Development of a system for 3D optical imaging of root systems in a heterogeneous, refractive index matched media

Helen Downie[1,2], Tracy Valentine[1], Wilfred Otten[2], Andrew Spiers[2], Lionel Dupuy[1]

1. SCRI, Invergowrie, Dundee, DD2 5DA, UK

2. SIMBIOS, University of Abertay Dundee, Dundee, DD1 1HG, UK

Introduction

• Plant root systems are import for capturing nutrient resources and water, for anchorage and in producing hormones that regulate plant processes.
• Relatively little is known about the mechanisms they use to explore the soil environment effectively.
• They are difficult to study because of the opacity of soil and so often plants are grown in highly unrealistic gel-based media in order to visualise the roots. The resulting root systems are very different from those of plants grown in soil and therefore new methods are required to allow realistic imaging of root systems.
• Here we present a new method using a system that incorporates some of the physical attributes of soil and yet is completely transparent and suitable for imaging root systems in 3D.

Aims

To create a medium where 3D root growth is more realistic than in gel.

To incorporate heterogeneous aspects of soil including solid particles, and free water and air in pore spaces.

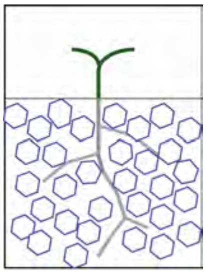

Figure 1. Diagram of 2D section of complex root growth in medium with solid particles

Root systems of plants grown in artificial, heterogeneous media are more similar to soil root systems than to those of plants grown in gel

Although shoot biomass is higher in plants grown in soil, primary root length is not significantly different in any of the treatments (figure 2a). Lateral root density, however, in artificial heterogeneous media is similar to that in plants grown in soil (figure 2b). Gel plants produce very few lateral roots.

Treatment group	Treatment number	Substrate constituents	Nutrients
Gel	1	3 g/L phytagel	MSR media
FEP particles	2	Granulates	MSR media
	3	Chunks	MSR media
	4	Shard 1-2 mm	MSR media
	5	Shards 2-4 mm	MSR media
Soil	6	Lower Pilmore soil, ≤ 2 mm, 1.1 gcm^{-3}, 20% MC.	No added nutrients
	7		MSR media

Table 1. Description of treatment groups

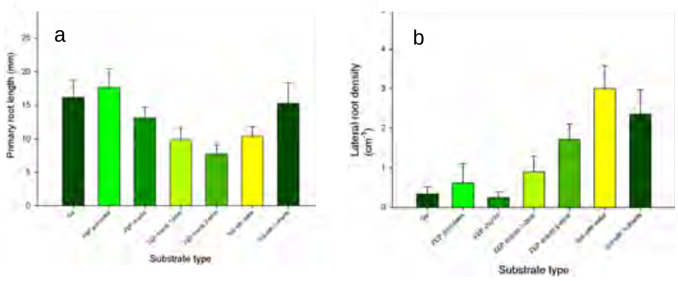

Figure 2. Graphs showing description of plants grown in gel, artificial heterogeneous medium and soil. a) primary root length and b) lateral root density.

Construction of a heterogeneous, RI matched medium for plant growth

Refractive Index (RI) matching

When the RIs of a solid and a liquid transparent material are matched, the edges of the solid material cannot be detected when immersed in the liquid (figure 3).

Figure 3. Pyrex rod immersed in RI matched oil solution. Edges of rod are invisible and solution is transparent

We used this principle to enable imaging of roots grown in solid particles of two different, low RI materials: Nafion (figure 4a) and FEP (figure 4b).

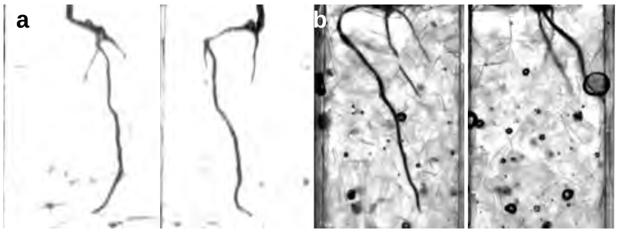

Figure 4. Projected light images of root systems in particles of a) nafion and b)FEP. Both show root systems from two angles.

3D imaging

Optical Projection Tomography (OPT) is a recently developed 3D imaging technique (Sharpe et al., 2002) that is useful for large biological samples. We can use OPT to generate 3D reconstructions of complex root systems.

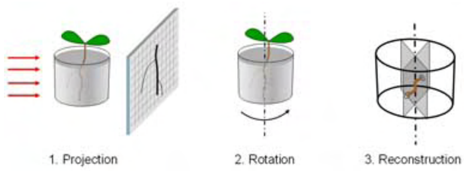

Figure 5. Steps involved in OPT

Potential future applications

• Analysis of root growth trajectories in a complex environment

• Studying root responses to a heterogeneous supply of nutrients

• Analysis of root-particle interactions

• Investigating localisation of root microbes throughout root development

References

SHARPE, J., AHLGREN, U., PERRY, P., HILL, B., ROSS, A., HECKSHER-SORENSEN, J., BALDOCK, R. & DAVIDSON, D. (2002) Optical projection tomography as a tool for 3D microscopy and gene expression studies. *Science,* 296, 541-5.