Site-Directed Mutagenesis and Gene Insertion in Wheat through Wheat x Maize Hybridization Coupled with Genome Editing Technology

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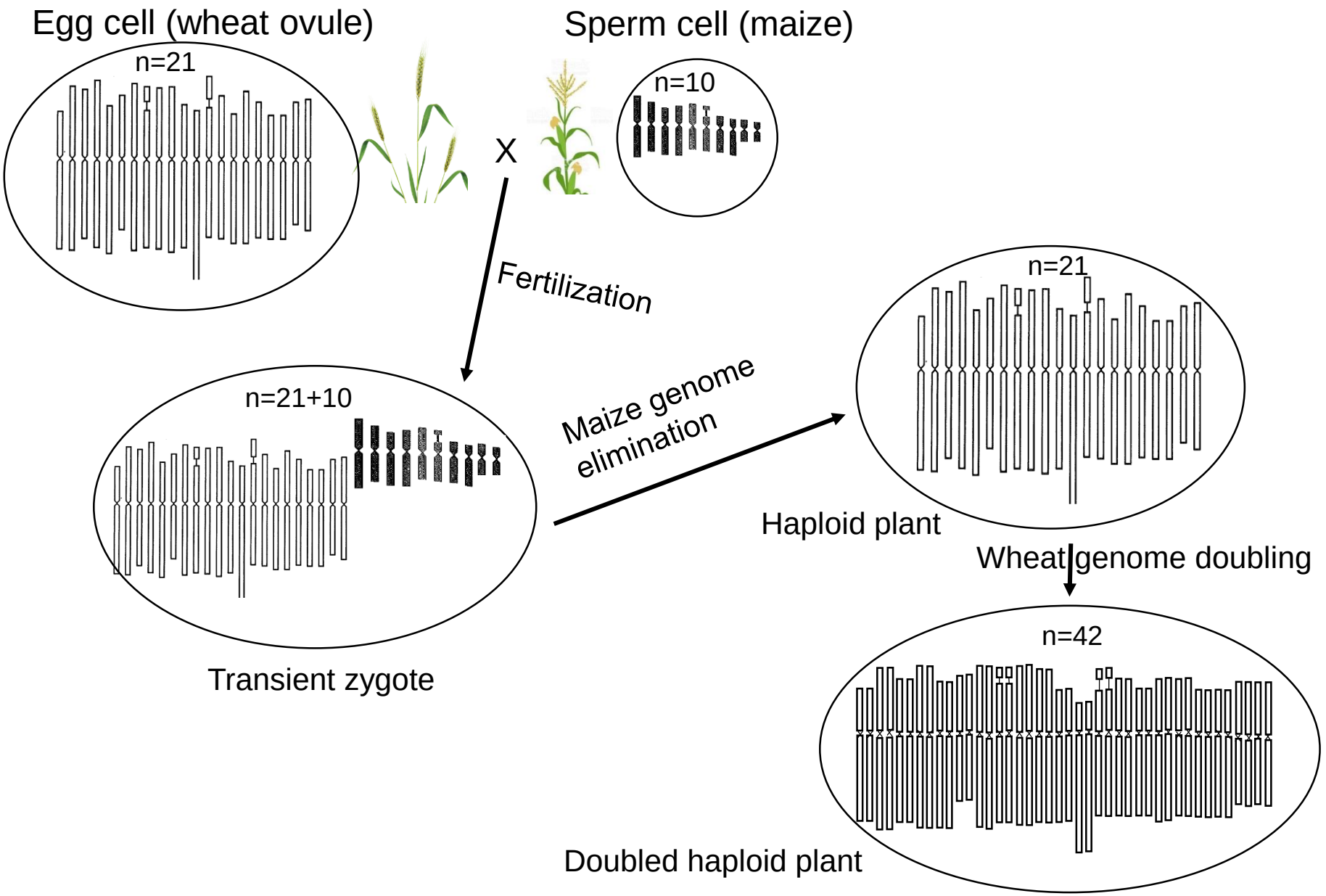
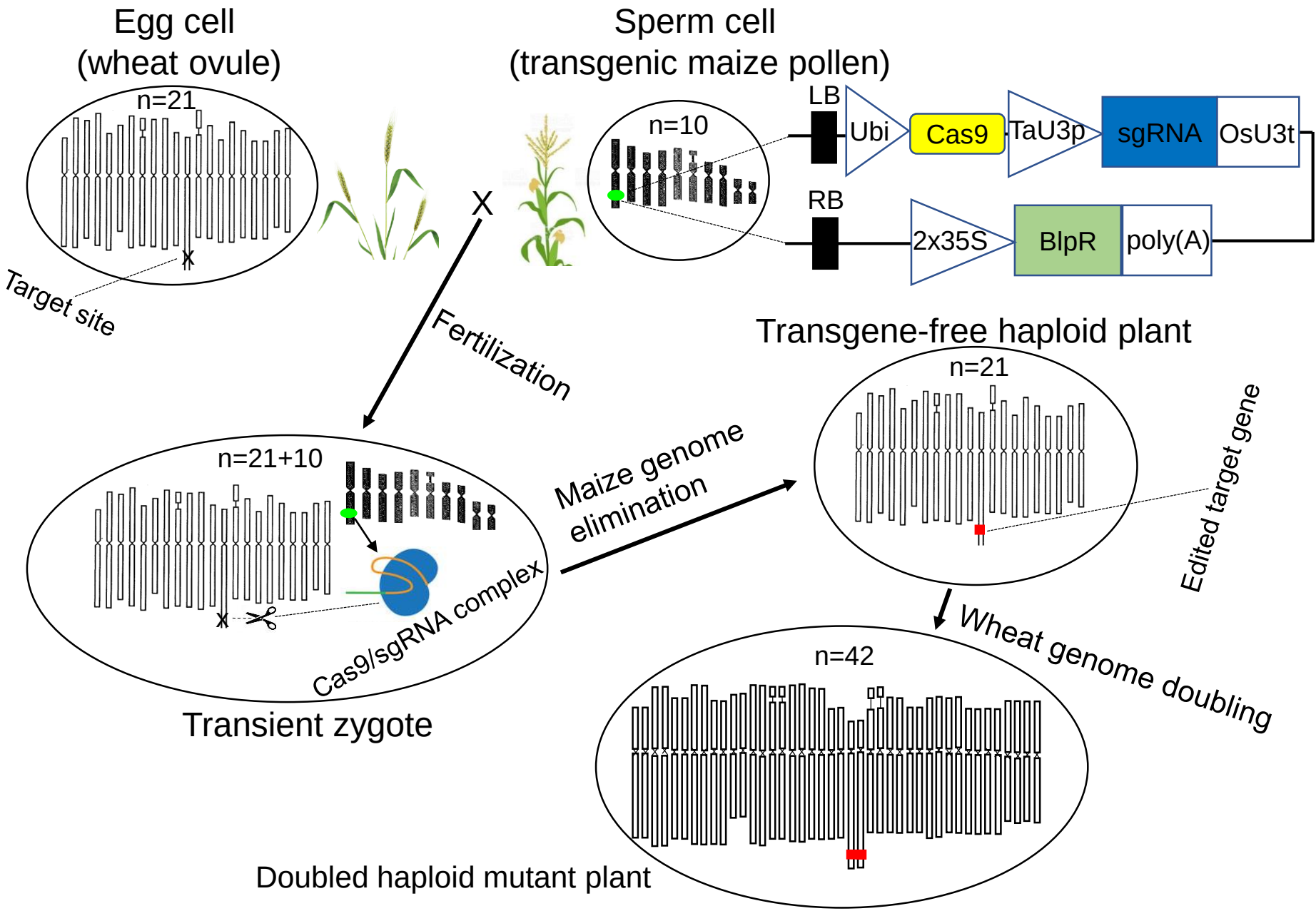
Use of Wheat x Maize Hybridization for Production of Doubled Haploid (DH) Lines in Wheat (Laurie and Bennett 1986)

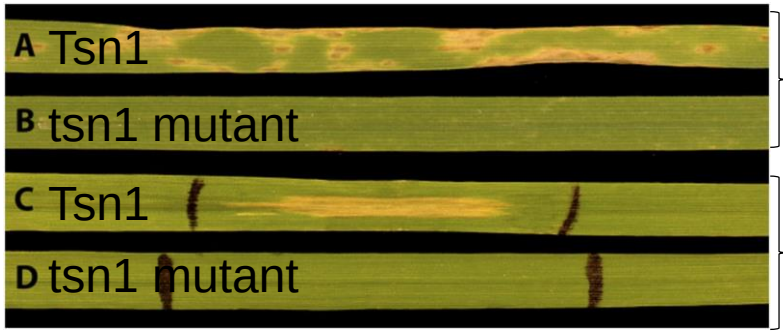


Use of Wheat x Maize Hybridization Coupled with Genome Editing Technology for Targeting Genes in Wheat (Kelliher et al. 2019)

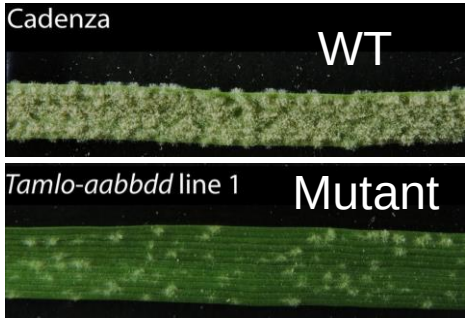


Targeting genes that confer susceptibility to diseases in wheat

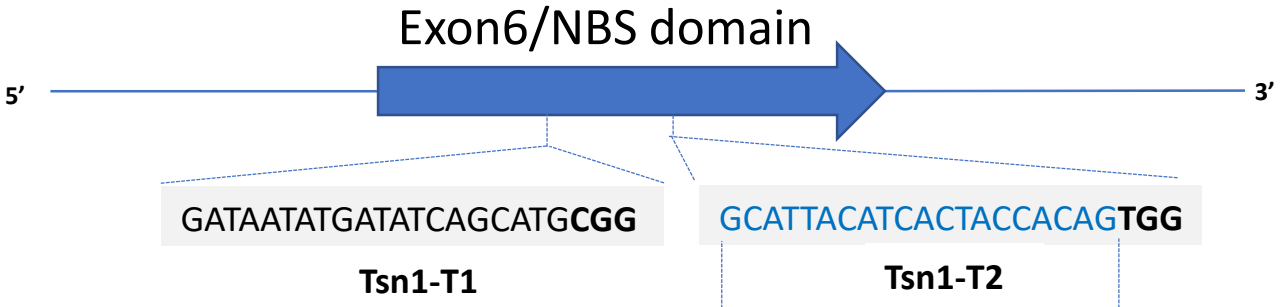
Tsn1 confers susceptibility to tan spot (*Pyrenophora tritici-repentis*) and sensitivity to the fungal toxin ToxA (Faris et al. 2010)



TaMLO makes wheat susceptible to wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) (Acevedo-Garcia et al. 2017; Li et al. 2022)

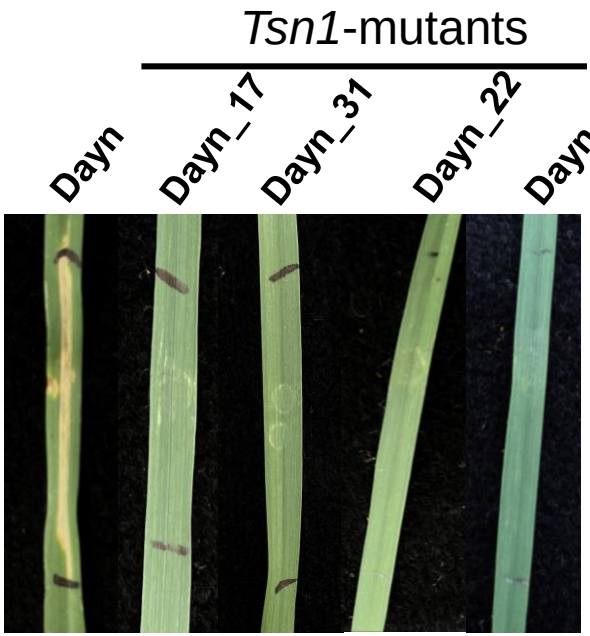


TaHRC-S has been reported to be responsible for susceptibility to FHB (*Fusarium graminearum*) in wheat (Su et al. 2019)



Dayn:	CTACAGGAATTAAAGCTTATGCATTGCAATAGCATTACATCACTACCACAG**TGG**TTTGGAGAACT	**WT**
Dayn_5:	CTACAGGAATTAAAGCTTATGCATTGCAATAGCATTACATCACTAC––CAG**TGG**TTTGGAGAACT	**(-2)**
Dayn_23:	CTACAGGAATTAAAGCTTATGCATTGCAATAGCATTACATCACTAC––CAG**TGG**TTTGGAGAACT	**(-2)**
Dayn_25:	CTACAGGAATTAAAGCTTATGCATTGCAATAGCATTACATCACTAC––CAG**TGG**TTTGGAGAACT	**(-2)**
Dayn_22:	CTA----------CAG**TGG**TTTGGAGAACT	**(-45)**
Dayn_31:	CTACAGGAATTAAAGCTTATGCATTGCAATAGCATTACAT----------ACT	**(-22)**
Dayn_17:	---------- ----------	**(-N)**

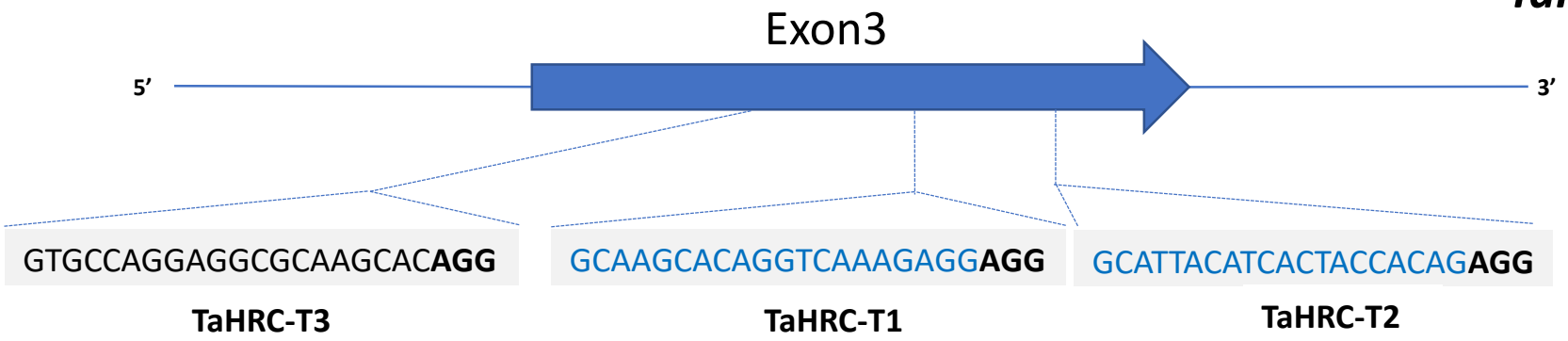
6 DHs



Infiltration with ToxA

Karmacharya et al. (2023)

TaHRC-S



	TaHRC-T1	TaHRC-T2	
Dayn:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG**…….CGCCGAAGGAAGAAGCACTCGCAC**AGG**		**WT**
Dayn-14-6:	AGGAGGCGCAAGCACAGGTCAAAG – - CAC**AGG**		**(-88)**
Dayn-15-6:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG** …… CGCCGAAGGAAGAAGCACTCGTCAC**AGG**		**(+1)**
Dayn-7-1:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG** …….CGCCG – – – – – – – – – – – – – – CGA**AGG**		**(-19)**
Dayn_19-15-3-19:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG**……..CGCCGAAGGAAGAAGCACTCGTCAC**AGG**		**(+1)**
Dayn_22-15-3-20:	AGGAGGCGCAAGCACAGGTCAAAG – –G**AGG**…….CGCCGAAGGAAGAAGCACTCGACAC**AGG**		**(-2/+1)**
Dayn_30-15-4-22:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG**……..CGCCGAAGGAAGAAGCACTCGTCAC**AGG**		**(+1)**
Dayn_32-7-1-28:	AGGAGGCGCAAGCACAGGTCAAAGGAGG**AGG**…..CGCCGAAGGAAGAAGCACTCGTCAC**AGG**		**(+1/+1)**
Dayn_35-15-4-22:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG**……..CGCCGAAGGAAGAAGCAC – – –AGG**AGG**		**(-7/+3)**
Dayn_36-15-4-23:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG**……..CGCCGAAGGAAGAAGCACTCGGCAC**AGG**		**(+1)**
Dayn_46-15-4-22:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG**……..CGCCGAAGGAAGAAGCACTCGACAC**AGG**		**(+1)**
Dayn_49-15-4-22:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG**……. CGCCGAAGGAAGAAG – – – – – CAC**AGG**		**(-7)**
Dayn_52-15-3-20:	AGGAGGCGCAAGCACAGGTCAAAGAGG**AGG**……..CGCCGAAGGAAGAAGCACTCGGCAC**AGG**		**(+1)**

12 DHs

Karmacharya et al. (2023)

TaPFT and *TaHRC/His* have been reported to be involved in *Fhb1* resistance (Rawat et al. 2016; Su et al. 2019; Li et al. 2019), but controversy still exists on identity and function of the gene for *Fhb1* resistance

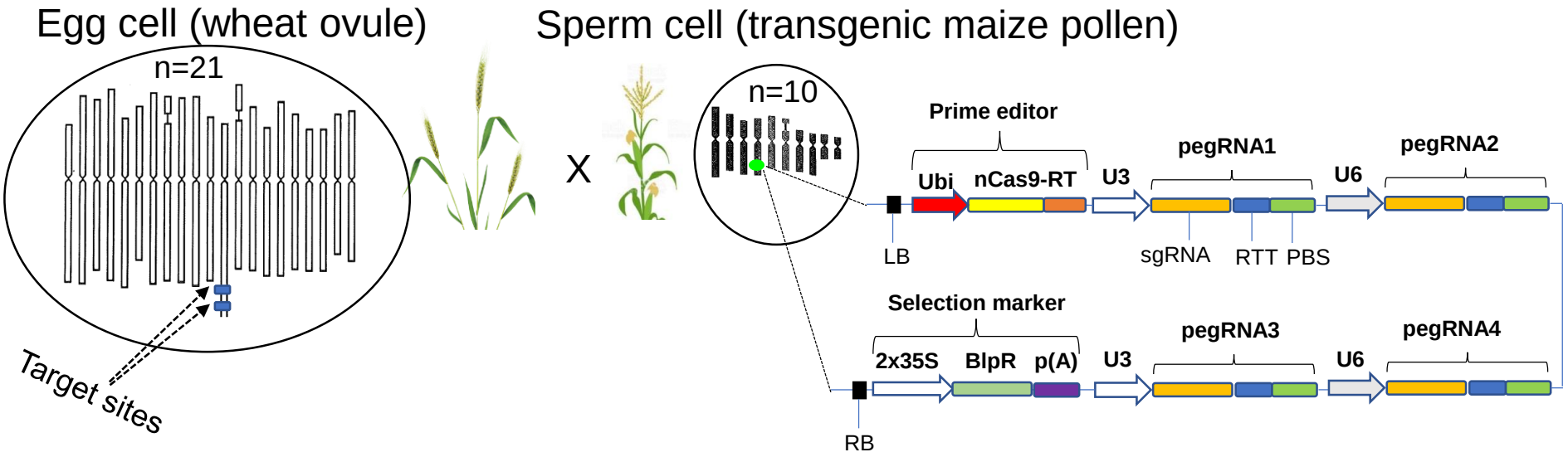
We have generated DH lines with mutations at *TaPFT* and *TaHRC* from different wheat varieties with and without *Fhb1* resistance using the wheat x maize hybridization method

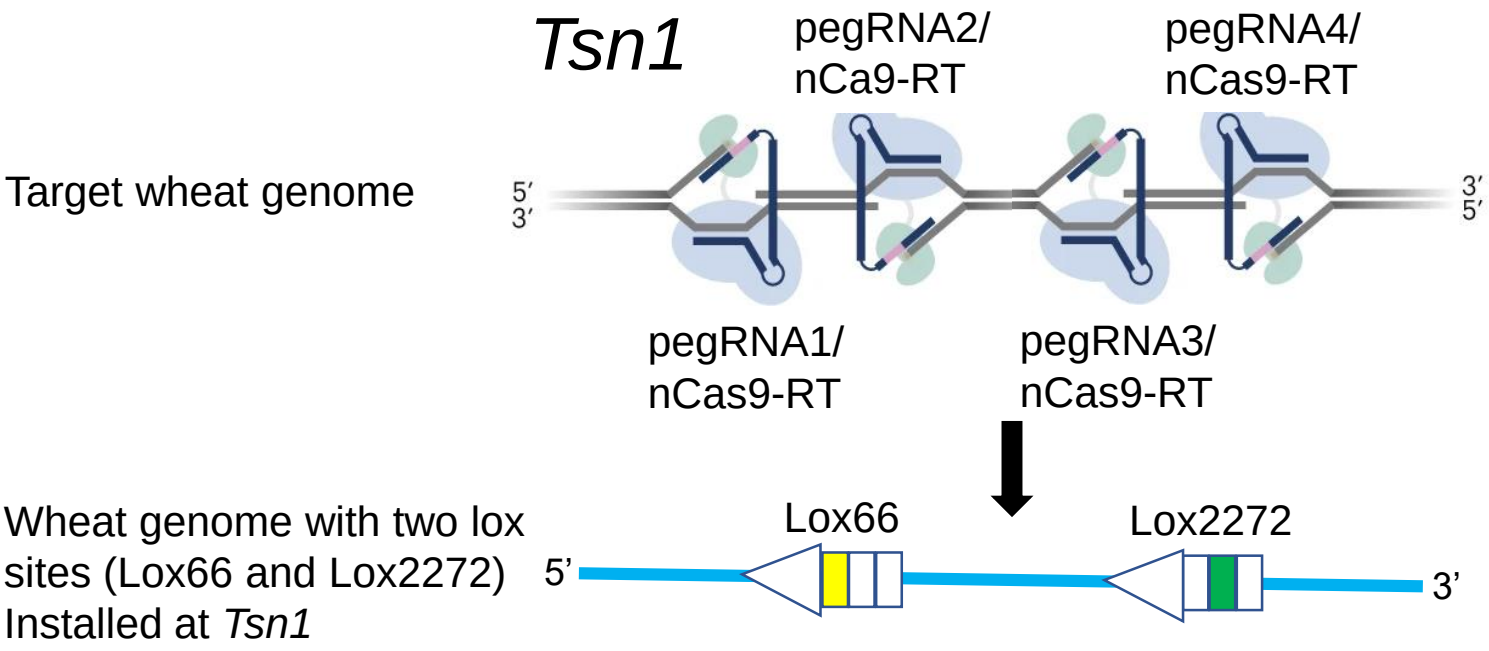
Evaluation of the DH lines for FHB resistance in greenhouse is in progress

Our data may provide more evidence to clarify the issues on the genes reported to be responsible for the *Fhb1* resistance

Use of Wheat x Maize Hybridization Coupled with Prime Editing and Cre-Mediated DNA Recombination for Targeted Gene Insertion in Wheat

Step 1: Install two lox sites (Lox66 and Lox2272) in wheat genome using wheat x maize hybridization coupled with dual-prime editing





Step 2: Insert large DNA fragment (*Fhb7*) into wheat genome using wheat x maize hybridization combined with Cre-mediated DNA recombination

Wheat lines carrying Lox66 and Lox2272

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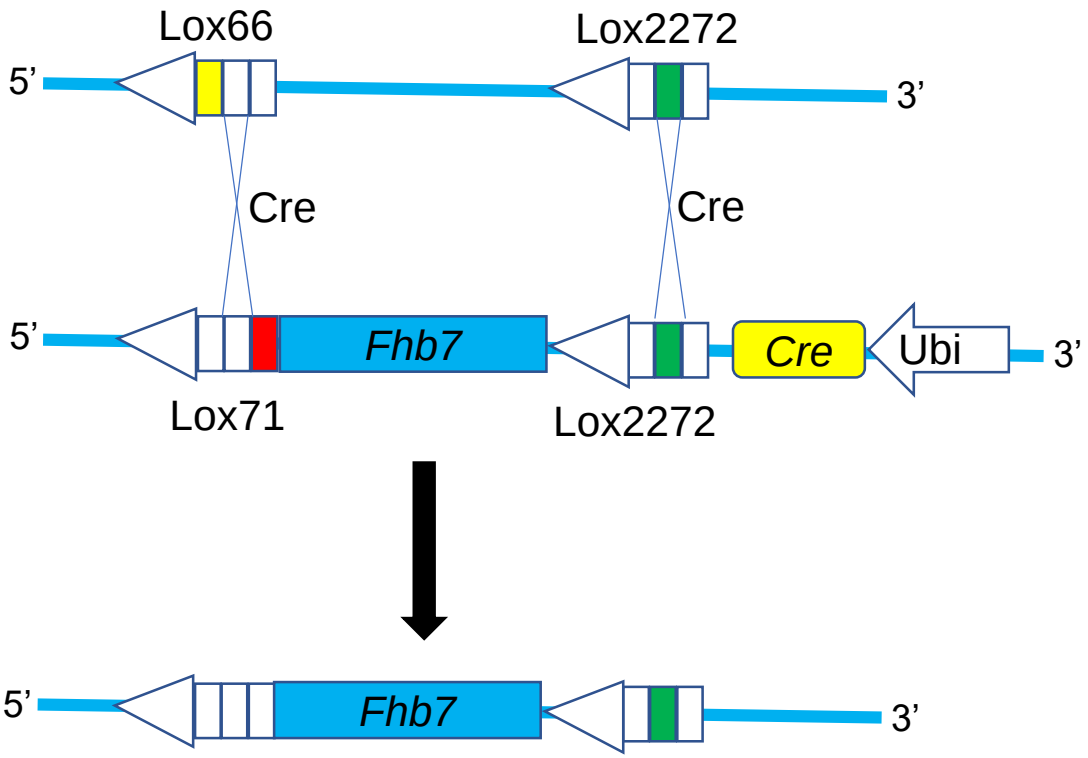
Transgenic maize plants carrying transgene (T-DNA) with the recombinase gene (*Cre*) and *Fhb7* flanked by lox sites (Lox71 and Lox2272)



DH line with *Fhb7* inserted

Summary

Three wheat disease susceptibility genes (*Tsn1*, *TaMLO*, *TaHRC*) were mutated using CRISPR/Cas9 genome editing through wheat × maize hybridization

15% of haploid plants had mutations at the target gene from wide crosses with T_0 maize plants as pollen donors

33% of haploid plants had mutations at the target gene from wide crosses with T_1 maize plants as pollen donors

Transgenic maize plants carrying prime editing components have been generated and will be used to install lox sites in wheat genome through wheat x maize hybridization

Transgenic maize with T-DNA carrying *Cre* and target gene flanked by lox sites will be generated and used for wide crosses with wheat lines carrying lox sites for targeted gene insertion

Wheat x maize hybridization coupled with genome editing technology is a simple, quick, and efficient tool for targeted mutagenesis and gene insertion in wheat

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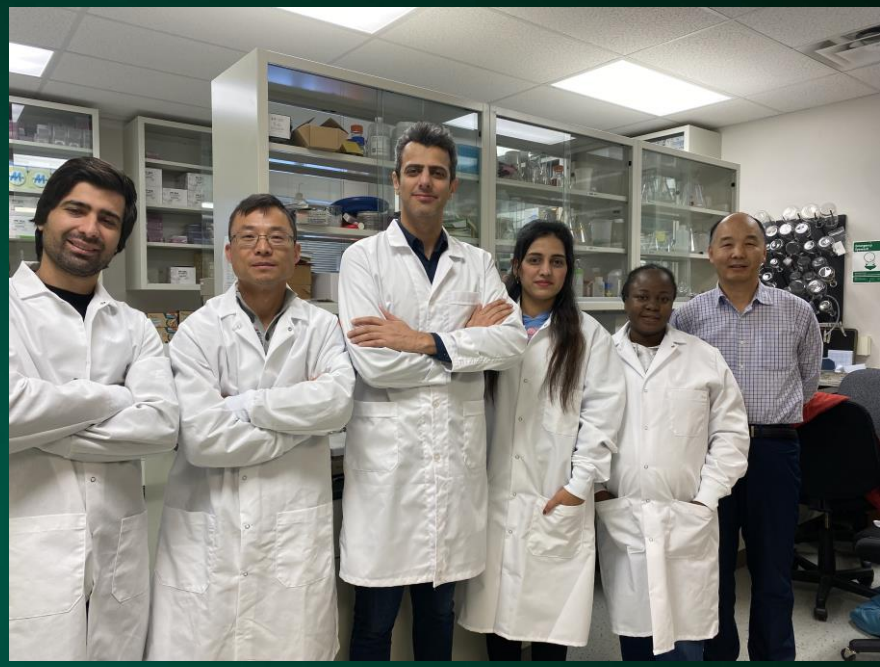

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Building Bigger Better Markets

Questions?