

1 **Characterization of pearl millet root architecture and anatomy**

2 **reveals three types of lateral roots**

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26

27 **Abstract**

28 Pearl millet plays an important role for food security in arid regions of Africa and India.
29 Nevertheless, it is considered an orphan crop as it lags far behind other cereals in terms of
30 genetic improvement efforts. Breeding pearl millet varieties with improved root traits
31 promises to deliver benefits in water and nutrient acquisition. Here, we characterize ~~of~~ early
32 pearl millet root system development using several different root phenotyping approaches that
33 include rhizotrons and microCT. We report that early stage pearl millet root system
34 development is characterized by a fast growing primary root that quickly colonizes deeper
35 soil horizons. We also describe root anatomical studies that revealed 3 distinct types of lateral
36 roots that form on both primary roots and crown roots. Finally, we detected significant
37 variation for **two** root architectural traits in pearl millet inbred lines. This study provides the
38 basis for subsequent genetic experiments to identify loci associated with interesting early root
39 development traits in this important cereal.

40

41 **Keywords**

42 Lateral root, root growth, metaxylem, root architecture, breeding

43

44 1. Introduction

45 | In Africa, most of the recent increase in agricultural production ~~have~~has been due to the
46 expansion of cultivated lands rather than an increase in yields (Bationo *et al.*, 2007).
47 Moreover, several climate models predict that global changes may reduce the potential
48 productivity of cereals (Berg *et al.*, 2013). For example, millets potential productivity is
49 predicted to decrease by 6% in the driest cultivated regions. In order to achieve future food
50 security in Africa, it is therefore necessary to improve crop productivity through breeding and
51 improved agricultural practices.

52 | Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the sixth most important cereal grain in
53 the world (FAO, 2014). It accounts for 6% of the total cereal production in Africa, and 14% in
54 West Africa alone (FAO, 2014). Pearl millet grain is a significant source of micronutrients
55 | such as iron and zinc with contents higher than those in other cereals (Souci *et al.*, 2000).
56 Both in sub-Saharan Africa and India, it potentially represents one of the cheapest food
57 sources of these micronutrients and proteins when compared with other cereals and
58 vegetables. In addition, pearl millet is well adapted to dry climates and is mostly grown in
59 areas with limited agronomic potential characterized by low rainfall, in the 200-500 mm
60 range, and marginal soils (Guigaz, 2002). These facts make millet an important food staple
61 over much of the African continent, especially in the semi-arid areas of the Western Sahel
62 where other crops tend to fail because of inadequate rainfall and poor soil conditions. Thus
63 pearl millet is an important cereal in arid and semi-arid regions where it contributes to food
64 security and is expected to have an increased importance in the future adaptation of
65 | agriculture to climate change in sub-Saharan Africa.

66 | Despite its importance, pearl millet is considered as an orphan crop because it has received
67 very little support from science, industry and politics while other crops such as wheat, rice or

68 maize were subjected to intense efforts of genetic and agronomic improvement. As a result, it
69 lags behind sorghum and far behind the other major cereals in its genetic improvement. Its
70 average grain yields barely reach 900 kg/ha, compared to 1500 kg/ha for sorghum (FAO,
71 2014). Moreover, production has increased by only 0.7% a year in West Africa during the last
72 two decades, the lowest growth rate of any food crop in the region and far less than the
73 population's growth rate of nearly 3% per year (United Nations Statistics Division, 2016).
74 However, its untapped genetic potential is vast and could be used to improve pearl millet
75 tolerance to some environmental factors that are the main limitations to its growth potential.
76 For instance, pearl millet is mostly grown in marginal soils such as sandy soils in Western
77 Sahel where low water and nutrient (particularly phosphate) availability are major limiting
78 factors. Moreover, root establishment in poor soil is essential to ensure efficient use of
79 available water.

80 | The importance of root architecture for water and nutrients acquisition has been well
81 | documented in both monocots and dicots, and could be successfully used for root trait-
82 | targeted genetic improvement. For example, targeted modifications of root architecture in pea
83 | to increase P acquisition efficiency were ~~achieved~~produced (Lynch, 2011). Pearl millet is a
84 | monocot species displaying a fibrous root system in which different categories of roots can
85 | contribute to a various extent in root system growth, branching and tropism dynamics as well
86 | as to water transport. Importantly, substantial differences in root traits were reported for 8
87 | pearl millet varieties grown in soil in Niger (Brück *et al.*, 2003) indicating a potential genetic
88 | diversity that could be used for breeding and selecting new varieties with improved root
89 | systems. However, the detailed structure and dynamics of pearl millet root system has not
90 | been described and very little is known about root growth and anatomy.

91 | Here, we analyzed root architecture during the early phase of pearl millet development.
92 | Furthermore, we identified and characterized the anatomy of the different root types. Finally,

93 we compared two root development parameters in 16 pearl millet inbred lines and show that
94 there is a large diversity of phenotypes that could be exploited in later breeding studies.

95

96 2. Material and Methods

97 2.1. Plant material

98 Pearl millet (*Pennisetum glaucum* (L.) R. Br.) inbred lines (Saïdou *et al.*, 2009) originating
99 from Indian, West and Central African landraces were used in this study. Seeds were surface
100 sterilized with 5% hypochlorous acid for two minutes, rinsed three times in sterile water, then
101 immersed in 70% ethanol for two minutes, rinsed three times again and kept for ten minutes
102 in sterile water. Seeds were put in Petri dishes containing wet filter paper for 24 hours in the
103 dark at 30°C for germination. The age of the plants are given in DAG (Days after
104 Germination) i.e. the number of days from the date of seed- transfer onto the filter paper for
105 germination.

106

107 2.2. Root phenotyping

108 For analysis of root development, rhizotrons were built according to Neufeld *et al.*
109 (Neufeld *et al.*, 1989). They were composed of a 400 x 700 x 20 mm aluminum frame, and,
110 from rear to front, a 5 mm extruded polystyrene layer, a 20 mm layer of substrate, a cellulose
111 acetate tissue layer (40 µm mesh) and a 5 mm plexiglass (**Figure 1A**). In this system, the root
112 system grows in two dimensions between the fabric and the plexiglass (**Figure 1B**). The
113 cellulose acetate was chosen because it is both non-deformable, preventing roots to grow
114 through (this was confirmed at harvest), and allows roots to remain hydrated. The water
115 content of the substrate was evaluated at the onset of the experiment and later maintained
116 above stressful threshold by daily weighing the rhizotrons and watering from the top. The
117 substrate used was composed of 30% fine clay, 25% peat fibers, 5% blond peat and 40%

118 frozen black peat (Klasmann-Deilmann France SARL). The average SWC (Soil Water
119 Content) of the substrate was 56% (w:w). At one DAG, one germinated seedling (displaying a
120 primary root of about 1 cm long) was transferred to the top of each rhizotron, in a layer of wet
121 sphagnum. This layer permanently maintained wet in order to prevent the seedlings from
122 drying out during the early stages of growth. The plants were placed in a 1 m² growth room
123 with a 14 hour photoperiod, a temperature of 28°C/ 24°C during days/nights and a VPD of 1.5
124 kPa. From the second day of growth onwards, rhizotrons were scanned (Epson Expression
125 10000XL) every day at a fixed time at a resolution of 600 DPI. Root system outlines were
126 then extracted using SmartRoot (Lobet *et al.*, 2011). These outlines comprised information on
127 all root lengths, branching position and angle for every scan.

128 For high-throughput root phenotyping, a paper-based system was used (**Figure 1C**)
129 according to **Atkinson et al.** (Atkinson *et al.*, 2015). One DAG-old seedlings were transferred
130 into pouches and then maintained in a growth room with a 14 hours photoperiod (28°C during
131 day and 24°C during night). Pictures of the root system were taken every 2 days for 6 days
132 with a D5100 DSLR camera (Nikon) at a resolution of 16 M pixels. The camera was fixed on
133 a holder to maintain the same distance between the lens and each root system. At six DAG,
134 the root tip of the “fastest-growing” plants reached the bottom of the pouches. The experiment
135 was repeated 4 times independently. Root traits (primary root length, lateral root density
136 along the primary root and number of crown roots) were extracted using RootNav (Pound *et*
137 *al.*, 2013).

138 2.3. Root sections and microscopy

139 One DAG-old seedlings were transferred in a hydroponic system containing quarter
140 strength Hoagland medium (Hoagland and Arnon, 1950) or put on the top of seed germination
141 paper (Anchor Paper Company, USA) rolled on itself with the base immersed in distilled
142 water (Hetz *et al.*, 1996). The plants were kept in a growth chamber (12 hour photoperiod, a

143 temperature of 27°C and an hygrometry of 60%) for 10 to 20 days. For sections of fresh
144 material, 1-cm long samples were collected at the root apex and every 5 cm along the root
145 and were embedded in agarose blocks (3% v/v in water) before sectioning, as described in
146 (Lartaud *et al.*, (2014). The sampling positions were recorded. Transverse root sections
147 (thickness 60 µm) were obtained using a HM 650V vibratome (Microm) and observed
148 directly under the epifluorescence microscope. Some section were stained with Safranin and
149 Alcian blue (FASGA, Tolivia & Tolivia, 1987).

150 For thin sections, samples were fixed and dehydrated as described by Scheres *et al.* (1994).
151 Samples were then embedded in Technovit 7100 resin (Heraeus Kulzer) according to the
152 manufacturer's instructions. Thin longitudinal sections (5 µm) were produced with a HM355S
153 microtome (Microm). Sections were stained for 15 min in aqueous 0.01% toluidine blue
154 (pH=6,8) solution and mounted in Clearium Mountant (Surgipath). Sections were visualized
155 using a Leitz DMRB epifluorescence microscope (objectives used: 10x, numerical aperture
156 (NA)=0,3; 20x, NA=0,5; 40x, NA=0,75). Pictures were taken using a Retiga SRV FAST
157 1394 camera (QImaging) and the QCapture Pro7 software (QImaging). Vessel dimensions
158 were measured using ImageJ.

159 2.4. X-ray microcomputed tomography

160 Plants were transferred to pots (50 mm diameter and 120 mm height) containing “Newport
161 Series Loamy Sand” soil (sand 83.2%, silt 4.7%, and clay 12.1%; organic matter 2.93%; pH=
162 7.13; Nitrate= 5.48 mg.L⁻¹; Phosphorus = Defra index of 3 (29.65 mg kg⁻¹)) one DAG. Plants
163 were maintained throughout the experiment at a soil water content of ~75.26% (w:w), which
164 corresponds to 75% of field capacity. The SWC was monitored daily by weighing the pots.
165 Plants were scanned with a v|tome|x M scanner (Phoenix/GE Systems), with a maximum
166 energy of 240 kV, 4 times over an 18 days period (4, 8, 14 and 18 DAG) to image the root

167 structure. Root systems were segmented manually from the image stacks using the VGStudio
168 Max software (Volume Graphics GmbH).

169 2.5 Statistical analyses and heritability estimates

170 Statistical analyses were performed using R (R Development Core Team, 2008). An
171 analysis of variance was performed to detect an effect of the line on the variability of the
172 different root traits measured. When an effect was detected, a Tukey's HSD (Honest
173 Significant Difference) test was used to group lines of homogeneous means for the trait of
174 interest.

175 Broad sense heritability was computed by dividing the variance associated with line with
176 the total variance of the character (variance associated with line + environmental variance +
177 residual variance).

178 Average seed weight for each line was evaluated and a Spearman's rank correlation
179 coefficient was computed to detect a putative correlation between seed weight and root trait.

180

181 3. Results

182 3.1. Early development of pearl millet root system

183 The emergence and development of different roots in pearl millet seedling was studied in
184 different growth conditions. Different roots observed at early stage are named according to
185 the nomenclature presented in **Figure 2A**, based on the nomenclature used for maize root
186 systems (Hochholdinger and Tuberosa, 2009). The first root to emerge from the seed, initially
187 called the radicle, is then called the primary root. A small segment, called the mesocotyl, links
188 the seed and the base of the shoot. At later stages of development, crown roots emerge from
189 the base of the shoot. Branches that appear on the primary or crown roots are called lateral
190 roots. The lateral roots can branch themselves, these ramifications being called secondary
191 lateral roots.

192 The developmental dynamics of the root system was studied more finely on pearl millet
193 line LCICMB1 (line 109 of the panel). In all of the plants that we analyzed in rhizotrons (n =
194 28), the early root system of pearl millet was made up of a single primary root that has
195 emerged from the seed 12 to 24 hours after seed rehydration. This primary root grew
196 vertically at an increasing rate during the first 6 DAG, reaching a maximum of 9.1 cm day^{-1} .
197 After that date, the primary root growth rate slightly slows down, but remains *ca.* 7 cm day^{-1}
198 at 11 DAG (**Figure 2C**). The average primary root length at 11 DAG was 66.3 cm. Crown
199 roots and lateral roots started to emerge respectively from the shoot base and on the primary
200 root at 6 DAG. The average number of crown roots per plant is shown in **Figure 2D**. Crown
201 roots started to emerge 6 DAG and were in average two per plant at the end of the
202 experiment. This number is quite low and this experiment only captured the very beginning of
203 crown root emergence period. Average crown root growth rate was 3.7 cm day^{-1} . The number
204 of lateral roots emerging each day on the primary root is shown on **Figure 2E**. Lateral roots
205 started to emerge on the primary root 6 DAG. Their emergence rhythm increased until the end
206 of the experiment, quickly up to 8 DAG and then slowly between 8 and 11 DAG. Lateral root
207 density on the primary root was 4.2 roots cm^{-1} . Lateral root growth rates were heterogeneous,
208 reaching up to 3 cm day^{-1} . Interestingly, crown roots and lateral roots started to appear at 6
209 DAG, when primary root growth rate reached its maximum, and correlates with the
210 emergence of the third leaf.

211 Early root development was also analyzed in 3D in soil using micro-computed x-ray
212 tomography (**Figure 3**). LCICMB1 plants were grown in small soil columns (5 cm diameter x
213 12 cm high) and scanned at 4, 8, 14 and 18 DAG. As in the rhizotrons, only primary root was
214 visible at 4 DAG and crown and lateral roots could be detected from 8 DAG onwards. This
215 indicated that these roots emerged between 4 and 8 DAG, but the time resolution was too
216 rough to identify a precise emergence date. However, this time interval is consistent with their

217 emergence time observed in rhizotron, of 6 DAG. This observation therefore supports the
218 hypothesis that rhizotrons provide a realistic assessment of root architecture development in
219 natural conditions. The 3D images also gave us information about the organization of the
220 different roots in space. The primary root, first to emerge, grew nearly vertically into the soil
221 volume. On the contrary, crown roots grew at an angle of between 20° and 40° to vertical.
222 This angle appeared conserved for the first centimeters of crown root growth, but the small
223 diameter of the pots scanned constraining root growth to just a few centimeters after
224 emergence, did not allow us to check whether this angle could be maintained. Crown root
225 emergence sites were distributed regularly in space around the stem base.

226 Hence, early root system development in pearl millet is characterized by a fast growing
227 primary root that quickly colonizes deeper soil horizons, while lateral and crown roots only
228 start to emerge 6 DAG.

229

230 3.2. Anatomy of the different root types

231 We next analyzed the cellular organization of primary, crown and lateral roots of young
232 pearl millet plants (LCICMB1 line) grown on germination paper or in hydroponics. Root
233 fragments were harvested at different positions along the root and transverse sections were
234 obtained using a vibratome. As root characteristics did not vary strongly in the zone we
235 sampled (SupFig1 for example ~~for~~ of stele diameter) we considered all the samples we had to
236 define the anatomical features of the different root types (Table 1).

237 Primary roots were characterized by a large diameter metaxylem vessel located at the
238 center of the stele (Figure 4). Their ground tissue contained 3 to 5 layers of cortical cells.
239 Aerenchyma differentiation was observed in mature parts of the root. Crown roots were
240 thicker than primary roots with a significantly larger stele that contained 2 to 5 (3 in most
241 cases) large metaxylem vessels separated by parenchyma cells (Figure 4, Table 1). They also

242 showed 3 to 5 layers of cortical cells and aerenchyma. In both cases, cell wall
243 autofluorescence was lower in the stele close to the root tip and increased particularly in the
244 endodermis as the root matures, presumably because of cell wall lignification and
245 ~~suberization~~suberification accompanying casparian strip formation.

246 In order to localize secondary deposition (lignin or suberin) in the cell wall, we performed
247 FASGA staining on transverse sections of primary and crown roots (Figure 5). The formation
248 of a typical horseshoe-shaped Casparian strip could be visualized in the endodermis of both
249 primary and crown roots as they differentiated. In addition, the FASGA staining revealed 6
250 xylem poles, alternating with 6 phloem poles in the primary root (Figure 5E), while we
251 observed 12 to 16 xylem poles in crown roots (Figure 5D). Mature parts of crown roots
252 displayed a sclerenchyma, surrounded by a hypodermis and a rhizodermis (Figure 5A).

253 Longitudinal sections (5 μm) through the primary root meristem revealed a closed
254 meristem organization with cell files converging to a small group of cells whose location and
255 size are consistent with those of quiescent center cells (Figure 6A). The metaxylem
256 differentiated and expanded radially close to the putative initial cells. Cortex parenchyma
257 cells accumulate metabolites, possibly starch grains, but further investigation is needed to
258 identify the nature of this deposit. Longitudinal sections through the crown root meristem
259 showed a similar closed meristem organization with a larger stele (Figure 6B).

260 Transverse sections through first order lateral roots ($n = 33$) branching from either primary
261 or crown roots revealed distinct organizations. Interestingly, lateral roots could be classified
262 into three types based on their anatomy (Figure 7, Table 1). Type 1 lateral roots are very thin
263 (68-140 μm diameter) with an anatomy characterized by a diarch (2 protoxylem poles) stele
264 without any central metaxylem vessel. Ground tissues include an endodermis, a bi-layered
265 cortex, and epidermis, but neither sclerenchyma nor aerenchyma (Figure 7A, D, G, J). Type
266 2 lateral roots have a medium diameter (235-291 μm), show one small (16 μm diameter in

267 average) metaxylem vessel and 3 layers of cortical cells. Like type 1, type 2 lateral roots have
268 no sclerenchyma or aerenchyma (Figure 7B, E, H, K). Finally, type 3 lateral root exhibit the
269 largest diameter (328-440 μm similar to primary root) and the same organization as primary
270 roots, independently of the root from which they emerge (i.e. primary root or crown root)
271 (Figure 7C, F, I, L). Hence our anatomical studies have revealed that there are 3 distinct
272 types of lateral roots that form on both the primary root and crown roots in pearl millet.

273

274 3.3. Diversity in pearl millet root development

275 We next addressed whether there was significant variation in pearl millet root architecture.
276 We selected 16 lines from a panel of pearl millet inbred lines (Saïdou *et al.*, 2009). As our
277 objective was to maximise diversity, these lines were sampled to represent the whole diversity
278 observed in the phylogenetic tree of 90 inbred lines (Saidou *et al.*, 2009), taking also into
279 account a sufficient seed set availability and good germination rate. We analysed the root
280 system of these plants using a germination-paper-based phenotyping platform (Atkinson *et*
281 *al.*, 2015).

282 We observed large variation in primary root growth and lateral root density along the
283 primary root among the individuals screened of this panel (Figure 8). In both cases, a
284 significant part of this variability was explained by the genetic line variable (ANOVA
285 $p < 0.01$). The lines could be separated into groups of homogeneous means with a Tukey's
286 HSD test. For primary root length, the group identification showed some clear outliers with
287 especially large or small values, associated with a group of lines with intermediate and quite
288 homogeneous values (Figure 8A). For lateral root density, no clear outlier was observed, the
289 values for all the lines forming a rather smooth continuum between small and large values
290 (Figure 8B). The broad-sense heritability was equal to 0.72 for primary root length and to
291 0.34 for lateral root density. We tested whether the variability in early primary root growth

292 was due to differences in available seed reserves by computing the Spearman's rank
293 correlation coefficient between average seed weight and primary root length for each line.
294 The Spearman's rank coefficient correlation was equal to 0.22. This value was not
295 significantly different to zero ($p = 0.21$), indicating that no correlation could be found
296 between seed weights and primary root growth in our experiments. As seed mainly contains
297 reserves, this result suggests that the differences we observed are not simply due to available
298 reserves.

299 ~~We conclude that we were able to detect significant variation in two root traits within a~~
300 ~~subset of pearl millet inbred lines. Our study therefore serves as a proof of concept that will~~
301 ~~form the basis of later genetic experiments designed to identify loci associated with early~~
302 ~~pearl millet root architecture traits.~~

303

304

305 4. Discussion

306 Here, we analyzed root system architecture at early stages of the pearl millet life cycle. We
307 named the different roots following the current standards in terms of monocotyledonous root
308 nomenclature (Hochholdinger *et al.*, 2004). One striking feature of early pearl millet root
309 development is the very rapid emergence and vertical growth of the primary root (7 cm day⁻¹
310 in our experimental conditions) compared to other cereals (3 cm day⁻¹ for maize and wheat ;
311 ~~(Muller *et al.*, 1998; Pahlavanian and Silk, 1988; Pritchard *et al.*, 1987)~~ ~~Pahlavanian et Silk~~
312 ~~1988 ; Muller et al 1998, PCE ; Pritchard et al 1987~~). In contrast, root branching started
313 relatively late after seedling germination (6 DAG). The X-ray CT experiment confirmed this
314 global dynamics of early root system formation. Traditionally, pearl millet is sown at the very
315 start of the rainy season. As it was domesticated in Sahel (Oumar *et al.*, 2008) and is mostly
316 grown in areas characterized by light soils with a low carbon content and water retention

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317 capacity, we hypothesize that the observed developmental pattern can be favorable to the
318 rapid colonization of deep soil horizons that retain some water. This might therefore be an
319 important adaptive strategy to deal with early drought stress. The observed anatomy of pearl
320 millet roots is consistent with those found in other cereals such as rice (Rebouillat *et al.*,
321 2009), wheat, barley and triticale (Watt *et al.*, 2008) or maize (Hochholdinger, 2009). A
322 striking difference between the different root types comes from the number of central
323 metaxylem vessels: one (or two) in the primary root, always more than two in the crown
324 roots, including the root emerging from the scutellar and coleoptile node. Interestingly, our
325 analyses identified three different lateral root types on the basis of their diameter and radial
326 anatomy. Variation in lateral root anatomy has been reported in other cereals, with numbers of
327 distinct types varying from two in rice (Rebouillat *et al.*, 2009) to five in wheat (Watt *et al.*,
328 2008). Recently, a more detailed characterization of cortex cell layers present in rice lateral
329 roots revealed that 3 types of lateral roots exist in rice (Henry *et al.*, 2016). These anatomical
330 distinctions share similar features across species, the smallest root type having a very simple
331 organization, with only two (or three) xylem vessels and no aerenchyma, and the bigger type
332 having an organization similar to a primary root. One can hypothesize that these different
333 lateral root types have different roles: type 1 lateral roots may be involved in the exploitation
334 of resources close to the root whilst type 3 lateral root could be involved in the branching of
335 the root system and the exploration of new soil volumes. The role of type 2 lateral roots is still
336 unclear. Nevertheless, the functional relevance of these differences in anatomy needs to be
337 explored. Similarly, it will be interesting to unravel how these different lateral roots develop
338 and how their formation is controlled by environmental factors. Whilst the molecular
339 mechanism controlling lateral root development has been extensively studied in the model
340 plant *Arabidopsis thaliana* (see Lavenus *et al.*, 2013 for review), how these mechanisms are
341 modified to form different types of lateral roots in Monocots is completely unknown.

342 Root phenotyping of different pearl millet inbred lines revealed a high variability for two
343 root traits within the panel, consistent with an earlier study (Brück, *et al.*, 2003). Here we
344 showed that this variability was also visible *in vitro* at a very early stage of growth (6 DAG).
345 This finding together with the high heritability of the primary root length could be exploited
346 to identify the genetic determinants of primary root growth, a potentially beneficial root trait
347 for pearl millet early establishment. For instance, screening of natural variability of the
348 primary root length have been done at the cellular level in *Arabidopsis thaliana* and led to the
349 identification of a root meristem regulator gene (Meijón *et al.*, 2014). Beside, it will be
350 interesting to exploit the large diversity we observed for primary root growth to test the
351 adaptive value of this character for early drought stress tolerance. We conclude that we were
352 able to detect significant variation in two root traits within a subset of pearl millet inbred
353 lines. Our study therefore serves as a proof of concept that will form the basis of later genetic
354 experiments designed to identify loci associated with early pearl millet root architecture traits.
355 In conclusion, our analysis opens the way to dissecting the genetic determinants controlling
356 key root phenes and the characterization of their impact on yield and stress tolerance in pearl
357 millet.

Comment [SP2]: J'ai déplacé ça là
mais c'est un peu redondant avec la
phrase d'après du coup.

358

359 5. Author contributions

360 SP, PG, DW, JLV, YV, YG, BM, LL designed the study. SP, FG, DM, ML, SG, BMO, JA,
361 MNB, LL performed the experiments. SP, ML, SG, BMO, MJB, DW, JLV, YG, BM, LL
362 analyzed the data. SP, JLV, YG, LL wrote the paper. All authors read and approved the
363 manuscript.

364

365 6. Conflict of interest statement

366 The authors declare that the research was conducted in the absence of any commercial or
367 financial relationships that could be considered as a potential conflict of interest.

368

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380

381 8. References

- 382 Atkinson, J. A., Wingen, L. U., Griffiths, M., Pound, M. P., Gaju, O., Foulkes, M. J., et al.
383 (2015). Phenotyping pipeline reveals major seedling root growth QTL in hexaploid
384 wheat. *J. Exp. Bot.* doi:10.1093/jxb/erv006.
- 385 Bationo, A., Kihara, J., Vanlauwe, B., Waswa, B., and Kimetu, J. (2007). Soil organic carbon
386 dynamics, functions and management in West African agro-ecosystems. *Agric. Syst.* 94,
387 13–25. doi:10.1016/j.agsy.2005.08.011.
- 388 Berg, A., De Noblet-Ducoudré, N., Sultan, B., Lengaigne, M., and Guimberteau, M. (2013).
389 Projections of climate change impacts on potential C4 crop productivity over tropical
390 regions. *Agric. For. Meteorol.* 170, 89–102. doi:10.1016/j.agrformet.2011.12.003.
- 391 Brück, H., Sattelmacher, B., and Payne, W. A. (2003). Varietal differences in shoot and
392 rooting parameters of pearl millet on sandy soils in Niger. *Plant Soil* 251, 175–185.
- 393 FAO (Food and Agriculture Organization of the United Nation) (2014). FAOSTAT database.
394 Available at: http://faostat3.fao.org/browse/Q/*/E.
- 395 Guigaz, M. (2002). *Memento de l'Agronome*. Quae, eds. CIRAD-GRET and Ministère des
396 Affaires Etrangères.
- 397 Henry, S., Divol, F., Bettembourg, M., Bureau, C., Guiderdoni, E., Périn, C., et al. (2016).

- 398 Immunoprofiling of Rice Root Cortex Reveals Two Cortical Subdomains. *Front. Plant*
399 *Sci.* 6, 1–9. doi:10.3389/fpls.2015.01139.
- 400 Hetz, W., Hochholdinger, F., Schwall, M., and Feix, G. (1996). Isolation and characterization
401 of *rctcs*, a maize mutant deficient in the formation of nodal roots. *Plant J.* 10, 845–857.
402 doi:10.1046/j.1365-313X.1996.10050845.x.
- 403 Hoagland, D. R., and Arnon, D. I. (1950). The Water-Culture Method for Growing Plants
404 without Soil. *Calif. Agric. Exp. Stn. Circ.* 347.
- 405 Hochholdinger, F., Park, W. J., Sauer, M., and Woll, K. (2004). From weeds to crops: Genetic
406 analysis of root development in cereals. *Trends Plant Sci.* 9, 42–48.
407 doi:10.1016/j.tplants.2003.11.003.
- 408 Hochholdinger, F., and Tuberosa, R. (2009). Genetic and genomic dissection of maize root
409 development and architecture. *Curr. Opin. Plant Biol.* 12, 172–177.
410 doi:10.1016/j.pbi.2008.12.002.
- 411 Lartaud, M., Perin, C., Courtois, B., Thomas, E., Henry, S., Bettembourg, M., et al. (2014).
412 PHIV-RootCell: a supervised image analysis tool for rice root anatomical parameter
413 quantification. *Front. Plant Sci.* 5, 790. doi:10.3389/fpls.2014.00790.
- 414 Lavenus, J., Goh, T., Roberts, I., Guyomarc'h, S., Lucas, M., De Smet, I., et al. (2013).
415 Lateral root development in *Arabidopsis*: Fifty shades of auxin. *Trends Plant Sci.* 18,
416 1360–1385. doi:10.1016/j.tplants.2013.04.006.
- 417 Lobet, G., Pagès, L., and Draye, X. (2011). A novel image-analysis toolbox enabling
418 quantitative analysis of root system architecture. *Plant Physiol.* 157, 29–39.
419 doi:10.1104/pp.111.179895.
- 420 Lynch, J. P. (2011). Root Phenes for Enhanced Soil Exploration and Phosphorus Acquisition:
421 Tools for Future Crops. *Plant Physiol.* 156, 1041–1049. doi:10.1104/pp.111.175414.
- 422 Meijón, M., Satbhai, S. B., Tsuchimatsu, T., and Busch, W. (2014). Genome-wide association
423 study using cellular traits identifies a new regulator of root development in *Arabidopsis*.
424 *Nat. Genet.* 46, 77–81. doi:10.1038/ng.2824.
- 425 Muller, B., Stosser, M., and Tardieu, F. (1998). Spatial distributions of tissue expansion and
426 cell division rates are related to irradiance and to sugar content in the growing zone of
427 maize roots. *Plant. Cell Environ.* 21, 149–158.
- 428 Neufeld, H. S., Durall, D. M., Rich, P. M., and Tingey, D. T. (1989). A rootbox for
429 quantitative observations on intact entire root systems. *Plant Soil* 117, 295–298.
- 430 Oumar, I., Mariac, C., Pham, J.-L., and Vigouroux, Y. (2008). Phylogeny and origin of pearl
431 millet (*Pennisetum glaucum* [L.] R. Br) as revealed by microsatellite loci. *Theor. Appl.*
432 *Genet.* 117, 489–97. doi:10.1007/s00122-008-0793-4.
- 433 Pahlavanian, A. M., and Silk, W. K. (1988). Effect of Temperature on Spatial and Temporal
434 Aspects of Growth in the Primary Maize Root. *Plant Physiol.* 87, 529–532.
- 435 Pritchard, J., Tomos, A. D., and Wyn Jones, R. G. (1987). Control of Wheat Root Elongation
436 Growth. *J. Exp. Bot.* 38, 948–959.
- 437 R Development Core Team (2008). *R: A language and environment for statistical computing*.
438 R Foundation for Statistical Computing, Vienna, Austria Available at: [http://www.r-](http://www.r-project.org)
439 [project.org](http://www.r-project.org).
- 440 Rebouillat, J., Dievart, A., Verdeil, J. L., Escoute, J., Giese, G., Breitler, J. C., et al. (2009).
441 Molecular genetics of rice root development. *Rice* 2, 15–34. doi:10.1007/s12284-008-

442 9016-5.

443 Saïdou, A. A., Mariac, C., Luong, V., Pham, J. L., Bezançon, G., and Vigouroux, Y. (2009).
444 Association studies identify natural variation at PHYC linked to flowering time and
445 morphological variation in pearl millet. *Genetics* 182, 899–910.
446 doi:10.1534/genetics.109.102756.

447 Scheres, B., Wolkenfelt, H., Willemsen, V., Terlouw, M., Lawson, E., Dean, C., et al. (1994).
448 Embryonic origin of the Arabidopsis primary root and root meristem initials.
449 *Development* 2487, 2475–2487. doi:10.1038/36856.

450 Souci, S. W., Fachmann, W., and Kraut, H. (2000). *Food composition and nutrition tables*.
451 6th editio. Medpharm Scientific Publishers Stuttgart.

452 Tolivia, D., and Tolivia, J. (1987). Fasga : a new polychromatic method for simultaneous and
453 differential staining of plant tissues. *J. Microsc.* 148, 113–117.

454 United Nations Statistics Division (2016). UNData. Available at:
455 <http://data.un.org/Data.aspx?q=population&d=PopDiv&f=variableID%3a12>.

456 Watt, M., Magee, L. J., and McCully, M. E. (2008). Types, structure and potential for axial
457 water flow in the deepest roots of field-grown cereals. *New Phytol.* 178, 135–146.
458 doi:10.1111/j.1469-8137.2007.02358.x.

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461 **Table 1.** Anatomical features of the different root types in pearl millet. Mean and standard
462 deviation of all sections. Letters correspond to groups formed by Tukey's Honest
463 Significant Difference test (alpha=0.05). n: sample size.

Root type	Root diameter (μm)	Stele diameter (μm)	# metaxylem vessels	Metaxylem vessel diameter (μm)	n
Primary root	429 ± 103^{ab}	181 ± 34^b	1	58 ± 11^a	10
Crown root	517 ± 76^a	229 ± 54^a	3	56 ± 9^a	8
LR type 1	112 ± 27^d	32 ± 8^e	0	NA	14
LR type 2	264 ± 22^c	74 ± 9^d	1	16 ± 2^b	7
LR type 3	367 ± 66^b	145 ± 16^c	1	50 ± 6^a	12

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467 Figure 1: A: Scheme of the rhizotron used. B: A rhizotron at the end of an experiment. Scale
468 bar: 5 cm. C: One of the pouches used in the high-throughput phenotyping system. ps:
469 plastic sheet, pr: plastic rod, gp: germination paper. These three elements are held
470 together by foldeback clips (not visible here)

471 Figure 2: A: Scheme of the various roots of a pearl millet seedling. B: Daily average length of
472 the primary root. C: Daily average primary root growth rate. D: Daily cumulative number
473 of lateral roots along the primary root. E: Daily cumulative number of crown roots. N =
474 mean +/- standard deviation.

475 Figure 3: Establishment of the architecture of a soil grown pearl millet root system using X-
476 Ray CT : 2D projection of a 3D image of the root system architecture. **Images at 4 DAG**
477 **(days after germination), 8 DAG, 14 DAG and 18 DAG.** Scale bar: 1 cm

478 Figure 4: Anatomical organization of a primary root (B-I) and a crown root (K-T), 11 and 15
479 days after germination respectively. Transverse sections were performed every 5
480 centimeter, from the root apex to the root basis. A: general view of a primary root with
481 the sampled zones marked by an arrow. B-E : transverse section of primary root observed
482 in transmitted light (scale bar: 100 μ m). F-I : transverse section of primary root focused
483 only on the root stele, observed in epifluorescence (natural autofluorescence at 460-480
484 nm) (scale bar: 50 μ m). J: general view of a crown root with the sample zones marked. K-
485 O : transverse section of crown root observed in transmitted light (scale bar: 100 μ m) P-T
486 : transverse section of crown root focused only on the root stele, observed in
487 epifluorescence (natural autofluorescence at 460-480 nm) (scale bar: 50 μ m) co: cortex,
488 ae: aerenchyma, MX: metaxylem, pX: peripheric xylem vessel, en: endodermis.

489 Figure 5: Transverse section of crown roots and primary root stained with FASGA. Sections

490 were performed at various level along the roots axis. A-C : transverse section of crown
491 root, after FASGA staining. D: transverse section of a crown root after FASGA staining,
492 focus on the stele. E: transverse section of primary root after FASGA staining, focused on
493 the stele (scale bar: 100 μ m) sc: schlerechyma, en: endodermis, X: xylem vessel, MX:
494 metaxylem vessel, ph: phloem vessel, ae: aerenchyma

495 Figure 6: Anatomical organization of primary root and crown apices observed on a
496 longitudinal section, stained with toluidine blue, sampled 5 days after germination. A:
497 Longitudinal section of a primary root apex. B: Longitudinal section of a crown root
498 apex. QC: quiescent center, cc: central cylinder, co: cortex, MX: metaxylem vessel. (scale
499 bar: 100 μ m)

500 Figure 7: Comparative anatomical organization of lateral roots (left: transmitted light, right:
501 autofluorescence). A-F : lateral root emerging from primary root. Picture F only shows
502 the root stele. G-L: lateral root emerging from crown root. 3 root types are identified,
503 independent of the mother root: **LR type 1**: small root diameter and no metaxylem (A, D,
504 G & J), **LR type 2**: medium root diameter and small diameter metaxylem vessel, (B, E, H
505 & K), **LR type 3**: large root diameter and large diameter central metaxylem vessel: (C, F,
506 I & L). Scale bar: 20 μ m

507 Figure 8: High throughput pearl millet root phenotyping: distribution of primary root length
508 (A) and lateral root density (B) among 16 pearl millet from a panel of inbred lines
509 covering a large genetic diversity. Error bars represent standard deviation, letters
510 represent Tukey's HSD groups.

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