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Plant-in-chip: Microfluidic system for studying root growth and pathogenic interactions in *Arabidopsis*

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We report a microfluidic platform for the hydroponic growth of *Arabidopsis* plants with high-resolution visualization of root development and root-pathogen interactions. The platform comprises a set of parallel microchannels with individual input/output ports where 1-day old germinated seedlings are initially placed. Under optimum conditions, a root system grows in each microchannel and its images are recorded over a 198-h period. Different concentrations of plant growth media show different root growth characteristics. Later, the developed roots are inoculated with two plant pathogens (nematodes and zoospores) and their physicochemical interactions with the live root systems are observed. © *2011 American Institute of Physics*. [doi:10.1063/1.3604788]

Plant development is greatly dependent upon its interactions with the environment, which may present challenges to its sessile lifestyle.[1] On one hand, plants need to search for essential resources such as light, water, and nutrients.[1,2] On the other hand, they need to compete with neighboring plants for limited resources and defend themselves against soil-borne pathogens.[3–8] In this context, the root system has been well-studied for elucidating the role of different genes,[2,4] proteins,[1,3] and phytohormones[1,5] in the plasticity of root development.[9] A number of important revelations have come to light from root studies such as how plants regulate organ growth rates,[8] foraging responses,[6] defense mechanisms,[3] and cell cycle machinery.[7] Observations of behavioral adaptability of root systems and associated genotype are providing insights into key biophysical and biochemical processes in plants (e.g., synthesis and transport of enzymes,[4] cell production and expansion in growing tissues,[7,8] nutrient sensing and transduction,[4] search and navigation strategies,[5] and evolution of phyllotactic patterns[10–12]) and the varied interrelationships (both symbiotic and parasitic) they establish with their surroundings.

In the past three decades, several techniques were developed to study root development of the model plant, *Arabidopsis thaliana*. Besides being the first plant system to be fully sequenced, *Arabidopsis* has been exploited for its smaller size, ever increasing database of genetic information, and the relative ease of screening mutants.[1–3] Most studies on root development were based on genetic analysis because of the difficulty of characterizing root growth in soil pots.[5] For real-time observation of root architecture, agarose plates have been used to grow *Arabidopsis* plants with controlled local environments.[10–13] However, such experiments on agarose plates have limited spatial resolution (in the millimeter range) and throughput (one experimental condition per plate).[10,14] Recently, a microfluidic device with multi-laminar flow was demonstrated to chemically stimulate a 10–20 μm section of a live *Arabidopsis* root, showing the possible advantages of improved spatial and temporal resolution.[14] In their work, *Arabidopsis* seeds were germinated, grown on agarose plates for 7 or 11 days, and then transferred to open microchannels in a polydimethylsiloxane (PDMS) (Ref. 15) mold. Subsequently, three converging laminar streams were flowed in the device to observe the effects of localized chemical stimulation on auxin transport and root hair growth over a 24-h period.[14]

We hypothesized that germinated *Arabidopsis* seedlings could be directly sown in microfluidic ports connected to microchannels filled with a suitable growth medium. Under optimum hydroponic growth conditions,[16] the shoots would grow upwards while the roots would grow into the microchannels. This could possibly allow the observation of growth kinetics of multiple roots over long time periods with the flexibility of testing plant responses to various abiotic and biotic stresses. Building on this hypothesis, we present a method of growing germinated *Arabidopsis* (*Col*-0) seedlings in a microfluidic platform (Fig. 1(a)). The device comprises eight parallel straight microchannels (length = 1 cm, width = 350 μm, height = 80 μm), each with its individual input and output ports (diameter = 2 mm). The SU-8 photoresist masters are made using standard soft lithography,[15] which are then used for rapid prototyping in PDMS. Each PDMS mold, comprising the desired parallel microchannels, is punched to create input/output ports and irreversibly bonded to a microscope glass slide (75 mm × 25 mm × 0.2 mm) by exposure to oxygen plasma. The microchannels are further connected with thin vertical side channels (length = 750 μm, width = 25 μm, height = 80 μm) to allow

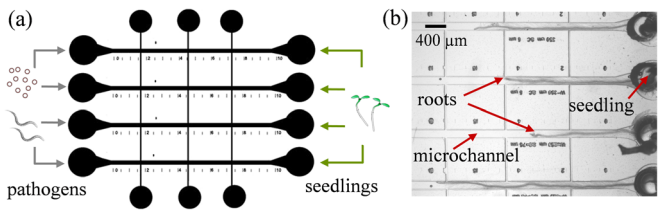

FIG. 1. (Color online) (a) Mask layout of the microfluidic device showing the parallel microchannels with ports for housing seedlings and inoculation of pathogens, (b) snapshot of multiple *Arabidopsis* roots growing in the microchannels filled with DI water. The image is taken after 60 h of planting the seedlings in the input ports.

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the application of chemicals and pathogens in the entire chip. *Arabidopsis* seeds are surface sterilized by treating in 5% sodium hypochlorite solution followed by washing three times with distilled water and putting at 4 °C for 48 h to synchronize germination.[9] After germination, seeds are transferred to half-strength Murashige and Skoog (MS) medium and incubated at 23 °C for 24 h.[9] The microchannels are filled with a pre-specified growth medium. Each seedling is hand-picked using sterile forceps and placed in individual input ports. The chip is put in a Petri dish having a moist wick (tissue paper), which is later sealed (with a parafilm) and punched with perforations for ventilation. The Petri dish is placed in a near vertical position under constant white light intensity (approximately 80–100 μE m^{-2} s^{-2}) at 23 °C.[16] Individual root systems in the entire chip are monitored and imaged for 198 h using a Leica MZ16 stereozoom microscope and QImaging camera (Fig. 1(b)).

Fig. 2 shows the growth parameters of *Arabidopsis* roots measured in four different fluid media (deionized (DI) water, 10% MS, 25% MS, and 50% MS). The transparent microfluidic chip is particularly useful in high-magnification imaging and analysis of root structures (Fig. 2(a)). The root length (L) is calculated as the distance from the root tip to the root apex and is measured for 198 h of growth time (Fig. 2(b)). Data are shown as mean ± SD of at least 12–15 samples in three replicates. In DI water, the growth rate (55.41 ± 9.6 μm/h) is steady during this time period. In 10%, 25% and 50% MS, the growth rate is higher in the first ~53 h (41.97 ± 17.0 μm/h, 29.47 ± 17.0 μm/h, and 13.45 ± 3.4 μm/h, respectively) and becomes slower at later times (12.48 ± 6.3 μm/h, 5.92 ± 2.1 μm/h, and 3.16 ± 2.9 μm/h, respectively). At the end of the growth period, the roots in DI water are significantly longer (L = 9344.95 ± 901.3 μm) and thinner than those in 10% MS (L = 4062.71 ± 450.0 μm), 25% MS (L = 2689.44 ± 588.7 μm), or 50% MS media (L = 1275.43 ± 53.9 μm). Furthermore, root hairs are thinner and longer in DI water compared to those in 25% and 50% MS media. This observation is in agreement with current literature[17,18] that suggests that root systems are short, compact, and densely branched in nutrient-rich growth media (e.g., 50% MS), while they are long, thin, and sparsely branched in more dilute media (e.g., DI water). In addition, the root diameter (Fig. 2(c)) and cell length (Fig. 2(d)) are measured along the root (for up to 4 mm from the root tip) at the end of the growth period. For these measurements, highly magnified images of the different root sections are recorded, and QImaging calibration tools are used to extract the various physical dimensions. In each media, the root diameter is roughly uniform throughout the root length (DI water: 100.86 ± 8.6 μm, 10% MS: 154.23 ± 10.9 μm, 25% MS: 111.53 ± 6.3 μm). However, there is significant difference between the roots thicknesses measured in different growth media ($p < 0.05$, one-way analysis of variance). The cell length is smaller close to the root tip (DI water: 21.57 ± 3.1 μm, 10% MS 13.05 ± 5.8 μm, 25% MS 28.0 ± 4.4 μm) and larger towards the root apex (DI water: 69.58 ± 17.4 μm, 10% MS 33.24 ± 5.8 μm, 25% MS 56.29 ± 5.1 μm).

Besides root development, we show that the microfluidic platform is useful for studying root-pathogen interactions. Both nematodes (e.g., sugarbeet nematode (SBN)) and oomycetes (e.g., *Phytophthora sojae*) are known to establish intimate relationships with numerous plants, resulting in several economically important diseases.[19–24] Currently, functional genomic strategies of plant pathosystems,[19,21] along with microarray analyses and polymerase chain reaction techniques,[22] are providing exciting leads into the role of specific genes in establishing biotrophic parasitism.[3,8] It is, however, difficult to visually observe early biophysical or chemical interactions between pathogens and root systems with real-time imaging.[19–21] We inoculated the developed roots in the microchannels with two pathogens: SBN and *P. sojae*. Upon inoculation, the SBNs migrated through the microchannels and found their way to the roots within the first 1–2 h. Subsequently, they probed the root surface along its length and started penetrating the cell layers in the next 12 h (Fig. 3(a)). No visual changes in the root were noticed in the initial few hours. As such, image recordings were continued every 24 h for the next 4 days. After 2–3 days, some

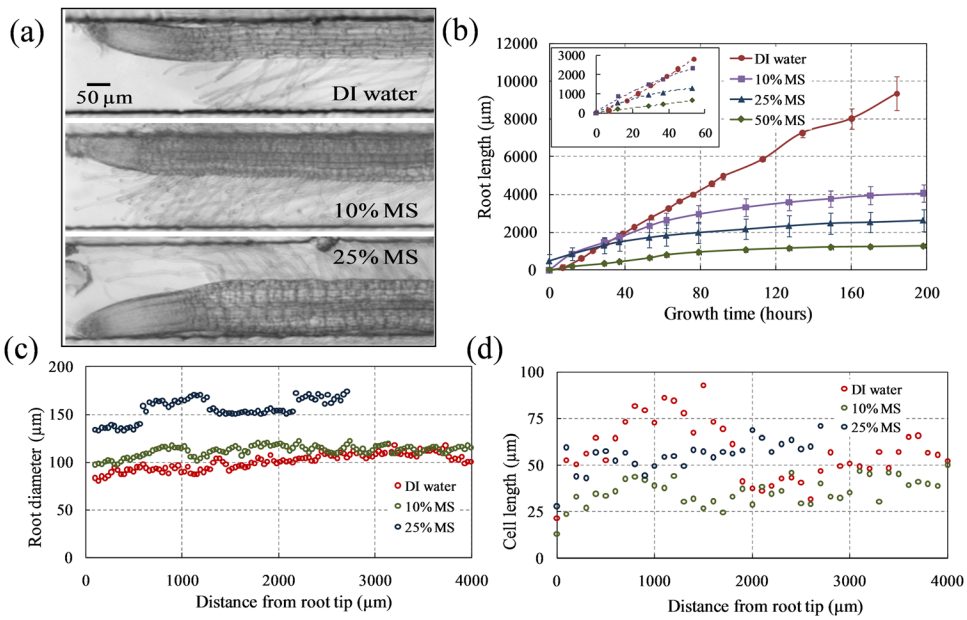

FIG. 2. (Color online) Root growth parameters measured during hydroponic growth of the *Arabidopsis* plants in the microfluidic device with different concentrations of growth media. (a) Snapshots of the root tip (at the end of the growth period) grown in DI water, 10% MS, and 25% MS media, (b) root length versus growth time, (c) root diameter, and (d) cell length along the root (for the first 4 mm from root tip) at the end of 198 h.

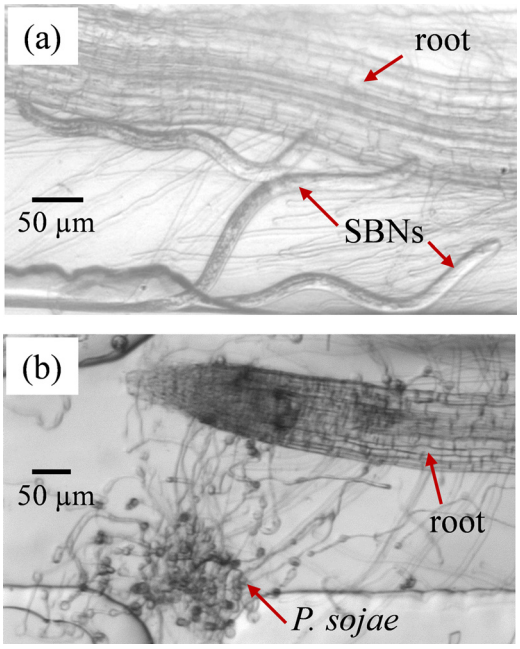

FIG. 3. (Color online) Illustration of interactions between the *Arabidopsis* roots grown in microfluidic device with two plant pathogens. (a) Sugarbeet nematodes, inoculated 2 h before, are probing the root surface (enhanced online) [URL: http://dx.doi.org/10.1063/1.3604788]. Later, some of them find their way to the root center and use their stylus to draw nutrients from the root (enhanced online) [URL: http://dx.doi.org/10.1063/1.3604788]. (b) *P. sojae* zoospores, inoculated 24 h before, cluster around the root tip and grow hyphae towards the root.

SBNs (n = 3–5 per root) were found inside roots (usually near the root apex) with their body aligned with the central vein of the root. The rhythmic motion of the stylet (i.e., hollow and feeding tube) was observed in the real-time videos.[25] This is characteristic of plant-parasitic nematodes that use their stylets to secrete substances and establish feeding sites within the root. Compared to SBNs (~400 μm long), *P. sojae* zoospores (i.e., motile spores) are much smaller in size (~3–5 μm in diameter) and show a different mode of interaction. Upon inoculation, the zoospores swim randomly in the microchannels and start settling at locations close to the root tip in the first 2 h.[22] They form clusters as they settle, encyst, germinate, and grow their hyphae[22] (i.e., germ tubes) in the next 6 h to penetrate the root tissues. In our control experiments (i.e., without roots), zoospores swell to form appressoria and the hyphae generally grow in random directions.[23] In the presence of roots, appressoria are also formed but with a majority of hyphae extending towards the root (Fig. 3(b)). In subsequent days, the root tip appears darker indicating possible localized cell death.[24,25]

In conclusion, this plant-in-chip platform harnesses the known advantages of microfluidics technology[15] to conduct experiments in root development and root-pathogen interactions. We showed reliable and steady growth of *Arabidopsis* roots in microchannels with imaging at cellular resolution. The root morphology was influenced by different MS concentrations and opens possibilities of testing other nutrients and stress factors.[6,14,18] The hydroponic growth of *Arabidopsis* seedlings in transparent, closed microenvironments over long time periods offers improved throughput in conducting parallel growth tests and eliminates the need of macroscopic agarose plates[14] for culturing seedlings. The real-time imaging of root-pathogen physicochemical interactions demonstrated here can significantly advance our knowledge about the plethora of physiological and molecular changes undergoing in the host or non-host system during pathogenic attack.[3,4] Phenotypic characterization of the complex interactions between these multi-kingdom organisms in such microfluidic platforms can complement existing genetic and proteomic screening tools[16] aimed at engineering nematode-resistant plant mutants and identifying the possible defense strategies (e.g., physical or chemical barriers)[8,23] employed by plant systems.

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[25]See supplementary material at http://dx.doi.org/10.1063/1.3604788 showing pathogens (SBNs and *P. sojae*) interacting with the *Arabidopsis* roots in microchannels.