# Root growth responses to soil physical properties.

Tracy A. Valentine, Kirsty Binnie, Joachim Hans, Blair M. McKenzie and A. Glyn Bengough
Scottish Crop Research Institute, Dundee.

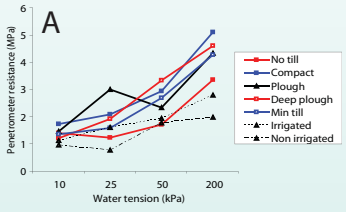
Scottish Crop Research Institute

## Background

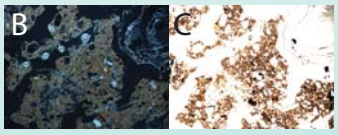
- **Roots experience a range of physical constraints including mechanical impedance, water stress and oxygen deficiency.**
- **We are linking studies of soil physical properties to root responses in *invitro* studies and in the field.**

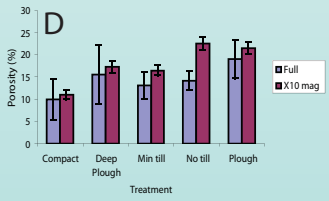
## Tillage effects on soil physical properties

***Soil strength depends on soil type, water content and soil structure (A). Soil structure e.g. soil porosity can be influenced by different tillage treatments (B-D)***




(A) Soil strength (penetrometer resistance) increases as soil dries, and is greatest in compacted soil.




Thin sections of ploughed soil: Pore space appears black (B; using polariser & analyser) or white (C; light field). Thus, porosity and its distribution can be quantified (D).

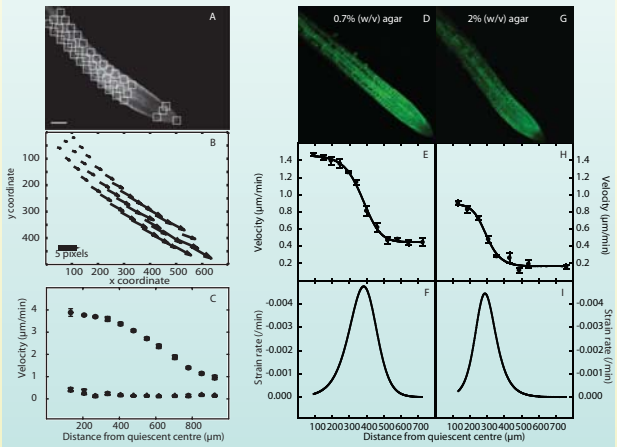
## Root phenotypic responses

***Mechanically impeded roots are shorter and fatter with more border cells.***

### Computer visualisation of root growth

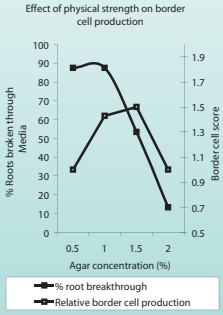
Aims: Quantify cell expansion and root growth over short time intervals to study control of root responses under stress.

Particle Image Velocimetry (P.I.V.) tracks pixel patches between images to measure growth, with a 2 min resolution.



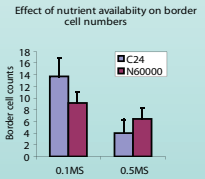

P.I.V. process (A-C). A: Patch placement on confocal image. B: Displacement vector field showing direction and relative movement of patches. C: Calculated velocity of patches verses distance from quiescent centre.

D-I: *Arabidopsis* growth on 0.7% agar (D-F) and 2% agar (G-I) using P.I.V. Confocal images of roots (D,G). Root tissue velocity as related to position along the root length (E,H) Strain rate calculated from velocity curves (F, I).



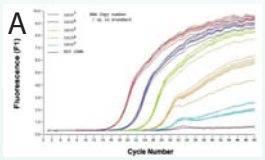

*Arabidopsis* induces border cell production under nutrient stress.

We are screening for mutants that have altered border cell responses under nutrient and physical stress.



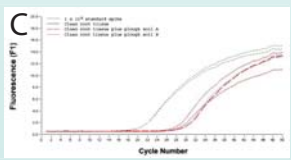

## Quantitative RT-PCR

***Expression of root genes related to the soil physical environment will be investigated in both agar and soil. Light Cycler quantitative PCR has been used to quantify transcripts from soil grown plants.***



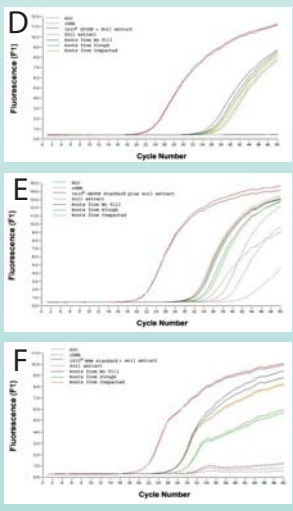

PCR calibration: A dilution series of known RNA standards were reverse transcribed, and used in LC-pcr reactions to establish calibration parameters for an *Arabidopsis* gene (At SPIKE, At3g54890, used to monitor extraction efficiency), Barley GAPDH (root internal standard), and a target gene Barley BPW (BPW is involved in water transport). BPW calibration curves (A), Crossing point vs Concentration for standards (B).

### Effect of soil on extraction and PCR reaction




Root RNA from seedlings germinated on filter paper were extracted in the absence or presence of 2 different soils. Soil had little effect on recovery of RNA from the samples.

### Field samples:




Root RNA extracted from 3 barley plants grown under 3 different tillage treatments. Samples were spiked by adding At SPIKE RNA during extraction. Graphs show PCR using Light cycler for AtSPIKE (D), GAPDH (E) and BPW (F) sequences.

## Future work

1. Assess functions of genes involved in border cell mutants.
2. Measure variation in root gene expression (including border cell genes), for *in vitro*, and soil grown roots.
3. Computer visualisation - development of a model to track cell features and extract cell expansion data, so that gene function can be related to growth in soil environment.

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