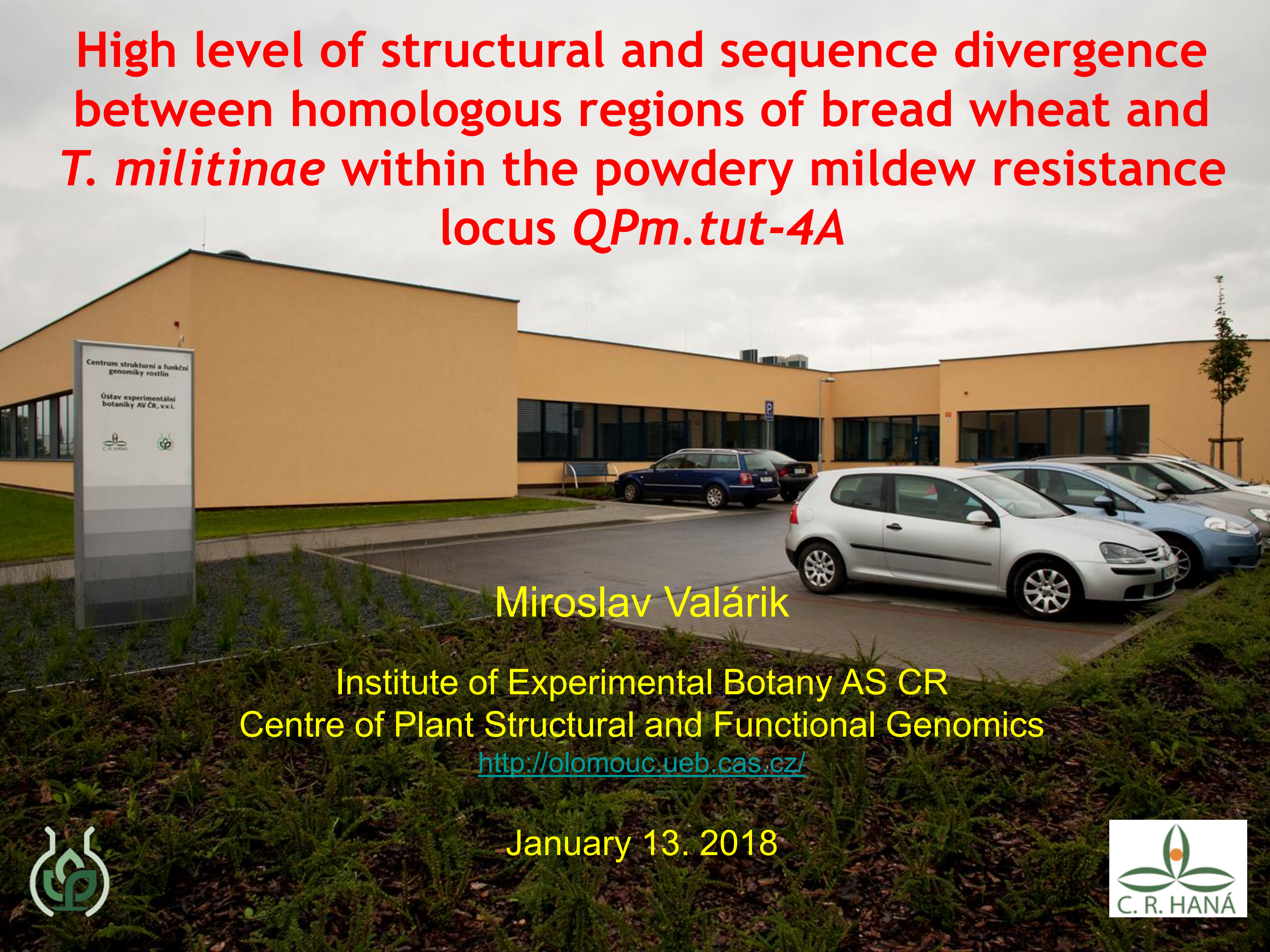
High level of structural and sequence divergence between homologous regions of bread wheat and *T. militinae* within the powdery mildew resistance locus *QPm.tut-4A*



Miroslav Valárik

Institute of Experimental Botany AS CR
Centre of Plant Structural and Functional Genomics
http://olomouc.ueb.cas.cz/

January 13. 2018

Cloning of powdery mildew resistance gene *QPm.tut-4A* introgressed to bread wheat from *T. militinae*

Triticum aestivum cv. Tähti

(AABBDD)

SUSCEPTIBLE TO POWDERY MILDEW

x

Triticum militinae

(AAGG)

RESISTANT TO POWDERY MILDEW

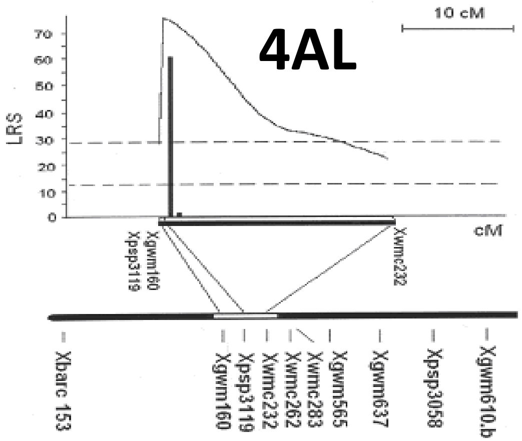
Backcross
to Tähti

Triticum aestivum line

8.1 (AABBDD*)

IMPROVED SEEDLING AND ADULT PLANT RESISTANCE

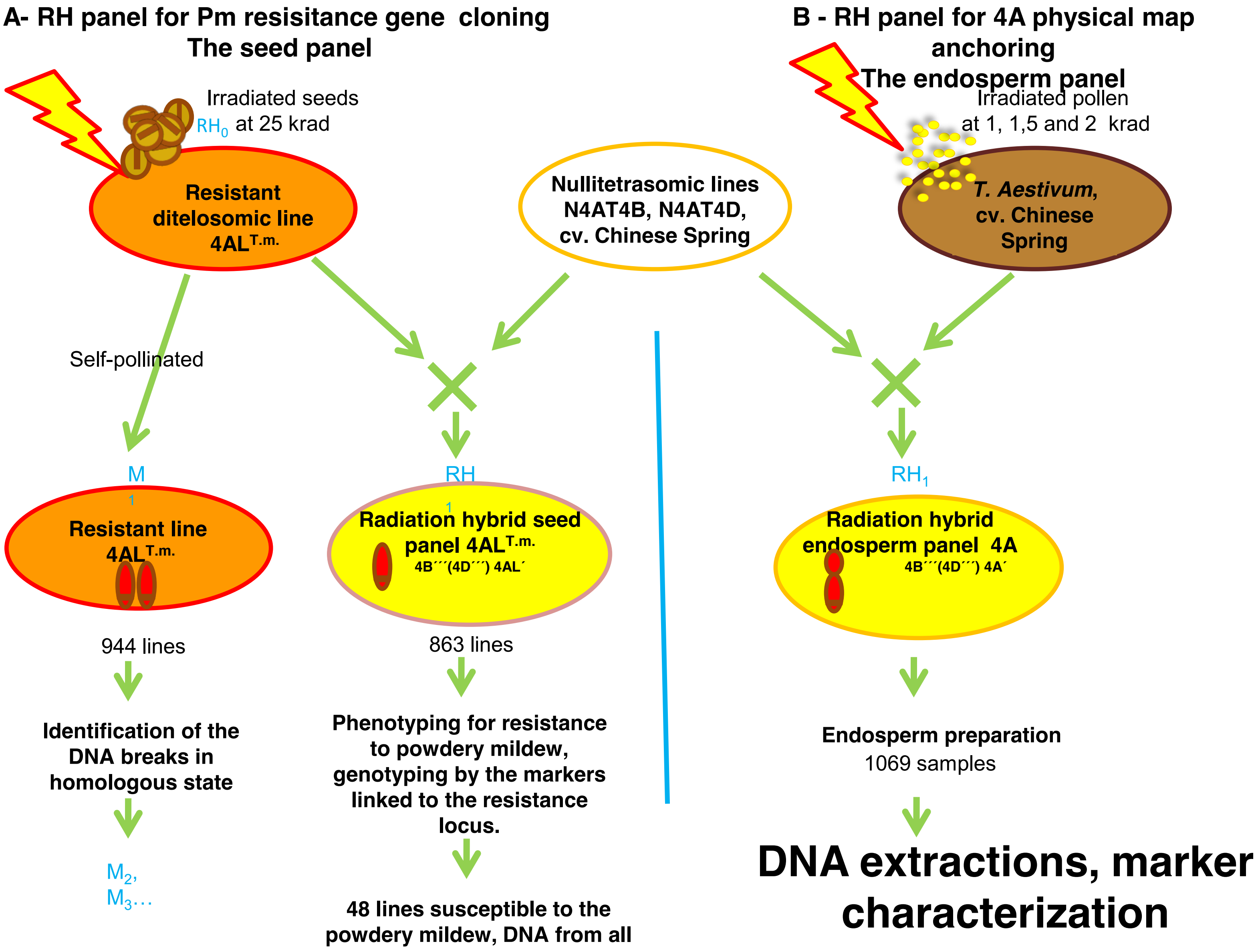
Introgressions detected in:
1A, 1B, 2A, 4A, 5A, 5B, 7A,



Major *Pm* resistance QTL identified on chromosomal arm 4AL and denominated as *QPm.tut-4A*

Minor Pm QTL identified on chromosomes 7AL, 5AL, 1A and 5B

Radiation hybrid panels



A- RH panel for Pm resisitance gene cloning
The seed panel

Irradiated seeds
RH_0 at 25 krad

Resistant ditelosomic line $4AL^{T.m.}$

Self-pollinated

M_1

Resistant line $4AL^{T.m.}$

944 lines

Identification of the DNA breaks in homologous state

M_2, M_3…

Nullitetrasomic lines N4AT4B, N4AT4D, cv. Chinese Spring

RH_1

Radiation hybrid seed panel $4AL^{T.m.}$

4B´´´(4D´´´) 4AL´

863 lines

Phenotyping for resistance to powdery mildew, genotyping by the markers linked to the resistance locus.

48 lines susceptible to the powdery mildew, DNA from all lines axtracted

B - RH panel for 4A physical map anchoring
The endosperm panel

Irradiated pollen
at 1, 1,5 and 2 krad

***T. Aestivum*, cv. Chinese Spring**

RH_1

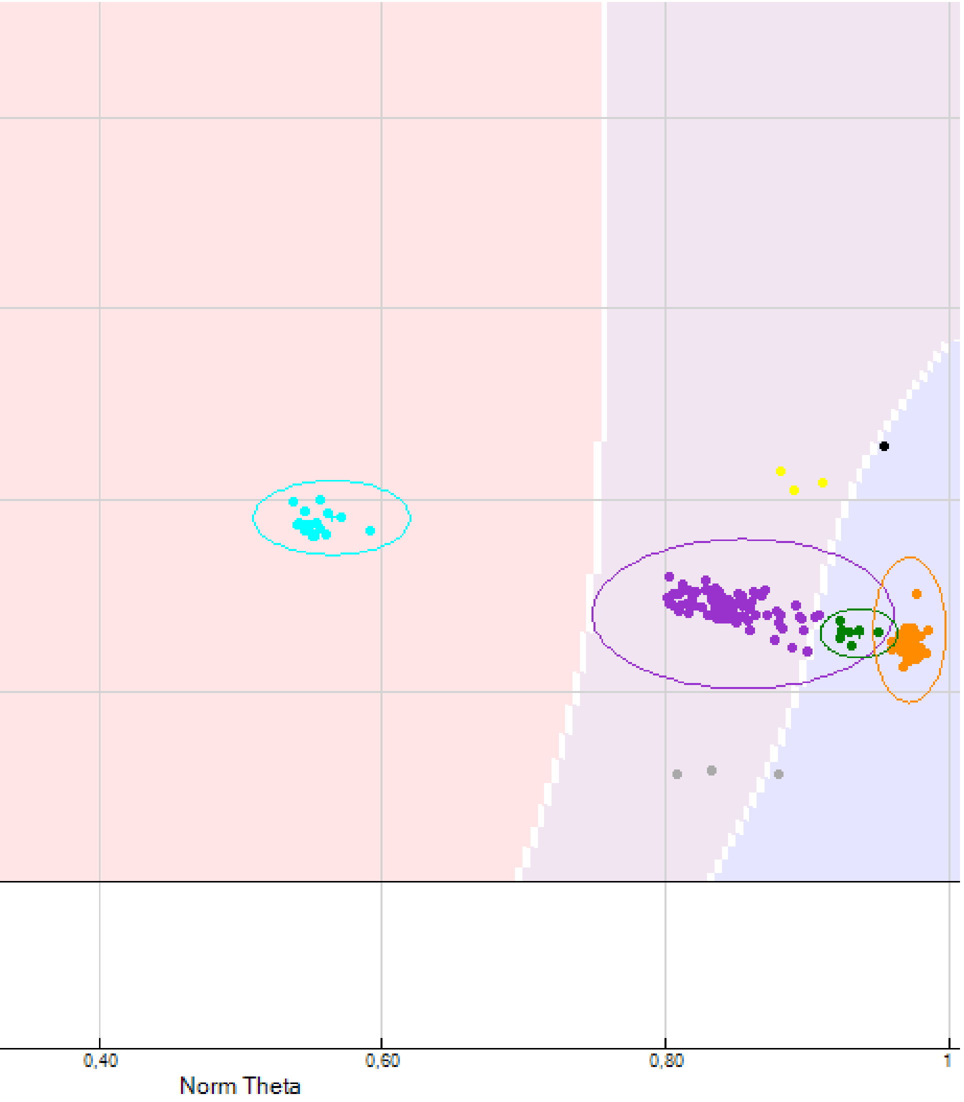
Radiation hybrid endosperm panel 4A

4B´´´(4D´´´) 4A´

Endosperm preparation
1069 samples

DNA extractions, marker characterization

4A Endosperm Rradiation Hybrid (ERH) map for physical map anchoring and genes loci saturation



414 DNA extracted / genotyped - 11 STS markers

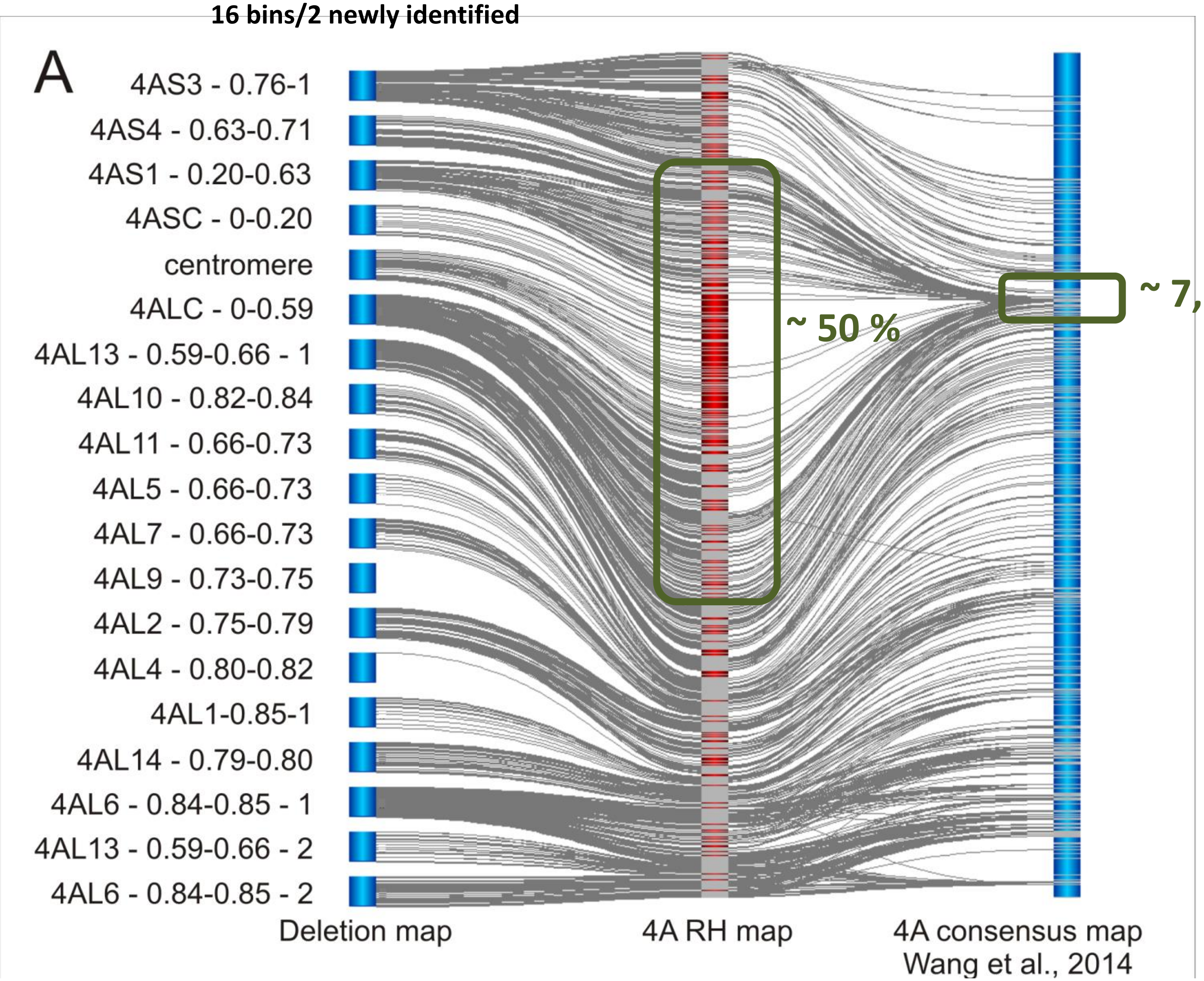
Selected 119 lines – 60 B / 59 D + 3 reps of control DNAs (15 4A deletion lines (4-S/11-L arms), 4AS/4AL MDA DNA, CS, NT4A4B/NT4A4D, 4A monosomics)

Genotyping 90K iSelect SNP array
81 587 genotypes

4A ERH SNP map

2687 SNPs **2308 SNPs** **973 common SNPs**

16 bins/2 newly identified



~ 50 %

~ 7,2 %

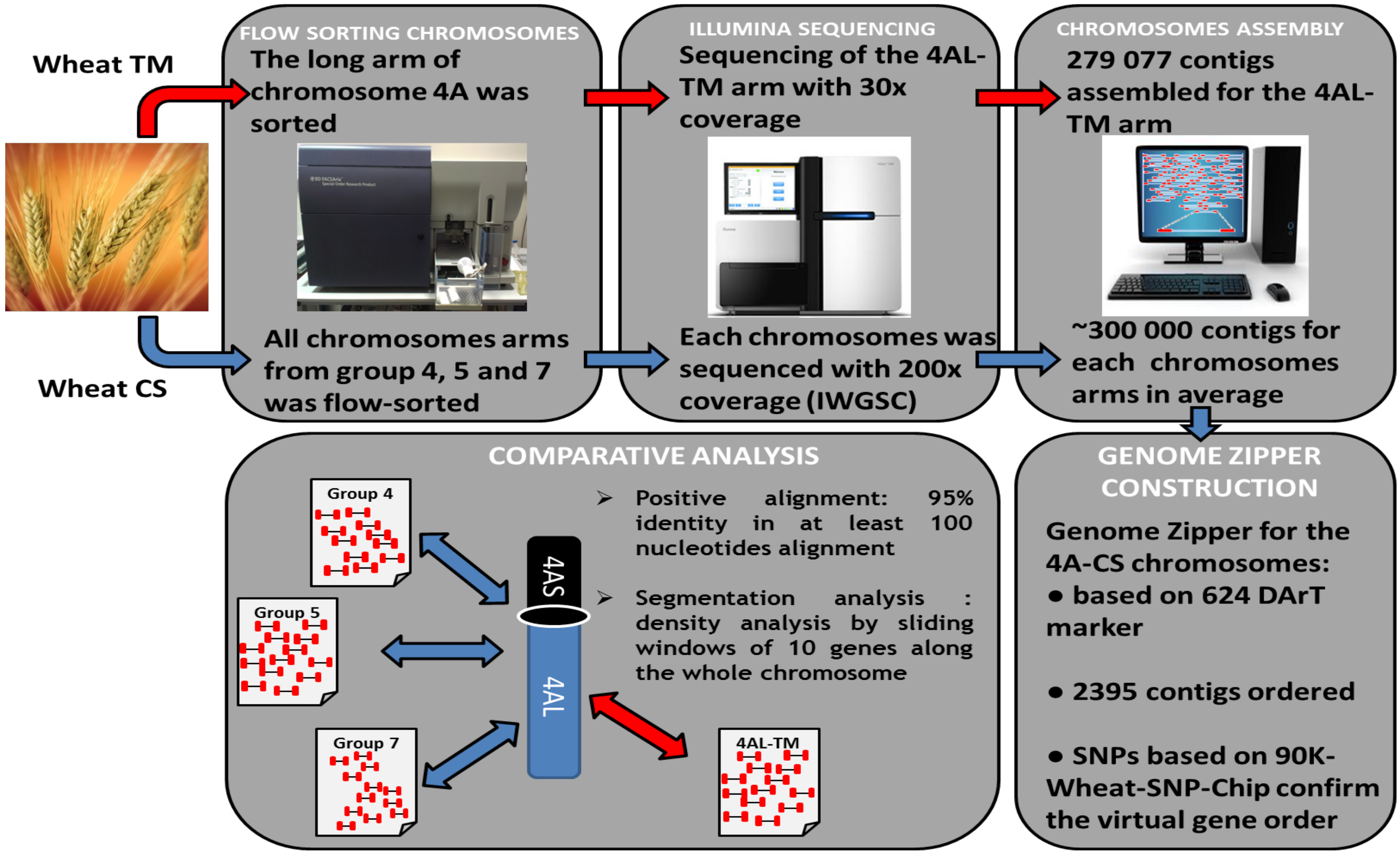
2711 4A spec. markers - retention fr. 72%

2687 in deletion map/16 bins 2 newly identified

2308 SNPs on RH map in 1080 mapping bins and map length 6550.9 cR

Balcarkova et al., 2017 *Front. Plant Sci., doi: 10.3389/fpls.2016.020*

In *silico* chracterization of introgression to bread wheat from *T. militinae*



Wheat TM

Wheat CS

FLOW SORTING CHROMOSOMES

The long arm of chromosome 4A was sorted

All chromosomes arms from group 4, 5 and 7 was flow-sorted

ILLUMINA SEQUENCING

Sequencing of the 4AL-TM arm with 30x coverage

Each chromosomes was sequenced with 200x coverage (IWGSC)

CHROMOSOMES ASSEMBLY

279 077 contigs assembled for the 4AL-TM arm

~300 000 contigs for each chromosomes arms in average

COMPARATIVE ANALYSIS

- ➢ Positive alignment: 95% identity in at least 100 nucleotides alignment
- ➢ Segmentation analysis : density analysis by sliding windows of 10 genes along the whole chromosome

GENOME ZIPPER CONSTRUCTION

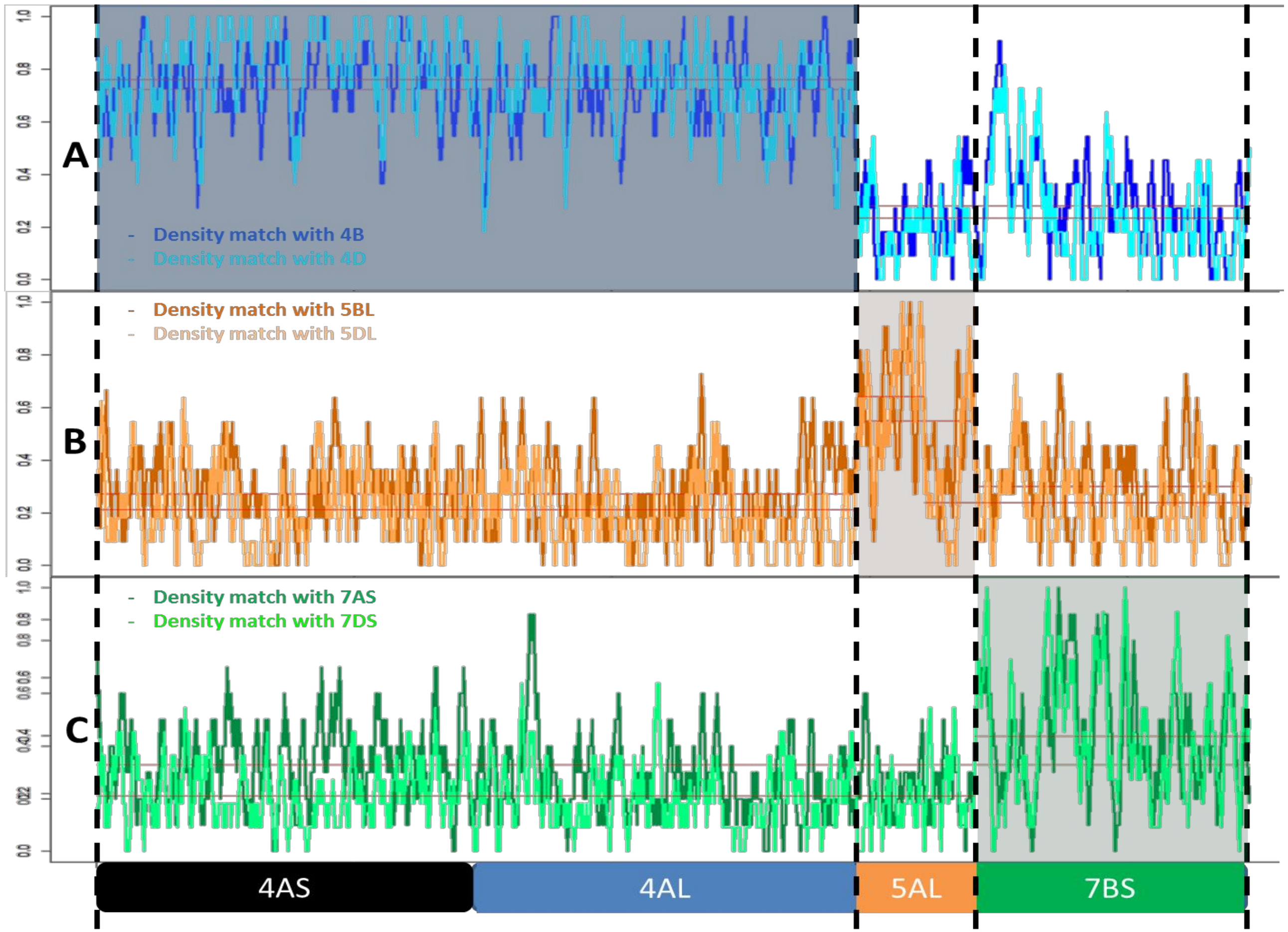
Genome Zipper for the 4A-CS chromosomes:

- ● based on 624 DArT marker
- ● 2395 contigs ordered
- ● SNPs based on 90K-Wheat-SNP-Chip confirm the virtual gene order

Abrouk 2017 Plant Biotech. J., doi: 10.1111/pbi.12610

In *silico* chracterization of introgression to bread wheat from *T. militinae*

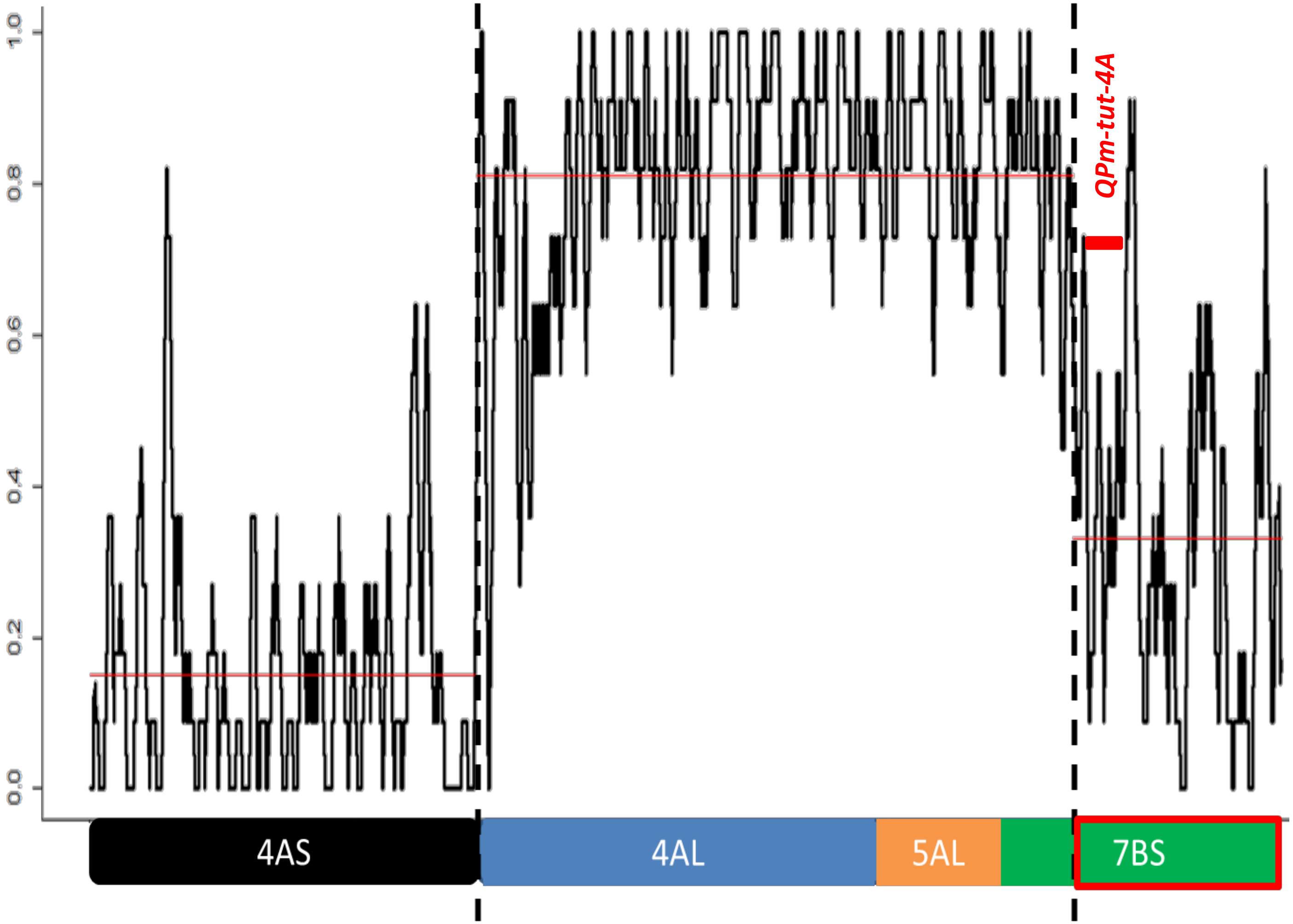
Chromosomal Rearrangement Identification and Characterization (RICh) - Identification of translocated regions



Abrouk 2017 Plant Biotech. J., doi: 10.1111/pbi.12610

In *silico* chracterization of introgression to bread wheat from *T. militinae*

RICh - Identification of introgressed region

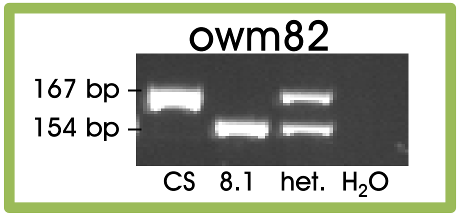


Abrouk 2017 Plant Biotech. J., doi: 10.1111/pbi.12610

QPm-tut-4A locus mapping in the introgressive line 8.1

Genetic maps:

new genetic markers

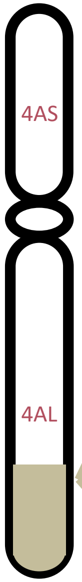


+

8519 plants of CS x 8.1 mapping population 0.012 cM

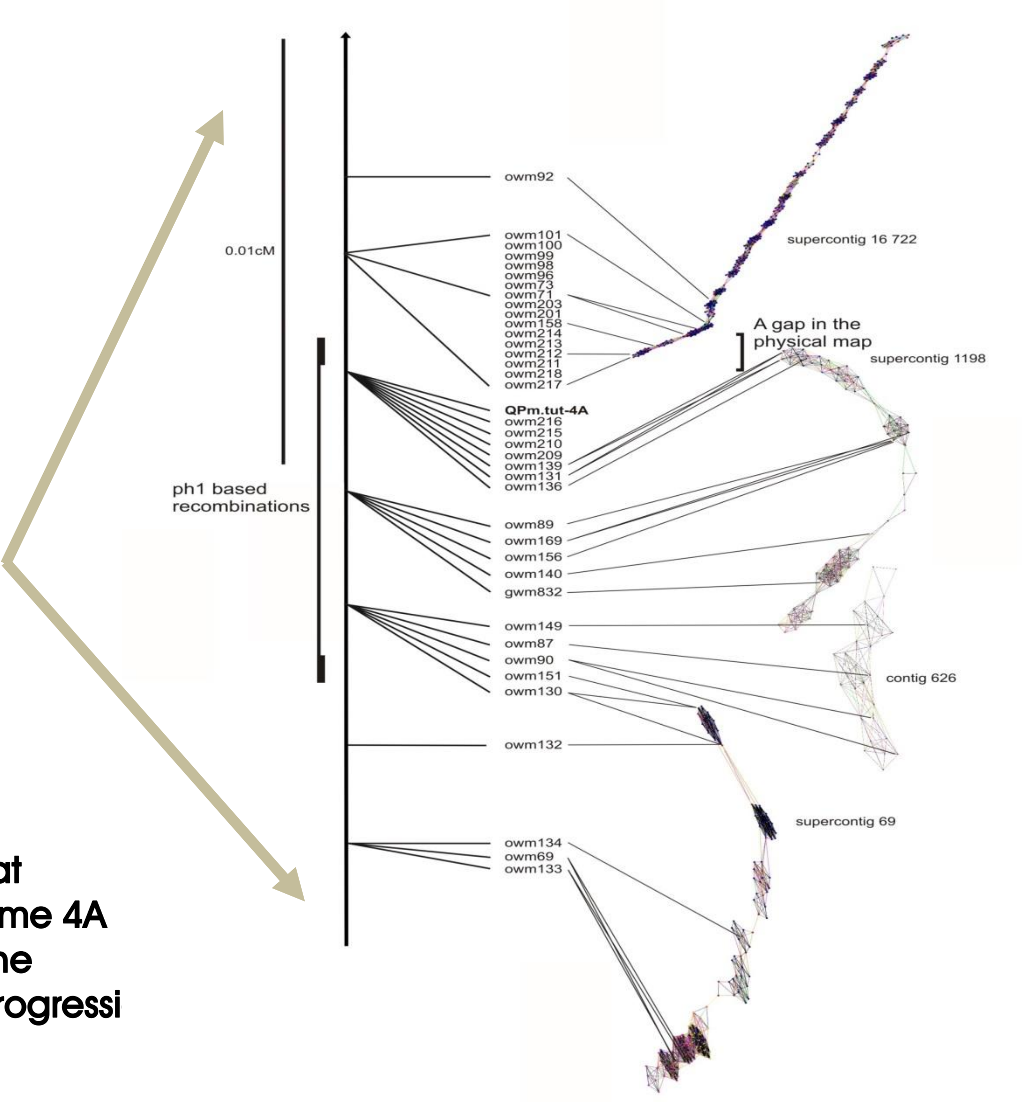
+

Lines of CS^{ph1} x 8.1 mapping population 4 rec



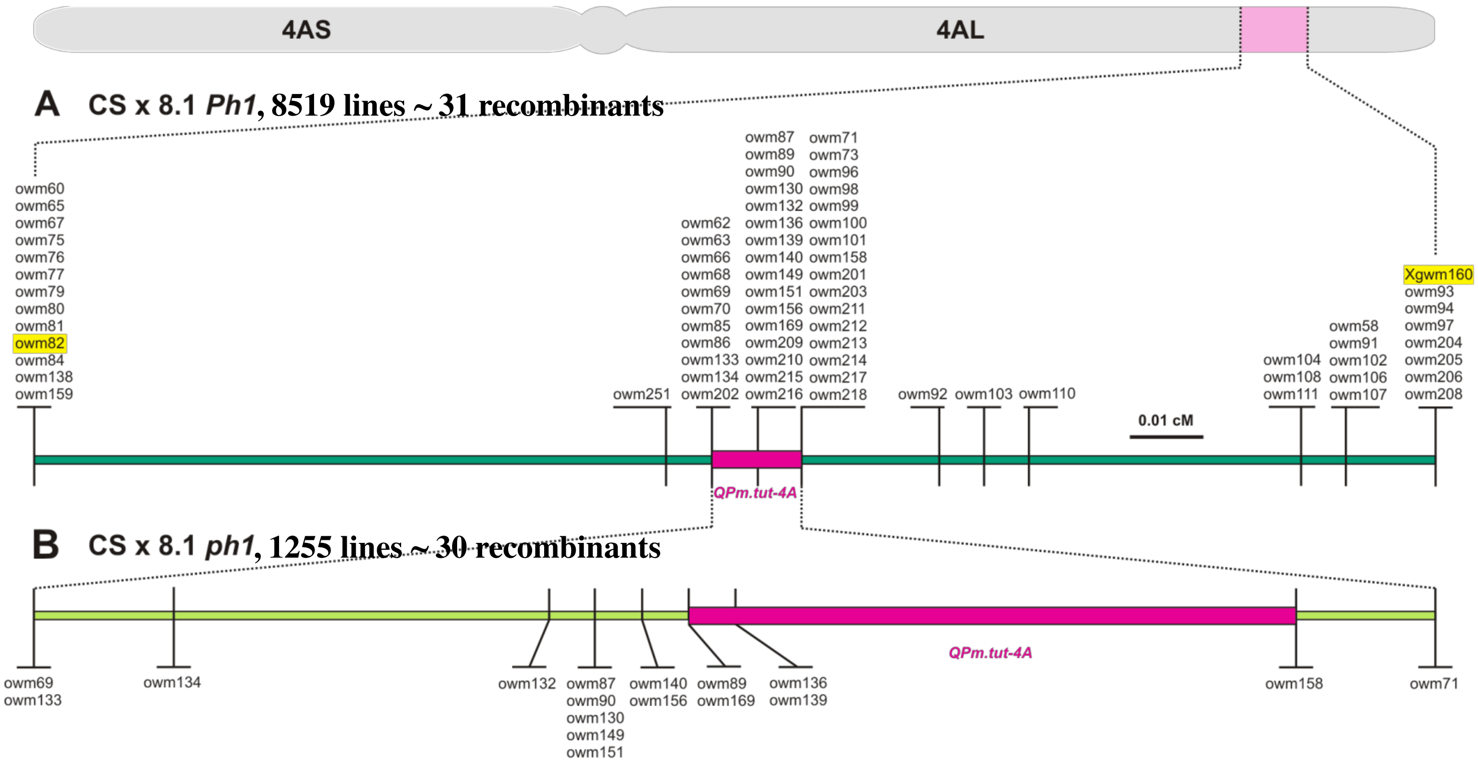
Wheat chromosome 4A with the *T. militinae* introgressi

Physical maps of 4A of CS and $4AL^{TM}$:



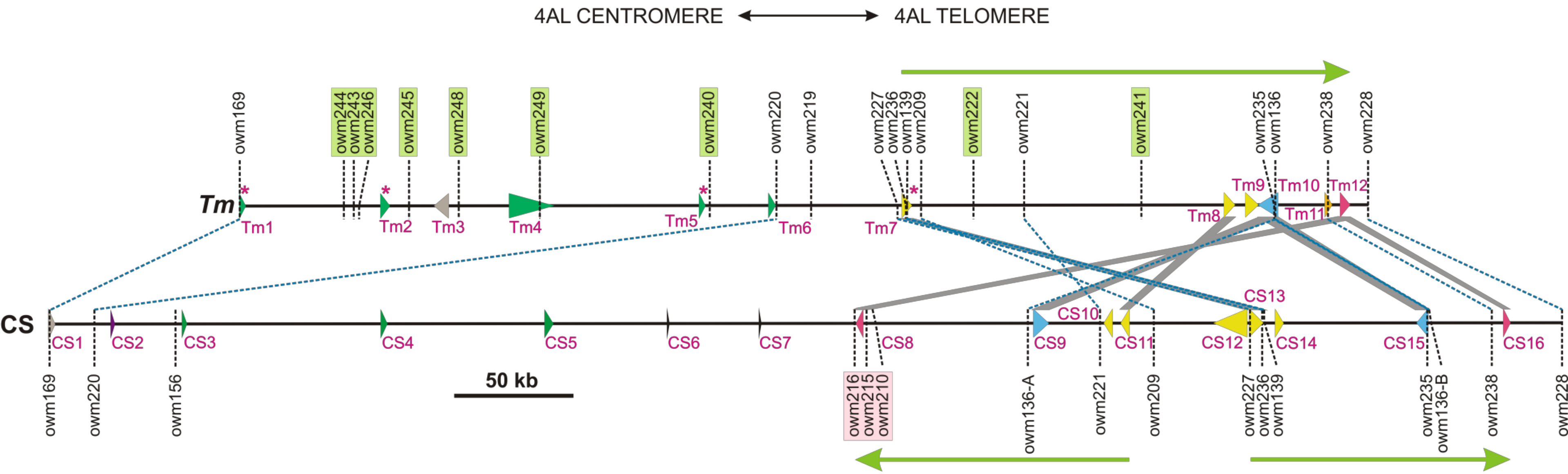
QPm-tut-4A locus high – density mapping

In total 102 markers developed and 75 mapped to the *QPm-tut-4A locus*



Physical map of the *QPm-tut-4A* locus

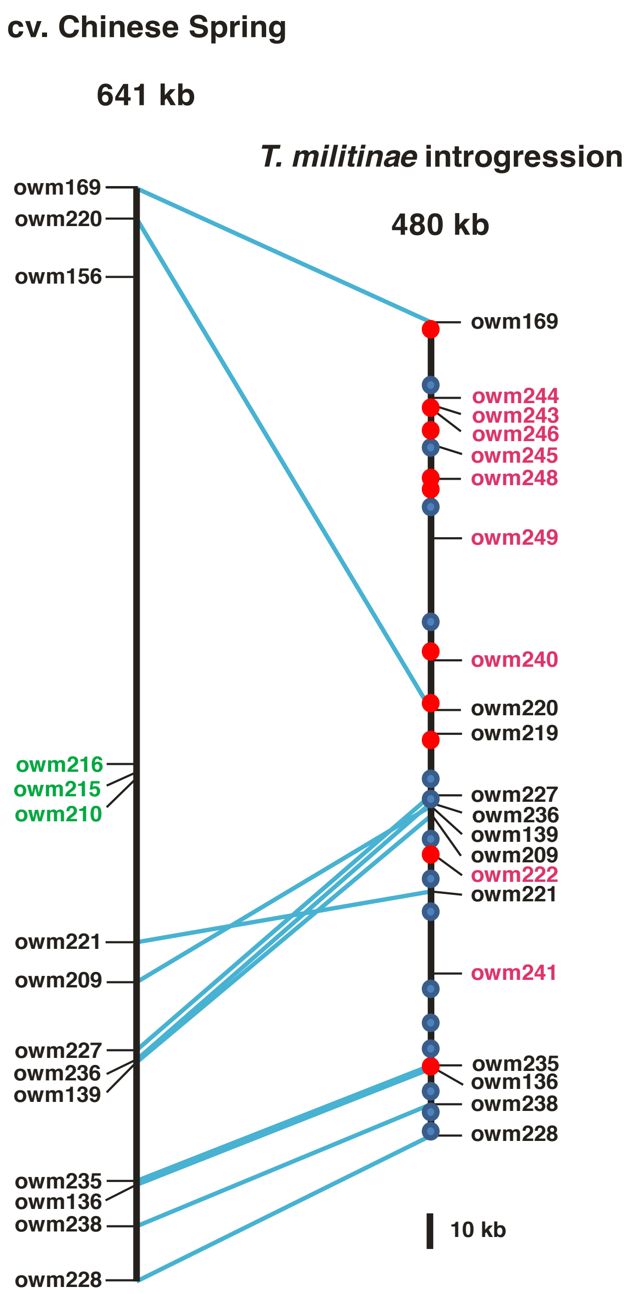
Four walking steps and 27 BAC sequenced and analyzed = 480 kbp, 12 HC genes



Applying CS reference sequence v1.0 = 641 kbp, 16 HC genes

- ▶ CC-(LRR)-NB-ARC domains
- ▶ PGG-domain-containing protein
- ▶ ANK- and PGG-domain containing protein
- ▶ LRR-malectin-(Pkinase) domains
- ▶ patatin-like protein
- ▶ PMEI-like domain
- ▶ uncharacterized protein

QPm-tut-4A locus mapping in the introgressive line 8.1



Eva Janáková

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Many thanks for your attention!