Transcriptional reprogramming activated by Wheat Tandem Kinase 1 in response to stripe rust infection

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1. Introduction

Durum wheat (*Triticum durum*) is the second most cultivated wheat, used for the production of pasta, couscous, burghul, etc., accounting for 8% of total world wheat production. Wheat production can be severely restricted by devastating diseases such as stripe rust, caused by the fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*) (*Fig. 1*).

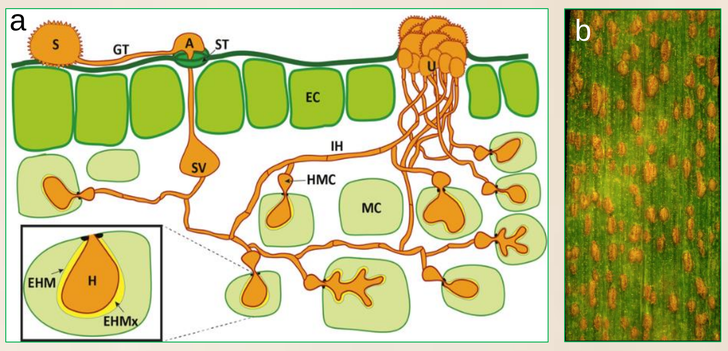


Figure 1. (a) Schematic representation of the developmental phases of stripe rust infection on wheat (Garnica et al., 2014). **(b)** *Pst* sporulation on susceptible wheat plant.

1.1 *WTK1*

***Yr15* is a wild emmer wheat (*Triticum dicoccoides*) (*Fig. 2a*) gene, conferring high resistance to a wide spectrum of *Pst* isolates (*Fig. 2b*). *Yr15,* cloned by our lab, encodes a tandem kinase-pseudokinase protein, designated as wheat tandem kinase 1 (WTK1) (*Fig. 2c*) (Klymiuk et al., 2018). Tandem kinase proteins (TKPs) represent a novel protein family that emerged as a new player in plant innate immunity.**

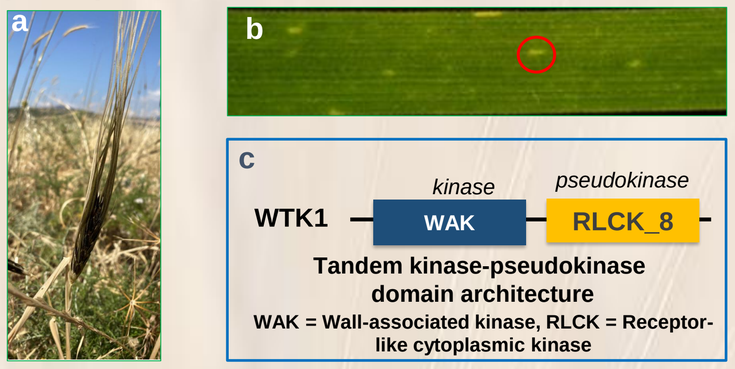
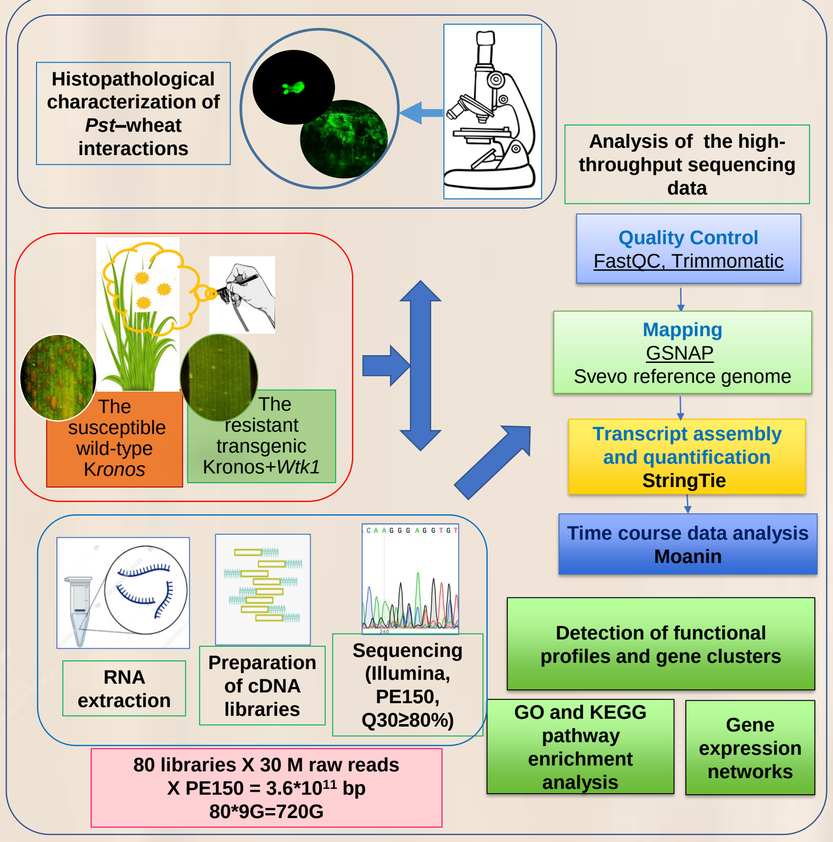


Figure 2. (a) Wild emmer wheat (*Triticum dicoccoides*). **(b)** Hypersensitive response to stripe rust infection on wheat carrying WTK1 resistance gene. **(c)** WTK1 tandem kinase-pseudokinase domain architecture (Klymiuk et al., 2018).

2. Methodology



3. RESULTS

3.1 Histopathological characterization

Wheat-*Pst* interactions were characterized using fluorescence microscopy. Fungal growth and symptom development were followed along 16 days post-inoculation (dpi) (*Fig. 3*). Leaf samples (1-7 dpi) were used for transcriptome analysis.

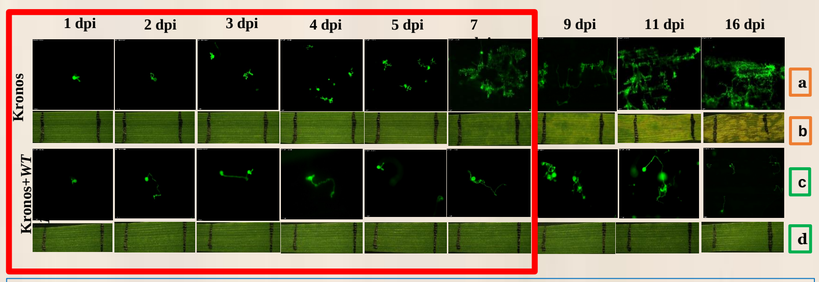


Figure 3. A comparison of the responses of the susceptible Kronos host plants (a, b) with the resistant Kronos+*Wtk1* plants (c, d) over 16 dpi inoculated with *Pst.* (a, c) Fungal development within leaf tissues followed by fluorescence microscopy. (b, d) Macroscopic observations of symptom development. The scale bar is 50 µm.

3.2 Analysis of differential gene expression

A total of 3357 differentially expressed (DE) genes (qvalues<0.05, LFC=+/-2) were detected between Kronos and Kronos+*WTK1* (*Fig. 4*), of which 1131 were upregulated and 2226 were downregulated. The 3357 DE genes were clustered into 10 different patterns of expression along 7 dpi (*Fig. 4*).

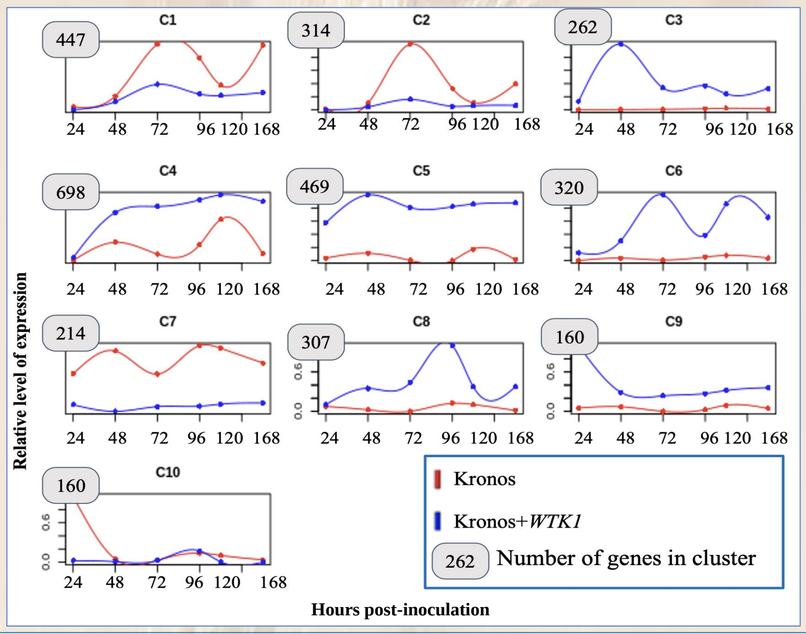


Figure 4. Differential expression analysis (global analysis over time). DE genes were filtered to represent only genes that showed significant DE with a large-fold change between groups. Spline clustering identified 10 clusters showing various regulation patterns of differentially expressed genes.

3.3 Transcriptional patterns

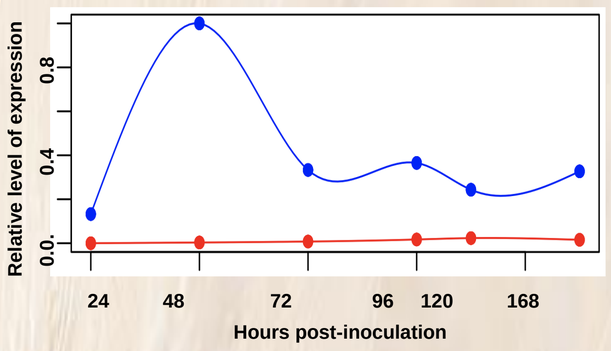


Figure 5. Cluster with up-regulation in Kronos+*WTK1.* This cluster grouped 262 DE genes that represent early events in the resistance response with a peak of upregulation in Kronos+*WTK1* at 48 h post-inoculation. This cluster included many genes that belong to pathways involved in the development of hypersensitive cell death responses.

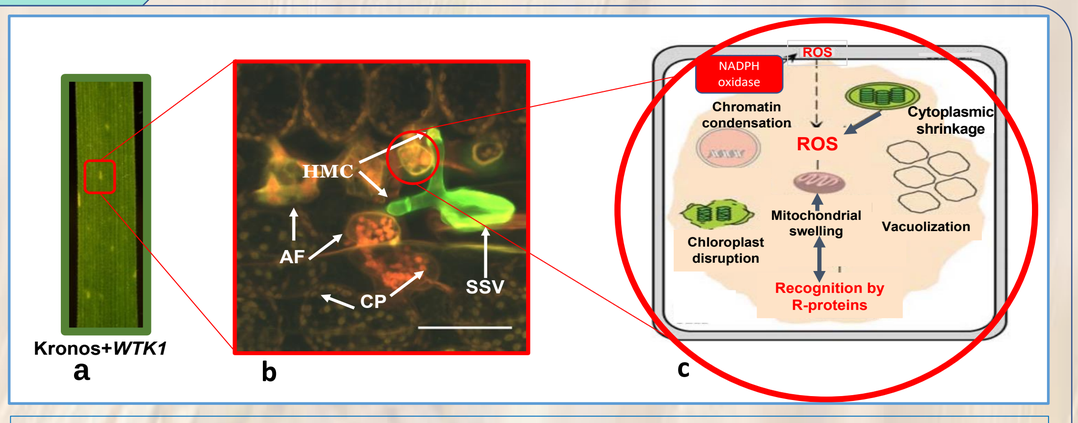


Figure 6. Resistance response in Kronos+*WTK1*. *(a)* Hypersensitive response to stripe rust infection on wheat carrying *WTK1* resistance gene. *(b)* Microscopic characterization of the interaction between wheat-*Pst* showed the formation of fungal feeding structures within leaf tissues, such as fungal hyphae (in green fluorescence) substomatal vesicles (SSV), and haustorial mother cells (HMCs). Host cells showing bright orange autofluorescence (AF, as an indicator of HR) appeared to be collapsed and distorted and contain aggregated chloroplasts (CP) (Klymiuk et al., 2018). *(c)* Schematic representation of the intracellular events occurring during the HR downstream defense responses (Modified from Coll et al., 2011) which fit and correspond to the microscopic observations as well as the gene expressions profile describe in *Fig. 5.*

3.4 Functional classification of DE genes

The key over-represented pathways identified by functional enrichment analysis in the gene clusters upregulated in the resistant Kronos+*WTK1* at 1-7 dpi are presented in *Fig. 7*.

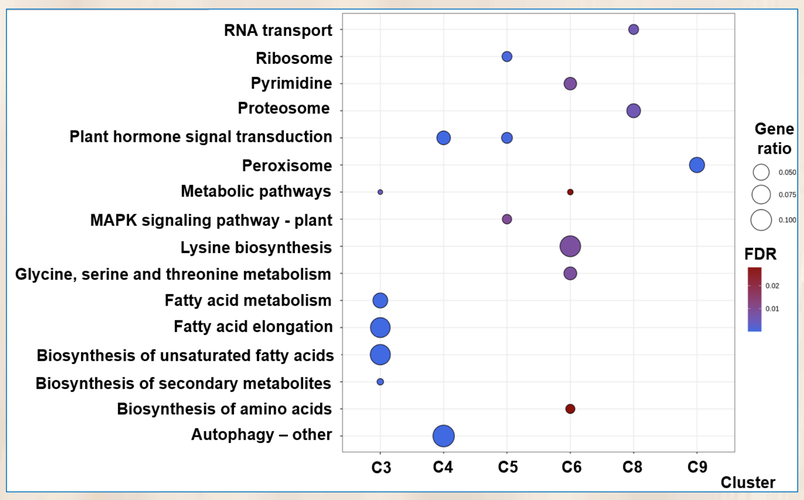


Figure 7. Over-represented biological pathways identified within upregulated gene clusters in the resistant Kronos+*WTK1* by Gene Ontology and KEGG functional pathway enrichment analysis

4. Conclusions

- **Our transcriptome analysis indicates that the key biological pathways involved in the resistance response of Kronos+*WTK1* are autophagy, homeostasis of lipids, amino acids and nucleic acids metabolic processes, and ubiquitin-proteasome systems.**
- **Additionally, key genes known to be involved in hypersensitive cell death response were up-regulated in the resistant Kronos+*WTK1* at the early stages after *Pst* inoculation and may play important roles in the resistance response activated by WTK1. These genes are encoding proteins related to signaling, phosphorylation, proteolysis, R-proteins, transcription factors, etc.**
- **Our whole transcriptome analysis revealed a gene cluster enriched with genes involved in cell death immune response that peaked at 48 hpi in the resistant line, therefore corresponding to HR cell death process detected by histopathological characterization.**
- **To conclude, the whole transcriptome analysis of wheat near-isogenic lines (NILs) revealed that transcriptional reprogramming was activated by *WTK1* upon pathogen infection and induced HR defense responses leading to disease resistance.**

5. References

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Acknowledgments