

Using Genebank diversity and state-of-the-art biotechnological approaches to improve breeding and food security

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BIOTECHNOLOGY
opportunity for intersectoral cooperation and technology transfer
Date: 19th September, 2018 ■ Venue: Vodňany, MEVPIS – Na Valše 207







The IPK sees itself as a **catalyst for the social transformation**, which aims at an efficient and sustainable supply of food, energy and raw materials. It creates solutions that are based on a knowledge-based conservation and exploration and exploiting of the **biodiversity of cultivated plants**.

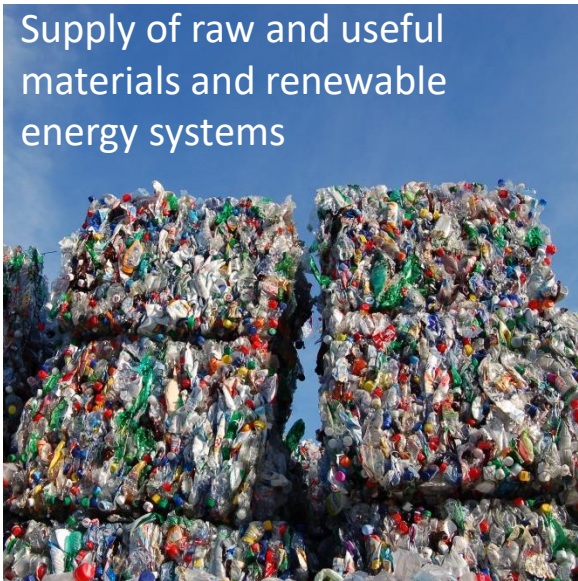
Reliable supply of food to a growing world population



Advancement of a sustainable, resource-sparing plant production



Supply of raw and useful materials and renewable energy systems



Adapt of agricultural products to the consequences of climate change



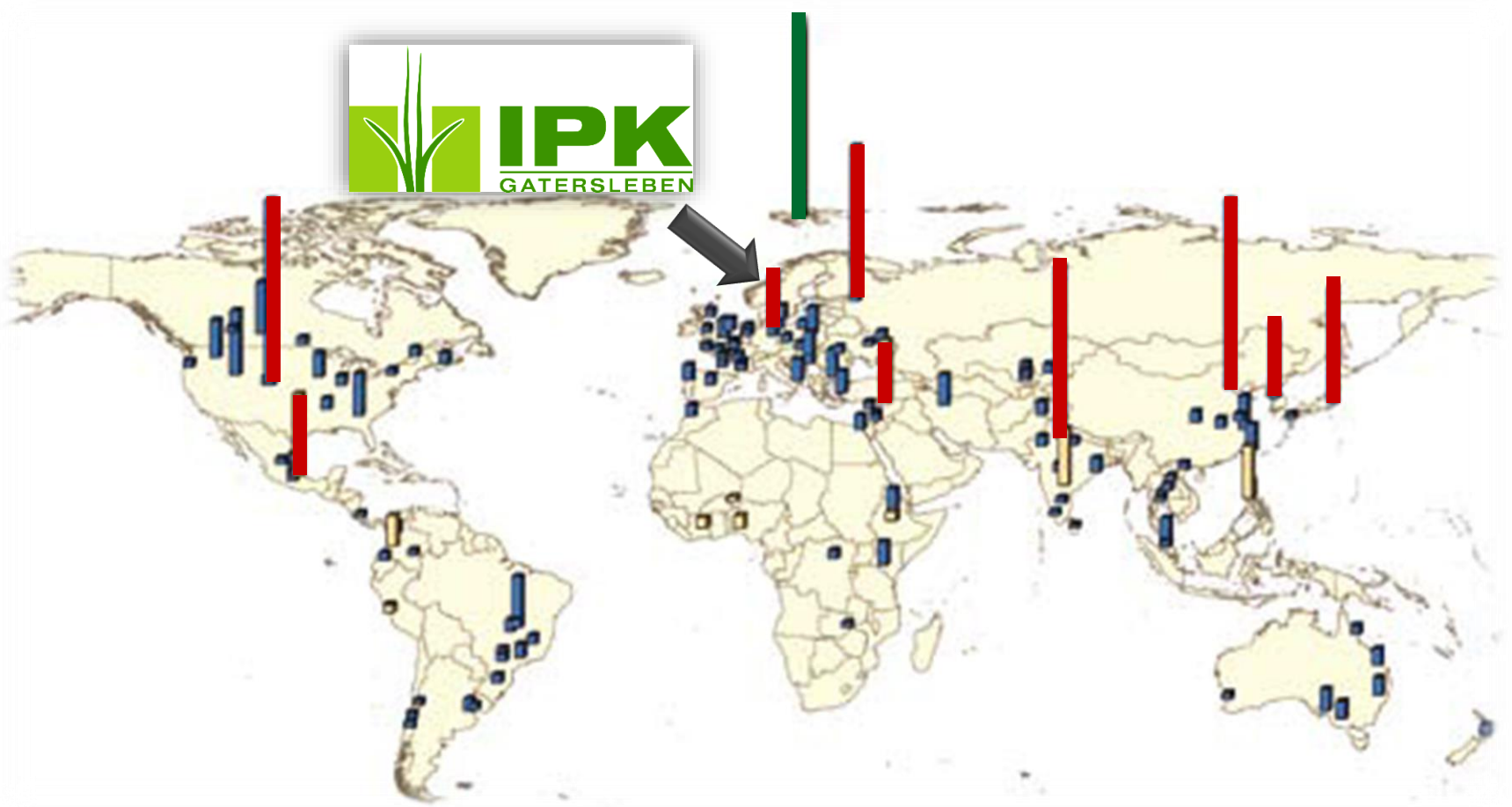
Establishment of IPK as

- a leading biological digital resource centre of knowledge-based use of biodiversity
- a Global trendsetter of genome analysis from Triticeae
- an innovation centre for the development of plant breeding technologies for wheat and barley
- an international beacon of elucidation of the molecular and physiological processes of agronomic characteristics



The **four scientific departments** of the IPK work on the elucidation of fundamental principles of the evolution, development and adaptability of important crops. On this basis the IPK develop innovative approaches for a knowledge-based conservation and exploitation of crop diversity to increase the resource efficiency and sustainability of plant-based production processes





Genebanks holding >10,000 accessions (blue); CGIAR Centers genebanks (beige); SGSV4 (dark green) (Source: FAO 2010)



Collections	Accessions
Cereals and Grasses	65,897
Wheat	28,206
Barley	23,607
Oat	4,849
Rye	2,410
Aegilops	1,526
Legumes	27,819
Vegetables	21,052
Oil-/ Fibercrops	5,478
Medicinal-/ Spiceplants	8,194
Forage crops	14,388
Potatoes	6,217
Total	150,751



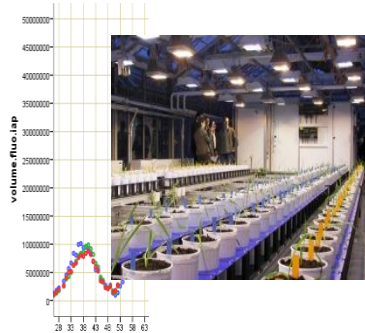




- Multiplication (6,164 acc)
- Cryopreservation (1,859 potatoes, garlic)
 - Field genebank (3,159 acc)
 - 420,000 voucher specimen
 - 154,000 reference samples (seed, fruits, spikes)



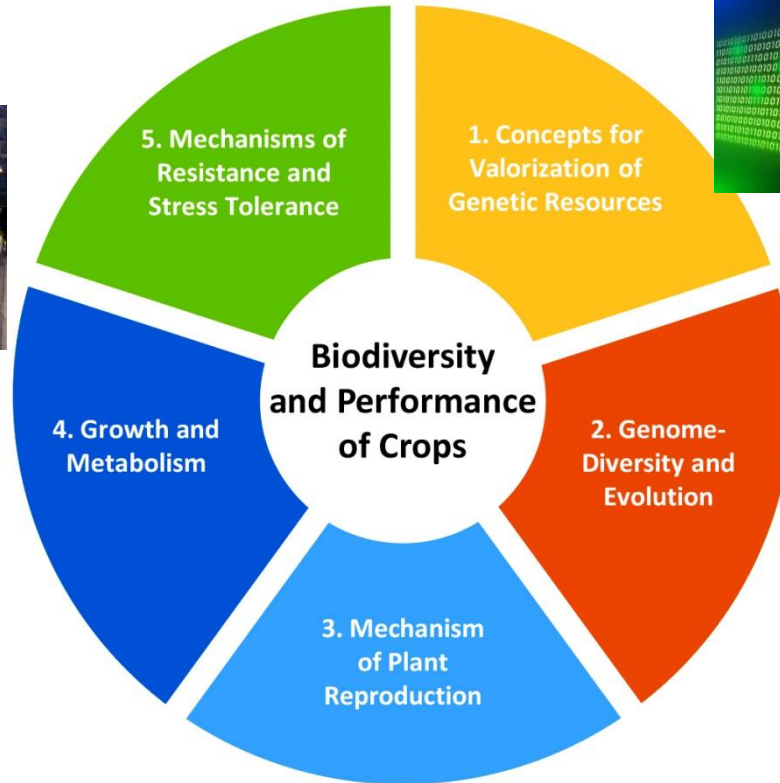
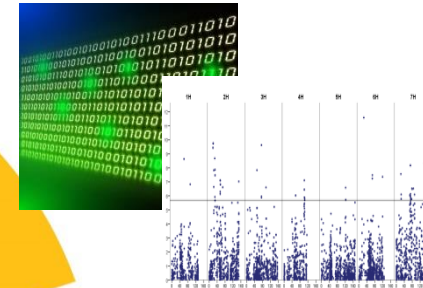
Sustainability and adaptation



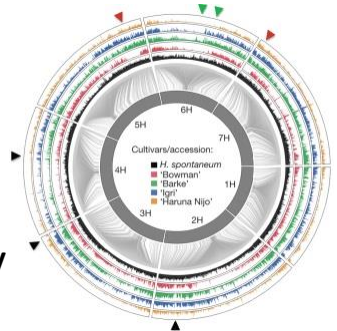
Plant performance



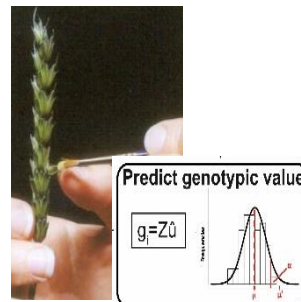
Delivery, valorization and utilization

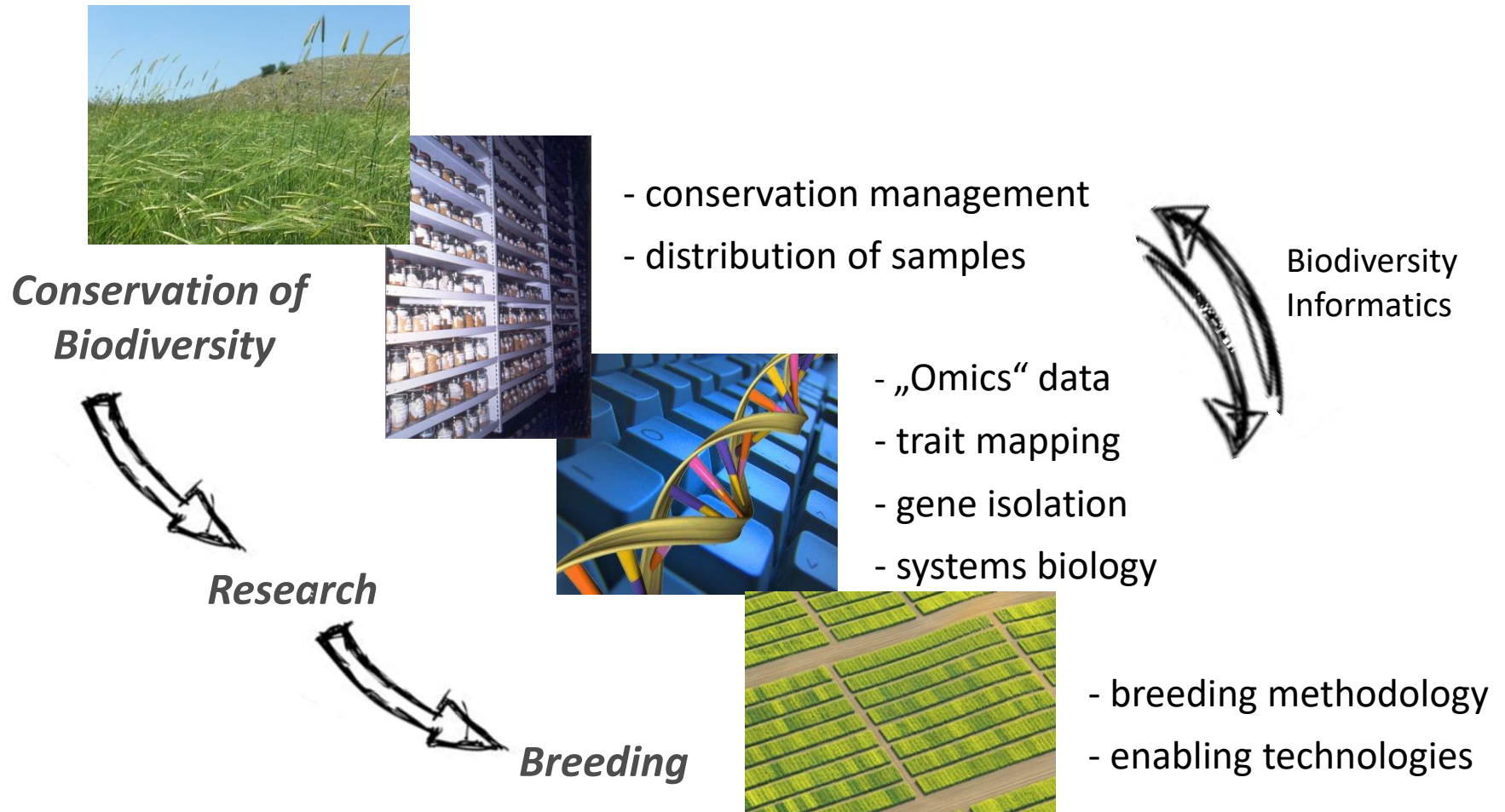


Genetic Diversity



Breeding technologies





Crop Plants



Barley



Wheat



Corn



Legumes



Rapeseed

Model Plants



Arabidopsis thaliana



Tobacco



St. John's wort



Cress

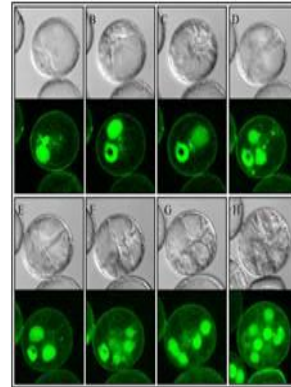
DNA Sequencing

- **ABI 3730 XL**
- **Roche 454 FLX**
- **Illumina HiSeq 2000**



Microscopy

- **transmission and scanning electron microscopy**
- **Confocal laser scanning microscopy**
- **Confocal Spinning Disc Mikroskopy**
- **High-resolution microscopy**



Computer systems

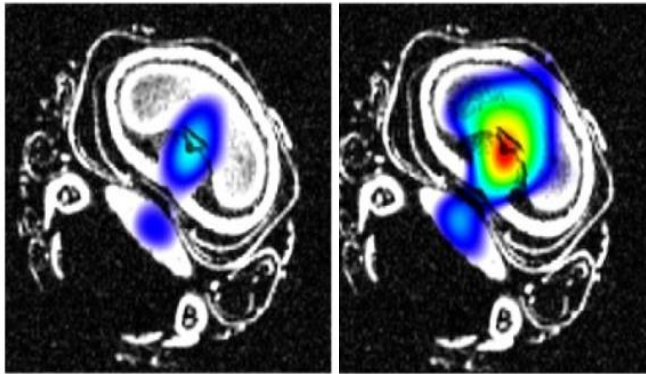
- **Cluster Computer with 200-core**
- **2 Server with main memory size (SMP)**
- **high-performance server**



Chromatography/Mass spectrometry

- **Proteins (Nano-HPLC + ESI-MS/MS, MALDI-TOF-MS)**
- **Metabolites (HPLC-MS, ICP-MS, EA-IRMS, GC-MS)**

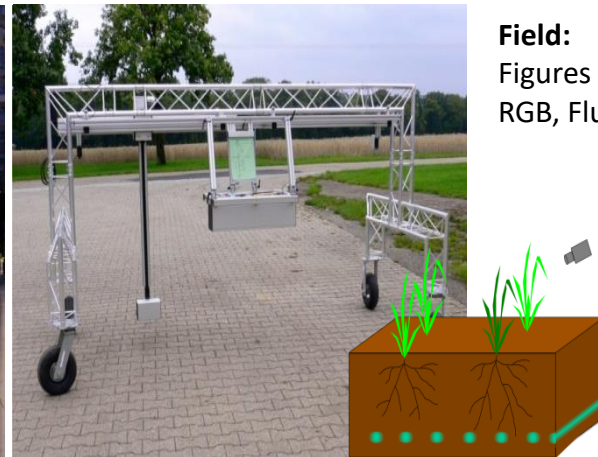
NMR:
Seed



Microscopy:
Leaves: Epidermis



Field:
Figures
RGB, Fluor



Greenhouse:
Figures

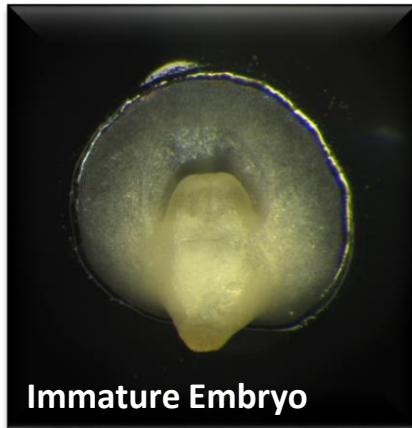
RGB, Fluor, NIR, IR



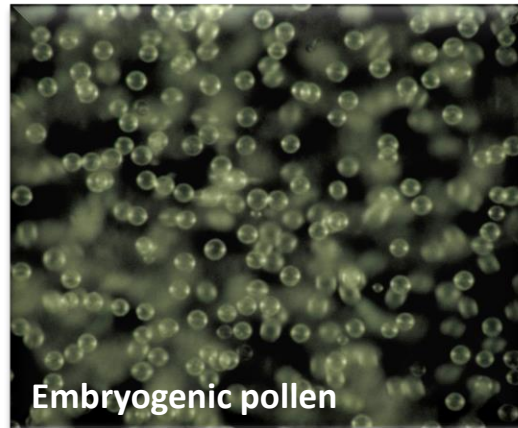
- Building high-quality technological platforms
- Protocol development for registration of plant parameters, growth and environmental conditions
- Image Analysis



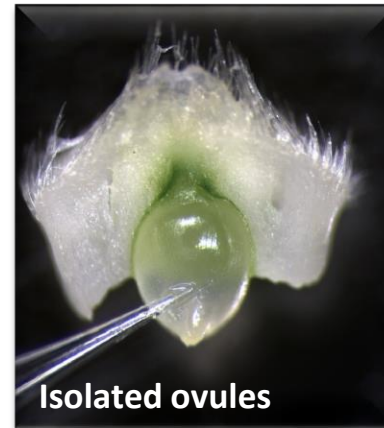
Transformation and regeneration



Immature Embryo



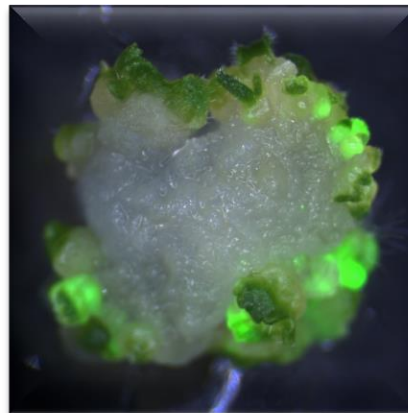
Embryogenic pollen



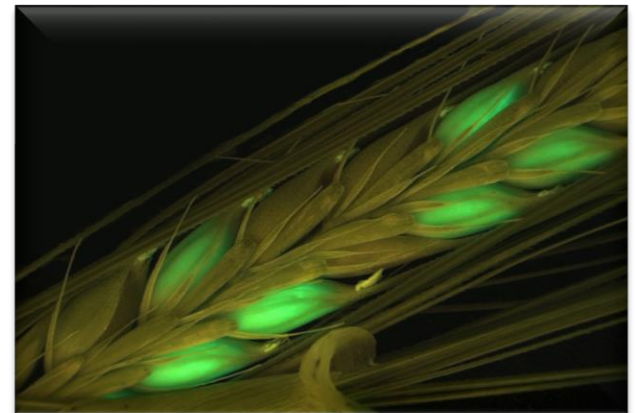
Isolated ovules



Shoot-segments



callus and shoot
development



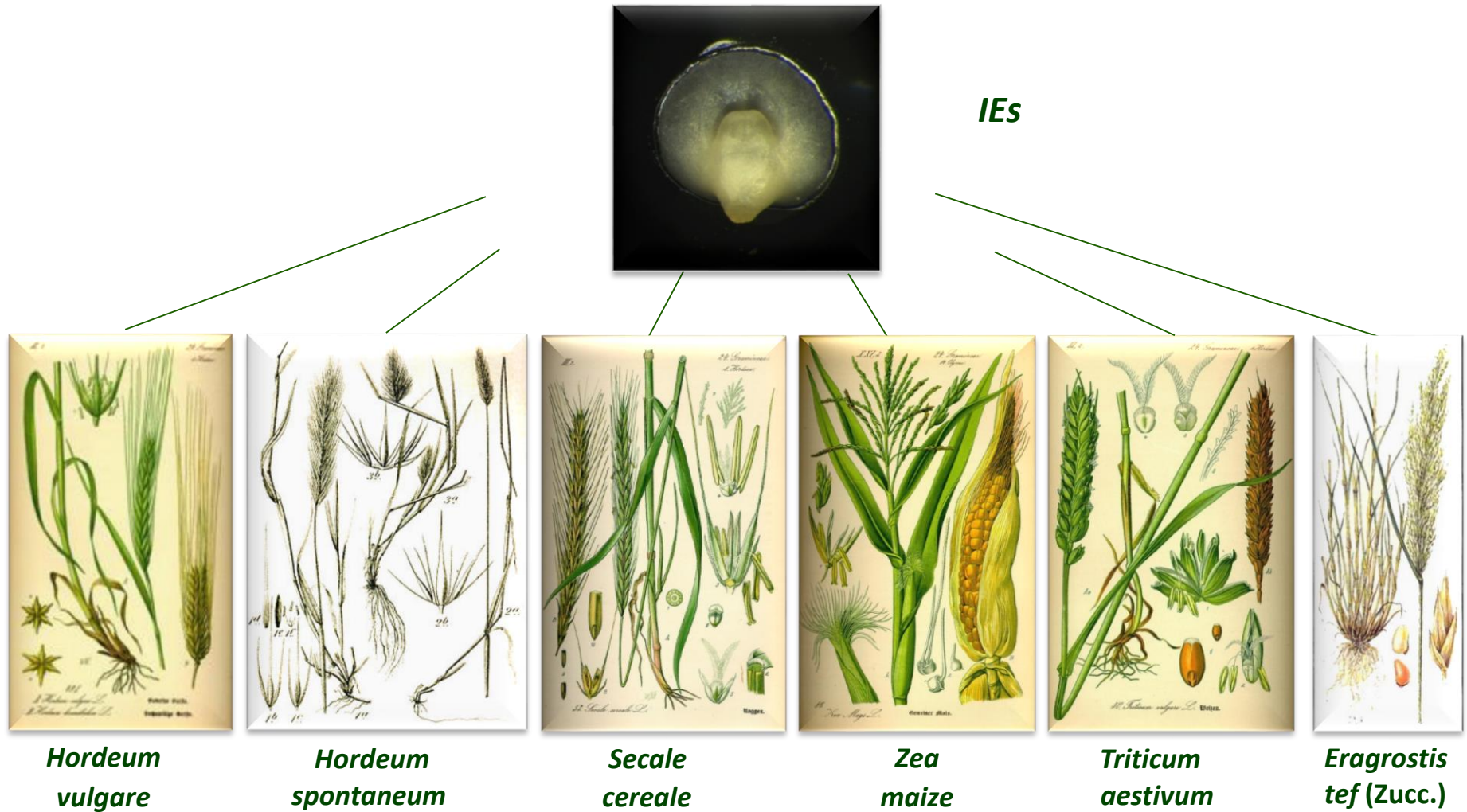
T₀ spike, genetic segregation



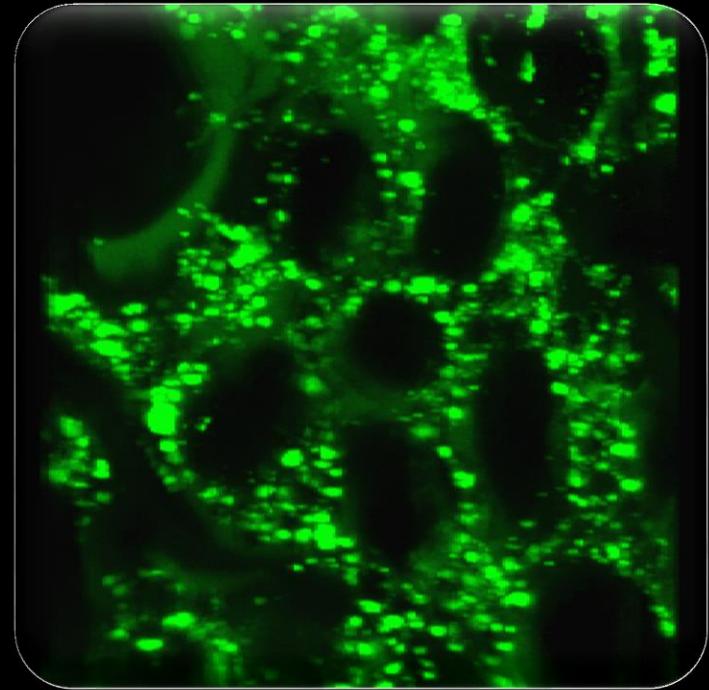
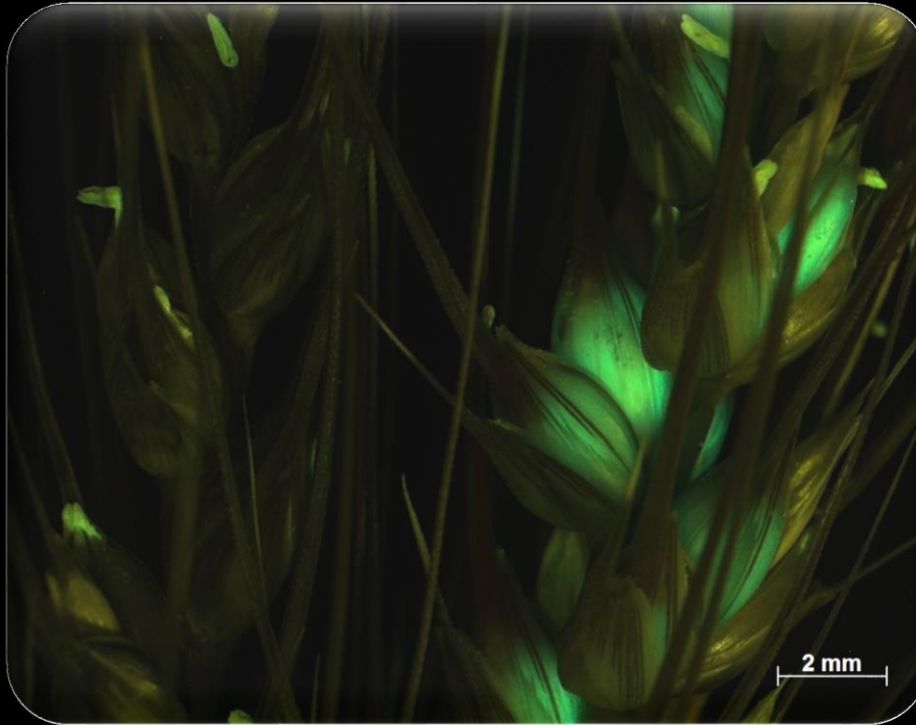
Agrobacterium-mediated gene transfer to cereals

Hensel et al. 2009, Int J Plant Genomics

L. Gugsä, unpublished

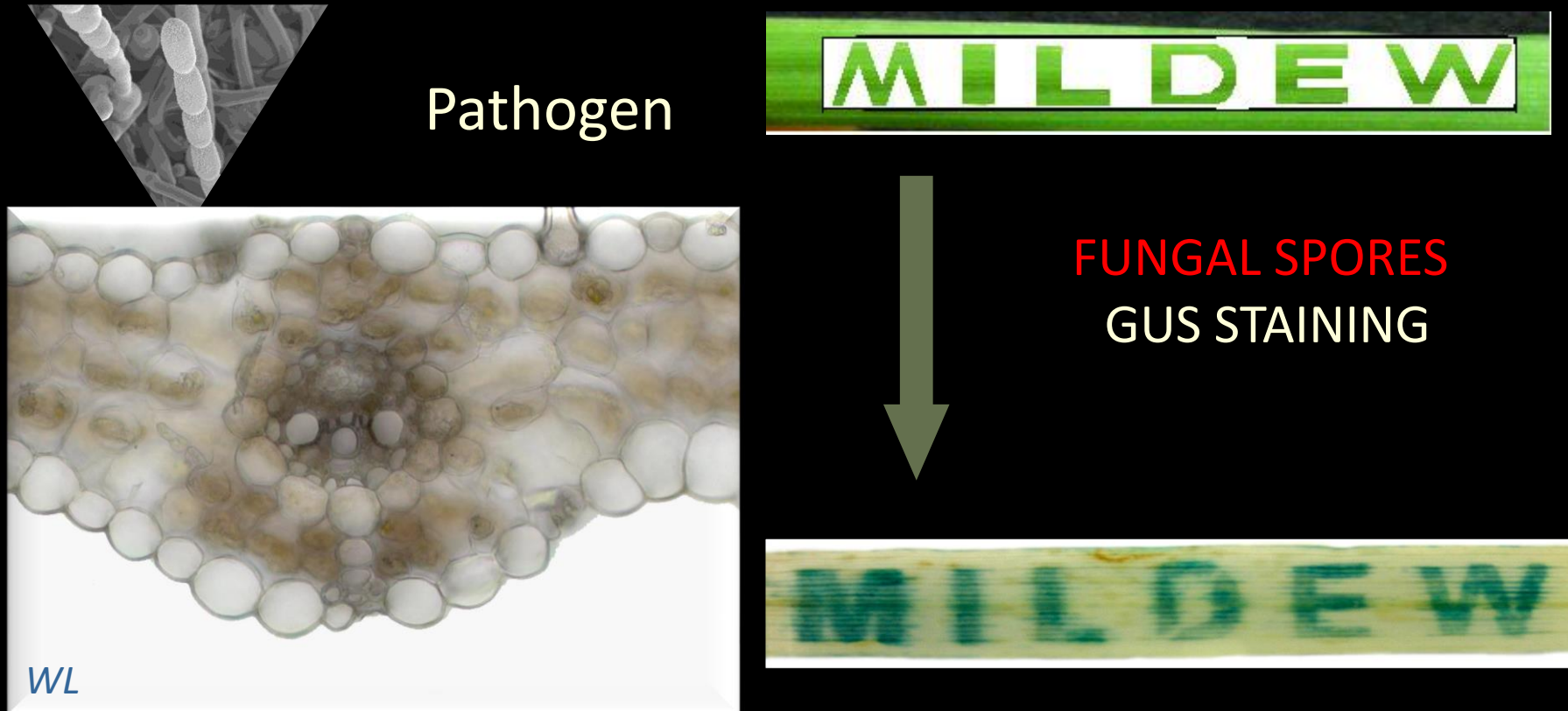


Endosperm-specific expression of AsGlo1P::gfp



- metabolic engineering (e.g. carbohydrates, proteins)
- production of recombinant proteins (enzymes, antibodies, vaccines etc.)

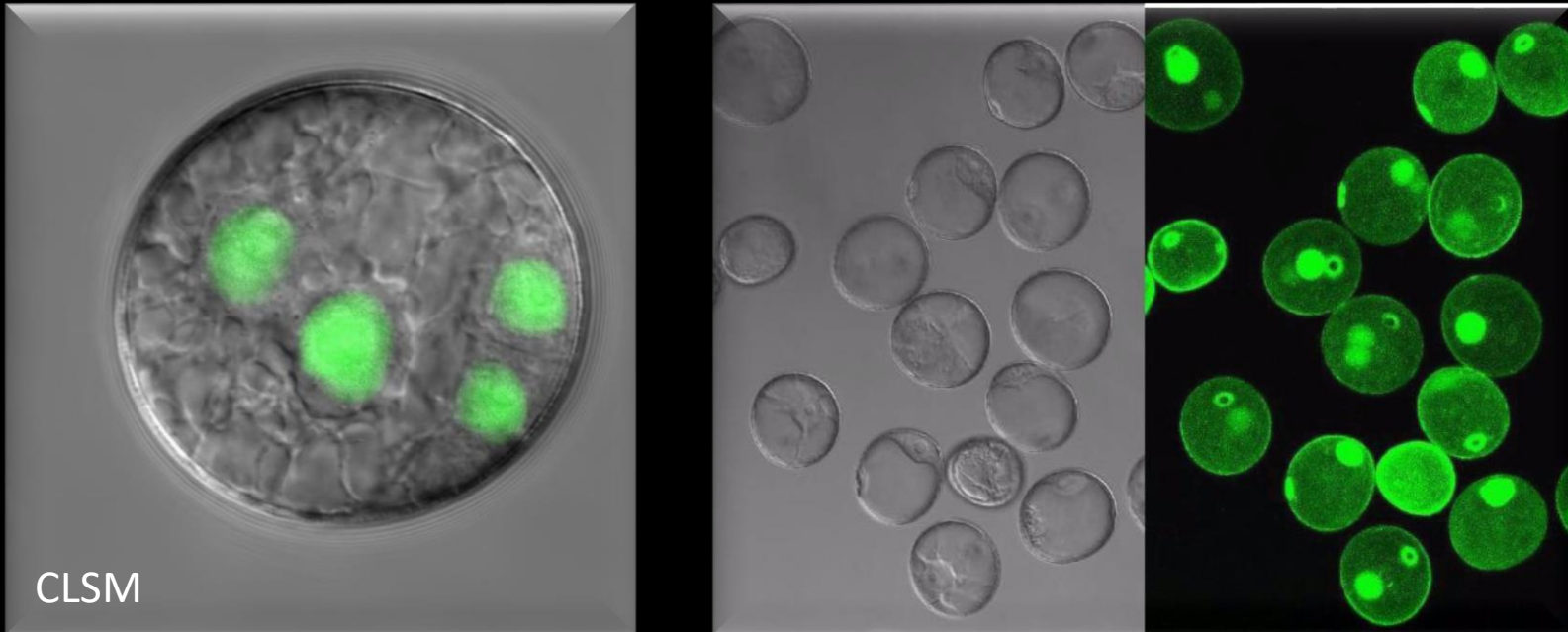
Epidermis-specific and pathogen-inducible expression of *GER4P::GUS*



- functional analysis of genes involved in plant – pathogen interactions
- inducible expression to avoid pleiotropic effects

Microspore-specific expression

ZmUbi1P:NLS::gfp

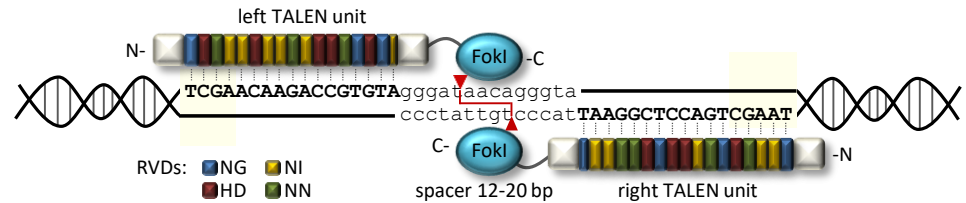


- functional analysis of POEM initiation
- production of doubled haploid plants for breeding processes

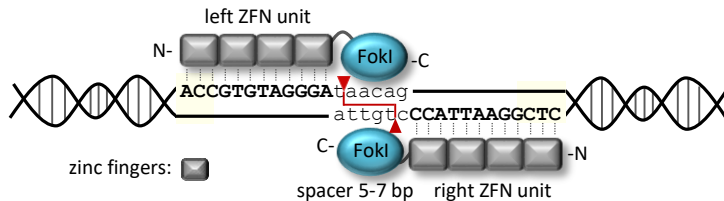
Meganucleases



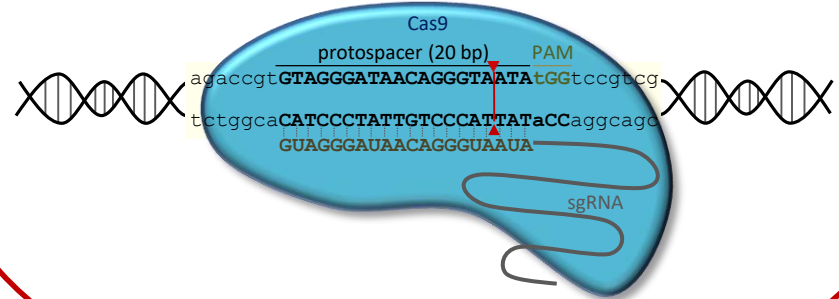
TALENs



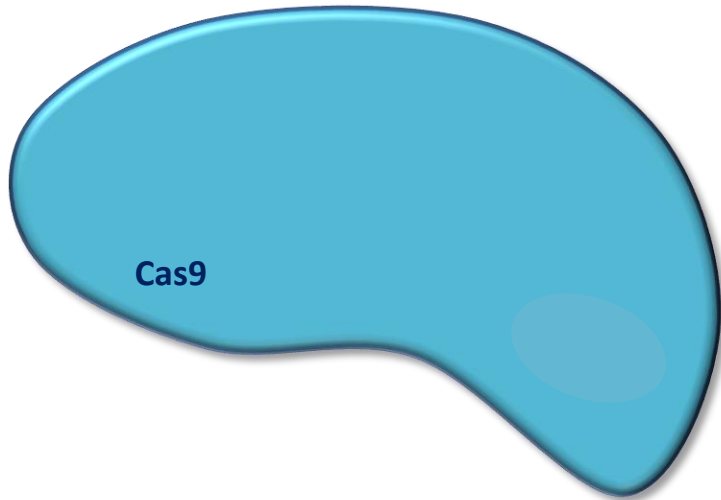
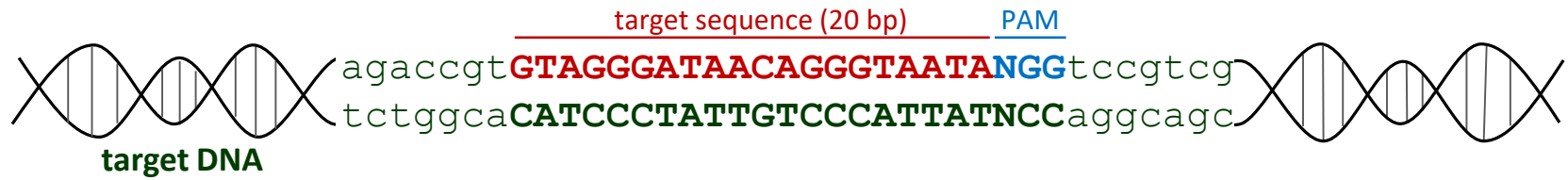
ZFNs



RGEnS (CRISPR RNA/Cas)

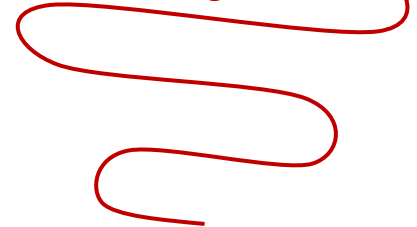


CRISPR RNA-guided/Cas9-mediated mutagenesis

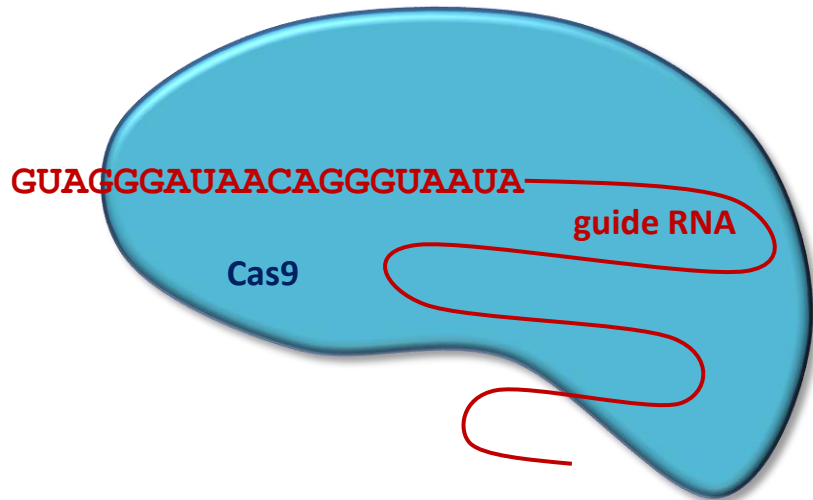
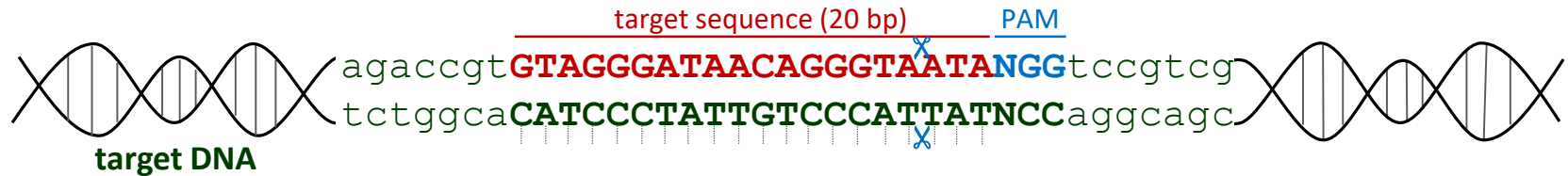


GUAGGGAUACAGGGUAUA

guide RNA



CRISPR RNA-guided/Cas9-mediated mutagenesis



CRISPR RNA-guided/Cas9-mediated mutagenesis



Repair

error-free

...C T T A C C T C A T C G C C A A G C T G G C A C C C T T G T T C A A G C G G A C A G C A A T A C C G A A T G G A A G T G...
...G A A T G G A G T A G C G G T T C G A C C G T G G G A A C A A G T T C G C C T G T C G T T A T G G C T T A C C T T C A C...

erroneous

...C T T A C C T C A T C G C C A A G C T G G C A C C C T T G T T - A A G C G G A C A G C A A T A C C G A A T G G A A G T G...
...G A A T G G A G T A G C G G T T C G A C C G T G G G A A C A A - T T C G C C T G T C G T T A T G G C T T A C C T T C A C...

Vrs1



2-rowed

→
domestication



vrs1

6-rowed



gRNA/Cas9-triggered
mutagenesis of *Vrs1*



Golden Promise WT, *Vrs1*

Golden Promise *vrs1*-KO
(awns of spikelets removed)

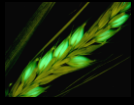
- KO lines show fully developed lateral spikelets

Comparison of different production systems

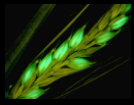
System	Overall costs	Production time	Scale-up capacity	Product quality	Contamination risk	Purification costs
Bacteria	low	short	high	low	medium	high
Yeast	low	short	high	medium	medium	high
Insects	medium	medium	medium	high	high	medium
Mammalian cells	high	long	medium	very high	very high	high
Plant cells	medium	medium	high	high	very low	medium
Whole plants	low	very long	very high	high	low	high

Source: Xu et al., (2011) 29:278-299, modified

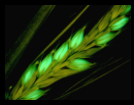
Advantages of barley



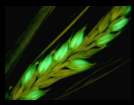
Experimental model for small-grain cereals and agronomically important



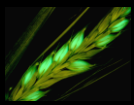
Exhibits a broad range of adaptability



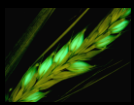
Relatively high protein content (12-15%)



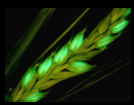
Caryopses are prone to be used as bioreactor



Self-pollinating nature and no outcrossing



G.R.A.S. (generally recognized as safe) status from FDA



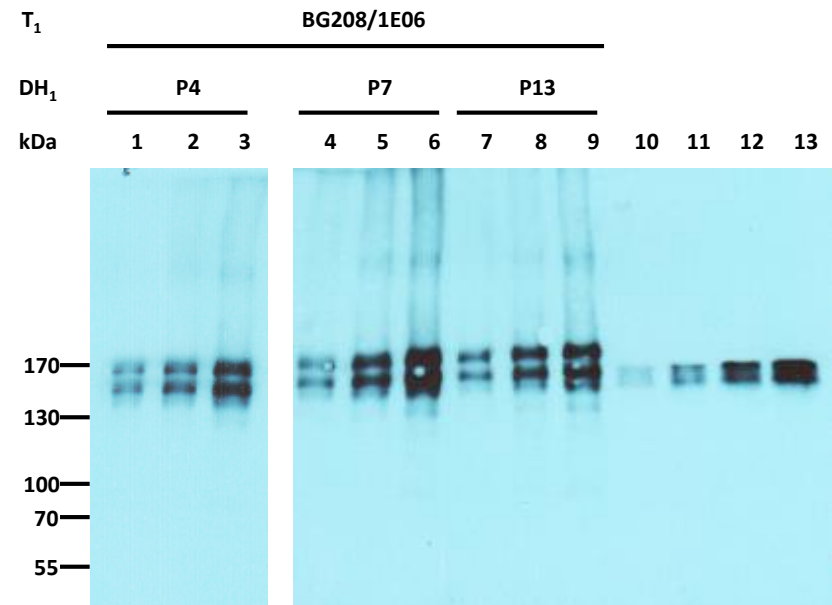
Low production costs due to a highly developed infrastructure for harvest,
transportation and storage

Bioproduction of Recombinant Protein in Barley Grains

Hensel et al. 2015 PLOS One

Endosperm-specific expression of the HIV neutralizing antibody 2G12

- cause of AIDS (acquired immunodeficiency syndrome)
- antibody neutralize the virus by binding to gp120
- 160 g antibody per kg mature grains produced
- biacore binding assays were promising



Thanks to the Plant Reproductive Biology group members

Head: J. Kumlehn



Cooperators at IPK

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T. Schnurbusch
N. Stein
P. Schweizer

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A. Hanemann (Breun Saatzucht)
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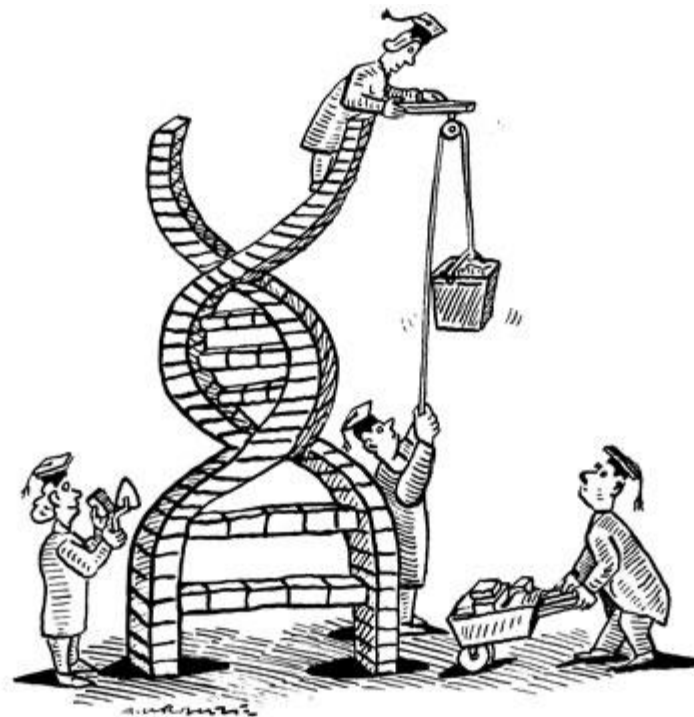
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Fayaz Shaikh

Thank you



<http://www.the-scientist.com/images/December2012/Genome.jpg>