ROOT-MICROBE INTERACTION VIA ROOT-ON-CHIP: A MICROFLUIDIC APPROACH

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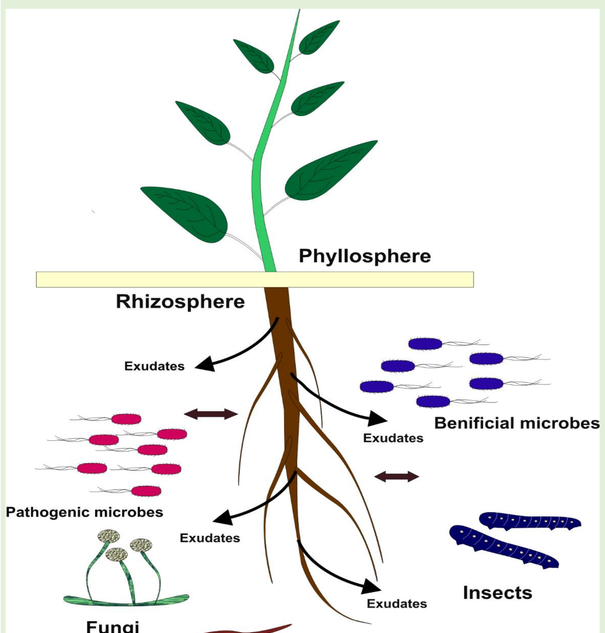
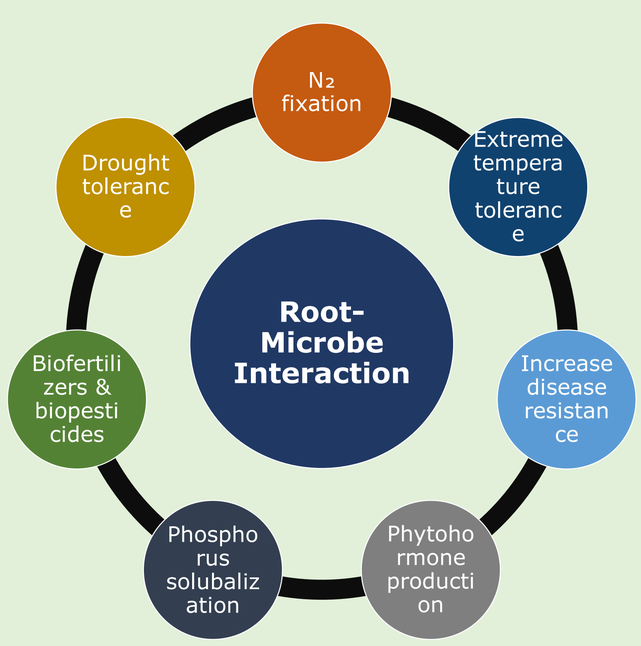
Introduction

Plant roots play a crucial role in shaping the rhizosphere where all the interaction between microbes and roots takes place. These interactions and the microenvironment around the root were major research areas for plant biologists across the world. The scientific community faced major challenges, like real-time imaging of the plant-microbe interaction, high throughput values, high spatiotemporal resolution, etc. Integrating microfluidics and microscopy provides a powerful approach to studying the dynamics of plant physiology, plant-microbe interaction, and the root microenvironment. Here we have developed a novel Rice Root-on-Chip (RROC) device to monitor the effects of biotic as well as abiotic stress on Oryza sativa root.

Significance





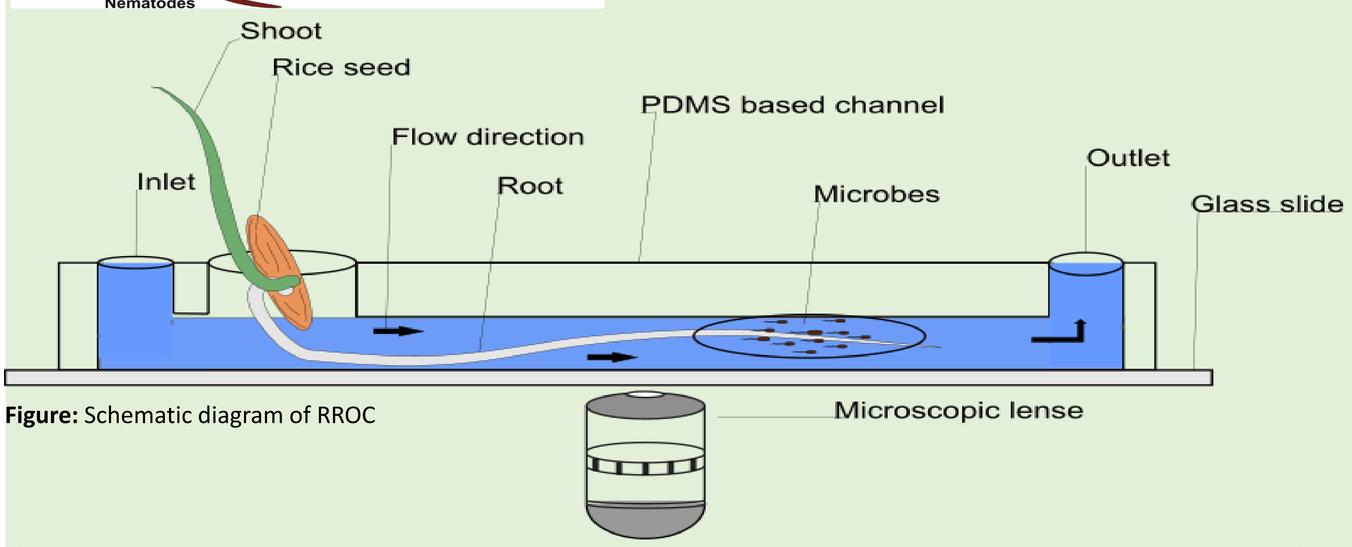


Figure: Schematic diagram of RROC

Materials & Methods

Inoculant preparation
- Rhizobium (Nutrient broth)
- Xanthomonas (N.B supplemented with sucrose)

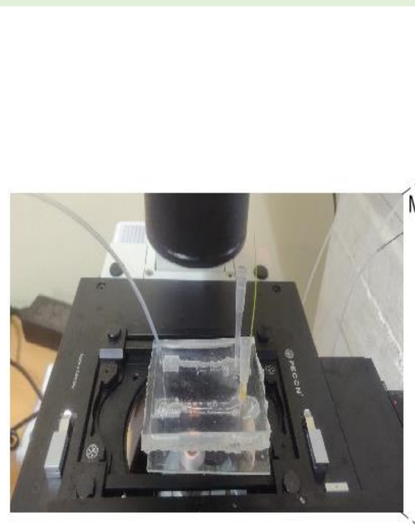
Abiotic stress
- Salinity stress by 80mM NaCl
- Osmotic stress by 20% PEG6000

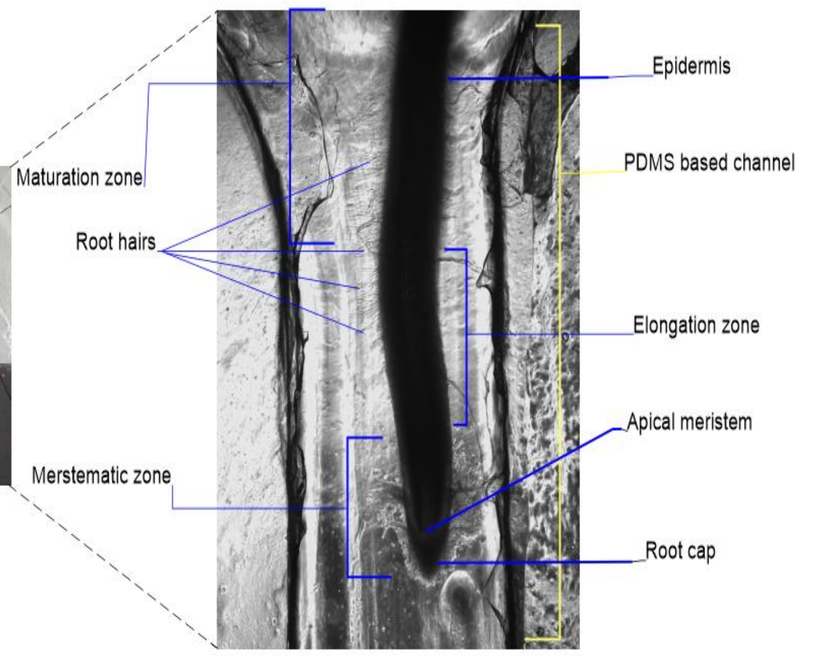
Oryza sativa prepartion
- Surface sterilization
- Germination in petri plate

Imaging
- Seedling transfer from petri plates to channel
- Microfluidic chip mounted on inverted microscope for imaging

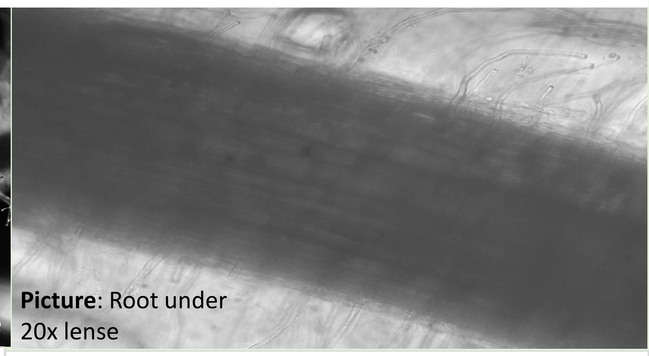
Results

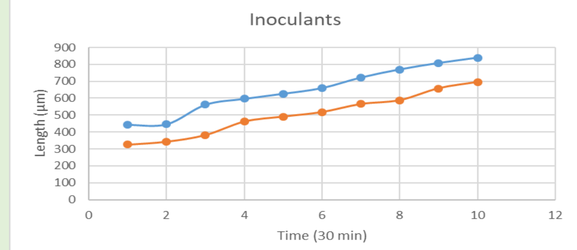
- Being free from any mechanical constraints, the root showed a growth rate at par with the Petri plate growth which suggests that the PDMS is not interfering with the plant root growth in any way.
- Rice roots showed optimum growth with tap water, microbial inoculants, and 20% PEG6000.
- With a 4x lens, change in root length can be observed whereas adhesion of microbes to the tip of root hairs was observed using a 40x lens.
- Notably, the adhesion of different microorganisms to the root cap was also seen when tap water was used for germination.

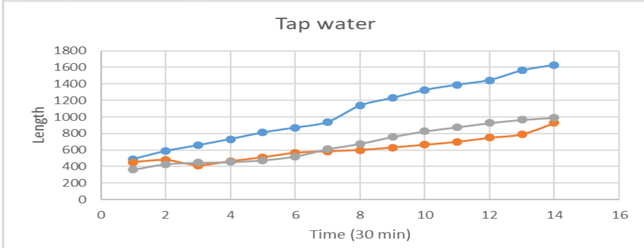


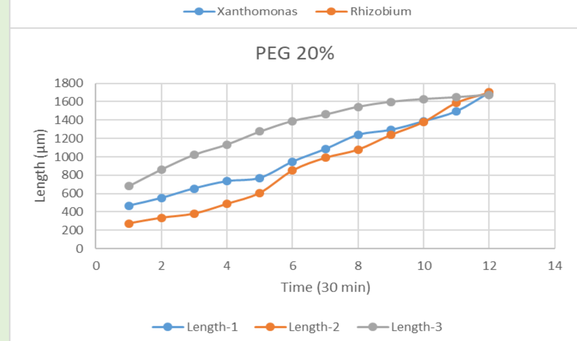


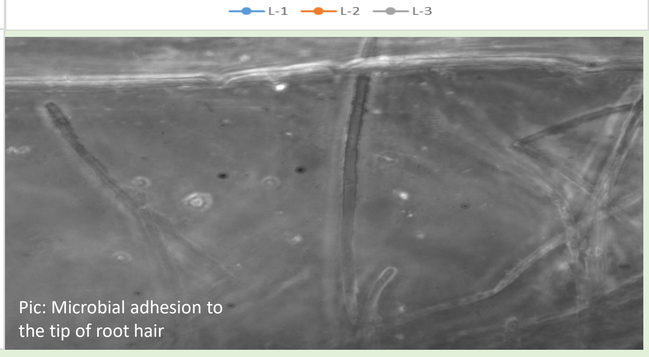

Picture: Root under 4x lense


Picture: Root under 20x lense



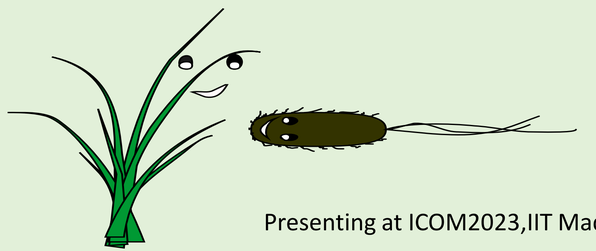





Pic: Microbial adhesion to the tip of root hair

Conclusion

- Here we conclude that the microenvironment of the seedling can be controlled and manipulated in real time using this ROC device.
- High spatial-temporal resolution images of the growing root can be obtained.
- Different plant species can be grown in the device with slight modifications in channel dimensions.
- ROC devices can be integrated with OMICS studies.



Presenting at ICOM2023,IIT Madras

Ref:
1. Claire E. Stanley,et al. (2016)
2. Hassan Massalha, (2017)
3. Kailash Chand Kumawat,.(2021)
4.Min-Jung Kwak,H.G-K.W.O.. 2018
5. Samuel J. Martins,.
6. Vishwakarma,K.K..Frontiers. 2020