# Genome-wide analysis, functional annotation and expression profiling of thioredoxin (Trx) gene family during resistance and susceptible interaction of leaf rust pathogen with wheat (*Triticum aestivum* L.)

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## INTRODUCTION

- Thioredoxins (TRX) are small (~12kDa), ubiquitous disulphide reductases, regulate the redox status of target proteins
- First study that implicating thioredoxin activity in the regulation of plant disease resistance demonstrated a thioredoxin-homologous, interacts specifically with Cf-9 (RLP), called CITRX (Cf-9-interacting thioredoxin) negatively regulates Cf-9/Avr9-mediated cell death and defense responses in tomato and tobacco.
- Trxs are also confirmed to be involved in the orchestration of SAR in plants by its redox activity that converts the oligomeric form of NPR1, a master regulator of SAR into a monomeric form
- Trx modulates ROS scavenging and actively participates in the regulation of cellular redox homeostasis and it was reported that functional loss of Trx leads to altered levels of ROS

## MATERIALS AND METHODS

HOST MATERIAL:
The seeds of following plant material are available in the Division of Genetics
- Chinese spring (CS)
- CS+*Lr9*

PATHOGEN:
Uredospore inoculum of leaf rust pathogen *Puccinia triticina* which is being maintained in the Division of Genetics
Pathotypes:
- 77-5



1. Sowing of test materials


2. Inoculation of one week old seedlings of test materials


4. Sampling for RNA isolation


3. Incubation of inoculated seedlings in humidity chamber for 48 hrs

Screening against leaf rust in glass house

Sampling for Expression analysis Cs and Cs+Lr9

| Pathogen | Duration(Hours after inoculation) | Chinese spring(CS) | CS+*Lr9* |
|---|---|---|---|
| *P. triticina* pathotype 77-5 | 0 HAI (control) | Sample 1 | Sample 2 |
| | 24 HAI | Sample 3 | Sample 4 |
| | 72 HAI | Sample 5 | Sample 6 |
| | 144 HAI | Sample 7 | Sample 8 |

- Biological replicate- 3
- Technical replicate-3

Collection of leaf sample → Extraction of RNA → cDNA → RT-PCR → Expression analysis

Expression analysis was done using $2^{-\Delta\Delta CT}$ method (Schmittgen et al.,2001)

## RESULTS

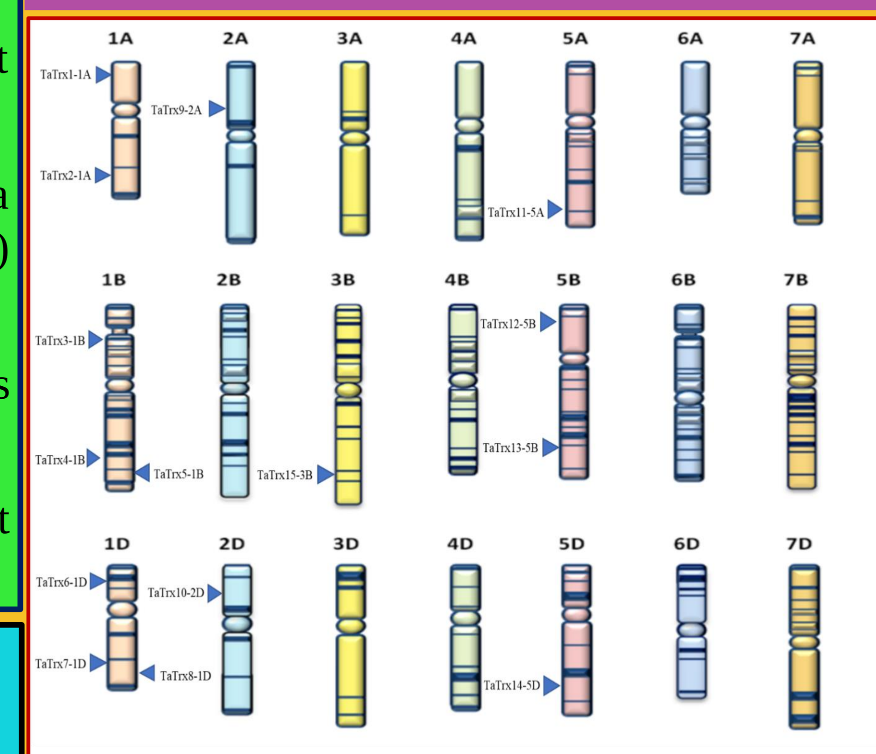
**Fig. 1** The physical positions of 15 *T. aTrx* genes to corresponding chromosomes

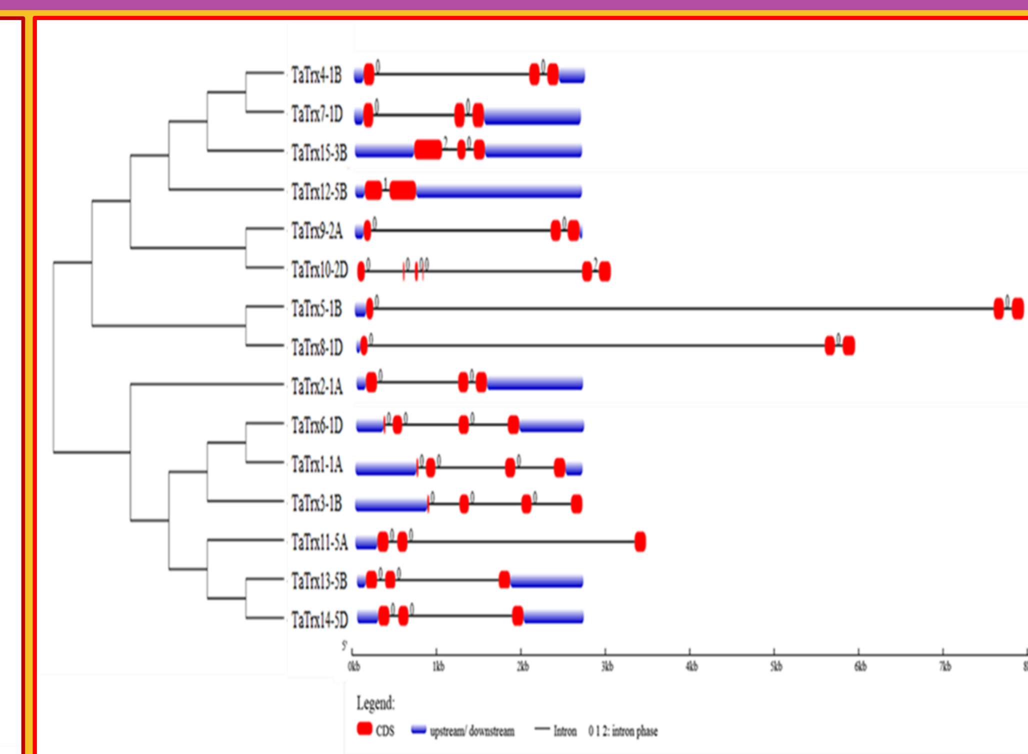
**Fig. 2** Phylogenetic grouping and gene structure of identified *TaTrx* genes.

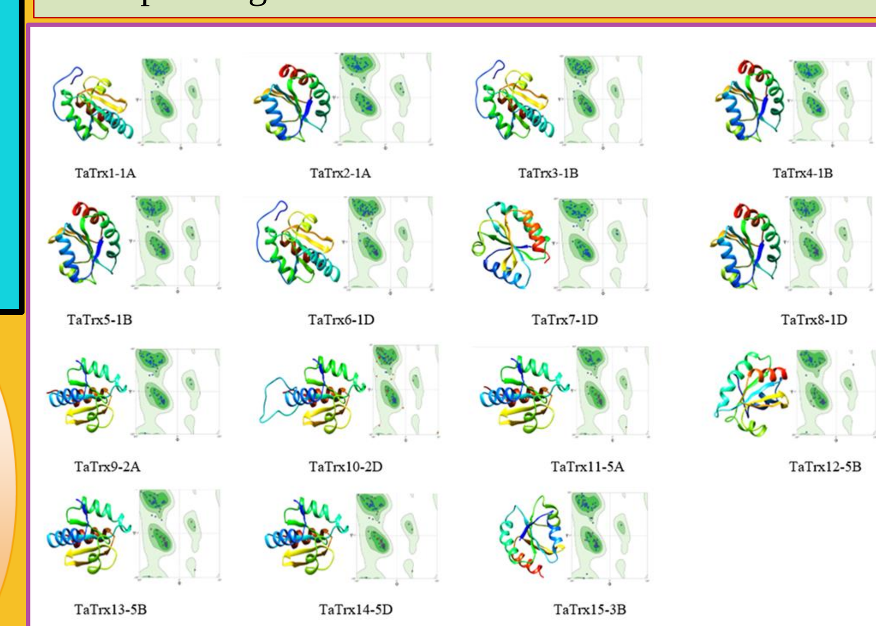
**Fig. 3** 3D structures and Ramachandran plot of TaTrx proteins.

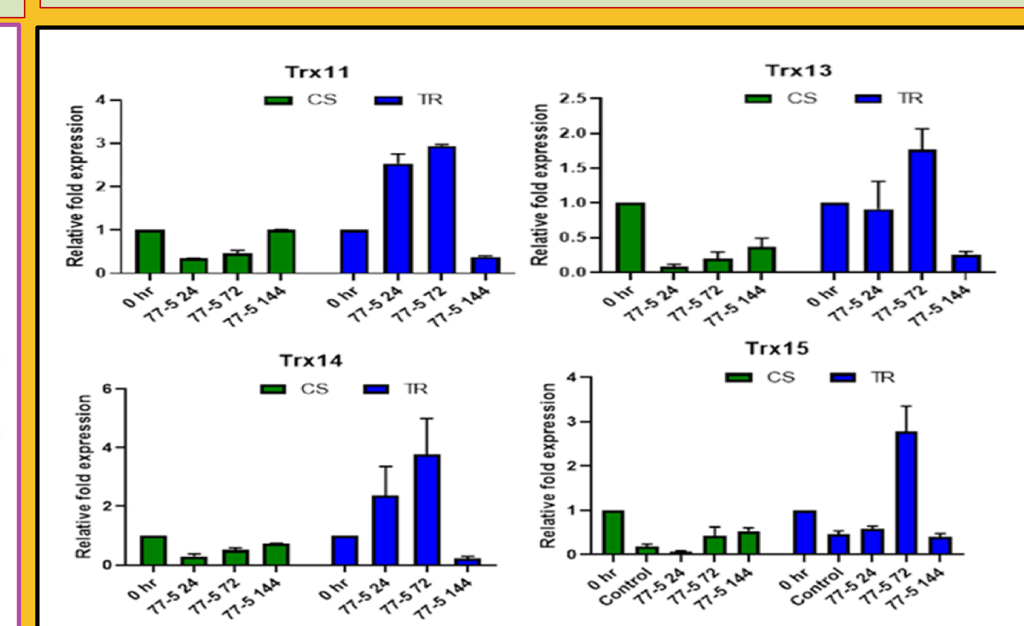
**Fig. 4** Differential expression of *TaTrx* genes against inoculation to leaf rust.

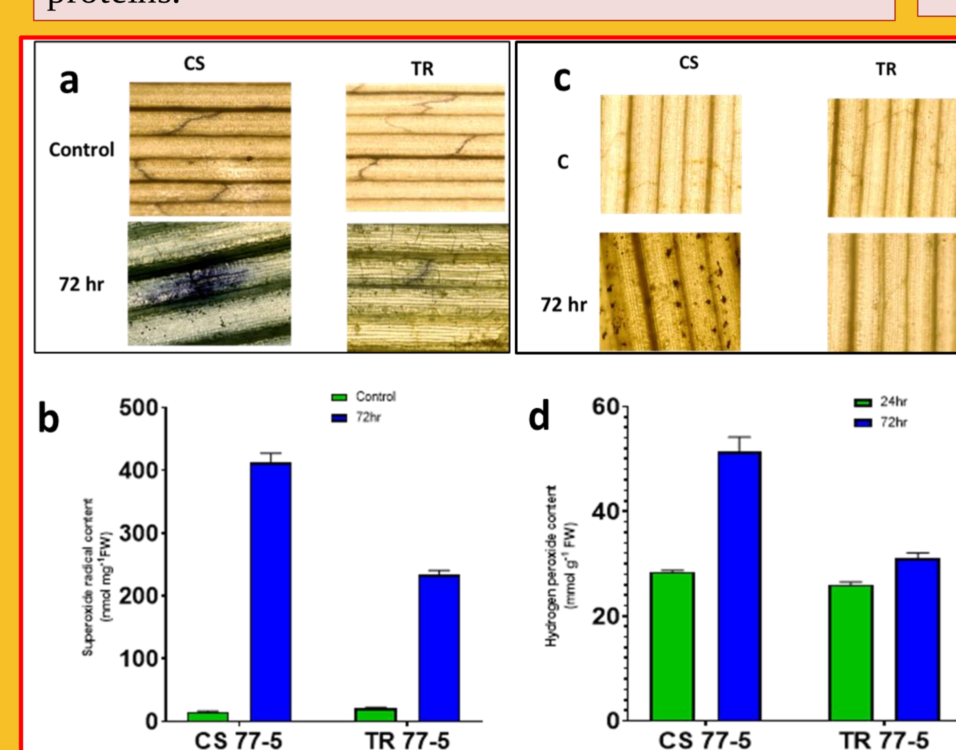
**Fig. 5** Effects of leaf rust pathogen on the localisation and quantification of reactive oxygen species, superoxide radical (a, b) and hydrogen peroxide (c, d) Chinese spring (CS) and Introgression line of leaf rust resistance gene (*Lr9*) in CS, Transfer (TR). Values are means (±SE) of 3 biological replicates.

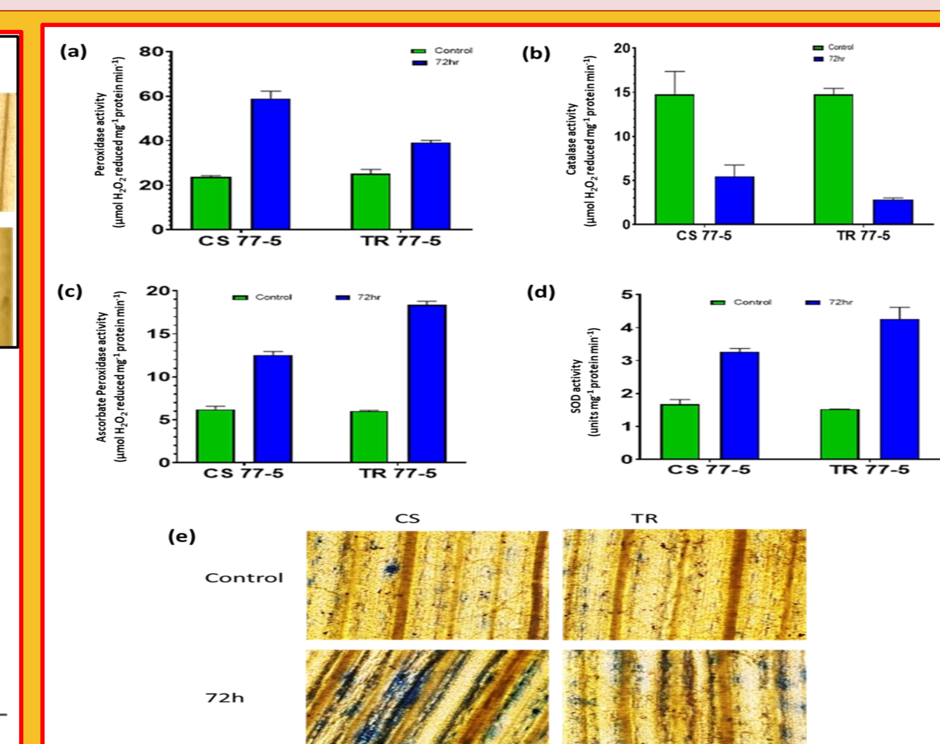
**Fig. 6** Effect of leaf rust pathogen on the activity of antioxidant enzymes **(a)** Peroxidase, **(b)** Catalase, **(c)** Ascorbate peroxidase, **(d)** Superoxide dismutase and **(e)** membrane injury visualised by Evans blue staining of leaves of wheat genotypes Chinese spring (CS) and Introgression line of leaf rust resistance gene (*Lr9*) in CS, Transfer (TR). Values are means (±SE) of 3 biological replicates.

## CONCLUSION

Our research is its first report conducted genome-wide analysis of *Trx* genes in wheat for leaf rust disease. Current outcomes not only strengthen earlier conclusions associated with the role of Trxs as a regulator of redox homeostasis (antioxidant). Expression profiles of selected 15Trxs, estimation and localization of ROS demonstrate that *Trx* gene family is associated with ROS homeostasis during biotic stress. Although previous studies indicated the participation of Trxs in various metabolic processes but role in plant immunity is so far not entirely understood. This provides an excellent opportunity to exploit Trxs in defence responses. Overall, this study provides insights into the function of *Trx* gene family in response to leaf rust and can be further utilized in establishing protein-protein interaction during defense response and immune signaling. As much of the wheat interactome data is still uncharacterized, in future characterization of wheat proteins will provide new insights into the immune pathways in-depth. Further we can target immune proteins to modulate plant immunity.

## OUTCOMES OF STUDY

- Expression of *TaTrx11-5A* , *TaTrx13-5B* , *TaTrx14-5D* and *TaTrx15-3B* genes were upregulated in incompatible interaction, indicating their role in resistance. Expression of *TaTrx1-1A, TaTrx4-1B, TaTrx7-1D, TaTrx8-1D, TaTrx9-2A, TaTrx12-5B* remained unaffected or were downregulated by leaf rust infection.
- Compatible interaction of leaf rust resulted in ROS burst as indicated by localisation and content of SOR and Hydrogen peroxide. Incompatible interaction could arrest the ROS burst by upregulating the activity of ROS scavenging enzymes APX and SOD.
- The perturbation in ROS homeostasis also indicate the involvement of thioredoxins (which has regulatory role in antioxidant enzyme activity) in leaf rust resistance.

## REFRENCES

- A. Holmgren, Thioredoxin. 6. The amino acid sequence of the protein from Escherichia coli B, *Eur. J. Biochem.* **6** (1968) 475–484.
- C. Mata-Pérez, S.H. Spoel, Thioredoxin-mediated redox signalling in plant immunity, *Plant Sci.* **279** (2019) 27–33.
- C. Vieira Dos Santos, P. Rey, Plant thioredoxins are key actors in the oxidative stress response., *Trends Plant Sci.* **11** (2006) 329–334.
- M. Moreau, M. Tian, D.F. Klessig, Salicylic acid binds NPR3 and NPR4 to regulate NPR1-dependent defense responses, *Cell Res.* **22** (2012) 1631–1633.

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