

1 **An organ-on-a-chip approach for investigating root-environment interactions in**
2 **heterogeneous conditions**

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21 **Keywords**

22 Root development, environmental sensing, host-microbe interactions, cell-cell
23 communication, calcium signalling, plasticity, microfluidics, organ-on-a-chip

24 **Abstract**

25

26 Plants adapt their root morphology in response to changing environmental
27 conditions, yet it remains largely unknown to what extent developmental adaptations
28 are based on systemic or cell-autonomous responses. We present the dual-flow-
29 RootChip (dfRootChip), a microfluidic organ-on-a-chip platform for asymmetric
30 perfusion of Arabidopsis roots to investigate root-environment interactions under
31 simulated environmental heterogeneity. Applications range from root guidance,
32 monitoring of physiology and development under asymmetric conditions, tracing
33 molecular uptake and selective drug treatments to local inoculation with microbes.
34 We measured calcium responses in roots treated with biotic and abiotic elicitors and
35 observed elicitor-specific signal propagation across the root from treated to
36 untreated cells. We provide evidence for non-autonomous positive regulation of hair
37 growth across the root upon exposure to unfavourable conditions on the opposite
38 side. Our approach sheds light on lateral coordination of morphological adaptation
39 and facilitates studies on root physiology, signalling and development in
40 heterogeneous environments at the organ level.

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42 INTRODUCTION

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44 The rhizosphere is a diverse ecosystem and an environment with great structural and
45 compositional complexity. Soil texture and density, as well as its mineral content or
46 oxygen availability, can vary in a way that a single root system may have to adapt to
47 a range of micro-environmental conditions. Due to both biological activity and abiotic
48 conditions, any local micro-environment is permanently subject to changes,
49 rendering rhizosphere conditions highly dynamic. The ability of plants to adapt their
50 root system architecture according to soil conditions is a prime example of
51 developmental plasticity and has attracted increasing attention over the past
52 decades. The emergence of lateral roots and root hairs can be triggered or inhibited
53 in response to environmental stimuli. Soil conditions such as water content, nutrient
54 concentrations or salinity result in characteristic root system architecture through
55 growth modulation of primary and higher order roots as well as the density and length
56 of root hairs (Gruber et al., 2013; Rellán-Álvarez et al., 2016; Williamson et al., 2001).
57 Root hairs are tip-growing protrusions of specialised root epidermal cells that play
58 important roles in soil penetration and absorption of phosphate and other mineral
59 nutrients (Brown et al., 2013; Haling et al., 2013). Root hair development has served
60 as a readout for the root's response to a lack of mineral nutrients (Bates and Lynch,
61 1996; Chandrika et al., 2013; Müller and Schmidt, 2004; Song and Liu, 2015).
62 Experiments on root systems exposed to different media conditions showed that
63 both lateral root formation and root hair development are regulated independently of
64 the global metabolic state, but rather responded to local conditions (Bates and Lynch,
65 1996). The finding that hair development can be suppressed or stimulated on the
66 same root due to variations in local water availability (Bao et al., 2014) highlights the
67 ability of roots to modify their architecture on a cellular level and underlines the need
68 to study developmental adaptation at cellular resolution.

69 The high sensitivity of root cells towards changing environmental conditions is also
70 illustrated by rapid physiological changes that precede the regulation of root growth
71 and development. Responses to biotic and abiotic stimulation commonly involve
72 calcium signalling (Dodd et al., 2010). The visualisation of cytosolic calcium transients
73 using luminescent or fluorescent sensors has revealed stimulus-specific calcium

74 signatures and long-distance communication in roots (Behera et al., 2015; Choi et al.,
75 2012; 2014; Keinath et al., 2015; Kiegle et al., 2000; Knight et al., 1997; Krebs et al.,
76 2012; Martí et al., 2013; Xiong et al., 2014). Many more studies have highlighted the
77 importance of numerous environmental factors in root development, but how locally
78 perceived signals are communicated to neighbouring cells, how roots differentiate
79 and prioritise between diverse environmental signals and how cell signalling
80 orchestrates root development in complex natural environments is still largely
81 unknown.

82 A technical challenge in the lab has been the simulation of environmental complexity
83 to reflect the soil conditions that roots are exposed to, whilst at the same time being
84 able to control and quantify this environmental complexity. For high-resolution
85 studies on root signalling and development, plants are commonly grown on synthetic
86 gelled or hydroponic media. This reductionist approach has, however, limitations as
87 environmental diversity may be a critical factor for root development. Indeed, it has
88 been shown that numerous physiological and developmental processes are
89 significantly different between soil-grown and media-grown plants (Downie et al.,
90 2014; Rellán-Álvarez et al., 2015). Hence, there is a lack of knowledge regarding how
91 environmental diversity influences root development. New approaches and
92 technologies are needed that enable root growth in defined, yet increasingly complex,
93 environments with the possibility to locally apply a stimulus to selected cells in a
94 single root.

95 Organ-on-a-chip technology, i.e. the cultivation of tissues in microfluidic devices, has
96 revolutionised experimental access to animal and plant organs by enabling
97 researchers to precisely control the specimen's micro-environment and image
98 biological processes within tissues at high spatiotemporal resolution (Bhatia and
99 Ingber, 2014; Sanati Nezhad, 2014; Zheng et al., 2016). This emerging field therefore
100 offers the potential to simulate the natural diversity found in soil. Indeed, the
101 significance of "soil-on-a-chip" microfluidic technologies for investigating complex
102 relationships between soil-dwelling organisms and their environment has been
103 recently highlighted (Stanley et al., 2015).

104 Herein, we present a microfluidic device, termed the dual-flow-RootChip
105 (dfRootChip), that allows cultivation of *Arabidopsis* roots in asymmetric micro-

106 environments. We guided root growth through an array of micro-pillars that centred
107 the root and allowed us to generate different conditions on either side of the root
108 using laminar flow. We demonstrate the versatility of the approach by following the
109 uptake of fluorescent molecules, by locally trapping plant-pathogenic bacteria at the
110 root surface and by imaging calcium signals upon selective application of abiotic and
111 biotic stresses. In addition, we studied the role of local environmental conditions on
112 the development of root hairs and found that root hair repression and stimulation can
113 be regulated both cell-autonomously and in a coordinated manner across the root.
114 The dfRootChip therefore provides a means for incorporating environmental
115 complexity into experimental design for studies on plant roots.

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119 **RESULTS**

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121 **Design and operation of the dual-flow-RootChip (dfRootChip)**

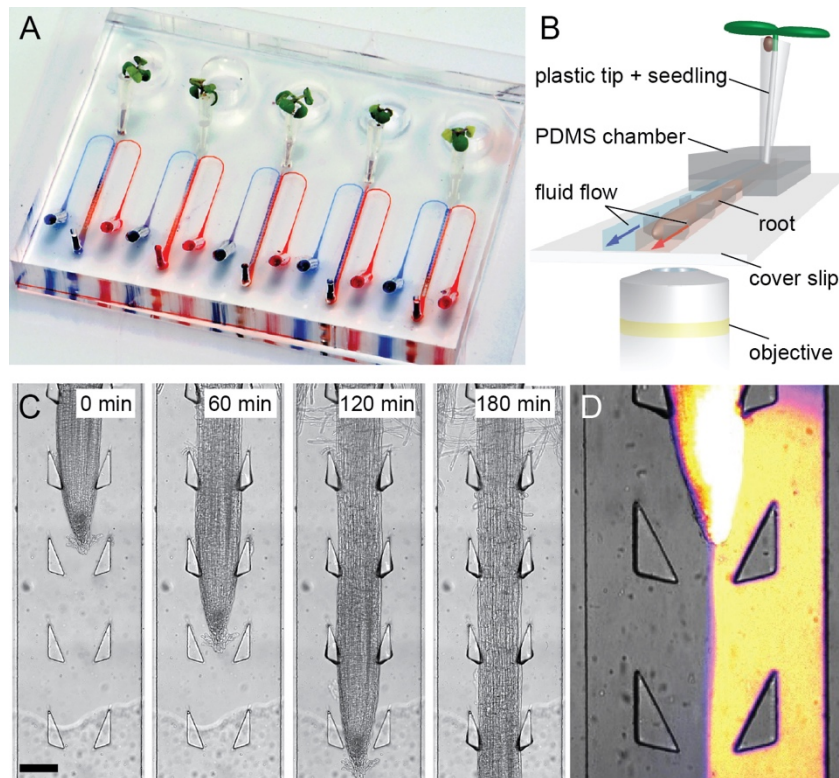
122 The dual-flow-RootChip (dfRootChip) consists of micron-sized root observation
123 chambers that feature a root guidance array and a perfusion system, mounted on
124 optical glass (**Figure 1A,B**). Each observation channel has a height, width and length
125 of 110 μm , 500 μm and 12 mm respectively. Arabidopsis seedlings are grown through
126 medium-filled plastic pipette tips that are inserted into the root inlet of the observation
127 channel (**Supplementary Figure S1B**). Once the primary root has entered the
128 observation channel via a guidance channel (approx. 7 days after germination,
129 **Supplementary Figure S1**), the length of the chamber permits continuous
130 observation of root growth for 2-3 days, depending on the growth rate of the root.
131 For seedlings (Col-0) grown in the dfRootChip we observed growth rates of
132 $1.7 \pm 0.4 \mu\text{m}/\text{min}$ ($n=15$), which falls within a similar range to growth rates observed
133 on gel media (Yazdanbakhsh et al., 2011). A reservoir, connected to the root inlet via
134 a small channel, acts as a means to provide additional medium to each root during
135 initial incubation in growth chambers (**Supplementary Figure S1**). The microchannels
136 were engineered to have a target height of 110 μm as this is the approximate diameter
137 of an Arabidopsis root. Hence, the growing root is confined in the z-direction, which

138 aids imaging of the roots. The compact design of the dfRootChip enables Arabidopsis
139 seedlings to be mounted and several experiments to be performed in parallel. We
140 exemplify this by combining five chambers in parallel on a single glass slide
141 **(Figure 1A)**.

142 One of the major components of the dfRootChip is the root guidance array, which
143 consists of sets of triangular-shaped micropillars distributed in a regular arrangement
144 throughout the root observation channel **(Figure 1C, Supplementary Figure S1)**.
145 This design specifically enables the direction of root growth to be controlled by
146 centring the root tip in the root observation channel **(Figure 1C)**. An inter-pillar
147 distance of 355 μm (along the observation channel) was chosen as this corresponds
148 to a size that is slightly smaller than the distance between elongation zone (EZ) and
149 Arabidopsis root apex. This distance prevents the touch-sensitive columella cells
150 from being stimulated by the pillars and thereby avoids excessive bending of the
151 flexible young shaft, hence avoiding divergence of the root from the desired path. A
152 triangular shape was found to be an appropriate pillar design for aiding the root tip
153 through the pillar array; the shallow angle of the triangular pillar guides the growing
154 root tip towards the centre of the observation channel **(Figure 1C, Supplementary**
155 **Movie 1)**. As the microfluidic devices are comprised of the elastomeric polymer
156 poly(dimethylsiloxane) (PDMS), the tips of the micropillars are flexible and deform
157 when the growing root passes them. Hence, the micropillar array does not only act
158 as a guidance mechanism, but also more accurately simulates the presence of
159 loosely packed soil particles.

160 Two inlet channels and one outlet serve each observation chamber **(Figure 1A,**
161 **Supplementary Figure S1)**. Laminar flow is dominant at these length scales, with a
162 boundary between two co-flowing liquids being evident beyond the root tip
163 **(Figure 1D, Supplementary Movie 2)**. Consequently, the dfRootChip design allows
164 a growing root to be subjected to two different flow conditions simultaneously by
165 utilising laminar flow to generate separate micro-environments, enabling either
166 symmetric or asymmetric perfusion of the root. The guidance channel, which
167 connects the “root inlet” to the observation channel, prevents fluid escaping into the
168 reservoirs and therefore disruption of the channel flow.

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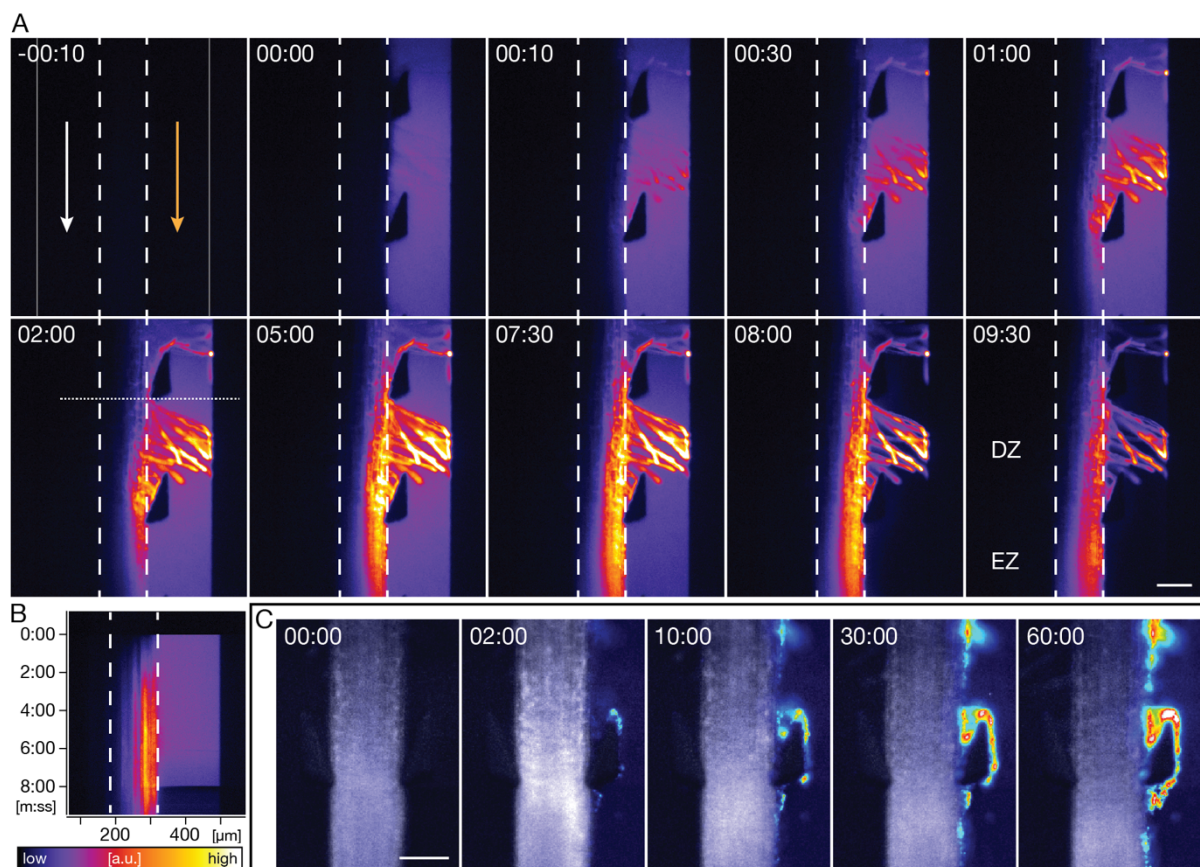
172 **Figure 1. The dual-flow-RootChip (dfRootChip).** (A) Photograph illustrating the dfRootChip mounted
173 with five Arabidopsis seedlings. Two inlet channels serve each chamber and are coloured red and
174 blue. (B) 3D schematic of the device illustrating the direction of flow within the two microchannels. The
175 blue and red colours indicate the ability to deliver two different reagents simultaneously to either side
176 of a growing root. (C) Time-series illustrating growth and guidance of an Arabidopsis root through the
177 array of flexible pillars (see also **Supplementary Movie 1**). Scale bar, 100 μm . (D) Generation of an
178 asymmetric micro-environment using a fluorescein-containing solution for visualisation purposes. At
179 these length scales laminar flow dominates, demonstrated by the presence of a boundary between
180 the two co-flowing liquids.

181

182 **Tracing uptake of small molecules into roots**

183 To test to what extent stable asymmetric availability of small molecules is reflected
184 by gradients inside the root we subjected roots grown inside the dfRootChip
185 to 1 $\mu\text{g/ml}$ fluorescein on one side and traced the uptake of the fluorescent dye over
186 time (**Figure 2A,B**). Within 30 seconds, we observed a fluorescence signal first in root
187 hairs, followed by a signal increase in cortical tissues on the side of treatment. The
188 signal intensities inside cells substantially exceeded the signal of the treatment
189 solution, which may indicate dye accumulation inside cells, but can also be explained
190 by pH-dependent quenching of fluorescein fluorescence in the plant growth medium

191 used (1/2x Hoagland's medium, pH 5.7) (1/2x HM). During the course of dye
192 application (8 min), the signal inside the root did not spread evenly across the organ
193 but instead remained largely retained to the treated side, which indicates that it is
194 possible to generate root-internal gradients and trace the uptake of small molecules
195 into the root. The degree of retention appears, however, dependent on the root zone.
196 Consistent with a maturing endodermis that becomes sealed by the Casparian strip,
197 the signal spread only slowly beyond the treated cortical tissues in the differentiation
198 zone (DZ) and spread more rapidly in the EZ and in zones towards the root tip
199 **(Figure 2A, last panel).**



200
201 **Figure 2. Establishing asymmetric root micro-environments in the dfRootChip. (A)** Uptake of
202 fluorescein upon asymmetric perfusion of an Arabidopsis root over time (time stamp format, mm:ss).
203 The dashed lines outline the root boundaries. Arrows depict the flow direction of 1/2x HM; the orange
204 arrow indicates the channel where a pulsed treatment using a fluorescein-containing solution (1 μg/ml
205 fluorescein in 1/2x HM) was performed on one side of the root between time points 0 and 08:00. **(B)**
206 Kymograph generated along the dotted line displayed in the image above (panel 02:00 in **(A)**). **(C)** Time
207 series of the association of GFP-expressing *Pseudomonas fluorescens* using the triangular pillars as
208 traps. Scale bars, 100 μm.

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210 **The dfRootChip facilitates investigation of root-microbe interactions**

211 Besides the patchy availability of solutes in natural soil, roots also interact with
212 numerous beneficial or pathogenic microbes, which locally associate with the root.
213 As the nature of this colonisation is for many cases still obscure, there is a high
214 demand for tools that facilitate the visualisation of this process *in vivo* over time. We
215 tested whether the pillar array can be utilised as traps for microbes by inoculating the
216 root with a live culture of the plant pathogen *Pseudomonas fluorescens* expressing
217 green fluorescent protein (GFP) (Haney et al., 2015). Within 10 minutes, we observed
218 a steady accumulation of bacterial cells at the pillar structures, in particular at the
219 pillar tips and in the niche between pillar and root (**Figure 2C**). This shows that the
220 pillar array can indeed be advantageous, when a local increase in microbe density is
221 desired to study the colonisation or infection of roots.

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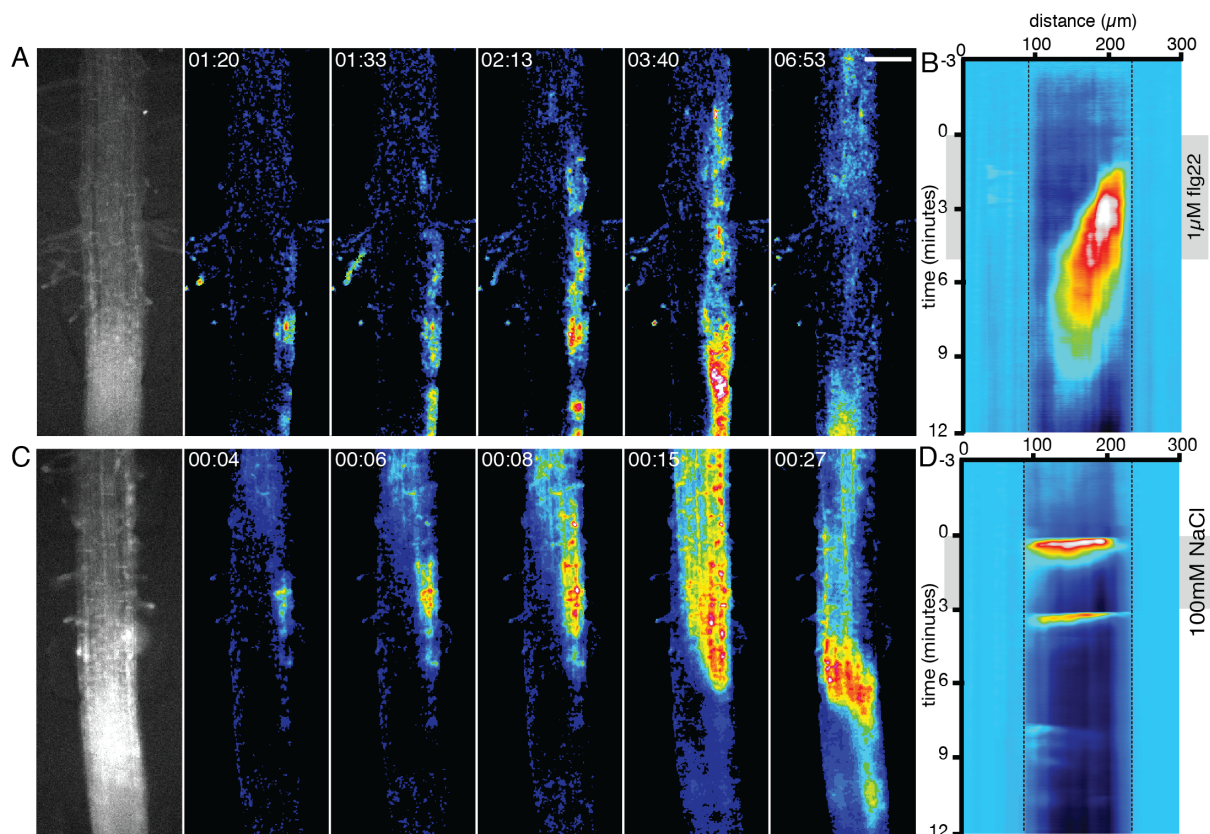
223 **Selective stimulation with calcium elicitors**

224 The observation that it is possible to generate asymmetric root micro-environments,
225 which result in solute gradients inside the root raises the question to what extent such
226 endogenous gradients are perceived by the root and reflected by intra- and
227 intercellular signalling. Cytosolic calcium elevations are among the first signals that
228 are evoked upon environmental stress and have been used as a readout for cell-to-
229 cell communication in plants (Choi et al., 2014; Dodd et al., 2010). To probe cytosolic
230 calcium elevations ($[Ca^{2+}]_{cyt}$) in roots we used Arabidopsis lines expressing the
231 intensimetric calcium indicator R-GECO (Keinath et al., 2015; Zhao et al., 2011). We
232 recorded the $[Ca^{2+}]_{cyt}$ response upon selective stimulation of the primary root by
233 performing a pulsed treatment with the elicitor of interest on one side (**Figure 3**).
234 R-GECO-expressing roots grown in the device were exposed to controlled
235 asymmetric perfusion with 1 μ M flagellin (flg22), a peptide widely used as biotic
236 elicitor that triggers plant immune responses (Felix et al., 1999). The $[Ca^{2+}]_{cyt}$ response
237 upon symmetric treatment with flg22 was reported to start in the epidermis and travel
238 inward to the vasculature from where it would travel rootwards and shootwards in the
239 root (Keinath et al., 2015). Upon applying asymmetric treatments we found that
240 $[Ca^{2+}]_{cyt}$ only rose in the epidermis on the treated side, after which it propagated into
241 deeper tissues with a velocity of $0.62 \pm 0.19 \mu\text{m s}^{-1}$ (n=5) (**Figure 3A, Supplementary**

242 **Movie 3).** The signal decreased substantially before reaching the untreated side of
243 the root, but, instead, propagated shootwards when reaching the vasculature
244 **(Figure 3B).**

245 To assess the $[Ca^{2+}]_{cyt}$ response upon asymmetric exposure to abiotic stress
246 conditions we exposed one side of the root to 100 mM NaCl to mimic salt stress.
247 NaCl treatment is known to generate long-distance $[Ca^{2+}]_{cyt}$ signals propagating
248 through the entire root system at velocities of approximately $400 \mu\text{m s}^{-1}$ when locally
249 applied to the tip of a lateral root (Choi et al., 2014). Under our asymmetric treatments
250 we observed that the $[Ca^{2+}]_{cyt}$ signals started in the epidermis on the treated side and
251 propagated across the root to the epidermis on the untreated side at a velocity of
252 $14.1 \pm 3.61 \mu\text{m s}^{-1}$ (n=6) **(Figure 3C,D, Supplementary Movie 4).**

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255 **Figure 3. Selective stimulation with calcium elicitors.** Calcium dependent signal changes upon
256 asymmetric stimulation with $1 \mu\text{M}$ flg22 **(A, B)** and 100 mM NaCl **(C, D)** in $1/2x$ HM. Elicitors were
257 applied on the right side using a pulsed treatment, while the left side was perfused continuously with
258 $1/2x$ HM. **(A)** and **(C)** depict time series of normalised R-GECO fluorescence images ($\Delta F/F$). **(B)** and **(D)**
259 depict kymographs generated from the left to the right of the entire image sequences represented in
260 **(A)** and **(C)** respectively averaging the intensity over the entire height of the image. Scale bar, $100 \mu\text{m}$.

261 Our results demonstrate that the dfRootChip enables selective treatment and
262 recording of $[Ca^{2+}]_{\text{cyt}}$ elevations in treated and untreated cells simultaneously. Based
263 on the differences in directionality and velocity of calcium signal propagation, we
264 further conclude there must be different mechanisms at play when biotic or abiotic
265 stresses are communicated among root tissues.

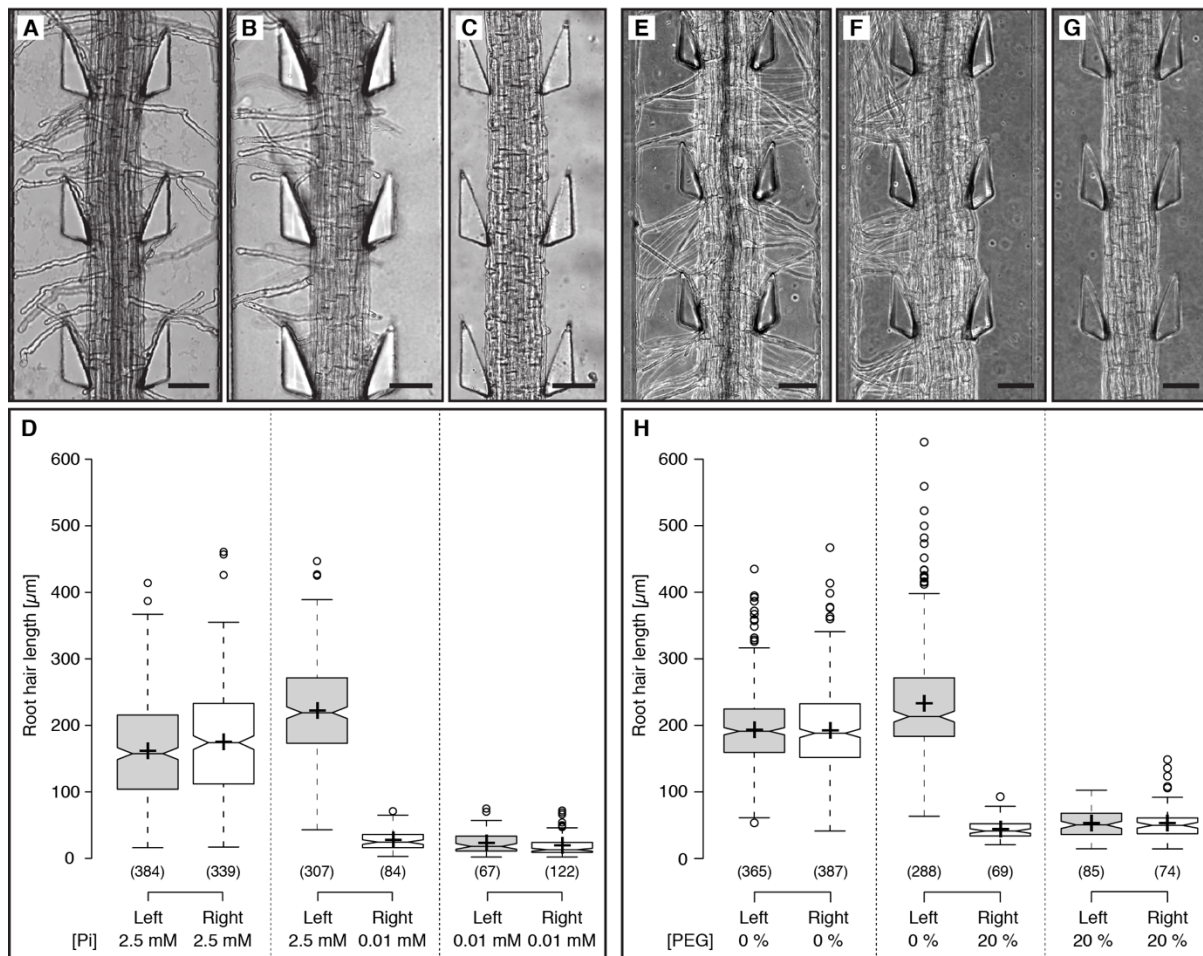
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267 **Root hair development in asymmetric growth environments**

268 Since our data support the notion that Arabidopsis roots are able to perceive and
269 process asymmetric stimuli, the question arose as to whether developmental
270 responses and their potential coordination could also be detected upon exposure to
271 asymmetric growth conditions in the dfRootChip. To test whether stimulation of root
272 hair growth is coordinated across the root or regulated cell-autonomously, we
273 compared the effects of asymmetric and symmetric treatments on root hair length in
274 the dfRootChip. One of the best studied conditions leading to altered root hair length
275 is phosphate deficiency (Ma et al., 2001). Numerous studies with seedlings growing
276 on solid media have shown that phosphate deficiency results in longer root hairs,
277 reduced primary root growth and, due to decreased cell elongation, increased root
278 hair density (Bates and Lynch, 1996; Chandrika et al., 2013; Ma et al., 2001; Song et
279 al., 2016). We therefore expected stimulated root hair growth on the phosphate-
280 deficient side. Surprisingly, when we applied different concentrations of phosphate
281 on the two sides of the root, we observed that root hair growth was inhibited and only
282 short bulges ($27.8 \pm 15.5 \mu\text{m}$) were formed in trichoblasts on the phosphate-deficient
283 (0.01 mM) side, while normal hair growth was observed on the side supplied with
284 2.5 mM phosphate ($222.0 \pm 71.3 \mu\text{m}$) (**Figure 4B,D**). Under symmetric phosphate
285 conditions, we observed that hair growth was also inhibited under phosphate-
286 deficient conditions ($20.6 \pm 16.0 \mu\text{m}$) as compared to symmetric rich conditions
287 ($167.9 \pm 79.8 \mu\text{m}$) (**Figure 4A,C,D**). We obtained similar hair growth phenotypes in
288 experiments using hydroponic growth conditions in medium-filled containers
289 (**Supplementary Figure S2**), which excludes that the unexpected finding was caused
290 by growth in the microfluidic device. Growth tests on vertical plates containing the
291 same medium supplemented with agar revealed, however, stimulated hair
292 development under phosphate-deficient conditions (**Supplementary Figure S2**),

293 which is consistent with published data (Ma et al., 2001).

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Figure 4. Root hair growth under asymmetric phosphate availability and water stress conditions.

(A-C) Primary roots of Arabidopsis Col-0 were grown under (A) symmetric high phosphate availability (2.5 mM KH_2PO_4), (B) asymmetric phosphate availability with high phosphate availability on the left side and low phosphate availability (0.01 mM KH_2PO_4) on the right side and (C) symmetric low phosphate availability. (D) Quantification of root hair length for the conditions given in (A-C). Centre lines depict the median values, crosses represent sample means; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots. Sample points (n) from at least 3 independent experiments are given in parentheses below each box. Note that the median values of root hair length under phosphate deficiency are overestimated due to the frequent complete suppression of root hairs or bulges. Only cells with characteristic hair or bulge morphology were included in the analysis. (E-G) Roots grown under (E) symmetric 1/2x HM, (F) asymmetric treatment with polyethylene glycol (PEG) solution (20% (w/v) PEG 8000 dissolved in 1/2x HM), and (G) symmetric treatment with PEG solution. (H) Quantification of root hair length for the conditions represented in (E-G). Box plot annotations are analogous to the graph in (D). The overestimation of root hair length under 20% PEG applies for the same reason as in (D). Scale bars in (A-C) and (E-G) represent 250 μm .

312 When we compared hair length of trichoblasts exposed to 2.5 mM KH_2PO_4 under
313 symmetric and asymmetric conditions we noticed an increase in hair length by 32%
314 ($p=10^{-23}$) when the opposite side was subjected to phosphate-deficient conditions
315 (0.01 mM KH_2PO_4) (**Figure 4D**). This indicates that hair length in cells exposed to rich
316 phosphate conditions can be influenced by the overall phosphate availability.

317 To test whether the observed asymmetric response in root hair regulation was
318 specific to phosphate availability or a more general phenomenon, we exposed roots
319 to water stress, which has a known inhibitory effect on root hair length (Schnall and
320 Quatrano, 1992). One side of a growing root was subjected continuously to a
321 polyethylene glycol (PEG) solution containing 20% (w/v) PEG 8000 dissolved in 1/2x
322 HM to investigate if root hair growth is influenced upon exerting a low water potential
323 (Ψ_w), i.e. simulating drought conditions (**Figure 4F**). 1/2x HM containing no PEG was
324 introduced continuously on the other side of the growing root. Typical values for a
325 “low Ψ_w stress” and “unstressed” conditions have been defined by Verslues *et al.* as
326 a plant cell being exposed to external water potentials equal to -1.0 MPa
327 and -0.2 MPa respectively (Verslues *et al.*, 2006). A 20% (w/v) PEG 8000 solution was
328 chosen as this equates to a water potential of ca. -0.5 MPa (Michel, 1983), thus
329 mimicking low Ψ_w stress conditions. After symmetric treatment with PEG for
330 16 hours, the average root hair length was found to be $53.3 \pm 22.9 \mu\text{m}$ (**Figure 4G,H**),
331 compared to $193.2 \pm 62.1 \mu\text{m}$ under symmetric 1/2x HM (**Figure 4E,H**). We observed
332 that under asymmetric conditions, root hair growth of the side in contact with the
333 PEG solution was arrested (**Figure 4F,H**) ($43.8 \pm 13.5 \mu\text{m}$), while root hairs continued
334 to grow on the side containing 1/2x HM only ($233.3 \pm 82.0 \mu\text{m}$). Under asymmetric
335 conditions, we further detected a stimulation of hair growth on the 1/2x HM side by
336 21% ($p=10^{-17}$), when compared to symmetric 1/2x HM conditions (**Figure 4H**). This
337 stimulation was in a similar range as observed for asymmetric phosphate availability.

338 Taken together, our experiments on roots under asymmetric phosphate and water
339 availability demonstrate that root hair growth inhibition can be controlled largely
340 cell-autonomously. On the other hand, local deficiencies can stimulate hair length in
341 distal cells, which points to systemic signals or intercellular communication. The
342 mechanisms in charge of these local and distal responses remain, however, to be
343 unveiled.

344 **DISCUSSION**

345

346 **The dfRootChip enables high-resolution studies of root interactions with** 347 **complex micro-environments**

348 Studies on root-environment interactions commonly involve experimental conditions
349 with changes in single parameters to reveal plant responses regarding signalling,
350 metabolism or development. In their natural habitat, plants are, however, exposed to
351 a complex interplay of environmental conditions. Consequently, mechanisms of
352 prioritisation must exist to maximise overall fitness. This includes, for example, the
353 finding that plant immunity responses are suppressed under insufficient light
354 conditions in favour of growth stimulation (Lozano-Durán et al., 2013), or that
355 adaptation of root system architecture to phosphate deficiency depends on the
356 availability of nitrate (Medici et al., 2015). A key to a better understanding of how
357 plants develop in complex environments will be to identify whether responses are
358 systemic or cell-autonomous and to reveal intercellular communication of
359 environmental signals within an organ. It has been recognised that a better
360 understanding of plant developmental plasticity depends on our ability to design
361 experiments that approximate the complexity and dynamics of natural environmental
362 conditions (Rellán-Álvarez et al., 2016).

363 Here we present an experimental tool that allows the simultaneous stimulation of a
364 single plant organ with spatially confined conditions. The dfRootChip represents an
365 adoption of organ-on-a-chip technology for plants and takes advantage of guided
366 root growth and laminar flow inside the observation channel to create separate
367 environments on both sides of the root. We also show that the triangular pillar design
368 cannot only efficiently guide root growth but is also suitable as traps for bacteria or
369 fungi in host-microbe interaction studies.

370 Over the past years, microfluidic devices have been developed for plant science to
371 facilitate microscopic access to Arabidopsis roots (Busch et al., 2012; Grossmann et
372 al., 2011; Jiang et al., 2014; Meier et al., 2010; Parashar and Pandey, 2011), pollen
373 tubes (Horade et al., 2013; Nezhad et al., 2013), or moss (Bascom et al., 2016).
374 RootChips have been particularly useful for measuring cellular concentration changes
375 of small molecules using genetically encoded nanosensors for nutrients (Grossmann

376 et al., 2011; Lanquar et al., 2014), phytohormones (Jones et al., 2014) or the second
377 messenger calcium (Denninger et al., 2014; Keinath et al., 2015). While test solutions
378 or changes in the root micro-environment could, so far, only be applied to the root as
379 a whole, the dfRootChip now provides the ability to apply treatments to one side of
380 the root and also study responses in cells that are not in immediate contact with the
381 test solution. In addition, combinations of two treatments can be tested without
382 exposing individual cells to both treatments. This substantially increases the
383 capabilities of the device for drug treatments, flux measurements, or local stimulation
384 to address cell-cell communication and identify mechanisms of cell autonomy and
385 intercellular coordination.

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387 **Roots are able to sense asymmetric environments and adapt root hair** 388 **development**

389 To reveal how roots perceive and respond to environmental heterogeneity, we used
390 the dfRootChip to provide an asymmetric micro-environment. Our data on fluorescein
391 uptake demonstrated that external gradients of small molecules can be reflected by
392 gradients throughout the root. In our dual flow perfusion system, such internal
393 gradients are stabilised likely by active depletion of freely diffusing, apoplastic
394 fluorescein molecules from the root side facing the flow of unstained solution.

395 We further tested whether asymmetric stimulation with stress elicitors results in
396 asymmetric signalling. Our measurements on roots expressing R-GECO
397 demonstrated that, as expected, calcium responses were first initiated on the side of
398 stimulation. We observed, however, that the propagation of calcium signals differed
399 substantially between responses to the biotic elicitor flg22 and the abiotic elicitor
400 NaCl. While the response to salt stress propagated almost without attenuation across
401 the root within less than 10 seconds ($14.1 \mu\text{m s}^{-1}$), the elicited calcium response to
402 flg22 treatment decayed beyond the vasculature and also propagated at an
403 approximately 20 times slower rate ($0.62 \mu\text{m s}^{-1}$). Recent studies used local
404 application of salt solutions at the root tip and reported shootward long-distance
405 propagation of calcium signals at approximately $400 \mu\text{m s}^{-1}$ (Choi et al., 2014; Evans
406 et al., 2016). It was suggested that electrical signals may act as a fast mode of
407 propagation along cells and the release of extracellular reactive oxygen species helps

408 crossing cell-cell boundaries, albeit at likely a slower rate (Gilroy et al., 2014). The
409 rate-limiting step would therefore be the number of cell-cell boundaries that are
410 crossed over a distance. In line with this model, length:width ratios of fully elongated
411 epidermis (13:1), cortex (10:1) and endodermis (18:1) cells (Hauser et al., 1995) could
412 explain the slower rate of transverse versus longitudinal signal propagation. It
413 remains, however, unclear why the calcium signals in response to flg22 only reached
414 the vasculature and not the untreated side, while salt stress seemed to be perceived
415 on both sides with little delay. A likely explanation for the localised calcium response
416 to flg22 is the involvement of plasma membrane-based receptors and downstream
417 signalling pathways, while NaCl may elicit calcium influx more directly due to mild
418 osmotic effects. It can also be speculated that this behaviour reflects a fundamental
419 difference between the two types of stresses, where biotic threats usually occur
420 locally and activate local defence mechanisms, while abiotic stresses such as
421 drought or salt affect the entire plant and require responses in distal cells to ensure
422 survival of the organism. On the other hand, since responses to biotic stress often
423 come at a significant cost to the plant, it may be advantageous to restrict responses
424 of innate immunity to infected cells and tissues in their close vicinity.

425 When it comes to developmental adaptation of the root system architecture on the
426 basis of nutrient availability the situation is more complicated. In soil environments
427 with patchy distribution of barely diffusing nutrients, e.g. phosphate, evading
428 depleted zones through growth requires some form of coordination as some cells
429 potentially have to invest more resources than what can immediately be absorbed
430 from the environment. Enhanced root hair growth is considered to be a typical
431 adaptive response to low phosphate availability (Bates and Lynch, 1996; Karthikeyan
432 et al., 2014; Ma et al., 2001; Song et al., 2016). The early finding that the transfer of
433 seedlings between media with different phosphate content always triggered a
434 phosphate concentration-specific response independent of the phosphate content in
435 the medium before transfer was taken as evidence that root hair length is locally
436 controlled (Bates and Lynch, 1996) and was later interpreted as a cell-autonomous
437 response (Karthikeyan et al., 2014). However, so far no experiments had been
438 performed where trichoblasts on the same root were exposed to different external
439 phosphate concentrations at the same time. We therefore used root hair growth as a

440 developmental readout under asymmetric phosphate supply. Instead of enhanced
441 growth of root hairs, we observed, however, repressed root hair growth under
442 phosphate deficient conditions. Although this was unexpected at first, it is in line with
443 a study showing that hair growth stimulation under low phosphate depends on
444 sucrose in the medium (Jain et al., 2007), which was part of the growth conditions in
445 the aforementioned studies on root hair length regulation but absent in our media.
446 We decided to not add sucrose to our media as we aimed to obtain root hair
447 responses under conditions that approximate natural habitats where sucrose is also
448 mostly absent. The apparent discrepancy between our results and previous work can
449 also be explained by the influence of gelling agents on root hair growth, which was
450 shown to have drastic effects with respect to phosphate-dependent hair growth
451 regulation (Jain et al., 2009) and other root traits (Gruber et al., 2013). While previous
452 work has usually employed agar-containing media to study root hair length under
453 phosphate deficiency, our perfusion system involves exclusively synthetic
454 hydroponic media. An additional concern, regarding root hair measurements on
455 vertical plates is the inherent asymmetry in the experimental growth conditions, with
456 one side of the root being in direct contact with the substrate, while the other side is
457 exposed to air. Recent work demonstrated that hair growth is stimulated on the air
458 side and repressed on the medium side (Bao et al., 2014), which means that hair
459 length measurements in many previous phosphate deficiency experiments may have
460 mostly evaluated trichoblasts that were not in direct contact with the medium.
461 Our experiments, using hydroponic perfusion in the dfRootChip demonstrated that
462 root hair growth under phosphate-deficient conditions can be repressed,
463 independent of the overall phosphate supply. This local response is evidence for a
464 cell- or cell-file-autonomous negative regulation of root hair growth. Yet, hair growth
465 on the phosphate-sufficient side was stimulated by approximately 32% when the
466 opposite side of the root was exposed to phosphate-deficient conditions. This
467 response of distal cells indicates the existence of intercellular communication in order
468 to positively regulate root hair growth. Ethylene signalling could present a potential
469 mechanism for this intercellular regulation, since recent studies showed an
470 involvement in root hair growth control under phosphate deficient conditions
471 (Nagarajan et al., 2011; Song et al., 2016; Song and Liu, 2015). Interestingly, a very

472 similar response was observed under asymmetric water-stress conditions; high
473 osmolarity of the perfusion medium containing 20% PEG (w/v) resulted in hair
474 repression and an approximately 21% stimulation of hair growth on the opposite side
475 of the root. We therefore conclude that root hair development can be regulated on
476 both, the cellular and the organ level. Future studies will unveil the mechanisms and
477 regulatory networks that allow plants to adapt their root morphology.

478

479 **Conclusions and perspectives**

480 Environmental complexity will remain a major challenge for studies on plant signalling
481 and development. Yet, we cannot ignore this characteristic feature of natural plant
482 habitats when aiming to understand plant development of model and crop plants
483 alike (Rellán-Álvarez et al., 2016). Advanced technologies for plant cultivation,
484 imaging and quantification will enable us to better mimic field conditions in the
485 laboratory and thereby provide the basis for new discoveries (Ehrhardt and Frommer,
486 2012). In recent years, the adaptation of microfluidics for organismal studies has
487 demonstrated great potential to create dynamic micro-environments and aid new
488 experimental approaches for a number of model organisms (Stanley et al., 2015). It
489 will be interesting to systematically test combinations of environmental conditions in
490 the dfRootChip and reveal further developmental trade-offs and decision-making.
491 Particularly the visualisation of proteins and small molecules using genetically
492 encoded nanosensors is benefitting from the defined conditions provided by Lab-on-
493 a-Chip devices (Jones et al., 2013; Uslu and Grossmann, 2016). In combination,
494 nanosensors and microfluidics offer countless possibilities for studies on plant-
495 environment interactions including tracing nutrient absorption, understanding
496 environmental sensing and stress signalling or investigating intercellular and inter-
497 organismal communication. With environmental complexity taken into account, these
498 studies will yield novel molecular mechanisms and genetic traits that will benefit crop
499 breeding and field research.

500

501 MATERIALS AND METHODS

502

503 Plant lines and growth conditions

504 *Arabidopsis thaliana* seeds (Col-0 and R-GECO) were prepared for imaging as
505 described previously (Grossmann et al., 2012) with some modifications detailed
506 herein. Briefly, the seeds were surface sterilised using 5% sodium hypochlorite
507 solution (Sigma Aldrich, Germany) by vortexing the seeds for 3-5 mins and rinsing
508 three times with sterile double distilled water. The seeds were stratified for 3 days at
509 4 °C and germinated on 10 µl pipette tips (cut to ca. 5 mm in length) filled with half
510 strength Hoagland's medium (1/2x HM) solidified with 0.7% (w/v) plant agar (Duchefa
511 Biochemie, Germany) (Supplementary Table S1). In all experiments the plants were
512 grown continuously under long day conditions (16 h light, 22 °C, 100 µE m⁻² s⁻¹,
513 50-70% relative humidity) except for those involving poly(ethylene glycol) (PEG)
514 treatments, which were grown under short day conditions (9 h light, 24 °C,
515 180 µE m⁻² s⁻¹, 53% relative humidity).

516 Microfluidic device design and mould fabrication

517 The device design was constructed in AutoCAD Mechanical 2011 (Autodesk) and
518 used to create a mylar film photolithography mask (Micro Lithography Services Ltd.,
519 UK). The master mold was manufactured using photolithography (Duffy et al., 1998).
520 Briefly, a 100 mm silicon wafer (Silicon Materials, Germany) was spin-coated with
521 SU-8 3050 photoresist (MicroChem, USA) by dispensing ca. 4 ml of photoresist onto
522 the wafer while spinning at 100 rpm for 5 s with acceleration of 10 rpm/s, then
523 spinning at 500 rpm for 10 s with acceleration of 5 rpm/s and finally at 500 rpm for
524 10 s with acceleration of 5 rpm/s. The resist was baked at 95°C for 20 minutes for
525 hardening and then exposed in an MA6 ultraviolet (UV) mask aligner (Suss Microtec,
526 Germany) using the mylar film photolithography mask, with an exposure energy dose
527 of 500 mJ/cm² ($\lambda = 365$ nm). The resist was again baked at 95°C for 12 minutes.
528 Finally, the SU-8 resist was developed using mr-Dev 600 developer solution
529 (Microresist Technologies, Germany) and characterised using a Dektak XT stylus
530 profilometer (Bruker, USA). The height of the SU-8 structures was found to be on
531 average 115 ± 10 µm in height. The master moulds were then silanised under vacuum
532 for 2 hours with 50 mL chlorotrimethylsilane (Fluka, Germany) per master.

533 **Device fabrication.**

534 PDMS was prepared using a 10:1 ratio of base to curing agent (Sylgard 184, Dow
535 Corning, USA). The base and the curing agent were mixed together thoroughly,
536 degassed for 1 hour under vacuum and poured on top of the master mold. Cover
537 slips (45 x 50 mm, #1, Menzel GmbH, Germany) were spin coated (Laurell
538 Technologies, USA) with approx. 0.02 mm PDMS. The PDMS was then cured
539 overnight at 70 °C, removed from the master mold and diced to size. Precision cutters
540 (Syneo, USA) were used to punch holes to form the root inlets (1.65 mm cutting edge
541 diameter), solution in- and outlets (1.02 mm cutting edge diameter) and reservoirs
542 (4.75 mm cutting edge diameter). The PDMS top layer, containing the microchannels,
543 as well as the PDMS-coated cover slip, were washed and dried as detailed elsewhere
544 (Stanley et al., 2014). Bonding of the PDMS top layer was sealed to the bottom layer(s)
545 of the device using a Diener ZEPTO plasma cleaner (Diener electronic, Germany;
546 conditions: power 50%; 1 min.)

547 **On-chip plant cultivation, media perfusion and treatments**

548 Microfluidic devices were prepared as described previously (Grossmann et al., 2012;
549 2011) with minor modifications. Briefly, the devices were sterilised under ultraviolet
550 light for 20 minutes and the microchannels were filled with 1/2x HM by manually
551 pipetting medium through the root inlet. The inlets and reservoirs were then topped
552 up with 1/2x HM to ensure that they were completely filled. The Arabidopsis seedlings
553 were visually inspected using a stereoscope 4-5 days after germination to select for
554 roots being at approximately the same growth stage. The pipette tips containing the
555 Arabidopsis plants were then inserted into the root inlets under sterile conditions.
556 Each device was then placed into a round plastic petri dish, ca. 15 ml of 1/2x HM
557 introduced to provide a humid environment and each petri dish sealed with parafilm.
558 The petri dishes containing the microfluidic devices and plants were then transferred
559 into the growth chamber, tilted at an angle of ca. 20° to encourage root growth in the
560 direction of the microchannels and left until the roots had grown into the main
561 observation channels. The device was then transferred to a homemade rectangular
562 chip carrier, fixed with an adhesive tape and subsequently mounted on the
563 microscope stage. To maintain a humid environment, the dfRootChip was
564 surrounded by moist tissues and covered with a transparent plastic lid.

565 **Poly(ethylene glycol) (PEG) experiments.**

566 A 20% (w/v) PEG solution was prepared as follows. 40 g of PEG 8000 (Sigma-Aldrich,
567 USA) was dissolved in 100 ml of autoclaved full strength Hoagland's Medium
568 (Supplementary Table S1) and made up to a final volume of 200 ml with double
569 distilled water. The pH was adjusted to 5.7. PEG solutions were always prepared
570 freshly and sterile filtered (0.22 µm filter) before use. Symmetric and asymmetric
571 treatments were performed using two Aladdin-220 syringe pumps (World Precision
572 Instruments, UK) to pump media into the microchannels. Specifically, two 60 ml BD
573 Plastipak sterile syringes containing the test solutions were held in the syringe pump
574 holders and each connected to a 7-port manifold (P-150, Ercatech AG, Switzerland)
575 using a flangeless fitting for 1/16" tubing, luer adapter (Ercatech AG, Switzerland) and
576 polytetrafluoroethylene (PTFE) tubing with inner and outer diameters of 0.25 mm and
577 1/16" respectively (Upchurch Scientific, Germany). Each manifold was used to split
578 the input flow into five separate flows, therefore serving five inlet channels (either
579 inlets 1A-5A or 1B-5B, Supplemental Fig. 1). Tubing from the manifold was directly
580 inserted into the device inlets. Before each experiment, the tubing was thoroughly
581 rinsed with 70% ethanol or autoclaved. Connection of the tubing to the device inlets
582 was always conducted in a sterile hood and fluid was pumped through the tubing
583 when connecting them to the device inlets to prevent the introduction of air bubbles.
584 The flow rate per channel was set to 8 µl/min. Treatments were conducted
585 immediately after removing the microfluidic devices from the plant growth chamber.

586 **Phosphate experiments.**

587 For perfusion experiments under phosphate rich (2.5 mM KH_2PO_4) or phosphate
588 deficient (0.01 mM KH_2PO_4 supplemented with 2.49 mM KCl) conditions, liquid media
589 were prepared as described elsewhere (Chandrika et al., 2013) without the addition
590 of sucrose (**Supplementary Table S1**). For experiments performed on agar plates,
591 the same media were supplemented with 0.7% plant agar (Duchefa, Germany).
592 Treatments were performed as detailed for PEG experiments with the following
593 modifications. The device was connected to a six-channel syringe pump (NE-1600,
594 New Era Pump Systems, USA) via Tygon flexible plastic tubing (ID 0.02 inch;
595 OD 0.06 inch; Saint-Gobain Performance Plastics, France) and stainless steel
596 connectors (0.025" OD, 0.013" ID, 0.5" long; New England Small Tube, USA). The

597 flow rate was set to 5 μ l/min per inlet channel. Initially, roots were perfused with
598 phosphate rich medium on both sides until the root tips had entered the observation
599 channels. The phosphate rich medium was then i) continued or ii) switched on either
600 one or both sides of the roots to perform an asymmetric or symmetric perfusion with
601 the deficient phosphate medium respectively.

602 **Rapid asymmetric treatments with calcium elicitors.**

603 A pressurised vial (10 ml volume) containing the 1/2x HM was connected to one of
604 the device inlets via Tygon tubing, stainless steel connectors and a luer-lock
605 stopcock valve (Vygon, Germany) set to an “open” configuration. The control solution
606 was introduced into the microchannels using a volumetric flow rate of 20 μ l/min until
607 the solution exited the outlet and second solution inlet. A second tubing prefilled with
608 treatment solution (1/2x HM containing either 100 mM NaCl or 1 μ M flg22) was
609 connected to a second pressurised vial via a stopcock valve in a “closed”
610 configuration. This treatment tubing is then connected to the second solution inlet of
611 the dfRootChip. The actively flowing control solution prevents the treatment solution
612 from entering into the device before the stopcock valve is opened. Imaging is started
613 and the treatment solution valve is opened at a given time point to initiate immediate
614 treatment. The control valve is kept open to achieve asymmetric treatment. If
615 symmetric treatment with the treatment solution is required, the control valve is
616 closed and treatment valve opened simultaneously.

617 **Treatments with *Pseudomonas fluorescens*.**

618 The bacterial strain *Pseudomonas fluorescens* WCS365 carrying a plasmid for
619 expression of green fluorescent protein (GFP) (Haney et al., 2015) was maintained on
620 lysogeny broth (LB) medium supplemented with 50 μ g/ml kanamycin and grown
621 aerobically at 28 °C. For experiments, WCS365 cells were inoculated in 20 ml LB
622 supplemented with kanamycin (50 μ g/ml), pelleted during the exponential growth
623 phase ($OD_{600} = 0.1-0.2$) and resuspended in 20 ml 1/2x HM. The *P. fluorescens* cell
624 suspension was directly used for treatments of RGECO roots in the dfRootChip.
625 Before treating the roots with *P. fluorescens*, they were first perfused with 1/2x HM
626 until the root tips had entered the observation channels. The perfusion set-up used
627 in this experiment was the same as described for the phosphate experiment. The flow
628 rate was set to 5 μ l/min per inlet channel for the duration of the experiment.

629 **Imaging and image analysis.**

630 Bright field and fluorescence images as well as time series of growing plant roots
631 were taken on a Nikon Ti-U inverted microscope, equipped with a x10 air immersion
632 N.A. 0.30 objective (Nikon, Switzerland), a Prior ProScan III motorised stage (Prior
633 Scientific, UK) and a CoolSNAP HQ2 camera (Photometrics, Germany) or a Nikon
634 Ti-E, equipped with 20x air immersion N.A. 0.7 and 60x water immersion N.A. 1.2
635 objectives (Nikon), a motorized XYZ stage (Applied Scientific Instrumentation, USA),
636 a filter wheel (Cairn Optospin, UK), a laser launch (Omicron, Light Hub, Germany)
637 housing 5 laser lines (440, 488, 515, 561, 638 nm), and an EMCCD camera
638 (Photometrics, Evolve Delta, USA). NIS-Elements Advanced Research imaging
639 software (Nikon, Switzerland) with autofocus or Micro-Manager 1.4 were used to
640 synchronise long-term, multi-position, time-lapse imaging experiments at an ambient
641 temperature of 24°C. The images were analysed using Fiji (Schindelin et al., 2012).
642 Root hair length was measured using the freehand line tool in Fiji and an Intuos pen
643 tablet (Wacom, USA). Statistical significance between data sets was calculated using
644 a Student's *t*-test. Root hair growth within ca. 1500 µm (phosphate experiments) or
645 1200 µm (PEG experiments) of the growing root tip was excluded during the analysis
646 to discount newly elongating or emerging root hairs. Box blot graphs were generated
647 in R with the help of the BoxPlotR web tool (<http://shiny.chemgrid.org/boxplotr/>). The
648 authors are grateful to the Tyers and Rappsilber labs for providing this helpful
649 resource.

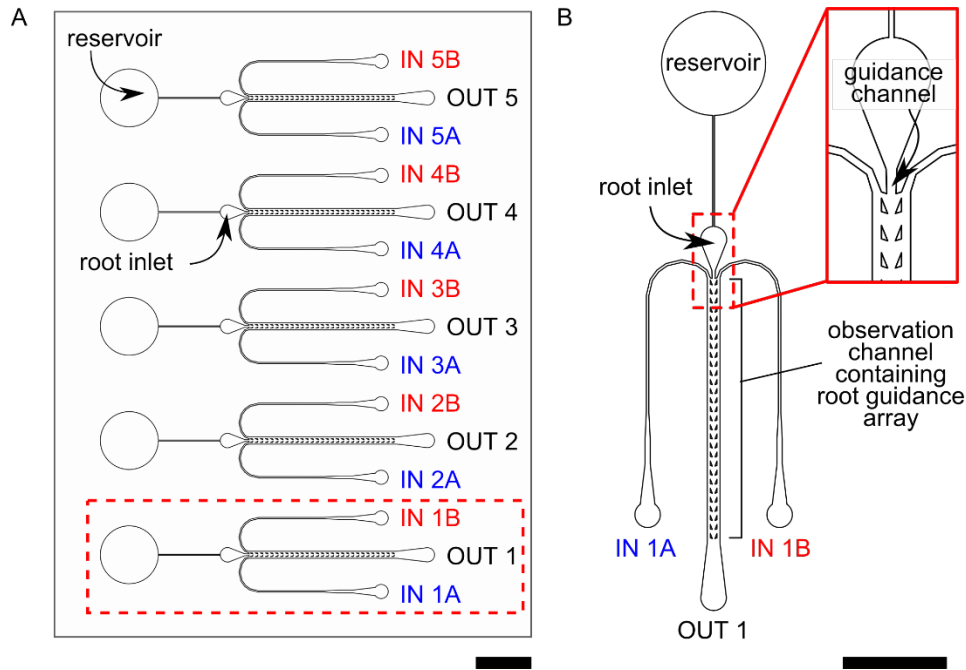
650

651 **SUPPLEMENTARY INFORMATION**

652

653 **Supplementary Figures**

654



657

657 **Supplementary Figure S1. Design master for the dual-flow-RootChip (dfRootChip).** (A) Overview

658 of the design master for the dfRootChip, in which a plant chamber (highlighted by the dotted red box)

659 can be replicated to enable several plant root experiments to be conducted in parallel. We exemplify

660 this by combining five chambers in parallel on a single glass slide. The dfRootChip consists of ten inlet

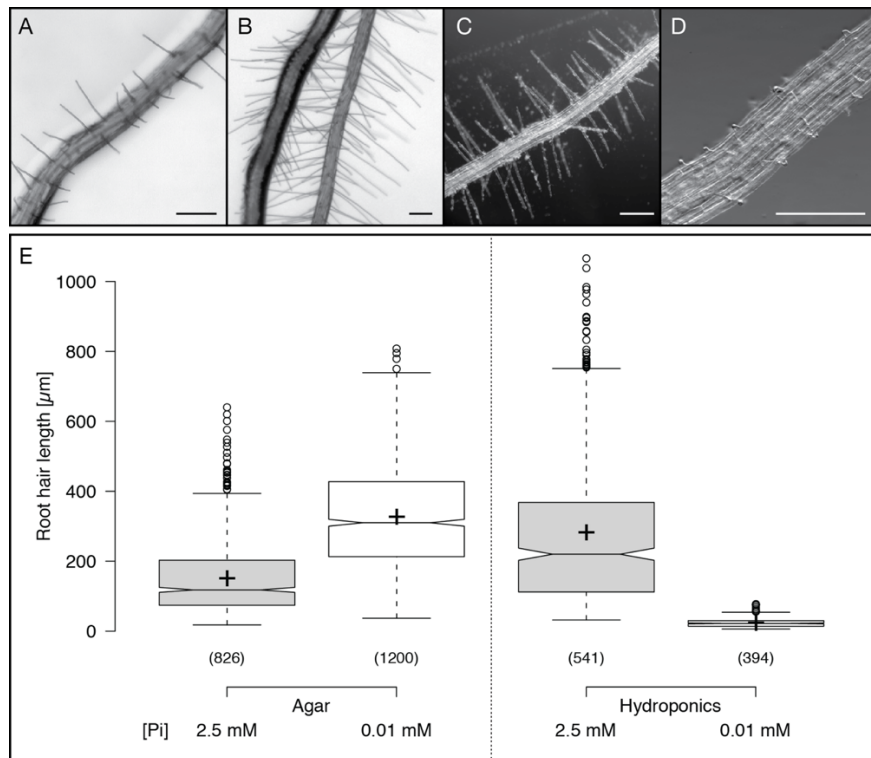
661 channels (two serving each plant chamber) and five outlets. A plant growth medium reservoir is

662 connected to each root inlet (and consequently to the plant root observation channel) via a small

663 channel. (B) An enlarged version of a single plant chamber, where the reservoir, root inlet containing a

664 guidance channel (dotted red box), observation channel and root guidance array are highlighted. Scale

665 bars represent 4 mm.



666

667

668 **Supplementary Figure S2. Root hair growth under high and low phosphate availability on agar**

669 **plates and in hydroponic medium. (A-D)** Representative images of primary roots of 10 days old Col-0
670 seedlings grown on solid medium containing (A) 2.5 mM KH_2PO_4 , (B) 0.01 mM KH_2PO_4 and in
671 hydroponic culture containing (C) 2.5 mM KH_2PO_4 and (D) 0.01 mM KH_2PO_4 . All scale bars represent
672 100 µm. (E) Quantification of root hair length for the conditions given in (A-D). Centre lines depict the
673 median values, crosses represent sample means; box limits indicate the 25th and 75th percentiles;
674 whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are
675 represented by dots. Sample points (n) from at least 2 independent experiments (agar plates) and 3
676 independent experiments (hydroponics), respectively, are given in parentheses below each box.

677

678

679 **Supplementary Movie Legends**

680

681 **Supplementary Movie 1.** An Arabidopsis root growing in the dfRootChip. The time
682 interval between successive frames is 2 minutes. The playback rate is 20 fps. Scale
683 bar, 200 µm.

684

685 **Supplementary Movie 2.** Laminar flow in the dfRootChip. Two co-flowing liquids
686 (water), one of which was supplemented with food colouring to visualise flow

687 separation. Bright field images were captured and false-coloured using the “Royal”
688 lookup table. At time point 8.9 s, the flow pressure is changed from 25 mbar to zero,
689 resulting in a breakdown of laminar flow. The playback rate is 20 fps (real-time
690 playback). Scale bar, 200 μm .

691

692 **Supplementary Movie 3.** Normalised R-GECO fluorescence images ($\Delta F/F$) of
693 calcium dependent signal changes upon asymmetric stimulation with 1 μM flg22 in
694 1/2x HM. The treatment was introduced on the right side. The time interval between
695 successive frames is 0.75 s. The playback rate is 60 fps. Scale bar, 100 μm .

696

697 **Supplementary Movie 4.** Normalised R-GECO fluorescence images ($\Delta F/F$) of
698 calcium dependent signal changes upon asymmetric stimulation with 100 mM NaCl
699 in 1/2x HM. The treatment was introduced on the right side. The time interval between
700 successive frames is 0.75 s. The playback rate is 60 fps. Scale bar, 100 μm .

701

702

703 **Supplementary Materials and Methods**

704

705 **Supplementary Methods S1 | Phosphate experiments conducted in containers**

706 For hydroponic phosphate experiments conducted in small containers, the
707 phosphate rich and deficient media were prepared as described in Supplementary
708 Table S1. Five-day-old Col-0 seedlings grown in pipette tips filled with 1/2x HM
709 medium were inserted into holes punched into a thin PDMS slab, which was then
710 mounted on a round float rack (VWR, America). This set-up was transferred to
711 sterivent high containers (107 x 94 x 96 mm; Duchefa, Germany) filled with 500 ml of
712 either phosphate rich or deficient medium under sterile conditions and the seedlings
713 grown under long day conditions (16 h light, 22 $^{\circ}\text{C}$, 100 $\mu\text{E m}^{-2} \text{s}^{-1}$, 50-70% relative
714 humidity) for 5-7 days. To acquire images, roots were cut, and gently placed on flat
715 glass slides (76 x 26 mm; Labsolute, Germany) and a cover glass slide (24 x 50 mm;
716 Roth, Germany) laid on top. Images were acquired using a SMZ18 Nikon stereoscope
717 (Nikon, Japan) equipped with a 2x objective and Orca Flash 4.0 camera (Hamamatsu,
718 Japan).

719

Supplementary Table S1 | Media used in this study

Medium	Details
1/2x Hoagland's medium (1/2x HM)	0.8 g/L Hoagland's no. 2 basal salt mixture, 0.5 g/L MES hydrate. Adjusted to pH 5.7 with KOH. Agar plates made with 1/2x HM were solidified with 0.7% w/v agar.
Medium containing Poly(ethylene glycol)	1/2x HM containing 20% w/v PEG 8000. Adjusted to pH 5.7 with KOH.
Media containing Ca ²⁺ elicitors	1/2x HM containing either 100 mM NaCl or 1 μM flg22
Phosphate rich medium (Chandrika et. al. 2013)	2.5 mM K ₂ H ₂₂ PO ₄ , 2 mM MgSO ₄ ·7H ₂ O, 5 mM KNO ₃ , 2 mM Ca(NO ₃) ₂ ·4H ₂ O, 0.04 mM Na-Fe-EDTA, 70 μM H ₃ BO ₃ , 0.5 μM CuSO ₄ ·5H ₂ O, 1 μM ZnSO ₄ ·7H ₂ O, 14 μM MnCl ₂ ·2H ₂ O, 0.2 μM Na ₂ MoO ₄ , 0.01 μM CoCl ₂ ·6H ₂ O, 1 g/L MES hydrate. Adjusted to pH 5.5 with KOH. Agar plates made with this medium were solidified with 0.7% w/v agar.
Phosphate deficient medium (Chandrika et. al. 2013)	0.01 mM KH ₂ PO ₄ , 2.49 mM KCl, 2 mM MgSO ₄ ·7H ₂ O, 5 mM KNO ₃ , 2 mM Ca(NO ₃) ₂ ·4H ₂ O, 0.04 mM Na-Fe-EDTA, 70 μM H ₃ BO ₃ , 0.5 μM CuSO ₄ ·5H ₂ O, 1 μM ZnSO ₄ ·7H ₂ O, 14 μM MnCl ₂ ·2H ₂ O, 0.2 μM Na ₂ MoO ₄ , 0.01 μM CoCl ₂ ·6H ₂ O, 1 g/L MES hydrate. Adjusted to pH 5.5 with KOH. Agar plates made with this medium were solidified with 0.7% w/v agar.
Lysogeny broth (LB) medium	1% w/v tryptone, 0.5% w/v yeast extract, 1% w/v NaCl

720

721

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732 to GG.

733

734

735 **AUTHOR CONTRIBUTIONS**

736 The study was conceived by GG, the device was designed by CES and GG, with help
737 from DS. CES and JS performed fluorescein, growth and root hair measurements.
738 Experiments involving fluorescent bacteria were conducted by JS. JS and RB
739 performed calcium measurements. CES, JS, RB and GG analysed the data. CES and
740 GG wrote the manuscript with input from JS and RB. All authors read and approved
741 the final version of the manuscript.

742

743

744 **COMPETING FINANCIAL INTEREST STATEMENT**

745 The authors declare no competing financial interests.

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