Crop Root Behavior Coordinates Phosphorus Status and Neighbors: From Field Studies to Three-Dimensional In Situ Reconstruction of Root System Architecture\[W\][OA]

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Root is a primary organ to respond to environmental stimuli and percept signals from neighboring plants. In this study, root responses in maize (Zea mays)/soybean (Glycine max) intercropping systems recognized soil phosphorus (P) status and neighboring plants in the field. Compared to self culture, the maize variety GZ1 intercropping with soybean HX3 grew much better on low P, but not in another maize variety, NE1. This genotypic response decreased with increasing distance between plants, suggesting that root interactions were important. We further conducted a detailed and quantitative study of root behavior in situ using a gel system to reconstruct the three-dimensional root architecture. The results showed that plant roots could integrate information on P status and root behavior of neighboring plants. When intercropped with its kin, maize or soybean roots grew close to each other. However, when maize GZ1 was grown with soybean HX3, the roots on each plant tended to avoid each other and became shallower on stratified P supply, but not found with maize NE1. Furthermore, root behavior in gel was highly correlated to shoot biomass and P content for field-grown plants grown in close proximity. This study provides new insights into the dynamics and complexity of root behavior and kin recognition among crop species in response to nutrient status and neighboring plants. These findings also indicate that root behavior not only depends on neighbor recognition but also on a coordinated response to soil P status, which could be the underlying cause for the different growth responses in the field.

The root is the major organ for plant nutrient and water acquisition (Barber, 1995). The developmental plasticity of the root system is influenced by environmental factors (Karban, 2008) including alterations in root architecture in response to heterogeneity of mineral nutrients, including phosphorus (P; Liao et al., 2001b, 2004; Fang et al., 2009) and nitrogen (Bouma et al., 2001; López-Bucio et al., 2003; Hodge, 2004). Besides responding to environmental stimuli, plants also respond to other plants growing in close proximity. Several studies show that roots respond to plant neighbors in a very specific manner depending on the identity of the neighbors (Maina et al., 2002; de Kroon et al., 2001; Falik et al., 2003; Dudley and File, 2007; Karban and Shiojiri, 2009). It was reported that some plant species respond differently to self and non-self neighbors (Kranzitt and Caldwell, 1995; Huber-Sannwald et al., 1996; Mahall and Callaway, 1996; Biedrzycki et al., 2010). Buchloe dactyloides plants could distinguish between self and nonself neighbors, and produced fewer and shorter roots when they grew with their siblings (Gruntman and Novoplansky, 2004). de Kroon (2007) found that root extension growth tended to be greater when roots grew into substrate containing nonself roots. Dudley and File (2007) reported that plants of the Great Lakes Sea Rocket (Cakile edentula) invested more biomass in fine roots when they competed with unrelated individuals than with siblings. It was also reported that Impatiens pallida plants were capable of kin recognition, but only in the presence of another plant’s roots (Murphy and Dudley, 2009). From these and other recent studies, there is increasing evidence that root behavior is much more sophisticated than was previously thought (Hodge, 2009).

Intercropping is a widely used agricultural system to which contributes to more than 50% of the agriculture productivity in developing countries (Andrews and Kassam, 1976; Willey, 1985; Li et al., 1999; Zhang and Li, 2003; Sun et al., 2009). But so far, no studies on plant recognition in this important agricultural system have been performed. What happens to the root architecture in the intercropping system? Are there any special root behaviors among intercropping plants in response to the availability of different nutrients? Since the root system is below ground, it is difficult...
to observe and quantify root growth in situ. There have been very few reports on in situ observations of root interactions (Fang et al., 2009). In this study, we examined plant growth responses to P stress of two maize (Zea mays) varieties intercropped with soybean (Glycine max) in the field, and then grew the same plant species in a transparent gel system combined with three-dimensional (3D) laser scanning to reconstruct the plant root system in 3D that revealed dynamic in situ root behavior in a nondestructive manner in response to different P treatments, to begin to understand how root interactions influence whole-plant behavior in the field.

RESULTS

Plant Growth Behavior in the Field

Different aspects of crop growth were significantly affected by soil P status, planting distance, and cropping combination in the field, based on the measurements of shoot biomass and P content made in this study. Interestingly, when plants were grown in close proximity (5-cm apart), different crop species responded differently to P status and neighboring plants (Table I). Compared to its monoculture, the soybean variety HX3 always had lower shoot biomass and P content when intercropped with maize, especially when intercropped with the maize cultivar GZ1. The two maize varieties used in this study, GZ1 and NE1, differed in growth responses to changes in soil P status when intercropped with soybean HX3. At two harvests in the field (15 and 60 d after sowing), maize GZ1 biomass and P content were 1.73 and 1.34, 1.61 and 1.58 times of those for GZ1 monoculture under low P (LP), respectively (Table I). But no such differences were found for the maize NE1 cultivar and also for wider planting distances (20 × 20 cm and 40 × 40 cm; Supplemental Tables S1 and S2). This indicated that maize root behavior in response to P status and neighbors was genotypically dependent.

Furthermore, the root interactions were significantly affected by soil P status, planting distance, and cropping combination as indicated by the total root length in the 20-cm upper soil layer of the 5-cm middle area between two plants at harvest II (Table II). In general, root interactions increased with decreasing planting distance. In monoculture, soybean had relatively less root interactions, but two maize varieties did not differ dramatically in each other in most cases. In intercropping, when soybean HX3 intercropped with maize GZ1, there were much less root interactions than with maize NE1, and the differences became larger with enlarged planting distance in both LP and stratified P (SP) conditions. The total root length in the 20-cm upper soil layer of the 5-cm middle area between HX3 and GZ1 were only 71.54%, 53.52%, 33.42%, 25.61% and 45.61%, 49.80%, 42.80%, and 8.31% of that between HX3 and NE1 at LP and SP level, respectively (Table II). This indicated that maize root behavior in response to neighbors was also genotypically dependent, and this genotypic difference might be based on root interactions.

Root Growth Behavior in Gel System

To further study whether root interactions and behavior were the main causes for the genotypic differences in maize growth in response to P status and the species and cultivar of its neighbors, soybean HX3, and maize NE1 and GZ1 were grown in the different cropping combinations in a transparent gel system at two P levels. As seen in Figure 1, the findings indicate that the root systems of soybean HX3 intercropped with GZ1 both under LP (Fig. 1A) and SP (Fig. 1B), grew in opposite direction to the GZ1 maize root systems, in what appears to be an avoidance response, while the root systems of HX3 intercropped with maize NE1 both under LP (Fig. 1C) and SP (Fig. 1D) grew toward the maize root system and had a higher frequency of crossing roots than those of the HX3/GZ1 intercropping. This tendency started with young

Table I. Shoot biomass and P content as affected by P status and neighbors in the field experiment when planting distance was 5 × 5 cm

<table>
<thead>
<tr>
<th>Species</th>
<th>Cropping System</th>
<th>Harvest I</th>
<th>Harvest II</th>
<th>P Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass</td>
<td>LP</td>
<td>SP</td>
<td>LP</td>
</tr>
<tr>
<td>Soybean</td>
<td>HX3/HX3</td>
<td>0.61 ± 0.05a</td>
<td>0.47 ± 0.08a</td>
<td>4.15 ± 0.63a</td>
</tr>
<tr>
<td></td>
<td>HX3/NE1</td>
<td>0.54 ± 0.04a</td>
<td>0.42 ± 0.01a</td>
<td>3.45 ± 0.33ab</td>
</tr>
<tr>
<td></td>
<td>HX3/GZ1</td>
<td>0.28 ± 0.02b</td>
<td>0.48 ± 0.03a</td>
<td>2.98 ± 0.33ab</td>
</tr>
<tr>
<td></td>
<td>NE1/NE1</td>
<td>0.71 ± 0.03a</td>
<td>1.04 ± 0.04a</td>
<td>56.04 ± 3.88a</td>
</tr>
<tr>
<td></td>
<td>NE1/GZ1</td>
<td>0.87 ± 0.11a</td>
<td>0.94 ± 0.09a</td>
<td>60.92 ± 7.62a</td>
</tr>
<tr>
<td></td>
<td>HX3/GZ1</td>
<td>0.66 ± 0.08a</td>
<td>0.84 ± 0.03a</td>
<td>46.31 ± 3.02a</td>
</tr>
<tr>
<td>Maize</td>
<td>NE1/NE1</td>
<td>0.38 ± 0.07b</td>
<td>0.66 ± 0.11a</td>
<td>28.80 ± 2.56a</td>
</tr>
<tr>
<td></td>
<td>GZ1/GZ1</td>
<td>0.37 ± 0.07b</td>
<td>0.66 ± 0.11a</td>
<td>28.80 ± 2.56a</td>
</tr>
</tbody>
</table>
plants when the soybean and maize roots had no
direct contact. Furthermore, root systems of the self-
culture treatments, HX3/HX3, GZ1/GZ1, and NE1/
NE1, grew close to each other under both LP and SP
treatments (Fig. 1, E–G).

Root Image Capture and Modeling

Supplemental Figure S1 depicts the original 3D im-
ages of maize/soybean roots under different P treat-
ments captured by a 3D laser scanner on the 12th d after
germination. The high-quality imaging results show
that maize and soybean root systems in the transparent
growth system can be imaged by the 3D laser scanner
successfully and the transparent root growth system
can be used to study not only a single root system (Fang
et al., 2009) but also root systems of multiple plants in
an intercropping system.

The image data obtained by the 3D laser scanner
provide the 3D coordinates of the root surface voxels.
The root system skeleton was then computationally
extracted using a skeleton extraction method based on
the Hough transformation, and mesh modeling using
Ball-B spline was employed (Fang et al., 2009). Mod-
eling results of the root systems of soybean plants
intercropped with maize showed that this method was
robust, with high accuracy and realistic visual effect
for mutliroot systems (Fig. 2).

Quantification of Root Traits

The total root lengths of different cropping combi-
nations from the 1st to 20th d of growth were calcu-
lated. According to the dynamics of the total root
length changes, the soybean HX3/HX3 combination
had the smallest total root length while GZ1/GZ1 had
the largest total root length compared to the other
four cropping combinations under both LP (Fig. 3A)
and SP (Fig. 3B) conditions. Using a curve fitting
(Motulsky and Christopoulos, 2004) to analyze the
total root length on a specific day after germination,
the total root length under LP or SP could be quan-
tified as follows:

\[
\text{Total root length} = \text{a} \times \text{d}^\text{b}
\]

Table II. Total root length in the 20-cm upper soil layer of the 5-cm middle area between two plants as affected by soil P status, planting distance, and cropping combination on 60 d after sowing (harvest II)

<table>
<thead>
<tr>
<th>Cropping System</th>
<th>Root Length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP</td>
</tr>
<tr>
<td></td>
<td>5 × 5 10 × 10 20 × 20 40 × 40</td>
</tr>
<tr>
<td>Intercropping</td>
<td></td>
</tr>
<tr>
<td>HX3/NE1</td>
<td>244.9 ± 18.7a 160.3 ± 8.5a 120.6 ± 11.2a 24.6 ± 1.3a 343.8 ± 23.4a 175.3 ± 13.2a 125.7 ± 9.1a 30.1 ± 4.3a</td>
</tr>
<tr>
<td>HX3/GZ1</td>
<td>175.2 ± 12.9c 85.8 ± 7.1c 40.3 ± 3.1d 6.3 ± 0.7c 156.8 ± 13.3e 87.1 ± 6.7c 54.0 ± 2.6c 2.5 ± 0.7c</td>
</tr>
<tr>
<td>Monoculture</td>
<td></td>
</tr>
<tr>
<td>HX3/HX3</td>
<td>164.5 ± 8.6c 125.7 ± 9.7b 82.2 ± 6.3c 14.1 ± 1.2b 104.7 ± 13.1d 135.8 ± 10.8b 91.6 ± 6.7b 6.4 ± 0.8b</td>
</tr>
<tr>
<td>NE1/NE1</td>
<td>257.6 ± 11.6a 133.9 ± 6.6b 99.6 ± 7.6b 23.0 ± 2.7a 232.5 ± 23.2c 129.5 ± 11.2b 119.3 ± 8.3a 16.1 ± 1.6b</td>
</tr>
<tr>
<td>GZ1/GZ1</td>
<td>211.2 ± 15.9b 126.2 ± 7.5b 94.4 ± 6.2bc 11.7 ± 0.5b 297.4 ± 17.6b 126.2 ± 8.6b 93.3 ± 5.5b 11.5 ± 1.0b</td>
</tr>
</tbody>
</table>

Figure 1. Root systems of soybean and maize in the different cropping combinations on the 12th d after germination in the gel. A to D, Intercropped cultures. A, HX3 and GZ1 at LP. B, HX3 and GZ1 at SP. C, HX3 and NE1 at LP. D, HX3 and NE1 at SP. E to G, Self cultures at LP. E, NE1. F, HX3. G, GZ1.
Figure 2. Modeling results of 3D root systems of intercropped plants on the 12th d after germination in the gel. A, HX3 (left) and GZ1 (right) at LP. B, HX3 (left) and GZ1 (right) at SP. C, NE1 (left) and HX3 (right) at LP. D, HX3 (left) and NE1 (right) at SP.

Changes in the root system architecture alteration were characterized by changes in the relative root length in the top layer, which is similar to the findings previously (Liao et al., 2001a; Fang et al., 2009). Figure 4 shows that P availability significantly affected plant root architecture in a gel-growth system. The roots in the three self combinations (HX3/HX3, NE1/NE1, and GZ1/GZ1) tended to be shallower under SP as indicated by higher relative root length in the top 0- to 5-cm layer, especially at harvest II and III (Fig. 4, D–F). However, the roots of the two nonself combinations had opposite architectural responses to P availability. On SP, the roots of HX3/GZ1 tended to be shallower with 5.39%, 13.95%, and 12.89% higher relative root length in the top layer on the 6th, 12th, and 18th d after germination than those under LP, respectively. On the other hand, the roots of the HX3/NE1 under SP tended to be deeper with 8.86%, 6.81%, and 9.94% lower relative root length in the top layer on the 6th, 12th, and 18th d after germination than those under LP, respectively (Fig. 4, D–F).

All of the cropping combinations grown at SP had significantly longer total root length than the plants grown at LP, showing the inhibitory effect of LP on root growth (Figs. 3 and 4). Furthermore, the two maize self combinations (NE1/NE1 and GZ1/GZ1) did not differ in total root length, and both of them had significantly longer roots than soybean in self culture (HX3/HX3) at the same P level. This indicates that there were no phenotypic differences between two maize root systems when its neighbors were siblings, and maize generally has a larger root system than soybean. Interestingly, HX3/GZ1 had significantly greater root length than HX3/NE1 under the same P treatment starting from the 6th d after germination, especially on LP (Fig. 3).

\[
Li = 1.1132x^2 - 2.2151x + 2.09\text{(HX3/NE1, LP)} \\
Li = 1.1035x^2 - 0.6691x - 1.49\text{(HX3/GZ1, LP)} \\
Li = 1.2562x^2 - 4.171x + 6.89\text{(NE1/NE1, LP)} \\
Li = 1.3115x^2 - 2.8997x + 2.42\text{(GZ1/GZ1, LP)} \\
Li = 1.3124x^2 - 9.898x + 24.08\text{(HX3/HX3, LP)} \\
Li = 1.3832x^2 - 4.1464x + 11.11\text{(HX3/NE1, SP)} \\
Li = 1.0843x^2 + 4.1184x - 11.18\text{(HX3/GZ1, SP)} \\
Li = 1.0539x^2 + 5.4273x - 11.42\text{(NE1/NE1, SP)} \\
Li = 0.9146x^2 + 9.2723x - 20.94\text{(GZ1/GZ1, SP)} \\
Li = 1.2209x^2 - 4.6586x + 8.52\text{(HX3/HX3, SP)}
\]

\(Li\) is the total root length (cm) in the different cropping combinations, and \(x\) is the growth day after germination. As you can see from the above equations, the different cropping systems differed in all the criteria, indicating the different root behavior in response to \(P\) status and cropping combination.

Figure 3. Total root length of intercropped plants from the 1st through the 20th d after germination in gel experiment. A, On LP. B, On SP. HX3/NE1: HX3 intercropped with NE1; HX3/GZ1: HX3 intercropped with GZ1; HX3/HX3: HX3 self culture; NE1/NE1: NE1 self culture; GZ1/GZ1: GZ1 self culture. The \(F\) value of cropping combination and \(P\) treatment were 32.75 and 128.85 (\(P < 0.01\)) on the 20th d after germination.

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Relationship between Root and Shoot Growth Parameters

The total root length and the relative root length in the top 0- to 5-cm layer obtained on three different days (the 6th, 12th, and 18th d) in gel were used to conduct a correlation analysis with shoot biomass and shoot P content in both the gel and field experiments. The results showed that total root length was significantly correlated with shoot biomass and P content for the two nonself and HX3/HX3 self combinations on each of the three different days examined, and for NE1/NE1 and GZ1/GZ1 self combinations on 12 and 18 d after sowing in the gel experiments (Table III; Supplemental Table S3), but only for the two nonself combinations in the field experiment (Table II; Supplemental Tables S3 and S4). This indicates that the root architecture changes in cropping systems containing maize GZ1 appear to involve neighbor plant interactions that contribute to plant growth and P uptake.

DISCUSSION

It has been well reported that there are kin recognitions in living organisms in nature. However, kin recognition in plants, especially in crop species has only caused attentions from biologists in recent years and many studies are speculative (Biedrzycki and Bais, 2010). In this study, dynamic root interactions between roots from neighboring plants were observed and quantified in situ using a nondestructive imaging of plant roots in a transparent gel-growth system. Using this novel system, we provide the direct evidence that crop plants could recognize kin, and this kin recognition varied in species and genotypes.

In the gel system, when the maize cultivar NE1 was intercropped with the soybean variety HX3, roots had an affinity for one another, like Arabidopsis (Arabidopsis thaliana) plants grew with strangers (Biedrzycki et al., 2010), showing the ability of nonkin recognition between maize and soybean (Figs. 1 and 2). Interestingly, when the maize NE1 and GZ1 cultivars, or soybean HX3, were grown with their respective siblings, their roots also grew toward each other. These results imply that breeding of modern maize and soybean varieties might have reduced their ability for kin recognition. In previous reports, kin and nonkin interactions did not follow the pattern we observed with maize NE1 and

Figure 4. The two root traits in different cropping combinations on the 6th (A and D), 12th (B and E), and 18th (C and F) d after germination under two P levels in gel. HX3/NE1: HX3 intercropped with NE1; HX3/GZ1: HX3 intercropped with GZ1; HX3/HX3: HX3 self culture; NE1/NE1: NE1 self culture; GZ1/GZ1: GZ1 self culture. Parameters with the same letter on the same day showed no significant difference (Duncan multiple comparison, P < 0.05).
soybean HX3 (Murphy and Dudley, 2009). In these previous studies, when grown with their kin, roots avoided each other, possibly to avoid direct resource competition. On the other hand, when grown with nonkin plants, roots overlapped perhaps to better compete for resources, which are consistent with the resource-partitioning hypothesis (Cheplick and Kane, 2004). The resource-partitioning hypothesis suggests that relatives are more phenotypically similar and so will compete more intensely for the same share of resources than would more dissimilar plants. However, most of the reports on kin recognition have been conducted with wild plants, but not crop species (Huber-Sannwald et al., 1996; Dudley and File, 2007; Murphy and Dudley, 2009). Therefore, the ability to discriminate kin from nonkin might be an important aspect of root behavior under more natural conditions where resources are limited. In crops, however, after thousands of years of agricultural domestication with monocultures grown under rich resource conditions (Wenke, 1980), roots may have gradually reduced their ability to recognize their kin. Because there was no need to avoid competition for resources such as nutrients, a strong kin recognition would not have conferred a selective advantage. Furthermore, physiological and molecular mechanisms of kin recognition have not yet been revealed, and it is possible that these mechanisms in wild plant species might not provide benefits to crop plants and/or might impose carbon costs that reduce yield (Biedrzycki et al., 2010). Therefore, during agricultural domestication based on selection for high yield, any unnecessary carbon cost, which might include kin recognition, might have been unconsciously diminished.

Interestingly, we found that the nonkin recognition between maize and soybean plants varied in genotypes. The roots in the maize GZ1 and soybean HX3 intercropping system acted as though they were kin. The results presented in Figures 1 and 2 show that the roots of HX3 and GZ1 clearly separated from each other whether P was limiting or not. Furthermore, this root segregation happened before individual roots came in direct contact with each other. Therefore, this response was not mediated by physiological co-ordination among roots as suggested by Gruntman and Novoplansky for *B. dactyloides* (Gruntman and Novoplansky, 2004). Mahall and Callaway (1996) reported that the roots of the desert shrub *Ambrosia dumosa* could respond differently to contact roots of the same specific plants from different regions, indicating that roots might be able to distinguish the origins of their neighbors. The maize variety GZ1 and the soybean variety HX3 both originated from South China while the maize NE1 variety is from North China. As discussed in previous studies, kin recognition is especially advantageous under competitive situations if it prevents costly competitive behavior toward kin or promotes cooperation among siblings to maximize use of resources (Axelrod and Hamilton, 1981; Waldman, 1988). The soil nutrient status, especially the soil P status in South China is much poorer than that in North China (Zhang et al., 2005). Therefore, root segregation in a maize/soybean intercropping system could be more important for efficient utilization under the nutrient-limited conditions in South China. This could also explain why GZ1 had much higher shoot biomass and P content at LP when planted close to its neighbors (5 × 5 cm) when intercropped with soybean HX3, but not at high P or for more widely spaced plantings (Table I; Supplemental Tables S1 and S2). Furthermore, maize and soybean are usually intercropped closely in South China while the maize NE1 variety is from North China. As discussed in previous studies, kin recognition is especially advantageous under competitive situations if it prevents costly competitive behavior toward kin or promotes cooperation among siblings to maximize use of resources (Axelrod and Hamilton, 1981; Waldman, 1988). The soil nutrient status, especially the soil P status in South China is much poorer than that in North China (Zhang et al., 2005). Therefore, root segregation in a maize/soybean intercropping system could be more important for efficient utilization under the nutrient-limited conditions in South China. This could also explain why GZ1 had much higher shoot biomass and P content at LP when planted close to its neighbors (5 × 5 cm) when intercropped with soybean HX3, but not at high P or for more widely spaced plantings (Table I; Supplemental Tables S1 and S2). Furthermore, maize and soybean are usually intercropped closely in South China; therefore, the advantages of root avoidance in maize/soybean intercropping for maize growth in short planting distance might also be a consequence of long-term domestication in an intercropping system under limited soil nutrient conditions and close planting distance.

More recently, Cahill et al. (2010) found that the roots of *Abutilon theophrasti* could not only sense neighboring plants and nutrient heterogeneity, but also integrate the information and respond coordinately. In our study, we used a SP treatment to simulate the heterogeneous P distribution along a soil profile in the field (Liao et al., 2001b). We found that root interactions also responded to changes in P status, and the maize GZ1 and NE1 cultivars differed in their responses to P availability when intercropped with

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**Table III. Correlation coefficients between root and plant growth parameters in nonself cropping combinations when planting distance was 5 × 5 cm**

<table>
<thead>
<tr>
<th>Gel Experiment</th>
<th>Harvest I</th>
<th>Harvest II</th>
<th>Field Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HX3/NE1</td>
<td>HX3/GZ1</td>
<td>HX3/NE1</td>
</tr>
<tr>
<td><strong>R Value</strong></td>
<td>Biomass</td>
<td>P Content</td>
<td>Biomass</td>
</tr>
<tr>
<td>Total root</td>
<td>0.9541**</td>
<td>0.9952**</td>
<td>0.9797**</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>0.8924*</td>
<td>0.9366*</td>
<td>0.8372*</td>
</tr>
<tr>
<td>Relative root</td>
<td>0.9666**</td>
<td>0.9169*</td>
<td>0.9186*</td>
</tr>
<tr>
<td>Length in</td>
<td>0.8881*</td>
<td>0.9131*</td>
<td>0.7741 ns</td>
</tr>
<tr>
<td>0- to 5-cm layer (%)</td>
<td>0.9094*</td>
<td>0.9143*</td>
<td>0.7510 ns</td>
</tr>
<tr>
<td></td>
<td>0.6551 ns</td>
<td>0.9649*</td>
<td>0.9692**</td>
</tr>
</tbody>
</table>

*Significant at P < 0.01; *, significant at P < 0.05; ns, not significant at P < 0.05.*

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soybean HX3. The segregating root system in the HX3/GZ1 intercropping system resulted in shallower root system architecture with more roots in the top layer where there is more available P, but the overlapping root system in the HX3/NE1 system tended to produce deeper root systems with fewer roots in the surface horizon (Fig. 4, D–F). A number of studies have demonstrated that a shallower root system is advantageous for the acquisition of limiting nutrients, especially for mineral nutrients that tend to be fixed in the soil such as P, resulting in more P fixed in the shallow soil horizons (Rubio et al., 2001). Therefore, these changes in root system architecture might greatly benefit plants grown in LP soils such as those found in South China, and could partially explain why there were strong correlations of relative root length in the top 0- to 5-cm layer with shoot biomass and P content in the field when plants were grown in close proximity to each other (Table III).

The kin recognition of plants has been speculated through a variety of mechanisms. Root secretions have been demonstrated to play an important role in plant kin recognition. Biedrzycki et al. (2010) found that Arabidopsis plants had more lateral roots when exposed to stranger secretions than to kin secretions. Micallef et al. (2009) reported that genetic influences within a species can produce unique root secretion cocktails. They compared the differences between root secretion profiles of eight different Arabidopsis accessions (ecotypes) and determined that root secretion profiles did indeed differ in the compounds present and in the relative abundance of many of these compounds. Therefore, we assume that the two maize varieties might differ in root exudates, thus consequently cause different root growth direction and architecture when intercropped with nonkin soybean HX3. But in our study, the roots of maize GZ1 acted oppositely to NE1 when intercropped with soybean HX3 (Figs. 1 and 2), and this tendency of root interactions also showed in field (Table II) with much less root length in the 20-cm upper soil layer of the 5-cm middle area between two plants in the larger planting distances (20 × 20 cm and 40 × 40 cm), where the root exudates could not diffuse from one plant root to another. This implied that besides root exudates, there might be some other mechanisms to cause these phenomena. Some reports stated that emission of a chemical signal from one plant could determine the growth performance. For example, maize released (Z)-3-hexen-1-ol (Z-3-ol) in response to leaf damage so as to help the neighbor plants to avoid an herbivore attack (Heil and Bueno, 2007; Heil and Karban, 2010). Therefore, we speculated that the genotypic-dependent maize/soybean recognition might also involve in chemical communication in above-ground parts and need to be further studied.

In conclusion, our study provides novel findings regarding the dynamics of root behavior in response to low soil P status and the identification of neighboring crop plants. We found that root behavior not only depends on neighbor recognition but also coordinately responds to P status, which might cause the different growth responses seen in the field for LP soils such as those in South China.

MATERIALS AND METHODS

Plant Growth in the Field

Soybean (Glycine max) variety HX3 and maize (Zea mays) varieties NE1 and GZ1 were grown in different cropping combinations on silty loam soil at Quzhou experimental farm of China Agricultural University in 2009. Basic soil chemical characteristics were as follows: pH, 7.72; organic matter, 1.26 g kg⁻¹; available P (Olsen method), 6.99 mg P kg⁻¹; nitrate nitrogen, 6.18 mg kg⁻¹; available potassium, 72.7 mg kg⁻¹. Two P levels were used with five cropping combinations. Among them, 120 kg P₂O₅ ha⁻¹ was added as calcium superphosphate to the topsoil by spread application as SP and no additional P was added as LP treatments. There were four planting distances including 5 × 5 cm, 10 × 10 cm, 20 × 20 cm, and 40 × 40 cm. Five cropping combinations included the two nonself combinations as HX3/NE1 and HX3/GZ1, and three self combinations as HX3/HX3, NE1/NE1, and GZ1/GZ1. Each treatment had four replicates in a split-block design. There were two harvests for the 5 × 5 cm planting distance, including 15 d (harvest I) and 60 d (harvest II) after sowing, and one harvest for the other planting distances, which was 60 d after sowing. Two representative plants from each cropping combination were harvested for every replicate. The shoot biomass and P content of all the plants were analyzed. The P concentration in plants was colorimetrically measured using the methods described by Murphy and Riley (1963).

Root interactions in field were analyzed following a modified version of the profile wall method described by Liao et al. (2004). Tangential trenches were dug in the middle from the row of plants. The walls were carefully scraped with a screwdriver to reveal the tips of the roots. Plastic transparent sheets (25 × 30 cm) were positioned adjacent to the exposed soil wall. The roots in the 20-cm upper soil layer were marked on the sheets, and the root length in the middle 5-cm area between two plants were quantitatively measured with root image analysis software (WinRhizo Pro, Régent Instruments; Supplemental Fig. S2).

Plant Growth in Gel

The same soybean and maize varieties were used as target plants for studying root growth in a transparent gel system. There were two P treatments: LP (no additional P added) and SP (top layer [0–5 cm], 1 mg P; lower layer [5–15 cm], no P added). The five cropping combinations were set as described in the field experiment. Three replicates were done for each treatment. Seeds were surface sterilized with 10% hydrogen peroxide for 30 min, and rinsed three times with sterile water. The sterilized seeds were planted in petri dish, which contained half-strength Hoagland media, in the dark at 25°C for 48 h to germinate. The germinated uniform seeds were transplanted to transparent polymethyl methacrylate cylinders with 20 cm in height and 12 cm in diameter, filled with transparent growth medium consisting of half-strength Hoagland solution and 0.15% phytagel (pH = 5.8). Aluminum foil was used to cover the gel surface and wrap the cylinder to keep the roots away from light. Because both the growth medium and cylinder were transparent, the root system could be exposed by removing the aluminum foil to obtain 3D root architecture images daily (Fang et al., 2009). The distance between two plants was 5 cm. Plants were grown at 12-h day/night, 28°C day, 19°C night, and were harvested on the 20th d after germination. The shoot biomass and P content of all plants were analyzed.

3D Root Architecture Image Reconstruction System

The 3D root architecture image reconstruction system included a 3D laser scanner (Roland LPV-1200, Roland DG Corporation) and a PC. The scanning precision of the 3D laser scanner is up to 0.1 mm, and the scanning range is 130.0 mm (diameter) × 203.2 mm (height). The plant root system is scanned by the 3D laser scanner without contact by using a laser beam, which enables high-speed scanning without damage to the root system. In this study, root
systems were scanned per 0.18° in circumference and per 0.1 mm in height as described by Fang et al. (2009).

Calculation Methods for 3D Parameters of Plant Root Architecture

The 3D parameters of root architecture including total root length and relative root length in different layers were calculated. The calculation methods for these parameters are briefly described as below and in more detail described in Zhu et al. (2006) and Fang et al. (2009).

Total Root Length

In this study, because there were two root systems in the cylinder, the total root length represented the two root systems’ combined total root length. Since the spatial distance between the points of seminal and adventitious roots is quite small, the sum of Euclidean distance of 3D spatial skeleton points, which are ordered as described by Miller et al. (2007), is used to calculate the length of each root. Based on the above scattered data and their skeletons, it is convenient to precisely calculate total root length. Suppose that the data point of one root \( R_i \) is \( V_i(x_i, y_i, z_i), i = 1, 2, ..., n \); therefore, the length of each root segment should be:

\[
D_i = \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2 + (z_{i+1} - z_i)^2},
\]

\( i = 1, 2, ..., n - 1, \)

The length of each root is:

\[
L_k = \sum_{i=1}^{n-1} D_i
\]

If there are \( m \) roots in the root systems, then the total root length of the two root systems is:

\[
L = \sum_{i=1}^{m} L_k
\]

Relative Root Length in Different Layers

In the above parts, total root length, length of each root segment could all be calculated. The relative root length in different layers could be obtained by vertically dividing the 3D space into several different depth layers. SAS software (SAS Institute Inc.) was used to do ANOVA analysis and multiple comparisons of 3D root architecture and plant growth parameters. Correlation analysis between root architecture parameters and plant growth parameters was done by STATISTICA 6.0 (StatSoft Inc.).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Original root system images captured by the 3D laser scanner on the 12th d after germination in the gel.

Supplemental Figure S2. Scheme of root interaction analysis in field.

Supplemental Table S1. Shoot biomass (g plant\(^{-1}\)) as affected by P status and planting distance to neighbors in field experiments.

Supplemental Table S2. P content (mg plant\(^{-1}\)) in shoots as affected by P status and planting distance to neighbors in field experiments.

Supplemental Table S3. Correlation coefficients between root and plant growth parameters in self-cropping combinations in gel experiment.

Supplemental Table S4. Correlation coefficients between root and plant growth parameters in self-cropping combinations in field experiments when planting distance was 5 × 5 cm.

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LITERATURE CITED


