Identification of the long sought-after *Ph2* gene, a step towards the control of homoeologous recombination in wheat

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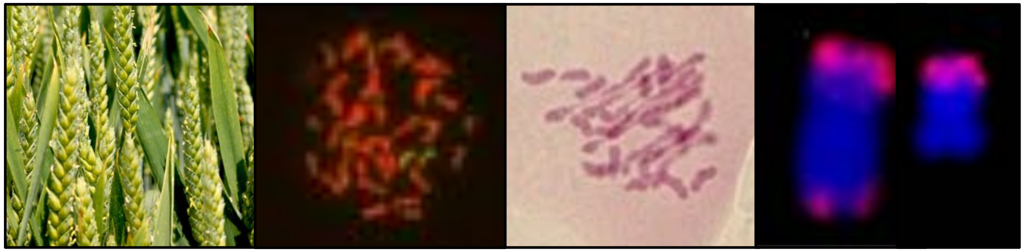

17th June 2021

Identification of the long sought-after *Ph2* gene, a step towards the control of homoeologous recombination in wheat

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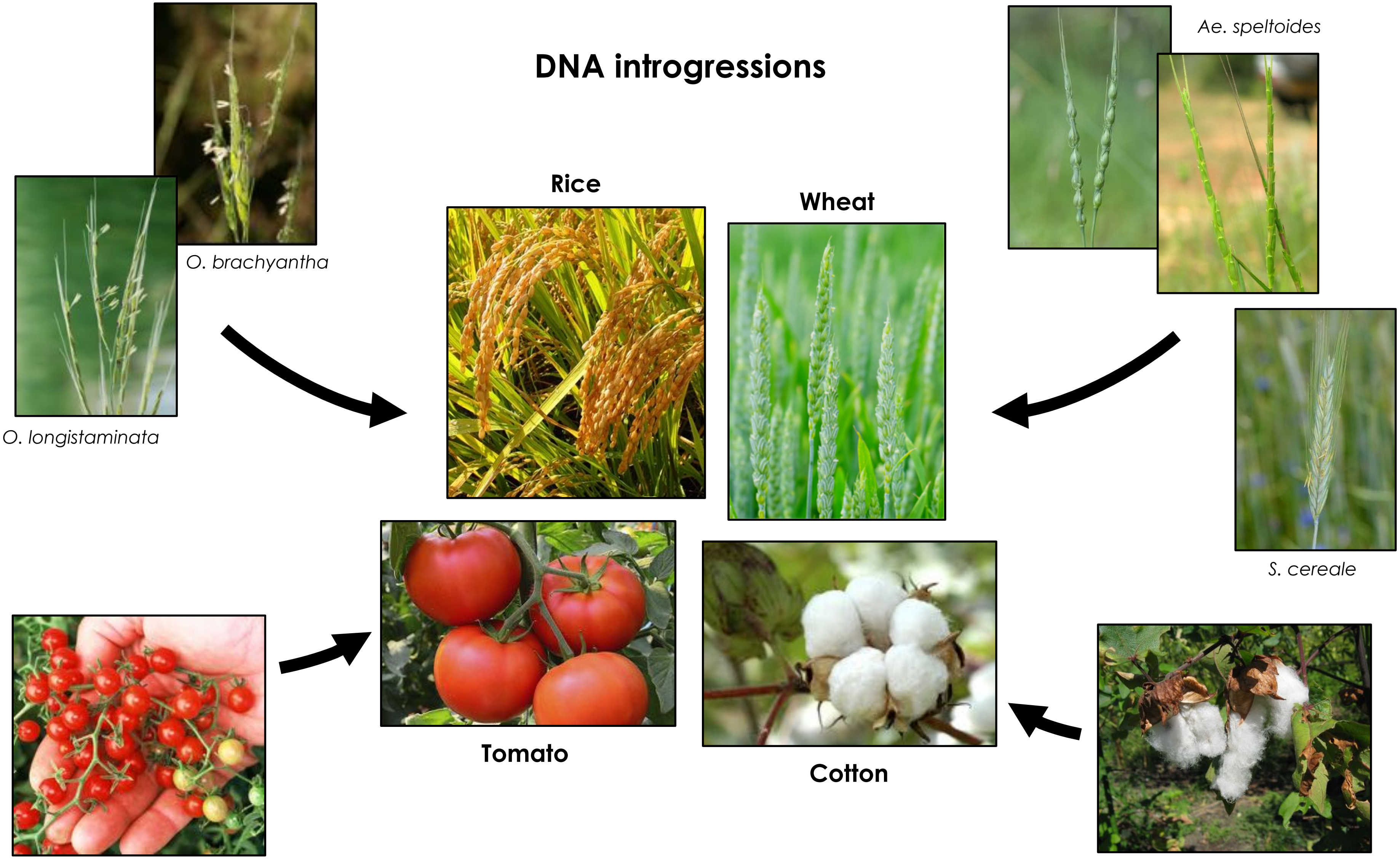
U. Baumann

THE UNIVERSITY of ADELAIDE
SUB CRUCE LUMEN

Relative species, a source of genetic diversity

DNA introgressions



O. brachyantha

O. longistaminata

Rice

Wheat

Ae. ventricosa

Ae. speltoides

S. cereale

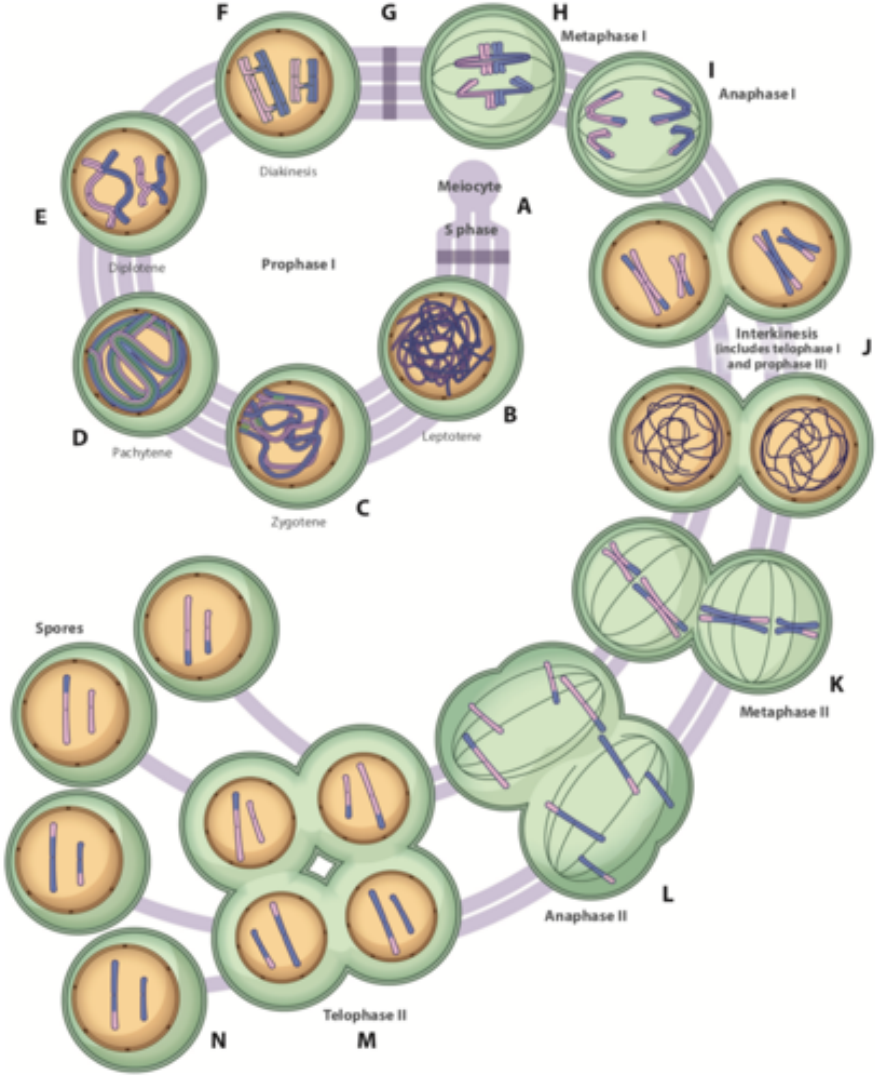
Tomato

S. pimpinellifolium

Cotton

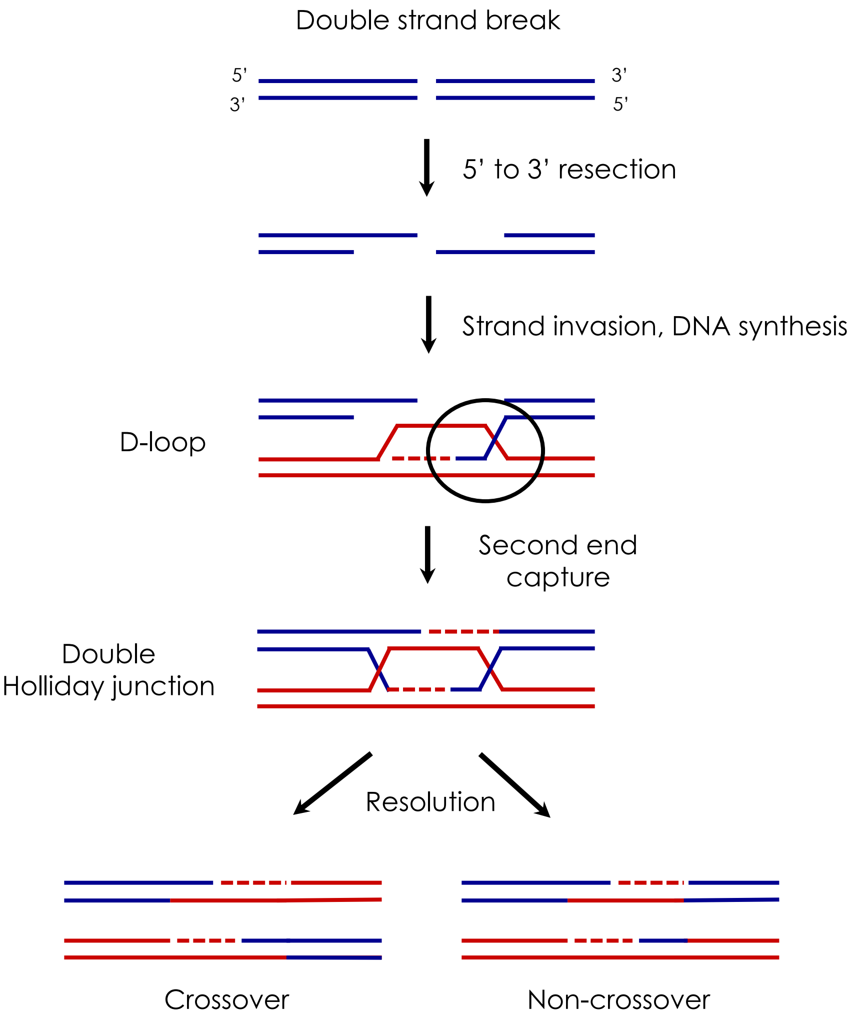
G. anomalum

Introgression relies on meiotic recombination



Mercier et al, 2018

Mechanisms of meiotic recombination



Double strand break

5’ 3’ 3’ 5’

5’ to 3’ resection

Strand invasion, DNA synthesis

D-loop

Second end capture

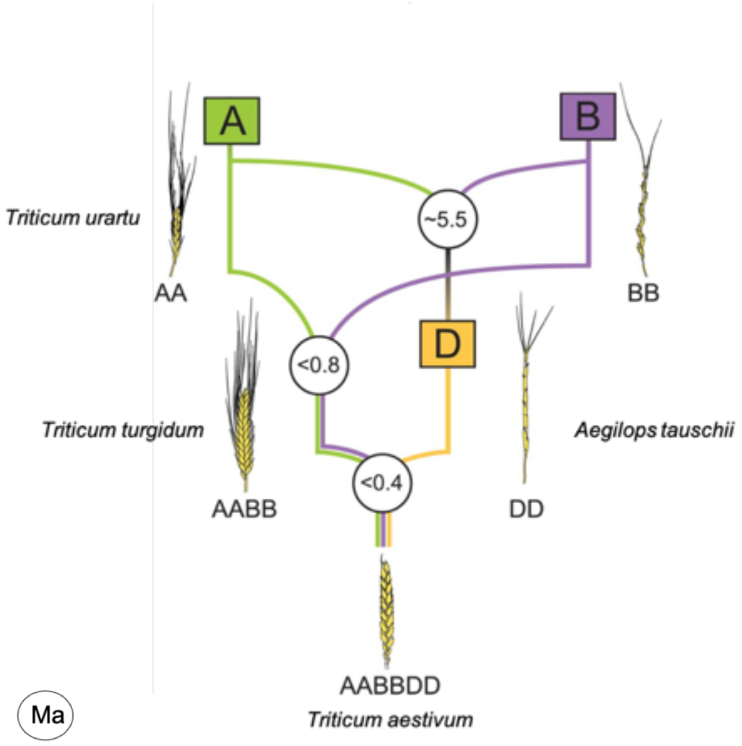
Double Holliday junction

Resolution

Crossover

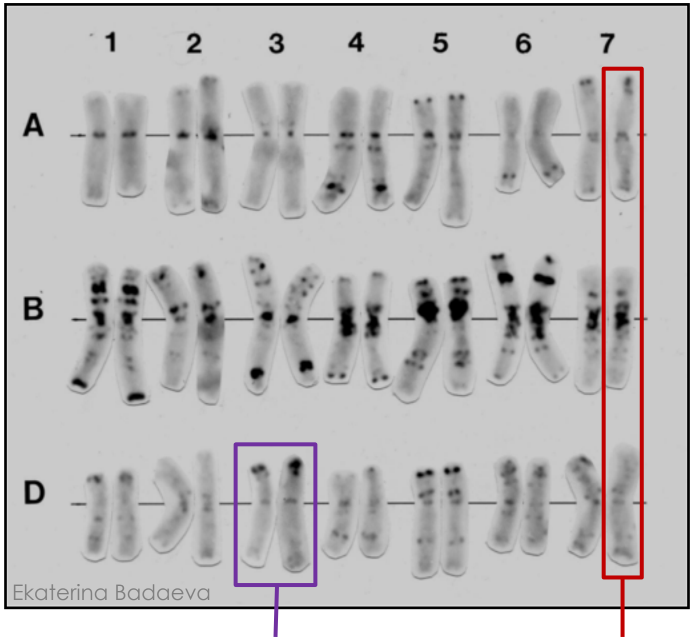
Non-crossover

Allopolyploid genome of bread wheat



Marcussen *et al*, 2014

Triticum aestivum AA BB DD
2n = 6x = 42 chromosomes

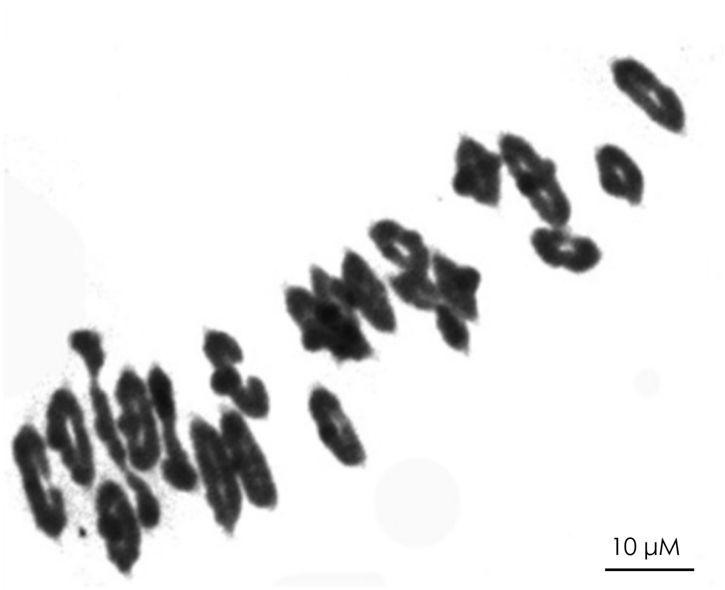


Homologous chromosomes

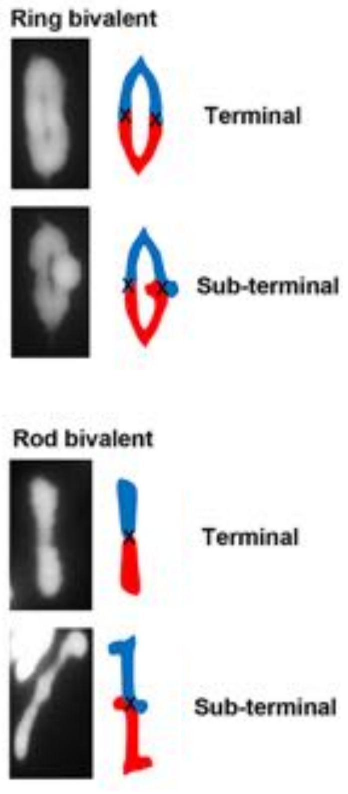
Homo**e**ologous chromosomes

Homoeologous recombination is inhibited during meiosis

Chromosome configurations at meiotic metaphase I



42 chromosomes,
21 bivalents



Wang *et al*, 2020; Osman *et al*, 2021

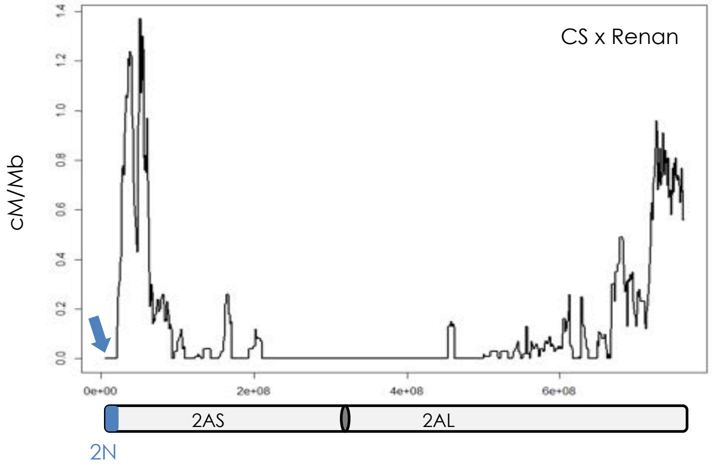
Unlocking homoeologous recombination to facilitate introgression

S. cereale

Ae. speltoides

Ae. ventricosa

Wheat



Introgression

Genes of interest
+
Potentially
deleterious genes

Linkage drag

Control of homoeologous recombination

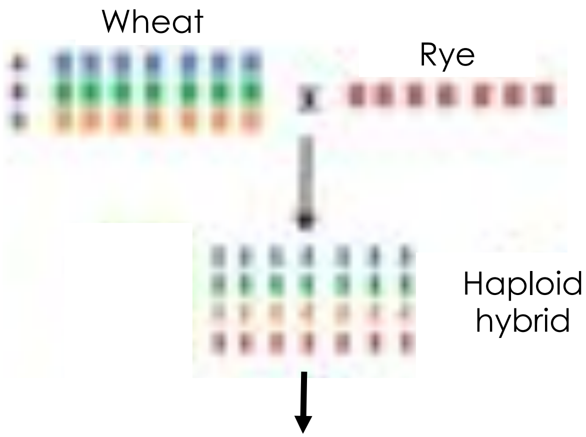
- One major locus: ***Ph1*** *(Pairing homoeologous 1)*
  - On chromosome 5BL (Riley & Chapman, 1958)
  - Cloning (Griffiths et al, 2006)
    - Heterochromatic block (3B)
    - ***TaZIP4-B2***
    - Complex of 4 *CDK2-L*
  - Mode of action (Martin et al, 2014)
    - Promotes homologous pairing
    - Prevents homoeologous recombination intermediates to become crossovers

Ph1+

Ph1-

Control of homoeologous recombination

- Locus ***Ph2*** *(Pairing homoeologous 2)*

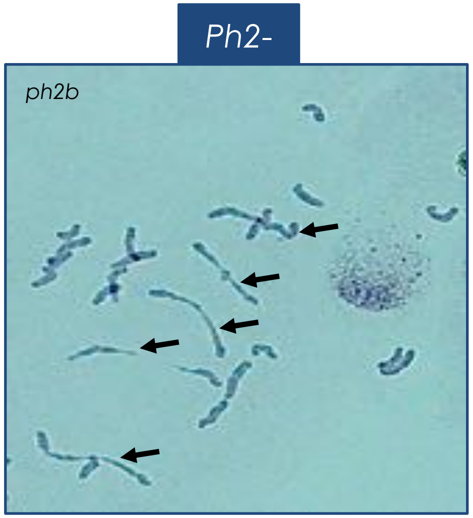


Metaphase I

28 univalents

Ph2+

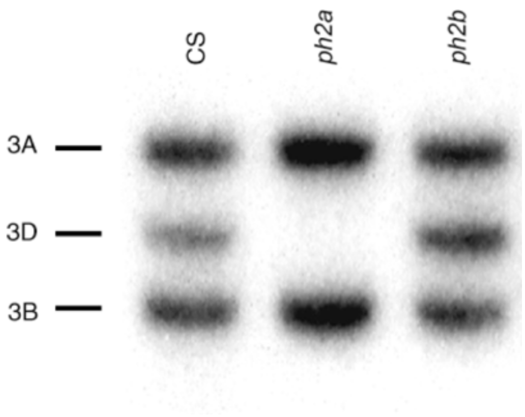
Ph2-



Control of homoeologous recombination

- Locus ***Ph2*** *(Pairing homoeologous 2)*
  - On chromosome 3DS (Mello-Sampayo et al, 1968)
  - Two mutants:
    - Irradiation mutant ***ph2a*** (Sears et al, 1982)

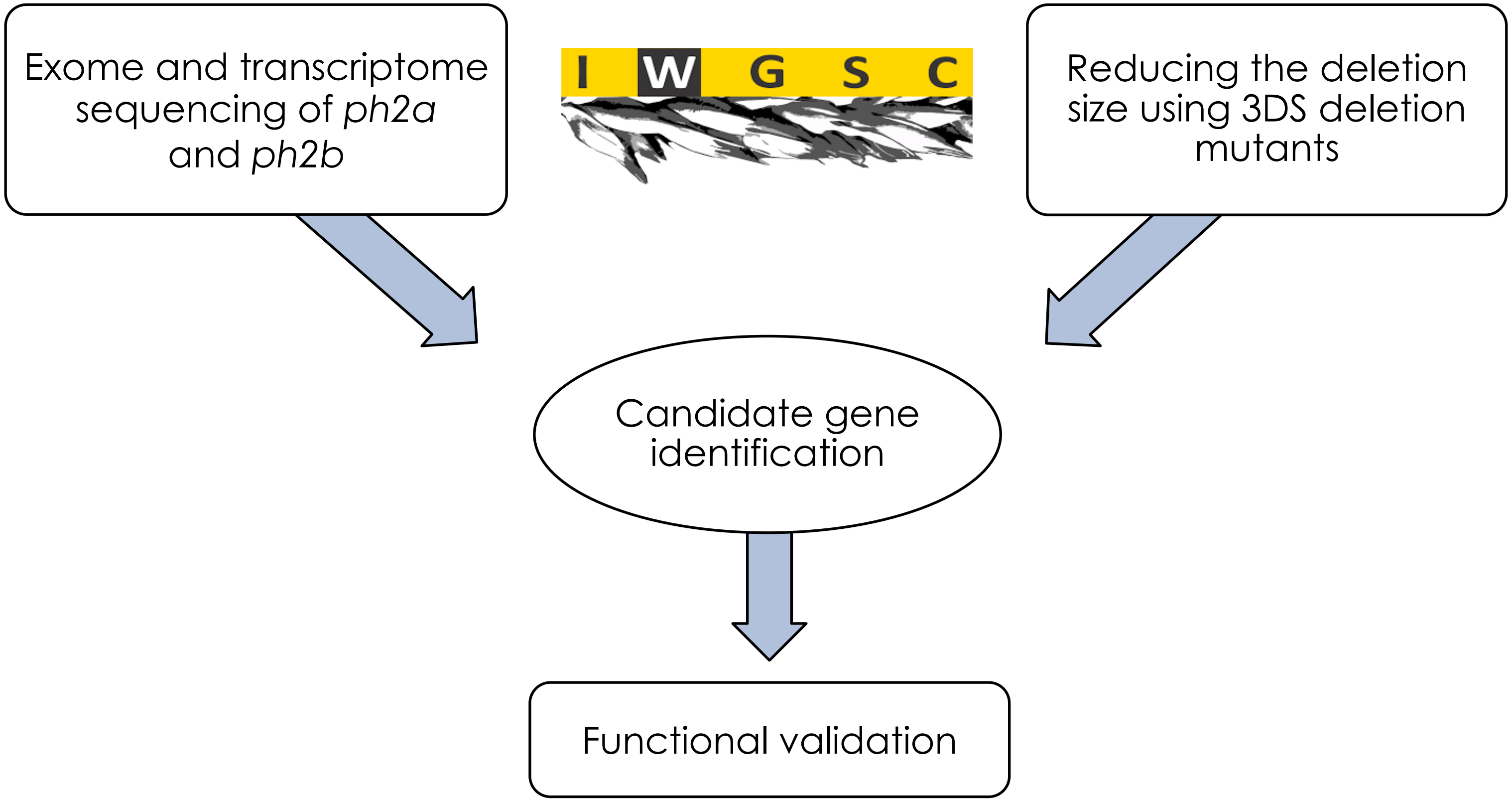
      Terminal deletion of ~80 Mb (Sutton et al, 2003)
    - EMS mutant ***ph2b*** (Wall et al, 1971)
  - Of particular interest:
    - Minimal disruption of endogenous homologous rec
    - Reinforces *ph1b*'s effect of promoting homoeologous rec
  - Candidate genes: WM1 gene family, WM3, WM5, *TaMSH7-3D*



Southern blot, probe on 3DS

Sutton et al, 2003

Positional cloning of *Ph2* : our strategy



Exome and transcriptome sequencing of *ph2a* and *ph2b*

IWGSC

Reducing the deletion size using 3DS deletion mutants

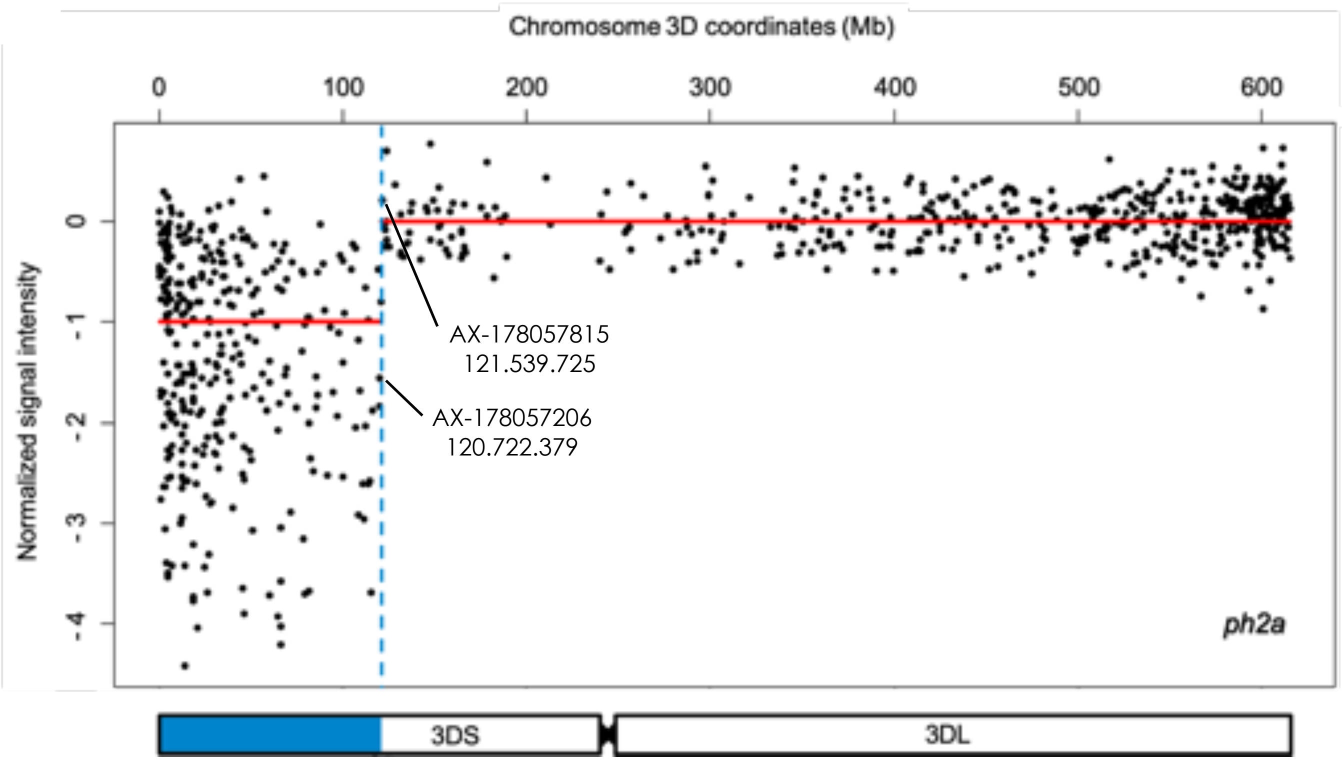
Candidate gene identification

Functional validation

Identification of *ph2a* deletion breakpoint

High-density SNP genotyping array

(35 K SNP Affymetrix Axiom®)

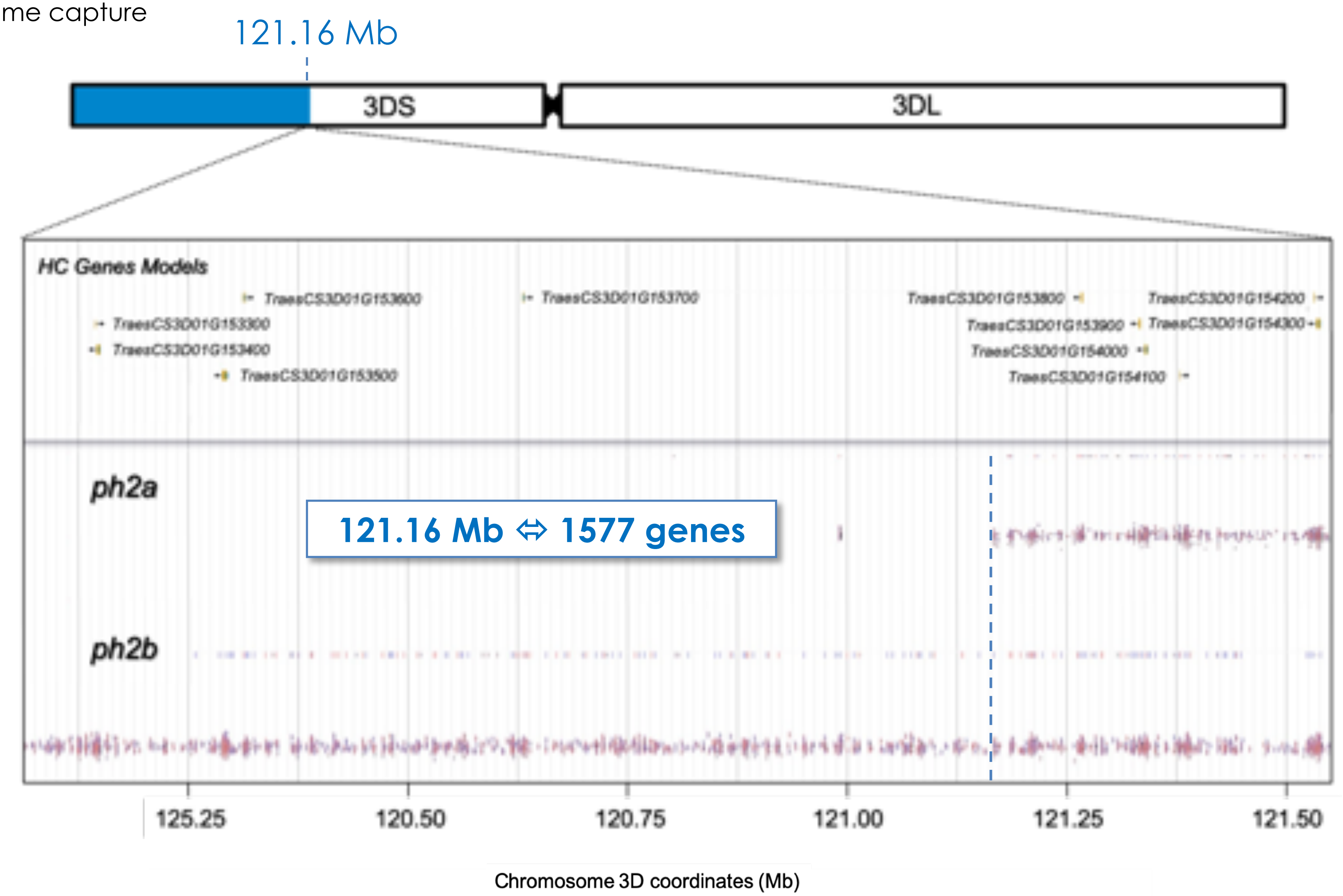


AX-178057815
121.539.725

AX-178057206
120.722.379

Identification of *ph2a* deletion breakpoint

Exome capture



121.16 Mb ⇔ 1577 genes

Identification of mutations in *ph2b*

Exome capture of EMS induced *ph2b* mutant vs Chinese Spring reference genome:

165 single nucleotide differences within *ph2a* deletion region

(mainly G-A and C-T transitions)

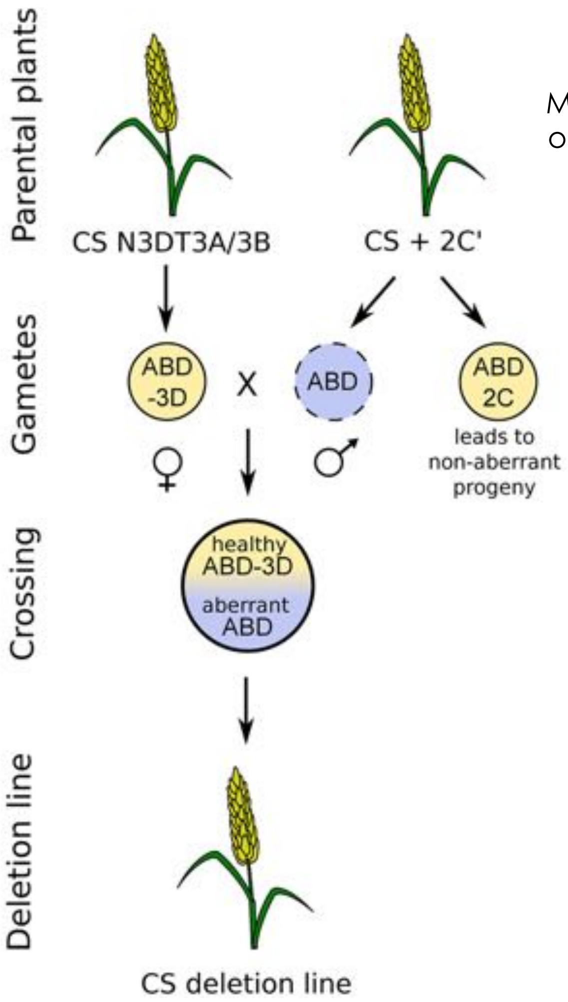
59 SNPs within genic regions (CDS, 5’ & 3’ UTR, promotor regions)

36 exonic mutations + **1** mutation affecting **splicing junction**

- 13 synonymous
- 21 non-synonymous
- 2 non-sense

***Ph2* candidate genes: 1577 => 24 genes**

Production of 3D deletion lines



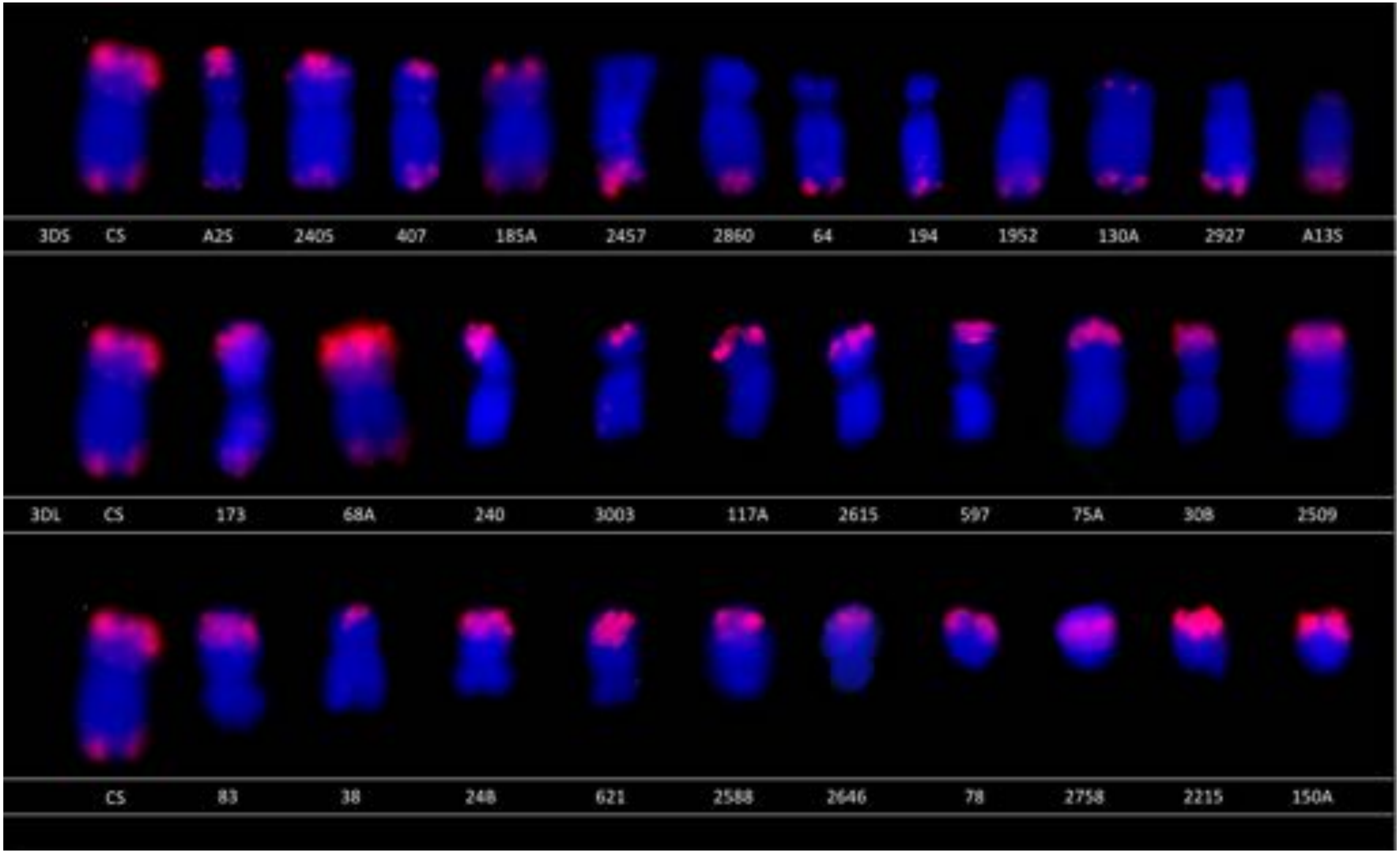
Monosomic addition line
of chromosome 2C from
Aegilops cylindrica

◌ aberrant gamete
○ non-aberrant gamete
◐ fertilized egg cell

- Breakages => mainly distal deletions
- Selection of 3D deletion lines using telomeric + centromeric markers => **113** deletion lines
- Characterization of the deletions using 3D-specific markers => Deletions ranged from **6.5** to **357 Mb**

Svacina et al, 2020

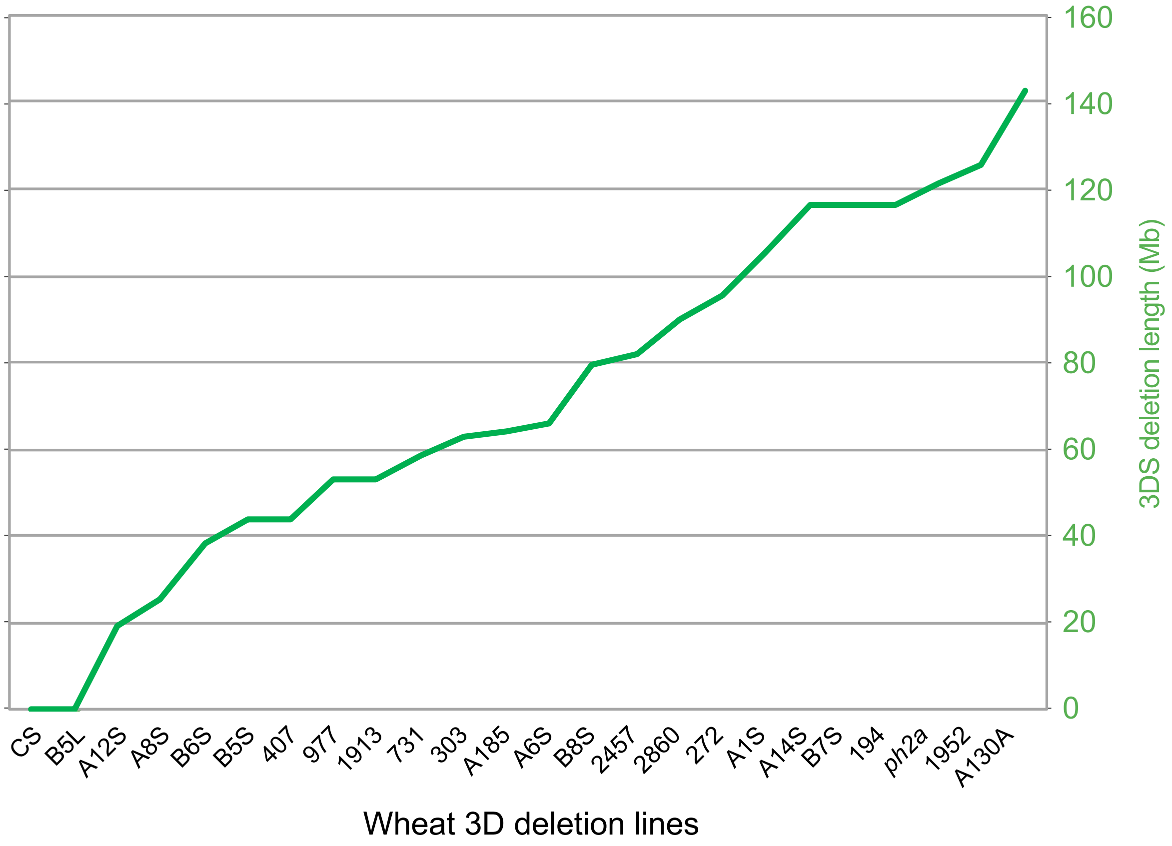
Production of 3D deletion lines

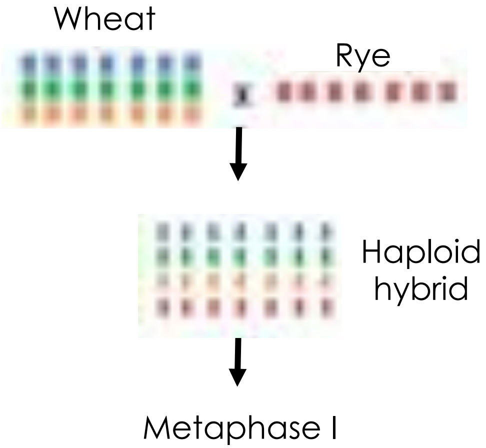


FISH analysis using Afa repeats

Svacina et al, 2020

Selection of lines carrying a terminal deletion on 3DS




Wheat

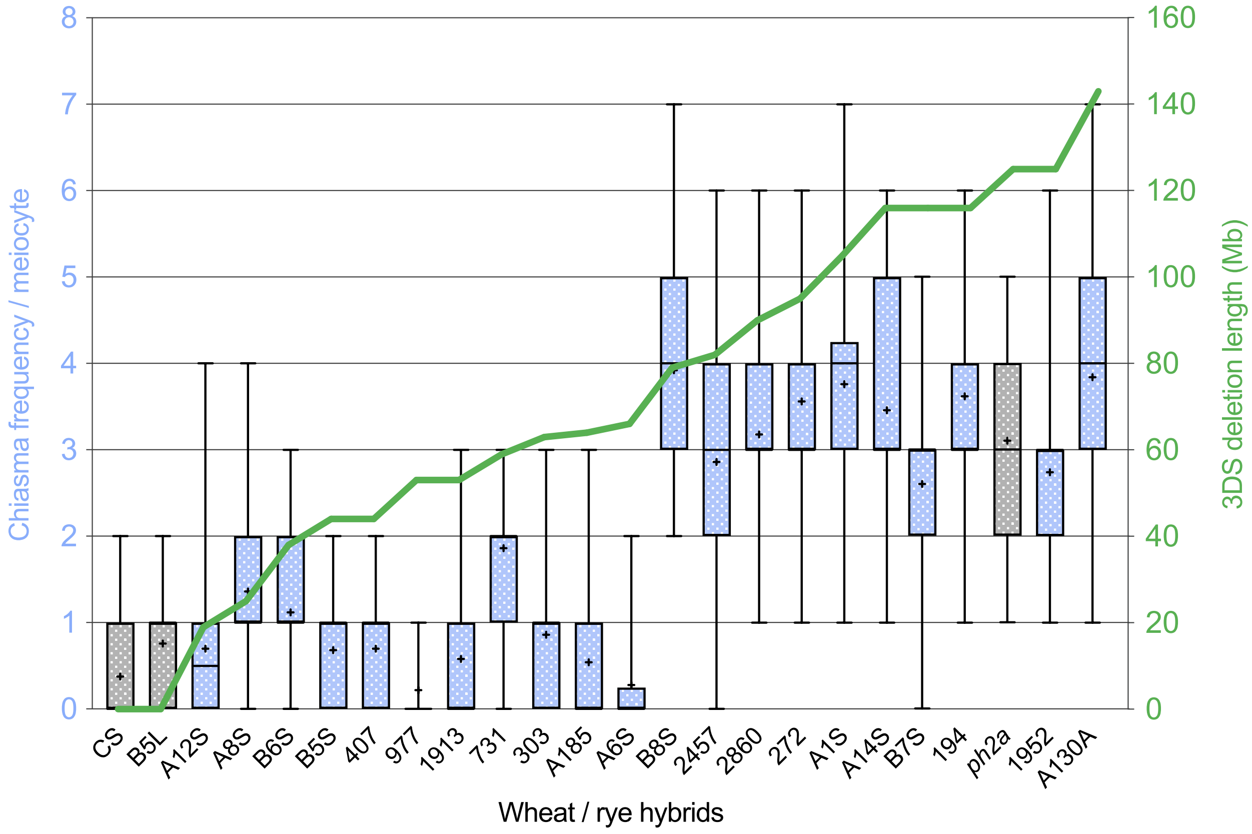
x

Rye

Haploid hybrid

Metaphase I

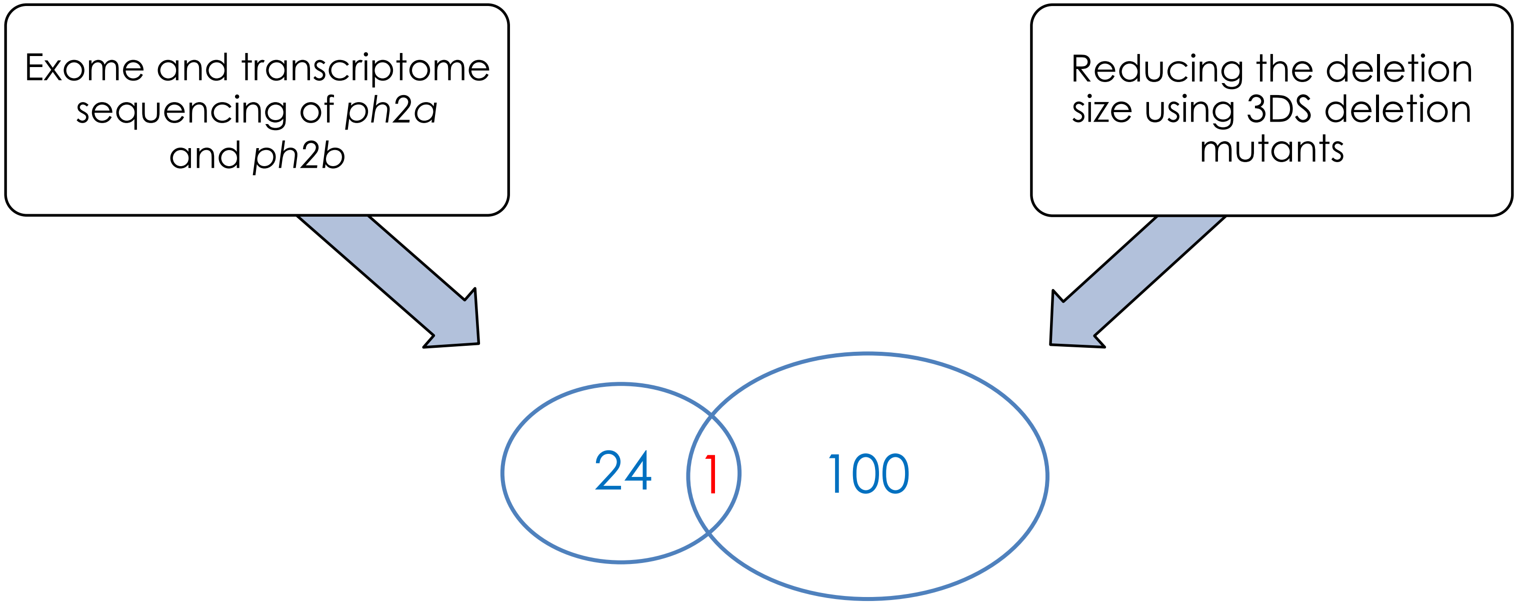
Ph2 locates within a 14.3-Mb region on 3DS



Ph2 locates between positions 64.9 Mb and 79.2 Mb

***Ph2* candidate genes: 100 genes**

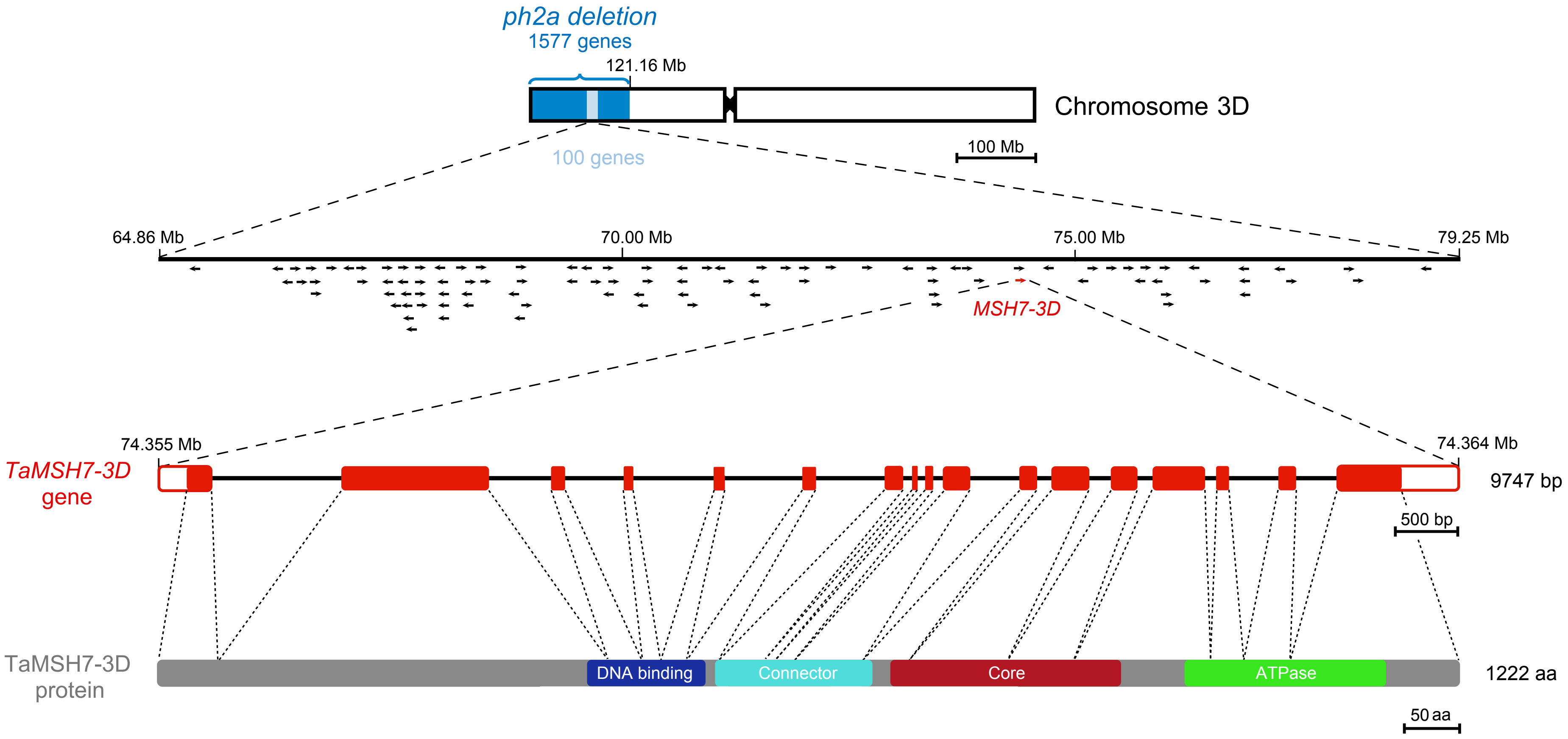
Candidate genes for *Ph2*



Exome and transcriptome sequencing of *ph2a* and *ph2b*

Reducing the deletion size using 3DS deletion mutants

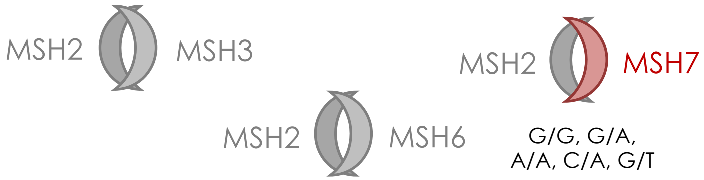
TaMSH7-3D is a unique candidate for *Ph2*



TaMSH7-3D, a DNA mismatch repair protein

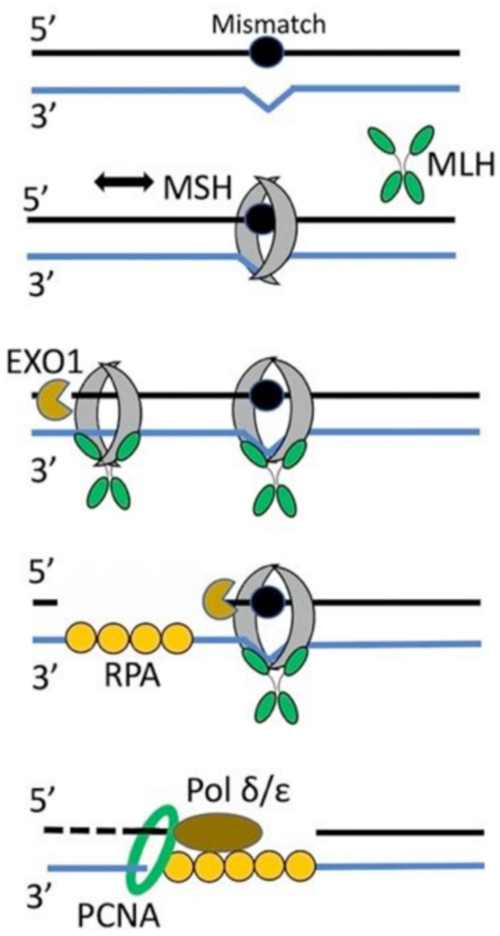
MSH7: *MutS homolog 7*

- Member of the DNA mismatch repair family with MSH2, MSH3 & MSH6
- Specific to plants
- Maintain genome stability by assuring DNA mismatch recognition in MMR pathway



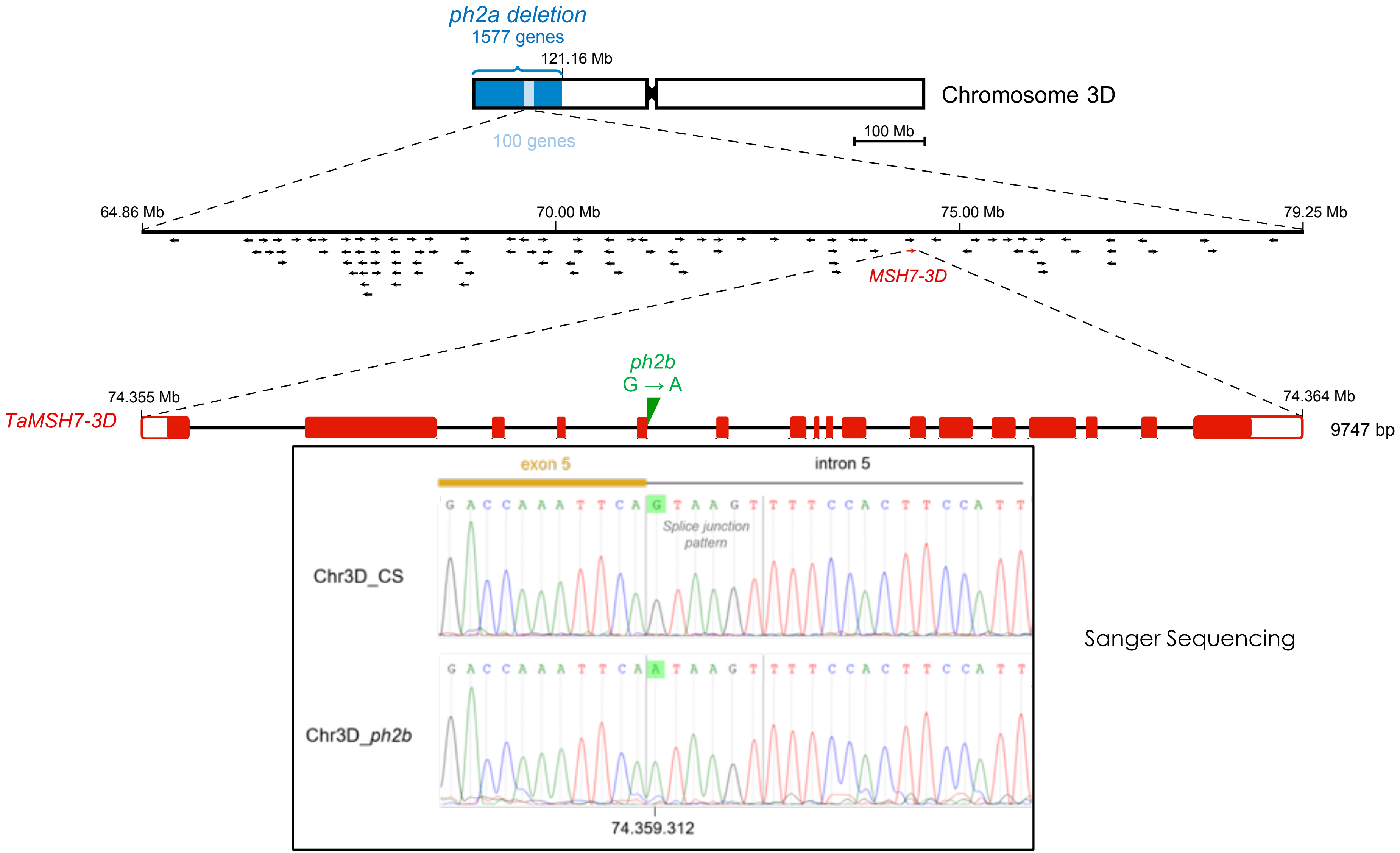
MSH7 supresses homoeologous recombination in tomato (Tam et al, 2011)

Mismatch Repair Pathway



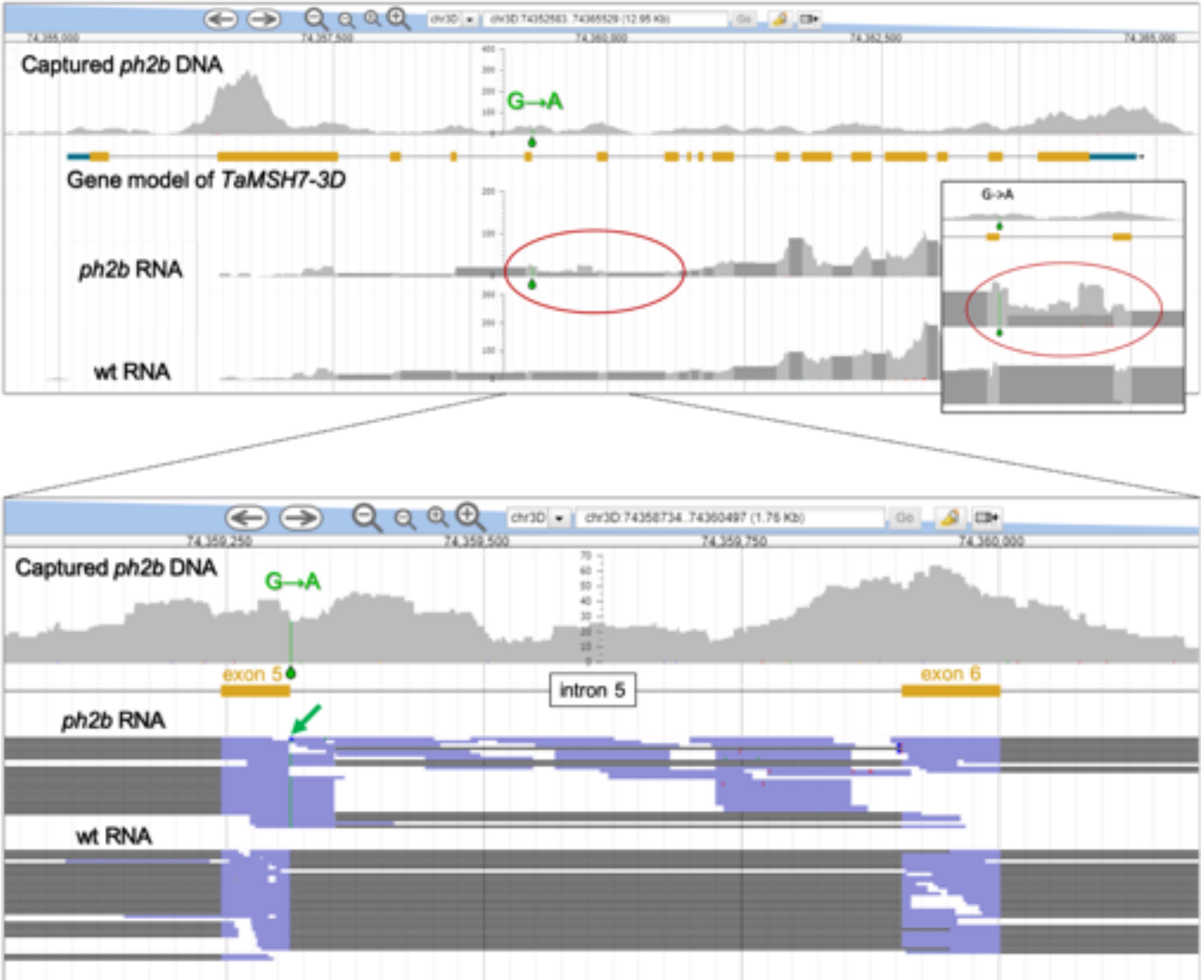
Karakaidos et al, 2020

TaMSH7-3D in *ph2b* mutant



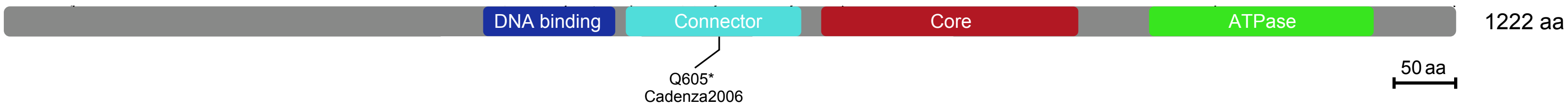
Sanger Sequencing

TaMSH7-3D in *ph2b* mutant



Dark shading: spliced reads

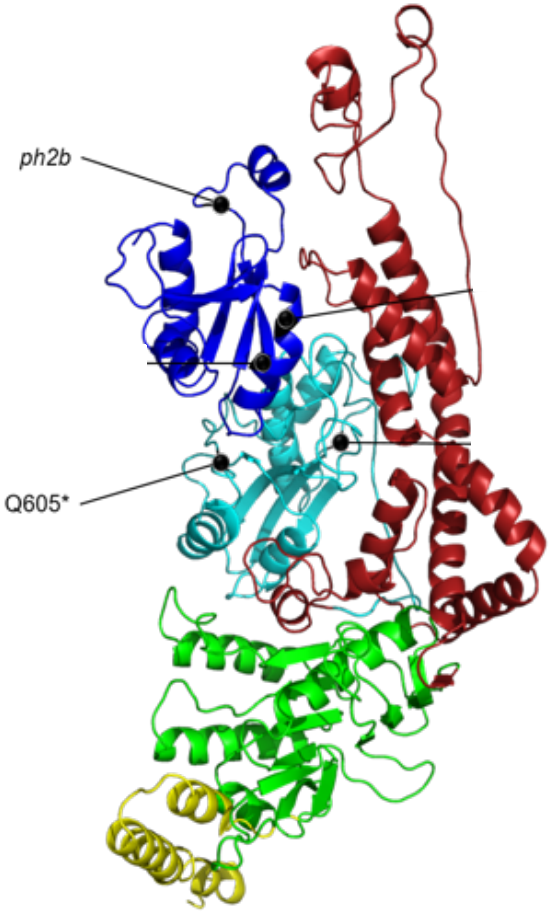
Tamsh7-3D Q605* mutant



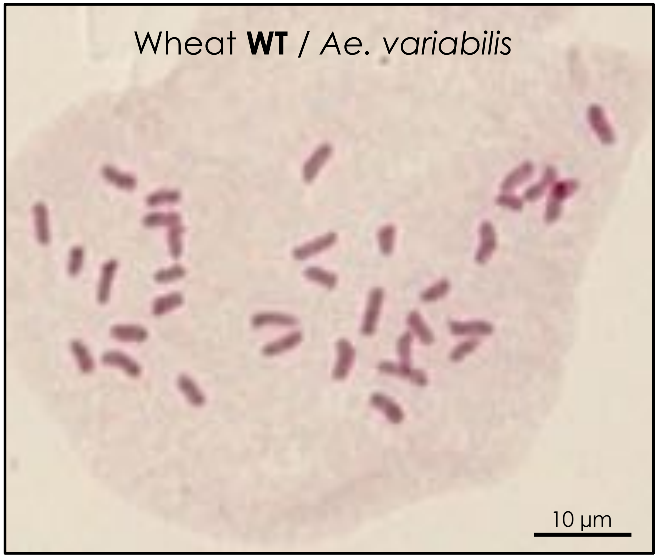
- EMS mutant from the Wheat TILLING population (JIC, UK)

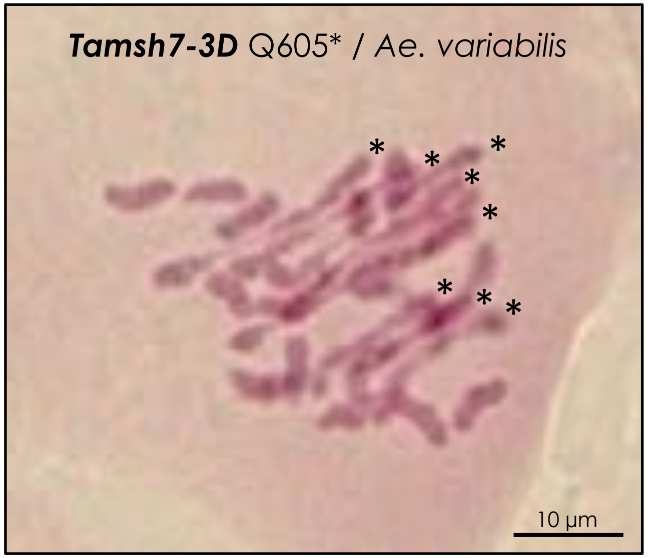
- Truncated TaMSH7-3D protein

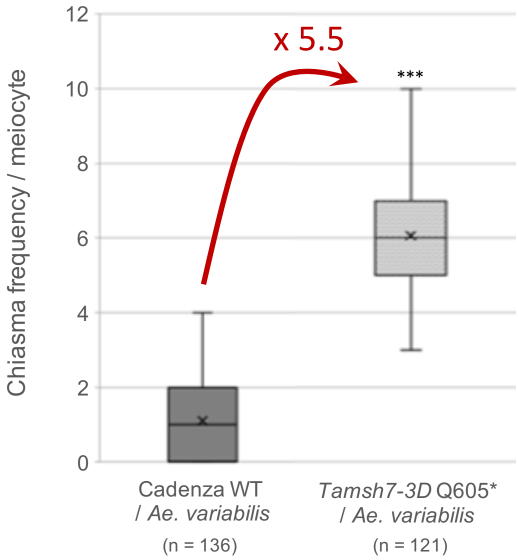
- No other mutated gene in the *ph2a* deletion



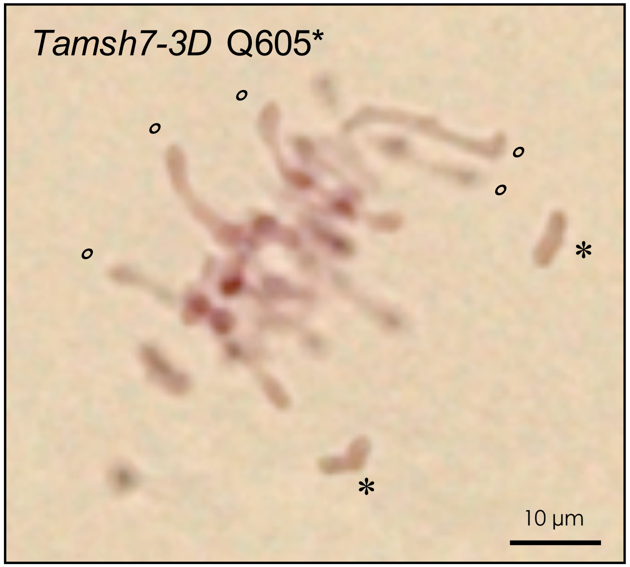
TaMSH7-3D inhibits homoeologous recombination

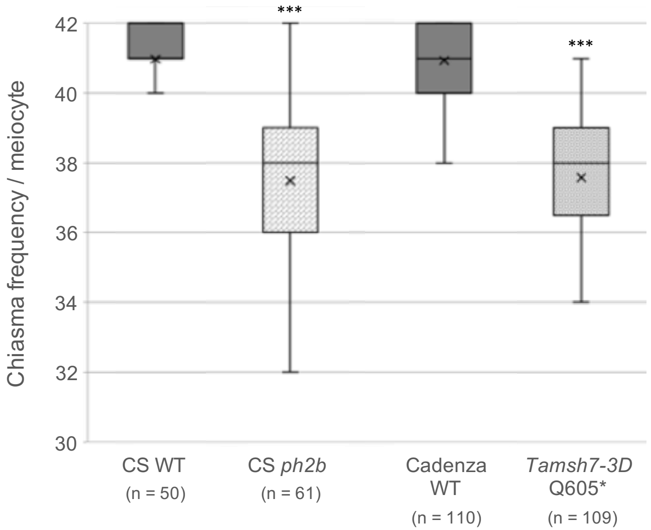




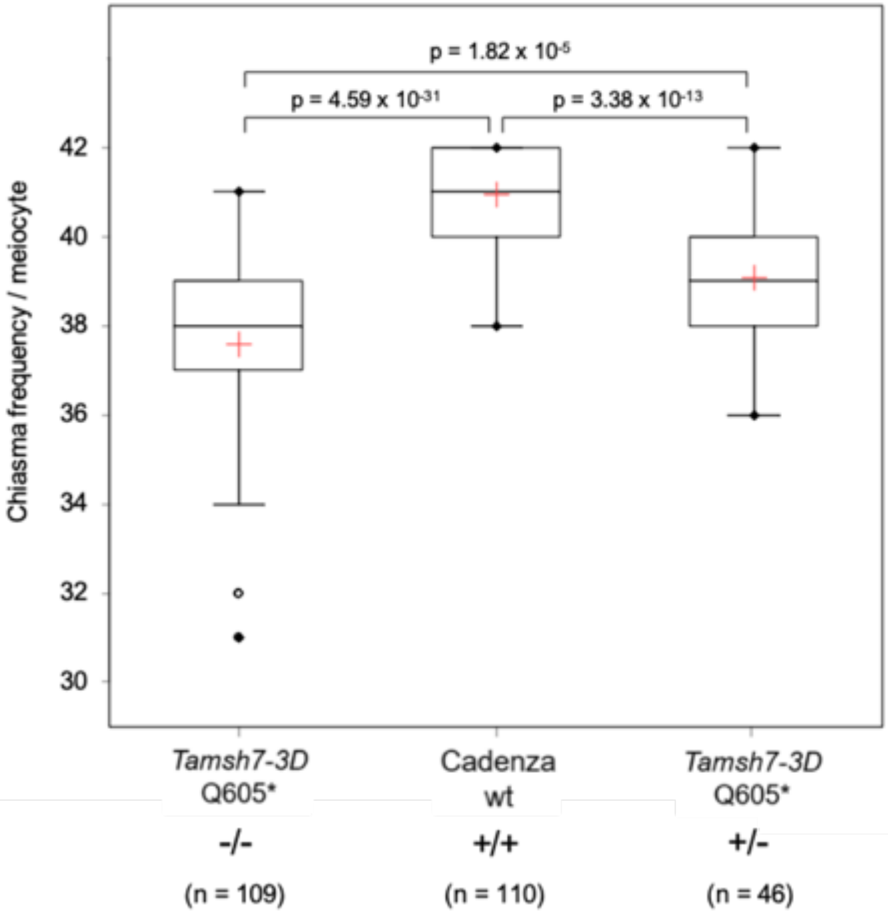


TaMSH7-3D promotes homologous recombination

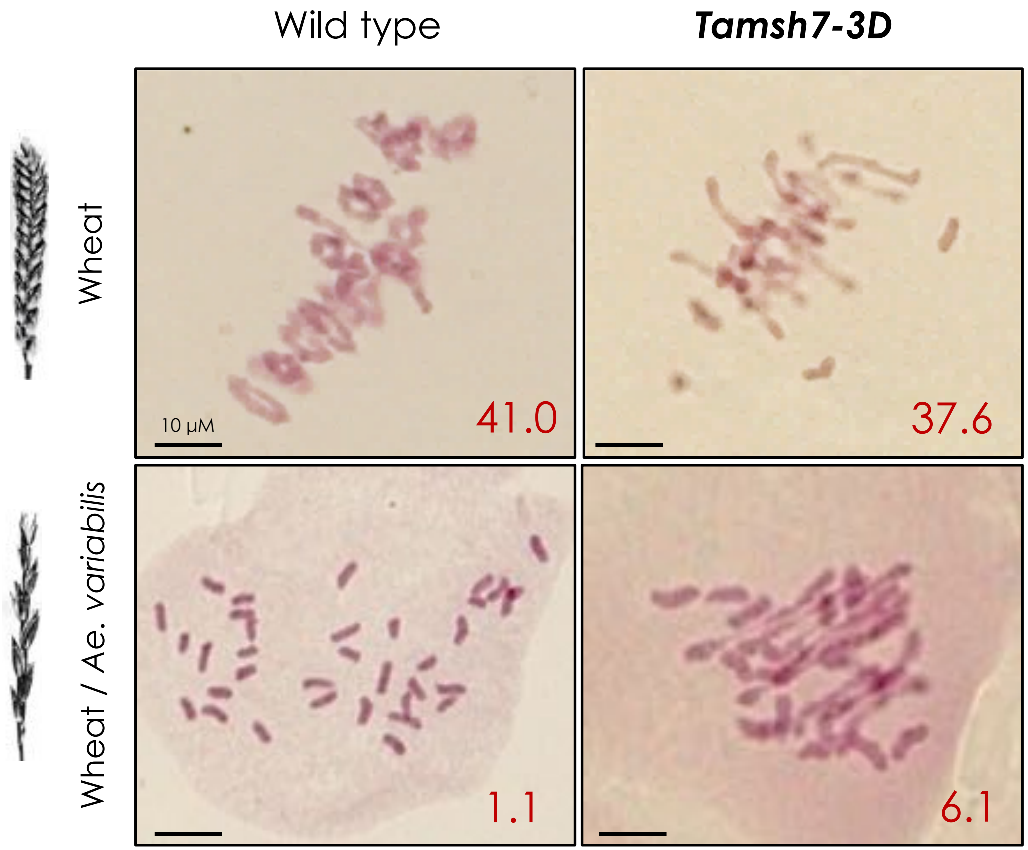




TaMSH7-3D is dosage sensitive



msh7-3D recapitulates the *ph2* phenotype

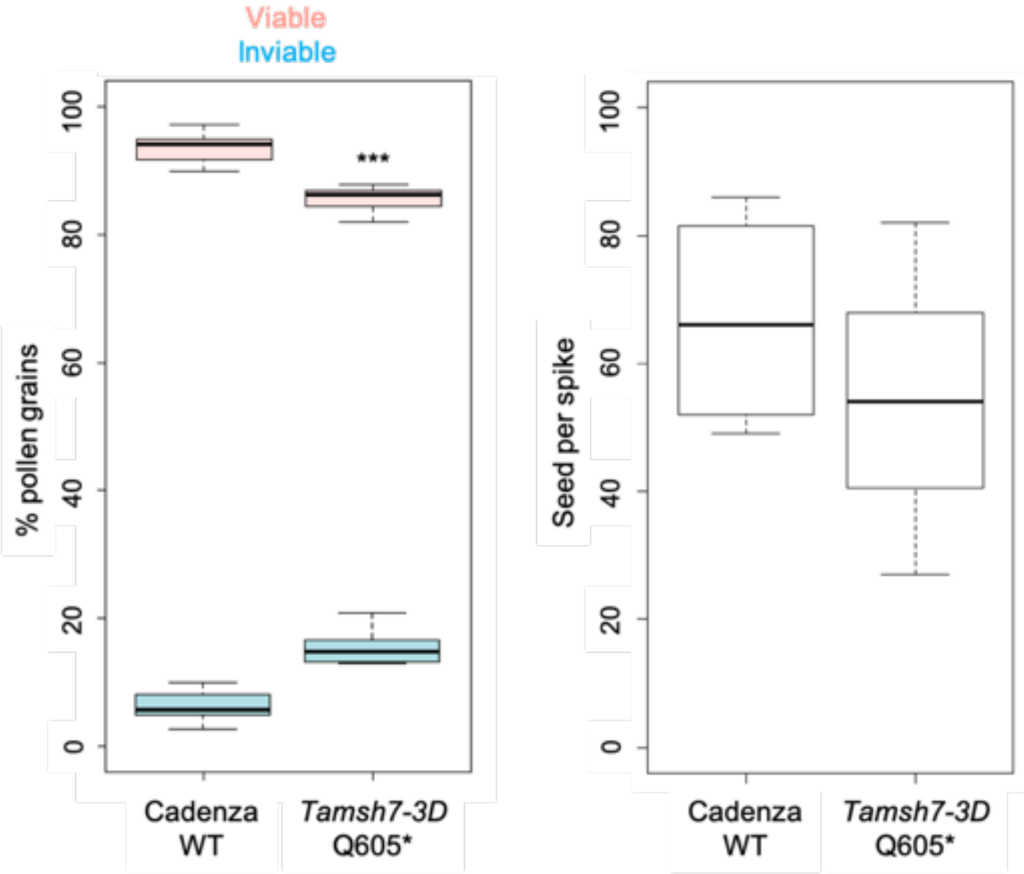


Homologous recombination ↘

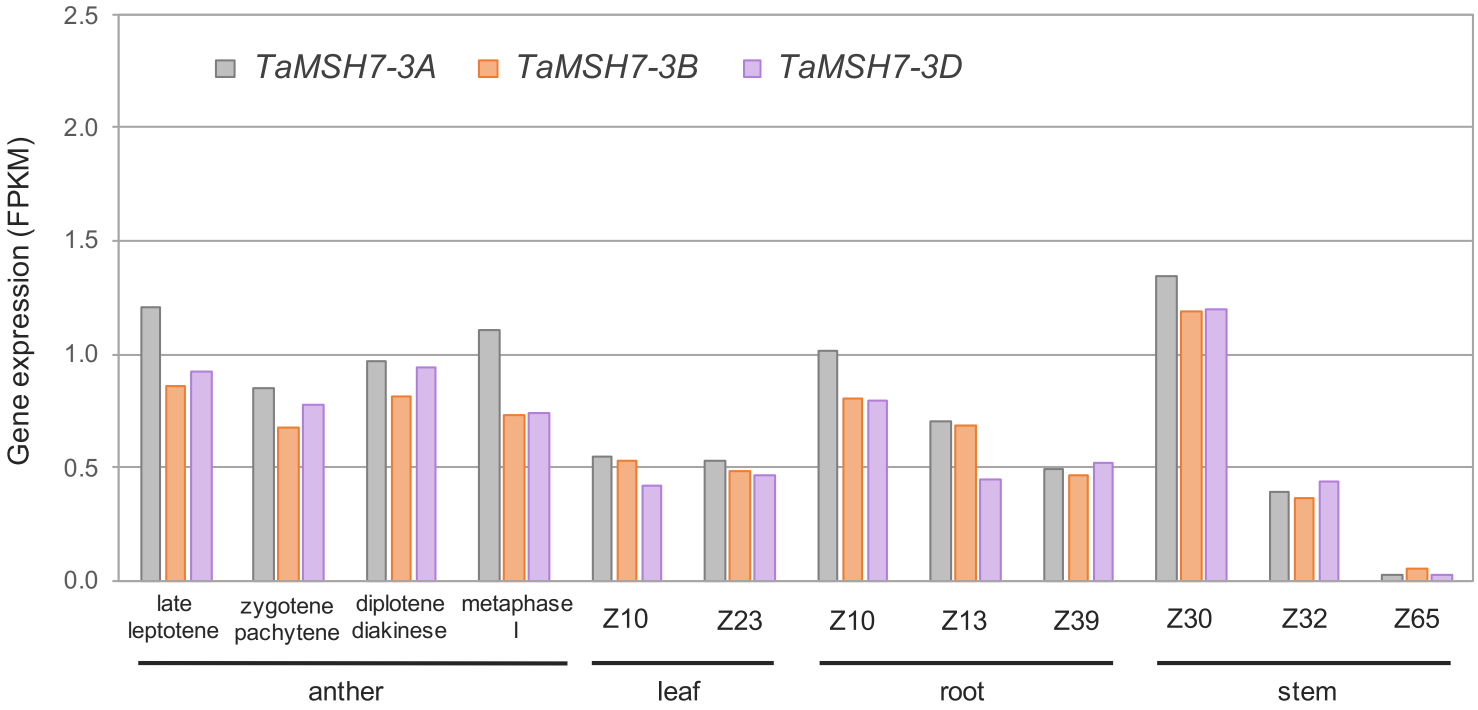
Homoeologous recombination ↗

Chiasma frequency / meiocyte

Tamsh-3D does not affect wheat fertility



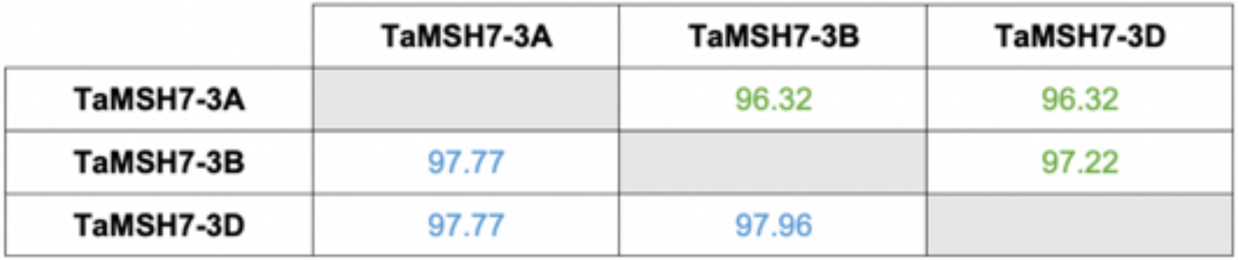
MSH7-3D is expressed in anthers during meiotic prophase I



Pingault *et al*, 2015

What about *TaMSH7-3A* and *TaMSH7-3B*?

Pairwise percentages of nucleotide and amino acid sequence identities



	TaMSH7-3A	TaMSH7-3B	TaMSH7-3D
TaMSH7-3A		96.32	96.32
TaMSH7-3B	97.77		97.22
TaMSH7-3D	97.77	97.96	

Minor suppressors of homoeologous recombination?

Summary

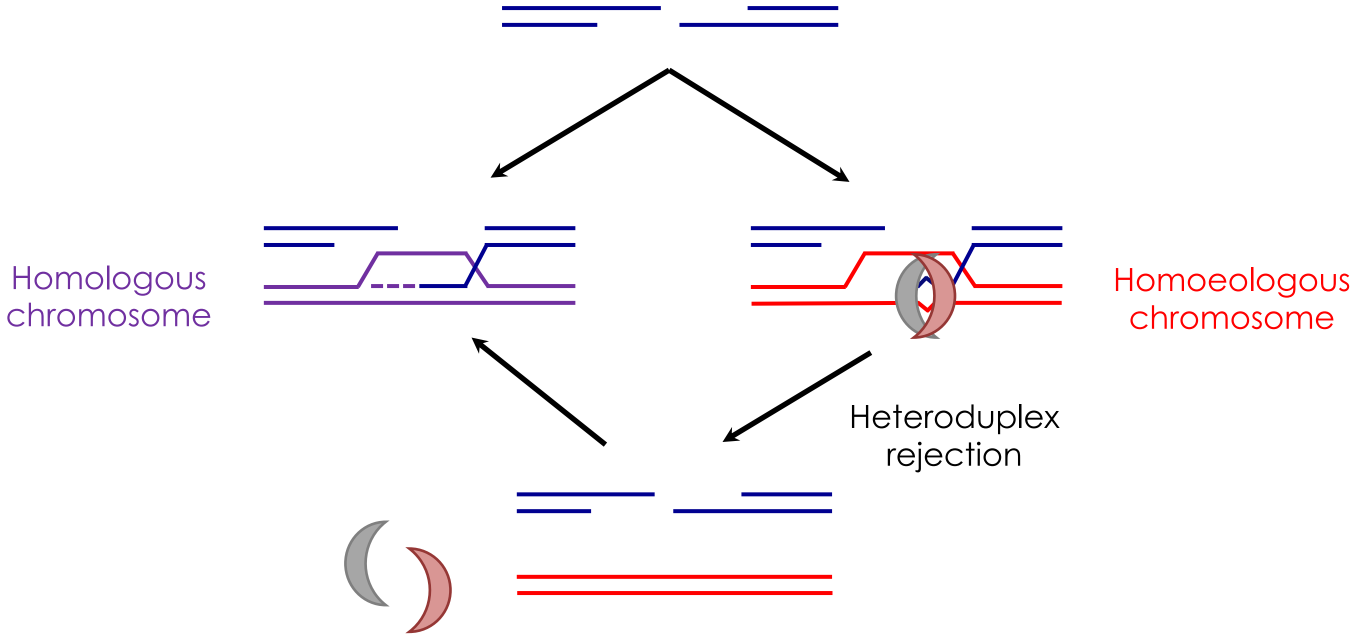
- *Ph2* locates within a **14.3-Mb region** ranging from 64.9 to 79.2 Mb **on 3DS**
- *TaMSH7-3D* is the only gene localised within this region that contains an EMS-derived SNP susceptible to affect protein sequence in *ph2b*
- An additional mutant of *TaMSH7-3D* **recapitulates the *ph2* phenotype** in regards to homologous and homoeologous recombination
- Exclusion of all previously proposed candidates for *Ph2 (*not located in the newly refined *Ph2* locus and not mutated in *ph2b*) except *TaMSH7-3D*

TaMSH7-3D, the causative gene for *Ph2*

Putative model

- *TaMSH7-3D* is a key **inhibitor of homoeologous recombination** in wheat

  Mode of action?



Putative model

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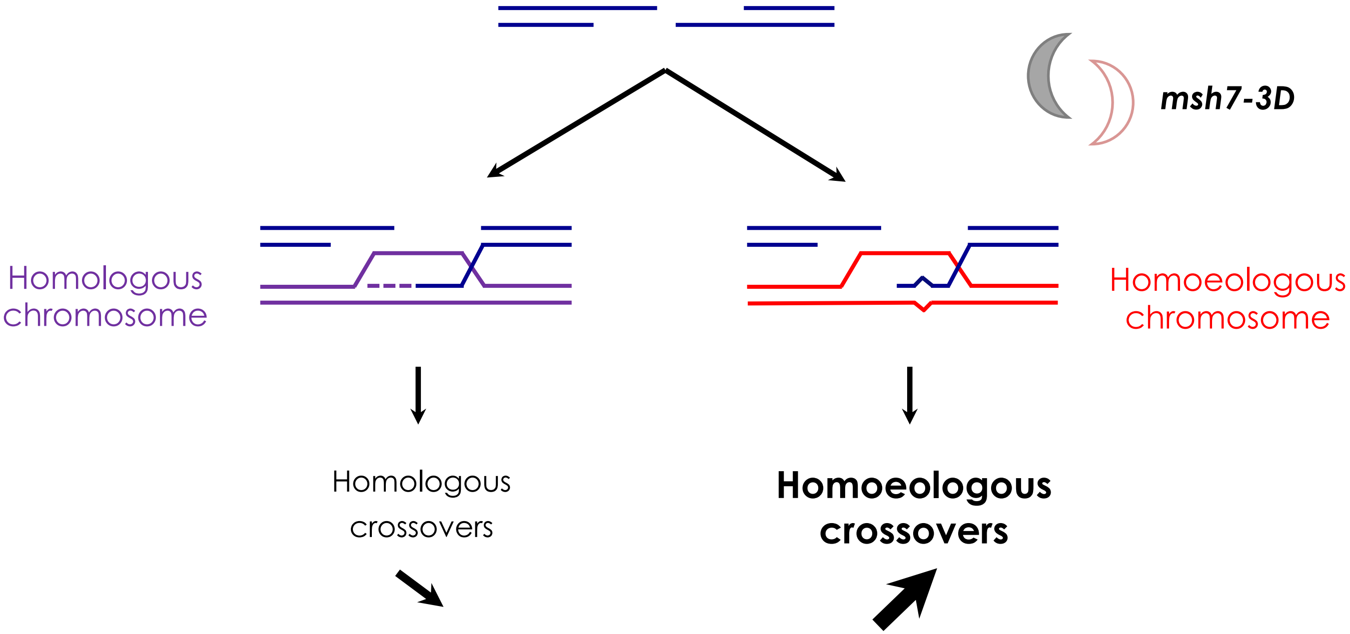

Perspectives…

- Decipher the **modes of action** and **interactions** of ***TaZIP4-B2*** and ***TaMSH7-3D***
- **Combine *Tazip4-B2* and *Tamsh7-3D* mutations** to further improve efficiency and ease of introgressions
- Investigate **relative impact** and **combinatorial effects of *TaMSH7* copies** on homoeologous recombination


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OPEN

Ph2 encodes the mismatch repair protein MSH7-3D that inhibits wheat homoeologous recombination

Heïdi Serra[1,5✉], Radim Svačina[2], Ute Baumann[3], Ryan Whitford[3], Tim Sutton[3,4], Jan Bartoš[2] & Pierre Sourdille[1✉]

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