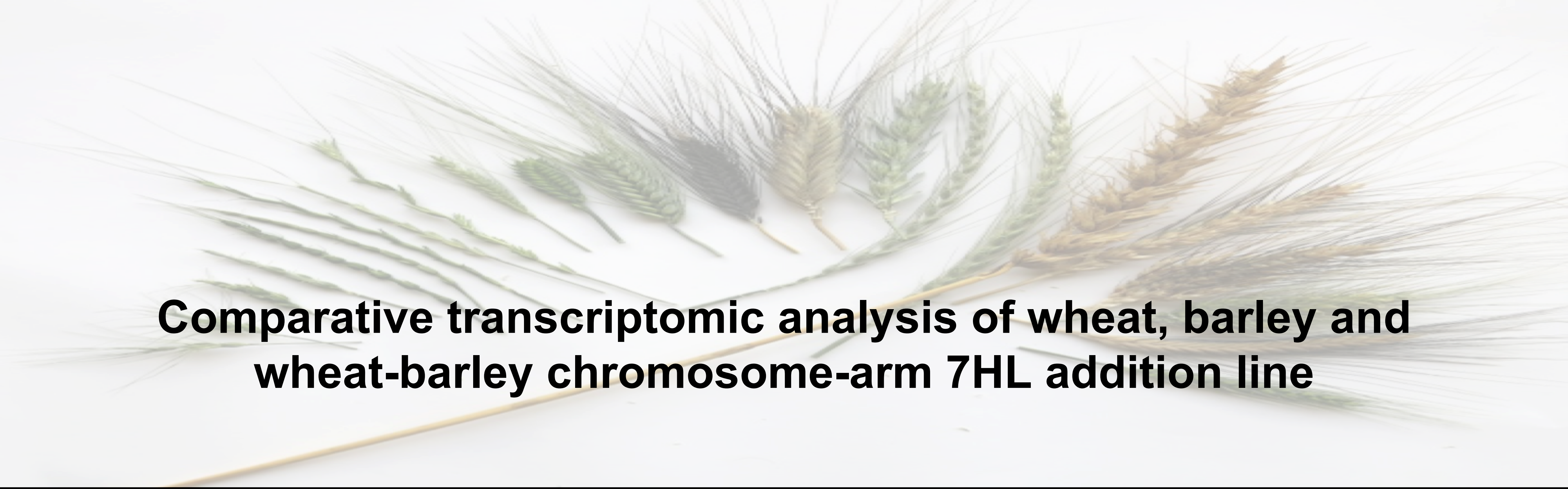

Comparative transcriptomic analysis of wheat, barley and wheat-barley chromosome-arm 7HL addition line

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Bread wheat (*Triticum aestivum* L.)

Important component of global food safety

- Cereal from the grass family (Poaceae)
- Staple food for more than 2.5 billion people from over 89 countries
- Grown in more land area than any other crop & important trade value

Concerns to sustain human needs

- World human population expected to reach 9.6 billion by 2050
- Need to increase wheat yields by ~60%
- Adverse environmental conditions (climate changes, erosion of arable lands, lower inputs)
- A need for improved bread varieties (introduction of new genes/alleles)

Wide hybridization

Transfer beneficial genes and alleles from related species into wheat through inter-species/genera crosses

Wheat-wide hybridization practiced for >140 years

Very little introgression lines made their way to agricultural use

- Numerous hurdles faced after hybridization
- Many events likely undiscovered and/or not described
- Poor knowledge/resources about donor genomes

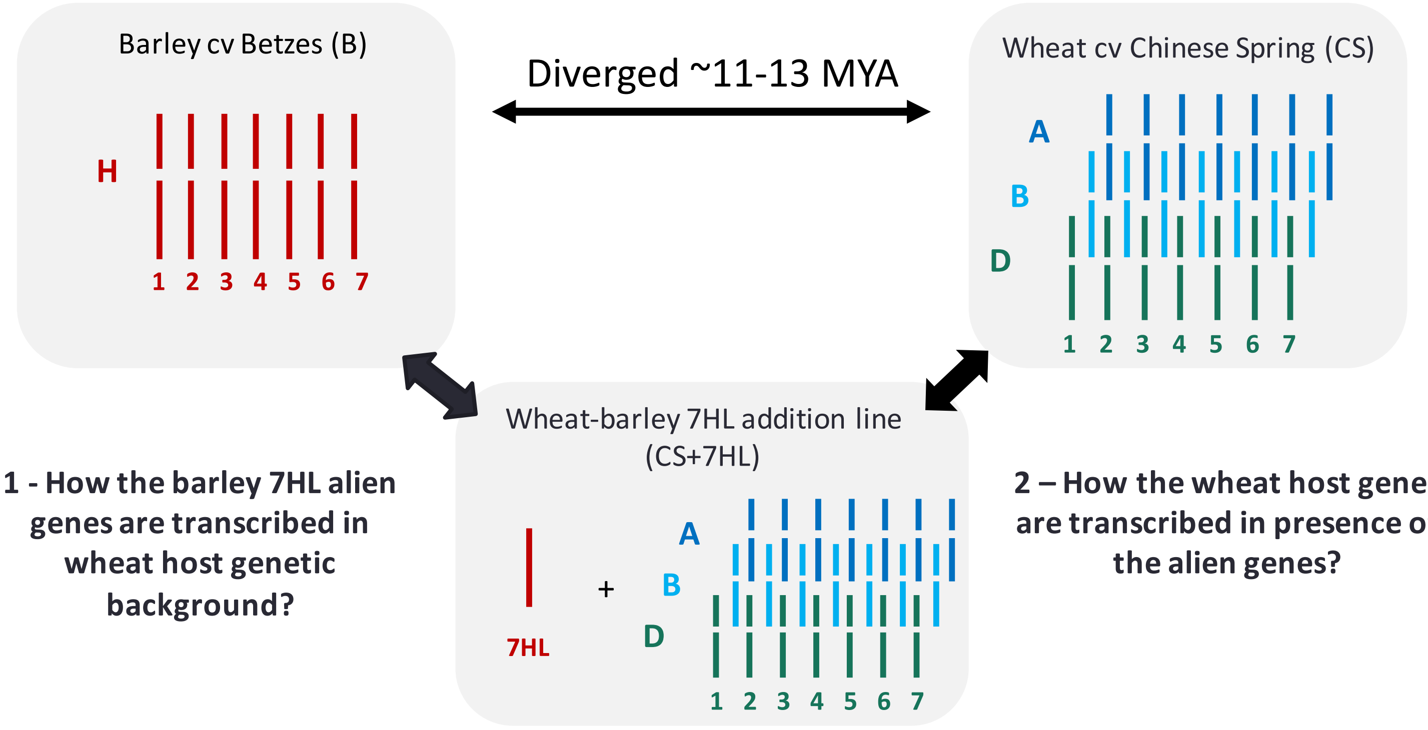
Need for better understanding of mechanisms underlying alien gene transfer

- Interactions between host and alien genomes
- Characterize beneficial/deleterious impact on alien/host genes functionality
- Mechanisms involved in inter-species incompatibility

Genetic diversity of wheat species: *picture from CIMMYT genebank*

Aim of the analysis

Investigate **transcriptomic changes** affecting host and alien genes as a result of interactions between the donor and host genomes



1 - How the barley 7HL alien genes are transcribed in wheat host genetic background?

2 – How the wheat host genes are transcribed in presence of the alien genes?

3 – What are the mechanisms underlying the differences in gene transcription between the parents and the alien addition line?

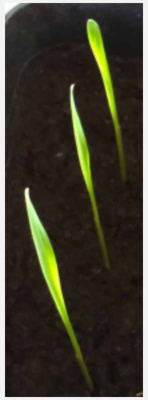
Islam, A. K. M. R. (1981). Heredity 46, 161–174

Chromosome-arm addition line:

- No physical interruption of the host chromatin
- Ideal set of genes to study inter-genomic interactions

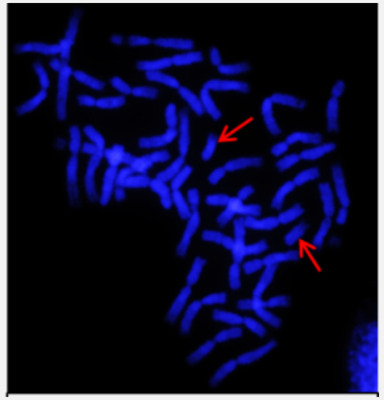
7HL provides salt-tolerance, earliness and increased β-glucan content in the grain (Molnár-Láng, Linc, and Szakács, 2014)

RNA-sequencing



Top leaf
collected at
Zadok 1.1
stage

→



Control of karyotype
2n = 42 + 7HL''
in the addition lines

→

RNA extraction
(Qiagen RNeasy
Plant Mini Kit)
-
mRNA selection
+ cDNA library
preparation
(illumina TruSeq®)

→

Sequencing
illumina HiSeq 2000

→

**~30M PE
reads/sample**

For wheat + barley parents and chromosome-arm addition line CS+7HL
6 bio-replicates of each genotype

RNA-seq analysis : transcriptome reconstruction

Library complexity - How to determine the possible origin(s) of the RNA-seq reads?

Barley cv Betzes	Wheat cv Chinese Spring	Wheat-Barley 7HL **addition line**
2n=2x=14 (HH) 1 genome	2n=6x=42 (AABBDD) 3 sub-genomes	(AA BB DD 7HL’’) 2n= 42 wheat chromosomes + 2 x 7HL telosomes
Paralogous copies of genes and duplicated conserved genic regions in the genome	Potentially 3 homoeologous **highly conserved copies** of gene between the **three sub-genomes**	**Orthologous** copies of genes between wheat and barley

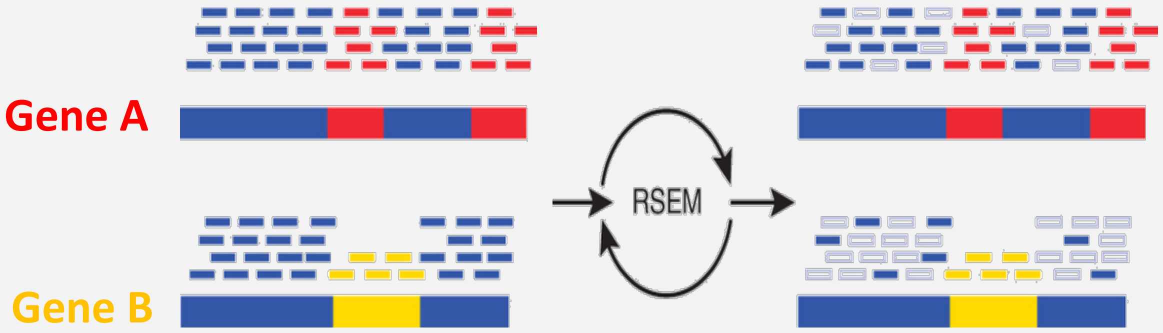
RNA-seq reads were mapped against **High Confidence CDS** of both
wheat (IWGSC RefSeq v1.0, see IWGSC, 2018) + **barley** (IBSC RefSeq, see Mascher et al., 2017)

RNA-seq analysis : transcripts abundance

- Significant proportion of multi-mapped reads → fine tuning of read mapping stringency and estimation of transcripts abundance

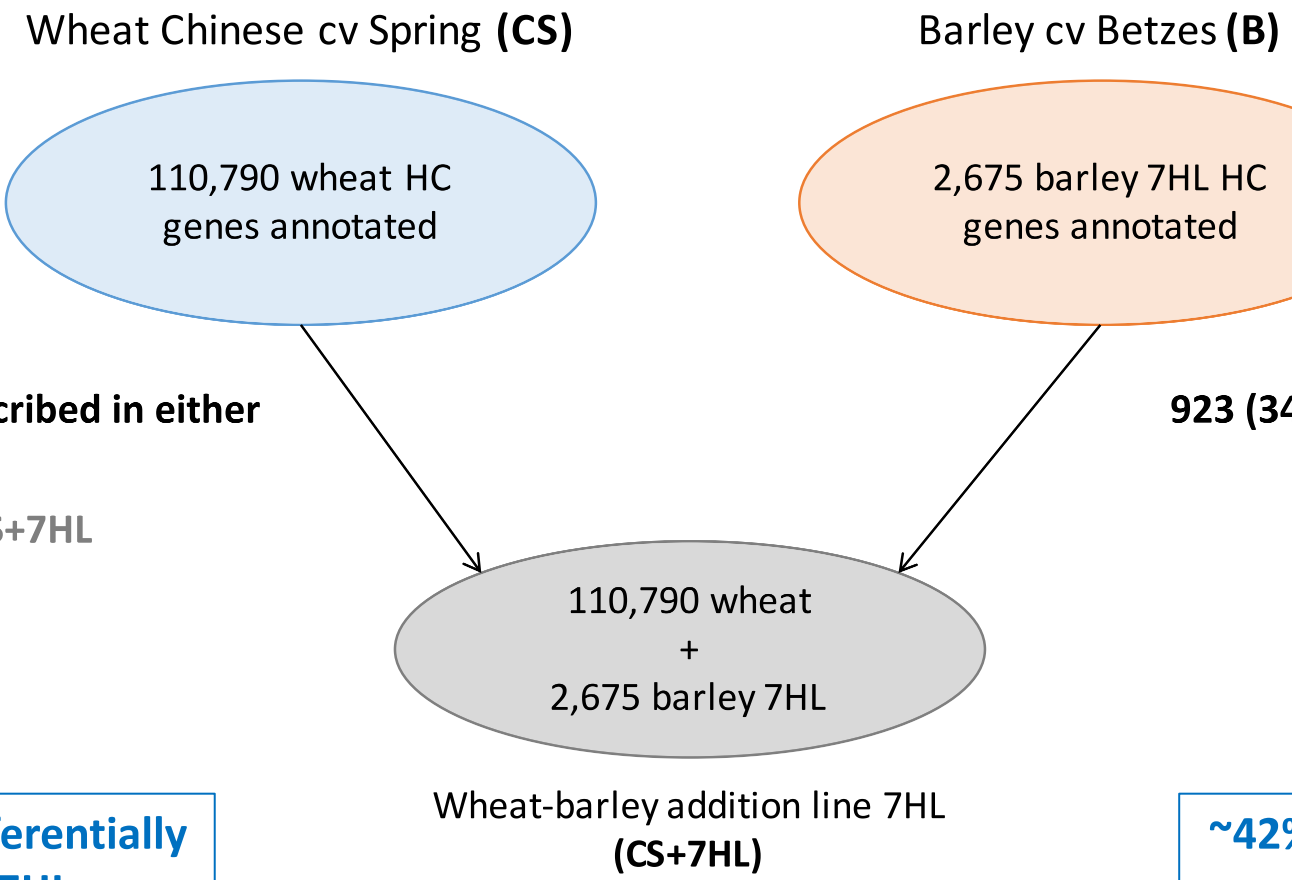
Stringent reads mapping
(0 mismatches in the seed using Bowtie)

Normalization of multi-mapped reads via maximization expectation (RSEM)



Differential Expression analysis
(GLM analysis using edgeR
–
DE genes at FDR 5%)

Differential Transcription: results



Wheat Chinese cv Spring **(CS)**

110,790 wheat HC genes annotated

Barley cv Betzes **(B)**

2,675 barley 7HL HC genes annotated

110,790 wheat
+
2,675 barley 7HL

Wheat-barley addition line 7HL
(CS+7HL)

35,301 (31.8%) genes transcribed in either wheat parent or CS+7HL

34,341 (97.2%) not-DT in CS+7HL

↑ **451 (1.2%) up-regulated**

↓ **509 (1.4%) down-regulated**

~3% of wheat genes differentially transcribed in CS+7HL

923 (34.5%) genes transcribed in either barley parent or CS+7HL

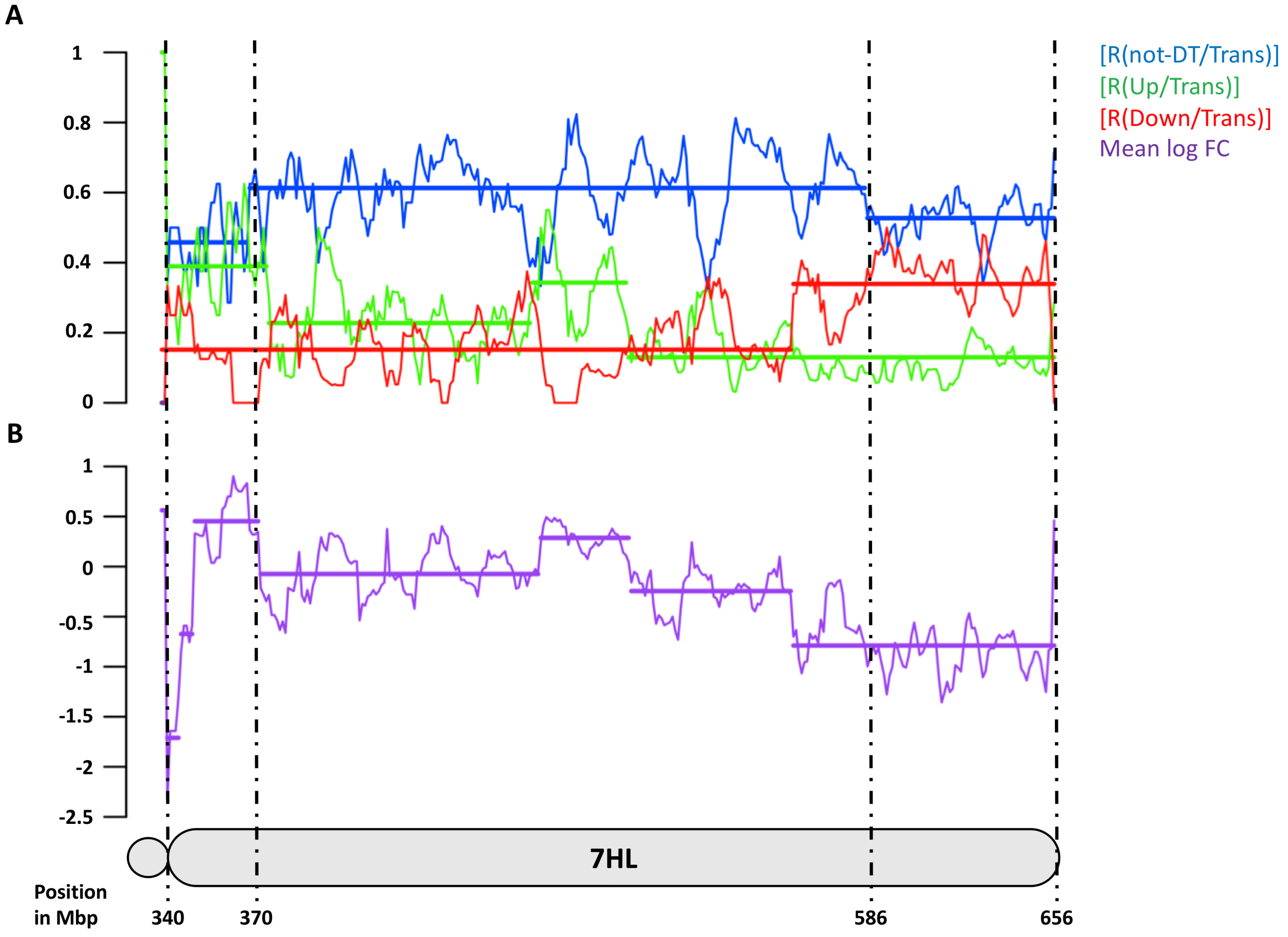
534 (57.8%) not-DT in CS+7HL

156 (16.9%) up-regulated ↑

233 (25.2%) down-regulated ↓

~42% of 7HL genes differentially transcribed in CS+7HL

Distribution of Differentially Transcribed (DT) genes along 7HL chromosome-arm in CS+7HL



Increasing centromere to telomere gradient of down-regulation

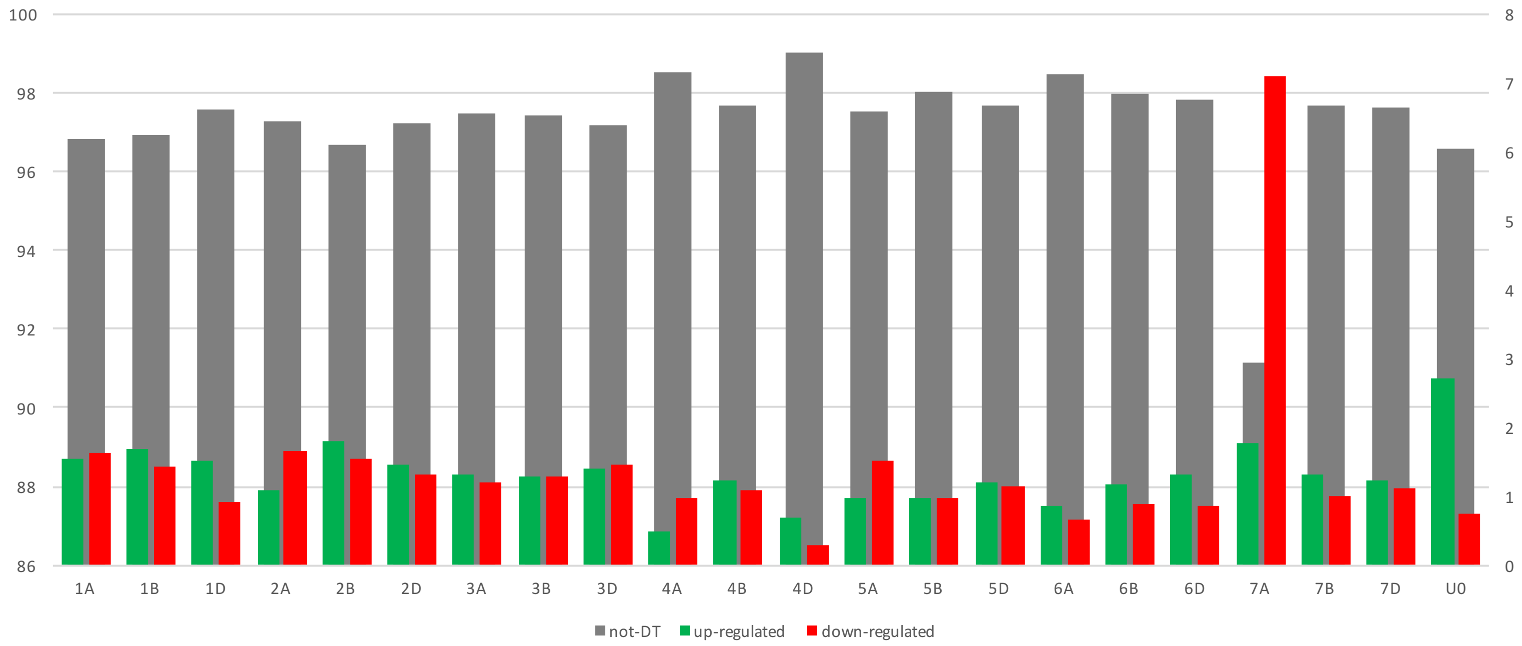
- Proximal region enriched for up-regulated genes
- Distal region enriched for down-regulated genes
- Interstitial region neutral

Gene Ontology enrichment analysis revealed:

- Up-regulated genes enriched in terms related to ‘protein synthesis’
- Down-regulated genes enriched in terms related to ‘cell recognition’, ‘sexual incompatibility’

Distribution of Differentially Transcribed (DT) genes along wheat chromosomes in CS+7HL

- Average of 24 down-regulated and 20 up-regulated genes per chromosome
- Except for 7A chromosome (**117 down-regulated** + **29 up-regulated** genes)

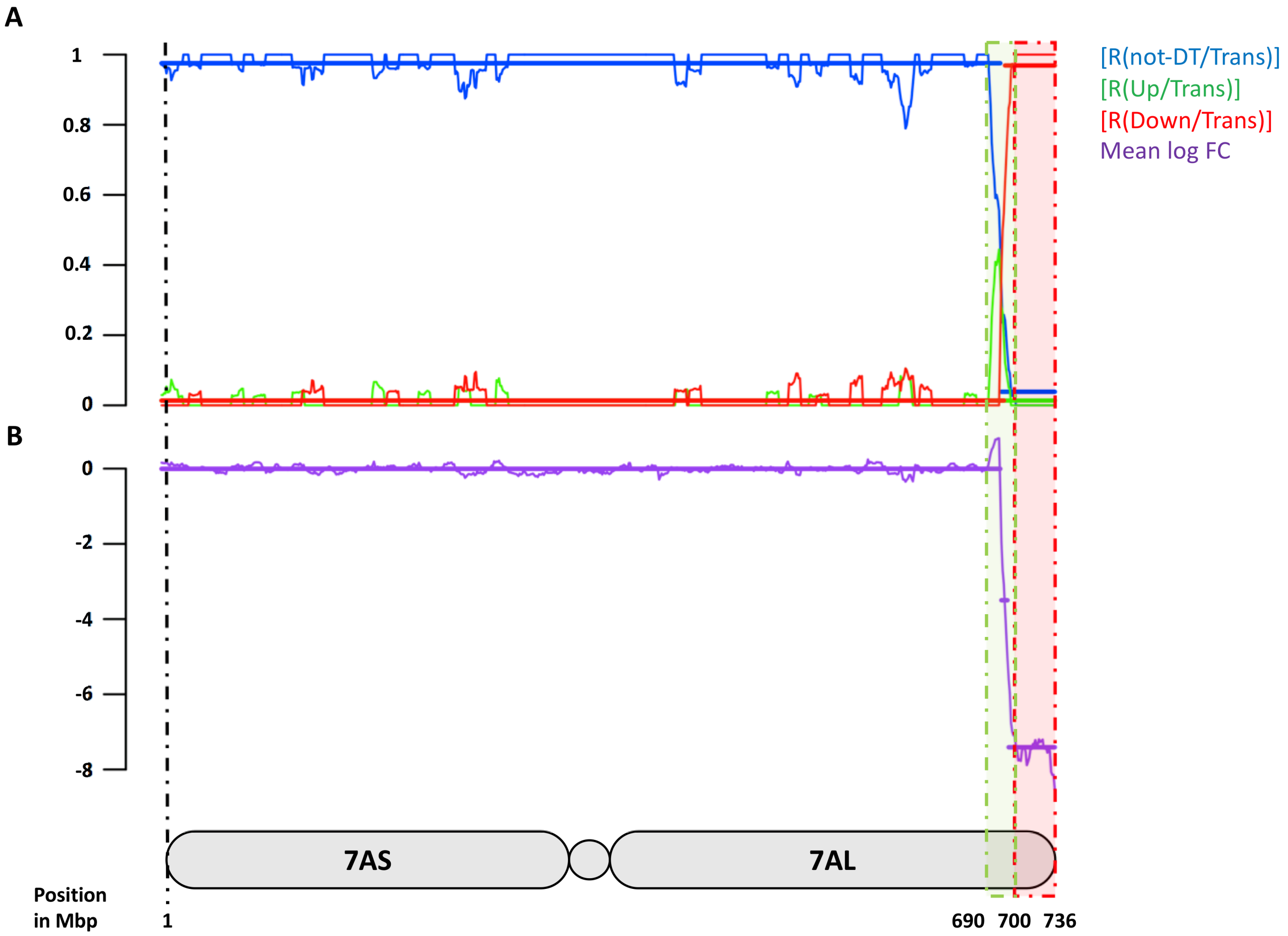


- Alterations to the wheat host transcriptome are **non-random**

  Up-reg = response to biotic, abiotic and oxidative stresses

  Down-reg = energy production
- Reflects a response to a **loss in homeostasis at the protein and metabolite levels**

Distribution of DT genes along wheat 7A chromosome



Up-reg ~10Mb region (690-699 Mb):

- 27 transcribed genes
- 12 are DT (all up-regulated)

Down-reg ~36Mb region (700-736 Mb):

- 101 transcribed genes
- 99 down-regulated
- Mean logFC = -8.2
- 25 genes completely switched-off
- Mean abundance = 0.25 FPKM

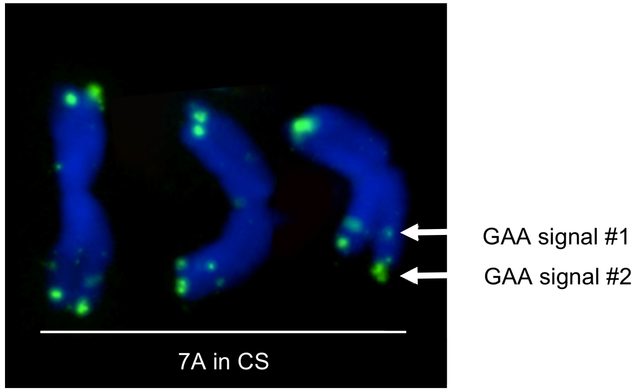
Cluster of down-regulated genes at the distal end of chromosome 7AL (700-736 Mb) strongly repressed

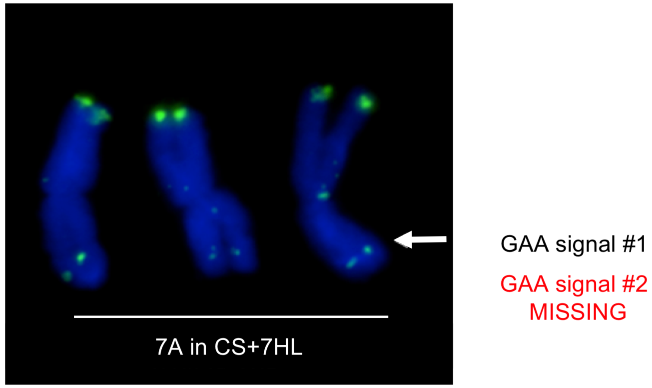
CS+7HL carries a 36Mb deletion in 7AL chromosome-arms

- **A 36Mb deletion found at the distal end of 7AL chromosome-arms in CS+7HL**

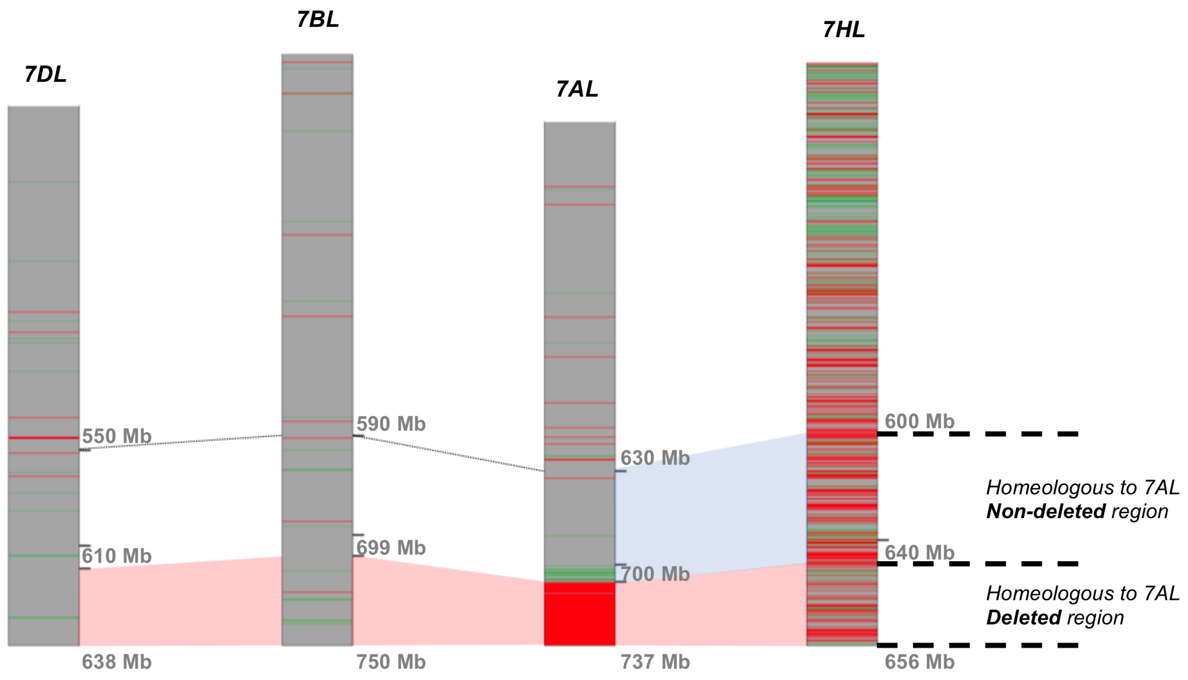
  → Confirmed by cytogenetics, flow-cytometry and molecular markers

- The 7AL distal deletion is shared by all CS/B chromosome addition lines
- The deletion was likely **created during hybridization** (present in the amphiploid) or **introduced by the CS parental line**



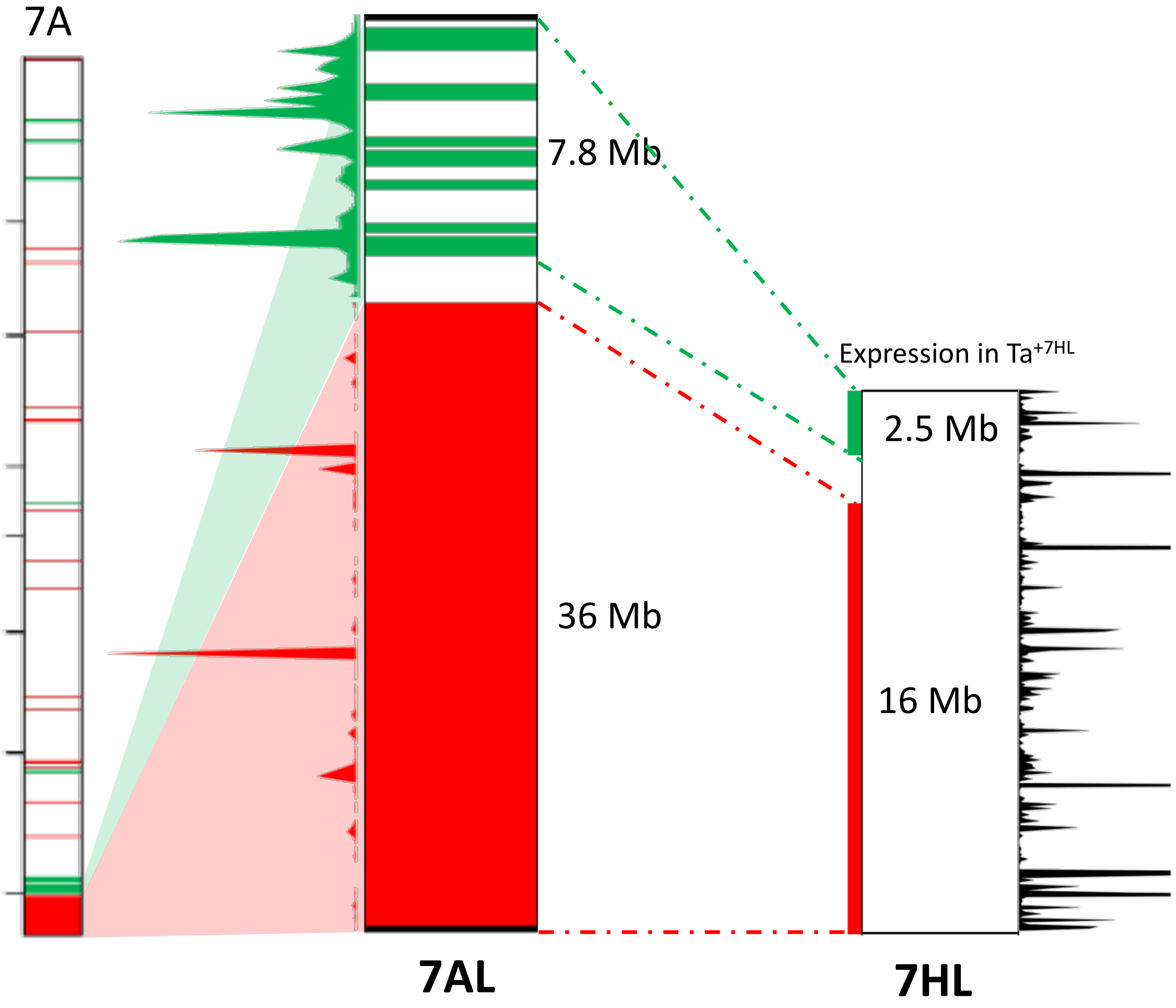


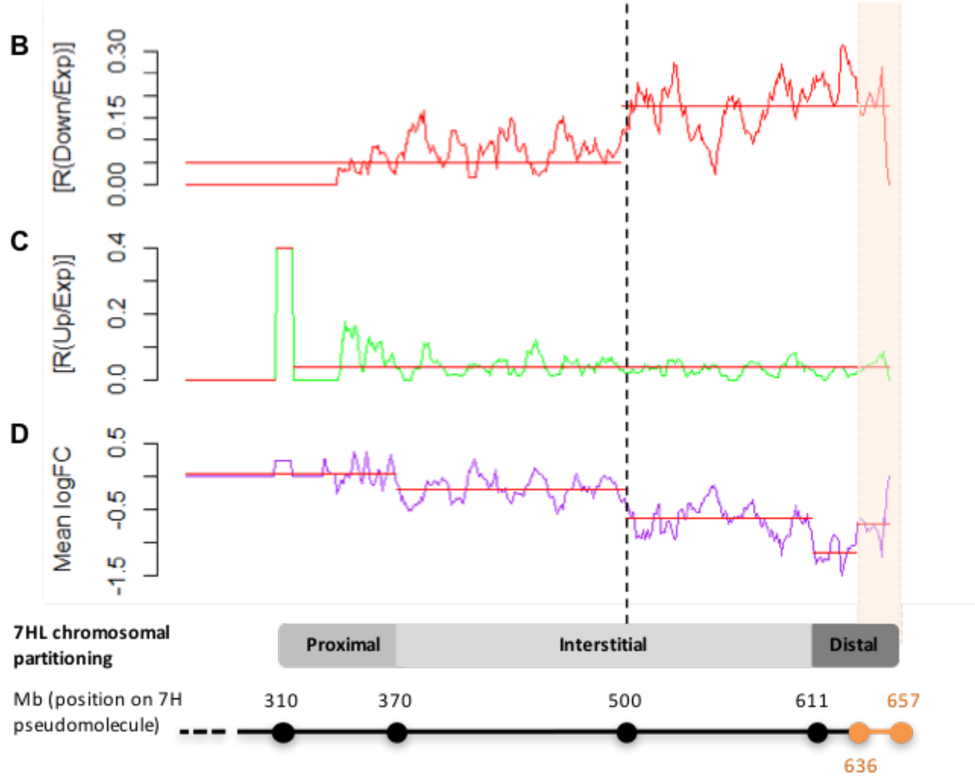
The distal deletion on 7AL does not induce differential transcription of homologous genes



- 7AL deletion does not induce differential transcription of 7BD homeologous genes
- No evidence for higher proportion of differential transcription of genes in 7HL homeologous fraction of genes
- Loss of 7AL at least partially compensated for by 7HL orthologous transcripts pool

Genome-guided transcripts assembly





7HL homologous region showed significantly higher expressed transcripts in CS+7HL vs B

Conclusions

- Established a protocol to assess DT in wheat-barley introgression line using the **latest genome sequence resources** for wheat and barley + **stringent criteria** for RNA-seq analysis
  - ➢ **Opens the way for further transcriptomic analysis** in other alien introgression lines, including breeding plant material
- The RNA-seq analysis revealed
  - **Unbalanced effect of inter-genome interactions** on the expression of **barley 7HL (~42% DT)** and **wheat (~3% DT) genes** in the addition line
  - Though the DT genes in the CS + 7HL line were **randomly distributed** across the genomes, they were **specific changes in relation to their function**
  - ➢ This set of DT genes provides a **resource for investigating the molecular basis of DT**, which may involve regulatory sequences, epigenetic changes or the organization of chromosome domains in the interphase nucleus
  - ➢ Identified a **set of genes of potential relevance for interspecific incompatibility** between wheat and barley
- Revealed an unexpected deletion in the host wheat genome → Should make us wary about the cytogenetic stocks

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