# *IN SITU* IMAGING OF ROOT SYSTEM ARCHITECTURE TO IMPROVE DROUGHT TOLERANCE AND YIELD IN WHEAT

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## Introduction

Drought is one of the principal abiotic stresses limiting crop productivity. Drought monitor data from Washington State shows that there has been a tremendous increase in area (percent) exposed to drought in the recent years 2015/16 (**Fig 1**). The root system is important for the uptake of water and essential nutrients, to provide anchorage support to plant while acting as interface between plant and several biotic and abiotic conditions. Breeding for improved root traits is an untapped source of crop improvement and a key to successful crop production. However, improving the crop performance in the past were highly limited to the study and modification of the shoot components while the root system was greatly overlooked due to difficulties in root phenotyping, limited understanding of root system in relevant crops, and laborious and time-consuming field-based studies. Field-based RSA studies examine partial root profiles either by excavation, soil coring and trenching. Laboratory-based methods for studying the root growth are well established. However, findings from seedling root growth in controlled environments may not necessarily reflect the highly developmentally plastic and complex root system in heterogeneous field conditions.

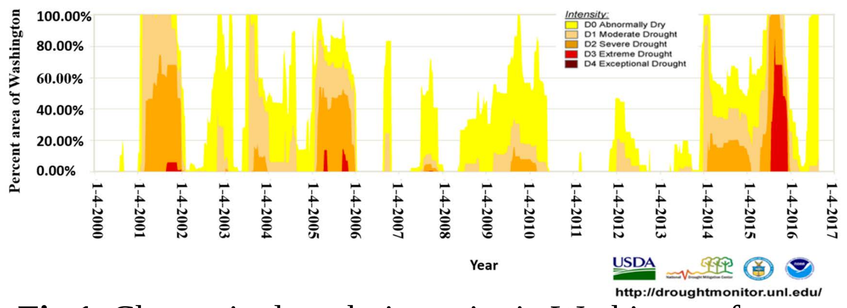


**Fig 1.** Change in drought intensity in Washington from 2000-2017

Thus, we are using *in situ* root imaging (mini-rhizotron) to assay spatiotemporal root system development of spring and winter wheat. First, RSA was evaluated in six spring wheat lines as well as a recombinant inbred lines of Hollis and Drysdale with the hopes of identifying root traits that correlate with yield and/or drought tolerance. Second, RSA was evaluated in two winter wheat lines to identify the impact of semi-dwarfing alleles, *Rht1* and *Rht2* on overall root architecture as their effect on winter wheat remains an underexplored opportunity. Third, we conducted a seedling root growth study to see if there is a correlation between early seedling root traits with adult plant root traits.

## Research Aims and Hypotheses

**Aims**

1. To study the impact of root system architecture (RSA) on drought tolerance in spring wheat
2. To study the impact of semi-dwarfing alleles (*Rht1*, Rht*2 & Rht1/Rht2)* on overall root architecture of winter wheat

**Hypotheses**

1.1 RSA traits differ in six parental lines of spring wheat

1.2 RILs (Hollis/Drysdale) yield correlates with root traits in drought conditions

2.1 Near isogenic lines (NILs) with semi-dwarfing alleles will have altered RSA traits compared to wild type siblings

## Methodology

- **Root phenotyping approach in the field and greenhouse**

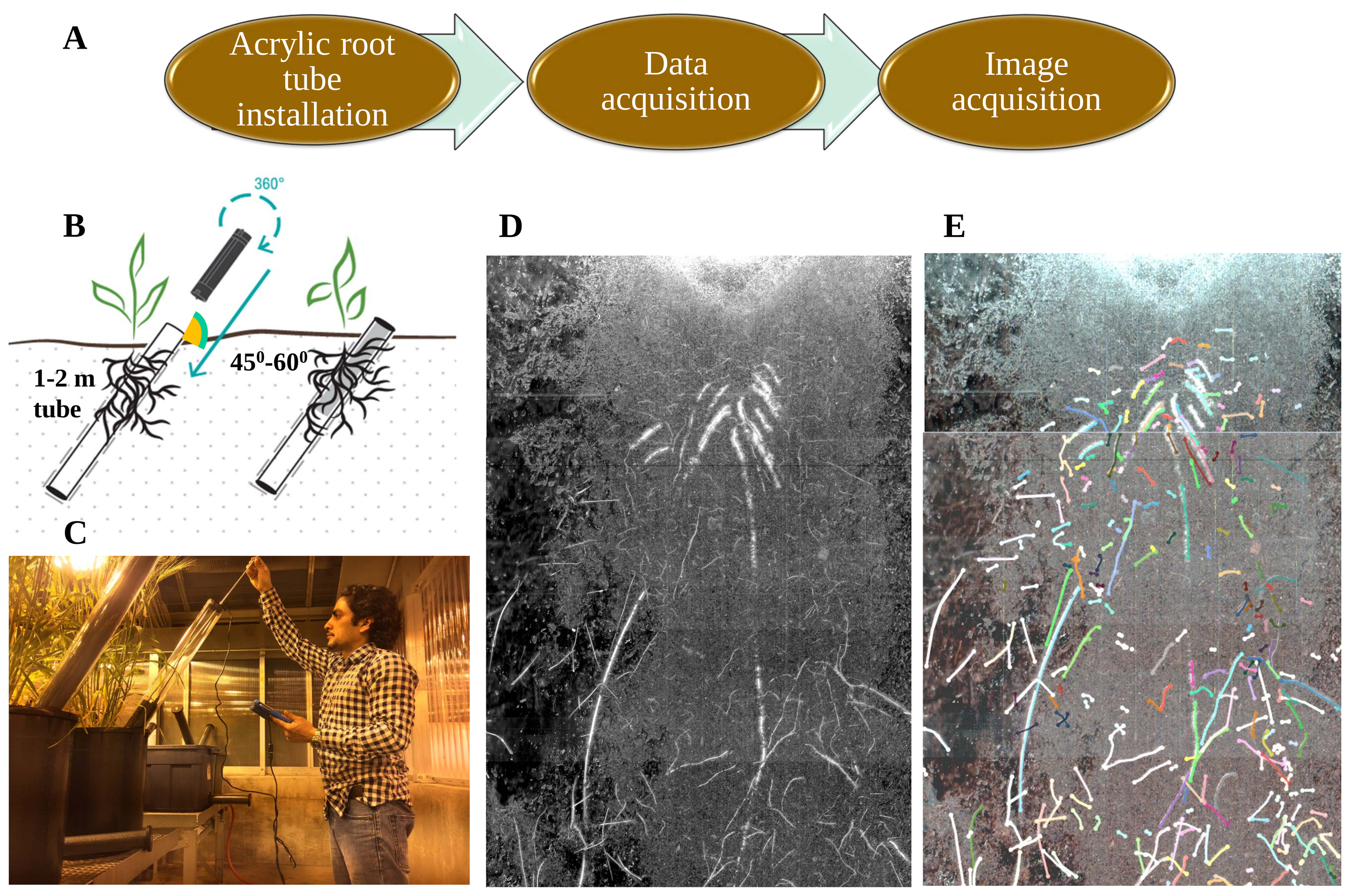


**Fig 2.** Flow chart and images showing the root phenotyping approach (A-E); A. Root imaging flow chart; B. Installation of root tube in the ground; C. Use of CI-600 *in situ* root imager in greenhouse D. Root image of 42 DAS Dharwar Dry taken with the CI-600 E. Image after mapping with CI-690 RootSnap software.

- **Planting materials:** *Spring wheat:* Alpowa, AUS28451, Dharwar Dry, Drysdale, Hollis and Louise; Recombinant inbred lines (RILs) of Hollis and Drysdale *Winter wheat:* Near Isogenic lines (NILs) of 'Golden' and 'Brevor' wheat harboring Rht1, Rht2, Rht1/Rht2 and wild type
- **Seedling root growth study:** Seeds were surface sterilized and grown on ½ MS media to record the primary and seminal root growth rate for 5 days. ImageJ was used to quantify the root length.
- **Data analysis:** Data from greenhouse and field-based studies were analyzed using a repeated measures experimental design and tested for assumption of normality before analyzing with a SAS Proc Mixed model. Best fit variance-covariance structure was used to find the best covariance model based on BIC value.

## Results

### I. Spring Wheat

**A. Root growth rate study of 5-day old seedlings of spring wheat genotypes**

*1. Primary root (PR)*

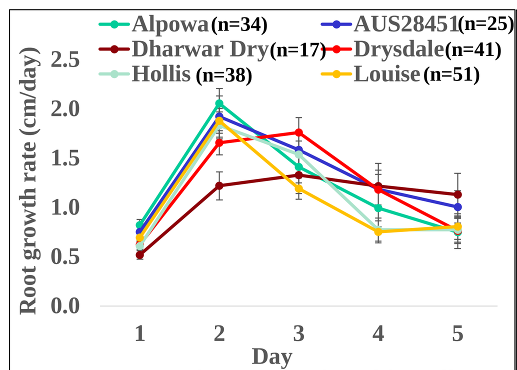


**Fig 3:** PR growth rate of 5-day old seedlings of spring wheat genotypes

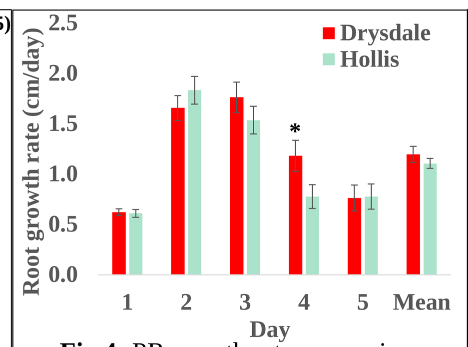


**Fig 4:** PR growth rate comparison between Drysdale and Hollis (** statistical significant at P<0.05)*

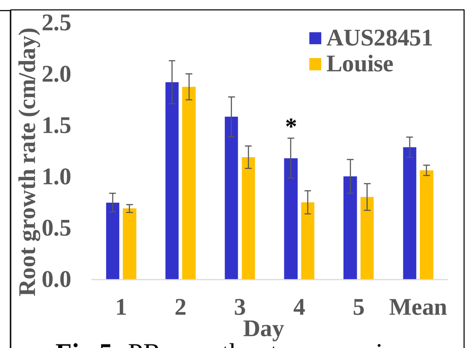


**Fig 5:** PR growth rate comparison between AUS28451 and Louise (** statistical significant at P<0.05)*

- Dharwar Dry showed a slower rate of growth initially, but it increased by day-4.
- Primary root growth rate was significantly higher in Drysdale and AUS28451 at day-4 when compared to Hollis and Louise respectively.

*2. Seminal root (SR)*

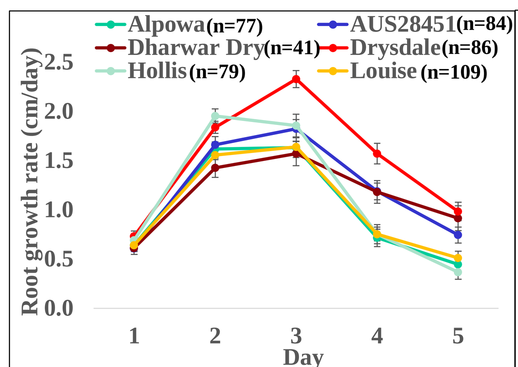


**Fig 6:** SR growth rate of 5-day old seedlings of spring wheat genotypes

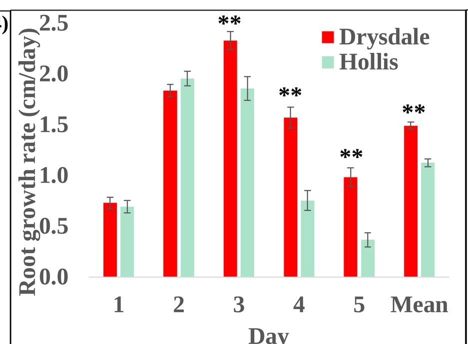


**Fig 7:** SR growth rate comparison between Drysdale and Hollis (*** statistical significant at P<0.01)*

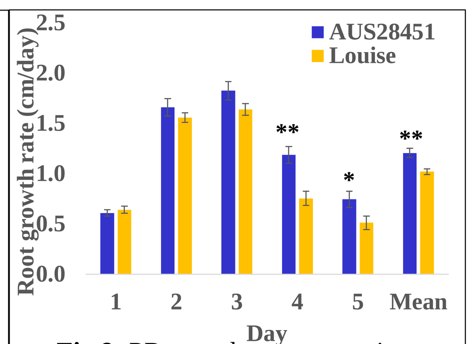


**Fig 8:** PR growth rate comparison between AUS28451 and Louise (**statistical significant at P<0.05 & **P<0.01)*

- Seminal root growth rate on day-5 was greatest in Drysdale and Dharwar Dry.
- Drysdale showed significantly higher seminal root growth compared to Hollis at 3, 4, and 5-days.
- Similarly, seminal root growth rate was higher in AUS28451 on day 4 and day 5 when compared to Louise.

**B. RSA traits in Hollis/Drysdale RILs in dryland field conditions (2015)**

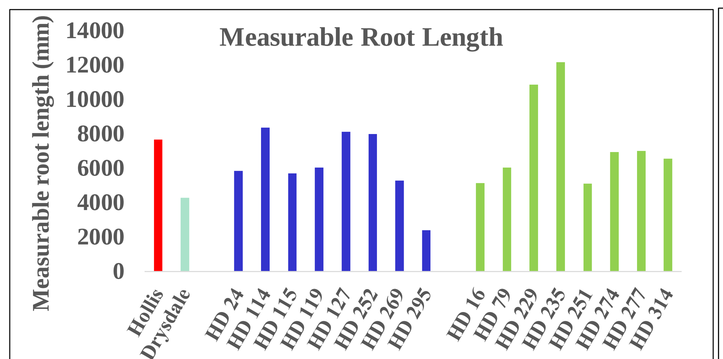


**Fig 9:** Measurable root length comparison among Hollis, Drysdale and their RILs (*Blue=high yielding, Green=low yielding)*

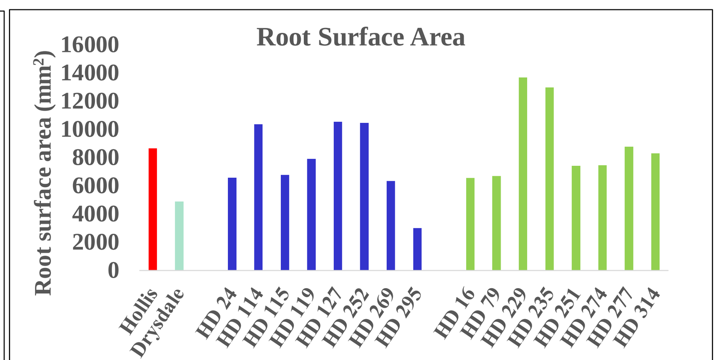


**Fig 10:** Root surface area comparison among Hollis, Drysdale and their RILs

- No significant differences in RSA traits between Hollis and Drysdale.
- RILs HD 229 and HD 235 have higher measurable root length and root surface area

**C. RSA in spring wheat in controlled greenhouse conditions (2015/16)**

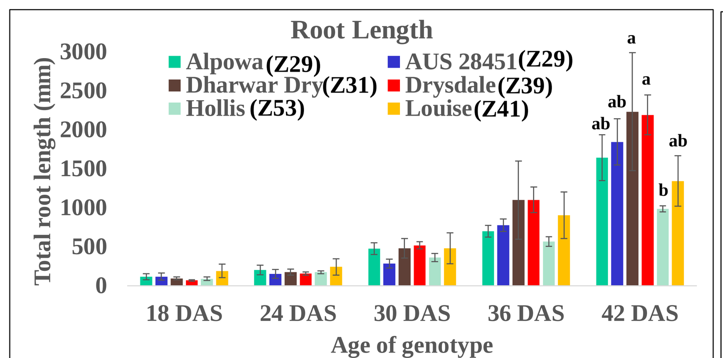


**Fig 11:** Total root length of different age old spring wheat genotypes *(Letter in the parenthesis are Zadoks growth scale at 42 DAS)*

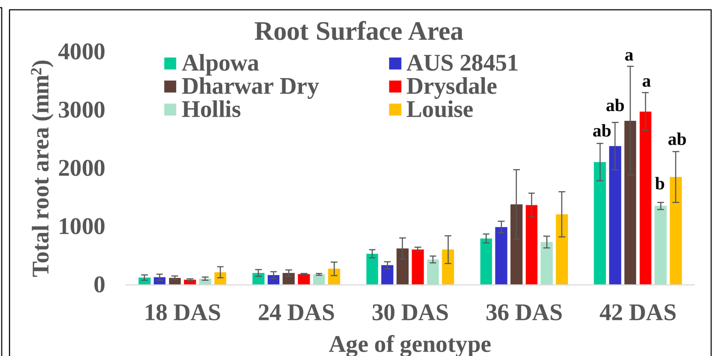


**Fig 12:** Root surface area of different age old spring wheat genotypes *(bars with same letters are not significantly different at α=0.05)*

- No significant difference was found between six genotypes until 36 DAS
- The age by genotype interaction was found to be non-significant for root number, root length, root surface area, volume and diameter.
- A linear and quadratic trend was found in mean root number, root length, root surface area and volume

**D. RSA in spring wheat in controlled greenhouse conditions (2016/17)**

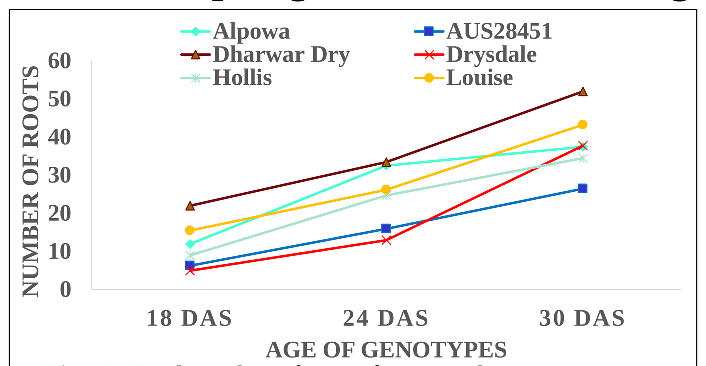


**Fig 13:** Total number of root of spring wheat genotypes at different age

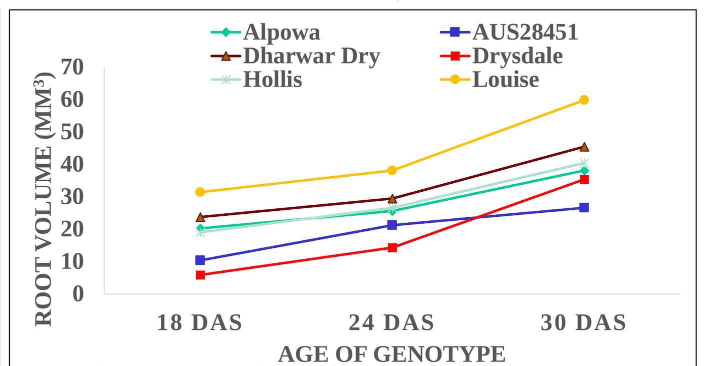


**Fig 14:** Root volume of spring wheat genotypes at different age

- No significant age by genotype interaction was found until 30 DAS.
- Dharwar Dry and Louise show increased root number and root volume, whereas Drysdale shows an accelerating growth rate.

### II. Winter Wheat

**A. Impact of *Rht1* and *Rht2* semi dwarfing alleles on RSA of winter wheat**

- *Rht1/Rht2* seems to have decreased root length compared to *Rht1* and wild type in early stage.
- Minimal root growth was recorded over winter in NILs of 'Golden' wheat (Dec-Feb).

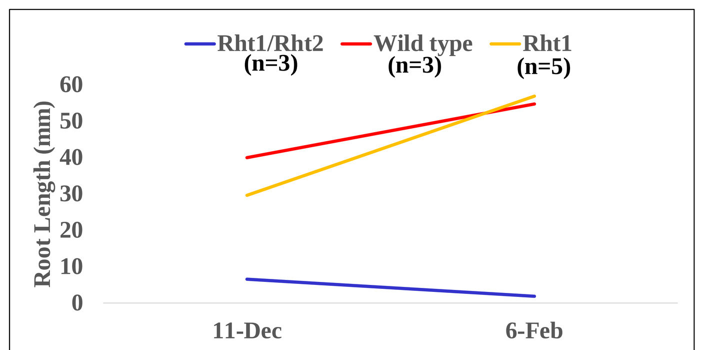


**Fig 15:** Root length comparison of different NILs of 'Golden' wheat at winter

**B. Deep hydraulic soil coring in winter wheat**

- Winter wheat roots penetrate deeper than 2 meters in the field

**Fig 16:** Hydraulic soil coring carried out in winter wheat at the grain filling stage

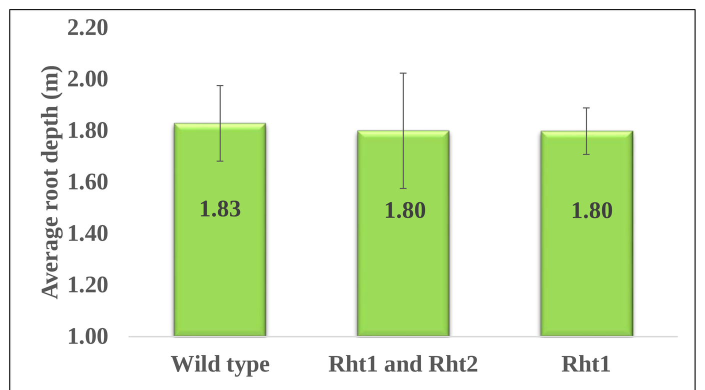


**Fig 17:** Rooting depth of NILs of 'Golden' wheat in the field

## Conclusions

- **Spring Wheat**

*Seedling root growth experiment*: **Drysdale, Dharwar Dry and AUS28451** are good candidates for increased root growth in field and greenhouse conditions

*Field experiment:* The use of 1m tubes was insufficient to track the root growth throughout the growing season. In year two, 2m tubes were used to track root growth at an increased depth.

*Greenhouse experiment:* **Dharwar Dry and Drysdale** are prospective candidates for improved root traits.

- ***Winter wheat***

Minimum root growth was observed during winter (December-February) and after the heading stage suggesting root systems develop until the reproductive stage after which metabolites accumulate for grain filling. The impact of dwarfing genes on RSA of winter wheat is still under study.

## Future Directions

- Seedling root length correlation with adult root phenotypes in greenhouse and field studies
- Combined analysis of RSA traits from two years of greenhouse experiments on spring wheat will help to detect trait differentials among tested genotypes.
- Quantification of RSA traits from spring wheat experiments at Lind Dryland Research Station with deeper 2m tubes will yield important data for later stages of root growth.
- RSA traits that differ between wild type and *Rht1/Rht2* semi-dwarfing alleles in winter wheat will be identified.
- Any desirable root traits identified in these studies will be used as an important selection criteria in subsequent breeding cycles and will be further utilized for the development of drought tolerant spring wheat varieties.

## Acknowledgements

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