



# CSIRO-GRDC canola establishment project

## National Canola Pathology Workshop Webinar

Matthew Nelson | 12 March 2021

Australia's National Science Agency





# Summary of talk



- What's the problem?
- Meet the team
- Understanding the problem
- Addressing the problem
- Where next?
- What's in it for the breeders?





What's the problem?



50-  
60%



\$\$\$!!



Poor establishment is a serious problem *globally*

# Project outcomes

2030

Growers in low-medium rainfall zones realise a 25% improvement in canola establishment

2022

Breeders have access to improved germplasm (averaging 75% establishment) and knowledge of how to deploy the material

2019

Project begins





# Meet the team



+





# Leveraging international connections





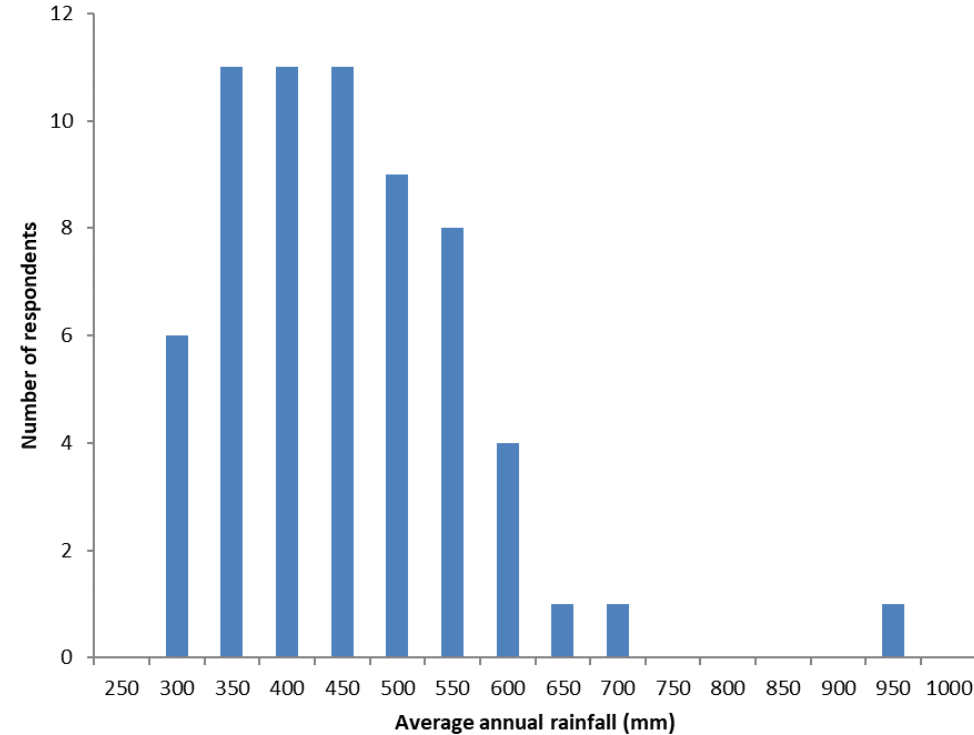
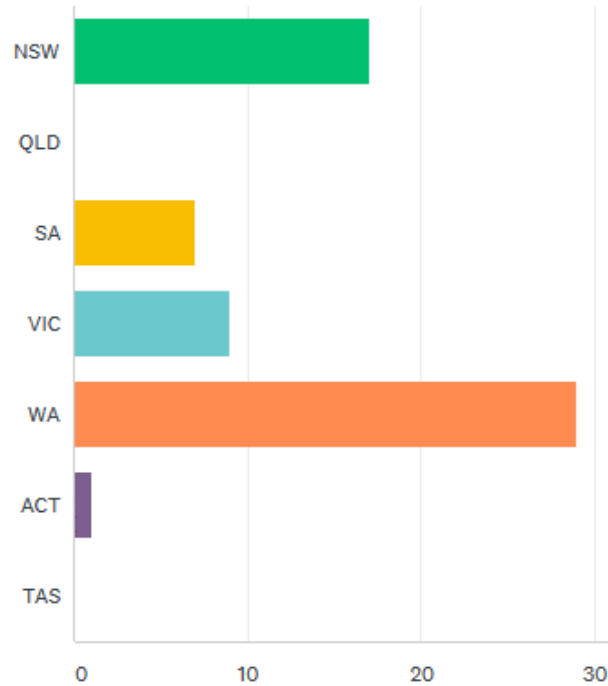
# Understanding the problem





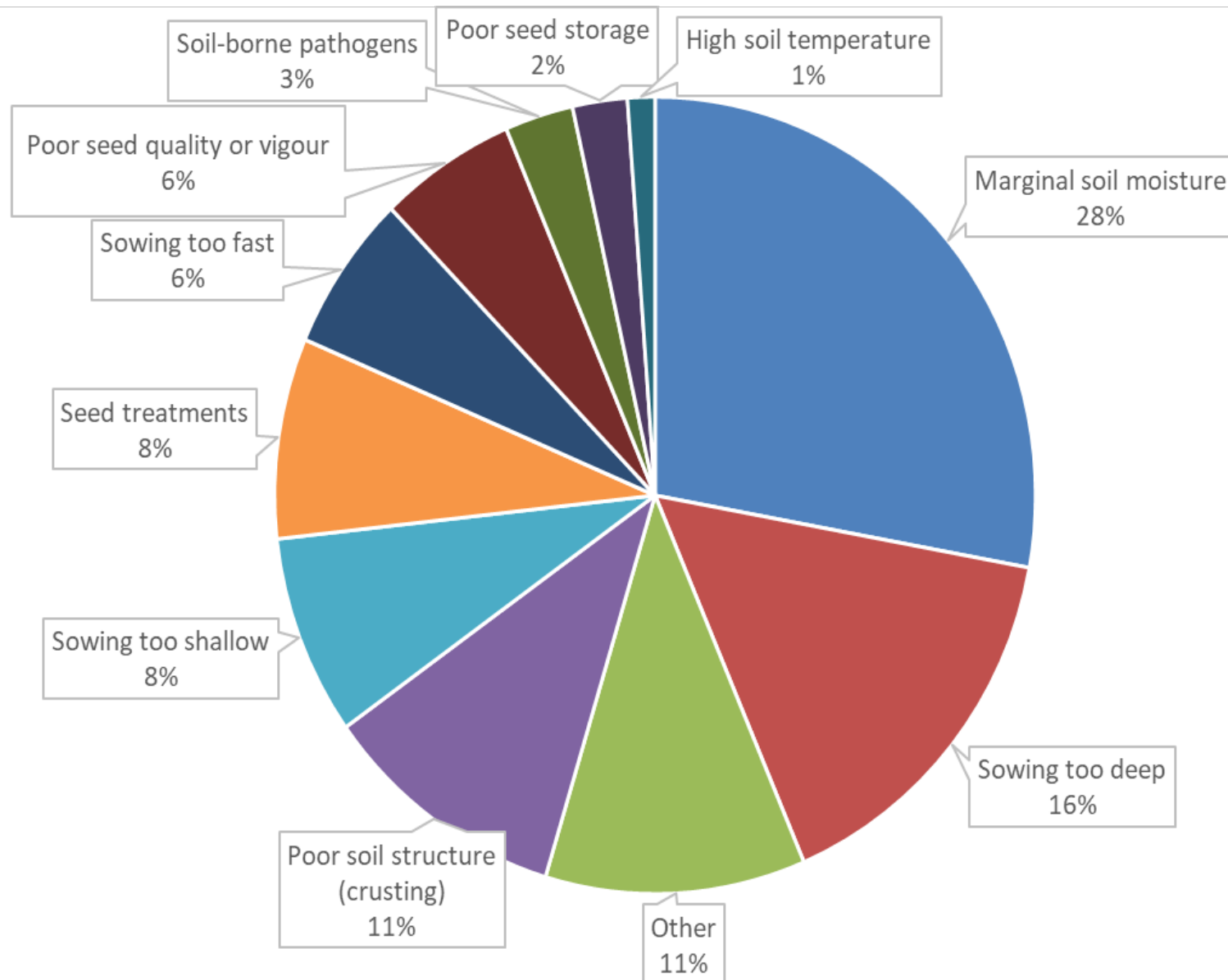
# Industry survey

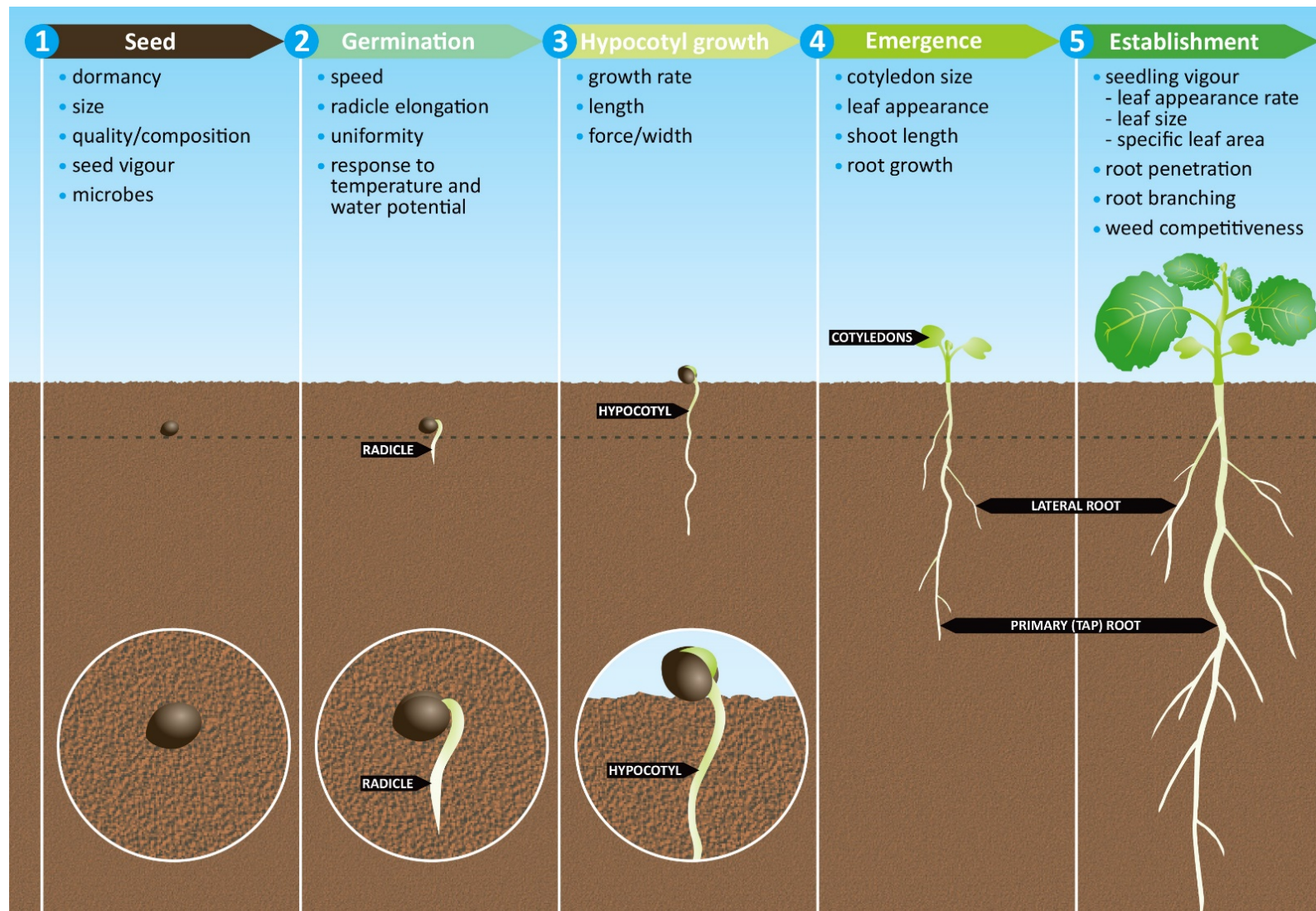
- Surveyed 63 growers & agronomists from the main canola growing areas





# Multiple factors in poor establishment





## KEY FINDINGS

- Unreliable canola establishment is a global problem
- Centrality of vigour
- Hypocotyl length / strength for deeper sowing

Nelson et al. (in preparation)

Figure credit: Carl Davies



# Fate of trial seeds in 2019



- 4 varieties x 4 sites in NSW
- Dug up 1.5m rows at establishment to determine fate of non-established seeds:
  - 62% seeds established
  - 10% ungerminated
  - 0% unemerged seedlings
  - 28% missing
- Difficult to see tiny black seeds in soil!





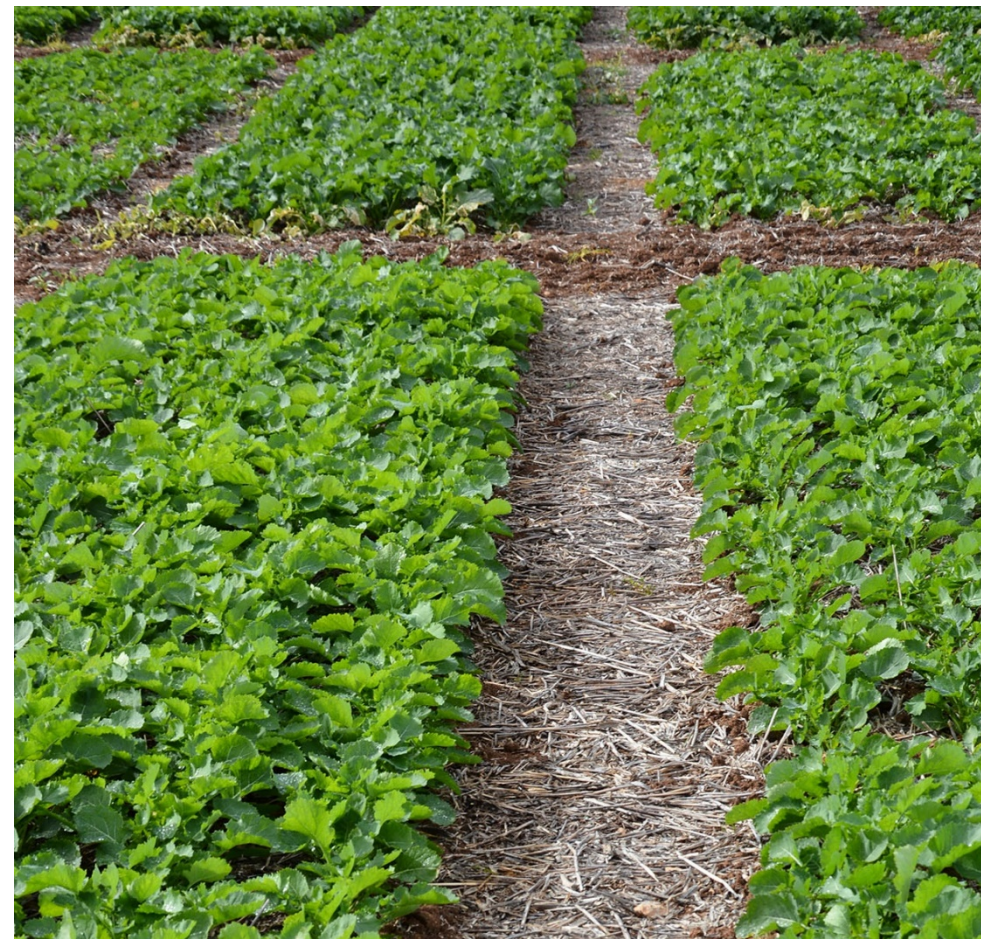
# Addressing the problem



# Our approach



- Identify genetic diversity for improving canola establishment
- Identify key contributing traits and develop high-throughput methods that we can use and pass onto breeders
- Understand the genetics
- Develop diagnostic markers for selection
- Benchmark the best germplasm against current varieties in field conditions





Addressing the problem:

Selecting a diversity panel

# Canola diversity panel



- 306 accessions from AGG, ASSYST and AAFC
- Seed multiplied in common garden expt. 2019
- Genotyped with 90K Infinium SNP array



# Selecting 100 accessions

- Spring, semi-winter, winter, swede, kale, fodder, winter veg, synthetic
- From 18 countries
- Each branch of diversity captured (90K SNP chip)
- Double low, single low, double high
- Range of germination and vigour behaviour under control, drought and heat treatments (published & unpublished work)
- 11 TT / 89 native cytoplasm
- Mapping population parents represented





Addressing the problem:

Focus traits

# Focus traits for HTP phenotyping

**Germination  
traits**

**Hypocotyl  
traits**

**Early  
vigour**

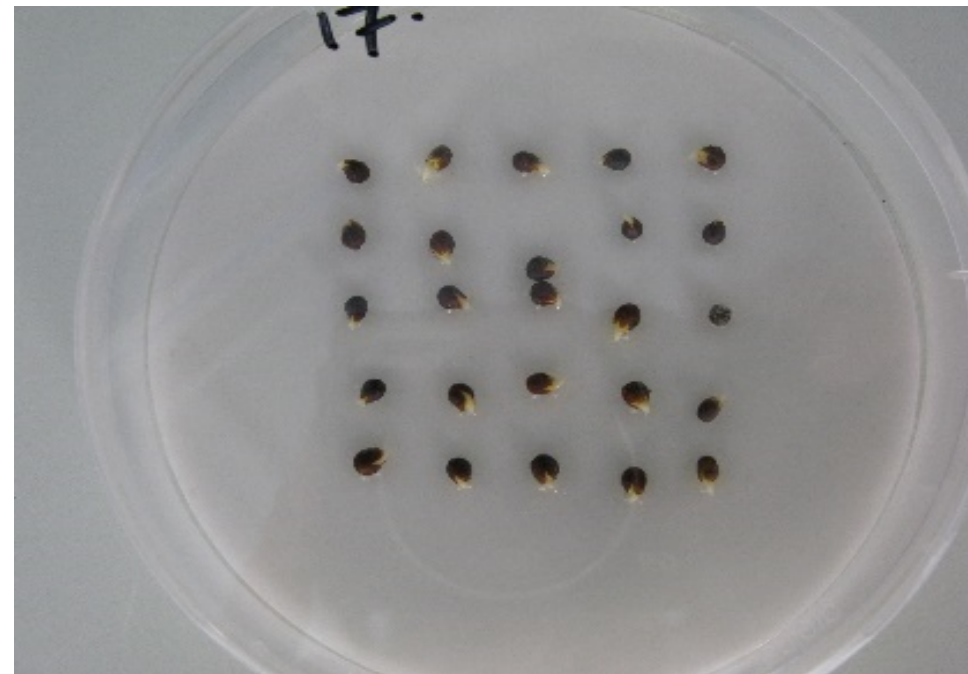


# Seed vigour (GI)



Germination  
traits

- 25 seeds x 3 reps x 100 accessions
- 10 °C in darkness
- Germination Index (GI) calculated by scoring each day for 7 days
- BLUPs calculated for Glsq

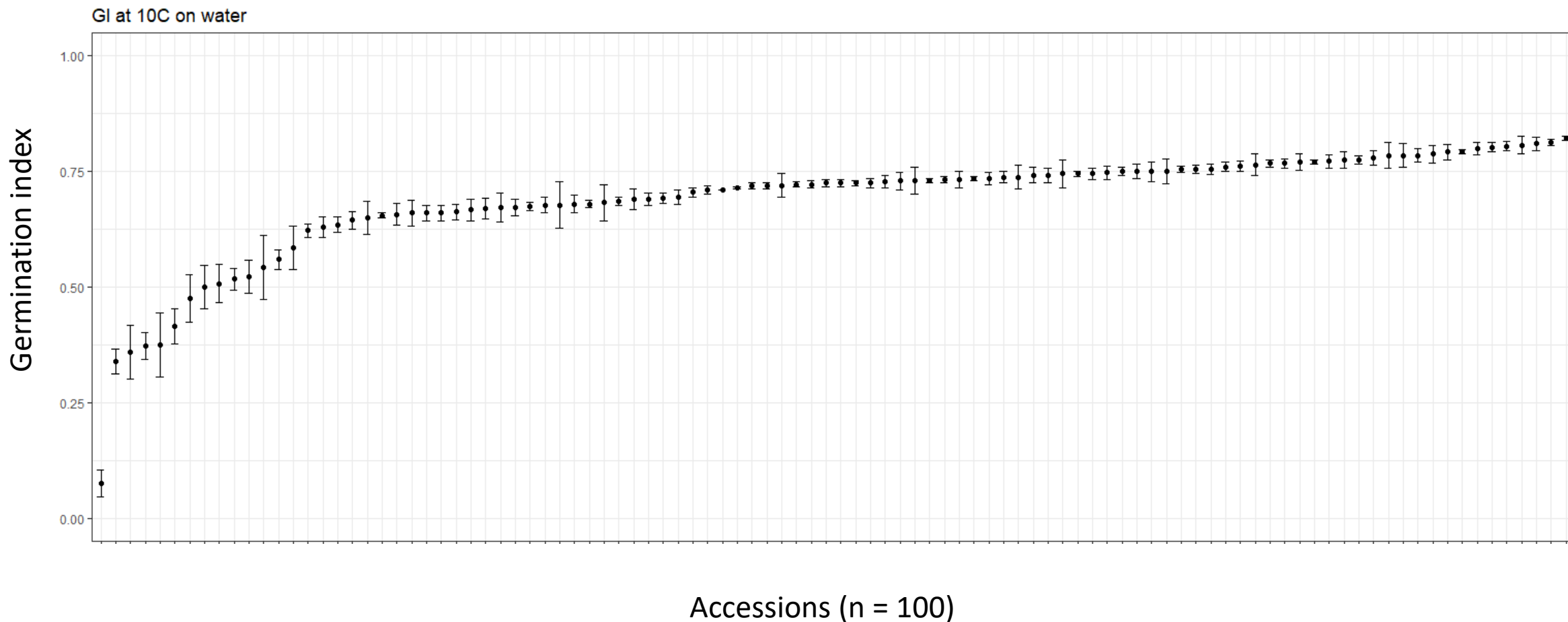




# Seed vigour (GI)



Germination traits



Heritability = 0.957



# Secondary dormancy



Germination traits

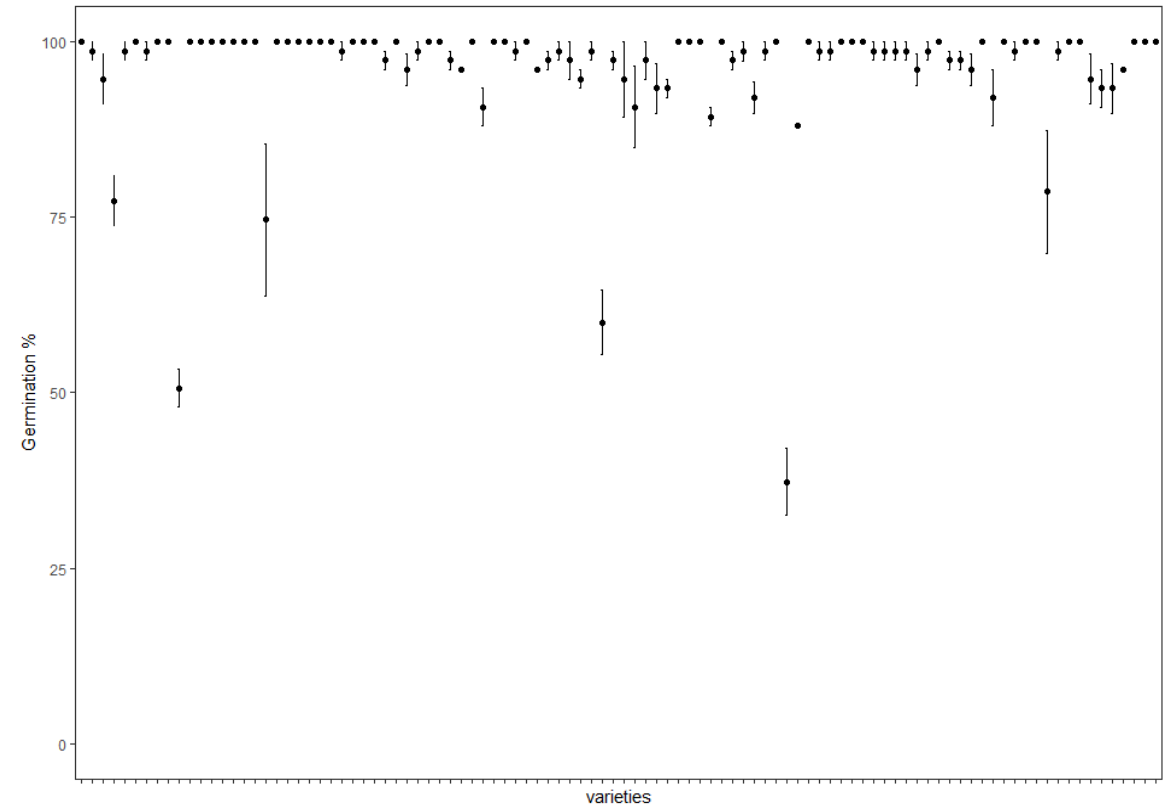
Induce dormancy by water deficit stress for 2 weeks (PEG6000 solution, -1.5MPa)



Transfer to water for 1 week



Score germination



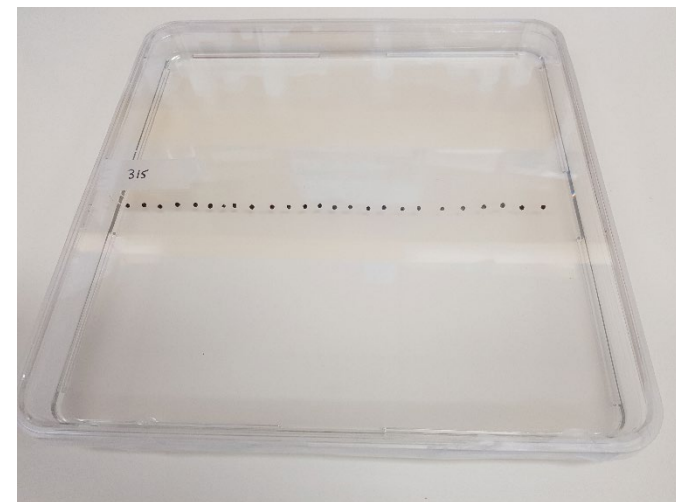
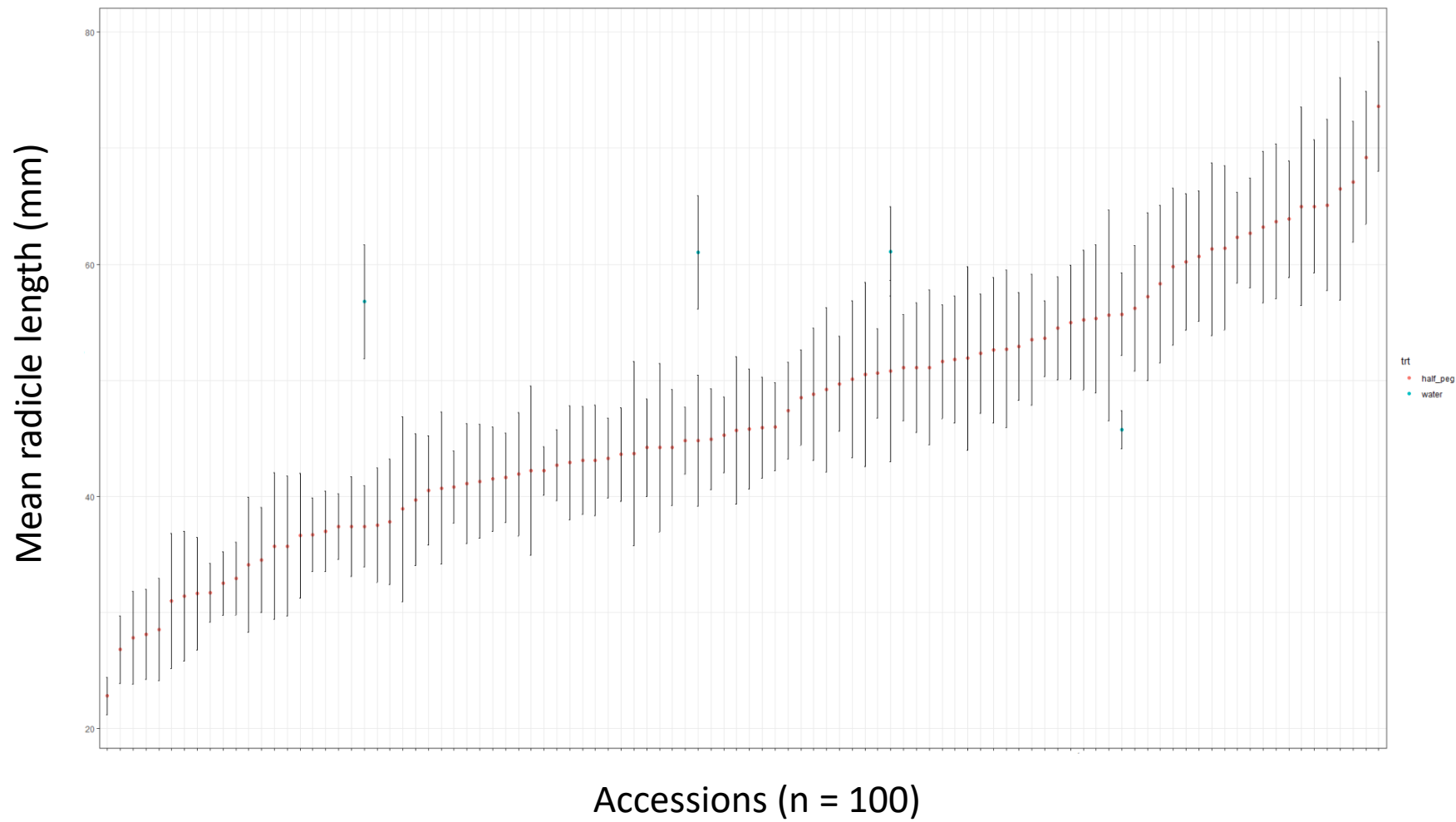
- 6 spring accessions with significant secondary dormancy
- Controls varieties only became available from AAFC recently
- Further testing in progress



# Radicle length



## Germination traits



# Focus traits for HTP phenotyping

**Germination  
traits**

**Hypocotyl  
traits**

**Early  
vigour**



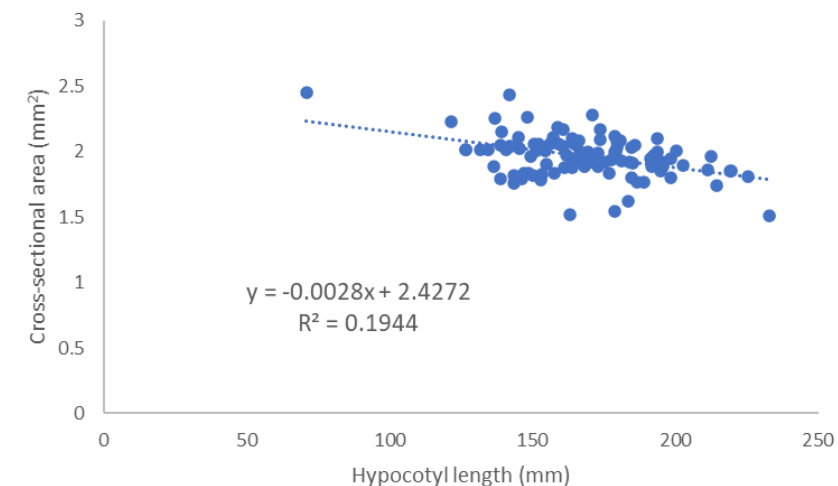
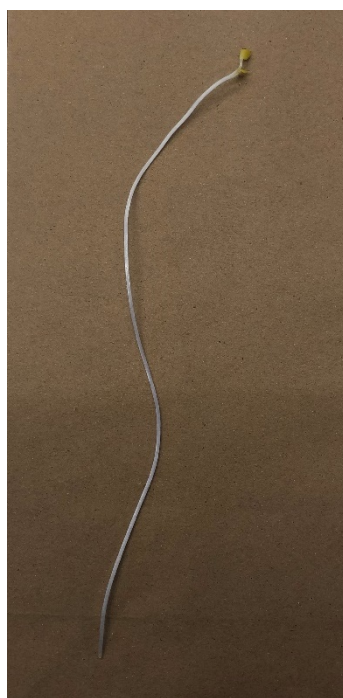


# Method development



## Hypocotyl traits

- Methods adapted from Greg's experience in long coleoptile wheat
- Seeds graded by size then individual seeds weighed
- Grown in dark at 16-18 °C until first true leaf (230 °Cd)
- Measure hypocotyl length and cross sectional area



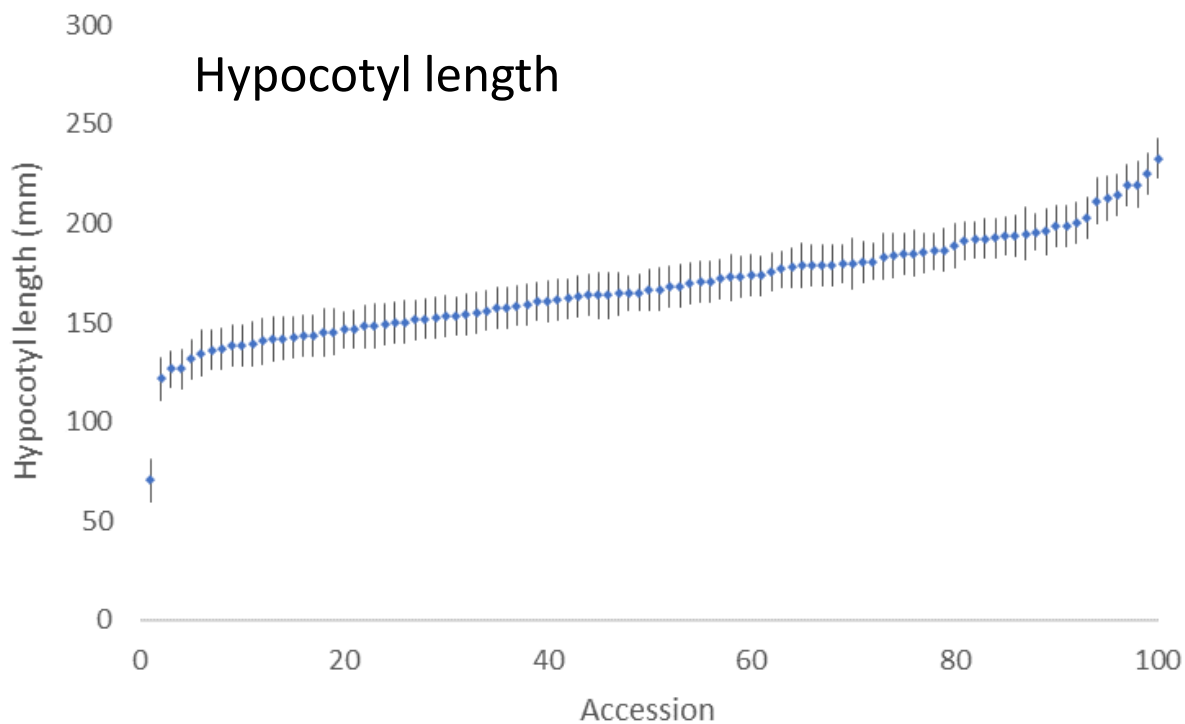


# Phenotyping 100 accessions

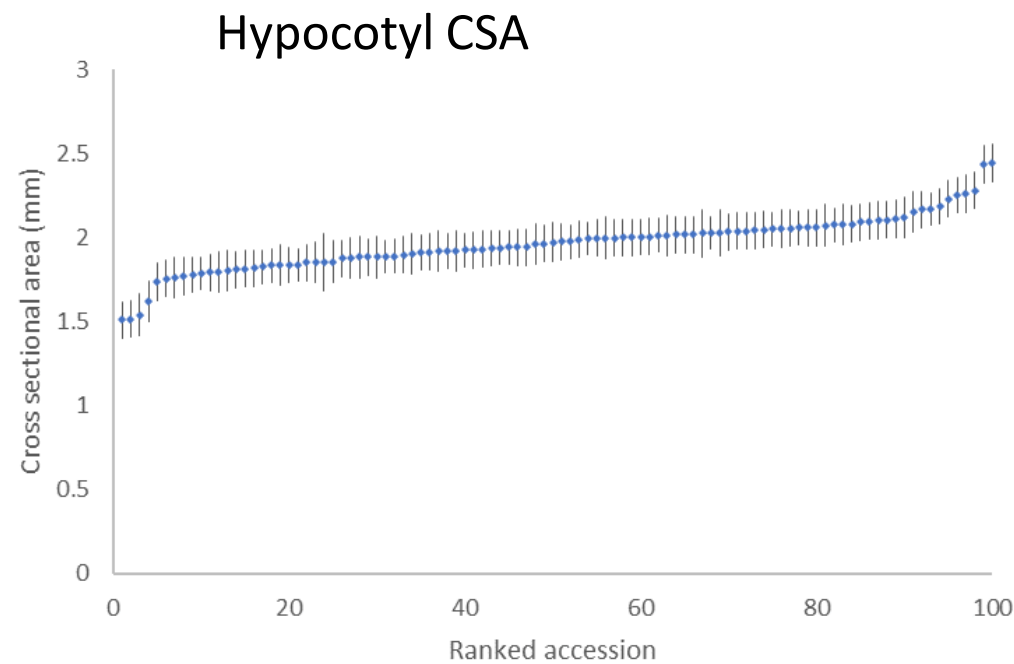


## Hypocotyl traits

- 100 accessions, p.rep (1.32x), spatially optimised design, 2 cabinets per run
- BLUPs calculated from two runs so far (5.28 reps)



Heritability = 0.85



Heritability = 0.50

# Focus traits for HTP phenotyping

**Germination  
traits**

**Hypocotyl  
traits**

**Early  
vigour**

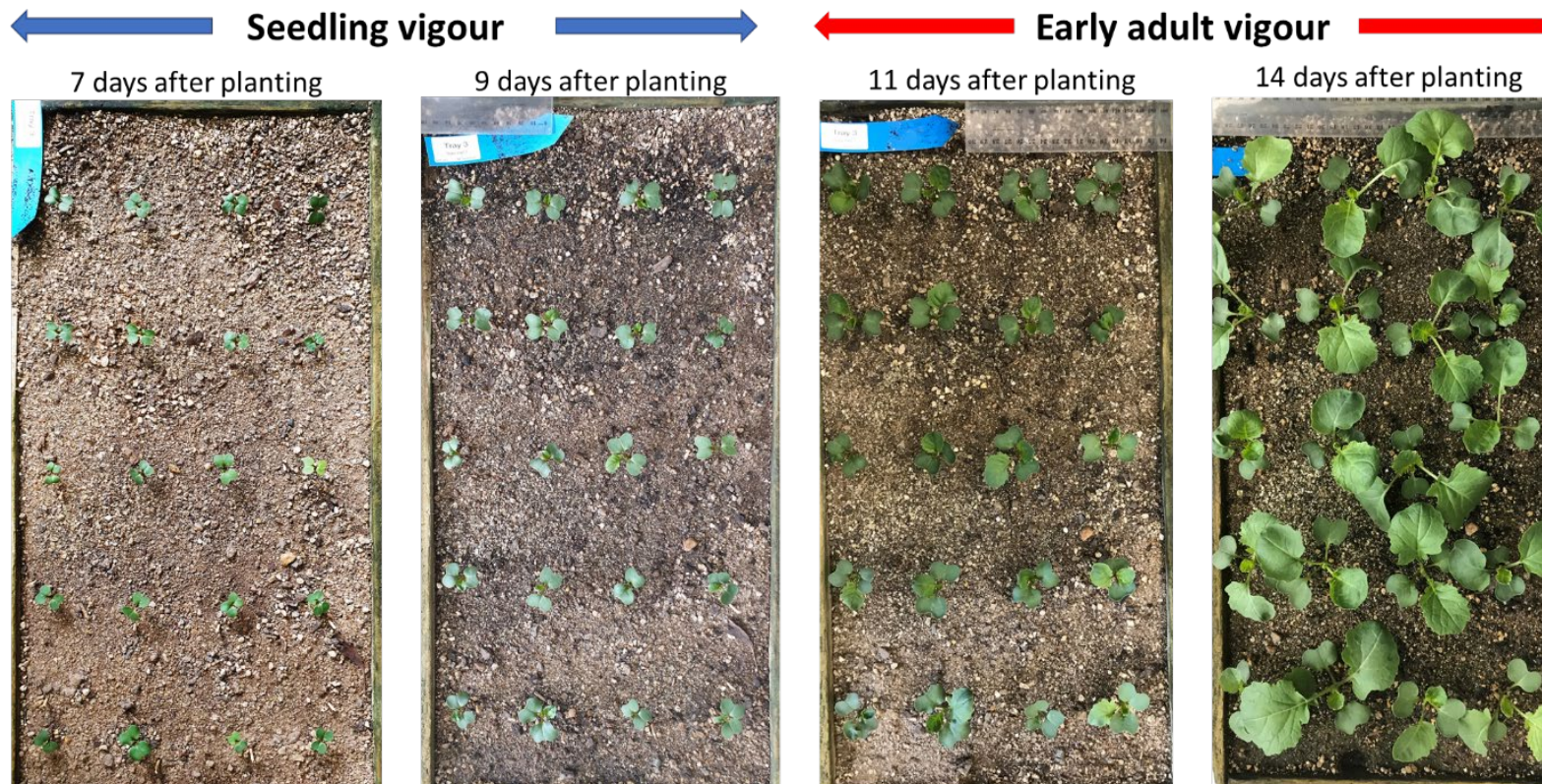


# Method development

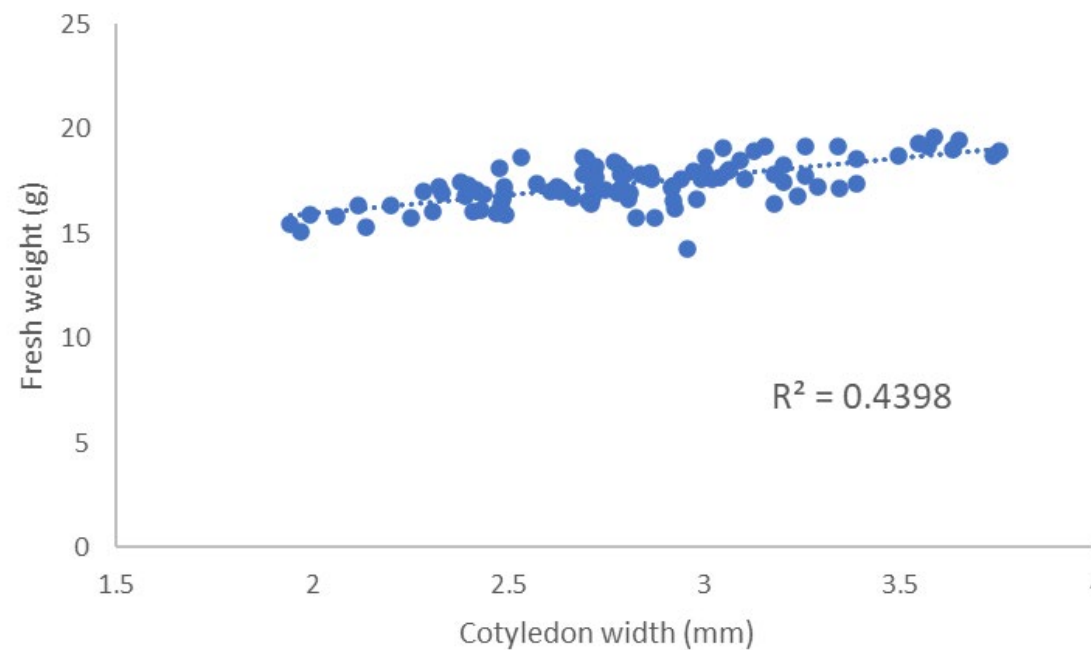


Early vigour

- Seeds graded by size then individual seeds weighed
- Grown on soil in trays (30 x 60 x 10cm) in 16 hr light 24°C / 8hr dark 15°C
- Several stages measured up to 3-leaf stage



- 100 accessions, p.rep (1.2x), spatially optimised design, 2 cabinets per run
- BLUPs calculated from two runs so (4.8 reps)



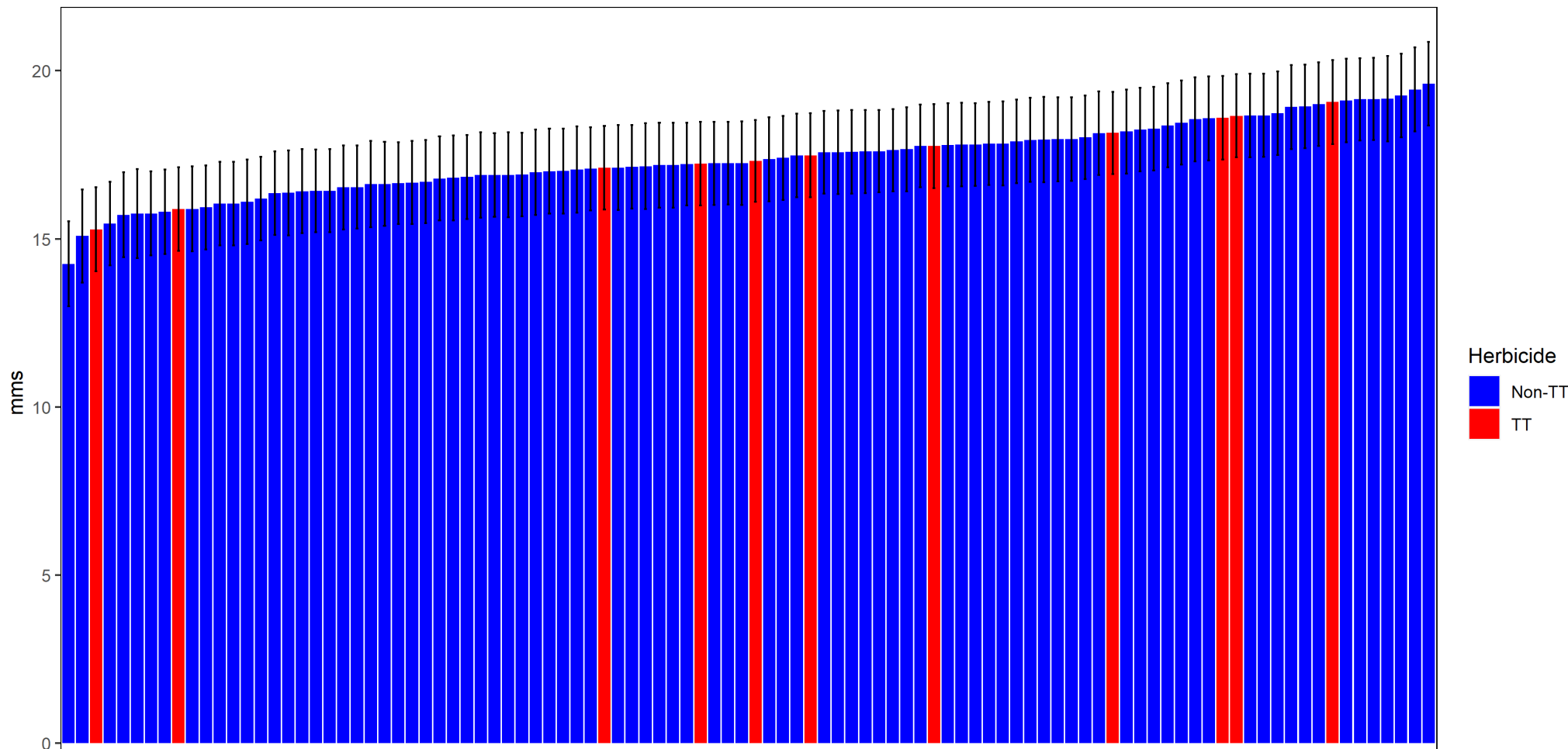
Cotyledon width @ 7 days (147 °Cd)



# Cotyledon width @ 147 °Cd



Early vigour



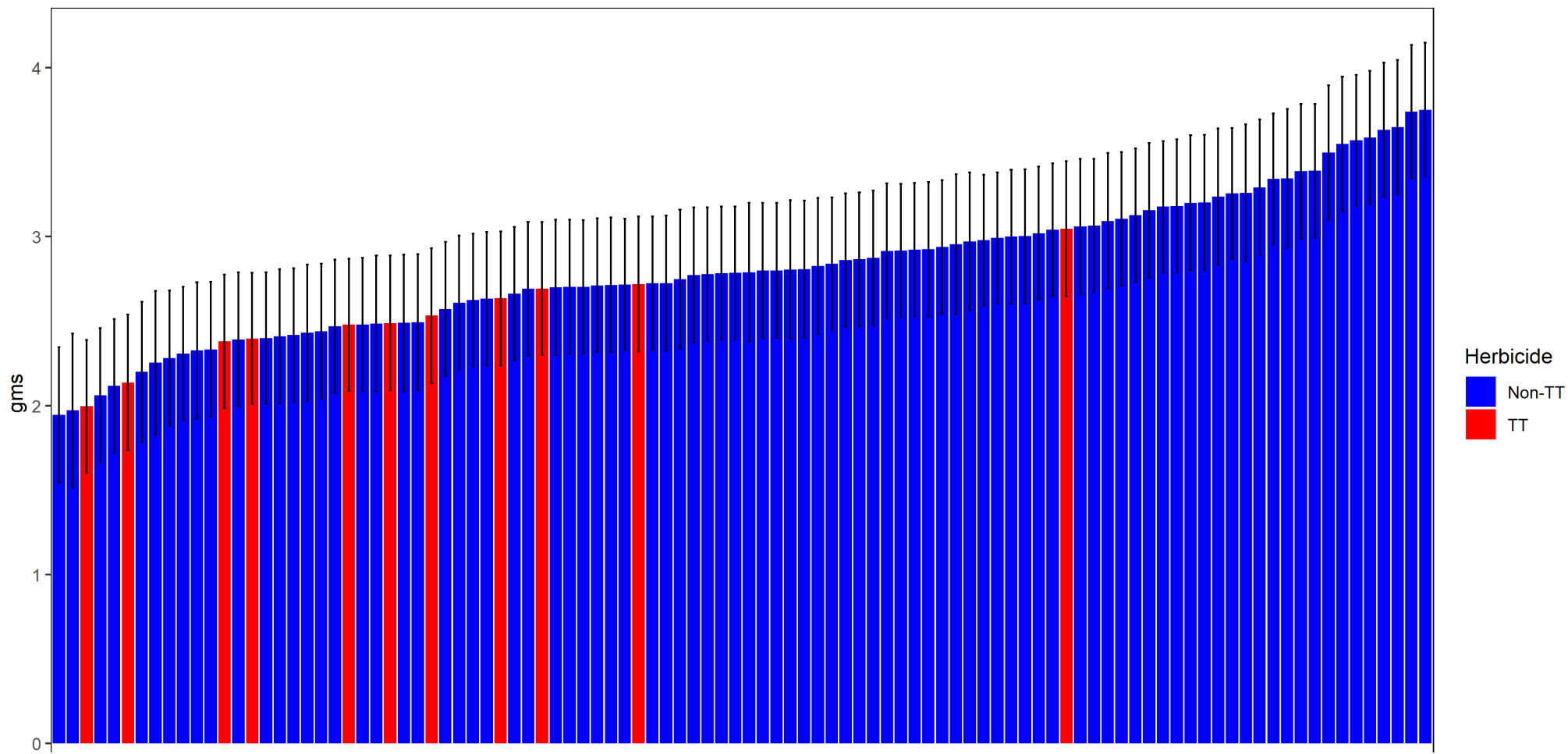
Heritability = 0.71



# Fresh weight @ 294 °Cd



Early vigour



Heritability = 0.78



Addressing the problem:

Field trials





# Ground-truthing lab-based phenotyping



- 20 extreme accessions selected
- Seed weight variation minimised within and among accessions



Accession	Type	Country of origin	TSW (g)
JIC_Samourai	winter	France	4
JIC_COUVENABICA	swede	Portugal	4.1
AGG_HAYA	spring	Japan	4.2
JIC_Tribune	spring	Australia	4.4
JIC_TANTAL	spring	France	4.5
JIC_MADRIGALxRECITALDHLNE	winter	GBR	4.5
AGG_6_06_3737	semi-winter	China	4.6
NUS_ZIRCON	spring	Australia	4.6
AGG_LIFURA	fodder	Germany	4.8
AGG_WILLI	spring	Denmark	4.8
AGG_RESTON	spring	Canada	4.8
JIC_KAROO-057DH	spring	Australia	4.9
AGG_QUINTA	winter	Germany	4.9
JIC_SURPASS400-024DH	spring	Australia	4.9
AGG_BC5TOWER	spring	Canada	5
JIC_NORIN	winter	Japan	5.2
JIC_Baltia	winter	USSR	5.3
JIC_ABUKUMANATANE	winter	Japan	5.6
JIC_SlovenskaKrajova	winter	Czechoslovakia	5.7
JIC_Capitol	winter	France	5.9

## Five commercial checks

Cultivar	Maturity	Type	Herbicide tol	Release date	Company
ATR-Bonito	Spring	OP	TT	2013	Nuseed
HyTTec Trident	Spring	Hybrid	TT	2019	Nuseed
InVigor R3520	Spring	Hybrid	RR	2019	BASF
InVigor T 4510	Spring	Hybrid	TT	2016	NPZA/BASF
SF Edimax CL	Winter	Hybrid	Clearfield	?	SeedForce



# Trial design, 2021



- 2 trials in WA (York & Cunderdin), 2 trials in NSW (Boorowa & Yanko)
- Spatially optimised, split-plot design with 2 sowing depths (20 & 50 mm)
- Score emergence and establishment rates, measure cotyledon width & fresh wt
- Forensic investigation of non-emerged seeds



**Background measurements:**  
Soil temperature (loggers)  
Soil moisture (loggers)  
Soil testing

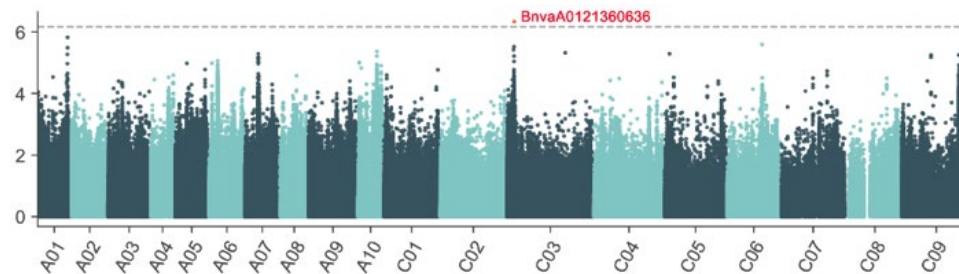


Addressing the problem:

Next steps

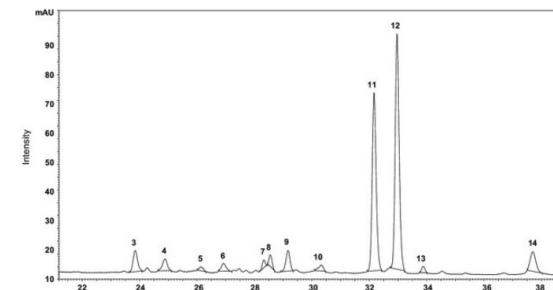
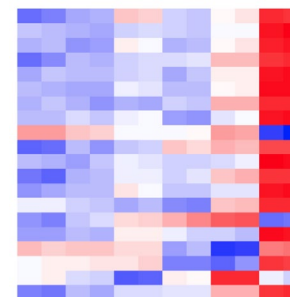
- With the full 100 accessions:

- Resequencing
- GWAS



- With the 20 extreme accessions:

- Detailed hormone analysis, 3 growth stages, 6 reps (ANU)
- RNAseq at 3 growth stages, 3 reps



- Phenotype company varieties / advanced breeding lines

Germination  
traits

Hypocotyl  
traits

Early  
vigour

## High-throughput phenotyping methods (2021)

Germination  
traits

Hypocotyl  
traits

Early  
vigour

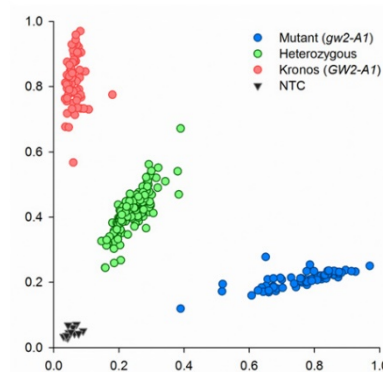
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<b>A</b>	<b>AA</b>	<b>Aa</b>
<b>a</b>	<b>Aa</b>	<b>aa</b>

Understand the genetics of establishment-related traits (2022)



Superior germplasm benchmarked against the best elite cultivars (2023)

## Develop diagnostic markers (2022)



Collaboration in establishment trials?

Understand your varieties relative to international germplasm  
\*\*\* STILL TIME TO CONTRIBUTE VARIETIES! \*\*\*



BASF, NPZ Australia, NuSeed and SeedForce for contributing varieties  
Francis Ogbonnaya and Justine Morgan, GRDC

