# Overcoming the root phenotyping bottleneck in cereals: Opportunities for breeding and agronomy


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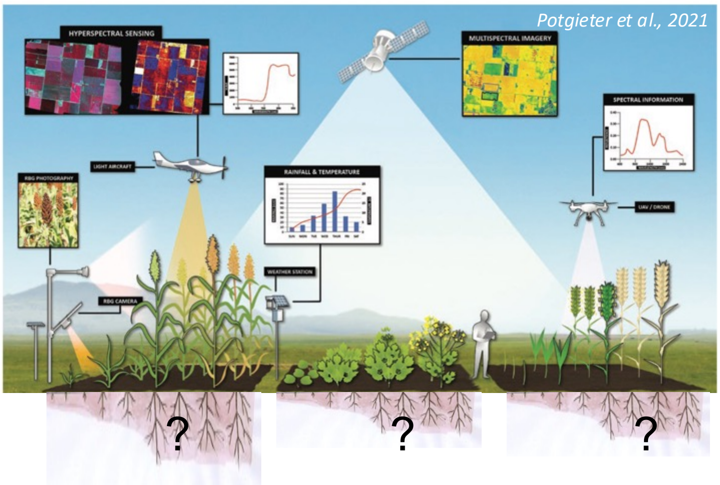
## What is the problem?



*Potgieter et al., 2021*

- Most phenotyping focuses on measuring canopy traits.
- The root system remains hidden to both breeding and agronomy.
- Root traits derived under control conditions have failed to be related to yield or yield stability traits.
- Most phenotyping tends to only measure the mean value of a trait, ignoring the presence of trait plasticity.

Over the coming decades, the main challenge for the Australian grains industry will be to do more (yield) with less (resources). This will require significant gains in resource use efficiency, primarily water and nitrogen. Given the primary role of the root system to access water and nutrient resources, the following questions become relevant:

- Do all cultivars/hybrids have the same type of root system?
- Does the root system of different cultivars/hybrids respond the same to stresses?

Here, we propose that (i) there is genetic variability in valuable root traits that affect yield and yield stability, and (ii) that measuring these traits can help design crops that can do more (yield) with less (resources) and that are more resilient to changes in the availability of soil resources.

## What are we trying to achieve?

We are a multidisciplinary team of breeders, agronomists, crop physiologists, crop modellers, soil scientists, molecular scientists and artificial intelligence experts working in collaboration with industry to:

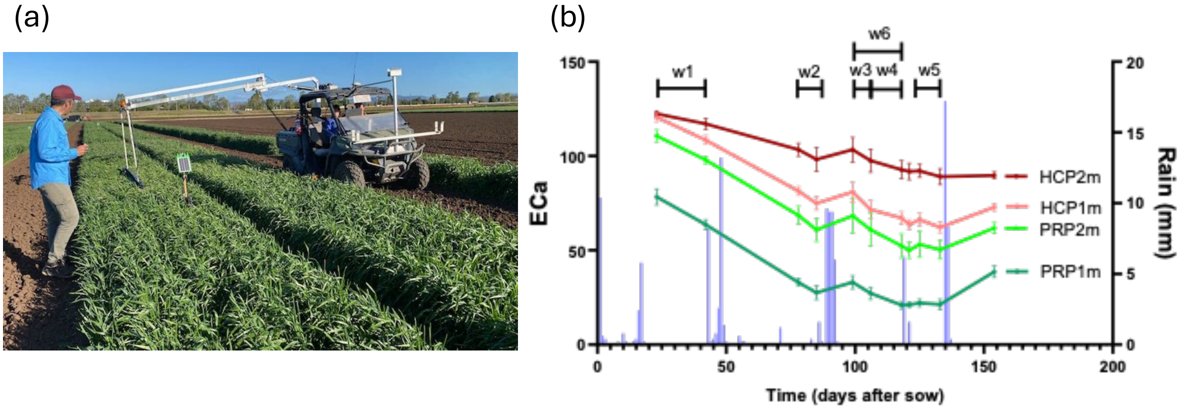
- *Develop accurate, fast and cost-effective high-throughput phenotyping tools for impactful root traits that affect yield, and yield stability.*
- *Quantify the genetic diversity of impactful root traits on elite and non-elite panels in sorghum, nested-association mapping populations in wheat, and commercial checks.*
- *Quantify the value of selecting for root traits across target production environments.*
- *Improve the algorithms in APSIM to simulate root growth and function.*

## How are we proposing to do it?

So far, root phenotyping approaches have included growing single plants in pots, containers or growth chambers, primarily under controlled conditions in glasshouses. In the field, approaches have used "shovelomics", soil coring, and rhizotrons to measure a small fraction of the root system. It is important to note that all these approaches assume that visualising the **form** of the root system can be used to infer its **function** e.g., water or nutrient uptake.

An important limitation in previous work is that it only characterizes the mean value of a root trait, which overlooks the fact that the rooting system is highly plastic and that different genotypes show different degrees of plasticity under stress. In addition, most of these approaches fail to meet the need for the approach to be accurate, fast, cost-effective, and to measure traits that are of value to breeding and agronomy.

Our approach, instead, is based on estimating proxies for **root activity** using accurate, fast, and cost-effective measurements of the changes in layered soil moisture in combination with canopy vegetative indices from drone imagery. Proxies for root function are derived from soil-layered crop water use from time-lapsed EMI surveys during short windows ~7-10 days with no rain during the crop's critical periods for yield formation and during grain filling (see figure below).

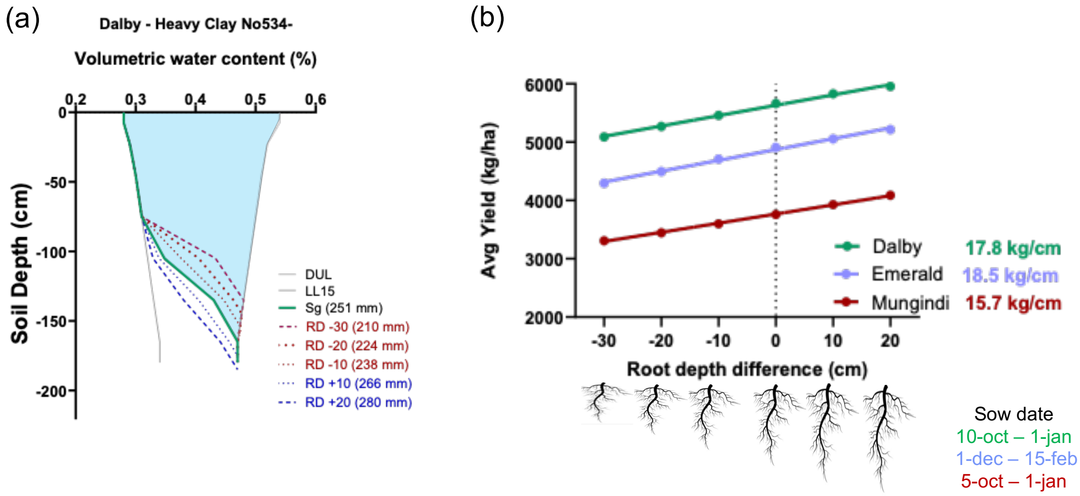



*UQ-QAAFI's RootPhenomobile surveying the 2024 wheat trial at Gatton, Qld (a), and (b) readings from the RootPhenomobile showing the decline in soil water during the trial for one plot at different integration depths 0-0.5m (dark green), 0-1m (light green), 0-1.5m (pink) and 0-3m depth (red). In (b) w1 to w6 shows the definition of dry windows during which root traits could be derived.*

## What have we done so far?

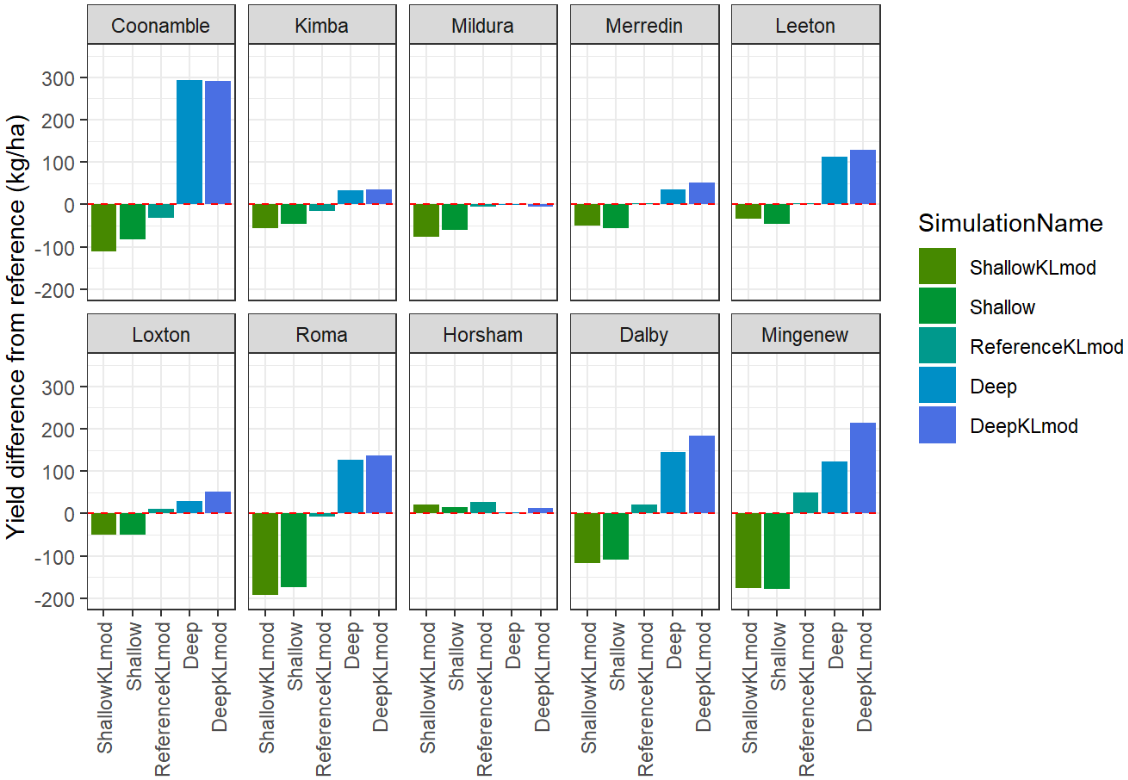
### *Ex-ante APSIM modelling*

APSIM Next Gen was used to ex-ante simulate the likely yield benefits of having wheat and sorghum germplasm, showing contrasting rooting patterns. These results need to be taken with caution as there are traits that APSIM is not able to simulate e.g., phenotypic plasticity on root traits. This modelling will be repeated later in the project once APSIM algorithms have been fully tested.




*Simulated results using APSIM Next Gen- Sorghum, from reducing i.e., -10 to -30 cm, or increasing i.e., +10 to +30 cm, the maximum rooting depth of a sorghum crop at Dalby, Emerald and Mungindi (a), and (b) changes in the simulated yield for Dalby (green), Emerald (blue) and Mungundi (red). Figure (b) shows yield changes of 15.7 to 18.5 kg / cm of additional root exploration.*

For wheat, the effect of variations in maximum rooting depth on grain yield and yield stability in APSIM Nxt Gen were generated by modifying the value of the crop lower limit, i.e., the extent to which a particular crop can extract water from a particular soil layer, for typical soils across the northern, southern and western grain growing regions




*Simulated yield distributions for a reference variety (Suntop), and artificial varieties showing contrasting root architectures and function, i.e., shallow or deeper root explorations with standard KL values i.e., maximum capacity to extract water per soil layer; and shallower or deeper with higher KL values for the deeper soil layers. Simulated results are shown for Loxton and Kimba in South Australia, Horsham and Mildura in Victoria, Coonamble and Leeton in NSW, Mingenew and Merredin in WA, Dalby and Roma in QLD.*

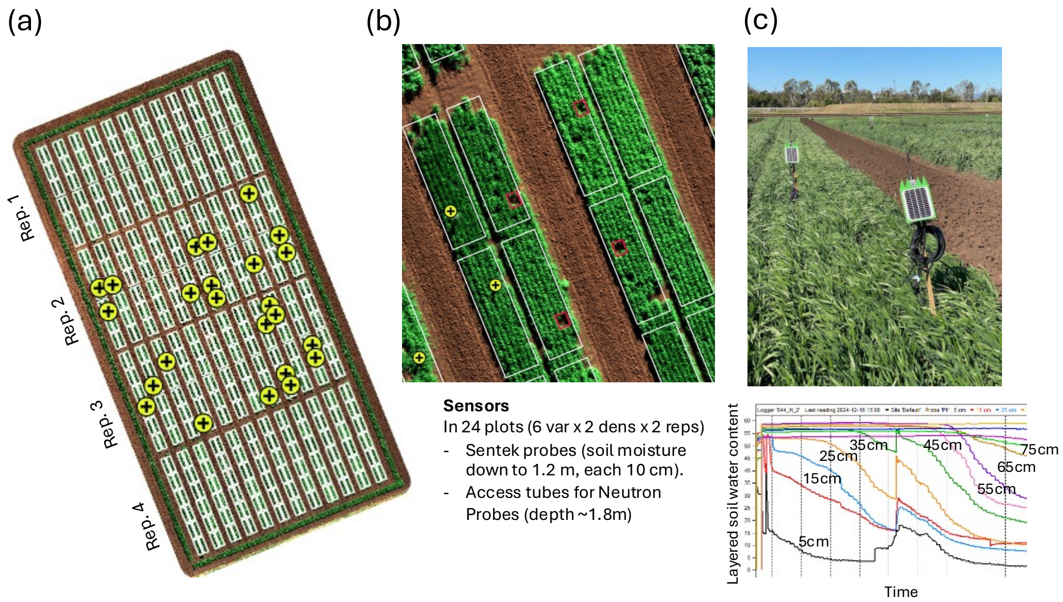
For wheat, shallower rooting systems across all sites than the reference cultivar (Suntop) reduced yield, and deeper rooting systems increased yield. The most significant yield increase was 300 kg/ha at Coonamble in NSW, and the largest yield loss ca. 200kg/ha at the hottest and driest site, Roma in QLD. Irrespective of the site, yield increases were associated to deep water use, indicating that varieties having deeper rooting systems would be beneficial across the whole of the Australian wheat belt. Note that these results are preliminary, simulations will be repeated once APSIM algorithms for root growth and function have been updated.

*Wheat field trials 2024*

The 2024 trials aimed to apply the developed high-throughput phenotyping tools and pipelines on wheat lines with contrasting root architectures.

The same experimental design was applied across three sites in Queensland (DPI Research Station at Gatton), CSIRO-Canberra (CSIRO Boorowa Research Station), and a farmer's field in Meckering (WA). The Boorowa site was discontinued as CSIRO decided to leave the project. AgVictoria has now replaced CSIRO and this trial will be sown at Horsham's Plant Breeding Centre during 2025.

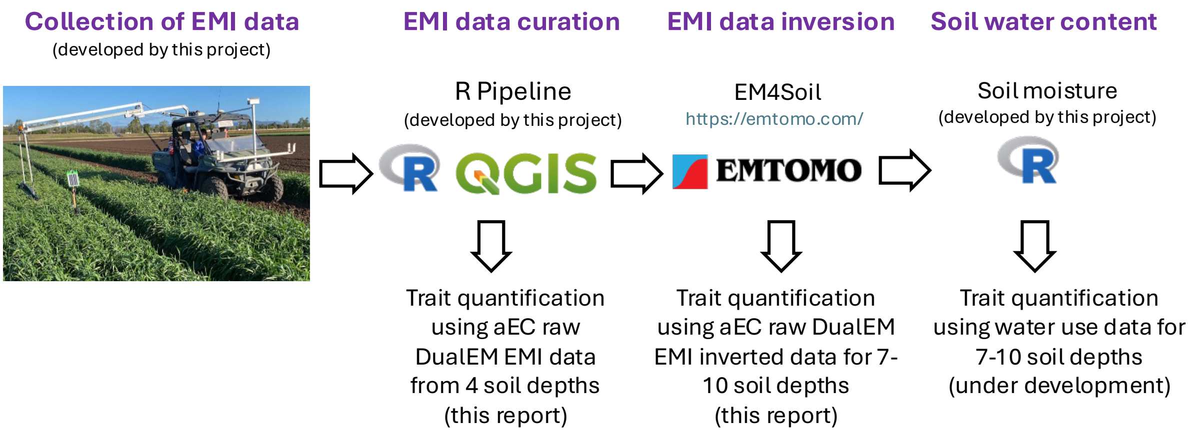
All trials were similar, to allow for a collective analysis using Factor Analytic variance structure for modelling GxE interactions in mixed models (R-ASREML). Treatments included 36 wheat lines from UQ's NAM population and commercial checks sown at two contrasting plant densities. Treatments were replicated four times. The two contrasting plant densities i.e., 60 and 200pl/m2, were used to generate contrasting intraspecific competition environments, which we expect will result in contrasting responses on the rooting system and allow us to detect differences in phenotypic plasticity on root traits between cultivars.




*Standard twin plot trial design across all researcher-managed trials for wheat during 2024, and additional continuous soil water monitoring at the Gatton trial using Sentek Drill&Drop sensors on 24 plots including 6 varieties, two plant densities and two replications.*

*Data pipeline*

A data pipeline was developed, and in-person training delivered to research and industry project partners at UQ St Lucia Campus between the 14th and 16th of January 2025. Participants included researchers from DPIRD, AgVictoria, UQ PhD students, UQ, and a service provider of digital agriculture products, Airborn Insight (Loren Otto). Training included data collection, data preparation for analysis, and data inversion using the EM4Soil software from Entomo. All training material has been uploaded to the MS Teams folder and is available to all partners.




*Developed pipeline for the analysis of electromagnetic indication data from DualEM sensors. The pipeline consists of data collection using DualEM surveying equipment, a R pipeline to clean and prepare the data to be exported to the EM4Soil software from ENTOMO for data inversion, and final calibration from apparent electrical conductivity data values to soil moisture data. We propose that root traits could be developed at each stage, using (i) raw EM apparent electrical conductivity data from the four DualEM channels, (ii) using inverted apparent electrical conductivity data for eight to ten soil layers, and/or (iii) layered (8 to 10 layers) soil moisture / water use data. We are assessing the value and trade-offs of each of these approaches.*

## What did we find (so far)?

**Wheat 2024**

- Plant density treatments created contrasting soil water extraction dynamics, with the higher-density plots showing deeper water extraction than lower-density plots.
- Wheat cultivars showed a wide range of yield stability / plasticity phenotypes.
- Yield plasticity was also positively related to yield. This is low-yielding cultivars tended to produce stable yields across environments, and high-yielding cultivars tended to produce high yields in high-yielding environments and low yields in poor-yielding environments.
- So far, the analysis identified cultivars having higher and lower than average values of phenotypic plasticity for water use, and high and low mean value of water use per unit of available water.
- After heading, late-flowering cultivars, such as Suntop, Denison, UQ45, UQ48, UQ49, and UQ57, consistently showed high water use and plasticity. These cultivars also showed higher-than-average yields.
- These initial results are encouraging and suggest that the approach
    - (i) can identify differences in root activity by different genotypes and
    - (ii) that there was large variability in the expression of contrasting phenotypes across environments.

These results will be later validated with the results from adding other trials – as results become available - and the results from the root sampling

**Sorghum 2024-2025**

- 53 inbreeds and commercial checks were sown at Hermitage Research Station in early November 2024 and harvested in mid-March 2025. Samples are being processed.

**Wheat 2025**

- A summer nursery with 400 wheat entries was recently harvested in Naracoorte SA, after a seed multiplication trial sown at Hermitage Sation in 2024 was lost to sprouting.

**PhD students**

- Two PhD students have started working on sorghum, and one student is due to start soon on wheat.

## What are we proposing to do next?

During 2025 activities will focus on:

- Completing the analysis of the 2024 wheat method validation trials and writing up the results.
- Repeating the 2024 method validation trial with wheat (36 entries x two densities and four replications) at a third site in Horsham, Victoria. AgVic will conduct this trial, that will complete the first-year method x 3 sites (6 environments) validation data set.
- Sowing the trials to characterise the existing diversity in root traits and their relationship with yield and yield stability in wheat. 400 entries from UQ's NAM population will be sown in a partially replicated trial at Gatton Qld, and Northam WA. This trial will be sown in Victoria during 2026.
- Sowing a trial to characterise the existing diversity in root traits and their relationship with yield and yield stability in sorghum. 400 entries will be selected from Hermitage Research Station's sorghum elite and non-elite panels.

## The RootBot: a new high throughput phenotyping platform

- UQ-QAAFI is commissioning the RootBot to be used during the 2025 wheat season. The RootBot is an autonomous high clearance vehicle used to collect electro magnetic induction soil data and hyperspectral canopy data.