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Tolerance to Lime–induced Iron Chlorosis of Asian Pear Rootstocks (*Pyrus* spp.)

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Summary

This study was conducted to evaluate the resistance of Asian pear rootstocks to lime–induced iron chlorosis when grown in calcareous soil. Two experiments were conducted in greenhouse condition, using three pear rootstock species: *Pyrus xerophila* Yü, *P. betulaefolia* Bunge, and *P. calleryana* Decne). Plants were grown over 90 days in pots filled with calcareous soil. Difference in iron chlorosis tolerance was estimated by chlorophyll contents by using chlorophyll meter readings, iron contents in leaves, and growth parameters of plants. Among the three pear rootstocks, *P. xerophila* showed higher chlorophyll and iron content in fully expanded apical leaves than did *P. betulaefolia* and *P. calleryana* in high pH, calcareous soil. It was further confirmed in the grafting experiment with the 'Housui' cultivar. These results confirmed that *P. xerophila* has a higher tolerance to lime–reduced iron chlorosis. However, further plant evaluation under field conditions is needed to verify whether *P. xerophila* can replace the widely used, *P. betulaefolia* in the Asian pear producing areas with high pH and calcareous soil, especially in the northwestern region of China.

Key Words: calcareous soil, chlorosis, pear rootstocks.

Introduction

Plants, grown on calcareous soils, are faced with a characteristic problem of iron acquisition. The total iron content in these soils is high, but the available fraction for the plants is insufficient. This is caused by the very low solubility of iron oxides under alkaline conditions, buffered by the presence of bicarbonate (Lindsay and Schwab, 1982). Iron is an essential micronutrient, linked to proteins that participate in different metabolic processes, such as chlorophyll synthesis and electron transport through the respiratory and the photosynthetic chains (Chaney, 1982; Korcak, 1987). The typical symptoms of iron deficiency consist of an interveinal yellowing or chlorosis of young leaves.

Lime–induced iron chlorosis affects numerous agricultural crops, grown on calcareous soils throughout the world. Approximately 25–30% of the world's arable land is estimated to be calcareous in the surface horizon; about 40% of the world's soil is known to be potentially iron–deficient (Vose, 1982). Economic losses from iron chlorosis in calcareous soil are difficult to estimate, due to irregular occurrences and the dynamic nature of crop response, but they may be severe. In extreme cases of iron chlorosis, supplementary applications of iron chelate, which are expensive, are needed to attain commercial yield and marketable products. The application of iron chelates does not represent a sustainable way to prevent or cure iron chlorosis because of the high cost and environmental risks associated with their use (Tagliavini and Rombolà, 2001).

One of the best alternatives to prevent iron chlorosis problems is the use of tolerant plant species or genotypes. Differences in the tolerance of iron chlorosis have been found in many herbaceous and woody species (Chaney et al., 1992; Hamzé et al., 1986; Kolesch et al., 1987; Tagliavini et al., 1993). Fruit crops generally do not tolerate a low availability of iron in calcareous and alkaline soils (Korcak, 1987). Among deciduous fruit crops, pear and peach are the most susceptible to lime–induced iron chlorosis (Tagliavini and Rombolà, 2001). Genotypic difference against iron chlorosis in pear rootstocks has also been long known (Hendrickson, 1924). In pear production, it is necessary to find rootstocks tolerant to different environmental conditions as well as being graft–compatible. The success of commercial varieties grafted on these tolerant rootstocks is mainly determined by the rootstock genotype (Alcántara et al., 2003; Byrne et al., 1989).

Bicarbonate is considered to be one of the most important factors that induce iron chlorosis (Mengel et al., 1982). Bunnag et al. (1996) who proposed the use of bicarbonate in the planting medium as a method of screening quince cultivars found a good correlation between pot tests and field results. The screening tech-

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niques for tolerance to lime-induced iron chlorosis have been applied more frequently in woody species (Hamzé et al., 1986; Gogorcena et al., 2000), but very few tests have been carried out in Asian pear rootstocks except some western pear rootstocks.

A significant part of the pear industry in Asia is located on calcareous or alkaline soils, especially in the northwest region of China, where iron chlorosis commonly occurs. Pear cultivars often show symptoms of iron chlorosis if grafted on *Pyrus betulaefolia*, which has been largely adopted in Asia. To overcome the problem, it is important to select rootstocks tolerant to high pH and lime soils. However, little information has been collected on genotypic differences among Asian pear species to tolerance in iron deficiency.

The objective of this study was to evaluate the tolerance of lime-reduced iron chlorosis on the main pear rootstock species used in the pear orchards in China and Japan, by growing plants in pots with a calcareous soil and high pH levels.

Materials and Methods

Plant materials

Seeds of the three pear rootstocks were collected in October 2000. *P. xerophila* Yü was obtained from Gansu province in China; *P. betulaefolia* Bunge and *P. calleryana* Decne were obtained from the orchard of Tottori University, Japan. Seeds were germinated in a soil mixture of sand: perlite: peat (1:2:1) in a greenhouse; and the resulting seedlings used for two experiments.

Greenhouse experiments

Experiment 1: After careful washing and ridding the soil from the roots, uniform plantlets (10 cm long) of the three pear rootstocks were transplanted to plastic pots (1.5 L), filled with a soil mixture amended with $CaCO_3$ until the three soil treatments of pH 5.5, 7.8 and 8.2 were attained. Pots were arranged into three blocks, and each block contained 15 plants and arranged randomly with five replications. The plants were harvested at 91 days after treatments.

Experiment 2: One-year-old, uniform rootstock seedlings of *P. xerophila*, *P. betulaefolia*, and *P. calleryana* were transplanted into 5-liter Wagner's pots containing 5.0 kg mixture soil which were supplemented with $CaCO_3$ in the spring of 2002; the pH was adjusted to 5.5 (control) and 8.2 (treatments). After planting, all rootstocks were immediately grafted with a scion of 'Housui' approximately 5 cm above the soil surface. Grafting was successful for all the scion-rootstock combinations. The pots were arranged in two blocks, according to pH difference. Each block contained 5 plants of *P. xerophila*, *P. betulaefolia* and *P. calleryana*. When plants became approximately 80 cm tall, chlorophyll contents and growth parameters were noted.

In all experiments, seedlings were grown in the greenhouse kept at approximately 35/18℃ (day/night). The seedlings were irrigated with half-strength Hoagland solution without iron once a week; the soil moisture was maintained with tap water.

Chlorophyll detection and growth parameters

Leaf chlorophyll content was estimated with the portable SPAD-502 chlorophyll meter (Minolta, Osaka) on three fully expanded apical leaves. Average values per plant were the mean of three readings per leaf. Low SPAD values correspond to a high degree of chlorosis. At the end of the experiment the plant height and dry weight of shoot were measured.

Iron measurement

The leaf samples were washed with tap water, followed by 0.1 M HCl, washed with distilled water, and then oven-dried at 70℃ for 7 days. The dried leaves were pulverized and an aliquot of the sample that was dry-ashed at 500℃ for 5 h, and dissolved in 1 M HCl. The iron content was determined by atomic absorption spectrophotometry; the iron content is expressed as $\mu g \cdot g^{-1}$ FW.

Results

Leaf chlorophyll content

In the first experiment, the leaf chlorophyll content of three pear rootstock seedlings decreased markedly with the increasing soil pH; severe leaf chlorosis occurred at the high pH, but the SPAD values differed in the three pear rootstocks (Fig. 1). The SPAD value of the seedlings of *P. xerophila* was more than 30; while for *P. betulaefolia* and *P. calleryana*, it was 26 and 25, respectively, at pH 8.2. Iron chlorosis was not observed in low soil pH 5.5 (control). The SPAD values were between 39 and 41 (Fig. 1). The highest SPAD values were found in *P. xerophila* for all treatments. In the second experiment, the SPAD value of three pear rootstocks-grafted plants was lower at pH 8.5 than at pH 5.5 (control, Fig.

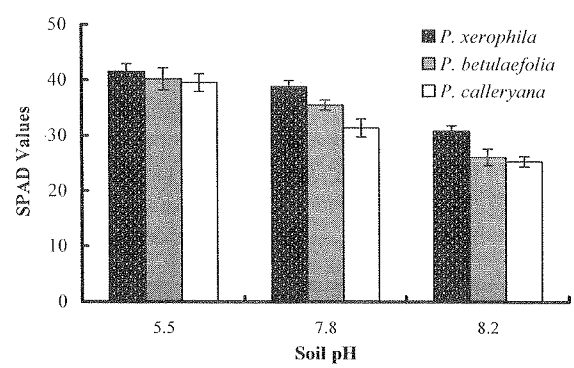

Fig. 1. Chlorophyll content of fully expanded apical leaves of *P. xerophila*, *P. betulaefolia* and *P. calleryana* rootstocks for different soils pH, as estimated by SPAD values. Vertical bars indicate SE (n=5).

2). Among the three pear rootstocks grafted with 'Housui', there were significant differences between chlorophyll content (SPAD value) in the high pH treatment (Fig. 3). However, the SPAD values were lower than in experiment 1 for all pear rootstocks. The data from experiments 1 and 2 indicated that the chlorophyll contents of *P. xerophila* in both of seedling and 'Housui'/ rootstock combination were higher than those of other pear rootstocks in the high pH treatment.

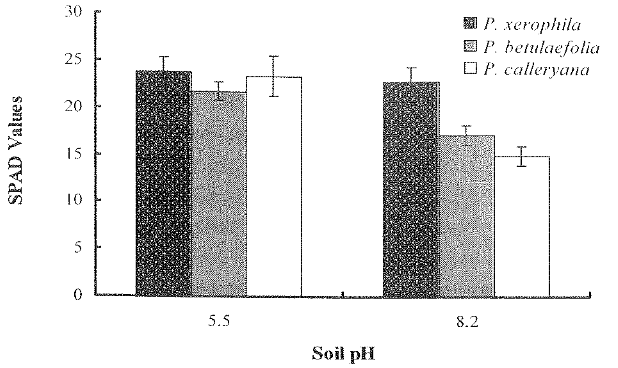

Fig. 2. Chlorophyll content of fully expanded apical leaves of 'Housui' grafted on *P. xerophila*, *P. betulaefolia* and *P. calleryana* rootstocks and planted in soils with pH 5.5 and 8.2. The SPAD values were obtained 56 days after grafting. Vertical bars indicate show SE (n=5).

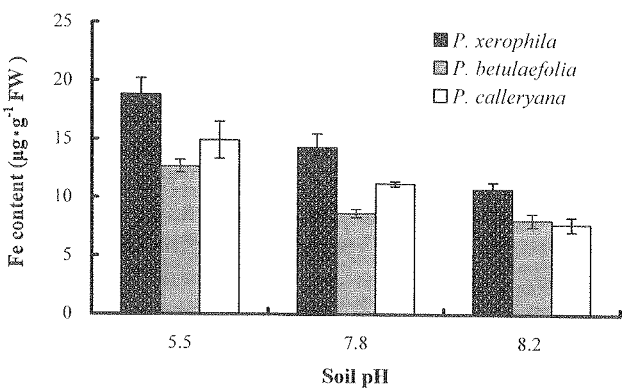

Fig. 3. Iron content of fully expanded apical leaves in *P. xerophila*, *P. betulaefolia* and *P. calleryana* rootstocks growing different soil pH. Vertical bars indicate SE (n=5).

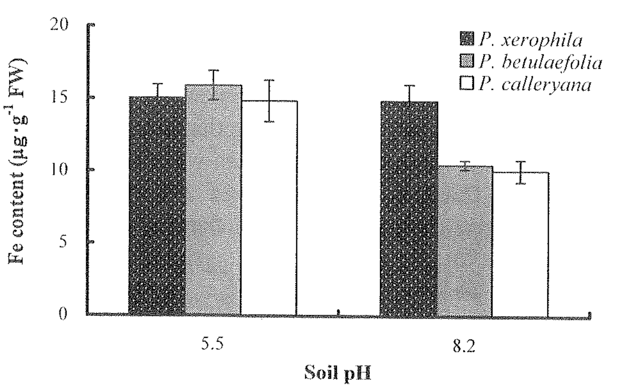

Fig. 4. Iron content of fully expanded apical leaves of 'Housui' grafted on *P. xerophila*, *P. betulaefolia* and *P. calleryana* rootstocks and planted in soils with different pH. Vertical bars indicate SE (n=5).

Leaf iron content

In experiment 1, iron content of three fully expanded apical leaves declined gradually as the soil pH increased (Fig. 3). Among all treatments, *P. xerophila* leaves contained more iron than comparable samples from other rootstock species on fresh weight basis. A similar pattern was observed in the experiment of grafting with 'Housui' (Fig. 4). Leaf SPAD value was linearly correlated to the leaf iron content. Correlation coefficients were revealed as 0.71 (Exp. 1) and 0.88 (Exp. 2) (Fig. 5).

Plant growth parameters under different soil pH

In experiment 1, shoot length was not significantly affected by soil pH (Table 1). Shoot dry weight showed significant reductions as the soil pH increased, but this varied with different pear rootstocks. Shoot dry weight was unaffected by soil pH in *P. xerophila*. The reductions in shoot dry weight caused by chlorosis were 25 and 42% for *P. calleryana* and *P. betulaefolia*, respectively (Table 1). In the second experiment, plant height and shoot dry weight of the grafted scion 'Housui' did

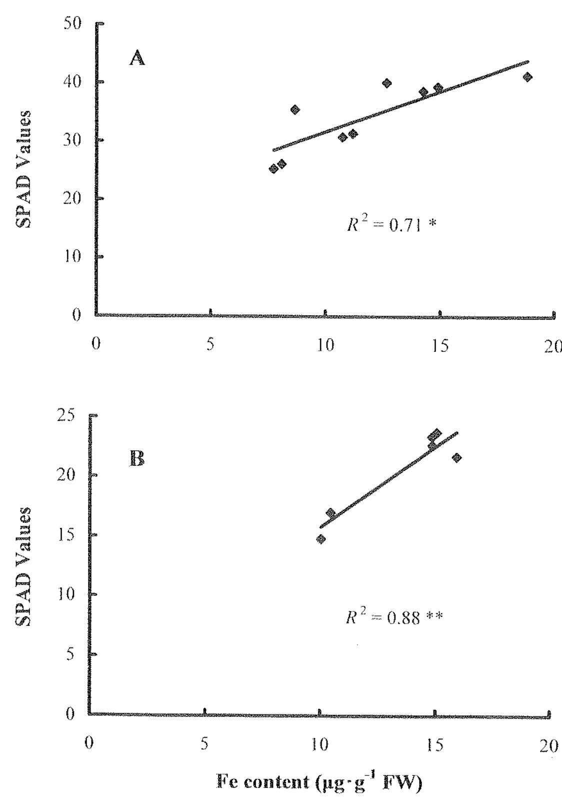

Fig. 5. Correlations between chlorophyll content (SPAD value) and iron content of leaves for the three pear rootstocks growing in mixture soils with pH 5.5, 7.8, 8.2 (A) and 'Housui' grafted on the three pear rootstocks growing in soils adjusted to pH 5.5 and 8.2 (B). *, ** significant at $P \leq 0.05$, 0.01, respectively.

Table 1. Effect of soil pH on plant growth for three pear rootstocks.

Soil pH	Rootstocks	Shoot length (cm)	Shoot DW (g)/plant	FW/DW
5.5		49.2 a[z]	2.73 a	2.91 a
7.8	*P. xerophila*	50.3 a	2.45 a	3.10 a
8.2		55.7 a	2.61 a	3.24 a
5.5		50.2 a	2.98 a	2.85 a
7.8	*P. betulaefolia*	45.2 a	1.70 b	3.40 b
8.2		45.0 a	1.72 b	3.45 b
5.5		62.5 a	3.54 a	2.84 a
7.8	*P. calleryana*	59.0 a	2.95 ab	2.96 a
8.2		59.8 a	2.66 b	3.27 b
Significance				
Rootstocks(R)		**	***	NS
pH		NS	**	**
R × pH		NS	NS	NS

NS, **, ***, non - significant, significant at $P<0.01$, 0.001, respectively.

[z] Different letters within the same rootstocks are significantly different ($P<0.05$) using Tukey's multiple comparison test (n=5).

Table 2. Effect of soil pH and rootstock on Japanese pear 'Housui' shoot growth 56 days after grafting.

Soil pH	Rootstocks	Shoot length (cm)	Shoot DW (g)/plant	FW/DW
5.5	*P. xerophila*	82.6 ± 4.8[z]	6.09 ± 0.56	2.44
8.2		75.2 ± 5.3	5.86 ± 0.66	2.65
5.5	*P. betulaefolia*	85.2 ± 7.1	6.95 ± 1.00	2.80
8.2		82.0 ± 3.2	6.83 ± 0.60	2.72
5.5	*P. calleryana*	80.8 ± 8.8	6.30 ± 1.30	2.74
8.2		74.4 ± 1.9	5.96 ± 0.30	2.73

[z] Mean ± SE (n=5). Mean are not significantly different between the rootstocks and the pH ($P<0.05$) using Tukey's multiple comparison test.

not showed any significant differences between the control and high pH treatment (Table 2).

Discussion

Iron chlorosis of pear leaves was identified as early as 1920 in the United States (Gile and Carrero, 1920). *P. pyrifolia* and quince were more sensitive than *P. communis* to iron chlorosis (Hendrickson, 1924). *P. calleryana* rootstocks were also more susceptible to lime - induced iron chlorosis than *P. communis* rootstock (Higdon, 1957). Recently, several rootstock species and varieties such as somaclonal quince variants IE - 1 and 5.7A (Bunnag et al., 1996; Marino et al., 2000) have been selected for their resistance to lime - induced iron chlorosis in field and greenhouse conditions. However, quince is graft - incompatible with Asian pears. Because Asian pear tree require rootstocks that give high vigor, no *P. communis* rootstocks are suitable as Asian pear rootstocks (Stebbins, 1995).

Several authors reported that plants with severe iron chlorosis had a significant reduction in shoot dry weight (Clark et al., 1982; Singh et al., 1986). Our data show that the reduction in shoot dry weight occurred in the ungrafted rootstock, but not in the grafted pear trees (Table 2). It is possible that grafting influenced the shoot dry weight. The grafting union plays an important role in regulating the rate of assimilate transport from shoots, which is necessary for root development. In experiment 1, plant heights were not decreased markedly as the soil pH was increased. Nevertheless, the shoot dry weight was significantly decreased in the *P. betulaefolia* and *P. calleryana* as soil pH was increased. That may be a consequence of the low vigor and thinness of shoots grown at pH 8.2 compared with those grown at pH 5.5. However, in *P. xerophila*, high soil pH had no influence on plant height and shoot dry weight compared with the low pH soil treatment. This difference is attributed to the possible ability of rootstocks to adapt to the soil environment. These results showed that *P. xerophila* may be well - adapted to high soil pH. However, in the grafted experiment, the 'Housui'/ *P. xerophila* graft combination had lower shoot dry weight than *P. betulaefolia* as rootstocks. The difference may be the consequence of the nutrient reserve levels that the roots accumulated the previous growing season.

P. xerophila is a wild species native to the Gansu and Shaanxi provinces in northwestern China. In Gansu province, a very historic pear production site, there are pear trees grafted on *P. xerophila* that are over 300 - years old, indicating that the scion/stock combinations are well adapted to local poor soil and environmental conditions. However, this rootstock species has not received much attention. This experiments, using mixtures of lime - induced iron - deficient soil, reveals that *P. xerophila* had higher chlorophyll contents (SPAD value) and iron content than other pear rootstocks, whereas the experiments with grafted plants exhibited that leaf iron contents decreased with the increasing iron stress with high pH (Fig. 3); the leaf iron content of *P. xerophila* did not significantly decrease under iron - deficiency condition. Although a similar pattern was observed in all rootstocks, the leaf iron content of *P. xerophila* was always higher than those in *P. betulaefolia* and *P. calleryana*. In all treatments, *P. xerophila* was more efficient in absorbing iron than other rootstocks. However, further evaluation of this species under field conditions is needed on vegetative growth and fruit quality because of the low dry weight accumulated by shoots of the grafted scion, we need to verify if *P. xerophila* may replace the widely used pear rootstocks, *P. betulaefolia* and *P. calleryana* in the areas with high pH and calcareous soils.

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石灰誘導性鉄欠乏クロロシスに対するアジアナシ台木種の耐性

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摘　要

石灰質土壌条件下で誘発される鉄欠乏クロロシスに対するアジアナシ台木種の耐性を比較，調査した．実験には3種のアジアナシ台木種 *Pyrus xerophila* Yü，*P. betulaefolia* Bunge および *P. calleryana* Decne を用い，pHの異なる土壌を充填したポットに移植した後の変化を，台木実生および‘豊水’を接木した場合のそれぞれについてハウス内で調査した．鉄欠乏クロロシスに対する耐性は移植90日後以降，上位葉のクロロフィル含量(SPAD値)，鉄含有率および成長量を測定することで評価した．その結果，石灰誘導性鉄欠乏クロロシスに対し，実生および‘豊水’を接木した場合のいずれも *P. xerophila* が他の2種よりも耐性を持ち，上位葉のクロロフィル含量，鉄含有率がともに高い値を示した．このように，*P. xerophila* は原産地の土壌条件を反映して石灰誘導性鉄欠乏クロロシスに対し，他の台木よりも耐性が強いことが示唆され，クロロシス耐性台木として中国西北部のような石灰過剰の土壌地帯で利用可能な有望な台木種とみなされたことから，その耐性機構を継続して調査する必要がある．