VISUALISING AND QUANTIFYING CEREAL ROOT RESPONSES TO PHOSPHORUS

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RICHARD JAMES FLAVEL
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School of Environmental and Rural Science
The university of New England
Armidale, New South Wales
Australia

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Statement of Originality

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree of qualification.

I certify that any help received in preparing this thesis and all sources used have been acknowledged in this thesis

Richard James Flavel

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Abstract

Phosphorus (P) and nitrogen macronutrient deficiencies remain a primary constraint to global agricultural production. Expectations for decreasing fertiliser availability and increased food demand provide impetus for improving plant nutrient efficiencies. While significant genetic advances have been made based on plant shoot characteristics, the root system traits have remained largely untargeted in breeding programs. This is largely due to the difficulties in rapidly assessing root system attributes. Since the spatial distribution of the root system (root architecture) determines the edaphic resources available to plants, it is logical to target root architecture in an effort to improve plant nutrient acquisition efficiency. Characteristics such as localised root proliferation in response to high nutrient patches could be improved to increase nutrient capture from point source fertiliser applications.

Accurate and rapid assessment of root system architecture remains the rate-limiting step for plant breeding with root system targets. X-ray Computed Tomography (CT) has significant potential for quantifying both the spatial and temporal dynamics of the edaphic environment. Despite a significant developmental history, X-ray CT technology has only recently reached a capacity for high throughput analysis of plant and soil samples with field relevant pot sizes at plant root relevant resolutions. The rate-limiting step is now in the processing of tomographs to extract the root system and the root architectural characteristics. This thesis firstly identified the optimal scanning conditions to capture high quality tomographs using the GE V|tome|xs X-ray CT equipment at The University of New England. This included quantifying the increased noise associated with high throughput X-ray CT techniques and the settings required to achieve high quality scans comparable to conventional CT techniques with "FastScan" mode (3 fold increase in scan rate).

Following experimental setup optimisation, a semi automated segmentation method was identified capable of accurately isolating the root system voxels from the remainder of the tomograph. This technique correlated well with conventional root washing analysis (best R^2 =0.96) and indicated a systematic underestimation of root

length of 8%. The underestimation appears to be common to the CT technique with other authors reporting similar factors on comparably smaller less complex root systems. The time requirement for segmentation was deemed impractical for larger trials and as such an automated method is presented in the form of an Image J toolbar. Firstly, the toolbar allows rapid import of the GE reconstructed volumes into Image J and some basic pre-processing. Secondly, the toolbar uses a sequential filtering process to segment the 'root' voxels from the remainder of the volume. While automatic, this method is less robust and produces a 'noisier' image than the previous method. Finally, the toolbar includes functionality to extract the root length, root length density, branching density and, with some manual input, branching angles from the binary segmented volume.

The X-ray CT method was then tested for the impact of repeated scanning (radiation exposure) on root development with no detectable effect on any root architectural characteristics measured. The spatial response of the root system to localised P application was examined, measuring the location of root length density, branching density and the branching angles both in the P band and external to the P application band. This study revealed the highly spatially specific response of young spring wheat (*Triticum aestivum* L. *cv*. Gregory) plants with branching density increasing at the band boundary (± 0.1mm) and root length density increasing steeply within 2mm of the band boundary and declining more slowly (14mm) below the band. The precise response of branching to the P band indicates that branching may be the driver of local root system plasticity, the sensing mechanism for which remains unknown. Branch angle analysis indicated no plastic trends with P status or within the P band regions for the cultivar tested. However, this technique has relevance for many root-modelling programs, providing empirical measures of branch angle.

The application of X-ray CT to a wider range of genetic material may allow the identification and inclusion of root architectural characteristics, such as the spatial specificity of root proliferation, into breeding programs. In addition to phenomics applications, the spatial and non-destructive character of X-ray CT allows temporal analysis of root responses that could help isolate the conditions required to stimulate morphological adaptions. While requiring further software development, X-ray CT

now provides significant potential for high-throughput root system analysis and the extraction of relevant root architectural information. Examination of the complex physio-chemical interactions at the root/soil interface is rapidly becoming a reality.

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Abbreviations

Aluminium	Al
Analysis of variance	ANOVA
Beam Hardening correction	BHC
Calcium	Ca
Carbon	C
Charge-coupled Device	CCD
Computed Tomography	CT
Computer numeric control	CNC
Copper	Cu
Days after sowing	DAS
Di-ammonium phosphate	DAP
General Electric	GE
Iron	Fe
Kilo-Volts	kV
Manganese	Mn
Micro- computed tomography (high resolution)	µ-CT
Micro-Amps	µA
Nitrogen	N
Nuclear magnetic resonance	NMR
Oxygen	O
Partial Volume effects	PVE
Percentage as weight by weight	w/w
Phosphate (inorganic)	P_i
Phosphate Buffer Capacity	PBC
Phospholipid fatty acid	PLFA
Phosphorus	P
Phosphorus uptake efficiency	PUPE
Phosphorus utilisation efficiency	PUTE
Poly-vinyl chloride	PVC
Positron emission tomography	PET
Potassium	K
Region of interest	ROI
Root length density	RLD
Root System Architecture	RSA
Root to shoot ratio	R:S
Scanning electron microscope -energy dispersive x-ray spectroscopy	SEM-EDX
Silicon	Si
Terminal restriction fragment length polymorphism	T-RFLP
Three dimensions	3D
Tin	Sn
Volumetric Water content	VWC
X-ray Fluorescence	XRF