

# Feasibility of new breeding techniques for organic farming

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Food and Agriculture  
Organization of the  
United Nations

*for a world without hunger*

## What is organic agriculture?



FAO INTER-DEPARTMENTAL  
WORKING GROUP ON  
**ORGANIC**  
AGRICULTURE

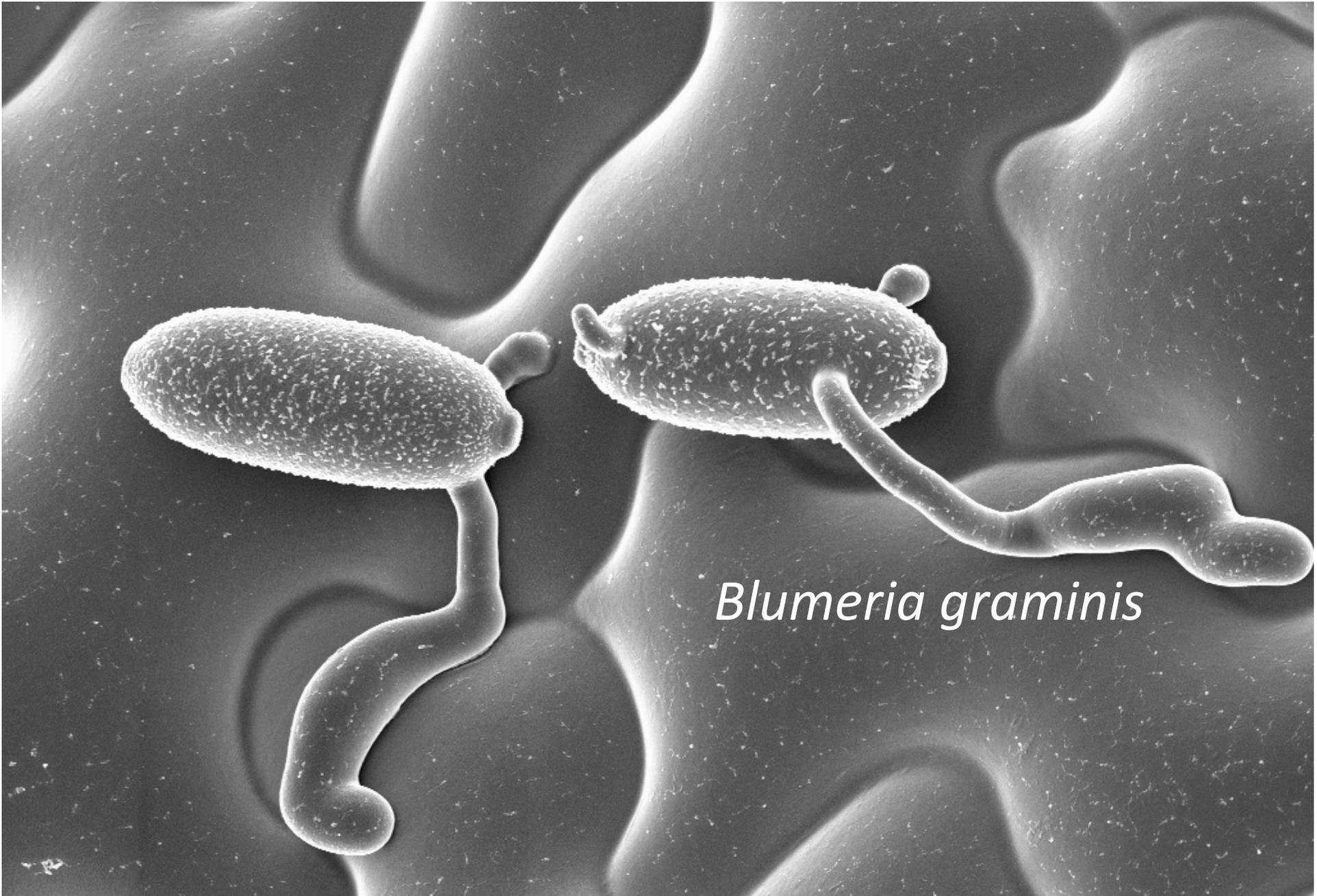
“There are many explanations and definitions for organic agriculture but all converge to state that it is a system that relies on ecosystem management rather than external agricultural inputs.”

FAO position paper on Organic Agriculture, 1999

An example how loss of function  
by the use of new breeding  
techniques have led to a crop  
that is less dependent on  
external inputs



Powdery mildew on wheat



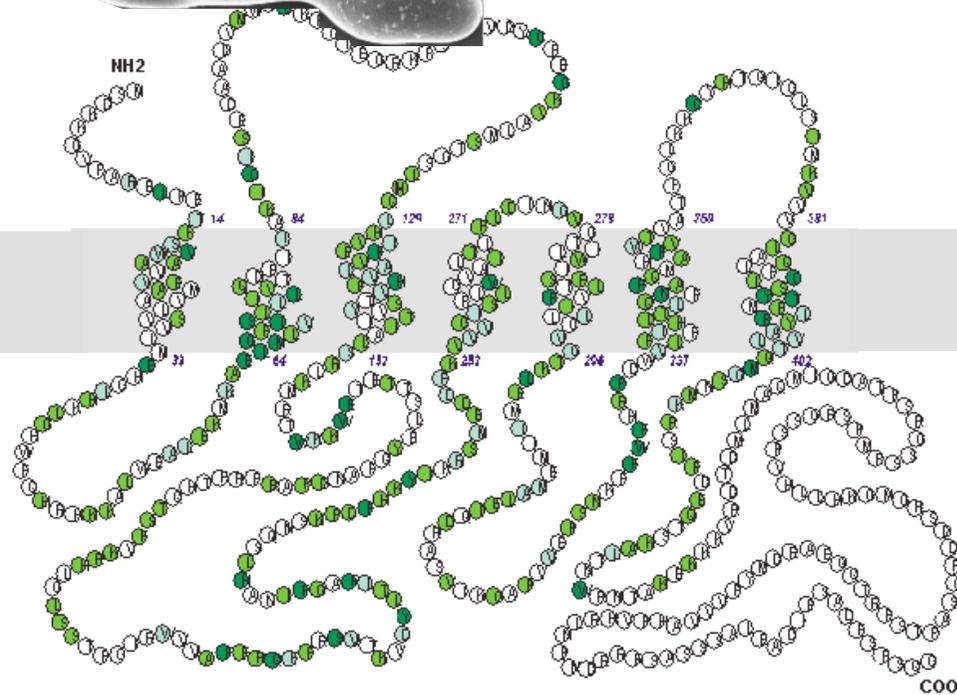
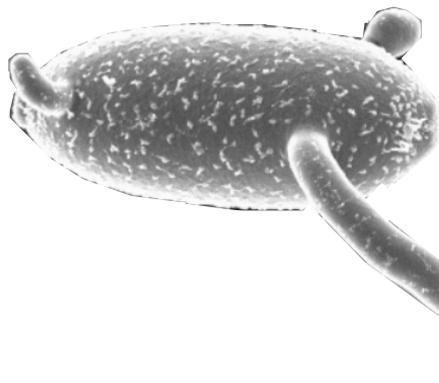
*Blumeria graminis*

10µm  
ACK

EHT = 6.87 kV    Mag = 1.06 K X    Signal A = InLens    WD = 3 mm  
Chamber Status = Pumping (HV)    Chamber = 1.19e-002 Pa



The powdery mildew fungus 'tastes' MLO proteins on the surface of the plant cell



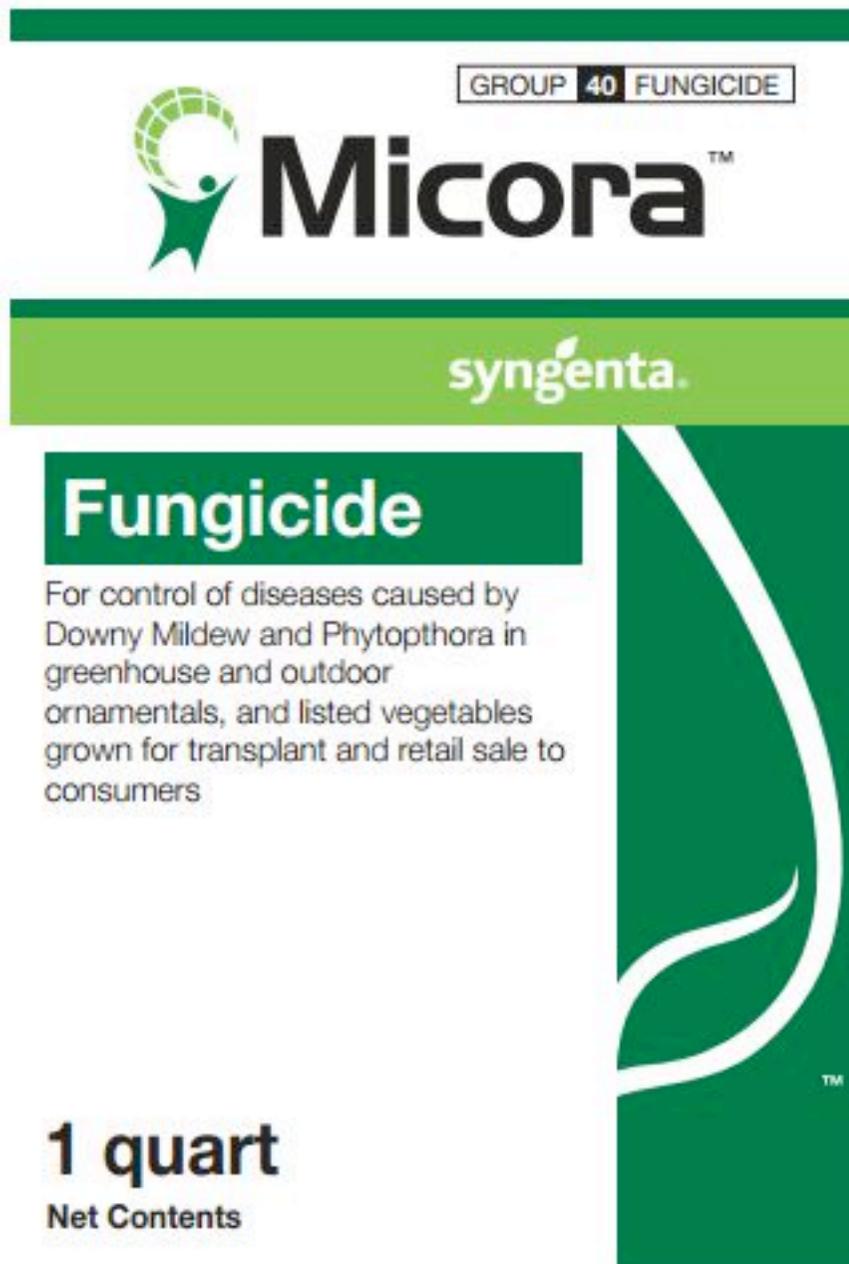
Cellens ydre

Cellens indre

**MLO protein**

Wheat has three *MLO* genes, each coding for a MLO protein that the fungus can recognize

In conventional agriculture powdery mildew is controlled by fungicides



PULL HERE ►  
TO OPEN

**Active Ingredient:**

Mandipropamid* .....	23.3%
Other Ingredients:	76.7%
<b>Total:</b>	<b>100.0%</b>

\*CAS No. 374726-62-2

Contains 1,2-benzisothiazolin-3-one at 0.017% as a preservative  
Contains 23.3% Mandipropamid equivalent to 2.08 pounds per gallon or 250 grams per liter of active ingredient

**KEEP OUT OF REACH OF CHILDREN.**

See additional precautionary statements and directions for use inside booklet.

EPA Reg. 100-1388

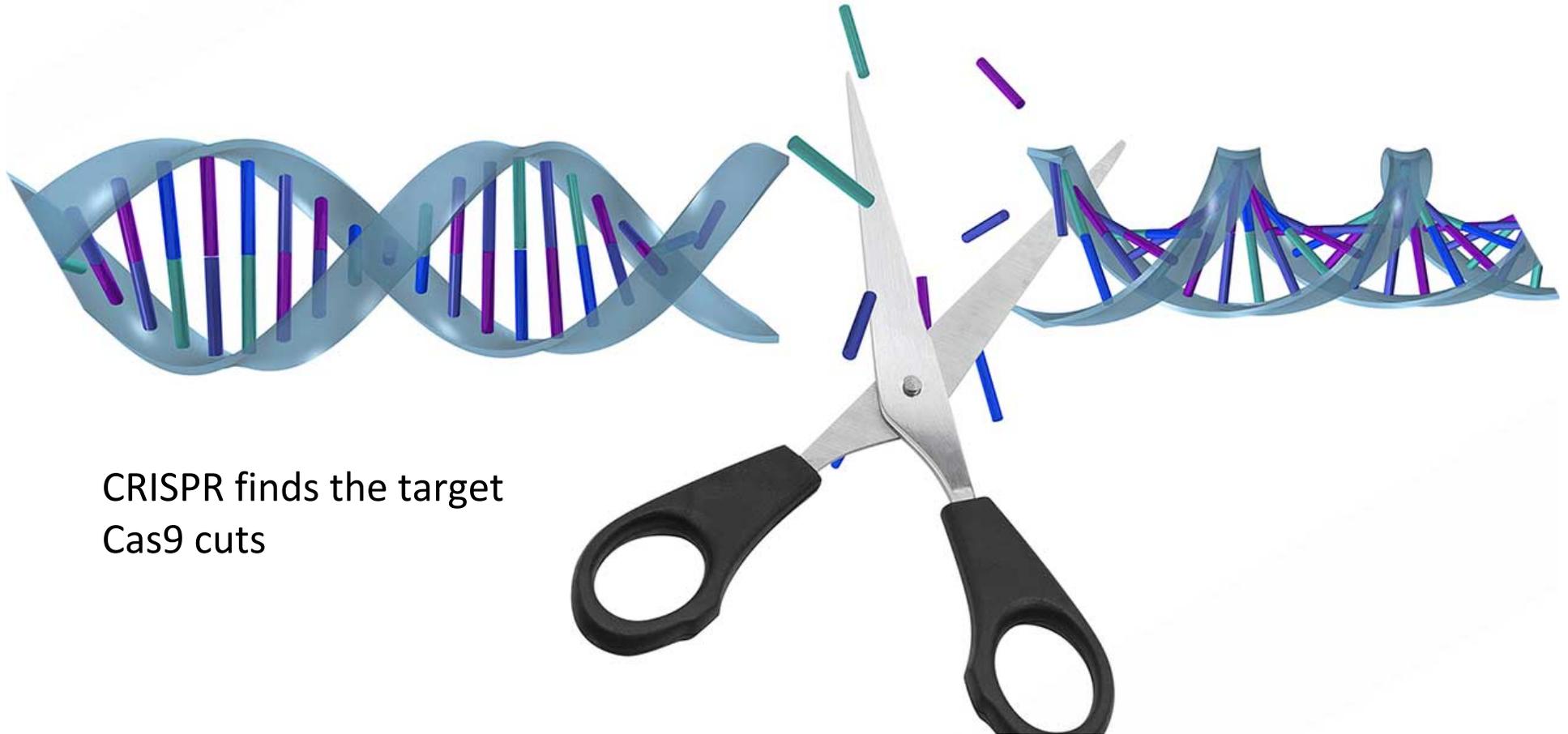
EPA Est. 100-NE-001

Product of Switzerland

Formulated in USA

SCP 1388A-L1 1111  
357008

# CRISPR/Cas9 is a powerful method for generation of targeted loss-of-function mutations



CRISPR finds the target  
Cas9 cuts

## Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew

Yanpeng Wang<sup>1,3</sup>, Xi Cheng<sup>2,3</sup>, Qiwei Shan<sup>1</sup>, Yi Zhang<sup>1</sup>, Jinxing Liu<sup>1</sup>, Caixia Gao<sup>1</sup> & Jin-Long Qiu<sup>2</sup>

Sequence-specific nucleases have been applied to engineer targeted modifications in polyploid genomes<sup>1</sup>, but simultaneous modification of multiple homoeoalleles has not been reported. Here we use transcription activator–like effector nuclease (TALEN)<sup>2,3</sup> and clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 (refs. 4, 5) technologies in hexaploid bread wheat to introduce targeted mutations in the three homoeoalleles that encode MILDEW-RESISTANCE LOCUS (MLO) proteins<sup>6</sup>. Genetic redundancy has prevented evaluation of whether mutation of all three *MLO* alleles in bread wheat might confer resistance to powdery mildew, a trait not found in natural populations<sup>7</sup>. We show that TALEN-induced mutation of all three *TaMLO* homoeologs in the same plant confers heritable broad-spectrum resistance to powdery mildew. We further use CRISPR-Cas9 technology to generate transgenic wheat plants that carry mutations in the *TaMLO-A1* allele. We also demonstrate the feasibility of engineering targeted DNA insertion in bread wheat through nonhomologous end joining of the double-strand breaks caused by TALENs. Our findings provide a methodological framework to improve polyploid crops.

breaks, which are then repaired mainly by either error-prone non-homologous end joining (NHEJ) or high-fidelity homologous recombination<sup>11</sup>. All three types of SSN have been used to create targeted gene knockouts in various plant species<sup>12</sup>. Although heritable gene modification has been demonstrated in *Arabidopsis*<sup>13–15</sup> and rice<sup>16</sup> using the SSNs, germline transmission of a mutation created by such genome editing strategies has not yet been achieved in hexaploid bread wheat. We have previously demonstrated genome editing in bread wheat using a transient protoplast expression system<sup>17</sup>. Here we report the use of TALENs and the CRISPR-Cas9 system to modify an endogenous wheat gene and produce newly introduced, stably transmitted genetic traits. Our findings underscore the potential of SSNs as efficient tools for wheat research and breeding.

We chose to target three *MLO* loci, which encode proteins that were shown to repress defenses against powdery mildew diseases in other plants<sup>18</sup>. Loss-of-function *mlo* alleles in barley<sup>19</sup>, *Arabidopsis*<sup>20</sup> and tomato<sup>21</sup> lead to broad-spectrum and durable resistance to the fungal pathogens that cause powdery mildew in these species. In wheat, powdery mildew is caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), which is one of the most destructive plant pathogens worldwide. Modification of *MLO* genes in wheat may provide the opportunity to breed varieties

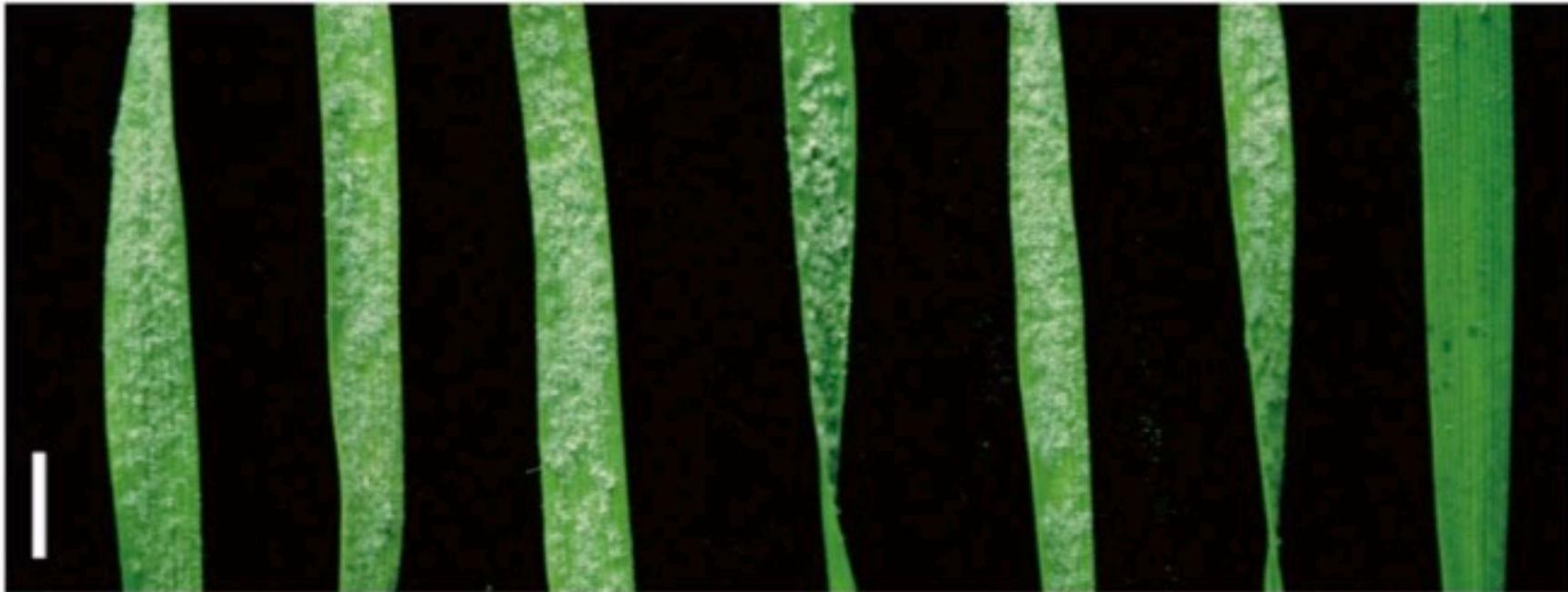
## Mutations in an *MLO* gene from wheat resulting from cuts by CRISPR/Cas9

MLO-A1: TCGCTGCTGCTCGCCGTCACGCAGGACCCAATCTCCGGATATGCATCTCCCA  
M3: TCGCTGCTGCTCGCCGTCA...AGGACCCAATCTCCGGATATGCATCTCCCA -3  
M6: TCGCTGCTGCTCGCCGTCA.GCAGGACCCAATCTCCGGATATGCATCTCCCA -1  
M11: TCGCTGCTGCTCGCCGTCA....GGACCCAATCTCCGGATATGCATCTCCCA -4  
M13: TCGCTGCTGCTCGCCGTCA<sup>T</sup>CGCAGGACCCAATCTCCGGATATGCATCTCCCA +1



Wheat with three *MLO* genes cut – *A*, *B* og *D* – is resistant towards mildew and need not be sprayed

	only <i>A</i>	only <i>B</i>	only <i>D</i>	<i>A</i> and <i>B</i>	<i>A</i> and <i>D</i>	all three
WT	cut	cut	cut	cut	cut	cut



# How can nature provide clues for a climate friendly sustainable agriculture?



**What to do?**

*University of Copenhagen KU2016  
Crossdisciplinary Program*

How can we use today's enormous knowledge on plants for the benefit of mankind in a societally acceptable way?

*“Plants for a changing world”*

# **Biotechnology and plants**

All agriculture and human  
nutrition are based on mutants!

**Some examples of 'traditional'  
mutant plants**

# The origin of agriculture



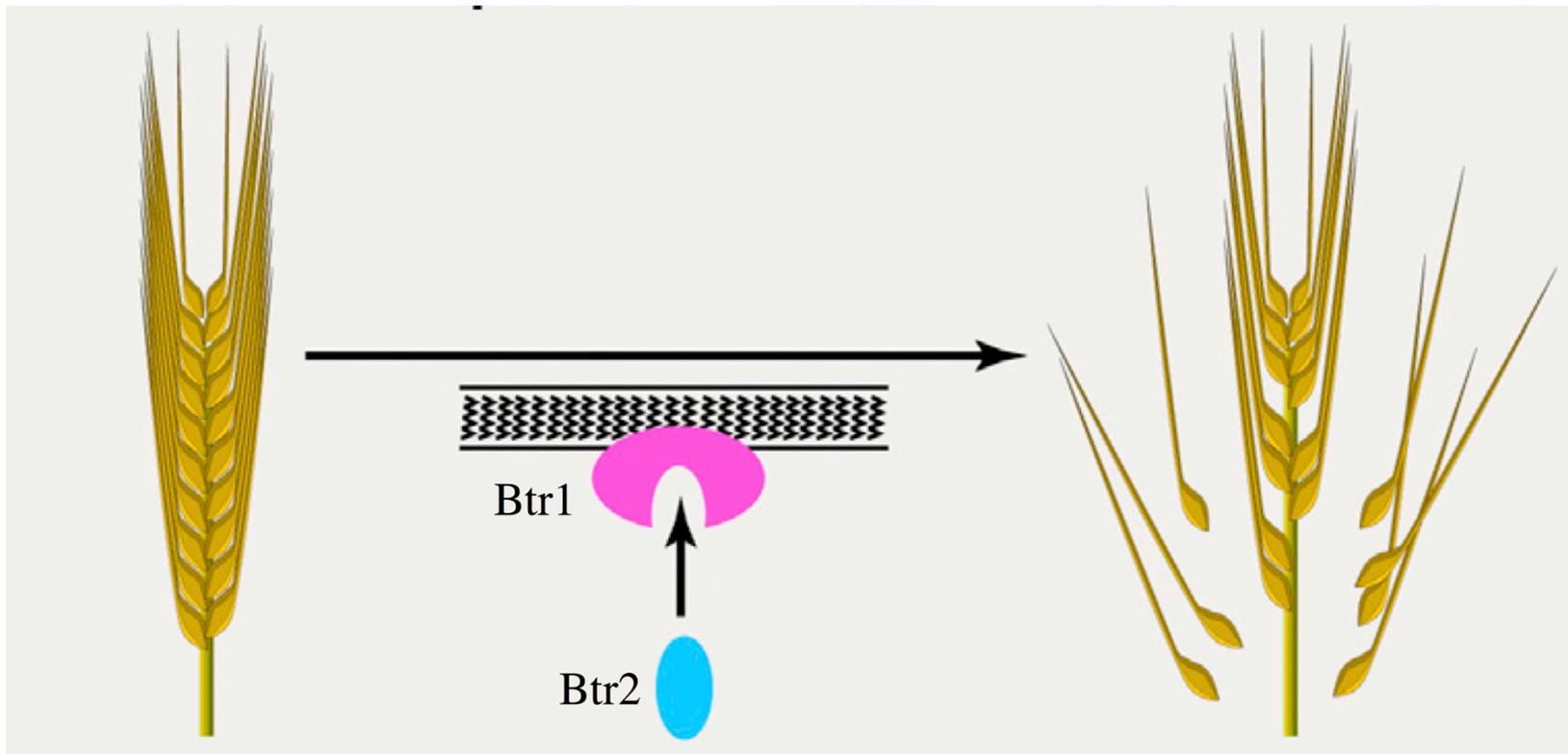
Agriculture is only about 10.000 years old

# Wheat harvest in Egypt $\approx$ 1.300 B.C.



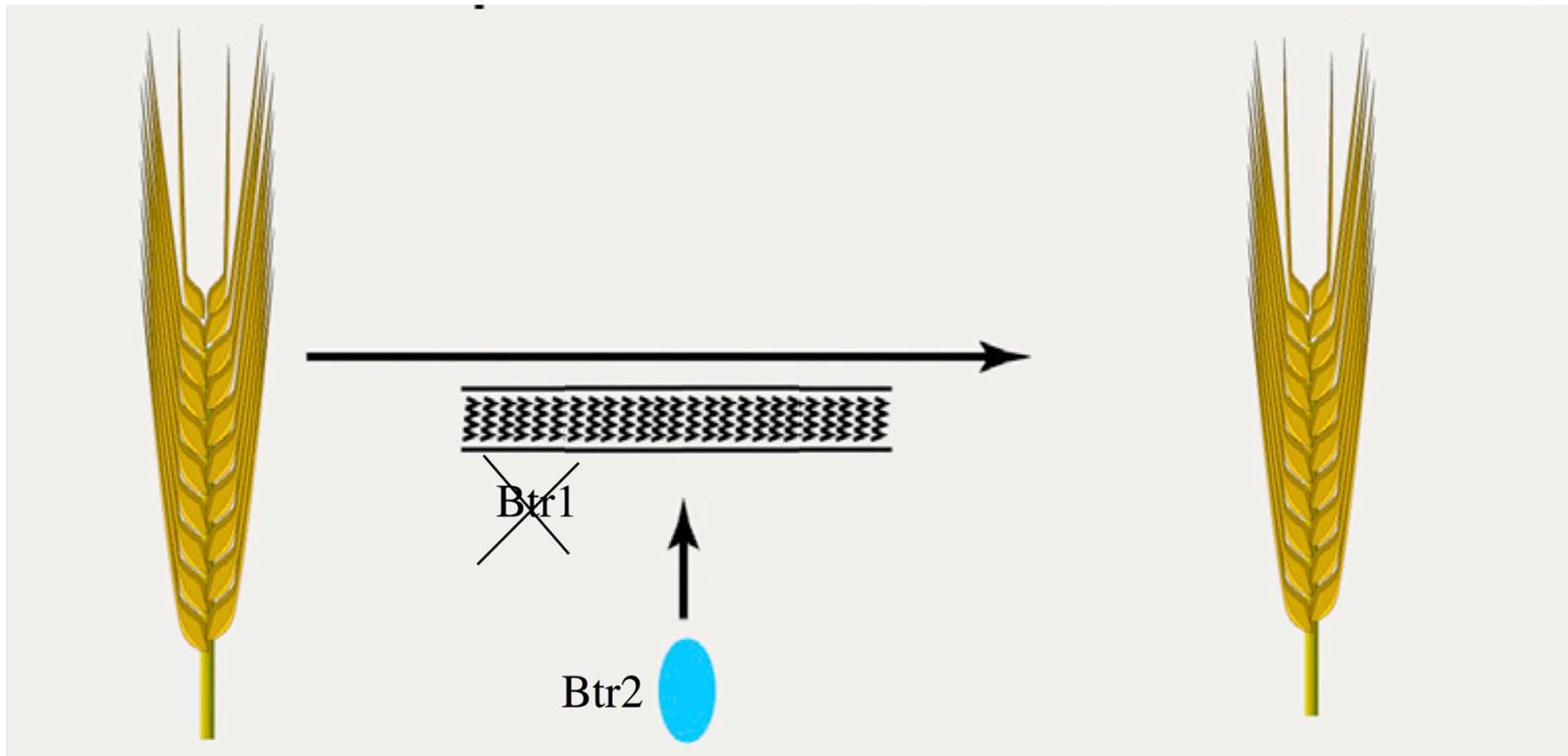
Mural painting; Tomb of Sennedjem, Ancient Thebes.

In the wild relative to barley does the spike splinter when mature to facilitate dispersal of the seeds



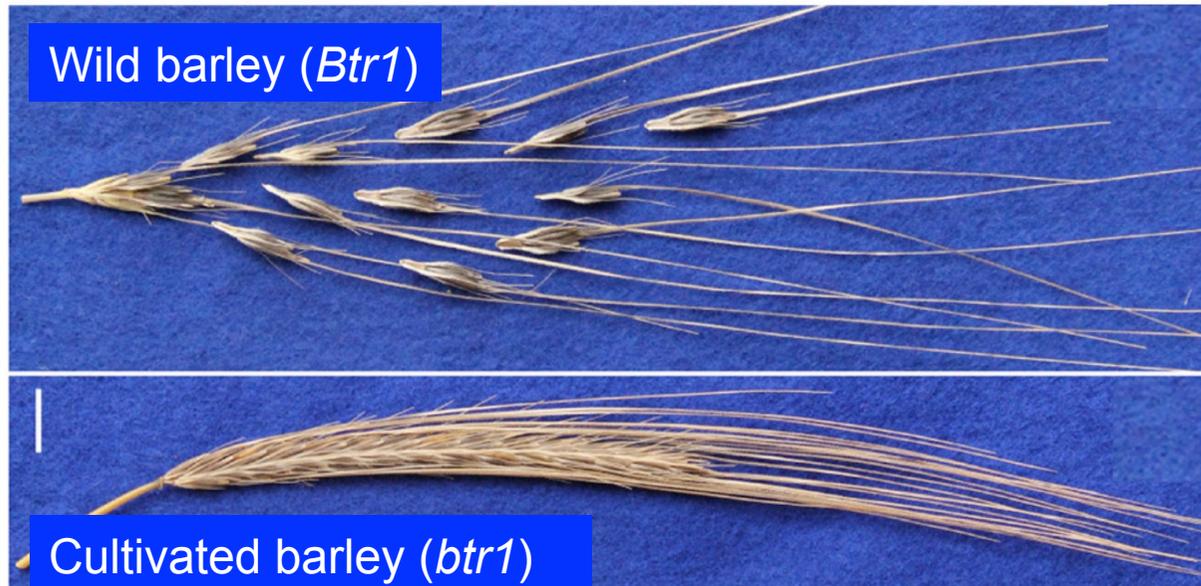
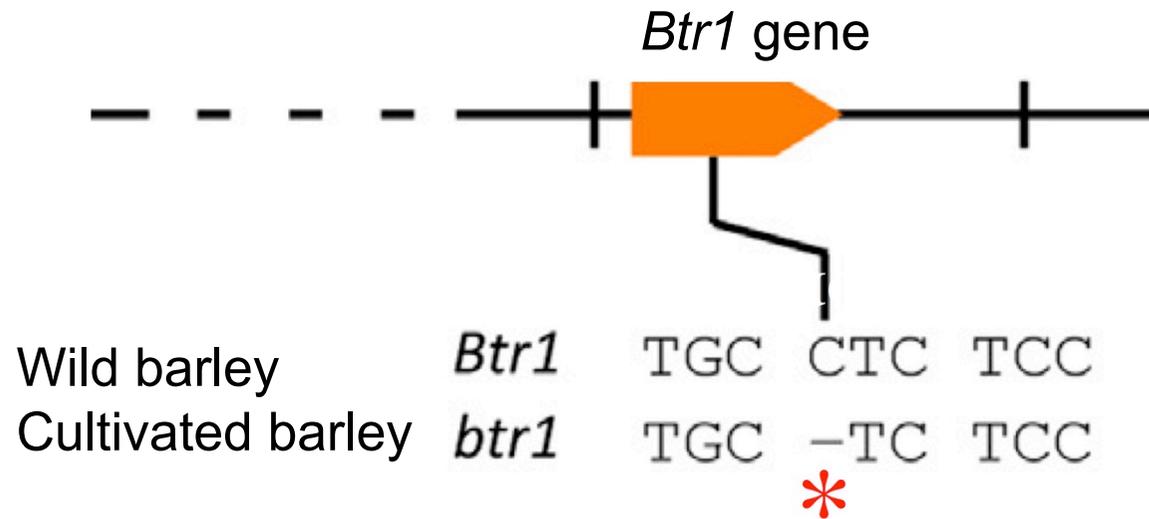
Wild barley  
(*Hordeum vulgare* subsp. *spontaneum*)

A mutation in the gene that codes for Btr1 protein lead to a defect in this process



Cultivated barley  
(*Hordeum vulgare*)

In the cultivated barley the *Btr1* gene is defect due to the deletion of a single DNA base in the gene



The defect in the cultivated barley can be 'repaired' by insertion of the non-mutated *Btr1* gene from wild barley



Golden promise  
(*btr1*)

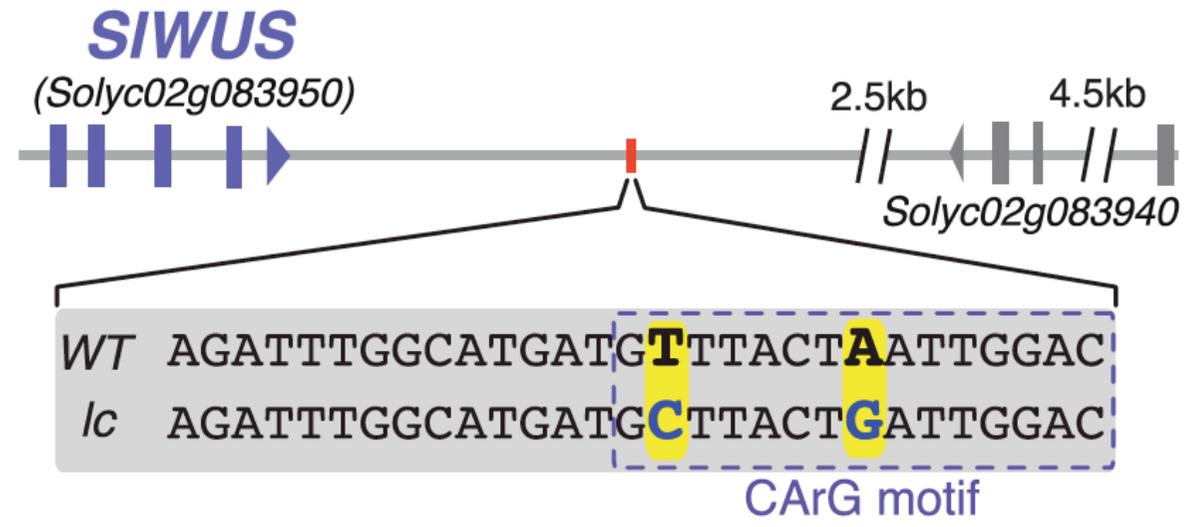
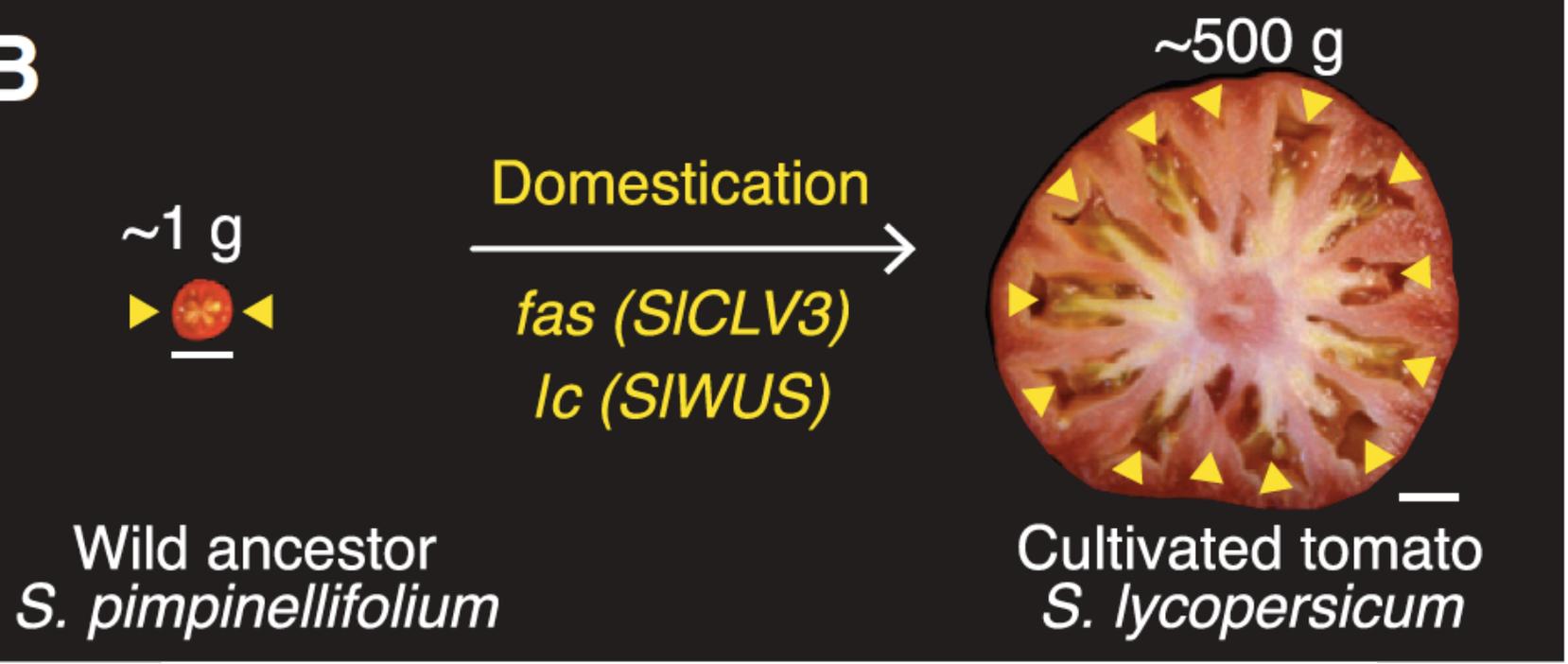


Golden promise  
(*btr1 + Btr1*)

# TRAITS GAINED BY BREEDING



**B**



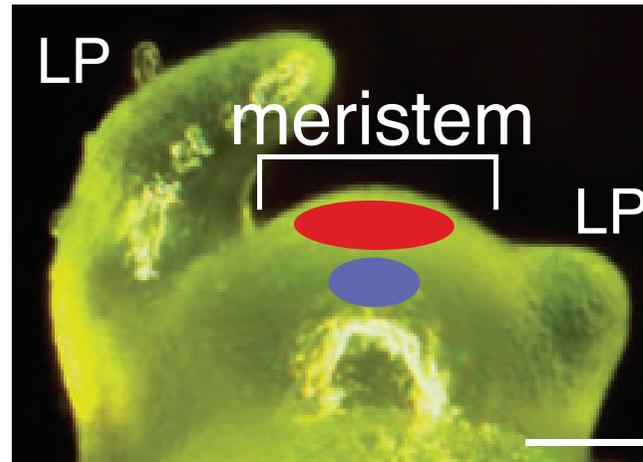
Rodríguez-Leal *et al.* (2017) *Cell* 171: 470-480.

## Tomato fruit size is controlled by two genes

■ CLAVATA3



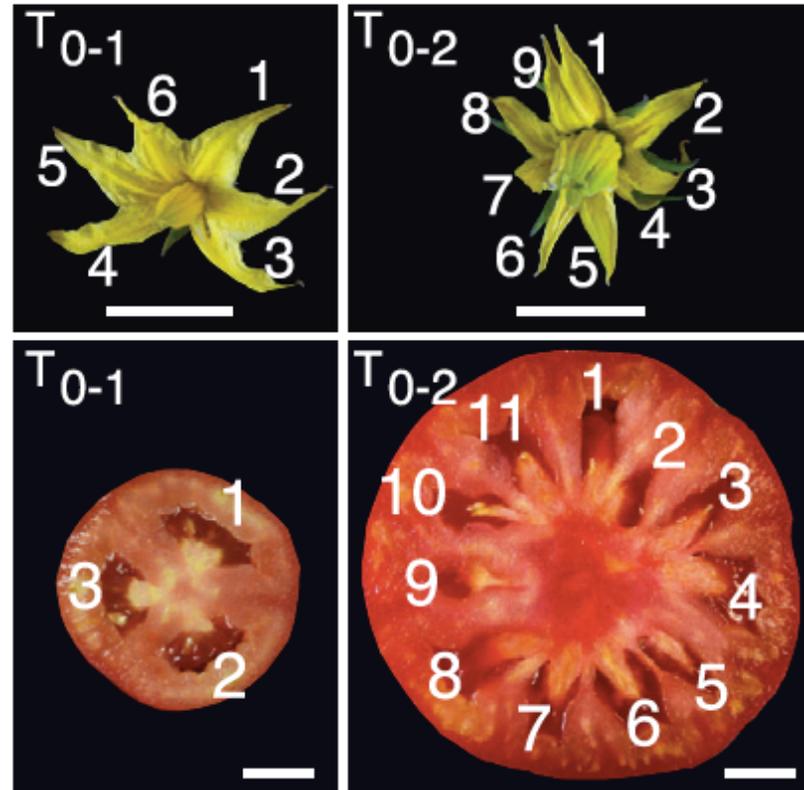
■ WUSCHEL



**WUSCHEL (WUS)** → Stem cell promoting transcription factor

**CLAVATA3 (CLV3)** → Differentiation promoting peptide

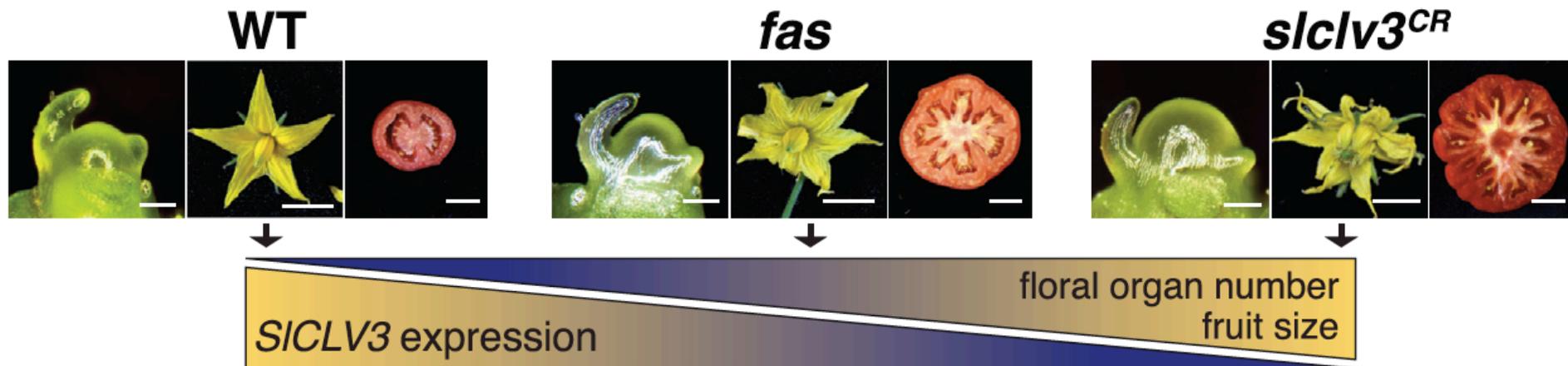
## Two different mutants of wild tomato made by CRISPR/Cas9



CRISPR/Cas9 cutting in *S/CLV3* promoter region mimics mutations that lead to domestication of tomato

Rodríguez-Leal *et al.* (2017) Engineering quantitative trait variation for crop improvement by genome editing. *Cell* 171: 470-480.

# *S/CLV3* functions as a brake that inhibits *S/WUS*, which stimulates stem cell division



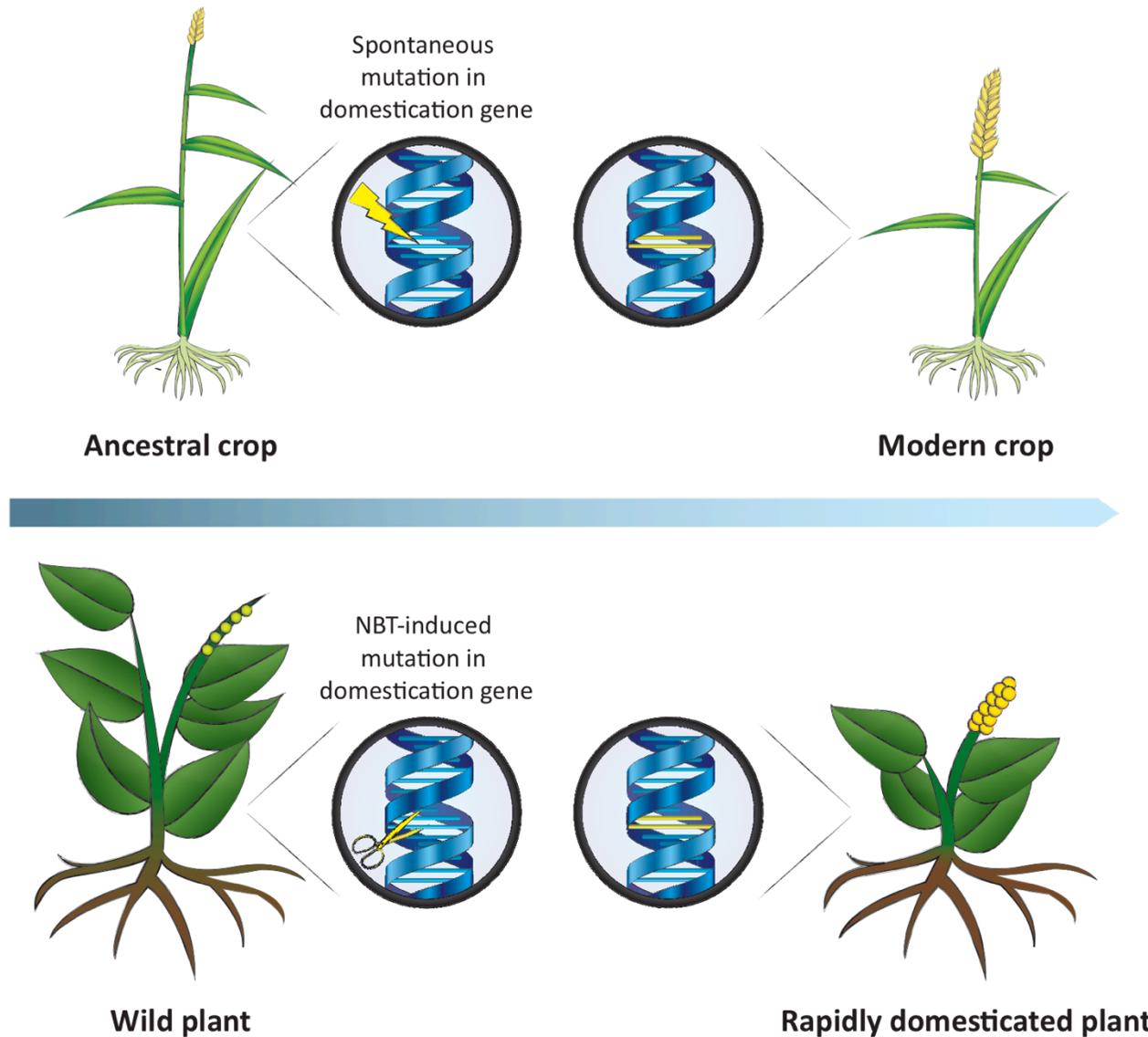
**More SICLV3 protein:**  
Small fruits  
Few locules

**Less SICLV3 protein:**  
Large fruits  
More locules

Rodríguez-Leal *et al.* (2017)  
*Cell* 171: 470-480.

- Many traits in crops that were crucial for their domestication are caused by mutations that can be produced by mutation techniques.
- This opens a path for accelerated domestication of wild plants

# Accelerating the Domestication of New Crops



# *De novo* domestication of wild tomato using genome editing

Agustin Zsögön<sup>1,7</sup> , Tomáš Čermák<sup>2,6,7</sup>, Emmanuel Rezende Naves<sup>1</sup>, Marcela Morato Notini<sup>3</sup>, Kai H Edel<sup>4</sup>, Stefan Weigl<sup>4</sup>, Luciano Freschi<sup>5</sup>, Daniel F Voytas<sup>2</sup>, Jörg Kudla<sup>4</sup>  & Lázaro Eustáquio Pereira Peres<sup>3</sup> 

**Breeding of crops over millennia for yield and productivity<sup>1</sup> has led to reduced genetic diversity. As a result, beneficial traits of wild species, such as disease resistance and stress tolerance, have been lost<sup>2</sup>. We devised a CRISPR–Cas9 genome engineering strategy to combine agronomically desirable traits with useful traits present in wild lines. We report that editing of six loci that are important for yield and productivity in present-day tomato crop lines enabled *de novo* domestication of wild *Solanum pimpinellifolium*. Engineered *S. pimpinellifolium* morphology was altered, together with the size, number and nutritional value of the fruits. Compared with the wild parent, our engineered lines have a threefold increase in fruit size and a tenfold increase in fruit number. Notably, fruit lycopene accumulation is improved by 500% compared with the widely cultivated *S. lycopersicum*. Our results pave the way for molecular breeding programs to exploit the genetic diversity present in wild plants.**

We previously identified a suite of loci that have shaped the morphology and agronomic potential of current cultivars of tomato, maize, rice and other crops and proposed a reverse genetic approach for the *de novo* domestication of novel crops<sup>9</sup> (**Table 1** and **Supplementary Table 1**). In tomato, at least six loci important for key domestication traits have been identified: general plant growth habit (*SELF-PRUNING*)<sup>10</sup>, fruit shape (*OVATE*)<sup>11</sup> and size (*FASCIATED* and *FRUIT WEIGHT 2.2*)<sup>12,13</sup>, fruit number (*MULTIFLORA*)<sup>14</sup>, and nutritional quality (*LYCOPENE BETA CYCLASE*)<sup>15</sup>. We set out to create a novel crop derived from *S. pimpinellifolium* by targeting this set of genes using a multiplex CRISPR–Cas9 approach to generate loss-of-function alleles. We constructed a single CRISPR–Cas9 plant transformation vector, pTC321 (**Supplementary Note 1**), which harbored six single guide RNAs (gRNAs) targeting specific sequences in the coding regions of all six genes (**Supplementary Fig. 1**). Using this vector, we generated ten primary T<sub>0</sub> lines, of which three were grown to maturity. T<sub>1</sub> seeds were harvested from plant 3, which showed an

Li *et al.* (2018) Nature Biotechnology  
Oct 1. doi: 10.1038/nbt.4273

## Domestication of wild tomato is accelerated by genome editing

Tingdong Li<sup>1,2,5</sup>, Xinping Yang<sup>3-5</sup>, Yuan Yu<sup>2-5</sup>, Xiaomin Si<sup>1,2</sup>,  
Xiawan Zhai<sup>3,4</sup>, Huawei Zhang<sup>1</sup>, Wenxia Dong<sup>3,4</sup>,  
Caixia Gao<sup>1,2</sup>  & Cao Xu<sup>2-4</sup>

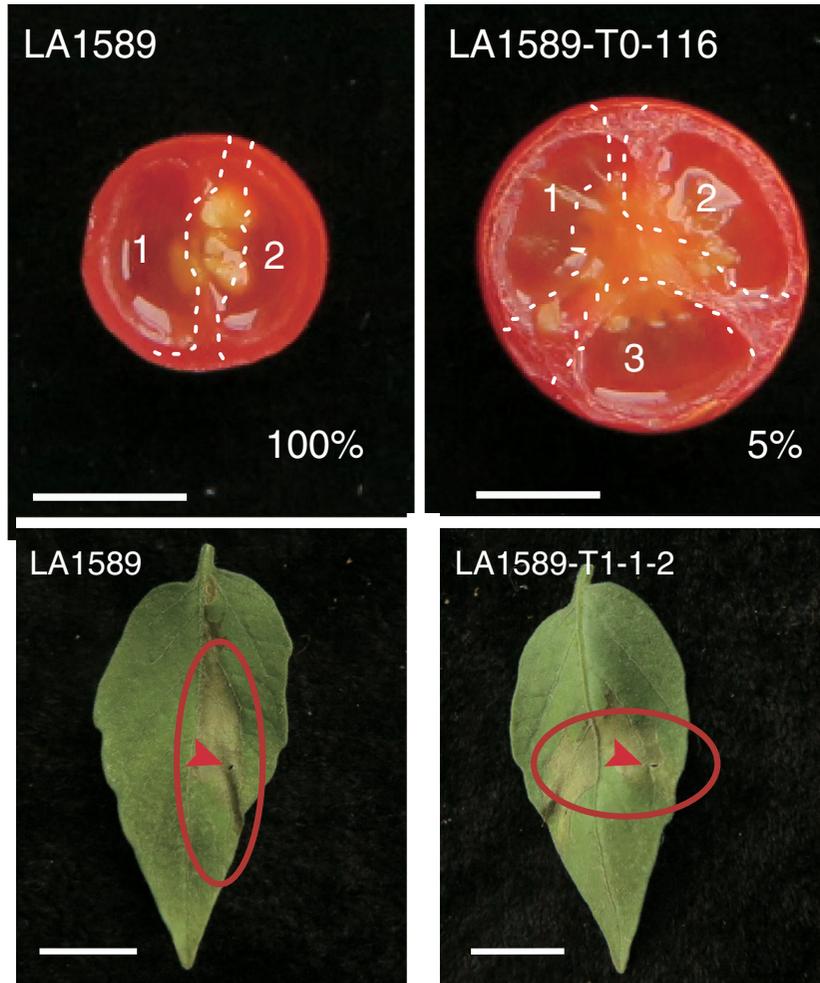
**Crop improvement by inbreeding often results in fitness penalties and loss of genetic diversity. We introduced desirable traits into four stress-tolerant wild-tomato accessions by using multiplex CRISPR–Cas9 editing of coding sequences, cis-regulatory regions or upstream open reading frames of genes associated with morphology, flower and fruit production, and ascorbic acid synthesis. Cas9-free progeny of edited plants had domesticated phenotypes yet retained parental disease resistance and salt tolerance.**

Conventional breeding to domesticate wild plants increases productivity but is often accompanied by decreased fitness and genetic diversity, thus hampering growth in challenging environmental conditions<sup>1</sup>. Introgression of stress-tolerance genes from wild relatives

Loss of the flowering repressor SELF-PRUNING 5G (*SP5G*, a member of the CETS protein family) in tomato confers day neutrality and thus could potentially extend the geographical cultivation range<sup>12</sup>. Mutations in the *SP* (*SELF PRUNING*) gene, another CETS family flowering repressor, change tomato indeterminate shoot architecture into determinate vines. This change enables high-density growth and mechanical harvesting, because *sp* variants are compact plants with intense inflorescence and almost synchronous fruit ripening<sup>12,13</sup>. Moreover, simultaneous mutation of *SP5G* and *SP* in tomato varieties can produce ‘double determinate’ plants that flower early on both primary and sympodial shoots, and permit early harvest<sup>12</sup>. Precise editing of *SP* and *SP5G* in wild tomato species might serve as a first step toward generating commercially cultivable lines. To that end, we designed one guide RNA (gRNA) for the first exon of each gene to induce mutations in *SP* and *SP5G* through genome editing (**Supplementary Fig. 1a,b**).

Fruit-size enlargement due to increased numbers of seed compartments (locules) is a crucial feature of domesticated tomato<sup>14</sup>, but *S. pimpinellifolium* has only tiny bilocular fruits. Two quantitative trait loci, *fasciated (fas)*<sup>15</sup> and *locule number (lc)*<sup>16</sup>, are responsible for increasing locule number and fruit size, and are correlated with mutations in the small-peptide-encoding gene *CLV3* (*CLAVATA3*) and the homeobox-encoding gene *WUS* (*WUSCHEL*), respectively. *CLV3* null mutations in tomato and *WUS* loss-of-function

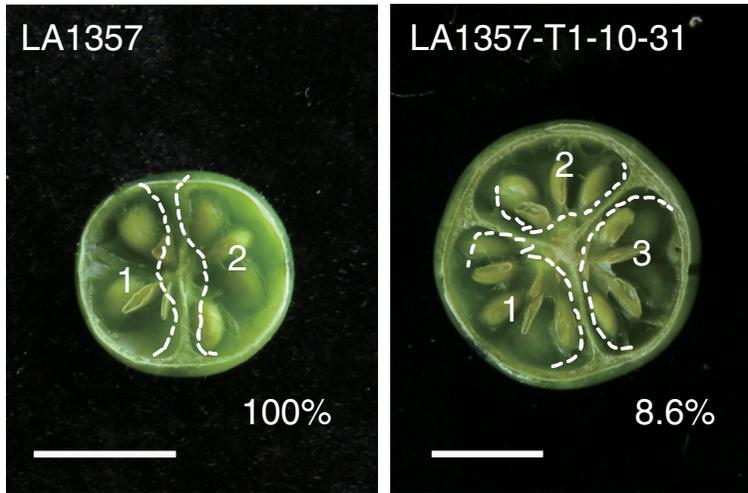
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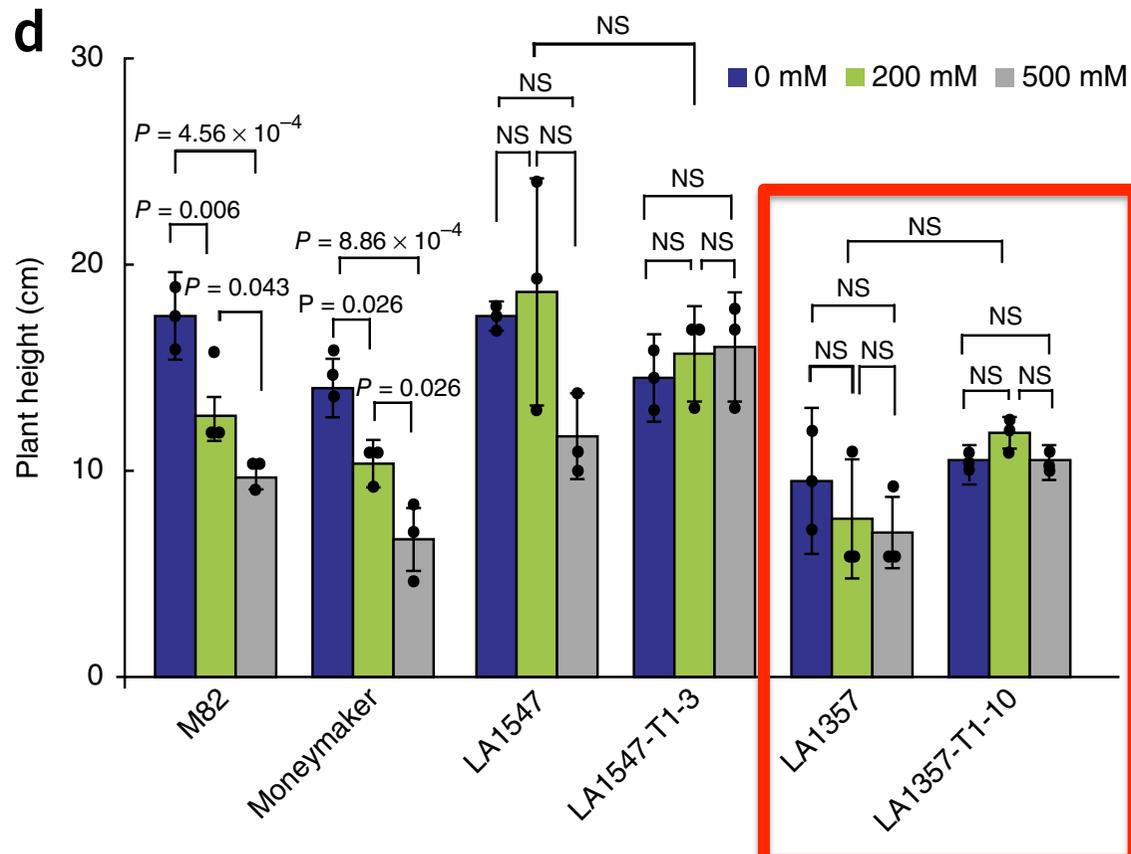
Accelerated  
domestication of a  
***Xanthomonas*-resistant  
line** of wild tomato

*Xanthomonas*-resistance  
is maintained after  
domestication

Li *et al.* (2018) *Nat. Biotechnol.*  
Oct 1. doi: 10.1038/nbt.4273



Accelerated domestication of a **salt-resistant line** of wild tomato



Salt-resistance is maintained after domestication

REVIEW

PLANT SCIENCE

# Revolutions in agriculture chart a course for targeted breeding of old and new crops

Yuval Eshed<sup>1\*</sup> and Zachary B. Lippman<sup>2,3\*</sup>

The dominance of the major crops that feed humans and their livestock arose from agricultural revolutions that increased productivity and adapted plants to large-scale farming practices. Two hormone systems that universally control flowering and plant architecture, florigen and gibberellin, were the source of multiple revolutions that modified reproductive transitions and proportional growth among plant parts. Although step changes based on serendipitous mutations in these hormone systems laid the foundation, genetic and agronomic tuning were required for broad agricultural benefits. We propose that generating targeted genetic variation in core components of both systems would elicit a wider range of phenotypic variation. Incorporating this enhanced diversity into breeding programs of conventional and underutilized crops could help to meet the future needs of the human diet and promote sustainable agriculture.

tem that directs toward flowering relationship is cr navigate between production of su leaves (source ti metabolic dema fruits, and seeds completion of g before growing

Although this florigen-antiflorig the dynamic evc family and specie plays between it generalization o chitecture and r Particularly rele foundation of thi for which the tra is followed by re and reproductive

- Out of the more than 350.000 plant species that exist, less than 200 are commercially important and three species, rice, wheat and maize, account for the major part of plant derived nutrients humans consume.
- Traits of importance for a sustainable and carbon neutral agriculture already exist in wild plants.
- Transform agriculture by learning from nature.

# **All current crops are developed for conventional agriculture**

**New breeding techniques allow for accelerated domestication of new crops for organic agriculture**

## **Plants that**

- require less input (water, nutrients, chemicals)**
- are more resilient (drought, salt stress, pests)**
- grow in soil-preserving perennial polycultures**
- give more output than wild plants**
- store carbon**

# Intermediate wheatgrass

*Thinopyrum  
intermedium*

## perennial

a potential  
sustainable  
grain crop but  
still not  
domesticated

Perennial  
grasses need not  
be sown every  
year and **store  
carbon in their  
deep root  
system** and **take  
up nutrients and  
water more  
efficiently**



# Wheat annual

reduced yield  
because of seed  
shattering



2 cm

Wheatgrass

Wheat



# NovoCrops Center (Novo Nordisk Foundation € 10 mio; 2020-26)

		Plant	Wild-type properties	Target traits
De novo	Domestication type	Barley 	Disease resistance Protein quality	Non-brittle rachis Hull-less grains Reduced height
		Potato 	Disease resistance Glycemic properties	Adaptation to long-day photoperiod Elimination of alkaloids in tubers
Accelerated	Domestication type	Alfalfa 	Wide environmental adaptability Resistance to frequent cutting	Delayed flowering Increased vegetative growth
		Lupin 	Herbivore resistance High content of good-quality protein	Early flowering Elimination of alkaloids in seeds
		Quinoa 	Resistance to water-related stresses Exceptional nutritional qualities	Increased grain size and yield Elimination of saponins in seeds
		Int. wheatgrass 	Perennial with efficient nutrient and water use Resistance to frequent cutting	Short stems, seed shattering and increased grain size

A group of people, including children and adults, are gathered in a field of tall green grass. Some are looking at plants, while others are talking. The sky is overcast with grey clouds. In the background, there are trees and a distant building.

**Thank you for your attention**

*Thinopyrum intermedium*  
A perennial grain crop  
awaiting domestication