

26 **Keywords**

27 Plant root system architecture; Data Analysis of Root Tracings (DART); Root System Markup Language (RSML);
28 2D dynamic analysis; root trait.

29

30 **Abstract**

31 *Background and Aims*

32 In order to analyse root system architectures (RSAs) from captured images, a variety of manual (e.g. Data Analysis
33 of Root Tracings, DART), semi-automated and fully automated software packages have been developed. These
34 tools offer complementary approaches to study RSAs and the use of the Root System Markup Language (RSML)
35 to store RSA data makes the comparison of measurements obtained with different (semi-) automated root imaging
36 platforms easier. The throughput of the data analysis process using exported RSA data, however, should benefit
37 greatly from batch analysis in a generic data analysis environment (R software).

38 *Methods*

39 We developed an R package (archiDART) with five functions. It computes global RSA traits, root growth rates,
40 root growth directions and trajectories, and lateral root distribution from DART-generated and/or RSML files. It
41 also has specific plotting functions designed to visualise the dynamics of root system growth.

42 *Results*

43 The results demonstrated the ability of the package's functions to compute relevant traits for three contrasted RSAs
44 (*Brachypodium distachyon* [L.] P. Beauv., *Hevea brasiliensis* Müll. Arg. and *Solanum lycopersicum* L.).

45 *Conclusions*

46 This work extends the DART software package and other image analysis tools supporting the RSML format,
47 enabling users to easily calculate a number of RSA traits in a generic data analysis environment.

48 **Introduction**

49 As water and nutrients are resources characterised by a heterogeneous spatial and temporal distribution in the soil,
50 the selection of plants able to develop a root system architecture (RSA) that optimizes water and nutrient uptake
51 in various growing conditions has been shown to be an important target for crop improvement in terms of
52 sustainable agriculture (Lynch 1995; de Dorlodot et al. 2007; Den Herder et al. 2010; Meister et al. 2014). In this
53 context, linking genotypes and environmental conditions to phenotypes requires the use of high-throughput plant
54 phenotyping platforms that allow a quantitative analysis of a large number of plants on which an increasing number
55 of traits can be accurately measured (Cobb et al. 2013; Fiorani and Schurr 2013). Due to its complexity and
56 belowground localisation, however, phenotyping a root system is challenging and numerous tools are being
57 developed to address this difficulty. These tools are part of a processing chain that consists of three main steps: (1)
58 high-resolution imaging of excavated (2D) or undisturbed (2D or 3D) root systems, (2) recognition of the relevant
59 biological structures in the captured images (Zhu et al. 2011), and (3) calculation of static and dynamic features
60 that describe the RSA either locally (for an individual root, a specific root class, etc.) or globally (at the whole root
61 system scale) (de Dorlodot et al. 2007; Clark et al. 2011; Nagel et al. 2012). Using high-resolution images acquired
62 with light or confocal microscopes (Wells et al. 2012; Slovak et al. 2014), flatbed scanners (Pagès 2014), digital
63 cameras (Le Marié et al. 2014; Mathieu et al. 2015) or non-invasive 3D techniques such as X-ray computed
64 tomography and magnetic resonance imaging (Zhu et al. 2011), software packages can be used for the detection
65 of roots on captured images prior to the calculation of relevant RSA traits. Depending on the level of interaction
66 between the user and the imaging software, root detection can be performed manually (Le Bot et al. 2010), semi-
67 automatically (Lobet et al. 2011; Clark et al. 2013; Pound et al. 2013) or fully automatically (French et al. 2009;
68 Iyer-Pascuzzi et al. 2010; Wells et al. 2012; Diener et al. 2013; Kumar et al. 2014; Leitner et al. 2014; Pace et al.
69 2014; Slovak et al. 2014; Bucksch et al. 2014; Cai et al. 2015). The use of a manual tracing software tool like Data
70 Analysis of Root Tracings (DART) is well suited for the 2D analysis of mature root systems with a high level of
71 root overlaps or for rhizotron images, but it is not suitable in a high-throughput phenotyping context for which
72 scientists will prefer automated or semi-automated tracing tools. As a consequence of the variety of 2D image
73 analysis software solutions dedicated to the analysis of RSAs [listed in the Plant Image Analysis Database, (Lobet
74 et al. 2013)], the comparison of measurements acquired with different platforms is often difficult, mainly because
75 these platforms do not compute the same RSA traits and the stored RSA data do not share a common structure. In
76 order to partly solve this problem, the Root System Markup Language (RSML) has been recently introduced as a
77 convenient way to store and exchange RSA data in a standardized format (Lobet et al. 2015). At the time of writing,

78 a RSML support has been implemented in two semi-automated (SmartRoot and RootNav) and three automated
79 (RootTrace, RhizoScan and RootSystemAnalyser) 2D root image analysis software tools. As manual, semi-
80 automated and fully automated root tracing solutions offer complementary approaches to study RSAs and generate
81 large datasets from which relevant RSA traits can be calculated, it would be of great interest to develop a tool
82 allowing the batch analysis of DART-generated and/or RSML files using automated procedures in a generic data
83 analysis environment that allows easy access to the calculated RSA traits for further statistical analyses (Table 1).
84 In order to achieve this goal, we developed an R package (**archiDART**) for the computation of global RSA traits
85 (root length, root number, etc.) at each observation date, root growth directions and trajectories (branching and
86 root tip angles, root curvature), and lateral root length and density distribution from DART and RSML files (Delory
87 et al. 2015). The package also enables the dynamics of root system growth and local root growth rate variations to
88 be visually assessed using specific plotting and mapping functions. In this paper, our objective was to present the
89 main functionalities of archiDART and demonstrate the added value of this package using contrasted RSAs.

90

91 **Materials and Methods**

92 In order to illustrate the functionalities of the R package archiDART, we performed a quantitative root system
93 architecture analysis of a model cereal (*Brachypodium distachyon* [L.] P. Beauv.) and two dicotyledonous plant
94 species (*Hevea brasiliensis* Müll. Arg. and *Solanum lycopersicum* L.) produced under various growing conditions
95 and we focused our analysis on selected root traits that were particularly relevant for each plant species.

96

97 **Example 1: Time series images of *Brachypodium* plantlets exposed to rhizobacterial volatiles *in vitro***

98 In order to show the impact of volatile compounds emitted by plant growth-promoting rhizobacteria (PGPR)
99 commonly found in the plant's rhizosphere on the RSA of a model cereal, eight root systems of *B. distachyon*
100 plantlets were produced *in vitro* in square petri dishes on a Hoagland medium and were either co-cultivated with
101 one PGPR strain (*Bacillus pumilus* C26, *Bacillus subtilis* AP305-GB03 or *Enterobacter cloacae* AP12-JM22) or
102 cultivated alone following the protocol described by Delaplace et al. (2015), except that the photoperiod used in
103 the growth chamber was 16 h/8 h – L/D and the lateral roots were detected without setting a minimal length
104 threshold. Monitoring the RSA of each plantlet started at the beginning of the experiment (day 0) and was
105 performed by scanning each root system at 400 dpi every 24 h for 11 days. At the end of the experiment (day 11),
106 a single composite image of each root system was constructed with Adobe Photoshop 7.0.1 (Adobe Systems

107 Incorporated, San Jose, CA, USA). In order to facilitate the vectorization with DART, the new root segments that
108 appeared at each observation date were drawn with a specific colour.

109

110 Example 2: Time series images of the root system of a rubber tree growing in a rhizotron
111 The root system of this rubber tree species was produced in a vertical root observation box filled with 2 mm-sieved
112 vermiculite. The development of the root system was monitored every 2 days by drawing the new growth
113 increments on a transparent plastic sheet using a unique colour for each observation date. The RSA used in this
114 work was that of a 37-day-old *H. brasiliensis* seedling (Thaler and Pagès 1996a).

115

116 Example 3: Time series images of a tomato root system produced hydroponically
117 As a third example, we used the root system of a tomato plant produced hydroponically in a horizontal flat box, as
118 described by Le Bot et al. (2010). Images of the entire root system were taken every 24 h using a digital camera.
119 The RSA used in this work was that of a 28-day-old tomato plant.

120

121 RSA input data

122 Two types of RSA input data can be used to compute RSA traits with the R package archiDART (Table 1): (1) the
123 files exported by Data Analysis of Root Tracings (DART) (Le Bot et al. 2010) and (2) RSA data encoded with the
124 Root System Markup Language (RSML) (Lobet et al. 2015). Although DART-generated and RSML files do not
125 share a common structure, the functions of our R package use the file extensions to discriminate between these
126 two file types and compute RSA traits for each independently, allowing the batch analysis of DART-generated
127 and RSML files in a single operation.

128 In this paper, the images showing the RSA of each plant species were processed with DART and the files
129 containing topological (file extension: .rac), temporal (file extension: .tps) and spatial (file extension: .lie)
130 attributes were saved into a specific folder for each plant species after the manual vectorization of each root system.
131 The RSAs were then analysed with the archiDART 1.1 package (Delory et al. 2015) using the 3.2.0 version of R
132 statistical software (R Core Team 2015).

133 Overview of the package's functions

134 The archiDART package (version 1.1) has five functions (*archidraw*, *archigrow*, *architect*, *latdist* and *trajectory*)
135 that allow the batch processing of many root systems by simply knowing (1) the path(s) to the folder(s) containing
136 the DART-generated and/or the RSML files exported by another root imaging software tool supporting this file
137 format, (2) the unit of length and unit of time that should be used by the functions in order to perform the
138 calculations and express the results, and (3) the resolution of the images used to vectorize the root systems (if
139 images were acquired with a flatbed scanner; resolution is expressed in dots/inch) or the ratio between the length
140 of a reference object located on the image expressed in pixels and the actual length of the same object expressed
141 in inches (if images were acquired with a digital camera) (Delory et al. 2015). An overview of the required files,
142 returned R objects and calculated RSA traits for each R function of the package is shown in Table 1. A full
143 description of the values taken by each function is in the documentation files provided with the R package
144 (<http://cran.r-project.org/package=archiDART>).

145 The graphical functions *archidraw* and *archigrow* allow the X-Y plotting of each vectorized root system for
146 selected observation dates. They both use the generic X-Y plotting functions of the R package 'graphics' (R Core
147 Team 2015) to plot the vectorization results of many root systems in a single operation. Whereas *archidraw* is a
148 plotting function that allows the graphical representation of a root system with a colour code depending on the
149 observation date at which a link is seen for the first time, *archigrow* allows both the exportation of growth rate
150 matrices and the X-Y plotting of vectorized root systems with a colour code depending on the growth rate value
151 of each link constituting the vectorized root systems. The *archigrow* function computes root growth rates following
152 the method described in Table 2. Using these functions, the user can easily customize the graphical outputs by
153 setting additional graphical parameters.

154 The *architect* function was designed for the one-step calculation of a number of integrated RSA traits in order to
155 provide an overall description of entire root systems at each observation date (Table 1). Root lengths, root numbers
156 and lateral root densities are computed by the *architect* function according to the methods described in Table 2. In
157 addition to these parameters, the secondary root distribution along a first-order root can also be studied with
158 *architect*. To do so, one has to delimit zones along the first-order root before running the function using the *rootdiv*
159 argument. The total secondary root length, the total number of secondary roots and the secondary root density will
160 then be computed for each zone on the first-order root. The calculated RSA parameters are stored in a data frame
161 that can easily be used for subsequent statistical analyses with R or other statistical software.

162 The purpose of the *latdist* function is to describe lateral root length and density distribution along each mother root
163 of a vectorized root system (Table 2). To do so, the function use the distance between each branching point to the
164 parent root base (DBase, Fig. 1a) in order to select the daughter roots to be used for calculating local lateral root
165 lengths and densities along each parent root. Using the *latdist* function comprises two main steps. First, the function
166 selects the points along the parent roots on which the calculation of RSA parameters should be performed.
167 Depending on user choice, these points can be selected with or without linear interpolation. The use of interpolated
168 points allows a more continuous evaluation of the lateral root length and density distribution on each parent root.
169 Lateral root density and a total lateral root length are then calculated at intervals of a length defined by the
170 experimenter and centred on each selected point. At the end of the calculation process, the results are stored in a
171 list, giving easy access to the computed RSA traits for each mother root of each analysed root system. A second
172 algorithm for measuring the inter-branch distances was also incorporated to *latdist* (Table 2).

173 With regard to the importance of root growth directions and trajectories in RSA, the *trajectory* function was
174 designed to calculate root growth angle (basal branching and root tip angles), orientation and tortuosity, as well as
175 statistical parameters (mean and standard deviation) that describe the curvature of each root of a vectorized root
176 system. These RSA traits are computed by the *trajectory* function following the methods described in Table 2. As
177 the orientation of the vectorized root system can be different from that of the natural plant root system depending
178 on the device used to acquire root images, one can specify an angle value that will be used by the function to rotate
179 the vectorized root system clockwise before calculating the RSA parameters. Because the angle values computed
180 by *trajectory* depend on the initial values of *l.brangle* and *l.tipangle* (Table 2), these must be carefully chosen
181 before running the function and will be notably linked to the plant species studied. The curvature of a root can be
182 evaluated by its tortuosity. In addition, *trajectory* also allows the computation of statistical parameters (mean and
183 standard deviation) that characterise the distribution of local angles measured at selected points linearly
184 interpolated along a root. The method used by *trajectory* to calculate local angles on a root is very similar to that
185 used in RootTrace (French et al. 2009) and comprises three main steps. First, the function will position
186 equidistantly spaced points along each root constituting a vectorized root system. The distance between the
187 interpolated points (*l.curv*) is set by the user before running the *latdist* function. Second, the angles (θ_{ij}) between
188 the direction vectors (\vec{H}_{ij}) of successive links along each root are calculated (Fig. 1d). As pointed out by French et
189 al. (2009), a high θ_{ij} value is associated with a pronounced local curvature. The mean and standard deviation of
190 the calculated angles are then determined for each root. As the user-defined distance between the interpolated
191 points (*l.curv*) affects the computed local angles between successive direction vectors, the initial value of this

192 parameter should be carefully chosen by the experimenter before carrying out the function in order to best fit the
193 RSA of the considered plant.

194

195 **Results**

196 *archidraw*: X-Y plotting of vectorized root systems

197 In order to qualitatively compare several RSA across time series experiments and export the vectorized root
198 systems as high-resolution images, we developed an R function allowing the X-Y plotting of each vectorized root
199 system for selected observation dates. As an example, we used *archidraw* to plot the RSA of a 12-day-old *B.*
200 *distachyon* plantlet produced *in vitro* and exposed to the volatiles emitted by *B. subtilis* AP305-GB03 (Example
201 1, Fig. 2a), the RSA of a 37-day-old rubber tree produced in a vertical rhizotron (Example 2, Fig. 2b) and part of
202 the RSA of a 28-day-old tomato plant produced hydroponically in a horizontal flat box (Example 3, Fig. 2c). The
203 last-mentioned RSA was plotted with a specific colour for each observation date.

204

205 *archigrow*: computing growth rate matrices and X-Y plotting of vectorized root systems

206 As the graphical outputs exported by *archidraw* did not allow the visual detection of the root system parts that
207 were characterised by high or low growth rate values, we developed a second mapping function (*archigrow*)
208 allowing both the exportation of growth rate matrices and the X-Y plotting of vectorized root systems for selected
209 observation dates with a colour code depending on the growth rate value of each link constituting the vectorized
210 root systems. Such a tool could be particularly useful for rapidly screening local growth rate variations between
211 analysed root systems. As an example, we used *archigrow* to study the growth rate variations between four root
212 systems of *B. distachyon* plantlets that were or not exposed to rhizobacterial-emitted volatiles (Example 1). The
213 results showed that plants exposed to bacterial volatiles were characterised by higher growth rate values for the
214 primary root and the first-order lateral roots (Fig. 3).

215

216 *architect*: computing parameters describing the global RSA

217 In order to quantitatively compare the architecture of many root systems, it is often useful to start the data analysis
218 process by calculating the RSA traits that provide a global description of each root system. In this way, *architect*
219 is an R function performing a one-step calculation of common RSA traits for each analysed root system at each
220 observation date. To illustrate this R function, we used *architect* on the DART files associated with the *B.*
221 *distachyon* root systems (Example 1) and we plotted the results for three RSA parameters showing contrasted

222 results between experimental treatments (Fig. 4). The use of *architect* allowed a rapid analysis of the effects of
223 rhizobacterial volatiles on the RSA of *B. distachyon* plantlets. As shown in the figures, the root system of plants
224 exposed to rhizobacterial volatiles had a higher growth rate (Fig. 4a), a higher number of lateral roots (Fig. 4b)
225 and a greater length of first-order lateral roots (Fig. 4c) than the control plants at the end of the experiment. These
226 results confirm the growth rate variations observed between experimental treatments using the mapping function
227 *archigrow* (Fig. 3). Both *architect* and *archigrow* can be used to compare the growth kinetics of root systems but
228 these two functions will not have the same spatial resolution when computing root growth rates. Whereas *architect*
229 allows the computation of growth rates for the entire root system or for each branching order at each observation
230 date, the growth rate matrices and the graphical outputs exported by *archigrow* allow an analysis of the entire root
231 system growth with a far higher spatial resolution because a 'root by root' analysis can be performed.

232

233 *latdist*: computing lateral root length and density distribution

234 As DART is a software package that can provide length and topological information for each root of a vectorized
235 root system (Le Bot et al. 2010), notably via a distance calculated between each branching point and the
236 corresponding parent root base (DBase, Fig. 1a), an algorithm that could convert this information into lateral root
237 length and density distribution on each mother root would be a valuable tool for RSA analysis. In order to illustrate
238 the outputs calculated by *latdist*, we used it on four root systems of *B. distachyon* plantlets that were or were not
239 exposed to rhizobacterial volatiles (Example 1, Fig. 5), as well as on the root system of a rubber tree that had
240 shown periodicity in lateral root development (Example 2, Fig. 6) (Thaler and Pagès 1996b). For clarity reasons,
241 the results plotted in Fig. 5 and 6 show only the evolution of lateral root density, the lateral root length and the
242 distance between neighbouring lateral roots for the primary root. For *B. distachyon*, a maximum lateral root density
243 of 11-13 roots/cm was reached in a zone localized between 1.2 and 1.8 cm from the primary root base and no clear
244 difference emerged when the four root systems were compared for that variable (Fig. 5, a-d) or for the distances
245 between neighbouring lateral roots (Fig. 5, i-l). As for the total length of the first-order lateral roots, this showed
246 a maximum value localized between 1.1 and 1.6 cm, which parallels the results obtained for lateral root density
247 distribution. When the results were compared for that primary root zone, it was evident that total secondary root
248 length was lower for the control plant (2.2 cm of secondary roots/cm of primary root) than for the plants exposed
249 to rhizobacterial volatiles (5.1-5.6 cm of secondary roots/cm of primary root), confirming that the lateral root
250 elongation rate was lower for the unexposed plant (Fig. 5, e-h). In a second example, we tested the ability of *latdist*
251 to detect a periodic pattern in the lateral root development of a rubber tree, as described by Thaler and Pagès

252 (1996b). Three first-order lateral root density maxima were visually detected along the taproot of the rubber tree
253 (Fig. 6a). The first maximum (9 roots/cm) was located between 0.7 and 1 cm from the taproot base. This local
254 maximum was associated with secondary roots that started to develop early (Le Roux and Pagès 1994). Two
255 additional local maxima associated with acropetal first-order lateral roots were also detected at 6.9 (8 roots/cm)
256 and 27.7 cm (8 roots/cm) from the taproot base. The secondary root length distribution along the taproot paralleled
257 the lateral root density distribution with high and low lateral root lengths calculated in zones with high or low
258 branching densities, respectively (Fig. 6b). The use of the *archigrow* function to study the evolution of lateral root
259 vigour along the taproot showed that highly branched areas with high lateral root growth rates alternated with
260 poorly branched areas characterised by low lateral root growth rates (Fig. 7). Taken together, these results
261 demonstrate the ability of *latdist* and *archigrow* to detect a periodic developmental process in a rubber tree for
262 first-order lateral root emergence and elongation, as previously demonstrated by Thaler and Pagès (1996b).

263

264 *trajectory*: computing RSA traits describing root growth directions and trajectories

265 The *trajectory* R function was designed for calculating root traits that describe root growth directions and
266 trajectories. To illustrate some outputs of *trajectory*, we used the function on the DART files associated with the
267 root system of a 37-day-old rubber tree (Example 2). First, we compared the branching angles of the main root
268 types identified by Le Roux and Pagès (1994) (Fig. 8a). The results showed that the branching angles of early and
269 acropetal secondary roots had median values of 59.2° and 70.8°, respectively. By contrast, the tertiary roots had a
270 higher median value (82.3°) and greater variability than the branching angles measured for the secondary roots.
271 Second, we studied the potential of *trajectory* to follow the root tip angle of selected roots growing in a vertical
272 rhizotron for 34 days (Fig. 8b). For clarity reasons, in the figure the evolution of the root tip angle during the
273 cultivation period is shown only for the taproot, the two longest early secondary roots and the two longest acropetal
274 secondary roots. The results showed that the taproot grew almost vertically with a root tip angle of $4.3^\circ \pm 4.5^\circ$
275 (mean \pm sd). Compared with the acropetal secondary roots, the two analysed early secondary roots seem to grow
276 with a steeper root tip angle. It can also be seen that one of the acropetal secondary roots grew with a negative
277 gravitropism at the end of the experiment because its registered root tip angle at the last observation date was
278 greater than 90° (92.7°).

279 **Discussion**

280 In this paper, we presented five functions of an R package (archiDART, version 1.1) designed for the automated
281 computation of RSA traits from data exported by manual (DART) or semi- and fully automated root image analysis
282 platforms that have been implemented with a Root System Markup Language (RSML) support. Considering the
283 increasing number of root image analysis tools, the RSML was introduced as a convenient way to store and
284 exchange RSA data between imaging software tools (Lobet et al. 2015; Lobet 2015). In order to allow the analysis
285 of RSML files with the archiDART package, an import function has been implemented in *architect*, *archidraw*,
286 *latdist* and *trajectory*. Briefly, this function converts the RSA data encoded in each RSML file into data frames
287 possessing the same structure as the files generated by DART before calculating the RSA traits. This import
288 function allows the processing of RSML files and extends the use of the archiDART package to RSA data that
289 were exported with root imaging software tools supporting this file format (Fig. 9). Therefore, as the functions of
290 the archiDART package can use the spatial and topological information stored in both DART and RSML files to
291 compute relevant RSA traits, this tool allows the comparison of root systems skeletonized with different root
292 tracing solutions and complements these software packages with the possibility to compute new RSA traits. When
293 coupled with DART or root imaging software tools supporting the RSML format, the archiDART package
294 improves the RSA analysis process by allowing the batch analysis of many root systems and the computation of
295 relevant parameters aimed at describing each analysed RSA at both a global and a local scale. To date, the
296 package's functionalities include the computation of RSA traits and graphical outputs that allow (1) a global and
297 dynamic analysis of root system growth (*architect*, *archidraw*), (2) an efficient description and mapping of growth
298 rate variations between components of a single or many root systems (*archigrow*), (3) a detailed analysis of lateral
299 root length and density distribution along the mother roots of each analysed root system (*latdist*), and (4) a
300 comprehensive analysis of root growth directions and trajectories via the calculation of branching and root tip
301 angles, as well as parameters characterising root curvature (*trajectory*).

302 The choice of a root tracing solution for the analysis of root system architectures is conditioned by the quality and
303 the number of root images, the level of automation required by the experimenter, as well as the RSA traits that
304 should be measured on captured images. Although most root imaging platforms deal with root number and root
305 length measurements, other RSA traits that are computed by a limited number of platforms can be more relevant
306 to analyse in certain situations. Among all the parameters that can be calculated by the archiDART package, lateral
307 root length and density distribution are two important RSA traits for at least two reasons. First, as roots compete
308 with each other for shared internal resources, the optimal number of lateral roots will be related to the balance

309 between their metabolic costs and the need for root development and resource acquisition (Lynch 2013). Second,
310 as mobile resources (e.g., water and nitrates) are more efficiently taken up by long but dispersed laterals and
311 immobile resources (e.g., phosphorus) are more efficiently acquired by fine but dense laterals, these RSA features
312 will determine the balance between mobile and immobile resource uptake (López-Bucio et al. 2003; Malamy 2005;
313 Lynch 2013). In addition, as resource acquisition by plant roots is challenged by a heterogeneous spatial and
314 temporal distribution of water and nutrients in the soil (Lynch 1995; Giehl et al. 2014), root growth direction and
315 trajectories also play an important role in the development of RSA, but only a few root imaging platforms are able
316 to compute such traits (Lobet et al. 2013). Among the possible parameters aimed at describing RSA, root growth
317 angles are of major importance in resource foraging and depend on root type, edaphic conditions and genotype
318 (Rich and Watt 2013; Wu et al. 2014). As shallow and steep root growth angles are associated with topsoil and
319 deep foraging strategies, respectively, root growth angles will condition the soil layers that will be explored by
320 plant roots (Forde and Lorenzo 2001; Lynch 2013; Uga et al. 2013). Root growth direction has also been shown
321 to be an important RSA feature in plant – plant interaction studies, which have revealed that individuals growing
322 next to each other can sense the presence of neighbours and are able to orient their root development accordingly
323 (de Kroon 2007; Gonkhamdee et al. 2010; Rascher et al. 2011; Faget et al. 2013; Schmid et al. 2015). Because of
324 its complementarity with several root tracing solutions, the use of archiDART can help scientists to choose the
325 most appropriate root image analysis software tool without uniquely focusing on the RSA traits computed by these
326 software packages.

327 In recent years, root models have become an important tool in root research (Godin and Sinoquet 2005). However,
328 the main limitation in using these models is often the lack of quantitative data. Our R package archiDART can
329 help overcome this issue by providing modellers with useful metrics. Typically, dynamic variables (e.g. growth
330 rates), often used as input parameters in root models (Pagès et al. 2013), can be generated using
331 archiDART. Moreover, density distribution, growth rate and direction can also be used in density-based models
332 (Dupuy et al. 2010).

333 The functions of the archiDART package have been shown to work efficiently for the computation and analysis
334 of RSA traits of both monocotyledonous (*B. distachyon*) and dicotyledonous plant species (*H. brasiliensis* and *S.*
335 *lycopersicum*) produced under various growing conditions. Considering that manual, semi-automated and fully
336 automated root tracing solutions offer complementary approaches for the analysis of RSAs, archiDART extends
337 the DART software package and other image analysis tools supporting the RSML format, enabling users to easily
338 calculate a number of RSA traits in a generic data analysis environment for further statistical analyses. As an R

339 package, it has a detailed documentation file for each function and is freely downloadable from the CRAN
340 repository (<http://cran.r-project.org/package=archiDART>).

341

342 **Conflict of Interest:** The authors declare that they have no conflict of interest.

343

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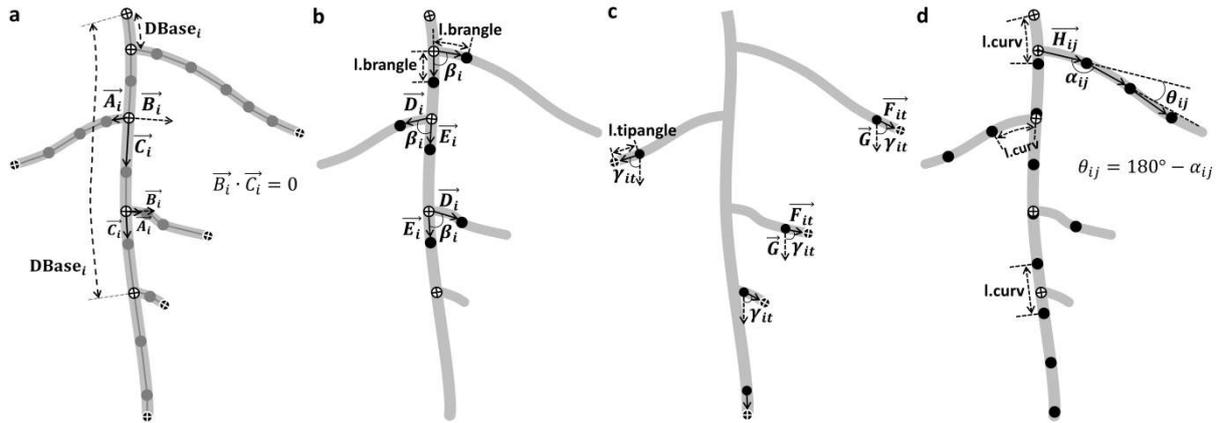
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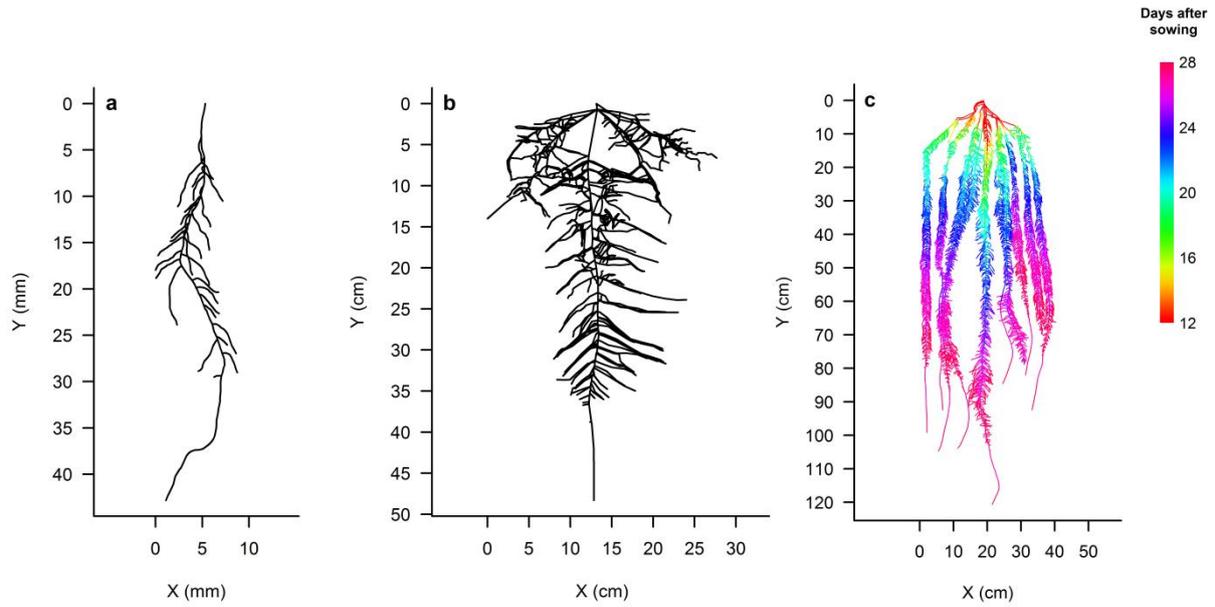
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456 **Fig. 1 Computation of branching angles (b), root tip angles (c) and root curvatures (d) by trajectory.** (a) A
 457 simple root system after vectorization with a root image analysis software package. In Fig. 1a, the distance between
 458 the branching point and the parent root base (referred to as $DBase$ in the text) is shown for two lateral roots. β_i , the
 459 branching angle of the root i on its corresponding mother root; γ_{it} , the tip angle of the root i at the observation date
 460 t ; α_{ij} , the angle j between two successive links of the root i ; θ_{ij} , the angle j between the direction vectors of two
 461 successive links of the root i . White dots with a black cross: branching points; black dots with a white cross: root
 462 tips; grey dots: points placed on the root system during the tracing of roots; black dots: new points linearly
 463 interpolated by trajectory

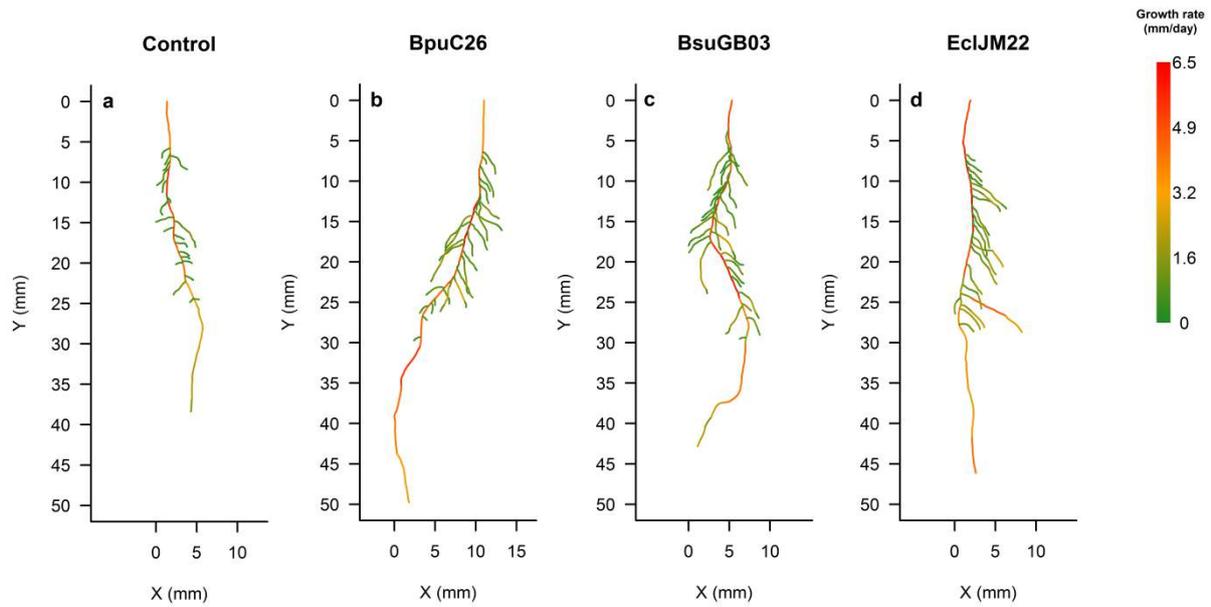
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466 **Fig. 2 X-Y plotting of vectorized root systems using *archidraw*.** (a) A *B. distachyon* root system produced *in*
467 *vitro* that was exposed for 11 days to volatiles emitted by *B. subtilis* AP305-GB03, (b) the root system of a 37-
468 day-old *H. brasiliensis* seedling produced in a vertical rhizotron, and (c) part of the root system of a 28-day-old *S.*
469 *lycopersicum* seedling produced hydroponically in a horizontal flat box. Each root system was plotted at the last
470 observation date. The *S. lycopersicum* root system is shown with a specific colour for each observation date

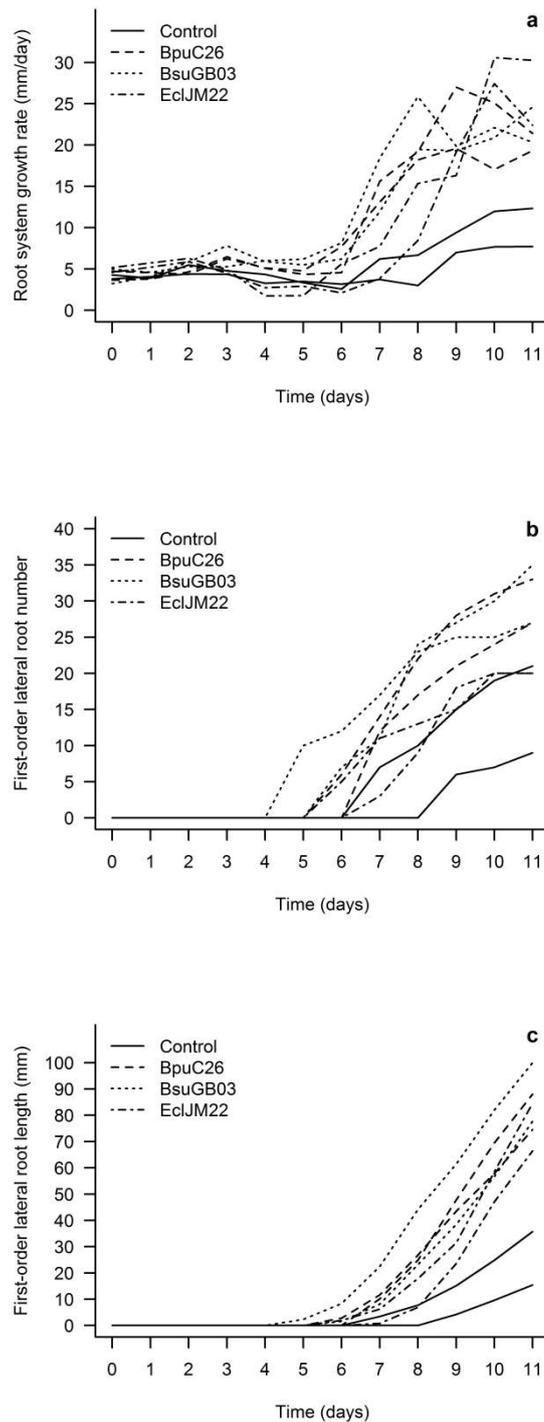
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473 **Fig. 3 X-Y plotting of vectorized *B. distachyon* root systems using *archigrow*.** Plants were either cultivated
474 without PGPR (control) or exposed to volatiles emitted by *B. pumilus* C26 (BpuC26), *B. subtilis* AP305-GB03
475 (BsuGB03) or *E. cloacae* AP12-JM22 (EclJM22) for 11 days. Each root system was plotted at the last observation
476 date. The colour code used for each link depends on its corresponding growth rate value and is shown at the right
477 side of the plot

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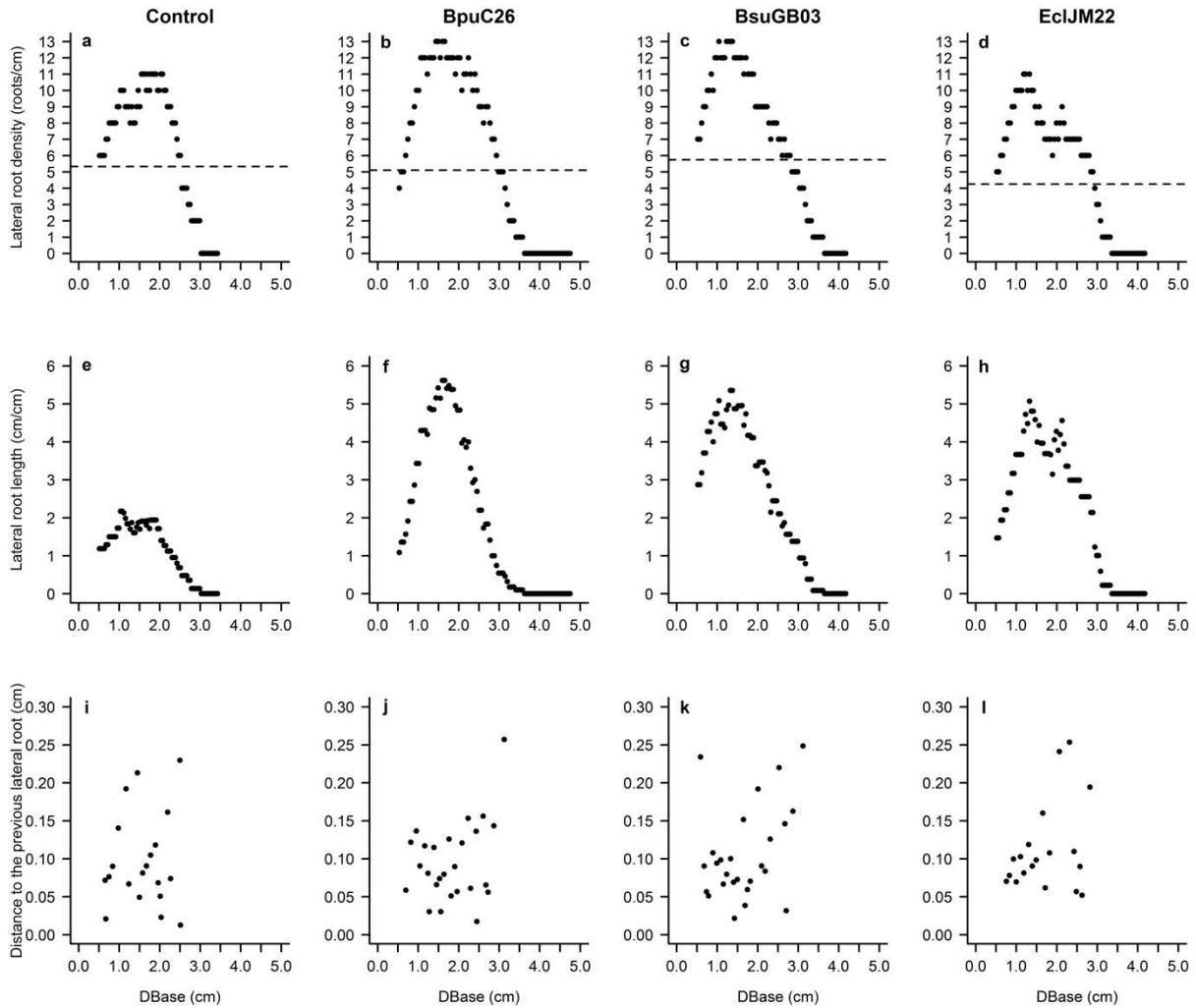
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480 **Fig. 4 Evolution of the root system growth rate (a), the first-order lateral root number (b), and the first-**
481 **order lateral root length (c) of representative *B. distachyon* plantlets co-cultivated or not with PGPR. Plants**

482 were either cultivated without PGPR (control) or exposed to volatiles emitted by *B. pumilus* C26 (BpuC26), *B.*

483 *subtilis* AP305-GB03 (BsuGB03) or *E. cloacae* AP12-JM22 (EclJM22) for 11 days. RSA parameters were

484 calculated using the function *architect*



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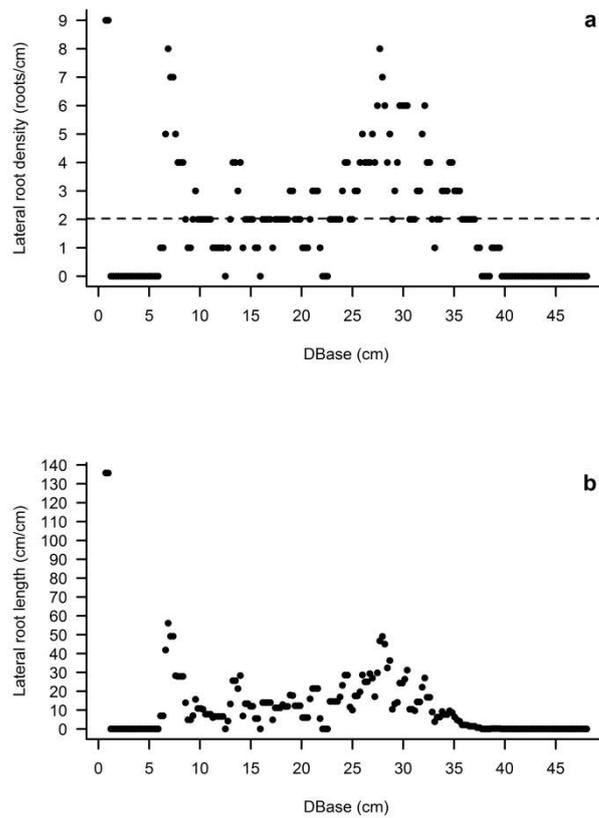
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Fig. 5 Analysis of the first-order lateral root density distribution (a-d), the first-order lateral root length distribution (e-h) and the inter-branch distance between neighbouring first-order lateral roots (i-l) on the primary root of representative *B. distachyon* plantlets co-cultivated or not with PGPR. Plants were either cultivated without PGPR (control) or exposed to volatiles emitted by *B. pumilus* C26 (BpuC26), *B. subtilis* AP305-GB03 (BsuGB03) or *E. cloacae* AP12-JM22 (EclJM22) for 11 days. DBase refers to the distance between the branching point of a first-order lateral root to the parent root base. Each horizontal dashed line refers to the mean secondary root density calculated by *latdist* along each primary root (Control: 5.3 roots/cm; BpuC26: 5.1 roots/cm; BsuGB03: 5.7 roots/cm; EclJM22: 4.3 roots/cm). Calculations were performed using the function *latdist* with an interval length of 1 cm and an interpolation value of 100

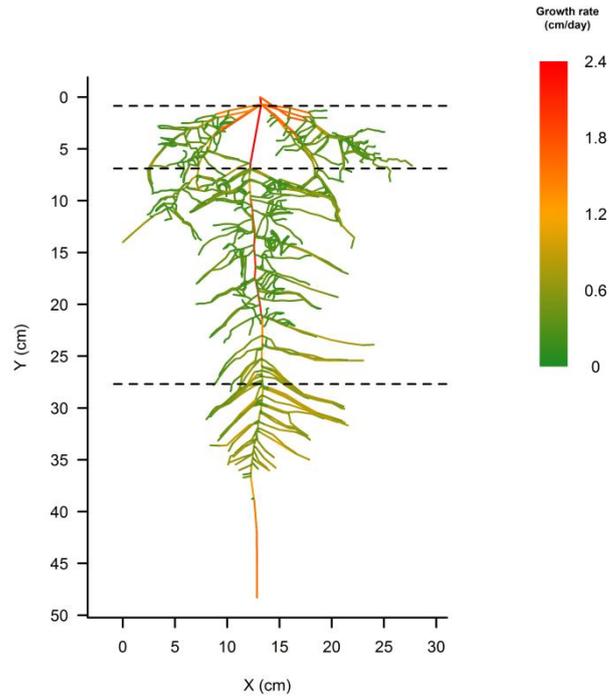


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497 **Fig. 6 Analysis of the first-order lateral root density distribution (a) and the first-order lateral root length**
498 **distribution (b) along the taproot of a 37-day-old *H. brasiliensis* root system produced in a vertical rhizotron.**

499 DBase refers to the distance between the branching point of a first-order lateral root to the parent root base. The
500 horizontal dashed line refers to the mean secondary root density calculated by *latdist* along the taproot (2.0
501 roots/cm)

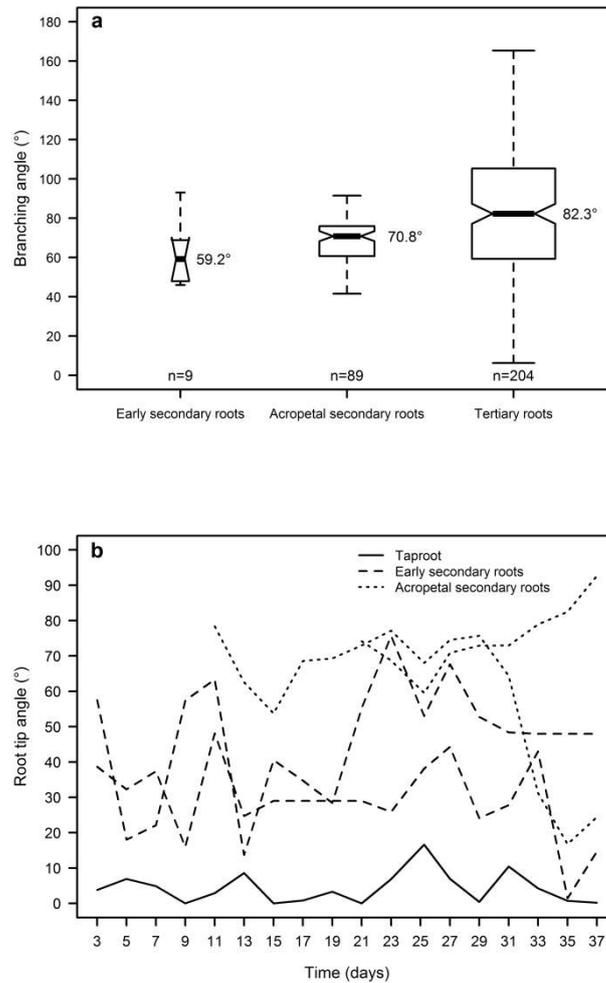
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504 **Fig. 7 X-Y plotting of a 37-day-old *H. brasiliensis* root system produced in a vertical rhizotron using**
505 ***archigrow*.** The root system was plotted at the last observation date. The colour code used for each link depends
506 on its corresponding growth rate value and is shown at the right side of the plot. Regions of the taproot associated
507 with high first-order lateral root densities are located at the intersection of the taproot and each horizontal dashed
508 line
509

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511 **Fig. 8 Analysis of the branching angles (a) and the gravitropism (b) of selected roots of a 37-day-old *H.***

512 ***brasiliensis* root system produced in a vertical rhizotron.** In Figure a, the branching angles were calculated

513 separately for the main root types identified by Le Roux and Pagès (1994). In each boxplot, the whiskers were

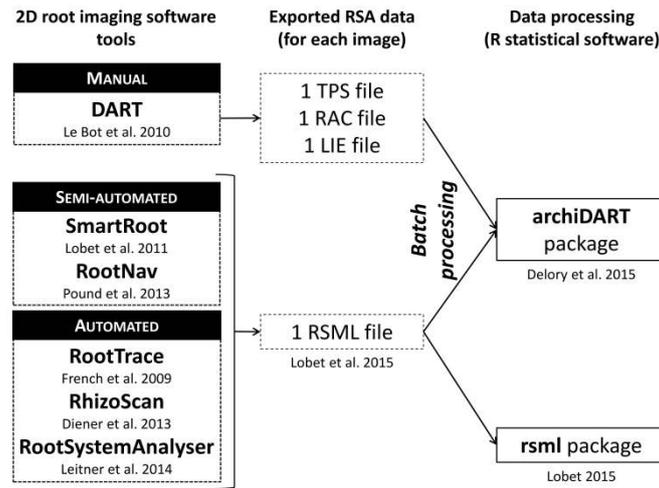
514 extended to the most extreme data points localized between the hinges of the box and 1.5 times the interquartile

515 range. For clarity reasons, Figure b shows only the evolution of the root tip angle for the taproot, the two longest

516 early secondary roots and the two longest acropetal secondary roots. Calculations were performed using the

517 *trajectory* function with the *l.brangle* and *l.tipangle* set at 0.5 cm

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520 **Fig. 9** Root system architecture analysis pipeline: from data acquisition to data processing

521 **Table 1 Overview of the required files, returned R objects and calculated RSA traits for each R function of**
522 **the archiDART package (version 1.1).** DART, Data Analysis of Root Tracings; RAC, DART-generated file
523 storing topological and length attributes for each individual root of a vectorized root system (file extension: .rac);
524 TPS, DART-generated file storing temporal attributes for each observation date (file extension: .tps); LIE, DART-
525 generated file storing spatial attributes for each point used to construct a vectorized root system (file extension:
526 .lie); RSML, Root System Markup Language; UT, the unit of time used by the experimenter; mm, millimetres;
527 cm, centimetres; px, pixels; d, degrees; r, radians

R function	DART/RSML files	Returned R objects	RSA traits	Units
<i>archidraw</i>	LIE RSML	Plot window	×	×
<i>archigrow</i>	LIE + TPS	Plot window List	×	×
			Individual root growth rate at each observation date	(mm, cm or px)/UT
			<i>For each observation date:</i>	
			Length of the root system	mm, cm or px
			Growth rate of the root system	(mm, cm or px) /UT
			Length of the first-order root ^a	mm, cm or px
			Growth rate of the first-order root ^a	(mm, cm or px) /UT
			Total number of lateral roots	-
			Total length of lateral roots	mm, cm or px
<i>architect</i>	RAC + TPS RSML	Data frame	Total number of lateral roots by branching order	-
			Total length of lateral roots by branching order	mm, cm or px
			Mean length of lateral roots by branching order	mm, cm or px
			Growth rate of lateral roots by branching order	(mm, cm or px) /UT
			Density of secondary roots on the first-order root ^a	mm ⁻¹ , cm ⁻¹ or px ⁻¹
			Secondary root number distribution on the first-order root ^a	-
			Secondary root length distribution on the first-order root ^a	mm, cm or px
			Secondary root density distribution on the first-order root ^a	mm ⁻¹ , cm ⁻¹ or px ⁻¹
			<i>For each mother root:</i>	
			Total number of lateral roots	-
<i>latdist</i>	RAC RSML	List	Mean lateral root density	mm ⁻¹ , cm ⁻¹ or px ⁻¹
			Lateral root density distribution	mm ⁻¹ , cm ⁻¹ or px ⁻¹
			Lateral root length distribution	mm/mm, cm/cm or px/px
			Distance to the previous lateral root (inter-branch distance)	mm, cm or px
			Tortuosity	-
			Orientation	-
			Branching angle of each daughter root on its corresponding mother root	d or r
<i>trajectory</i>	RAC + LIE + TPS RSML	List	The mean and the standard deviation of the local angle values calculated between the direction vectors of the successive links constructed using equidistantly spaced interpolated points along each root of a vectorized root system	d or r
			<i>For each observation date:</i>	
			Root tip angle relative to a vertical direction vector	d or r

528 ^a When analysing the topology of a root system, a first-order root is defined as a root that is directly connected to
529 the shoot.

530 **Table 2 Description of the RSA traits computed by the functions of the R package archiDART (version**
531 **1.1)**

RSA traits	Description
Root length	The length of a root is calculated as the length of the polyline (i.e. a succession of linear segments) constituting the skeletonized root. Root length calculations are performed using the spatial and topological information exported from DART or using the geometry elements (SCENE > PLANT > ROOT > Geometry > polyline) stored in each RSML file.
Root number	The number of root elements constituting a skeletonized root system.
Root growth rate	For the first observation date, it is calculated as the ratio of the root length to the root system age. For other observation dates (t), it is calculated as the difference between the root length at time t and t-1 divided by the difference between the root system age at time t and t-1.
Lateral root density	For each mother root, it is calculated as the total number of daughter roots directly connected to the parent root divided by the length of the mother root. When evaluating the lateral root density distribution, the <i>latdist</i> function calculates local lateral root densities at intervals of a length defined by the experimenter and centred on points located at various distances from the parent root base.
Inter-branch distance	The distance separating a lateral root from its closest neighbour located at the greatest distance from the apex of the mother root.
Tortuosity	The ratio of the root length to the Euclidean distance between the root base and the apex.
Orientation (Fig. 1a)	Does a daughter root emerge at the left ($\vec{A}_i \cdot \vec{B}_i > 0$) or the right ($\vec{A}_i \cdot \vec{B}_i < 0$) side of the parent root? \vec{A}_i is a direction vector going from the branching point to the following point on the daughter root <i>i</i> . \vec{B}_i is a vector orthogonal to a direction vector going the branching point to the following point on the mother root of the root <i>i</i> . In this paper, we consider that the normal vector that is orthogonal to a direction vector (a,b) will have the following coordinates: (b,-a).
Branching angle (Fig. 1b)	The branching angle β_i of the root <i>i</i> on its parent root is calculated according to the following equation: $\beta_i = \cos^{-1} \left(\frac{ \vec{D}_i \cdot \vec{E}_i }{\ \vec{D}_i\ \cdot \ \vec{E}_i\ } \right)$ \vec{D}_i is a direction vector going from the branching point to a linearly interpolated point located at a user-specified distance (l.brangle) from the branching point on the daughter root <i>i</i> . \vec{E}_i is a direction vector going from the branching point to a linearly interpolated point located at a user-specified distance (l.brangle) from the branching point on the parent root of the root <i>i</i> .
Root tip angle (Fig. 1c)	The tip angle γ_{it} of the root <i>i</i> at the observation date <i>t</i> is calculated according to the following equation: $\gamma_{it} = \cos^{-1} \left(\frac{ \vec{F}_{it} \cdot \vec{G} }{\ \vec{F}_{it}\ \cdot \ \vec{G}\ } \right)$ \vec{F}_{it} is a direction vector going from a linearly interpolated point placed along the root <i>i</i> at a user-defined distance from the apex (l.tipangle) to the root tip. \vec{G} is a vertical direction vector.
Root curvature (Fig. 1d)	The curvature of the root <i>i</i> is evaluated by statistical parameters (mean and standard deviation) describing the distribution of local angles (θ_{ij}) measured along each root following the method proposed by French et al. (2009).

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