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Insight Into Wheat Spike and Grain Development by Learning From Natural Variation And Chemically-Induced Mutants

John Innes Centre
Unlocking Nature's Diversity

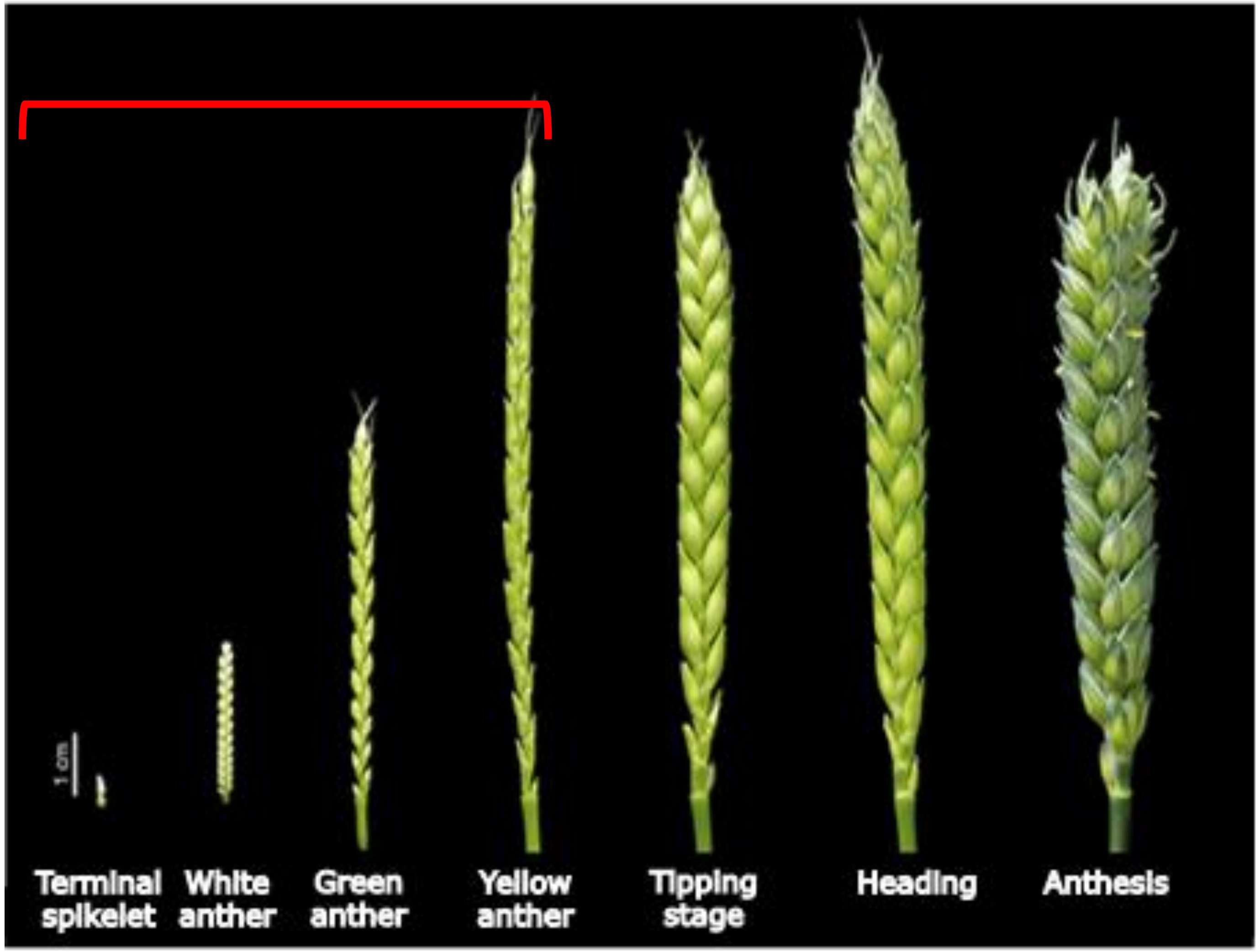
@AndyChen_Wheat

Andy Chen (andy.chen@jic.ac.uk)

Wheat spike architecture

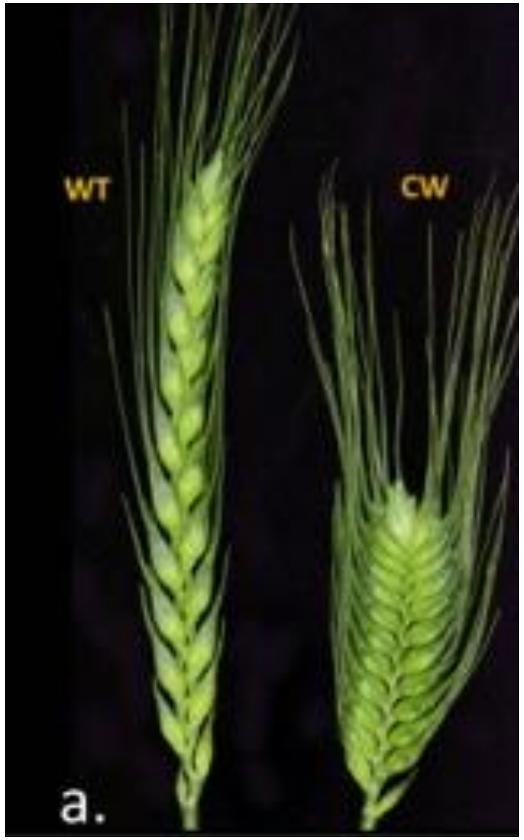
Spike

Wheat spike development



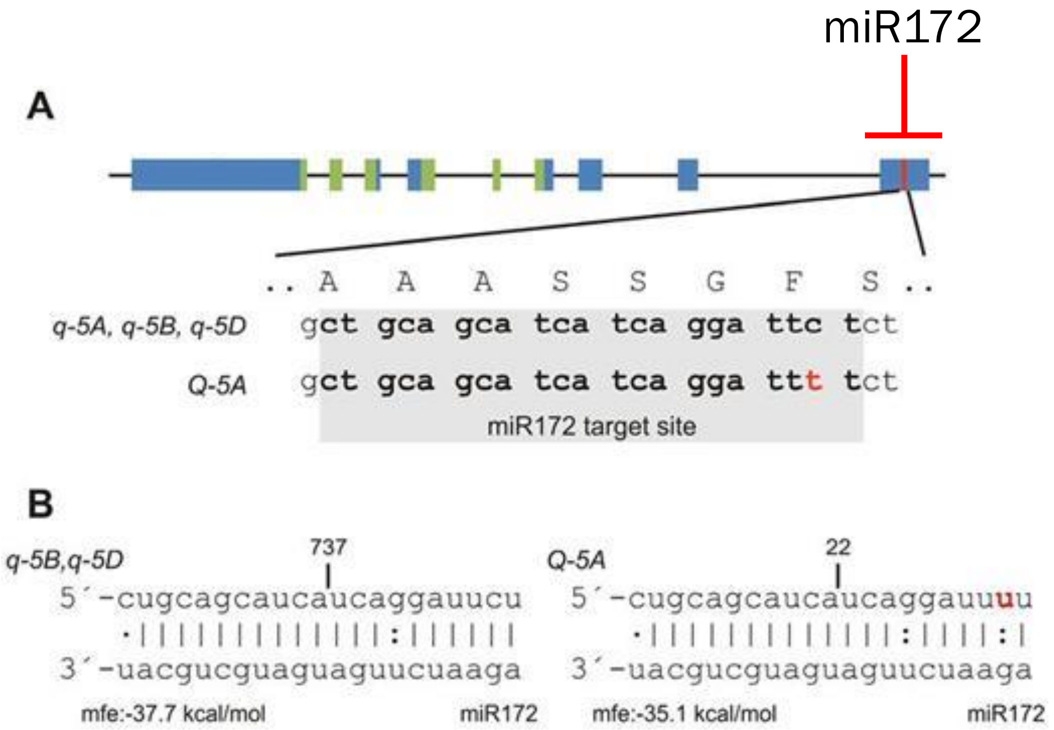
Modified from Guo *et al* 2018

Major loci influencing spike morphology



Compactum (*C*)
2D: 231 to 242 Mb
(Kajla et al. 2023)

qq ***QQ***

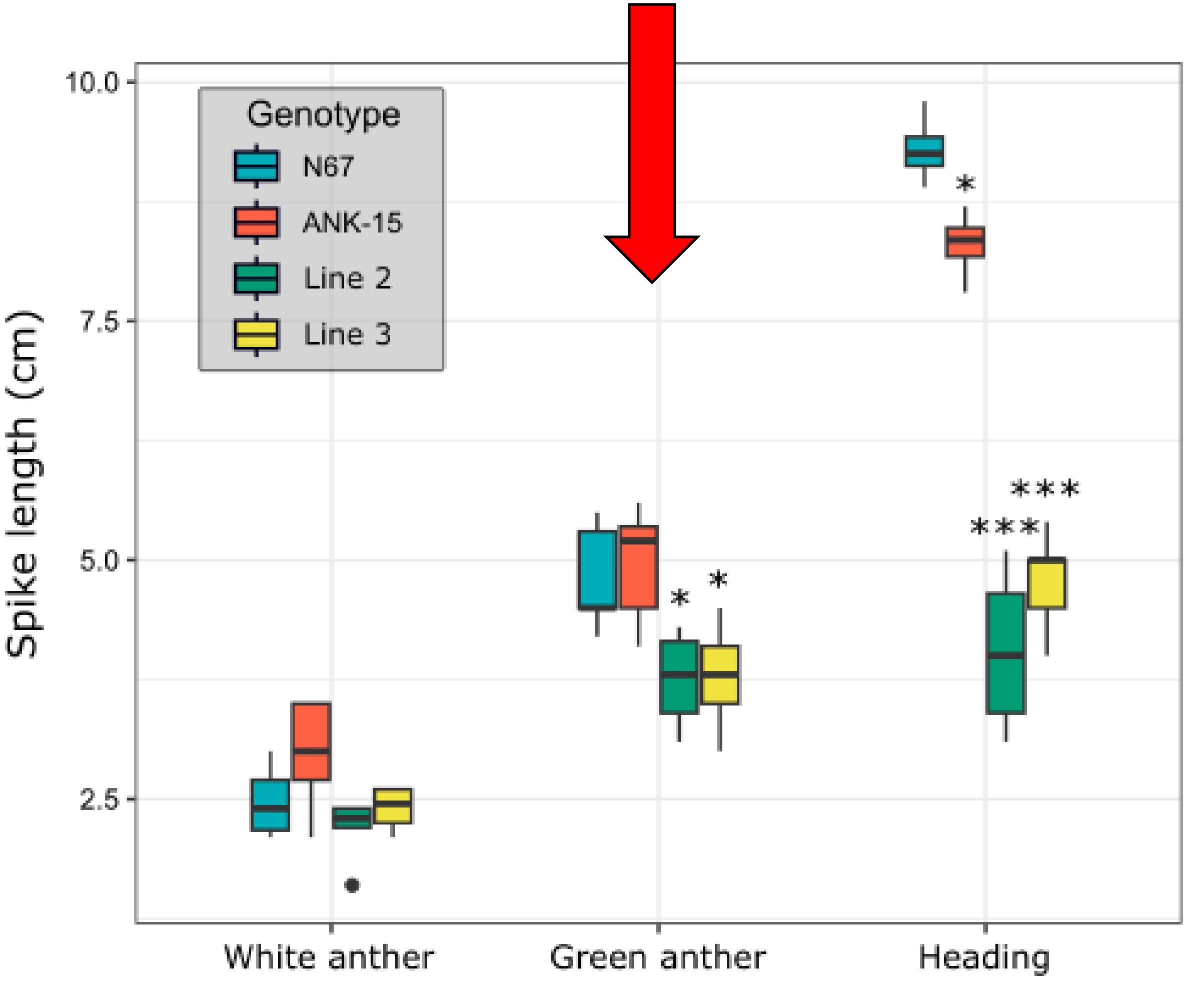


Q (*AP2L-A5*)
(Zhang et al. 2011; Debernardi et al 2017)

Compact spike near-isogenic lines

(Koval et al. 1988)

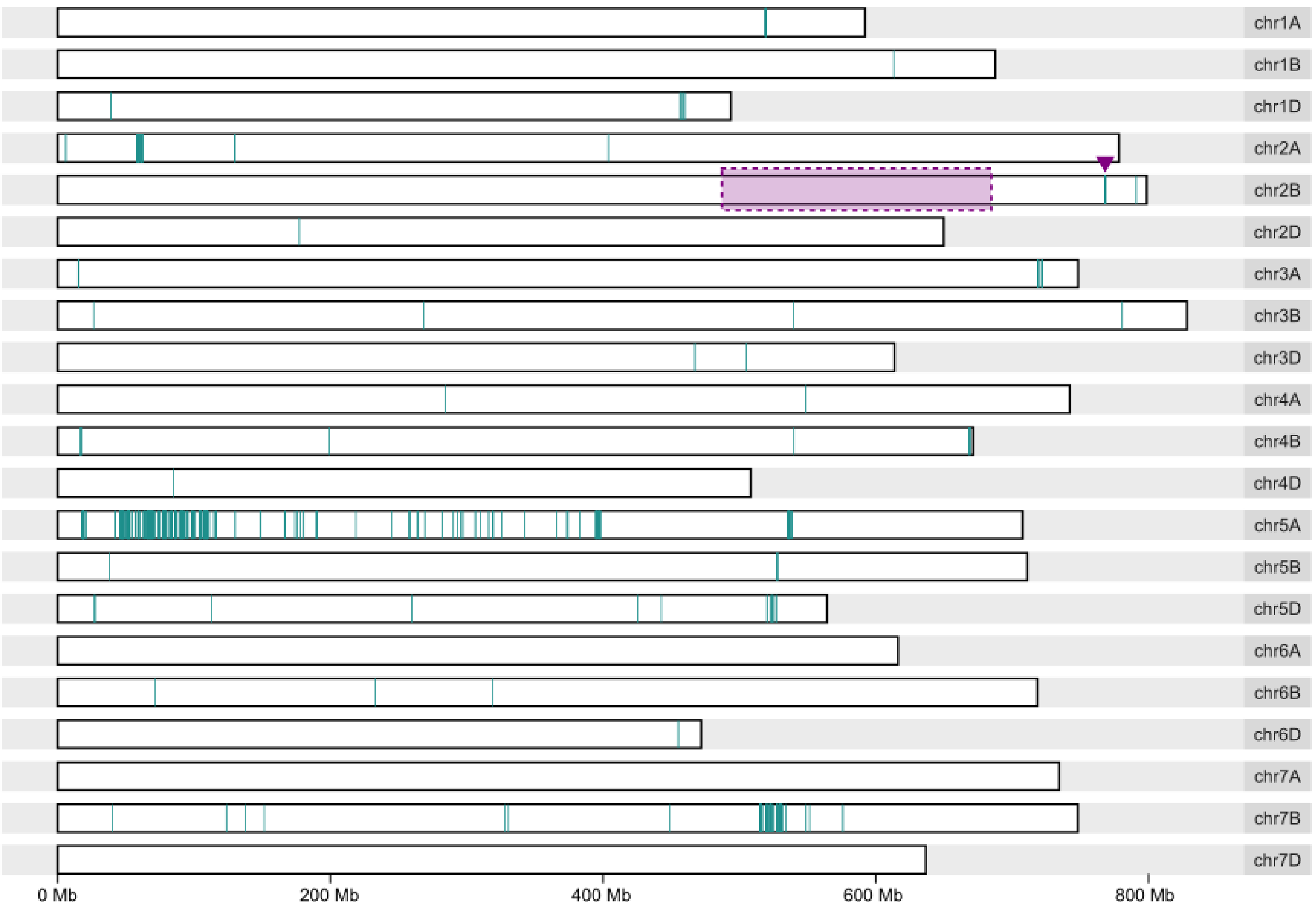
Rachis collected (n = 4 plants)



ANK-15

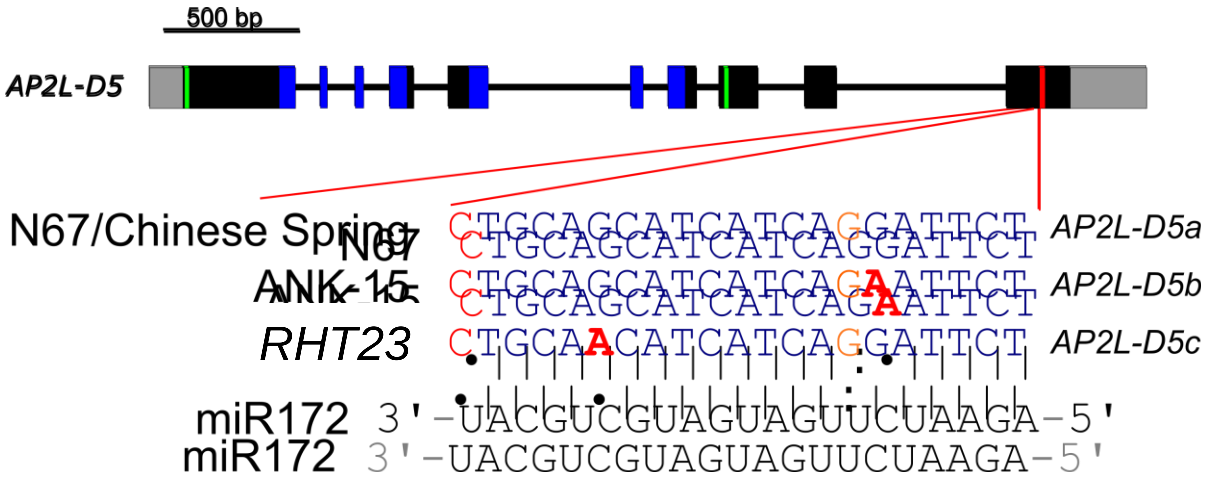
- Compact spike locus was introduced from a mutant line of wheat cultivar Skala (Koval et al. 1988)
- Compact spike phenotype has been proposed to be controlled by a locus on chromosome 2B (Amagai et al. 2016)

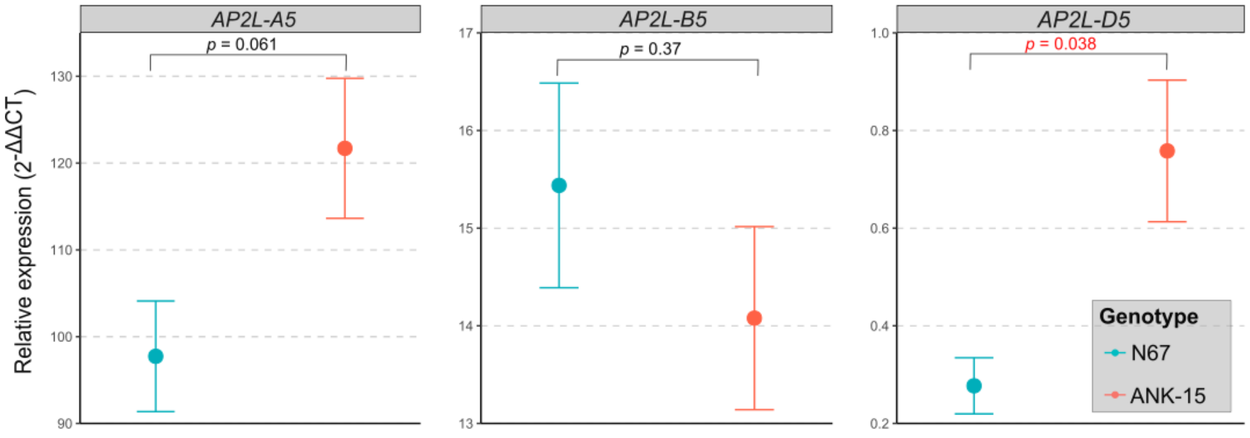
No detectable SNP in putative 2B region



RNA-seq aligned against RefSeq v1.0

SNP in *AP2L-D5* of ANK-15



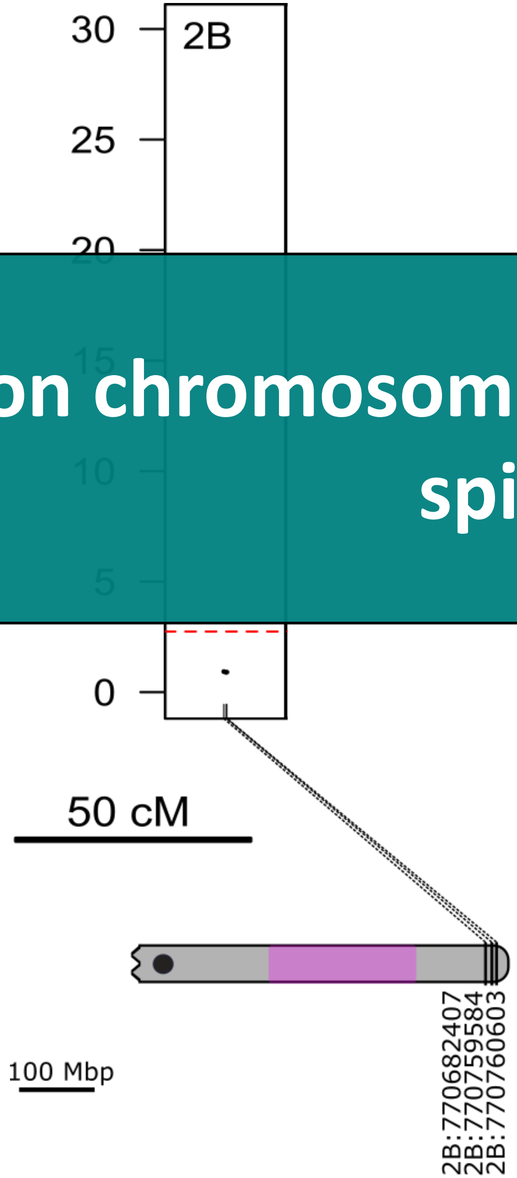


- SNP introduces mismatch with miR172
- *AP2L-D5* suppressed speltoid phenotype (Zhang et al. 2011)
- *AP2L-D5* was the underlying gene for *REDUCED HEIGHT 23* (Zhao et al. 2018)

t al 2014

Zeng et al. 2023 *bioRxiv*

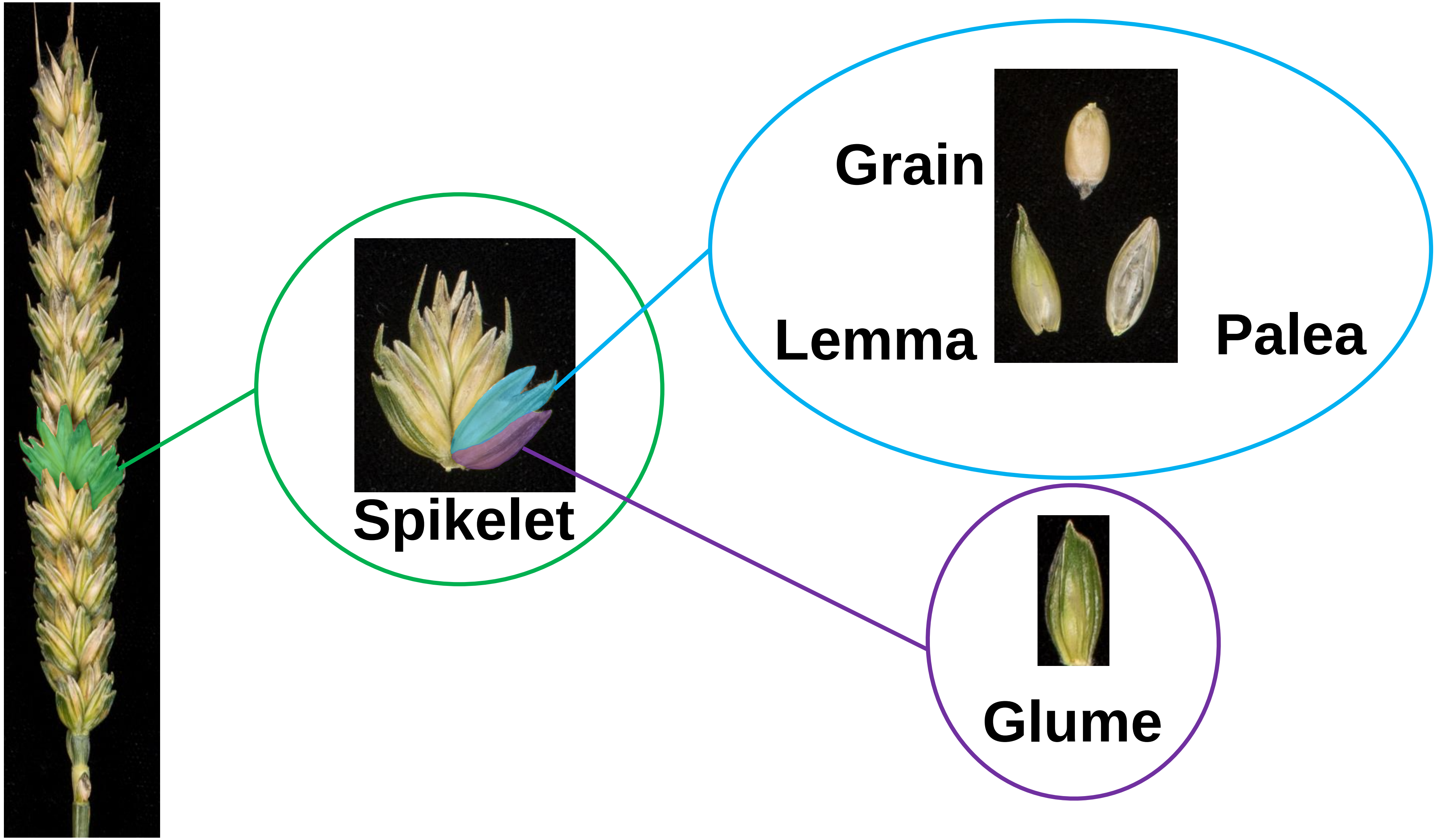
ANK-15’s compact spike locus is on 5D



AP2L-D5 **on chromosome 5D is likely the gene underlying the compact spike phenotype of ANK-15**

Zeng et al. 2023 *bioRxiv*

Wheat spike structure



Spike

Spikelet

Grain

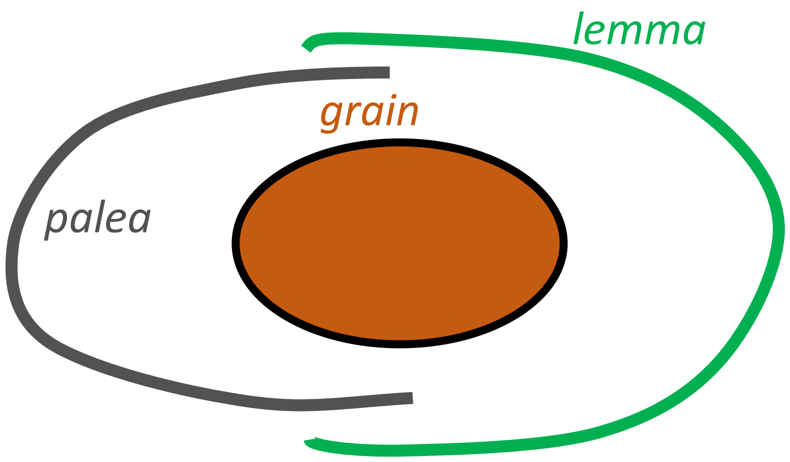
Lemma

Palea

Glume

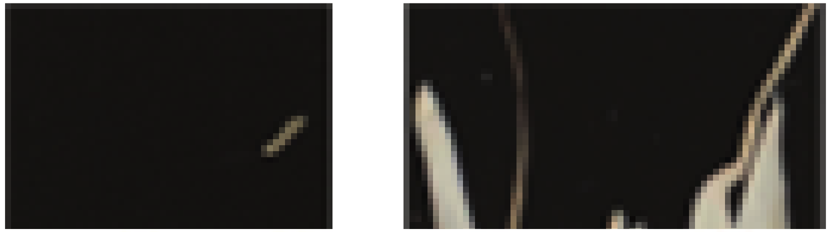
Size of maternal floral organs influences grain morphology

- Lemma and palea envelop the grain during development
- The size of floret cavity correlate positively with grain size in wheat and rice (Millet *et al*. 1986; Li *et al*. 2015)



Wheat

Natural variation loci for glume size



Solving a 100 year old mystery: Cloning the P1 locus in Triticum polonicum

401 views • 1 year ago

International Wheat Genome Sequencing Consortium

IWGSC webinar 02/24/2022 Presenter: Nikolai Adamski, John Innes Centre, UK Outline: The tetraploid wheat Triticum polonicum ...

Tunicate locus in “pod corn”
ZMM19

Han *et al.* 2012
Wingen *et al.* 2012

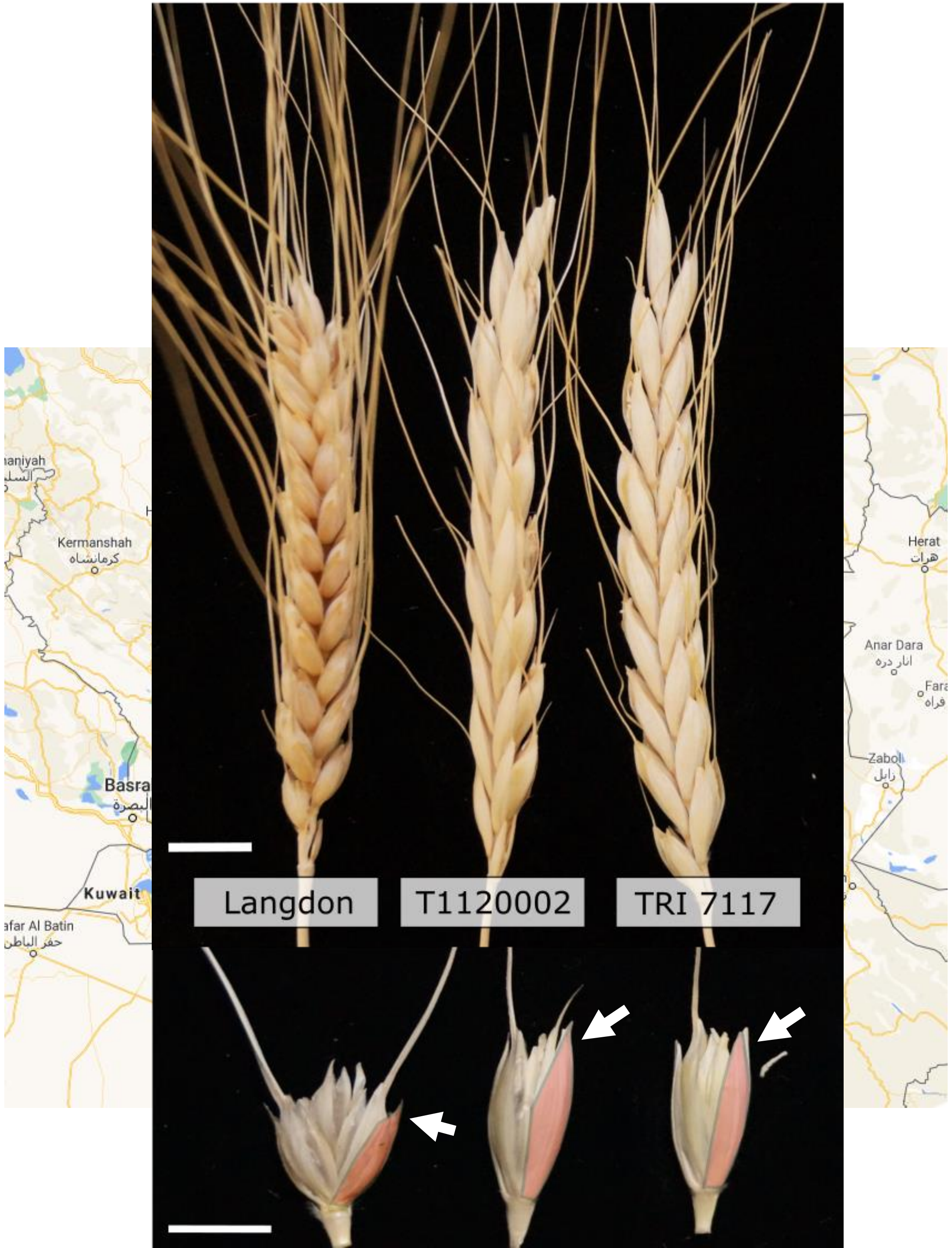
P1 locus in *T. polonicum*
VRT-A2

Adamski *et al.* 2021
Liu *et al.* 2021

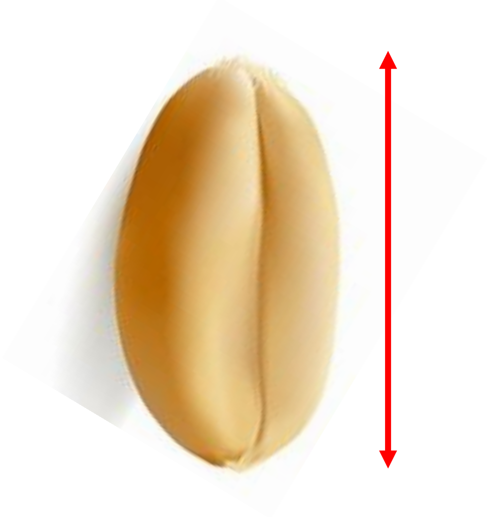
P2 locus in *T. ispahanicum*

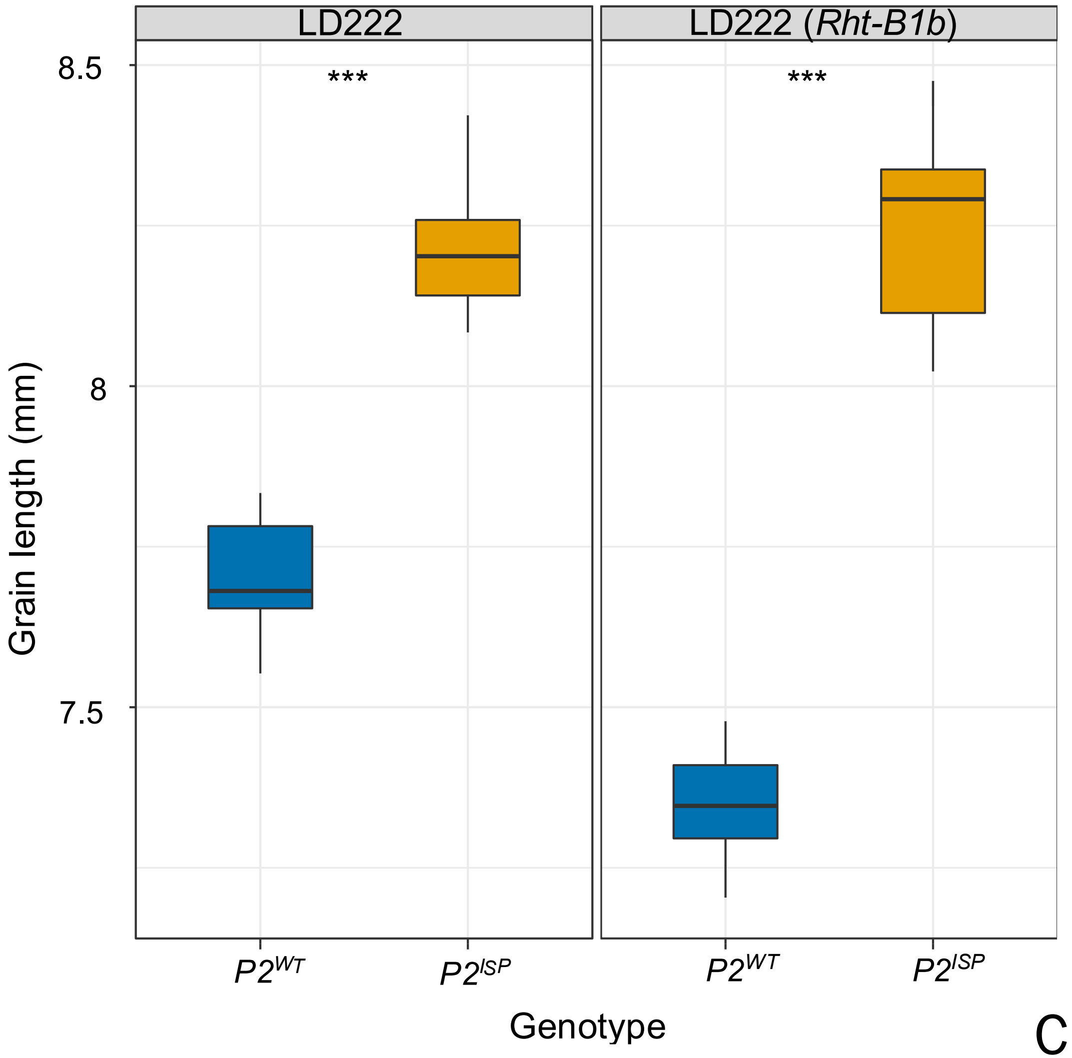
T. ispahanicum (AABB)

- Identified in the Isfahan province of Iran (Heslot 1958)
- Elongated glume controlled by a major QTL, *P2*



P2 increases grain length

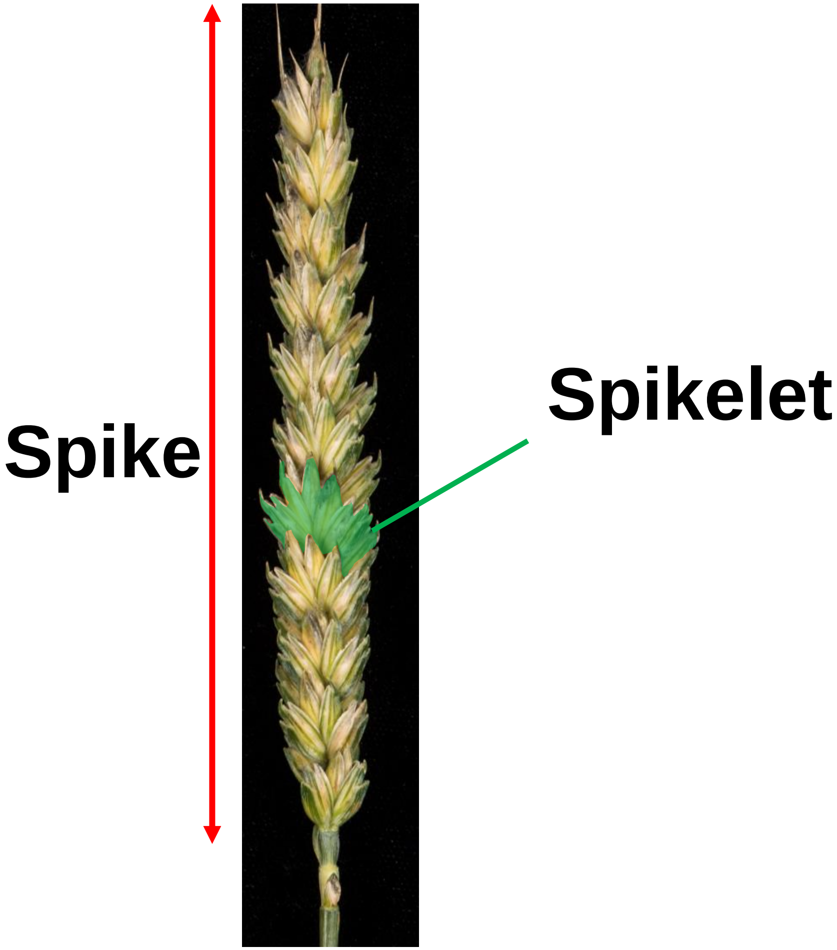




NIL provided by Professor Watanabe

Chen *et al* 2022

P2 increases spike length and spikelet number



NIL provided by Professor Watanabe

Chen *et al* 2022

P2 decreases number of grains per spike via reducing number of fertile florets

NIL provided by Professor Watanabe

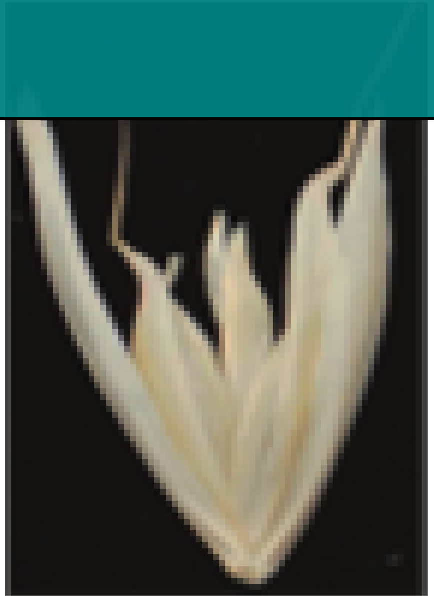
Chen *et al* 2022

P2 of *T. ispahanicum* was located to 7B

- *P2* was assigned to chromosome 7B (Watanabe *et al*. 1999)
- *VRT-A2* on chromosome 7A was the underlying gene of the long glume locus, *P1*, from *T. polonicum*

Is *VRT-B2* the gene underlying the *T. ispahanicum P2* locus?

T. aestivum



T. polonicum

Adamski *et al* 2022

P2 is on chromosome 6A!

- F2 mapping Langdon × *T1120002* (*T. ispahanicum*) (*n* = 93)
- Marker designed using published RNA-seq dataset (Zou et al. 2015)
- No association on chromosome 7B
- Significant association on chromosome 6A

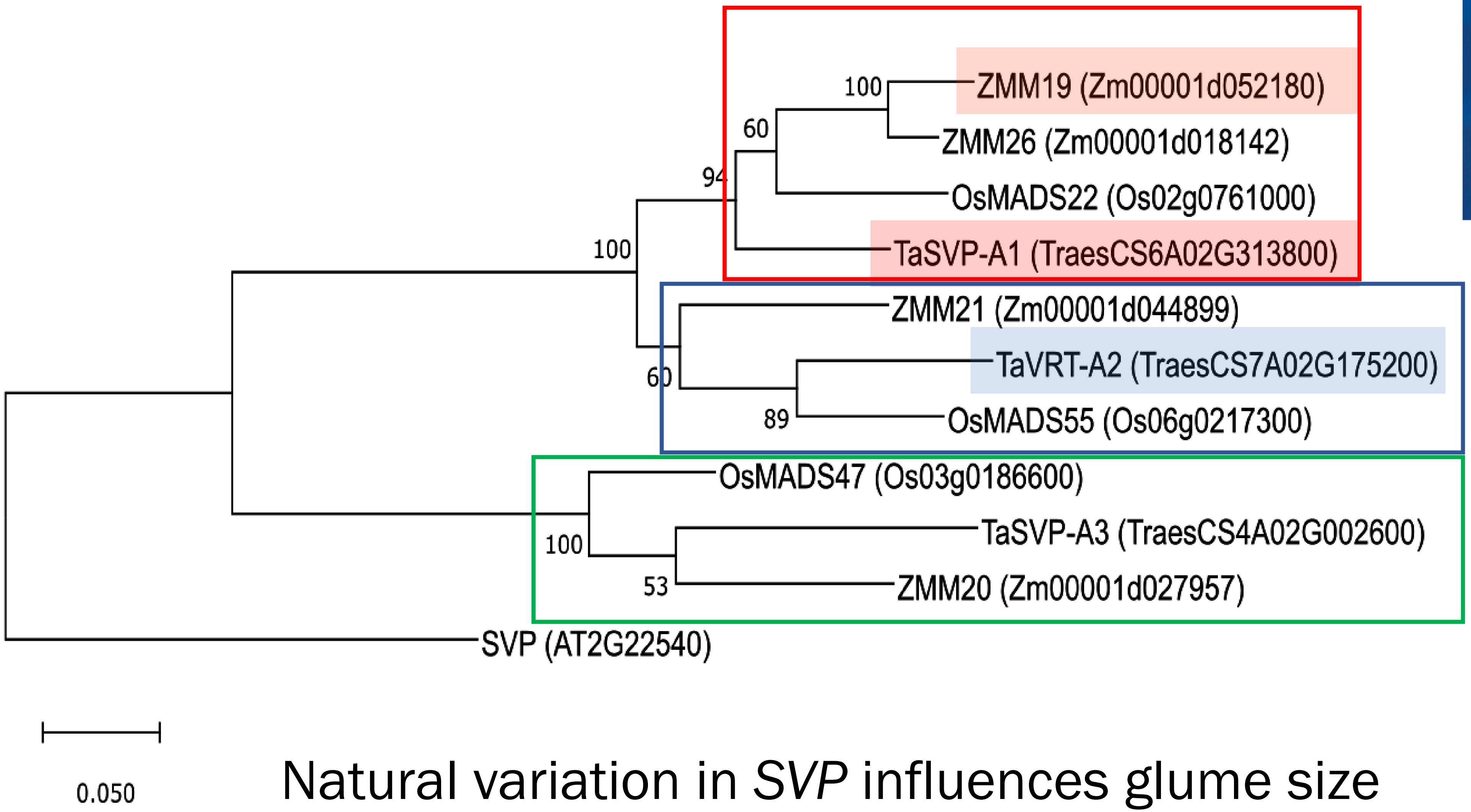
VRT-B2

Chen *et al* 2022

SVP-A1 is a likely candidate within *P2*

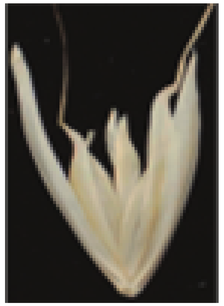
- We fine-mapped *P2* to a region of 1.68 Mbp (549 Mbp - 550.7 Mbp)
- Identified ***SVP-A1*** (*TraesCS6A02G313800*) as a strong candidate gene
- *Short Vegetative Phase* (*SVP*) genes belong to a subfamily of MADS-box transcription factor

Phylogenetic tree of *SVP* proteins in rice, wheat, and maize



Han *et al.* 2012
Wingen *et al.* 2012

T. aestivum



T. polonicum

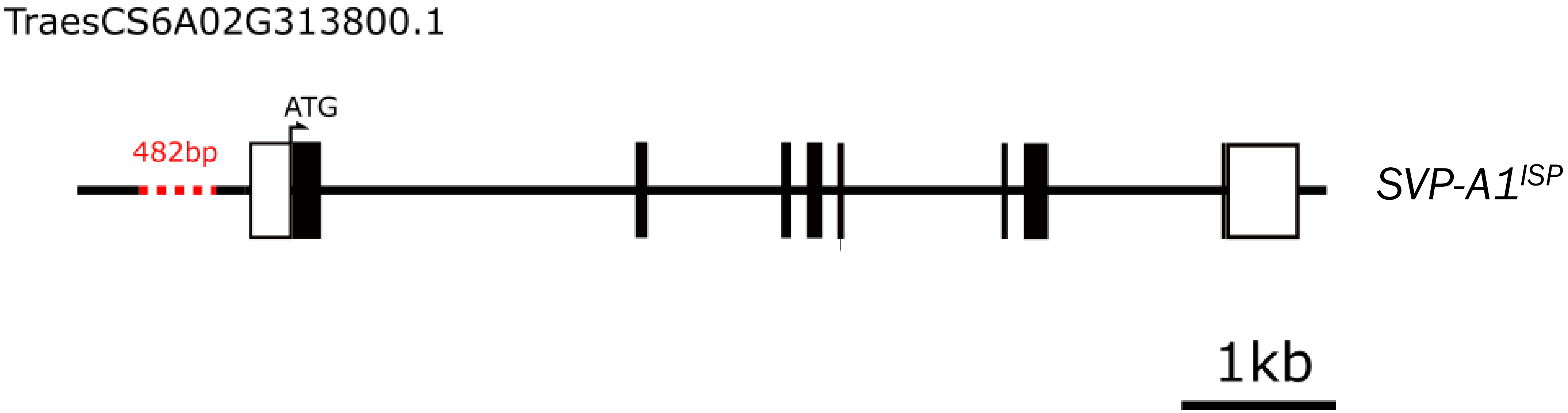
Adamski *et al.* 2021
Liu *et al.* 2021

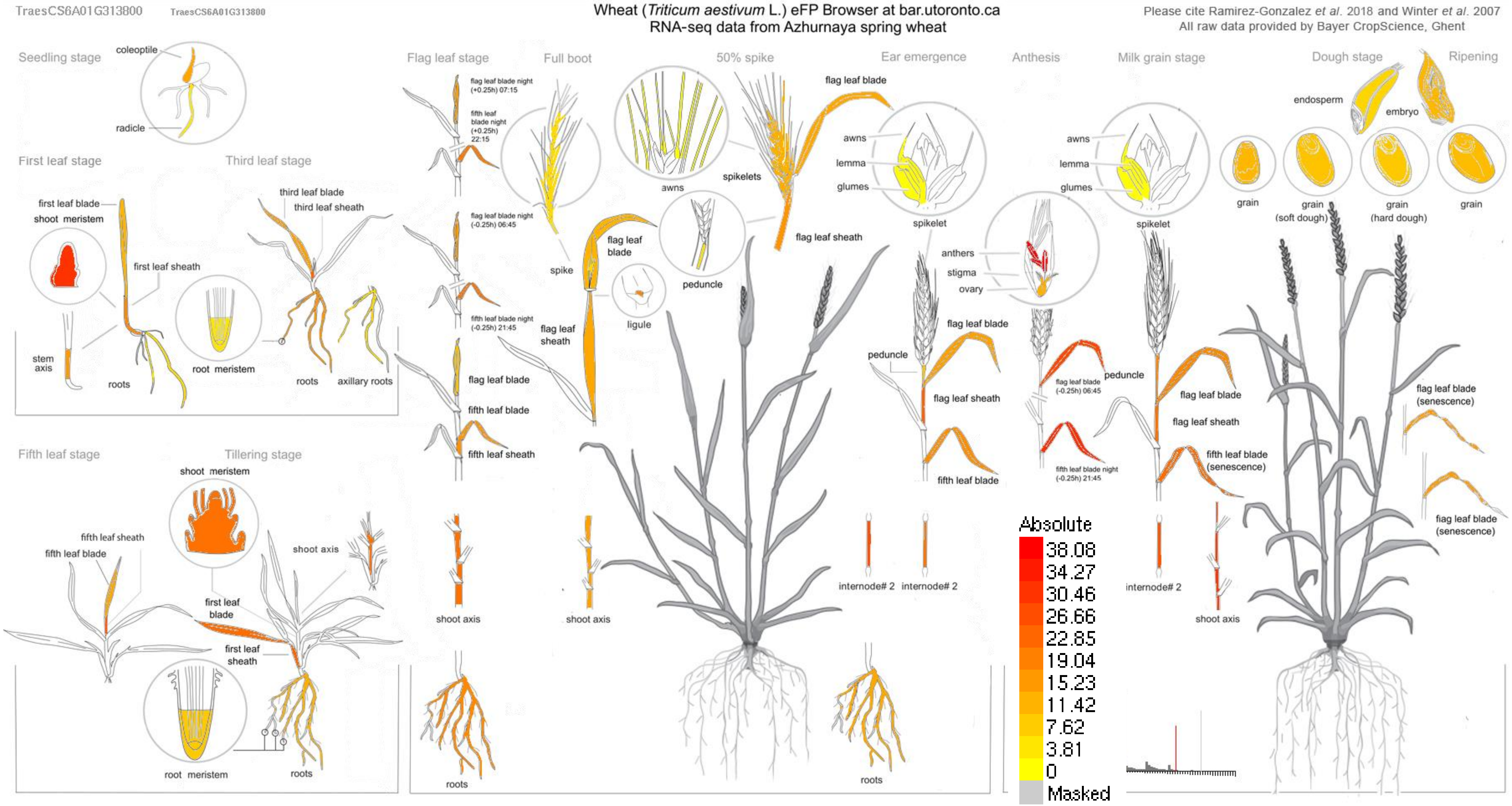
Natural variation in *SVP* influences glume size

SVP-A1 variation in *T. ispahanicum*

Whole-genome sequencing data of *T. ispahanicum* (Zhou et al. 2020)

- Promoter deletion (482-bp)
- 53 polymorphisms (SNP and small indel)
- One missense mutation (exon 5)



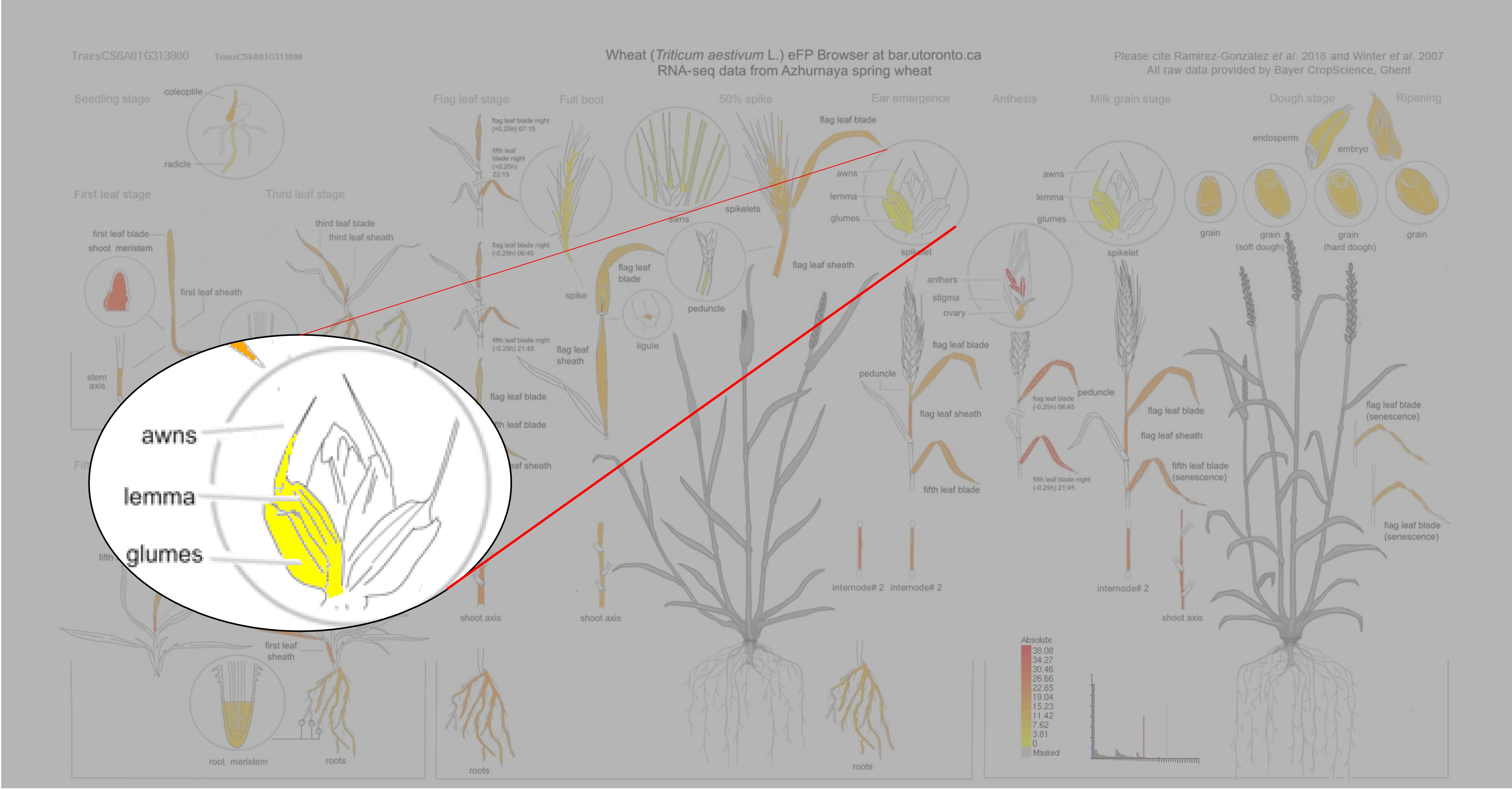


TraesCS6A01G313800 TraesCS6A01G313800

Wheat (*Triticum aestivum* L.) eFP Browser at bar.utoronto.ca
RNA-seq data from Azhurnaya spring wheat

Please cite Ramirez-Gonzalez *et al*. 2018 and Winter *et al*. 2007
All raw data provided by Bayer CropScience, Ghent

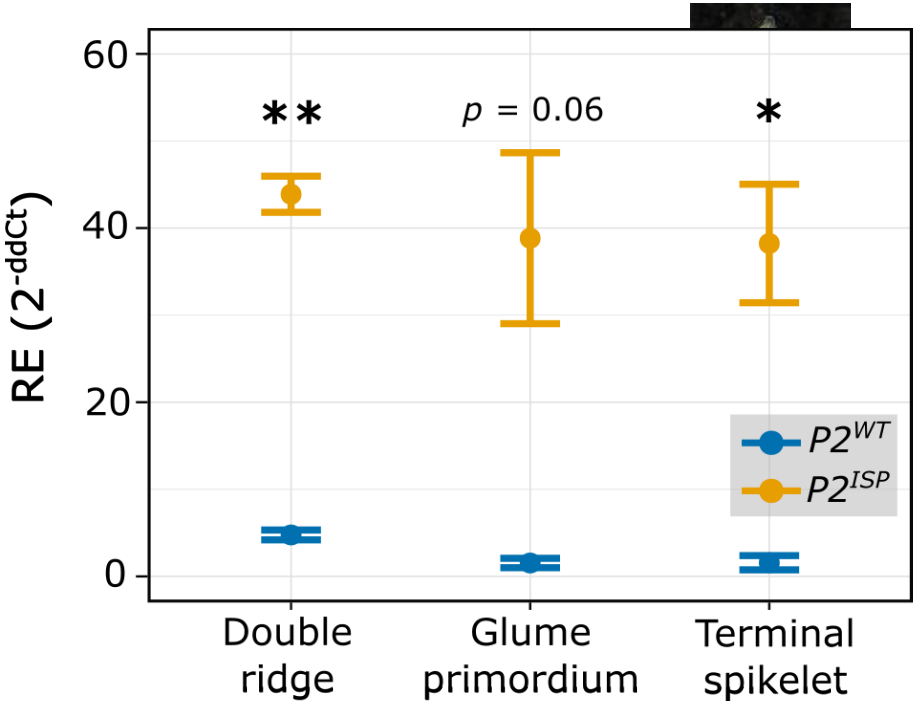
Ramirez Gonzalez *et al* 2018



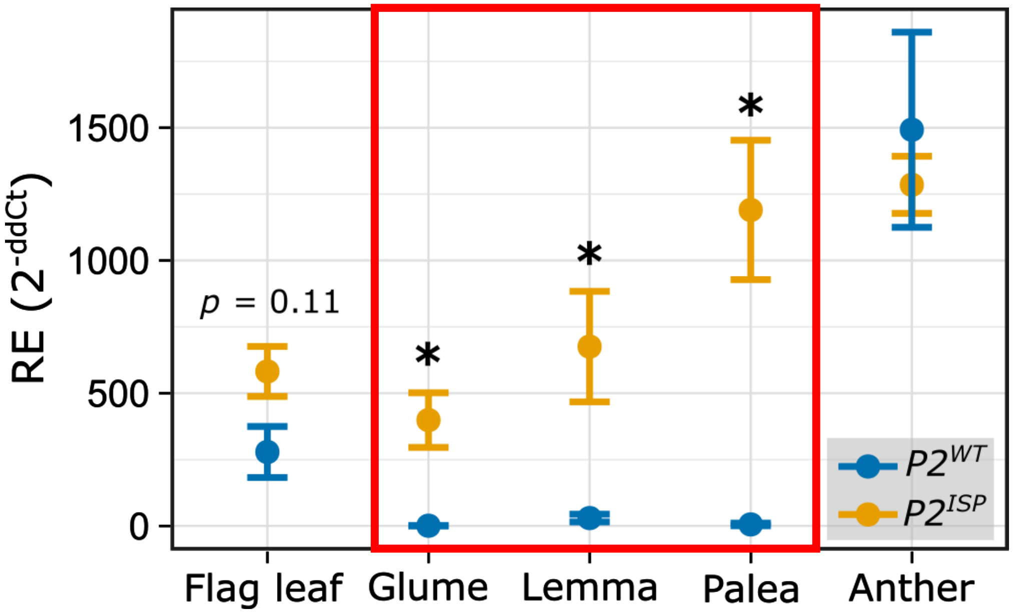
SVP-A1 has no/low expression in lemma and glume

Ramirez Gonzalez *et al* 2018

$P2^{ISP}$ has higher *SVP-A1* expression than $P2^{WT}$



N = 3

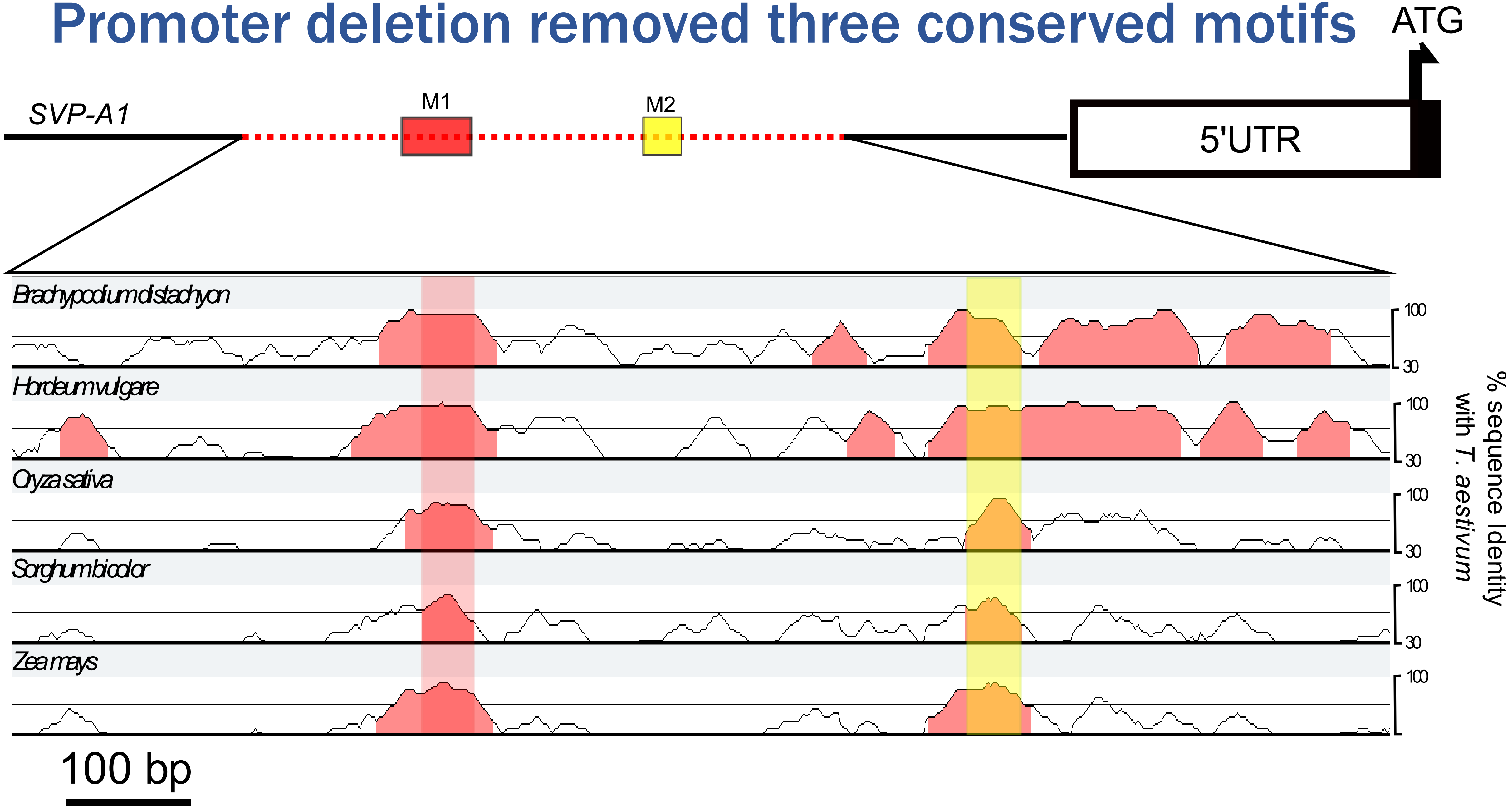


Tissue collected at late booting, N=4

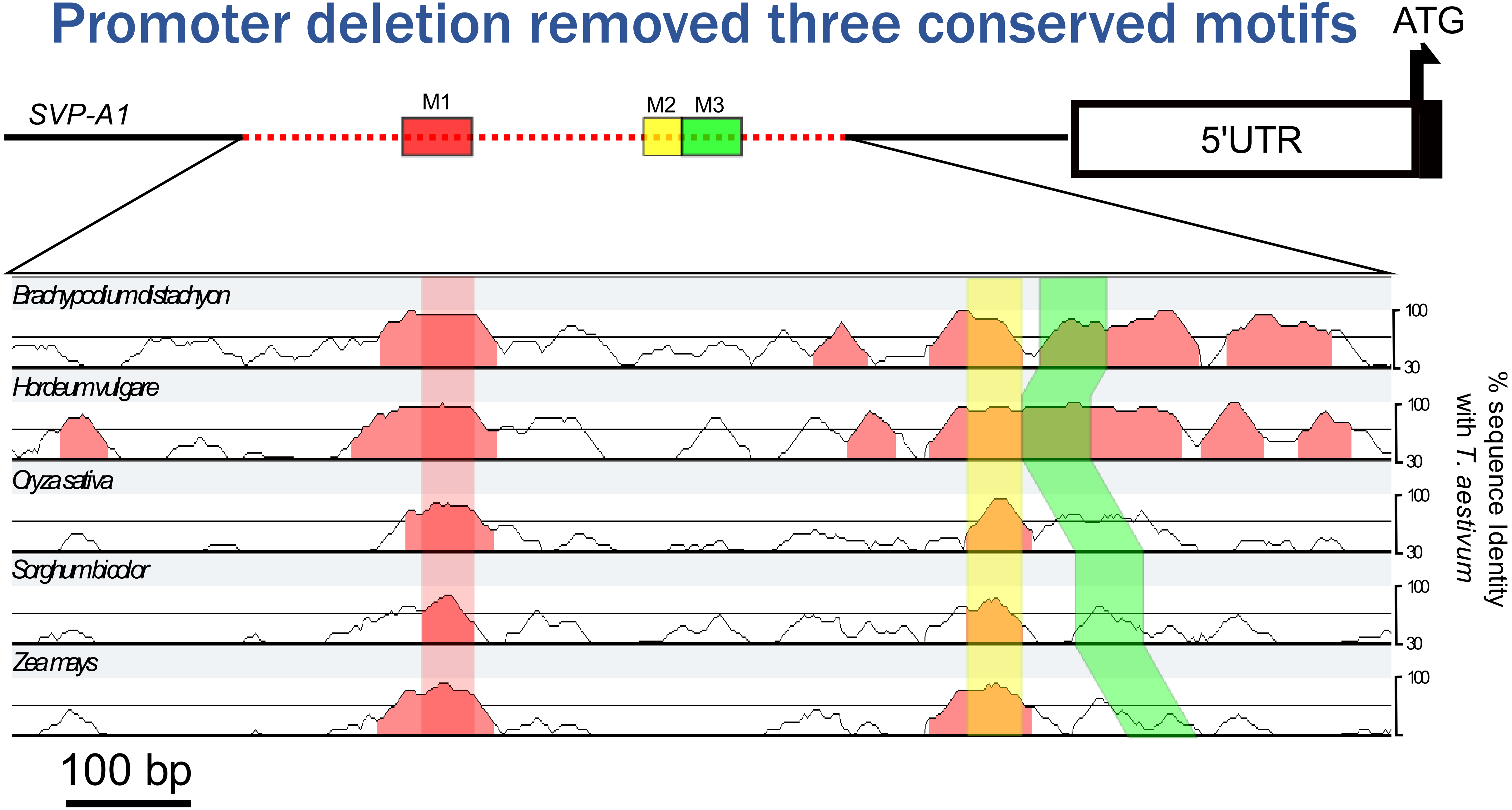
Chen *et al* 2022


100 bp
SVP-A1
5'UTR
ATG

Promoter deletion removed three conserved motifs

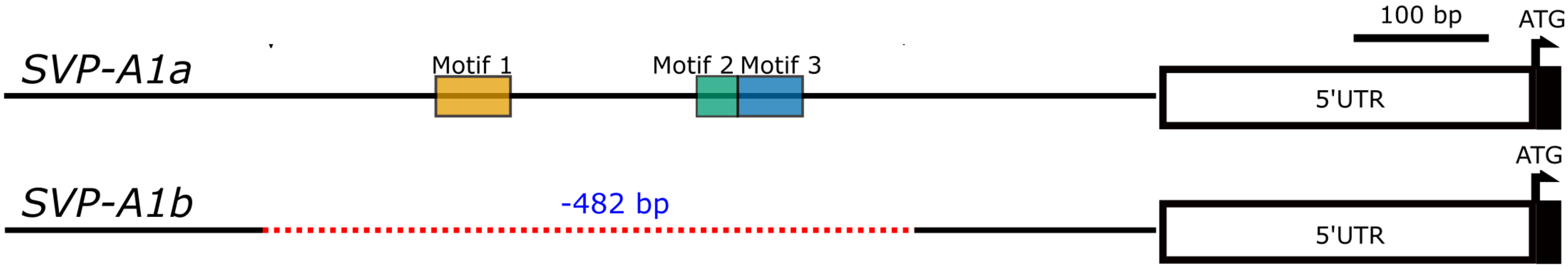


Promoter deletion removed three conserved motifs

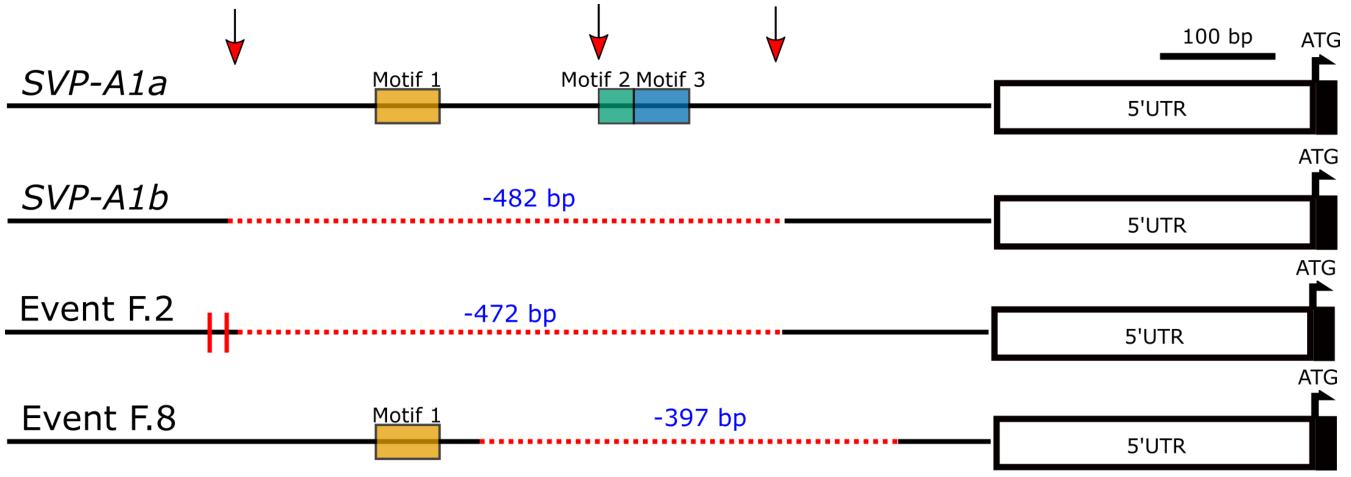


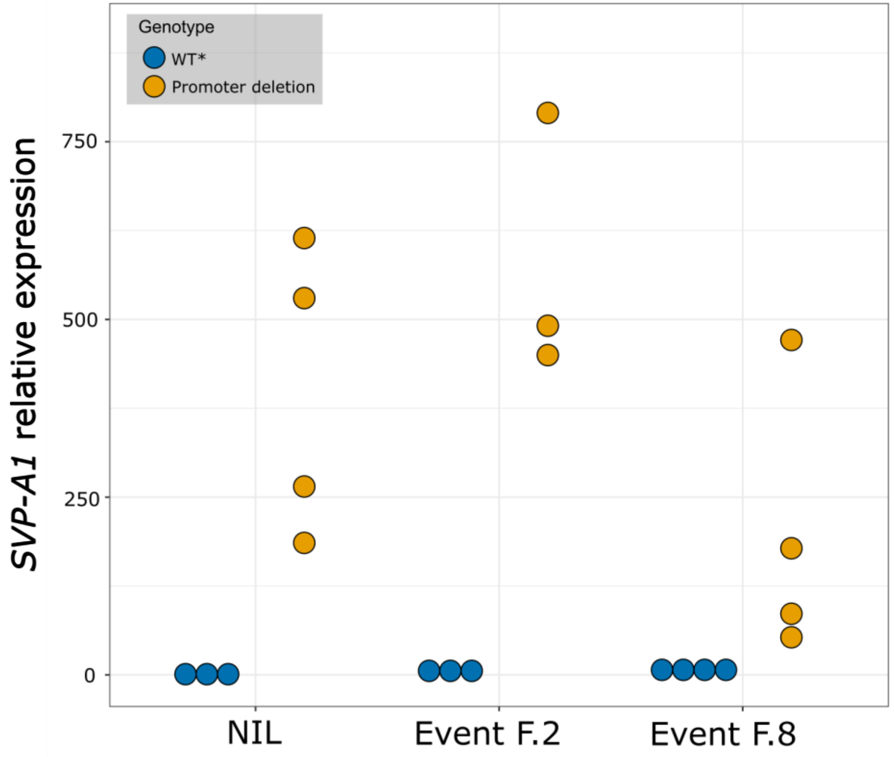
Generation of novel *SVP-A1* alleles via promoter editing

- CRISPR-Cas9 gene editing of the promoter region of *SVP-A1* (Sadiye et al. 2019; Smedley et al. 2021)
- Chimeric protein of Growth-regulating factors 4 and GIF-interacting factor 1 (GRF/GIF) to improve regeneration efficiency (Debernardi et al. 2017)



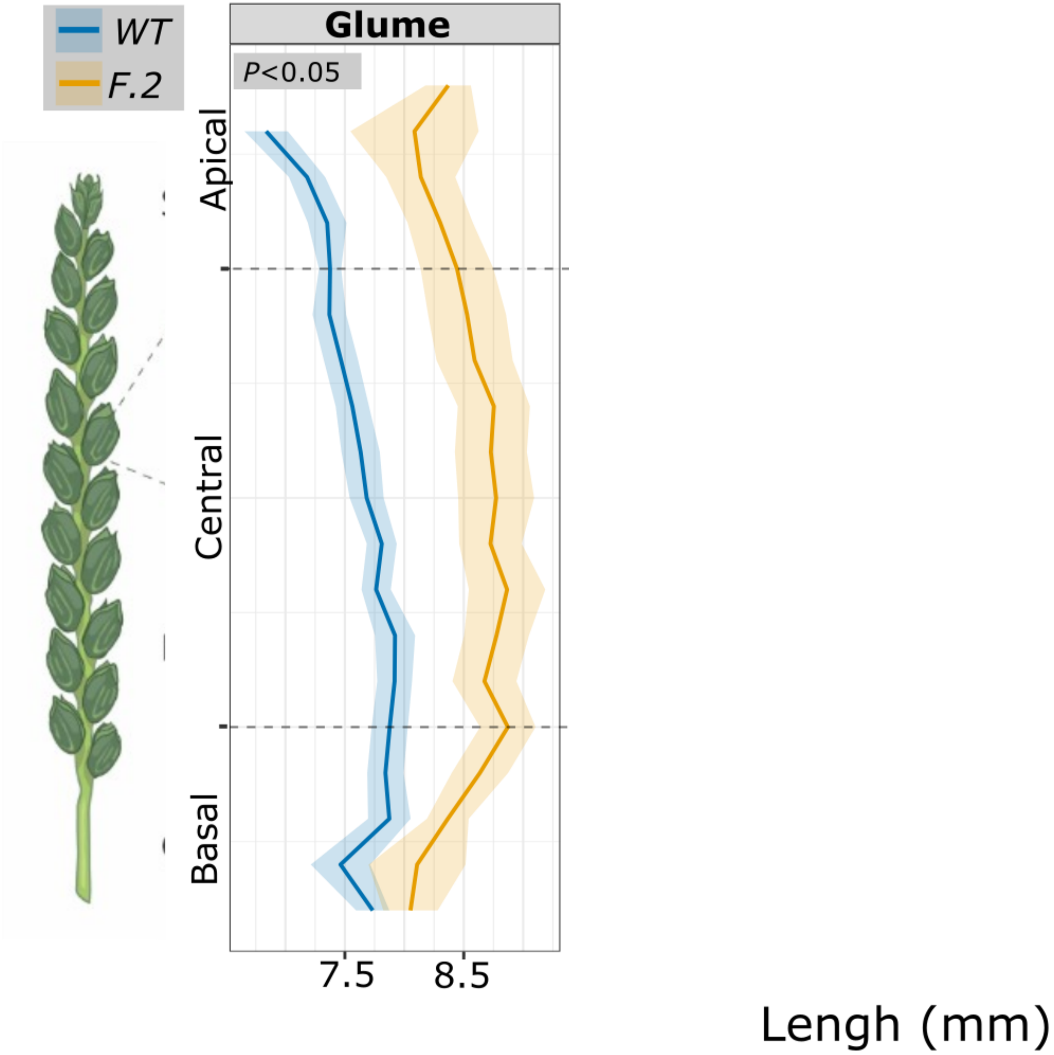
Promoter deletion affects *SVP-A1* expression (cv. Fielder, hexaploid)

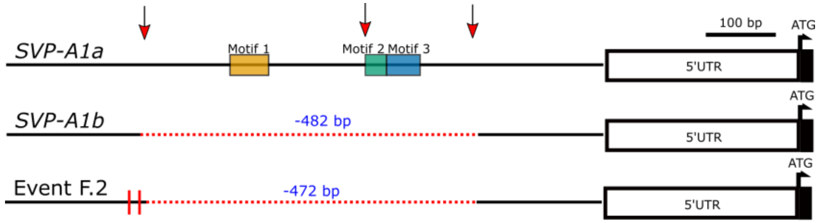




Chen et al *unpublished*

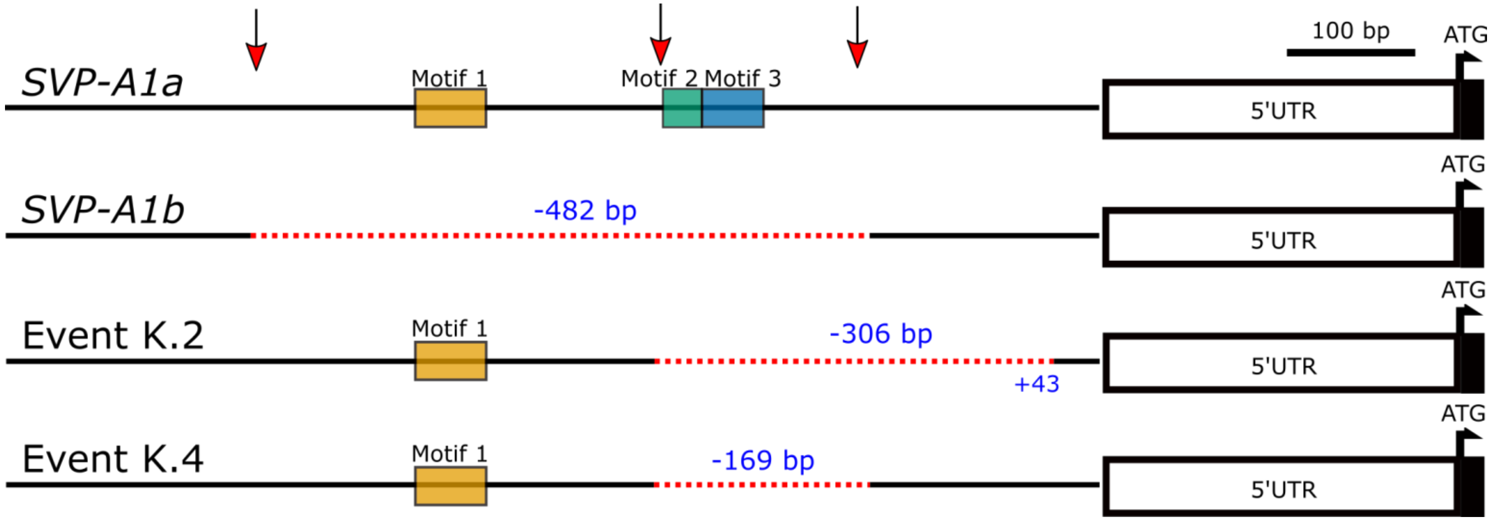
Promoter deletion increases organ length (cv. Fielder, hexaploid)

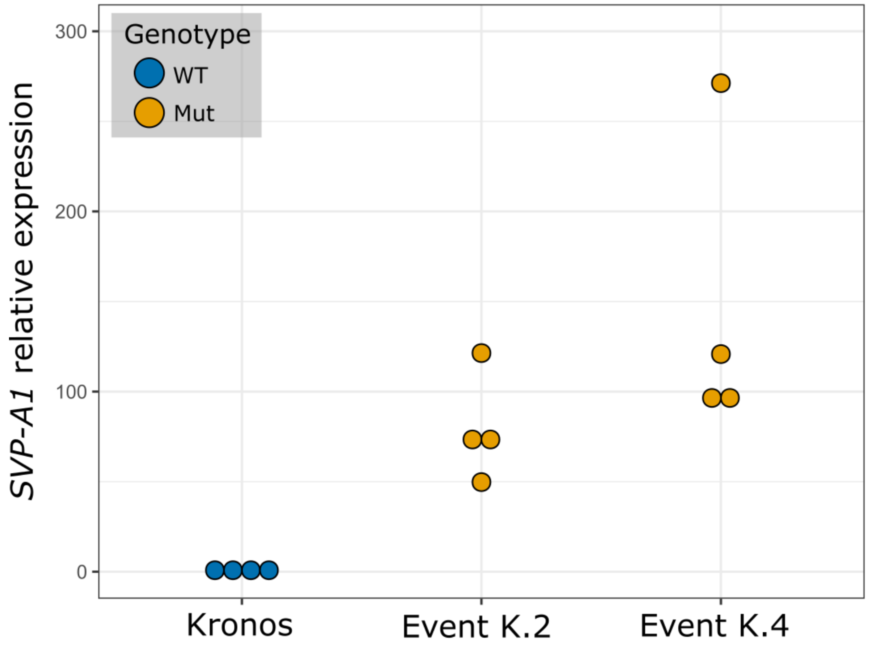




Chen et al *unpublished*

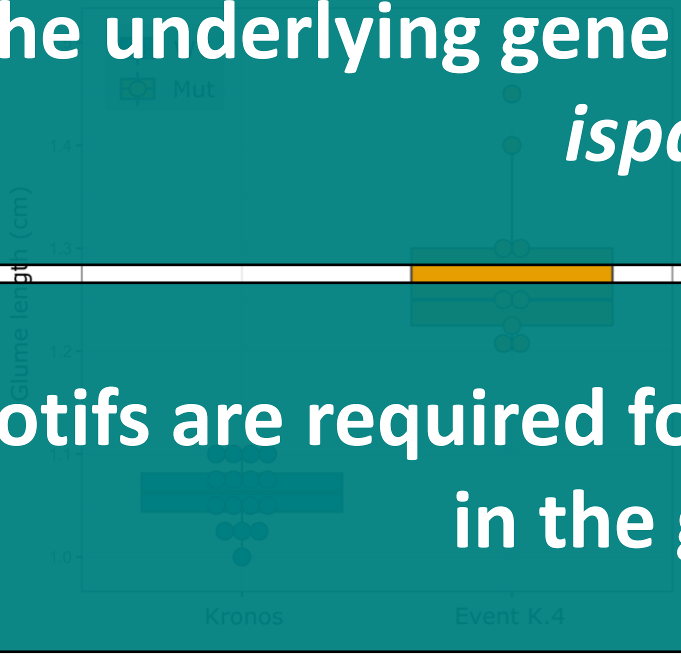
Promoter deletion affects *SVP-A1* expression (cv. Kronos, tetraploid)





Chen et al *unpublished*

Promoter deletion increases glume length (cv. Kronos, tetraploid)

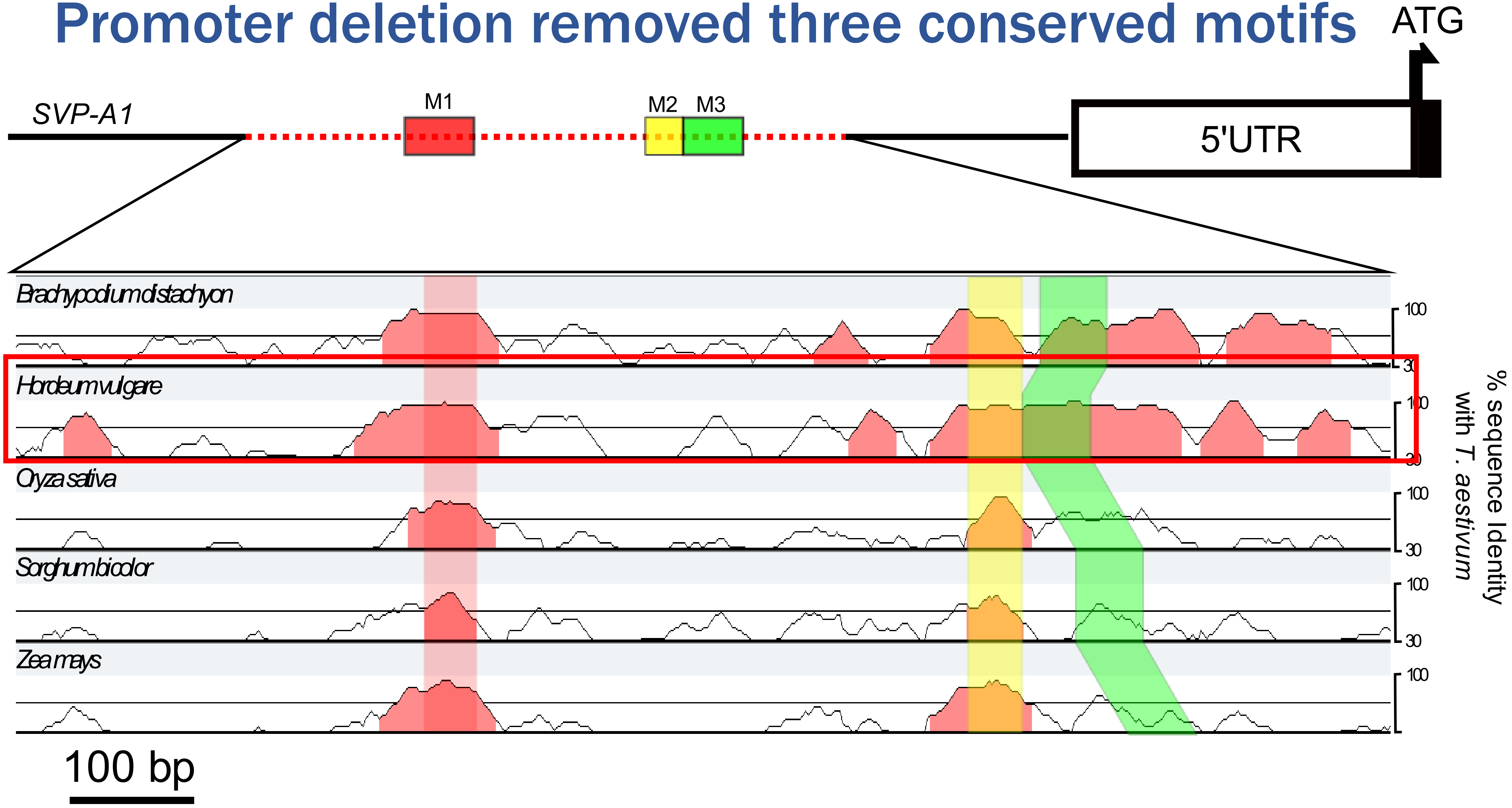


SVP-A1 **is the underlying gene for the long glume locus,** ***P2*****, from** ***T. ispahanicum***

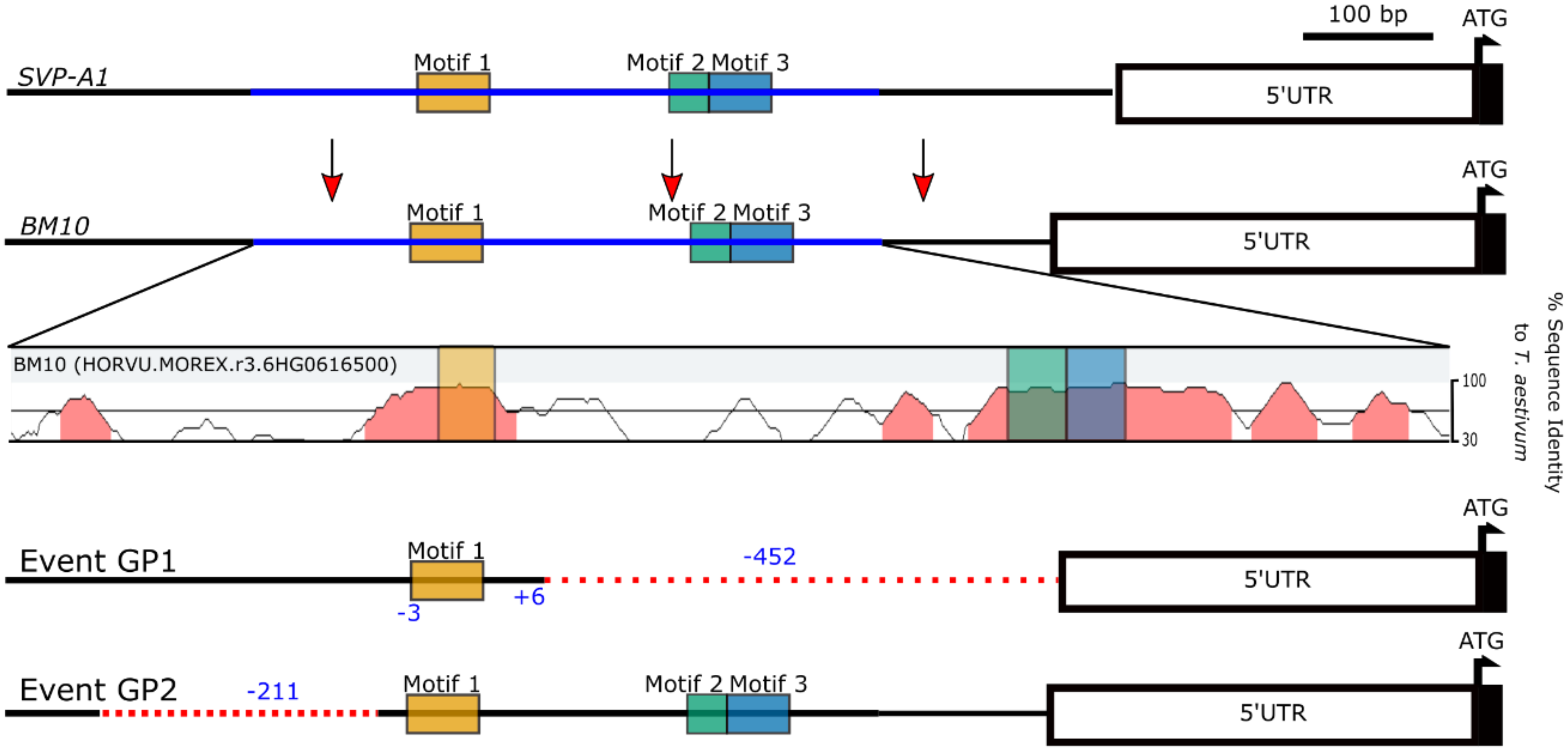
Identified motifs are required for the regulation of ***SVP-A1*** **expression in the glume tissue**

Chen et al *unpublished*

Promoter deletion removed three conserved motifs

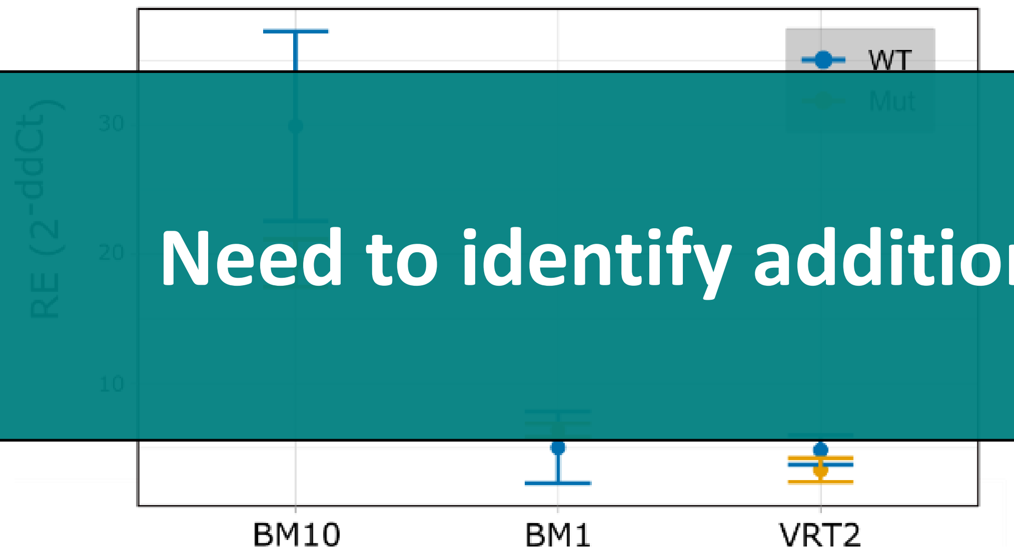


CRE function conservation: Barley

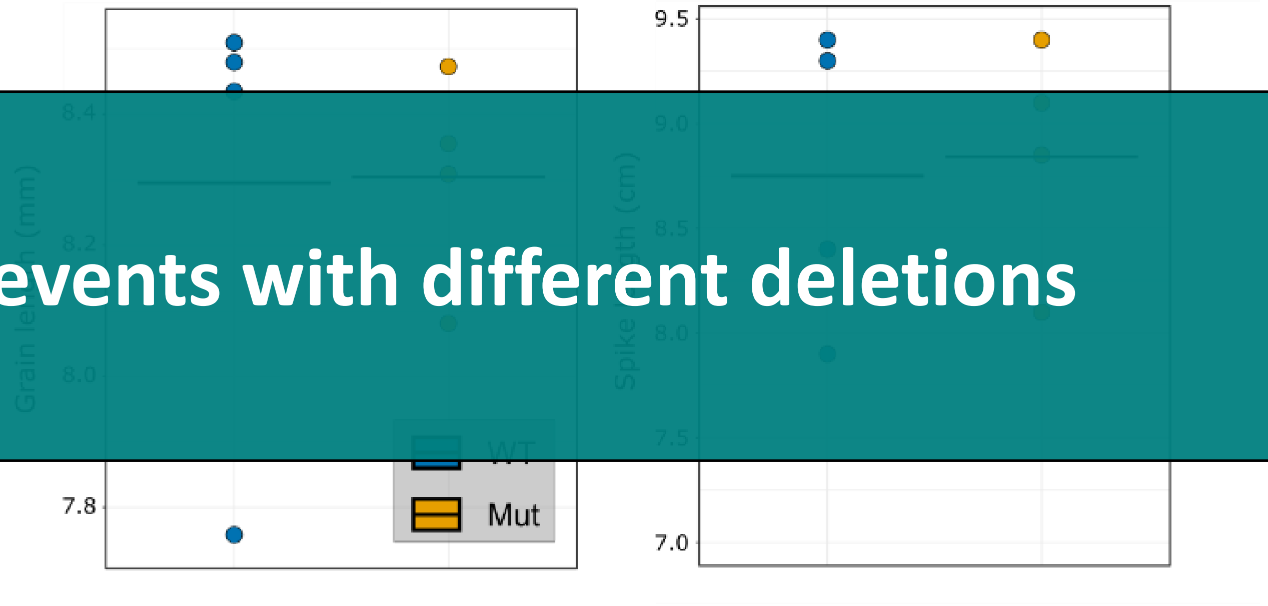


Chen et al *unpublished*

No change in expression/phenotype



Lemma, N=4

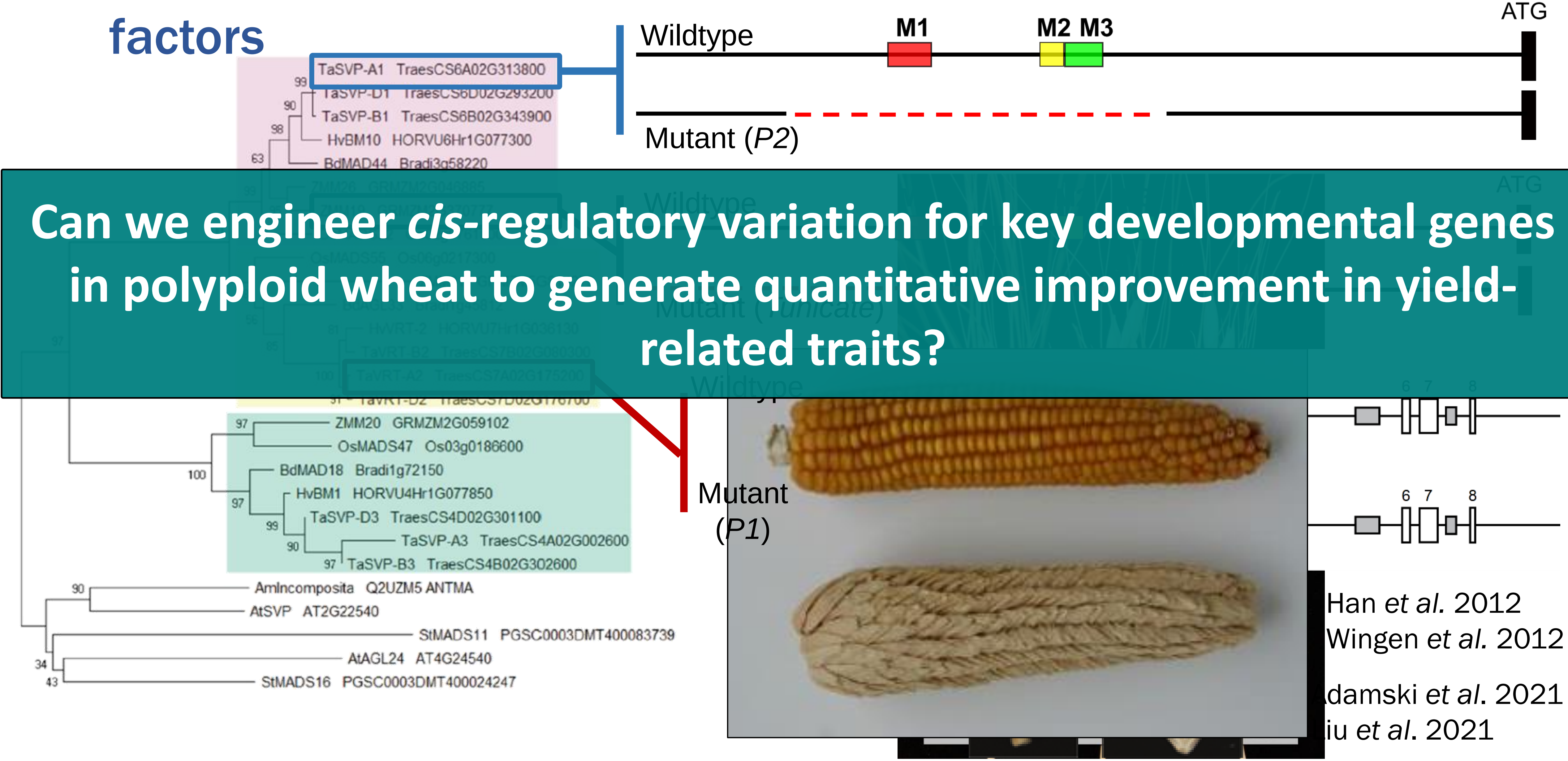


Need to identify additional events with different deletions

*BM10 is the ortholog of *SVP1* in barley

Chen et al *unpublished*

Natural variation at *cis*-regulatory region of *SVP* transcription factors



Can we engineer *cis*-regulatory variation for key developmental genes in polyploid wheat to generate quantitative improvement in yield-related traits?

Han *et al*. 2012
Wingen *et al*. 2012

damski *et al*. 2021
iu *et al*. 2021

Cis-regulatory variations underlie agronomically important alleles

- Promoter sequence of EMS-population of Kronos (Zhang et al. 2023)
- Public dataset characterizing open chromatin regions (Debernardi et al. 2023; Lu et al. 2020) and histone modification (Wang et al. 2021)

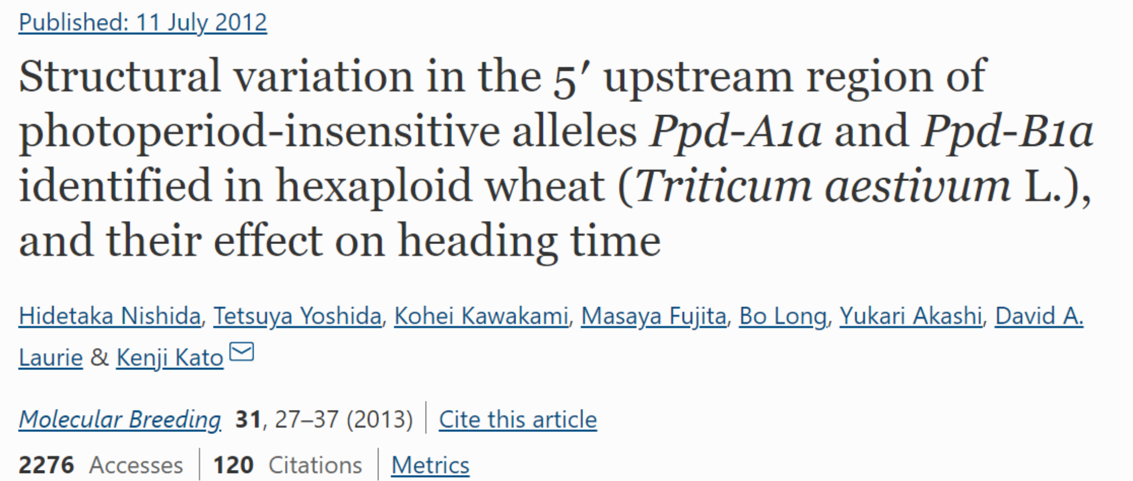
Original Paper | Published: 03 February 2005

Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat

Daolin Fu, Péter Szűcs, Liuling Yan, Marcelo Helguera, Jeffrey S. Skinner, Jarislav von Zitzewitz, Patrick M. Hayes & Jorge Dubcovsky

Molecular Genetics and Genomics **273**, 54–65 (2005) | Cite this article

4222 Accesses | **487** Citations | **3** Altmetric | Metrics



Published: 11 July 2012

Structural variation in the 5′ upstream region of photoperiod-insensitive alleles *Ppd-A1a* and *Ppd-B1a* identified in hexaploid wheat (*Triticum aestivum* L.), and their effect on heading time

Hidetaka Nishida, Tetsuya Yoshida, Kohei Kawakami, Masaya Fujita, Bo Long, Yukari Akashi, David A. Laurie & Kenji Kato

Molecular Breeding **31**, 27–37 (2013) | Cite this article

2276 Accesses | **120** Citations | Metrics

Legislation changes around gene-edited plants

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Defra in the media

Organisations: Department for Environment, Food & Rural Affairs

Genetic Technology Act becomes law

Defra Press Office, 24 March 2023 - Farming

Legislation changes around gene-edited plants

… it is Health Canada's scientific opinion that gene-edited plant products should be regulated like all other products of plant breeding, by focusing on their final characteristics and **not** the method of product development.

-Health Canada, August 2022

… gene edited plants product should be regulated like all other products of plant breeding. Namely, by the traits they exhibit and how these traits impact the safety of environmental and human health.

-CFIA, May 2023

Acknowledgement

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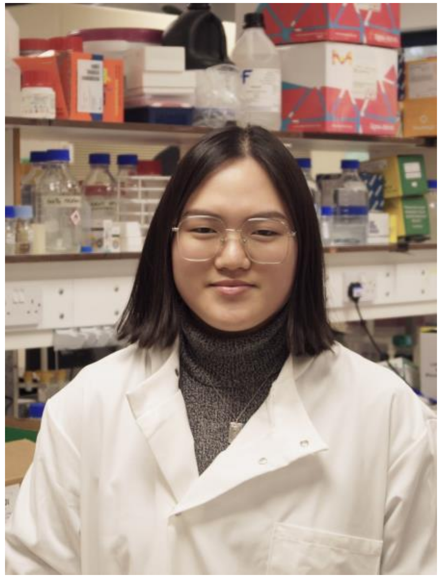
Uauy lab members

IWGSC Community



Victoria Zeng

Zeng et al. 2023 *bioRxiv*

Chen et al. 2022 *TAG*

NSERC CRSNG

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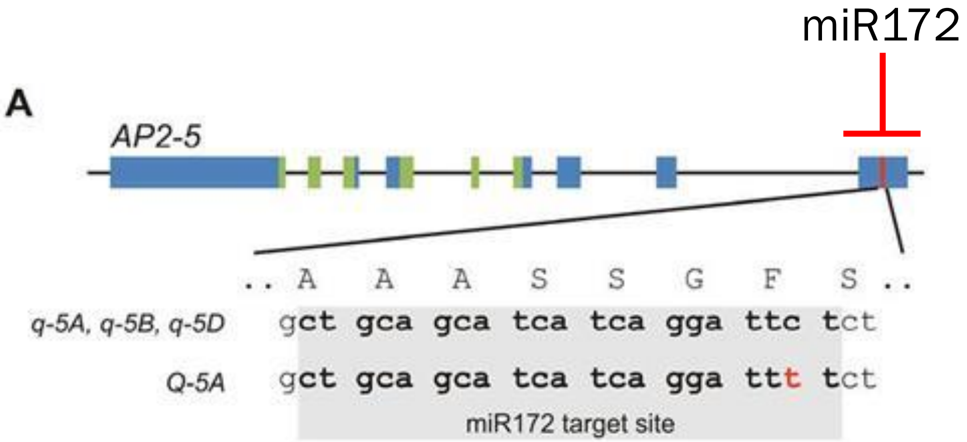
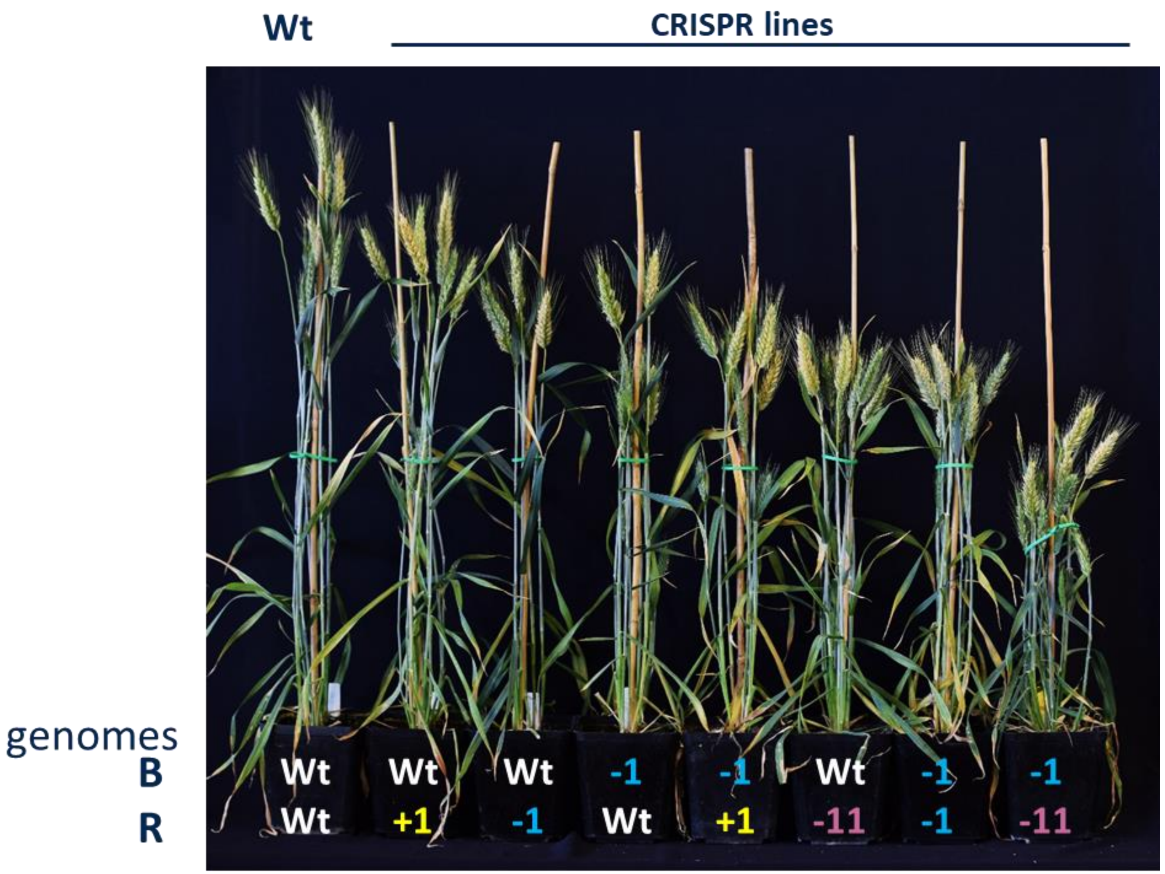
Targeting miRNA binding site of *Q* in *Triticale* to modify height





Credit to Juan M Debernardi, Chaozhong Zhang, and Joshua M Hegarty

Phenotype	ANOVA *P* value		Lsmeans		
	Genotype	**Block**	**Fielder**	**F.2**	**F.8**
Glume and grain morphology					
Glume length (cm)	< 0.01	0.62	0.89 a	0.92 b	0.86 a
Grain area (mm^2)	0.19	0.10	17.9 a	18.3 a	17.8 a
Grain width (mm)	0.12	0.09	3.40 a	3.39 a	3.46 a
Grain length (mm)	< 0.01	0.18	6.55 a	6.76 b	6.47 a
TGW (g)	0.63	0.18	44.1 a	43.2 a	43.1 a
Hectolitre Weight (kg/hl)	0.24	0.06	79.6 a	80.0 a	80.4 a
Grains per Spike	0.08	0.76	63.3 a	61.0 a	69.5 a
Yield estimation*	< 0.01	0.66	765 b	474 a	533 a
Phenology					
Heading time	< 0.01	0.20	79.7 a	78.0 b	80.5 c
Height (cm)	0.23	0.70	84.7 a	86.3 a	81.4 a
Spike morphology					
Spike length (cm)	< 0.01	0.45	11.1 a	12.5 c	11.7 b
Spikelet number	< 0.01	0.79	18.8 a	19.3 b	19.6 b
Basal infertile spikelets	< 0.01	0.81	0.17 a	1.24 b	0.20 a
Floret Number	0.08	0.77413	4.80 a	4.79 a	5.03 a