High-throughput three-dimensional visualization of root system architecture of rice using X-ray computed tomography

CURRENT STATUS: UNDER REVIEW

Plant Methods  BMC

Shota Teramoto
NARO

Satoko Takayasu
NARO

Yuka Kitomi
NARO

Yumiko Arai-Sanoh
NARO

Takanari Tanabata
Kazusa DNA Research Institute

Yusaku Uga  yuga@affrc.go.jp
NARO
Corresponding Author
ORCID: 0000-0003-4006-954X

DOI: 10.21203/rs.2.18397/v1

SUBJECT AREAS

Plant Physiology and Morphology  Plant Molecular Biology and Genetics

KEYWORDS

Image processing, Oryza sativa, Plant root, Root plasticity, RSAvis3D, X-ray CT
Abstract

**Background:** Plants adjust their root system architecture (RSA) against changing environments to optimize their growth. Nondestructive phenotyping of roots beneath the soil not only reveal the response of RSA against environmental stimuli but also allow for designing an ideal RSA for crop cultivation. Generally, roots beneath the soil surface are three-dimensionally visualized using X-ray computed tomography (CT). However, root isolation from X-ray CT images involves a longer time; in addition, CT scanning and reconstruction processes require longer periods. For large-scale root phenotyping, a shorter image acquisition time is required. Thus, the objective of this study is to develop a high-throughput pipeline to visualize rice RSA consisting of radicle and crown roots in the soil, from X-ray CT images.

**Results:** We performed the following three processes to develop the pipeline. First, we used calcined clay with uniform soil particle size as the soil substrate. The size of voids between the soil particles was less than the scanning resolution, resulting in a clear root shape in the CT images. Second, we optimized the parameters for rapid X-ray CT scanning. Higher tube voltage and current produced the highest root-to-soil contrast images. Third, we used a 3-D median filter to reduce noise, and an edge detection algorithm to isolate the root segments. The detection limits of the root diameters of the pots of diameters 16 cm and 20 cm were 0.2 mm and 0.3 mm, respectively. Because the crown root diameter of rice is generally higher than 0.2 mm, almost all crown roots could be visualized. Our condition allows for simultaneously performing CT scanning and reconstruction by a high-performance computing technology. Consequently, our pipeline visualizes rice RSA in the soil, requiring less than 10 min (33 s, if a rough image is acceptable) for CT scanning.
and reconstruction, and 2 min for image processing to visualize rice RSA. We scanned the roots of the upland rice (considered in this study) daily, and our pipeline successfully visualized the root development dynamics over three weeks.

**Conclusions:** We developed a rapid three-dimensional visualization method to visualize rice RSA in the soil using X-ray CT and a fully automated-image processing method known as RSAvis3D. Our methodology allows for high-throughput measuring and requires no manual operators in image processing, thereby providing a potentially efficient large-scale root phenotyping.

**Background**

Roots are essential organs for taking up water and nutrients in the soil. To effectively absorb water and nutrients, plants develop various roots of different forms and functions, such as primary and lateral roots, and root hairs. The spatial distribution of such different root types in the ground is defined as the root system architecture (RSA) [1]. Plants adjust the RSA according to the changing environments. For example, in the case of nutrient absorption, when roots enter the soil zones or patches containing rich nutrients, such as nitrate, ammonium, phosphate, and potassium, plants increase the lateral root biomass in the area to increase the nutrient uptake capacity [2]. On the contrary, plants inhibit root growth locally to avoid entering undesirable regions containing poor phosphorus [3] or heavy metals at toxic levels [4]. In addition, the deficiency of nutrients other than phosphorus causes various RSA changes as well [5, 6]. In the case of abiotic stress responses, a meta-analysis of the effect of drought stress on root phenotype revealed that the drought stress decreases the root length and root length density, and increases the diameter, root:shoot mass ratio, and root cortical aerenchyma...
Such plasticity of RSA against environmental stimuli is considered to optimize plant growth and development [2–7]. Understanding the response of plants to the stimuli is beneficial in developing crop cultivars that adapt to several environmental stresses.

Isolating the genes or quantitative trait loci (QTL) affecting the RSA plasticity is one of the useful methods for crop breeding [8]. Many RSA phenotyping methods have been developed and employed in the fields to date. Digging up the roots in the soil [9] is a simple method, but loses a significant amount of information, such as spatial distribution of roots in the soil and the sequential change of RSA response against environmental stimuli. Other popular field methods, such as monolith, auger, and profile wall methods [10], face a similar problem as well. Although the glass wall method enables the observation of the sequential change of RSA by providing for observation of root growth through glass windows placed against the soil profile [10], the process is laborious. All these methods are difficult to apply for large-scale root phenotyping required to isolate genes or QTLs for RSA plasticity. Therefore, the related genes or QTLs are mostly isolated under artificially controlled conditions using a small model plant known as Arabidopsis (Arabidopsis thaliana).

For example, a split-root experimental system using segmented vertical agar plates revealed that the nitrate transporter NRT1.1 [11] and the ammonium transporter AMT1;3 [12] play an important role in nitrate- and ammonium-induced root colonization, respectively. Two robust QTLs affecting the RSA response to osmotic stress have been detected in a growth assay with mild osmotic stress using a recombinant inbred population between two Arabidopsis ecotypes, namely, Landsberg erecta and Columbia [13]. Unlike small model plants, such as Arabidopsis, in the case of crops, soil culture would be required rather than
hydroponic and gel cultures to examine the RSA plasticity to natural environment. In soybeans (Glycine max), waterlogging stress tests under field and greenhouse conditions revealed a QTL associated with waterlogging tolerance [14]. Although a few such reports are available, RSA phenotyping in soil remains a difficult task, and the dynamics of RSA response triggered by stresses could not be monitored. Recently, X-ray computed tomography (CT) and magnetic resonance imaging (MRI) are being employed to observe the root growth dynamics in soil [15]. X-ray CT has the potential for a wide range of applications in plant phenotyping because non-medical X-ray CT scanners are available at lower equipment cost compared with MRI [15]. X-ray CT visualizes roots in the soil based on the attenuation differences caused by X-rays between the materials. For imaging in X-ray CT, the signal data of multi-angle projections are used to compute densitometric slice images (reconstruction), which are stacked to construct 3-D densitometric volumes. By these processes, the RSA in the soil becomes nondestructively observable [16]. X-ray CT has been used in various studies through nondestructive root observation in the soil. For example, X-ray CT imaging revealed the interaction of two individual plant roots in Populus tremuloides and Picea mariana [17], development of porous architecture at the root-soil interface in tomato (Solanum lycopersicum) [18], and interaction of roots and phosphate fertilizer in wheat (Triticum aestivum) [19]. However, large-scale root phenotyping using X-ray CT for investigating the time-course of 3-D or 4-D root development has not been intensively studied yet, despite its importance in revealing the RSA plasticity in the soil. A few reasons for this are long scanning and reconstruction times, small scanning area, and laborious processes involved in the root segmentation in X-ray CT imaging [20]. Because a 4-D study requires repeated scanning of identical samples, shorter scanning and
processing times are required for large-scale analysis.

In the recent decades, the scanning and reconstruction times have been reduced by optimizing both hardware and software [21, 22]; however, the root segmentation process remains laborious. Root segments in the 3-D densitometric data are mainly isolated by three methods, namely, manual, semi-automatic, and fully automatic methods. In the manual method, root segments in the 3-D data are selected using a simple algorithm, such as thresholding and seeded region growing [19, 21, 23]. This method is simple and applicable for analyzing soil with complex texture but involves longer periods to determine the parameters—depending on the soil texture surrounding the interests and its location in the 3-D images—to select the region of interest. The semi-automatic method employs a more complex algorithm, which is mostly based on the particle tracking approach, to isolate the interest segments [24, 25, 26]. Because most plant roots penetrate the soil vertically or along the z-axis, this method regards the roots in horizontally-sliced images as particles and tracks the particles along the z-axis. The fully automatic method automatically isolates the root segments without any manual operation; requiring no operators, this method is potentially applicable to large-scale studies of root development dynamics. To date, there have been a few applications of the fully automatic method for plant root segmentation except for a method based on deep neural networks for root segmentation [27] and feature detection of tubular shape of roots [28]. The former is a developing technique for root segmentation in soil, and has a few applications in 4-D root phenotyping [27]. The latter was designed based on a medical image analysis method for detecting blood vessels [28], which is widely used in the medical field [29]. Because the target of the latter method is roots, including laterals, the pot diameter (70 mm) is relatively small and the X-ray dose
(2.5 Gy) for scanning one plant sample is relatively high. Therefore, this method is not suitable for 4-D analysis of RSA plasticity in crops. Thus, the objective of this study is to develop a pipeline for rapid RSA visualization that is suitable for 4-D RSA phenotyping. To develop the high-throughput method, we determined the CT imaging condition for soil substrate, scanning parameters, and X-ray doses, and developed an image processing program. Using the developed pipeline, we demonstrate the visualization of 4-D RSA development of an upland rice over three weeks. We focused on radicle and crown roots, which form the main skeleton of RSA. We studied rice as a representative monocotyledonous crop with fibrous root system [30].

Results

Conditions for X-ray CT scanning

Assuming the application for large-scale root phenotyping of crops, a scanning time of 10 min for each plant sample was determined as the X-ray CT condition. Because additional time was required for machine operation, start-up of X-ray generator, and saving the CT images, the actual time was 15 min for a single sample. Therefore, 32 samples could be processed in a day, provided a working time of 8 h a day. For example, weekly scanning could process 160 individuals per week, which is sufficient to perform large-scale phenotyping. To observe the RSA development of rice continuously before the roots reached the pot wall, the pot diameter and depth were set as 20 cm and 25 cm, respectively, based on the maximum size that can be scanned by the detector of the CT scanner used in this study. The scanning conditions were as follows: each scan digitally obtained 1200 projections using a signal averaging of two frames over 360° without binning (pixel detector resolution:
3000 × 3000) at 4.0 frames per second (fps). Finally, 860 horizontal slices of pixel resolution 1024 × 1024 were computed. The final spatial resolution was 300 μm, corresponding to a total volume of 30.72 × 30.72 × 25.8 cm³. These conditions can simultaneously process the scanning and the reconstruction steps in the CT system we used, and are thus advantageous for a rapid CT scan.

To obtain images containing a clear root shape, we determined the soil substrate for CT scanning. We used the upland rice cultivar Kinandang Patong (KP) as the test sample to satisfy the requirement of CT scanning. Moreover, KP was expected to be relatively easy in isolating the root segments from CT images because upland rice usually has thicker roots than lowland rice [31]. To select the suitable soil substrate for CT scanning of rice roots, we examined the CT images of the roots of KP grown in five different soil substrates, namely, calcined clay, volcanic ash soil, andosol, alluvial soil, and sand (Additional file 1). Among these five types, calcined clay exhibited the clearest root shape in the CT images (Additional file 2). Based on this result, we decided to use calcined clay for CT scanning of rice roots.

Because the tube voltage and the current in X-ray CT scanning affect the CT image quality, we determined them such that they exhibit the highest root-to-soil contrast. Calcined clay was packed into the pot and saturated with a hydroponic solution based on Kimura B solution, which is used for rice cultivation [32]; nitrate and ammonium concentrations were adjusted for upland condition. Further, KP was grown in the pot for five weeks in a growth chamber, and was subjected to CT scanning. The representative CT images are shown in Fig. 1. The inside of the pot was invisible in the 3-D reconstructed volumes (Fig. 1a). In the horizontal and the vertical slices (Fig. 1b and 1c, respectively), the roots are visible as dark pixels. The pixels of lower values were colored in black, which indicates lower X-ray absorbance
in the rice roots compared with that of calcined clay. To evaluate the influence of tube voltage and current on the CT image quality, we scanned the pot with the tube voltages of 125 kV, 150 kV, 175 kV, 200 kV, and 225 kV and tube currents of 100 μA, 200 μA, 300 μA, 400 μA, and 500 μA. The scaled-up images of all these combinations are shown in Fig. 2. Apparently, higher voltage and current produced the images highest contrast. This was supported by the fact that the peak signal to noise ratio (PSNR) increased with higher tube voltage and current, attaining the highest value at 225 kV and 500 μA. These results conclusively indicated that the voltage of 225 kV and the current of 500 μA were the best combination for rice root scanning under our conditions.

**Image processing**

To visualize the rice root segments automatically, we developed the image processing pipeline. The developed pipeline involves the following two steps: (1) a 3-D median filter process to increase the root-to-soil contrast and (2) an edge detection process to dilute the soil-like texture but retain the root-like structure. The first step is to increase the root-to-soil contrast to reduce noise in the CT images. Noise is caused by mineral particles, void in the soil, and short scan time because we selected a short scan time in CT imaging for high-throughput imaging.

To reduce the noise level, we applied a 3-D median filter to the CT images. Fig. 3a shows vertical slices with five different kernel sizes of 1, 3, 5, 7, and 9. An image processed with the kernel size of 1 is equivalent to a non-filtered image. We calculated the PSNR of each condition and found that the PSNR was the highest for the 3-D median filter of kernel size 7. The image with the kernel size of 9 was the most blurred. Thus, we determined the kernel size as 7 for this process based on these results.
The images were processed by the following steps to segment the root area in the images. First, the value intensity of the CT images was inverted because the CT images had soil voxels with higher value intensity and root voxels with lower value intensity. Next, we subtracted the blurred slices from their corresponding non-blurred counterparts to zero soil value intensity. The pixels, whose image brightness changes sharply would be isolated as root segments, which is a simple algorithm of edge detection. Fig. 3b shows vertical slices subtracted with blurred images with various kernel sizes. Because an image processed with the kernel size of 1 is the same as a non-filtered image, it results in all-zero images. In the image processed with a kernel size higher than 5 (Fig. 3b), signals were observed at the positions where the root is located in Fig. 3a. The areas of root became larger with the increase in the kernel size (Fig. 3b). Furthermore, the area of signals in the image with the kernel size of 21 was the same as the area of root in Fig. 3a with the kernel size of 1. Thus, we decided to use the kernel size of 21 based on these results.

After cropping the region of the inside of the CT images to eliminate the effects of pot wall on RSA development, all the processed slices were stacked to construct 3-D volumes. The horizontal projection and 3-D animation of the 3-D rendered volume are shown in Fig. 3c and Additional file 3, respectively. Furthermore, the RSA in the soil was successfully visualized; however, non-root segments were additionally recognized in the image and the movie. Because the root segmentation depends on contrast difference, all the voids in the soil are visualized. The non-root segments at the bottom were caused by collapsing of soil, and the segments at the top were cracks caused by plant growth and incompletely packed soil close to the ground surface. Small particles appearing everywhere were voids or water gradient in the soil. To remove small non-root segments, we used a thresholding and size opening
method. Thresholding cut off connections of each segment, and the small segments were removed by a size opening filter. Filtered horizontal projection and 3-D animation are shown in Fig. 3d and Additional file 4, respectively. Root segments were unaffected by these processes in this case, but the risk of erasing of small root segments by thresholding and size opening existed. For this reason, we did not use the thresholding and size opening filter in this study.

We implemented the algorithm by python script (Additional file 5) and measured the processing time with different hardware (Table 1). All the hardware we tested took less than 8 min for image processing. The processing time depended on the central processing unit, and the fastest processing time of 2 min was achieved using Intel® Xeon® E5-2650 v4. Because python is an interpreter language, batch operation is easily executable.

**Scanning time and metal filters to reduce X-ray doses**

X-rays affect the plant growth [22], which is a problem for 4-D root phenotyping with X-ray CT systems, because repeated CT scanning increases the cumulative X-ray dose. To reduce the X-ray doses, we investigated the influence of scanning time and metal filters on the CT image quality.

The scanning time is determined by the projection number, signal averaging number, exposing time, and binning size. Under our conditions, the projection number, signal averaging number, and binning size can be changed to reduce the scanning time. In a scanning time of 10 min, each scan digitally obtains 1200 projections using a signal averaging of two frames over 360° without binning (pixel detector resolution: 3000 × 3000) at 4.0 fps. At the fastest scanning, each scan digitally obtains 600 projections using no signal averaging over 360° with 3 × 3 binning (pixel detector resolution: 1000 × 1000) at 18.0 fps. In this condition,
scanning is performed in 33 s. We scanned a pot in which KP was cultivated for eight weeks under eight scanning conditions, and obtained 600 or 1200 projections using no signal averaging or a signal averaging of two frames over 360° with or without binning. The horizontal projections of the processed CT images are shown in Fig. 4. We observed similar RSA in all conditions, despite the degraded image quality at faster scanning conditions. Based on the result, the decision to shorten the CT scanning time for low X-ray doses was rejected.

Another method to reduce the X-ray doses is to use metal filters. The X-ray beams have a range of wavelengths. Because X-rays of longer wavelength have lower energy, their penetration ability is low, and are thus absorbed at the material surface, resulting in high X-ray dosage. Generally, metal filters are used to reduce the proportion of low-energy X-rays. To reduce the X-ray doses to plants, we evaluated the influence of copper (Cu) filters on the CT image quality. The scaled-up horizontal slices of unprocessed CT images without and with 0.5 mm, 1.0 mm, and 2.0 mm Cu filters are shown in Fig. 5. The noise level increased with the increase in the thickness of the filters. On the contrary, the quality of the image-processed horizontal projection using Cu filters were very similar to those with no filters; however, we observed a small increases in noise in the CT image when using the 2.0 mm Cu filter (Fig. 5). These results indicated that the 1.0 mm Cu filter is effective in reducing the X-ray dosage on plants under our conditions. Therefore, we used the 1.0 mm Cu filter in this study.

Influence of X-ray dose on rice growth

To evaluate the influence of X-ray CT exposure on rice growth, we estimated the X-ray doses using Rad Pro Dose Calculator (http://www.radprocalculator.com/). When a tube voltage of 225kV and current of 500 μA were applied to the material placed at
900 mm from the X-ray source using a 0.5 mm Cu filter, the X-ray dose of the material was estimated as 0.55 Gy/hr. At a scanning time of 10 min using an 1.0 mm Cu filter, the dose to rice plants was estimated as less than 0.09 Gy per scan. Because 0.09 Gy is sufficiently lower than 33 Gy, which is the threshold affecting plant growth [22, 33], it was considered that sequential X-ray scanning does not pose a problem for rice growth. In the case of rice, it was revealed that daily scanning with a dose of 1.4 Gy for nine days, i.e., total dose of 12.6 Gy, did not negatively impact the rice growth [22]. A simple arithmetic calculation indicated that 140 scanning procedures are permissible in our scanning condition, if 12.6 Gy is the upper limit for X-ray CT exposure.

To evaluate the influence of sequential X-ray doses on plant growth, KP was cultivated for two weeks and subjected to daily CT scanning for seven days. The results indicated no apparent differences between shoot and root shape of mock- and X-ray-treated plants at 21 DAS (Fig. 6a–b); also in addition, we quantified the shoot and the root traits. There was no significant difference in plant height, total root length, shoot dry weight, and root dry weight. (Fig. 6c–f). These results indicated that X-ray doses in our scanning condition do not constitute any problem for rice growth.

**Four-dimensional visualization of root development**

To evaluate the fully automatic visualization method in this study, we monitored the dynamics of root development of KP for three weeks. We cultivated KP for one week, and KP was subjected to daily CT scanning for three weeks. The processed horizontal projections and 3-D movie are provided in Additional files 6 and 7, respectively. From seven to 13 days after sowing (DAS), the root shape in the image was hazy but the daily root growth was observed. From 14 to 20 DAS, the root
shape became bolder and the root length increased rapidly. Many root tips went outside the scope at 20 DAS. From 21 to 27 DAS, the root shape became increasingly bolder but the general shape of RSA remained unchanged. They indicated that our pipeline visualized the root development dynamics of KP for three weeks from seven DAS.

**Verification of root fragments in the processed CT images**

To verify the length of root diameter detectable with our pipeline, we implemented the wired basket method. The basket method is used to evaluate the rooting angle of rice cultivars by counting the proportion of roots penetrating the bottom of the basket [34, 35]. As a modified approach, we used the basket, whose inside was wired with nylon monofilament at intervals of 1.5 cm, to keep *in situ* RSA when the basket was unearthed from the ground and the soil was removed. More information on the procedure is available in the Methods section. We cultivated rice plants for 21 days and then unearthed the basket. Because we observed that 21-DAS KP has many roots of different thicknesses (Additional file 6), 21-DAS rice was suitable for verifying the length of the detectable root diameter. To exclude the influence of root distribution, we used three genotypes that had different RSAs, [36], namely, KP (thick and deep-root type), IR64 (thin and shallow-root type), and Dro1-NIL (thin and intermediate-root type). To visualize the roots in the soil, we scanned the rice plants using the X-ray CT scanner and performed image processing. The vertical projections are shown in the left column in Fig. 7a. After scanning, to obtain a visible root image keeping *in situ* RSA, we unearthed the baskets and removed the soil from the baskets by washing with tap water. The images shot from directly above the basket are shown in the middle column in Fig. 7a. We compared the CT and the camera images, and traced the crown and the radicle roots in the projection
images (right column in Fig. 7a). There were 68 detectable (solid line) and 12 non-detectable (dash line) roots in the processed X-ray CT images, compared with the camera images. We collected the root segments, scanned them with a 2-D scanner, and measured the root diameters using ImageJ plug-in SmartRoot [37]. We compared the root diameters of detectable and non-detectable roots, and found that many roots with a diameter of less than 0.3 mm were not visualized by our method, irrespective of the RSA type (Fig. 7b). To visualize all radicle and crown roots, we used a smaller pot of diameter 16 cm, and adjusted the source-detector distance and source-rotation axis distance to be 800 mm and 407 mm, respectively. We performed the wired basket assay again, and found 82 detectable roots but did not find any non-detectable roots (Fig. 7c). The diameter of the detectable roots was higher than 0.2 mm (Fig. 7d). These results indicated that the detection limit of roots can be determined by adjusting the pot diameter, source-detector distance, and source-rotation axis distance. In this condition, the X-ray dose per scan was estimated to be 0.44 Gy. A simple arithmetic calculation indicated that 28 scanning procedure are permissible in our scanning condition, if 12.6 Gy is the upper limit for X-ray CT exposure.

Discussion

Rapid, nondestructive, and less-laborious root visualization method is required for 4-D large-scale root phenotyping. X-ray CT system is one of the most promising equipment for this purpose. However, X-ray CT has a few disadvantages in root visualization, such as long scanning time, limited scanning area, long reconstruction time, and laborious procedure. In this study, we propose a pipeline for rapid 3-D rice root visualization in X-ray CT images. The CT parameters used in this study are as
follows: each scan digitally obtained 1200 projections using a signal averaging of
two frames over 360° without binning (pixel detector resolution: 3000 × 3000) at
4.0 fps. Finally, 860 horizontal slices of pixel resolution 1024 × 1024 were
computed. To harden the X-ray beam, a 1.0 mm Cu filter was used. The total
scanning time was 10 min. Table 2 provides the details of recent studies on rice
roots with X-ray CT [38–44]. The table clearly indicates that our method provides
rapid scanning, large scanning area, and fully automated rice root visualization.
To scan a large volume of the soil, we used the longest source-rotation axis
distance in our CT system, thereby obtaining the largest scanning area (Table 2).
Because we focused on the RSA consisting of radicle and crown roots, the resolution
using only the radicle and the crown roots can be detected is sufficient. However,
we used the highest tube voltage and current to enhance the root-to-soil contrast
(Table 2, Fig. 2, and Fig. 3). Previous studies employed a pot diameter of less than
80 mm for X-ray CT scanning of rice (Table 2). In the case of pots with smaller
diameter, the root tips easily touched the inner wall of the pot and changed the
growth direction. Our system used a pot of diameter 200 mm, and rice RSA was thus
visualized well (Fig. 3c and 3d, Additional files 3, 4, 6, and 7); therefore, RSA
development for longer periods can be analyzed.
Because of the high-performance computing technology, we performed scanning
and reconstruction simultaneously, thereby achieving acquisition of CT images in 10
min. If a small degradation of image quality is not considered a problem, acquisition
time of 33 s is useful as well (Fig. 4). This is the fastest acquisition time among the
available protocols published for rice root visualization (Table 2). Because we used
a strong 3-D median filter in image processing, an increase in noise level caused by
rapid scanning was minimized. However, the 3-D median filter loses some
information of roots and erases thin root-like laterals. For example, the rice root diameter within 10 mm from the root tip is smaller than the diameter of other regions [45], leading to a possibility that the root tips are difficult to be observed. Accordingly, our method is optimized monitoring the overall RSA development, and not for detailed response, such as root tip angle of each root.

Our image-processing method is fully automatic and requires no operators (Table 2); thus, consistent results are obtained even if a person processes the images, thereby saving significant time required for manual processing. Because a 4-D root study generates huge volume of scanning data, the simplicity of the fully-automated method is advantageous for image processing. Compared with the fully manual and semi-automatic methods, the fully automatic method has a possible risk of not detecting the obscure roots because it performs automatic processing, and there are no chances of intervention by a person. Although this is not a major problem in observing the overall development of RSA, some information of RSA is lost.

Further, doses are one of the most considerable problems in X-ray CT imaging of plant roots. It was revealed that 33 Gy is the threshold affecting plant growth [22, 33], and that 12.6 Gy does not negatively impact the rice growth [22]. The doses in our system were estimated under 0.09 Gy (Table 2), which is 140 times smaller than the value reported to have no influence on rice growth [22]. Moreover, daily CT scanning for seven days indicated no significant differences in root and shoot traits (Fig. 6). Compared with other studies, the X-ray doses under our condition was relatively smaller because of fast scanning and long source-rotation axis distance (Table 2), because the X-ray dose is directly proportional to the scanning time and inversely proportional to the square of the distance. These results additionally indicated that our method is applicable for repeated CT scanning, which is required
for 4-D root phenotyping.

The detection limits of the root diameter in our method are 0.2 mm and 0.3 mm when pots of diameters 16 cm and 20 cm, respectively, are used (Fig. 8). Because the threshold of the root diameter to distinguish the radicle and the crown roots from lateral roots is known to be approximately 0.2 mm [46], our method can detect almost all radicle and crown roots. Under the condition with pots of diameter 20 cm, we could not detect 15% of roots of 21-DAS rice plants (Fig. 7a–b). The root diameter generally increases with the growth of plants. Thus, losing some thinner roots would not be a serious problem in observing the RSA development dynamics; however, it could be a potential problem in analyzing the RSA of young seedlings (Additional files 6 and 7).

To apply our image-processing method for other crops, the conditions of soil and root type need to be considered. Further, soil substrates with high root-to-soil contrast for other crops are necessary. Because this method employs intensity change to enhance the root segments, root-to-soil contrast is an important factor for efficient root isolation. Furthermore, the diameter of the soil particles must be considered as well, because voids between the soil particles are visualized, and roots of diameter less than that of soil particles are not visualized. To obtain results with low noise levels, uniform soil particles are preferable for all CT analyses. In the case of crop roots with large diameter, e.g., radish (Raphanus raphanistrum), our method is not suitable because it recognizes the large area with flat intensity at the inner region of primary root as soil.

Conclusions

In this study, we developed a pipeline for rapid 3-D visualization of rice RSA in soil
using X-ray CT (RSAvis3D). Various pots of large diameter, rapid scanning, low X-ray doses, and fully automatic image processing enabled large-scale phenotyping of 4-D RSA dynamics required for studying the RSA plasticity. In image processing, enhancing the root value intensity is useful for not only visualizing RSA but also its quantification.

Methods

Plant materials

Three rice (Oryza sativa) lines, namely, IR64 (IRGC #66970), Kinandang Patong (KP, IRGC #23364), and Dro1-NIL [36], were used in this study. KP has deep and thick roots, and IR64 has shallow and thin roots. Further, Dro1-NIL is a near-isogenic line of IR64 with KP-type DRO1 allele, having intermediate and thin roots.

Growth condition

We used Profile® Greens Grade™ (PROFILE Products, Buffalo, Illinois, USA) as a soil-like root growth substrate. Profile is calcined clay and has characteristics similar to those of Turface® (PROFILE Products, Buffalo, Illinois, USA) [47], which is a popular substrate for root imaging with X-ray CT [43, 48]. Profile has the following advantages in studying plant roots: (1) It is hard and its volume is not affected by water content, keeping RSA in the soil both under dry and well-watered conditions. (2) It is easily removed from the root surface, and root samples are effectively collected. (3) It can retain sufficient water and nutrients for plant growth. Before using, Profile was rinsed with tap water three to five times and dried because it has a large variation of labile (readily desorb-able or readily plant-available) nutrient content [47]. The dried Profile was packed in the pot, and saturated with a modified Kimura B hydroponic solution [32] consisting of 1.23 mM of NO₃⁻, 0.41 mM of NH₄⁺,
0.18 mM of H$_2$PO$_4^-$, 1.00 mM of SO$_4^{2-}$, 1.78 mM of K$^+$, 0.55 mM of Mg$^{2+}$, 0.37 mM of Ca$^{2+}$, and 8.9 μM of Fe$^{3+}$. The pH was adjusted to be 5.5 with HCl and KOH. The ratio of nitrate to ammonium was based on the ratio of these compounds in the soil collected from an upland field in the Institute of Crop Science (National Agriculture and Food Research Organization, Ibaraki, Japan; 36°02'89'' N and 140°09'97'' E) on 2018 May 24, which we usually use for characterization of rice plant roots [31].

Except where indicated, we used custom-made pots of diameter 20 cm and height 25 cm (TSP2530P, TecS, Itako, Ibaraki, Japan). Rice seeds were immersed in water for a day at 15 °C with a fungicide, and in water for 2 days at 30 °C. The germinated seeds were sown in the pot. Deionized water was supplied at the bottom of the pots during cultivation.

The rice plants were grown in a custom-made growth chamber (Nippon Medical & Chemical Instruments Co., Tennoji-ku, Osaka, Japan), whose temperature, light intensity, and humidity were strictly controlled. The light condition was 14 h of day light, and the light intensity was set as a photosynthetic photon flux density (PPFD) of approximately 500 μmol m$^{-2}$ s$^{-1}$ at the top of the pot, with the exception of PPFD of 250 μmol m$^{-2}$ s$^{-1}$ during dawn and dusk. The diurnal program of light intensity is as follows: ZT0, PPFD of 250 μmol m$^{-2}$ s$^{-1}$; ZT1, PPFD of 500 μmol m$^{-2}$ s$^{-1}$; ZT13, PPFD of 250 μmol m$^{-2}$ s$^{-1}$; and ZT14, PPFD of 0 μmol m$^{-2}$ s$^{-1}$. The diurnal program of temperature was based on the average of diurnal temperatures in Tsukuba in July 2017, which was obtained from the data of the Weather Data Acquisition System of Institute for Agro-Environmental Sciences, NARO. The diurnal program is as follows: ZT0, 25 °C; ZT2, 26 °C; ZT3, 27 °C; ZT4, 28 °C; ZT5, 29 °C; ZT6, 30 °C; ZT12, 29 °C; ZT13, 28 °C; ZT14, 27 °C; ZT15, 26 °C; and ZT16, 25 °C. The humidity condition was
set as 50% at light and 60% at dark conditions. To maintain the CO₂ concentration, a part of air in the chamber was periodically exchanged with the outside air. Because of the large space of the chamber (86.4 m³), the range of CO₂ concentration during cultivation was 400–500 ppm.

**Soil chemical analysis**

The ammonium and nitrate concentrations of the field soil were respectively quantified by the indophenol method and a colorimetric method based on diazotization with nitrate reduction. Four samples from different locations in the field were subjected to ammonium and nitrate quantification by a commercial service (Katakura & Co-op Agri Corporation, Chiyoda-ku, Tokyo, Japan).

**X-ray CT imaging and 3-D reconstruction**

The rice root in the soil was imaged using the X-ray CT system inspeXio SMX-225CT FPD HR (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan). Except where indicated, each scan digitally obtained 1200 projections using a signal averaging of two frames over 360° without binning (pixel detector resolution: 3000 × 3000) at 4.0 fps. Finally, 860 horizontal slices of pixel resolution 1024 × 1024 were computed. The final spatial resolution was 300 μm, corresponding to a total volume of 30.72 × 30.72 × 25.8 cm³. Further, tube voltages of 150 kV, 175 kV, 200 kV, and 225 kV and tube currents of 100 μA, 200 μA, 300 μA, 400 μA, and 500 μA were used. The source-detector distance and the source-rotation axis distances were 1200 mm and 900 mm, respectively. Three Cu filters of different thickness (0.5 cm, 1.0 cm, and 2.0 cm) were used to harden the X-ray beam. Beam hardening was approximately corrected by the operation software with a correction table calculated with metal material.
Image Processing

The CT images were processed using the python script. The CT slices were loaded as 16-bit grayscale images in the memory. The gray scale range of the CT images was normalized by setting the modes of air and soil fractions as 0 and 1024, respectively. The images were stacked into a 3-D numpy array [49], normalized, filtered with a 3-D median, inverted, and subtracted by its blurred slices. To remove small objects, 3-D size opening was applied to the computed 3-D array. The processed images were saved as 8-bit grayscale images. The median, blur, and size opening filters were imported from scipy [50], opencv [51], and skimage [52] modules, respectively. Furthermore, parallel processing was performed with a multiprocessing package [53]. The cylindrical shaped area of height 25 cm and diameter 18 cm was trimmed to remove the roots that touched the pot wall, based on the results.

Visualization of CT slices and 3-D volume

The CT slices were visualized using the VG Studio MAX 3.2 software (Volume Graphics, Heidelberg, Germany) or python scripts with numpy and matplotlib [54] modules. The 2-D images rendered by 3-D data were obtained with VG Studio MAX or maximum intensity projection using python scripts. The 3-D movies were constructed with VG Studio MAX.

Growth test against X-ray exposure

KP was cultivated for two weeks and subjected to daily CT scanning for seven days. For X-ray treatment, KP was exposed to a 10 min X-ray scanning, receiving approximately 0.09 Gy per scan. For mock treatment, KP was loaded on the turn table in the CT machine, where it was left for 10 min without X-ray exposure. The positions of all plants in the growth chamber were shifted daily to buffer the
influence of position on growth. At 21 DAS, shoot and root traits were quantified. The plant height was measured using a ruler, and the shoot sample was collected and dried at 80 °C for three days to measure the dry weight. Root samples were collected from the soil and scanned using a scanner of 400 dpi (Expression 12000XL, Seiko Epson Corporation, Suwa, Nagano, Japan). The total root length was measured using the WinRHIZO™ Pro 2017a software (Regent Instruments, Quebec, Canada). The root sample was dried at 80 °C for three days to measure the dry weight.

**Wired basket method**

To keep RAS in the soil *in situ*, the inside of the plastic mesh baskets (diameter: 15 cm, height: 6 cm) was wired with φ0.148 mm nylon monofilaments. Two wire layers were designed at 1 cm and 3 cm under the top of the basket. Each wire layer consisted of seven parallel and seven perpendicular wires. The interval between each parallel or perpendicular wire was approximately 1.5 cm. The wired mesh basket was buried in the pot, and germinated rice seeds were sown on the top of the basket. After cultivating for three weeks, the roots in the soil were scanned with the X-ray CT scanner, and the wired basket was excavated from the soil. All excavations were performed in water to avoid damaging the roots. Subsequently, soil was dropped from the mesh of the basket. The picture was shot from directly above using a digital camera (Xperia Z5 Compact, Sony Corporation, Shinagawa-ku, Tokyo, Japan). To enhance the root colors, the chroma of blue and cyan colors, which are the colors of the basket, were adjusted using the GIMP software (version 2.8.22, https://www.gimp.org/). By comparing the CT image and the picture of the wired basket method, we categorized all crown roots and radicles as detectable and
undetectable by X-ray CT. The radicle and the crown roots were cut at approximately 2 cm and 5 cm from the shoot-root junction, and the resulting 3 cm root fragments were sampled. Each fragment was scanned by a scanner of 600 dpi (Expression 12000XL). The average root diameter of each fragment was calculated using ImageJ plug-in, SmartRoot (version 4.21, [37]).

**Statistical analysis**

Student’s t test was performed with the “t.test()” function in the R software (version 3.5.1), and a P value under 0.05 was considered significant.

**Declarations**

1. Lynch J. Root architecture and plant productivity. Plant Physiol. 1995; 109:7-13.

2. Hodge A. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 2004; 162:9-24.

3. Péret B, Desnos T, Jost R, Kanno S, Berkowitz O, Nussaume L. Root architecture responses: in search of phosphate. Plant Physiol. 2014; 166: 1713-23.

4. Khare D, Mitsuda N, Lee S, Song WY, Hwang D, Ohme-Takagi M, et al. Root avoidance of toxic metals requires the GeBP-LIKE 4 transcription factor in *Arabidopsis thaliana*. New Phytol. 2017; 213: 1257-73.

5. Gruber BD, Giehl RF, Friedel S, von Wirén N. Plasticity of the arabidopsis root system under nutrient deficiencies. Plant Physiol. 2013; 163: 161-79.

6. Shahzad Z, Amtmann A. Food for thought: how nutrients regulate root system architecture. Curr Opin Plant Biol. 2017; 39: 80-87.

7. Zhou G, Zhou X, Nie Y, Bai SH, Zhou L, Shao J, et al. Drought-induced changes in root biomass largely result from altered root morphological traits: Evidence
from a synthesis of global field trials. Plant Cell Environ. 2018; 41: 2589–99.

8. de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X. Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci. 2007; 12: 474–81.

9. Trachsel S, Kaeppler SM, Brown KM, Lynch JP. Shovelomics: high throughput phenotyping of maize (Zea mays) root architecture in the field. Plant Soil. 2011; 341: 75–87.

10. Böhm W. Methods of Studying Root Systems. Vol. 33. Springer Science & Business Media, Germany, 2012.

11. Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E, et al. The Arabidopsis1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. Proc Natl Acad Sci USA. 2006; 103: 19206–11.

12. Lima JE, Kojima S, Takahashi H, von Wirén N. Ammonium triggers lateral root branching in Arabidopsis in an AMMONIUM TRANSPORTER1; 3-dependent manner. Plant Cell. 2010; 22: 3621–33.

13. Gerald JNF, Lehti-Shiu MD, Ingram PA, Deak KL, Biesiada T, Malamy JE. Identification of quantitative trait loci that regulate Arabidopsis root system size and plasticity. Genet. 2006; 172: 485–98.

14. Ye H, Song L, Chen H, Valliyodan B, Cheng P, Ali L. et al. A major natural genetic variation associated with root system architecture and plasticity improves waterlogging tolerance and yield in soybean. Plant Cell Environ. 2018; 41: 2169–82.

15. Atkinson JA, Pound MP, Bennett MJ, Wells DM. Uncovering the hidden half of plants using new advances in root phenotyping. Curr Opin Biotechnol. 2019;
16. Heeraman DA, Hopmans JW, Clausnitzer V. Three dimensional imaging of plant roots in situ with X-ray Computed Tomography. Plant Soil. 1997; 189: 167-179.

17. Paya AM, Silverberg JL, Padgett J, Bauerle TL. X-ray computed tomography uncovers root-root interactions: quantifying spatial relationships between interacting root systems in three dimensions. Front Plant Sci. 2015; 6: 274.

18. Helliwell JR, Sturrock CJ, Mairhofer S, Craigon J, Ashton RW, Miller AJ, et al. The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. Sci Rep, 2017; 7: 14875.

19. Ahmed S, Klassen TN, Keyes S, Daly M, Jones DL, Mavrogordato M, et al. Imaging the interaction of roots and phosphate fertiliser granules using 4D X-ray tomography. Plant Soil. 2016; 401: 125-34.

20. Pfeifer J, Kirchgessner N, Colombi T, Walter A. Rapid phenotyping of crop root systems in undisturbed field soils using X-ray computed tomography. Plant Methods. 2015; 11: 41.

21. Hodge A. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 2004; 162: 9-24.

22. Zappala S, Helliwell JR, Tracy SR, Mairhofer S, Sturrock CJ, Pridmore T, et al. Effects of X-ray dose on rhizosphere studies using X-ray computed tomography. PLoS One. 2013; 8: e67250.

23. Helliwell JR, Sturrock CJ, Mairhofer S, Craigon J, Ashton RW, Miller AJ, et al. The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. Sci Rep. 2017; 7: 14875.

24. Mairhofer S, Zappala S, Tracy SR, Sturrock C, Bennett M, Mooney SJ, et al. RooTrak: automated recovery of three-dimensional plant root architecture in
soil from x-ray microcomputed tomography images using visual tracking. Plant Physiol. 2012; 158: 561–9.

25. Mairhofer S, Sturrock CJ, Bennett MJ, Mooney SJ, Pridmore TP. Extracting multiple interacting root systems using X-ray microcomputed tomography. Plant J. 2015; 84: 1034–43.

26. Perret J, Al-Belushi M, Deadman M. Non-destructive visualization and quantification of roots using computed tomography. Soil Biol Biochem. 2007; 39: 391–9.

27. Douarre C, Schielein R, Frindel C, Gerth S, Rousseau D. Transfer learning from synthetic data applied to soil–root segmentation in x-ray tomography images. J Imaging. 2018; 4: 65.

28. Gao W, Schlüter S, Blasé SRGA, Shen J, Vetterlein D. A shape-based method for automatic and rapid segmentation of roots in soil from X-ray computed tomography images: Rootine. Plant Soil. 2019; 441: 643–55.

29. Sato Y, Nakajima S, Shiraga N, Atsumi H, Yoshida S, Koller T, et al. Three-dimensional multi-scale line filter for segmentation and visualization of curvilinear structures in medical images. Med Image Anal. 1998; 2: 143–68.

30. Khush G. Productivity improvements in rice. Nutrition reviews. 2003; 61: S114–S116.

31. Uga Y, Ebana K, Abe J, Morita S, Okuno K, Yano M. Variation in root morphology and anatomy among accessions of cultivated rice (Oryza sativa) with different genetic backgrounds. Breed Sci. 2009; 59: 87–93.

32. Yoshida S, Douglas AF, James HC, Gomez KA. Laboratory Manual for Physiological Studies of Rice. 3rd ed. Manila: International Rice Research Institute; 1976.
33. Johnson EL (1936) Susceptibility of seventy species of flowering plants to X-radiation. *Plant physiology* 11(2): 319.

34. Kato Y, Abe J, Kamoshita A, Yamagishi J. Genotypic variation in root growth angle in rice (*Oryza sativa*) and its association with deep root development in upland fields with different water regimes. Plant Soil. 2006; 287: 117-129.

35. Uga Y, Ebana K, Abe J, Morita S, Okuno K, Yano M. Variation in root morphology and anatomy among accessions of cultivated rice (*Oryza sativa*) with different genetic backgrounds. Breed. Sci. 2009; 59: 87-93.

36. Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, et al. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet. 2013; 45: 1097-102.

37. Lobet G, Pagès L, Draye X. A novel image-analysis toolbox enabling quantitative analysis of root system architecture. Plant Physiol. 2011; 157: 29-39.

38. Kirk GJ, Boghi A, Affholder MC, Keyes SD, Heppell J, Roose T. Soil carbon dioxide venting through rice roots. Plant Cell Environ. 2019; doi: 10.1111/pce.13638.

39. Fang H, Rong H, Hallett PD, Mooney SJ, Zhang W, Zhou H, et al. Impact of soil puddling intensity on the root system architecture of rice (*Oryza sativa*) seedlings. Soil Tillage Res. 2019; 193: 1-7.

40. Wang L, Guo M, Li Y, Ruan W, Mo X, Wu Z, et al. LARGE ROOT ANGLE1, encoding OsPIN2, is involved in root system architecture in rice. J Exp Bot. 2018; 69: 385-97.

41. Giri J, Bhosale R, Huang G, Pandey BK, Parker H, Zappala S, et al. Rice auxin influx carrier *OsAUX1* facilitates root hair elongation in response to low
42. Huang G, Liang W, Sturrock CJ, Pandey BK, Giri J, Mairhofer S, et al. Rice actin binding protein RMD controls crown root angle in response to external phosphate. Nat Commun. 2018; 9: 1408.

43. Rogers ED, Monaenкова D, Mijar M, Nori A, Goldman DI, Benfey PN. X-ray computed tomography reveals the response of root system architecture to soil texture. Plant Physiol. 2016; 171: 2028–40.

44. Zappala S, Mairhofer S, Tracy S, Sturrock CJ, Bennett M, Pridmore T, et al. Quantifying the effect of soil moisture content on segmenting root system architecture in X-ray computed tomography images. Plant Soil 2013; 370: 35-45.

45. Yamauchi T, Abe F, Tsutsumi N, Nakazono M. Root cortex provides a venue for gas-space formation and is essential for plant adaptation to waterlogging. Front Plant Sci. 2019; 10: 259.

46. Henry A, Cal AJ, Batoto TC, Torres RO, Serraj R. Root attributes affecting water uptake of rice (Oryza sativa) under drought. J Exp Bot. 2012; 63: 4751–63.

47. Adams C, Jacobson A, Bugbee B. Ceramic aggregate sorption and desorption chemistry: implications for use as a component of soilless media. J Plant Nutr. 2014; 37: 1345–57.

48. Piñeros MA, Larson BG, Shaff JE, Schneider DJ, Falcão AX, Yuan L, et al. Evolving technologies for growing, imaging and analyzing 3D root system architecture of crop plants. J Integr Plant Biol. 2015; 58: 230-41.

49. Van Der Walt S, Colbert SC, Varoquaux G. The NumPy array: a structure for efficient numerical computation. Comput Sci Eng. 2011; 13: 22.

50. Jones E, Oliphant T, Peterson P. SciPy: Open source scientific tools for Python.
2001; https://www.scipy.org.

51. Bradski G, Kaehler A. Learning OpenCV: Computer vision with the OpenCV library. O'Reilly Media Inc, USA. 2008.

52. Van der Walt S, Schönberger, JL, Nunez-Iglesias J, Boulogne, F, Warner JD, Yager N, et al. scikit-image: image processing in Python. PeerJ, 2014; 2: e453.

53. Palach J. Parallel Programming with Python. Packt Publishing Ltd, England. 2014.

54. Hunter JD. Matplotlib: A 2D graphics environment. Comput Sci Eng. 2007; 9: 90.

Abbreviations

RSA: root system architecture
CT: computed tomography
QTL: quantitative trait locus
Dro1: DEEPER ROOTING 1
KP: Kinandang Patong
3-D: three-dimensional
4-D: four-dimensional
fps: frames per second
DAS: days after sowing
PPFD: photosynthetic photon flux density
ZT: zeitgeber time

References

1. Lynch J. Root architecture and plant productivity. Plant Physiol. 1995; 109:7-
2. Hodge A. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 2004; 162:9-24.

3. Péret B, Desnos T, Jost R, Kanno S, Berkowitz O, Nussaume L. Root architecture responses: in search of phosphate. Plant Physiol. 2014; 166: 1713-23.

4. Khare D, Mitsuda N, Lee S, Song WY, Hwang D, Ohme-Takagi M, et al. Root avoidance of toxic metals requires the GeBP-LIKE 4 transcription factor in Arabidopsis thaliana. New Phytol. 2017; 213: 1257–73.

5. Gruber BD, Giehl RF, Friedel S, von Wirén N. Plasticity of the arabidopsis root system under nutrient deficiencies. Plant Physiol. 2013; 163: 161–79.

6. Shahzad Z, Amtmann A. Food for thought: how nutrients regulate root system architecture. Curr Opin Plant Biol. 2017; 39: 80–87.

7. Zhou G, Zhou X, Nie Y, Bai SH, Zhou L, Shao J, et al. Drought-induced changes in root biomass largely result from altered root morphological traits: Evidence from a synthesis of global field trials. Plant Cell Environ. 2018; 41: 2589-99.

8. de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X. Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci. 2007; 12: 474–81.

9. Trachsel S, Kaeppler SM, Brown KM, Lynch JP. Shovelomics: high throughput phenotyping of maize (Zea mays) root architecture in the field. Plant Soil. 2011; 341: 75–87.

10. Böhm W. Methods of Studying Root Systems. Vol. 33. Springer Science & Business Media, Germany, 2012.

11. Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E, et al. The Arabidopsis1 transporter participates in the signaling pathway triggering root
colonization of nitrate-rich patches. Proc Natl Acad Sci USA. 2006; 103: 19206–11.

12. Lima JE, Kojima S, Takahashi H, von Wirén N. Ammonium triggers lateral root branching in Arabidopsis in an AMMONIUM TRANSPORTER1; 3-dependent manner. Plant Cell. 2010; 22: 3621–33.

13. Gerald JNF, Lehti-Shiu MD, Ingram PA, Deak KI, Biesiada T, Malamy JE. Identification of quantitative trait loci that regulate Arabidopsis root system size and plasticity. Genet. 2006; 172: 485–98.

14. Ye H, Song L, Chen H, Valliyodan B, Cheng P, Ali L. et al. A major natural genetic variation associated with root system architecture and plasticity improves waterlogging tolerance and yield in soybean. Plant Cell Environ. 2018; 41: 2169–82.

15. Atkinson JA, Pound MP, Bennett MJ, Wells DM. Uncovering the hidden half of plants using new advances in root phenotyping. Curr Opin Biotechnol. 2019; 55: 1–8.

16. Heeraman DA, Hopmans JW, Clausnitzer V. Three dimensional imaging of plant roots in situ with X-ray Computed Tomography. Plant Soil. 1997; 189: 167–179.

17. Paya AM, Silverberg JL, Padgett J, Bauerle TL. X-ray computed tomography uncovers root-root interactions: quantifying spatial relationships between interacting root systems in three dimensions. Front Plant Sci. 2015; 6: 274.

18. Helliwell JR, Sturrock CJ, Mairhofer S, Craigon J, Ashton RW, Miller AJ, et al. The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. Sci Rep, 2017; 7: 14875.

19. Ahmed S, Klassen TN, Keyes S, Daly M, Jones DL, Mavrogordato M, et al. Imaging the interaction of roots and phosphate fertiliser granules using 4D X-
20. **Pfeifer J, Kirchgessner N, Colombi T, Walter A.** Rapid phenotyping of crop root systems in undisturbed field soils using X-ray computed tomography. Plant Methods. 2015; 11: 41.

21. **Hodge A.** The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 2004; 162: 9–24.

22. **Zappala S, Helliwell JR, Tracy SR, Mairhofer S, Sturrock CJ, Pridmore T, et al.** Effects of X-ray dose on rhizosphere studies using X-ray computed tomography. PLoS One. 2013; 8: e67250.

23. **Helliwell JR, Sturrock CJ, Mairhofer S, Craigon J, Ashton RW, Miller AJ, et al.** The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. Sci Rep. 2017; 7: 14875.

24. **Mairhofer S, Zappala S, Tracy SR, Sturrock C, Bennett M, Mooney SJ, et al.** RooTrak: automated recovery of three-dimensional plant root architecture in soil from x-ray microcomputed tomography images using visual tracking. Plant Physiol. 2012; 158: 561–9.

25. **Mairhofer S, Sturrock CJ, Bennett MJ, Mooney SJ, Pridmore TP.** Extracting multiple interacting root systems using X-ray microcomputed tomography. Plant J. 2015; 84: 1034–43.

26. **Perret J, Al-Belushi M, Deadman M.** Non-destructive visualization and quantification of roots using computed tomography. Soil Biol Biochem. 2007; 39: 391–9.

27. **Douarre C, Schielein R, Frindel C, Gerth S, Rousseau D.** Transfer learning from synthetic data applied to soil-root segmentation in x-ray tomography images. J Imaging. 2018; 4: 65.
28. Gao W, Schlüter S, Blasé SRGA, Shen J, Vetterlein D. A shape-based method for automatic and rapid segmentation of roots in soil from X-ray computed tomography images: Rootine. Plant Soil. 2019; 441: 643–55.

29. Sato Y, Nakajima S, Shiraga N, Atsumi H, Yoshida S, Koller T, et al. Three-dimensional multi-scale line filter for segmentation and visualization of curvilinear structures in medical images. Med Image Anal. 1998; 2: 143–68.

30. Khush G. Productivity improvements in rice. Nutrition reviews. 2003; 61: S114–S116.

31. Uga Y, Ebana K, Abe J, Morita S, Okuno K, Yano M. Variation in root morphology and anatomy among accessions of cultivated rice (Oryza sativa) with different genetic backgrounds. Breed Sci. 2009; 59: 87–93.

32. Yoshida S, Douglas AF, James HC, Gomez KA. Laboratory Manual for Physiological Studies of Rice. 3rd ed. Manila: International Rice Research Institute; 1976.

33. Johnson EL (1936) Susceptibility of seventy species of flowering plants to X-radiation. Plant physiology 11(2): 319.

34. Kato Y, Abe J, Kamoshita A, Yamagishi J. Genotypic variation in root growth angle in rice (Oryza sativa) and its association with deep root development in upland fields with different water regimes. Plant Soil. 2006; 287: 117–129.

35. Uga Y, Ebana K, Abe J, Morita S, Okuno K, Yano M. Variation in root morphology and anatomy among accessions of cultivated rice (Oryza sativa) with different genetic backgrounds. Breed. Sci. 2009; 59: 87–93.

36. Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, et al. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet. 2013; 45: 1097–102.
37. Lobet G, Pagès L, Draye X. A novel image-analysis toolbox enabling quantitative analysis of root system architecture. Plant Physiol. 2011; 157: 29-39.

38. Kirk GJ, Boghi A, Affholder MC, Keyes SD, Heppell J, Roose T. Soil carbon dioxide venting through rice roots. Plant Cell Environ. 2019; doi: 10.1111/pce.13638.

39. Fang H, Rong H, Hallett PD, Mooney SJ, Zhang W, Zhou H, et al. Impact of soil puddling intensity on the root system architecture of rice (Oryza sativa) seedlings. Soil Tillage Res. 2019; 193: 1-7.

40. Wang L, Guo M, Li Y, Ruan W, Mo X, Wu Z, et al. LARGE ROOT ANGLE1, encoding OsPIN2, is involved in root system architecture in rice. J Exp Bot. 2018; 69: 385-97.

41. Giri J, Bhosale R, Huang G, Pandey BK, Parker H, Zappala S, et al. Rice auxin influx carrier OsAUX1 facilitates root hair elongation in response to low external phosphate. Nat Commun. 2018; 9: 1408.

42. Huang G, Liang W, Sturrock CJ, Pandey BK, Giri J, Mairhofer S, et al. Rice actin binding protein RMD controls crown root angle in response to external phosphate. Nat Commun. 2018; 9: 2346.

43. Rogers ED, Monaenkova D, Mijar M, Nori A, Goldman DI, Benfey PN. X-ray computed tomography reveals the response of root system architecture to soil texture. Plant Physiol. 2016; 171: 2028-40.

44. Zappala S, Mairhofer S, Tracy S, Sturrock CJ, Bennett M, Pridmore T, et al. Quantifying the effect of soil moisture content on segmenting root system architecture in X-ray computed tomography images. Plant Soil 2013; 370: 35-45.
45. Yamauchi T, Abe F, Tsutsumi N, Nakazono M. Root cortex provides a venue for
gas-space formation and is essential for plant adaptation to waterlogging. Front Plant Sci. 2019; 10: 259.

46. Henry A, Cal AJ, Batoto TC, Torres RO, Serraj R. Root attributes affecting water uptake of rice (Oryza sativa) under drought. J Exp Bot. 2012; 63: 4751-63.

47. Adams C, Jacobson A, Bugbee B. Ceramic aggregate sorption and desorption chemistry: implications for use as a component of soilless media. J Plant Nutr. 2014; 37: 1345–57.

48. Piñeros MA, Larson BG, Shaff JE, Schneider DJ, Falcão AX, Yuan L, et al. Evolving technologies for growing, imaging and analyzing 3D root system architecture of crop plants. J Integr Plant Biol. 2015; 58: 230-41.

49. Van Der Walt S, Colbert SC, Varoquaux G. The NumPy array: a structure for efficient numerical computation. Comput Sci Eng. 2011; 13: 22.

50. Jones E, Oliphant T, Peterson P. SciPy: Open source scientific tools for Python. 2001; https://www.scipy.org.

51. Bradski G, Kaehler A. Learning OpenCV: Computer vision with the OpenCV library. O'Reilly Media Inc, USA. 2008.

52. Van der Walt S, Schönberger, JL, Nunez-Iglesias J, Boulogne, F, Warner JD, Yager N, et al. scikit-image: image processing in Python. PeerJ, 2014; 2: e453.

53. Palach J. Parallel Programming with Python. Packt Publishing Ltd, England. 2014.

54. Hunter JD. Matplotlib: A 2D graphics environment. Comput Sci Eng. 2007; 9: 90.

Tables
Table 1. Image-processing time involved in this study.

| Central processing unit                  | Process count | Memory size | Processing time |
|------------------------------------------|---------------|-------------|-----------------|
| Intel® Core™ i5-6500 CPU @ 3.20 GHz      | 4             | 16.0 GB     | 8 min           |
| Intel® Xeon® E3-1270 v5 @ 3.60 GHz       | 8             | 32.0 GB     | 6 min           |
| Intel® Xeon® E5-2650 v4 @ 2.20 GHz       | 48            | 192.0 GB    | 2 min           |

The processing time included the time from loading the raw files to saving the processed images. Image processing was performed using python 3.7. All the CPUs were used with the multiprocessing module.

Table 2. Information of literatures using X-ray CT for rice root visualization in the past decade.

| Detectable root | Detectable root diameter | Detectable root size | Other parameters | Scan time | Filter | Dose | Soil type | Reference |
|-----------------|--------------------------|----------------------|------------------|-----------|--------|------|-----------|-----------|
| NA              | 100 (80)*1              | mm                   | mm               | NA        | 282    | NA   | paddy soil, sieved | [38]      |
| NA              | 80                      | mm                   | mm               | NA        | NA     | NA   | paddy soil, sieved | [39]      |
| NA              | 80                      | mm                   | mm               | NA        | 150    | NA   | sandy clay loam soil, sieved | [40]      |
| NA              | 55                      | mm                   | mm               | NA        | 1.5    | NA   | clay loam, sieved | [41]      |
| NA              | 72                      | mm                   | mm               | NA        | NA     | NA   | play sand, sieved, or mixture of peat-based substrate and calcined clay | [43]      |
| NA              | 72                      | mm                   | mm               | NA        | NA     | NA   | play sand, sieved, or mixture of peat-based substrate and calcined clay | [44]      |
*1) Scanning area was narrowed down to the diameter indicated in the parentheses by cropping the images.

*2) Source-rotation axis distance.

*3) There was a contradiction that the source-rotation axis distance was longer than the source-detector distance.

*4) The value was calculated with the exposure time and the projection and averaging number.

*5) Profile® Greens Grade™, PROFILE Products, USA.

*6) Fafard #52 soil mix, Conrad Fafard Inc, USA.

*7) Turface®, PROFILE Products, USA.

Figures

Figure 1

Representative X-ray CT images of the pot with various soils and a rice plant. (a)
Figure 2

Influence of tube voltage and current on the X-ray CT images. Scaled-up vertical:

| Tube Voltage | Tube Current |
|--------------|--------------|
| 100 μA       | 200 μA       | 300 μA | 400 μA | 500 μA |
| 125 kV       | 3.1          | 3.6    | 4.3    | 4.6    | 4.6    |
| 150 kV       | 3.1          | 3.9    | 4.2    | 4.9    | 5.6    |
| 175 kV       | 3.7          | 3.8    | 5.1    | 4.6    | 5.0    |
| 200 kV       | 4.0          | 4.4    | 5.4    | 5.8    | 5.6    |
| 225 kV       | 4.9          | 4.9    | 4.9    | 5.7    | 6.2    |
Figure 3

Image processing of X-ray CT images. (a) Influence of 3-D median filter with the kernel size.
Figure 4

Various conditions for rapid CT scanning. Kinandang Patong was scanned with X-r
Influence of Cu filters on the X-ray CT images. The pot with Kinandang Patong was scanned using X-ray CT images, at four weeks after sowing. Scaled-up vertical slices of unprocessed and processed CT images are shown.

Influence of X-ray dose on plant growth. Kinandang Patong was cultivated for two
Verification of the detection limit of roots using the wired basket method. Kinando:

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to
download.

Additional file 7.wmv
Additional file 3.wmv
Additional file 4.wmv
Additional file 6.pptx
Additional file 2.pptx
Additional file 5.py
Additional_file_1.docx