Cloning of *TaCol-B5* and its applications in wheat breeding

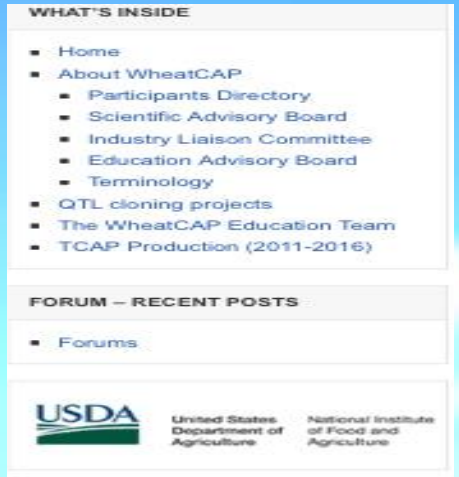
Liuling Yan
Plant and Soil Sciences
Oklahoma State University
liuling.yan@okstate.edu

DEPARTMENT OF
Plant &
Soil Sciences

WHEAT CAP
Coordinated Agricultural Project

Cloning of yield QTLs in the wheat CAP3

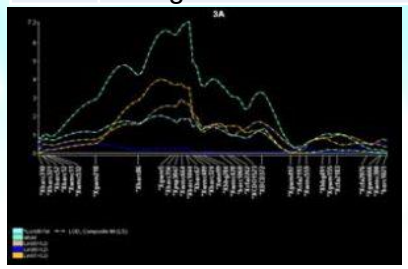
https://www.triticeaecap.org/qtl-cloning-projects

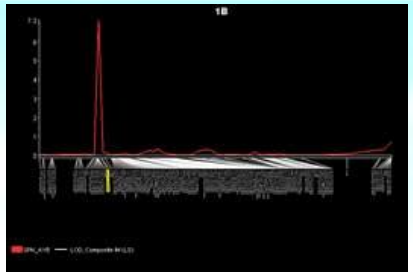


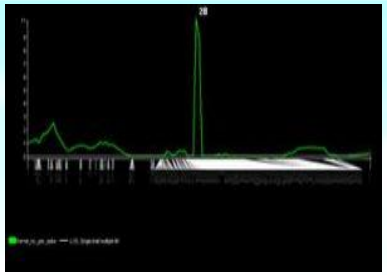
1	University of California, Davis	Spikelets per spike	7AL (160-165 Mb)
2	Colorado State University	Grain width, thousand kernel weight	6BL
3	Cornell University	Seed size	5BS
4	University of Idaho	Spikelets per spike	5AL
5	Montana State University	Tiller number	6BS (30 Mb)
6	Texas A&M AgriLife Research	Yield, thousand kernel weight and harvest index	2BS (18 Mb)
7	USDA-ARS, Fargo, ND	Grain yield, Spikelets per spike, grains per spike	3A, 1BS, 2BS
8	USDA-ARS and Kansas State University	Yield	2DL (111 Mb)
9	South Dakota State University	Yield	7DS
10	University of Minnesota	Kernel weight and kernel width	2A
11	**Oklahoma State University**	Yield	**1BS**
12	North Carolina State University and USDA-ARS	Kernel weight	6A
13	University of Arkansas	Yield, thousand-kernel weight	1A, 3AL
14	Washington State University	Spike length, kernels per spike and thousand kernel weight	4AL
15	Michigan State University	Yield	2DL (637-640 Mb)

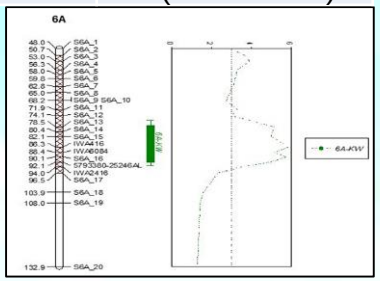
USDA-NIFA-IWYP
2016-2021
$10M

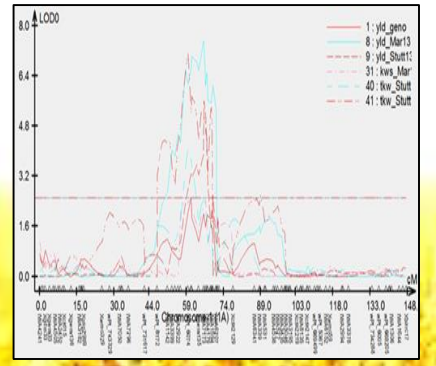
Dr. Jorge Dubcovsky
UC Davis

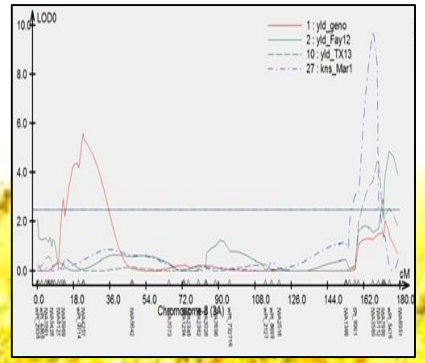


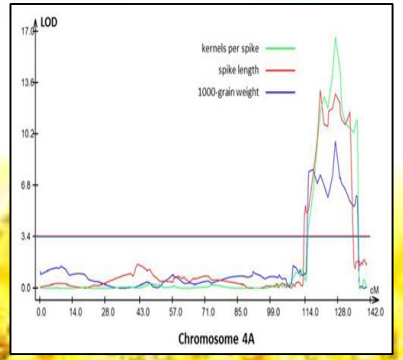


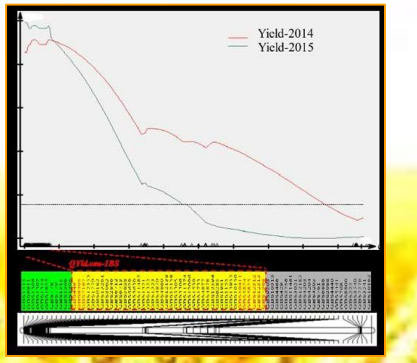












A major QTL *QYld.osu-1BS* for grain yield

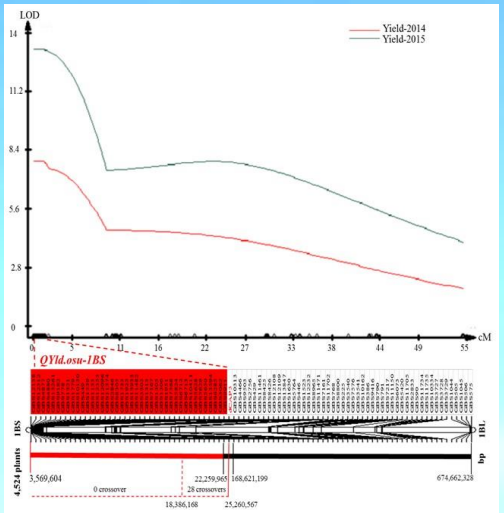
(Initially proposed gene cloning project in Oklahoma)

Results:

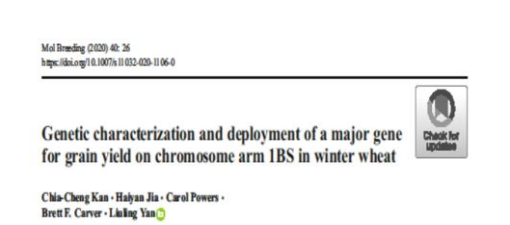
- Duster and Billings, two locally adapted winter wheat cultivars.
- 260 doubled haploid (DH) lines.
- GBS markers for the DH population.
- Field-based grain yield from breeding plots for two years.
- A major QTL on chromosome arm 1BS (*QYld.osu-1BS*) for 13.9% and 23.5% of the total phenotypic variation.
- No crossover in the targeted region from the telomere to 18.4 Mb among >4,000 individual $F_{2:4}$ plants.
- KASP markers for unique sequences in the *QYld.osu-1BS* region in the Duster allele.

Conclusion:

- *QYld.osu-1BS* cannot be cloned in the short term.
- The KASP markers can be used in molecular breeding programs.



Forrest Kan



Mol Breeding (2020) 40: 26
https://doi.org/10.1007/s11032-020-1106-0

Genetic characterization and deployment of a major gene for grain yield on chromosome arm 1BS in winter wheat

Chia-Cheng Kan · Haiyan Jia · Carol Powers · Brett F. Carver · Liuling Yan

Kan *et al.*, Mol. Breed. 40: 26 (2020)

A back-up plan for the WheatCAP3 project in Oklahoma

Science AAAS

RESEARCH

PLANT SCIENCE

TaCol-B5 modifies spike architecture and enhances grain yield in wheat

Xiaoyu Zhang[1]†, Haiyan Jia[1,2]†, Tian Li[1,3]†, Jizhong Wu[1,4], Ragupathi Nagarajan[1], Lei Lei[1], Carol Powers[1], Chia-Cheng Kan[1], Wei Hua[5], Zhiyong Liu[6], Charles Chen[7], Brett F. Carver[1]*, Liuling Yan[1]*

Xiaoyu Zhang

Haiyan Jia

Tian Li

Science 376, 180-183 (2022)

https://www.science.org/doi/10.1126/science.abm0717

Two parental lines used for gene cloning

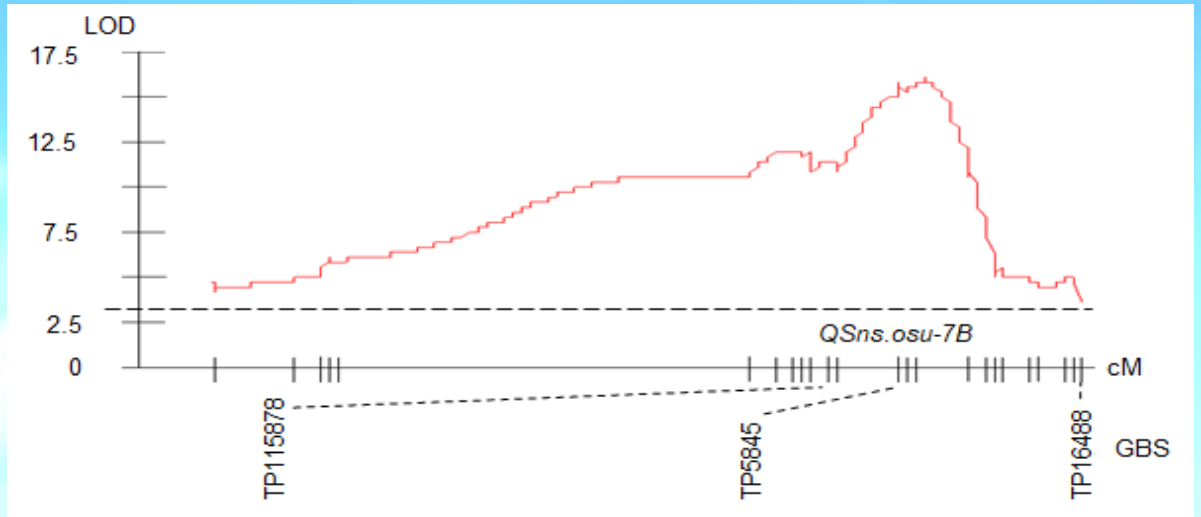
CItr 17600 (L20) Yangmai18 (Y18)

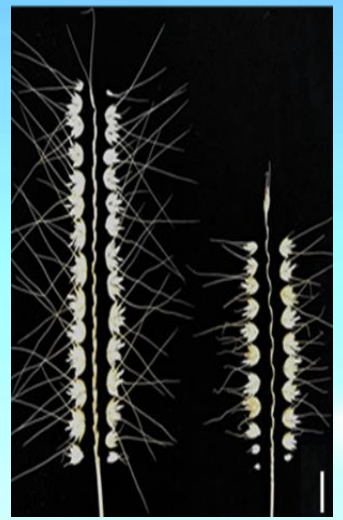
CItr 17600 (L20) Yangmai18 (Y18)

CIMMYT

Yangzhou

A major QTL for the number of spikelet nodes per spike





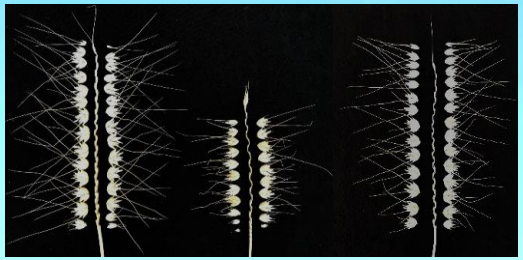
QSns.osu-7B on chromosome arm 7BL

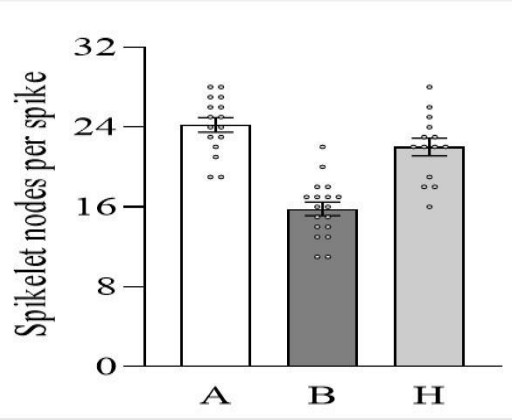
LOD value:15.3

Effect: 43.1% of the total phenotypic variation

The number of spikelets per spike was controlled by a single gene in the sub-population

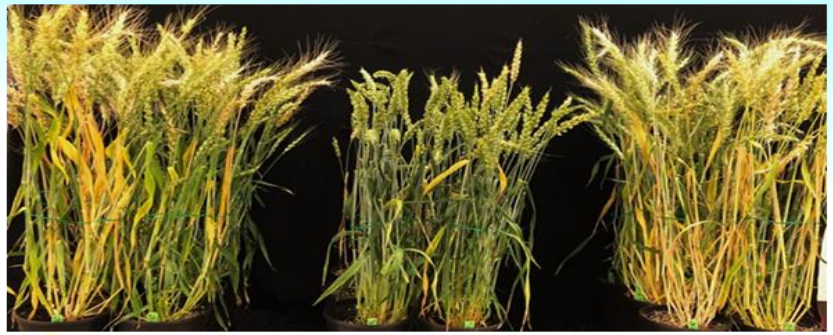
- WF112 F_4 population showed **3:1** segregation ratio for the number of spikelets per spike.
- L20 carried the dominant allele, whereas Y18 carried the recessive allele.
- 1,857 individual plants were screened for crossovers.



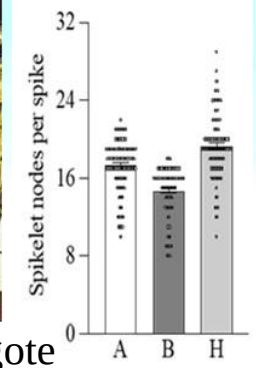


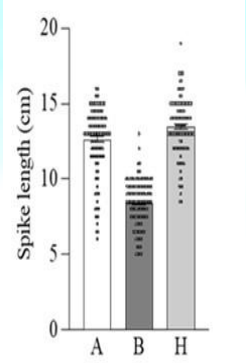
Recombinant lines used for cloning of *TaCol-B5*

Marker	Physical location	P11-49	P11-628	P11-718	P19-237	P19-460	P19-1121	P11-719	P11-29	P19-469	P19-885	P19-1138	P11-589	P11-63	P11-648	P11-687	P19-221	P19-308	P19-962	P11-590	P11-58	P19-236
SNS-M12	672,853,843	H	H	H	H	H	H	A	H	H	H	H	H	H	B	A	A	A	A	B	B	H
		X	X	X	X	X			X	X	X	X	X			X	X	X	X	X	B	H
SNS-M7	673,741,413	A	A	A	A	A	H	A	B	B	B	B	B	H	B	H	H	H	H	H	B	H
SNS-M1	**673,890,507**	A	A	A	A	A	H	A	B	B	B	B	B	H	B	H	H	H	H	H	B	H
							X							X							X	
SNS-M2	673,988,986	A	A	A	A	A	A	A	B	B	B	B	B	B	B	H	H	H	H	H	H	H
SNS-G4M4	674,022,583	A	A	A	A	A	A	A	B	B	B	B	B	B	B	H	H	H	H	H	H	H
																						X
SNS-G2M3	**674,209,292**	A	A	A	A	A	A	A	B	B	B	B	B	B	B	H	H	H	H	H	H	B
								X							X							
BACR258	677,789,941	A	A	A	A	A	A	H	B	B	B	B	B	B	H	H	H	H	H	H	H	B



A allele B allele Heterozygote





Two candidate genes in the targeted genomic region

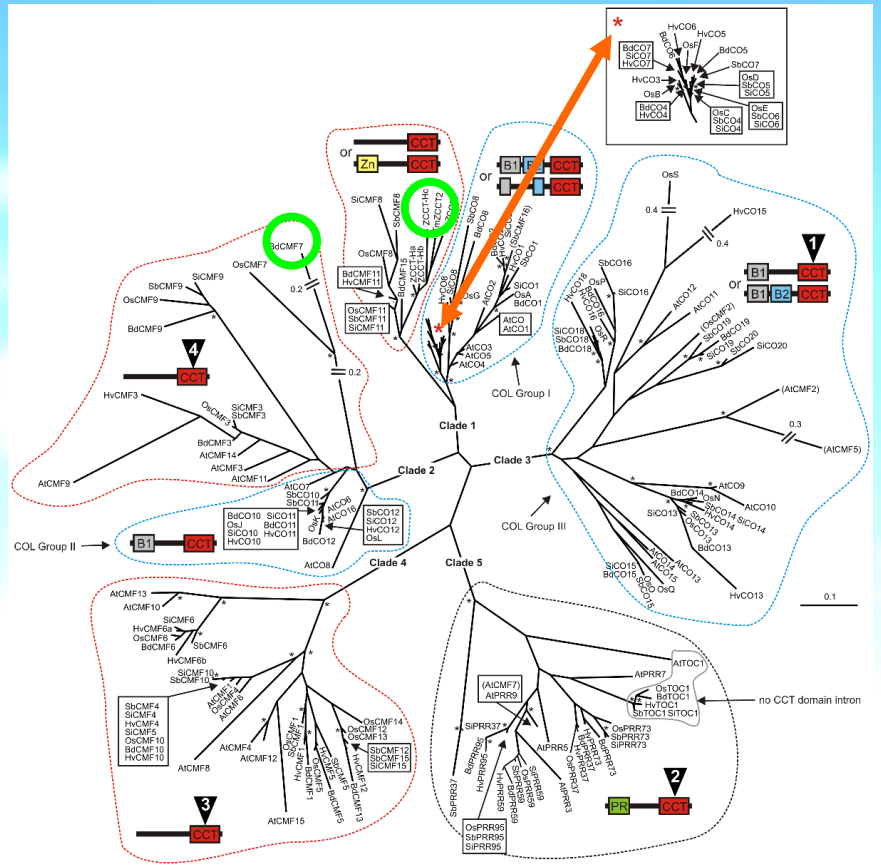
TraesCS7B02G400600 **encodes CONSTANS-like protein (*Ta*Col-B5)**

10 SNPs between two alleles

TraesCS7B02G400700 **encodes F-box domain containing protein**

TraesCS7B02G400700**: no difference between two alleles**

Phylogenetic analysis of CMF, COL, PRR and ZCCT proteins, based on the CCT domain



Cockram *et al*., PLoS ONE 7: e45307 (2012)

CCT: Constans, Constans-like, TOC1

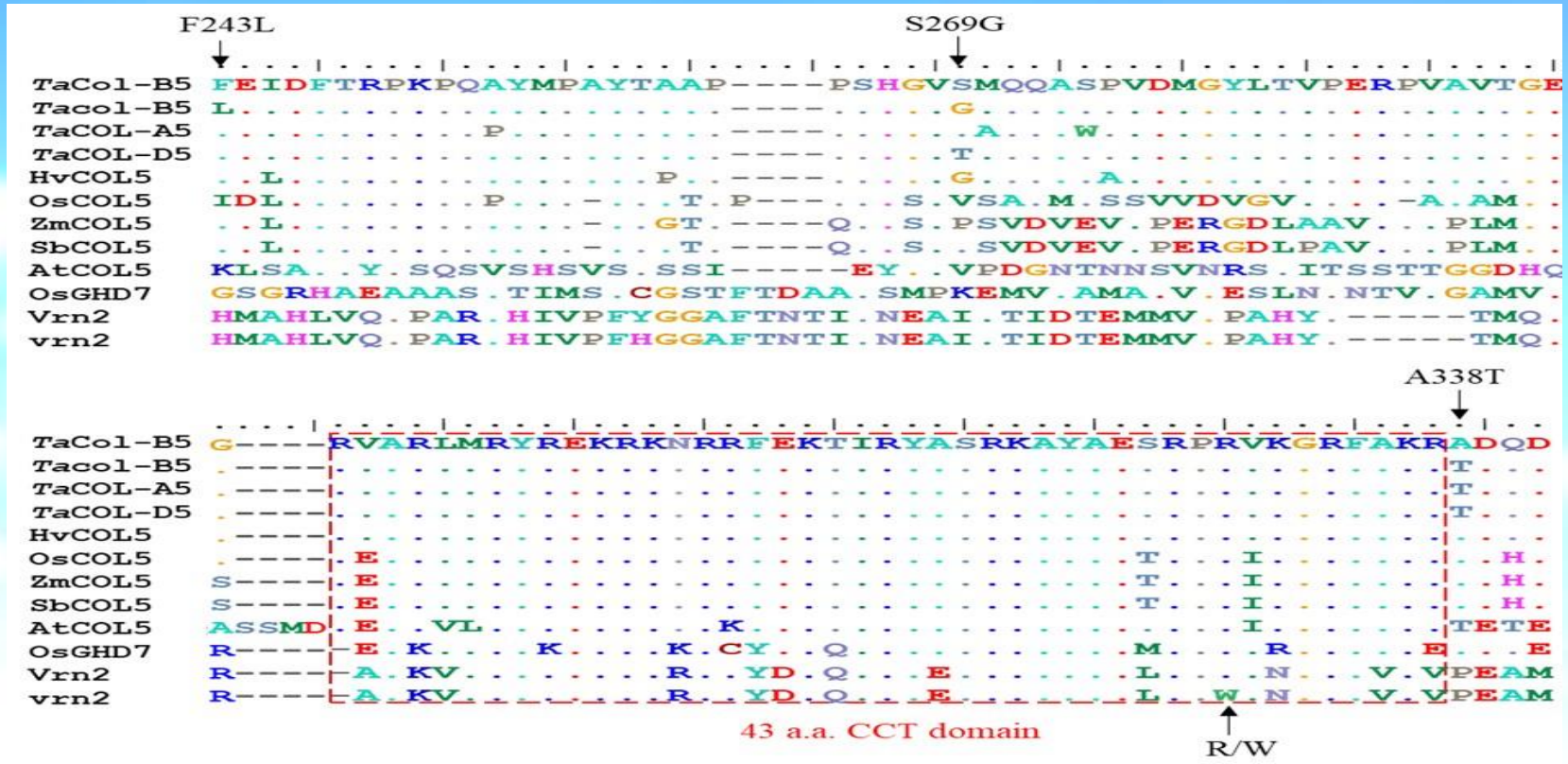
Constans: A mutant flowers in a ‘constant’ manner regardless of photoperiod.

TOC: Timing of CAB expression

CAB: Chlorophyll a/b binding protein.

CMF: CCT Motif Family
COL: Constans-Like
PRR: Pseudo Response Regulator
ZCCT: Zinc CCT

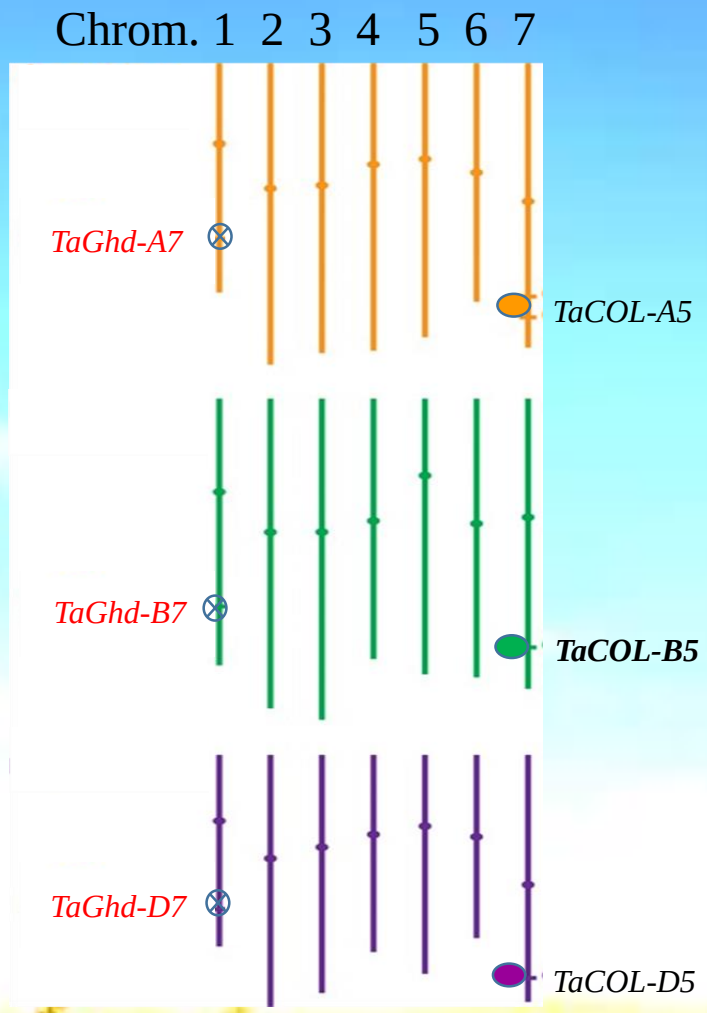
Multiple sequence alignment of *Ta*COL-B5 and related proteins



VRN2: Vernalization gene 2, ZCCT1 and ZCCT2.
Yan *et al*., Science. 303: 1640-1644 (2004)

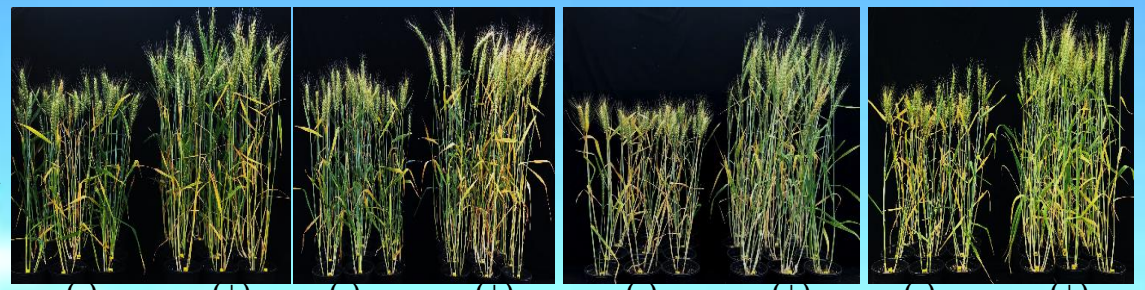
***Os*Ghd7:** **G**rains per panicle, plant **h**eight, heading **d**ate.
Xue *et al*., Nat. Genet. 40: 761-767 (2008)

TaCol-B5 was not orthologous to *OsGhd7*



Phenotypic effects *of* overexpressed *TaCol-B5* in Yangmai18

T_1



(-) (+) (-) (+) (-) (+) (-) (+)

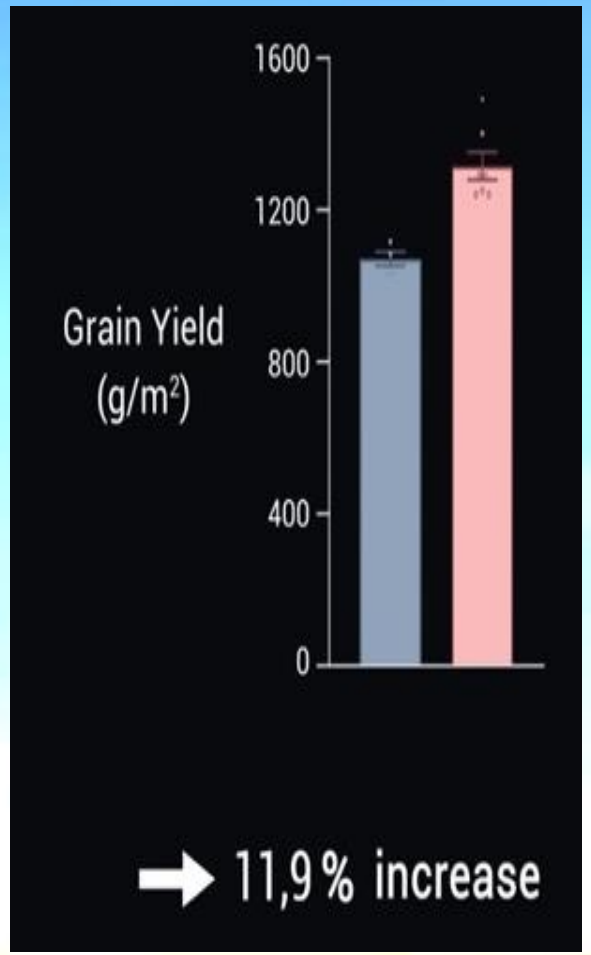
T_2

(-) (+)

(-) (+)

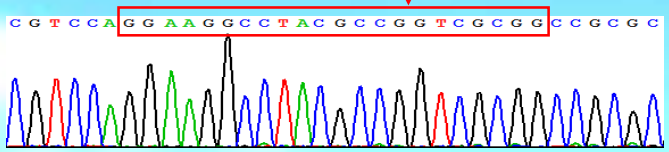
T_3

(-) (+) (-) (+)

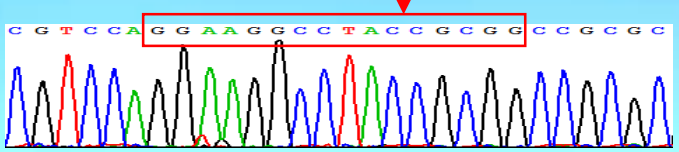


Pleiotropic effects of edited CCT domain in *Ta*col-B5 protein

Tacol-B5-ED1 GGAAGGCCTACGCCGAGTCGCGG



Tacol-B5-ED12 GGAAGGCCTACGCCGAGTCGCGG



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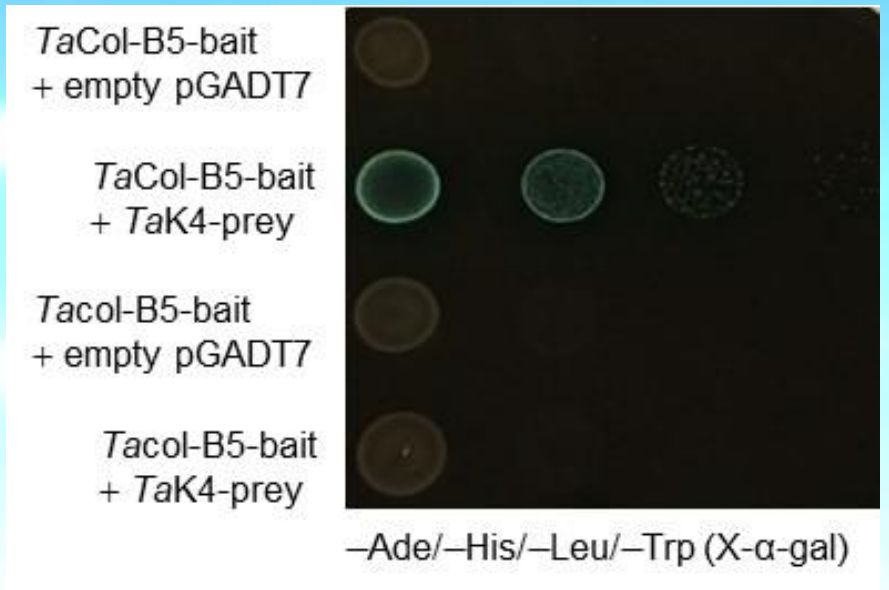
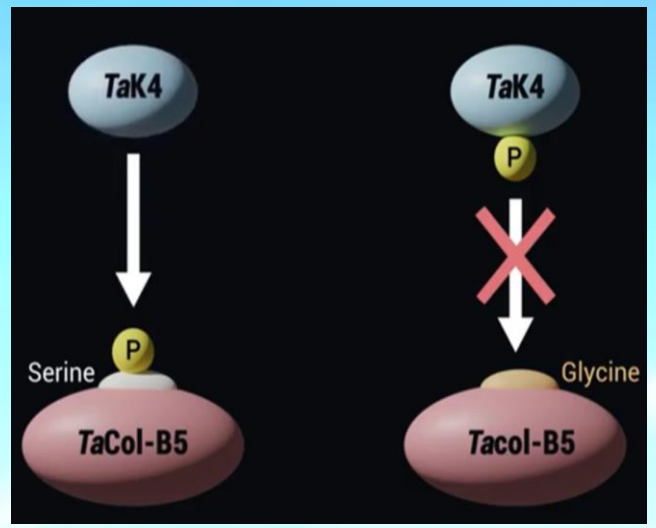
Mechanisms of *TaCol-B5* underlying the traits

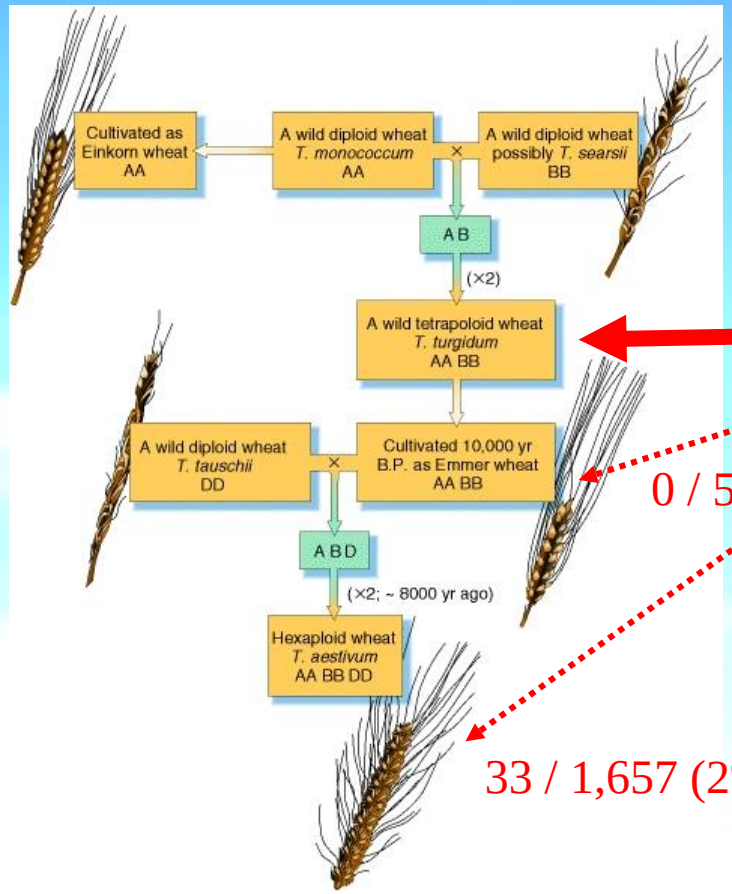


Interactions of *Ta*COL-B5 with *Ta*K4



Phosphorylation of *Ta*COL-B5 by *Ta*K4

Origin and distribution of *TaCol-B5*



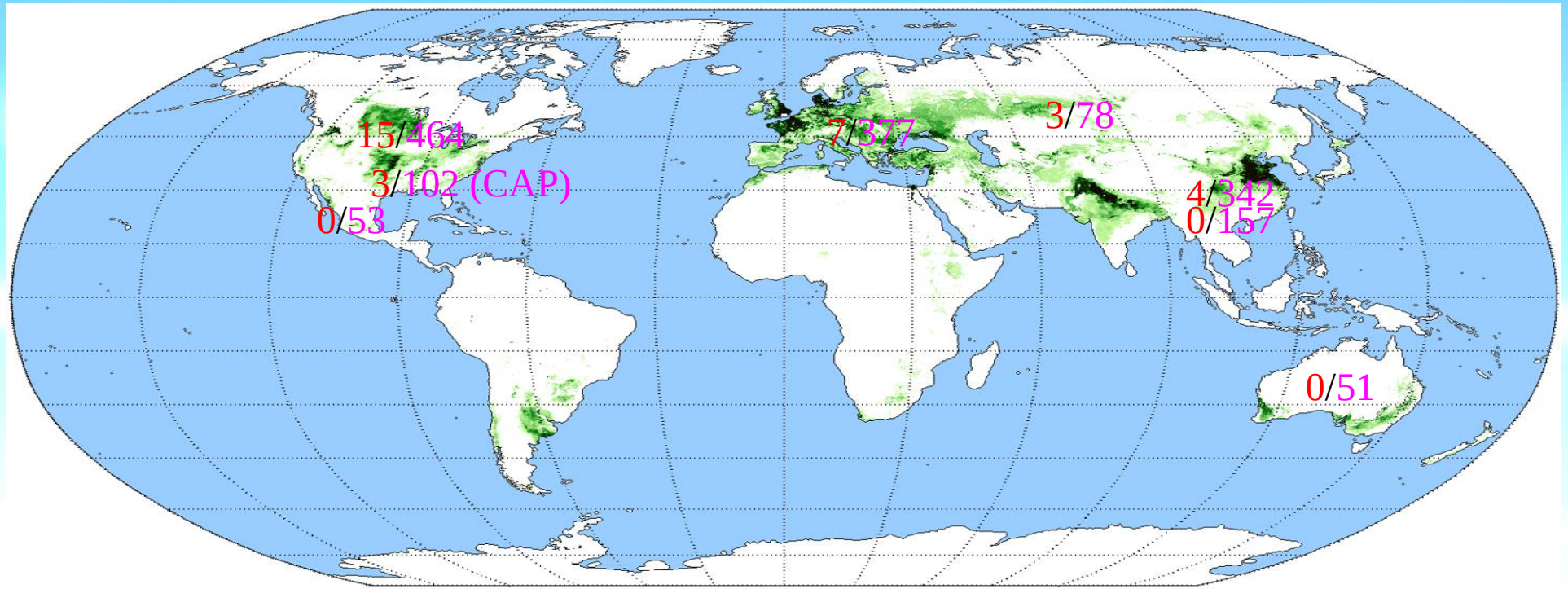
TaCol-B5 in 'Zavitan'

0 / 5

33 / 1,657 (2%)

https://www.cerealsdb.uk.net/cerealgenomics/WheatBP/Documents/DOC_Evolution.php

The distribution of *TaCol-B5*/*Tacol-B5* alleles in global wheat accessions



WheatCAP: McNeal, RSI5 (PI584453), Billings