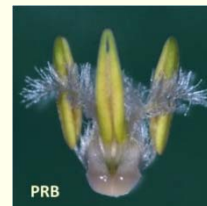


# Site-directed genome modification in barley and wheat

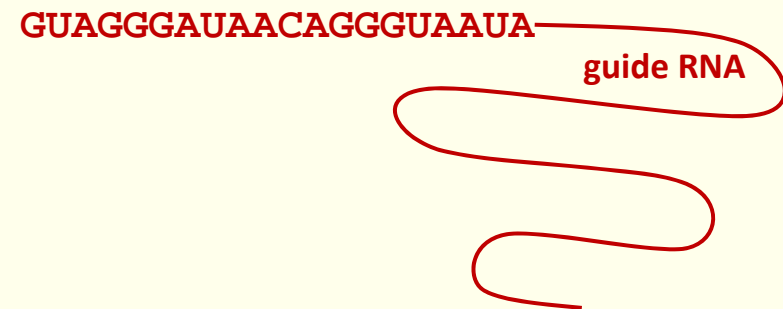
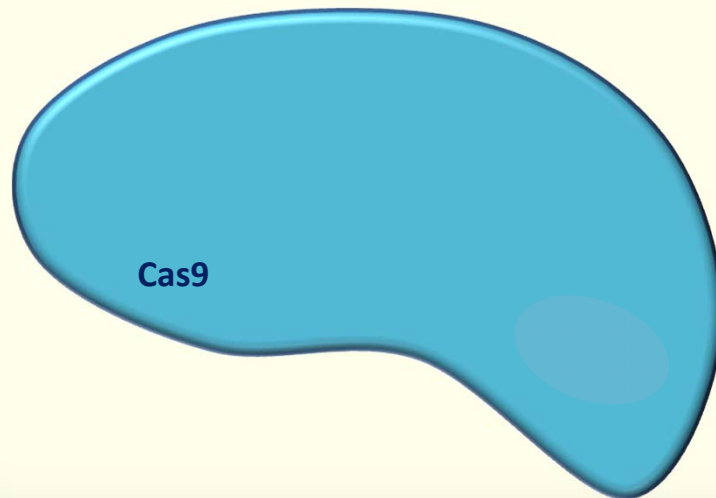
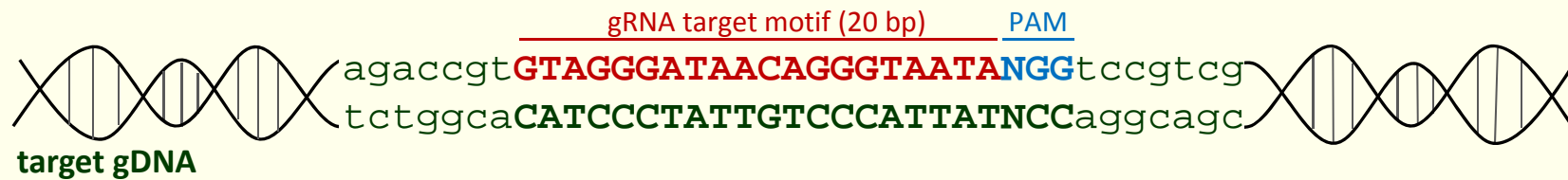
*Jochen Kumlehn*

**Plant Reproductive Biology**

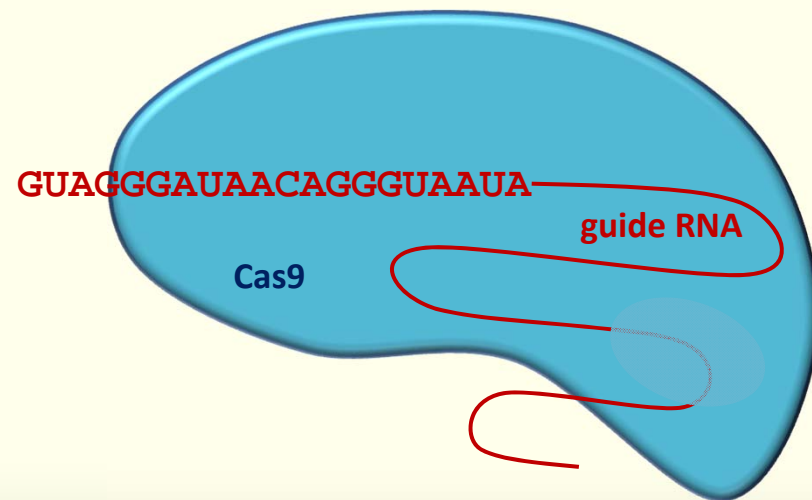
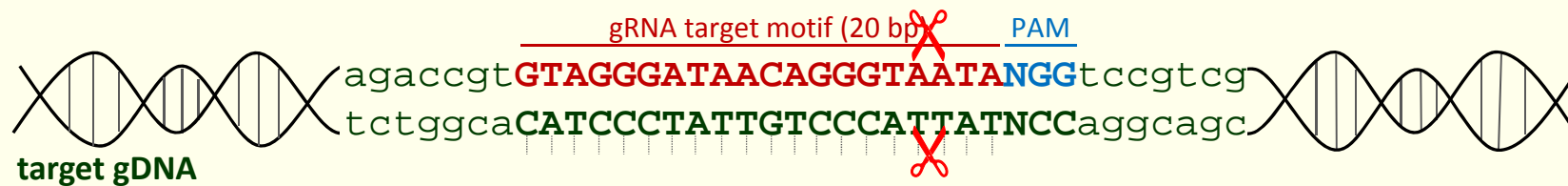
**Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben**



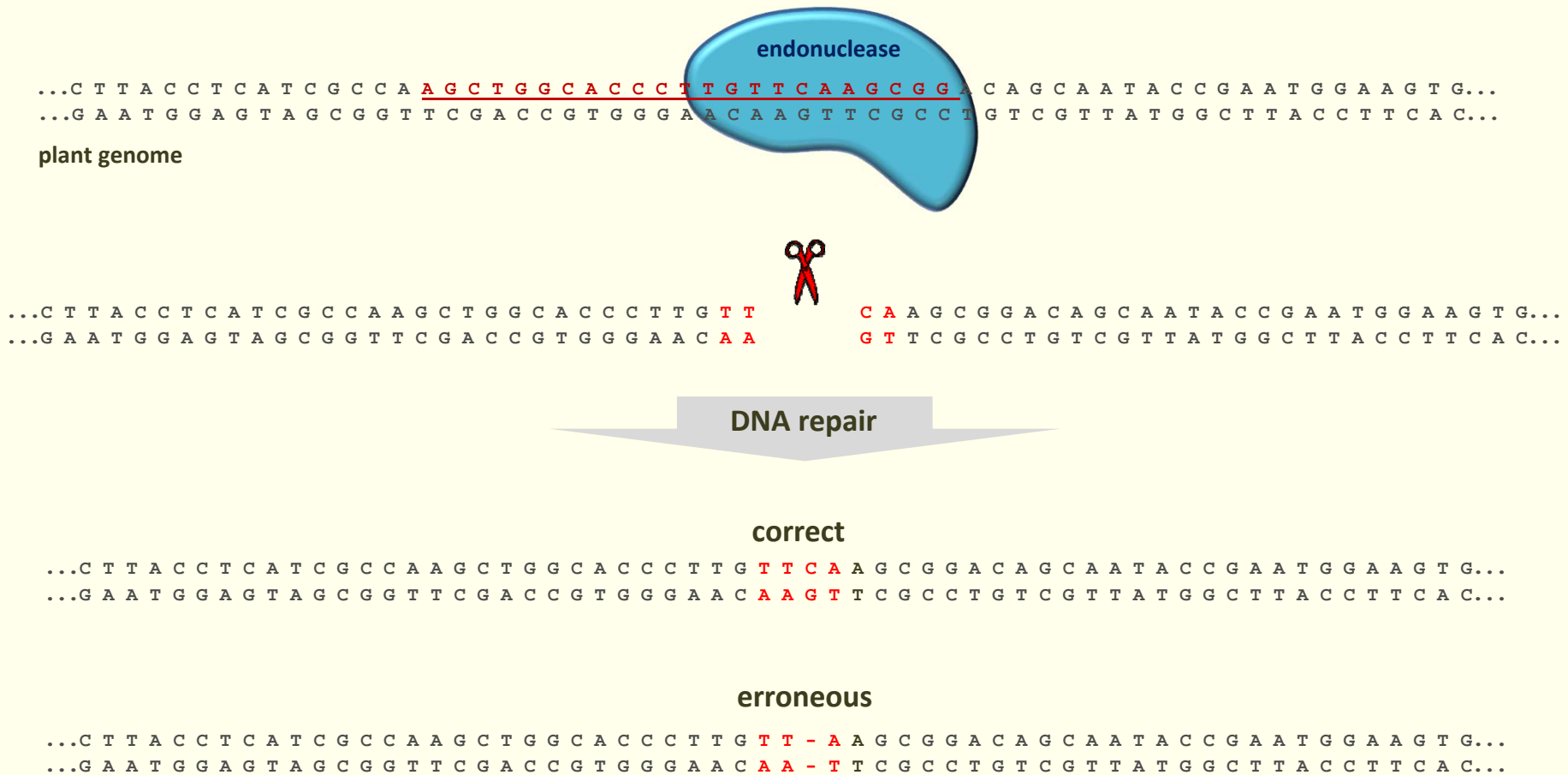
# RNA-guided Cas9 endonuclease



# RNA-guided Cas9 endonuclease



# Site-directed mutagenesis using customized Cas endonucleases



➡ Any genomic sequence of choice can be mutated *in planta*.

# Improvement of beer quality via KO of *LIPOXIGENASE1* (*Lox1*)

## LOX1 oxidizes linoleic acid

- breakdown end products cause off-flavour and foam instability



# Site-directed mutagenesis of barley *Lox1*

Götz Hensel, Sabine Sommerfeld, Carola Bollmann, Ingrid Otto

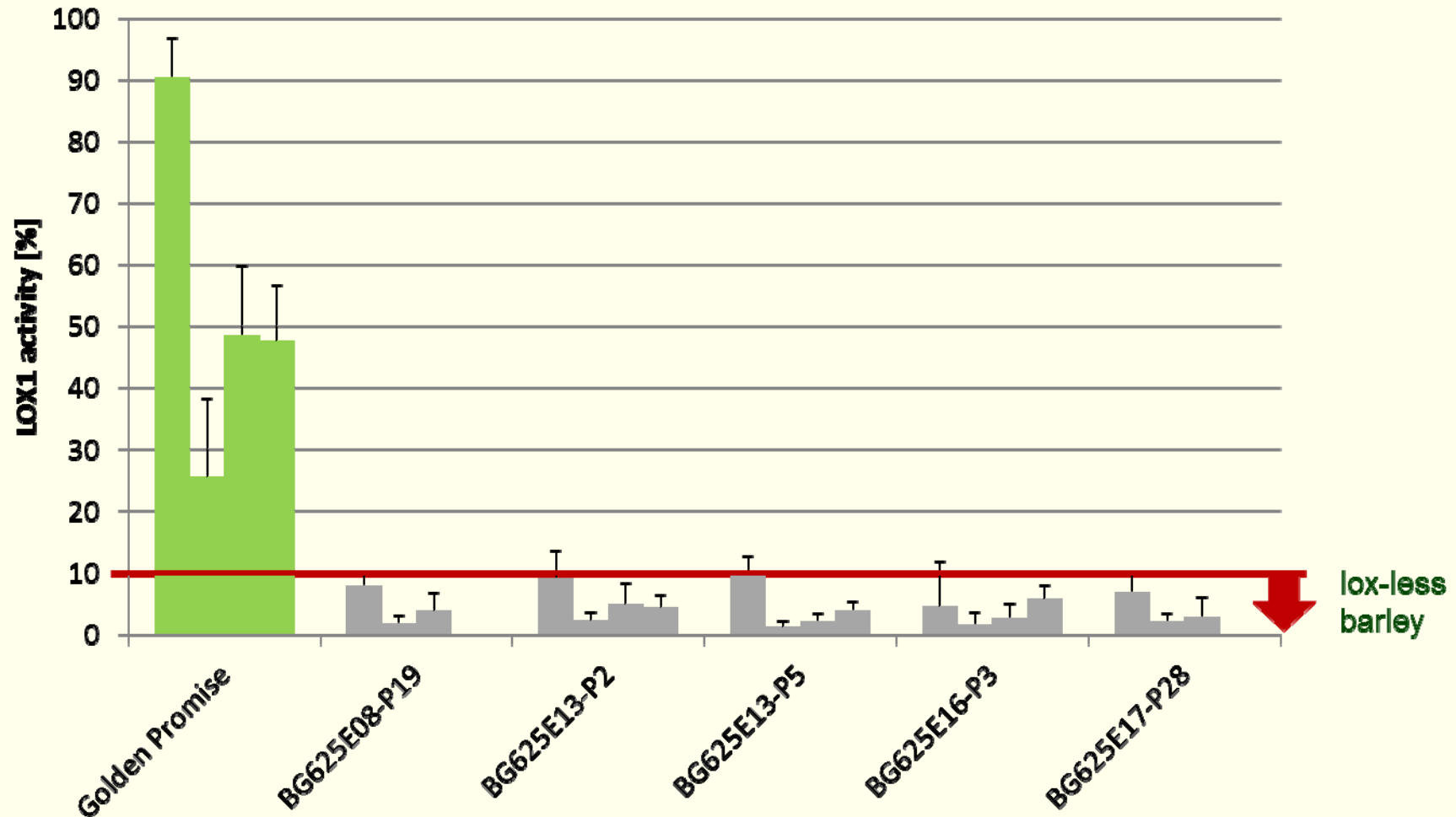
## simultaneous expression of 3 different gRNAs

Plant ID	gRNAs expressed	TARGET MOTIF 1 ✂ (100%)	TARGET MOTIF 2 ✂	
Golden Promise		GGAGGCCAAGAACAAGATGCTGCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	GGAGGCCAAGAACAAGATGCTGCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	
<b>T0</b>	BG625E02	gRNA1 and 2	GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3
<b>DHT1</b>	BG625E02-P3		GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCA---GGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3, -2
	BG625E02-P7		GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCA---GGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3, -1
	BG625E02-P24		GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3
<b>T0</b>	BG625E08	gRNA1	GGAGGCCAAGAACAAGATGCG---GCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-1
			GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3
<b>DHT1</b>	BG625E08-P12		GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCACGGGGCGAGCAAGAGCGCCCGGCTCAAGGG	-3
	BG625E08-P15		GGAGGCCAAGAACAAGATGCG---GCTGGGAGGGCTGATCGACACCCTCACGGGGCGAGCAAGAGCGCCCGGCTCAAGGG	-1
<b>T0</b>	BG625E13	gRNA1 and 2	GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCA---GGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3, -1
			GGAGGCCAAGAACAAGATGCT-----CGGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-26
<b>DHT1</b>	BG625E13-P2		GGAGGCCAAGAACAAGATGCG---GCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-1
	BG625E13-P4		GGAGGCCAAGAACAAGATGCT-----CGGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-26
	BG625E13-P11		GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3
Golden Promise			GGAGGCCAAGAACAAGATGCTGCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	
<b>T0</b>	BG625E16	gRNA1, 2 and 3	GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCA---CGGGGGCGAGCAAGAGCGCCCGGCTCAAGGG	-3
			GGAGGCCAAGAACAAGATGCTGCTGGGAGGGCTGATCGACACCCTCAACGGGGCGAGCAAGAGCGCCCGGCTCAAGGG	+1
<b>DHT1</b>	BG625E16-P3		GGAGGCCAAGAA-----AGGG	-60
	BG625E16-P9		GGAGGCCAAGAACAAGATGCTGCTGGGAGGGCTGATCGACACCCTCAACGGGGCGAG-----CAAGGG	+1, +1, -15
	BG625E16-P12		GGAGGCCAAGAACAAGATGCT-----CAAGGG	-51
	BG625E16-P13		GGAGGCCAAGAACAAGATGCT-----CGGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-26
			GGAGGCCAAGAA-----CAAGGG	-60
			GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCAACGGGGCGAGCAAGAGCGCCCGGCTCAAGGG	-3, +1
<b>T0</b>	BG625E17	gRNA1, 2 and 3	GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCA---GGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3, -2
			GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCACGGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3
<b>DHT1</b>	BG625E17-P28		GGAGGCCAAGAACAAGAT---GCTGGGAGGGCTGATCGACACCCTCA---GGGGCGAACAAGAGCGCCCGGCTCAAGGG	-3, -2
	BG625E17-P30		GGAGGCCAAGAACAAGATGCT-----CAAGGG	-51

# Site-directed mutagenesis of barley *Lox1*

Phillip Reuter, Steffen Baier

## Lipoxygenase activity in grains of mutant plants

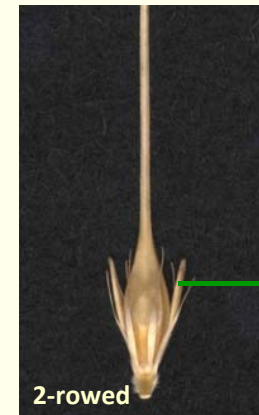


- *Lox1* mutants were also generated in an elite line, micro-brewing experiment planned

# Manipulation of the row-type in barley

## *2-rowed spike*

- derived from wild ancestor *H. spontaneum*
- lateral florets do not develop
- just one floret/one grain per rachis node



Lateral spikelets reduced

## *6-rowed spike*

- only found amongst cultivated barleys (*H. vulgare*)
- lateral florets fully developed
- associated with loss of function in *Vrs1/Hox1*
- confirmed by *Vrs1*-RNAi causing partial restoration of lateral florets



Sakuma et al., *Funct. Integr. Genomics* 2009  
Sakuma et al., *New Phytol.* 2013



# Conversion of 2-rowed into 6-rowed barley via KO of *Vrs1*

Götz Hensel, Sabine Sommerfeld, Sibylle Freist

## *gRNA/Cas9-triggered mutagenesis of Vrs1*



Golden Promise WT (*Vrs1*)



Golden Promise *Vrs1*-KO



... with some awns removed

# Optimization of plant height in barley and wheat



Pieter Bruegel d.Ä. 1565



Harvest 2017

## Green Revolution

- lodging resistance thanks to reduced plant height
- hence, more effective use of N-fertilizer
- and improved relation of grain and straw
- most plant height genes play a role in GA biosynthesis or perception

same principle in all cereals  
nutritional basis of modern society

# Generation of new genetic diversity in plant height

## BRASSINOSTEROID-INSENSITIVE 1

- encodes a brassinosteroid receptor
- *bri1* mutants cannot implement the hormonal signal into shoot growth
- the *uzu* mutant has reduced *Bri1* function (used in Asia)



Bowman (WT)



*uzu1*

# Generation of allelic diversity in the *Bri1* gene

Nagu Budhagatapalli, Stefan Hiekel, Götz Hensel, Sabine Sommerfeld, Sibylle Freist

## gRNA/Cas9-triggered mutagenesis of *Bri1* in barley



WT (GP)

*Bri1* KO mutants

WT

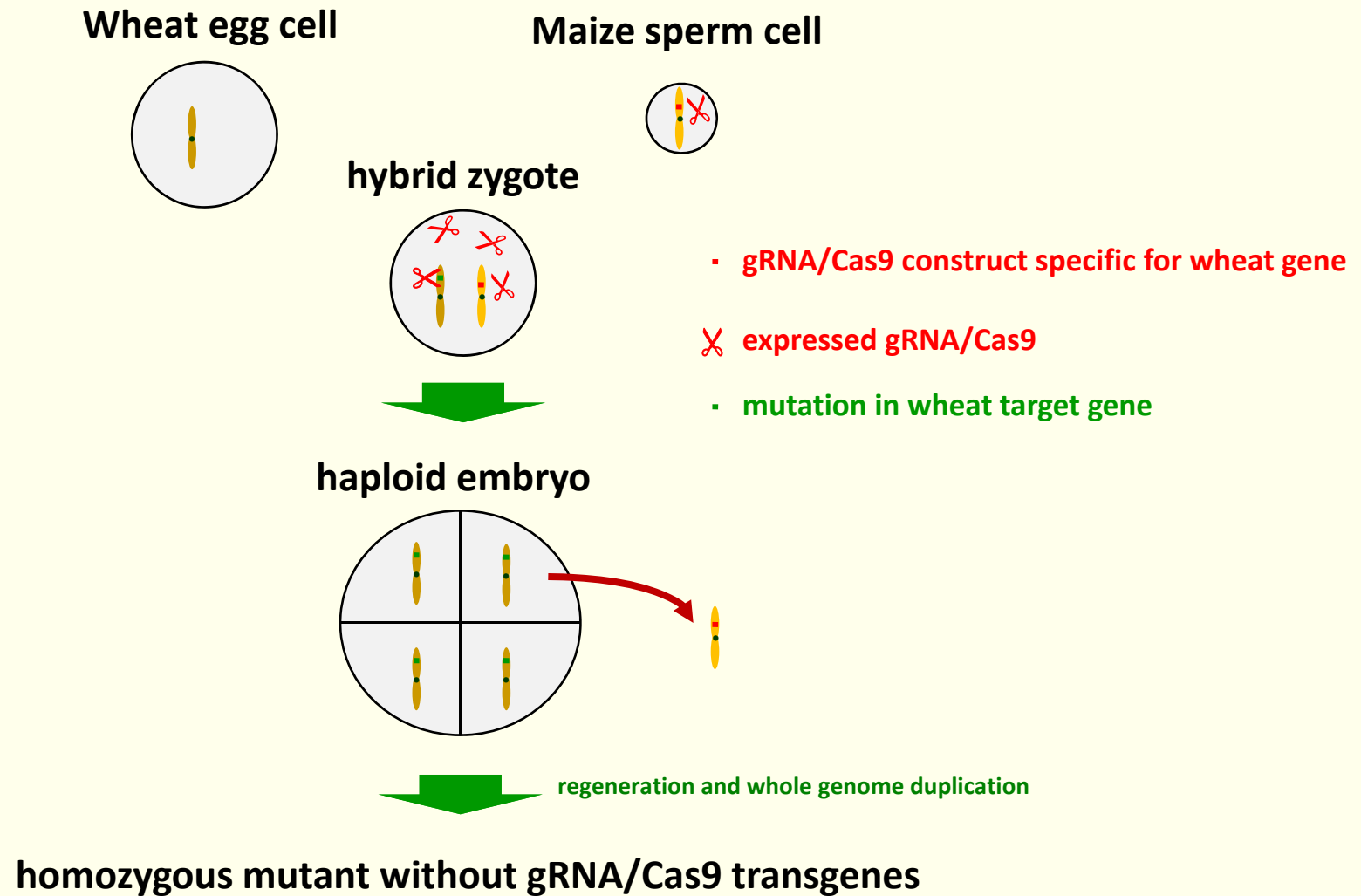
*Bri1* in-frame mutant

WT

*Bri1* in-frame mutant

- site-directed mutagenesis in elite material may provide new plant height diversity for breeders

# Targeted mutagenesis in wheat triggered by maize-encoded gRNA/Cas9



# Identification of gRNA/Cas9-triggered *Bri1* mutations in wheat

Nagu Budhagatapalli, Stefan Hiekel, Heike Büchner, Conny Marthe

## Wheat pollinated with *TaBri1* gRNA/Cas9-transgenic maize

### gRNA/Cas9 target motifs

WT\_B GGAGGGTTGTTGCAATCAAGAAGCTAATACATGTGAGTGGCCAGGGTGACCGGGAGTTCACaGCGGAAATGGAGACCATT

BH\_117B GGAGGGTTGTTGCAATCAAGAAGCTAATACATGTGAGTGGCCAGGGTGACCGGGAGTTCACAGCGGAAATGGAGACCATT +1 bp

WT\_D GGAGGGTTGTTGCAATCAAGAAGCTAATACATGTGAGTGGCCAGGGTGACCGGGAGTTCACgGCGGAAATGGAGACCATT

BH\_43D GGAGGGTTGTTGCAATCAAGAAGCTAATACATGTGAATGGCCAGGGTGACCGGGAGTTCACGGCGGAAATGGAGACCATT +1 bp

BH\_72D GGAGGGTTGTTGCAATCAAGAAGCTAATACATGTGAGTGGCCCGGGTGACCGGGAGTTCACGGCGGAAATGGAGACCATT +/-1 bp

BH\_99D GGAGGGTTGTTGCAATCAAGAAGCTAATACATGTGAGTGGCTAGGGTGACCGGGAGTTCACGGCGGAAATGGAGACCATT +/-1 bp

BH\_105D GGAGGGTTGTTGCAATCAAGAAGCTAATACATGTGGTGGCCAGGGTGACCGGGAGTTCACGGCGGAAATGGAGACCATT +/-1 bp

**Insertions**

**Substitutions**

# Identification of gRNA/Cas9-triggered *Bri1* mutations in wheat

Nagu Budhagatapalli, Stefan Hiekel, Heike Büchner, Conny Marthe  
Thomas Halbach (Strube Research)

## Durum wheat pollinated with *TaBri1* gRNA/Cas9-transgenic maize

### gRNA/Cas9 target motifs

**WT\_A** TCAAGCTCTCATTGTGGAAGCCATTGGTGGCCTCAACGAGATCACCCAAGGTGAGTTTCTGCAGTGGCTTCTCAAATGCAGCCA

**D7 2-57 M1** TCAAGCTCTCATTGTGGAAGCCATTGGTGGCCTCAAC-----GAGTTTCTGCAGTGGCTTCTCAAATGCAGCCA -15 bp

**D7 2-57 M2** -----GAGTTTCTGCAGTGGCTTCTCAAATGCAGCCA -391 bp

**WT\_B** TACAAGGCAACGCTCAAGGATGGGAGGGTTGTTGCAATCAAGAAGCTAATACATGTGAGTGGCCAGGGTGACCGGGAGTTCACA

**D6 1-51 M1** TACAAGGCAACGCTCAAGGATGGGAGGGTTGTTGCAATCAAGAAGCTAATACATGTGAG-----CAGGGTGACCGGGAGTTCACA -4 bp

**D6 1-51 M2** -----CCAGGGTGACCGGGAGTTCACA -199 bp

### Deletions

# Summary

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## *Site-directed genome modification*

- improved beer quality through reduction of lipoxygenase activity during the malting process
- improved yield potential via conversion of 2-rowed into 6-rowed barley
- new allelic diversity in plant height of barley and wheat

## Establishment of a novel principle for the induction of site-specific mutations

- pollination of wheat by gRNA/Cas9-transgenic maize
- instantaneous generation of true-breeding mutants
- site-directed mutagenesis without gRNA/Cas9 transgene integration in the target genome
- just one transgenic maize plant can be used to produce many independent wheat mutants
- the principle of haploidy induction is far less genotype-dependent than transgenesis



# Plant Reproductive Biology



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