Studying root development in soil using DNA technology: idea to impact

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Abstract

Quantitative DNA assays for measuring root distribution and seed banks in soil are under development for wheat, barley, ryegrass, subterranean clover, phalaris and lucerne. These assays have potential to determine root distributions in soil profiles in either monoculture or mixed sward systems. The assays quantify DNA from live cells only and many of these cells will be in fine roots, prone to loss during soil washing with conventional root assessment procedures. In pot experiments the ryegrass DNA assay showed a rapid decline in soil root DNA over 10 days following plants being either defoliated or sprayed with glyphosate. This response is most likely due to DNA degrading in dying root cells. DNA assays for wheat and barley have also been used to assess genetic differences in root architecture under drought conditions and at field sites with hostile subsoils. The assays for pasture species have been used to measure differences in root distribution of pasture species in lime amended plots at the MASTER site (NSW DPI, Wagga Wagga).

Results from the above studies show that quantitative DNA assays combined with high throughput DNA extraction systems are potentially useful to determine distribution of root systems under field conditions. This paves the way for high-throughput root phenotyping and incorporation of root architecture traits into breeding programs to improve drought tolerance as one possible role for the technology. In pasture trials the assays have the capacity to measure the impact of different management systems on specific species in mixed swards.

Key Words

Root distribution, quantitative DNA assays, root sampling

Introduction

The ability to study root growth in the field is limited by the lack of fast and reliable techniques. Currently most techniques require plant roots to be washed from the soil (Metcalfe *et al.*, 2007). This is a laborious task especially when working with soils that are difficult to disperse and often fine roots are lost during the process. Separating remnant roots of previous crops from the current crop is also difficult (Watt *et al*., 2008). Because of these issues root growth studies are often conducted in glasshouses using sand columns, but such studies may do not necessarily predict root growth under field conditions.

Studying root distribution in mixed plant populations is especially difficult and requires a different approach. Linder *et al.,* 2000, used DNA assays to test sections of root found in caves from 5 to 65 m deep, to identify the plant species. Moore and Field (2005) used digestion of amplified internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) to detect multiple plant species in mixed root samples. Mommer *et al*., 2008 used real-time PCR to determine the proportions of individual plant species in mixed root samples washed from sand cultures.

Since 1997, quantitative DNA assays have been used to assess populations of soilborne pathogens before seeding using a soil DNA extraction system designed to process 500 g samples (Ophel Keller *et al.,* 2008).

This paper reports the use of quantitative DNA assays to study root DNA concentrations in relation to root mass, vertical growth of barley roots into hostile subsoils, root distribution of drought tolerant wheat genotypes and seasonal changes in soil DNA concentrations of individual pasture species in mixed plant populations. The potential applications of this technology are discussed.

Methods

Assaying plant DNA in soil

DNA was extracted from soil by SARDI Root Disease Testing Service (RDTS) and the concentration of target DNA determined using quantitative (TaqMan?) DNA assays (Ophel Keller *et al.*, 2008). Probes and primers for the assays were designed in the ITS region of the rDNA. Assays were designed to detect different taxonomic levels depending on the target plant. The ability of the assays to quantify plant DNA in soil was confirmed by addition of known quantities of both freeze-dried roots and seed to 500 g samples of field soil (data not shown).

Decay of plant DNA in soil

A TaqMan? assay for ryegrass (*Lolium* spp.) was used to assess the changes in root systems following defoliation or herbicide treatment to monitor subsequent changes in the relationship between DNA and root mass. Sixty pots of perennial ryegrass plants were grown in a growth room at 20/16?C with a 12 h light/dark cycle for 4 weeks, each pot contained 500 g of sandy loam from Winulta, SA. The pots were then randomly divided into 3 groups of 20, one group was defoliated (regrowth removed daily), another sprayed with glyphosate and the third left untreated. A randomised block design was used to arrange the pots. On days 0, 1, 4, 7 and 10 following treatment, 2 pots from each treatment were washed to remove soil for root dry weight assessment, and the entire soil from a further 2 pots assayed for *Lolium* DNA.

Barley roots - vertical distribution

A TaqMan? assay for *Hordeum vulgare* was used to monitor vertical root distribution of two different barley cultivars (Mundah and Keel) in a field experiment at Whitwarta (SA) in 2007. The experiment was a randomised block design with 4 replicates. The soil type was an alkaline clay loam with subsoil salinity. The EC sampled mid growing season in the 0-30 cm and 30-60 cm depths was 3.6 and 8.5 dS/m respectively. At Zadoks stage 50, soil cores were collected using Dig Sticks (Spurr Soil Probes, Adelaide, Australia) to a depth of 60 cm and these were divided into 4 consecutive layers, each 15 cm deep. In each plot, 4 pairs of cores were collected, 2 pairs from within, and 2 pairs from between, crop rows. To reduce spatial variability, the corresponding layers of each pair, were combined. One composite bulk was used to determine DNA concentration and the other root mass. The composite root mass samples were washed over a 500 micron sieve, roots picked out and oven dried at 65?C for 72 hours. The samples for DNA assessment were submitted to SARDI RDTS and dried overnight in a dehydration oven at 40?C. DNA was then extracted and the TaqMan? assay used to quantify barley DNA in each composite soil sample.

DNA data were log transformed for analysis. The upper two (0-30 cm) and lower two (30-60 cm) layers were then analysed separately by ANOVA with sample position nested within cultivar in the treatment stratum and sample depth nested with replicate within the block stratum, effectively combining the sample depths to improve the residual degrees of freedom and resolution of the analysis. A small number of values with large residuals were excluded from the analysis. Mean DNA data were back transformed for presentation.

Wheat roots - horizontal distribution

A TaqMan? assay for *Triticum* spp. (*Triticum aestivum*, *T durum* and *Triticosecale*) was developed to measure the concentration of wheat DNA in soil to study root distribution. The assay was evaluated using soil samples collected in the top 10 cm between the rows of nine reference lines; Babax, Berkut, Drysdale, Excalibur, Gladius, Krichauff, Kukri, RAC875 and Seri, which had been included in a drought tolerant mapping population field experiment established at Roseworthy in 2007. The experiment, which had two replications, was sampled in mid October, 2007. A single composite sample was collected from each plot by combining 20 individual randomly placed 1 ? 10 cm cores (AccuCore from Spurr Soil Probes, Adelaide, Australia). DNA from each line was tested to check for variation in the target sequence and results used to standardise soil concentrations relative to Krichauff.

Mixed population roots – changes in time

TaqMan? assays were used to assess changes in root distribution between spring and autumn for subterranean clover (*Trifolium subterranean*) an annual pasture species, phalaris (*Phalaris aquatica*) a summer dormant perennial species and lucerne (*Medicago sativa*) a summer active perennial species. Soil samples were collected from limed perennial pasture plots at the MASTER site, Book Book near Wagga Wagga, NSW. The spring samples were collected in the first week of October 2007, and the plots were sampled to a depth of 50 cm using Dig Sticks. Twenty cores were collected per plot. Each core was divided into 10 cm layers and the corresponding layers were combined for each plot. Subterranean clover was dominated on both limed (60%) and unlimed pastures (56%). The proportion of phalaris was low on both limed (11.7%) and unlimed pastures (8.7%). There was only 5% of lucerne on the limed pastures, but no lucerne was observed on the unlimed pastures. Phalaris and lucerne populations were low, less than 15 plants per m^2. Autumn samples were collected in mid March 2008 to 40 cm deep (the soil was too hard to sample deeper at that time). The soil samples were spread thinly in trays to rapidly air dry in the field and then submitted to the RDTS for DNA analysis.

Results

Decay of plant DNA in soil

The *Lolium* assay measured an 80% decline in soil DNA over a 10 day period after ryegrass grown in pots was either sprayed with glyphosate or defoliated. Root mass (determined from separate pots) did not change significantly over the same period. These results indicate DNA degrades quickly in dying cells (Figure 1).

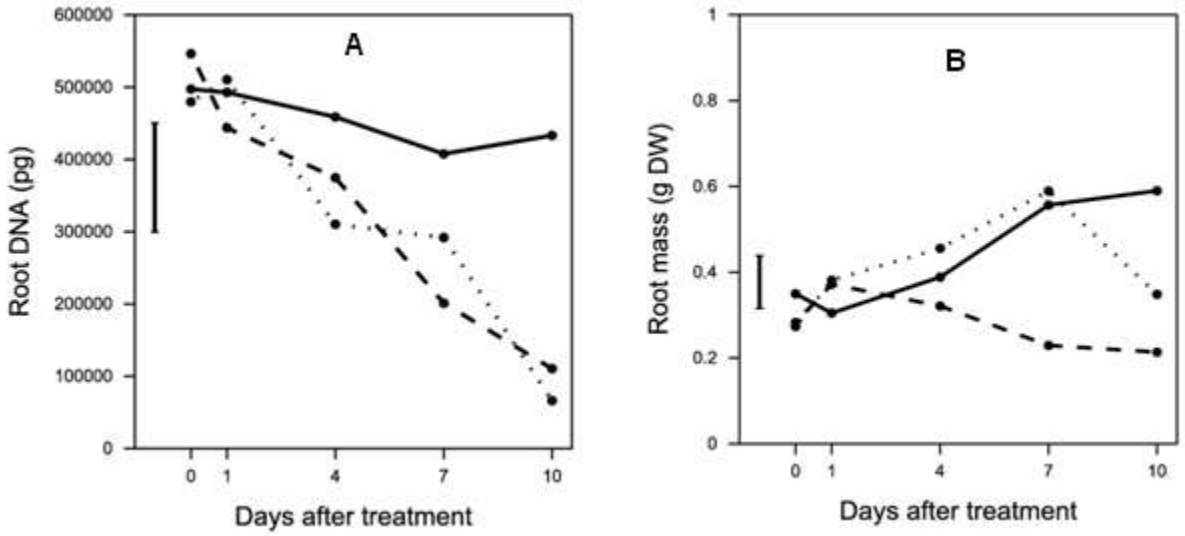

Figure 1. Part A soil DNA (pg DNA / g soil). Part B root dry weight (g / g soil), changes in perennial ryegrass roots 0 to 10 days after treatment, •- - -• defoliation, •…..• glyphosate and — untreated plants, I = standard error of mean.

Barley roots - vertical development

Mundah had greater DNA concentration and root mass than Keel in the 30 to 60 cm soil layer, but not in the 0 to 30 cm layer. Barley DNA concentrations were also greater in row than between rows in the 0-30 and 30 to 60 cm layer, whilst root mass was not assessed to be different in either zone by conventional root washing.

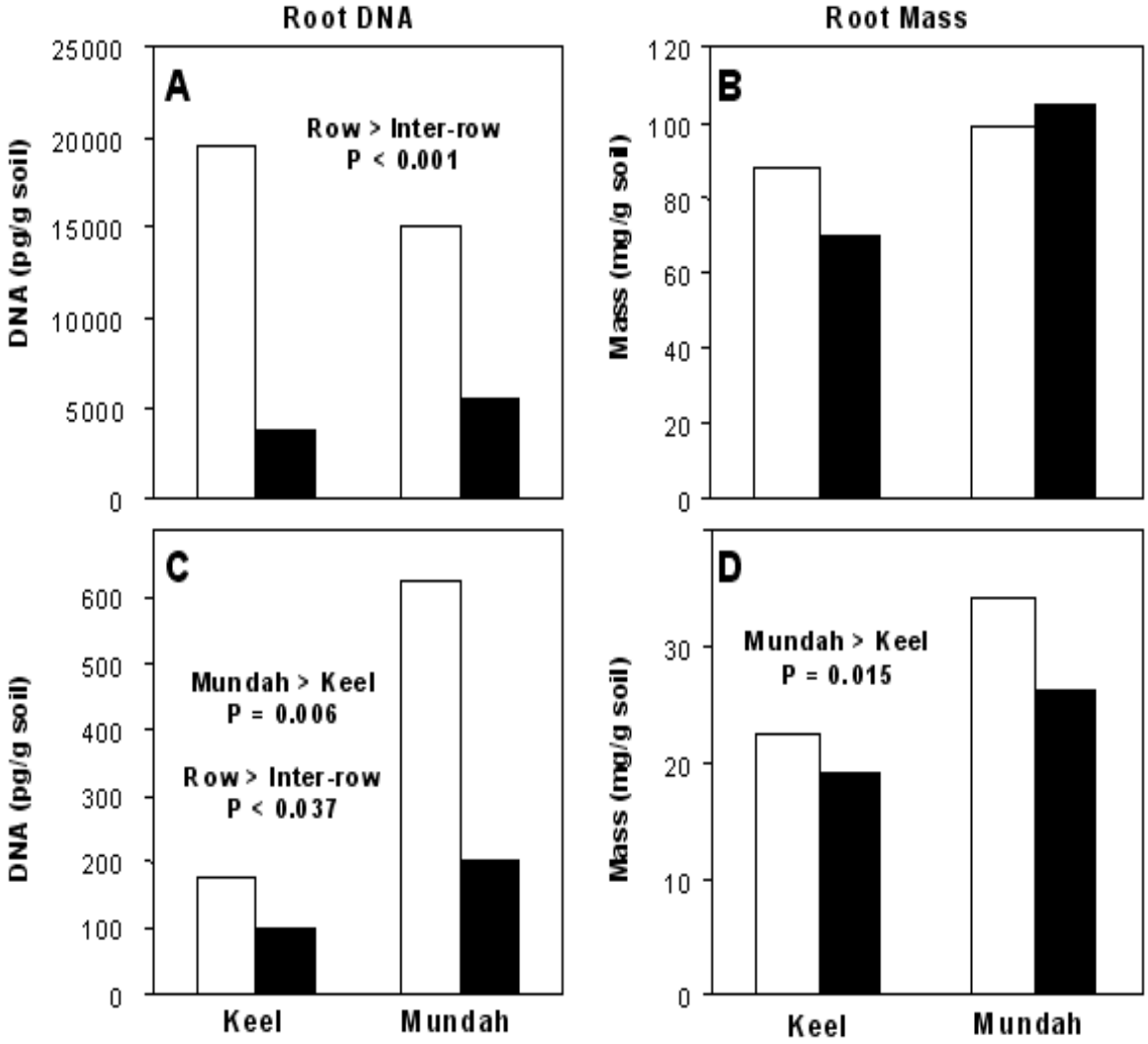

Figure 2: Mundah and Keel barley root density within row □ and between row ■ at Zadoks 50 developmental stage, Whitwarta SA, 2007 estimated as DNA and root dry mass in the 0-30 cm (A and B) and 30-60 cm soil layers (C and D). Statistically significant (P<0.05) treatment effects are noted in each graph.

Wheat roots - horizontal distribution

In September, 2007 two cultivars with contrasting root architecture, Berkut and Krichauff, were sampled within and between the rows to a depth of 40 cm in a separate study. DNA data indicated a difference in

root development in the top 10 cm between the rows (data not shown). On the basis of this result soil DNA concentrations were measured in the top 10 cm midway between the rows for the nine reference lines in a drought tolerant experiment (Figure 3). DNA concentrations of the lines clustered into two main groups. Excalibur, Badax, Drysdale and Gladius had the lowest and Krichauff, RAC875 and Kukri had the highest soil DNA concentrations in the top 10 cm between the rows. The other lines Berkut and Seri had intermediate DNA concentrations.

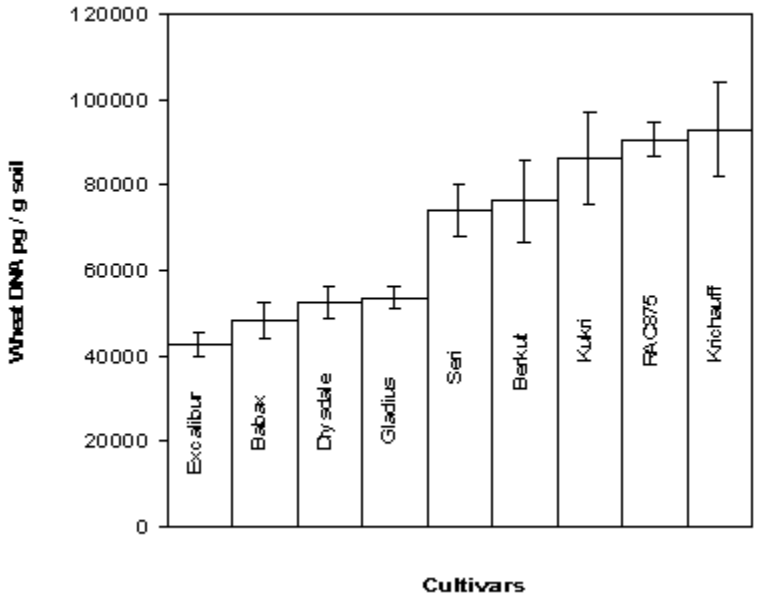

Figure 3. Wheat DNA concentrations (pg g soil) in the top 10 cm of soil between the rows of wheat lines in a field experiment at Roseworthy, mid October 2007.

Mixed population roots – changes in time

Root DNA concentrations in soil for all three species of pasture plants were highest in spring (Figure 4). Subterranean clover DNA was concentrated in the top 10 cm and declined sharply down to the 40-50 cm layer. Phalaris and lucerne DNA concentrations in spring were also highest in the top 10 cm, but from 10-50 cm, were similar.

By autumn, subterranean clover DNA concentration in the top 10 cm had declined to 1 % of that detected in spring (calculated using back-transformed data) and even lower in 20 to 40 cm layers. Phalaris DNA declined to 1% in the top 10 cm, and 10% between 20 to 30 cm and 4% in the 30 to 40 cm layer. Lucerne DNA had dropped to 2% in the top 10 cm, and 55%, 65% and 85% in the 10-20, 20-30 and 30-40 layers respectively. Lucerne DNA concentrations below 10 cm were similar to those detected in the previous spring (Figure 4).

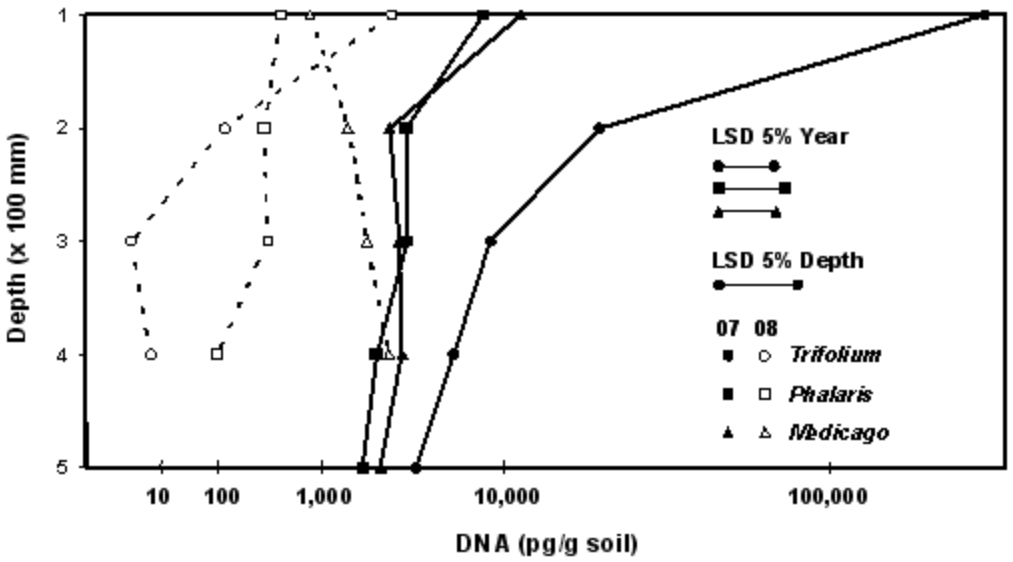

Figure 4. Distribution of plant DNA in spring 2007 (—) and autumn 2008 (- - -) at the MASTER field site, Book Book near Wagga Wagga, NSW. ● *Trifolium* (subterranean clover), ■ *Phalaris aquatica*, ▲ *Medicago* (Lucerne). Depth 1 = 0-10 cm, 2 = 10-20cm etc.

Discussion

DNA assays provide a new technique to study root distribution under field conditions. DNA concentrations in soil are a measure of live cells associate with plant roots (and seed) in the soil sample. Root distribution can be determined by testing soil samples from different positions in the soil profile. DNA results are not a measure of root mass, as DNA concentrations vary in different parts of the root system depending on the age and amount of structural components, sugar content etc (results not presented).

The results summarised in this paper show quantitative DNA assays have significant potential to support detailed studies on root development under field conditions. The advantages of this method include:

- Total soil sample is assayed, therefore no loss of fine roots as with root washing methods,
- Field soil can be cored to depth and assayed with minimal disturbance to the study site,
- Individual plant species can be studied in mixed plant communities, and
- DNA assays do not detect dead roots.

The rapid decline (80% over 10 days) in ryegrass DNA in soil after the plants were either sprayed with glyphosate or defoliated shows DNA degrades quickly in dying root cells, while root mass over the same period were not significant. A high proportion of the plant DNA in roots is likely to be in the live fine roots, which are important for water and nutrient uptake. The fine roots are likely to be the most susceptible to soilborne pathogens, selective herbicides and environmental stress, and the most responsive to beneficial organisms, seed treatments and fertiliser placement.

The barley DNA assay had greater resolution to detect differences in root architecture both between rows and at depth than root mass measurements. The increased DNA concentrations and root mass under Mundah in the 30-60 cm soil layer are consistent with the findings of previous studies (Long, 2003). Increasing sampling intensity would enable smaller differences to be detected by both methods, especially below 30 cm where root mass is low. The sampling strategy in this experiment produced composite soil samples averaging 110 g. The DNA extraction system can cope with 500 g samples, so there is scope to increase sampling intensity. Processing costs could be reduced by specifically sampling areas in the soil profile where differences are expected, but this strategy risks missing unexpected root responses between sites and seasons. Further investigations are needed to determine the size of

differences in root architecture that need to be detected to support phenotyping experiments and to optimise soil sampling strategy.

Wheat root architecture in drought tolerant cultivars is expected to differ between those adapted to grow on moisture stored deep in the soil profile or shallow from in season rain events. The cultivars with high DNA concentrations between the rows in the experiment at Roseworthy 2007 were all developed for southern Australia environments where most available moisture comes from growing season rainfall. However, Excalibur, which is adapted to the low rainfall Mallee regions in SA, and Gladius a new cultivar adapted to southern Australia had low soil DNA concentrations midway between the rows, similar to Badax and Drysdale, which are adapted to grow on stored moisture. Further studies are underway to determine if these traits are stable across sites and seasons, or vary possibly in response to available moisture, fertility and/or incidence of soilborne diseases.

DNA assays for lucerne, phalaris and subterranean clover detected significant spatial and temporal differences in pasture plots. Lucerne growth during summer and autumn appears to be supported by maintenance of the root system below 10 cm, where DNA concentrations were similar to that detected in the previous spring. Dormant phalaris plants in autumn appear to be supported by a root system with 90% less viable cells below 10 cm compared to that detected in previous spring. The subterranean clover results indicate this plant has a predominately shallow root system that degrades over summer. The low subterranean clover DNA in the top 10 cm in autumn is probably associated with the seed bank and was equivalent to 2 seeds/500 g soil or approximately 500 seeds/m^2. The low phalaris and lucerne DNA levels in autumn in the 0-10 cm layer suggest these plants have few active roots in this zone over summer/autumn. Presumably this means they need to regenerate roots in this layer to benefit from summer/autumn rainfall. These results suggest soil DNA concentration is a potentially useful measure of root activity and is also a reminder that the seed bank should be considered when using DNA assays to study root architecture in field trials.

The use of DNA assays has potential to provide a better understanding of root growth under field conditions, and support multidisciplinary research to develop new cultivars and farming systems that are better adapted to climate variability and soil chemical, physical and biological constraints.

Conclusions

The results summarised in this paper indicate DNA assays provide new opportunities to study root distribution in the field. The approach overcomes many of the limitations of existing methods and can provide a measure of live roots which should be more closely linked to root activity than can be achieved by quantifying root mass and length. It is acknowledged that considerable further work is required to validate the technology and investigate the relationship between root DNA concentration in soil and root function. Further investigations are also needed to develop and optimise efficient sampling strategies to compensate for spatial variability, while minimising DNA degradation in the samples before delivery to the laboratory.

Applications of the technology potentially include assessing the impact on amount and distribution of live root cells associated with, seasonal variation in available water, herbicides, row spacing, fertiliser placement, sub-soil chemical, physical and biological constraints, and G x E affects in pre-breeding and breeding programs. The ability to detect individual species in mixed plant communities, fine live roots and not detect dead roots are key features distinguishing DNA assays from measures of root dry weight and root length that require the roots to be washed from the soil. The potential benefits of this technology to farming systems and plant breeding justify further investment in this area.

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