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Genetics of Root Architecture

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ABSTRACT

Different varieties of *Arabidopsis thaliana* show variation in their density of root branching from the primary root. We have taken advantage of the polymorphism between varieties of the plant to generate a QTL map of regions of the genome that associate with a change in root branching. This data has been combined with the known positions of polymorphisms (determined by DNA sequencing) and tissue specific microarray expression data in order to prioritise genes as root branching candidates. We will present our investigations of the role played by candidate root architecture genes in the physiology of the root system in a model plant and in a crop plant.
KEYWORDS: root architecture, quantitative trait loci, root branching

1. INTRODUCTION

Roots mediate a plant's ability to take up water and nutrients, they provide anchorage in soil, resist pathogens, avoid obstacles and respond to gravity and light. Root system architecture describes the spatial arrangement of the entire root system of a plant in soil, which provides an idea about the shape and structure of the root system of the corresponding plant. Different configurations of root system architecture may arise as a consequence of breeding, selection and plastic responses to the environment. Indeed, roots show remarkable plasticity under environmental flux. The plant, *Arabidopsis thaliana*, has been widely used by geneticists in order to elucidate the genetic components of plant physiological processes (Alonso-Blanco and Koornneef, 2000). In our laboratory, assays have been developed to measure root architecture traits in *A. thaliana*, *Gossypium hirsutum* (upland cotton) and *Gossypium barbadense* (dryland cotton). Genes have been found that are involved in determining root system size, root plasticity and root branching in *A. thaliana* (Casimiro et al, 2003, Himanen et al, 2004, De Smet et al, 2006, Fitz Gerald et al, 2006, Reymond et al, 2006, Saleeba, unpublished).

Here we report our observations of the root architecture of *A. thaliana* and cotton in simple two-dimensional and three-dimensional assays designed to investigate some of the genetic and environmental components of root architecture

2. METHODS

For *A. thaliana* growth on petri dishes, surface sterilised seeds were placed approximately 1.5 cm apart and approximately 1 cm from the top of 10 cm x 10 cm x 2 cm square petri dishes containing Gamborg's B5 medium with minimal organics (Sigma-Aldrich) with 0.05 % w/v 2-(N-Morpholino)ethanesulfonic acid, 2 % w/v sucrose, 0.7 % w/v agar, pH 5.8. The plates were then mounted vertically in a controlled environment chamber under cool white fluorescent light at 80 $\mu molm^{-2}s^{-1}$ for 16 hour long day cycle. The temperature was maintained at 21 °C during the day and 19 °C at night. The roots grew along the surface of the agar medium until the seedlings reached the 4 – 6 true leaf stage. The plants were scored by measuring the length of the

straightened primary root and by counting the number of lateral root branches along the primary root. Student’s T-test was used to determine significant difference at $p < 0.05$ between genotypes.

For *A. thaliana* grown in pots of sand, low feldspar river sand was passed through a 750 µm mesh and retained by a 150 µm mesh to obtain uniform sand particle size. The sand was washed 20 times in water to remove silt. Seeds were germinated on the surface of pots of 5 cm x 5 cm x 10 cm that were filled with the sieved sand then moistened with 0.5 x Hoagland’s solution (Hoagland and Arnon, 1938). Pots were incubated in ambient filtered natural light at 200 $\mu molm^{-2}s^{-1}$ in a glasshouse at approximately 20 ^{o}C. Pots were watered from below using 0.5 x Hoagland’s solution until the seedlings reached 4 – 6 true leaves. The seedlings were gently removed from the sand by washing in water and scored for their root branching density.

For cotton experiments, pre-germinated seeds were planted in pots (4 cm x 4 cm x 12 cm) containing a soil mix (2 parts perlite, to one part each of soil, river sand and peat). Plants were grown for 4 weeks in a controlled environment cabinet (Conviron, Model E15) set to a 16 h long day cycle at 200 $\mu molm^{-2}s^{-1}$, 22 ^{o}C day and 18 ^{o}C night temperature. Plants were watered on alternate days with Hoagland’s solution. At harvest, the plants were washed free of soil, the primary root length was measured and the number of primary branch points was counted. Data was analysed using the Kruskal-Wallis Test.

3. RESULTS

The density of lateral root branch points along the primary root varied between different ecotypes of *A. thaliana*, including the Col-4 and L*er*-0 genotypes. Col-4 showed a lower density of lateral root initiation sites along the primary root than L*er*-0 when grown in two-dimensions along the surface of sterile medium in petri dishes and also when grown in three dimensions in sand (Figure 1, Table 1). There was no significant difference in the root branching density of Col-4 grown in either two or three-dimensions. The same result was obtained for L*er*-0.

Figure 1. *A. thaliana* seedlings grown in two-dimensions on agar medium in petri dishes.

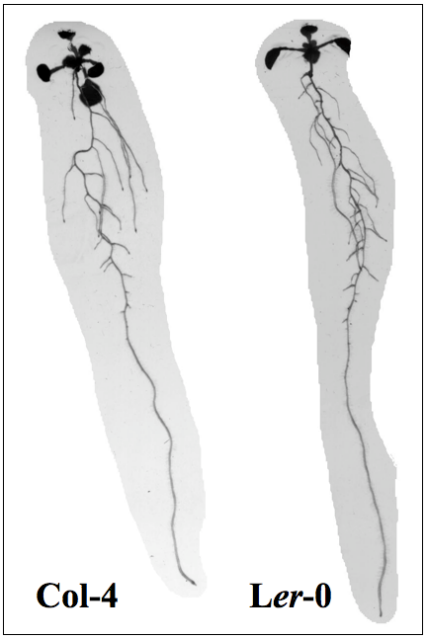

Table 1. *A. thaliana* root branching density on different media.

Growth Medium and Light	n	Growth Dimensions	Col-4 No. Primary Root Branch Points/mm Primary Root Length (SE)	L*er*-0 No. Primary Root Branch Points/mm Primary Root Length (SE)	Trait Differs by Genotype ($p < 0.05$)
Gamborg's B5, 80 $\mu molm^{-2}s^{-1}$	23	2	0.34 (0.01)	0.42 (0.02)	Yes
Sand, 200 $\mu molm^{-2}s^{-1}$	16	3	0.36 (0.01)	0.42 (0.02)	Yes

Growth of cultivars of cotton showed that root branching density varied with cotton genotype (Table 2). One *hirsutum* genotype showed the lowest density of lateral roots growing from the primary root. The highest density was seen in a *barbadense* genotype.

Table 2. Cotton root branching density in soil.

Cotton variety	n	No. Primary Root Branch Points/cm Primary Root Length (SE)	Trait Differs From Each Other Genotype ($p < 0.05$)
G. hirsutum 1	37	3.05 (0.20)	Yes
G. hirsutum 2	29	4.18 (0.56)	Yes
G barbadense 1	39	3.97 (0.31)	Yes
G. barbadense 2	29	8.23 (1.12)	Yes

4. DISCUSSION

We have confirmed that root branching density varies with plant genotype in both *A. thaliana* and in cotton. As well as being affected by a plant's underlying genetics, the root architecture of a plant is highly responsive to environmental flux. In order to dissect the genetic and environmental components of root branching away from each other we needed to develop a simple assay that was somewhat buffered to minor environmental change and thus would give repeatable growth of root systems. We achieved this goal by keeping environmental change to a minimum by the use of controlled environment chambers, by restricting our measurements to root branching density and by using homozygous plant genotypes. Remarkably, we found specific conditions for two-dimensional and three-dimensional plant growth that gave the same degree of root branching despite the differences in the conditions experienced by the plant.

A robust assay for root branching gives geneticists the accuracy of measurement required to dissect individual root branching genes from a complex genetic system. In our laboratory, and elsewhere (Fitz Gerald et al, 2006), geneticists have found the approximate locations of genes encoding components of root architecture. While a genetic understanding of root architecture may first come to fruition in model plant systems such as *A. thaliana*, the application of this knowledge to crop plants will be important in deciphering the contribution that root architecture makes to crop yield.

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