HIGH-THROUGHPUT PHENOTYPING OF MORPHOLOGICAL TRAITS AND NUTRIENT UPTAKE OF PLANTS USING MICROFLUIDIC DEVICES

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ABSTRACT

Microfluidics and microsystems technologies are relatively under-researched for applications in agriculture and plant sciences that have huge social and economic impacts. This presentation gives a few examples of our recent research results on designing and utilizing microfluidic plant chips and integrated sensors for plant analysis.

KEYWORDS: Plant analysis, Plant phenotyping, Microfluidics, Agriculture

INTRODUCTION

Next-generation sequencing and microarray technologies have recently enabled obtaining data on genotypes of plants [1]. However, the obtained genotype information becomes useful insofar as it allows efficient prediction of traits of plants. Due to large genome sizes and various responses of genes to different environments, it has been a grand challenge to systematically characterize phenotypes of plants [1]. Although *Arabidopsis thaliana*, a small model plant, is easy to grow and has a small genome, rapid phenotyping of *Arabidopsis* under various biotic and abiotic stresses is considerably difficult. This is due, in part, to the lack of a large set of growth conditions using traditional growth chambers, and, in part, to the inconvenience in dynamically tracking changes of traits over time. Therefore, high-throughput plant phenotyping is so far difficult to achieve.

In addition, nutrient stress is one of the major factors to cause unhealthy plant growth. Continuous monitoring of nutrient availability during growth of plants is critical to fertilizer management and environmental sustainability [2-5]. Current practices to apply nitrogen and phosphorus fertilizers are uneconomical and can cause ecological damage [2]. However, there is a significant lack of quantitative information about dynamic variations of N and P contents availability and nutrient uptake of plants. Therefore, it is highly desirable to realize a new ability to characterize nutrient use efficiency, an important trait, of plants in a real-time manner.

While microfluidics and microsystems technologies have enabled numerous miniaturized devices, and have extensively been applied to biology, medical, and life sciences, they have been relatively under-researched for applications in agriculture and plant sciences that have huge social and economic impacts [5]. This presentation gives a few examples of our recent research results on using microfluidic devices and integrated sensors for plant sciences and sustainable agriculture.

INTEGRATED PLANT CHIPS

We have developed microfluidic devices for high-throughput phenotyping of *Arabidopsis* plants (Fig. 1a-c) [1, 5-8]. The device allows multiple plants to grow in different growth sites of the device. A hydrodynamic trapping method is used to load *Arabidopsis* seeds into the seed sites. Seeds are carried by a liquid medium into the chip [1]. Each seed site allows only one seed to come and settle in under a sucking pressure. The seeds are germinated inside the seed sites. The plant roots grow downward into a tapered channel, while the shoots grow upward into a holizontal channel. The plants can grow within the chip for about eleven days [1]. The vertical arrangement of the chip makes it convenient to image seed germination, and emergence and growth of root, hypocotyl, cotyledon, and the first true leaves. By opening up the horizontal channel, the plants can grow out of the chip over more than two weeks [1]. This chip facilitates easy and high-quality observation of plant phenotypes at the whole plant level, as well as at the cellular level [1]. Clear visible phenotypes of the *immutans* mutant of *Arabidopsis*, and phenotypic changes at different developmental stages due to plant-pathogen interactions are observed (Fig. 1d). For *Arabidopsis* plants grown in the chips, the phenotypic variations and the timeline for different developmental stages are consistent with *a priori* data and highly comparable to growth in agar plates. Based on this device, we further developed a phenotyping system [6], consisting of improved vertical plant chips, a programmable robotic arm, low-cost gravity driven pumps, and microfluidic concentration gradient generators. We demonstrated the ability of the system in phenotyping mutants of *Arabidopsis* under chemical (e.g., hormone) stresses [6].

In addition, the ability to generate controllable humidity conditions is of significant benefit for assaying the role of air water contents in studying plant-pathogen interactions [5, 9]. We developed an economical and accessible approach to generate different discrete relative humidity conditions in separated wells of a modified multi-well plate (Fig. 1e) [9]. The device consists of a freeway channel in the top layer, multiple compartmented wells in the bottom layer, a water source, and a drying agent source. The effects of evaporation, diffusion, and convection are utilized to realize a stable discrete humidity gradient. The formation of the humidity gradient can be realized in just a few minutes and maintained over a few days inside the device. The device has been employed to study visible and molecular disease phenotypes of soybean in responses to infection by *Phytophthora sojae*, an oomycete pathogen, under a set of humidity conditions, with two near-isogenic soybean lines, Williams and Williams 82, that differ for a *Phytophthora* resistance gene (Rps1-k) [9]. Our result has showed that at 63% relative humidity, the transcript level of the defense gene GmPR1 was at minimum in the susceptible soybean line Williams and at maximal level in the resistant line Williams 82 following *P. sojae* CC5C infection (Fig. 1f) [9]. This device will benefit many laboratories in the area of seed science and plant-microbe biology, where humidity is an important factor that influences disease infection, establishment, and development [9].

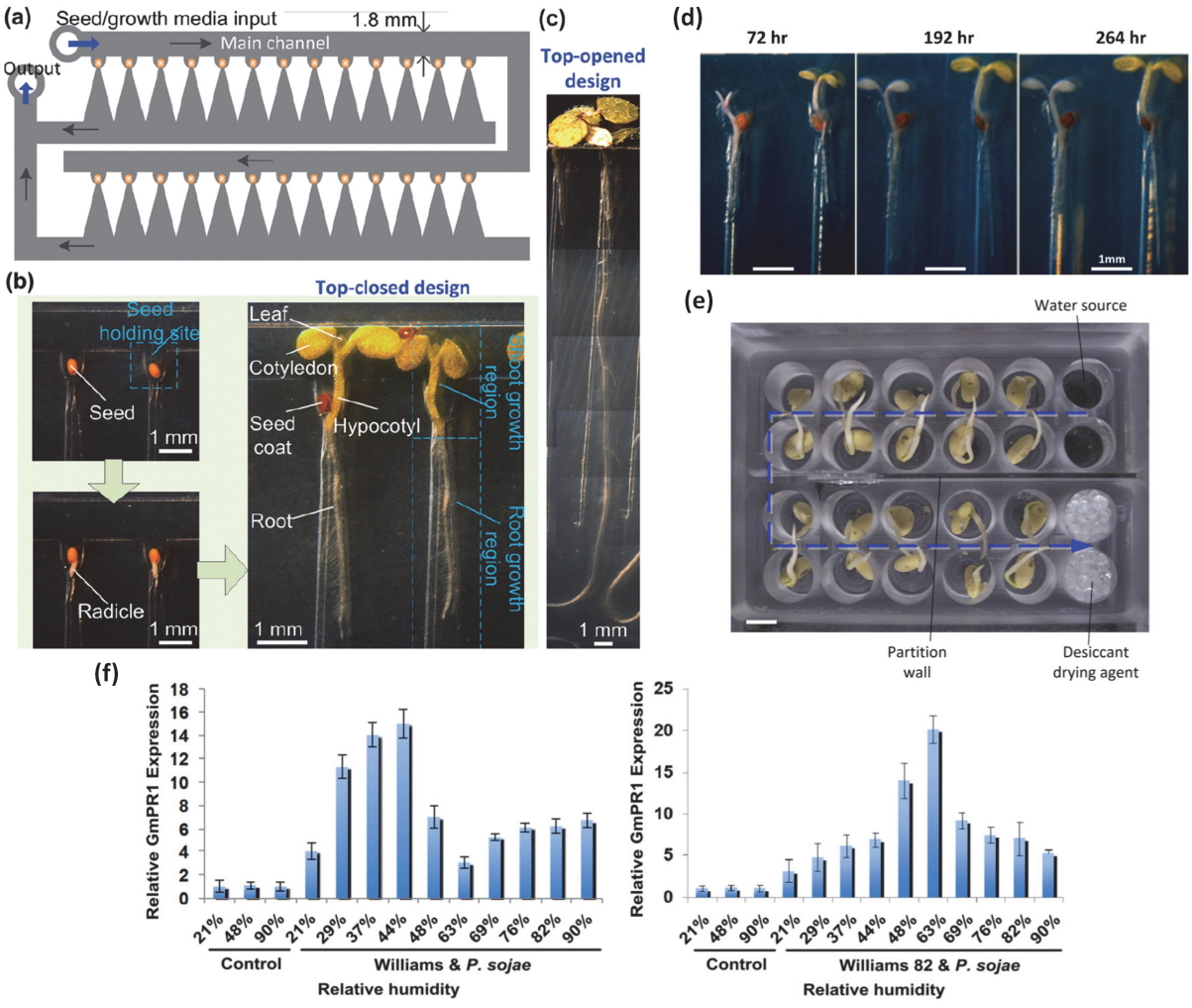

Fig. 1. (a)-(c) Microfluidic plant chip design [1]. (d) Time course study of growth and development of WT Arabidopsis and immutans plants growing in a standard medium in the microfluidic device [1]. (e) Humidity assay for soybean-pathogen interaction [9]. (f) Relative expression of soybean defense GmPR1 gene in infected roots of etiolated soybean seedlings with P. sojae under different humidity conditions as compared to that in the control.

Further, we developed a miniature plant growth device integrated with ion-specific nutrient sensors for real-time monitoring of P and N uptake of plants (Fig. 2a) [10]. The device consists of a poly(methyl methacrylate)

(PMMA) chamber filled with growth medium, ion-specific nutrient sensors formed on the bottom surface of the chamber, and a plastic mesh emplaced on top of the sensors to avoid direct contact of the roots with the sensors (Fig. 2b-e). Rice plants are used as a model system in this study. The sensor is constructed by two Ag/AgCl electrodes, with one coated by an ion-selective membrane (ISM) to form a working electrode (WE) and the other uncoated as a reference electrode (RE) [10]. Due to the nitrogen (here, nitrate) and phosphorus (here, dihydrogen phosphate) uptake by the plants, their concentrations in the growth medium will reduce. This can lead to measurable changes in the potential difference (i.e., electromotive force or EMF) between the WE and RE. As the initial compositions and concentrations of the medium are well defined, the detected reduction in the concentrations of specific ions indicate the corresponding uptake by the plant, thus enabling accurate quantification of nutrient use efficiency [10].

The embedded sensors were used to measure $H_2PO_4^-$ (0.625 mM KH_2PO_4) and NO_3^- (10 mM NH_4NO_3 and 9.4 mM KNO_3) in the growth medium of the plants [10]. The $H_2PO_4^-$ and NO_3^- concentrations were monitored over five successive days (Fig. 2f). The longer the plant growth time, the less the $H_2PO_4^-$ and NO_3^- concentrations were observed in the medium, resulting from the nutrient uptake by the plants (Fig. 2g). Further, as the growth chamber was replenished with the refresh medium once every 5 days over 15 days, the plants were found to routinely absorb $H_2PO_4^-$ and NO_3^- (Fig. 2g). Fig. 2h compares the concentration changes measured using the integrated sensors with that obtained using the expensive and labor intensive ion chromatography method, showing the two methods provide consistent results.

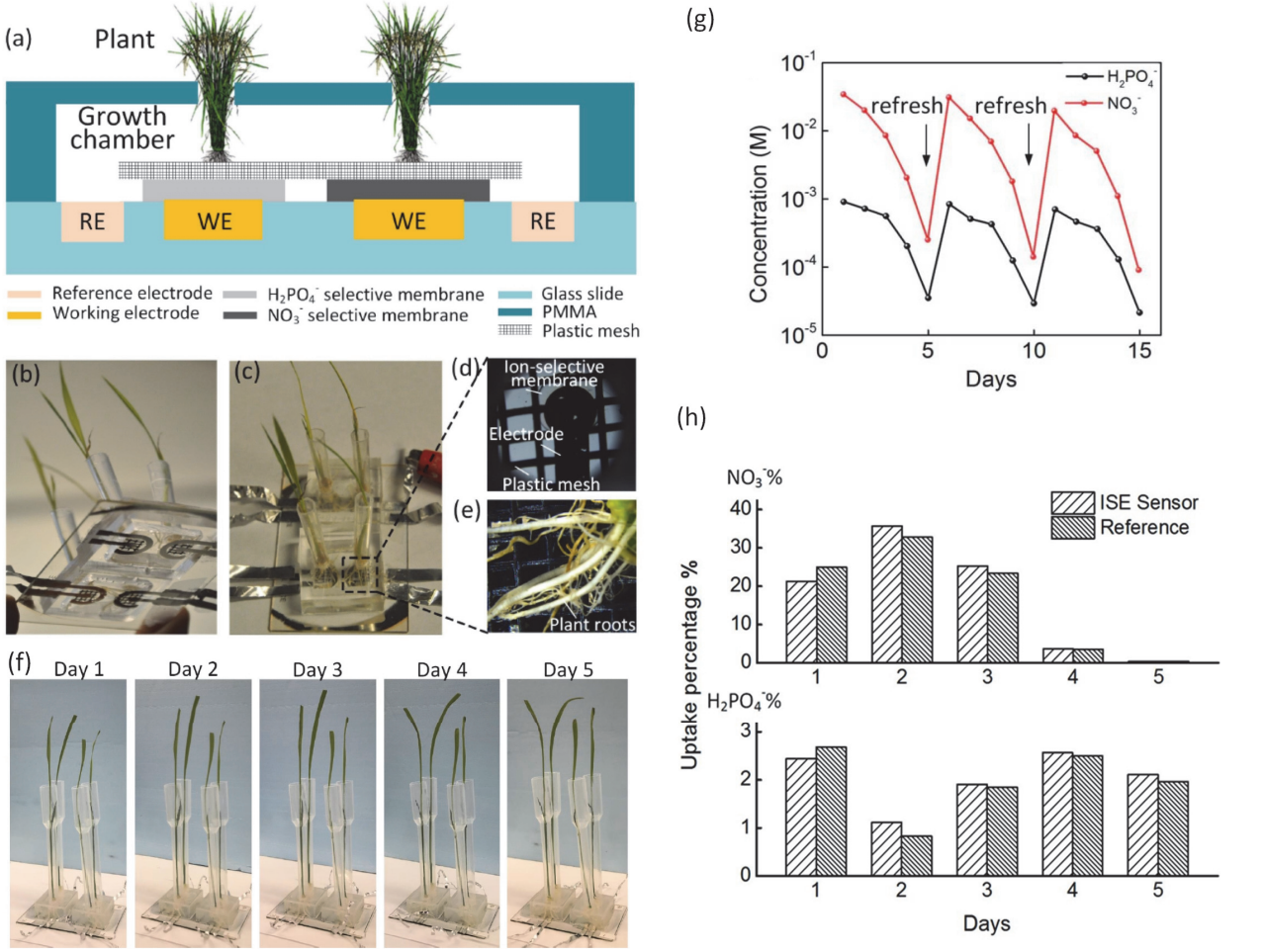

Figure 2: (a) Schematic cross-sectional view of the plant chip with integrated sensors. (b)-(c) Photos of the integrated device, (d)-(e) photos of the fabricated ion-selective electrode and plant roots inset the growth chamber. (f) Photos of the plants grown in the plant chip with integrated sensors. (g) Repeatability study of the $H_2PO_4^-$ and NO_3^- selective sensor with the plants grown for 15 days in the growth chambers. (h) Comparison of the concentration changes measured using the integrated sensors with that obtained using ion chromatography technique.

CONCLUSION

We demonstrate that microfluidic plant chips and integrated sensors are useful for plant analysis. We believe that the microfluidic plant chip technology, in combination with various embedded sensors, can contribute towards establishing a powerful experimental framework for high-throughput and precise plant phenotyping, and it will create a paradigm shift in the plant phenomics and precision agriculture areas [1, 5].

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