

Short title

Lateral roots types revealed in two cereals

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Title

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

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One sentence summary

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

34

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.

45

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59

60 **Abstract**

61 Recent progress in root phenotyping has focused mainly on increasing throughput for
62 genetic studies while identifying root developmental patterns has been comparatively
63 underexplored. We introduce a new phenotyping pipeline for producing high-quality
64 spatio-temporal root system development data and identifying developmental patterns
65 within these data. The SmartRoot image analysis system and temporal and spatial
66 statistical models were applied to two cereals, pearl millet (*Pennisetum glaucum*) and
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
78 distance from this root. This random branching pattern of primary roots was
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.

82

83

Introduction

Plant breeding has long ignored the belowground part of the plant but it is now acknowledged that the root system represents an opportunity for improving plant efficiency and tolerance to abiotic stresses (Bishopp and Lynch, 2015). A better knowledge of root system structure and function is thus needed for root system oriented crop improvement. Phenotyping, as the evaluation of heritable plant traits in a given environment and in a reproducible manner, is one key approach to extend this knowledge. Recent progresses in plant phenotyping platforms, including plant handling automation and computer assisted data acquisition, have allowed an increase 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 throughput, another strategy chosen in some phenotyping platforms is to improve data dimensionality and structure (Dhondt et al., 2013). In these platforms, the amount of data collected on a single plant is increased, either by measuring several traits that can be of different natures or by measuring the same trait at successive time points to focus on physiological processes (Fahlgren et al., 2015a).

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

116 In addition, the analysis of structured data such as root growth rate profiles is more
117 challenging 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 annuus*), 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

of similar statistical models. This model-based clustering of growth rate profiles led us to identify three types of lateral root. We also applied this clustering approach to the *rootless concerning crown and seminal roots (rtcs)* maize mutant affected in auxin signaling and to maize plants exposed to severe shading. We then investigated the relationships between lateral root types and morphological (apical diameter profile in maize) and anatomical (stele diameter and central xylem tracheary element diameter in pearl millet) traits. In a second step, potential dependencies in the succession of lateral root types along the primary root were analyzed using variable-order Markov chains leading to a precise characterization of the primary root branching pattern. Our phenotyping pipeline opens the door to a quantitative, model-assisted characterization of developmental patterns of lateral roots to support root system oriented crop improvement.

161

162 **Results**

163

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

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

2. *spatial analysis*: The intervals between consecutive lateral roots and the succession 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
185 rate profiles are essentially divergent after root emergence and the growth rate
186 dispersion increases with the root age. Hence, lateral roots can be roughly ordered
187 according to their growth rate profiles. This raised the question of a stronger
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

mechanism associated with a SMS-LM can be described as follows: A growth state is randomly selected according to an initial distribution. This initial distribution represents the lateral root type proportions. A growth phase duration is then randomly selected according to the corresponding distribution of the selected growth state. The growth rate then changes with time according to the linear trend model associated with the selected growth state until the end of the growth phase and the transition to the growth arrest state. In such SMS-LM, the transitions from the growth arrest state to a growth state are not possible and each state can be visited at most once (see illustration in Fig. 2 and Supplemental Fig. S1).

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

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.
265 S3A) and 94% above 0.8 and 81% above 0.8 for maize (Supplemental Fig. S3B) –
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

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

298

The apical diameter profiles partially match the different root types identified in maize

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

312

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
324 between anatomical traits and lateral root types for pearl millet ($\rho = 0.81$ between
325 stele diameter and lateral root types and $\rho = 0.91$ between central XTE diameter and
326 lateral root types).

327

328 **The definition of lateral root types is neither affected by a mutation altering auxin** 329 **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 ≥ 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 ≥ 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
344 growth rate profiles of length ≥ 5 to lateral root types, using the SMS-LMs previously
345 estimated on the basis of these learning samples, were most often high – 91% above

0.8 and 87% above 0.9 for the *rtcs* mutant, and 93% above 0.8 and 80% above 0.9 for the shaded plants – indicating a clear between-cluster separation. Growth rate profiles of length < 5 (test sample corresponding to 603 lateral roots of cumulative length 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 growth rate profiles to lateral root types were most often high despite the limited information conveyed by these profiles: 85% above 0.8 and 50% above 0.9. These independent assignments constitute clear elements of validation of the clustering assumption for the *rtcs* mutant. The results of independent assignments are not reported for the shaded plants since there were only 32 growth rate profiles of length < 5 in this case. Both for the *rtcs* mutant and the shaded plants, the growth rate profiles were assigned to lateral root types using both the SMS-LM built on the basis of these lateral roots and the SMS-LM built on the basis of the unshaded wild-type plants. We obtained a match of 94% (938 lateral roots among 994) between the two independent assignments for the *rtcs* mutant and a match of 91% (492 lateral roots among 540) for the shaded plants indicating that the lateral root types are robustly defined. The estimated growth phase duration distributions are similar for each lateral 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 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 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 for the shaded plants, (ii) the higher proportion of type B lateral roots compensated by a lower proportion of type C lateral roots for the *rtcs* mutant and a higher proportion of type C lateral roots for the shaded plants (Table 2). Results concerning the shaded plants are consistent with the expectation of an inhibition of root growth in relation with a restriction in the supply of carbohydrates. Moreover, we could observe (iii) larger diameters compared to wild type for all root types in the *rtcs* mutant and for type C in the shaded plants (Fig. 8B) and (iv) a hierarchy in apical diameter profiles between types B and C for the *rtcs* mutant whereas type B and C apical diameter profiles are confounded in both wild-type and shaded plants (see Fig. 8B and Supplemental Table S3).

379

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

primary roots were roughly stationary. For some individuals (one for pearl millet and 4 for maize), the autocorrelation function fluctuates around the positive confidence limit (Supplemental Fig. S8 E,F) indicating a slight non-stationarity. We further investigated primary root branching sequences applying a statistical modeling approach. To this end, we modeled potential dependencies between successive lateral root types along 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 selected (Csiszár and Talata, 2006) for each primary root branching sequence and for samples of branching sequences corresponding to each species. For all plants and for both species, a zero-order Markov chain was selected. This confirmed that the type of a lateral root was independent of the type of the previous lateral roots. Hence, our results indicate that there is no influence of the lateral root growth pattern on the 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
429 species. The mean interval lengths were not equal in all plants (ANOVA,
430 $p < 10^{-5}$ for pearl millet and $p < 10^{-6}$ for maize). Plants were thus classified according
431 to Tukey's honest significant difference test. Two overlapping groups were found,
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
435 both for pearl millet and maize (Kruskal-Wallis test, $p < 10^{-10}$ and $p < 10^{-15}$
436 respectively, Fig. 9). For pearl millet, the 8 plants were separated into three groups
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

444 plants in terms of lateral root type proportions, the stationary random branching pattern
445 is 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
452 primary root branching pattern and applied it to explore the diversity of lateral roots in
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
466 some lateral roots were still growing at the end of the experiment. Our approach
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.

477

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

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 (Varney 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?**

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

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
561 roots. They could also constitute a pool of potentially fast growing roots if conditions
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

576 analyses showed that there was no relationship between the length of the interval
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).

609

610 **An avenue for considering the diversity of lateral roots in future high-throughput**
611 **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
617 overlooked although they represent the best example of the overall structural plasticity
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.

625

626

627 **Materials and Methods**

628

629 **Plant growth**

630 Pearl millet (*Pennisetum glaucum* (L.) R. Br.) inbred lines LCICMB1 (Passot et al.,
631 2016), maize (*Zea mays*) hybrid B73xUH007 (referred to as wild type) and *rtcs*
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
641 substrate used was composed of 30% fine clay, 25% peat fibers, 5% blond peat and

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

647 Maize seeds were surface sterilized with 6% hypochlorite for five minutes and rinsed
648 in distilled water for one minute. Seeds were then germinated on moistened filter paper
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
660 Instruments, Llandrindod Wells, Powys, UK). In our conditions, photosynthetic photon
661 flux density (PPFD) was 300 $\mu\text{mol}/\text{m}^2/\text{s}$. Temperature and air humidity were recorded
662 (HC2-SH, Rotronic, Bassersdorf, CH) for each growth room. The sphagnum was
663 watered twice a day at the beginning of the experiment and from 6 days after
664 germination onward, rhizotrons were watered daily using a 1/10 Hoagland solution to
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**

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

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).

The SmartRoot software (Lobet et al., 2011) was used to extract root system architecture at successive dates as well as root growth parameters because it supports time-lapse images and focuses on the analysis of individual root behavior. SmartRoot needs images where roots appear darker than background. An ImageJ (Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA) macro was developed to automatically invert and adjust the contrast of the rhizotron images by scaling the 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 when using the algorithm for automatic lateral root tracing provided by SmartRoot using a subset of scan images, and was applied to the whole set of images using the macro tool.

SmartRoot enables semi-automatic root tracing. The primary root was drawn on the 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, using automatic tracing. Crown and lateral roots were added as they appeared, either manually or using automatic detection. Their lengths increased progressively on the successive scans as for the primary root. When all roots were traced, the data were extracted with the batch export tool of SmartRoot. This tool provides several measurements including the length, the insertion position and the diameter for each root. The growth rates were directly extracted from successive length measurement. Because the resolution was not sufficient for pearl millet lateral roots, we only considered root diameter for maize. On average, it took about two days to process the data of one plant.

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

correction algorithm aiming at identifying implausible growth rate profiles that derive from errors in image analysis. The most typical errors were defaults in alignment, one-day missing root length increments or non-visible root tips in the case of roots encountering an obstacle. This kind of error results in implausible trajectories for the root length at some time point, which can be better identified by examining growth rate profiles. Depending on the type of error, growth rate profiles were either corrected or truncated before the first implausible growth rate. The data correction algorithm is described in Supplemental Methods S3.

Statistical models

Definition of semi-Markov switching linear models

Semi-Markov switching linear models are two-scale models that generalize hidden semi-Markov chains by incorporating linear regression models as observation models. They are formally defined in Supplemental Methods S1. In our context, the succession and duration of growth phases (coarse scale) are represented by a non-observable semi-Markov chain while the growth rate trend within a growth phase (fine scale) are represented by observation linear models attached to each state of the semi-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:

1. Initial probabilities $(\pi_j; j = 0, \dots, J - 1)$ to model which is the first phase occurring in a growth rate profile,
2. Transition probabilities $(p_{ij}; i, j = 0, \dots, J - 1)$ to model the succession of growth phases,
3. Occupancy distributions attached to non-absorbing states (a state is said to be absorbing if, after entering this state, it is impossible to leave it) to model the growth phase duration in number of days. We used, as possible parametric state occupancy distributions binomial distributions $B(d, n, p)$, Poisson distributions $P(d, \lambda)$ and negative binomial distributions $NB(d, r, p)$ with an additional shift parameter $d \geq 1$.

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

740 successive growth rates for pearl millet and up to 17 successive growth rates
741 for 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

773 being fixed. Stationary variable-order Markov chains are formally defined in
774 Supplemental 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

788 Images were taken using a Leica DMRB microscope equipped with an epifluorescence
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 ≥ 5 to a cluster.

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 in
825 pearl millet.

826 **Supplemental Table S5.** Length of the intervals between successive lateral roots in
827 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 lateral
831 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

Table 1. Characteristics (mean and standard deviation (s.d.) in days; and censoring level (c.l.; %)) of growth phase duration distributions estimated for the three root types within the semi-Markov switching linear models for pearl millet and maize (wild-type plants, *rtcs* mutant and wild-type plants exposed to shading).

Root type	Pearl millet			Maize								
	mean	s.d.	c.l.	Wild type			<i>rtcs</i> mutant			Shading		
				mean	s.d.	c.l.	mean	s.d.	c.l.	mean	s.d.	c.l.
A	17.3	7.6	96.2	15.2	7.7	80.3	15.7	8.6	89.7			
B	7.6	4.6	53.6	6.9	5	36.3	5.8	3.3	37	5.8	3	25.6
C	3.2	2.6	13.9	3	2.4	9.7	2.9	1.9	5.1	2.5	1.7	2.5

Table 2. Proportions (%) of lateral root types in the pearl millet and maize (wild-type plants, *rtcs* mutant and wild-type plants exposed to shading).

Root type	Pearl millet		Maize		
			Wild type	<i>rtcs</i> mutant	Shading
A	13.3		8.1	8	
B	23.8		27.3	34.4	26
C	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).

Root type	Pearl millet			Maize		
	A	B	C	A	B	C
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

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 ≥ 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 Kruskal-Wallis 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).

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

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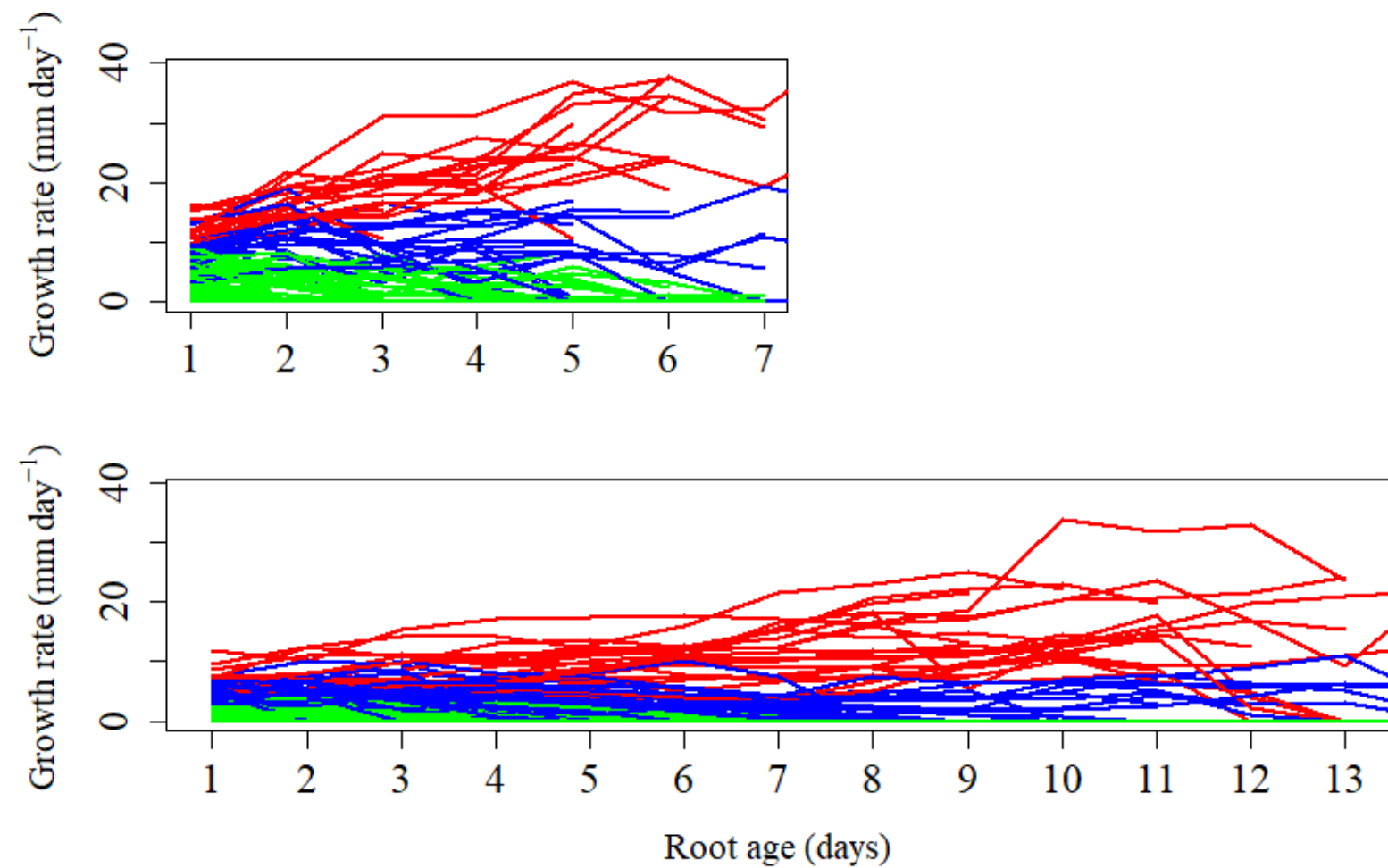
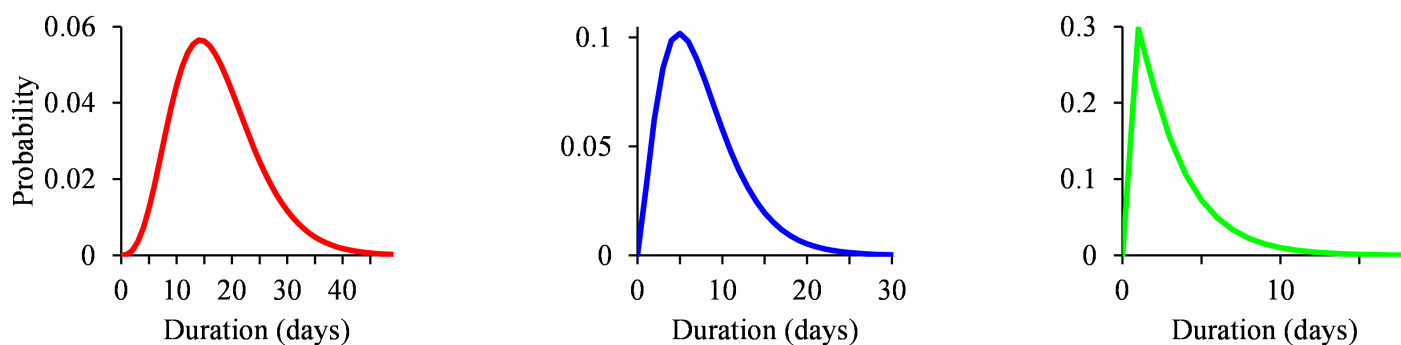


Figure 1. Growth rate profiles of lateral roots

A Growth phase duration distributions



B Graph of transitions

C Linear trend models

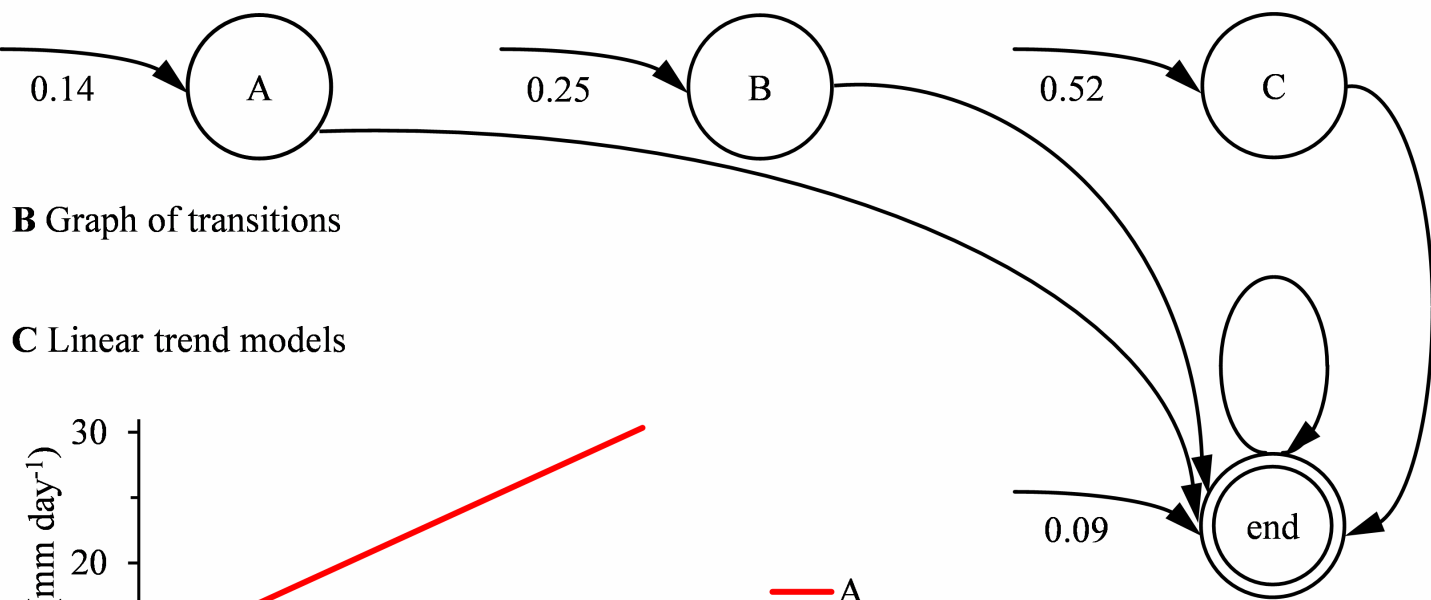
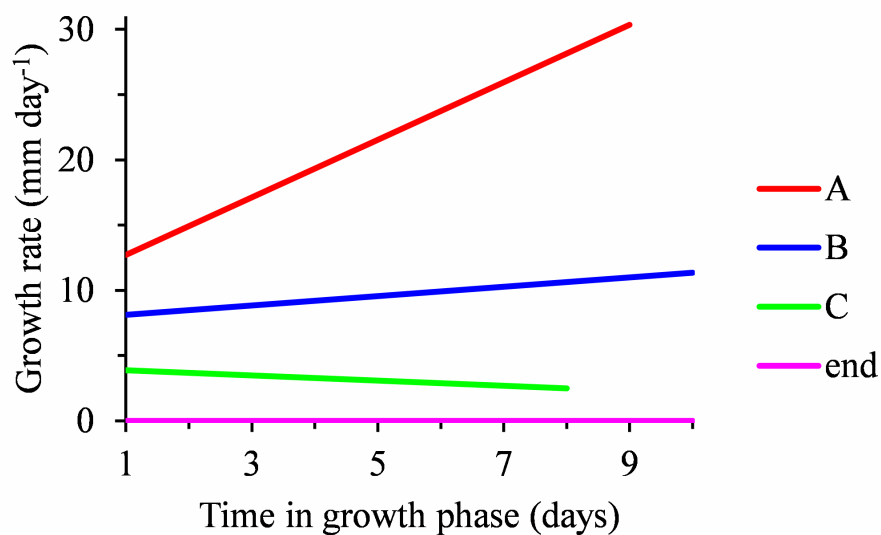
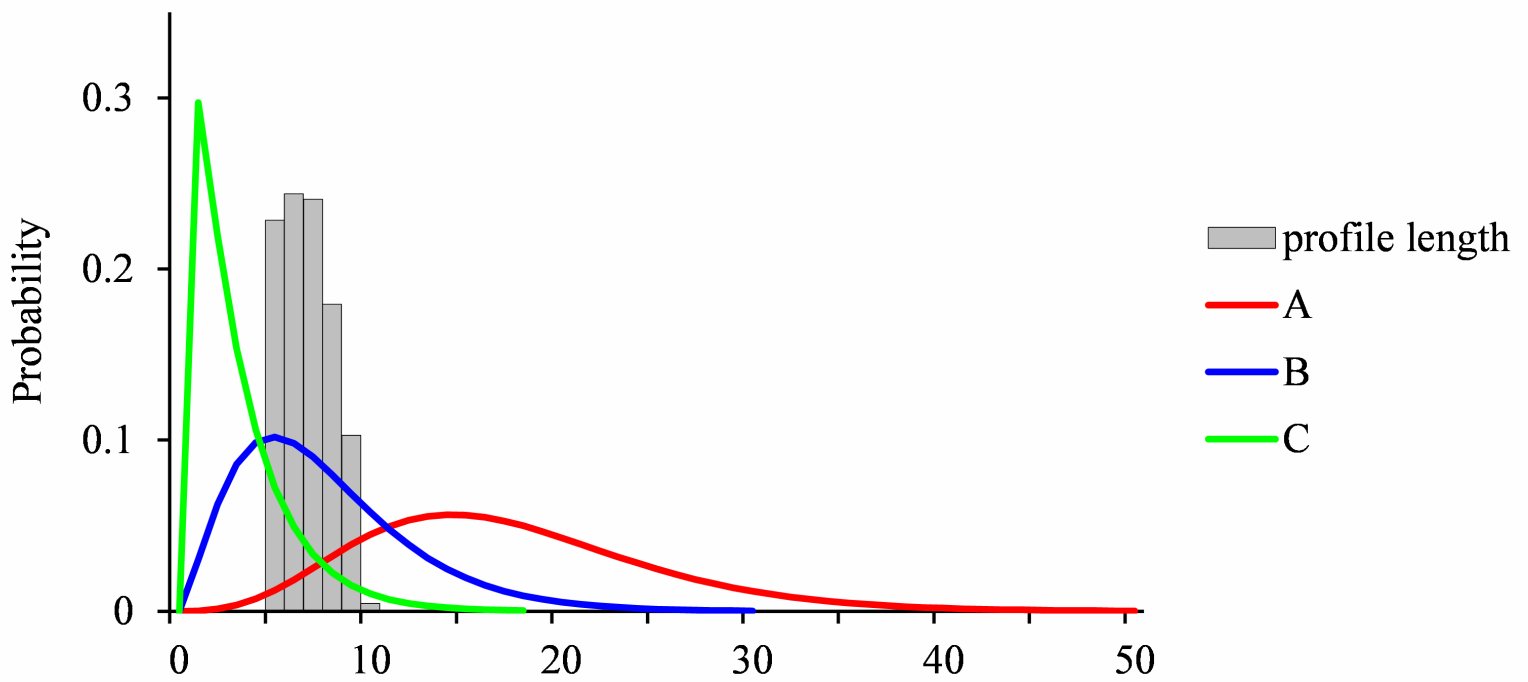


Figure 2. Four-state semi-Markov switching linear model estimated on the basis of pearl millet lateral root growth rate profiles.

A Pearl millet



B Maize

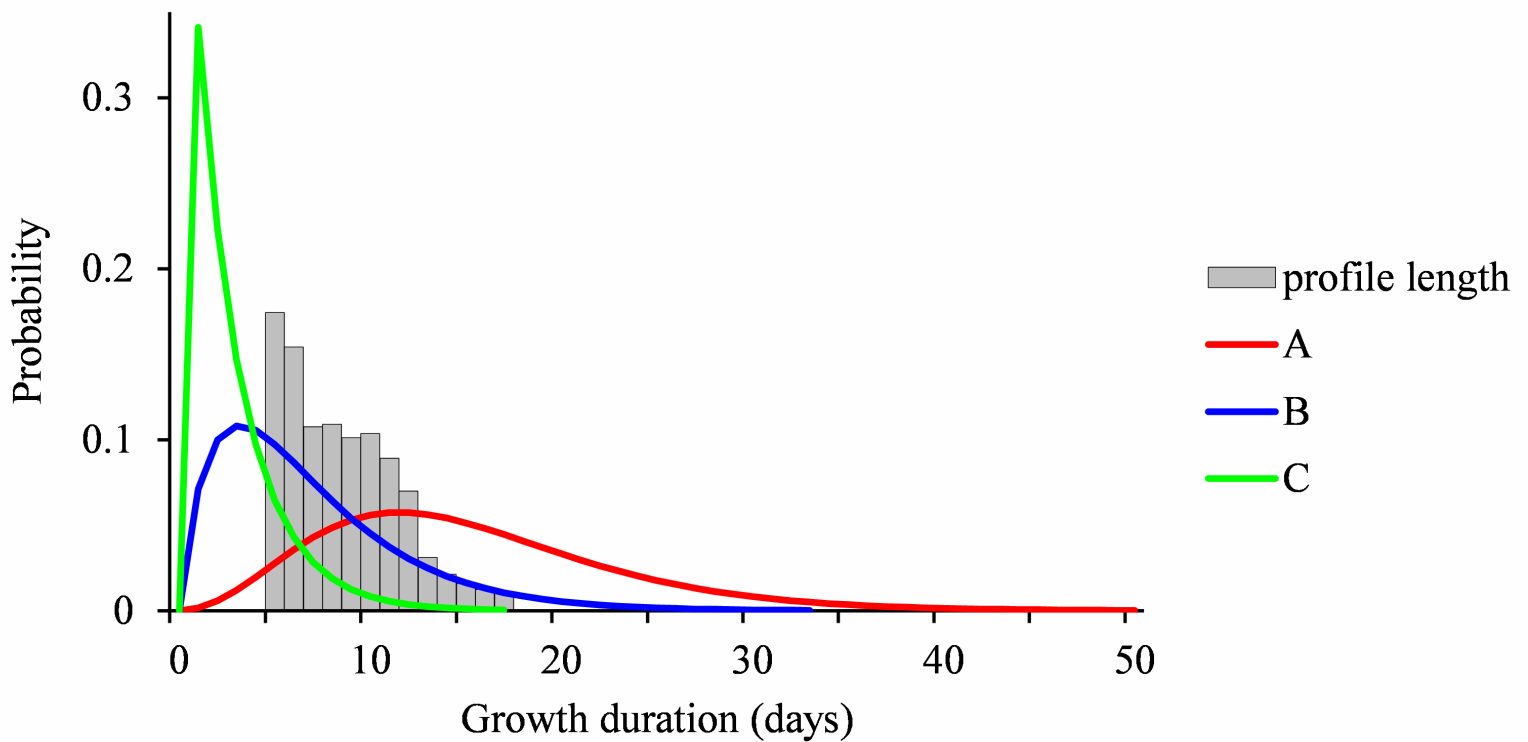


Figure 3. Growth phase duration distributions in pearl millet and maize.

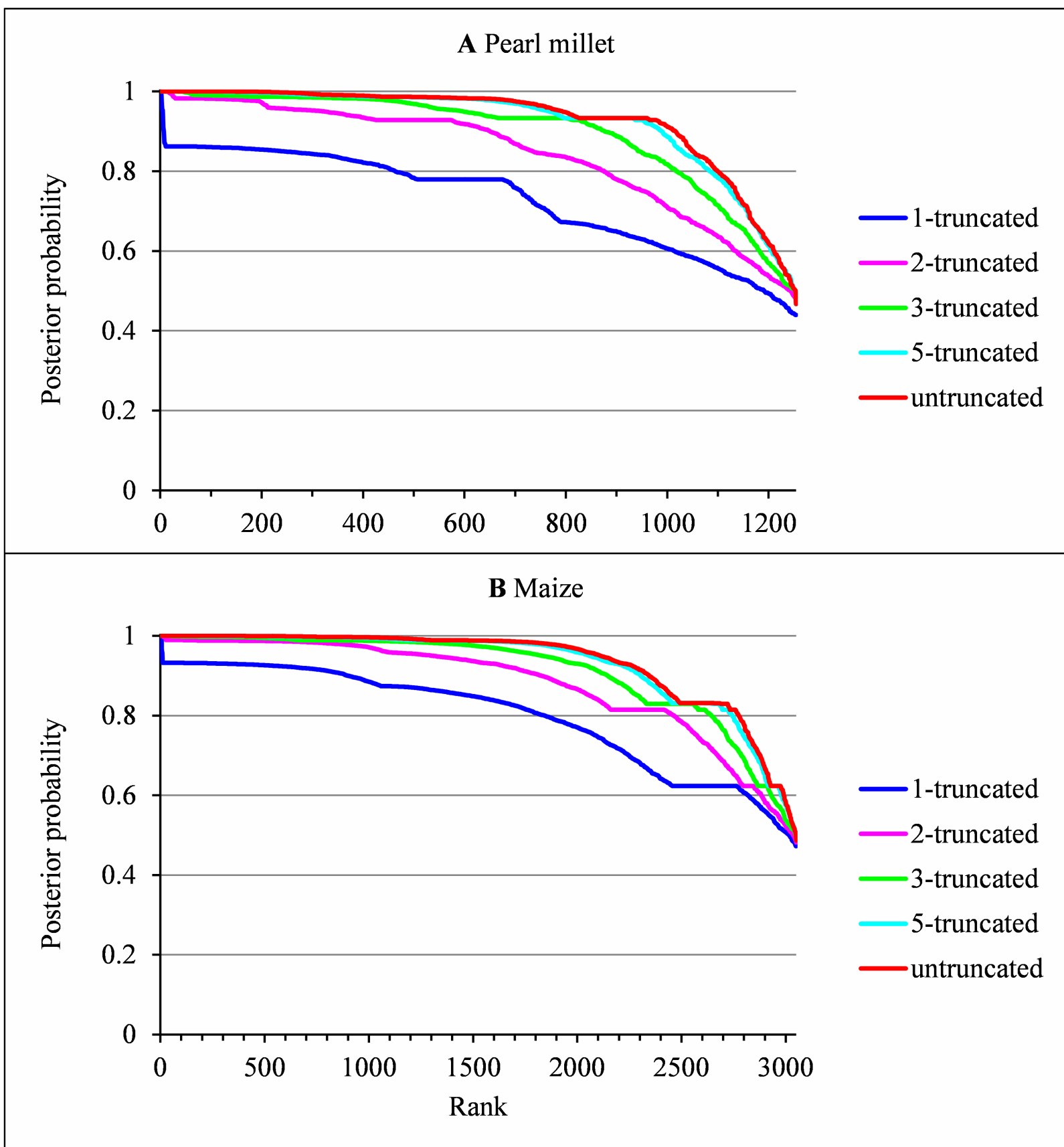


Figure 4. Ranked posterior probabilities of the optimal assignment of each lateral root growth rate profile to a cluster.

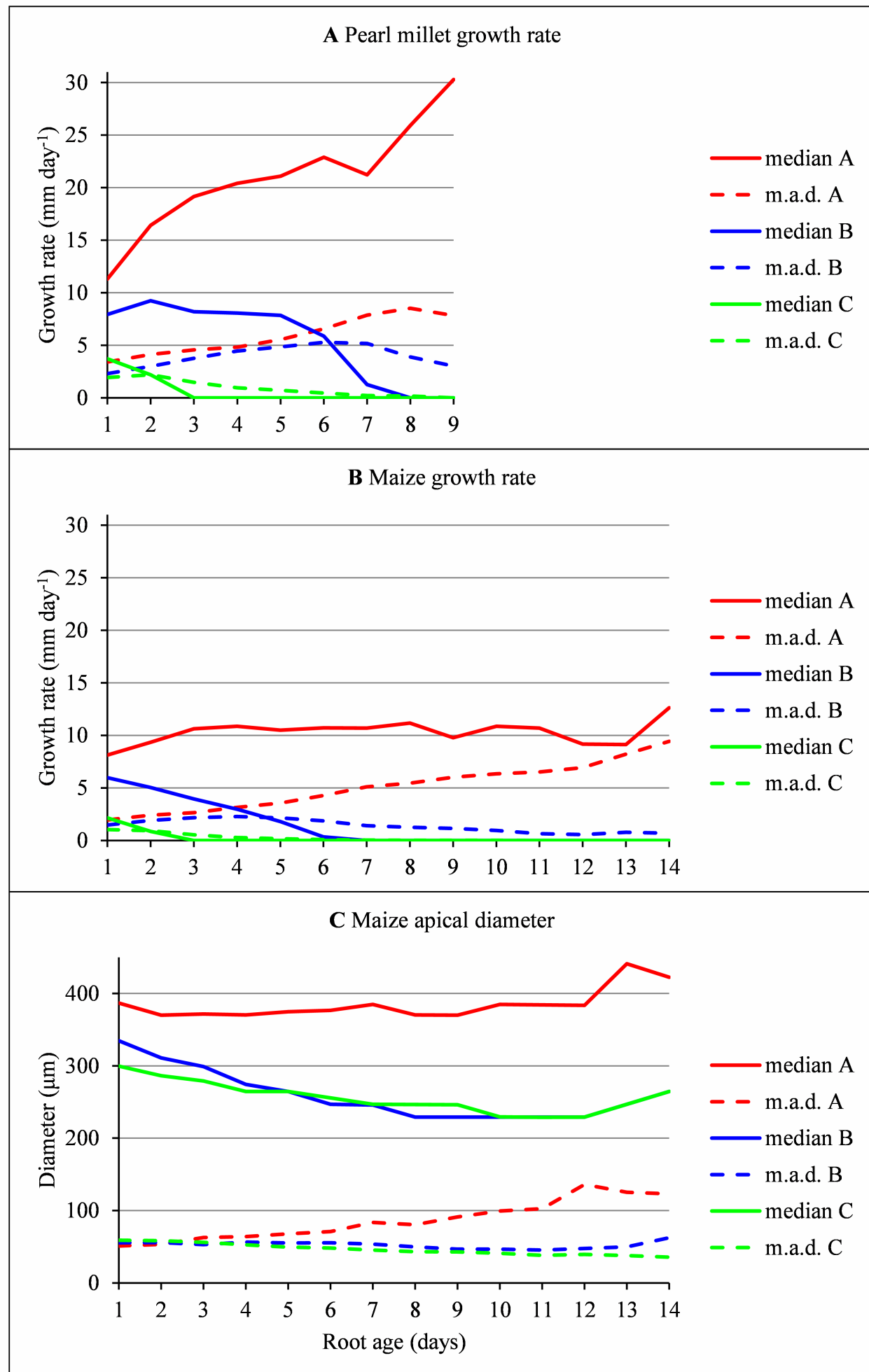


Figure 5. Daily median growth rates and apical diameters of lateral roots in pearl millet and maize.

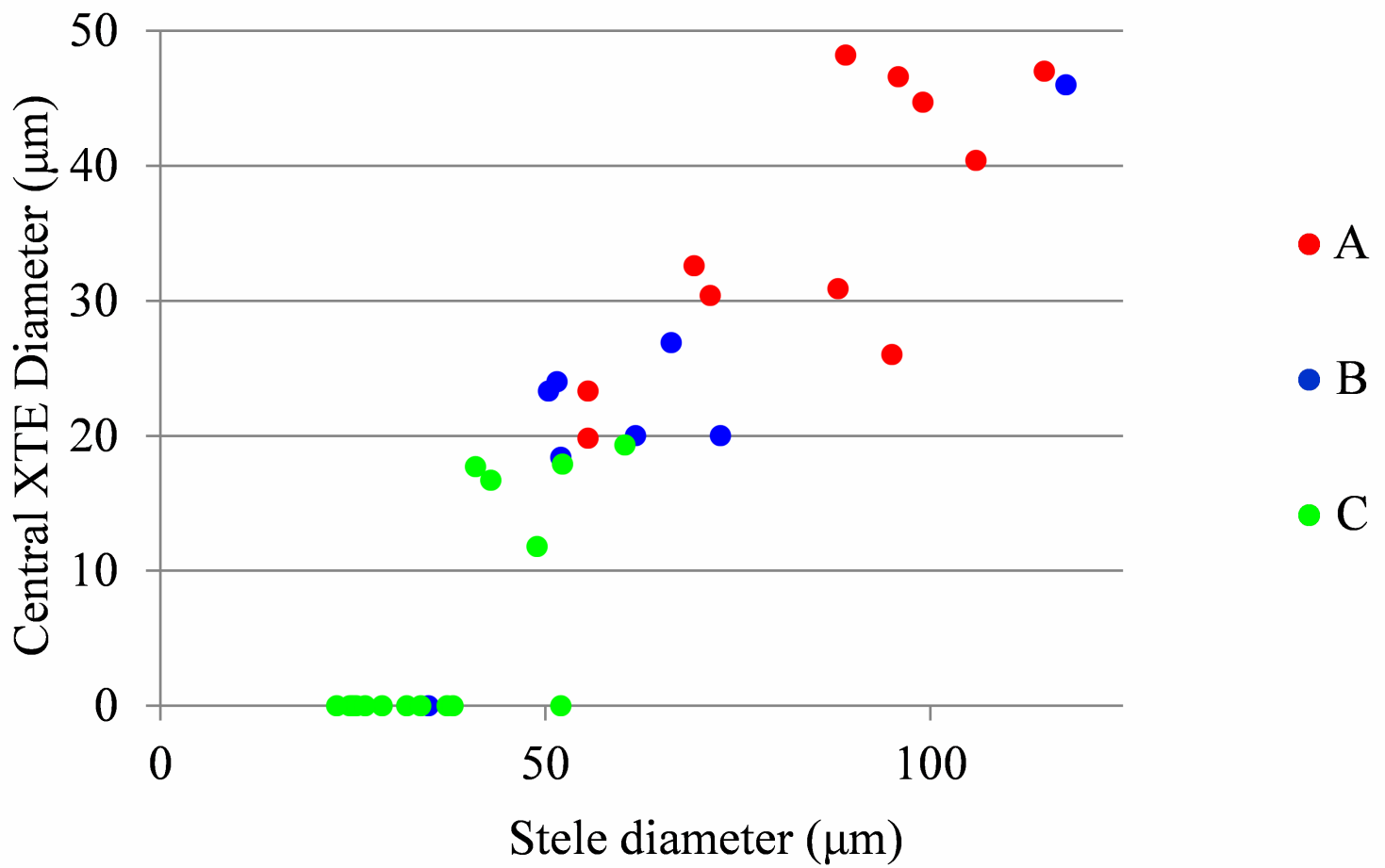


Figure 6. Relationship between stele and central xylem tracheary element (XTE) diameter of lateral roots in pearl millet.

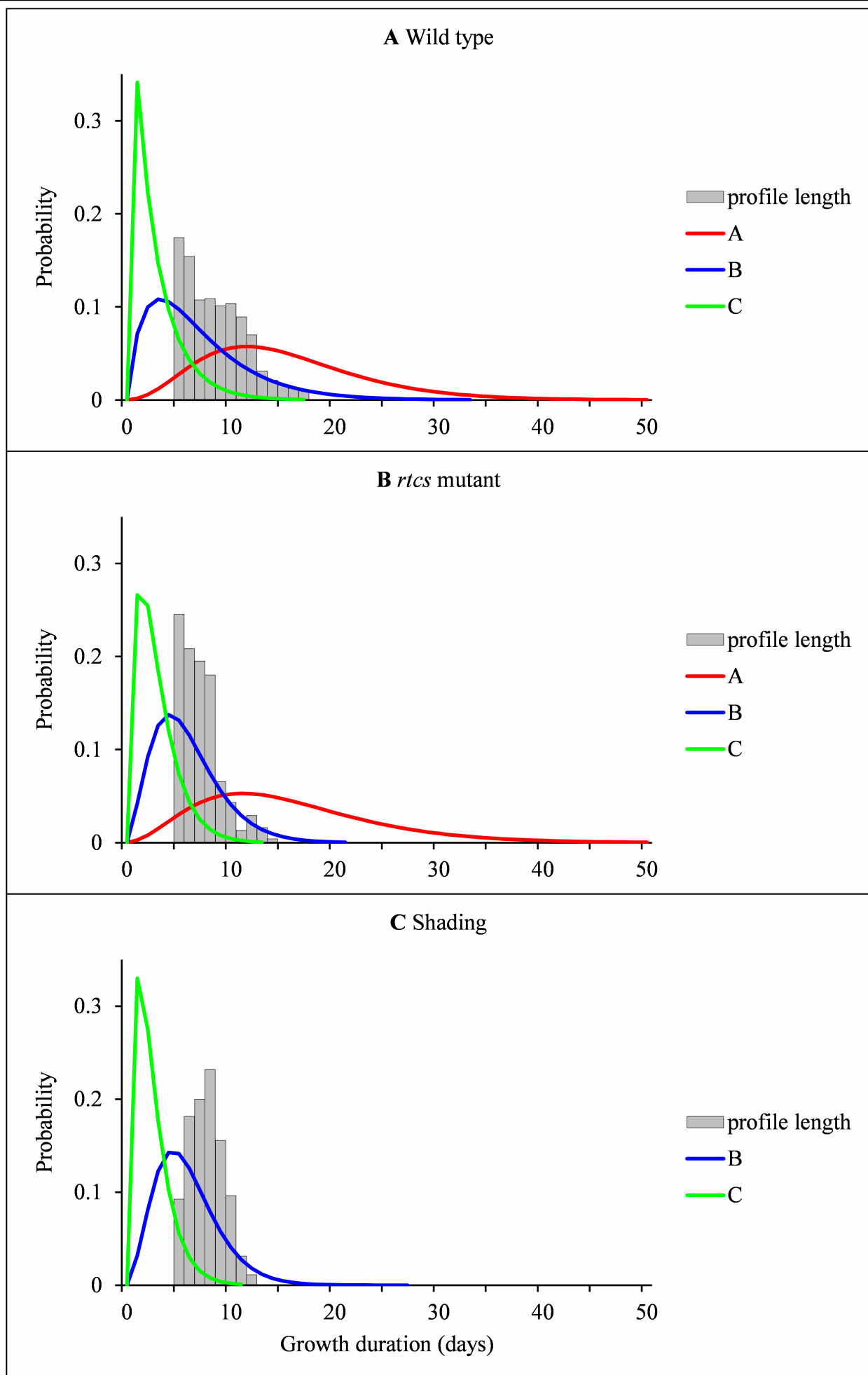


Figure 7. Growth phase duration distributions in wild-type, *rtcs* mutant and shaded maize.

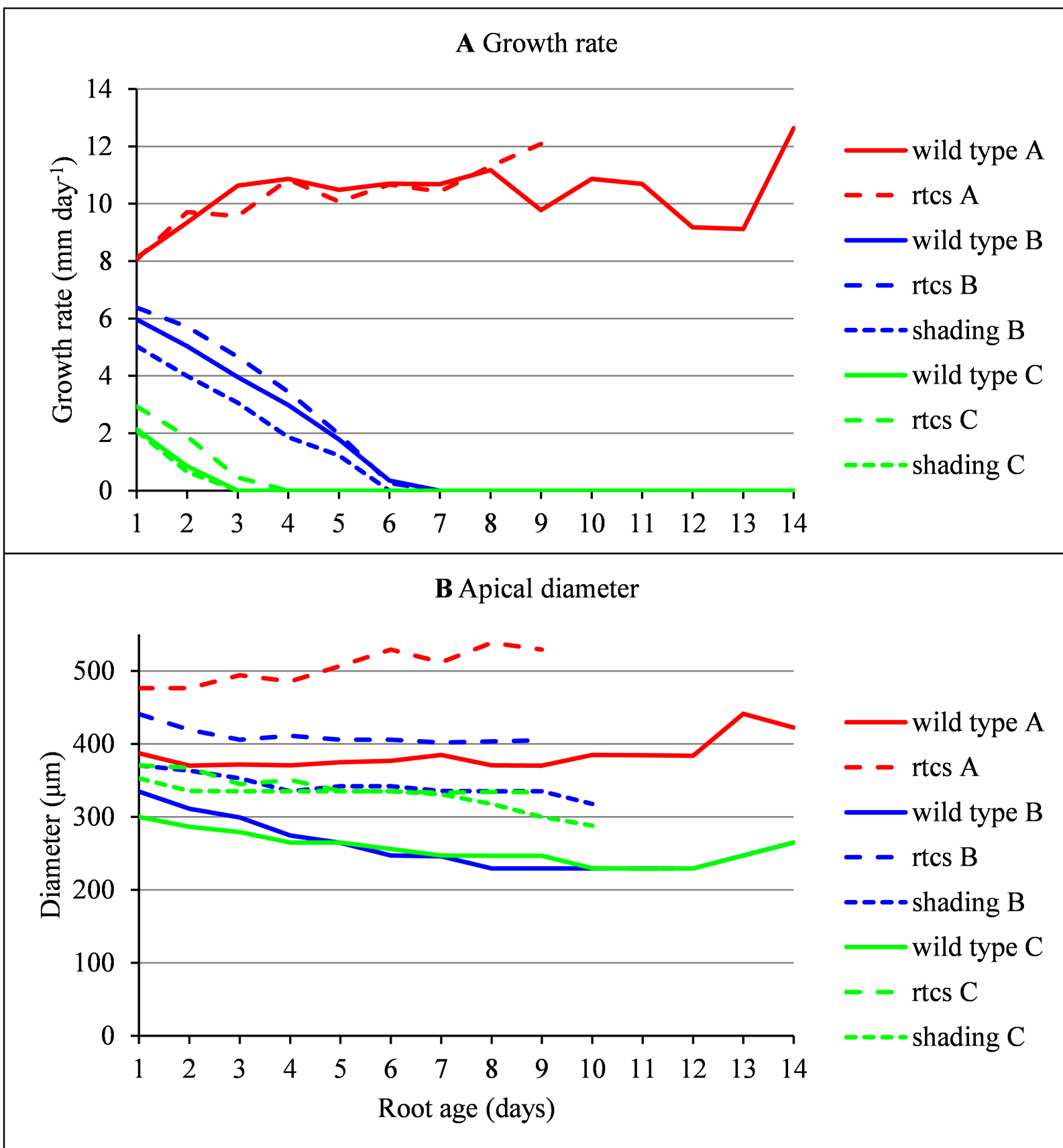
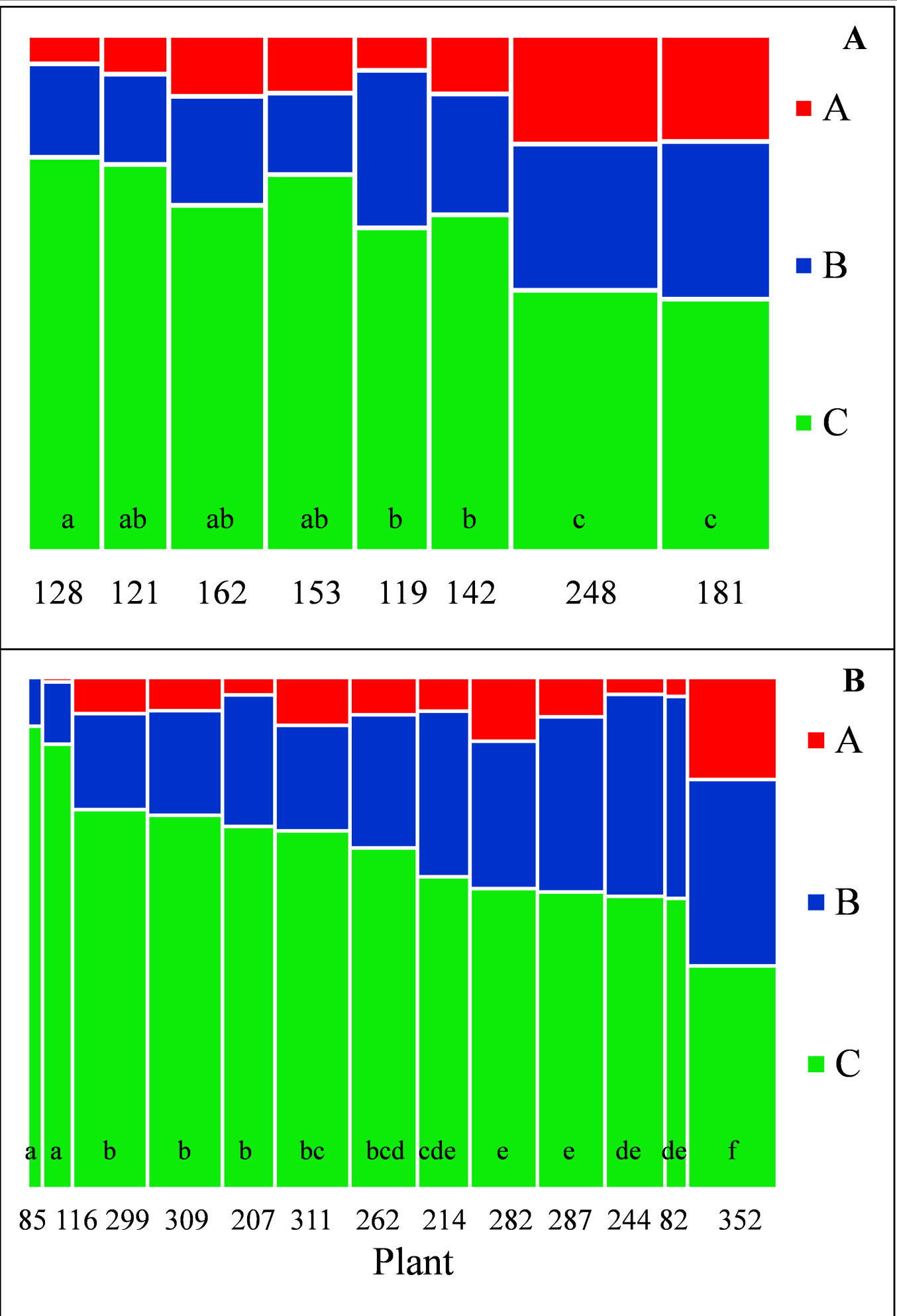


Figure 8. Daily median growth rates and apical diameters of lateral roots in wild-type, *rtcs* mutant and shaded maize.



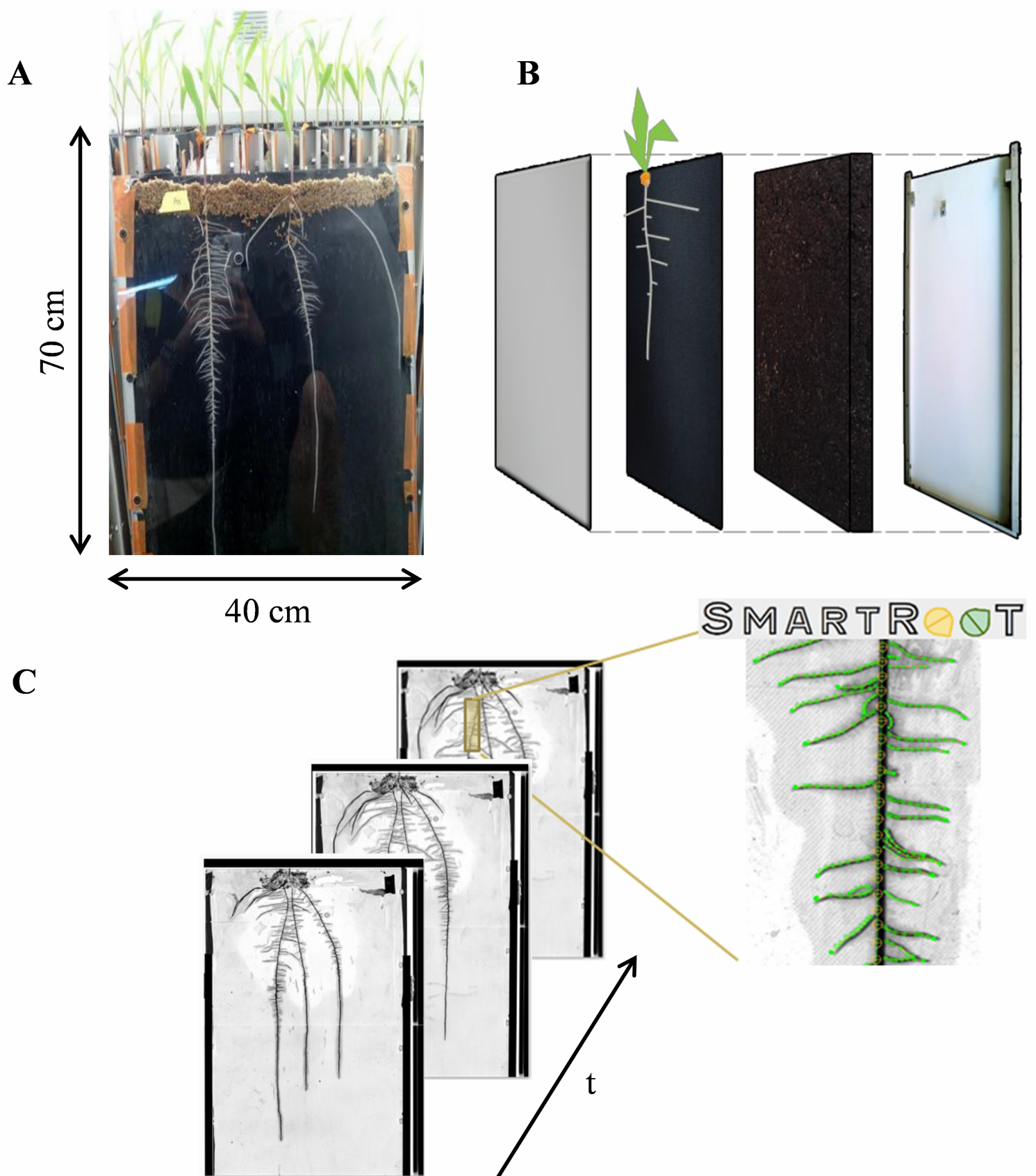


Figure 10. Rhizotron development and root system measurement.

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