‘Overgrowth’ mutants of wheat: many new alleles at the ‘Green Revolution’ dwarfing locus

Isolation of overgrowth mutants in barley

Overgrowth mutants in wheat

Applications in wheat breeding

The ‘Green Revolution’ resulted from *semidwarf* varieties:

- better yield (less assimilate goes to stem growth)
- reduced lodging, especially under high input conditions

The *semidwarfing* genes involve the hormone gibberellin (GA)

Crop	Semidwarfing gene(s)	Function
Rice	*sd1 (GA20ox2)*	GA biosynthesis
Wheat	*Rht1,2 (Rht-B1b, Rht-D1b)*	GA signalling (DELLA proteins)

Sln1 mutants of barley

sln1c *WT* *Sln1d*

Slender1 is the principal DELLA gene of barley (orthologous to *Rht-1* of wheat)

'*Elongated slender*' and '*dwarf slender*' differ from *WT* by single nucleotide changes

DELLA proteins:

- function as growth repressors
- involved in GA signalling (DELLA degradation)

Many dwarf mutants:

- GA signalling (GA receptor, DELLA)
- GA biosynthesis (5 loci)
- Are they of any use in breeding?

Mutagenise dwarf lines, and select for phenotypic reversion

Dwarf mutant → **Tall(er) lines**

- Novel genes/alleles involved in growth regulation
- Phenotypes closer to *WT* than in a typical ‘forward’ screen

Search for ‘overgrowth’ mutants

Three different starting lines:

- dwarf mutants (GA biosynthesis; GA receptor; DELLA)
- mutations defined at nucleotide level
- M_1 grains sown in the field, M_2 grains harvested, screened in flats

Plus elongated “slender” types

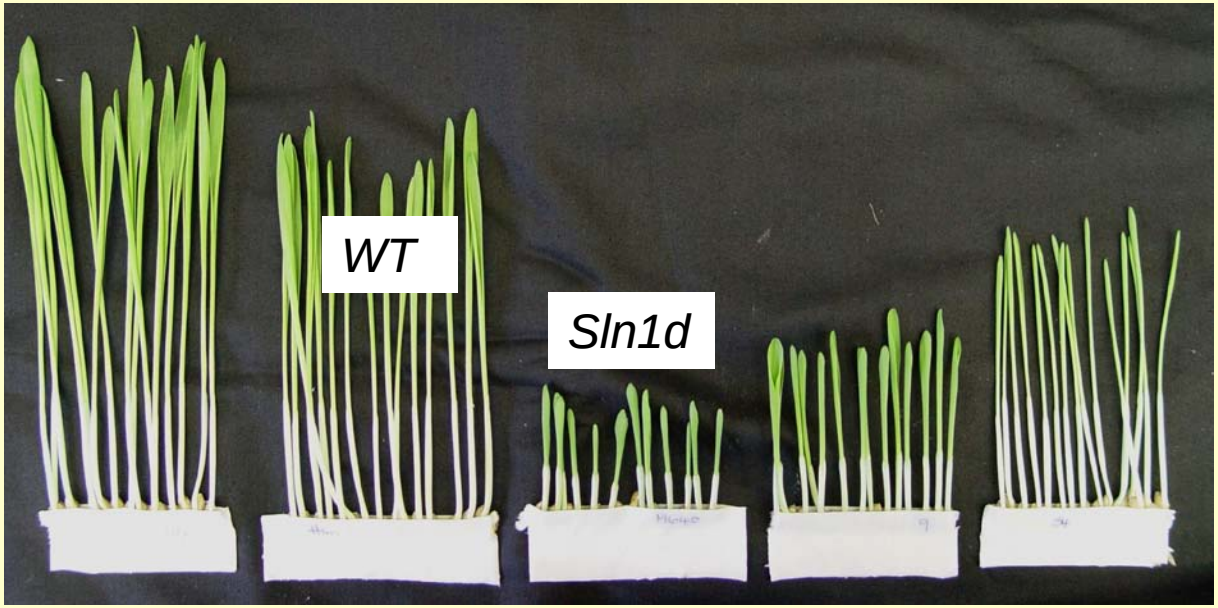
Recovered 13 independent mutants

Properties:

Partial to full (= *WT*) recovery of growth

All retain the original dwarfing allele (not contaminants!)

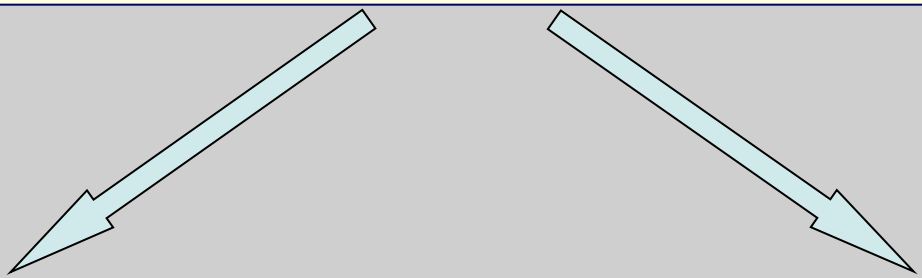
Plants are self-fertile and phenotype is stable



Most overgrowth mutants are altered in the *Sln1* gene

11/13: new mutations in *Sln1* ORF (three different dwarfing backgrounds)

2/13: no new mutation in *Sln1* ORF (TR26, TR103)



Unlinked to *Sln1*
Mutation in *Spy1*

100% linkage to *Sln1*
(promoter mutation?)

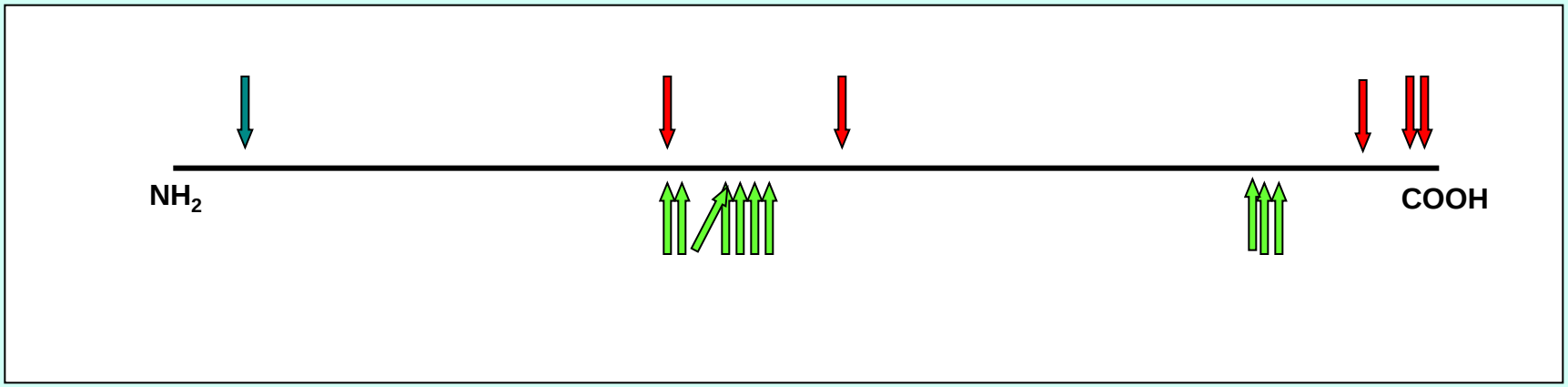
Overgrowth mutations → enhanced GA signalling

Mutations in the SLN1 protein

dwarf
slender

elongated
slender,
sterile

overgrowth
slender



Two regions of the SLN1 protein involved in *ovg* phenotype

Overgrowth mutants in wheat

Wheat — challenging for mutation studies:

3 genomes, so redundancy of many genes

Rht1, *Rht2*: DELLA mutants

insufficiently dwarfed for a good screen

Rht3 is a more severe DELLA dwarfing allele

nature of mutation unknown

- a ‘gain of function diploid’?
  Isolate ‘loss of function’ derivative alleles

rht *Rht-B1c (Rht3)*

Maringa BC6 NILS

Grains of Maringa *Rht-B1c* treated with sodium azide, sown in the field, and M_2 generation screened in flats

Overgrowth mutants in wheat

Screening: flats (2008); mature plants in field (2009)

≈ 400 mutants, from about 1 x 10^5 M_1 spikes

≈ 200 are derivatives of *Rht-B1c*

≈ 200 have probable deletions of the *Rht-B1* gene

Rht3 *rht* *ovg* (3 mutants)

Height variation in *ovg* mutants

>50% are intermediate in height between *rht* and *Rht3*

Some variation in:

- leaf morphology
- tillering
- anthesis date
- ear morphology
- grain properties

Molecular nature of '*Rht3*' (=*Rht-B1c*) mutation

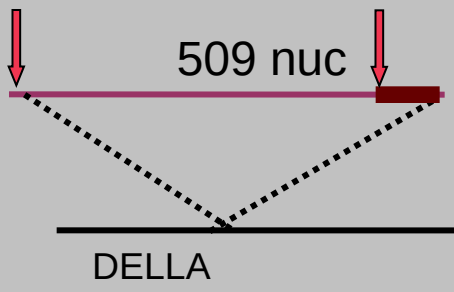
Collaborative studies with Stephen Pearce, Steve Thomas, Peter Hedden at Rothamsted Research, UK.

RT-PCR results of Stephen Pearce:

- predicted mRNA with 90 nucleotide (30 aa) insertion,

Genomic DNA PCR studies:

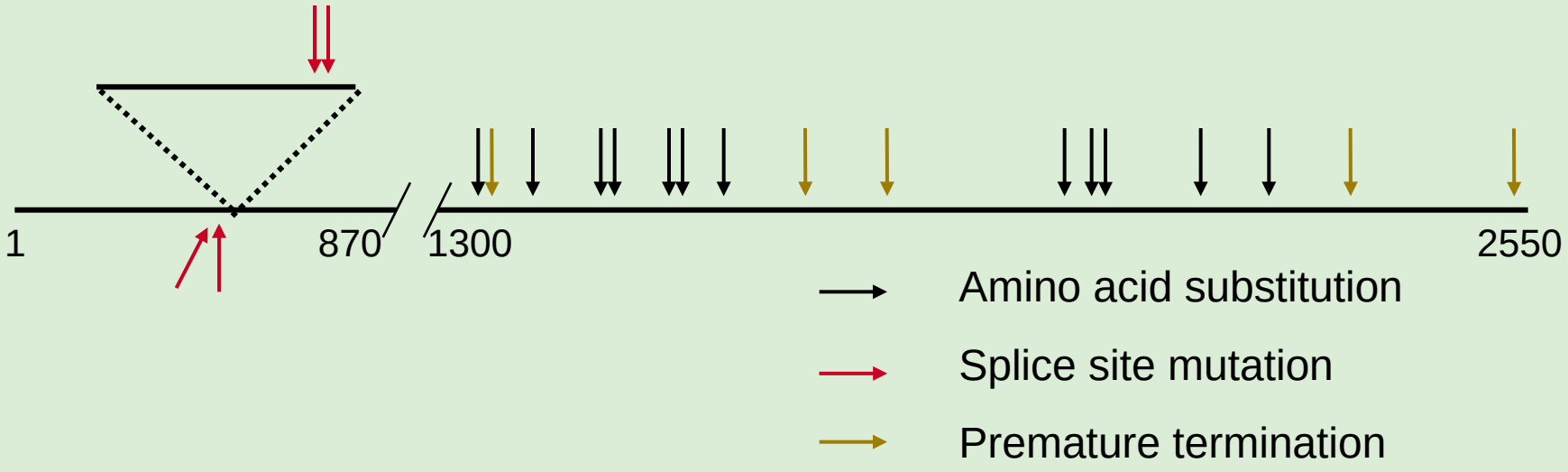
- predicted a much larger insertion



Most of insert excised as an intron,
but 90 nucleotides at 3' end remain

DNA sequencing studies (≈ 50 mutants)

98-99% nucleotide sequence identity between the A, B, D genes in 3' half



Estimate 50-75 new *Rht-B1* alleles will be present in our collection – all derived from *Rht-B1c* in the dwarf parent

Question: Why so many new alleles?

Answer: Exploit allelic variation in GA responses

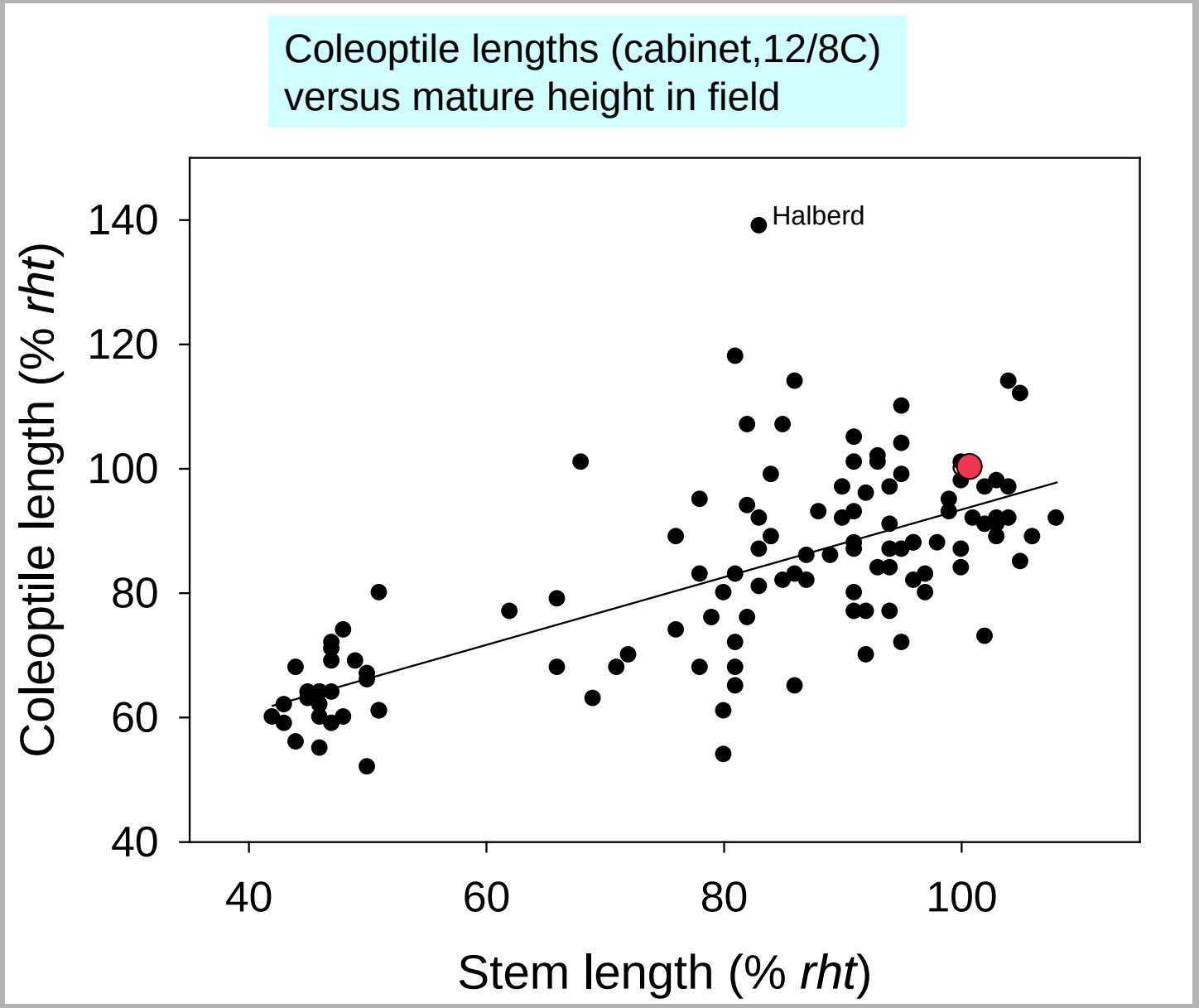
'GA traits' of interest to breeders & physiologists:

- germination vigour, early establishment
- changes in time to floral initiation, length of pre-anthesis spike development, rate and duration of grain growth
- mature plant height
- grain quality traits (low α-amylase content/high dormancy)

Barley mutants – a degree of independence between traits

– not just volume control!

Allele specificity of wheat overgrowth mutants?



Ongoing studies in wheat

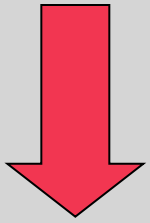
Complete the identification of all new mutant alleles

'Proof-of-concept' crosses with selected alleles

Field-based assessment of emergence, anthesis, height, HI, yield

Accurate phenotyping:

- early vigour/coleoptile length (well-watered, water-limited)
- grain dormancy (6 weeks difference *Rht-B1a* vs *Rht-B1c*)
- other traits? (altered leaf architecture, etc.)



For breeders:

New alleles at *Rht-B1* to replace *Rht1* and *Rht2*

- greater flexibility in degree of dwarfing
- improved early vigour and/or grain quality, other??
- better suited to conservation agriculture
- perfect molecular markers
- non-transgenic

For hormone biologists:

- many new alleles in an important “GA gene”
- share a common genetic background

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