UNIVERSITY OF COPENHAGEN
DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES

# Drought tolerance in white clover as a future breeding target

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## Background and objective

Agriculture faces growing threats due to **climate change**. Forage legumes, such as **white clover (*Trifolium repens L.*)**, aid adaptation and mitigation by providing high-protein feed, reducing emissions, and enriching the soil. However, their **drought sensitivity** has a severe impact on **yield and quality**.

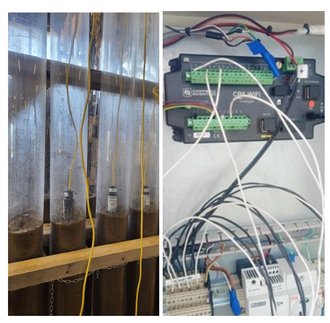
We conducted this study to determine cultivar differences in **deep root development, deep water use** and **drought tolerance**, and the impact of drought on **biological $N_2$ fixation in white clover**.

## Methodology

**Rhizotube experiment**
2 m tall and Ø: 10 cm

| Cultivars |
|---|
| Brianna |
| Coolfin |
| DLF TRF- 3536 |
| Galway |
| Rivendel |
| Silvester |

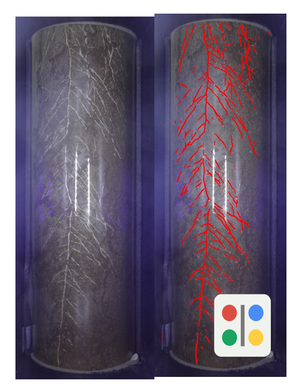
Filling tubes with field top- and sub-soil



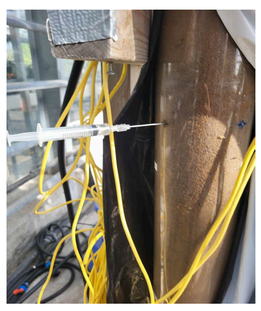
TDR Water sensors and CR6 data logger to record soil VWC%

Roots in tube and soil interface

Root depth measurement and imaging



Root segmentation with RootPainter



Deuterium tracer injection

Well watered Water stressed

**Leaf sampling**

Transpiration water collection

**Plant and water sample analysis for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{2}H$**

## Results

### (1) Root depth

- Deeper roots of **Brianna**, **Rivendel** and **Silvester** compared to **DLF-3536** and **Galway**
- Tendency towards deeper roots under drought

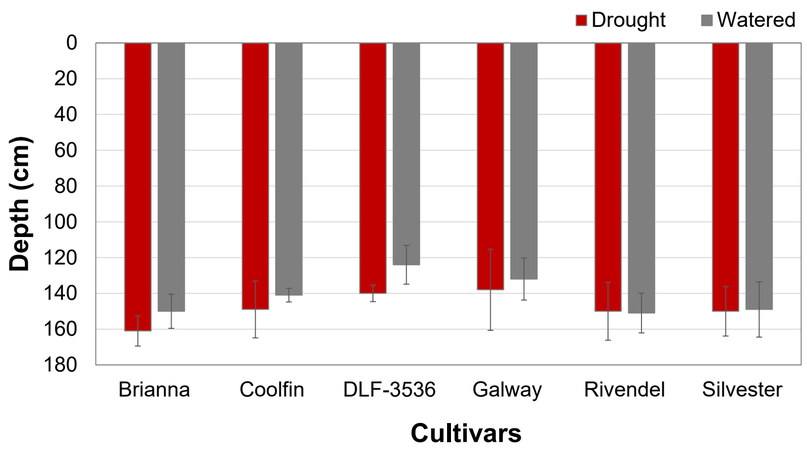



***Fig:*** *Maximum root depth observed at 134 DAS*

### (2) Planar root length density (pRLD)

- Negative effect of **drought** on **pRLD**
- Cultivar differences in pRLD across soil depths and in response to drought

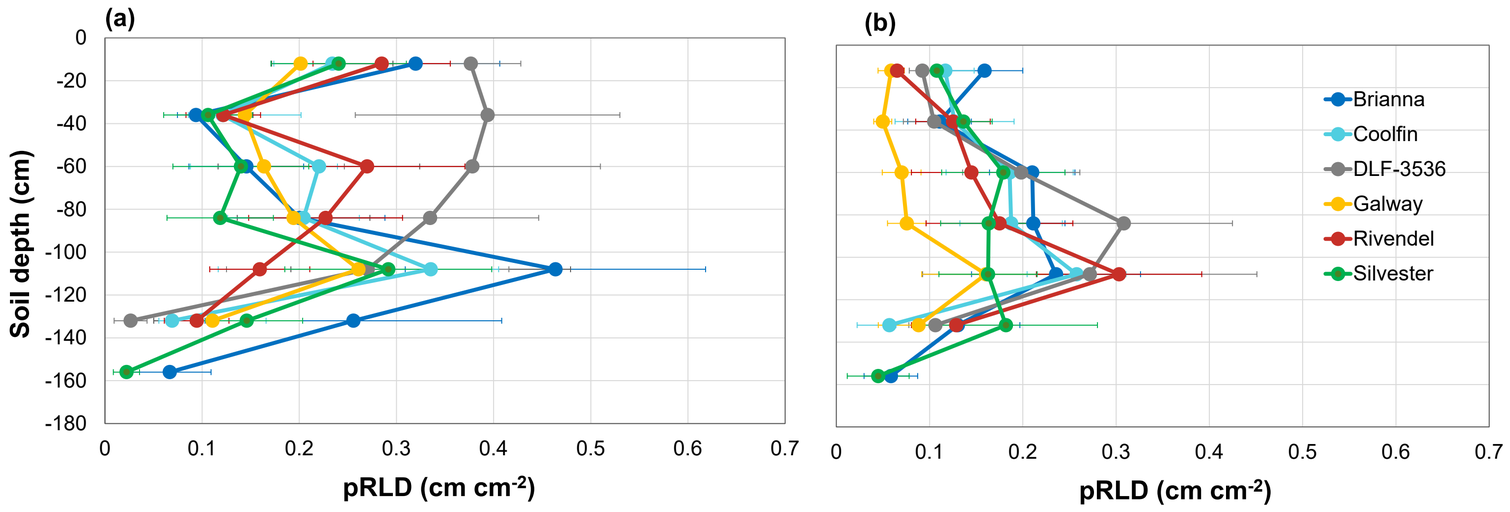



***Fig: pRLD*** *determined from the root images taken at 134 DAS grown under well watered* ***(a)*** *and drought* ***(b)*** *conditions*

### (3) Tracer uptake in transpiration water

- Active use of deep water under drought, except Brianna at 16 DATA (Brianna > Rivendel > Silvester)

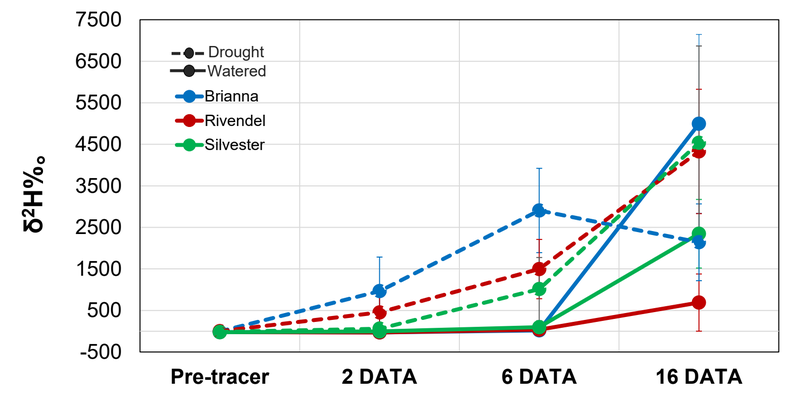



***Fig:*** *Detection of deuterium tracer in transpiration water collected before and 2, 6 and 16 days after injection at a 120 cm soil depth*

### (4) Tracer uptake in plant tissues

- Greater uptake of deep water in Rivendel under drought

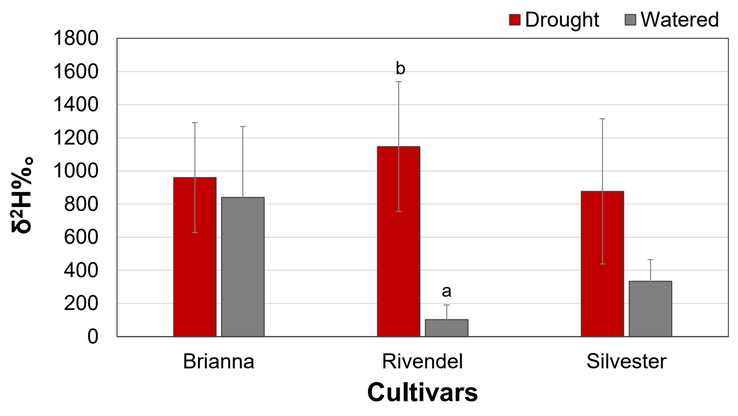



***Fig:*** *D*euterium tracer uptake in young plant leaves 18 days after injection at a 120 cm soil depth

**DAS:** Days after sowing; **DATA:** Days after tracer application

### (5) Drought effect on $N_2$ fixation

- Significant drought effect in **Coolfin** and **Galway** and no effect in **Silvester**

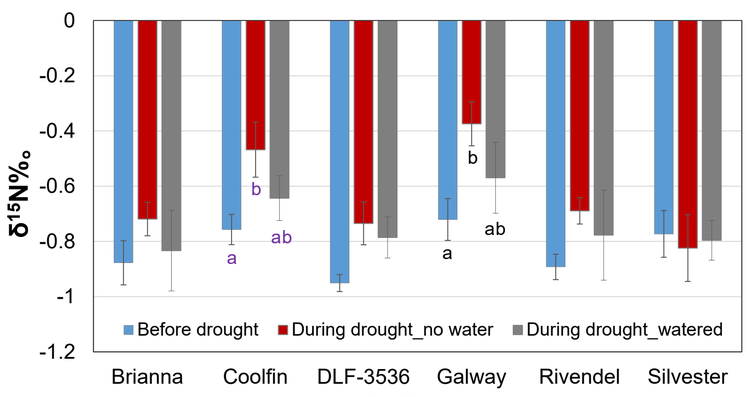



***Fig:*** *δ¹⁵N in leaf samples measured before drought initiation and under drought and well-watered conditions thereafter*

## Conclusion and perspectives

- The study provides insights into cultivar differences in **deep-root development** and **water use** in white clover.
- The results highlight the **role of deep roots in water uptake and drought tolerance**, and the **impact of drought on biological $N_2$ fixation**, with implications for breeding more **resource-efficient** and **climate-resilient** white clover varieties.
- Parallel studies in 4-meter rhizoboxes and field conditions investigate the effect of **cultivar differences** and **intercropping** with grass on deep-root development, drought tolerance and $N_2$ fixation.

**References**
[1] Smith et al. (2022) RootPainter: deep learning segmentation of biological mages with corrective annotation. *New Phytologist*

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