Rhizosphere acidification of faba bean, soybean and maize

L.L. Zhou a,c, J. Cao b, F.S. Zhang a, L. Li a,⁎

a College of Resources and Environmental Sciences, China Agricultural University, Key Laboratory of Plant and Soil Interactions, Ministry of Education, Beijing, 100094, PR China
b School of Life Science, Key Laboratory of Arid and Grassland Ecology, Lanzhou University, Lanzhou 730000, PR China

c Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, 100094, PR China

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A B S T R A C T

Interspecific facilitation on phosphorus uptake was observed in faba bean/maize intercropping systems in previous studies. The mechanism behind this, however, remained unknown. Under nitrate supply, the difference in rhizosphere acidification potential was studied by directly measuring pH of the solution and by visualizing and quantifying proton efflux of roots between faba bean (Vicia faba L. cv. Lincan No.5), soybean (Glycine max L. cv. Zhonghuan No.17) and maize (Zea mays L. cv. Zhongdan No.2) in monoculture and intercrop, supplied without or with 0.2 mmol L−1 P as KH2PO4. The pH of the nutrient solution grown faba bean was lower than initial pH of 6.0 from day 1 to day 22 under P deficiency, whereas the pH of the solution with maize was declined from day 13 after treatment. Growing soybean increased solution pH irrespective of P supply. Under P deficiency, the proton efflux of faba bean both total (315.25 nmol h−1 plant−1) and specific proton efflux (0.47 nmol h−1 cm−1) was greater than that of soybean (21.80 nmol h−1 plant−1 and 0.05 nmol h−1 cm−1, respectively). Faba bean had much more ability of rhizosphere acidification than soybean and maize. The result can explain partly why faba bean utilizes sparingly soluble P more effectively than soybean and maize do, and has an important implication in understanding the mechanism behind interspecific facilitation on P uptake by intercropped species.

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1. Introduction

Interspecific facilitation was observed in Fabaceae/maize intercropping systems (Li et al., 1999). Total P uptake was increased by 20.3% when no P was fertilized and 37.7% when P was fertilized at 33 kg ha−1 in faba bean/maize intercropping system, comparing to corresponding monoculture in the field experiment (Li et al., 2003a). However, no similar result was observed in the soybean/maize intercropping system (Li et al., 2003b). Therefore, the mechanism behind the interspecific facilitation on P uptake was what we concern with. Li (2004) found that the capacity of faba bean to mobilize insoluble soil P is much greater than that of maize, and considered that a decrease of rhizosphere pH played an important role in P mobilization.

P deficiency can increase rhizosphere acidification that has been proved in white lupin (Lupinus albus L.) (Dinkelaker et al., 1989; Hinsinger and Gilkes, 1995; Neumann and Römheld, 1999), rape (Brassica napus L.) (Hoffland, 1992), maize (Zea mays L.), soybean (Glycine max L.), sorghum (Sorghum bicolor L.), sordan (soybean (S. bicolor L.), sudangrass (Sorghum sudanense L) hybrid), wheat (Triticum aestivum L.), oats (Avena sativa L.) and barley (Hordeum vulgare L.)(Gollany and Schumacher, 1993). Hedley et al. (1983) had showed that rape (Brassica napus var emerald) would acidify the soil if deprived of an adequate supply of phosphate. P deficiency strongly increased the net release of proton from the roots of tomato (Lycopersicon esculentum L., cv. Moneymaker), chickpea (Cicer arietium) and white lupin (L. albus L. cv. Amiga) and it probably determined their ability to utilize acid soluble Ca-phosphates in calcareous soils or rock phosphate fertilizers (Neumann and Römheld, 1999). Faba bean (Vicia faba L.) is an important grain legume in temperate area. Bolland et al. (1999) found that faba bean had higher yields with no added P and lower yield response to added P than wheat did in both greenhouse and field experiments. Nuruzzaman et al. (2005) also showed that faba bean had higher P concentration and contents than field pea (Pisum sativum L.), white lupin (L. albus L.) and wheat did. However, there was no published result on rhizosphere acidification available for faba bean. Furthermore, there were few results that focused on understanding interspecific facilitation on P uptake by intercropped faba bean and maize in terms of rhizosphere acidification.

Gillespie and Pope (1989) found that alfalfa rhizosphere acidification could enhance P uptake by walnut tree seedling in alfalfa (Medicago sativa L.) /black walnuts (Juglans nigra L.) intercropping system. However, the mechanism of faba bean that facilitates P uptake by maize (Li et al., 2003a) remains unclear. The objective of present study was to test the hypothesis that faba bean has more ability of rhizosphere acidification than maize does, and therefore provides some evidence on interspecific facilitation on utilization of sparingly soluble P in soil.

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* Corresponding author.
E-mail address: lilong@cau.edu.cn (L. Li).

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2. Materials and methods

2.1. Plant materials and culture conditions

2.1.1. Experiment 1

Hydroponic culture, a three-factor design of experiment with 4 replicates, was conducted in culture room at 23–25 °C day/18–20 °C night with 14 h photoperiod (200 μmol m⁻² s⁻¹). Three factors were P supply [−P (without P supply) and +P (supplying with 0.2 mmol L⁻¹ P)], plant species (Faba bean (V. faba L cv. Lincan No.5), soybean (G. max L cv. Zhonghuan No.17) and maize (Z. mays L cv. Zhongdan No.2)], and planting pattern (monocultured and intercropped). Seeds of faba bean, soybean and maize were surface-sterilized in 10% (v/v) H₂O₂ for 30 min and rinsed thoroughly in distilled water. Afterwards, the seeds had been germinated in quartz sand for 7–8 days. Four plants of maize, faba bean and soybean (as monoculture), or 2 plants of maize plus 2 plants of faba bean or 2 plants of maize plus 2 plants of soybean (as intercropping) were transplanted to 2 L of container with 1/2 concentration of full nutrient solution. The seedlings were cultivated at the 1/2 of concentration of full nutrient solution for 4 days. Then the plants were changed into full nutrient solution either without P addition or P supplied at 0.2 mmol L⁻¹ P in the form of KH₂PO₄. The nutrient solution contained the following minerals (mol L⁻¹): K₂SO₄ 0.75×10⁻³, MgSO₄ 0.65×10⁻³, KCl 0.1×10⁻³, Ca(NO₃)₂ 2.0×10⁻³, KH₂PO₄ 0.20×10⁻³, H₃BO₃ 1×10⁻⁶, MnSO₄ 1×10⁻⁶, CuSO₄ 1×10⁻⁷, ZnSO₄ 1×10⁻⁶, (NH₄)₆Mo₇O₂₄·4H₂O 5×10⁻⁶, Fe-EDTA 1×10⁻⁴. The pH of the nutrient solution was adjusted to 6.0 (i.e. initial pH of the solution was 6.0) by 0.01 mol L⁻¹ HCl or 0.01 mol L⁻¹ NaOH. The nutrient solution was renewed every 3 days.

2.1.2. Experiment 2

The experimental conditions and treatments were the same as experiment 1. Seeds of faba bean, soybean and maize were surface-sterilized in 10% (v/v) H₂O₂ for 30 min and washed thoroughly using distilled water before germination in the dark. Germinated seeds were placed on 28 cm-long and 20 cm-wide filter paper and nylon mesh layers were added by two. Afterwards, the seeds were placed on 28 cm-long and 20 cm-wide filter paper where nylon mesh (30 μm) was placed beneath to avoid the roots penetrating the filter paper. An additional layer of filter paper and nylon mesh were added on top. The filter papers and nylon mesh layers were fixed by two parallel clear plastic plates using two elastic clips. Four plants of faba bean, soybean and maize were transplanted as monoculture, or 2 plants of faba bean plus 2 plants of maize, or 2 plants of soybean plus 2 plants of maize as intercropping. These seedlings were grown in 1/4 concentration of full nutrient solution (the composition of nutrient solution were the same as in the experiment 1) either without P addition or P supplied in the form of KH₂PO₄ in the first 3 days, afterwards they were grown in 1/2 concentration of full nutrient solution. On the seventh day from transplanting, they were placed into full nutrient solution [except for P treatments where there was −P and +P (0.2 mmol L⁻¹ P) treatment] for a week.

2.2. pH of nutrient solution (Experiment 1)

The pH change of the nutrient solution was obtained through a pH meter (pH-HJ90, China) from the first day after transplanting and afterwards it was measured every 2 days, i.e., just before the solution will be substituted for new nutrient solution.

2.3. Visualization of rhizosphere acidification (Experiment 2)

The visualization of rhizosphere acidification was based on the method of Rao et al. (2000). After grown for 14 days in treatment solution, the intact plants were carefully taken from the nylon mesh and washed in 0.2 mmol L⁻¹ CaSO₄ solution for a few minutes, and then rinsed in distilled water with pH 5.5. In intercropping treatment, two plants grew together for two weeks, on filter paper, so their roots tend to intertwine partially, and can be separated easily by using a nipper. After that, the whole plant roots were placed on a 3 mm thick agar-gel (9.0 g L⁻¹) film with pH 5.5 containing 0.1 g L⁻¹ pH indicator (bromocresol purple) and complete nutrient solution without P addition and P supplied with 0.2 mmol L⁻¹. At least 3/4 of the root surface was embedded in the agar-gel by placing a transparent film on the roots and forcing the roots into the agar-gel gently. The transparent film was then removed using a plastic plate placed on the agar-gel film 3 mm above the surface to provide aeration to the roots. The agar-gel and the plastic plate were wrapped with aluminium foil to avoid light penetration into the root zone and was placed at a 45° angle for 6 h.

2.4. Quantification of roots proton efflux (Experiment 2)

Quantification of proton exudation was based on Rao’s method (Rao et al., 2000). The agar-gel film described above was scanned using a scanner (Artix Scan™ 2500, China) with the settings at 90 dpi, full colour and brightness level 4. These digitized images were further processed using image-analysis software Adobe Photoshop 7.0 and Scion image to obtain grey level values (0 to 255) for each pixel. Before these, a pH versus grey level value calibration was established, the actual pH (from 4.4 to 7.3) at each band was measured using a pH meter (Horiba B-212, Japan) in the area of 3-mm thick agar-gel. These digitized images were further processed using image-analysis software Adobe Photoshop 7.0 and Scion image to obtain grey level values (0 to 255) for each pixel. Before these, a pH versus grey level value calibration was established, the actual pH (from 4.4 to 7.3) at each band was measured using a pH meter (Horiba B-212, Japan) in the area of 3-mm thick agar-gel films without roots and processed in the same way to obtain grey level values. So the standard curve was established

\[ y = 36.739x - 86.446 \]  

where y is grey level value and x is pH. The final pH of rhizosphere was calculated based on the standard curves for relationship between color density and the corresponding pH value, and the proton flux was calculated according to the difference between final pH and initial pH

\[ H^+ = \left(10^{-pH_i} - 10^{-pH_f}\right) \times 10^9 - P_v / 1000 \]  

where \( H^+ \) is amount of protons (nmol), \( pHi \) and \( pHf \) are the initial pH and final pH at each pixel, respectively. \( P_v \) refers to the volume of pixel area and is 0.01875 cm³ (Versel and Pilet, 1986; Rao et al., 2000). The negative values indicated alkalinization and the positive values indicated acidification (Rao et al., 2000). Proton efflux of whole

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
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<tbody>
<tr>
<td>Effects of interspecific root interactions and P supply on the shoot and root dry weights (g plant⁻¹) of faba bean, soybean and maize in hydroponics (Experiment 1).</td>
</tr>
<tr>
<td>P Cropping level type</td>
</tr>
<tr>
<td>P₀ Monoculture</td>
</tr>
<tr>
<td>P₀ Faba bean/maize intercropping</td>
</tr>
<tr>
<td>P₀ Soybean/maize intercropping</td>
</tr>
<tr>
<td>P₀ Average</td>
</tr>
</tbody>
</table>

Footnotes:
1 Mean values of the different planting patterns with the same P source followed by different lower case letters (a, b) are significantly different (p<0.05).
2 Mean values of P level treatment followed by different capital letters (A, B) are significantly different (p<0.05).
Table 2
Effects of interspecific root interactions and P supply on the shoot and root dry weights (g plant⁻¹) of faba bean, soybean and maize in filter paper culture (Experiment 2).

<table>
<thead>
<tr>
<th>P level type</th>
<th>Cropping type</th>
<th>Shoots (g plant⁻¹)</th>
<th>Roots (g plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Faba bean/maize</td>
<td>0.39a</td>
<td>0.25a</td>
</tr>
<tr>
<td></td>
<td>Soybean/maize</td>
<td>0.37a</td>
<td>0.25a</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>0.38A</td>
<td>0.25A</td>
</tr>
<tr>
<td>P₀ Monoculture</td>
<td></td>
<td>0.09a</td>
<td>0.04a</td>
</tr>
<tr>
<td>P₀ Intercropping</td>
<td></td>
<td>0.10a</td>
<td>0.03a</td>
</tr>
<tr>
<td>P₀ Average</td>
<td></td>
<td>0.09A</td>
<td>0.04A</td>
</tr>
</tbody>
</table>

1 Mean values of the different planting patterns with the same P source followed by different lower case letters (a, b) are significantly different (p<0.05).

2 Mean values of p level treatment followed by same capital letters (A, A) showed no significant different (p>0.05).

The experiments were set up in a completely randomized design. Statistical significance of differences between treatments was analyzed by analysis of variance (ANOVA) and LSD (least significant difference) multiple comparison (SAS Institute, 1985).

3. Results

3.1. Plant growth and biomass

In experiment 1, the shoot dry weight of intercropped faba bean was significantly greater than that of corresponding monocultured faba bean under P deficiency (Table 1). However, both the shoot and root dry weights of soybean were not affected by intercropping regardless of P supplying or not (Table 1). Both the shoot and root dry weights of maize, regardless of being intercropped with faba bean or with soybean, were not significantly different from corresponding monocultured maize (Table 1). The shoot dry weight of faba bean, soybean and both shoot and root dry weights of maize supplied with 0.2 mmol L⁻¹ P were significantly greater than those without P supply after growing 22 days (Table 1). The root dry weight of both faba bean and soybean were not affected by P supplying (Table 1).

In experiment 2, the shoot and root dry weights of faba bean, soybean and maize were not affected significantly by intercropping under without P supplying condition (Table 2). However, with the supply of 0.2 mmol L⁻¹ P, the shoot and root dry weights of soybean were greater than those of the corresponding monocultured soybean. The shoot and root dry weights of the maize intercropped with faba bean were decreased and the maize intercropped with soybean was not affected (Table 2). The shoot and root dry weights of faba bean were greater than those of soybean and maize, regardless of P supply (Table 2).

3.2. pH in nutrient solution grown different species (Experiment 1)

At very early growth stage (from day 1 to day 4), plants were grown in half concentration of full nutrient solution, which supplied with 0.1 mmol L⁻¹ P, the pH of nutrient solution grown faba bean and soybean for 4 days was decreased to 4.4 and 5.4, respectively, whereas the pH of the solution grown maize alone was increased to 7.2 from initial 6.0 [Fig. 1(i) and (ii)]. After day 4, the plants were grown in the full nutrient solution without supplying with P, the pH of the nutrient solution grown faba bean was lower than initial 6.0 during the whole cultivated growth stages from day 4 to day 22, although the decline of the pH was a little bit alleviated at day 22 [Fig. 1(i)], indicating that faba bean roots always acidified the rhizosphere in 22 days of growth.

Fig. 1. The pH of nutrient solution after grown sole faba bean, soybean, and maize for 3 days (i.e. just before the solution will be substituted for new nutrient solution) (i) without P supply; (ii) supplied with 0.2 mmol L⁻¹ P; and (iii) faba bean/maize, soybean/maize intercropping without P supply and supplied with 0.2 mmol L⁻¹ P.
after transplanting under phosphorus deficiency condition. The pH of nutrient solution grown maize was increased to and retained to a range of 7.0–7.5 before day 13 then decreased rapidly to 4.7 on day 19 and maintained around 4.5 [Fig. 1(i)], which showed that maize could also acidify the rhizosphere, but at more late growth stage than faba bean could. The pH of the solution grown soybean was always higher

going to 7.8 on day 19 and maintained around 7.0 [Fig. 1(j)], which showed that soybean could also acidify the rhizosphere, but at more late growth stage than faba bean could. The pH of the solution grown maize was always lower than that of faba bean because the former had a stronger root system than the latter. The pH of the solution grown faba bean was always lower than that of soybean because the former had a stronger root system than the latter.

![Fig. 2](image)

**Fig. 2.** Visualization of rhizosphere acidification of (a) faba bean, (b) soybean, (c) maize, (d) faba bean intercropped with maize, (e) soybean intercropped maize, (f) maize intercropped with faba bean, (g) maize intercropped with soybean under P deficiency, (h) faba bean, (i) soybean, (j) maize, (k) faba bean intercropped with maize, (l) soybean intercropped maize, (m) maize intercropped with faba bean, (n) maize intercropped with soybean, supplied with 0.2 mmol L^{-1} P for 14 days. The roots were imbedded in agar-gel containing a pH indicator (bromocresol purple) for 6 h.

![Fig. 3](image)

**Fig. 3.** Total proton efflux of whole plant roots (nmol h^{-1} plant^{-1}) for (i) monocropped faba bean, soybean and maize; (ii) maize grown alone, intercropped with faba bean, or intercropped with soybean; (iii) soybean grown alone, or intercropped with maize; (iv) faba bean grown alone or intercropped with maize, under P deficiency and supplying with 0.2 mmol L^{-1} P; Result of ANOVAS with significant effect of main factor (P level) and sub-factor (planting patterns): Bars with different capital letters (A, B) mean significant difference between P sources (p<0.05). Bars with different lower case letters (a, b) mean significant difference between monoculture and intercropping (p<0.05).
than the initial 6.0 of new nutrient solution, even under P deficiency condition [Fig. 1(i)].

When the plants were grown in full nutrient solution with P supplied at 0.2 mmol L\(^{-1}\) P [Fig. 1(ii)], the pH of the nutrient solution grown faba bean and soybean tended to increase and exceeded 7.0 from day 16 and day 10, respectively, whereas the pH of the nutrient solution grown maize was around 7.0–7.5 from day 4 to day 22 [Fig. 1(ii)]. These results showed that faba bean alkalize the rhizosphere latest and maize earliest during their growth progress under sufficient phosphorus supply conditions. In this experiment, phosphorus supply seems to have less effect on acidification of soybean roots, in which there was no significant difference in the pH of nutrient solution grown soybean between the added P and without P supply treatments [Fig. 1(i) and (ii)].

The pH of nutrient solution grown faba bean and maize together in the same pot (intercropping of faba bean and maize) was lower than that of intercropping of soybean and maize during early growth stage, regardless of P supply or not [Fig. 1(iii)], indicating that acidification of faba bean roots was exerted with faba bean and maize intercropping. In addition, pH of the nutrient solution grown faba bean and maize intercropping was reduced by associated faba bean, compared to monocultured maize, especially under phosphorus deficiency condition [Fig. 1(i) and (iii)].

3.3. Visualization of rhizosphere acidification (Experiment 2)

The difference in ability of rhizosphere acidification among faba bean, soybean and maize was distinguished easily by visualization method [Fig. 2]. Although there were no significant effects of P supply and planting patterns on all of three plants species, a significant difference was observed between faba bean, soybean and maize. Faba bean rhizosphere turned bright yellow after its roots were placed in agar-gel with pH indicator (bromocresol purple) and designed nutrient solution for 6 h, indicating faba bean had very intensive ability of rhizosphere acidification in both monoculture and intercropping, regardless of supplying with P or not (Fig. 2a, d, h and k). Soybean presented a rhizosphere acidification too, but was much less than faba bean. Furthermore, soybean seems to alkalize its rhizosphere in its root tips whether P was supplied or not (Fig. 2b and i). Soybean with different planting treatment showed the similar trends (Fig. 2e and l). In contrast, the whole roots of maize alkalized the rhizosphere in both monoculture and intercropping, regardless of supplying with P or not (Fig. 2c, f, g, j, m and n).

3.4. Proton efflux of whole plant roots (Experiment 2)

The proton is exudated apparently from roots if the efflux values are positive, and the hydroxy is released apparently from roots if the efflux values are negative. The amount of proton efflux of the faba bean was significantly greater than soybean for the whole plant roots [Fig. 3(i)]. Total proton efflux by faba bean roots was 14 times that by soybean under P deficient treatment, and 5 times that by soybean when 0.2 mmol L\(^{-1}\) P was supplied. The total proton efflux was significantly enhanced by P deficiency, compared to that when P was supplied with 0.2 mmol L\(^{-1}\) P as KH\(_2\)PO\(_4\) for all of three tested plant species [Fig. 3(i)]. When no P was supplied, the alkalization of maize...
roots was less under monoculture than under intercropping. However, when P was supplied, the alkalization of maize roots was greater than intercropped with faba bean than when maize was monocultured and intercropped with soybean. The alkalization of maize rhizosphere was increased by P deficiency [Fig. 3(ii)]. P supplying did not significantly affect proton efflux of soybean roots. However, the amount of proton efflux in intercropped soybean was greater than monocropped soybean under P deficiency [Fig. 3(iii)]. The rhizosphere acidification of faba bean was significantly greater under P deficiency than under supplying with 0.2 mmol L\(^{-1}\) P. There was no significant difference in total proton efflux between the intercropped and monocultured faba bean [Fig. 3(iv)].

3.5. Specific proton efflux (Experiment 2)

The specific proton efflux, which was expressed by proton exudation per hour and per centimeter root length, showed an intensity index of proton exudation by roots. The specific proton efflux of faba bean roots was significantly greater than those of soybean and maize (Fig. 4). The specific proton efflux of faba bean roots was 9-fold higher than soybean under P deficiency, and 2-fold under supplying with 0.2 mmol L\(^{-1}\) P. The results proved that faba bean had stronger acidification ability than other species did, in terms of intensity of rhizosphere acidification.

4. Discussions

We tested the hypothesis that faba bean has more capacity of rhizosphere acidification than maize. The results explain partly why faba bean could mobilize sparingly soluble phosphorus in soil. Many reports have shown that plant rhizosphere acidification can enhance the effectiveness of phosphate rocks utilization such as white lupin (Sas et al., 2002), clover and ryegrass (Hinsinger and Gilkes, 1996) and lowland rice (Saleque and Kirk, 1995). Zoya (1997) showed that the effectiveness of phosphate rock fertilizer utilization was increased with decreasing rhizosphere pH of camellia plants. Hinsinger and Gilkes (1996) reported that the excretion of protons by roots of clover and ryegrass was the driving force for the root-induced dissolution of phosphate rock. Using a mathematical model, Saleque and Kirk (1995) concluded that solubilization by acidification account for at least 80% of the P taken up for lowland rice.

In experiment 2, although faba bean has a large root system (Table 2) comparing to maize and soybean, its proton efflux of whole plants and the specific proton efflux was the most among the three species. Therefore, the intensive acidification ability of faba bean was not only due to its large root system at early stage, but also the rate of proton efflux per unit root length.

N forms can affect rhizosphere pH, which had been testified in many plants, such as soybean (Riley and Barber, 1971), ryegrass (Gahoonia et al., 1992), sunflower (Römheld et al., 1984), rice (Kirk et al., 1999) and tea (Zoya et al., 1998), in which the increased rhizosphere pH was associated with nitrate-N and rhizosphere acidification was associated with ammonium-N. In contrast, although nitrate-N was supplied in the present experiment, faba bean showed a strong acidification of its rhizosphere (Fig. 2a, d, h and k) and its pH can decrease to 4.0–4.5 in hydroponic culture supplied with nitrate-N [Fig. 1(i) and (ii)]. This further proved that faba bean had an important trait of rhizosphere acidification.

The present study demonstrated a considerable difference in the ability of rhizosphere acidification of three plants species, even between two legume species, faba bean and soybean. In the presence of nitrate source, alkalization of the maize rhizosphere may be due to the nitrate uptake (Nye, 1981). Legume absorbed more cations than anions through N\(_2\) fixation and released proton (Raven et al., 1990; Tang et al., 1998). So some N\(_2\)-fixing legume even fed by nitrate, they also can acidify rhizosphere (Marschner and Römheld, 1983). In our experiment conditions, the legume plants were not inoculated with rhizobium, therefore the biological nitrogen fixation probably was not one of the reasons that caused rhizosphere acidification. Dinkelaker et al. (1989) also had showed that NO\(_3\) -fed white lupin decrease rhizosphere soil pH in a P-deficient calcareous soil due to the excretion of a large amount of citric acid. Although Nuruzzaman et al. (2005) did not detect significant amount of carboxylates in the rhizosphere of faba bean [V. faba L. cv. Ascot (an Australia cultivar)], Li et al. (2007) showed that faba bean [V. faba L. cv. No 5 Lincan (a Chinese cultivar)] can exude much higher amount of malate and citrate into rhizosphere than maize or soybean. This probably is owing to genotype difference in carboxylation exudation between Chinese and Australian cultivars. In another study, Tang et al. (2007) showed that soybean released very little organic acids and that P mobilization from sparingly soluble sources did not correlated with exudation of organic acids nor rhizosphere pH.

Intercropping did not influence the proton efflux of faba bean roots. However, intercropping enhanced the proton efflux in soybean roots when no P was supplied. On the other hand, rhizosphere alkalization of maize was intensified by intercropping with faba bean under P\(_{200}\) treatment, comparing to maize grown alone or intercropping with soybean. The mechanisms behind these phenomena need to be further studied.

The shoot biomass of faba bean was 80% greater in intercropping than in monoculture where no P was applied, in Experiment 1. An improved P acquisition cannot explain this as there was absolutely no P to be mobilized in this treatment. One of reasons may be that individual plant has more resources in intercropping treatment (2 faba bean plus 2 maize plants per pot) than in monocropped treatment (4 faba bean plants).

In conclusion, through comparing rhizosphere acidification between faba bean, soybean and maize in our experiments, we concluded that faba bean can release significant amounts of proton, comparing to soybean and maize. This may be one important mechanism of interspecific facilitation on P uptake between intercropped faba bean and maize, especially grown on calcareous soils.

Acknowledgments

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