Measuring root system architecture: Opportunities and challenges

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ABSTRACT

Root architectural traits are important for the selection of crops and cultivars that are most efficient in the acquisition of nutrients and water from soil. Increasingly laboratory-based methods are being used to screen large numbers of plants and to measure selected architectural traits. Our research with temperate cereals demonstrates that while such methods may allow numbers of root axes and angular spread to be determined reliably, the ranking of other traits such as length may be markedly influenced by the growing medium. For example, root length of dwarfing wheat lines grown on agar plates was increased by about 40% relative to wildtype and semi-dwarfing lines, while in a sandy loam soil under well-watered conditions it was decreased (by 24-33 %). Similarly while the ranking of particular growth traits (root number, root angular spread) of 5 barley genotypes grown in gel plates, soil sacs and small soil-filled pots was similar, those grown in gel chambers had a different order of ranking for root length to the soil-grown plants. Recent developments in x-ray microtomography have facilitated the 3D non-invasive measurement of small root systems grown in solid media allowing angular distributions to be obtained in addition to numbers and length. Developments in software and instrumentation mean that quantifying root architectural traits is becoming easier but the significant soil environment-genotype interactions mean that it is important to consider the typical soil conditions and stresses present in the environment where a crop is to be grown.
Keywords: barley, genotypic variation, root length, wheat, x-ray tomography.

INTRODUCTION

Water and nutrients are heterogeneously distributed in soil so that the distribution of roots (their architecture) will markedly affect the ability of a plant to secure these soil-based resources (Lynch, 1995). Quantifying the architecture of root systems is important as a means of determining the availability and accessibility of water and nutrients and hence improving crops and managing soils to improve crop yields. For example, Ge *et al*. (2000) demonstrated that root angle and the resulting orientation of the root system of *Phaseolus vulgaris* was influenced by the availability of soil P and hypothesised that the more surface-oriented root system found in low P soils was a positive adaptive response. With regard to soil water, Manschadi *et al*. (2006) demonstrated a relation between the angular orientation of wheat seminal roots, root system architecture and the subsequent extraction of soil water, while Hammer *et al*. (2009) also showed that change in root system architecture and exploitation of soil water had a direct effect on the accumulation of maize biomass and contributed to the increasing yield trend of the U.S. Corn Belt.

Measuring the architectural characteristics of root systems growing in soil is often time-consuming and tedious, so that a range of techniques has emerged to measure different elements of the root system with models used to synthesis the information into more explicit accounts of root system behaviour. While washing roots from soil has been widely employed to estimate the size and distribution of roots in a soil profile, the large number of samples required to account for

spatial variability and the failure to recover all fine roots has limited the amount of architectural information that can be collected (Gregory, 2006).

This paper briefly reviews some techniques for assessing root architectural traits and outlines opportunities for future improvements.

MEASUREMENT OF ARCHITECTURAL TRAITS

Measurements of root architectural traits have increasingly moved from the field to the laboratory in order to gain the detail, replication and standardization that allow ready comparison between species and genotypes. Many studies of root growth have been carried out in controlled conditions using young seedlings grown in homogenous media such as gels, hydroponics or sand because seedling roots can be screened rapidly in such media, and root length and elongation rates of individual roots can be determined relatively easily. For example, gel chambers were used to demonstrate the evolution of the barley (*Hordeum vulgare*) root system during domestication and subsequent breeding (Bengough *et al*. 2004; de Dorlodot *et al*. 2007). The wild progenitor had only a few (mainly two) highly positively geotropic seminal axes whereas modern cultivars had several axes (typically five or six) which exhibited a range of angles; landraces had intermediate properties. Hargreaves *et al*. (2009) found similar differences in the number of seminal axes of barley with the landrace Mehola having only four axes compared with six in the cultivar Chime. In this comparison of five different barley genotypes, there were also significant differences in root system length and vertical spread of axes between genotypes with both measures being smaller in Mehola than the mutant line GSH01915 (a near-isogenic line of Bowman containing the *cur4* gene producing coiled or bent leaves together with bent stems that was also found to produce curved roots). Wojciechowski *et al*. (2009) employed gel chambers to study the effects of dwarfing and semi-dwarfing genes in seven near-isogenic lines of wheat (*Triticum aestivum* cv. Mercia) and found that while there no significant effects of semi-dwarfing genes of total root length at the two-leaf stage, length of the dwarf lines (*Rht-B1c, Rht-D1c and Rht12*) was significantly increased by about 40%. However, Wojciechowski *et al*. (2009) also found that in contrast to the gel chamber results, dwarfing genes in wheat significantly reduced root length when the plants were grown to Zadoks growth stage 20 in soil-filled columns and to Zadoks stage 12 in a field experiment (Table 1). As in the gel chamber experiment, there was no significant effect of semi-dwarfing genes. The reason for these different results is not known but might lie in the different availability of nutrients in the different media.

Measuring the architecture of root systems growing in soil is difficult and often involves the destruction of the soil habitat. Recent developments in x-ray microtomography allow roots growing in soil to be imaged non-invasively, and for growth to be monitored by sequential measurements (Gregory *et al*. 2003). Because air, water and soil solids attenuate energy differently, such imaging can be used to characterise the relative abundance of soil components and, if attenuation is measured at different angles in a plane, their spatial distribution. (Jenneson *et al*. 2003) designed a scanner specifically to image roots growing in soil. This instrument is based on a third-generation cone-beam scanner and uses a filtered silver anode to produce a quasi-monoenergetic x-ray beam with optimum energy to achieve about 3 mean free path lengths when passing through 25 mm thickness of soil. The design specifications for the instrument allow voxel resolution of about 100 x 100 x 100 μm, a scanning time of about 40 minutes for a sample 25 mm in diameter and 30 mm high, and a radiation dose in the centre of the column of 0.1 Gy (well below the level of significant cell damage). The scanner has been used to image growing roots of wheat (*Triticum aestivum*) and rape (*Brassica napus*) in soil columns (Gregory *et al*.

2003), and the movement of the soil insect, *Sitona lepidus*, to host roots of white clover (*Trifolium repens*) in soil (Johnson *et al*. 2004).

Table 1. Root length (cm plant^{-1}) of near-isogenic lines of wheat (cv. Mercia) grown to Zadoks growth stage 12 in gel chambers and in soil in the field, and to growth stage 20 in soil-filled columns in a glasshouse. Values in brackets are 1 S.E. Based on Wojciechowski *et al*. (2009).

Genotype	Gel chamber	Soil-filled column	Field
rht (control)	30.1 (2.89)	1932 (167.0)	53.3 (4.16)
Rht-B1b	36.6 (2.78)	1691 (157.2)	53.3 (4.12)
Rht-D1b	34.1 (2.84)	1775 (157.2)	51.3 (3.36)
Rht-B1c	42.2 (2.78)	1388 (157.2)	39.9 (3.52)
Rht8c	36.4 (2.84)	1852 (157.2)	51.1 (3.26)
Rht-D1c	39.0 (2.83)	1291 (157.2)	38.0 (3.65)
Rht12	44.5 (3.02)	1456 (157.2)	40.5 (3.59)

Figure 1 shows 2D images of the growth of a wheat root growing through a solid medium consisting of three bands. The upper and lower layers consisted of acid-washed sand (10 g, sieved <250 μm, made up to a gravimetric water content of 20% with deionised water) and the middle layer of Bullionfield soil (collected from the Scottish Crop Research Institute; 2.5 g forming a layer 7 mm thick, sieved <250 μm, made up to a gravimetric water content of 20% with 0.5 g l^{-1} KH_2PO_4 solution). The images were constructed from projections taken over 360^o, so although 2D images in the same orientation are shown here, it was possible to construct a 3D image that could be viewed from any angle. Within 2 days of germination, three root axes contacted the soil band and 4 days later, lateral roots were visible. After 8 days after germination, an extensive array of lateral roots were visible on the axis within the soil band but not in the upper and lower sand layers. Visualisation of the roots was easier in soil than sand because the difference in relative attenuations was clearly defined. In sand, the boundary between the root and the surrounding matrix was more diffuse, leading to apparently thicker roots in the sand layers.

CONCLUDING REMARKS

Current techniques for assessing root traits are limited but improving rapidly as digitisation and software improve. Some traits differ between growing media meaning that architecture in field soils may differ from that determined in laboratory tests. While non-invasive techniques are emerging, scanning times mean that only a small number can be screened (typically <10 per day, not including analysis time of the large spatial datasets generated) and, depending on sample size, limited resolution may mean that fine roots remain unresolved. A further complication is that, to date, most screening has examined only external features. However, differences in the internal structure, such as the number and arrangement of xylem elements, may have profound effects on root functioning in relation to water uptake (Watt *et al*. 2008). These differences between lateral roots remain to be captured in descriptions of root architecture.

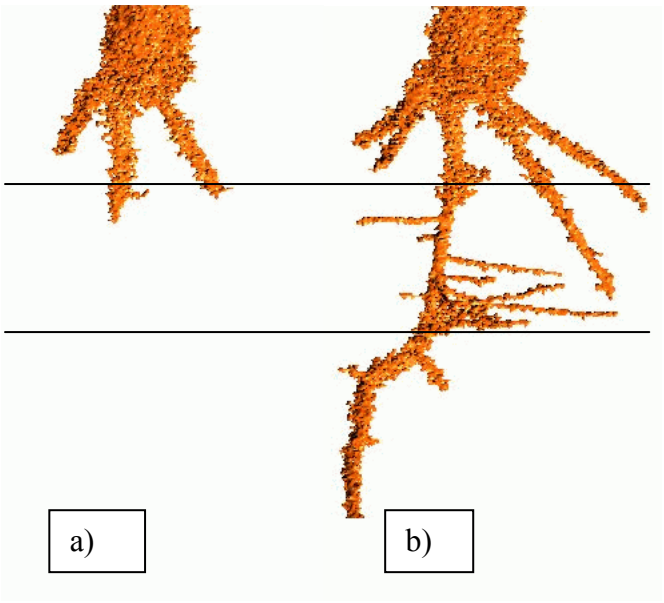

Figure 1: Growth of a wheat seedling through a soil band: (a) two days after germination, three root axes are visible, two of which have penetrated into the soil band; (b) after 8 days, an extensive system of lateral roots has been produced by the main root axis, within the soil band. The horizontal lines indicate the upper and lower boundaries of the soil band.

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