Effects of Fe-deficient conditions on Fe uptake and utilization in P-efficient soybean

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Abstract

Phosphorus (P)-efficient soybean (*Glycine max*) plants absorb and utilize P with high efficiency. To investigate the effects of iron (Fe)-deficient conditions on the absorption and utilization of Fe in P-efficient soybean plants, two soybean cultivars with different P efficiency, the 03-3 (P-efficient variety) and Bd-2 (P-inefficient variety), were used in this study. The two soybean cultivars were grown in nutrient solution containing Fe concentrations of 0 (Fe0), 20 (Fe20), 40 (Fe40), or 80 (Fe80) μM for 7 days. The Fe reductase activity of roots was higher in 03-3 plants grown under the Fe0, Fe20, and Fe40 treatments than in Bd-2 plants and the total Fe uptake was greater in 03-3 plants under the Fe40 treatment. *GmFRD3a* was much more highly expressed in the stem of 03-3 than in that of Bd-2, and significantly more iron was transported to 03-3 plant shoots during Fe0 treatment. Chlorosis in young leaves caused by Fe deficiency under the Fe0 and Fe20 treatments was alleviated by increased Fe concentration in shoots.

Increased levels of active Fe in young 03-3 leaves under Fe-deprivation conditions (Fe0) and maintenance of stable Fe concentrations in 03-3 shoots subjected to Fe20, Fe40, and Fe80 treatments suggested that the P-efficient 03-3 cultivar is also Fe-efficient. It is suggested that 03-3 soybean cultivar should be a good resource for application to farm field.

Keywords

Soybean, chlorosis, nutrient deficiency, hydroponic, Fe and P interaction

1. Introduction

Soybean (*Glycine max* (L.) Merr.) contains high levels of protein, fat, a variety of mineral elements, and physiologically active substances that play important roles in human health. Soybean is important globally as an oil crop, a health food, and as an essential industrial raw material (Carrera et al., 2014). Nutrient deficiency is one factor that limits soybean yield, and insufficient levels of phosphorus (P) and iron (Fe) are responsible for the most common nutritional deficiencies worldwide (Liang et al., 2010; Vasconcelos and Grusak, 2014). Both P and Fe are important elements for plant growth. P serves as the energy currency of living cells in the form of ATP and as a component of the genetic materials, DNA and RNA, which control protein synthesis in both plants and animals (Brady and Weil, 2002). P deficiency occurs when only limited amounts of inorganic P (Pi) are available in the soil for crops. Crop yields from 30–40% of the arable land worldwide may be limited by P deficiency (Aziz et al., 2011;

Runge-Metzger, 1995; Vance et al., 2003). Since Fe is an essential element involved in fundamental biological processes, such as chlorophyll biosynthesis and photosynthesis, when this micronutrient is unavailable to the plants, they frequently exhibit chlorosis in new leaves, and stunted growth (Jeong and Connolly, 2009). Soybean is a globally-produced legume crop, and iron deficiency has become a yield-limiting factor to soybean production in areas with predominantly calcareous soils (Rodríguez-Lucena et al., 2010). With increasing Fe deficiency in arable land, Fageria et al. (2008) suggested that nutrient-efficient plants would play an important role in increasing crop yields in 21st century agriculture.

Lynch (1998) developed a method of evaluating P efficiency that was subsequently used by Xu et al. (2003) to classify the P efficiency of many soybean cultivars by evaluating biomass, yield, P-acquisition efficiency, P-utilization efficiency, and other indices to classify soybean varieties as P-efficient or P-inefficient. The 03-3 soybean cultivar is a P-efficient variety with a higher level of P absorption and utilization and a larger root system than the Bd-2 cultivar (Xu et al., 2003). P absorption can be represented by total P (or P uptake). A soybean cultivar with high P-utilization efficiency can grow normally at low P concentrations. However, little is known about how P-efficient soybean cultivars respond to conditions of Fe deficiency.

Fe deficiency is another important factor that limits plant growth. To cope with Fe-deficient conditions, several biochemical and physiological mechanisms have evolved in plants. Plants are divided into two groups based on the strategies employed to take up soluble Fe under Fe-deficient conditions: strategy I plants, which include

nongraminaceous monocot and dicot species, and strategy II plants, which include graminaceous monocots (Römheld and Marschner, 1986). Soybean is a dicot species belonging to the strategy I plant group, which can acidify the soil by secreting protons (H^+) from the roots, thereby increasing the concentration of soluble Fe in the rhizosphere (Zocchi et al., 2007). Plant roots synthesize ferric reductase, which converts ferric Fe to ferrous Fe by a reduction reaction. The ferrous Fe is then absorbed into root tissues by divalent metal transporters on the root surface (Römheld and Marschner, 1986). Therefore, root ferric reductase capacity is the key component of Fe uptake in strategy I plants.

The interaction between P and Fe in plants has been demonstrated in many studies (Ladouceur et al., 2006; Ward et al., 2008). P deficiency resulted in higher Fe content in *Arabidopsis* (Hirsch et al., 2006), due to iron accumulation within chloroplasts. In addition, increased Fe availability has been found in rice seedlings under P-deficient conditions (Zheng et al., 2009). The application of P aggravated chlorosis induced in chickpea by Fe deficiency, irrespective of soil type (Sánchez-Rodríguez et al., 2014a). These results revealed a strong interaction between P and Fe, but the relationship cannot be predicted simply in all plant species. In sorghum, the leaf chlorophyll concentration was enhanced by P fertilization of soils poor in available P, but the opposite effect was generally observed for P-rich soils (Sánchez-Rodríguez et al., 2014b). Because Fe-induced chlorosis can be aggravated by P application, the plant and soil nutrient status should be taken into consideration. However, the specific mechanisms of Fe absorption and utilization at the physiological and molecular level in

various P-efficient soybean cultivars bred in China remain unknown.

In this study, we investigated the effects of various levels of Fe nutrition on the P-efficient soybean cultivar 03-3 and the P-inefficient cultivar Bd-2 over a time course. We also considered possible regulatory effects on Fe uptake and translocation in P-efficient soybean plants and sought to identify the mechanisms underlying the improved Fe status in P-efficient soybean plants under specific conditions of Fe availability. Based on our results, we attempt to provide feasible technologies and approaches to improve the Fe and P nutritional status in soybean and to increase the productivity of staple crops in low-input agricultural systems.

2. Materials and methods

2.1. Plant culture and growth

Soybean seeds of the 03-3 and Bd-2 cultivars were kindly provided by Hong Liao of the South China Agriculture University. The 03-3 cultivar is P-efficient and the Bd-2 cultivar is P-inefficient. After germination, sets of four seedlings were grown in a 2.5-L pot containing complete nutrient solution with continuous aeration. And each treatment contained four pots. The composition of the nutrient solution was as follows: 0.5 mM KH_2PO_4 , 0.4 mM NH_4NO_3 , 0.025 mM MgCl_2 , 0.3 mM $(\text{NH}_4)_2\text{SO}_4$, 1.5 mM KNO_3 , 1.2 mM $\text{Ca}(\text{NO}_3)_2$, 0.5 mM MgSO_4 , 25 μM H_3BO_3 , 15 μM MnSO_4 , 1.5 μM ZnSO_4 , 0.05 μM CuSO_4 , 0.0016 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, and 40 μM Fe-EDTA. After 7 days, seedlings without cotyledons were transferred to solution containing various levels of iron (Fe). The Fe concentrations used were 0 μM (Fe0, Fe deprivation), 20 μM (Fe20, low Fe), 40

134 μM (Fe40, normal Fe), and 80 μM (Fe80, excess Fe). The plants were grown at 28°C in
135 the light and at 22°C in the dark under a 14-h day/10-h night cycle with a relative
136 humidity of 70–75%. At 3 and 7 d after treatment, plant samples were harvested and the
137 chlorosis symptoms in young leaves caused by Fe-deficiency were photographed.

138 In another experiment, the 03-3 and Bd-2 cultivar soybeans were used. After
139 germination, seedlings were grown in complete nutrient solution with continuous
140 aeration. The composition of the nutrient solution has been described above. After 7
141 days, seedlings without cotyledons were transferred to a normal nutrient solution with P
142 (0.5 mM) or without P (0 mM). Sets of four seedlings were grown in a 2.5-L pot; each
143 treatment contained four pots. The plants were grown in the same climate chamber
144 described above. At 7 d after treatment, plant samples were harvested and chlorosis
145 symptoms in young leaves were photographed.

146 **2.2. Plant analysis**

147 Soil and Plant Analyzer Development (SPAD) values were measured in the middle
148 region of young soybean leaves to determine the relative chlorophyll content using a
149 method described previously (Bienfait et al., 1985; Takkar and Kaur, 1984). A Minolta
150 SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd., Osaka, Japan) was used to
151 determine SPAD values. Average values of 10 leaves per replicate plot were recorded.
152 Pairs of soybean plants from each pot were harvested separately as root and shoot
153 samples, and immediately dried at 105°C for 30 min. In addition, soybean roots, stems,
154 and leaves were harvested separately for the Fe0 treatment. Then plant samples were
155 dried at 65°C for 3 days and weighed. Portions of approximately 100 mg were digested

with 4 ml of 4.4 M HNO₃ and 6.5 M H₂O₂ for 30 min at 220°C using a MarsXpress oven (CEM, <http://www.cem.com/>). Fresh young leaves from soybean plants in each treatment were collected with three replicates for determination of HCl-extractable Fe (so-called 'active Fe') levels according to the procedure of Takkar and Kaur (1984). Metal concentrations were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES, Perkin-Elmer Optima 3300DV).

The P-absorption efficiency, defined by Xu et al. (2003) as the total P uptake under certain available P concentrations, reflected the P-absorption capacity from the medium. The Fe-absorption efficiency was derived from the P-absorption efficiency and was defined as the total Fe uptake under certain available Fe concentrations. Fe translocation to shoots was expressed as the percentage of shoot Fe content relative to the Fe content of the whole plant. The Fe-utilization efficiency of shoot was defined as the shoot dry weight produced under each Fe concentration.

2.3. Measurement of ferric reductase activity

Root samples were used to determine ferric chelate reductase activity according to a modified version of the procedure reported by Bienfait et al. (1983). Soybean roots were washed and dipped in saturated CaSO₄ solution for 30 min and then washed three times with deionized water. The roots were then transferred to a reaction solution containing 0.4 mM 2,2'-bipyridyl and 0.1 mM Fe(III)-EDTA. The absorbance of Fe(II)-bipyridyl complex was measured after 2 h (MEC at 520 nm: 8650).

2.4. Quantitative real-time PCR

Total RNA was extracted from soybean roots, stems, and leaves in the Fe0

treatment using the TRIzol reagent (Invitrogen) and treated with RNase-free DNase I (Takara) to remove genomic DNA contamination. First-strand cDNA was synthesized with M-MLV reverse transcriptase (Promega). Quantitative real-time PCR was performed using the SYBR Green PCR Master Mix reagent (Applied Biosystems) and an ABI7500 Fast Real-Time PCR System (Applied Biosystems). The *GmFRD3a* primers were used for quantitative real-time PCR as described by Rogers et al. (2009). The *GmIRT1*, *GmFRO2*, *ferretin* gene, and soybean control gene *18S* primers were used for qRT-PCR, in accordance with the work of Santos et al. (2015).

2.5. Statistical analysis

Data were analyzed using the SAS software and expressed as means of three replicates with standard errors. The statistical significance of differences between treatments was analyzed using analysis of variance (ANOVA) and the least significant difference (LSD) at the 5% probability level (SAS Institute, 1985). We performed two separate two-way ANOVA analyses to separate the effects of soybean genotype and Fe treatment on plant biomass. The differences between genotypes under the same Fe concentration were assessed by the independent-samples *t*-test method. Single or two asterisks indicate $P < 0.05$ or <0.01 .

3. Results

3.1. Effects of Fe concentrations on Fe nutrition status and growth of soybean plants

There was a highly significant difference in biomass of the two soybean genotypes (03-3 and Bd-2) at 3 and 7 days after Fe treatment (DAT) (Table 1). No significant difference in shoot dry weight was shown until 7 DAT because Fe treatment. However, the difference in shoot dry weight due to Fe treatment was observed from 3 DAT in 03-3. The shoot dry weight of 03-3 plants was markedly higher than that of Bd-2 plants at 3 and 7 DAT with Fe concentrations of 0 μM (Fe0, Fe deprivation), 20 μM (Fe20, low Fe), 40 μM (Fe40, normal Fe), and 80 μM (Fe80, excess Fe), but the Bd-2 plants exhibited less biomass variation under the various Fe concentrations at 3 DAT. No distinct chlorosis was induced in young leaves of the P-efficient 03-3 plants by Fe deficiency until 7 DAT, with the most severe chlorosis caused by the Fe0 treatment (Fig. 1). The degree of chlorosis at 7 DAT under the Fe0 treatment was reflected by significantly higher SPAD values of young leaves of the 03-3 plants than the Bd-2 plants (Fig. 2). During the treatment period, the SPAD values of the young 03-3 leaves remained persistently higher than those of the young Bd-2 leaves.

Under the Fe0 treatment, the active Fe concentration at 3 DAT in the young leaves of 03-3 plants was significantly higher than that in Bd-2 plants (Fig. 3A). After treatment for 7 days, the active Fe concentration in the young leaves of both cultivars increased as the Fe concentration increased from 0 to 40 μM , but decreased rapidly in the 03-3 plants subjected to the Fe80 treatment (Fig. 3B).

3.2. Ferric reductase activity and Fe-absorption efficiency in soybean roots

The root ferric reductase activity was affected by the various Fe concentrations at 7 DAT (Fig. 4A). The root ferric reductase activity of the 03-3 plants was highest under

the Fe20 treatment and then declined with increasing Fe concentration to the lowest level under the Fe80 treatment. In contrast, the root ferric reductase activity of the Bd-2 plants was significantly higher in response to the Fe0 treatment than that of Fe20 or Fe80. In addition, the root ferric reductase activity of the 03-3 plants remained higher than that of the Bd-2 plants across Fe treatments.

Fe-absorption efficiency refers to the total Fe uptake by a plant under a particular available Fe concentration and it reflects the capacity for Fe absorption from the growth medium. The total Fe uptake increased in both soybean cultivars at 7 DAT with increasing Fe concentration in the medium (Fig. 4B). The Fe uptake of the 03-3 plants peaked with the Fe40 treatment and was significantly higher than the total Fe uptake of the Bd-2 plants. As Fe concentrations reached excess conditions (Fe80), total Fe uptake exhibited no significant difference compared to Fe40 treatment in 03-3 plants, whereas Fe uptake by Bd-2 plants continued to increase.

3.3. Fe translocation and utilization efficiency

The Fe translocation from root to shoot at 7 DAT in 03-3 plants was highest under the Fe0 and Fe20 treatments and decreased at the higher Fe concentrations (Fig. 4C). A significantly greater quantity of Fe was transferred to 03-3 shoots than to Bd-2 shoots growing in medium containing 0 or 20 μ M Fe. The proportion of Fe transported to shoot was greater in Bd-2 plants only when grown under a normal Fe concentration of 40 μ M, but was less than in the 03-3 shoots when grown in medium containing 0 or 20 μ M Fe.

The Fe-utilization efficiency of the shoot of both soybean cultivars was highest

under the Fe0 treatment (Fig. 4D). The Fe-utilization efficiency of the 03-3 shoots was significantly lower than that of the Bd-2 shoots under Fe-deprivation conditions (0 μ M). This phenomenon corresponded to the low translocation of Fe to shoot.

3.4. P and Fe contents in soybean plants

The P concentration in the 03-3 shoots was not affected by the various Fe levels (Table 2), but it decreased with increasing Fe concentration in the Bd-2 shoots. Under the Fe0 and Fe20 treatments, the P concentration of the Bd-2 shoots was significantly higher than that in the 03-3 shoots.

In the Bd-2 plants, Fe concentration in shoots decreased significantly in response to the Fe-deficient (0 μ M) medium, compared to the Fe40, while P concentration increased (Table 2). In contrast, in the 03-3 shoots, while Fe concentration decreased in the Fe0 treatment, the P concentration exhibited no significant difference. In P treatment experiment, where the two genotypes were treated with or without P under the same Fe conditions, the Fe concentration in 03-3 leaves did not change (Fig. 6). However, it decreased significantly in Bd-2 leaves under the plus P treatment. In the Fe0 treatment, Fe concentration varied greatly in different tissues (Fig. 5D). Fe concentration was much higher in the Bd-2 roots than in 03-3. However, Fe concentration was lower in the Bd-2 leaves than in the 03-3 leaves, while no significant difference was observed in the stem.

Fe accumulation exhibited a trend similar to that of Fe concentration in the 03-3 shoots (Table 2). Fe and P accumulation in 03-3 plants subjected to the Fe20, Fe40, and Fe80 treatments were markedly higher than in the Bd-2 plants. In addition, to the Fe0

treatment, Fe and P content in 03-3 shoots was significantly higher than in Bd-2 when an independent comparison of genotypes was conducted at the Fe0 level. The P accumulation is consistent with the quality of the P-efficient soybean, 03-3 cultivar, which demonstrates higher P accumulation under low or suitable Fe concentrations.

3.5. Fe-deficiency-related gene expression patterns

Four known genes involved in the iron deficiency response, *GmIRT1*, *GmFRO2*, *GmFRD3a*, and *ferritin*, were examined using real-time PCR (Fig. 5A, B, and C). These genes were studied separately from different organs, such as roots, stems, and leaves, under the Fe0 treatment. The *GmIRT1* or *GmFRO2* gene was expressed at the same level in the roots of both soybean genotypes. The *GmFRD3a* expression level showed no difference between the two soybean root genotypes. However, the expression level of the *GmFRD3a* gene was enhanced in the 03-3 stems compared to that in Bd-2. No expression was observed in old leaves and very little in the new leaves (data not shown). Higher levels of *ferritin* expression were seen in the 03-3 leaves. Interestingly, this gene was downregulated in the 03-3 roots and stems compared to Bd-2.

4. Discussion

4.1. P-efficient 03-3 soybean plants also exhibit Fe-efficient

The P-efficient and -inefficient soybean varieties were used in this study for comparison of Fe efficiency. The P-efficient soybean 03-3 showed greener phenotype than P-inefficient Bd-2 soybean cultivar when treatment with low amount of Fe (Fig. 1).

Moreover, the SPAD value (Fig. 2) and active iron concentration (Fig. 3) were significantly higher in 03-3 soybean with Fe deficiency for 3 or 7 days. These results suggested that the P-efficient soybean variety 03-3 is more resistance to Fe deficiency.

Although there was no difference in the expression of the *GmIRT1* or *GmFRO2* genes in roots between genotypes in the Fe0 level, ferric reductase activity was enhanced in 03-3 roots. Similarly, ferric reductase activity in *35S-FRO2 Arabidopsis* was elevated only under the Fe-deficient conditions, indicating that *FRO2* was controlled by post-transcriptional regulation (Connolly et al., 2003). In plants subjected to the Fe treatments, the ferric reductase activity of roots was higher in the 03-3 plants, indicating that more Fe was available to the plants. As a consequence, a considerably greater quantity of Fe was absorbed by the 03-3 plants grown under the Fe40 treatment (Fig. 4B). As a P-efficient cultivar, 03-3 exhibited a higher P-acquisition efficiency, which was reflected by the level of P accumulation (Xu et al., 2003), and maintained a stable P concentration in shoots subjected to various levels of Fe. In this study, the 03-3 plants exhibited high levels of Fe and P accumulation in shoots comparing to that of Bd-2 plants (Table 2). Taken together, the 03-3 cultivar is Fe-acquisition efficient and thus it may be a Fe-efficient soybean cultivar based on the evaluation method of Lynch (1998).

4.2. The Fe distribution is optimized in 03-3 soybean

The encoding of a citrate transporter by *GmFRD3a* was important for Fe translocation from root to shoot; expression was induced by Fe deficiency (Rogers et al., 2009). In the stems of 03-3, higher expression of *GmFRD3a* indicated that much more

Fe could be transported to shoots in 03-3 plants across the Fe0 treatment (Fig. 5B). Associated with gene expression, the translocation of Fe from root to shoot was markedly higher in 03-3 plants than in Bd-2 plants (Fig. 4C). Consequently, Fe concentration was higher in leaves and lower in roots (Fig. 5D), indicating that the 03-3 cultivar is highly adapted to severe Fe-deficient conditions. In addition, the Fe-utilization efficiency in 03-3 shoots decreased sharply as biomass greatly increased under Fe0 conditions. Fe may be transported to aboveground parts of the plant and stored in chloroplasts (Hirsch et al., 2006). As the Fe concentration in the nutrient solution increased to a normal level (Fe40), the translocation of Fe decreased in the 03-3 plants. The translocation of Fe to shoots might be inhibited by a high level of P accumulation in shoots (Ladouceur et al., 2008). Beginning with the Fe20 treatment, Fe accumulation in the 03-3 shoots reached a high level and remained stable. High levels of Fe accumulation in the shoots may downregulate the concentration of citrate in the xylem (Durrett et al., 2007) which transports Fe from root to shoot. These results suggested that 03-3 plants can optimize the Fe distribution such that the Fe-P antagonistic effect is effectively delayed.

4.3. Fe-P antagonistic effect

The P concentration in shoots was higher in the Bd-2 plants under the Fe0 and Fe20 treatments (Table 2), similar to phenomena reported in rice (Zheng et al., 2009) indicating that a Fe-P antagonistic effect occurs under Fe-deficient conditions (Hirsch et al., 2006; Ladouceur et al., 2008; Li et al., 2009). On the other hand, 03-3 plants were able to maintain the P concentration at a particular stable level under the various Fe

treatments. In addition, specific changes (lower Fe but higher P concentration) in the Bd-2 shoots under the Fe0 treatment did not occur in 03-3, suggesting that the interaction between Fe and P may be delayed or prevented. The results of the P treatment experiment, namely, no change in the Fe concentration of 03-3 leaves under different P treatments, also support this suggestion. Ward et al. (2008) reported that the level of active Fe was more important than the level of available P in the interaction of Fe with P. In this study, compared to Bd-2, the level of active Fe in young 03-3 leaves was not influenced by the marked accumulation of Fe and P in shoots at 7 days under the Fe deficiency treatment (Fig. 3B). However, *ferritin* was much more highly expressed in 03-3 leaves than in those of Bd-2, suggesting that more Fe was stored to avoid the Fe–P interaction caused by higher Fe content (Santos et al., 2015). Sánchez-Rodríguez et al. (2014b) recently reported that Fe-induced chlorosis could be aggravated by application of P and that this effect might be dependent on plant species and soil nutrient status. Similarly, our results indicated that the degree of chlorosis could vary with the levels of P-efficiency in different soybean cultivars.

4.4. The P and Fe-efficient soybean 03-3 would be a valuable variety for agricultural practice

Acid soils usually exhibit low P availability for binding to cations and deposit in an insoluble form (Liang et al., 2013). In order to adapt to P deficiency in acid soils, plants have evolved adaptive strategies such as the exudation of organic acids (Vance et al., 2003). 03-3 soybean, as a P-efficient cultivar, could adapt to acid soils for its higher level of P absorption, utilization and a larger root system than Bd-2 cultivar (Xu et al.,

2003). In contrast, high pH value in calcareous soils always cause a barrier for iron uptake, especially for strategy I plants (Guo et. al., 2014). Since soybean is a dicot plant which belongs to strategy I for iron absorption, usually it exhibited iron deficiency induced chlorosis on calcareous soils (Santos et. al., 2016). Although 03-3 showed chlorosis under the Fe0 treatment at 7 d (Fig. 1), it still possessed higher SPAD value and iron concentration in leaves than that of Bd-2 soybean. Besides the P-efficient cultivar, 03-3 soybean is also Fe-efficient. In soybean commercial plantings, the identification and use of Fe-efficient genotypes is an important form of managing the soil-related plant stress (Peiffer et al., 2012). Taken together, 03-3 soybean cultivar should be a good resource for application to farm field in calcareous soil. Additional research on the effect of Fe deficiency on more P-efficient soybean varieties is needed in soil conditions.

5. Conclusions

In this study, we demonstrated that the 03-3 cultivar, a P-efficient soybean variety, also exhibits efficient Fe absorption under hydroponic condition. The stable Fe levels in shoots and the higher concentration of active Fe under Fe-deficient conditions suggest that 03-3 may also be a Fe-efficient cultivar. The advantages of the interaction between P and Fe in 03-3 cultivar may improve the ecological adaptation of the plants to calcareous soil. Substantial efforts are being made to improve P and Fe nutrition efficiency at the molecular, cellular, whole-plant and ecological population levels on different soil conditions. It is suggested that screening and breeding soybean variety

based on a better understanding of the molecular, physiological and ecological processes and interactions, at the individual level and right up to the agroecosystem level, is vital for a sustainable agriculture with high yields, high nutrient-use efficiency and environmental health.

Contributions

Wei Qiu, Xiaotong Guo, and Yuanmei Zuo designed the experiments. Wei Qiu performed most of the experiments, including plant analysis, gene expression analysis, and part of the soil analysis. Xiaoli Zhang participated in the sample collection and measurement of the ferric reductase activity. Nanqi Wang and Jing Dai conducted part of the plant nutrient analysis and analyzed the data. Wei Qiu wrote the manuscript and Yuanmei Zuo revised the paper. All of the authors discussed the results and commented on the manuscript. Yuanmei Zuo provided funding for this work as corresponding author.

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Figure captions

Fig. 1. Fe nutrition of young leaves of 03-3 (P-efficient) and Bd-2 (P-inefficient)
soybean plants at 3 and 7 days after treatment (DAT) with 0, 20, 40, and 80 μ M Fe. The
young leaves of Bd-2 plants exhibited obvious chlorosis induced by Fe deficiency
under the 0, 20, and 40 μ M Fe treatments after 7 days.

Fig. 2. SPAD values of young leaves of 03-3 (P-efficient) and Bd-2 (P-inefficient)

soybean plants at 3 and 7 days after treatment (DAT) with 0, 20, 40, and 80 μ M Fe. The results are presented as the means $\pm$ SE of triplicate samples. Different letters above the bars indicate a significant difference ($P < 0.05$) between the treatments.

Fig. 3. Active Fe concentration in young leaves of 03-3 (P-efficient) and Bd-2 (P-inefficient) soybean plants at (A) 3 and (B) 7 days after treatment (DAT) with 0, 20, 40, and 80 μ M Fe. The results are presented as the means $\pm$ SE of triplicate samples. Different letters above the bars indicate a significant difference ($P < 0.05$) between the treatments.

Fig. 4. (A) Root ferric reductase activity, (B) total Fe uptake, (C) Fe translocation from root to shoot and (D) Fe-utilization efficiency in the shoots of 03-3 (P-efficient) and Bd-2 (P-inefficient) soybean plants at 7 days after treatment (DAT) with 0, 20, 40, and 80 μ M Fe. The results are presented as the means $\pm$ SE of triplicate samples. Different letters above the bars indicate a significant difference ($P < 0.05$) between the treatments. Single or two asterisks indicate $P < 0.05$ or <0.01 by t-test.

Fig. 5. Expression pattern of *GmIRT1*, *GmFRO2*, *GmFRD3a*, *ferritin* gene and Fe concentration in 03-3 (P-efficient) and Bd-2 (P-inefficient) soybean plants at 7 days

after treatment (DAT) with 0 μ M Fe. (A) Relative expression of *GmIRT1* and *GmFRO2* gene in soybean roots. (B) Relative expression of *GmFRD3a* gene in soybean roots and stems. (C) Relative expression of *ferritin* and (D) Fe concentration in soybean roots, stems and leaves. The results are presented as the means $\pm$ SE of triplicate samples. Different letters above the bars indicate a significant difference ($P < 0.05$) between the treatments.

Fig. 6. Iron concentration in the leaves of 03-3 (P-efficient) and Bd-2 (P-inefficient) soybean plants at 7 days after treatment (DAT) with or without P. The results are presented as the means $\pm$ SE of triplicate samples. Different letters indicate a significant difference ($P < 0.05$) between the treatments.

Table 1. Biomass of 03-3 (P-efficient) and Bd-2 (P-inefficient) soybean plants at 3 and 7 days after treatment (DAT) with 0, 20, 40, and 80 μ M Fe. The results are presented as the means $\pm$ SE of triplicate samples. Different letters indicate a significant difference ($P < 0.05$) between the treatments. The asterisks represent the differences between genotype under the same Fe concentration. Single or two asterisks indicate $P < 0.05$ or <0.01 by t-test.

Table 2. Nutrient concentration and accumulation in the shoots of 03-3 (P-efficient) and Bd-2 (P-inefficient) soybean plants at 7 days after treatment (DAT) with 0, 20, 40, and 80 μ M Fe. The results are presented as the means $\pm$ SE of triplicate samples. Different letters indicate a significant difference ($P < 0.05$) between the treatments. The asterisks represent the differences between genotype under 0 μ M Fe concentration. Single or two asterisks indicate $P < 0.05$ or <0.01 by t-test.

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Author contribution

Wei Qiu, Xiaotong Guo, and Yuanmei Zuo designed the experiments. Wei Qiu performed most of the experiments, including plant analysis, gene expression analysis, and part of the soil analysis. Xiaoli Zhang participated in the sample collection and measurement of the ferric reductase activity. Nanqi Wang and Jing Dai conducted part of the plant nutrient analysis and analyzed the data. Wei Qiu wrote the manuscript and Yuanmei Zuo revised the paper. All of the authors discussed the results and commented on the manuscript. Yuanmei Zuo provided funding for this work as corresponding author.

Table 1

Genotype	Iron concentration (μM)	3 DAT DW		7 DAT DW	
		Shoot (g pot^{-1})	Root (g pot^{-1})	Shoot (g pot^{-1})	Root (g pot^{-1})
03-3	0	1.40 $\pm$ 0.03c**	0.13 $\pm$ 0.02	2.73 $\pm$ 0.23b**	0.23 $\pm$ 0.07
	20	1.50 $\pm$ 0.08bc**	0.11 $\pm$ 0.02	3.59 $\pm$ 0.61ab*	0.30 $\pm$ 0.10
	40	1.83 $\pm$ 0.14a**	0.19 $\pm$ 0.02*	3.59 $\pm$ 0.57ab*	0.43 $\pm$ 0.07
	80	1.73 $\pm$ 0.04ab**	0.22 $\pm$ 0.04*	4.69 $\pm$ 0.83a*	0.37 $\pm$ 0.06
Bd-2	0	0.85 $\pm$ 0.09	0.07 $\pm$ 0.03	1.17 $\pm$ 0.14	0.08 $\pm$ 0.01
	20	0.83 $\pm$ 0.05	0.07 $\pm$ 0.02	1.41 $\pm$ 0.29	0.12 $\pm$ 0.04
	40	0.83 $\pm$ 0.06	0.07 $\pm$ 0.02	1.07 $\pm$ 0.19	0.27 $\pm$ 0.03
	80	0.83 $\pm$ 0.13	0.08 $\pm$ 0.02	2.13 $\pm$ 0.37	0.24 $\pm$ 0.04
ANOVA: Genotype, Fe		<i>P</i> - value of biomass			
Genotype		0.000	0.000	0.000	0.002
Fe treatment		0.100	0.073	0.047	0.441
G X F		0.080	0.550	0.666	0.011

Table 2

Genotype	Iron concentration (μM)	Nutrient concentration in shoot		Nutrient accumulation in shoot	
		Fe (mg kg^{-1} DW)	P (g kg^{-1} DW)	Fe ($\mu\text{g plant}^{-1}$)	P (mg plant^{-1})
03-3	0	48.9 $\pm$ 0.8bc	5.1 $\pm$ 0.2b	33.4 $\pm$ 2.5b**	3.5 $\pm$ 0.4bc*
	20	96.9 $\pm$ 6.5a	4.8 $\pm$ 0.1b	86.6 $\pm$ 14.2a	4.3 $\pm$ 0.8ab
	40	101.3 $\pm$ 5.3a	4.8 $\pm$ 0.1b	96.9 $\pm$ 6.9a	4.6 $\pm$ 0.5ab
	80	90.6 $\pm$ 4.2a	4.8 $\pm$ 0.2b	107.3 $\pm$ 26.7a	5.7 $\pm$ 1.4a
Bd-2	0	30.0 $\pm$ 1.7c	5.8 $\pm$ 0.1a	8.9 $\pm$ 1.5b	1.7 $\pm$ 0.2cd
	20	78.7 $\pm$ 2.4a	5.6 $\pm$ 0.1a	27.9 $\pm$ 5.9b	2.0 $\pm$ 0.4cd
	40	81.5 $\pm$ 2.4a	5.1 $\pm$ 0.1b	21.8 $\pm$ 4.1b	1.4 $\pm$ 0.3d
	80	55.1 $\pm$ 19.8b	4.9 $\pm$ 0.1b	25.7 $\pm$ 7.2b	1.8 $\pm$ 0.3cd

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6