# Assessment of root growth by perennial pasture grasses in an acid soil using novel DNA-based methods

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## Abstract

The potential of species-specific DNA-based assays (using quantitative real-time PCR) for identifying and quantifying root growth in soil was examined as part of a study of the root responses of acid-soil resistant and sensitive perennial grasses and their persistence and productivity on acid soils. Root and shoot growth of acid-soil resistant (cv. Advanced AT) and sensitive (cv. Sirosa) phalaris (*Phalaris aquatica*) were compared during establishment at a field site with an acid and aluminium toxic subsoil ($pH_{CaCl2}$ 3.9; Al 25 mg $kg^{-1}$ $CaCl_2$ extractable). At 6 weeks after planting, the phalaris genotypes did not differ in plant density, shoot dry matter per plant or root mass to a depth of 0.1m. However, 10 months after planting, ground coverage and shoot dry matter yield of Sirosa were only 73% and 51% of that of Advanced AT, respectively. Roots were sampled at 0.1 m increments to a depth of 0.6 m and were washed from soil for dry matter determination. Root DNA was also extracted directly from intact soil cores and quantified using real-time PCR. Advanced AT had significantly greater root growth in the acid subsoil than Sirosa as shown by either root mass or root DNA content of soil cores. However, when converted to root mass (based on calibration samples), the DNA assays indicated a larger mass of roots than was measured after root washing. The cause of this difference is presently unknown. Better root development by Advanced AT was associated with improved plant persistence and dry matter production. Results from glasshouse studies indicate that differences in root growth between the genotypes are likely to be due mainly to differences in lateral root growth.

## Introduction

Perennial pasture species are important for sustainable pasture production. The deep roots and persistence of perennial species are important for the management of soil acidity, nutrient leakage, salinity and erosion, and consequently help to improve the productivity of pasture systems (Kemp and Dowling 2000). However, adverse soil and climatic conditions, and poor grazing management may lead to lack of persistence of perennial species and a subsequent loss of these benefits (Li et al. 2004).

Soil acidity is a major constraint to agricultural production. In soils with a $pH_{CaCl2} \leq 5.5$, $Al^{3+}$ ions move into soil solution and inhibit root growth. Phalaris is a common perennial grass species in the pasture systems of south-eastern Australia however different lines demonstrate variable resistance to acid soils (e.g Culvenor 1985; Culvenor et al. 2004). While application of lime is widely accepted as integral to the management of soil acidity, acid-soil resistant genotypes are also necessary, as surface applications of lime may take years to ameliorate subsoil acidity. Understanding how the effect of acidity on root growth and morphology affects plant establishment, persistence and productivity is important for selecting appropriate genotypes for acid soils. This paper compared root growth during establishment, and shoot growth of an acid-soil resistant (cv. Advanced AT) and sensitive (cv. Sirosa) phalaris at an acid soil field site. The potential application of DNA-based assays to quantify roots in field soils was also investigated. Traditional methods for studying roots, such as root washing and core-break methods, are limiting in both their cost, ability to accurately identify roots of specific species when growing in mixed swards, and may also severely under-estimate fine root length (Pierret et al. 2005). In recent years, molecular methods have been proposed that allow accurate identification of roots of individual plants or species, but have only qualitative (e.g. rooting depth) or semi-quantitative (e.g. relative abundance) applications (Mommer et al. 2008). More recently, quantification of root biomass of individual species directly from soil samples has been reported (Riley et al. 2010) in which DNA is extracted from soil and DNA of individual species is quantified using real-time PCR (qRT-PCR) with species specific Taqman probes. DNA content of the soil

may then be related to root biomass. The current paper compares the results of root growth measurements obtained by washing roots from soil, and by quantitative DNA-based assays of intact soil cores.

## Methods

Two phalaris cultivars, an acid-soil sensitive cultivar (cv. Sirosa) and a cultivar bred for acid-soil resistance (cv. Advanced AT) were sown on an acidic Kurosol (subsoil pH 3.9; Al 25 mg $kg^{-1}$ $CaCl_2$ extractable) at a pasture site near Rye Park (34°31′22″ S, 148°55′36″ E), southern New South Wales. The experiment was a randomised block design with ten replicate plots (1.2 x 5 m). Plant establishment, shoot dry matter and root growth were assessed over a 15 month period. Root growth was assessed by taking soil cores (32 mm diameter), directly beneath live plant bases, at 0.1 m increments to a maximum depth of 0.6 m. Six soil cores were taken per replicate, of which, five were combined for DNA analysis (~500g air dry soil) and one was washed to recover roots. Soil cores for DNA analysis were immediately frozen in the field with dry ice for transportation back to the laboratory where they were freeze-dried and DNA quantified using qRT-PCR with Taqman probes as outlined by Riley et al. (2010). Soil cores collected for washing were stored at 4°C during transport and roots washed from soil within 6 hours. Roots were immediately scanned and root length analysed with WinRHIZO (Regent Instruments). Roots were then freeze-dried, weighed and added back to 100 g of soil (collected from a plot in which no phalaris had been grown) for DNA analysis. A linear correlation between root mass and total DNA was determined for each 0.1 m depth increment and used as a conversion factor for DNA quantity to root mass. Root and DNA data were log-transformed (1000+K or ln1+K) and analysed in Genstat 11th edition (VSN International) using either 1- or 2-way ANOVA with genotype and where relevant, depth, as factors.

## Results

### *Shoot biomass and persistence*

Six weeks after sowing, there was no significant difference ($P$<0.05) between Sirosa and Advanced AT for root dry mass (washed to 0.1 m), plant density or shoot dry matter (Table 1). At 10 months after sowing, Advanced AT had 27% more “live base” ground coverage and 100% more shoot dry matter than Sirosa.

### *Calibration between root DNA and mass*

A significant linear relationship was found between washed root mass and its DNA content. Regressions were initially fitted for individual phalaris genotypes at the individual soil depths. Regressions differed significantly between several of the soil depths as DNA concentration per unit root mass significantly increased with depth. However, DNA concentration per unit root mass did not differ significantly between the genotypes, with the exception of the 0.1 to 0.2 m layer. Consequently, a single linear regression was fitted for each depth and used as a conversion between DNA and root mass for both phalaris genotypes (Table 2).

### *Profiles of root DNA concentration and mass*

Figure 1 shows the root mass and DNA distribution of Advanced AT and Sirosa as determined by both DNA-based (Figure 1a, c) and root washing (Figure 1b) methods. For all methods, there was no significant ($P$<0.05) interaction between genotype and depth. However, there was a significant main effect of both genotype and depth. Advanced AT had on average a larger root mass per soil core and a higher concentration of DNA per g of soil compared with Sirosa. Root mass and/or DNA concentration of both genotypes declined incrementally between 0 and 0.3 m depth. Some differences in the estimates of roots were revealed when analysing differences between Advanced AT and Sirosa in each soil layer with a one-way ANOVA. Root DNA concentration in soil and root mass was the same for Advanced AT and Sirosa in the 0 to 0.1 m and 0.5 to 0.6 m layers. Significantly higher DNA concentrations and, consequently, higher predicted root mass for Advanced AT relative to Sirosa were found in all other

layers. Significant differences were not detected by the washed-root method. However, a significant positive linear correlation (y=2.77x; $R^2$=0.98) was found between washed- (x; Fig 1b) and predicted-root mass (y; Fig 1c).

**Table 1. Root dry mass, plant count/ persistence and shoot dry matter of an acid-soil sensitive (Sirosa) and resistant (Advanced AT) phalaris at 6 weeks and 10 months after sowing at an acid field site[1]. Values are mean ? 1 standard deviation; LSD = least significant difference; ns = not significant.**

| | 6 weeks | | | 10 months | |
|---|---|---|---|---|---|
| *Genotype* | Root dry mass (mg/core) | Plant count (count/m row) | Shoot dry matter (mg/plant) | Plant persistence (% live bases) | Shoot dry matter (t/ha) |
| Sirosa | 4.2 ? 1.9 | 10.9 ? 3.5 | 21.1 ? 9.3 | 28.7 ? 8.6 | 0.48 ? 0.23 |
| Advanced AT | 3.8 ? 0.9 | 9.1 ? 2.5 | 19.2 ? 4.6 | 36.4 ? 12.6 | 0.96 ? 0.50 |
| LSD (*P*=0.05) | ns | ns | ns | 7.4 | 0.35 |

[1] Roots determined from a composite of 5 cores (3.2 mm diam.) to a depth of 0.1 m. Six week counts and dry matter based on 4 x 0.3 m row lengths. Ten month persistence and dry matter based on % live base in a 1 x 1 m grid or 2 x 0.5 $m^2$ quadrats. (n=10)

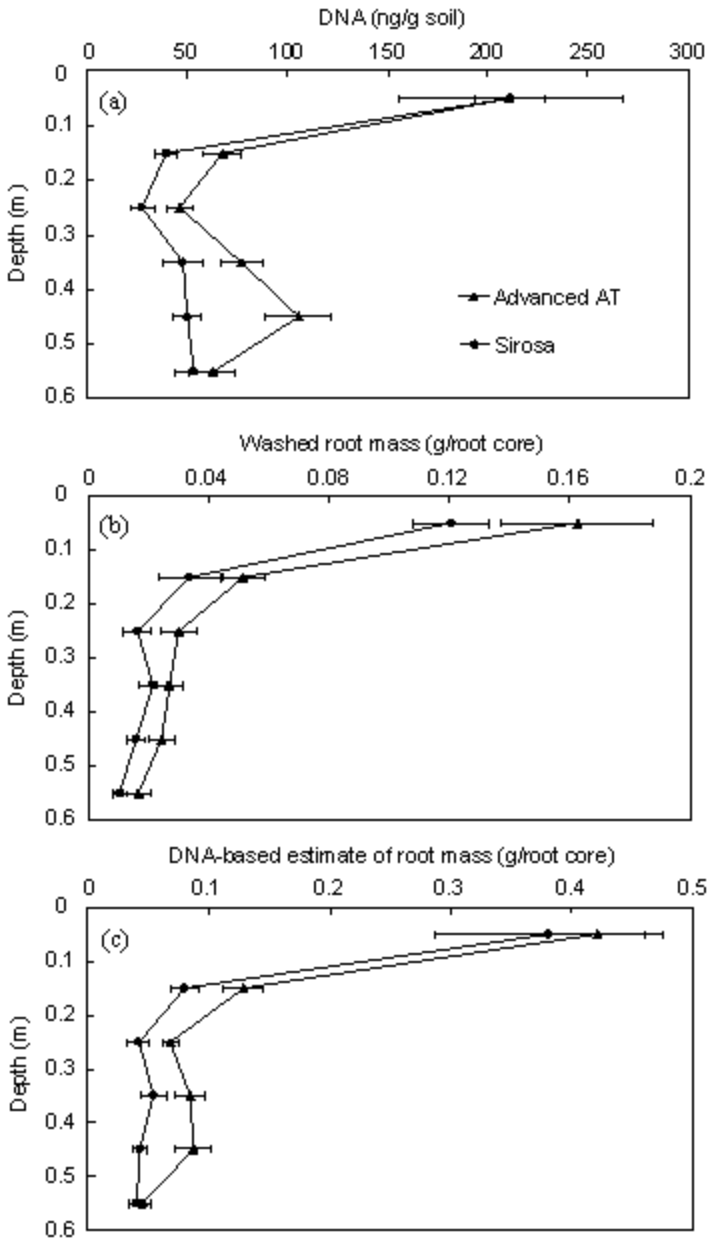


**Figure 1. Distribution of roots in an acid soil for acid-sensitive (cv. Sirosa; circle) and acid-tolerant (cv. Advanced AT; triangle) phalaris genotypes at 15 months after planting. Figures show the distributions of: (a) root DNA extracted per g of soil, (b) root mass washed from soil cores and (c) root mass predicted from DNA content of soil cores after applying calibrations of root mass per unit DNA determined for each soil layer (Table 2). Error bars show 2xSE, n=10.**

**Table 2. DNA concentrations of the dry mass of roots of phalaris cvv. Advanced AT and Sirosa that had been washed from soil cores in 0.1 m depth intervals down the soil profile; n=10. $R^2$ refers to the regressions from which the DNA concentrations were obtained.**

| Depth (m) | ug DNA/g dry root | $R^2$ |
|---|---|---|
| 0.0 - 0.1 | 38.2 | 0.64 |
| 0.1 - 0.2 | 58.0 | 0.60 |
| 0.2 - 0.3 | 76.2 | 0.68 |
| 0.3 - 0.4 | 96.6 | 0.60 |
| 0.4 - 0.5 | 118.1 | 0.76 |
| 0.5 - 0.6 | 117.1 | 0.82 |

**Discussion**

This work demonstrated that DNA-based assays may be used for quantitative studies of roots in the field and have good agreement with results from washed out samples. However, our findings indicate that DNA measurements alone are inadequate to compare root growth. Average DNA concentration per unit root mass increased with soil depth (Table 2). Previous studies (Haling, unpublished) demonstrate that the DNA concentration of roots varies between root types and decreases with older plants. It is likely that with increasing depth, an increasing percentage of roots were younger and had a higher average DNA concentration. For this reason, it was necessary to apply individual calibrations between DNA and root mass for each soil layer. As a result, the average proportion of roots in the 0.5 to 0.6 m layer was 9 and 11 % respectively that of the washed and predicted roots in the 0 to 0.1 m layer but would have appeared to be 27% if uncorrected DNA measurement alone had been used for roots.

The DNA concentration per unit root dry mass did not vary significantly between Advanced AT and Sirosa, presumably because roots of the same species do not vary in DNA concentration and because within each layer roots were of a similar age and type. Comparisons of the direct DNA measurements within soil layers were therefore valid. Differences between Advanced AT and Sirosa within soil layers were detected by the DNA measurements. On average, Sirosa had approximately 75% of the root mass or DNA of Advanced AT. While the washed-root method detected an overall difference between the genotypes, a difference could not be detected for the individual soil layers. This is likely to be due to an inadequate sample size of 1 soil core per replicate compared to 5 for the DNA-based results. This reflects the time, labour and hence cost limitations of root-washing methods compared to DNA-based methods. However, a major difference existed between the washed- and predicted-root masses, with 2.8-fold more root mass predicted per soil core by the DNA-assay than was measured after washing roots from soil (Fig. 3c cf 3b). It is unclear if DNA degradation during processing of the calibration samples (i.e. transport, storage and washing), or loss of fine roots during washing is the cause. The source of the difference is currently being investigated.

The acid-soil resistant (cv. Advanced AT) and sensitive phalaris (cv. Sirosa) did not differ in their initial (6 weeks after sowing) root growth, plant density or dry matter per plant. However, by 10 to 15 months after planting, clear differences had emerged with better ground coverage, dry matter production and root growth by the acid-soil resistant genotype. Based on glasshouse studies (Haling et al. 2010), we deduce that the better root growth of Advanced AT (relative to Sirosa) is most likely due to better lateral root growth, which would benefit acquisition of water and nutrients, and consequently growth.

**Conclusion**

Quantitative DNA-based assays may be used to measure root biomass directly from soils. This method presents advantages over previously proposed molecular methods that are limited in their ability to directly quantify roots of mixed species in soil. Direct DNA measurements may provide valid absolute comparisons of root biomass but only when it is known that roots do not differ in their average DNA concentration per unit root mass. Calibrations using washed roots, to relate root biomass and DNA, are still subject to the traditional errors and limitations of root washing and possibly losses of DNA. Nevertheless, application to a field experiment demonstrated that better persistence and dry matter production of an acid-soil resistant genotype at an acid field site was linked to better root growth.

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