Genotypic recognition and spatial responses by rice roots

Suqin Fang[a,b], Randy T. Clark[c,d], Ying Zheng[e], Anjali S. Iyer-Pascuzzi[a], Joshua S. Weitz[f,g], Leon V. Kochian[d], Herbert Edelsbrunner[e,h], Hong Liao[b,1], and Philip N. Benfey[a,1]

[a]Department of Biology, Duke Center for Systems Biology, Duke University, Durham, NC 27708; [b]State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Root Biology Center, South China Agricultural University, Guangzhou 510642, China; [c]Department of Biological and Environmental Engineering and [d]The Robert W. Holley Center for Agriculture and Health, US Department of Agriculture-Agricultural Research Service, Cornell University, Ithaca, NY 14853; [e]Department of Computer Science, Duke University, Durham, NC 27708; [f]School of Biology, Georgia Institute of Technology, Atlanta, GA 30332; [g]School of Physics, Georgia Institute of Technology, Atlanta, GA 30332; and [h]Institute of Science and Technology Austria, 3400 Klosterneuburg, Austria

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Root system growth and development is highly plastic and is influenced by the surrounding environment. Roots frequently grow in heterogeneous environments that include interactions from neighboring plants and physical impediments in the rhizosphere. To investigate how planting density and physical objects affect root system growth, we grew rice in a transparent gel system in close proximity with another plant or a physical object. Root systems were imaged and reconstructed in three dimensions. Root–root interaction strength was calculated using quantitative metrics that characterize the extent to which the reconstructed root systems overlap each other. Surprisingly, we found the overlap of root systems of the same genotype was significantly higher than that of root systems of different genotypes. Root systems of the same genotype tended to grow toward each other but those of different genotypes appeared to avoid each other. Shoot separation experiments excluded the possibility of aerial interactions, suggesting root communication. Staggered plantings indicated that interactions likely occur at root tips in close proximity. Recognition of obstacles also occurred through root tips, but through physical contact in a size-dependent manner. These results indicate that root systems use two different forms of communication to recognize objects and alter root architecture: root-root recognition, possibly mediated through root exudates, and root-object recognition mediated by physical contact at the root tips. This finding suggests that root tips act as local sensors that integrate rhizosphere information into global root architectural changes.

3D reconstruction | imaging | kin recognition

Plants interact with the environment in a number of ways (1, 2). Aboveground tissues may identify volatile cues that provide information about their neighbors (3, 4) and detect irradiance, directional light, and light quality (5), whereas belowground tissues, such as roots, can detect changes in soil moisture, nutrient availability, and physical obstacles (6–8). Plants not only detect but also respond to changes in their environment, exhibiting adaptation in their morphology and physiology in response to environmental stimuli (9–14), such as alteration in total root length, root system volume, and root depth (15, 16). Phenotypic plasticity of plants in response to environmental heterogeneity may have consequences for plant fitness.

Communication among plants is mediated by interactions that take place aboveground (17, 18) and belowground (2, 19–21). Aboveground interactions have been studied in greater detail, in part because of the accessibility of aerial tissue. However, there is growing interest in root-system architecture and its effect on plant function and fitness (12, 15). Studies of root-system architecture suggest that root systems develop differently in the presence of other root systems. For example, when exposed to the roots of a neighboring plant, common bean plants altered the vertical and horizontal distribution of roots, placing fewer roots in soil domains occupied by roots of a neighbor (12). In another study, analysis of root placement in *Abutilon theophrasti* suggested a hierarchical set of decision rules dependent on the presence or absence of a neighbor. For example, if a plant grows alone, it adopts a broad foraging strategy that is independent of resource distribution. However, if neighbors are present, a restricted foraging strategy is adopted, which is modified by resource distribution. This effect was most pronounced when nutrients were more abundant in the same soil zone as the competitor (22).

There is evidence that the outcome of belowground interactions between plants, of the kind illustrated in the previous set of examples, can be mediated by identity recognition, including species-specific responses (23–26), kin/stranger responses (27–30), and self/nonself responses (2, 3). Mahall and Callaway (23) found that root systems of the desert shrub *Ambrosia dumosa* appear to be capable of detecting and avoiding other *Ambrosia* root systems. In contrast, roots of another desert shrub species, *Larrea tridentata*, inhibit both *Larrea* and *Ambrosia* roots in their vicinity. Tosti and Thorup-Kristensen (7) reported that the root system of the red beet showed much faster and deeper growth than that of legumes grown in the same soil, with the result that red beet became the dominant root system and the legume roots were confined to the shallower soil layers. Another study showed that *Cakile edentula* plants were capable of kin recognition through root interactions. Allocation to roots increased when groups of strangers shared a common pot, but not when groups of siblings shared a pot (27). However, in another species, *Impatiens pallida*, groups of strangers had lower root allocation than groups of siblings (28). Further evidence for differential responses came from observing root systems grown in growth medium and soil environments. Maize and soybean root systems grew differently when grown alone or with the other species; and even for the same soybean variety, differences in root behavior were observed when intercropped with different maize varieties (15).

Hence, although there is substantial evidence suggesting that roots can recognize and respond to neighboring root systems, there has been little effort to isolate and quantify belowground interactions in situ and determine how such interactions manifest themselves at the level of individual roots. This lack of effort is in part because of the difficulty in observing root interactions of plants growing in real time. To address this issue, we grew rice root seedlings in close proximity in a translucent gel-based growth environment (16, 31). Using 3D reconstructions of the root systems (32, 33), we quantified the extent of overlap of roots from the same or different genotypes. For the rice varieties that were evaluated, we found that rice roots of different genotypes tended to avoid each

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[1]To whom correspondence may be addressed. E-mail: hliao@scau.edu.cn or philip.benfey@duke.edu.

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other, but those of the same genotype tended to grow closer to each other. When grown in isolation, root systems grew larger, as measured by multiple root traits, than when grown with a neighbor of the same or a different genotype. We investigated the possible means of interaction (i.e., aboveground or belowground) and present cumulative evidence that plant interactions likely occur at root tips over small spatial distances. Further experiments showed that roots recognize physical obstacles in the environment via a different mechanism than they use to recognize other root systems. Our results suggest that root systems have different spatial sensors: root-root recognition through root communication, and root-object recognition mediated through physical contact by individual root tips.

Results

Root Systems of Plants Grown in Isolation Are Larger Than When Grown with a Neighbor. We quantified a range of root system architectural traits (31, 34) (Table S1) for plants grown: (*i*) in isolation, (*ii*) with plants of the same genotype, or (*iii*) with plants of a different genotype. Plants were grown in a transparent gel system using the rice genotypes Azucena, Caiapo, and IR64 (*Materials and Methods*). Micropore tape was used to cover the growth cylinder to allow adequate light for plant growth. Representative images from intragenotype and intergenotype experiments on the seventh day after germination (DAG) are shown in Fig. 1. For all three genotypes, plants grown in isolation had larger total root length, surface area, root system volume, convex area, median root number (MedR), and reduced bushiness than plants grown with neighbors, regardless of genotype (Table S2). Thus, plants grown with a neighbor appear to recognize a smaller available environment and inhibit root system growth. Measurements of root and shoot biomass showed that plants have larger root and shoot biomass when grown alone, but there is no difference in shoot/root allocation (Table S3). In line with this finding, root systems of plants grown in isolation in half-size containers were smaller than those grown alone in full-size containers (Table S4).

Root Architecture Traits Are Differentially Altered When Plants Are Grown with the Same or Different Genotypes. Most root traits exhibited no significant difference when plants were grown with the same genotype or with different genotypes. However, several traits were dependent on the genotypes paired. For example, Azucena had significantly smaller surface area, increased bushiness, and smaller median root number when grown with Caiapo (734.0 mm^2, 4.0 and 2.6, respectively) than when paired with another Azucena plant (988.8 mm^2, 2.9 and 3.4, respectively); IR64 had larger maximum root number (MaxR = 12.9 and 13.1 when grown with Azucena and Caiapo, respectively) and median root number (MedR = 5.1 when grown with Azucena) when its neighboring plant was of a different genotype compared with when grown with the same genotype (MaxR = 10.2, MedR = 4.1); Caiapo had significantly smaller median root number when paired with IR64 (MedR = 2.6) than when grown with another Caiapo plant (MedR = 3.2). Thus, both the type of root trait and the degree of change for a given trait are dependent on which genotypes are grown together.

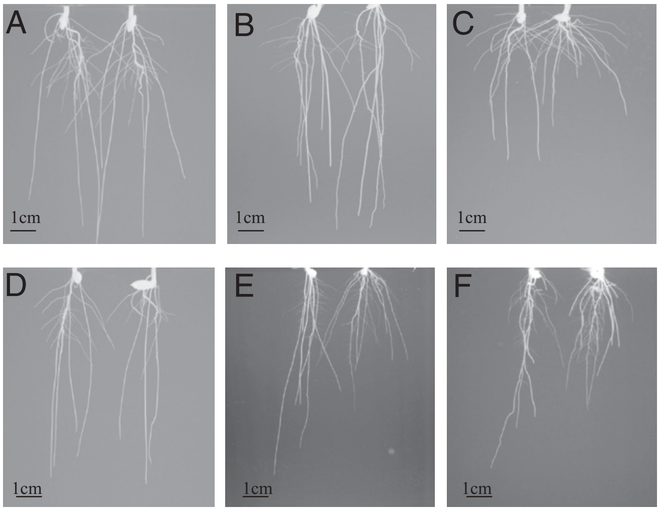

Fig. 1. Intragenotype and intergenotype rice root interaction on the seventh DAG. (*A*–*C*) Intragenotype exhibiting high frequency of overlap between root systems of the same genotype: (*A*) Azucena, (*B*) Caiapo, (*C*) IR64. (*D*–*F*) Intergenotype showing lower frequency of overlap between root systems of different genotypes: (*D*) Azucena (*Left*) -Caiapo (*Right*); (*E*) Azucena (*Left*) -IR64 (*Right*); (*F*) Caiapo (*Left*) -IR64 (*Right*).

Root Systems of the Same Genotype Show More Overlap Than Root Systems of Different Genotypes. We reasoned that root systems could recognize smaller environments either by physically contacting an object or by signaling to other root systems. To examine these possibilities, we observed the interaction of neighboring roots of different genotypes compared with neighboring roots of the same genotype. Surprisingly, roots of neighbors never physically touched, regardless of genotype (0 of 156 pairs, including both intra- and intergenotype). However, root systems of the same genotypes overlapped in the same space to a greater degree than those of different genotypes. To quantify the extent of overlap, we performed 3D reconstructions using two different approaches (32, 33) (Fig. 2). We used software to digitally extract one of the two root systems from each 3D reconstruction (32) and calculate the convex hull (analogous to the volume within a shrink-wrapped root system) for each root system. We then calculated the overlap, Q, of the two root systems for each of the combinations of roots (Fig. 3*A*) (*Materials and Methods*). Overlap is an index that ranges from 0, when root systems are completely separate (i.e., each root system does not intersect with the other convex hull) to 1, when two root systems are completely intertwined (i.e., both root systems are completely contained in the convex hull of the other). The overlap of the root systems of the same genotype was significantly higher than that of root systems from different genotypes (average $Q_{\text{same}} = 0.1303$ and average $Q_{\text{different}} = 0.0299$, $P = 0.00015$, t test). These results suggest that rice plants may signal to each other and recognize like genotypes in a form of kin recognition.

Separation of the Aerial Portion of the Plant Has No Significant Effect on Root Interactions. The recognition described above could have occurred through roots or shoots. To investigate the possibility that the observed genotype-specific root interactions were caused by signaling between the aerial organs of the neighboring plants, we placed a transparent barrier that separated the aerial portions of the two plants as they grew and imaged the roots at 7 DAG (Fig. S1). We found that there was no significant effect on the root interactions when plants were grown with the aerial barrier (Fig. 3 *B* and *C*) (average $Q_{\text{same}} = 0.1324$, $P = 0.95$ for intragenotype, t test and average $Q_{\text{different}} = 0.0255$, $P = 0.71$ for intergenotype). These results indicate that aerial signals are unlikely to be the cause of the differential root interactions we observed, and suggest that root systems may have kin recognition.

Seeds Planted on Different Days Demonstrate That Root Proximity Is Important for Interaction Responses. We observed that rice plants planted 4-cm apart exhibited the interaction phenotype significantly later (∼10 d) compared with rice plants planted 2-cm apart. This finding suggests that interaction between roots might take place only when roots approach each other within a critical

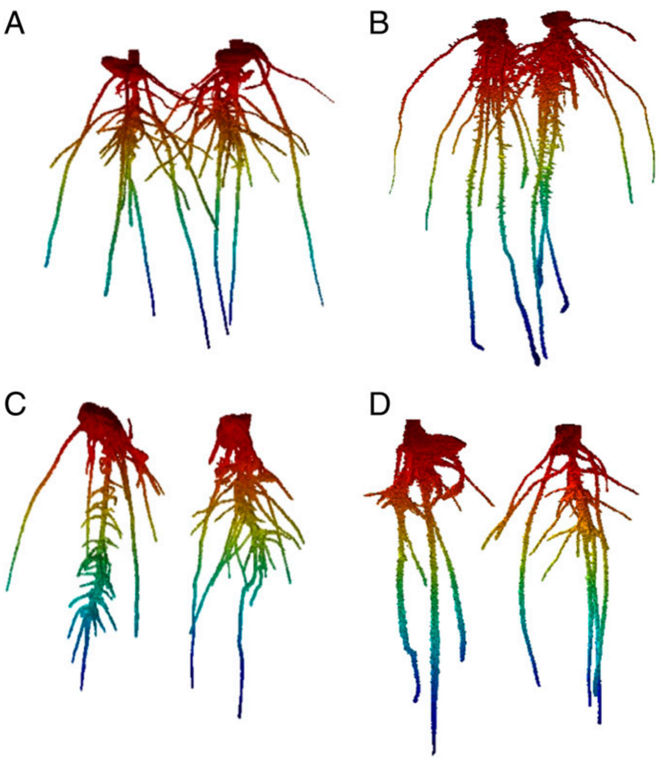

Fig. 2. Three-dimensional reconstruction results of rice root interaction on the seventh DAG. (*A* and *B*) Intragenotype, Azucena. (*C*) IR64 (*Left*) -Azucena (*Right*). (*D*) Caiapo (*Left*) -Azucena (*Right*). Coloring represents root depth.

distance. In particular, we noticed that interactions usually take place when the root tips from the two root systems come into close proximity. To further investigate this possibility, we planted IR64 6 d before planting Azucena 2-cm away. Imaging over consecutive days showed that initially the Azucena root appeared to grow toward the IR64 root (e.g., day 3 after Azucena was planted) (Fig. 4*A*). However, as the Azucena root continued to grow and the distance between the root systems diminished, the IR64 root appeared to bend downward, thus growing away from the Azucena root system (e.g., day 5 after Azucena was planted) (Fig. 4*A*). In contrast, when IR64 was grown with IR64, and the second IR64 seed was planted 6 d after the first, the root system of the older plant continued to elongate horizontally, even when its root system was close to the younger IR64 root system (Fig. 4*B*). This result was similar to the root growth of an IR64 plant grown in isolation (Fig. 4*C*). Comparison of the change in the angle of the root of IR64 that was closest to a neighboring Azucena or to the younger IR64 root system from day 3 to day 5 showed that the change in the angle is larger in the intergenotype combination (Fig. 4*D*). Specifically, the average change in the angle for IR64-Azucena is 25°, but the average change in the angle for IR64-IR64 is 12° (P = 0.0216, t test). These results suggest that root interactions likely occur at root tips and that close proximity between the two root systems is essential for kin recognition.

Root Growth Differs with Different Size Obstacles in Medium. Our results suggest that root systems are smaller when grown with neighbors because they recognize the presence of another plant and adjust their growth to a smaller physical region. However, this does not explain why plants grown in isolation have larger root systems when grown in large containers compared with small containers. Although root systems of neighboring plants do not physically touch, we hypothesized that root recognition of container size could occur through physical contact. To test this theory, we inserted a thin piece of plastic of different sizes (1-, 3-, 5-, and 7-cm wide by 8-cm deep) 1-cm away from the seed in the cylinder (Fig. 5*A*). During the analysis period (7 DAG), as expected, a smaller number of roots hit the smaller obstacles. Unexpectedly, when a small number of roots hit the 1-cm obstacle, root growth was induced. For example, bushiness of all three genotypes, surface area, and maximum root number of Azucena and Caiapo, total root length of Azucena, and median root number of Caiapo all increased (Fig. 5*B* and Table S5). More roots hit the obstacle as the size of the obstacle increased to 7 cm. At this point, compared with when grown without an obstacle or with a small obstacle, total root length, root system volume, and convex area were smaller for all three genotypes, and median root number and surface area were inhibited in one or two genotypes, respectively. Thus, root-system size was inhibited by larger obstacles (Fig. 5 *C*–*E*). The number of roots within a root system that touched a physical object was size-dependent: an average of 1.2 for a 1-cm wide object (15 pairs), 2.7 for a 3-cm object (15 pairs), 4.1 for a 5-cm object (15 pairs), and 6.5-times for a 7-cm object (15 pairs). These results show that roots were able to recognize an obstacle in a size-dependent manner through the number of roots physically in contact with the object. This finding suggests that root systems integrate information from local points, such as root tips into global root architectural changes.

Discussion

There is substantial evidence that roots can respond to neighboring plants and physical obstacles in the medium (1, 28). However, for neighboring plants, the response is not always consistent across species or genotypes. For example, several studies have shown that root growth is facilitated by kin interactions and inhibited by stranger interactions (28), yet the

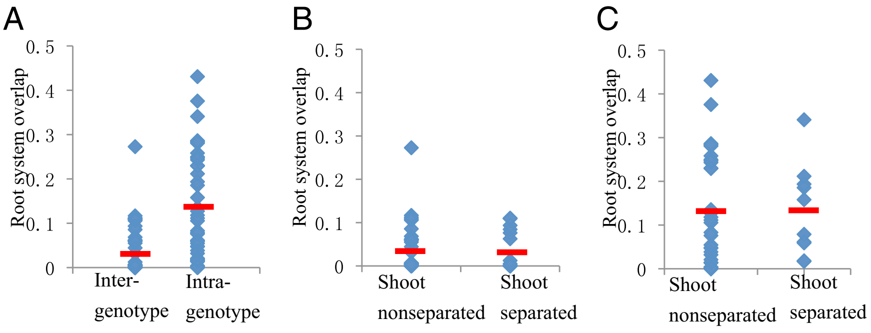

Fig. 3. Interaction analysis based on 3D voxels of rice root systems on the seventh DAG. (*A*) Intragenotype vs. intergenotype planting significantly affects rice root interaction ($P = 0.0011$, t test). (*B*) Comparison of interaction within intergenotype plantings with shoots not separated and separated ($P = 0.71$, t test). (*C*) Comparison of interaction within intragenotype planting with shoots not separated and separated ($P = 0.95$, t test). Bars represent mean of root system overlap.

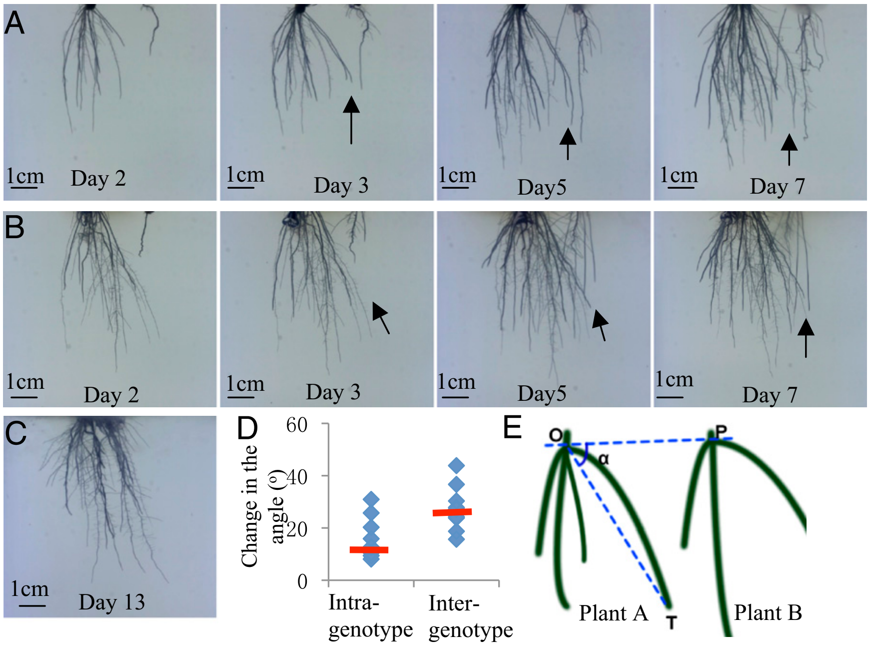

Fig. 4. Seeds planted on different days provide insight into root interaction. (*A*) IR64 (*Left*, day *n*+6)-Azucena (*Right*, day *n*), Azucena was planted 6 d after IR64; day 2: 2 d after the younger seedling was planted. (*B*) IR64 (*Left*, day *n*+6)-IR64 (Right, day *n*), the IR64 seedling on the right was planted 6 d after the IR64 seedling on the left. (*C*) IR64 grown alone on the 13th DAG. (*D*) Comparison of the change in the angle of the root of IR64, which is the closest to the other root system from day 3 to day 5 ($P = 0.021$, *t* test). Bars represent mean of the change in the angle. (*E*) The angle between *OT* and *OP* is calculated as the angle of the closest root of plant A.

converse has also been reported (27). Because of the difficulty of imaging below ground, previous studies of root–root interactions have not combined in situ observation and quantification of root interactions on a whole root system basis. In this study, we used an integrated transparent gel growth-imaging and analysis platform to observe and quantify root–root and root–object interactions. Our experiments suggest that root systems may use two different forms of communication to detect territory available for growth. First, they respond to neighboring roots by a genotype-specific signal likely recognized by the root tips.

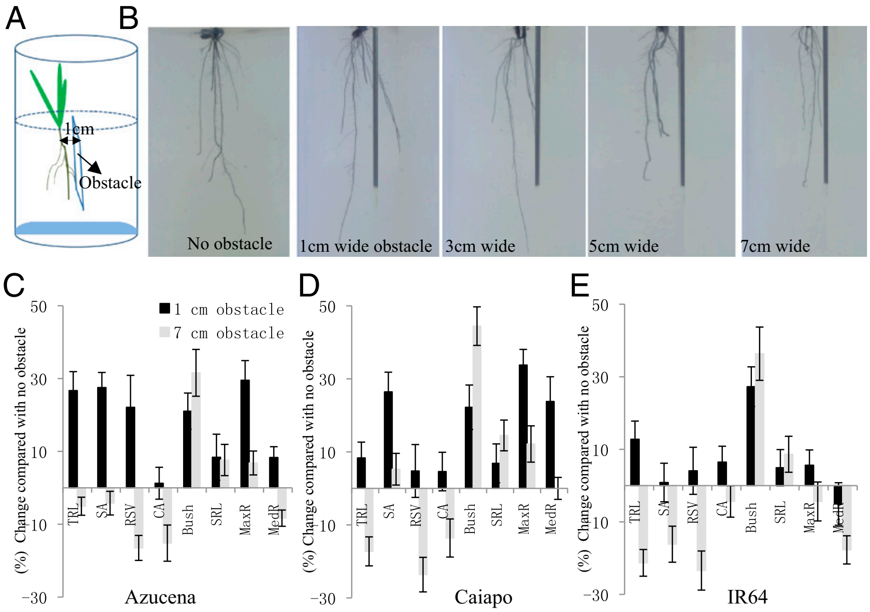

Fig. 5. Root growth differs with different size obstacles in the medium. (*A*) Depiction of obstacle experiment where thin plastic pieces of different sizes (1-, 3-, 5-, or 7-cm wide by 8-cm deep) were placed 1-cm away from the seed in the cylinder. (*B*) Comparison of Azucena root growth on the seventh DAG with obstacles of different sizes. (*C*–*E*) Change of root traits when grown with 1-cm wide or 7-cm wide obstacles compared with when grown alone with no obstacle. TRL, total root length; SA, surface area; RSV, root system volume; CA, convex area; Bush, bushiness; SRL, specific root length; MaxR, maximum root number; MedR, median root number. Bars represent mean of 15 replicates with SE.

Second, they recognize obstacles in a size-dependent manner mediated by the number of root tips in contact with the obstacle.

Kin Recognition in Rice Likely Occurs at Root Tips in Close Proximity. Our results showed that rice roots in the vicinity of a neighbor of a different genotype grew away from the neighboring roots. Quantification of the spatial overlap between two neighboring root systems indicated that there was significantly more overlap between two root systems of the same genotype than of different genotypes (Fig. 3*A*). Because the presence of other plants can affect the microclimate, in part through volatile cues (3), we separated the aerial portion of neighboring plants. Separation had no effect on root interaction (Fig. 3*B*), revealing that rice plants are capable of detecting the presence of a neighboring plant and of kin recognition through root systems. This finding is consistent with previous reports on kin recognition through root interactions in *C. edentula* (27).

Delayed planting experiments in which a second seedling was planted 6 d after the first suggest that the root–root interactions occur at root tips and are dependent on the physical distance between the roots. The interactions between root systems were not evident when roots were not physically close together. Thus, close proximity between two root systems appears to be essential for kin recognition. This finding is consistent with our preliminary observations that when seedlings were planted further apart (4 cm), a longer time was required to observe the interaction phenotypes. Dye diffusion experiments indicate that diffusion occurs in the gel growth medium (Fig. S2); hence, we hypothesize that interactions are induced by the concentration of root exudates diffusing in the medium rather than by physical contact. However, the biochemical basis for the apparent exudate-mediated interaction remains unknown.

Rice Roots Recognize an Object in the Medium in a Size-Dependent Manner. We found that root systems of plants grown in small pots are smaller than those of plants grown in large containers. Several other studies (35–38) have reported similar results. Gersani et al. (37) and O'Brien et al. (38) reported that the root system was larger when grown in two connected pots shared with the other plant than when grown alone in one pot. Our results showing that the size of the root system increases when a small number of roots physically contact an obstacle, but overall root size decreases as greater numbers of root tips touch the object, suggest that root impedance may be one factor in this phenomenon. However, there are several alternative hypotheses for why plants have a smaller root system size when grown with a competitor. First, there could be volatile cues from competitors, such that plants allocate more biomass to stem elongation and less to roots. Our measurements of root and shoot biomass showed that although these were both larger when plants were grown alone, there was no difference in root allocation. Additionally, separation of the aerial portion of neighboring plants also showed no effect on root systems. Second, plants could be smaller because of reduced or different types of resources (reduced light, or nutrients). Although we cannot entirely eliminate this possibility, plants were grown in a concentrated nutrient solution (39) and in growth chambers with consistent lighting, making it unlikely that either of these resources were lacking. Taken together, our results showing that overall root size decreases as greater numbers of root tips touch the object, suggest that root impedance may be one factor in why plants grown in smaller pots have smaller root systems.

Interestingly, one trait—bushiness—was always significantly larger with an obstacle present. Larger bushiness values indicate greater numbers of shallow roots. Thus, although the overall size of the root system decreased when root tips contacted large obstacles, root systems produced greater numbers of shallow roots, possibly to better explore available growth space.

We observed both genotype-dependent and trait-dependent differences in the inhibition of root system size in response to large obstacles in the medium (Table S5). For example, the convex area of Azucena decreased by nearly 15% when grown with a 7-cm obstacle, but that of IR64 was nearly the same as when grown without an obstacle. In contrast, the total root length and median root number of Azucena changed little when roots were impeded by a large 7-cm obstacle, but were each reduced by ∼20% in IR64. Interestingly, Azucena appears to be more resistant to compact soils than IR64 (40). In root penetration assays, more Azucena roots penetrated a wax layer, and those that did penetrate were longer than IR64 (40). It will be interesting to investigate whether the genes underlying growth responses to large obstacles are similar to those controlling root-penetration ability.

In conclusion, our data suggest that rice roots are able to recognize and identify objects in their vicinity through two mechanisms: genotype-specific root recognition likely mediated by signaling through root tips, and physical object recognition via size-dependent root tip contact. Collectively, these data suggest the existence of a coordinated root system response that can integrate local rhizosphere signals into global root architecture.

Materials and Methods

Plant Materials. Three rice genotypes with differing root architectures were selected for study: Azucena (*tropical japonica* with deep root systems), Caiapo (*tropical japonica* with deep root systems), and IR64 (*indica* with short root systems). For most treatments in this study, the seeds were from Susan McCouch's laboratory at Cornell University in Ithaca, New York.

Plant Growth. Three intragenotype growth combinations (Azucena-Azucena, Caiapo-Caiapo, IR64-IR64) and three intergenotype growth combinations (Azucena-Caiapo, Azucena-IR64, Caiapo-IR64) were tested with more than 13 replicates per combination. Individual plant-growth experiments (one plant in one cylinder) were performed with 10 replicates.

The seeds were surface-sterilized with 10% H_2O_2 for 30 min, followed by 70% alcohol (vol/vol) for 5 min, and then rinsed with sterile water three to five times. The seeds were then sown in a sterilized plate with agar medium and germinated in the dark at 28 °C for about 48 h. The germinated seedlings were then transplanted to transparent cylinders, which were 27 cm in height and 10 cm in diameter, and each filled with 1.3 L transparent growth medium made from sterile Yoshida rice solution (39) with 0.25% Gelzan (pH = 5.7). The distance between the seeds of the two plants was 2 cm. Micropore tape was used to cover the cylinder to allow light penetration and gas exchange and prevent the medium from becoming contaminated by external microbes. Shoots and roots were harvested separately and dried at 75 °C for 48 h, after which shoots and roots were weighed to determine biomass.

To test the hypothesis that root interactions might be caused by the aerial portion of the plant, a transparent barrier was placed between the aerial portions of the two plants to totally separate the shoot components. To study the effect of container size on root growth, the cylinder was completely separated by an acrylic divider installed between the two root systems. Plants were grown 2-cm apart, regardless of container size. For root-obstacle studies, thin plastic pieces of different sizes (1-, 3-, 5-, or 7-cm wide by 8-cm deep) were inserted into the medium 1-cm away from the seed in one cylinder (Fig. 5*A*). Each set had 15 replicates.

Imaging Platform. Plants were imaged using a PhotoCapture360 turntable and software (Ortery Technologies) that was connected to a Canon digital camera (EOS 50D, EFS 60 mm) and a computer. During imaging, the cylinders were placed in a water tank to reduce cylindrical surface refraction (32). A light box was set behind the water tank to improve image quality (31). Plants were imaged every 24 h from the third to the 15th DAG. Images were taken every 9° of rotation and, hence, 40 images were taken per cylinder per day. Each cylinder required about 5 min to image.

Image-Preprocessing and 3D Reconstruction. Images were cropped first to remove the sides of the cylinders. The cropped images were then converted to binary images using an adaptive thresholding method coded in Matlab. The image sequences were then reconstructed using the RootWork software (33) (Fig. S3), where the root system reconstructions had the same resolution as the preprocessed image sequences. The voxels of the two root systems were semiautomatically separated using RootReader3D (32).

Calculation of Root Traits. GiA Roots software [General Image Analysis of Roots, www.giaroots.org (34)] was used to calculate the following root traits based on the 2D images: total root length, surface area, root system volume, convex area, bushiness, specific root length, median root number, and maximum root number (31) (Table S1).

Calculation of Root Interaction Strength. ***Root system overlap, Q.*** The overlap of root systems was calculated in a sequence of steps. First, root systems were reconstructed in 3D using RootWork (33). Next, reconstructions were provided to RootReader3D, which was used to identify the voxel coordinates of each root system in a pair (32). Then, the convex hull of both root systems was calculated using Matlab (convhulln command). The convex hull is the smallest convex set of pixels that contains all other pixels in the root system. We used the convex hull and voxel coordinates to calculate Q as follows. Let H_a and H_b denote the two respective convex hulls. By definition, all root voxels of plant A are contained inside H_a and all root voxels of plant B are contained inside H_b. Denote the total number of voxels of the root systems as V_a and V_b. We then calculated the number of voxels of plant A that are contained inside H_b, which we denote as S_{ab}. Similarly, we calculated the number of voxels of plant B that are contained inside H_a, which we denote as S_{ba}. Note that the number of "unique" voxels of plant A (i.e., the voxels that are inside the convex hull H_a but not inside H_b) is equal to: $U_a = V_a - S_{ab}$. Similarly the number of "unique" voxels of plant B is: $U_b = V_b - S_{ba}$. The overlap can then be defined such that it is 0 when there are only unique voxels of plant A and B. Similarly, the overlap should be 1 when root voxels of both systems are contained within the convex hull of the other system. Formally, we use a similarity index to calculate the overlap of convex hull (Q) of the paired root systems:

$$Q = 1 - (U_a + U_b)/(V_a + V_b) \qquad [1]$$

For example, if the two convex hulls are completely separate (the two root systems do not intertwine), then $U_a = V_a$ and $U_b = V_b$ (because all voxels are uniquely within only one convex hull). In which case, $Q = 1 - (V_a + V_b)/(V_a + V_b) = 1 - 1 = 0$. Similarly, if the two root systems completely overlap, then $U_a = 0$ and $U_b = 0$, and so $Q = 1$.

Change in the angle of the closest root. The calculation of angles is based on the reconstructed 3D root systems. The root tip of the root of plant A, which is closest to a plant B root (or distributed furthest into the plant B's root system area) is denoted T and the hypocotyl of plant A as O and the hypocotyl of plant B as P. The angle α between OT and OP is calculated as the angle of the closest root of plant A (Fig. 4*E*). The difference in α on different days is defined as the change in the angle.

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