Chapter

Microbes for Iron Chlorosis Remediation in Peach

Saurabh Kumar Singh

Abstract

Peach \textit{(Prunus persica (L.) Batsch)} suffers from iron chlorosis when grown in calcareous soils due to low iron availability. Traditionally, soil and foliar application of ferrous sulphate, Fe-EDTA, Fe-EDDHA chelates, etc. is used as a corrective measure of chlorosis. The latter practice is quite effective. However, variable responses have been reported. Therefore, foliar spray cannot yet be considered as a reliable method to control lime-induced chlorosis. Bioremediation constitutes innovative approaches for chlorosis correction. Iron fixations in calcareous soil, iron uptake by plants, and advance detection techniques and correction strategies in plants for iron chlorosis have been discussed in this chapter. The microbe-mediated correction strategies are identified as eco-friendly.

Keywords: peach, \textit{Prunus persica}, calcareous soil, iron chlorosis, bioremediation

1. Introduction

Peach \textit{(Prunus persica (L.) Batsch)} is one of the most common temperate region fruit crops of the world. China, Italy, the USA, Greece, Spain, Turkey, Iran, Chile, etc. are the major producing countries [1]. This stone fruit crop belongs to the family Rosaceae. Peach \textit{(Prunus persica var. vulgaris} Maxim.) with round and fuzzy fruit, the nectarine \textit{(Prunus persica var. nectarina} (Aiton) Maxim.) with round fruit but without pubescence (fuzz), and the flat peach \textit{(Prunus persica var. platicarpa} Bailey) with flat-shaped fruit are the three categories [2]. Iron, the fourth most prevalent element preceded by O, Si, and Al in the earth's crust and soils, is classified as an essential micronutrient for plant growth. It is a multifunctional element [3], required for the different physicochemical processes of plants, and plays an important role in chlorophyll activation, chloroplast membrane structure, photosynthesis, respiration, and synthesis of many heme proteins and iron–sulphur (Fe-S) clusters as cofactors of proteins that function in the fundamental life of plants [4–6]. Higher plants use two general mechanisms (strategies I and II) for iron acquisition with low iron availability in soil [7]. Calcareous soil gives lower iron availability abreast with a diminishing uptake efficiency by plant roots specially of a plant that depends on ferric reductase activity, because of higher soil pH and bicarbonate concentration [8, 9]. Out of a total of 13.4 billion ha global land surface, 1.5 billion ha is used in crop production, including arable lands plus lands under permanent crops [10, 11]. 30% of the soils in the world are calcareous in nature. They limit the iron availability for plant growth and development, not due to the iron status of the soil but due to their solubility [12].
Iron chlorosis in calcareous soils is often termed as lime-induced iron chlorosis [13]. Applications of iron sources either in soil or as foliar spray are generally practised to correct the iron chlorosis in peach. An overview of the causes, detection methods, and control measures is given in Table 1. Kloepper et al. [14] gave a pioneer verification of iron-depriving microflora in soil and reported the plant growth-promoting activity of rhizobacteria pertaining to the iron-chelating siderophores. These are low-molecular-weight metabolites having a high affinity for Fe(III). Involvement of siderophore and proton production resulted in improved iron bioavailability in the root zone of plants [15, 16]. This chapter addresses the current trend of detection methods and control measures of iron chlorosis in peach and gives attention to bioremediation techniques for the correction of lime-induced iron chlorosis.

2. Iron fixation in calcareous soil

Calcareous soils have often more than 15% CaCO₃. Soil with high CaCO₃ belongs to calcisols and related calcic subgroup of other soils, dominantly found in dried areas of the earth [17]. Plants show iron stress when grown in calcareous soil due to lower concentration of available iron [18]. The two oxidation states of iron are the reduced form, i.e. ferrous iron (Fe²⁺), and the oxidized form, i.e. ferric iron (Fe³⁺), in all living forms. CaCO₃ directly participates in the reactions that decrease the iron availability to the plants. The reactions of iron fixation are as follows:

\[ \text{Fe}^{2+} + \text{CaCO}_3 \rightleftharpoons \text{FeCO}_3 + \text{Ca}^{2+} \quad (1) \]

\[ 4\text{FeCO}_3 + \text{O}_2 + \text{Ca(HCO}_3)_2 \rightleftharpoons 2\text{Fe}_2(\text{CO}_3)_2 + \text{Ca(OH)}_2 \quad (2) \]

\[ \text{Fe}_2(\text{CO}_3)_3 + 3\text{H}_2\text{O} \rightleftharpoons \text{Fe}_2\text{O}_3 + 3\text{H}_2\text{CO}_3 \quad (3) \]

Ferrous iron (Fe²⁺) is fixed as ferric oxide (Fe₂O₃) and becomes unavailable to plant roots.

3. Mechanism for iron uptake in higher plants

The iron uptake mechanisms of higher plants can be categorized into two groups as plant strategy and microbe mediated. In plant strategy mechanism, plants use two strategies, viz. strategy I and strategy II of iron uptake [19], whereas in microbe
mediated through Fe siderophore complexes [14]. A brief description of uptake mechanisms is given in the following subheadings.

3.1 Plant strategies for iron uptake

From small seasonal cereal crops like rice, wheat, etc. to the perennial tall fruit crops, two strategies are recognized for iron uptake: strategy I (for dicots and nongraminaceous monocots) and strategy II (for graminaceous species) [7, 8, 20, 21].

Dicots and nongraminaceous monocots use strategy I for iron destabilization in the root zone of the plants. The reduction of Fe$^{3+}$ to Fe$^{2+}$ at the root surface, increased proton (H$^+$) extrusion, and release of reducing and/or chelating substances are the three main mechanisms in the plants that use strategy I [20], whereas strategy II is expressed only in the grass family. Exudation of iron-chelating compounds, i.e. phytosiderophores (non-proteinogenic amino acids), from the roots helps in mobilizing Fe(III) as Fe phytosiderophore complexes. Finally, the Fe phytosiderophore complexes are absorbed by plant roots [7]. Peach suffers from iron chlorosis due to lower efficiency of iron chelation at the root zone in calcareous soils. The ferric-chelate reductase (FC-R) ability of the roots can be used for Fe$^{3+}$ tolerance screening tool [22].

3.2 Microbe-mediated iron uptake

Besides strategies I and II of the plants to absorb iron under limiting conditions, there is also microbial solubilization of iron in the soil. Evidence of plant growth-promoting rhizobacteria (PGPR)-mediated iron bio-solubilization was reported by Kloepper et al. [14]. A number of microbes that predominantly belong to *Pseudomonas* and *Trichoderma* genera of bacterial and fungal groups, respectively, have been reported for bio-solubilization of iron. They release siderophore, like the phytosiderophores of the plants of strategy II group. Siderophores are low-molecular-weight (500–1500 daltons) iron-chelating compounds [15], synthesized by micro-organisms, i.e. *Pseudomonas*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Serratia*, *Azospirillum*, *Rhizobium*, *Trichoderma*, *Cenococcum geophilum*, and *Suillus granulatus* [23–29]. Microbial siderophores are structurally diverse low-molecular-mass (200–2000 Da) [30, 31] compounds, with distinctive characteristics of Fe siderophore complex formation. Siderophores are usually classified by the ligands used to chelate the ferric iron by moieties donating the oxygen ligands for Fe(III) coordination and its specific chemical property. The major groups of siderophores include the catecholates, hydroxamates, and carboxylates. The catecholate is a dominating siderophore produced by bacteria, whereas the hydroxamate is produced by fungi [15, 32, 33]. They make stable complex with iron as Fe siderophore soluble complex, in soil solution and at the mineral surface, and then become available for uptake by the cell membrane of plant roots. Further, upon absorption, siderophores of Fe siderophore complexes are either recycled or destroyed [34–36]. Due to complex formation property of siderophores with iron, the Fe siderophore form of soil iron which can be utilized in controlling chlorosis of peach grown in calcareous soil has been little explored hitherto.

4. Markers for advance detection of Fe chlorosis

Chlorophyll content [37, 38], SPAD index [38–42], chlorophyll fluorescence [43, 44], thylakoid membrane lipids [45], photosynthetic rate [46], physiologically active iron [47–50], Fe/Mn ratio [51, 52], and transformed reflectance spectra [53]
are important physiological parameters used for the detection of iron chlorosis in different crops. Literature supports the possibility of using physiological and molecular markers as advance detection technique of iron chlorosis.

4.1 Physiological markers

Brown [54] emphasized to study the biochemical basis of iron chlorosis and its contributing factors. Efficiency of iron uptake depends on plant species [55]. Iron status of different plant parts like leaves, bark, flowers, vegetative buds, and floral buds has been reported by using tissue index in different crops for predicting the iron chlorosis. Floral analysis is reported as a tool for prediction of iron deficiency in peach [56, 57].

Iron plays an important role in chlorophyll formation [58, 59]. The reduction in the number of granal and stromal lamellae per chloroplast and in the number of thylakoids per granum under iron stress condition was reported by Spiller and Terry [60]. In parallel, Terry [61] also reported a decrease in chlorophyll (Chl) a and Chl b contents of sugar beet (Beta vulgaris L.) leaves under Fe stress condition and no effect on the number of chloroplast per unit area. The quantitative reduction (75%) in chlorophyll content per unit area and role of iron in chloroplas development were also noted in sugar beet [62, 63]. The findings showed that there is a quantitative decrease in chlorophyll content of leaves under iron stress condition. Chlorophyll fluorescence and iron concentration in the flowers of peach, root apoplastic iron in soya bean, and morphological changes of plant root coupled with alteration in citrate concentration in the phloem of castor bean are found to be directly correlated with chlorosis under iron stress condition [64–67]. So, chlorophyll content of leaves, SPAD index reading, chlorophyll fluorescence, concentration of iron in plant parts, and change in root morphology can be used as markers for advance detection of iron stress. These predictions will be helpful in managing the iron chlorosis in peach. Foliar iron application could be used for remediation of chlorosis problem [68]. Nicotianamine (a non-proteinogenic amino acid), nitric oxide levels, and concentration of nutrients in the reproductive buds need extensive research to be used as markers for the selection of Fe efficient genotypes in Prunus sp. [69–73].

4.2 Molecular markers

The need to search for the blueprint of iron transport, molecular mechanism of genes controlling iron uptake, and intracellular storage was emphasized by Briat and Lobreaux [74]. Current researches clarified that in different micro-organisms, a small regulatory RNA, RyhB, plays an essential role in the metabolism of iron. Numerous data on the molecular level of iron transport in plants are published, and there is a need for a comprehensive research on iron homeostasis [19, 75, 76]. Arabidopsis thaliana (arabidopsis), Lycopersicon esculentum (tomato), and Pisum sativum (pea) are used as model plants to study strategy I of iron absorption. Iron is translocated as a ferric citrate complex in the xylem with the help of FRD3 effluxes of citrate, from root to shoot portion of plants [77]. A lot of information for molecular basis of iron transport and compartment have been decoded. There is a need to spell out each Fe translocation step, iron chelator complex, Fe flux, signal, and receptor regulating the Fe nutritional status [78, 79].

Fe is concentrated in the vacuoles of cells. A group of co-expression genes is involved in iron deficiency regulation [80, 81]. In iron translocation, there are functional links between Fe loading in vacuoles (AtVIT1 gene) and remobilization (AtVIT1 and AtNRAMP3 genes) in arabidopsis, and iron accumulation in vascular
globoids is obstructed with mystifying genes [80]. Gonzalo et al. [82] studied P 2175 (myrobalan plum) and Felinem (peach-almond hybrid) for the differential expression of genes involved in homeostasis. The genes PFRO2 (for reductase activity), PIRT1 (for transport in roots), and PAHA2 (for proton release) were expressed, and can be used as molecular markers in screening and developing cultivars as well as the rootstocks of fruit crops for iron tolerance. Molecular advancement of iron regulation and decoding of iron regulatory gene will be helpful in managing iron chlorosis in peach.

4.3 Index tissue

Based on nutrient status of a specific plant part, the fertilizer rate may be recommended to correct the nutritional deficiency. In sampling, the age of selected plant part and time of sampling should be considered. Concurrently, the sampling of plants damaged due to insect pest infestation, pathogen attacks, and mechanical injuries must be avoided [83]. Details in plant analysis principles, sampling procedure, and laboratory analysis are given by Jones [83]. In peach, the leaves near the current year growth should be sampled during mid-season of growth, with a sample size of 50–100 selected plants. The best sampling time in peach with correlation to yield was found at 60 days after full bloom [84].

5. Microbes for iron chlorosis remediation

Soil and foliar application of synthetic iron sources is used for controlling iron chlorosis in peach. The latter practice is quite effective. Foliar Fe fertilization is a widespread agricultural strategy to control lime-induced iron chlorosis [68, 85]. However, variable responses to Fe sprays have often been described, and foliar Fe fertilization cannot yet be considered a reliable strategy to control plant Fe deficiency [86, 87]. Soil applications of iron sources have their own limitation. Due to its oxidized form as ferric state in soil, it forms very insoluble minerals. In addition to their practical applicability and intricacy, these chelated chemicals are too expensive. Due to the limitation of application of iron source, microorganism-mediated bioavailability of iron can be an effective way to control iron chlorosis.

Crowley et al. [88] confer the existence of a microbial siderophore iron transport system in oat (Avena sativa cv. Victory). Application of bacterial siderophore of the two siderophore-producing bacterial strains, namely, Chryseobacterium spp. C138 from the rhizosphere of Oryza sativa and Pseudomonas fluorescens N21.4 from the rhizosphere of Nicotiana glauca, in iron-starved tomato plants grown in hydroponic culture resulted significantly in higher plant yield and chlorophyll and iron content [89]. The findings indicated that siderophores are helpful in providing iron to plant. Another experiment on red bean under greenhouse condition showed an increase in bean plant growth factors, significantly inoculated with 7NSK2, UTPF5, and UTPF 76 strains of fluorescent Pseudomonas [90]. The beneficial effects of microbial siderophores have potential to correct lime-induced chlorosis in peach.

6. Conclusions

Peach is unexplored in terms of application of bioremediation. It is therefore necessary to evaluate the response of microorganism for controlling iron chlorosis in peach, grown in calcareous soils. Microbial iron mobilization needs vast research for identifying efficient strains regarding iron mobilization and their effect on
plant growth, nutritional status, and yield. Bioremediation will help in reducing the dependency on chemical measure of controlling chlorosis in addition to eco-friendly remediation as a long-term solution.

Author details

Saurabh Kumar Singh
Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

*Address all correspondence to: saurabh3596@gmail.com

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