

Functional characterization of *TaHRC* in regulating FHB resistance in wheat

Hui Chen

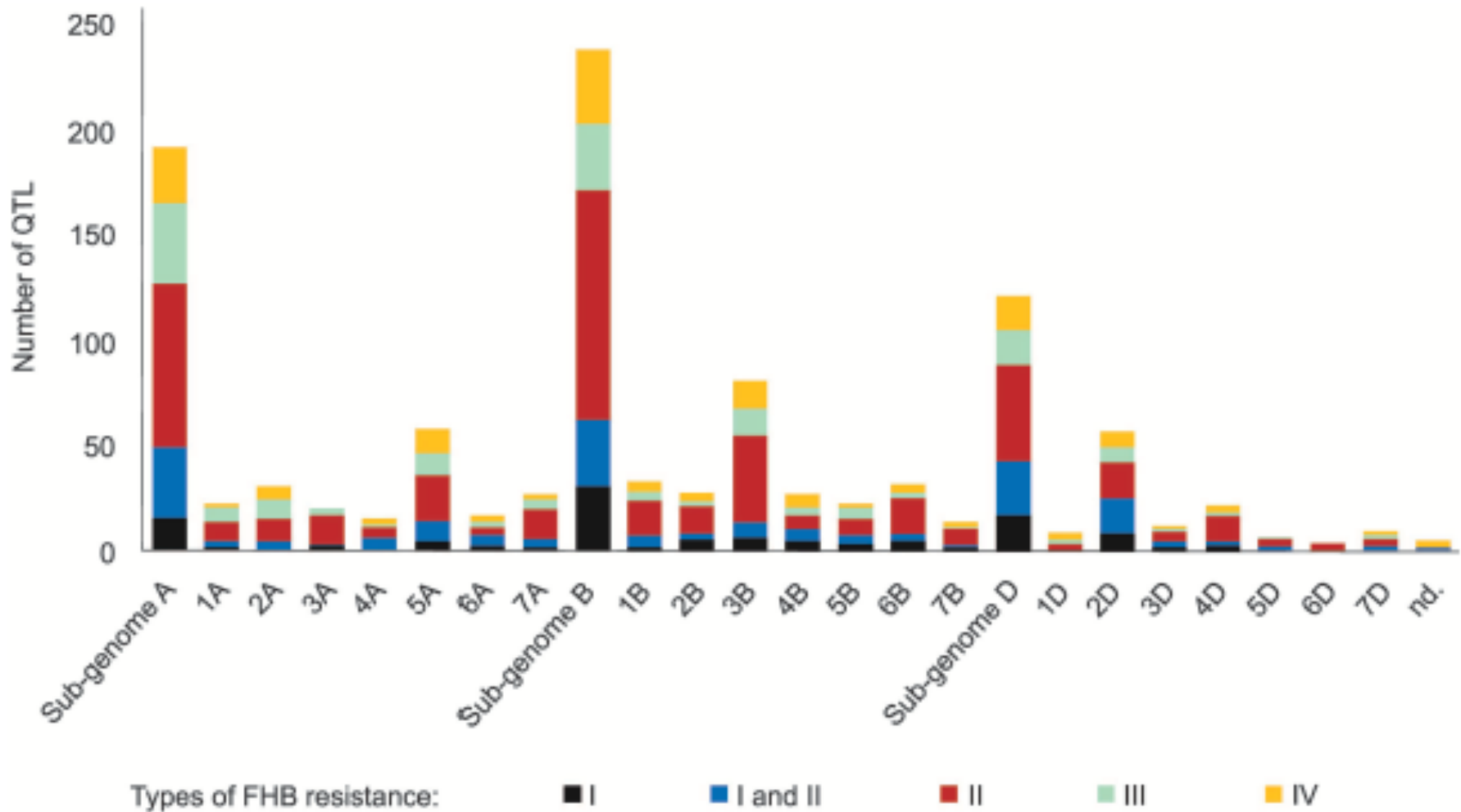
Department of Agronomy, Kansas State University

Guihua Bai

USDA Central Small Grain Genotyping Lab

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> 500 QTLs associated with wheat FHB resistance



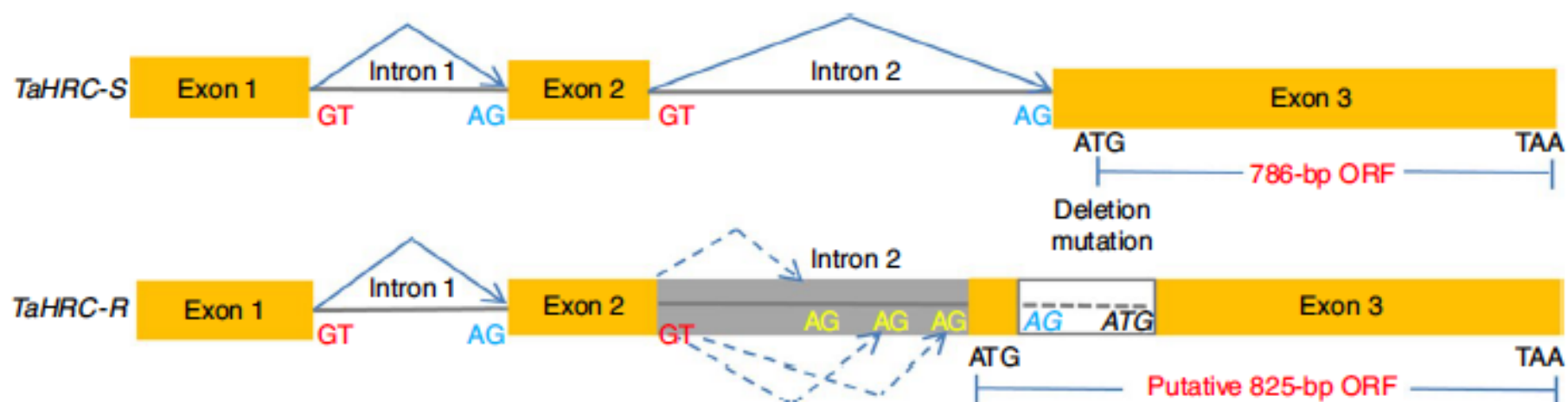
Venske, E. et al. *Frontiers in plant science* 10 (2019): 727

Fhb1 is a major QTL for Type II resistance



Bai, G. et al. *Can. J. Plant Pathol.* 2018, 40: 336-346

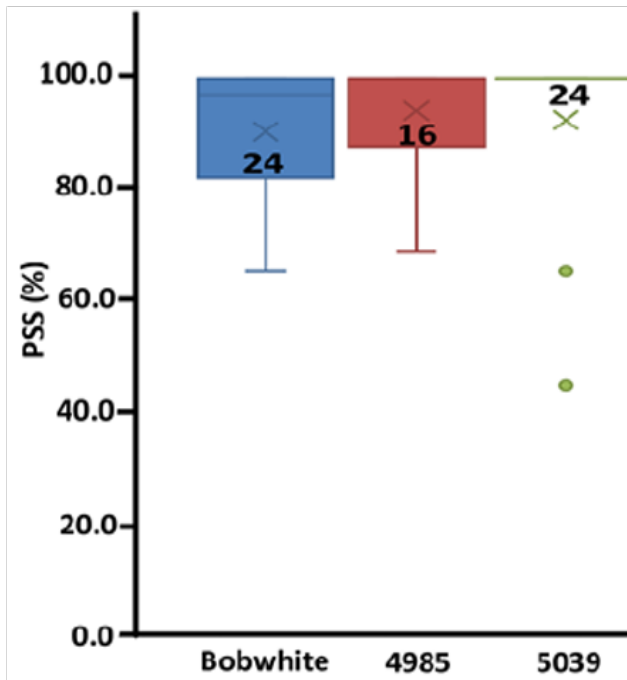
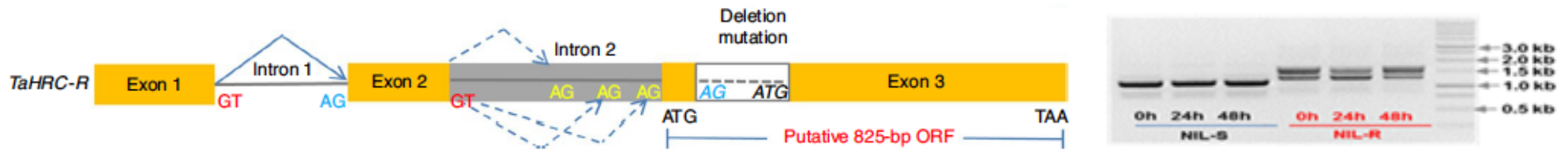
Wild-type allele of *TaHRC* conditions FHB susceptibility



❖ A large deletion in the start codon region of *TaHRC* in resistant lines

Su, Z. et al. *Nat. Gen.* 2019, 51: 1099-1105

TaHRC-R did not gain a new function conferring FHB resistance



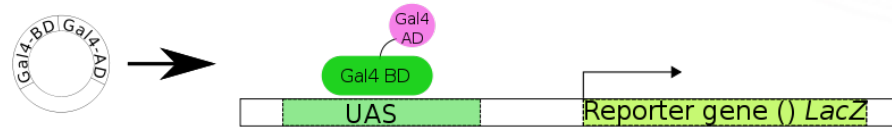
- ❖ Two Bobwhite transgenic lines overexpressing *TaHRC-R* are FHB susceptible as non-transgenic Bobwhite.

Su, Z. et al. *Nat. Gen.* 2019, 51: 1099-1105

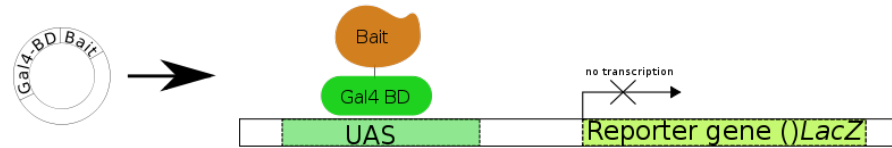


- **How is *TaHRC-S* involved in regulating FHB susceptibility in wheat?**

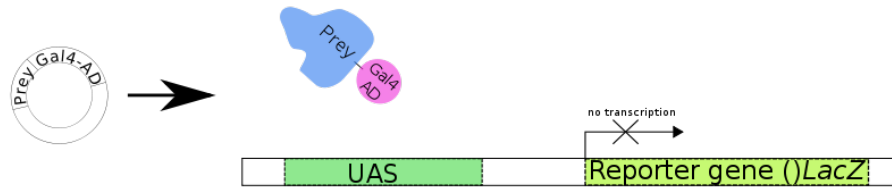
Yeast two-hybrid (Y2H) screening system



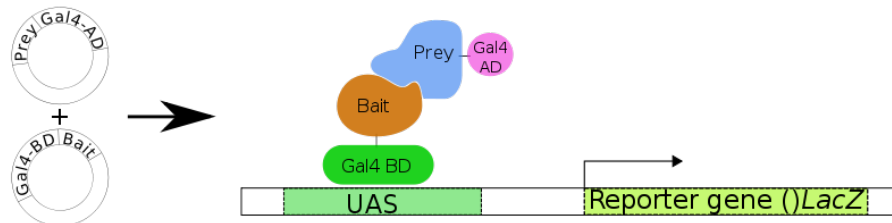
A. Regular transcription of the reporter gene



B. One fusion protein only (Gal4-BD + Bait) - no transcription



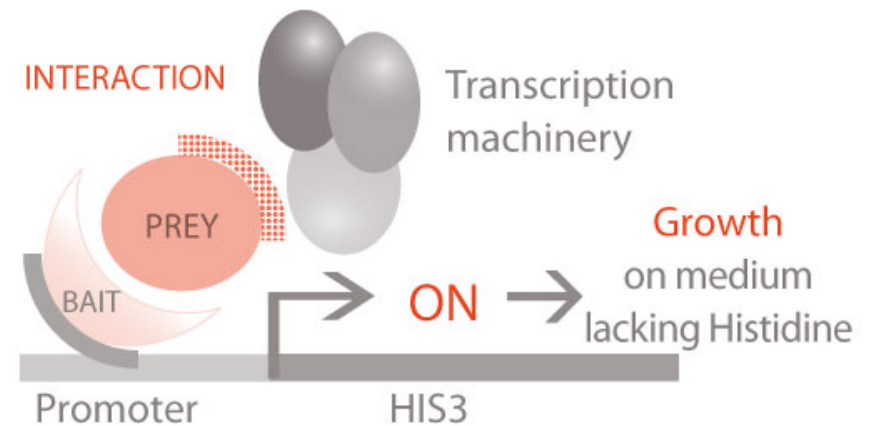
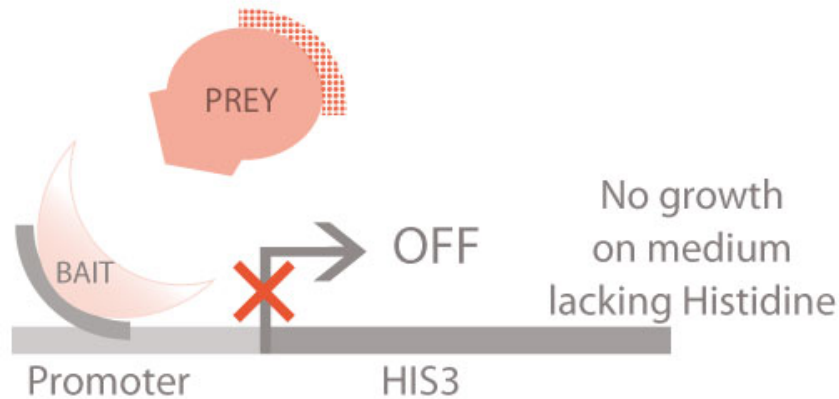
C. One fusion protein only (Gal4-AD + Prey) - no transcription



D. Two fusion proteins with interacting Bait and Prey

https://www.wikiwand.com/en/Two-hybrid_screening

ULTimate Y2H screening with wheat cDNA library



- TaHRC_S cDNA clone in pGBKT7 bait vector
- Wheat cDNA library in pP6 prey vector
- Using ULTimate Y2H screening protocol

LexA or Gal4
DNA Binding
Domain

Gal4
Activation
Domain

The interaction of 2 proteins reconstitutes an active transcription factor and enables yeast growth

BAIT = your protein of interest
PREY = protein partner of the bait

<http://www.hybrigenics-services.com>

Results of ULTimate Y2H screen

130 million clones were screened.

84 positive clones were identified.

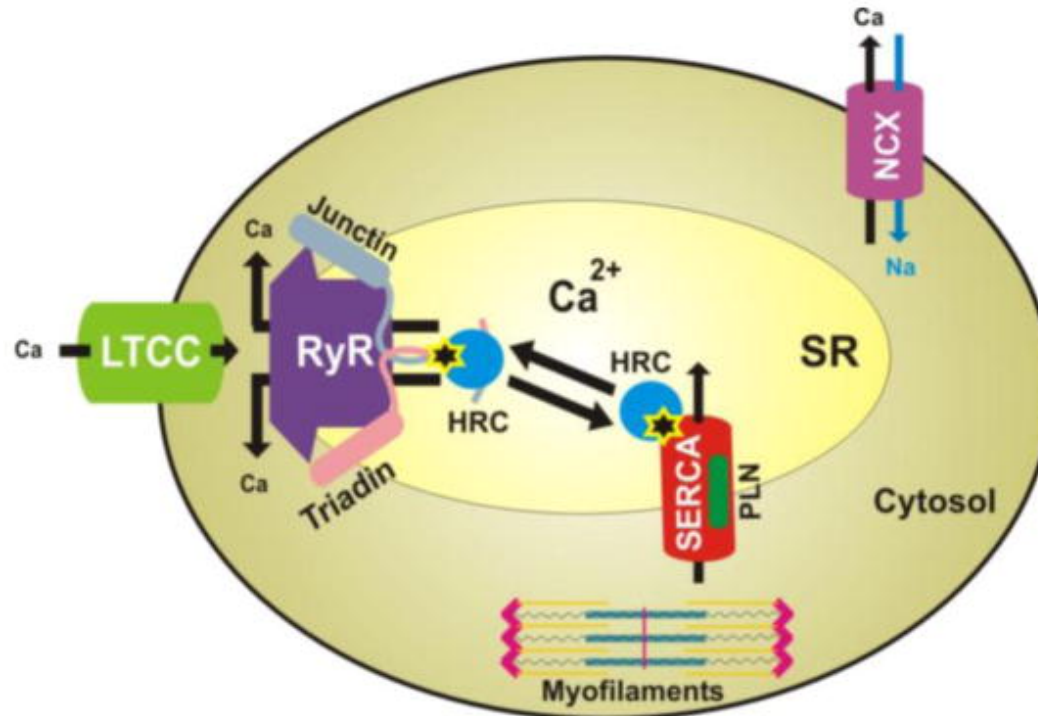
20 clones with high-confidence interactions.

Screen Parameters

Nature	cDNA
Reference Bait Fragment	Triticum aestivum - TaHRC (aa 1-261) ; hgx4861v1
Prey Library	Wheat Head Leave Root_RP1
Vector(s)	GBKT7 (N-GAL4-bait-C fusion)
Processed Clones	318 (GBKT7_A)
Analyzed Interactions	130 millions (GBKT7_A)

Putative candidate protein: TaCXIP4

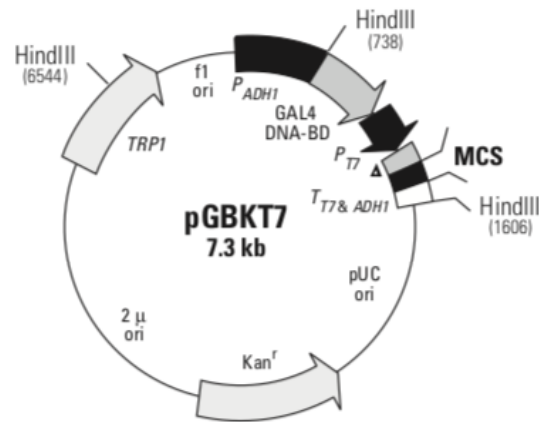
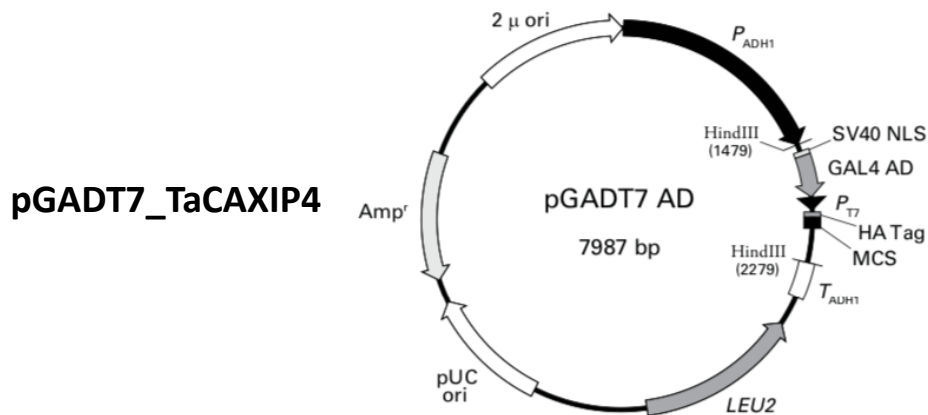
HRC plays a critical role in Ca^{2+} -homeostasis: regulation of both Ca^{2+} -uptake and Ca^{2+} -release.



- ❖ Cation Exchanger (CAX)-interacting protein 4 (TaCAXIP4) is unique candidate protein that interacts with TaHRC_S.

Arvanitis, et al, *J Mol Cell Cardiol*, 2011,50(1):43-9

Coexpression with TaHRC-S and TaCXIP4 in yeast



pGBKT7-TaHRC

pGBKT7-TaHRC_N (NLS)

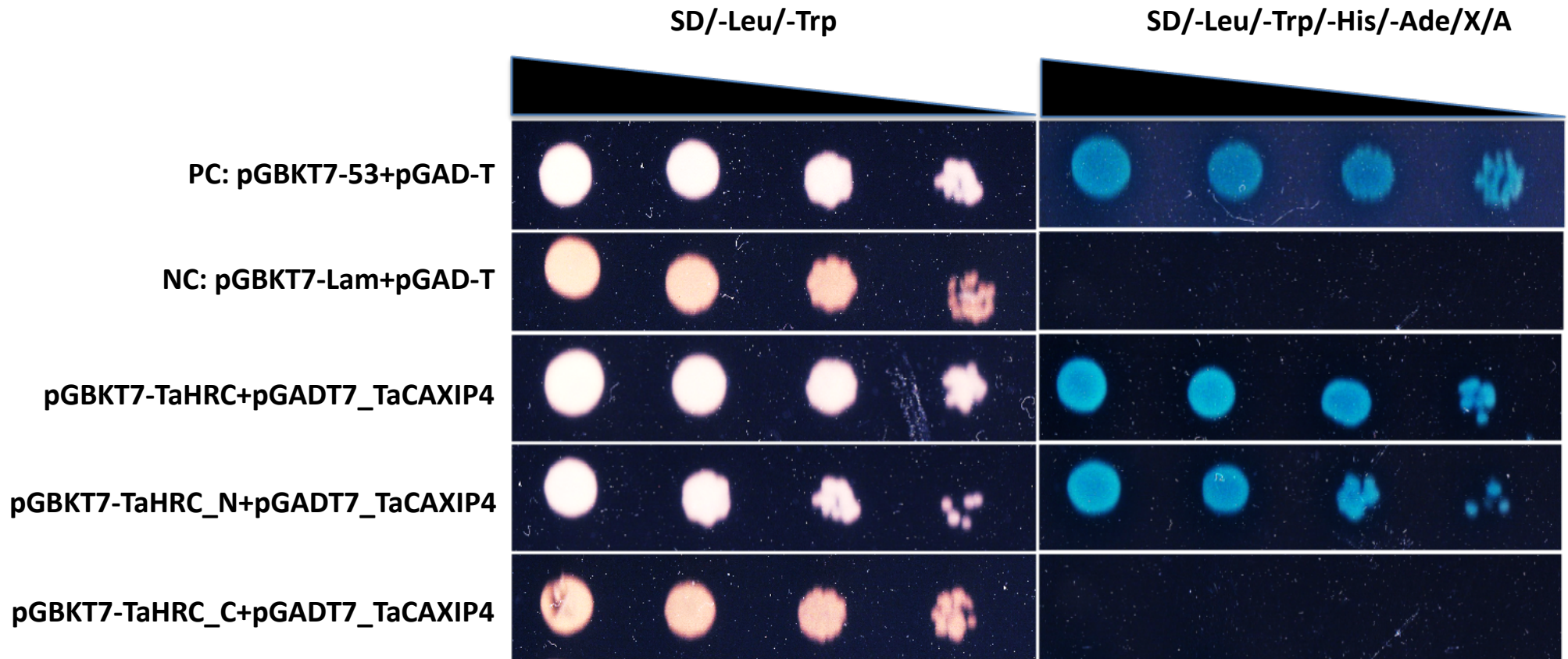
pGBKT7-TaHRC_C (no NLS)

Y2HGold (Mating Partner) reporter gene constructs

G1 Promoter	HIS3	Histidine
G2 Promoter	ADE2	Adenine
M1 Promoter	AUR1-C	Toxic drug resistance (Aureobasidin A)
M1 Promoter	MEL1	Galactose (colonies turn blue in the presence of X-Gal)

www.takarabio.com

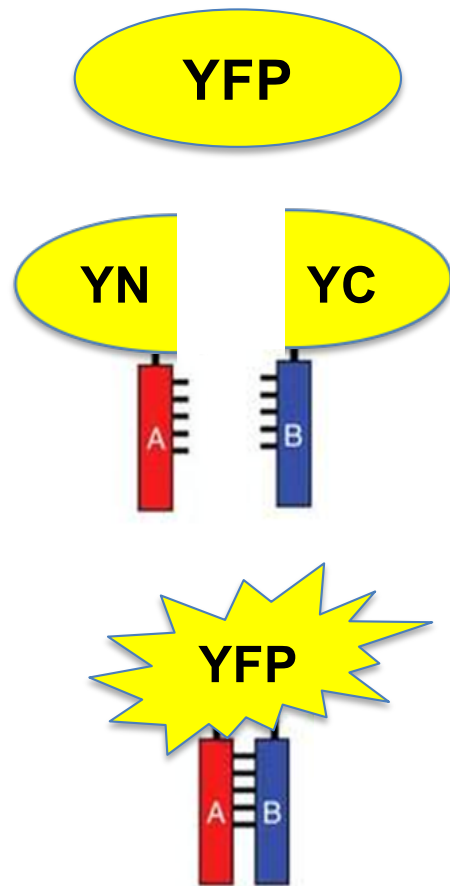
Interaction between TaHRC and TaCAXIP4 in yeast



- ❖ TaCAXIP4 showed a strong interaction with TaHRC-S and only N-terminus with a nuclear localization signal (NLS) domain of TaHRC-S plays an essential role during the interaction.

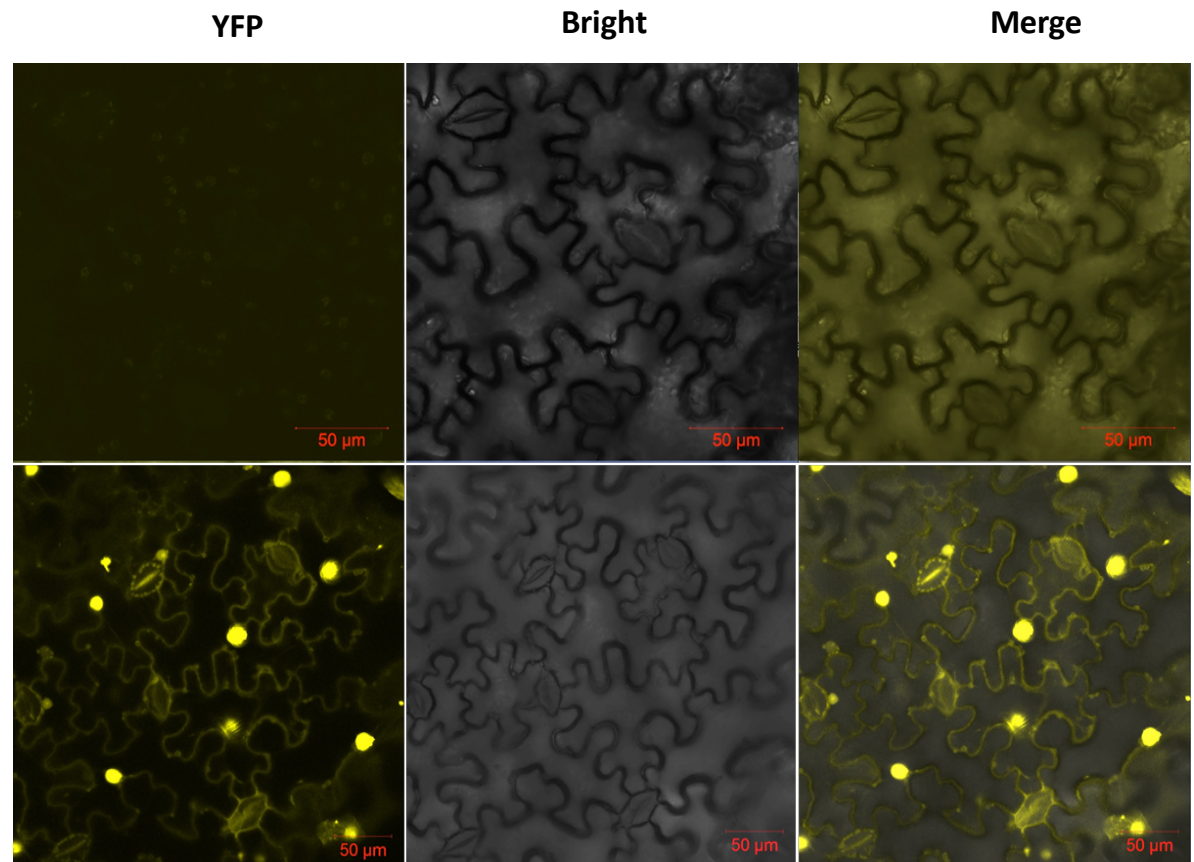
Interaction between TaHRC and TaCAXIP4 in planta

Bimolecular fluorescence complementation (BiFC) assay



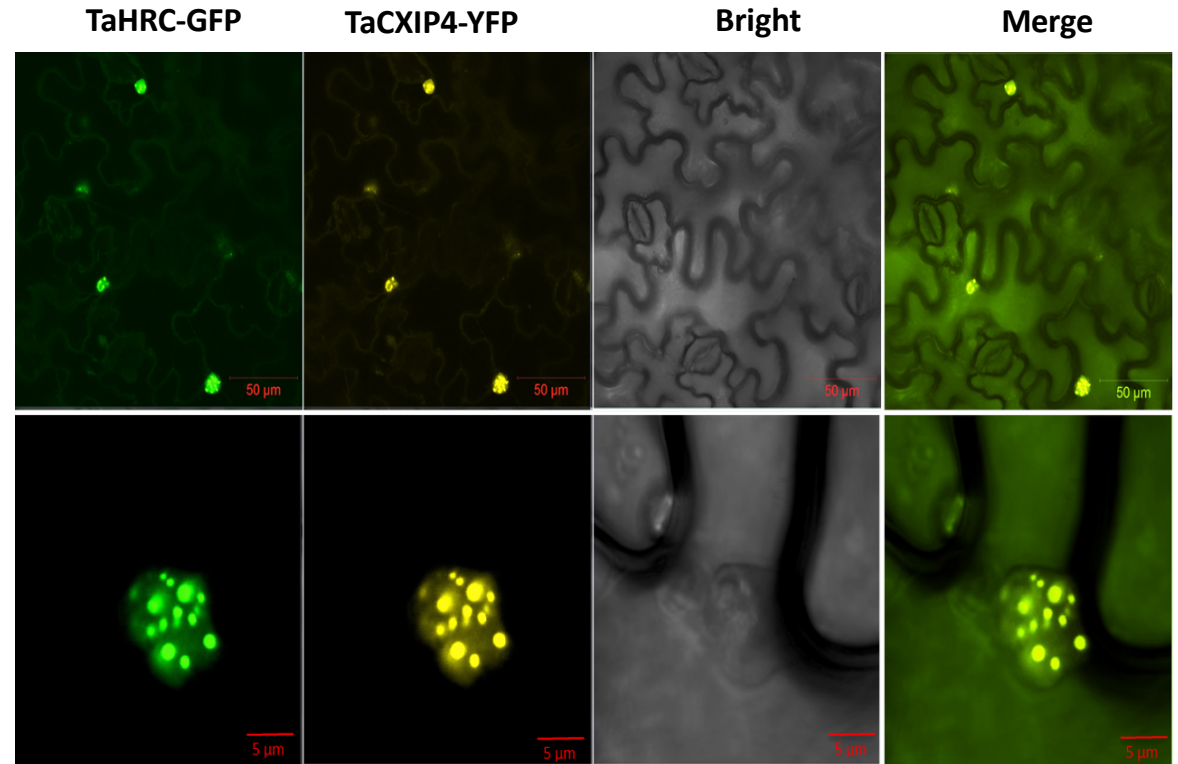
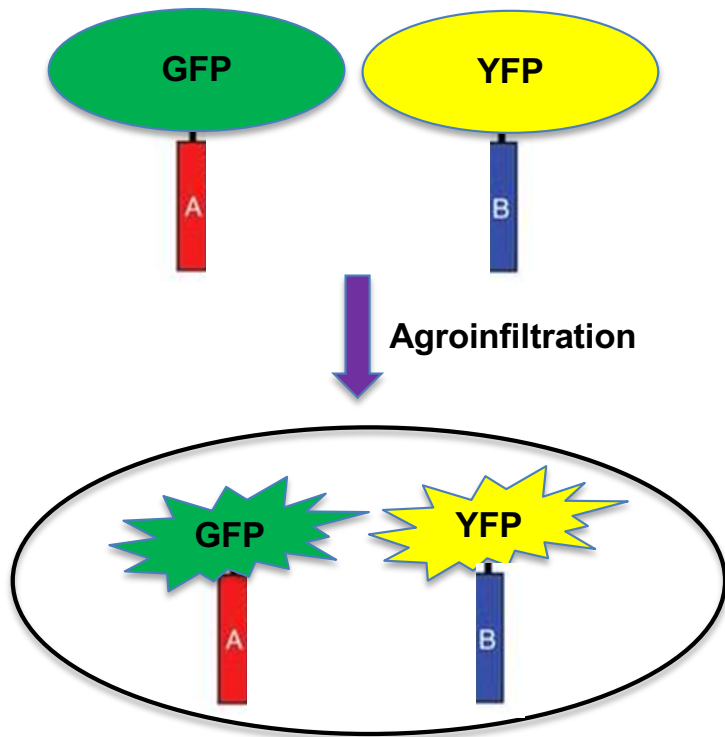
YN-Negative+
YC-Negative

YN-TaHRC+
YC-TaCXIP4

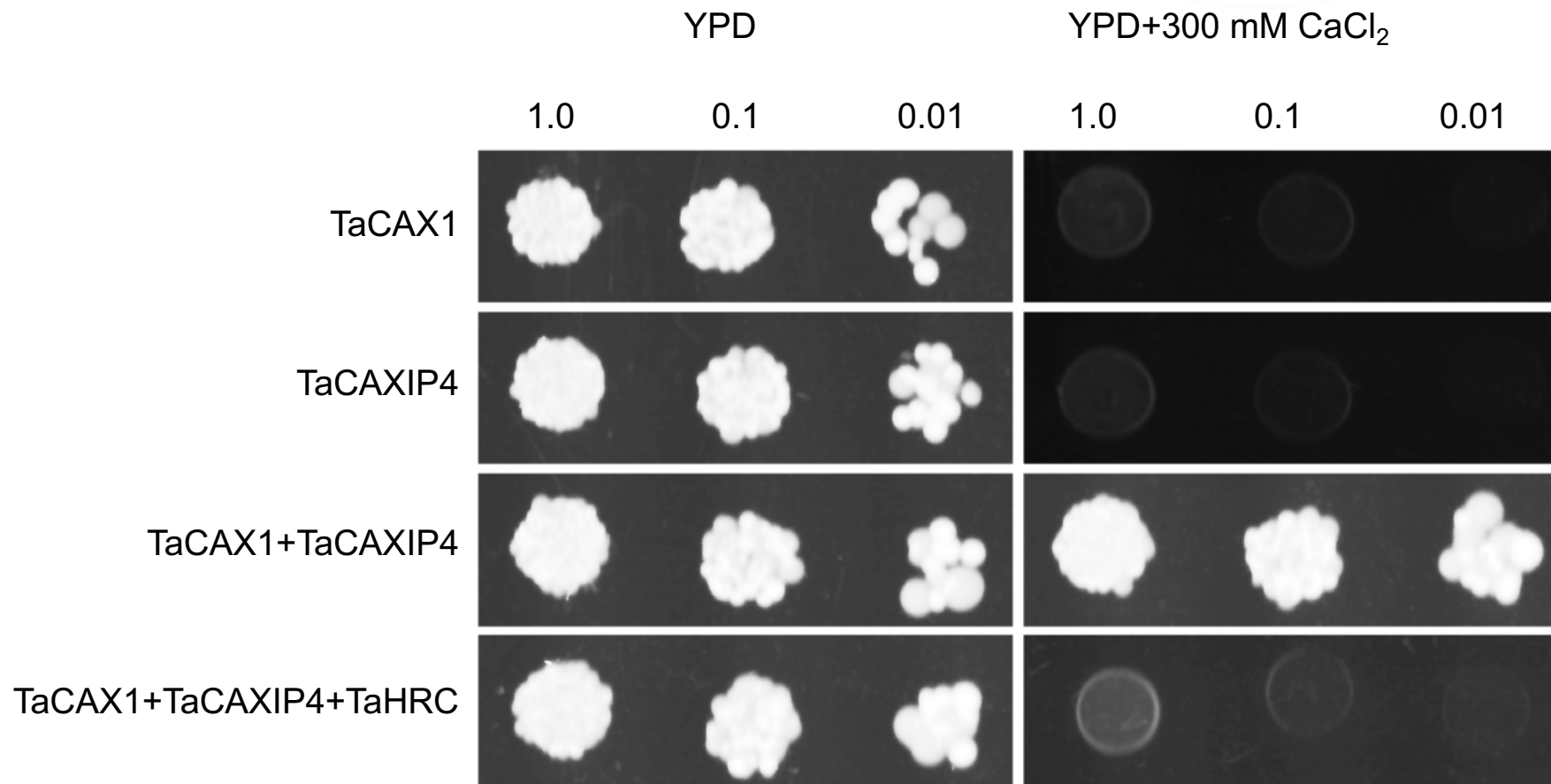


Interaction between TaCXIP4 and TaHRC_S occurred in the nucleus

Colocalization Assay



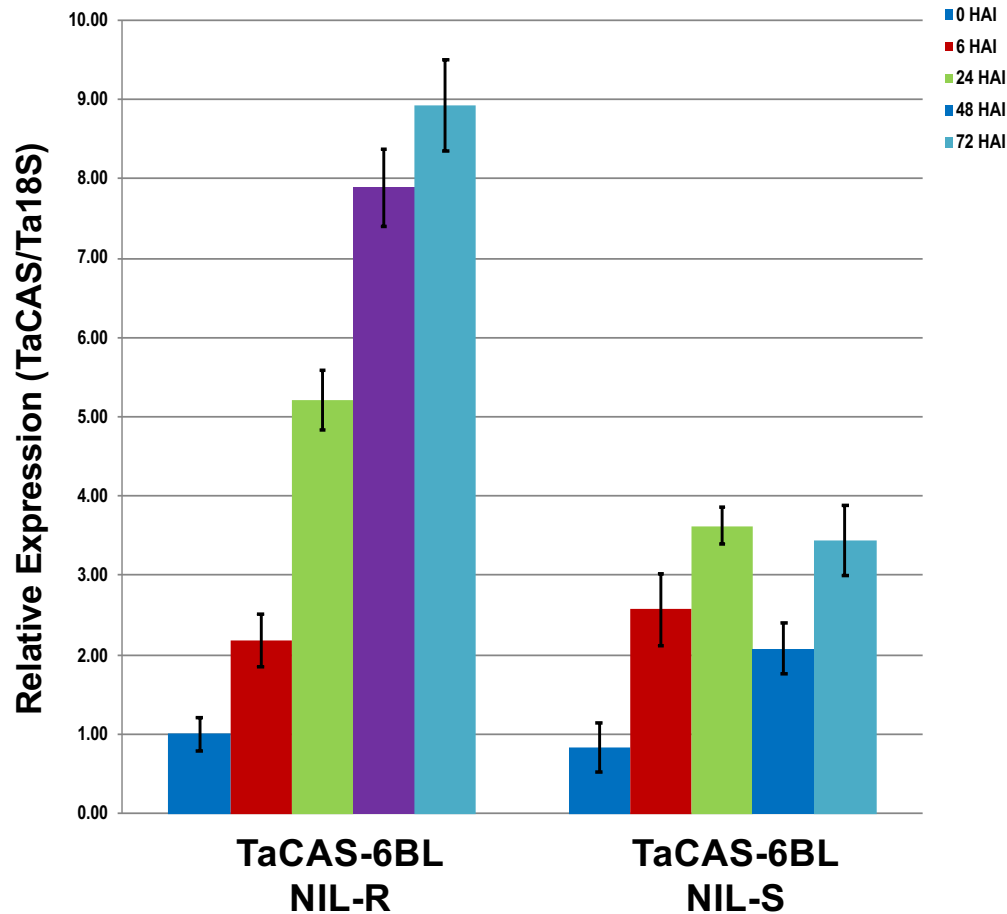
Calcium suppression assay



Ca²⁺ sensitive yeast strain K667

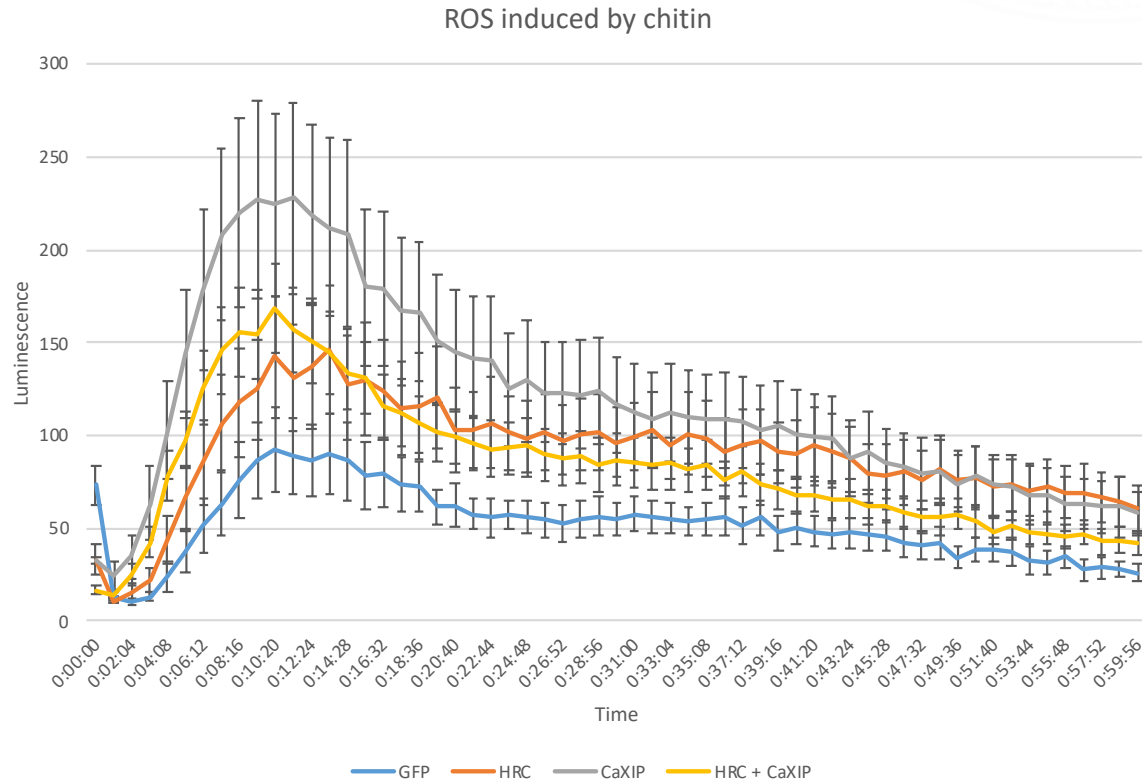
- ❖ TaHRC_S may sequester TaCAXIP4 to suppress the expression of TaCAX1, while TaCAX1 may activate Ca²⁺ antiporter to affect calcium signaling transduction during FHB infection.

Suppression of calcium-sensing receptor (*TaCAS*) by *TaHR_S*



- ❖ Lower expression of *TaCAS* in the NIL-S than those in the NIL-R suggests that suppression of CAS expression by *TaHRC_S* might contribute to FHB susceptibility in wheat.

ROS assay with TaHRC and TaCXIP4 in *N. benthamiana* plants



ROS induced by chitin (100µg/ml) in leaves expressing HRC, CaXIP4, HRC+CaXIP4 and GFP control

- ❖ TaHRC_S may suppress chitin-triggered plant immune responses during FHB infection by sequestering TaCaXIP4 to maintain FHB susceptibility.

* ROS (Reactive Oxygen Species)

Summary

- ❖ Y2H screening wheat cDNA library identified TaCAXIP4 protein as a candidate to interact with TaHRC-S.
- ❖ Both TaCAXIP4 and TaHRC-S proteins co-localize in the plant cell nucleus where they interact.
- ❖ *TaHRC-S* may sequester TaCAXIP4 to inhibit calcium-mediated defense responses and facilitate pathogen spread within a wheat spike to maintain FHB susceptibility.
- ❖ This work provides further insights into molecular mechanisms of *TaHRC* in regulating FHB resistance in wheat.

USDA CENTRAL SMALL GRAIN GENOTYPING LAB



Collaborator: Dr. Guixia Hao, NCAUR, USDA-ARS, Peoria, IL, USDA-ARS

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USDA- US Wheat and Barley Scab Initiative





Thank You

Questions?