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

**Rodriguez D**[1*], Zhao D[1], de Voil P[1], Gimenez R[1], Chenu K[1], Hickey L[1], Jordan D[1], Mace E[1], Singh V[1], Khan H[2], Wasson A[3], Schnider H[4], Jones D[4]

[1*] Centre for Crop Sciences, Queensland Alliance for Agriculture and Food Innovation (QAAFI) The University of Queensland, DigitalAg Building (#8115) Gatton Campus University of Queensland, Gatton QLD 4343, d.rodriguez@uq.edu.au
[2] DPIRD, Northam WA
[3] CSIRO Black Mountain ACT
[4] IPK, Germany

## Abstract
There is no doubt that the main challenge to food production, now and in the decades to come, is the expected increase in the frequency and intensity of drought stresses. Adaptation to these stresses should be based on matching better-adapted genotypes (G) and crop managements (M) to mitigate the impact of abiotic stresses across a wide range of growing environments (E). Here we propose that the architecture, anatomy and function of the root system offer untapped opportunities for crop adaptation. However, so far, the lack of quick, cheap, accurate and functional high throughput root phenotyping approaches in the field has limited the capacity of breeding, agronomy and precision agriculture to develop valuable traits and products. In this thumbnail article, we present a new functional approach to phenotype rooting systems in sorghum.

## Keywords
Drought tolerance, phenotypic plasticity

## Introduction
Given that grain production in Australia is mainly limited by water availability (Rodriguez et al., 2005), it is rather perplexing how little we know about the rooting system, the most critical plant organ to access soil water and nutrients. Root traits are hard to measure and the lack of quick, cheap, accurate and functional root phenotyping approaches in the field, has limited the capacity of breeding, agronomy, and precision agriculture to develop valuable traits and services for the grains industry (Tracy et al., 2020).

Even though genetic variability in root architecture (i.e., root structure) is known to exist in sorghum (Borrell et al., 2014; Mace et al., 2012; Singh et al., 2012), and wheat (Alahmad et al., 2019; Hassouni et al., 2018; Richard et al., 2015), rarely these traits have been functionally related to differences in crop water use, yield, or yield stability in the field. Lack of success can be attributed to multiple factors including:

- the presence of interactions and seasonal stress dynamics between genotypes (G) and environment (E) on root structural traits limit the value of glasshouse, tubes, root chamber, pots, and lysimeter studies.
- that root structural traits determined early in the season on plants in vegetative stages tend to show poor correlation with the same traits determined later in the season.
- that there has been a focus on characterising root structural traits rather than understanding how the rooting system functions and responds to environmental and management cues i.e., functional traits.
- that the phenotyping of structural root traits is expensive, only feasible on a small number of treatments, and subject to large errors when sampled in the field; and
- that the predominant focus of approaches has been limited to characterise the average value of a trait, which overlooks the fact that the rooting system is highly plastic and that different genotypes show different degree of such as plasticity under stress.

Here we present an example of a new approach to field non-destructive high throughput phenotyping for functional root traits. The approach was originally developed by the GRDC-funded Optimising Sorghum Agronomy project, and now is being further developed by a multidisciplinary team of researchers from Australia and Germany, in collaboration with breeding and digital agriculture companies. The project started in January 2024 and will run for four years. Project products can be expected to help breeding companies characterise cultivars for more drought-tolerant rooting systems, and providers of precision agriculture deliver new services for the grains industry.

## Methods

The proposed functional phenotyping approach was applied here to screen functional root and canopy traits in sorghum in a panel of twenty commercial and experimental hybrids from Pioneer Seeds Australia.

*Field trial:* Twenty - experimental and commercial - sorghum hybrids were sown at a farm site in Brookstead Qld during 2022. The trial design was a completely randomised block design with three replications. Plots were four 1m rows and 10m long. From flowering to maturity canopy and root traits were derived from drone imagery and five consecutive electromagnetic induction (EMI) surveys, conducted about ten days apart. Drone imagery was collected four times during grain filling by flying a multispectral MicaSense Altum PT sensor (USA). A green leaf index (GLI, Liedtke et al., 2020) was derived to characterise canopy senescence dynamics during grain filling. Canopy senescence dynamics was characterised as the slope of the value of the GLI from flowering to maturity and termed “Stay green” (Liedtke, et al., 2020). The time-lapse electromagnetic induction surveys were conducted using a DUALEM-21S (Dualem Inc., Milton, ON, Canada). The instrument was dragged 3m to the right of a four-wheel all-terrain vehicle that traversed the field along the transect in the middle of each plot (Fig. 1). After calibration, the surveyed values of layered water use were used to calculate two root traits, (i) a root activity factor for each soil layer, down to the maximum rooting depth (as in Zhao et al., 2022 and 2024), and (ii) the maximum rooting depth estimated as the deepest depth that showed no change in soil moisture between the two consecutive surveys. At maturity, grain yield and test weights were determined for each plot. The data was analysed using linear mixed models with the REML procedure in ASReml-R  (The VSNi Team (2023) (version 4). The significance of the hybrid fixed effect term was evaluated using a Wald test with a conditional F-statistic (Kenward & Roger 1997). The assessment of differences between treatment levels within fixed effect terms was performed using Fishers least significant difference (LSD) test. All testing was completed using a significance level of 5%.


**Figure 1.** Example of the set up applied to the trial at Brookstead QLD, as described in Zhao et al., (2022).

*Root chamber trial:* The same 20 hybrids from the field experiment were grown in root chambers to characterise nodal root angles at 6 expanded leaves (Singh et al., 2010). The root chambers were 30cm wide, 50cm deep and 4cm wide, and had a translucid front so that nodal roots could be photographed for image processing (Fig. 2). The average value of the left and right nodal roots was used as root trait.

**Figure 2.** Example of the set up for the determination of nodal root angles in sorghum.

## Results

*Yield and root traits*

Yields were low due to terminal water stress conditions, from flowering to maturity the crop received 7mm of rainfall. Environmental conditions were then highly favourable for studying root traits during grain filling. The hybrid effect on yield was significant ($P<0.001$) with yields varying between 2.4 and 4.6t/ha (dry basis).

The tested hybrids showed a large distribution of nodal root angles (Fig. 3a) that varied between ~30° to ~45°. Smaller values of nodal root angles i.e., steeper roots, were associated with higher-yielding hybrids, while deeper rooting systems at flowering were associated with higher values of canopy stay green (Fig. 1 b). Also, hybrids that had deep root systems by flowering showed small values of root depth growth between flowering and maturity.

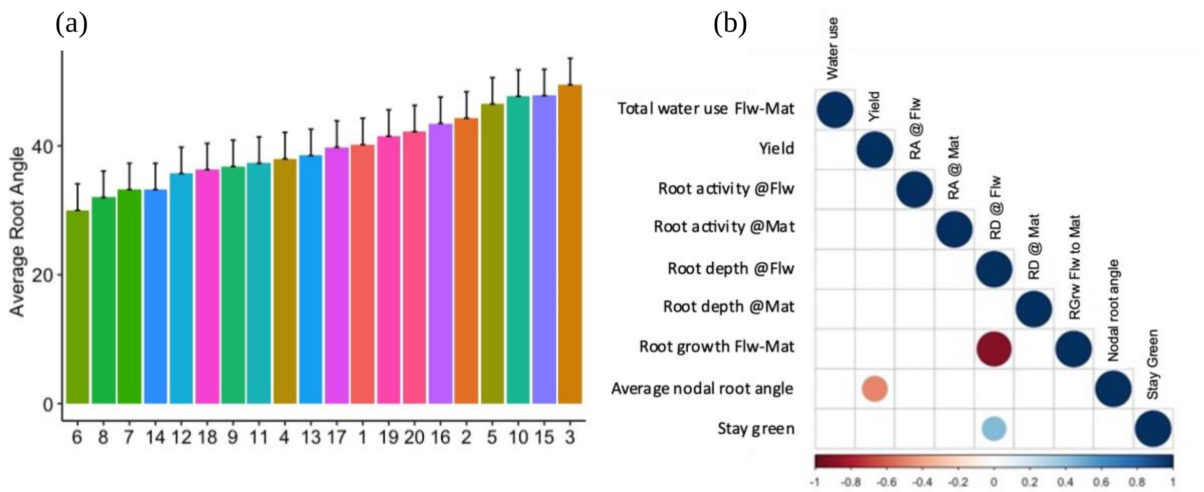


**Figure 3.** Average nodal root angles determined at 6 leaves in sorghum for twenty Pioneer commercial and experimental hybrids determined using root chambers (a), and (b) correlation matrix between total crop water use between flowering and maturity, grain yield, and shoot (Stay green) and root traits i.e., Root activity at flowering, Root activity at maturity, Maximum rooting depth at flowering, Maximum rooting depth at maturity, Root growth between flowering and maturity and Average nodal root angles, from a field trial sown at Brookstead, Qld.

## Conclusion

- The use of EMI technologies to functionally phenotype root systems shows the opportunity to quantify difficult-to-measure root traits in breeding programs and agronomic research.
- Hybrids showing narrow nodal root angles at 6 leaves had higher yields in a season characterised by a severe terminal water stress.
- Hybrid root depth at flowering was related to stay-green traits during grain filling.

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