (51.51 mg kg^{-1} DW) and ferric iron (39.06 mg kg^{-1} DW) content of the leaves were observed in plant sprayed with water (F_0). A significant interaction between *Pseudomonas fluorescens* treatment and foliar spray of the ferrous sulphate was observed for iron contents in leaves. The maximum physiologically active iron (25.66 mg kg^{-1} DW), total iron (79.90 mg kg^{-1} DW) and ferric iron (53.99 mg kg^{-1} DW) content of the leaves were observed with *Pseudomonas fluorescence* (I_1) application along with foliar spray of ferrous sulphate (0.5%) thrice (F_3) whereas, minimum content of active iron, total iron and ferric iron were recorded with no inoculation (I_0) along with water spray (F_0) *i.e.,* 9.90 mg kg^{-1} DW, 49.36 mg kg^{-1} DW and 38.66 mg kg^{-1} DW. It is evident from the data (Table 1) that iron content of peach leaves increased with increase in treatment levels due to the fact that the soil of experimental plot have low available iron and inoculation of *Pseudomonas fluorescence* and foliar spray of ferrous sulphate improve the iron level of peach leaves. Chakraborti *et al.* (2012) reported that exogenous application of different iron sources significantly improved both physiologically active iron (Fe^{2+}) and total iron concentration of leaves. The role of microbial siderophores on iron acquisition by roots has been demonstrated by studies by Crowley *et al.* (1991) and Sah *et al.* (2017).

Inoculation of *Pseudomonas fluorescens* (I_1) and foliar spray of ferrous sulphate were effective for increasing fruit weight and fruit volume, whereas, interaction between *Pseudomonas fluorescence* inoculation and foliar spray of ferrous sulphate was failed to exert significant effect. It is evident from the data (Table 2) that inoculation of *Pseudomonas fluorescence* (I_1) resulted a significantly higher fruit weight (49.98 g) and fruit volume (43.82 ml) over the weight and volume recorded without inoculation of the *Pseudomonas fluorescence* (I_0) *i.e.,* 47.22 g and 41.19 ml, respectively. Three (F_3) foliar spray of the ferrous sulphate resulted in maximum fruit weight (50.90 g) and fruit volume (44.17 ml), whereas, minimum fruit weight (45.96 g) and fruit volume (40.96 ml) were recorded with water spray (F_0). Inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate increased fruit weight because of the fact that bacterial inoculation enhanced soil iron availability to roots that helps in improving iron status of plants (Crowley *et al.*, 1991) and foliar application of ferrous sulphate also improved iron status of peach leaves that helped in higher fruit growth (Policarpo *et al.*, 2002). Our findings are in agreement with Ram and Bose (2000) who also reported that foliar spray of 0.25% ferrous sulphate increased average fruit weight of orange. Fruit volume increased with the treatments *Pseudomonas fluorescens* and ferrous sulphate because the treatment is directly correlated with size (length and breadth) of fruit. Chaturvedi (2003) also stated that micronutrients improved the physical characteristics of strawberry fruits.

The perusal of data in Table 2 indicated that ascorbic acid content in fruit pulp was significantly affected by inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate. The interaction between inoculation of *Pseudomonas fluorescence* and foliar spray of ferrous sulphate was also significant for ascorbic acid content in fruit. The inoculation of *Pseudomonas fluorescens* (I_1) resulted in significantly higher ascorbic acid content (5.78 mg) over the content recorded without inoculation of the *Pseudomonas fluorescence* (I_0) *i.e.*, 5.03 mg). Maximum ascorbic acid content (6.23 mg 100 g^{-1}) was recorded with three (F_3) foliar spray of ferrous sulphate (0.5%). The minimum ascorbic acid content (4.51 mg 100 g^{-1}) was recorded with water spray (F_0). Data showed significant interaction between inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate for increasing ascorbic acid content. The maximum ascorbic acid content (6.80 mg 100 g^{-1}) was recorded with inoculation of *Pseudomonas fluorescence* (I_1) along with three foliar spray of ferrous sulphate (0.5%). The minimum ascorbic acid content (4.16 mg 100 g^{-1}) was recorded in the combination of no inoculation of *Pseudomonas fluorescence* (I_0) and water spray (F_0). These findings are in agreement with the results of Kumbhar and Deshmukh (1993) who reported that application of ferrous sulphate significantly improved the ascorbic acid content in tomato cv. Rupali over control.

The data presented in Table 3 indicated that reducing sugar, non-reducing sugar and total sugar were not significantly influenced by inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate. The interaction between inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate also failed to exert significant change in sugars viz., reducing sugar, non-reducing sugar and total sugar. The higher reducing sugars (5.66%)

Table 2: Effect of the inoculation of *Pseudomonas fluorescens* PBAP-27 and foliar spray of ferrous sulphate on weight, volume and ascorbic acid content of fruits

Foliar spray of ferrous sulphate (F)	Inoculation (I)								
	Fruit weight (g)			Fruit volume (ml)			Ascorbic acid (mg/100g)		
	I_0	I_1	Mean	I_0	I_1	Mean	I_0	I_1	Mean
F_0	45.45	46.47	45.96	40.62	41.30	40.96	4.16	4.87	4.51
F_1	46.43	49.51	47.97	41.33	43.33	42.33	4.99	5.30	5.15
F_2	48.23	50.91	49.57	41.55	44.77	43.16	5.29	6.16	5.73
F_3	48.75	53.05	50.90	42.48	45.87	44.17	5.67	6.80	6.23
Mean	47.22	49.98		41.49	43.82		5.03	5.78	
	S.Em.±	CD at 5%		S.Em.±	CD at 5%		S.Em.±	CD at 5%	
To compare two I mean	0.25	1.14		0.49	2.21		0.07	0.34	
To compare two F mean	0.61	1.83		0.35	1.06		0.06	0.18	
To compare two F mean at a given I	0.86	NS		0.50	NS		0.08	0.26	
To compare two I mean, either at a given F or at different F	0.79	NS		0.65	NS		0.10	0.40	

F_0: No iron spray (water spray), F_1: Foliar spray of ferrous sulphate (0.5%) once, F_2: Foliar spray of $FeSO_4.7H_2O$ (0.5%) twice, F_3: Foliar spray of $FeSO_4.7H_2O$ (0.5%) thrice, I_0: No inoculation, I_1: Inoculation of P. fluorescens PBAP-27. S.Em.±: Standard error mean, CD: critical difference.

Table 3: Effect of the inoculation of *Pseudomonas fluorescens* PBAP-27 and foliar spray of ferrous sulphate on ascorbic acid and reducing, non-reducing and total sugar content in fruit pulp

Foliar spray of ferrous sulphate (F)	Inoculation (I)								
	Reducing sugars (%)			Non-reducing sugars (%)			Total sugars (%)		
	I_0	I_1	Mean	I_0	I_1	Mean	I_0	I_1	Mean
F_0	5.33	5.64	5.48	1.96	1.42	1.69	7.29	7.06	7.17
F_1	5.36	5.72	5.54	1.84	1.41	1.62	7.19	7.14	7.16
F_2	5.43	5.70	5.56	1.84	1.63	1.74	7.27	7.33	7.30
F_3	5.48	5.61	5.54	1.82	1.63	1.73	7.29	7.24	7.27
Mean	5.40	5.66		1.86	1.52		7.26	7.19	
	S.Em.±	CD at 5%		S.Em.±	CD at 5%		S.Em.±	CD at 5%	
To compare two I mean	0.06	NS		0.08	NS		0.01	NS	
To compare two F mean	0.07	NS		0.12	NS		0.08	NS	
To compare two F mean at a given I	0.10	NS		0.15	NS		0.03	NS	
To compare two I mean, either at a given F or at different F	0.11	NS		0.16	NS		0.01	NS	

F_0: No iron spray (water spray), F_1: Foliar spray of $FeSO_4.7H_2O$ (0.5%) once, F_2: Foliar spray of $FeSO_4.7H_2O$ (0.5%) twice, F_3: Foliar spray of $FeSO_4.7H_2O$ (0.5%) thrice, I_0: No inoculation, I_1: Inoculation of P. fluorescens PBAP-27. S.Em.±: Standard error mean, CD: critical difference.

was recorded with inoculation of *Pseudomonas fluorescence* (F_1), whereas, lower reducing sugars (5.40%) was recorded in without inoculation of *Pseudomonas fluorescence* (F_0). Higher non-reducing sugar and total sugar (1.86% and 7.26%, respectively) were recorded with no inoculation of *Pseudomonas fluorescens* (F_0) over per cent non-reducing sugar, and total sugar recorded with inoculation of *Pseudomonas fluorescens* (F_1) *i.e.*, 1.52% and 7.19%, respectively. Among the foliar spray of ferrous sulphate, reducing sugar, non-reducing sugar and total sugar varied from 5.48 to 5.56%, 1.62 to 1.74 and 7.16 to 7.30 respectively. In peach, sucrose (reducing sugar) accumulation at maturity (Vizzotto *et al*., 1996; Cantín *et al*., 2009) depends on de novo synthesis in the mesocarp (Morandi *et al*., 2008), thus being likely independent of leaf photosynthetic rates.

CONCLUSION

Inoculation of *Pseudomonas fluorescens* and foliar spray of ferrous sulphate were effective in improving the leaf iron status and fruit quality. Thus, based on present findings it can be concluded that inoculation of *Pseudomonas fluorescens* along with foliar spray of ferrous sulphate could be strategy for iron fertilization in peach grown under iron limiting soil condition.

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