

Root shape phenotyping using three-dimensional root system vector data in rice

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Abstract

Purpose

Alternation of root distribution in the soil is a method for improving root system architecture (RSA) in crops. If the root is straight, root distribution has been altered by regulating the angle of root growth in the vertical direction. However, the root shape should be curved and winding. This study aimed to define parameters reflecting the actual root shape.

Methods

We used three-dimensional vector data of rice (*Oryza sativa* L.) RSA derived from an X-ray computed tomography image to compile two sets of two-dimensional vector data for horizontal and vertical components. In the vertical component, we defined the dropping angle θ_d , which is calculated assuming that the rice roots are bent upward. In the horizontal component, we defined the polar angle θ_p , which is the direction in which the roots grow when viewing the plant from above, and the winding degree $\log \sigma_w$, which is calculated by assuming that root elongation is in a random walk.

Results

Assuming that θ_p is distributed uniformly in all varieties, there should be no varietal differences in this angle. We measured θ_d and $\log \sigma_w$ of three rice varieties with different root distributions: shallow, deep, and intermediate. We found significant varietal difference in θ_d and $\log \sigma_w$.

Conclusions

We have shown that θ_d and $\log \sigma_w$ are useful parameters for comparing RSA in rice varieties. We have named this methodology RSAParam3D, and it is freely available to researchers.

37

38 **Keywords**

39 root system architecture model, radicle, seminal roots, crown roots, nodal roots

40

41 **Declarations**

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44

45 *Competing interests*

46 The authors declare that they have no conflicts of interest.

47

48 *Availability of data and materials*

49 The plant materials and raw data of the figures used in this study are available from the corresponding
50 author on reasonable request.

51

52 *Authors' contributions*

53 Shota Teramoto designed the study, acquired the data, constructed and evaluated the code for calculating
54 RSA parameters, and wrote the manuscript. Yusaku Uga coordinated the project and revised the
55 manuscript.

56

57 *Code availability*

58 Available from the GitHub repository (<https://github.com/st707311g/RSAParam3D.git>) or the project
59 homepage (<https://rootomics.dna.affrc.go.jp/en/>).

60

61 *Ethics approval*

62 Not applicable.

63

64 *Consent to participate*

65 Not applicable.

66

67 *Consent for publication*

68 Not applicable.

69

70

71 **Background**

72 Root system architecture (RSA) refers to the spatial distribution of various types of roots in the soil
 73 (Lynch 1995). In monocots, RSA is the fibrous root system comprising a radicle, crown roots, and lateral
 74 roots (Steffens and Rasmussen 2016). For example, in rice (*Oryza sativa* L.), the radicle is the embryonic
 75 root that first emerges from the seed (Itoh et al. 2005); it has many lateral roots and plays important roles
 76 in the plant taking root and growing during the juvenile stage. Crown roots are nodal roots that have many
 77 lateral roots and constitute the main skeleton of a fibrous root system (Itoh et al. 2005). RSA determines
 78 the availability of nutrients and water distributed heterogeneously in the soil (Lynch 1995; de Dorlodot et
 79 al. 2007). Therefore, RSA is an important crop characteristic that greatly influences productivity (de
 80 Dorlodot et al. 2007; Thorup-Kristensen et al. 2020). RSA modification is considered to be a useful
 81 approach to facilitate crop improvement (Uga et al. 2015a; Uga 2021). However, carrying out RSA
 82 improvement is difficult and laborious because it requires considerable effort to dig up roots from the soil
 83 (Böhm 1979). Moreover, digging up roots from the soil often causes loss of three-dimensional (3D)
 84 information such as root distribution in the soil (Trachsel et al. 2011).

85 The RSA skeleton is determined by four main parameters: growth, branching, surface area, and
 86 angle (Morris et al. 2017). In monocots, these four parameters are determined by root number, root
 87 growth angle (RGA), and root density. Root number determines growth and surface area, RGA
 88 determines angle, and root density determines growth, branching, and surface area. Generally, root
 89 number indicates the number of nodal roots (Obara et al. 2014; Zhang et al. 2018), RGA indicates an
 90 angle of vertical nodal root elongation when the horizontal is 0° (Oyanagi et al. 1993), and root density
 91 indicates total root length including lateral roots per unit area (Yoshida and Hasegawa 1982). Of these,
 92 RGA alteration is employed widely for RSA improvement (Uga et al. 2013; Kitomi et al. 2020) because
 93 RGA exerts a considerable effect on root distribution in the soil (Abe and Morita 1994) and is easy to
 94 measure with appropriate equipment (Oyanagi et al. 1993), such as a mesh basket. This procedure entails,
 95 planting the plants in soil-filled baskets and measuring RGA by connecting the planting point and the
 96 point where the roots protrude from the mesh with a straight line (Oyanagi et al. 1993; Kato et al. 2006).
 97 However, the actual shape of the roots should be curved and winding, not straight (Morris et al. 2017).
 98 There are few examples of such architecture being measured because simple methods for measuring 3D
 99 shapes are lacking. Accurate quantification of the root shape enables a more detailed evaluation of various
 100 characteristics.

101 In recent years, information on the 3D deployment of roots in the soil has become easy to
 102 obtain using X-ray computed tomography (CT), magnetic resonance imaging, and other techniques
 103 (Atkinson et al. 2019). For phenotyping, root density (van Dusschoten et al. 2016) or root length (Gao et
 104 al. 2019b, a) in each soil layer is generally calculated by isolating root segments from 3D images because
 105 root segmentation is relatively easy to carry out. However, segmentation cannot represent the root shape.
 106 Another phenotyping method with a 3D volume image is root vectorization, which represents root shape

as a vector; any position on a root has corresponding sets of coordinates (Lobet et al. 2011; Teramoto et al. 2021). We assumed that we could use 3D vector data to measure not only RGA calculated based on the roots being straight (Teramoto et al. 2021) but also root shape, whether straight, curved, or winding. This study demonstrates comparison of the differences in RSA by extracting parameters that represent 3D shapes from 3D vector data of rice RSA.

Materials and methods

Plant materials

In this study, we used three rice varieties: IR64 (IRGC #66970), Kinandang Patong (KP, IRGC #23364), and Dro1-NIL (Uga et al. 2013; Teramoto et al. 2019). KP has deep RSA, whereas IR64 has shallow RSA. Dro1-NIL is a near-isogenic line of IR64 with a KP-type *DRO1* allele, and it has intermediate RSA.

Growth conditions

We adopted the growing conditions used by Teramoto et al. (2020). We sowed rice plants in pots measuring 25-cm depth and 20-cm diameter (TSP2530P, Tecs, Itako, Ibaraki, Japan) filled with a soil-like root growth substrate (Profile® Greens Grade™, PROFILE Products, Buffalo, Illinois, USA) and cultivated the plants in a growth chamber for 4 weeks. Before sowing, we saturated the substrate with a hydroponic solution and supplied deionized water from the bottom of the pots during cultivation. The hydroponic solution consisted of 1.23 mM of NO_3^- , 0.41 mM of NH_4^+ , 0.18 mM of H_2PO_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+} . We adjusted the pH to 5.5, using HCl and KOH, as required. The light conditions we used simulated 14 h of daylight. Details of other conditions have been described in Teramoto et al. (2020). To buffer the positional effect, we adopted a block design. Six blocks were used, with each block having three pots: IR64, Dro1-NIL, and KP.

X-ray computed tomography scanning

We adopted the X-ray CT scanning and root segmentation conditions used in Teramoto et al. (2020). We imaged rice roots in the soil using the X-ray CT system inspeXio SMX-225CT FPD HR (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan). Each scan was obtained digitally; 1,200 projections, using a signal averaging two frames over 360° without binning (pixel detector resolution: 3,000 × 3,000), at 4.0 fps. We computed 860 horizontal, 1,024 × 1,024 pixel-resolution slices. The final spatial resolution was 300 μm , corresponding to a total volume of 30.72 × 30.72 × 25.8 cm^3 . The tube voltage was 225 kV, and the tube current was 500 μA . Source-detector and -rotation axis distances were 1,200 and 900 mm, respectively. To harden the X-ray beam, we used a 1.0-mm Cu filter. Beam hardening was corrected approximately using the operating software, with a correction table calculated using the correct metal material.

143

144 *Root segmentation and vectorization*

145 We imaged root segments in X-ray CT images using RSAvis3D (Teramoto et al. 2020). The parameters of
146 RSAvis3D were as follows: median filter size was 7, edge detection filter size was 21, and cylinder radius
147 was 9 cm. We vectorized segmented volume data using the “COG tracking” method in RSAtace3D
148 (Teramoto et al. 2021). We saved vectorized data in a JavaScript Object Notation (JSON) format.

149

150 *Parameter estimation for vertical root system architecture phenotype*

151 Given that the i -th coordinate of a vector is $c_i(x_i, y_i, z_i)$, where x_i , y_i , z_i are Euclidean coordinates for
152 the x , y , and z axes, respectively, and that n is the number of coordinates in the vector, the vector is
153 represented as Eq. (1).

$$vect = [c_1(x_1, y_1, z_1), c_2(x_2, y_2, z_2), \dots, c_n(x_n, y_n, z_n)] \#(1)$$

154 Based on Eq. (1), the i -th vertical local angle θ_{vi} is defined as Eq. (2).

$$\theta_{vi} = \tan^{-1} \left\{ \frac{|z_{i+1} - z_i|}{\sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2}} \right\} \#(2)$$

155 Given that the dropping angle is θ_d and the decay rate is α (0.005), the modeled local angle θ_{vsi} is
156 represented as Eq. (3).

$$\theta_{vsi} = \theta_d \times (1 - \alpha)^{i-1} \#(3)$$

157 Based on Eqs. (2) and (3), θ_d is determined to minimize Eq. (4) using Python3
158 (<https://www.python.org/>) with the “least_sequence” function in the “scipy.optimize” module (Virtanen et
159 al. 2020).

$$\sum_{i=1}^{n-1} \left(\sum_{k=1}^i \sin \theta_{vk} - \sum_{k=1}^i \sin \theta_{vsk} \right)^2 \#(4)$$

160

161 *Parameter estimation for horizontal root system architecture phenotype*

162 Based on Eq. (1), the i -th horizontal local angle θ_{hi} ($0^\circ \leq \theta_{hi} \leq 360^\circ$) is defined as Eqs. (5) and (6).

$$\theta_{hi} = \tan^{-1} \left\{ \frac{y_{i+1} - y_i}{x_{i+1} - x_i} \right\} (x_i \neq x_{i+1}) \#(5)$$

$$\theta_{hi} = 90^\circ (x_i = x_{i+1}) \#(6)$$

163 Based on Eqs. (5) and (6), $\Delta\theta_{hi}$ ($|\theta_{hi}| \leq 180^\circ$) is defined as Eq. (7).

$$\Delta\theta_{hi} = \theta_{hi+1} - \theta_{hi} \#(7)$$

164 For $\Delta\theta_{hi}$, where i is from 1 to $n-1$, we calculated winding degree σ_w using Python3 with the
165 “norm.fit” function in the “scipy.stats” module (Virtanen et al. 2020).

166

167 *Statistical analysis*

168 We carried out Student's t-tests and Tukey–Kramer tests using R software (<https://www.r-project.org/>) and

169 the “multcomp” package (Bretz et al. 2016). A P value ≤ 0.05 was considered significant.

170

171 **Results**

172 The overall diagram of the phenotyping method with vectorized RSA data is shown in Fig. 1. Vectorized
173 3D RSA comprised the vectorized radicle and crown roots (Fig. 1a). We defined the direction of root
174 elongation as the longitudinal axis and the axes horizontally and vertically perpendicular to the
175 longitudinal axis as the horizontal and vertical axes, respectively (Fig. 1b). To simplify the model, we
176 decomposed the 3D data into two sets of 2D data (Figs. 1c and d). To define the vertical movement of
177 roots, we offset the movement along the horizontal axis (Fig. 1c). One end of each root was gathered at
178 the center of the image (Fig. 1c). We constructed a vertical 2D root model using two parameters: the
179 direction of root growth from the center (similar to the concept of RGA) and bending degree of the roots.
180 To define the horizontal movement of roots, we offset the movement along the vertical axis (Fig. 1d). The
181 root is represented by a single curve connecting the center and end of the root (Fig. 1d). We constructed a
182 horizontal 2D root model using two parameters: the direction of root growth from the center (polar angle)
183 and winding degree of the left–right wobble of the root shape. Finally, we calculated frequency
184 distributions representing the RSA phenotype (Figs. 1c and d).

185

186 *Parameters for vertical root shape*

187 We used vectorized RSA data of an upland rice variety, KP (Teramoto et al. 2020, 2021). Each root is
188 represented by an unbranched vector with the distance between nodes being approximately four voxels.
189 To parameterize the vertical root shape, we converted the 3D RSA vector to a 2D vector by offsetting the
190 horizontal axis (Fig. 2a). Most roots extended downward to the right, with a slight upward bend (Fig. 2a).
191 We defined an angle against the ground surface of each node as the local angle and assumed the root
192 shape to be represented by the initial local angle and decay rate α , whereby the local angle decays by α
193 over nodes to a small value close to zero (Fig. 2a). We converted the vector data to node index–depth data
194 (Fig. 2b left) and then estimated the initial local angles by fitting with mean square error as a loss function,
195 with 0.005 of α (Fig. 2b right). We defined the fitted initial local angle as a vertical root shape parameter,
196 the dropping angle θ_d .

197

198 *Parameters for horizontal root shape*

199 To parameterize the horizontal root shape, we converted the 3D RSA vector to a 2D vector by offsetting
200 the vertical axis (Fig. 3a). We defined an angle on the x–y plane of each node as the local angle (Fig. 3a)
201 and the initial local angle as the polar angle θ_p . We calculated the normalized local angle by offsetting
202 the θ_p (Fig. 2b) from the local angles of all nodes. The curve in Fig. 3b took the form of a random walk,
203 likely because the roots elongate by random bumps into soil particles. As the random walk is represented
204 by the accumulation of random numbers, we assumed that the standard deviation of a random number

205 should be a parameter representing the horizontal root shape. We calculated the change in the normalized
206 local angle and its frequency distribution (Figs. 3c and d). We obtained a parameter σ_w as standard
207 deviation of the normal distribution (Fig. 3d). The parameters obtained— θ_p and σ_w —represent the
208 horizontal root shape.

209

210 *Root system architecture phenotyping based on the frequency distribution of root parameters*

211 To ascertain whether the obtained parameters can be used to phenotype root shape, we parameterized
212 three RSA types: IR64, Dro1-NIL, and KP. In IR64, the roots are concentrated close to the surface of the
213 ground, producing a shallow-rooting RSA phenotype, whereas KP has a deep-rooting RSA phenotype
214 (Uga et al. 2011). Dro1-NIL is a near-isogenic line of IR64 with a KP-type allele of the *DEEPER*
215 *ROOTING 1 (DRO1)* gene that controls the angle of root growth (Uga et al. 2013). Dro1-NIL has
216 intermediate RSA types of IR64 and KP (Uga et al. 2015b; Teramoto et al. 2019). IR64, Dro1-NIL, and
217 KP are suitable for testing the phenotyping capacity of the model in this study because previous studies
218 have used them as three different representative varieties of RSA (Teramoto et al. 2019; Teramoto and
219 Uga 2020). The vector data we use in this study are illustrated in Fig. 4.

220 In terms of the dropping angles, θ_d , the histogram of KP appears symmetrical, whereas those
221 of IR64 and Dro1-NIL do not appear symmetrical because of the large numbers of θ_d of approximately
222 0° (Figs. 5a–c). As the roots of IR64 and Dro1-NIL are close to the surface of the ground, they are likely
223 to have more θ_d of approximately 0° . In each of the three RSA types, the θ_d of the radicle was
224 apparently higher than was the θ_d of the crown roots. In terms of winding degrees, σ_w , the σ_w of the
225 radicle was also apparently higher than was the σ_w of the crown roots in the three RSA types (Figs.
226 5d–f). The distributions were long-tailed in shape (Figs. 5d–f), indicating that they were close to
227 log-normal distributions. We log-transformed the σ_w and adjusted the long tails to be close to
228 symmetrical (Figs. 5g–i).

229 We calculated the horizontal and vertical RSA parameters—mean of θ_d ($\overline{\theta_d}$) and mean of
230 $\log \sigma_w$ ($\overline{\log \sigma_w}$)—of IR64, Dro1-NIL, and KP (Fig. 6). The $\overline{\theta_d}$ and $\overline{\log \sigma_w}$ of the radicles were
231 significantly higher than those of the crown roots (Figs. 6a and b), indicating that the radicle elongates
232 more deeply and has greater horizontal movement. In both vertical (Fig. 6a) and horizontal parameters
233 (Fig. 6b), $\overline{\theta_d}$ and $\overline{\log \sigma_w}$ of IR64 in both crown roots and radicles were significantly lower than those of
234 KP (Fig. 6a, b). Dro1-NIL was the intermediate type and $\overline{\theta_d}$ and $\overline{\log \sigma_w}$ of IR64 were significantly lower
235 than those of Dro1-NIL in both radicles and crown roots (Figs. 6a and b), meaning that the *DRO1* gene
236 may not only affect the angle of growth of the vertical root but also horizontal movement. However, we
237 found a correlation between $\overline{\theta_d}$ and $\overline{\log \sigma_w}$, suggesting that the deeper the root, the greater the
238 horizontal movement (Fig. 6c). Further experiments are required to ascertain the relationship between $\overline{\theta_d}$
239 and $\overline{\log \sigma_w}$. Using RSA parameters of each variety, we simulated the RSA of each (Fig. 7). The
240 simulation yielded shallow, deep, and intermediate RSAs, indicating that the measured parameters reflect

241 the varietal characteristics.

242

243 *Root growth angle is influenced by the horizontal parameter*

244 RGA is affected by a range of factors including soil compaction (Correa et al. 2019), temperature (Nagel
245 et al. 2009), and root diameter (Materechera et al. 1992). In this study, we examined the effect of the
246 horizontal parameter $\log \sigma_w$ and distance in RGA measurement (distance between the planting site and
247 the point of measurement of RGA [Fig. 8a]), which is considered to have an impact, combined with
248 $\log \sigma_w$, on RGA. Fig. 8b shows the effect of distance in RGA measurement and $\log \sigma_w$ on RGA
249 estimated via simulation, and the raw data are provided in Online Resource 1. We conducted the
250 simulation with θ_d fixed at 60°. Irrespective of $\log \sigma_w$, when distance in RGA measurement is 0, the
251 RGA was simulated as 60°. As the distance in RGA measurement increased, the RGA was simulated
252 smaller, reducing RGA to 40°. This is because the roots are bent upward (Fig. 2). Conversely, when
253 $\log \sigma_w$ was high, the RGA was large. In Fig. 8 the maximum RGA is 69°. This was because undulation of
254 the roots increased the time taken to reach the length to measure RGA. To summarize, the calculation of
255 RGA assuming the roots are straight is affected by both distance in RGA measurement and $\log \sigma_w$, and
256 θ_d , which eliminates both effects, is a more accurate vertical parameter.

257

258 Discussion

259 Three-dimensional RSA phenotyping is one of the key processes in modification of RSA in crops. In this
260 study, we quantified the 3D vectorized RSA of rice by using three parameters—dropping angle θ_d , polar
261 angle θ_p , and logarithmic winding degree $\log \sigma_w$ —to describe the shapes of the radicles and crown roots.
262 Assuming that θ_p is distributed uniformly in all varieties, the varietal differences in RSA are explained
263 by θ_d and $\log \sigma_w$. We defined the means of θ_d ($\overline{\theta_d}$) and $\log \sigma_w$ ($\overline{\log \sigma_w}$) in each individual as
264 representative values and discovered that these two parameters could be used to characterize three rice
265 cultivars with different RSA phenotypes (Fig. 6). These findings suggest that θ_d and $\log \sigma_w$ are useful
266 parameters to compare the RSA of different varieties.

267 RGA is one of the most popular parameters in the vertical RSA phenotype (Oyanagi et al.
268 1993; Kato et al. 2006; Kitomi et al. 2015, 2020; An et al. 2017; Alahmad et al. 2019). The basket method
269 of measuring the RGAs of individual roots is the most informative approach (Oyanagi et al. 1993; Kato et
270 al. 2006). However, the basket method estimates RGA under the assumption that the roots are straight. In
271 this study, we have shown that RGA calculated under this assumption is affected by distance in RGA
272 measurement (distance between the planting site and point of measurement of RGA) and the horizontal
273 parameter $\log \sigma_w$ (Fig. 8). For example, when θ_d is 60° and the distance in RGA measurement is 10 cm,
274 RGA is overestimated by approximately 12% (7.1/60) in $\log \sigma_w$ of 3 and underestimated by 13%
275 (7.8/60) in $\log \sigma_w$ of 2 (Fig. 8b, Online Resource 1). The difference is 25%, and this error will become
276 problematic when making exact measurements of roots with various values of $\log \sigma_w$. In three rice

277 varieties (IR64, Dro1-NIL, and KP), the $\log \sigma_w$ values of radicles and crown roots were close to 3 and 2,
278 respectively (Fig. 6b). These findings suggested that RGA calculated under that assumption that the roots
279 are straight would be overestimated in radicles and underestimated in crown roots.

280 To our knowledge, few studies have calculated the horizontal RSA phenotype while focusing
281 on the shapes of individual roots. We assume that this is because most root phenotypings have been
282 performed in 2D (Atkinson et al. 2019; Jia et al. 2019; Wang et al. 2019; Martins et al. 2020) and even if
283 it has been conducted in 3D, root distribution parameters such as root length density and horizontal
284 percentage area coverage have been focused on, whereas individual root shapes have not (Chen et al.
285 2019; Gao et al. 2019a, b; Teramoto and Uga 2020). In this study, we calculated $\log \sigma_w$ as a horizontal
286 parameter and showed that three rice varieties displayed varietal differences of $\log \sigma_w$. These findings
287 also suggest that $\log \sigma_w$ may be genetically alterable. However, we found correlation between θ_d and
288 $\log \sigma_w$ in the three rice varieties (Fig. 6c), indicating simple links of vertical and horizontal phenotypes.
289 Further study is required to ascertain whether θ_d and $\log \sigma_w$ are independent.

290 We have considered that our methodology will be used in studying the biological process of
291 root development. In this study, we found that θ_d and $\log \sigma_w$ differed between radicles and crown roots
292 (Figs. 6a and b), suggesting that radicles and crown roots may have different mechanisms of
293 physiological development. Many studies have carried out root physiological experiments, such as
294 gravitropism tests and stress response tests, on radicles (Uga et al. 2013; Kitomi et al. 2015; Sun et al.
295 2018). This is because the radicle is the first root to develop and it is easy to control both the angle and
296 elongation of developing roots. Our methodology enables the physiological differences between seed and
297 crown roots to be quantified, and this is expected to bring new insights.

298 The methodology of this study has three limitations: (1) it is not designed for roots that grow
299 upward. We observed few roots extending from the lower soil layer to the upper soil layer. We considered
300 that such roots may appear if the variety, plant species, or pot size are changed. If the conditions change,
301 the equation should be reexamined. (2) It cannot accommodate roots that change their shape during
302 cultivation; for example, when a plant is grown under normal conditions during the first half of its growth
303 and then moved to grow under stress conditions. In these cases, the equation should be changed by
304 introducing a variable representing time (Pagès et al. 2004). (3) As our model ignores lateral roots, it
305 cannot be used to either evaluate or simulate whole root density in the soil. In other words, a simulation
306 study of water and nutrient uptake (Postma et al. 2017; Daly et al. 2018; Takahashi and Pradal 2021)
307 could not be conducted. Conversely, root distribution in the soil could be simulated, as shown in the case
308 of RSA breeding by modifying the shapes of radicles and crown roots (Uga et al. 2013; Kitomi et al.
309 2020).

310 We named this script for calculating θ_d , θ_p , and $\log \sigma_w$ “RSAParam3D”. RSAParam3D are
311 available from the GitHub repository (<https://github.com/st707311g/RSAParam3D.git>). It was developed
312 as an extension module of RSATrace3D (Teramoto et al. 2021). Thus, it is very easy to calculate

parameters; RSAttrace3D automatically calculates θ_d , θ_p , and $\log \sigma_w$ when the root system is vectorized.

Conclusion

We have demonstrated RSAparam3D, RSA parameterization software that calculates vertical and horizontal RSA phenotypes separately. With root system data vectorized by RSAttrace3D, various parameters can be calculated automatically. As RSAparam3D can be applied not only to phenotypic analysis but also to simulation analysis, it is expected to further promote root research.

References

- Abe J, Morita S (1994) Growth direction of nodal roots in rice: its variation and contribution to root system formation. *Plant Soil* 165:333–337
- Alahmad S, El Hassouni K, Bassi FM, Dinglasan E, Youssef C, Quarry G, Aksoy A, Mazzucotelli E, Juhász A, Able JA, Christopher J, Voss-Fels KP, Hickey LT (2019) A major root architecture QTL responding to water limitation in durum wheat. *Front Plant Sci* 10:436
- An H, Dong H, Wu T, Wang Y, Xu X, Zhang X, Han Z (2017) Root growth angle: an important trait that influences the deep rooting of apple rootstocks. *Sci Hortic (Amsterdam)* 216:256–263
- Atkinson JA, Pound MP, Bennett MJ, Wells DM (2019) Uncovering the hidden half of plants using new advances in root phenotyping. *Curr Opin Biotechnol* 55:1–8
- Böhm W (1979) *Methods of studying root systems*. Springer, Berlin
- Bretz F, Hothorn T, Westfall P (2016) *Multiple comparisons using R*. CRC Press, New York
- Chen X, Ding Q, He R, Li H (2019) A protocol of field-based phenotyping procedure for no-till wheat root system architecture based on data-driven model-assist. *Artif Intell Agric* 3:33–41
- Correa J, Postma JA, Watt M, Wojciechowski T (2019) Soil compaction and the architectural plasticity of root systems. *J Exp Bot* 70:6019–6034
- Daly KR, Tracy SR, Crout NMJ, Mairhofer S, Pridmore TP, Mooney SJ, Roose T (2018) Quantification of root water uptake in soil using X-ray computed tomography and image-based modelling. *Plant Cell Environ* 41:121–133
- de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends Plant Sci* 12:474–481
- Gao W, Blaser SRGA, Schlüter S, Shen J, Vetterlein D (2019a) Effect of localised phosphorus application on root growth and soil nutrient dynamics in situ – comparison of maize (*Zea mays*) and faba bean (*Vicia faba*) at the seedling stage. *Plant Soil* 441:469–483
- Gao W, Schlüter S, Blaser SRGA, Shen J, Vetterlein D (2019b) A shape-based method for automatic and rapid segmentation of roots in soil from X-ray computed tomography images: Routine. *Plant Soil* 441:643–655

349 Itoh JI, Nonomura KI, Ikeda K, Yamaki S, Inukai Y, Yamagishi H, Kitano H, Nagato Y (2005) Rice plant
350 development: from zygote to spikelet. *Plant Cell Physiol* 46:23–47

351 Jia Z, Liu Y, Gruber BD, Neumann K, Kilian B, Graner A, von Wirén N (2019) Genetic dissection of root
352 system architectural traits in spring barley. *Front Plant Sci* 10:400

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

356 Kitomi Y, Hanzawa E, Kuya N, Inoue H, Hara N, Kawai S, Kanno N, Endo M, Sugimoto K, Yamazaki T,
357 Sakamoto S, Sentoku N, Wu J, Kanno H, Mitsuda N, Toriyama K, Sato T, Uga Y (2020) Root
358 angle modifications by the *DRO1* homolog improve rice yields in saline paddy fields. *Proc Natl*
359 *Acad Sci U S A* 117:21242–21250

360 Kitomi Y, Kanno N, Kawai S, Mizubayashi T, Fukuoka S, Uga Y (2015) QTLs underlying natural
361 variation of root growth angle among rice cultivars with the same functional allele of DEEPER
362 ROOTING 1. *Rice* 8:16

363 Lobet G, Pagès L, Draye X (2011) A novel image-analysis toolbox enabling quantitative analysis of root
364 system architecture. *Plant Physiol* 157:29–39

365 Lynch J (1995) Root architecture and plant productivity. *Plant Physiol* 109:7–13

366 Martins SM, de Brito GG, Gonçalves W da C, Tripode BMD, Lartaud M, Duarte JB, Morello C de L,
367 Giband M (2020) Phenoroots: An inexpensive non-invasive phenotyping system to assess the
368 variability of the root system architecture. *Sci Agric* 77:1–8

369 Materechera SA, Alston AM, Kirby JM, Dexter AR (1992) Influence of root diameter on the penetration
370 of seminal roots into a compacted subsoil. *Plant Soil* 144:297–303

371 Morris EC, Griffiths M, Golebiowska A, Mairhofer S, Burr-Hersey J, Goh T, von Wangenheim D,
372 Atkinson B, Sturrock CJ, Lynch JP, Vissenberg K, Ritz K, Wells DM, Mooney SJ, Bennett MJ
373 (2017) Shaping 3D root system architecture. *Curr Biol* 27:R919–R930

374 Nagel KA, Kastenholz B, Jahnke S, Van Dusschoten D, Aach T, Mühlich M, Truhn D, Scharr H, Terjung
375 S, Walter A, Schurr U (2009) Temperature responses of roots: impact on growth, root system
376 architecture and implications for phenotyping. *Funct Plant Biol* 36:947–959

377 Obara M, Ishimaru T, Abiko T, Fujita D, Kobayashi N, Yanagihara S, Fukuta Y (2014) Identification and
378 characterization of quantitative trait loci for root elongation by using introgression lines with
379 genetic background of *indica*-type rice variety IR64. *Plant Biotechnol Rep* 8:267–277

380 Oyanagi A, Nakamoto T, Wada M (1993) Relationship between root growth angle of seedlings and
381 vertical distribution of roots in the field in wheat cultivars. *Japanese J Crop Sci* 62:565–570

382 Pagès L, Vercambre G, Drouet J-L, Lecompte F, Collet C, Le Bot J (2004) Root Typ: a generic model to
383 depict and analyse the root system architecture. *Plant Soil* 258:103–119

384 Postma JA, Kuppe C, Owen MR, Mellor N, Griffiths M, Bennett MJ, Lynch JP, Watt M (2017)

385 OpenSimRoot: widening the scope and application of root architectural models. *New Phytol*
386 215:1274–1286

387 Steffens B, Rasmussen A (2016) The physiology of adventitious roots. *Plant Physiol* 170:603–617

388 Sun H, Tao J, Bi Y, Hou M, Lou J, Chen X, Zhang X, Luo L, Xie X, Yoneyama K, Zhao Q, Xu G, Zhang
389 Y (2018) OsPIN1b is involved in rice seminal root elongation by regulating root apical meristem
390 activity in response to low nitrogen and phosphate. *Sci Rep* 8:13014

391 Takahashi H, Pradal C (2021) Root phenotyping: important and minimum information required for root
392 modeling in crop plants. *Breed Sci* 71:109–116

393 Teramoto S, Kitomi Y, Nishijima R, Takayasu S, Maruyama N, Uga Y (2019) Backhoe-assisted monolith
394 method for plant root phenotyping under upland conditions. *Breed Sci* 69:508–513

395 Teramoto S, Takayasu S, Kitomi Y, Arai-Sanoh Y, Tanabata T, Uga Y (2020) High-throughput
396 three-dimensional visualization of root system architecture of rice using X-ray computed
397 tomography. *Plant Methods* 16:66

398 Teramoto S, Tanabata T, Uga Y (2021) RSAtrace3D: robust vectorization software for measuring
399 monocot root system architecture. *BMC Plant Biol* 21:398

400 Teramoto S, Uga Y (2020) A deep learning-based phenotypic analysis of rice root distribution from field
401 images. *Plant Phenomics* 2020:3194308

402 Thorup-Kristensen K, Halberg N, Nicolaisen M, Olesen JE, Crews TE, Hinsinger P, Kirkegaard J, Pierret
403 A, Dresbøll DB (2020) Digging deeper for agricultural resources, the value of deep rooting. *Trends*
404 *Plant Sci* 25:406–417

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

407 Uga Y (2021) Challenges to design-oriented breeding of root system architecture adapted to climate
408 change. *Breed Sci* 71:3–12

409 Uga Y, Kitomi Y, Ishikawa S, Yano M (2015a) Genetic improvement for root growth angle to enhance
410 crop production. *Breed Sci* 65:111–119

411 Uga Y, Kitomi Y, Yamamoto E, Kanno N, Kawai S, Mizubayashi T, Fukuoka S (2015b) A QTL for root
412 growth angle on rice chromosome 7 is involved in the genetic pathway of *DEEPER ROOTING 1*.
413 *Rice* 8:8

414 Uga Y, Okuno K, Yano M (2011) *Dro1*, a major QTL involved in deep rooting of rice under upland field
415 conditions. *J Exp Bot* 62:2485–2494

416 Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue
417 H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, Yano M
418 (2013) Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under
419 drought conditions. *Nat Genet* 45:1097–1102

420 van Dusschoten D, Metzner R, Kochs J, Postma JA, Pflugfelder D, Bühler J, Schurr U, Jahnke S (2016)

421 Quantitative 3D analysis of plant roots growing in soil using magnetic resonance imaging. *Plant*
422 *Physiol* 170:1176–1188

423 Virtanen P, Gommers R, Oliphant TE, Haberland M, Reddy T, Cournapeau D, Burovski E, Peterson P,
424 Weckesser W, Bright J, van der Walt SJ, Brett M, Wilson J, Millman KJ, Mayorov N, Nelson ARJ,
425 Jones E, Kern R, Larson E, Carey CJ, Polat İ, Feng Y, Moore EW, VanderPlas J, Laxalde D,
426 Perktold J, Cimrman R, Henriksen I, Quintero EA, Harris CR, Archibald AM, Ribeiro AH,
427 Pedregosa F, van Mulbregt P, SciPy 1.0 Contributors (2020) SciPy 1.0: fundamental algorithms for
428 scientific computing in Python. *Nat Methods* 17:261–272

429 Wang T, Rostamza M, Song Z, Wang L, McNickle G, Iyer-Pascuzzi AS, Qiu Z, Jin J (2019) SegRoot: A
430 high throughput segmentation method for root image analysis. *Comput Electron Agric* 162:845–854

431 Yoshida S, Hasegawa S (1982) The rice root system: its development and function. In: International Rice
432 Research Institute (ed) Drought resistance in crops with emphasis on rice. International Rice
433 Research Institute, Los Baños, pp 97–134

434 Zhang Z, Zhang X, Lin Z, Wang J, Xu M, Lai J, Yu J, Lin Z (2018) The genetic architecture of nodal root
435 number in maize. *Plant J* 93:1032–1044

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Figure legends

Fig. 1 Root system architecture (RSA) phenotyping methodology from vectorized rice RSA. **a** Three-dimensional rendering of vectorized rice RSA. Each root is colored. **b** Definition of the axis for root movement. Three-dimensional rendering of vectorized rice RSA (left) and a two-dimensional line graph (right); **c** horizontal movement offset; and **d** vertical movement offset

Fig. 2 Fitting of vertical movement of vectorized rice root system architecture. **a** Line graph with horizontally offset data. **b** Line graph with data converted into node index and depth (left), and line graph with data generated by the fitted model (right)

Fig. 3 Fitting of horizontal movement of vectorized rice root system architecture. **a** Line graph with vertically offset data. **b** Line graph with data converted into a node index and local angles of nodes normalized so that the polar angle is zero. **c** Line graph with node index and amounts of normalized local angle changes. **d** Histogram with amounts of normalized local angle changes fitted to a normal distribution with mean 0 and variance σ_w

Fig. 4 Vector data of root system architecture in IR64, Dro1-NIL, and Kinandang Patong. The overhead view is illustrated. The color scale represents the root depth when the depth at which the seed was planted is set to zero

Fig. 5 Frequency distribution of root parameters. Six individuals of each variety were bulked. Histograms of radicle and crown roots: **a–c** dropping angles θ_d ; **d–f** winding degrees σ_w ; **g–h** logarithmic winding degrees $\log \sigma_w$; **a, d, g** IR64; **b, e, h** Dro1-NIL; **c, f, i** Kinandang Patong. Six individuals of each variety were used

Fig. 6 Comparison of $\overline{\theta_d}$ and $\overline{\log \sigma_w}$ of IR64, Dro1-NIL, and Kinandang Patong. Six individuals of each variety were used. Bar graphs of radicles and crown roots; **a** mean of θ_d ($\overline{\theta_d}$); **b** mean of $\log \sigma_w$ ($\overline{\log \sigma_w}$). The letters above the bars indicate a significant difference calculated using the Tukey–Kramer test. *: significant difference (Student's t-test). The error bars indicate the standard deviation. The raw data making up the bar graph are indicated by small circles. **c** A scatter plot of $\overline{\theta_d}$ and $\overline{\log \sigma_w}$. The straight line was drawn using a linear regression

Fig. 7 Simulated root system architecture with the measured parameters. Twenty-five roots were simulated

Fig. 8 Influence of distance in RGA measurement and $\overline{\log \sigma_w}$. **a** Schematic diagram of RGA measurement. When measuring the RGA, the roots are assumed to be straight. **b** Simulation analysis of the effect of distance in RGA measurement and $\overline{\log \sigma_w}$ on RGA. θ_d was fixed at 60 degrees. The plots were smoothed by the “bicubic” method

Figures

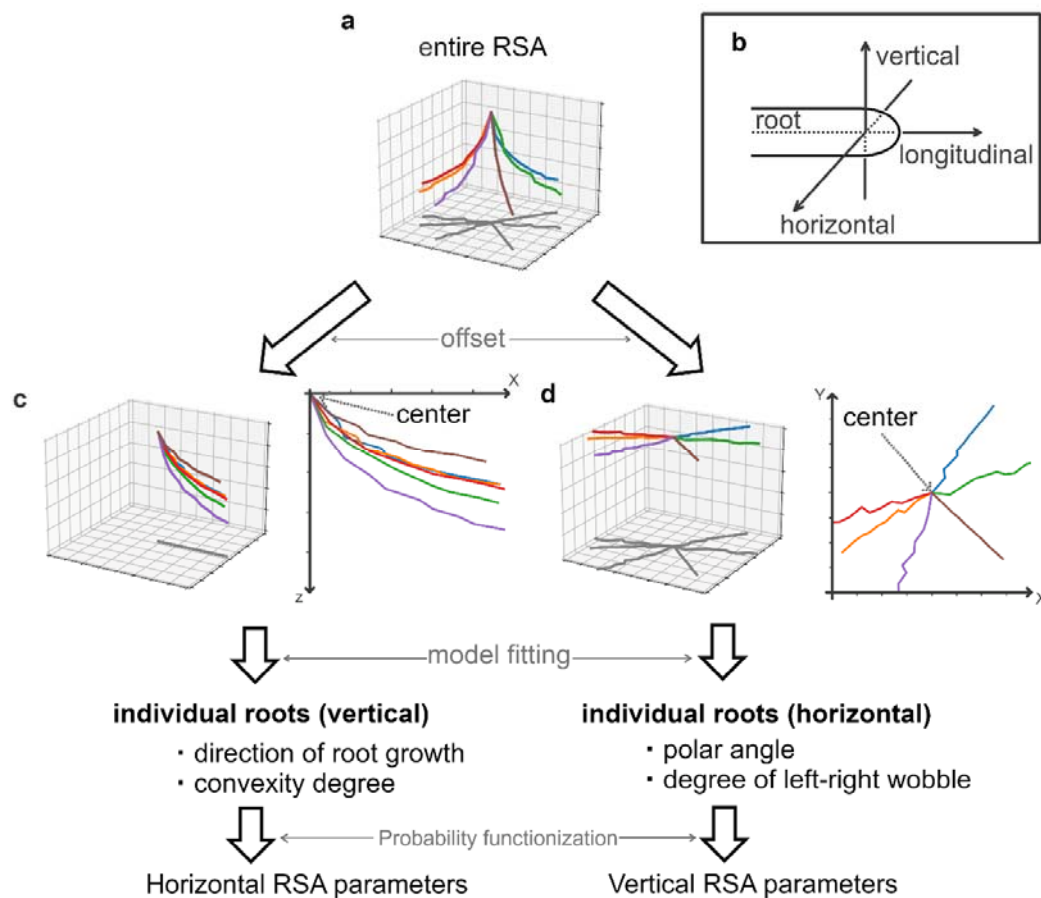


Fig. 1

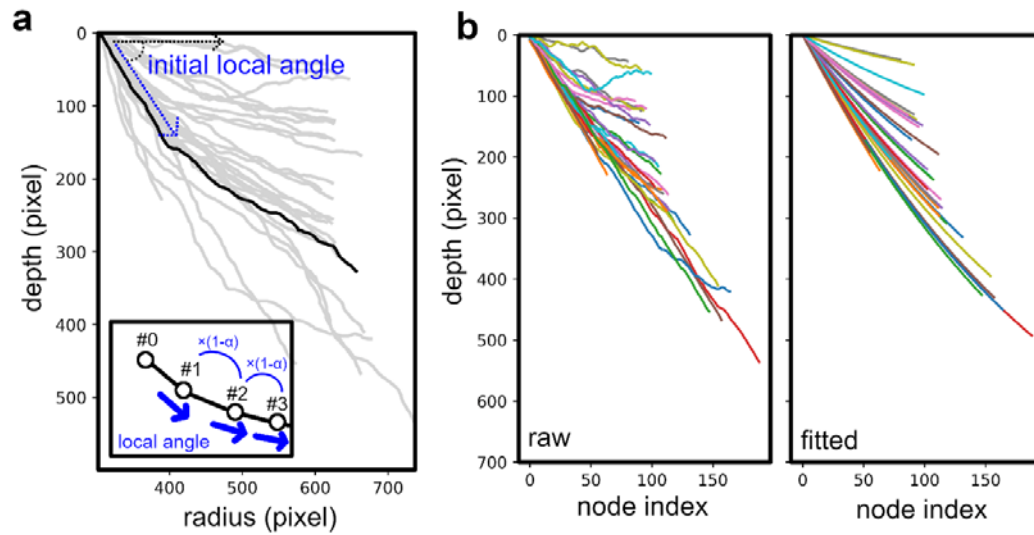
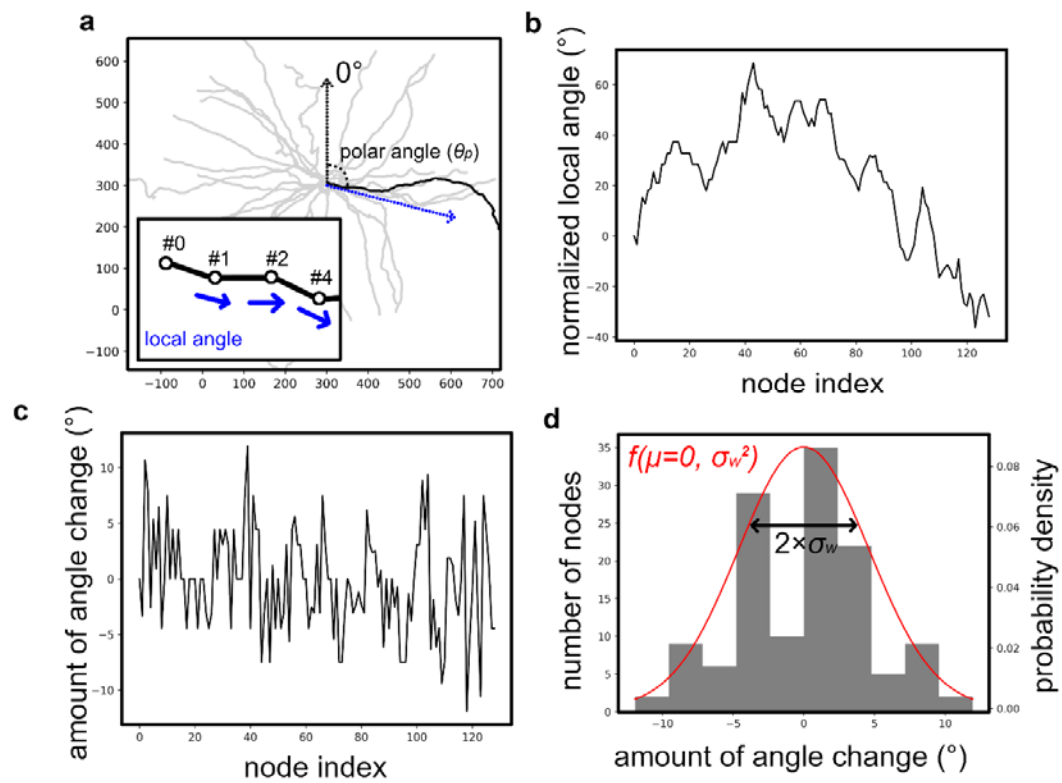


Fig. 2

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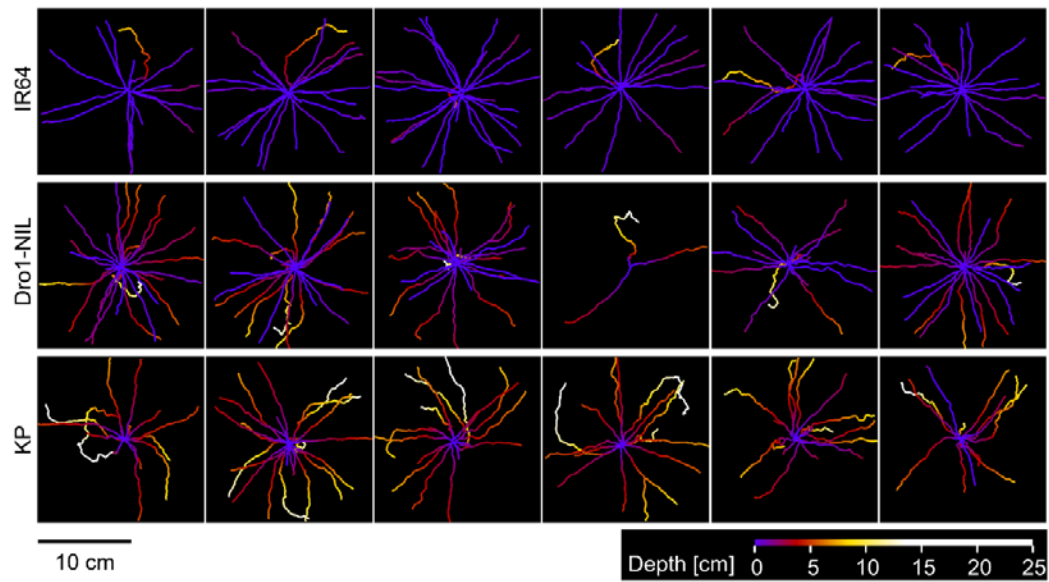
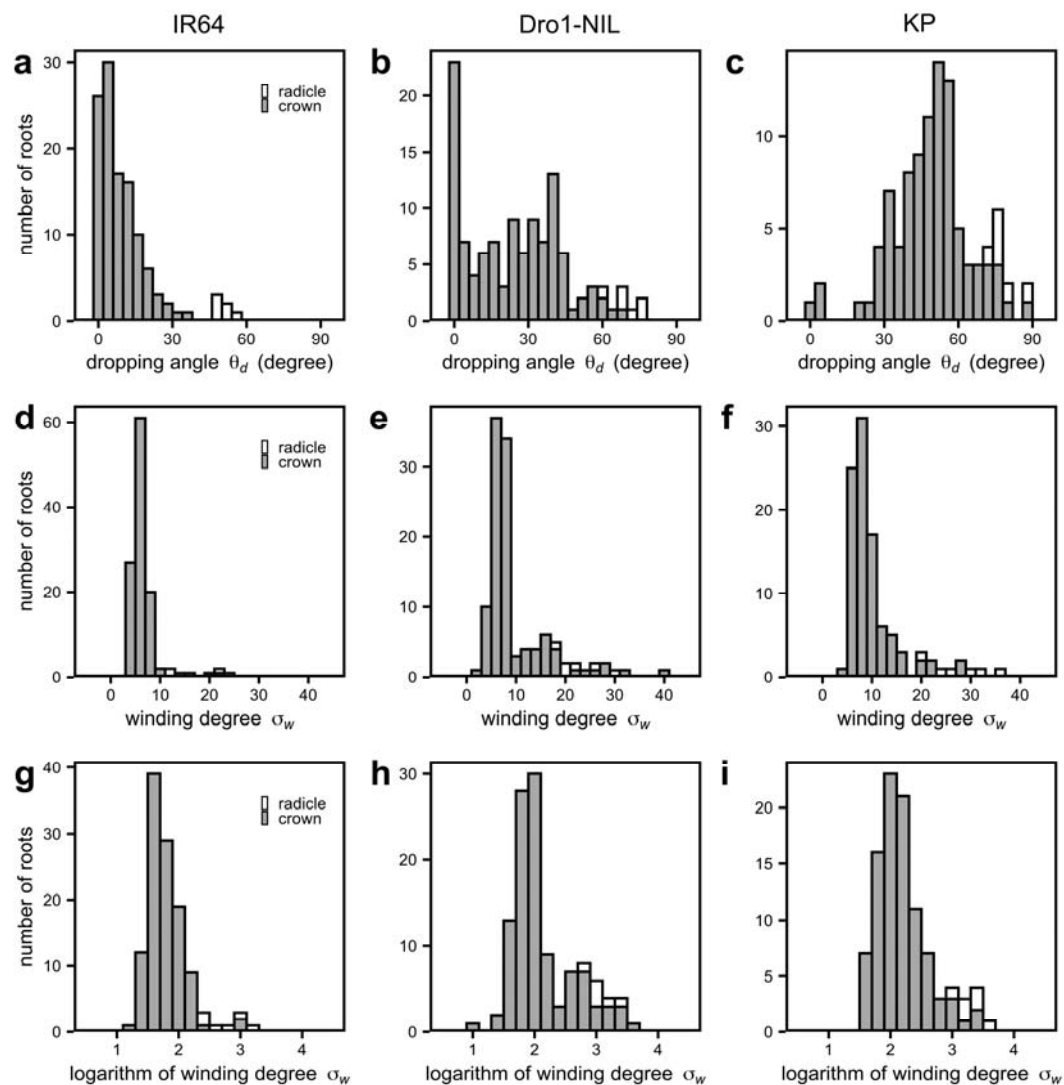


Fig. 4

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558 **Fig. 5**

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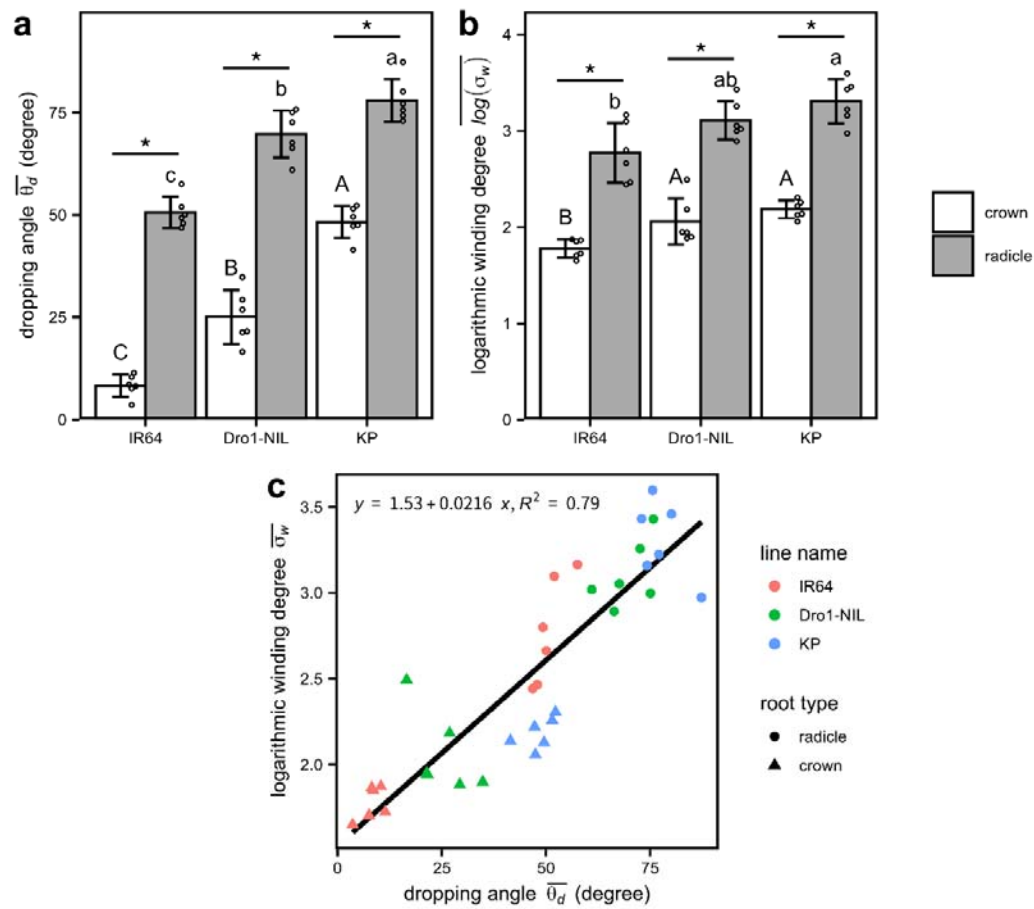
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570 **Fig. 6**

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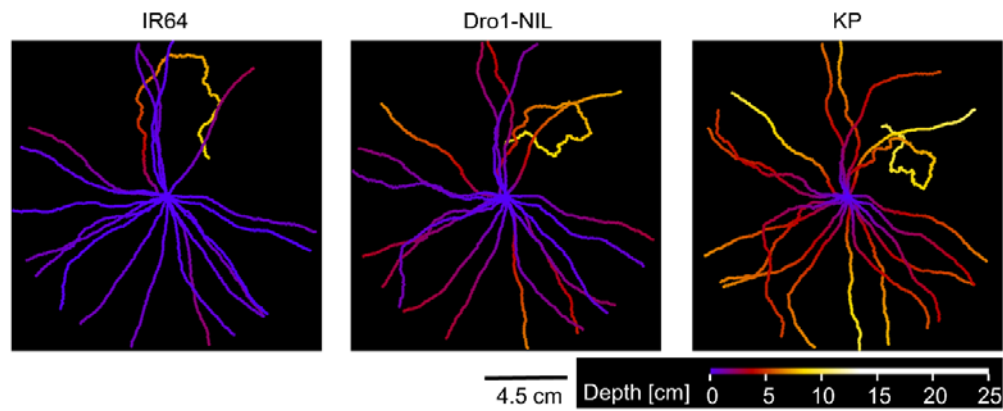
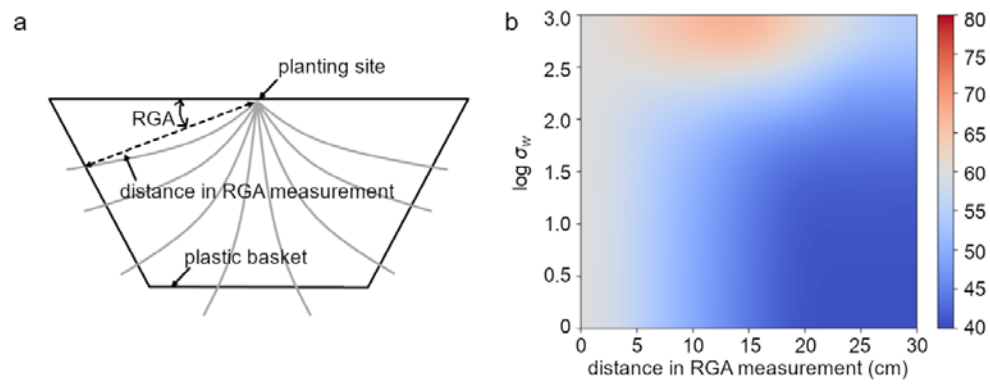


Fig. 7

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610 **Fig. 8**