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# Branching patterns of root systems: comparison of monocotyledonous and dicotyledonous species

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## Abstract

**Background and aims** Acropetal root branching is a major process which increases the number of growing tips and distributes their growth potential within the whole root system.

**Methods** Using a method presented in a recent paper (Pagès, 2014) we estimated the defined branching traits in 140 different species, and compared the branching patterns of monocots (45 species) and dicots (95 species).

**Results** We checked that the method also applied to monocots (not considered in the previous paper), and that all traits could be estimated in each species. Variations of most traits were even larger for monocots than for dicots. Systematic differences appeared between these two groups: monocots tended to have a larger range in apical diameters (stronger heterorhizy), with both finer and thicker roots; the diameters of their lateral roots were also more variable; their roots exerted a stronger dominance over lateral branches.

Altogether, species exhibited two main dependencies among their traits that we illustrated using two axes: (i) the “fineness-density” axis separated the species which develop very fine roots and branch densely, from species without fine roots which space out their branches; (ii) the “dominance-heterorhizy” axis separated the species according to the range in their apical diameter which was positively correlated to the level of dominance of mother roots over their branches. Both axes and correlations were remarkably similar for monocots and dicots.

**Conclusions** Beyond the overall typology, this study went on to validate the phenotyping method *in Natura*, and showed its potential to characterize the differences in groups of species.

**Keywords:** phenotyping; modelling; quantitative method; monocotyledon; dicotyledon;

## 30 **Introduction**

31 The dynamics of the root system architecture result from the combination of several developmental  
32 processes. Among them, acropetal branching has a key role in the drastic increase of the number of  
33 root tips, and also in allotting growth potential to these new growing tips (Pagès, 2014).

34 The branching density -i.e. the number of lateral roots per unit length of mother root- is the most  
35 direct way of adjusting the number of new lateral meristems. Both genetic and environmental factors  
36 interact to modulate this variable, as shown by many authors (reviewed by Hodge, 2009; Malamy,  
37 2005). Less obvious is the role of the branching process in defining the balance between the growth  
38 of a mother root and the growth of its daughter laterals. There is usually a hierarchy (or dominance)  
39 between a mother root and its lateral roots, laterals usually being finer and shorter than their  
40 mother. In his quantitative survey on different species, Pagès (2014) modelled this dominance using  
41 the relationship between the diameter of the lateral roots and that of their mother. Beyond its  
42 importance from a geometrical and economical point of view, the diameter of the root (measured in  
43 the young apical zone) is a key criterion to obtain a synthetic evaluation of its structure and fate. It  
44 can be measured on excavated roots and correlations have been shown for many species and  
45 conditions between the diameter, internal structure and size of the apical meristem (e.g. review by  
46 Coutts, 1987; Varney et al., 1991). Regarding developmental dynamics, tip diameter has been linked  
47 to several aspects of growth: rate, duration, potential and even tropism (Coutts, 1987; Cahn et al.,  
48 1989; Pagès, 1995; Lecompte and Pagès, 2006; Wu et al., 2015; Wu et al., 2016). The production of  
49 branches by lateral roots is also related to their diameter in maize (Pagès and Pellerin, 1994; Wu et  
50 al., 2016). Furthermore, neighbouring lateral roots emerging along the same mother may exhibit  
51 important variations in their diameters and growth characteristics (Varney et al., 1991; Pagès, 1995;  
52 Lecompte et al., 2005). Several authors have defended the functional interest of such structural and  
53 growth variations along the same parent root (Forde, 2009; Pagès, 2011).

54 Considering the importance of these different aspects of root branching (i.e. number, growth  
55 potential and variation of laterals), Pagès (2014) proposed to regard acropetal branching through a  
56 set of traits dedicated to the simultaneous analysis of all these characteristics. Since the proposed  
57 method relies on excavated peripheral parts of the root system, namely tips with young laterals, it is  
58 rather easy to use, provided a careful excavation, scanning at high resolution and specific  
59 measurements on the obtained images are carried out. Like other methods, such as that of Fitter  
60 (1982; 1987) or that of Spek and Van Noordwijk (1994), it does not require the monitoring of the  
61 developmental processes dynamics. Therefore, it can be applied to isolated samples observed at a

62 given stage, after excavation. However, in contrast with previous methods, it focuses on acropetal  
63 branching occurring in the young parts of the roots, not far from the growing tips. The considered  
64 diameters are those of the young and turgescient primary structures, before the influence of later  
65 radial growth or shrinkage related to desiccation and ageing further away from the tips.

66 In the reference paper (Pagès, 2014) the method was assessed on a set of 45 dicotyledonous species.  
67 The chosen traits were quantified for each species, and large interspecific variations were shown.  
68 Moreover, co-variations of traits were evidenced, which revealed trade-offs and branching types.  
69 This work legitimated the initial choice of regarding several attributes describing both branching  
70 density and apical diameters, which are associated with the growth potential of the laterals.

71 The applicability of the method, as well as the main results, deserves to be extended to a larger set of  
72 species and to monocotyledonous species. This is the main objective of this paper, in which several  
73 questions are addressed: (1) Is the method also applicable and valuable for these species? (2) What  
74 are the main differences between dicots and monocots regarding their root branching patterns? (3)  
75 Are the between-trait correlations similar in this new set? The studied species were sampled from 4  
76 different environments with 2 sites *in Natura* and 2 sites in pots.

77

## 78 **Material and methods**

### 79 *Sampling species and environments*

80 The studied population contained 178 different sampling cases, each case being a species or cultivar  
81 (140 different species and cultivars) observed in a given environment (4 different environments). We  
82 hereafter call this combination SE (Species\*Environment). Each SE (among the 178) was sampled by  
83 excavating the roots of 3 to 6 different plants. Table 1 contains the information on the species  
84 (according to Tela botanica, [www.tela-botanica.org](http://www.tela-botanica.org), related to the French flora), cultivars and  
85 collection sites. Our aim was to characterize, through the set of quantitative traits defined in Pagès  
86 (2014), the root phenotype of each SE.

87 The first environment (for 13 different species, described in Pagès and Picon-Cochard, 2014) was  
88 represented by foam-insulated cylindrical pots (150 cm in length, 10 cm in diameter) filled with a  
89 natural sieved and fertilized soil (brown acid soil) and located outside under natural climate  
90 conditions near Clermont-Ferrand (Auvergne region, latitude: 45°77'; longitude: 3°14'; altitude:  
91 339 m). These plants were sampled on two dates: mid-June and mid-July 2013.

92 The second environment (for 2 species and 2 rice cultivars) was also represented by insulated  
93 cylindrical pots (50 cm in length, 12 cm in diameter) filled with a mixture of sepiolite and sieved peat  
94 (60:40 in volume) located in a greenhouse near Avignon (Provence region, longitude: 43°92', latitude:  
95 4°88', altitude: 33 m). For each species and cultivar, we sampled 3 different plants on two dates  
96 during June and July 2013. The sampled roots in pots had not reached the bottom of the pots.

97 The third and fourth environments were *in Natura*. The sampled species grew spontaneously in  
98 kitchen gardens and cultivated fields as weeds, in two different zones with uniform soils (described in  
99 Pagès, 2014). The third site was near Thouzon, in the south-east of France (Provence region, latitude:  
100 43°57'; longitude: 4°59'; altitude: 50 m). The soil was a deep calcareous silty soil developed on loess.  
101 The fourth site was near Nozeyrolles, located in the Massif Central (Auvergne region, latitude: 44°59';  
102 longitude: 3°24'; altitude: 1100 m). The soil was a sandy brown acid soil developed on granitic arena.

103 Thus, most SE (159 out of 178) were observed *in Natura* (sites 3 and 4). The site of Thouzon  
104 represented 63 % of SE, while Nozeyrolles represented 26 %. The proportions of monocots and dicots  
105 were similar at the two sites, (Thouzon: 27 % of monocots and 73 % of dicots; Nozeyrolles: 22 % of  
106 monocots and 78 % of dicots).

107

#### 108 *Root system excavation*

109 When sampling *in Natura* (sites 3 and 4), we favoured isolated plants grown in recently cultivated  
110 soils (kitchen gardens or fields) or on recent mole-hills (in the case of pastures). A garden fork was  
111 used to demarcate a monolith around the chosen plant (radius 15 to 20 cm around the collar, 30 to  
112 50 cm deep), and to extract it before putting it in a large bucket with water. Then, the monolith was  
113 gently washed with running water. The same washing procedure was used for pot-grown plants.  
114 When the root system was nearly free of soil and organic debris, it was left for several minutes to  
115 one hour in a tray with salt water (5 g/l) and liquid soap to complete cleaning.

116

#### 117 *Measurements*

118 Root systems were carefully separated and spread with mounted needles in a layer of water several  
119 mm deep contained in a transparent plastic tray. The densest root systems were cut into several  
120 pieces in order to minimize root overlap in the tray. Then they were scanned with a flatbed scanner  
121 (EPSON perfection V700) at a resolution of 1200 – 4800 dots per inch, using the transparent mode.  
122 The resolution was adjusted for each species (in this range) in order to get at least 8 pixels

123 transversally to the finest roots and to measure them with sufficient accuracy. Previous tests had  
124 shown that this adjustment did not induce any bias, since we obtained the same values (on average)  
125 when measuring the same objects at these various resolutions.

126 Measurements were made manually (by eye) on the images using the ImageJ software  
127 (<http://rsbweb.nih.gov/ij/>) on the young branched part of the root, where acropetal branching  
128 occurs. On each identified substructure, we measured the diameter of the mother root, the diameter  
129 of the laterals, and the distance along the mother root from each lateral to its proximal closest  
130 neighbour (see Fig. 1 in Pagès, 2014). Depending on the architectural position of the mother root, the  
131 substructures studied had 1 to 15 laterals. We were careful, through a meticulous visual inspection,  
132 to sample roots with extreme diameters (finest and thickest) because these roots make it possible to  
133 estimate extreme diameters (parameters  $D_{min}$  and  $D_{max}$ ) according to Pagès (2014). For each SE, we  
134 measured from 131 to 805 lateral roots, for a total number of 42139 roots.

135 All diameters (also called “apical diameter” hereafter) were measured on the young part of the root,  
136 close to the tip, at a location where it was nearly cylindrical. The distance from this position to the  
137 very tip was typically between 5 and 50 mm on the thickest roots, and from 2 to 20 on the finest  
138 ones. The youngest lateral roots, less than 3 mm long, were discarded from these measurements.  
139 Zones of local thickening were observed along some roots (supposedly due to mechanical  
140 constraints), and were systematically discarded for diameter measurements. A number of root tips  
141 were broken by the procedure, but even on broken roots it was generally easy, on the high  
142 resolution pictures, to recognize the young parts thanks to several criteria (colour, transparency of  
143 the structure, root hairs, lateral primordia).

144

#### 145 *Data analysis*

146 All data treatments, plots and analyses were carried out with the R software (R Core Team, 2013;  
147 <http://www.r-project.org/>). Linear models were estimated with the “lm” function in order to  
148 estimate parameters and conduct analyses of variance and covariance. In these analyses, SE was the  
149 qualitative factor. The diameter of the parent roots was the covariable to estimate the dominance  
150 slopes for each SE (see below). To study the shape of the trends between two variables graphically,  
151 we used the “lowess” smoothing function of R, with the flexibility parameter at default value ( $f=0.6$ ).  
152 Principal component analyses (PCA) were performed with the “ade4” R package (Chessel et al.,  
153 2004). The PCAs were all centred and normalized.

154 The names and precise meanings of the considered traits are the same as in Pagès (2014). They are  
155 recalled in Table 2 and below with the results on their estimates. Each trait was estimated on each  
156 SE.

157

158

## 159 **Results**

### 160 *Diameter distribution*

161 We observed a 10-fold difference **in extreme diameters among SE**: between 0.044 and 0.45 mm for  
162  $D_{\min}$  and between 0.24 to 2.6 mm for  $D_{\max}$ .

163 The correlation between minimal and maximal diameter across cases was not significant at the 5%  
164 level, showing that the range in diameters was also highly variable. Some species have both fine and  
165 thick roots (e.g. *Setaria verticillata*, *Sorghum bicolor*), while others have relatively homogeneous and  
166 intermediate diameters (e.g. *Clematis vitalba*, *Euonymus europaeus*). These variations legitimated  
167 the use of the trait called Drange (relative range in diameters) and its inclusion in the PCA below.

168

### 169 *Inter-branch distance (IBD)*

170 Within several SE (i.e. species in its sampling environment, as defined in the Material and Methods  
171 section), the distance between neighbouring laterals was dependent on the mother root diameter,  
172 since we obtained low but significant correlations. Three examples are illustrated in fig 1. In the first  
173 one (fig 1A), the distance decreased with the mother-root diameter. In others, it was rather  
174 independent of the mother root diameter (fig 1B) or even tended to increase (fig 1C). Therefore, the  
175 IBD trait was calculated for each SE as the mean inter-branch distance on the thick roots, i.e. those  
176 thicker than the middle of the range in diameters for the given SE (Table 2). IBD exhibited large  
177 variations, between 0.51 and 7.0 mm (ca. 14-fold variations).

178

### 179 *Relationship between diameters of mother and lateral roots*

180 The Pearson correlation coefficients between the diameters of a mother root and those of its laterals  
181 were highly significant for all SE. P-values were between 0.000 and 0.002. Moreover, a systematic  
182 visual inspection of each SE graph showed that the trends (represented on fig. 2 by the smooth lines)

183 were approximately linear. Some small deviations from the linearity were observed, but they did not  
184 show any common shape from one SE to another.

185 Regression lines always intersected the bisecting line near the point of coordinates ( $D_{min}$ ,  $D_{min}$ ).  
186 Moreover, we observed on the images that the finest roots, when they branched, gave rise to  
187 laterals which had about the same diameter as themselves. Thus, the hypothesis that the regression  
188 lines passed through this point ( $D_{min}$ ,  $D_{min}$ ) was tested independently for all SE. We used the test of a  
189 zero intercept for the regression of the lateral root diameter (with  $D_{min}$  subtracted) on that of its  
190 mother (with  $D_{min}$  subtracted). This test was rejected only 19 times out of 178 at the 0.001 p-level. In  
191 these cases, the estimated intercepts were very small (less than 0.02 mm) and were either positive or  
192 negative. Therefore, we decided to force the regression lines to pass through this particular point  
193 ( $D_{min}$ ,  $D_{min}$ ) for all SE, in order to get a more robust estimate of the slope (called DIDm hereafter)  
194 following the same protocol. We also checked that we did not obtain a negative correlation between  
195  $D_{min}$  and DIDm.

196 We also used a covariance model (the parent root diameter being the covariate, the SE being the  
197 interaction factor) to test the effect of the SE on the slope. It was shown to be highly significant,  
198 when compared to the simpler model considering a single common slope. The slope estimates  
199 (DIDm) varied within a 6-fold range with extreme species being *Setaria verticillata* on the one hand,  
200 exhibiting a strong hierarchy of the mother to its laterals (slope: 0.062), and *Nigella damascena* on  
201 the other hand, exhibiting a low hierarchy (slope: 0.39).

202 Because both the average and the standard deviation of the diameter of laterals depended on the  
203 parent root diameter (see Fig. 2), we also estimated the coefficient of variation of the lateral root  
204 diameter (VarD, table 2). For this estimation, we made successive classes of mother root diameter  
205 with the same number of lateral roots (around 10 in each class). For each class, we calculated the  
206 average and standard deviation of the lateral root diameter. We fitted a linear model without  
207 intercept (standard deviation versus average of diameter). Its slope was the coefficient of variation  
208 (VarD). VarD exhibited large (4-fold) inter-specific variations, with species having homogeneous  
209 laterals (e.g. *Allium porrum*) and others having highly variable laterals (e.g. *Echinochloa crus-galli*).

210

### 211 *Distribution of traits between monocots and dicots*

212 Fig. 3 presents the synthesis of these comparisons through boxplots. All traits exhibited different  
213 distributions for the two sub-populations, and the median values were significantly different. The  
214 relative diameter range (Table 2; fig.3A) was much higher for monocots. This was due both to the



215 minimal diameter (fig. 3B), which tended to be lower for monocots (excepted for some species), and  
216 to the maximal diameter (fig. 3C), which tended to be higher for monocots. The inter-branch  
217 distance (fig. 3D) was higher for dicots (median: 2.7) than for monocots (median: 1.4). The  
218 dominance (fig. 3E) was usually higher for monocots (lower slope values) and the variation  
219 coefficients (fig. 3F) were also clearly higher. These coefficients were more homogeneously  
220 distributed among dicots than among monocots.

221

### 222 *Relationships between traits*

223 PCA were achieved for monocots and dicots separately (fig. 4A and fig. 4B) and together (fig. 4C). The  
224 data sets contained the different SE (table 1) as individuals and the 6 traits as variables (table 2).

225 Fig. 4A and 4B show the same co-variation pattern obtained for the two sub-populations. The arrows  
226 were just rotated from one figure to the other. The first plane (containing the first two components)  
227 explained 81% (49 plus 32%) of the total variation on monocots and 73% (40 plus 33%) on dicots.  
228 Because of obvious similarities, we included all species and made a third synthetic PCA (fig. 4C). As  
229 expected, this last PCA exhibited the same pattern. The first plane explained 80% (48 plus 32%) of  
230 the total variation and represented all traits correctly, since they were all close to the correlation  
231 circle.

232 On this plane, we can distinguish 2 groups of correlated variables ( $D_{\min}$  and IBD on the one hand,  
233 Drange, VarD, DIDm on the other hand) and an isolated one ( $D_{\max}$ ). In the first group, IBD and  $D_{\min}$   
234 were significantly and highly correlated ( $R=0.81$  for the overall population;  $R=0.74$  for dicots and  
235  $R=0.88$  for monocots). In the second group, correlations were lower, but still highly significant  
236 (between Drange and DIDm:  $R=-0.59$  for the overall population;  $R=-0.34$  for monocots;  $R=-0.51$  for  
237 dicots).

238

### 239 *Definition of two main axes of variations*

240 These two groups of variables, which are nearly perpendicular in the PCA plane, defined two axes  
241 which explained most variations. These two axes are presented in fig. 5 and fig. 6 to show their  
242 general shape and the relative position of species (monocots and dicots).

243 The relationship between  $D_{\min}$  and IBD (fig. 5) was tight and common for the two groups (monocots  
244 and dicots). Monocots had the largest range for both variables, while dicots occupied more

245 intermediate positions. This axis was called the “fineness – density” axis, since it characterizes the  
246 species simultaneously for these two visual criteria.

247 The second main axis of variation (DIDm vs Drange; fig. 6) was also common to the groups which  
248 followed approximately the same trend. However, they were more discriminated: monocots had  
249 larger ranges in diameters and stronger dominances. This axis was called the “heterorhizy –  
250 dominance” axis. Species with a strong heterorhizy (*sensu* Sutton and Tinus, 1983) regarding  
251 diameter also had a strong dominance, and vice-versa: species with a weak dominance had  
252 homogeneous roots.

253

## 254 **Discussion**

### 255 *Validation of the method for phenotyping root branching*

256 We have further validated the new method proposed by Pagès (2014) for characterizing the  
257 acropetal branching patterns, which includes protocols for data acquisition and treatment in order to  
258 obtain a defined set of quantitative traits for each phenotype. It was applied to a large set of  
259 different species from several families, on which we could estimate all traits and checked the validity  
260 of the main correlations between the measured variables. Moreover, we demonstrated the  
261 possibility of obtaining the required data from plants sampled in various environmental conditions  
262 (*In Natura* and in containers). In comparison to the previous paper, we have simplified the data  
263 analysis and quantification by keeping only 6 different traits (those presented in Table 2) because we  
264 wanted to make it as simple as possible, and the selected traits were considered to be the essential  
265 ones. Thus, we confirm that the method is rather easy to apply, although it requires meticulous  
266 measurements on high quality images. Thus, it can be integrated with various types of research  
267 approaches to characterize roots in ecosystems or plants (phenotyping, see for example Walter et al.,  
268 2015). At the present stage, the throughput is rather low because the different steps (excavation,  
269 sampling and measurements) are carried out manually. However, each step can be improved in the  
270 future. In the present study, our aim was first to confirm the feasibility and interest of this  
271 quantitative approach. The next steps should be dedicated to the optimization of root collection,  
272 data acquisition and treatment.

273

### 274 *Main points of this method*

275 The most important and original points are: (1) to focus on the peripheral parts of the root system  
276 which extend and branch; (2) to consider branching and growth indicators (diameters)  
277 simultaneously; (3) to consider extreme apical diameters instead of average ones; (4) to make a  
278 bridge between static observations and dynamic simulations of root systems.

279 The first point is crucial, since the young parts exhibit several morphological indicators which can  
280 reveal the developmental processes which occurred just before excavation. For example, inter-  
281 branch distance on young roots serves to characterize acropetal branching density. Branching density  
282 can be modified later (on older roots) by other developmental processes when root decay and self-  
283 pruning occur. This approach is in line with the recommendations of several authors (Guo et al.,  
284 2008; Pagès et al., 2010; McCormack et al., 2015) who pointed out the interest of focusing on the  
285 peripheral parts of root systems. This focus might also appear as a weakness since the young parts  
286 which are concerned by the sampling procedure are the most fragile. Excavation methods are  
287 important in this context: getting medium sized monoliths instead of small auger samples is an  
288 important means to get branched root samples in good condition. Therefore, this approach should  
289 be associated with suitable methods to extract and wash such monoliths, such as that proposed by  
290 Wu and Guo (2014). Application of the method to very strong or stony soils would be very  
291 challenging if not impossible.

292 The second point was confirmed in this work by several strong correlations between diameters and  
293 branching attributes (e.g. fineness – density axis presented on Fig. 5). It shows that these two aspects  
294 of branching are coordinated, and should be considered together for a better comprehension of the  
295 branching process and its consequences in root foraging.

296 The third point is related to the heterogeneity of root tip diameters in root systems (called  
297 “heterorhizy” for brevity). This is a major aspect for the development and functioning of root systems  
298 that cannot be captured by considering only average or median diameters. Many species which are  
299 similar regarding their mean diameter are very different regarding their range, and consequently  
300 have different foraging characteristics. The differences between monocots and dicots exemplified  
301 this point, since monocots usually exhibited large Drange values (e.g. *Sorghum bicolor*, between  
302 0.075 and 2.4 mm), while most dicot species have much more homogeneous diameters (e.g.  
303 *Medicago lupulina*, between 0.160 and 0.480 mm).

304 Moreover, when making the observations, we found it relatively easy (with magnifying glasses) to  
305 sample the extreme roots when the root systems were correctly disentangled and laid out in a large  
306 tray with water, and it was thus possible to characterize extreme diameter quantiles. Conversely,

307 from our experience, average values are not easy to obtain in such populations, and the risks of bias  
308 are important because of the usual huge asymmetry of diameter distributions.

309 The fourth point is important as well, because we know how difficult it is to get dynamic data on root  
310 systems. Therefore, connecting static data that can be obtained in relevant environmental conditions  
311 to models of the root system architecture is an interesting task, in order to synthesize the dynamic  
312 branching patterns *in silico*. The link is in line with some recent models (Pagès, 2011; Pagès and  
313 Picon-Cochard, 2014) since several traits which were measured following this method are also  
314 parameters of the dynamic simulation models. For example, Pagès and Picon-Cochard (2014) showed  
315 how the architecture model could serve to interpolate data between sampling dates or to link traits  
316 at different scales.

317

#### 318 *Variations of root traits in the SE population*

319 The 6 considered traits were highly variable from one SE to another. For example, we observed 14-  
320 fold variations for the inter-branch distance and 11-fold variations for the maximal diameter. SE  
321 variations were phenotypes, which mixed species and environmental effects. Since the study was a  
322 survey of existing species on several sites, only a limited number of species was sampled from several  
323 sites. Therefore, this work could not be strictly devoted to the study of the species effects on the  
324 traits. However, we noticed on the PCA graphs that SE of the same species usually had close positions  
325 (data not shown). A systematic study on this topic will require a suitable sampling schema with a  
326 sufficient number of species on several sites.

327 In comparison with previous works using similar traits (Pagès, 2014; Bui et al., 2015), we observed  
328 that the addition of new species in the population extended the range of variation for all traits and  
329 confirmed the main conclusions of Pagès (2014). Conversely, the correlations that were pointed out  
330 by Bui et al. (2015) in Solanaceae species were slightly different from those presented here, probably  
331 because the genetic background of their study was much narrower. For example, the variations in  
332 minimal diameter ( $D_{\min}$ ) in Solanaceae species were very low in comparison to ours.

333

#### 334 *Comparison of monocots and dicots*

335 Regarding the monocot and dicot groups, our survey was nearly balanced regarding the number of  
336 samples *in Natura*, which represented 90% of the total SE population. Therefore, this 2-level-factor  
337 (monocot and dicot) was not confused with the environmental effect. Its effect could thus be

338 presented and qualitatively tested. For this purpose, we used exploratory tools such as the “boxplot”  
339 function of the R software.

340 The addition of monocots to the population of SE (from that of Pagès, 2014) largely contributed in  
341 extending the range of variation for several traits. A number of species, especially among the  
342 Poaceae family, exhibited very low values for their minimal diameter and inter-branch distance.  
343 Several of them were also among the species with the largest maximal diameters. Thus, we found  
344 the highest levels of heterorhizy among monocots. High heterorhizy levels were associated with a  
345 high dominance level and high coefficients of variation of laterals. Strong dominance tends to give  
346 “herringbone patterns”, as noticed by other authors in various Poaceae species (e.g. Fitter and  
347 Stickland, 1991; Taub and Goldberg, 1996; Roumet et al., 2006; Arredondo and Johnson, 2011), and it  
348 often produces laterals with variable diameter, length and structure. The variability among laterals  
349 (VarD) could not be quantified satisfactorily by the Fitter’s method, although it was already  
350 mentioned as a particular feature in several Poaceae species (Varney et al., 1991; Pagès and Pellerin,  
351 1994; Lecompte and Pagès, 2006; Arredondo and Johnson, 2011). We hypothesize that the high  
352 levels of heterorhizy and dominance that we observe in monocots might be due to the inability of  
353 these species to make radial growth. Radial growth is a process which enables a later adjustment of  
354 the capacity of roots for water transport and anchorage strength. Species which cannot undergo  
355 radial growth must build variable roots from the beginning, i.e. from their primary structures. We  
356 can also say that lateral roots are constrained by the structure of their parent root. Their  
357 subordination can be seen as a mean to cope with the fixed structure in which they appear.

358

### 359 *Correlations between traits, and main axes of branching types*

360 We have confirmed the correlation structure (with essentially 2 groups of correlated traits) that we  
361 discovered and discussed in the previous paper (Pagès, 2014). Moreover, we have shown that the  
362 same structure appeared in both groups of species, confirming the robustness of the underlying  
363 correlations and their significance in terms of developmental coordination. Thanks to these stable  
364 correlations, trait variations could be summarized through two axes which represent trade-offs:  
365 “*fineness – density*” on the one hand, “*heterorhizy – dominance*” on the other hand. These two axes  
366 are interesting because they are nearly independent and they represented most of the variation  
367 extracted by the first plane of the PCA (ca 80% of the total variation). They are even simpler and  
368 clearer than the branching types which were defined in the previous companion paper (Pagès, 2014).  
369 In the latter, we discussed the possible functional significance of these two axes regarding foraging  
370 strategies. Future work, using this method, could be more specifically dedicated to defining these

371 strategies and testing the efficiency of these various root systems for different types of resources  
372 (water and nutrients). The present characterisation also opens new avenues to study the associations  
373 of these species with mycorrhizae. For example, interesting questions could be investigated on the  
374 relationships between fineness and mycorrhizal association.

375

#### 376 *Conclusion*

377 The proposed method devoted to the quantification of branching patterns was validated and its  
378 interest was confirmed by this study in a large set of species coming from monocots and dicots. The  
379 branching typology was made simpler and clearer thanks to the definition of two main and  
380 independent axes of variation which can be seen as developmental trade-offs. The differences  
381 between the two groups (monocots and dicots) were evidenced and quantified using several criteria.  
382 The genetic and environmental determinism of these traits should be investigated in more detail.

383

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391

392 **Captions of figures**

393

394 **Figure 1:** Relationships between inter-branch distance and diameter of the mother root for three  
395 different species. A. *Anisantha sterilis*; B: *Poa Pratensis*; C: *ArrhenatherumElatius*. The thick solid lines  
396 represent the trend between these variables (calculated by the “lowess” smoother of R). The three  
397 graphs illustrate the three cases that we observed: (A) decrease in inter-branch distance with mother  
398 root diameter; (B) independence; (C) slight increase.

399

400 **Figure 2:** Relationships between lateral and mother diameter for three different monocots. A. *Setaria*  
401 *verticillata*; B: *Sorghum halepense*; C: *Vulpia myuros*. The thick solid lines represent the trend  
402 between these variables (calculated by the “lowess” smoother of R). The bisecting line is also drawn  
403 as well as the particular point with coordinates ( $D_{min}, D_{min}$ ), to show that the trends would intersect  
404 the bisecting line near this particular point.

405

406 **Figure 3:** Boxplots showing the distribution of the traits between the two subpopulations (monocots  
407 (n=57) and dicots (n=121)) among all SE (i.e. Species in a given Environment). Horizontal lines  
408 represent quantiles at probability levels of 0.05, 0.25, 0.50, 0.75, 0.95. Notches in the boxes  
409 represent the confidence intervals on the medians.

410

411 **Figure 4:** Principal component analysis. Variables (i.e. traits) located on the plane defined by  
412 component 1 (horizontally) and 2 (vertically). A: Monocots only; B: Dicots only; C: All species  
413 together. See the text and Table 2 for the explanations of the traits.

414

415 **Figure 5:** Fineness vs density relationship. Open symbols represent monocots and black symbols  
416 represent dicots.

417

418 **Figure 6:** Heterorhizy vs dominance **slope** relationship. Open symbols represent monocots and black  
419 symbols represent dicots.



420 **Table 1:** List of species and sampling sites

421

Species	Clermont	Avignon greenhouse	Nozeyrolles	Thouzon
Achillea millefolium			X	
Agrostis capillaris			X	
Agrostis vinealis			X	
Alliaria petiolata				X
Allium cepa				X
Allium porrum			X	X
Alopecurus pratense	X			
Alyssum simplex				X
Amaranthus hybridus				X
Amaranthus retroflexus				X
Ambrosia artemisiifolia				X
Anisantha sterilis				X
Anthoxanthum odoratum	X		X	
Aquilegia vulgaris				X
Arabidopsis thaliana			X	
Arenaria serpyllifolia				X
Arrhenatherum elatius	X			
Artemisia annua				X
Artemisia vulgaris			X	X
Arum italicum				X
Arundo donax				X
Atriplex hortensis			X	X
Avena barbata				X
Brachypodium pinnatum			X	
Brachypodium sylvaticum				X
Bromopsis erecta				X
Bromus hordeaceus				X
Bryonia cretica				X
Capsella bursa-pastoris			X	X
Cardamine hirsuta			X	X
Ceratochloa cathartica				X
Chelidonium majus				X
Chenopodium album			X	X
Chenopodium album			X	X
Cicer arietinum				X
Cirsium vulgare				X
Clematis vitalba			X	
Cynosurus cristatus	X		X	X
Dactylis glomerata				X
Datura stramonium			X	
Digitalis purpurea				X
Echinochloa crus-galli	X			X
Elytrigia campestris				
Elytrigia repens				X
Erigeron canadensis				X

Erodium moschatum				X
Euonymus europaeus				X
Euonymus japonicus				X
Eupatorium cannabinum			X	X
Euphorbia helioscopia				X
Euphorbia peplus			X	X
Fallopia convolvulus	X			X
Festuca rubra			X	
Fragaria vesca			X	
Galeopsis segetum			X	X
Galium aparine			X	
Geranium molle				X
Glaucium flavum				X
Hedera helix				X
Helianthus annuus				X
Hemerocallis species	X		X	X
Holcus lanatus			X	X
Hordeum murinum				X
Hypomea learii				X
Impatiens balfouri				X
Iris germanica				X
Lactuca serriola			X	
Lamium album				X
Lamium amplexicaule			X	X
Lapsana communis	X		X	X
Lolium perenne				X
Lunaria annua				X
Lycopsis arvensis			X	X
Lysimachia arvensis				X
Malva neglecta			X	
Matricaria discoidea			X	X
Medicago lupulina				X
Melilotus albus				X
Melissa officinalis				X
Mercurialis annua				X
Minuartia hybrida				X
Nigella damascena				X
Oenothera biennis		X		
Oryza sativa 'Azucena'		X		
Oryza sativa 'IAC165'				X
Oxalis corniculata				X
Panicum capillare				X
Panicum miliaceum				X
Papaver rhoeas				X
Parietaria judaica				X
Parthenocissus inserta				X
Phalaris arundinacea	X			
Phleum pratense				X
Picris hieracioides		X		
Pisum sativum 'Kayanne'			X	X
Plantago lanceolata			X	X
Plantago major				X

Platycapnos spicata				X
Poa annua	X			
Poa pratensis	X			X
Poa trivialis				X
Polygonum aviculare				X
Portulaca oleracea			X	X
Potentilla reptans		X		
Prunus persica 'GF'		X		
Quercus suber				X
Ranunculus repens				X
Rubus species	X			
Schedonorus arundinaceus			X	X
Senecio vulgaris				X
Setaria italica				X
Setaria verticillata				X
Silene latifolia				X
Silene vulgaris				X
Solanum lycopersicon				X
Solanum nigrum			X	X
Sonchus oleraceus				X
Sorghum bicolor				X
Sorghum halepense			X	X
Stellaria media				X
Tanacetum parthenium			X	X
Taraxacum officinale				X
Torilis nodosa			X	X
Trifolium repens	X			
Trisetum flavescens				
Triticum aestivum			X	X
Urtica dioica				X
Urtica urens			X	
Verbascum nigrum				X
Verbascum sinuatum				X
Verbena officinalis				X
Veronica arvensis			X	X
Veronica hederifolia			X	X
Veronica persica				X
Vicia faba			X	X
Vinca major			X	X
Vinca minor			X	X
Viola odorata				X
Vulpia myuros		X		X
Zea mays 'Palqui'				

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429 **Table 2:** Abbreviations, significance, units and extreme values of the different traits (one value per  
 430 species was estimated for each trait)

431

Parameter abbreviation	Significance	Unit	Range
$D_{\min}$	Minimal diameter (quantile 2%)	mm	0.044 - 0.45
$D_{\max}$	Maximal diameter (quantile 100%)	mm	0.24 – 2.6
Drange	Relative diameter range $2.0 \cdot (D_{\max} - D_{\min}) / (D_{\max} + D_{\min})$	---	0.89 - 1.9
IBD	Inter-branch distance on the thick roots (with a diameter above the middle value)	mm	0.51 – 7.0
DIDm	Slope of the regression of lateral diameter versus mother diameter (regression passing through the point $(D_{\min}, D_{\min})$ )	---	0.062 - 0.39
VarD	Coefficient of variation of the diameter of laterals	---	0.099 – 0.41

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