

A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals

Sixtine Passot, Beatriz Moreno-Ortega, Daniel Moukouanga, Crispulo Balsera, Soazig Guyomarc'H, Mikael Lucas, Guillaume Lobet, Laurent Laplaze, Bertrand Muller, Yann Guédon

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A new phenotyping pipeline reveals three types of lateral roots and a 12

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31 **One sentence summary**

- 32 Lateral roots in both pearl millet and maize can be categorized into three types based
- 33 on growth rate profiles.

Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists. All rights reserved. G., Laplaze, L., Muller, B Copyright © 2018 American Society of Plant Biologists All rights reserved. of lateral roots and a random reserved.

35 Author contributions

36 S.P. contributed to design, ran the pearl millet experiments, analyzed the data and 37 wrote the manuscript; B.M.-O. contributed to design, ran the maize experiments, 38 analyzed the data and wrote the manuscript; D.M. performed histological sections for 39 pearl millet and maize and contributed to the pearl millet experiment; C.B. 40 manufactured the rhizotrons and contributed to experiments; S.G. and M.L. 41 contributed to pearl millet experiments; G.L. updated SmartRoot and added specific 42 functions for this study; L.L. contributed to the design of the study and wrote the 43 manuscript; B.M. designed the study and wrote the manuscript; Y.G. designed and 44 implemented the statistical models, analyzed the data and wrote the manuscript.

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60 Abstract

Recent progress in root phenotyping has focused mainly on increasing throughput for 61 62 genetic studies while identifying root developmental patterns has been comparatively 63 underexplored. We introduce a new phenotyping pipeline for producing high-quality spatio-temporal root system development data and identifying developmental patterns 64 65 within these data. The SmartRoot image analysis system and temporal and spatial statistical models were applied to two cereals, pearl millet (Pennisetum glaucum) and 66 67 maize (Zea mays). Semi-Markov switching linear models were used to cluster lateral 68 roots based on their growth rate profiles. These models revealed three types of lateral 69 roots with similar characteristics in both species. The first type corresponds to fast and 70 accelerating roots, the second to rapidly arrested roots, and the third to an intermediate 71 type where roots cease elongation after a few days. These types of lateral roots were 72 retrieved in different proportions in a maize mutant affected in auxin signaling, while 73 the first most vigorous type was absent in maize plants exposed to severe shading. 74 Moreover, the classification of growth rate profiles was mirrored by a ranking of 75 anatomical traits in pearl millet. Potential dependencies in the succession of lateral root 76 types along the primary root were then analyzed using variable-order Markov chains. 77 The lateral root type was not influenced by the shootward neighbor root type or by the distance from this root. This random branching pattern of primary roots was 78 79 remarkably conserved, despite the high variability of root systems in both species. Our 80 phenotyping pipeline opens the door to exploring the genetic variability of lateral root 81 developmental patterns.

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84 Introduction

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Plant breeding has long ignored the belowground part of the plant but it is now 86 87 acknowledged that the root system represents an opportunity for improving plant 88 efficiency and tolerance to abiotic stresses (Bishopp and Lynch, 2015). A better 89 knowledge of root system structure and function is thus needed for root system 90 oriented crop improvement. Phenotyping, as the evaluation of heritable plant traits in a 91 given environment and in a reproducible manner, is one key approach to extend this 92 knowledge. Recent progresses in plant phenotyping platforms, including plant 93 handling automation and computer assisted data acquisition, have allowed an increase 94 in phenotyping throughput (i.e. the number of plants analyzed; Fahlgren et al., 2015b) which is critical for association studies and gene discovery. Besides increasing 95 96 throughput, another strategy chosen in some phenotyping platforms is to improve data 97 dimensionality and structure (Dhondt et al., 2013). In these platforms, the amount of 98 data collected on a single plant is increased, either by measuring several traits that can 99 be of different natures or by measuring the same trait at successive time points to focus 100 on physiological processes (Fahlgren et al., 2015a).

101

102 The phenotyping of root systems presents specific challenges compared to the 103 phenotyping of aerial parts of plants. The root system is by nature hidden and root 104 phenotyping platforms have to make a compromise between the relevance of growth 105 conditions and trait measurement feasibility. Most root phenotyping platforms focus 106 on measurements at high throughput of selected root traits on a large number of plants, 107 with the objective of detecting quantitative trait loci (OTL) usable in breeding 108 (Kuijken et al., 2015). For example, Atkinson et al. (2015) reported a phenotyping 109 platform where root systems grow in 2D on a filter paper for a few days. Platforms 110 where root systems grow in 3D have also been developed (Iver-Pascuzzi et al., 2010) 111 and used for QTL detection (Topp et al., 2013). Most of these platforms generate traits 112 that give a global view of the root architecture. By contrast, the development of 113 individual roots during long periods of time is rarely studied, whereas temporal 114 analyses are more developed for the aerial parts (see e.g. Lièvre et al., 2016). These 115 studies have been hampered by the difficulty of collecting individual root growth data.

Passot, S., Moreno-Ortega, Comment citer of document - Published by www.plantphysiol.org G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), In addition, the analysis of structured data such as root growth rate profiles is morechallenging than the analysis of simple root traits.

118

119 The variability of lateral root length along a primary root is a widely observed feature 120 of root systems (reviewed by Forde (2009)), having been reported for annual dicots 121 (e.g. sunflower (Helianthus annus), Aguirrezabal et al., 1994), annual monocots (e.g. 122 maize (Zea mays), Jordan et al., 1993; Varney et al., 1991; Wu et al., 2016), and 123 perennials (oak (Quercus robur), Pagès, 1995; rubber tree (Hevea brasiliensis), Thaler 124 and Pagès, 1996; banana (Musa acuminata), Lecompte et al., 2005). It has also been 125 observed in the model species Arabidopsis (Arabidopsis thaliana) (Freixes et al., 126 2002). Although variability in length likely originates from variability in growth rate, 127 most of these descriptions do not consider growth dynamically. When they do (Pagès, 128 1995; Thaler and Pagès, 1996), they generally consider that the variability of growth 129 rate profiles forms a continuum but do not investigate a possible structuring into 130 distinct classes. Nevertheless, different lateral root types have been described, in 131 particular in cereals, but these classifications are based on anatomical traits or 132 diameter. Accordingly, three to four lateral root types have been reported in maize 133 (Varney et al., 1991), three in pearl millet (Pennisetum glaucum) (Passot et al., 2016) 134 and rice (Oryza sativa) (Gowda et al., 2011; Henry et al., 2016), and five in wheat 135 (Triticum aestivum), barley (Hordeum vulgare), and triticale (Triticale hexaploide) 136 (Watt et al., 2008).

137

138 The variability of lateral root growth is likely an important lever of root system 139 efficiency (Forde, 2009; Pagès, 2011). In order to provide an efficient frame to study 140 this phenomenon and to characterize genetic and/or environmental impacts on that 141 variability, we designed a phenotyping pipeline for producing high-quality spatio-142 temporal root system development data with a focus on lateral roots. This pipeline 143 combines the SmartRoot image analysis system (Lobet et al., 2011), which is able to 144 reconstruct consistent spatio-temporal data on the basis of successive snapshots of root 145 system architecture, with temporal and spatial statistical models. We used it to 146 characterize the early developmental patterns of root systems in two cereals, pearl 147 millet and maize. In a first step, lateral root growth rate profiles were analyzed using 148 semi-Markov switching linear models; see Lièvre et al. (2016) for another application

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Passot, S., Moreno-Ortega, Comment citer ce document : Downloaded from on May 15, 2018 - Published by www.plantphysiol.org G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 - DOI: 10.1104/pp.17.01648 149 of similar statistical models. This model-based clustering of growth rate profiles led us 150 to identify three types of lateral root. We also applied this clustering approach to the 151 rootless concerning crown and seminal roots (rtcs) maize mutant affected in auxin 152 signaling and to maize plants exposed to severe shading. We then investigated the 153 relationships between lateral root types and morphological (apical diameter profile in 154 maize) and anatomical (stele diameter and central xylem tracheary element diameter in 155 pearl millet) traits. In a second step, potential dependencies in the succession of lateral 156 root types along the primary root were analyzed using variable-order Markov chains 157 leading to a precise characterization of the primary root branching pattern. Our 158 phenotyping pipeline opens the door to a quantitative, model-assisted characterization 159 of developmental patterns of lateral roots to support root system oriented crop 160 improvement.

161

162 **Results**

163

Daily images of growing root systems were recorded for 15 and 21 days in a rhizotron 164 165 system to analyze early root system development and architecture, in pearl millet and 166 maize respectively. These temporal limits were imposed by the root systems reaching 167 the bottom of the rhizotron. The difference between the two species was a consequence 168 of a higher average elongation rate in pearl millet compared to maize. The ability of 169 SmartRoot (Lobet et al., 2011) to cross-link information corresponding to different 170 time points was then used to build consistent spatio-temporal data of root system 171 development and architecture on the basis of the corresponding series of images. We 172 chose to decompose the analysis of these spatio-temporal data into two steps:

173 1. *temporal analysis*: we first analyzed growth rate profiles of lateral roots using semi174 Markov switching linear models. Lateral roots were classified into types as a
175 byproduct of this longitudinal data analysis.

176 2. *spatial analysis*: The intervals between consecutive lateral roots and the succession

- 177 of lateral root types along the primary root were then analyzed.
- 178

179 A model-based clustering of lateral root growth rate profiles reveals three classes

180 of lateral root in pearl millet and maize

181 The dataset was composed of growth rate profiles of 1254 lateral roots from 8 plants in 182 pearl millet and of 3050 lateral roots from 13 plants in maize. The exploratory analysis 183 of these growth rate profiles highlighted a strong longitudinal organization with 184 growth rates either increasing or decreasing with lateral root age (Fig. 1). The growth rate profiles are essentially divergent after root emergence and the growth rate 185 dispersion increases with the root age. Hence, lateral roots can be roughly ordered 186 according to their growth rate profiles. This raised the question of a stronger 187 188 structuring of these longitudinal data beyond a simple ordering of lateral root growth 189 rate profiles. We thus chose to investigate a model-based clustering of these 190 longitudinal data. This raised two types of difficulties: (i) the growth rate profiles were 191 short and highly variable among lateral roots (1 to 10 and 1 to 17 successive growth 192 rates for pearl millet and maize, respectively) and (ii) a high proportion of lateral roots 193 were still growing the last date of measurement. We thus designed a statistical model 194 for clustering growth rate profiles, using only profiles lasting at least 5 days, based on 195 the following assumptions: (i) a growth rate profile is modeled by a single growth 196 phase either censored (to take into account lateral roots still growing the last date of 197 measurement) or followed by a growth arrest and (ii) changes in growth rate within a 198 growth phase are modeled by a linear trend. This strong parametric assumption is a 199 consequence of the short length of growth rate profiles. Hence, linear trend models 200 should be viewed as instrumental models for clustering growth rate profiles and not as 201 models for fitting accurately each growth rate profile.

202

203 The proposed statistical model is composed of growth states, each corresponding to a 204 lateral root growth rate profile type. A distribution representing the growth phase 205 duration (in days) and a linear model representing changes in growth rate during the 206 growth phase are associated with each of these growth states. Growth states are 207 systematically followed by a growth arrest state. The overall model is referred to as a 208 semi-Markov switching linear model (SMS-LM; see Materials and Methods and 209 Supplemental Methods S1 for a formal definition and Fig. 2 and Supplemental Fig. 210 S1 for pearl millet and maize SMS-LMs, respectively). The number of states of a 211 SMS-LM is thus the number of lateral root types plus one (the single growth arrest 212 state). This kind of integrative statistical model makes it possible to estimate growth 213 phase duration distributions combining complete and censored growth phases. The

Passot, S., Moreno-Ortega, Comment citer ce document - Published by www.plantphysiol.org G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new pneriotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 - DOI: 10.1104/pp.17.01648 214 mechanism associated with a SMS-LM can be described as follows: A growth state is 215 randomly selected according to an initial distribution. This initial distribution 216 represents the lateral root type proportions. A growth phase duration is then randomly 217 selected according to the corresponding distribution of the selected growth state. The 218 growth rate then changes with time according to the linear trend model associated with 219 the selected growth state until the end of the growth phase and the transition to the 220 growth arrest state. In such SMS-LM, the transitions from the growth arrest state to a 221 growth state are not possible and each state can be visited at most once (see illustration 222 in Fig. 2 and Supplemental Fig. S1).

223

224 We next had to select the number of growth states (i.e. the number of lateral root 225 types). Because of the specific structure of the model where each state can be visited at 226 most once, the usual model selection criteria such as the Bayesian information 227 criterion do not apply. We thus designed an empirical model selection method for 228 selecting the number of growth states which is detailed in Supplemental Methods S2 229 and illustrated by Supplemental Tables S1 and S2 for pearl millet and maize, 230 respectively. We selected for both species three lateral root types that correspond to the 231 best compromise between (i) the proportion of unambiguously assigned lateral roots 232 (between-cluster criterion) and (ii) the relative dispersion of growth rate profiles 233 particularly for the most vigorous root type (within-cluster criterion). Having two 234 classes only would dramatically increase the dispersion for the most vigorous root type 235 (Supplemental Fig. S2 for pearl millet) whereas having four classes would increase the 236 proportion of ambiguously assigned roots (Supplemental Fig. S3). Hence, clustering of 237 lateral roots based on their growth rate profiles revealed three lateral root types in both 238 pearl millet and maize. These three types ordered in decreasing vigor will be referred 239 to as A, B and C.

240

241 Growth phases are similar in both species

The estimated growth phase duration distributions are similar for each lateral root type between pearl millet and maize (Table 1; Fig. 3). The censoring level is defined as the proportion of growth phase incompletely observed for a given lateral root type (the corresponding lateral roots were still growing the last date of measurement). The censoring level is high for type A, intermediate for type B and rather low for type C (Table 1). The higher censoring level for pearl millet compared to maize is a direct consequence of the shorter average growth rate profiles for pearl millet (in relation to the faster root growth in this species) since the growth phase duration distributions are similar for the two species. It should be noted that the length of the growth rate profiles (i.e. the number of successive growth rates) are similar for the different lateral root types of a given species (Supplemental Fig. S4) suggesting no preferential location of the lateral roots of the different types along the primary root.

254

255 Comparison of growth rate profiles between the two species

256 Only growth rate profiles of length ≥ 5 (corresponding to 652 lateral roots of 257 cumulative length 4367 for pearl millet and 2029 lateral roots of cumulative length 258 17257 for maize) were used for the building of SMS-LMs. Once a SMS-LM was built, 259 the growth rate profiles belonging to the learning sample were assigned to lateral root 260 types (see Fig. 1A for examples of clustering of lateral roots in pearl millet and Fig. 1B 261 for examples of clustering of lateral roots in maize). The posterior probabilities of the 262 optimal assignment of growth rate profiles of length ≥ 5 to lateral root types (i.e. 263 weights of the optimal assignment among all the possible assignments) were most 264 often high – 92% above 0.8 and 88% above 0.9 for pearl millet (Supplemental Fig. S3A) and 94% above 0.8 and 81% above 0.8 for maize (Supplemental Fig. S3B) -265 266 indicating a clear between-cluster separation; see Supplemental Methods S2. Growth 267 rate profiles of length < 5 (test sample corresponding to 602 lateral roots of cumulative 268 length 1945 for pearl millet and 1021 lateral roots of cumulative length 2958 for 269 maize) were also assigned to lateral root types using the previously estimated SMS-270 LM. The posterior probabilities of the optimal assignment of these growth rate profiles 271 to lateral root types were most often high despite the limited information conveyed by 272 these profiles: 83% above 0.8 and 73% above 0.9 for pearl millet (Supplemental Fig. 273 S5A) and, 85% above 0.8 and 68% above 0.9 for maize (Supplemental Fig. S5B). 274 These independent assignments constitute clear elements of validation of the clustering 275 assumption. In order to assess the separation of lateral root types during growth, we 276 compared the classification accuracy between growth rate profiles (whatever their 277 length) truncated at successive lengths. Growth rate profiles truncated at length 1, 2, 3 278 and 5, and untruncated growth rate profiles were assigned to lateral root types (Fig. 4). 279 The posterior probabilities of the optimal assignment of truncated growth rate profiles

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280 show that the classification accuracy improves rapidly the very first days of growth 281 before stabilizing around day 5 consistently with the divergence of growth rate profiles 282 after root emergence (Fig. 1). In both species, daily median growth rates (computed 283 from all the growth rate profiles whatever their length) are divergent between the three 284 types of lateral roots (Fig. 5 A,B). Type A median growth rate stays positive at all ages 285 in both species. Type B median growth rate reaches zero at day 8 in pearl millet and at 286 day 6 in maize while type C median growth rate reaches zero at day 3 in both species. 287 The main differences between the two species, apart from differences in growth rates, 288 concern (i) type A lateral roots for which median growth rate continues to increase in 289 pearl millet whereas it stays nearly constant after a few days in maize and (ii) type B 290 lateral roots for which median growth rate stays nearly constant up to day 5 in pearl 291 millet whereas it starts to decrease immediately after emergence in maize. Dispersions 292 in growth rate profiles are rather similar between the two species for types B and C; 293 see the mean absolute deviation profiles in Fig. 5 A,B. A regular increase in mean 294 absolute deviation with root age can be observed for type A lateral roots in maize. This 295 may be due to the mixing at the later ages within this class of lateral roots whose 296 growth rate started to decrease with lateral roots whose growth rate continued to 297 increase.

298

The apical diameter profiles partially match the different root types identified inmaize

301 The optimal assignment of lateral roots to types computed using the estimated SMS-302 LM was used to analyze the link between growth rate types and root apical diameter in 303 maize (the lower apical diameter of lateral roots combined with the image resolution 304 did not allow this analysis in pearl millet). Apical diameter profiles (Fig. 5C) clearly 305 distinguish type A (higher diameters) from type B and C lateral roots but not type B 306 from type C lateral roots (see the overlaps between apical diameter distributions for the 307 successive ages in Supplemental Table S3). Apical diameter gradually decreases with 308 root age for types B and C and converges towards median apical diameter around 309 230 µm. This corresponds to a high proportion of arrested roots and suggests the 310 occurrence of a threshold value for the apical diameter, below which roots will 311 ultimately stop growing.

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Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists. All rights reserved.¹, G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 DOI: 10.1104/op.17.01648

313 Linking lateral root growth rate profile with anatomy in pearl millet

314 Previous studies have shown that different lateral root types can be defined based on 315 their anatomy in pearl millet (Passot et al., 2016). To explore the links between root 316 growth and root anatomy, we observed cross sections in 35 pearl millet lateral roots 317 with contrasting growth rate profiles. Lateral roots were assigned to one of the three 318 types previously defined, based on their growth rate profile. Stele diameter and central 319 xylem tracheary element (XTE) diameter, previously shown to be contrasting among 320 individual roots in pearl millet (Passot et al., 2016) were measured. The classification 321 of growth rate profiles in types A, B and C is mirrored by a ranking of both stele 322 diameter and central XTE diameter, although there is some overlap between types 323 (Fig. 6). This is confirmed by the high Spearman's rank correlation coefficients between anatomical traits and lateral root types for pearl millet ($\rho = 0.81$ between 324 325 stele diameter and lateral root types and $\rho = 0.91$ between central XTE diameter and 326 lateral root types).

327

The definition of lateral root types is neither affected by a mutation altering auxin signaling nor by a shading treatment in maize

330 One of the aims of this study was to provide a pipeline of analysis able to account for 331 genetic and environmental effects. We thus analyzed the growth rate profiles of lateral 332 roots of a maize mutant with altered auxin signaling (rtcs mutant; RTCS encodes a 333 LOB-domain transcription factor and carries auxin-responsive elements in its 334 promoter; Taramino et al., 2007) and of wild-type maize plants exposed to severe 335 shading. The datasets were composed of growth rate profiles of 1597 lateral roots from 336 9 rtcs mutant plants, and of 572 lateral roots from 6 shaded plants. Growth rate profiles 337 of 994 lateral roots of length \geq 5 (length up to 14 and cumulative length of 7022) were 338 used for the building of SMS-LMs for the rtcs mutant. Growth rate profiles of 540 339 lateral roots of length \geq 5 (length up to 12 and cumulative length of 4129) were used 340 for the building of SMS-LMs for shaded plants. Applying the empirical model 341 selection procedure previously described, we obtained three lateral root types for the 342 rtcs mutant and two for the shaded plants corresponding to types B and C identified in 343 unshaded wild-type plants. The posterior probabilities of the optimal assignment of growth rate profiles of length \geq 5 to lateral root types, using the SMS-LMs previously 344 estimated on the basis of these learning samples, were most often high -91% above 345

Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists: All rights reserved t, G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 DOL: 10.1104/pp.17.01548

0.8 and 87% above 0.9 for the rtcs mutant, and 93% above 0.8 and 80% above 0.9 for 346 347 the shaded plants – indicating a clear between-cluster separation. Growth rate profiles 348 of length < 5 (test sample corresponding to 603 lateral roots of cumulative length 349 1499) were also assigned to lateral root types using the SMS-LM previously estimated for the *rtcs* mutant. The posterior probabilities of the optimal assignment of these 350 351 growth rate profiles to lateral root types were most often high despite the limited 352 information conveyed by these profiles: 85% above 0.8 and 50% above 0.9. These 353 independent assignments constitute clear elements of validation of the clustering 354 assumption for the *rtcs* mutant. The results of independent assignments are not 355 reported for the shaded plants since there were only 32 growth rate profiles of 356 length < 5 in this case. Both for the *rtcs* mutant and the shaded plants, the growth rate 357 profiles were assigned to lateral root types using both the SMS-LM built on the basis 358 of these lateral roots and the SMS-LM built on the basis of the unshaded wild-type 359 plants. We obtained a match of 94% (938 lateral roots among 994) between the two 360 independent assignments for the rtcs mutant and a match of 91% (492 lateral roots 361 among 540) for the shaded plants indicating that the lateral root types are robustly 362 defined. The estimated growth phase duration distributions are similar for each lateral 363 root type between the *rtcs* mutant and the wild-type SMS-LM as well as between the shaded and the unshaded SMS-LM for types B and C (Fig. 7, Table 1). The median 364 365 growth rate profiles are only slightly affected by the mutation and shading treatment (Fig. 8A, Supplemental Figs. S6 and S7). In summary, the definition of lateral root 366 367 types remains robust irrespective of the genotype or the environmental treatment. Differences with the control wild type concerned (i) the absence of type A lateral roots 368 369 for the shaded plants, (ii) the higher proportion of type B lateral roots compensated by 370 a lower proportion of type C lateral roots for the *rtcs* mutant and a higher proportion of 371 type C lateral roots for the shaded plants (Table 2). Results concerning the shaded 372 plants are consistent with the expectation of an inhibition of root growth in relation 373 with a restriction in the supply of carbohydrates. Moreover, we could observe (iii) 374 larger diameters compared to wild type for all root types in the *rtcs* mutant and for type 375 C in the shaded plants (Fig. 8B) and (iv) a hierarchy in apical diameter profiles 376 between types B and C for the *rtcs* mutant whereas type B and C apical diameter 377 profiles are confounded in both wild-type and shaded plants (see Fig. 8B and 378 Supplemental Table S3).

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380 Identification of a stationary random primary root branching pattern

381 In order to characterize the primary root branching pattern in both pearl millet and 382 maize, we first analyzed the length of the intervals between consecutive lateral roots 383 and then, the potential dependencies between successive lateral root types (A, B and 384 C) along the primary root. We first evaluated the impact of the root type on the length 385 of the interval between a lateral root and its nearest neighbor in the rootward direction. 386 No difference was found between the mean interval length for the three root types in 387 both species (ANOVA, p-value = 0.83 and 0.33 for pearl millet and maize, 388 respectively; see Table 3). The same type of analysis was conducted splitting intervals 389 into 9 groups, depending on the types of the two lateral roots delimiting the interval 390 (Supplemental Tables S4 and S5 for pearl millet and maize, respectively). No 391 effect of the lateral root types was found on the interval lengths (ANOVA, p-392 value = 0.52 and 0.15 for pearl millet and maize, respectively). Hence, there is no 393 influence of the root types on the length of the interval between two consecutive lateral 394 roots.

395

396 We then analyzed the potential dependencies within lateral root type sequences 397 ignoring the length of the intervals between consecutive lateral roots (the branching 398 sequences from the collar rootward were thus simply indexed by the rank of the 399 successive lateral roots along the primary root). We first computed the Spearman rank 400 autocorrelation function for these sequences (the lateral root type can be considered as 401 a categorical ordinal variable with the three possible ordered categories A, B and C). 402 This autocorrelation function measures the correlation between ordinal variables at 403 different distances apart. The autocorrelation function for positive lags is within the 404 confidence interval corresponding to the randomness assumption for most of the plants 405 (Supplemental Fig. S8 A-D), indicating that the distribution of the lateral root types 406 along the primary root was stationary and suggesting no marked dependencies between 407 successive lateral root types. This finding is consistent with the similar frequency 408 distributions of the length of growth rate profiles for the three lateral root types 409 (Supplemental Fig. S4). Since the length of a growth rate profile directly depends on 410 the time of emergence of the lateral root and is thus related to its position along the 411 primary root, this suggests that the proportions of the three lateral root types along the

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Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists: All rights reserved.^t, G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 866-910. DOI: 10.1104/pp.17.01648

412 primary roots were roughly stationary. For some individuals (one for pearl millet and 4 413 for maize), the autocorrelation function fluctuates around the positive confidence limit 414 (Supplemental Fig. S8 E,F) indicating a slight non-stationarity. We further investigated 415 primary root branching sequences applying a statistical modeling approach. To this 416 end, we modeled potential dependencies between successive lateral root types along 417 the primary roots. Three-state variable-order Markov chains, each state corresponding to a lateral root type, were built. The memories of variable-order Markov chains were 418 419 selected (Csiszár and Talata, 2006) for each primary root branching sequence and for 420 samples of branching sequences corresponding to each species. For all plants and for 421 both species, a zero-order Markov chain was selected. This confirmed that the type of 422 a lateral root was independent of the type of the previous lateral roots. Hence, our 423 results indicate that there is no influence of the lateral root growth pattern on the 424 distance to or on the growth pattern of the next lateral root in pearl millet and maize.

425

426 Inter-individual variability of the branching pattern

427 We evaluated the variability of the length of the interval between consecutive lateral 428 roots and of the proportions of lateral root types among individual plants for each species. The mean interval lengths were not equal in all plants (ANOVA, 429 $p < 10^{-5}$ for pearl millet and $p < 10^{-6}$ for maize). Plants were thus classified according 430 to Tukey's honest significant difference test. Two overlapping groups were found, 431 432 both for pearl millet and maize (Supplemental Fig. S9), with average interval length 433 ranging from 0.21 to 0.31 cm in pearl millet, and from 0.14 to 0.25 cm in maize. 434 Significant differences among plants were also found for lateral root type proportions both for pearl millet and maize (Kruskal-Wallis test, $p < 10^{-10}$ and $p < 10^{-15}$ 435 respectively, Fig. 9). For pearl millet, the 8 plants were separated into three groups 436 437 with two groups overlapping and the type A lateral root proportion ranging from 0.06 438 to 0.21. The 13 maize plants were separated into 6 groups, with some overlaps 439 between groups and the type A lateral root proportion ranging from 0 to 0.2. These 440 results indicate that both species show significant between-individual differences in 441 terms of length of intervals between consecutive lateral roots and lateral root type 442 proportions. These differences were neither related to plant biomass, seed biomass, nor 443 to total root system length (results not shown). However, despite differences between

Passot, S., Moreno-Ortega, Comment citer ce document - Published by www.plantphysiol.org G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 - DOI: 10.1104/pp.17.01648 plants in terms of lateral root type proportions, the stationary random branching patternis markedly conserved in all plants.

446

447 **Discussion**

448

449 A new longitudinal data analysis approach to identify lateral root types based on 450 growth rate profiles

451 In this study, we designed a pipeline for analyzing lateral root growth rate profiles and primary root branching pattern and applied it to explore the diversity of lateral roots in 452 453 two cereals, maize and pearl millet. Previous efforts to classify lateral roots in cereal 454 species have been reported (Varney et al., 1991; Watt et al., 2008; Rebouillat et al., 455 2009; Henry et al., 2016; Passot et al., 2016) but these classifications were often based 456 on anatomical traits, mainly root diameter and vasculature. A first difficulty comes 457 from the fact that some morphological traits change along lateral roots, typically root 458 diameter (Wu et al., 2016), which was confirmed in our maize data. A different 459 classification method, based on growth rates, was reported in rice (Rebouillat et al., 460 2009) for which growth rates were highly contrasting among lateral roots but 461 assignment to classes was based on expert knowledge. In oak, lateral roots were 462 classified based on empirical thresholds applied to individual growth rates (Pagès, 463 1995). Here we assigned lateral roots to classes based on their growth rate profiles 464 using dedicated statistical models. A strength of these statistical models is the 465 capability to optimally combine complete and censored growth rate profiles since some lateral roots were still growing at the end of the experiment. Our approach 466 467 revealed three similar types of lateral roots in two different cereal species. In previous 468 studies, three anatomical types of lateral roots were identified in maize (Varney et al., 469 1991; Moreno-Ortega et al., 2017) and pearl millet (Passot et al., 2016) and our results 470 in pearl millet confirm that these types are well related to the types obtained from our 471 model-based clustering of growth rate profiles. In maize, the root apical diameter 472 profiles were at least partly linked to growth rate profiles, confirming a behavior 473 already seen in other species such as oak or rubber tree (Pagès, 1995; Thaler and 474 Pagès, 1996) where diameter and growth rate change in parallel. Taken together, our 475 results thus suggest that these changes observed at the macroscopic scale are at least 476 partly linked to temporal changes in root anatomy and vasculature.

15

Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists: All rights reserved^t, G., Laplaze, L., Muller, B., Guedon, T. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 DOI: 10.1104/pp.17.01648 478 Variability between plants is known to be particularly high for root systems 479 (Williamson et al., 2001; Ashehoug and Callaway, 2014) and this, in principle, 480 hampers our capacity to identify patterns within a given architecture. Our classification 481 was sufficiently robust to accommodate the high inter-individual variability among 482 replicate plants within the same experimental set-up. We failed to identify the sources 483 of such variability since neither seed mass, nor leaf area were able to account for 484 differences among individuals in line with the idea that inter-individual variability 485 could be an intrinsic property of root systems (Forde, 2009). Our classification was 486 also sufficiently robust to accommodate genetic (mutation in auxin signaling) and 487 environmental variation (severe shading) showing that these sources of variation 488 translate into altered proportions of lateral root types, not into a redefinition of each 489 type. The strong reduction of mean lateral root length upon shading was expected 490 (Freixes et al., 2002; Muller et al., 1998) but our analysis revealed that this effect was 491 the result of the complete disappearance of vigorous, fast growing lateral roots and a 492 large increase in the proportion of lateral roots showing a rapid growth cessation. A 493 mutation in auxin signaling which controls the formation of nodal roots (Hetz et al., 494 1996) only had a limited impact on the proportion of the three lateral root types 495 whereas it had great impact on lateral root diameter. This suggests that our pipeline of 496 analysis can be used to identify and characterize growth patterns within complex and 497 variable architectures and to evaluate related functional hypotheses such as the impact 498 of hormone signaling (De Smet et al., 2003; Lavenus et al., 2013) or carbohydrate 499 availability (Bingham and Stevenson, 1993).

500

501 Origin and fate of the three lateral root types

502 The identification of three lateral root types raises questions about their origin during 503 development. In rice, fast-growing lateral roots are also thicker and additional 504 periclinal cell divisions in the endodermal cell layer producing additional mesodermal 505 cell layers during the process of primordia establishment have been reported in these 506 large lateral roots (Rebouillat et al., 2009). Variability in size of different lateral root 507 primordia has been reported in maize (MacLeod, 1990) and could account for 508 differences in apical diameter, at least at emergence. Accordingly, it has been proposed 509 that lateral root variability would be determined early in development and would be

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Passot, S., Moreno-Ortega, Comment citer ce document : Downloaded from on May 15, 2018 - Published by www.plantphysiol.org G., Laplaze, L., Muller, B., Guedon, Y. (2018) American Society of Plant Biologists: All rights reserved: of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 510 tightly associated with morphology and anatomy (Thaler and Pagès, 1996). The 511 relationship between root anatomy (stele and central XTE diameter) and types based 512 on growth rate profile, evidenced in pearl millet, goes in this direction. In maize, 513 lateral root types could also be well defined based on stele and vessel diameters 514 (Varmey et al., 1991) and it will be necessary to explore the match between anatomy 515 and growth patterns in this species. Root diameters at emergence are also ranked 516 according to growth rate profiles in maize but this ranking is not strict as shown by the 517 large overlap of apical diameters between lateral root types. Another possibility is that 518 growth rate variability would be determined after emergence in order to adapt in a 519 more plastic manner to the local environmental conditions the roots encounter. Such 520 plasticity is commonplace in root systems and lateral roots are the most dynamic place 521 for this plasticity (Drew, 1975; Farrar and Jones 1986). The parallel between apical 522 root diameter profiles and growth rate profiles in maize is also in favor of a 523 progressive assignment of roots into one of the three types. These two hypotheses may 524 not be exclusive and growth patterns may result from a combination of these two 525 influences that would occur pre- and post-emergence. Factors influencing initial 526 growth rate, growth maintenance and growth arrest could also be different, therefore 527 rendering the picture more complex and leading to developmental patterns of lateral 528 roots globally more plastic to face a variability of external and internal clues (Malamy, 529 2005). Interestingly, we showed recently that the typology of growth patterns found in 530 the present study, matches a typology of meristem length being far longer in type A 531 lateral roots compared to type B lateral roots (mean of 450 µm and 280 µm, 532 respectively) and lacking in type C lateral roots (Moreno-Ortega et al., 2017). 533 Moreover, meristem length variation was associated with a variation of the length of 534 the elongation zone suggesting that growth variation is the result of coordinated 535 variations in both cell production and cell expansion (Moreno-Ortega et al., 2017).

536

537 Which role for the three lateral root types?

The sharing of tasks between the three lateral root types could contribute to the overall root system efficiency in front of a changing and unpredictable environment. In maize, short roots (i.e. type C roots) with xylem differentiated down to the tip are suspected to facilitate water uptake (Varney and McCully, 1991; Wang et al., 1994). In rice, the presence of short, thin and abundant lateral roots has been interpreted functionally as

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Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists: All rights reserved t, G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 DOI: 10.1104/op.17.01648

543 'super root hairs' (Nestler et al., 2016), increasing the root surface area and nutrient 544 uptake, in particular for immobile ions such as phosphorus (P) while at the same time 545 having a low carbon cost as compared to long lateral roots. By contrast, long lateral 546 roots (i.e. type A roots) may contribute to widen the exploration in the horizontal 547 dimension beyond the limited horizon explored by nodal roots in opposition to 548 exploration in depth covered by the primary root. They also contribute to the build-up 549 of the overall root architecture since only long lateral roots participate in higher levels 550 of branching (Gowda et al., 2011). In perennials, only long lateral roots contribute to 551 the perennial structure of the plant (Coutts, 1987). The functional efficiency of the root 552 system may also be linked to the proportions of these different lateral root types. The 553 production of too many long lateral roots is likely counterproductive because of their 554 carbon cost, as well as competition among individual roots for the capture of mobile 555 soil resources such as water or nitrate (Lynch, 2018). Finally, the plasticity of the 556 proportions of these different lateral root types could also contribute to the efficiency 557 of the root system. In rice, the proportions of short and long lateral root are highly 558 plastic in response to low P and there exists some genetic variability of this plasticity 559 (Vejchasarn et al 2016). The role of type B lateral roots is more speculative but they 560 could constitute a trade-off between the two opposite strategies of type A and C lateral roots. They could also constitute a pool of potentially fast growing roots if conditions 561 562 are favorable. The added value of this overall variability and plasticity to enhance root 563 foraging capacity was already suggested (Forde, 2009) while its cost/benefit advantage 564 compared to more homogenous lateral root patterns was demonstrated using simulated 565 root systems (Pagès, 2011). In our case, the type A roots, the most expensive roots to 566 construct (because of their length and diameter), represented only 13.3% and 8.1% of 567 the lateral roots in pearl millet and maize, respectively. Thus, early growth cessation 568 could well be an important strategy to avoid an excessive cost of root system therefore 569 increasing the efficiency of each carbon molecule invested.

570

571 The positioning of the three lateral root types is random along the primary root

572 One benefit of the proposed approach is that it enables a model-based architectural 573 analysis. All lateral roots were assigned to types and precisely positioned along the 574 primary root. We showed that, both in pearl millet and maize, the longitudinal spacing 575 of lateral roots was highly variable, both within and between root systems. Our

Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists. All rights reserved to G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3),

analyses showed that there was no relationship between the length of the interval 576 577 between two successive lateral roots and the types of these lateral roots and that the 578 succession of lateral root types was random along the primary root. If confirmed, this 579 would tend to rule out the hypothesis of a negative signal emanating from e.g. fast 580 growing roots to prevent another fast growing root to grow in its neighborhood. 581 Moreover, the absence of relationship between lateral root spacing and growth rate 582 suggests that lateral root initiation and later development are regulated independently. 583 These various hypotheses could be challenged using mutants affected in some of the 584 hormonal (e.g., auxin or cytokinin)checkpoints associated with initiation (Lavenus et 585 al., 2013).

586

587 A new experimental design is required for studying the whole growth profile of 588 type A lateral roots

589 The experiment duration constrained by the rhizotron dimensions restricted 590 observations to the beginning of type A lateral root growth. Hence, most of the growth 591 rate profiles assigned to type A lateral roots were censored for both species. This 592 makes a marked difference with type B and C lateral roots for which the whole growth 593 profile, up to growth arrest, was observed for a large proportion of individuals. Hence, 594 it would be useful to design larger rhizotrons or to change the growth conditions in 595 order to study the whole growth of type A lateral roots and in particular the transition 596 from increasing or stationary growth rate to decreasing growth rate. In order to capture 597 such behavior, the proposed modeling framework can be directly extended by adding 598 states in series for modeling successive growth phases for type A lateral roots. Such 599 extension of semi-Markov switching models with states in series was recently 600 developed for modeling successive developmental phase in Arabidopsis rosettes in 601 Lièvre et al. (2016). We may expect a single state with decreasing growth rate 602 following the current increasing growth rate state A or an intermediate roughly 603 stationary growth state between the increasing and decreasing growth rate states. This 604 would shed light on the future of "indeterminate" lateral roots which is to date not 605 documented, contrary to the mechanisms associated with growth arrest (e.g. Varney 606 and McCully, 1991). In particular, it would be interesting to see if this future interferes 607 with the decay of primary root system reported in cereals, occurring for example 608 within two months in pearl millet (Maiti and Bidinger, 1981).

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 G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 DOI: 10.1104/pp.17.01648

An avenue for considering the diversity of lateral roots in future high-throughput phenotyping and genetic analyses

612 To date, genetic improvement based on structural features of the root system has 613 essentially concentrated on its overall architecture such as deep vs. shallow rooting 614 (Saengwilai et al., 2014) and on anatomy such as the presence of aerenchyma in maize 615 roots, which are suspected to decrease the carbon construction cost of roots without 616 affecting their function (Zhu et al., 2010). Lateral roots have been comparatively overlooked although they represent the best example of the overall structural plasticity 617 618 of the root system to face the variable and unpredictable nature of the soil environment 619 encountered (Drew, 1975). Therefore, there could exist a mine of genetic variation to 620 exploit (and not only in cereals) if relevant phenotyping methods for characterizing the 621 diversity of lateral roots were available. Depending on the environments for which 622 genotypes are bred, it could be worth favoring or limiting the variability of lateral fates 623 (Lynch 2018). By combining image analysis and statistical modeling, our phenotyping 624 pipeline is a step in this direction.

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626

627 Materials and Methods

628

629 Plant growth

630 Pearl millet (Pennisetum glaucum (L.) R. Br.) inbred lines LCICMB1 (Passot et al., 2016), maize (Zea mays) hybrid B73xUH007 (referred to as wild type) and rtcs 631 632 mutants in the B73 background (Taramino et al., 2007; kindly provided by Frank 633 Hochholdinger, University of Bonn, Germany) were used in this study. Root 634 observation boxes (rhizotrons) were built according to Neufeld et al. (1989). The size 635 of the frame was 40 cm x 70 cm so that they could be imaged with 2 contiguous A3 636 images using a scanner (Fig. 10). The root system was sandwiched against a plexiglass 637 surface by a layer of viscose that was impermeable to roots, but permeable to water 638 and nutrients. Rhizotrons were made of (back to front) a 5 mm thick extruded 639 polystyrene plate, a 2 cm layer of substrate, a layer of viscose and a 5 mm thick 640 plexiglass plate, all joined together using aluminum U frames held by screws. The substrate used was composed of 30% fine clay, 25% peat fibers, 5% blond peat and 641

Passot, S., Moreno-Ortega, Downloaded from on May 15, 2018 - Published by www.plantphysiol.org G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 - DOI: 10.1104/pp.17.01548 642 40% frozen black peat (Klasmann-Deilmann France SARL). The substrate was sieved
643 before using. The rhizotrons were weighed individually before and after filling to
644 determine the weight of substrate contained in each one and later to manage daily
645 irrigation.

646

Maize seeds were surface sterilized with 6% hypochlorite for five minutes and rinsed 647 in distilled water for one minute. Seeds were then germinated on moistened filter paper 648 649 in Petri-dishes (20 x 20 cm) and placed vertically in a growth chamber in the dark at 650 20°C. Pearl millet germination was performed with a similar protocol, except that 651 seeds were also cleaned with ethanol solution (70%) for 5 minutes after the first 652 rinsing and germination temperature was set to 30°C. Germinated seedlings with 653 similar primary root length were transferred individually in the rhizotrons. A layer of 654 wet sphagnum on the top of the rhizotrons maintained the seedlings and prevented 655 them from drying. Rhizotrons were placed in a growth room with climatic conditions 656 adapted to each species: a temperature of 28°C during the day and 24°C during the 657 night for pearl millet and a constant temperature of 20°C for maize, with a 14-hour-658 photoperiod for both species. Light was provided by 6 mercury lamps (HQI, 250 W, 659 Osram, Munich, Germany) and measured by a light sensor (SKP215, Skye Instruments, Llandrindod Wells, Powys, UK). In our conditions, photosynthetic photon 660 flux density (PPFD) was 300 µmol/m²/s. Temperature and air humidity were recorded 661 (HC2-SH, Rotronic, Bassersdorf, CH) for each growth room. The sphagnum was 662 663 watered twice a day at the beginning of the experiment and from 6 days after germination onward, rhizotrons were watered daily using a 1/10 Hoagland solution to 664 665 maintain the humidity of the substrate. The amount of watering was monitored by a 666 daily weighting of the rhizotron. In one experiment, plants were shaded by installing a 667 net above the plants that reduced light intensity by 75%.

668

669 Imaging and image processing

From the second day of growth, rhizotrons were scanned with an A3 scanner (Epson Expression 10000XL Pro, Japan) at 600 or 720 dpi. The histogram of the gray level intensities was adjusted to optimize the contrast on fine roots. As rhizotrons are twice the size of the scanner, two images (upper part and lower part of the rhizotron) were taken and aligned using Align_4 (http://www.mecourse.com/landinig/software/

Passot, S., Moreno-Ortega, Comment citer ce document Downloaded from on May 15, 2018 - Published by www.plantphysiol.org G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-910 DOL: 10.1104/pp.17.01648 software.html) to recover an image of the entire root system, thanks to landmarks
visible in both parts. These landmarks were either added intentionally on the rhizotron
or were naturally present (water drops, the root system itself).

678

The SmartRoot software (Lobet et al., 2011) was used to extract root system 679 680 architecture at successive dates as well as root growth parameters because it supports 681 time-lapse images and focuses on the analysis of individual root behavior. SmartRoot 682 needs images where roots appear darker than background. An ImageJ (Rasband, W.S., 683 U. S. National Institutes of Health, Bethesda, Maryland, USA) macro was developed 684 to automatically invert and adjust the contrast of the rhizotron images by scaling the 685 image intensity histogram on a fixed range. The optimal contrast (min and max values of the intensity range) was determined empirically to reduce the number of errors 686 687 when using the algorithm for automatic lateral root tracing provided by SmartRoot 688 using a subset of scan images, and was applied to the whole set of images using the 689 macro tool.

690

691 SmartRoot enables semi-automatic root tracing. The primary root was drawn on the 692 first image. For the next days, the root system traced on the previous day was imported and aligned, in such a way that the trace of the primary root elongated progressively, 693 694 using automatic tracing. Crown and lateral roots were added as they appeared, either 695 manually or using automatic detection. Their lengths increased progressively on the 696 successive scans as for the primary root. When all roots were traced, the data were 697 extracted with the batch export tool of SmartRoot. This tool provides several 698 measurements including the length, the insertion position and the diameter for each 699 root. The growth rates were directly extracted from successive length measurement. 700 Because the resolution was not sufficient for pearl millet lateral roots, we only 701 considered root diameter for maize. On average, it took about two days to process the 702 data of one plant.

703

704 **Correction of growth rate profiles**

In spite of manual supervision of root tracings, the exported dataset contained some digitalization errors. It was therefore necessary to characterize the implausible data points resulting from such errors and to clean out the dataset. We designed a data

Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists. All rights reserved to G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 708 correction algorithm aiming at identifying implausible growth rate profiles that derive 709 from errors in image analysis. The most typical errors were defaults in alignment, one-710 day missing root length increments or non-visible root tips in the case of roots 711 encountering an obstacle. This kind of error results in implausible trajectories for the 712 root length at some time point, which can be better identified by examining growth 713 rate profiles. Depending on the type of error, growth rate profiles were either corrected 714 or truncated before the first implausible growth rate. The data correction algorithm is 715 described in Supplemental Methods S3.

716

717 Statistical models

718 Definition of semi-Markov switching linear models

719 Semi-Markov switching linear models are two-scale models that generalize hidden 720 semi-Markov chains by incorporating linear regression models as observation models. 721 They are formally defined in Supplemental Methods S1. In our context, the 722 succession and duration of growth phases (coarse scale) are represented by a non-723 observable semi-Markov chain while the growth rate trend within a growth phase (fine 724 scale) are represented by observation linear models attached to each state of the semi-725 Markov chain. Hence, each state of the semi-Markov chain represents a growth phase. A J-state semi-Markov chain is defined by three subsets of parameters: 726

/.

1. Initial probabilities $(\pi_j; j = 0, ..., J - 1)$ to model which is the first phase occurring in a growth rate profile,

- 729 2. Transition probabilities $(p_{ij}; i, j = 0, ..., J 1)$ to model the succession of 730 growth phases,
- 7313. Occupancy distributions attached to non-absorbing states (a state is said to be732absorbing if, after entering this state, it is impossible to leave it) to model the733growth phase duration in number of days. We used, as possible parametric state734occupancy distributions binomial distributions B(d, n, p), Poisson distributions735 $P(d, \lambda)$ and negative binomial distributions NB(d, r, p) with an additional shift736parameter $d \ge 1$.
- 737 A SMS-LM adds observation linear models to the non-observable semi-Markov chain:
- 4. We chose to model growth rate trends within growth phases using simple linear
 regression models because of the short length of growth phases (up to 10

23

Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists: All rights reserved.^{1,} G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), successive growth rates for pearl millet and up to 17 successive growth ratesfor maize).

742 A SMS-LM composed of parallel transient states followed by a final absorbing state 743 was estimated on the basis of growth rate profiles corresponding to a given species. A 744 state is said to be transient if after leaving this state, it is impossible to return to it. The 745 parallel transient states represent alternative growth phases. The final absorbing state 746 represents the growth arrest and a degenerate linear model corresponding to a constant 747 null growth rate is associated with this state. The censoring level was computed for 748 each growth state as a by-product of the estimation of the corresponding growth phase 749 duration distribution within SMS-LM. This censoring level takes into account all the 750 possible assignments of growth rate profiles of length ≥ 5 incorporated in the learning 751 sample. Each estimated model was used to compute the most probable state series for 752 each observed growth rate profile (Guédon, 2003). This restored state series can be 753 viewed as the optimal segmentation of the corresponding observed series into at most 754 two sub-series corresponding to a given growth phase either censored or followed by a 755 growth arrest. Because of the transient growth states in parallel, this restoration can be 756 interpreted as a classification of the lateral roots on the basis of their growth rate 757 profiles. In the case of the maize *rtcs* mutant and the shading treatment, the growth rate 758 profiles were segmented using both the model estimated on the corresponding growth 759 rate profiles and the unshaded wild-type model.

760

761 Definition of stationary variable-order Markov chain

762 Most of the methods for analyzing local dependencies in discrete sequences rely on 763 high-order Markov chains. However, the number of free parameters of a Markov chain 764 increases exponentially with its order, i.e. with the memory length taken into account. 765 For instance, in the case of three states (corresponding to three lateral root types), the 766 number of free parameters is 2 for a zero-order, 6 for a first-order, 18 for a second-767 order Markov chain, etc. Since there are no models "in between", this very 768 discontinuous increase in the number of free parameters causes the estimated high-769 order Markov chains to be generally over-parameterized. This drawback can be 770 overcome by defining sub-classes of parsimonious high-order Markov chains such as 771 variable-order Markov chains (Ron et al., 1997; Bühlmann and Wyner, 1999) where 772 the order is variable and depends on the "context" within the sequences, instead of

Passot, S., Moreno-Ortega, Copyright © 2018 American Society of Plant Biologists: All rights reserved. G., Laplaze, L., Muller, B., Guedon, Y. (2018). A new phenotyping pipeline reveals three types of lateral roots and a random branching pattern in two cereals. Plant Physiology, 177 (3), 896-810 - DOI: 10.1104/pp.17.01648 being fixed. Stationary variable-order Markov chains are formally defined inSupplemental Methods S4.

775

776 Pearl millet root anatomy

777 Plants were grown in rhizotrons as previously described. Lateral root growth rate 778 profiles were extracted before sampling, to determine the type of each root. Sampling 779 was performed around 15 days after germination. Selected roots were harvested and 780 fixed overnight in an acetic acid: ethanol solution (1:9) and conserved in 70% ethanol. 781 Segments were taken around the middle of each lateral root. Root segments were 782 gently dried on a filter paper and imbibed in warm (30-45°C) liquid 3% agarose 783 solution (SeaKem GTG Agarose, Lonza). Then, 55 µm-thick sections were obtained 784 from solidified agarose blocks using a vibratome (Microm HM 650V, Thermo 785 Scientific, speed 30, frequency 60). Individual root sections were then collected, 786 transferred to microscope slides and covered with a coverslip for direct observation.

787

Images were taken using a Leica DMRB microscope equipped with an epifluorescence 788 789 filter (excitation range: UV; excitation filter: 460-480 nm). Two pictures were taken 790 for each root section: one under visible light using Nomarsky optics and another using 791 epifluorescence that takes advantage of the natural fluorescence of cell walls with 792 secondary deposits. Images were taken using a Retiga SRV FAST 1394 camera and 793 the QCapture Pro7 software. The RGB images were opened in ImageJ using the 794 Bioformats importer plugin and transformed in gray level 8-bit images. A scale-bar 795 was added to the images according to their magnification. Measurements of the 796 diameter, stele and metaxylem and the number of xylem poles and vessels were 797 recorded for each root section.

798

799 Supplemental Data

800

801 Supplemental Figure S1. Four-state semi-Markov switching linear model estimated
802 on the basis of maize lateral root growth rate profiles.

803 Supplemental Figure S2. Daily median growth rates of lateral roots in pearl millet.

804 Supplemental Figure S3. Ranked posterior probabilities of the optimal assignment of

805 each lateral root growth rate profile of length \geq 5 to a cluster.

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- 806 Supplemental Figure S4. Cumulative distribution functions of the length of lateral
 807 root growth rate profiles.
- 808 Supplemental Figure S5. Ranked posterior probabilities of the optimal assignment of
 809 each lateral root growth rate profile of length < 5 to a cluster.
- 810 Supplemental Figure S6. Daily median growth rates and apical diameters of lateral
 811 roots in maize *rtcs* mutants.
- 812 Supplemental Figure S7. Daily median growth rates and apical diameters of lateral
 813 roots in shaded maize.
- 814 Supplemental Figure S8. Spearman rank autocorrelation functions in pearl millet and
 815 maize.
- 816 Supplemental Figure S9. Distributions of the length of intervals between successive
- 817 lateral roots.
- 818 **Supplemental Table S1.** Overlaps between growth rate distributions corresponding to
- 819 lateral root clusters for pearl millet.
- 820 Supplemental Table S2. Overlaps between growth rate distributions corresponding to
 821 lateral root clusters for maize.
- 822 **Supplemental Table S3.** Overlaps between growth rate distributions and apical 823 diameter distributions corresponding to lateral root types for maize.
- 824 Supplemental Table S4. Length of the intervals between successive lateral roots in825 pearl millet.
- 826 Supplemental Table S5. Length of the intervals between successive lateral roots in827 maize.
- 828 Supplemental Methods S1. Definition of semi-Markov switching linear models and
- 829 associated statistical methods.
- 830 Supplemental Methods S2. Empirical selection of the number of clusters of lateral831 roots.
- 832 Supplemental Methods S3. Algorithm for correcting growth rate profiles.
- 833 Supplemental Methods S4. Definition of stationary variable-order Markov chains and
- 834 associated statistical methods.
- 835
- 836

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837 Table 1. Characteristics (mean and standard deviation (s.d.) in days; and censoring level (c.l.;

838 %)) of growth phase duration distributions estimated for the three root types within the semi-

839 Markov switching linear models for pearl millet and maize (wild-type plants, *rtcs* mutant and

840	wild-type plants	exposed t	o shading).
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Pearl millet				Maize								
				Wild type		rtcs mutant		Shading				
Root type	mean	s.d.	c.l.	mean	s.d.	c.l.	mean	s.d.	c.l.	mean	s.d.	c.l.
А	17.3	7.6	96.2	15.2	7.7	80.3	15.7	8.6	89.7			
В	7.6	4.6	53.6	6.9	5	36.3	5.8	3.3	37	5.8	3	25.6
С	3.2	2.6	13.9	3	2.4	9.7	2.9	1.9	5.1	2.5	1.7	2.5

841

842 Table 2. Proportions (%) of lateral root types in the pearl millet and maize (wild-type plants,

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843	rtcs mutant and	wild-type plants	exposed to	shading)
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	Pearl millet		Maize	
Root type		Wild type	rtcs mutant	Shading
А	13.3	8.1	8	
В	23.8	27.3	34.4	26
С	62.9	64.6	57.6	74

Table 3. Length of the intervals between successive lateral roots classified according to the type of the lateral roots delimiting the interval in the shootward direction (sample size, mean and standard deviation (s.d.) in mm for each type). No significant differences between the means were found (ANOVA, p = 0.83 and p = 0.33 for pearl millet and maize, respectively).

	Pearl millet				Maize		
Root type	А	В	С	А	В	С	
Sample size	165	296	785	249	830	1958	
Mean	2.2	2.1	2.1	1.6	1.6	1.7	
s.d.	2.7	2.7	1.9	1.6	1.5	1.5	

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851 List of Figures

Figure 1. Growth rate profiles of lateral roots. Lateral roots of (A) one pearl millet and (B) one maize plant were assigned to the lateral root types, A, B and C using the estimated semi-Markov switching linear models. Root age refers to the number of days following emergence.

Figure 2. Four-state semi-Markov switching linear model estimated on the basis of pearl millet lateral root growth rate profiles. The three growth states correspond to the lateral root types, A, B and C, and the "end" state to growth arrest. (A) Growth phase duration distributions; (B) Graph of transitions. The possible transitions between states are represented by arcs (the attached probabilities are always equal to 1). The arcs entering in states indicate initial states and the attached initial probabilities are noted nearby. (C) Linear trend models estimated for each state.

Figure 3. Growth phase duration distributions in pearl millet and maize. Distributions were estimated for lateral root types A, B and C within the 4-state semi-Markov switching linear model for (A) pearl millet and (B) maize. The relative frequency distributions of the length of growth rate profiles are drawn for illustrating the censoring level. Only lengths ≥ 5 corresponding to lateral roots used to build the semi-Markov switching linear models are shown.

Figure 4. Ranked posterior probabilities of the optimal assignment of each lateral root growth rate profile to a cluster. Growth rate profiles were truncated at length 1, 2, 3 and 5, or left untruncated for (A) pearl millet and (B) maize.

Figure 5. Daily median growth rates and apical diameters of lateral roots in pearl millet and maize. Daily median growth rates and associated mean absolute deviations (m.a.d.) were computed for (A) pearl millet and (B) maize lateral root types A, B and C. (C) Daily median apical diameters and associated m.a.d. were computed for maize lateral root types A, B and C. Figure 6. Relationship between stele and central xylem tracheary element (XTE) diameter of lateral roots in pearl millet. Colors indicate the lateral root types determined on the basis of the assignment of growth rate profiles using semi-Markov switching linear models.

Figure 7. Growth phase duration distributions in wild-type, *rtcs* mutant and shaded maize. Distributions were estimated for lateral root types A, B and C within the semi-Markov switching linear model for the (A) wild-type, (B) *rtcs* mutant and (C) shaded maize. The relative frequency distributions of the length of growth rate profiles are drawn for illustrating the censoring level. Only lengths \geq 5 corresponding to lateral roots used to build the semi-Markov switching linear models are shown. **Figure 8.** Daily median growth rates and apical diameters of lateral roots in wild-type, *rtcs* mutant and shaded maize. (A) Daily median growth rates and (B) apical diameters were computed for lateral root types A, B and C of wild-type, *rtcs* mutant and shaded maize.

Figure 9. Proportions of lateral root types. Proportions are shown for each (A) pearl millet
and (B) maize plant. Plants were assigned to groups (indicated by letters) using the KruskalWallis test. Bin areas are proportional to the number of lateral roots of each type. Bin widths
are proportional to the total number of lateral roots per plant (indicated below each bin).

891 Figure 10. Rhizotron development and root system measurement. Root observation boxes 892 (rhizotrons) were built according to Neufeld et al. (1989). (A, B) Rhizotrons were made of 893 (back to front) an extruded polystyrene plate, a layer of substrate (sieved peat and compost), a 894 layer of viscose (impermeable to roots, but permeable to water and nutrients) and plexiglass 895 plate, all joined together using aluminum U frames held by screws. Germinated seedlings 896 with similar primary root length were transferred individually. A layer of wet sphagnum on 897 the top of the rhizotrons maintained the seedlings and prevented them from drying. 898 Rhizotrons were placed in a growth room with climatic conditions adapted to each species. 899 Rhizotrons were daily scanned with an A3 scanner. (C) The SmartRoot software (Lobet et al., 900 2011) was used to extract root system architecture at successive dates and compute root 901 growth parameters.

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Figure 1. Growth rate profiles of lateral roots





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Figure 8. Daily median growth rates and apical diameters of lateral roots in wild-type, *rtcs* mutant and shaded maize.





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