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A Preliminary Study on Physiological and Molecular Effects of Iron Deficiency in Fuji/Chistock 1

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A PRELIMINARY STUDY ON PHYSIOLOGICAL AND MOLECULAR EFFECTS OF IRON DEFICIENCY IN FUJI/CHISTOCK 1

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□ In order to get experimental data on apple rootstock with iron-efficient genotypes capable of improving scion resistance to iron deficiency, this experiment was conducted on the physiological and molecular characteristics of Fuji/ Chistock 1 (F/C) under different iron conditions and compared it to Fuji/ *M. Baccata* (F/B). F/C was less sensitive to iron deficiency than F/B. F/B showed chlorosis after 25 days under iron-deficient conditions, but F/C showed no phenotypic changes, even after 40 days. The shoot and leaf area growth of F/C were respectively 5 cm and 1000 mm² higher than those of F/B, regardless of the iron-deficient or iron-efficient conditions. The young leaf chlorophyll and active iron of F/C were 5 SPAD and 5 mg kg⁻¹ higher than those of F/B, either in iron-deficient or iron-efficient conditions. The expression of YSL5 and CS1 showed the same pattern. The enhancement expression of iron transport genes may be one explanation for these findings.

Keywords: graft, *Malus*, response to iron deficiency, rootstock

INTRODUCTION

Iron (Fe) is essential to the growth and development of fruit trees. Iron deficiency is particularly important to fruit trees, causing decreases in tree vegetative growth, marked decreases in fruit yield, and losses in quality (Álvarez-Fernández et al., 2006, Rombolà and Tagliavini, 2006, Hansch and Mendel, 2009). Iron deficient induced chlorosis is a major nutritional disorder in plants grown on calcareous soils in arid and semiarid regions. Plants

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grown on saline-alkali soil also show symptoms of iron deficiency (Jimenez et al., 2011). It has been estimated that 40% of soil from all over the world is poor in iron (Miller et al., 1984). This is especially true in northern China, where aerobic and calcareous soils are widespread. Iron deficiency is a large-scale problem in this area, so iron deficient induced chlorosis has been a hot topic in the study of plant nutrition since the beginning of this century (Yan et al., 2006). To avoid Fe deficiency, plants have developed two strategies for the efficient absorption and use of Fe (Sun et al., 2007). Stage I is carried out by all higher plants except for the Gramineae, which use strategy II (Romheld and Marschner, 1986; Curie and Briat, 2003; Briat et al., 2007).

Plants mainly acquire iron through soil, therefore, the most directly method of correcting iron deficient induced chlorosis involves Fe fertilizers. These can improve the iron content of soil and fruit trees. In all cases, Fe-fertilization leads to episodes of high Fe concentration in the rhizosphere and the roots (in cases of soil or growth substrate fertilization) or in plant shoot tissues (in cases of foliar fertilization and fertilizer injections) (Abadía et al., 2011). However, the effects of these measures have been unstable. They are also costly and prone to causing environmental pollution, so they do not constitute a practical solution to iron deficient induced chlorosis in fruit trees.

Ever since iron deficient induced chlorosis symptoms were first documented in plants in 1843, the screening and training Fe-efficient genotypes of plants has been recognized as the most effective way to solve the iron deficiency problem. *Malus xiaojinensis* was screened from more than 40 species or ecotypes and it was to be the first Fe-efficient genotype to be applied (Han et al., 1998). A series of iron-related gene are induced in *Malus xiaojinensis* when iron is scarce. These include *IRT1* and *FRO2*, which are expressed mainly in the roots responsible for transport and absorption (Han et al., 1994). They also include *YSL* and *CS*, which are responsible for transport above ground (Han et al., 2012; Curie et al., 2001; Durrett et al., 2007; Jeong and Guerinot, 2009). Chistock 1 is a clonal rootstock screened from the open pollinated seedlings of *Malus xiaojinensis* with iron efficient, semi-dwarfing, and apomictic. However, the ability of Chistock 1 to improve tolerance to iron deficiency in scions lacks systematic experimental data. In this study, two materials and two different iron supplements to determine the influence of rootstock on the scion were used.

MATERIALS AND METHODS

Plant Material and Treatments

The plant materials used for this study were seedlings of F/B and F/C annuals grown in sand slots 90 × 30 × 30 cm in size. Three seedlings were planted in each sand slot, which was drip-irrigated three times per day with

Hoagland solution. The duration of each irrigation period was 15 minutes, and the average of irrigation by each tree was $5.5 \times 10^{-4} \text{ m}^3$. The sand and seedling roots were washed after 1 week. The control group received a normal iron supplement [$40 \mu\text{M L}^{-1}$ ethylenediaminetetra acetic acid (EDTA)-Fe] and the deficiency group was given $4 \mu\text{M L}^{-1}$ EDTA-Fe. Each treatment was repeated three times.

Chlorophyll Measurement

For chlorophyll measurement, young leaves from two plants grown under normal iron conditions and iron deficient conditions were detected using a SPAD-502 Plus Chlorophyll Meter (Samar et al., 2007).

Measurement of Active Iron Content

For active iron measurement, about 10 pieces of young leaf were taken from the plant and cleaned of dust using wet gauze. Then the leaves were washed with distilled water for 1 min, and pieces were cut while the leaves were drying. Weighted one-gram mixtures were dissolved in 10 ml hydrochloric acid (HCl) (1 mol L^{-1}) and filtered after shaking for 5 hours. Active iron content was detected using an atomic absorption spectrophotometer.

RNA Extraction and Analysis of Gene Expression

RNA was extracted from young leaves by the cetrimonium bromide (CTAB) method and gene expression was analyzed by semi-quantitative reverse transcription- polymerase chain reaction (PCR) according to the methods described by Berczky et al. (2003) and Bauer et al. (1994). Briefly, $1 \mu\text{g}$ total RNA was treated with DNase (Takara, Japan) and reverse-transcribed into cDNA using an M-MLV RTase cDNA Synthesis Kit (Takara, Japan). Specific oligonucleotides were used to amplify *YSL5* (5'-CCTCCTCAACCAGCCCTTCACC-3' and 5'-GCGTTCGCTCATTCCAAACAGATA-3'), *CSI* (5'-TTCTCGTCTCGGGCAACTGTC-3' and 5'-TTCCTCTCATTCCACCAATCACC-3'). The actin gene (5'-TGGTGAGGCTCTATTCC AAC-3' and 5'-TGGCATATACTCTGGAGGCT-3') served as a control.

RESULTS

Phenotypic Expression of Chlorosis

After 25 days, the leaves of F/C showed no chlorosis, regardless of iron conditions (Figure 1a). The young leaves of F/B began to yellow after 25 days under iron-deficient conditions (Figure 1b). Chlorosis appeared increasingly serious as time passed. This phenomenon did not appear on the leaves under

FIGURE 1 A) Chlorosis of different scion/rootstock combinations after 25 days of Fe-deficient conditions. B) Chlorosis of different scion/rootstock combinations after 25 days of Fe-deficient conditions.

normal iron conditions. After 40 days of iron-deficient conditions, the young leaves of F/B showed very serious chlorosis and the old leaves had become yellow. The young leaves of F/C still showed no chlorosis. This phenomenon indicated that, as a rootstock, Chistock 1 could significantly improve the tolerance of the scion to iron deficiency under iron-deficient conditions, relative to *Malus baccata*.

Response on Plant Growth

Iron-deficient conditions treated by $4 \mu\text{M L}^{-1}$ EDTA-Fe were found to significantly repress the growth of young shoots (Figure 2a). The average young shoots grown by F/B increased by less than 10 cm, about 5 cm less

FIGURE 2 Growth of A) young shoots and B) leSaves under different iron conditions.

than under normal iron conditions. These trends were visible in the leaf area, but the average growth of young shoots under iron-deficient conditions was significantly higher than that of F/B and closer to the growth of F/B under normal conditions.

Response on Leaf Area Change

The growth of plant leaves is suppressed by iron deficiency. This is the result of the decreased leaf area. The leaves grown by F/B and F/C under iron-deficient conditions were smaller than those grown under normal conditions (Figure 2b). The average leaf area of F/B under iron-deficient conditions were 400mm² less than 1700 mm² of F/C and the same trends in the iron-efficient plants, where the average leaf area of F/B was 700 mm² less than 2000 mm² of F/C. These results shows that Fuji grafted onto Chistock 1 presented a stronger response to iron deficiency than those grafted to *Malus baccata*.

Response on Leaf Chlorophyll

Iron is the essential element component for chlorophyll and iron deficiency could affect the chlorophyll synthesis. Under iron-deficient stress conditions, the chlorophyll content of F/B and F/C showed no tendency to increase, unlike under iron-efficient conditions. The chlorophyll content of F/B decreased rapidly after ten days of iron deficient stress and then decreased slowly. The chlorophyll content of F/C decreased stably during iron deficiency stress and consistently remained higher than that of F/B. The chlorophyll content of F/B and F/C increased under normal iron conditions, but the ascending range of F/C was significantly higher than that of F/B (Figure 3). These data showed that the chlorophyll content of young F/C leaves was consistently higher than that of young F/B leaves, regardless of iron stress conditions.

Response on Active Iron Content

The total iron content of plant leaves is affected by many factors, so it is not a reliable indicator of iron nutrition in plants (Takkar and Kaur, 1984). There is, however, far better correlation between active iron content and chlorophyll content (Pierson and Clark, 1984), so active iron content is a more reliable index than total iron content for the diagnosis of iron nutrition status in fruit trees. The measurement of the active iron content of young F/B and F/C leaves under iron efficient and deficient conditions supported this conclusion. As shown in Figure 3, the active iron content of F/B and F/C correlated well with chlorophyll content, showing a similar curve to that of chlorophyll content. This provides another

FIGURE 3 The A) chlorophyll content and B) active iron content of young leaves under different iron supply conditions.

demonstration that Chistock 1 can enhance resistance to iron deficiency by increasing the active iron content of scion leaves under iron-deficient conditions.

Response on Gene Expression

The expression of genes *CSI* and *YSL5* in the young leaves of Chistock 1 and *Malus Baccata* were first up-regulated and then down-regulated under iron deficient conditions. They peaked on day six, with 3.5 fold and 2.7

FIGURE 4 Expression of A) *CSI* and B) *YSL5* in young leaves under iron-deficient conditions.

fold up-regulation, respectively. The same trend appeared with F/C, for which *CS1* and *YSL5* were up-regulate 5.9-fold and 4.1-fold, respectively (Figure 4). However, *CS1* and *YSL5* expression in F/C were up-regulated nearly twice as much during the same period under iron deficient conditions. This indicates that the Fe-efficient rootstock Chistock 1 can improve the iron nutrition condition of scions by up-regulating iron transport genes under iron deficiency stress conditions.

DISCUSSION

Malus xiaojinensis is the first apple Fe-efficient genotype. It was screened from about 40 *Malus* plants and its high-efficiency mechanism of iron assimilation and transport within tissue structures, the rhizosphere, and plant physiological metabolism has been clarified (Han et al., 1998). Under iron deficiency stress conditions, *Malus xiaojinensis* showed a typical Strategy I response. Compared with the Fe-deficient genotype *Malus baccata*, *Malus xiaojinensis* not only showed higher electrical conductivity in root exudates, higher ATP enzymatic activity in roots, higher affinity for and assimilation of iron, and a root reductive ability three times that of *Malus baccata*, but it also had more free space iron accumulation in the roots, an ability to use iron effectively, and higher cation exchange capacity in the roots (Han et al., 1994). Chistock 1 is screened from the natural seedling offspring of *Malus xiaojinensis* by examination of the rootstock type. So Chistock 1 should also have the Fe-efficient ability.

Grafting involved *Malus xiaojinensis* and *Malus baccata* had been done and the growth situation of the scions was observed. The results showed that *Malus baccata* grafted onto *Malus xiaojinensis* expressed typical chlorosis, while *Malus xiaojinensis* grafted onto *Malus baccata* showed iron deficiency phenomenon. A similar phenomenon appeared in the incubation and field experiments where *Malus xiaojinensis* and *Malus baccata* were used (Li et al., 2002). But So grafting Fuji plants onto Chistock 1 rootstock is expected to improve the ability of these plants to efficiently process iron in the field.

According to the results of the current study Chistock 1 can improve iron processing efficiency ability better than F/B. This was indicated not only by indexes of modality, such as the period of leaf chlorosis, young shoot growth, and leaf area but also by physiological indexes, such as the chlorophyll and active iron content of young leaves. Chistock 1 can therefore be said to be more suitable than *Malus baccata* for planting in iron-deficient areas. By assessing the expression of the iron transport gene, it can be concluded that Chistock 1 can improve resistance to iron deficiency by enhancing iron transport from roots to shoots. This also explains the efficiency with which Chistock 1 processes iron.

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