



Simposio: Mejoramiento genético por resistencia a enfermedades e interacciones planta-patógeno

# GENETICS OF LEAF RUST RESISTANCE IN THE BRAZILIAN WHEAT CULTIVAR TOROPI

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- **LEAF RUST** (caused by *Puccinia triticina*) is a major problem to wheat production.
- The southern region of Latin America is a suitable environment for the disease to develop.
- *P. triticina* has a high rate of physiological specialization and rapidly overcome resistance genes.



So the knowledge of durable resistance genes became an important alternative to the rusts problem.

- Breeding for leaf rust resistance is complicated by the high level of genetic variation within the Brazilian *P. triticina* population.



- Under favorable environmental conditions it causes heavy losses on susceptible wheat cultivars.

Ideal conditions: 15-20 °C;  
leaf wetness: 20 °C – 3h  
+ 10 °C – 10-12 h



- Genetic resistance is an ecologically and economically sound approach to disease control in crop plant.

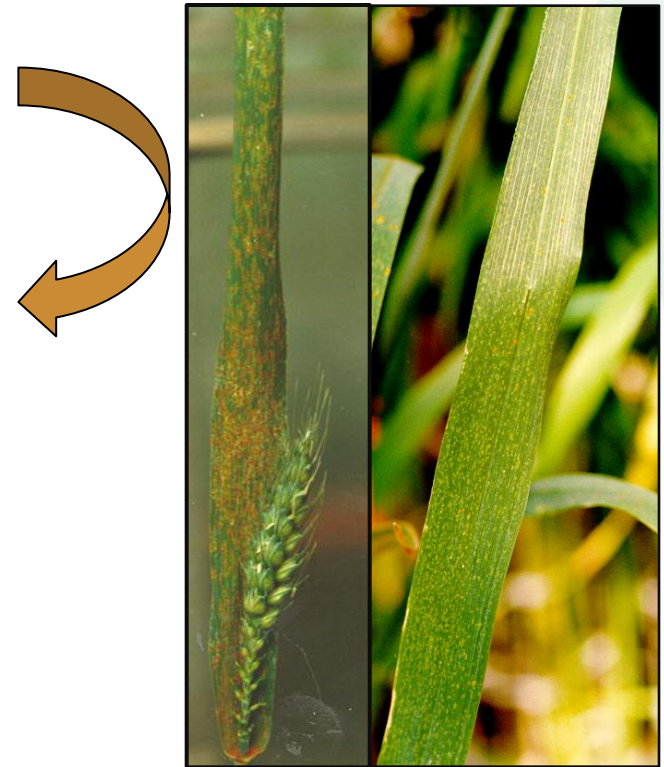


S.P. Brammer

In Brazil, the severity of the damages caused by leaf rust in bread wheat shows varying intensity, depending on weather conditions, genetic resistance of cultivars, and chemical control.

**Genes for resistance** can be effective in both seedling and adult stages of plant development.

Some genes express their resistance only in the adult plant growth stage. This type of resistance has been associated with **longer durability resistance**.



S.P.Brammer

# Wheat Culture

**Cultivar Frontana:** 1940 – Fronteira/Mentana:

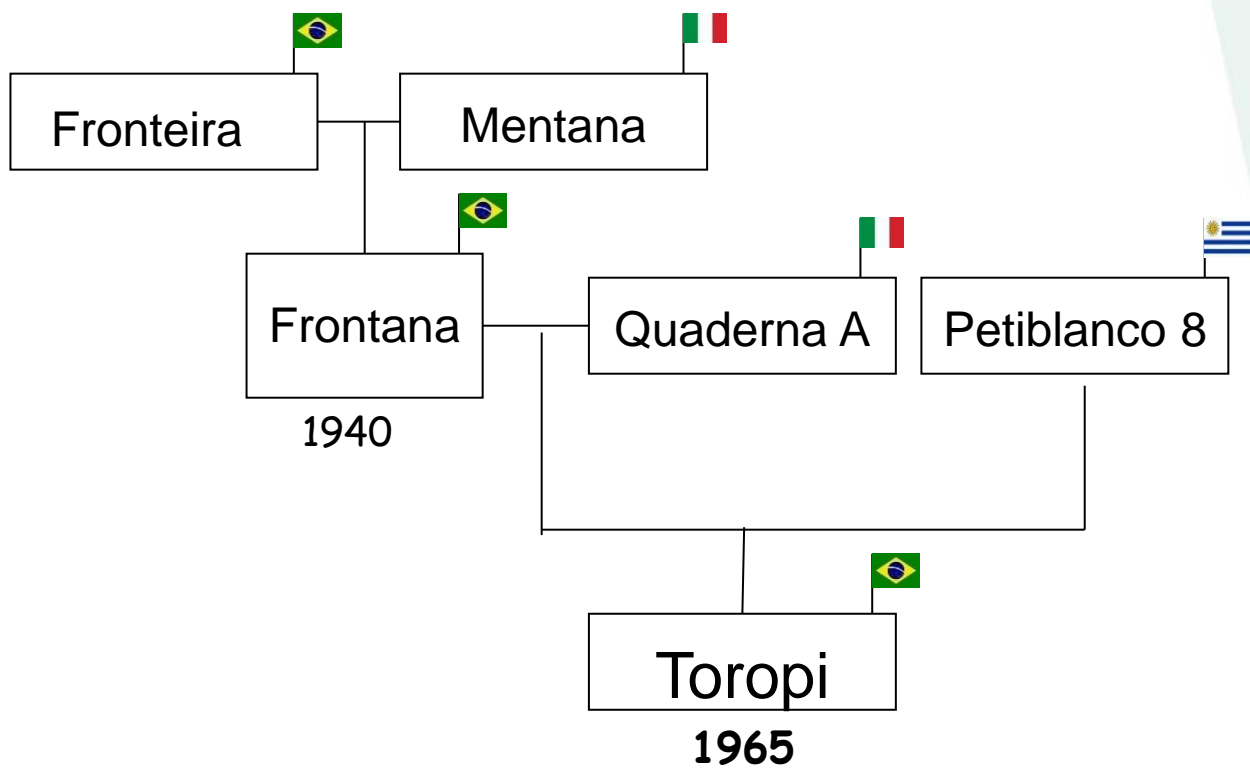
- short cycle;
- leaf rust resistance (*Lr13* and *Lr34*);
- yellow rust resistance;
- intermediate resistance to Fusarium Head Blight;
- short stature.



# CULTIVAR TOROPI

Montevideo  
9 y 12 de octubre de 2016  
Radisson Victoria Plaza

Congreso  
Latinoamericano de  
Genética

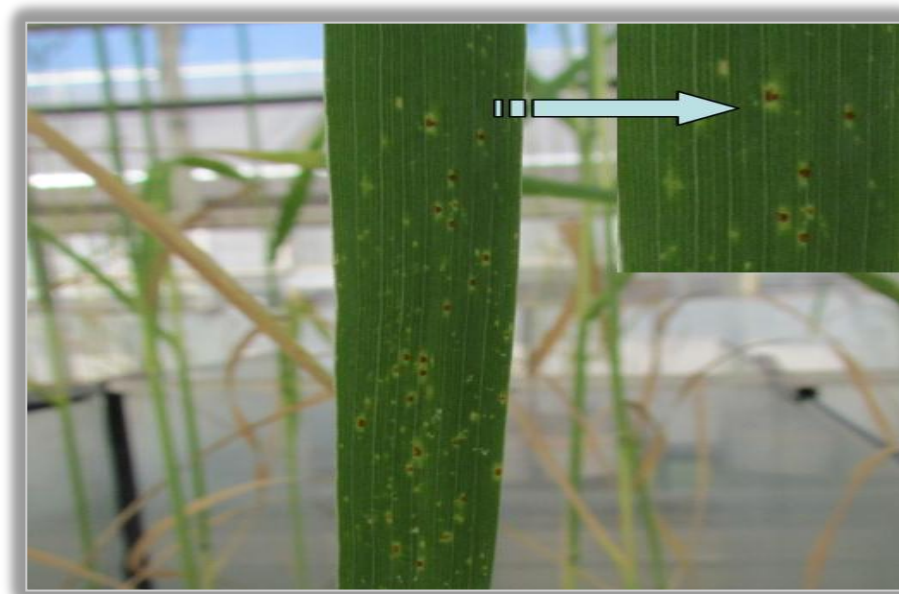




- The Brazilian cv. **Toropi** expresses a unique, durable source of leaf rust adult plant resistance (APR) that has remained effective for more than 50 years.
- Toropi has two recessive genes with additive effects: *Trp1* (1AS chromosome) and *Trp2* (4DS chromosome), and shows a pre-haustorial resistance phenotype.
- In addition, Toropi also has resistance to stripe and stem rust, carries moderate resistance to Fusarium Head Blight, tolerance to aluminium and exhibits good phosphate-use efficiency

## Leaf rust phenotype on the wheat cv. Toropi

The adult plant leaf rust resistance in Toropi is characterized by a mixture of small, off-white to yellow flecks characteristic of necrotic and chlorotic plant reactions, and by the occasional leaf rust pustule.



A. Casassola



## Gene Expression in wheat in response of *Puccinia triticina* infection



A. Casassola

- Objective: To identify, characterize and validate differential expressed sequences involved in the adult plant resistance against leaf rust of the cultivar Toropi.

## Activities

### PhD A.Casassola:

- Toropi inoculated (MDT-MR) x mock: 0, 6, 12, 24 hpi (three biological replicates) – Embrapa Trigo
- RNA-Seq - National Institute of Agricultural Botany (NIAB), Cambridge and The Genome Analysis Centre (TGAC), Norwich.
- Comparison to the available Chinese Spring (CS42) transcriptome – Embrapa Trigo / Embrapa Informática Agropecuária
- Analysis of differentially expressed genes were made via bioinformatics tools: Trinity software (mounting the transcriptome) and RSEM protocol and Edger software for calculation of differentially expressed genes.
- The functional annotation was performed by BLAST software and Blast2GO®

## RNA seq

In total: we obtained **2,798,981,764** reads



**463,371** contigs were assembled - CS42 reference transcriptome



Differentially expressed: **19.633**

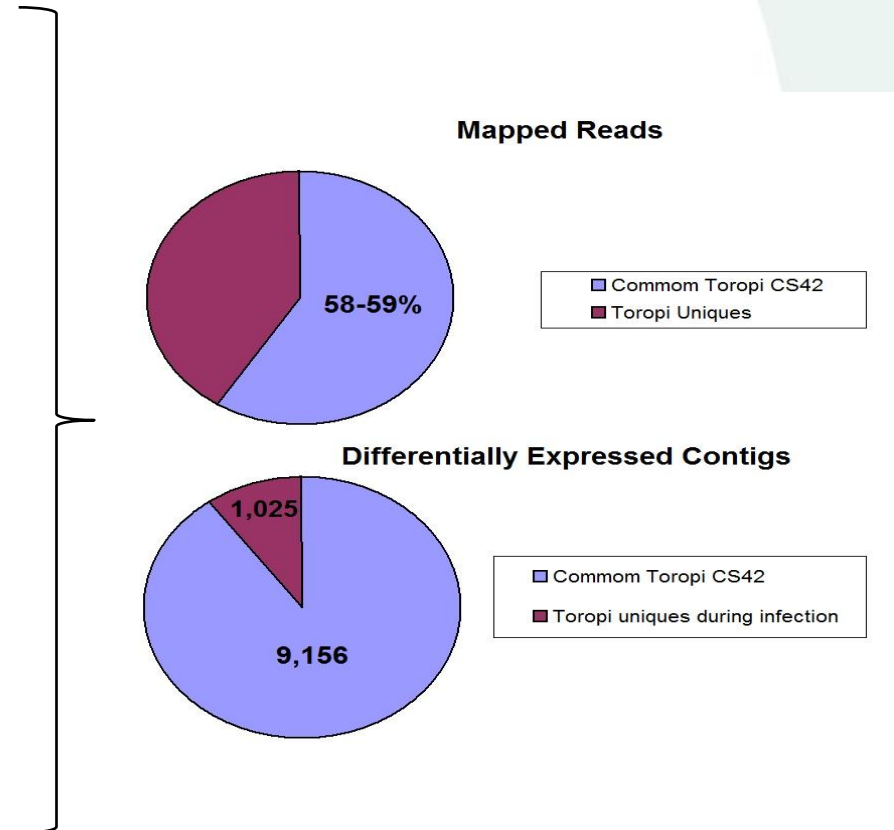
- ✓ A bulk of the Toropi libraries was used to build a reference transcriptome via de novo assembly.
- ✓ Contigs from the Toropi de novo assembly were submitted for similarity analysis using Blast against the CS42 transcriptome to identify contigs unique to Toropi.

# Toropi x Chinese Spring

Around 58-59% of Toropi reads were mapped to the CS42 transcriptome, while around 66K contigs presented no homology.

Among the 10,181 DE contigs, 9,156 were present in both Toropi and CS42,

while **1,025 contigs** which were **differentially expressed in Toropi** in the presence of the fungus had no hits in CS42



- Similarity: *Oriza sativa*, *Zea mays*, *Aegilops tauschii* e *Hordeum vulgare*
- **24 hai: most sequences are expressed in Toropi.** However, the increased expression has been observed 6 hai.
- Increased production of metabolic precursors and energy: production of defense compounds;
- Increased expression of genes related to the transport of molecules: supply for synthesis and regulation



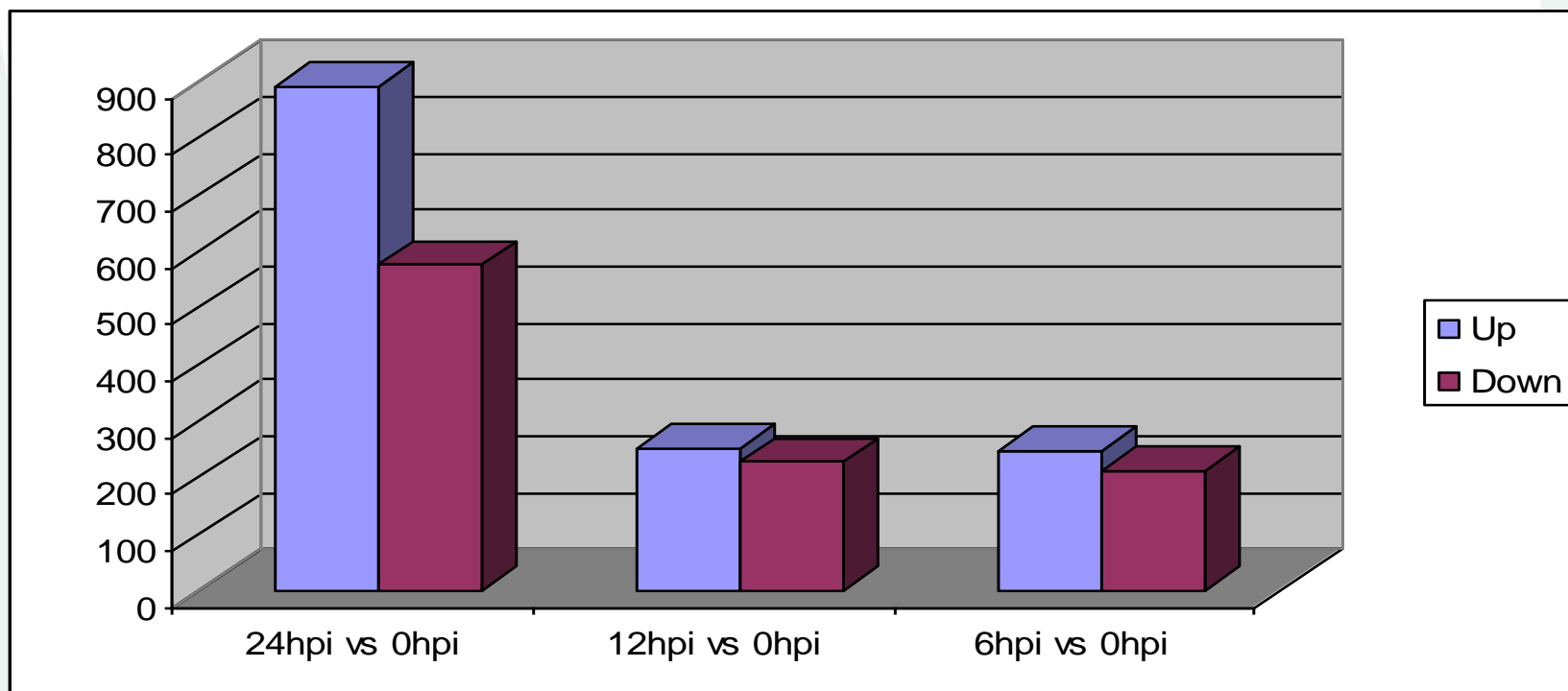
A. Casassola

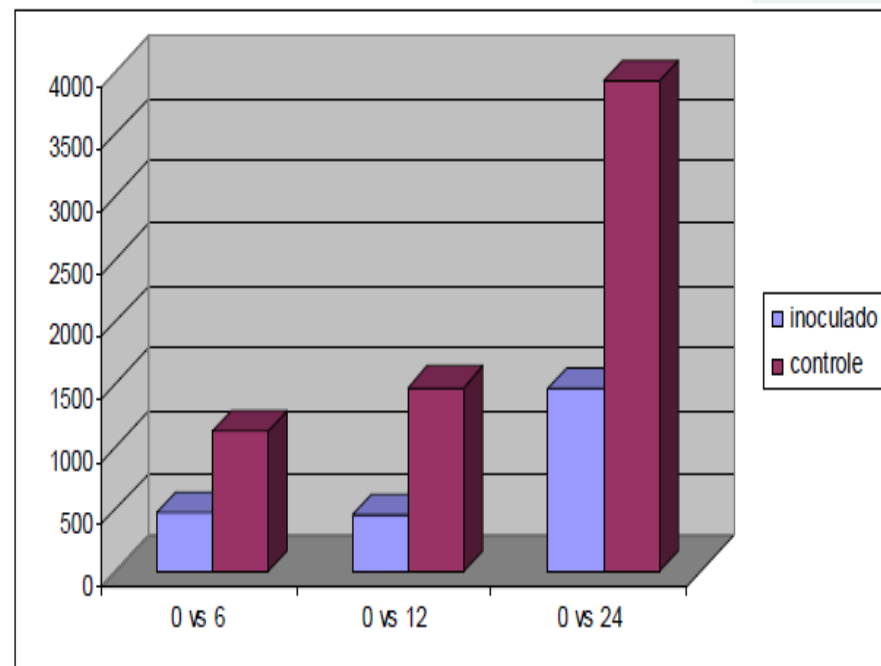
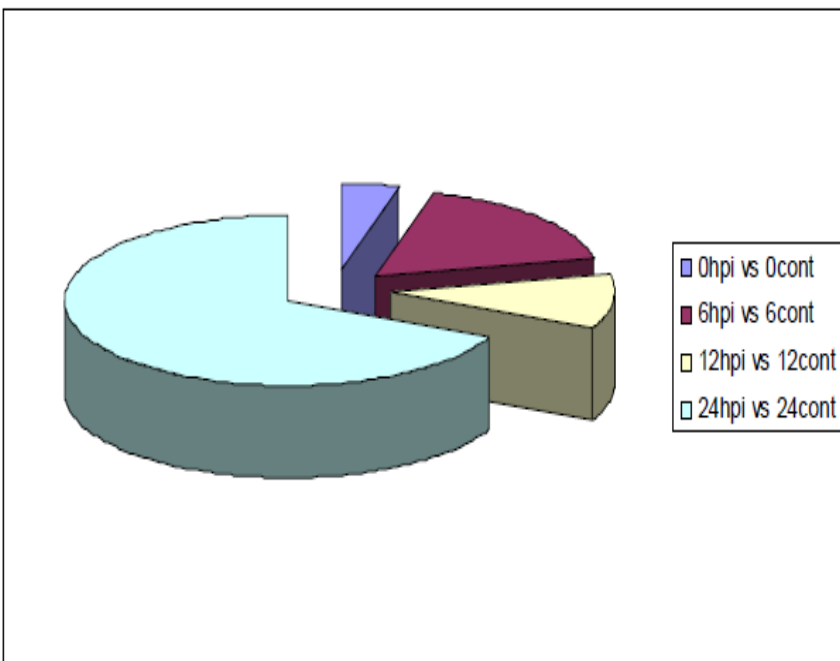
# RNA seq

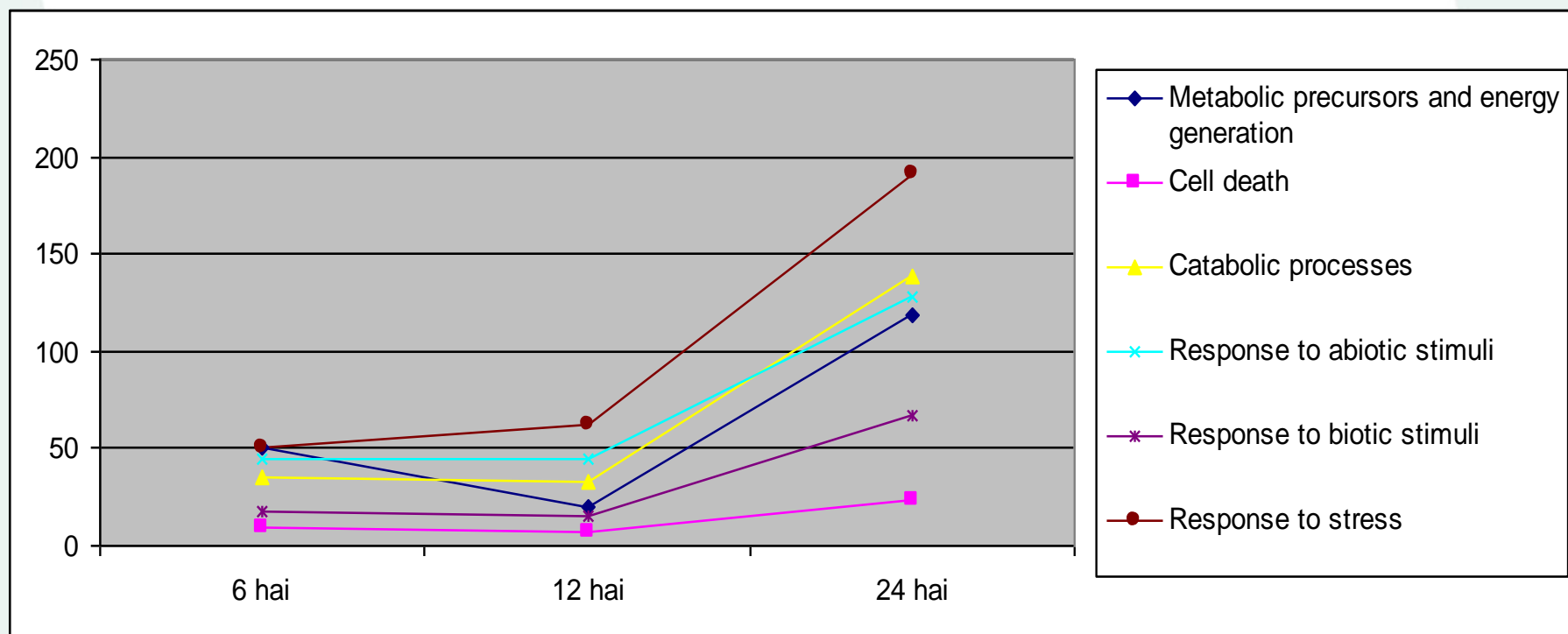
- 463.371 contigs;
- 19.633 differently expressed;
  - Inoculated versus control
    - 6 hpi – 100 contigs;
    - 12 hpi – 250 contigs;
    - 24 hpi – 1.631 contigs.
  - Inoculated *versus* zero
    - 6 hpi – 479 contigs;
    - 12 hpi – 458 contigs;
    - 24 hpi – 1.469 contigs.
  - Control *versus* zero
    - 6 hpi – 1.122 contigs;
    - 12 hpi – 1.475 contigs;
    - 24 hpi – 3.928 contigs.



- More contigs were up-regulated







## Validation

Similar behavior RNA-Seq and qPCR - mean correlation of 0.69

### Difficulties - Wheat:

- Different times after inoculation;
- Wide Genome, hexaploid and highly repetitive;
- Tools have been developed for species models;
- No reference genome.

## Validation selected contigs

- Cysteine-rich receptor-like protein kinase 26: catalytic activity, releases energy;
- Indole-2-monooxygenase: formation of DIBOA, allelopathic activity and defense;
- CBL – interacting protein kinase 32: catalytic and cofactor activity - defense signaling;
- O-acyltransferase WSD1: cutin biosynthesis
- Transcription factor Y (NF-YA-7): increase resistance in Arabidopsis and maize in response to biotic stress

- These Toropi-specific sequences could represent new resistance candidate genes to be used in wheat breeding and need further study to determine their role in plant defense.
- These results will be of interest to the broader plant-pathogen research community.





## Research in progress

A high-density genetic map of the Toropi x IAC 13 RIL population using DArTseq SNP and KASPar technology is currently being generated between Embrapa Trigo, Universidade Federal do Rio Grande do Sul (Brazil) and National Institute of Agricultural Botany (UK).

The current proposal focuses on identifying and functionally characterising the genes responsible for the unique leaf rust APR.



## Research in progress

In addition, to help understand the mode of action and biology underlying these leaf rust APR genes we will study the development of the leaf rust fungal pathogen in wheat at the microscopic level, infecting wheat plants at different stages of its growth and when grown at different temperatures

We also aim to incorporate these Toropi Brazilian wheat varieties, aided by the development of markers for Marker-Assisted Selection.



## TEAM

- Sandra Patussi Brammer - Embrapa Trigo/Passo Fundo, RS.
- Márcia Soares Chaves - Embrapa Clima Temperado/Pelotas, RS.
- Alice Casassola – Faculdades Ideau/Passo Fundo, RS.
- Lesley Boyd – NIAB/Cambridge UK.
- Antônio Martinelli – Universidade Federal do Rio Grande do Sul/Porto Alegre
- Paulo Roberto da Silva – Universidade do Centro Oeste/Gurapuava, PR.
- Caroline Wesp-Guterres – CCGL Tech
- Paula Regina Kuser Falcão and Adhemar Zerlotini - Embrapa Informática Agropecuária/Campinas, SP.
- Gerarda Beatriz Pinto - Universidade Federal do Rio Grande do Sul
- Andréia Caverzan, Paula Wiethölter, Antonio Nhani Junior, Alceu José Vicari, Marcos Copetti, Andréa Morás, Valdirene Volpato - Embrapa Trigo
- Francesca Stefanato – NIAB/UK.



**Thank you**  
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MINISTÉRIO DA  
**AGRICULTURA, PECUÁRIA  
E ABASTECIMENTO**

