



On-chip Analytical Methods for Investigating Plant-Soil Interactions

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(Degree)

博士 (政治学)

(Date of Degree)

2025-09-25

(Date of Publication)

2027-09-25

(Resource Type)

doctoral thesis

(Report Number)

甲第9322号

(URL)

<https://hdl.handle.net/20.500.14094/0100498362>

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(別紙様式3)

論文内容の要旨

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論文題目 (外国語の場合は、その和訳を併記すること。)

On-chip Analytical Methods for Investigating Plant-Soil
Interactions

(マイクロデバイスを用いた植物と土壌の相互作用の解
析手法に関する研究)

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1. Background

Optimizing plant growth environments remains critical challenges in both scientific researches and agricultural applications. To analyze the interactions between the plants and soil, the conventional research approaches often struggle to simulate natural conditions and accelerate experimental cycles. As the demand for experimental efficiency increases, Micro-Electro-Mechanical Systems (MEMS) applications have attracted significant attention. Beyond conventional applications including physical and chemical sensors, MEMS technologies have shown great potential in emerging fields like environmental sensing and sample monitoring. Although the application of MEMS in biological research remains limited, their miniaturization offers substantial advantages for enhancing experimental precision and efficiency. To promote the practical application of MEMS in plant biology, we focused on the development of MEMS-based devices to investigate various aspects of plant – soil interactions. In this study, we developed three types of MEMS devices with distinct purposes: (1) to study the chemotaxis of plant-parasitic root-knot nematodes (RKNs), (2) to measure the protrusive force generated by roots penetrating the soil during elongation, and (3) to analyze root gravitropism during bending growth. These polydimethylsiloxane (PDMS)-based microdevices are compact and transparent, thus we can simulate natural soil environments, greatly improving both observation accuracy and experimental efficiency using the devices.

2. Challenges and Objectives

Many researchers have investigated plant – soil interactions, such as pest control and root nutrient acquisition. However, studies on environmentally friendly nematode control strategies and the mechanical properties of root growth in soil remain limited. To advance the study of plant – soil interactions, several challenges need to be addressed:

- (1) Current methods for studying nematode chemotaxis mainly include agar plate assays and, more recently, microfluidic device-based approaches. Traditional agar plate assays face challenges in controlling the formation of concentration gradients, making it difficult to accurately quantify chemotactic behavior. Although microfluidic devices offer improved gradient control, previous studies still require significant time for nematode loading and gradient formation, which limits research efficiency.
- (2) The depth of roots in the soil directly affects plant anchorage and nutrient absorption. To penetrate the soil, especially under drought conditions or in heterogeneous environments, roots

must overcome mechanical resistance by exerting protrusive forces. However, conventional methods such as soil- or agar-based cultivation offer limited spatial resolution and are not suitable for high-throughput analysis, making it difficult to quantitatively assess root mechanical forces.

- (3) Gravitropism is a key adaptive mechanism that directs root growth downward, optimizing water and nutrient uptake. Root gravitropic responses involve complex mechanical forces, such as bending moments. Previous research on gravitropism has mainly focused on mathematical modeling or force measurements in stems, while the direct quantification of bending moments generated by roots during bending growth remains largely unexplored.

As a solution to the mentioned challenges, this study proposes three microfluidic-based approaches to quantitatively analyze plant-soil interactions. The objectives of this doctoral dissertation are outlined as follows:

- (1) To address the limitations of traditional agar plate assays in chemotaxis research, this study develops a microfluidic device designed to investigate the chemotaxis of root-knot nematodes and evaluate the effects of various chemicals on their movement. The device is fabricated using a dual-layer polydimethylsiloxane (PDMS) structure that integrates a porous membrane with a pore size of 10 μm . This membrane restricts nematode movement while permitting the diffusion of chemicals, thereby establishing a stable concentration gradient for precise chemotaxis analysis. By introducing nematodes into the microchannel using vacuum suction, the device significantly reduces loading time, enhances data accuracy, and shortens experimental cycles. Using this device, we will assess the effects of KNO_3 , cadaverine, and putrescine on nematode behavior. The findings will not only advance the understanding of chemotaxis in plant-parasitic nematodes but also have broader applications in bacterial and fungal research, offering a novel analytical tool and new strategies for environmentally friendly nematode control research.
- (2) To overcome the limitations of conventional methods in quantify root mechanical interactions with soil, this study develops a sensing micropillar-based device to quantitatively measure the mechanical stiffness (Young's modulus) and protrusive force generated by plant roots during growth. The objective is to gain a deeper understanding of how root systems adapt to and overcome soil mechanical resistance. To achieve this, we have designed a PDMS micro device integrated with sensing micropillars, capable of measuring the protrusive force exerted by growing roots. By Integrating physical modeling, we will calculate the apparent Young's

modulus to assess the biomechanical properties of root growth. The findings will provide a theoretical foundation for optimizing root morphology and enhancing crop adaptability to various soil conditions.

- (3) To address the lack of direct measurements in root gravitropism research, this study quantitatively analyzes the mechanical responses of plant roots during bending motion, with a particular focus on bending moments and root stiffness. We have designed and developed a microdevice based on a micropillar array to measure the mechanical forces exerted by roots under gravitational stimuli. Constructed from PDMS, the device offers high optical transparency and excellent biocompatibility, enabling clear visualization of root growth while providing high-resolution mechanical measurements. The results will not only deepen our understanding of the mechanobiology underlying root gravitropism but also offer theoretical insights for future crop improvement.

3. Experiments and results

Chapter 2 investigates the chemotaxis of plant-parasitic nematodes, focusing specifically on *Meloidogyne incognita* (*M. incognita*), a major agricultural plant-parasitic nematodes that significantly affects crop health.

- (1) Distribution of chemicals in the microchannel

We measured the variation in fluorescence intensity over time within the microchannel and calculated the correlation between fluorescence intensity and the distance from the chemical inlet, confirming that a stable concentration gradient was formed after five minutes. This significantly reduced the gradient formation time and improved experimental efficiency.

- (2) Chemotaxis assay

In the chemotaxis experiments, we first tested solutions of KNO_3 at three different concentrations (1 mM, 10 mM, and 100 mM) to assess the nematode's response. The results showed a concentration-dependent repellent response in *M. incognita* to KNO_3 , with stronger repulsion observed at higher concentrations. We then tested the effect of cadaverine solutions on *M. incognita*. The nematodes were clearly attracted to the cadaverine solution, with the attractive response becoming more pronounced as the concentration increased. Finally, we compared the responses of the nematodes to 100 mM solutions of cadaverine and putrescine. Both chemicals showed an attractive effect, but cadaverine demonstrated a significantly

stronger attraction, indicating that *M. incognita* is more sensitive to cadaverine than to putrescine.

(3) Effect of KNO₃ and putrescine on nematodes movement speed

In analyzing the effects of 100 mM KNO₃ and 100 mM putrescine on the movement speed of *M. incognita*, we observed significant differences in their impacts. KNO₃ was found to significantly inhibit nematode activity, with the average movement speed decreasing from 3.71 $\mu\text{m/s}$ to 3.26 $\mu\text{m/s}$ over the course of the experiment. In contrast, in the putrescine environment, the nematode movement speed initially decreased but gradually recovered to normal levels within 5 to 10 minutes after exposure. This indicates that KNO₃ may induce physiological changes in *M. incognita*, leading to a prolonged reduction in movement speed, while putrescine, although causing temporary inhibition, allows the nematodes to adapt and restore normal activity. These findings highlight the different response mechanisms of *M. incognita* to various chemicals, offering valuable insights for future chemotaxis research.

Chapter 3 introduces a novel microfluidic device designed to quantify the protrusive force of *Arabidopsis* roots. By incorporating sensing micropillar within microchannels, this device enables high spatial resolution, live observation, and efficient measurement of mechanical properties, such as Young's modulus and protrusive force. Understanding root protrusive force is a fundamental aspect of plant biology, providing valuable insights into growth dynamics, development, and responses to environmental factors. This study makes a significant contribution to the field of plant biomechanics by offering a new method for root force measurement, thereby advancing research on plant growth and mechanical properties.

(1) Development of an efficient measurement device

This study focuses on the design of a microfluidic device that stabilizes *Arabidopsis* root growth while providing precise force measurements during the growth process. This device enhances the ability to monitor and measure the mechanical forces exerted by the roots in real-time, improving both the accuracy and reliability of the data.

(2) Root growth assay

In the growth experiments, we found that after the root tip contacted the sensing micropillar, the root's growth rate significantly decreased, indicating that encountering obstacles during growth imposes notable mechanical resistance that slows down elongation. Additionally, this phenomenon showed noticeable variation among individual roots.

(3) Force measurement and analysis

In the experiments, we used micropillars with different side lengths of cross-sectional area ($a=0.1\text{ mm}$, 0.15 mm , and 0.2 mm) to measure the protrusive force exerted by the root tip and analyze the effects of different micropillar stiffness on root growth. The results showed that the spring constant of the micropillars was proportional to their cross-sectional area ($0.2\text{ mm} > 0.15\text{ mm} > 0.1\text{ mm}$). After contact, the root's growth rate decreased with increasing micropillar stiffness. Despite the varying conditions, the average measured protrusive force remained consistent at approximately $1.8 \times 10^{-3}\text{ N}$, suggesting that roots maintain a relatively stable force when facing obstacles of different hardness. The calculated Young's modulus of the roots was approximately 867 kPa .

Chapter 4 introduces the development of a micro-pillar device designed to measure the forces generated by plant roots during gravitropism using microfabrication technology. Growth experiments were conducted with the model plant *Arabidopsis thaliana*, and micro-pillar devices were placed around the roots in the direction of gravitropic growth to measure the forces exerted by the roots. Through these experimental measurements, key mechanical parameters, such as Young's modulus and the bending moment of the roots, were determined. The study successfully developed the device and established a reliable method for measuring and analyzing these mechanical properties. These findings establish a vital theoretical and experimental foundation for plant gravitropism research, with implications for crop breeding and root environmental adaptability.

(1) Determination of mechanical parameters

This study calculated key mechanical parameters of roots during gravitropism, including the Young's modulus and bending moment, providing essential data for understanding root biomechanics. The Young's modulus was approximately 1.40 MPa , while the bending moment was approximately $22.9 \times 10^{-9}\text{ N}\cdot\text{m}$.

(2) Method validation

The micro-pillar device, along with the measurement and analysis methods developed in this study, was proven to be highly feasible and accurate. The growth experiment results indicated that the device effectively measures the forces generated by roots during gravitropism and accurately determines mechanical parameters such as Young's modulus and bending moment. Thus, this study provides a reliable experimental method and technical means for similar future research.

4. Future works

(1) Nematode management and challenges in agricultural production

This study demonstrates that the introduction of KNO_3 , cadaverine, and putrescine into the soil environment surrounding plant roots can effectively control plant-parasitic nematode (PPN) populations. However, challenges remain in the existing experimental conditions and the use of chemicals, particularly in the establishment and control of multiple chemical gradients. Future research should focus on optimizing the design of microfluidic devices to enable the simultaneous control of various chemical gradients, improving experimental efficiency. Additionally, the development of technologies capable of maintaining stable gradients in complex environments will be essential to support more refined and diverse nematode management strategies.

(2) Enhancing the accuracy and expanding the application of root mechanical measurements

This study successfully developed a microfluidic device to measure the root protrusive force and Young's modulus of plant roots. However, this study currently focuses on *Arabidopsis thaliana* as a model plant. Future research should aim to extend this method to multiple plant species, enhancing its applicability across different plants and improving the generalizability of the findings.

(3) Optimizing device design for complex environmental conditions

This study successfully measured the mechanical properties of plant roots during gravitropic growth using a micropillar device, but limitations remain in simulating complex environmental conditions, such as variations in humidity, temperature, and soil types. Future research should focus on optimizing device designs to enhance adaptability in multivariable environments, better reflecting root growth dynamics in natural settings. Furthermore, integrating this technology with real-time monitoring systems and advanced biosensing tools will enable deeper insights into the dynamic changes in plant roots under external stimuli, thereby aiding the development of strategies to optimize plant growth.