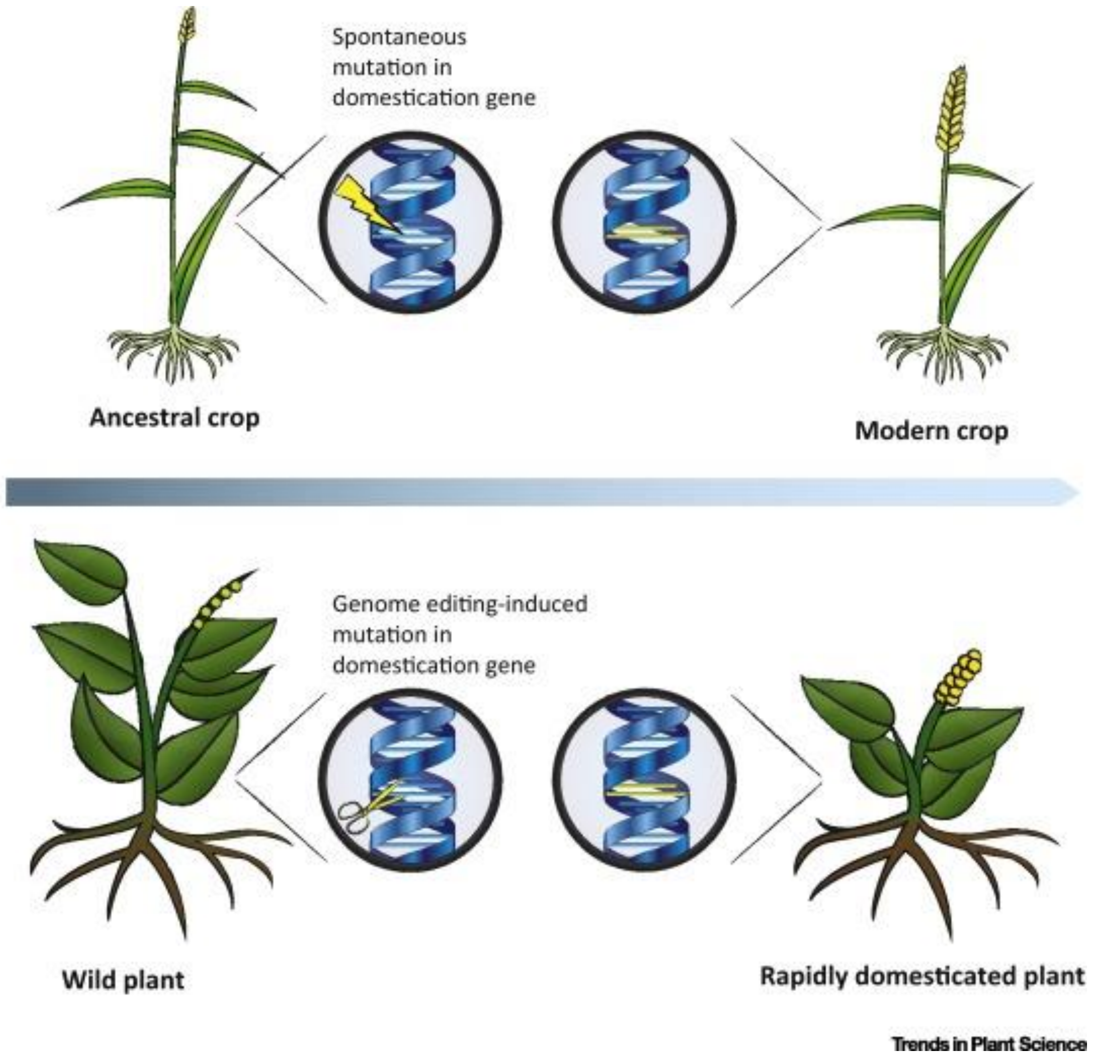
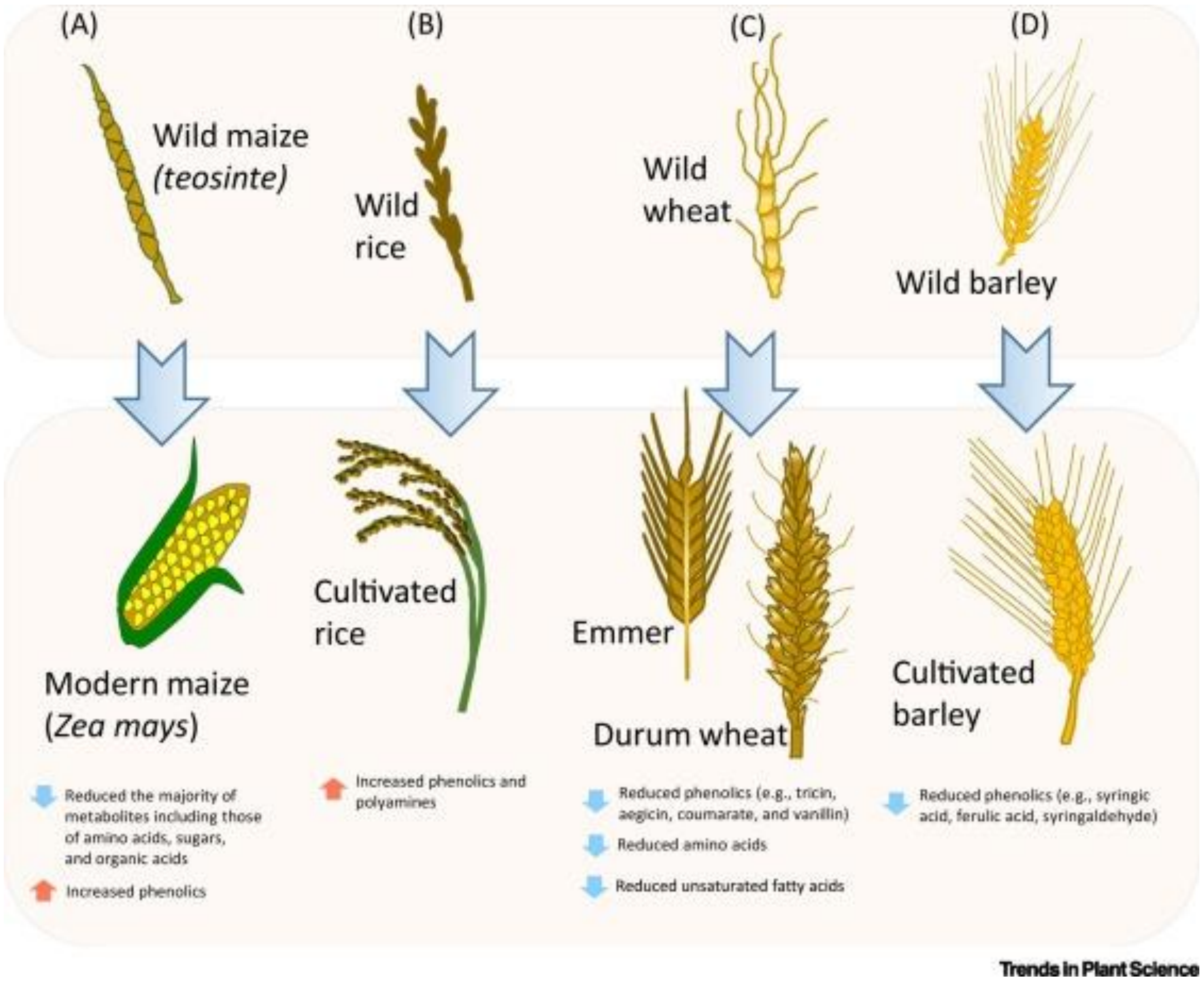
CRISPR technology can accelerate crop domestication.

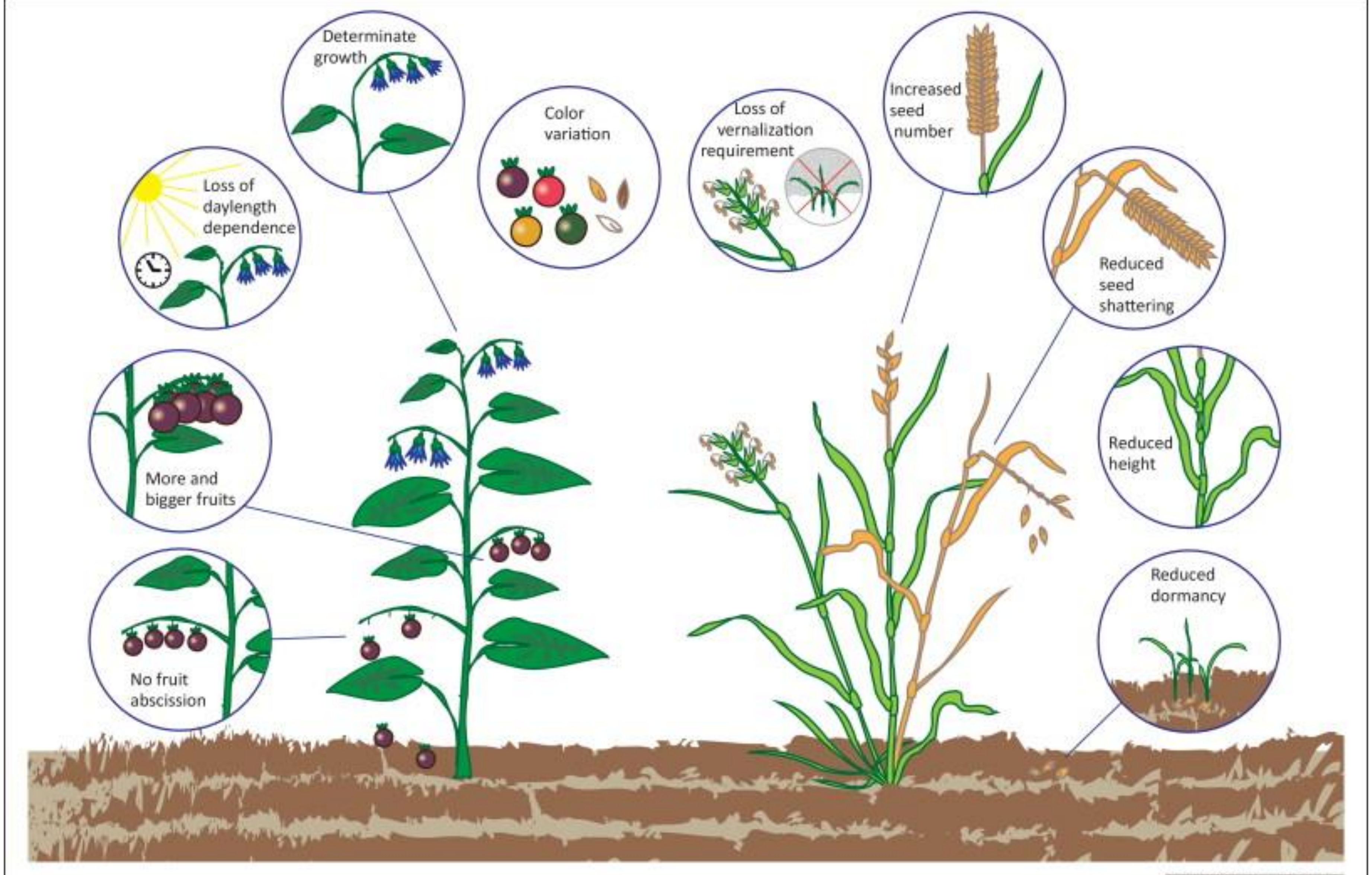
Plant domestication is a time- and labour-intensive process involving altering a plant from its wild state to a new form that can serve **human needs**.



Trends in Plant Science



Trends in Plant Science



TRENDS in Plant Science

- **Thousands of years ago**, ancient farmers initiated the domestication of all major crops, including rice, wheat and maize.
- However, our ancestors used only **a limited number of progenitor species** during the domestication process, and simply selected plants with improved traits such as **high yield and ease of breeding, culture, harvest and storage, resulting** in the **loss of genetic diversity and reduced nutritional value and taste** of our current food crops.
- Increasing current crop diversity is one of the most powerful approaches for promoting sustainable agricultural systems, and the **domestication of neglected, semi-domesticated or wild crops** would increase such diversity.

Solanum pimpinellifolium, which is remarkably stress tolerant but is defective in terms of fruit production

Considera questi articoli disponibili

SEMI POMODORO LYCOPERSICON PIMPINELLIFOLIUM (POMODORO RIB...

★★★★☆ 1

€5^{00}

SOLANUM PIMPINELLIFOLIUM - POMODORO RIBES, 25 SEMI

Marca: semiraridalmondo

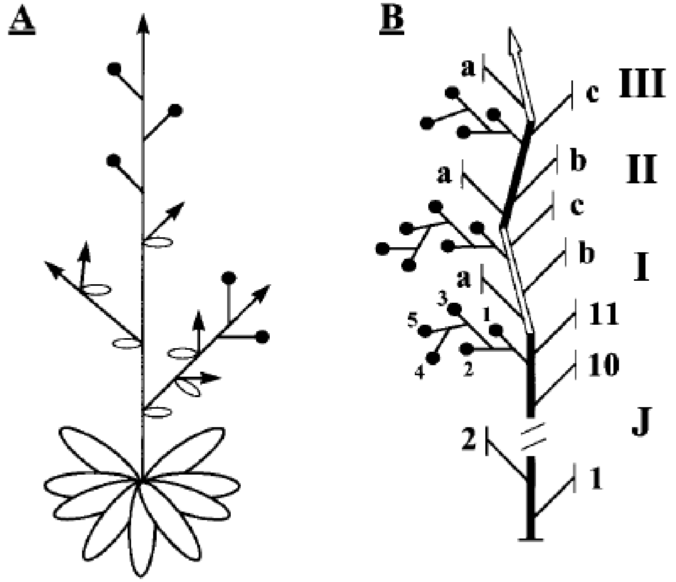
nature biotechnology

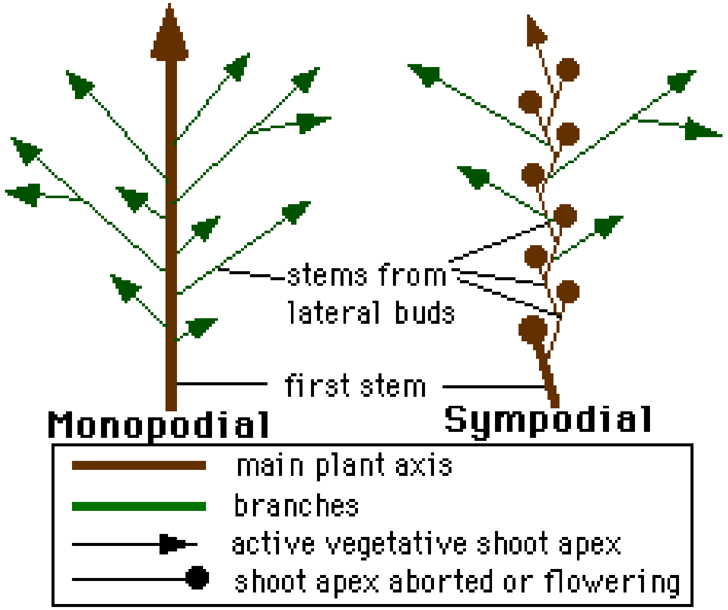
De novo domestication of wild tomato using genome editing

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

- In one study, six loci that are important for yield and productivity were targeted, and the engineered lines displayed increased fruit size, fruit number and fruit lycopene accumulation
- In tomato, at least six loci important for key domestication traits have been identified: general plant growth habit (SELFPRUNING), fruit shape (OVATE) and size (FASCIATED and FRUIT WEIGHT 2.2), fruit number (MULTIFLORA), and nutritional quality (LYCOPENE BETA CYCLASE)

general plant growth habit (SELFPRUNING)

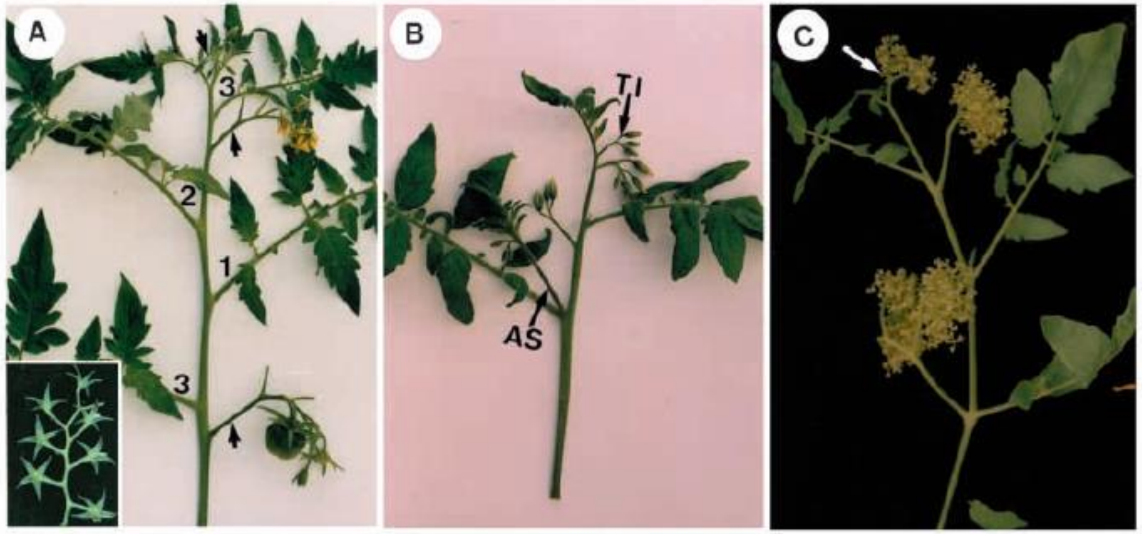




Shoot architecture of Arabidopsis and tomato. (A) Monopodial organization of Arabidopsis shoots. The indeterminate vegetative apex generates leaves on its flanks before changing to an indeterminate floral apex that extends indefinitely (arrow) as flowers are now generated in succession upon its flanks. Side arrows indicate coflorescences arising in the axils of cauline leaves and black circles represent solitary flowers. (B) Sympodial organization of tomato shoots. The primary vegetative shoot (J, leaves 1-11 in this example) is terminated by a flower. Subsequently, a vegetative shoot arises in the axil of the leaf just below the inflorescence. This first sympodial segment unites with the basal part of the leaf that subtends it thus placing it above the inflorescence and in addition displacing the inflorescence sideways. Reiterated units consisting of three nodal leaves (a, b, c in sympodial sections I and II) and a terminal inflorescence, are then generated indefinitely. New flowers (black circles) arise successively to the side of each earlier arising flower in a zig-zag pattern to generate the scorpioid inflorescence.

general plant growth habit (SELFPRUNING)

- Wild tomatoes display indeterminate growth, resulting from a sequential addition of modules (sympodial units) formed by three leaves and an inflorescence.
- spontaneous recessive mutant with a compact, bushy growth habit
- a single-nucleotide substitution in the SELF-PRUNING (SP) gene
- Breeding the SP mutation into industrial tomato cultivars was instrumental in the advent of mechanical harvest

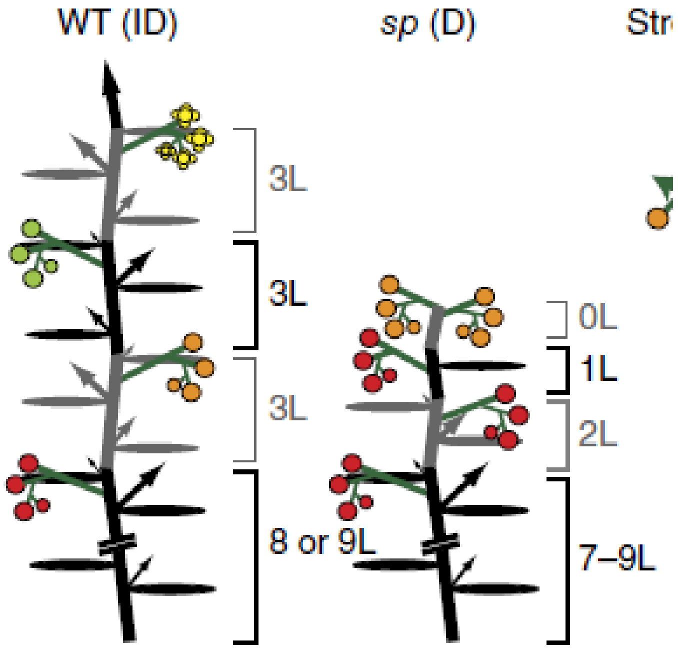


(A) Indeterminate (*SP*) shoot
(B) 'Determinate' (sp/sp) shoot. Only one nodal leaf separates the first two inflorescences
(C) Shoot of an sp double mutant

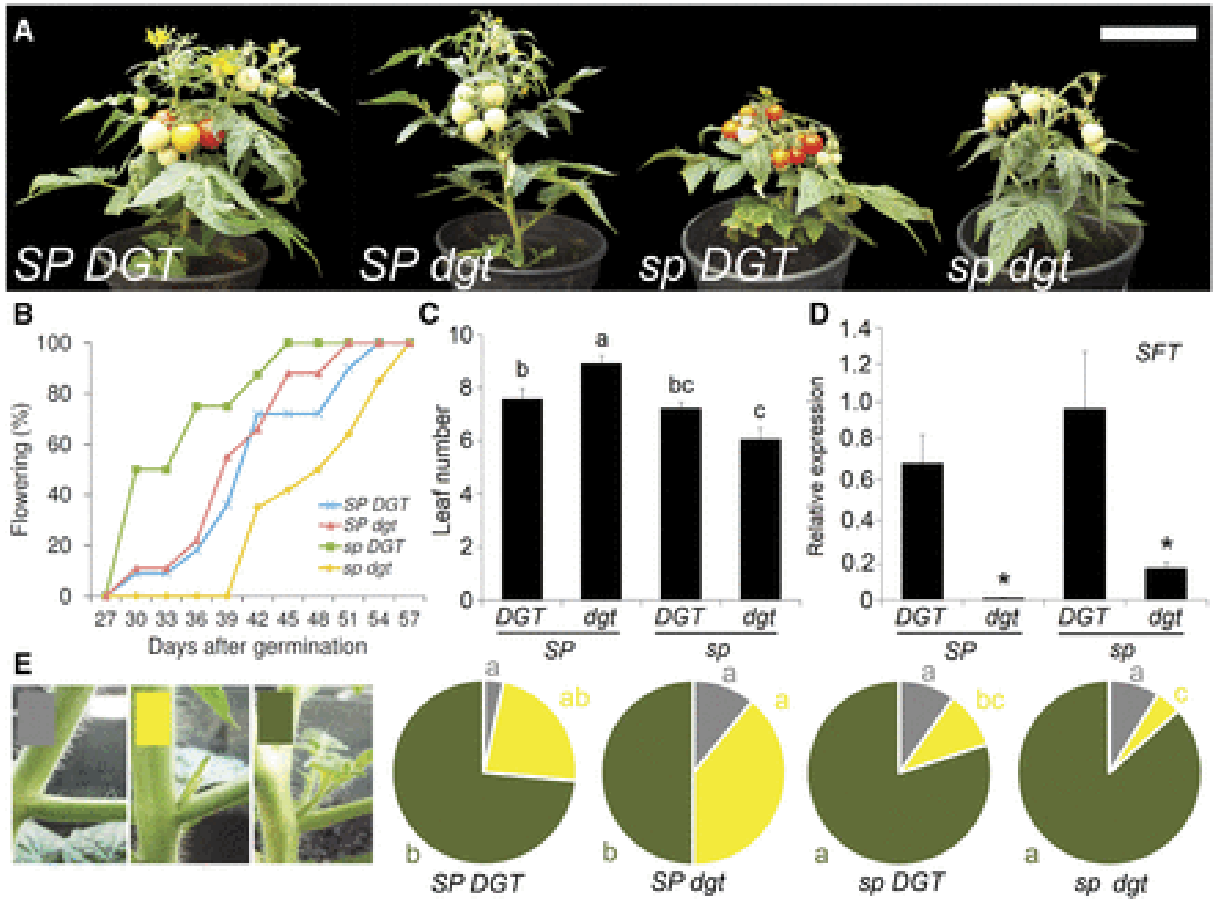
nature genetics

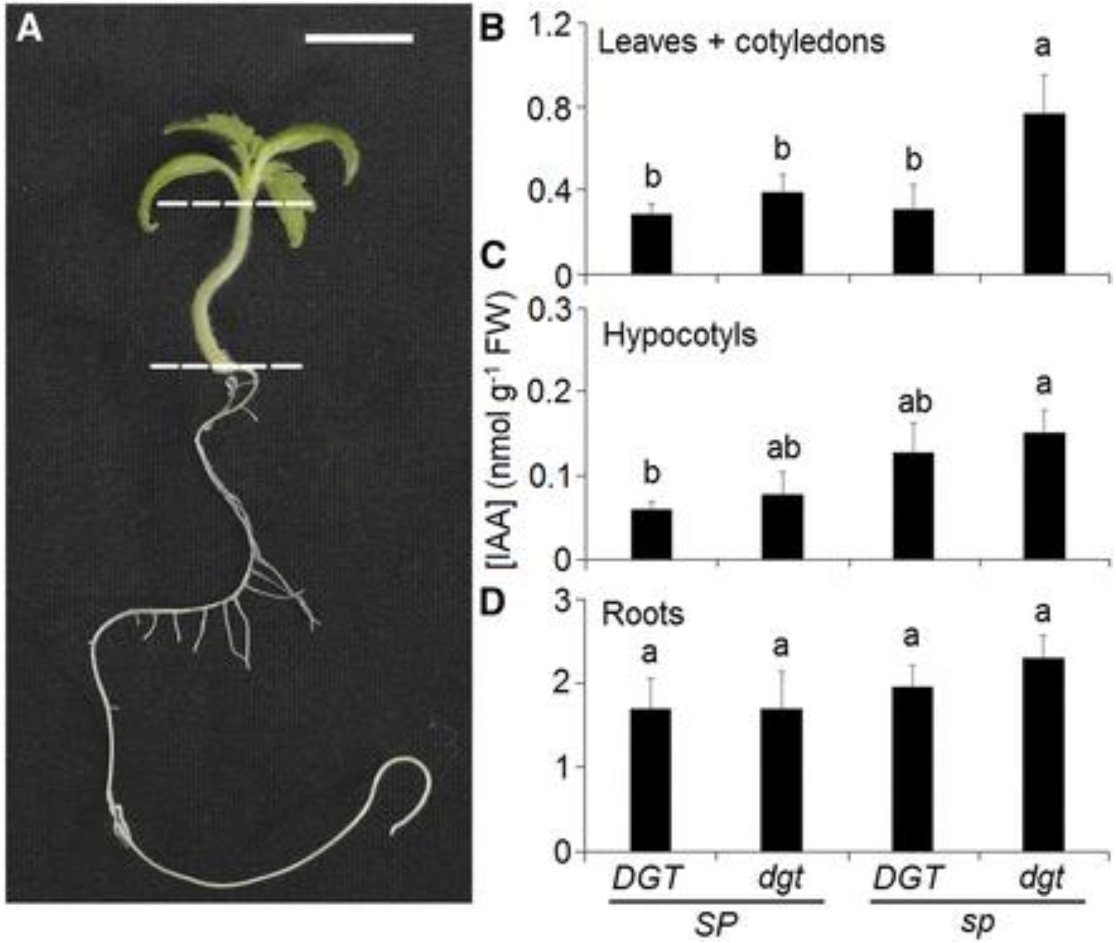
Optimization of crop productivity in tomato using induced mutations in the florigen pathway

Soon Ju Park[1], Ke Jiang[1], Lior Tal[2], Yoav Yichie[3], Oron Gar[3], Dani Zamir[3], Yuval Eshed[2] & Zachary B Lippman[1]



Auxins are involved





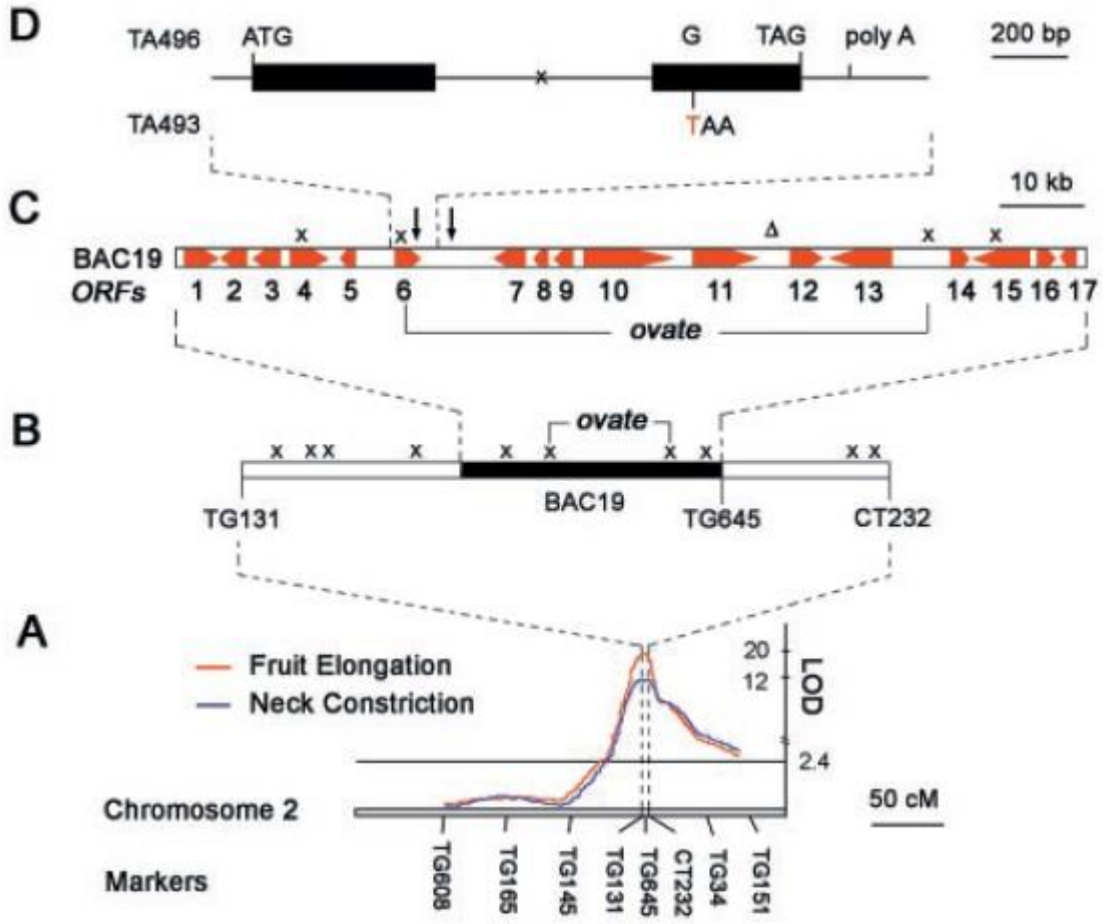
Dgt gene encodes a component of a specific auxin signaling pathway.

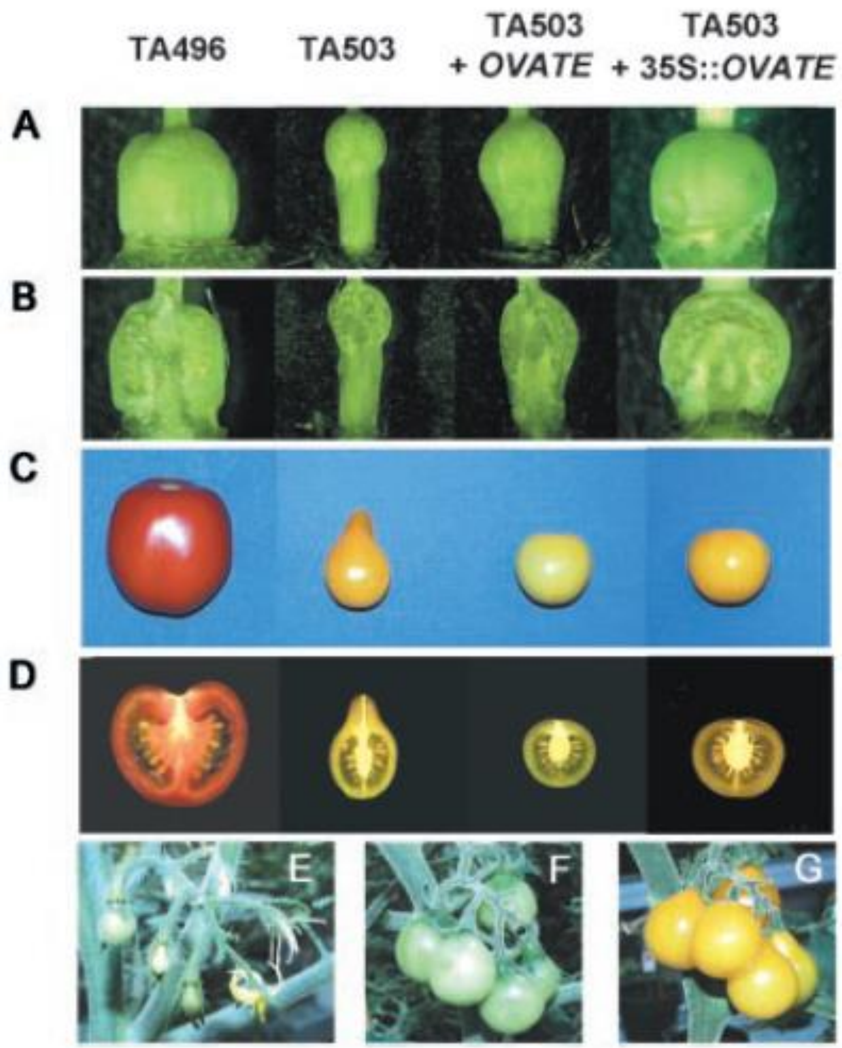
- A major quantitative trait locus (termed ovate) controlling the transition from round to pear-shaped fruit has been cloned from tomato.

A new class of regulatory genes underlying the cause of pear-shaped tomato fruit

Jiping Liu*, Joyce Van Eck†, Bin Cong*, and Steven D. Tanksley*‡

*Departments of Plant Breeding and Plant Biology, Cornell University, Ithaca, NY 14853; and †Boyce Thompson Institute for Plant Research, Tower Road, Ithaca, NY 14853





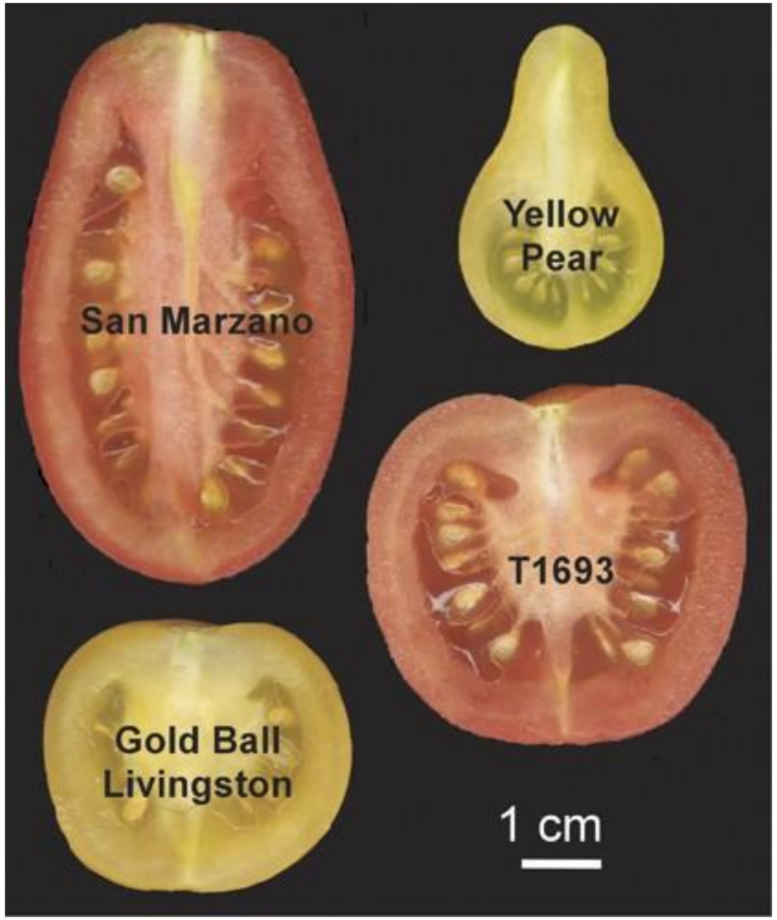
Heredity (2013) 111, 256–264

www.nature.com/hdy

ORIGINAL ARTICLE

Mapping of two suppressors of *OVATE* (*sov*) loci in tomato

GR Rodríguez[1], HJ Kim and E van der Knaap



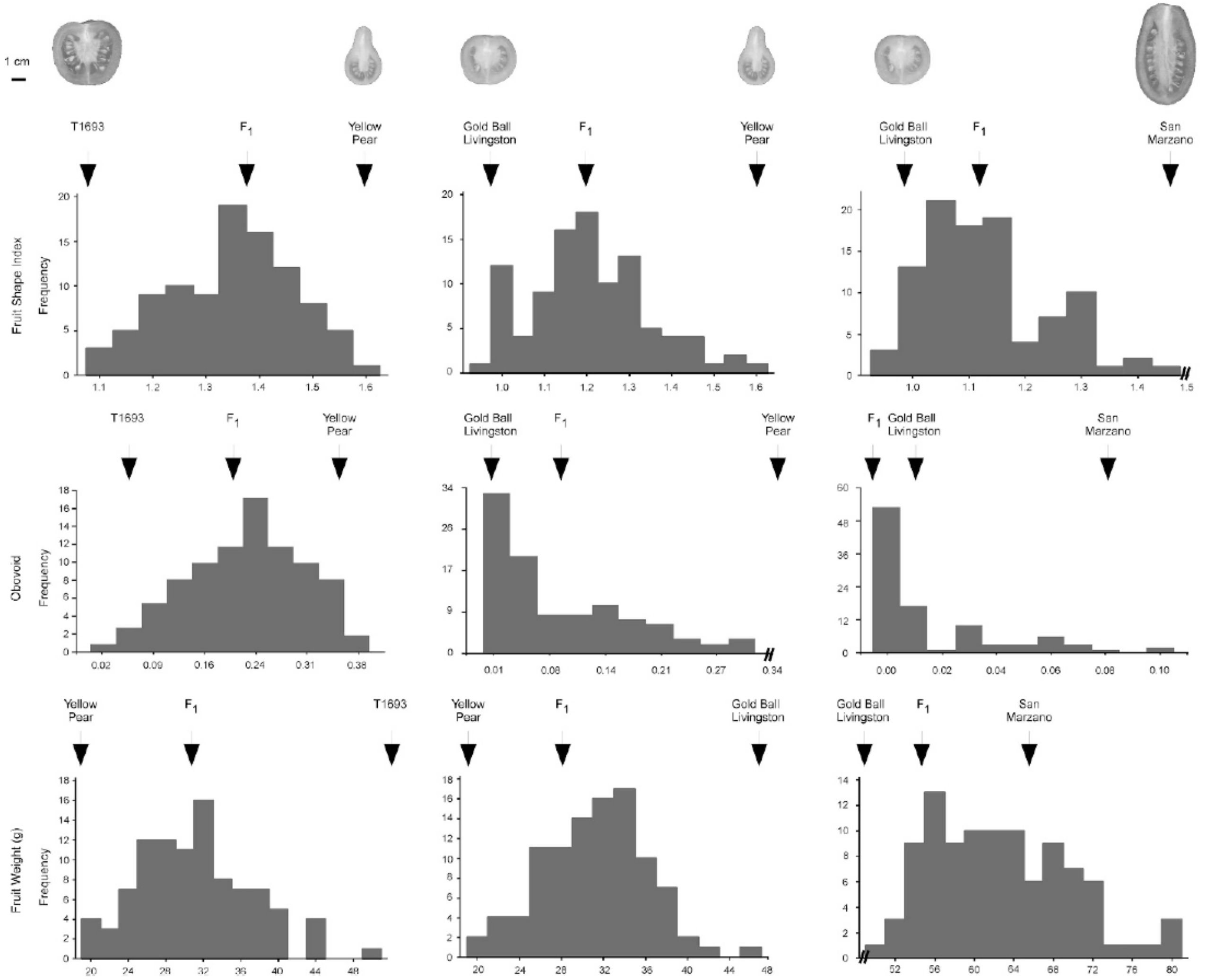


Figure 2 Phenotype distribution for fruit shape index, obovoid and fruit weight in three F_2 analyzed populations. The mean values of the parental genotypes and their F_1 are indicated by arrows.

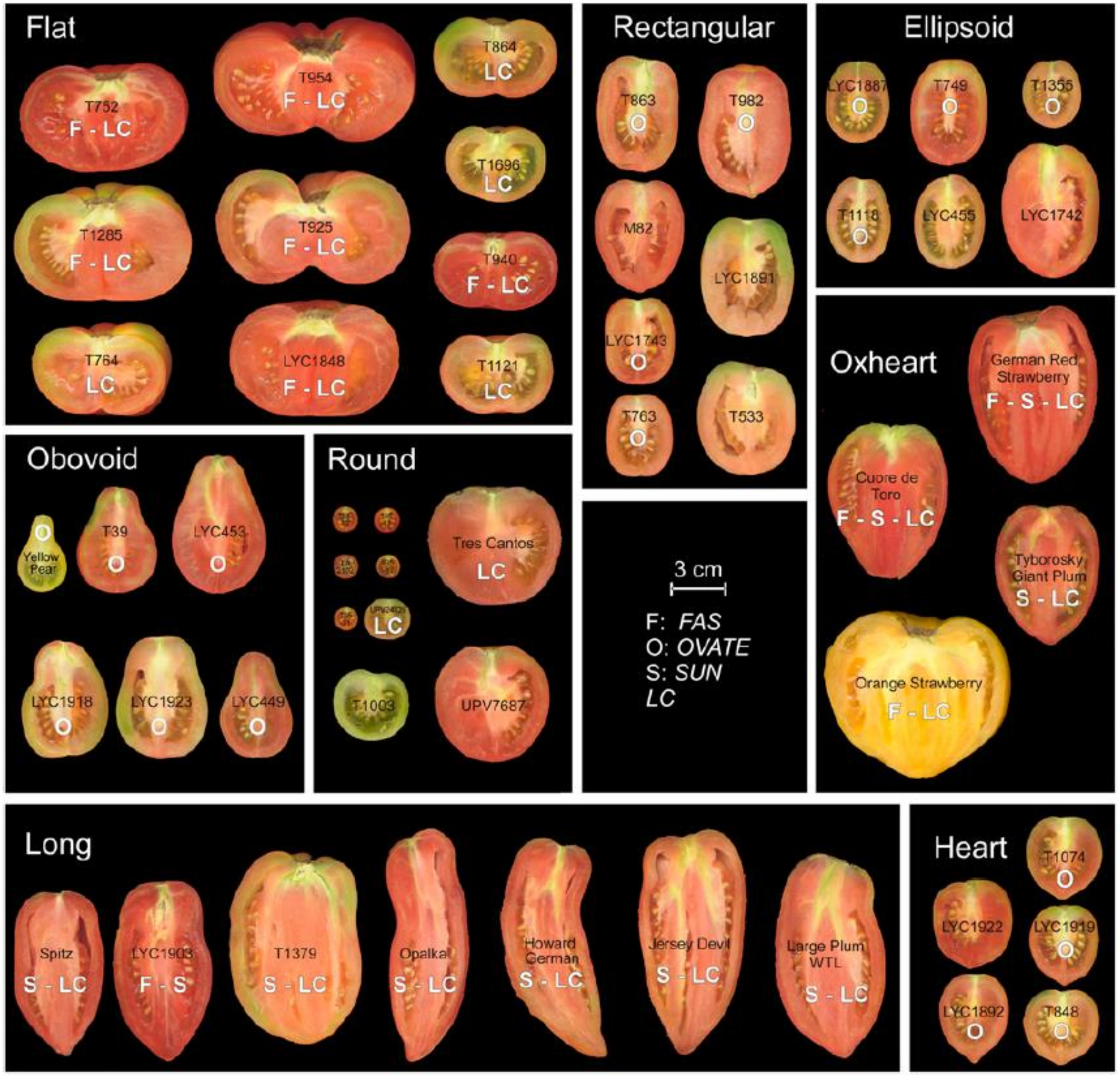
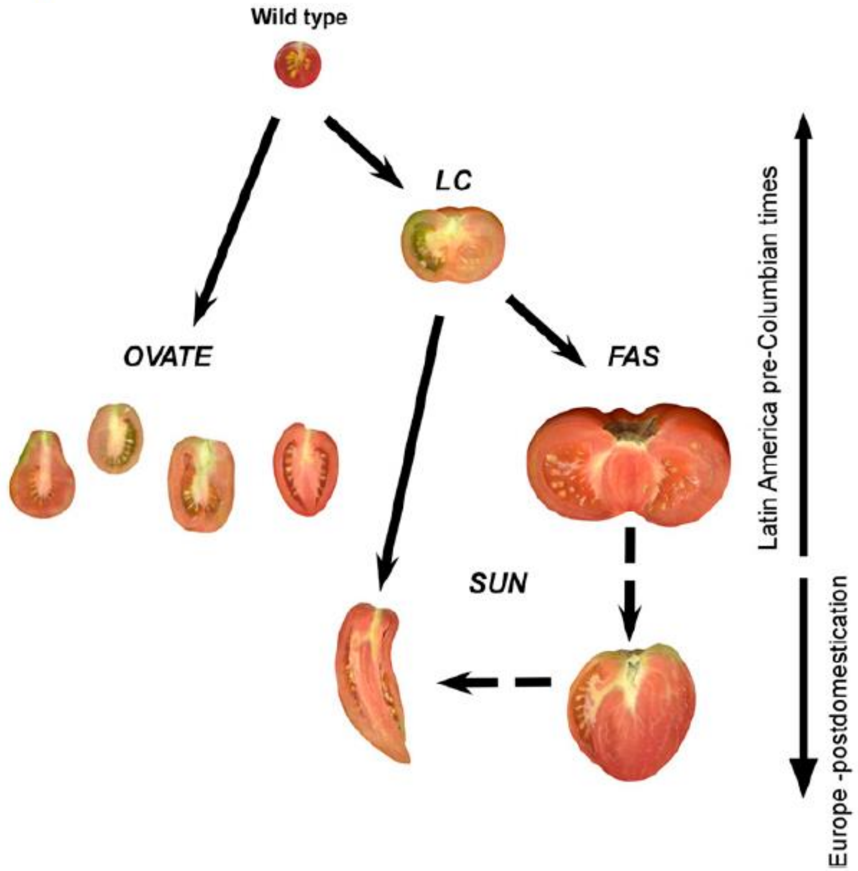


Figure 1. Tomato fruit shape categories adapted from UPOV (2001) and IPGRI (1996). Each fruit is identified by variety name (information available at http://solgenomics.net/) and presence of mutation in the *SUN*, *OVATE*, *LC*, and/or *FAS* genes (abbreviated as S, O, LC, and F, respectively).



SUN and OVATE control elongated shape whereas FASCIATED (FAS) and LOCULE NUMBER (LC) control fruit locule number and flat shape.

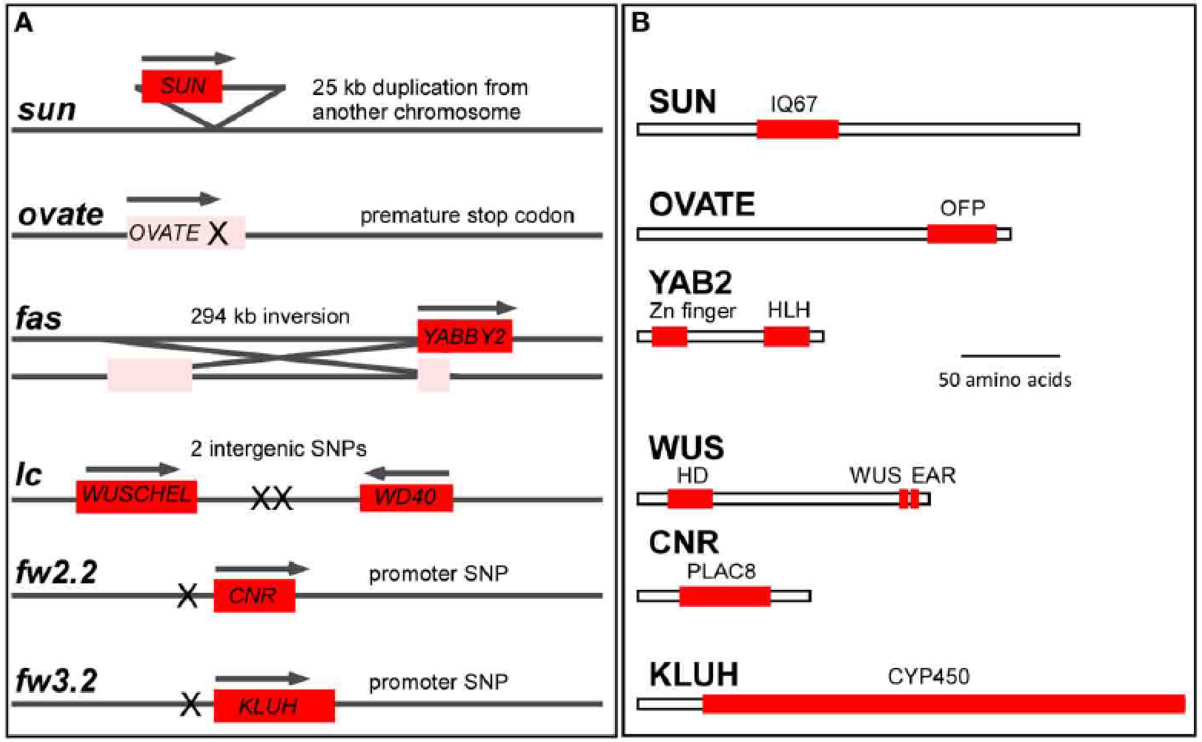
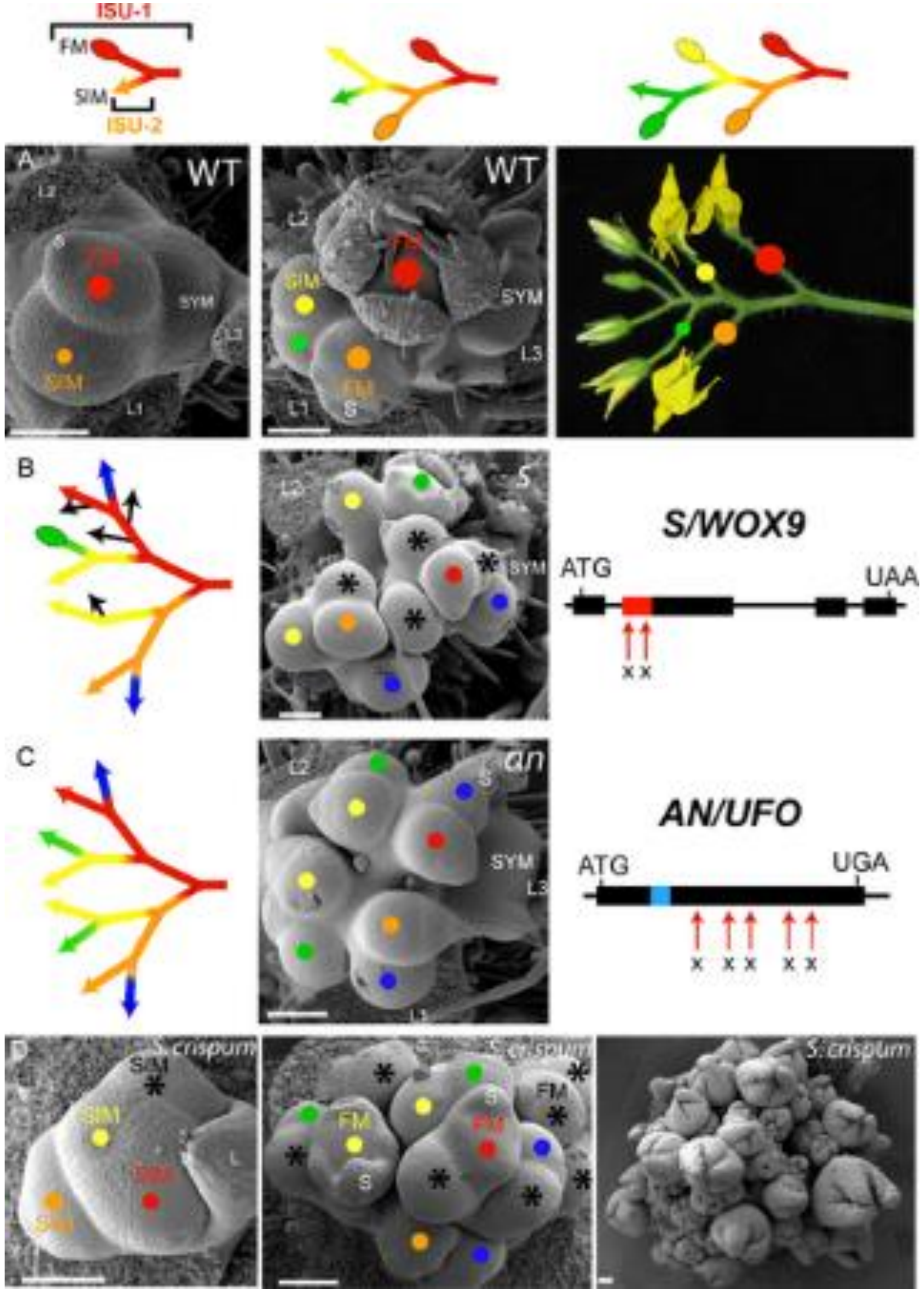


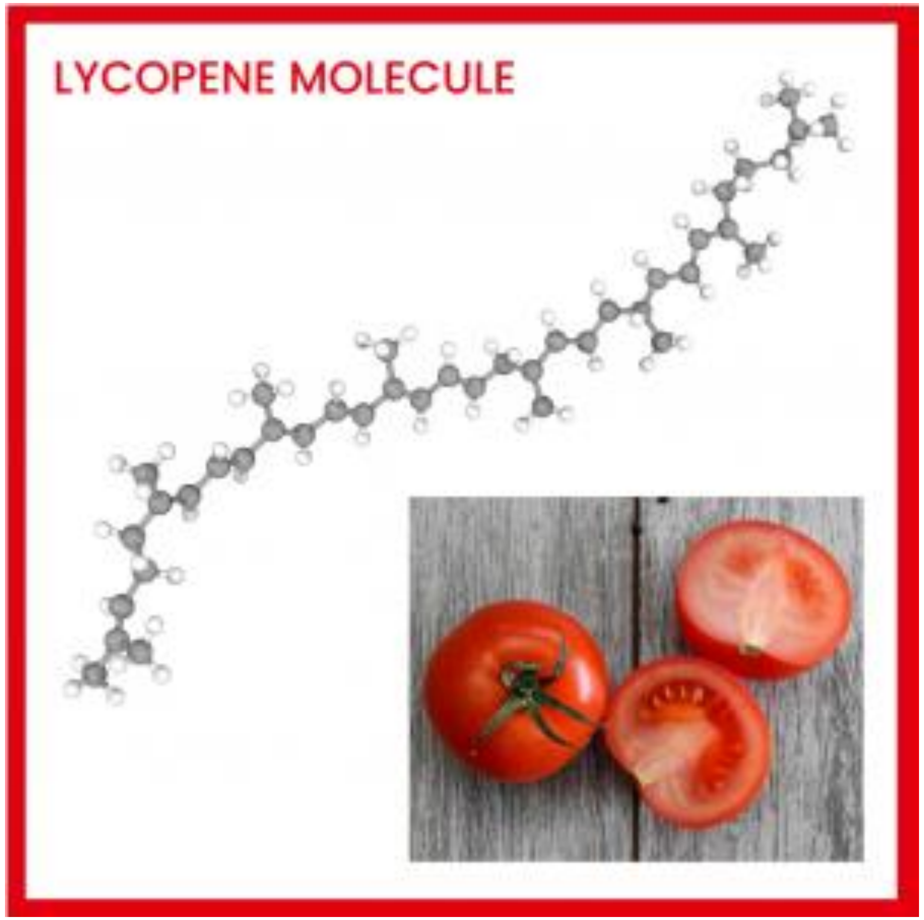
FIGURE 3 | The molecular basis of tomato fruit shape and weight variation. (A) Genome structure of the fruit shape and weight loci and the underlying mutations. Red box indicates the coding region of a functional gene whose regulation is altered by the mutation (denoted by X). Pink indicates a loss-of-function mutation of the gene. The size of the loci are not drawn to scale. **(B)** Protein features of the fruit shape and weight proteins. The box represents the coding region. The most important domains are listed as red boxes. IQ67, CaM binding domain of 67 amino acid and containing IQ; OFP, Ovate Family Protein motif of unknown function; HLH, YABBY type of DNA binding domain featuring a helix-loop-helix structure; HD, DNA binding homeodomain of the helix-loop-helix-turn-helix structure; WUS, essential for proper functioning of WUSCHEL; EAR, transcriptional repressor function; PLAC8, similarity to the placenta-specific gene 8 protein; CYP450, cytochrome P450. Size bar = 50 amino acids.

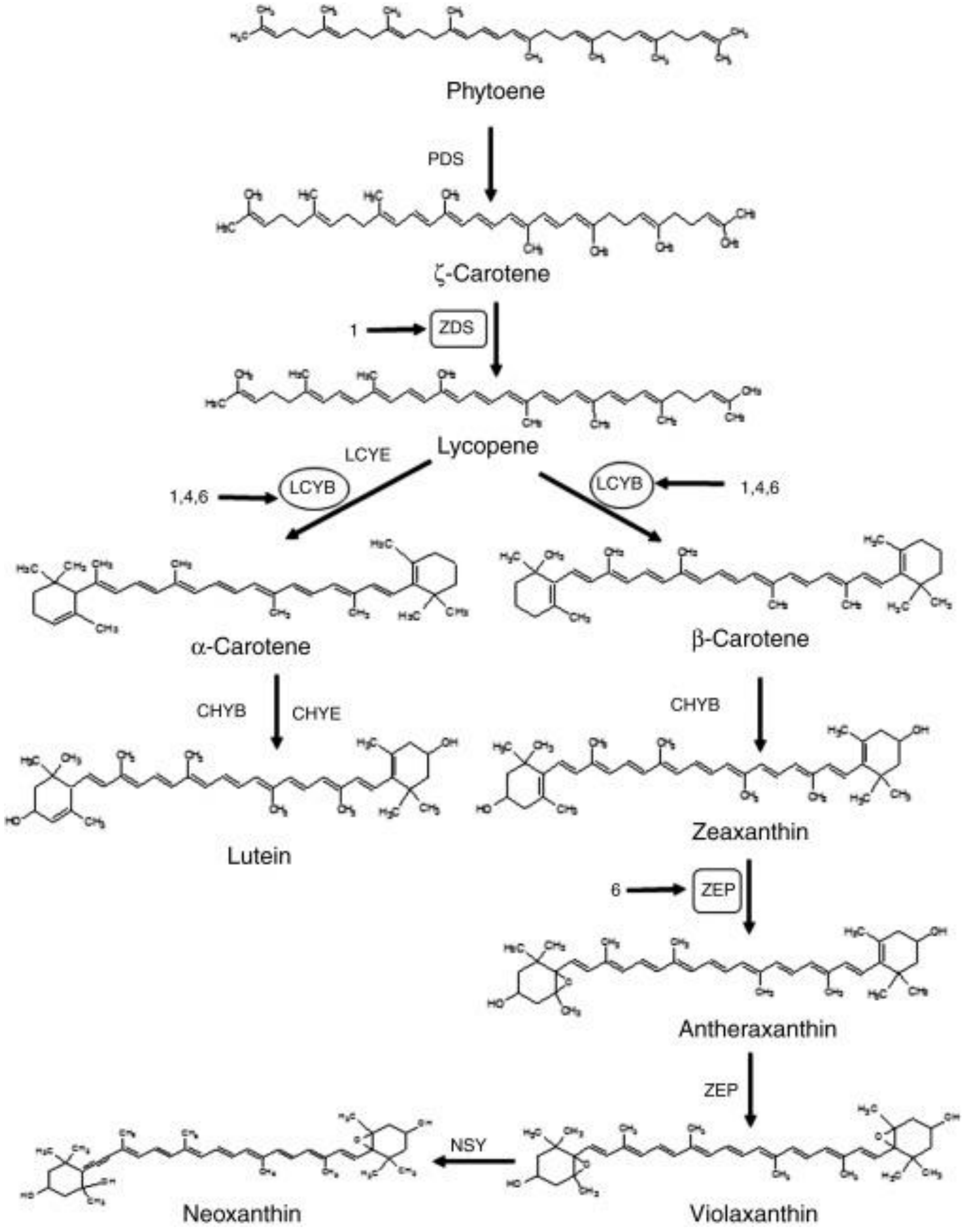
fruit number (MULTIFLORA)

- Gene function: WUSCHEL-homeobox (WOX) transcription factor; homologue to the AtWOX9/STYP gene Gene effect: plants with the mutated allele delay the differentiation of inflorescence meristem into flower meristem.

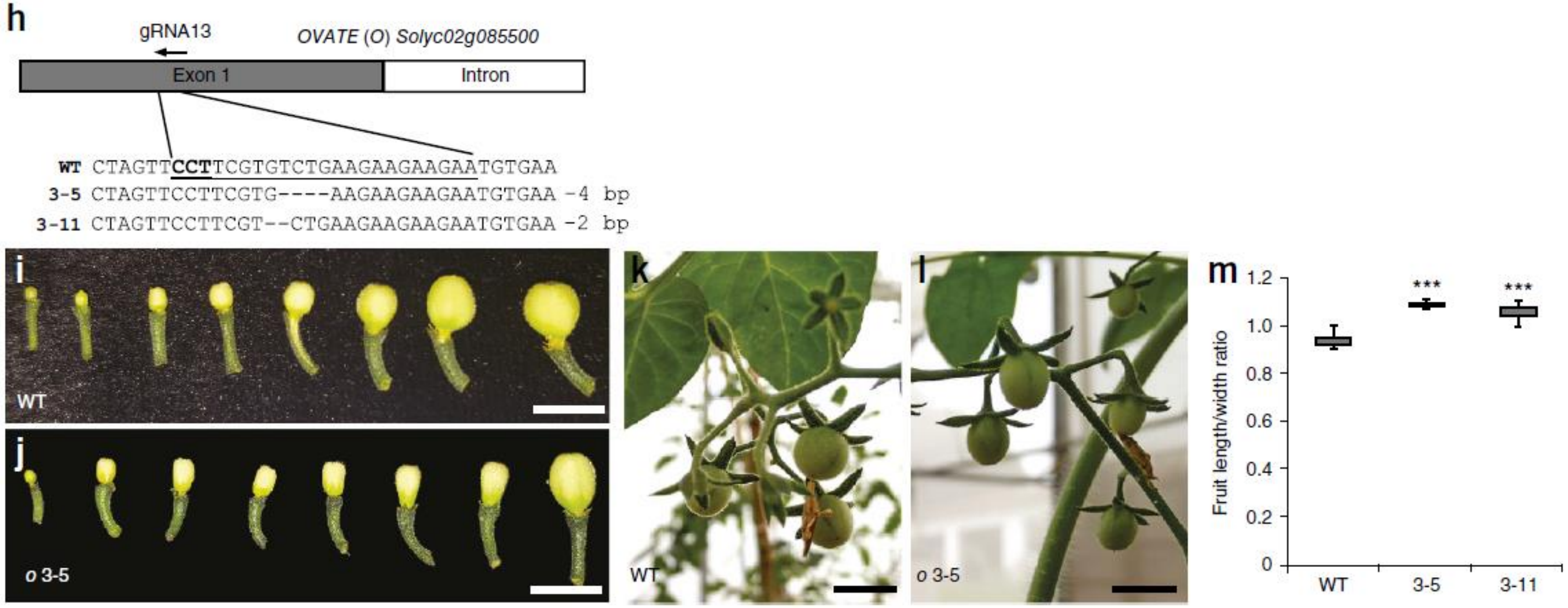


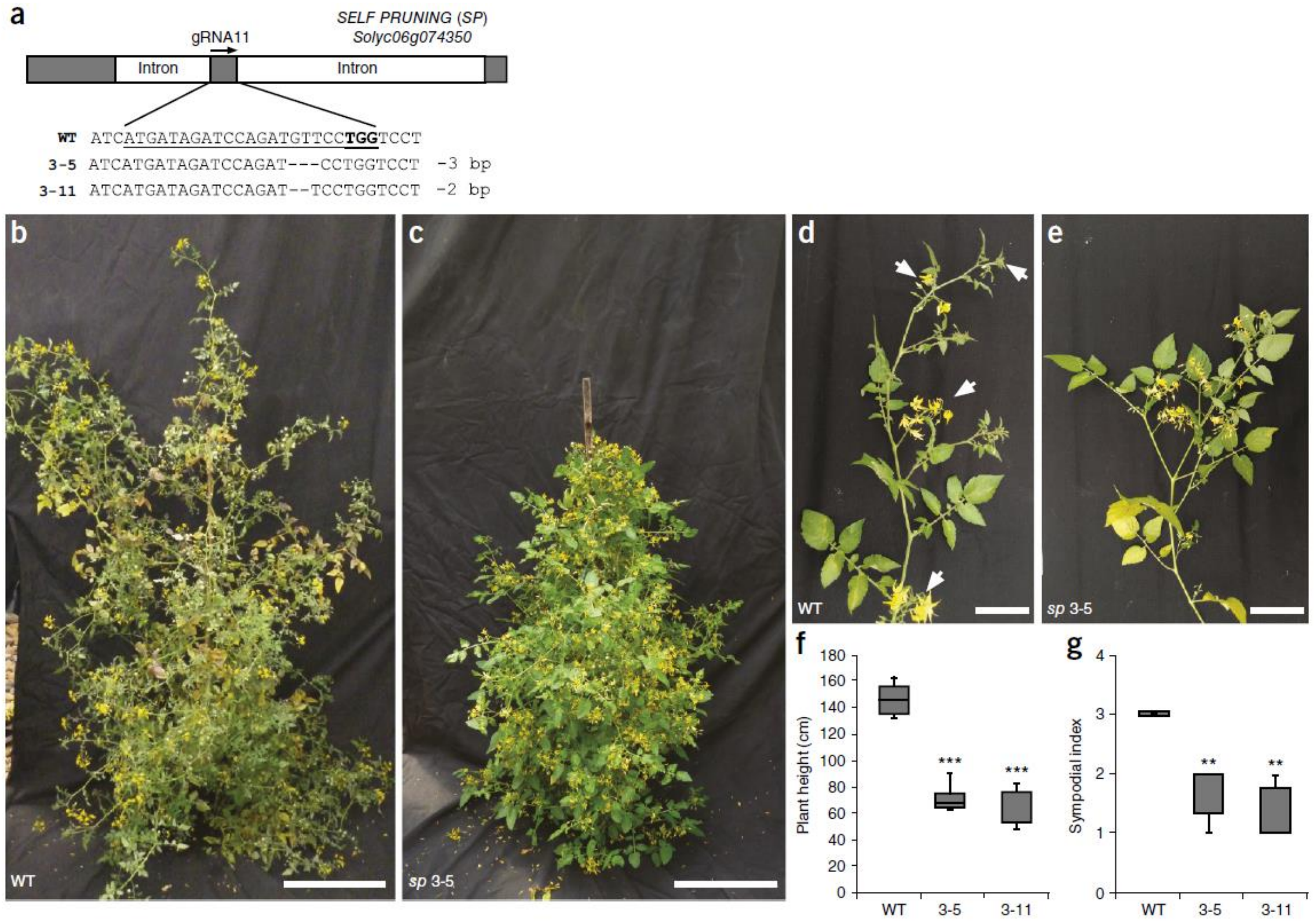
LYCOPENE BETA CYCLASE

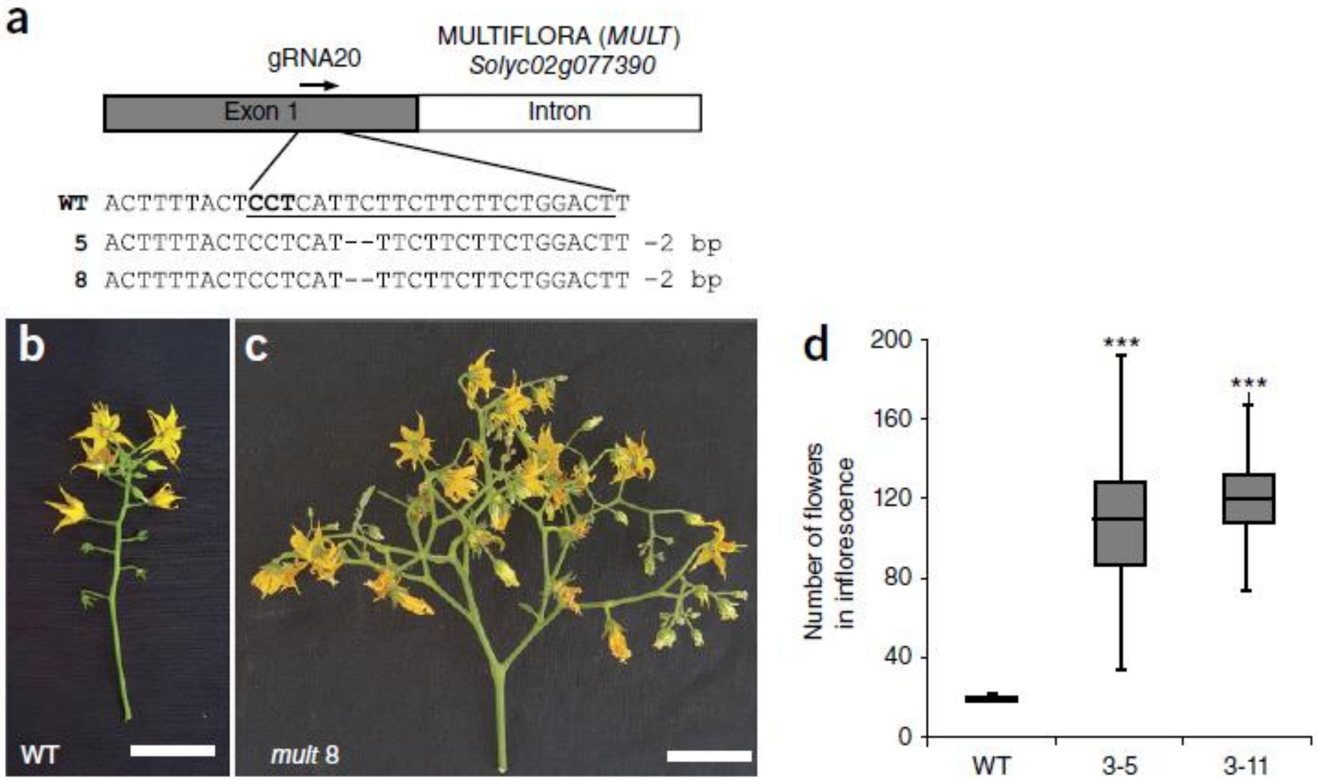




- CRISPR–Cas9 approach to generate loss-of-function alleles.
- constructed a single CRISPR–Cas9 plant transformation vector, pTC321, which harbored six single guide RNAs (gRNAs) targeting specific sequences in the coding regions of all six genes

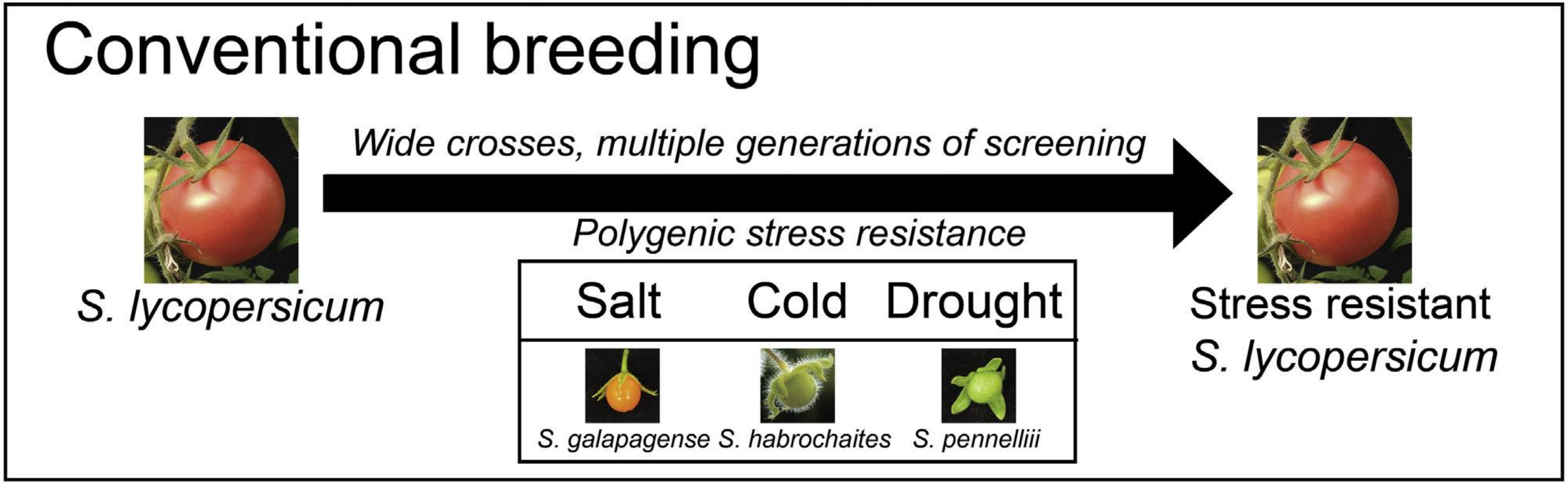



a
SELF PRUNING (SP)
Solyc06g074350
gRNA11
Intron
Intron
WT ATCATGATAGATCCAGATGTTCCTGGTCCT
3-5 ATCATGATAGATCCAGAT---CCTGGTCCT -3 bp
3-11 ATCATGATAGATCCAGAT--TCCTGGTCCT -2 bp
b
WT
c
sp 3-5
d
WT
e
sp 3-5
f
Plant height (cm)
180
160
140
120
100
80
60
40
20
0
***
***
WT
3-5
3-11
g
Sympodial index
4
3
2
1
0
**
**
WT
3-5
3-11


a
gRNA20
MULTIFLORA (*MULT*)
Solyc02g077390
Exon 1
Intron
WT ACTTTTACT**CCT**CATTCTTCTTCTTCTGGACTT
5 ACTTTTACTCCTCAT--TTCTTCTTCTGGACTT -2 bp
8 ACTTTTACTCCTCAT--TTCTTCTTCTGGACTT -2 bp
b
WT
c
mult 8
d
Number of flowers in inflorescence
200
160
120
80
40
0
***
***
WT
3-5
3-11

- study used CRISPR to modify coding sequences, cis regulatory regions and uORFs of genes associated with day-length sensitivity, shoot architecture, flower/fruit production and ascorbic acid synthesis, and the desirable traits were successfully introduced into wild tomatoes

Conventional breeding



S. *lycopersicum*

Wide crosses, multiple generations of screening

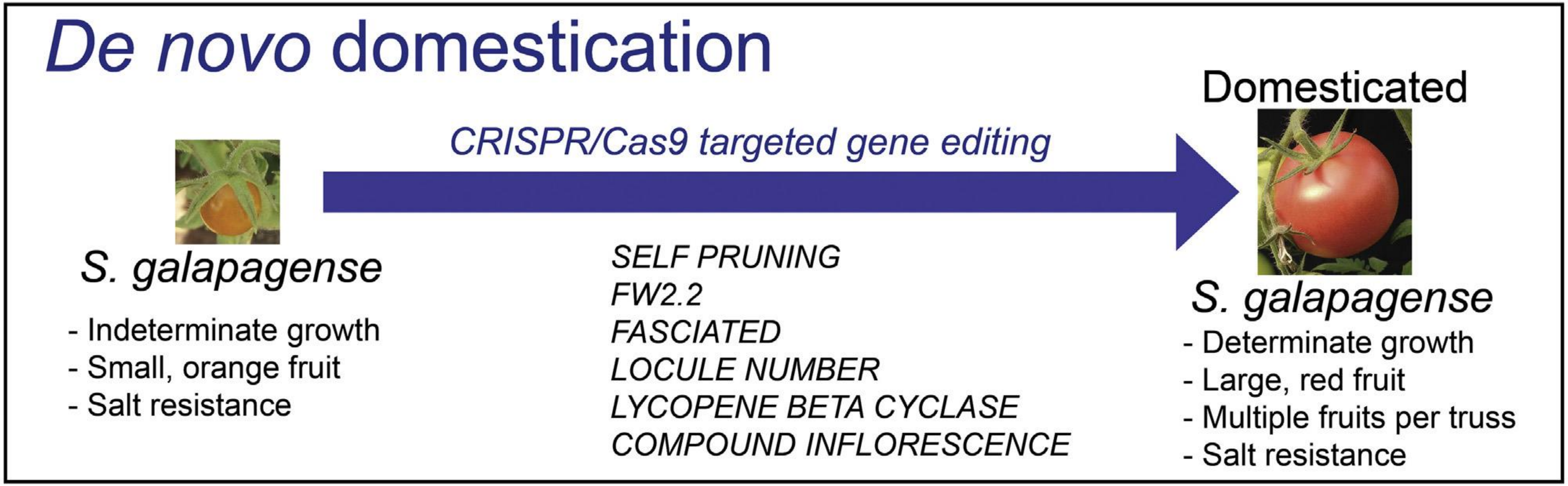
Polygenic stress resistance

Salt	Cold	Drought
S. galapagense	*S. habrochaites*	*S. pennelliii*

Stress resistant

S. lycopersicum

De novo domestication



S. galapagense

- Indeterminate growth
- Small, orange fruit
- Salt resistance

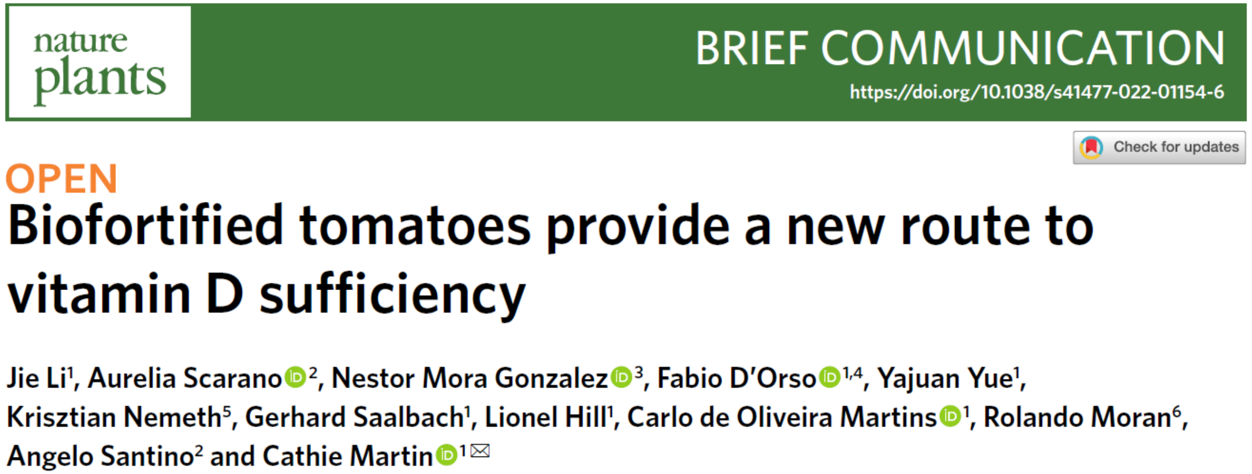
CRISPR/Cas9 targeted gene editing

SELF PRUNING
FW2.2
FASCIATED
LOCULE NUMBER
LYCOPENE BETA CYCLASE
COMPOUND INFLORESCENCE

Domesticated

S. galapagense

- Determinate growth
- Large, red fruit
- Multiple fruits per truss
- Salt resistance



nature plants

BRIEF COMMUNICATION

https://doi.org/10.1038/s41477-022-01154-6

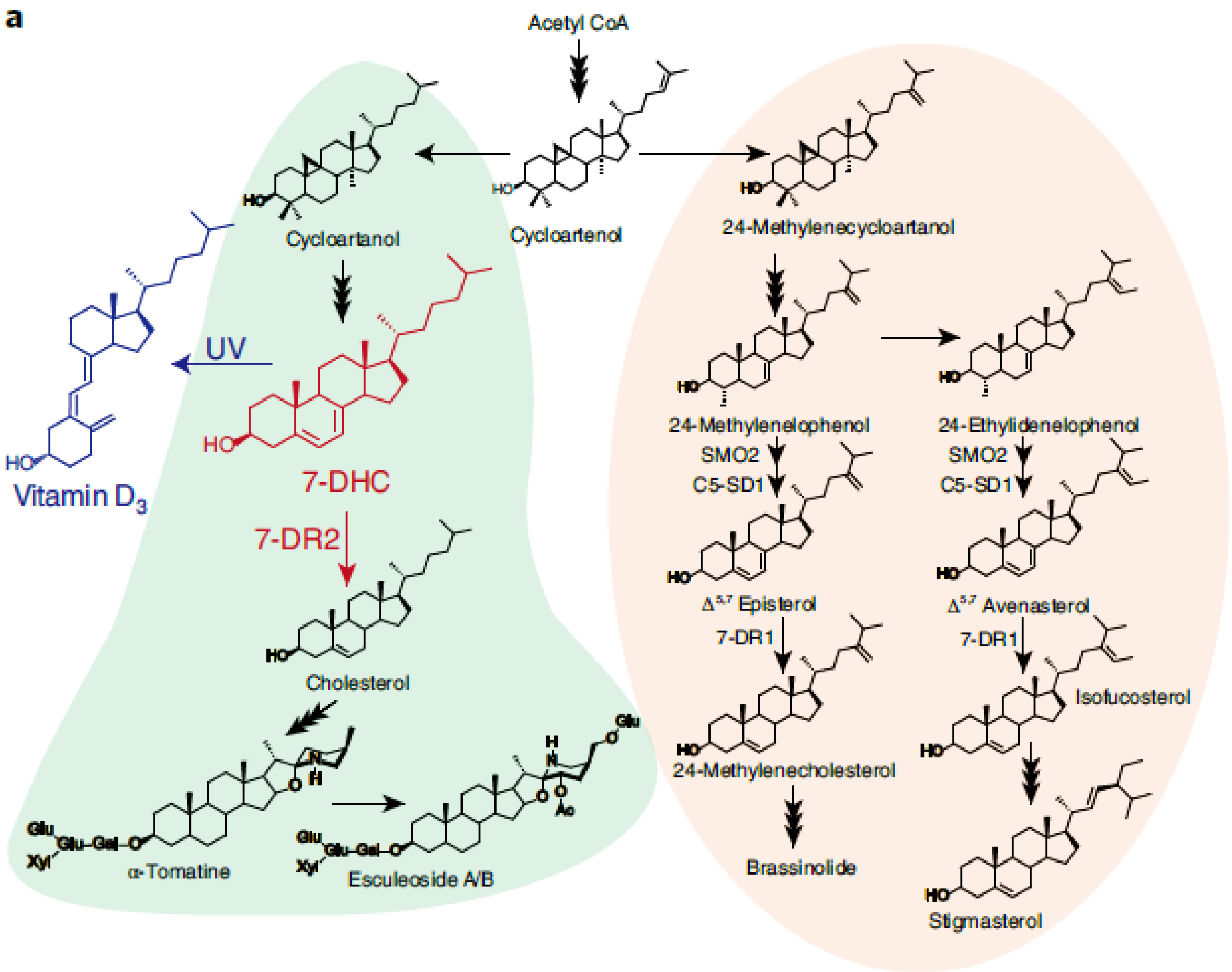
OPEN

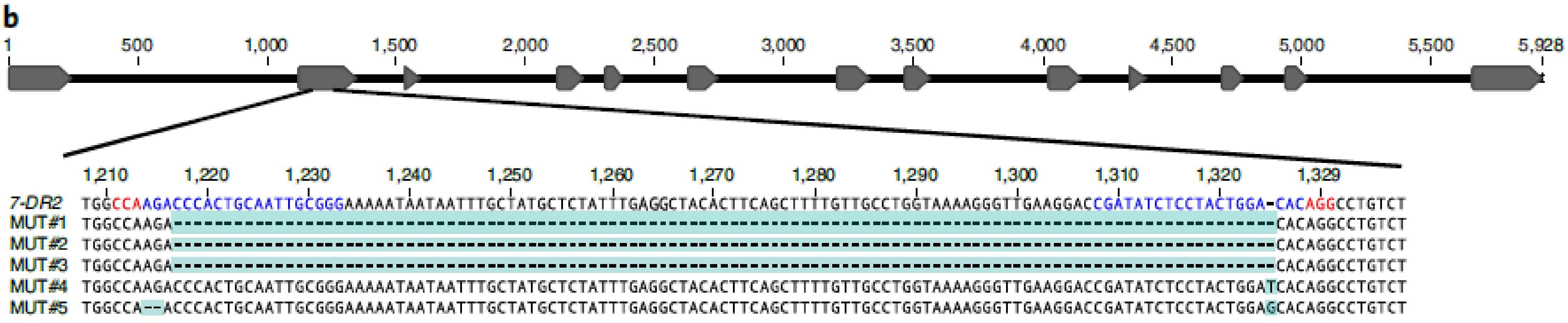
Biofortified tomatoes provide a new route to vitamin D sufficiency

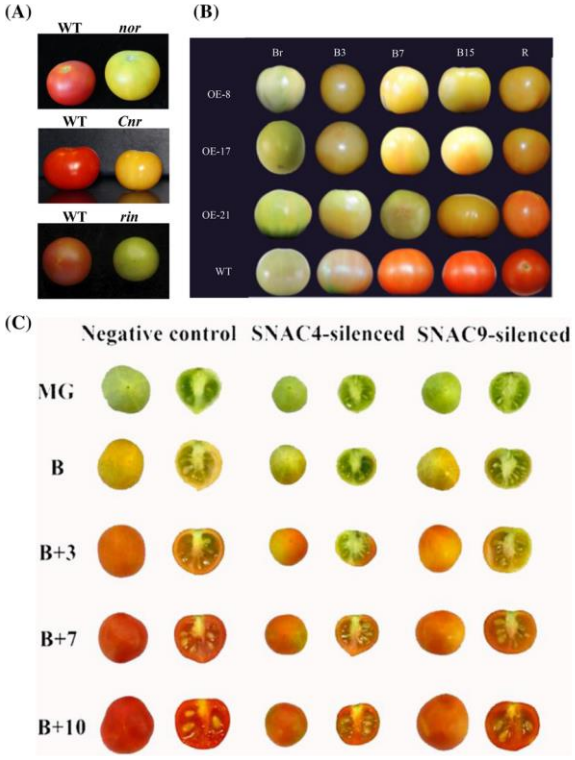
Jie Li[1], Aurelia Scarano[2], Nestor Mora Gonzalez[3], Fabio D'Orso[1,4], Yajuan Yue[1], Krisztian Nemeth[5], Gerhard Saalbach[1], Lionel Hill[1], Carlo de Oliveira Martins[1], Rolando Moran[6], Angelo Santino[2] and Cathie Martin[1] ✉

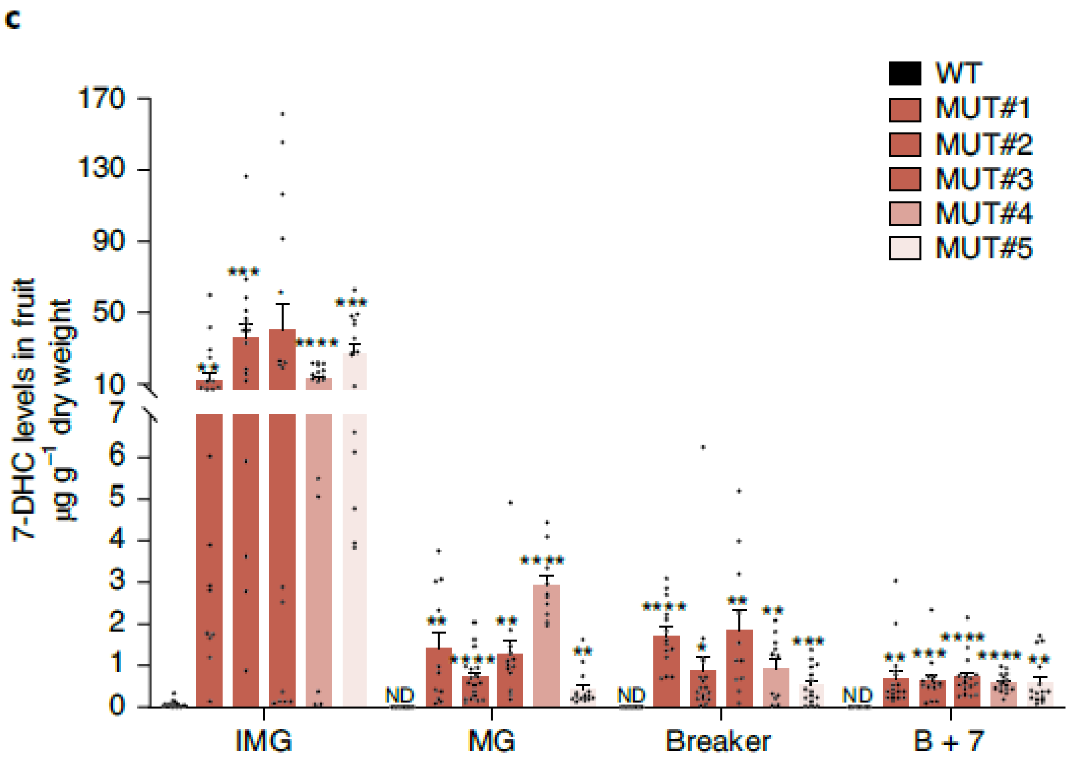
- Poor vitamin D status is a global health problem; insufficiency underpins higher risk of cancer, neurocognitive decline and all-cause mortality. Most foods contain little vitamin D and plants are very poor sources. We have engineered the accumulation of provitamin D3 in tomato by genome editing, modifying a duplicated section of phytosterol biosynthesis in Solanaceous plants, to provide a biofortified food with the added possibility of supplement production from waste material.

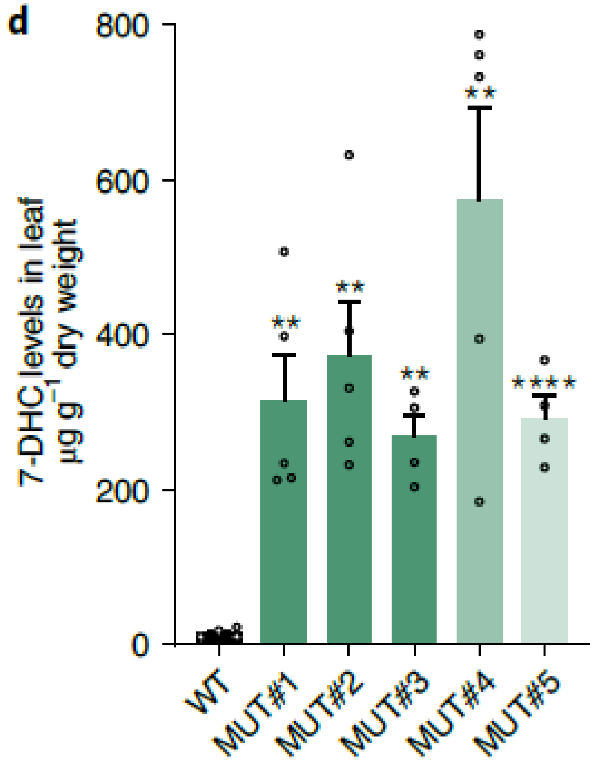
- Vitamin D can be synthesized by humans from 7-dehydrocholesterol (7-DHC), also known as provitamin D3, following exposure of skin to ultraviolet B (UVB) light, but the major source is dietary
- 7-DHC is synthesized by some plants such as tomato, on route to cholesterol and steroidal glycoalkaloid (SGA) synthesis, predominantly in leaves. UVB exposure of leaves of tomato produces vitamin D3



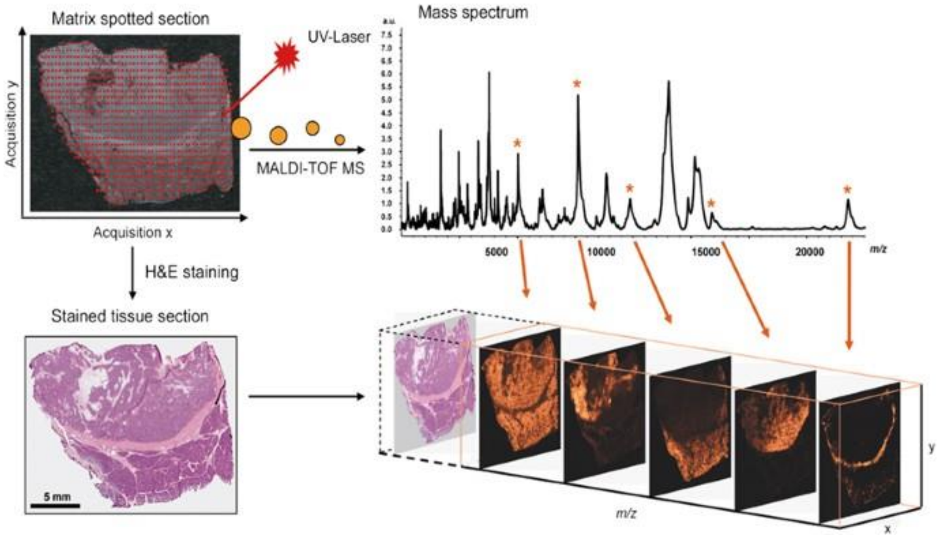

b
1
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
4,500
5,000
5,500
5,928
1,210
1,220
1,230
1,240
1,250
1,260
1,270
1,280
1,290
1,300
1,310
1,320
1,329
7-DR2 TGGCCAAGACCCACTGCAATTGCGGGAAAAATAATAATTTGCTATGCTCTATTTGAGGCTACACTTCAGCTTTTGTTGCCTGGTAAAAGGGTTGAAGGACCGATATCTCCTACTGGA-CACAGGCCTGTCT
MUT#1 TGGCCAAGA---CACAGGCCTGTCT
MUT#2 TGGCCAAGA---CACAGGCCTGTCT
MUT#3 TGGCCAAGA---CACAGGCCTGTCT
MUT#4 TGGCCAAGACCCACTGCAATTGCGGGAAAAATAATAATTTGCTATGCTCTATTTGAGGCTACACTTCAGCTTTTGTTGCCTGGTAAAAGGGTTGAAGGACCGATATCTCCTACTGGATCACAGGCCTGTCT
MUT#5 TGGCCA--ACCCACTGCAATTGCGGGAAAAATAATAATTTGCTATGCTCTATTTGAGGCTACACTTCAGCTTTTGTTGCCTGGTAAAAGGGTTGAAGGACCGATATCTCCTACTGGAGCACAGGCCTGTCT

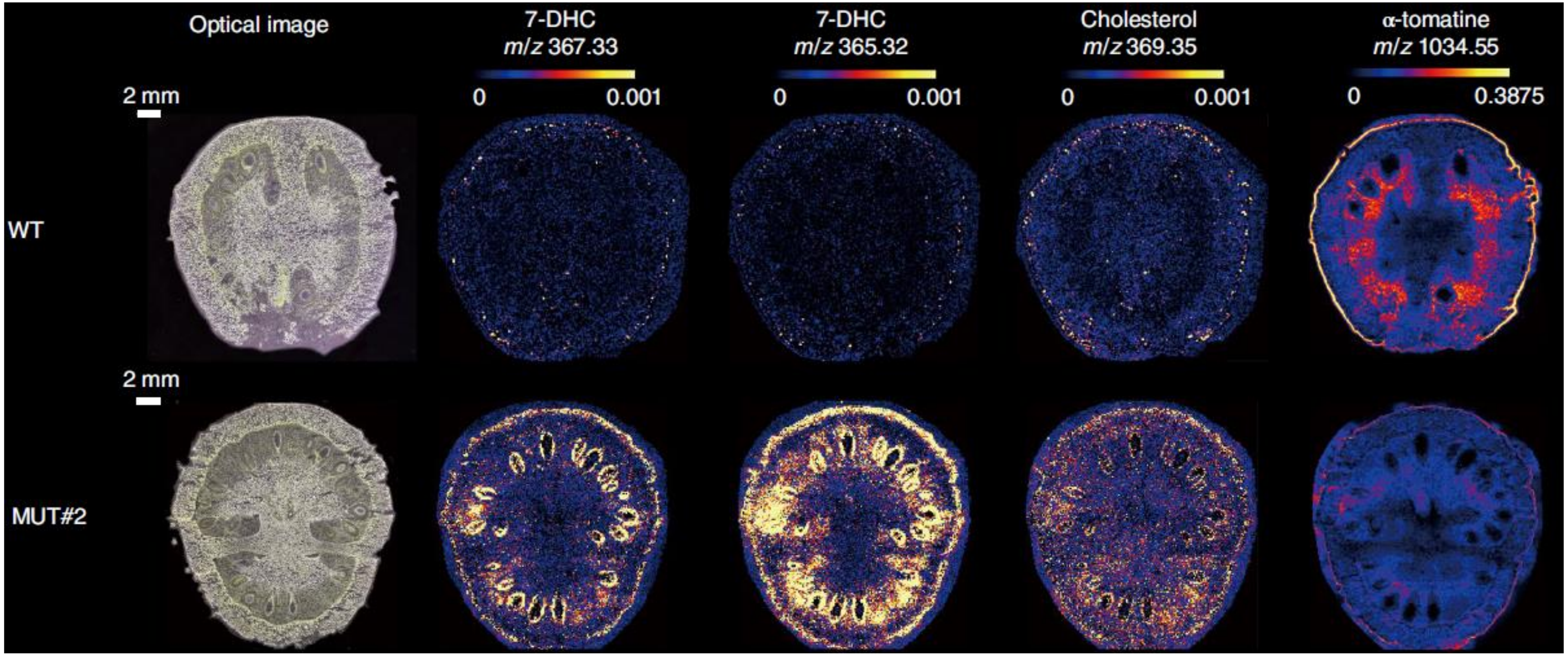

(A)
WT
nor
WT
Cnr
WT
rin
(B)
Br
B3
B7
B15
R
OE-8
OE-17
OE-21
WT
(C)
Negative control
SNAC4-silenced
SNAC9-silenced
MG
B
B+3
B+7
B+10

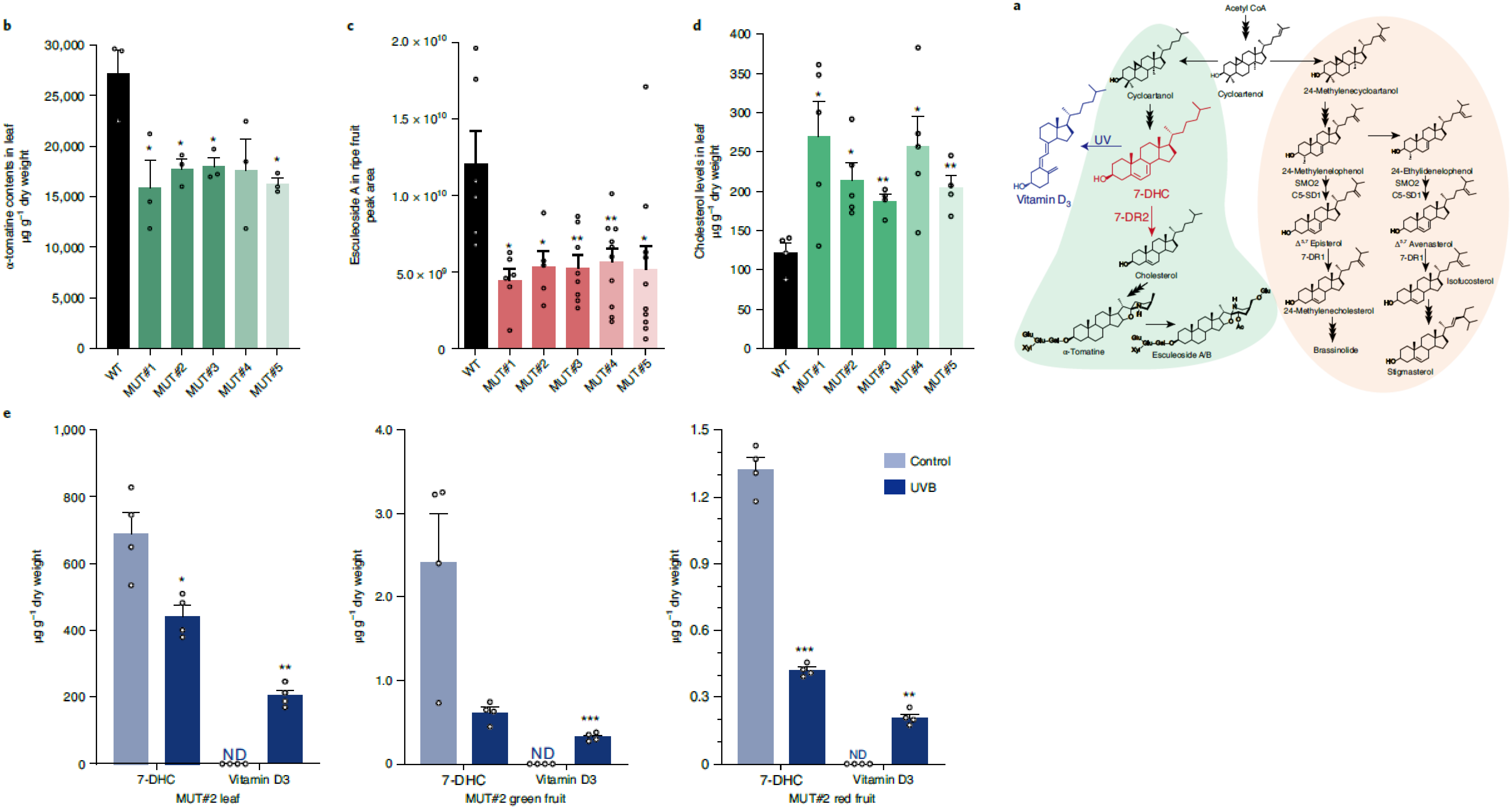

c
7-DHC levels in fruit
μg g⁻¹ dry weight
170
130
90
50
10
7
6
5
4
3
2
1
0
WT
MUT#1
MUT#2
MUT#3
MUT#4
MUT#5
**
***
****
***
ND
**
****
**
****
**
ND
****
*
**
**
***
ND
**
***
****
****
**
IMG
MG
Breaker
B + 7


d
7-DHC levels in leaf
μg g⁻¹ dry weight
800
600
400
200
0
**
**
**
**
****
WT
MUT#1
MUT#2
MUT#3
MUT#4
MUT#5

MALDI images of 7-DHC (*m/z* 367.33) and its laser-induced derivative ion (*m/z* 365.32), cholesterol (*m/z* 369.35) and α-tomatine (*m/z* 1,034.55).





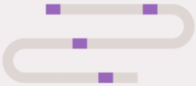

a
Acetyl CoA
Cycloartenol
Cycloartanol
24-Methylenecycloartanol
Vitamin D3
UV
7-DHC
7-DR2
Cholesterol
α-Tomatine
Esculeoside A/B
Glu
Glu–Gal–O
Xyl
Ac
24-Methylenelophenol
24-Ethylidenelophenol
SMO2
C5-SD1
Δ5,7 Episterol
Δ5,7 Avenasterol
7-DR1
24-Methylenecholesterol
Isofucosterol
Brassinolide
Stigmasterol
b
α-tomatine contents in leaf μg g−1 dry weight
30,000
25,000
20,000
15,000
10,000
5,000
0
WT
MUT#1
MUT#2
MUT#3
MUT#4
MUT#5
c
Esculeoside A in ripe fruit peak area
2.0 × 10^10
1.5 × 10^10
1.0 × 10^10
5.0 × 10^9
0
d
Cholesterol levels in leaf μg g−1 dry weight
400
350
300
250
200
150
100
50
0
e
μg g−1 dry weight
1,000
800
600
400
200
0
7-DHC
Vitamin D3
ND
MUT#2 leaf
4.0
3.0
2.0
1.0
0
MUT#2 green fruit
1.5
1.2
0.9
0.6
0.3
0
MUT#2 red fruit
Control
UVB
*
**
***

- **Orphan crops**, such as sweet potato, groundnut, cassava, banana and quinoa, are locally important crops that have good nutritional attributes and adaptations. However, despite their great potential for improving food and nutrition security, the undesirable characteristics (such as low yield, sprawling growth and fruit drop,) prevent orphan crops from wider cultivation. CRISPR technology, which is cheap, fast, precise and capable of editing multiple sites and modifying gene regulation, provides a powerful method for accelerating the domestication of orphan crops. It was recently used to target genes that control plant architecture, flower production and fruit size in groundcherry, a semi-domesticated orphan crop, and the modified plants showed improved domestication traits

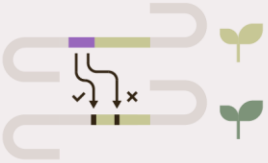
Criteria of equivalence of NGT plants to conventional plants

A NGT plant is equivalent to conventional plants when it differs from the recipient/parental plant by **no more than 20 genetic modifications** of types **1 to 5**.

1 Insertion or substitution of **no more than 20** nucleotides



3 On the condition that the genetic modification does **not interrupt an endogenous gene**:



Targeted insertion of a contiguous DNA sequence existing in the breeder's gene pool

Targeted substitution of an endogenous DNA sequence with a contiguous DNA sequence existing in the breeder's gene pool

2 Deletion of **any number** of nucleotides

4 Targeted inversion of a sequence of **any number** of nucleotides

5 Any other targeted modification of any size, on the condition that the **resulting DNA sequences already occur** (possibly with modifications as accepted under points **1** and/or **2**) in a species from the breeders' gene pool

GAIN4 CROPS

This project has received funding from the EU's Horizon 2020 research and innovation programme under the Grant Agreement 862087.

A second chance for plant biotechnology in Europe

Europe tilts towards gene-edited plants, but progress could be derailed over who owns the patents.

By Cormac Sheridan

On 7 February the European Parliament voted in favor of a legislative proposal to markedly relax rules for certain gene-edited plants. But it also added several amendments to the draft legislation, originally proposed by the European Commission, that, if adopted, would also ban patents for all CRISPR–Cas9-edited plants, a stance likely to discourage companies from investing in new plant products.

The European Union has long history of opposition to genetically modified crops, but CRISPR and other genome editing technologies have prompted a rethink of the rules. A genetically modified plant or organism is obtained by inserting genetic material from

CRISPR editing can alter the plant genome precisely, without adding foreign DNA, to breed plants with useful traits.

Table 1 | Selected gene-edited plants undergoing experimental release in Europe

Country	Institution	Species	Edit	Purpose	First year of release or proposed release
Italy	University of Milan	*Oryza sativa* (rice)	CRISPR–Cas9-mediated deletions in three genes: *Pi21*, *HMA1* and *HMA2*	Resistance to rice blast (*Magnaporthe grisea*)	2024
Belgium	Flanders Institute of Biotechnology	*Zea mays* (maize)	CRISPR–Cas-mediated disruption of three genes involved in lignin biosynthesis	Improved digestibility of animal feed	2024
Spain	National Agri-Food Technology Centre (CTAEX), Badajoz	*Nicotiana tabacum* (tobacco)	CRISPR–Cas9 edits of *MPO* genes, encoding methyl putrescine oxidase, to lower nicotine production	Enhanced production of the anti-inflammatory anatabine	2024
Belgium	Inari Agriculture (Cambridge, Mass., USA)	*Zea mays* (maize)	CRISPR–Cas edits of undisclosed genes encoding a transcription factor and a transcriptional coactivator that influence plant height	Improved biomass productivity	2023
Denmark	KMC (Brande)	*Solanum tuberosum* (potato)	CRISPR–Cas disruption of the *StDMR6-1* gene, which is associated with susceptibility to blight infection	Improved blight resistance	2023
Denmark	KMC	*Solanum tuberosum* (potato)	CRISPR–Cas disruption of the *StGBSS1* gene, which encodes granule-bound starch synthase	Modified starch content	2023
Sweden	Swedish University of Agricultural Sciences (Umeå)	*Solanum tuberosum* (potato)	CRISPR–Cas-mediated mutations in three genes: *GBSS*, *SSS*, and *SBE*	Modified starch content	2023
Spain	Grupo Lucas (Murcia)	*Brassica oleracea* (broccoli)	CRISPR–Cas9-mediated disruption of *ABI1*, *HAB1*, and *GSTU17*, which regulate the abscisic acid signaling pathway	Improved drought and salinity tolerance	2022
Sweden	SweTree Technologies (Umeå)	*Populus* × *canescens* (gray poplar)	CRISPR–Cas9-mediated disruptions of the *CCR2* gene, to reduce production of cinnamoyl CoA reductase 2	Reduced lignin content and increased sugar yield for improved biomass-to-energy conversion	2022
Sweden	Swedish University of Agricultural Sciences (Alnarp)	*Solanum tuberosum* (potato)	Generation of three different edited strains, with deletions in either the *DMR6* + *CHL1*, *AsS1* or *PiS1* genes	Altered resistance to pathogens	2021
Spain	Institute of Molecular and Cellular Biology of Plants (Valencia)	*Nicotiana tabacum* (tobacco)	CRISPR–Cas9-based disruption of the *SPL* family of transcription factor genes	Delayed flowering	2020
Sweden	Lyckeby Starch (Kristianstad)	*Solanum tuberosum* (potato)	Crispr–Cas9-mediated deletions in the *GBSS*, *SSS3* and *SSS2* genes	Altered starch content	2019

Source: European Commission GMO Register Part B Notifications.

Per questa pagina è disponibile unicamente una traduzione automatica in italiano. Tradurre la pagina in italiano

Your career at EFSA

Food is an important part of daily life, and at EFSA our mission is to keep it safe. We are located in Parma, and our team is just as diverse as the food that we assess. Do you have the ingredients to join our international team?

1. What is a hazard ? A risk ?
2. Risk analysis in the EU law
3. Molecular characterization of GM plants : what, how and why ?
4. The future : new avenues for the genetic modification of plants and possible impacts on risk assessment

Hazard and Risk

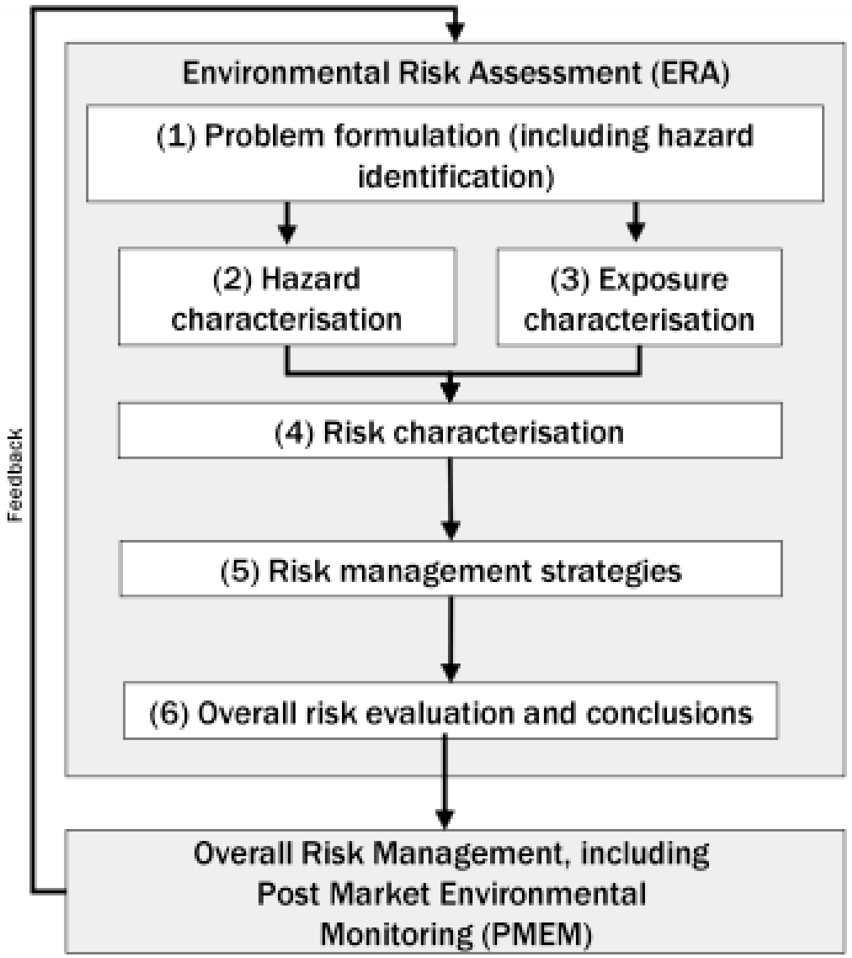
- **Hazard** : something capable of causing harm (*i.e.* adverse effects to health or the environment)
- **Risk** = hazard *x* exposure

Hazard and Risk

- **Hazard** : something capable of causing harm (*i.e.* adverse effects to health or the environment)
- **Risk** = hazard *x* exposure

➢ **Probability (likelihood) of adverse effects depends on exposure.**

From hazards to risks (and back)

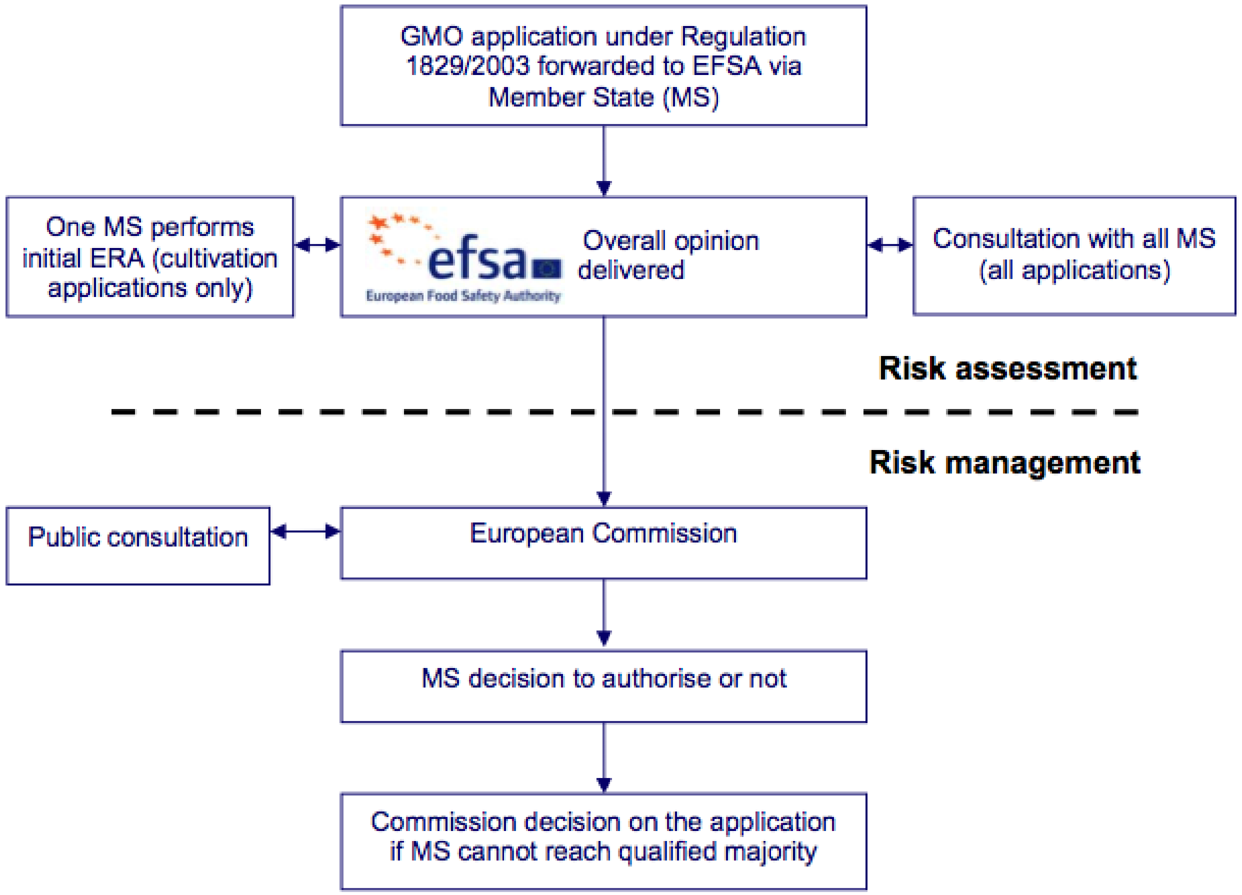


Six steps within the environmental risk assessment (ERA) and relationship to risk management including monitoring according to Directive 2001/18/EC and Regulation (EC) No. 1829/2003.

RA: three pillars

The three pilars of Risk analysis :
Risk assessment, risk management, risk communication

Authorisation procedure under Regulation (EC) No 1829/2003 (centralised procedure)



GMO application under Regulation 1829/2003 forwarded to EFSA via Member State (MS)

One MS performs initial ERA (cultivation applications only)

efsa European Food Safety Authority Overall opinion delivered

Consultation with all MS (all applications)

Risk assessment

Risk management

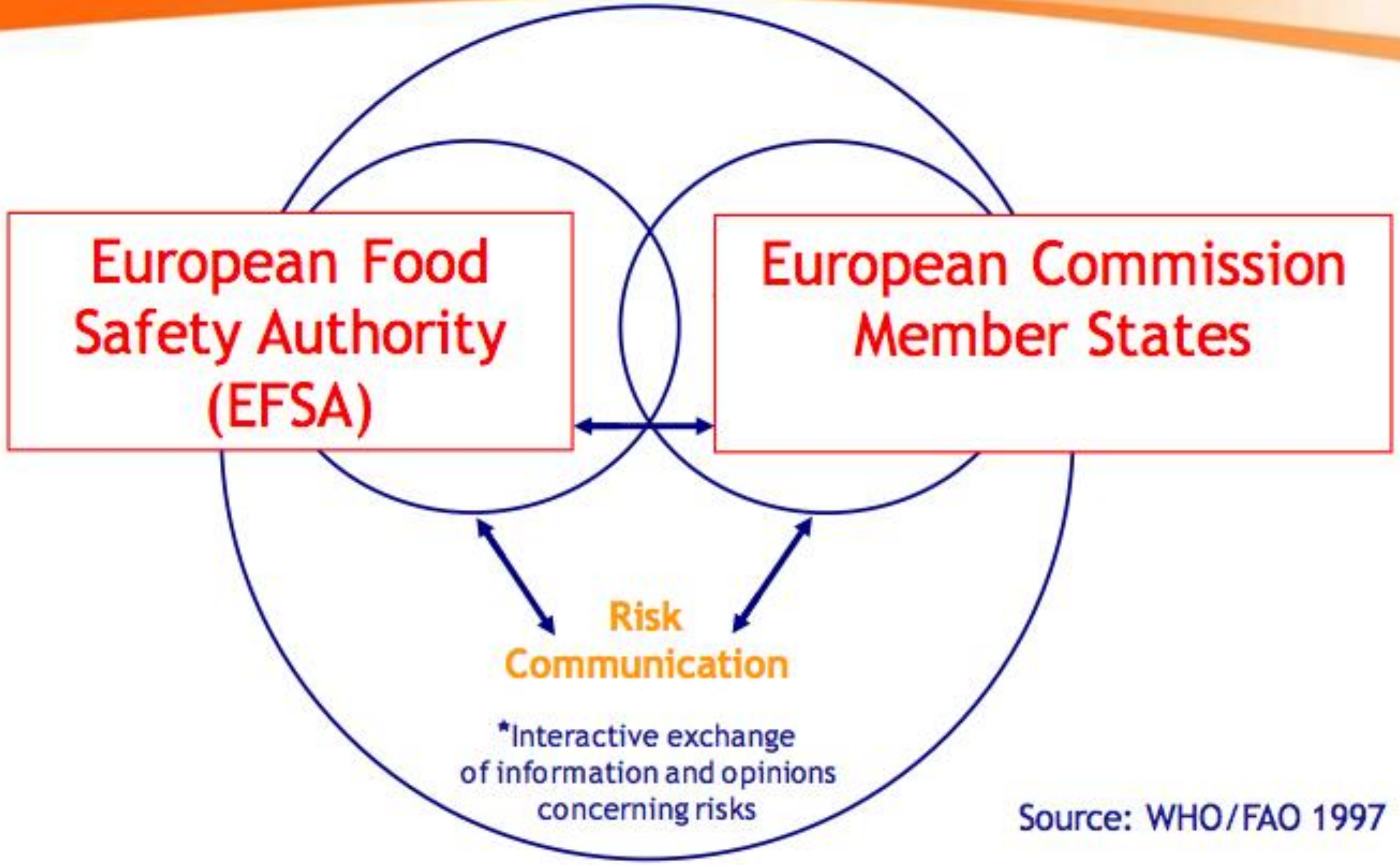
Public consultation

European Commission

MS decision to authorise or not

Commission decision on the application if MS cannot reach qualified majority

Actors of Risk Analysis in the EU



European Food Safety Authority (EFSA)

European Commission Member States

Risk Communication

*Interactive exchange of information and opinions concerning risks

Source: WHO/FAO 1997

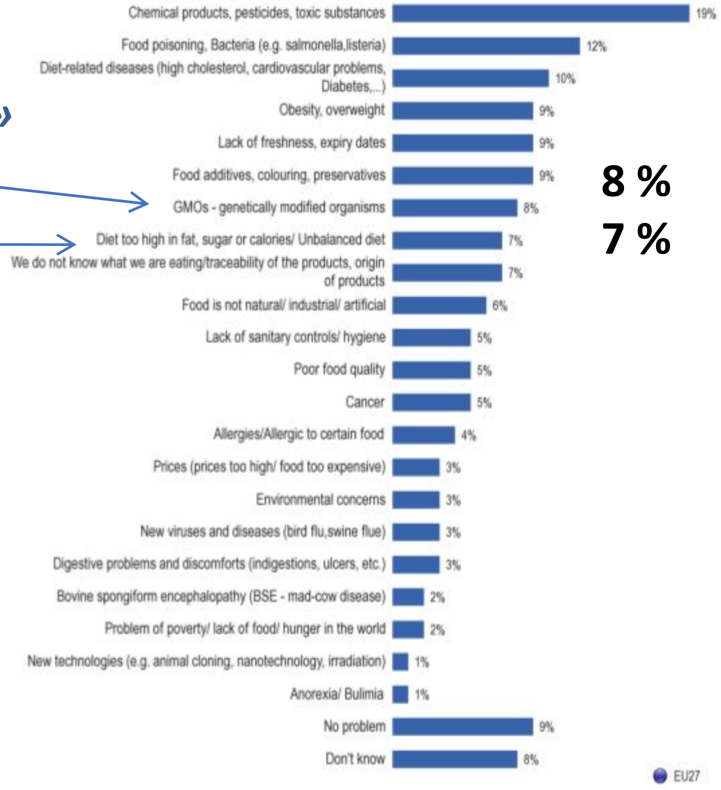
➡ Need for close cooperation between risk assessor and risk manager

Risk communication

Risk communication :
Scientific risk is not perceived risk.

Eurobarometer 2010 on Food-related risks:
« What are all the things that come to your mind when thinking about possible problems or risks associated with food and eating ? »

« GMOs - genetically modified organisms »
« Diet too high in fat, sugar or calories / Unbalanced diet »



8 %

7 %

Different regulatory frameworks in the EU *vs.* USA

EU	**USA**
« Process-based approach », *i.e.* which regulation applies depends on the technology	« Product-based approach », *i.e.* which regulation applies depends on the trait
Specific legislation for GMOs	Use of existing legislation for GMOs
Horizontal and sectorial regulations	Sectorial rules
European Food Safety Authority not competent for deciding on authorization and adoption of risk management measures.	Federal agencies (USDA, EPA, FDA) competent for deciding on « deregulation » (= authorization) and adoption of risk management measures.

What is a GMO in Europe

European Food Safety Authority

An organism is "genetically modified" if its genetic material has been changed in a way that does not occur under natural conditions through cross-breeding or natural recombination.

Definition by Directive 2001/18/EC (Art. 2)

In the EU, products that are, contain, or are produced from Genetically Modified Organisms (GMOs) must have an authorisation prior to entering the market.

What EFSA does

EFSA's Mission*

1. Provide scientific advice, opinions, information, and technical support for Community legislation and policies

2. Collect and analyse data to allow characterisation and monitoring of risks (DCM Unit)

3. Promote and coordinate development of uniform risk assessment methodologies (Guidance Documents)

4. Communicate risks related to all aspects of EFSA's mandate

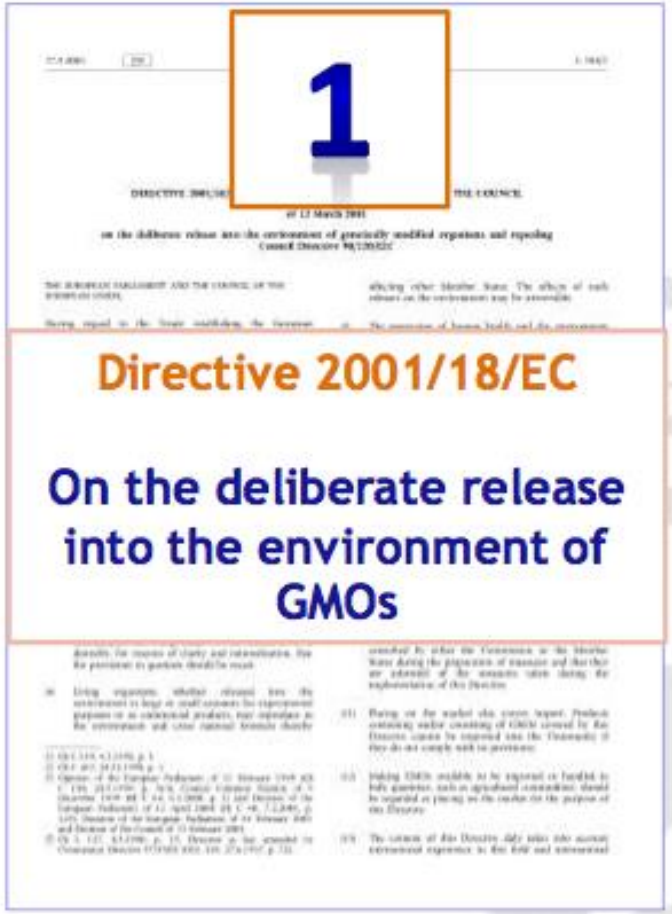
* As laid down in Regulation (EC) 178/2002

EFSA cannot

- **Be responsible for food safety legislation** (give authorisations for products such as GMOs, feed additives, food additives, pesticides etc)
- **Take charge of food safety/quality controls** (sampling, labelling) or other risk management issues such as co-existence measures
- **Substitute National Competent Authorities**

Legal framework for GMO risk assessment

EFSA's role is to carry out scientific Risk Assessment on GMOs under two regulatory frameworks:



1

Directive 2001/18/EC

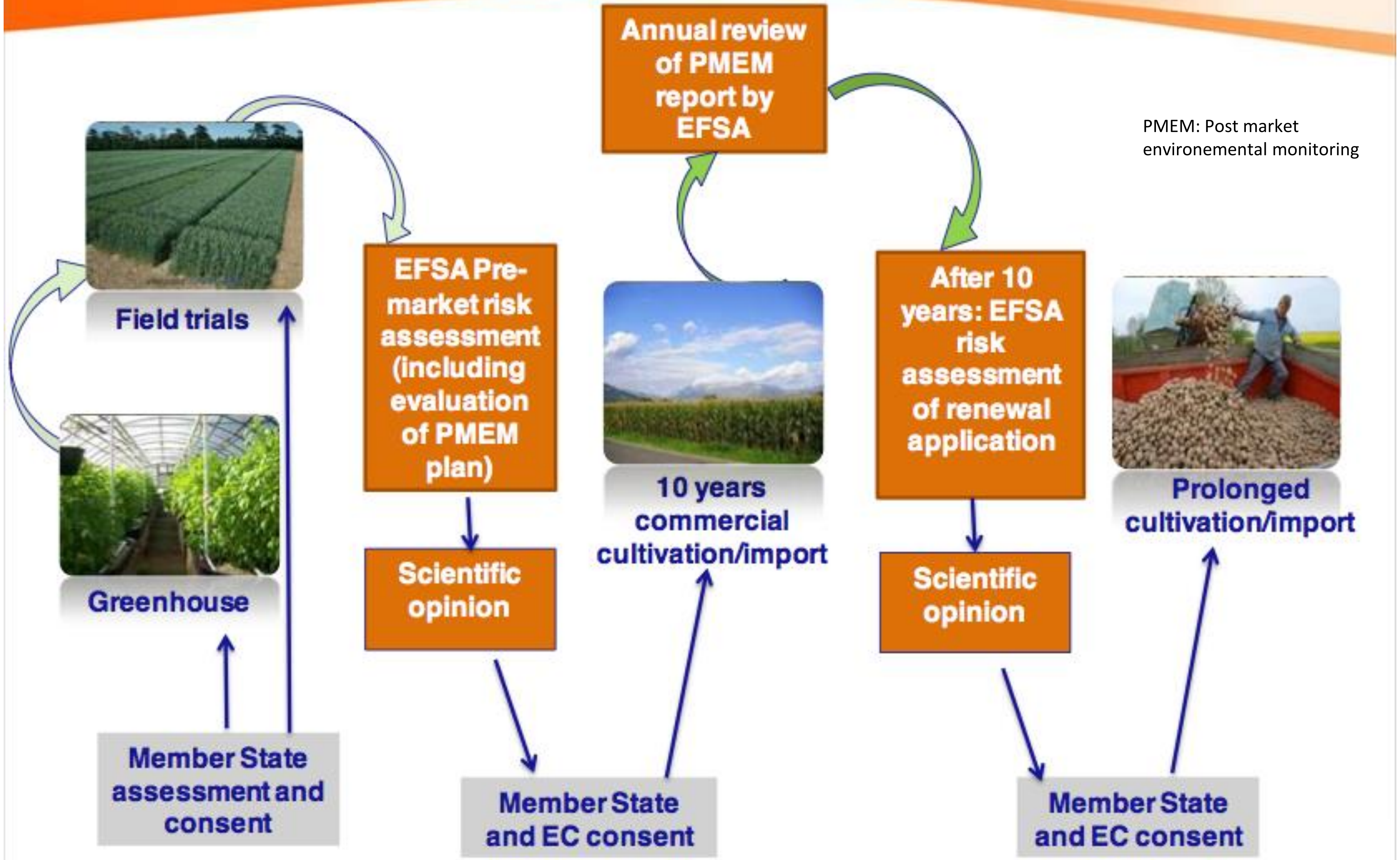
On the deliberate release into the environment of GMOs

2

Regulation (EC) No 1829/2003

On GM food and feed including derived products

The Risk Assessment cycle of GMOs



Greenhouse

Field trials

Member State assessment and consent

EFSA Pre-market risk assessment (including evaluation of PMEM plan)

Scientific opinion

Member State and EC consent

10 years commercial cultivation/import

Annual review of PMEM report by EFSA

After 10 years: EFSA risk assessment of renewal application

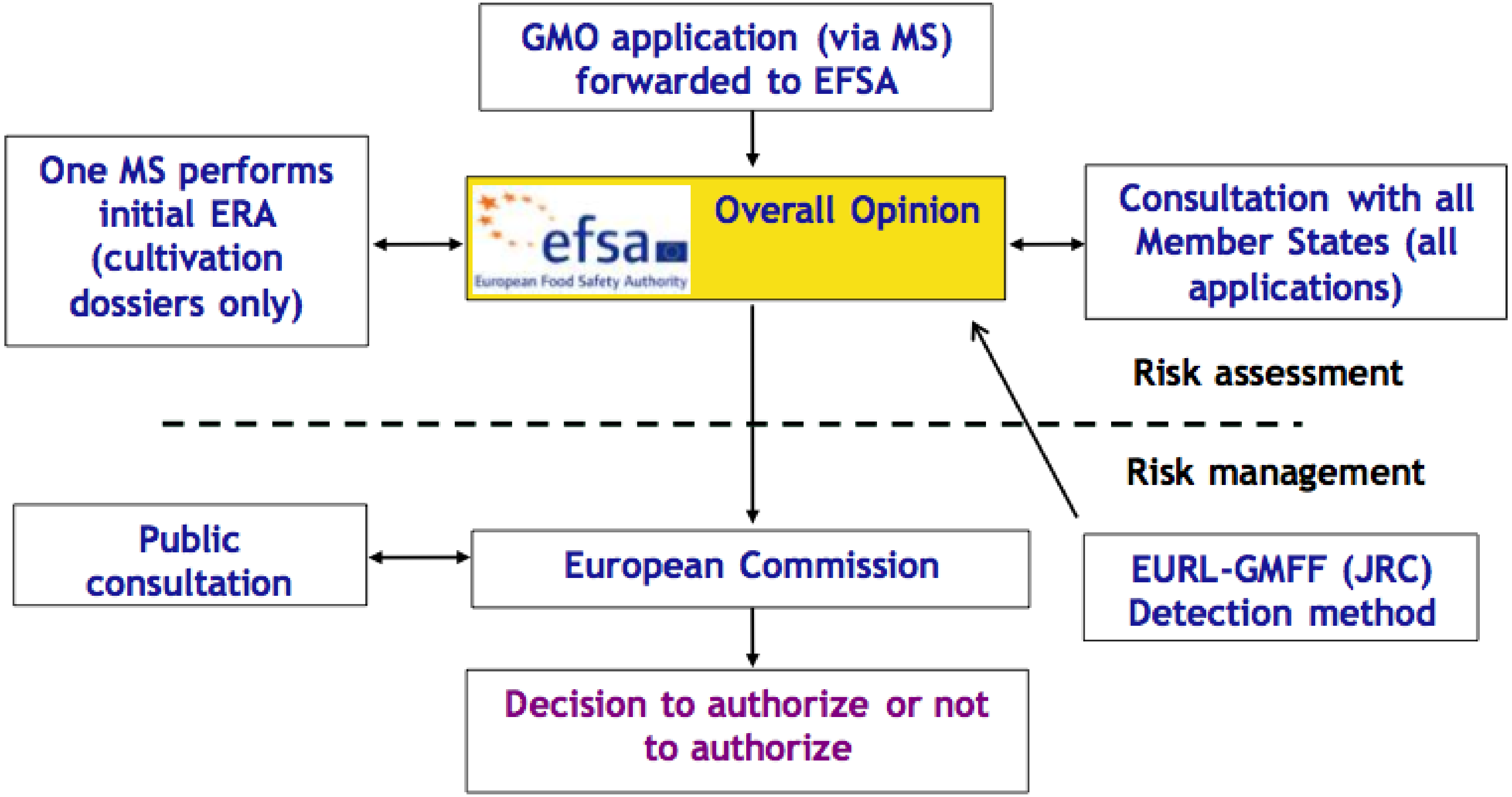
Scientific opinion

Member State and EC consent

Prolonged cultivation/import

PMEM: Post market environemental monitoring

Regulatory framework: Regulation (EC) No 1829/2003 for GM food & feed



GMO application (via MS) forwarded to EFSA

One MS performs initial ERA (cultivation dossiers only)

Overall Opinion

Consultation with all Member States (all applications)

Risk assessment

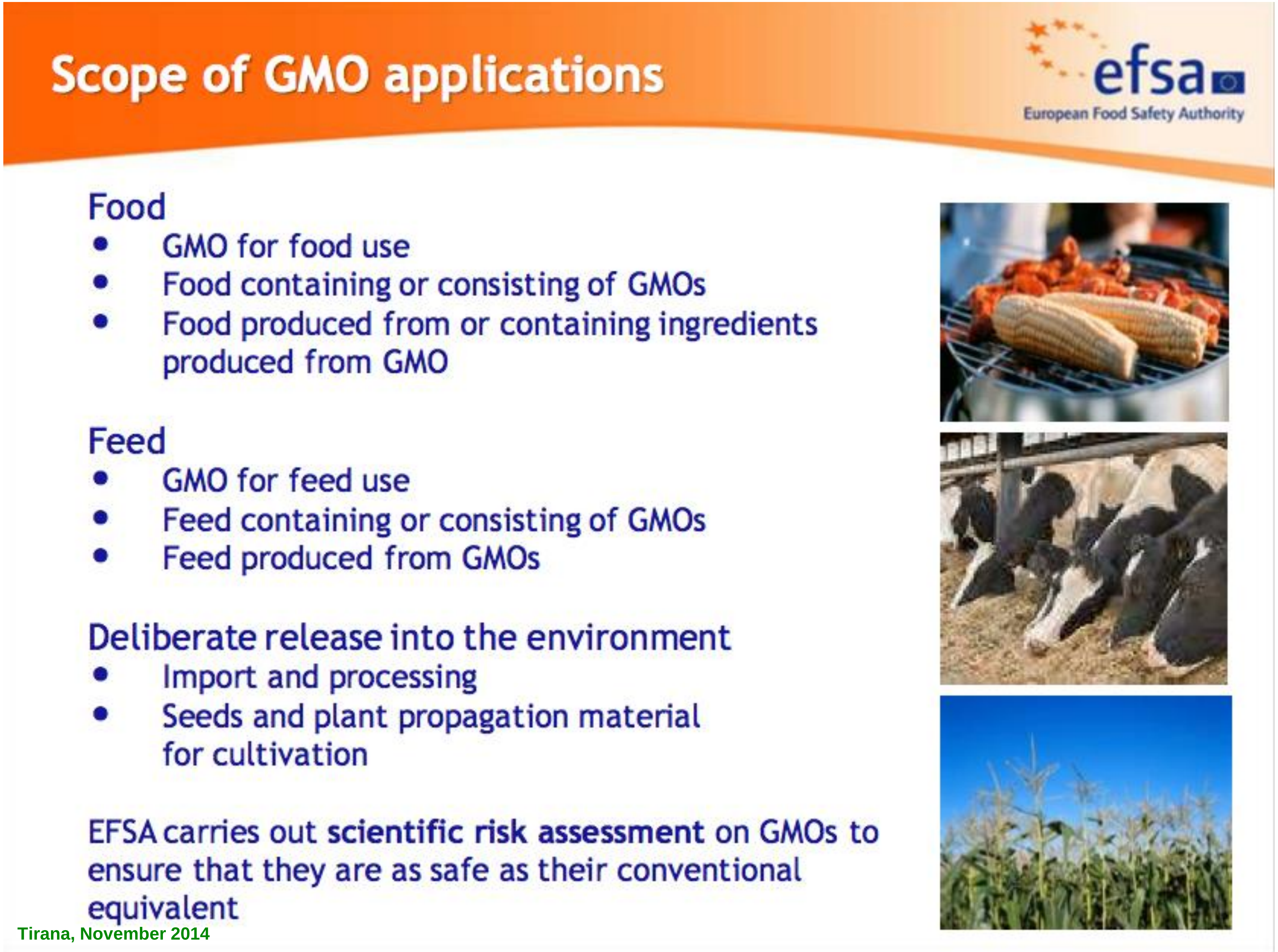
Risk management

Public consultation

European Commission

EURL-GMFF (JRC) Detection method

Decision to authorize or not to authorize



Scope of GMO applications

efsa
European Food Safety Authority

Food

- GMO for food use
- Food containing or consisting of GMOs
- Food produced from or containing ingredients produced from GMO

Feed

- GMO for feed use
- Feed containing or consisting of GMOs
- Feed produced from GMOs

Deliberate release into the environment

- Import and processing
- Seeds and plant propagation material for cultivation

EFSA carries out **scientific risk assessment** on GMOs to ensure that they are as safe as their conventional equivalent

Risk assessment performed by

- **The GMO Panel**
  - elaborates guidance documents
  - delivers scientific opinions on applications for market authorisation regarding GMOs

- **Plenary meetings** 8 times a year, for adoption of opinions and other discussions

- **40 Ad-hoc experts** support the GMO Panel in **Working groups**

- **13 GMO Unit scientists** provide support to the GMO Panel and its Working Groups

The Principle Logic

COMPARATIVE APPROACH

Compare the GMO and derived products to their non-GM counterparts (history of safe use, familiarity)

Assessment of the identified differences regarding:

Environmental impact

Food/Feed safety

Nutritional impact

- **Intended effects**: those occurring because of the genetic modification
- **Unintended effects**: additional effects which were NOT the objective of the genetic modification

The Principle Logic

Intended effects

- **Intended effects**: those occurring because of the genetic modification

The Principle Logic

Intended effects

CRY1 expression against lepidopteran pests

- **Intended effects**: those occurring because of the genetic modification

The Principle Logic

Intended effects

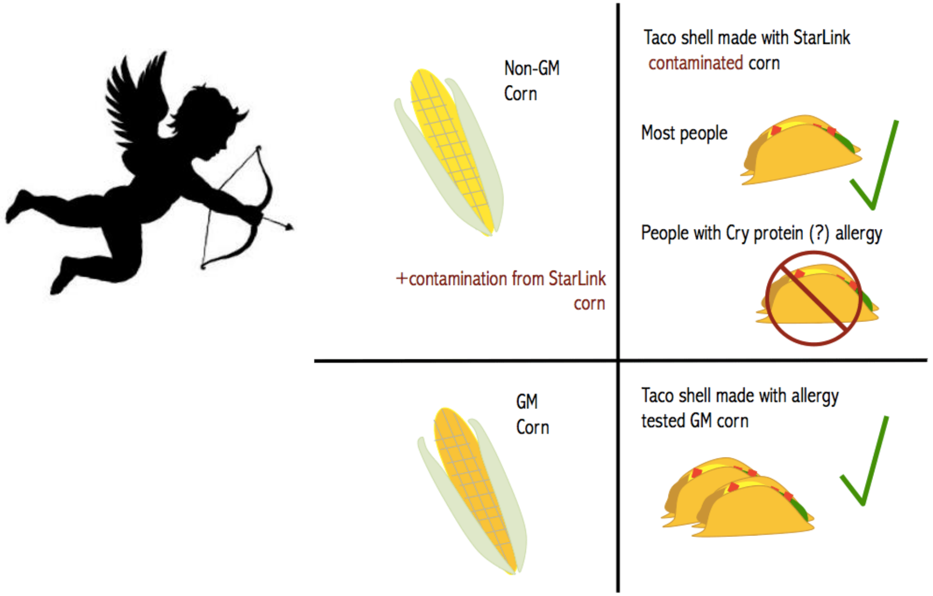
... and unintended effects

- **Intended effects**: those occurring because of the genetic modification
- **Unintended effects**: additional effects which were NOT the objective of the genetic modification

The Principle Logic

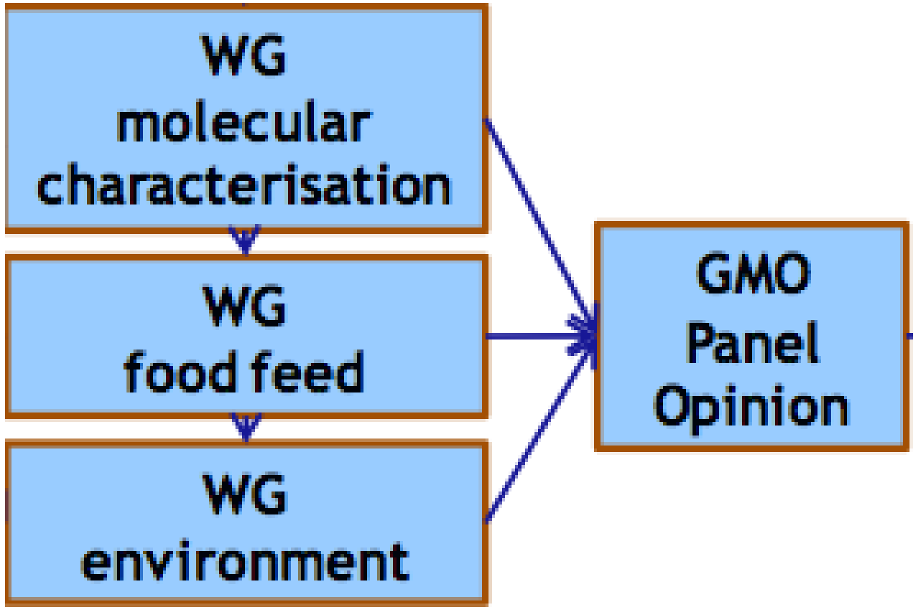
Intended effects

... and unintended effects



- **Intended effects**: those occurring because of the genetic modification
- **Unintended effects**: additional effects which were NOT the objective of the genetic modification

Elaboration of the opinion



- **Strong interactions between the different Working Groups in order to reach a consensus and give an opinion**

Molecular characterization of the GM plant : practical contribution to hazard identification

- **Newly expressed proteins** : bioinformatic search for similarities with toxins and allergens
- **New ORFs** : bioinformatic search for similarities of their (putative) translation products with toxins and allergens
- Possible **disruption of endogenous genes** at the insertion site
- **Similarities of the T-DNA with microbial DNA** and their possible impact on Horizontal Gene Transfer from plants to bacteria.

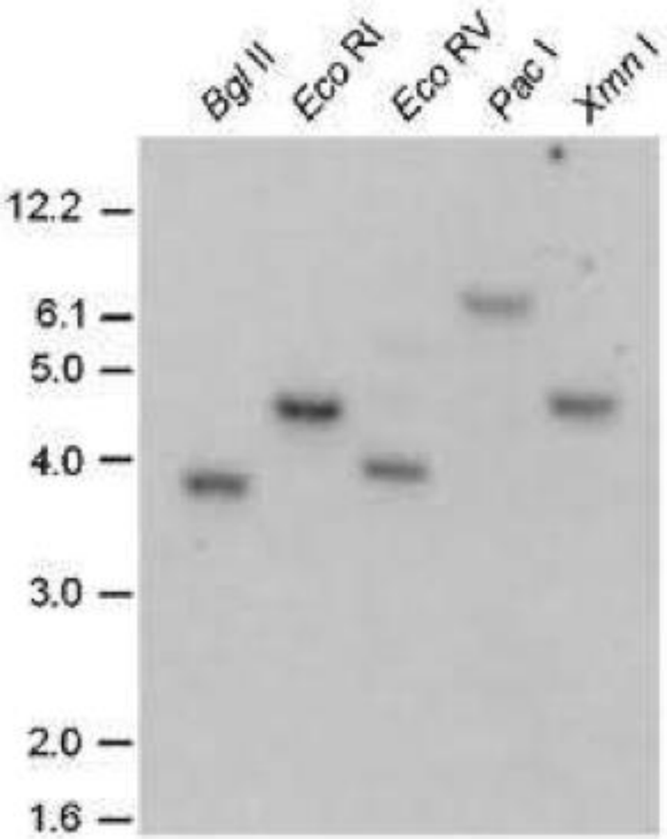
Molecular characterization :
analysis of the structure of the insert

- **The rationale**:
  - Authorization will bear on the «transformation event », *i.e.* the new DNA in its insertion locus (but possibly in multiple genetic backgrounds).
  - This event needs to be precisely defined for the purposes of risk assessment (task of EFSA) and of risk management (*e.g.* detection methods, task of COM JRC- Ispra).
- **The aims**:
  - To determine the number and structure of all detectable inserts, complete or partial.
  - To determine the sub-cellular location of the inserts
  - To determine the flanking regions of the recipient genome

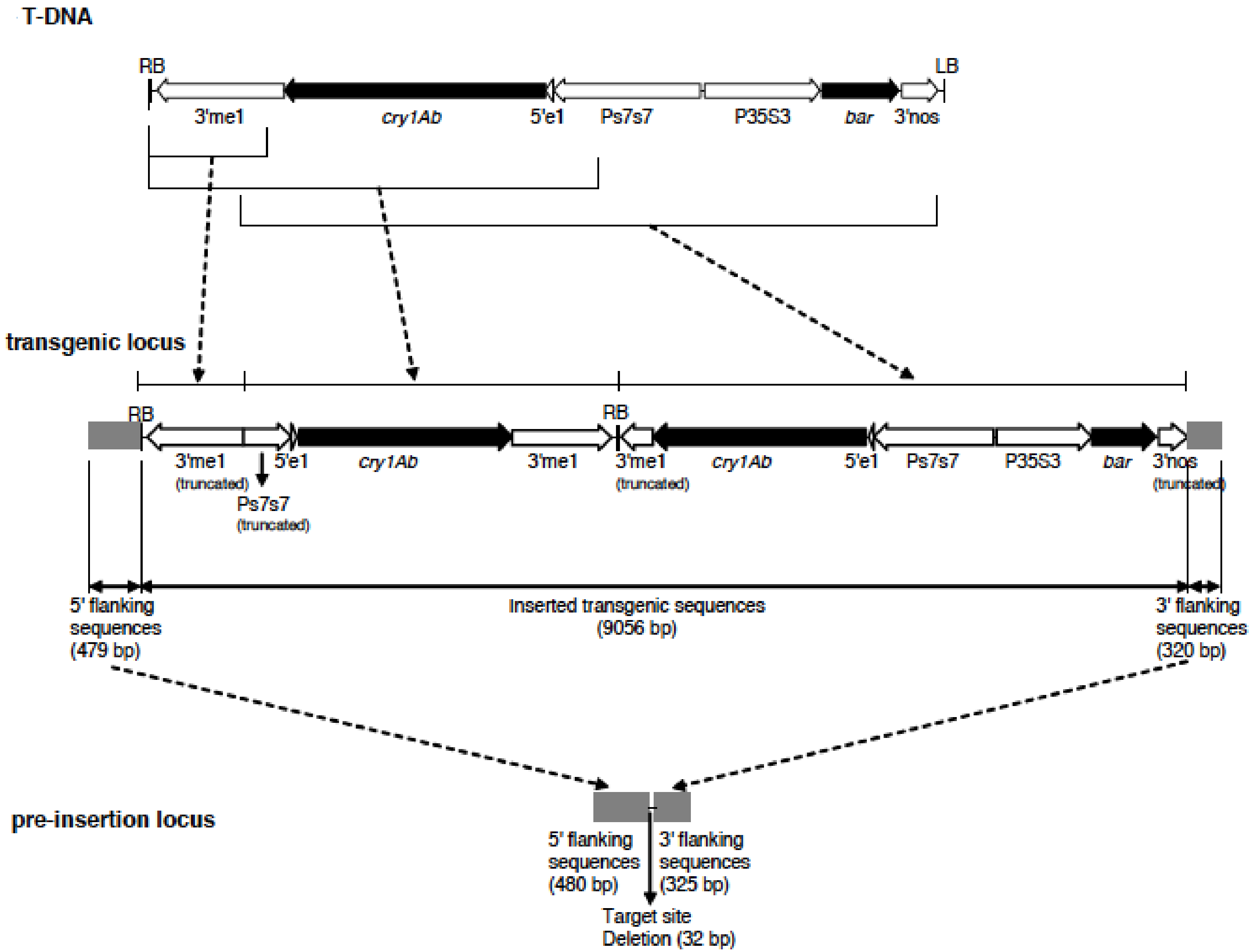
Molecular Characterisation

- Information on the genetic modification: materials and methods, results of insertion:
  - modification method
  - source of inserted DNA
  - vector used

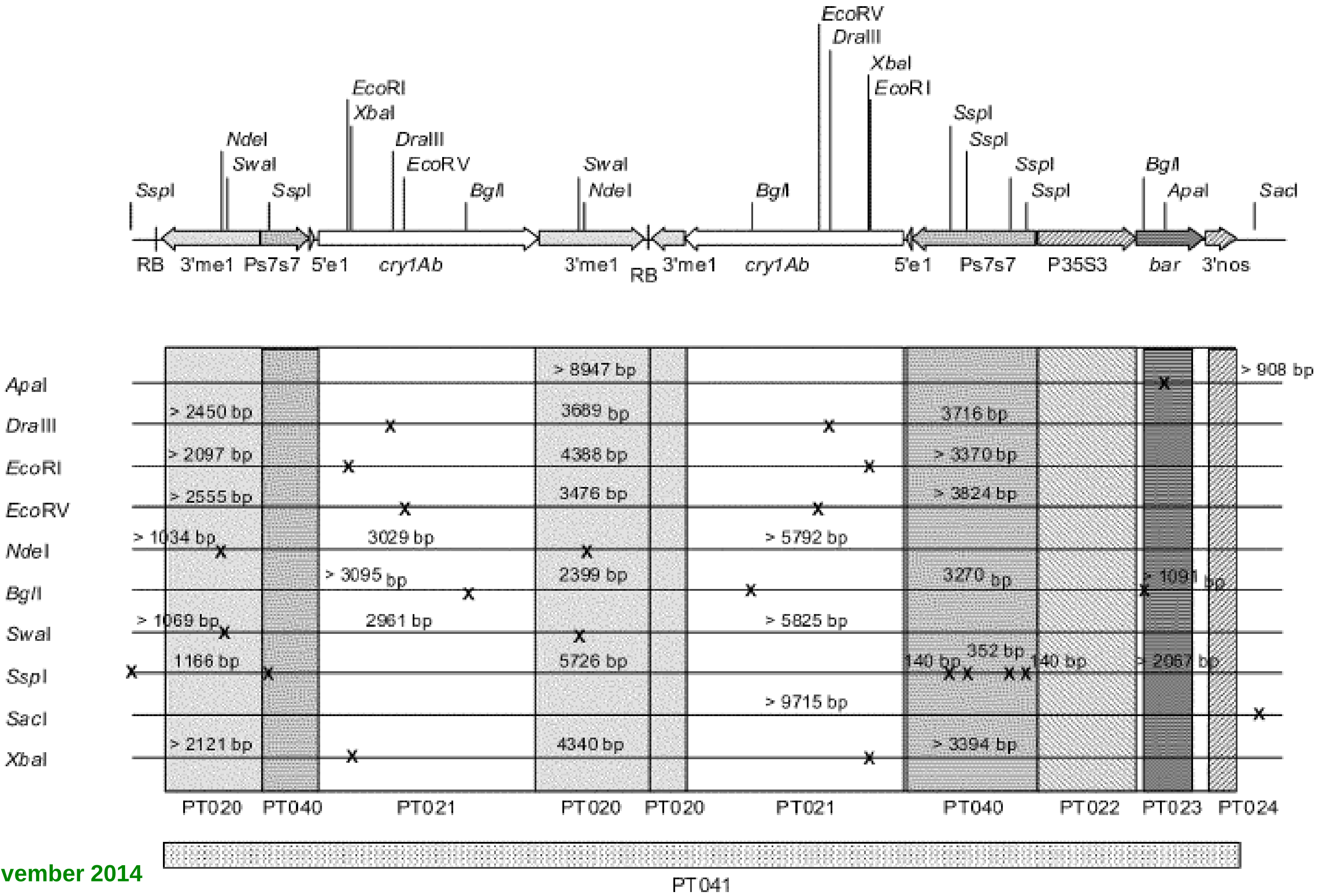
- Information on the GM plant:
  - Description of the trait
  - Information on the sequences actually inserted (or deleted)
  - Information on expression
  - Genetic and phenotypic stability



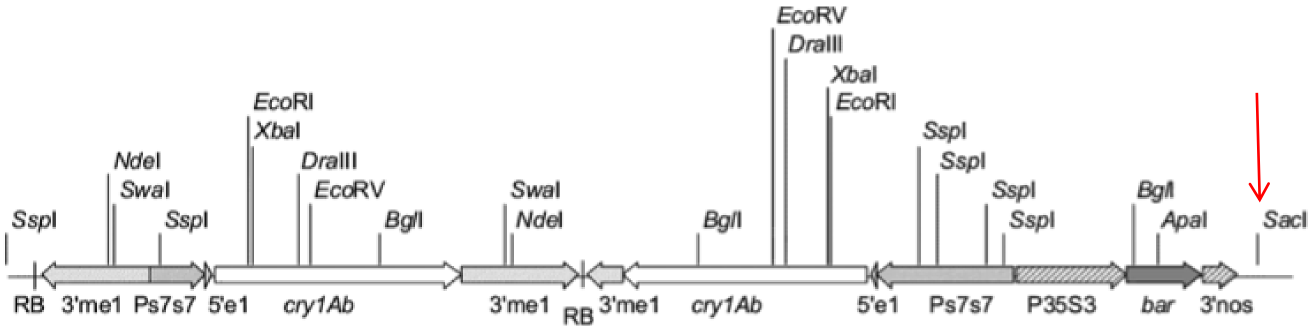
Analysing the transgenic locus by DNA sequencing : example :

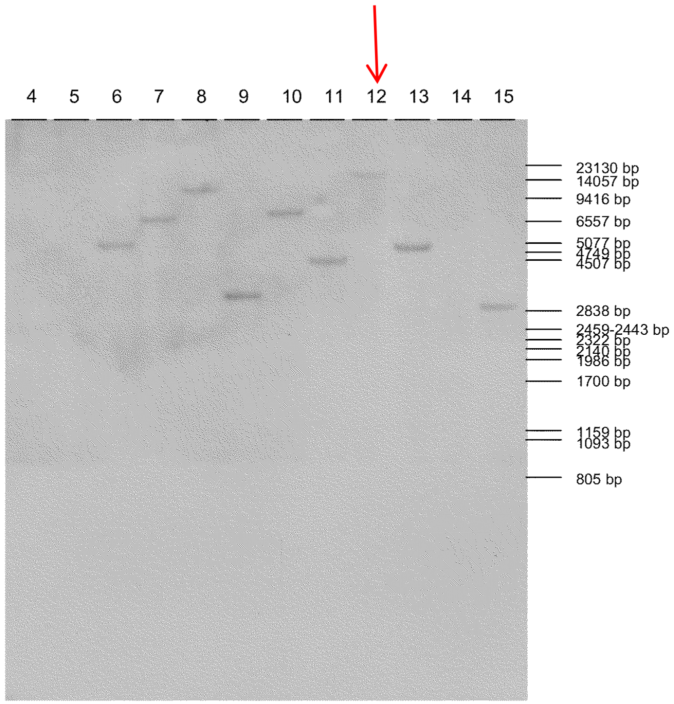


Southern blot analysis is extensively used for analysing insert structure.



Southern analysis of insert number and structure





Lane 4: *Gossypium hirsutum* elite event [redacted] *Apa*I digested
Lane 5: *Gossypium hirsutum* elite event [redacted] *Dra*III digested
Lane 6: *Gossypium hirsutum* elite event [redacted] *Eco*RI digested
Lane 7: *Gossypium hirsutum* elite event [redacted] *Eco*RV digested
Lane 8: *Gossypium hirsutum* elite event [redacted] *Nde*I digested
Lane 9: *Gossypium hirsutum* elite event [redacted] *Bgl*I digested
Lane 10: *Gossypium hirsutum* elite event [redacted] *Swa*I digested
Lane 11: *Gossypium hirsutum* elite event [redacted] *Ssp*I digested
Lane 12: *Gossypium hirsutum* elite event [redacted] *Sac*I digested
Lane 13: *Gossypium hirsutum* elite event [redacted] *Xba*I digested
Lane 14: *Gossypium hirsutum* wild type va[redacted]66 – *Xba*I digested
Lane 15: *Gossypium hirsutum* wild type va[redacted]66 – *Xba*I digested + an equimolar amount of pTDL008 – *Xba*I digested

Figure 5: Southern blot analysis

NB : SacI allows insert number determination.

Southern analysis of the absence of the vector backbone : checking for the absence of (*e.g.*) antibiotic resistance marker genes

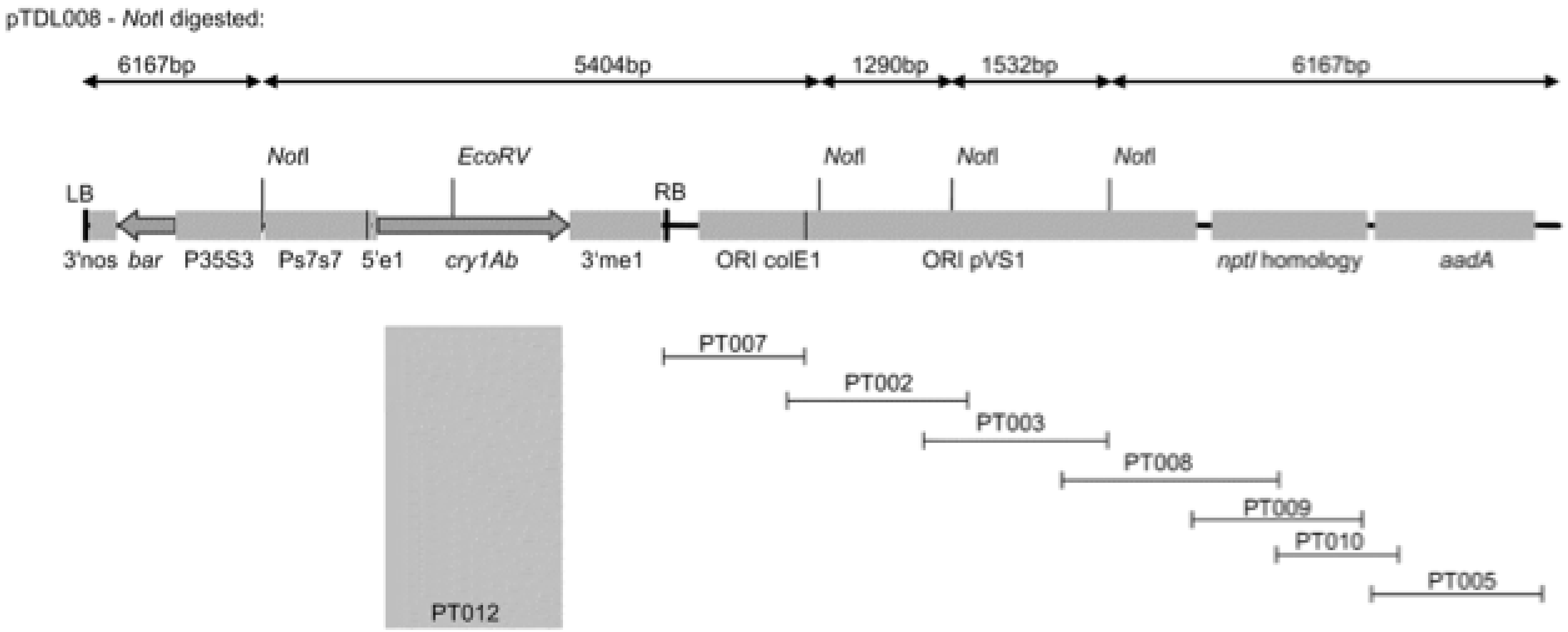
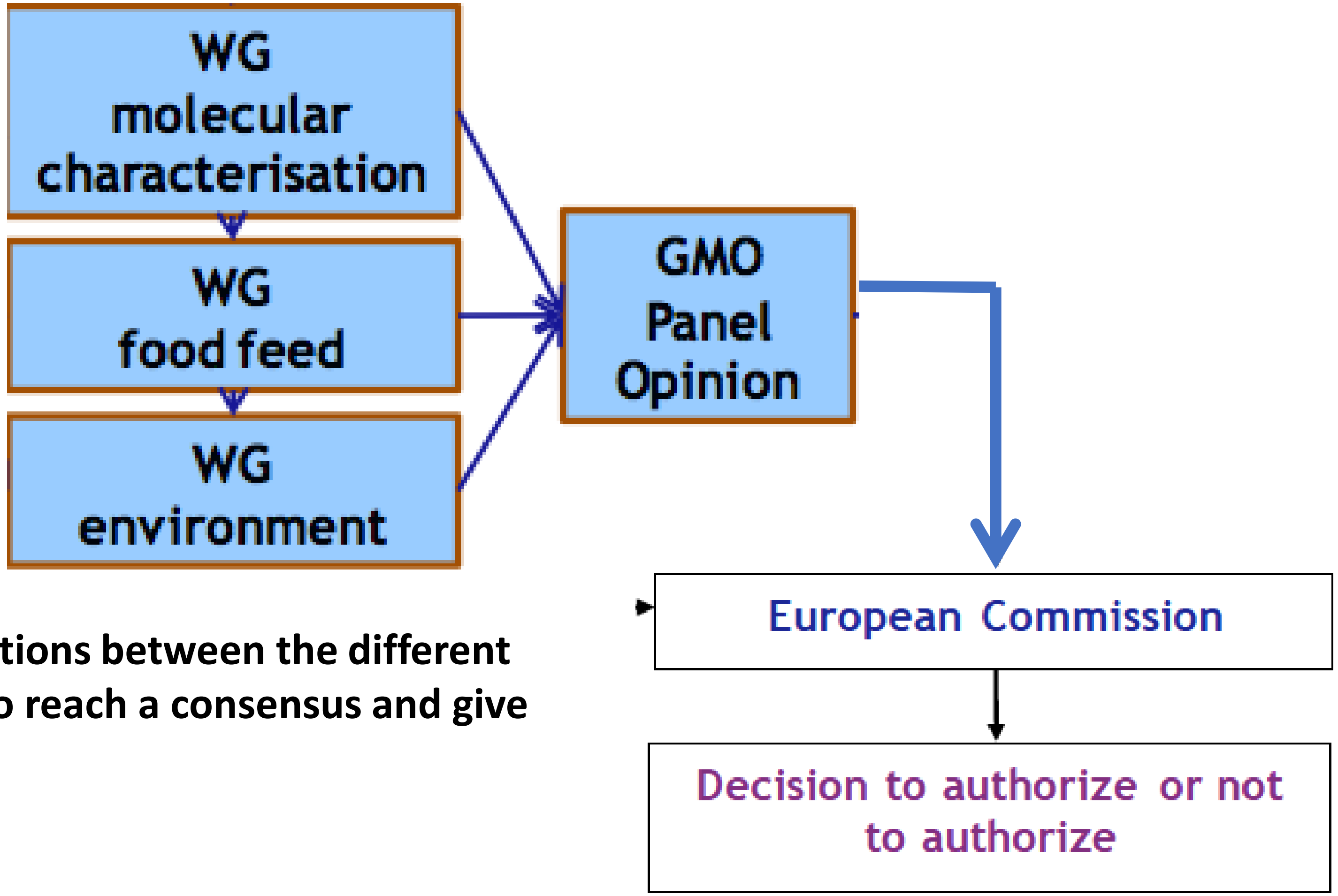


Figure 2: Schematic drawing of pTDL008 with indication of relevant restriction sites and position of the probes used.

Molecular characterization of the expression of the insert

- Determination of the **levels of the newly expressed proteins** (in a range of tissues depending on the scope of the application)
- Phenotypic data confirming generational **stability** of the trait / expression of the inserted genes
- **Methods** : typically ELISA

Elaboration of the opinion



WG molecular characterisation

WG food feed

WG environment

GMO Panel Opinion

European Commission

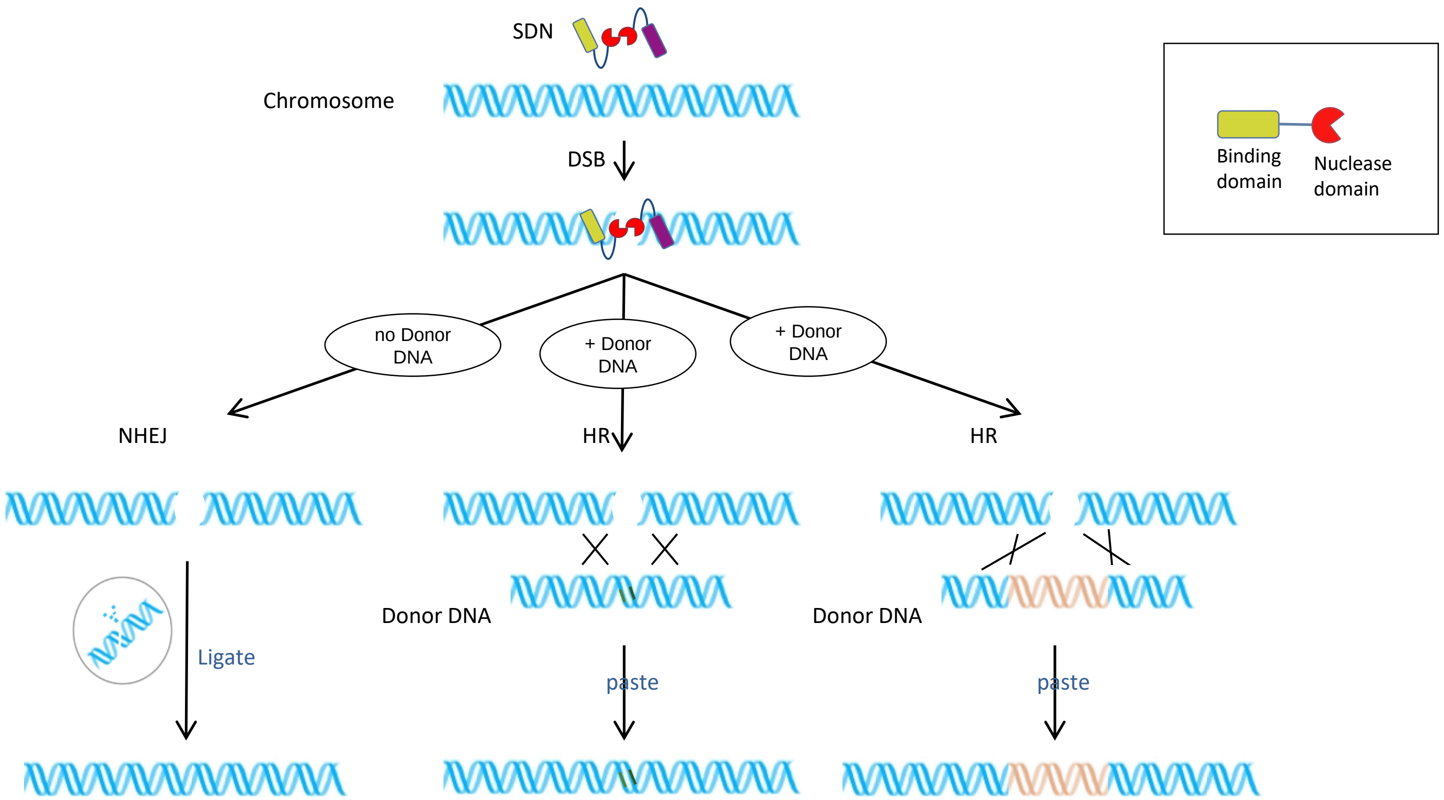
Decision to authorize or not to authorize

- Strong interactions between the different WG in order to reach a consensus and give an opinion

The future : new avenues for the genetic modification of plants (and possible impacts on risk assessment)

- New « breeding » techniques are being developed for the targeted genetic modification of plants.
- They do not necessarily involve addition of transgenes.
- Whether they will be considered as GMOs in the sense of the EU law is still unclear.

What about site directed nucleases?



Random repair with gain or loss of base pairs
SDN1
Gene off

Gene modification at one or more positions
SDN2
Gene edit

DNA insertion
SDN3
Add gene

GMO in Europe

European Food Safety Authority

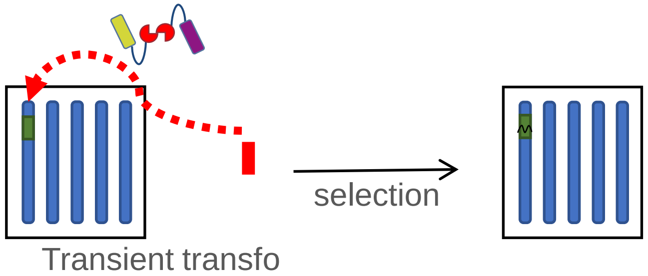
Case of the Amflora potato (root tubers contain only one type of starch), making it ideal for paper and textile production.

Amflora potato from BASF

🟩 Target gene (GBSS in this case)

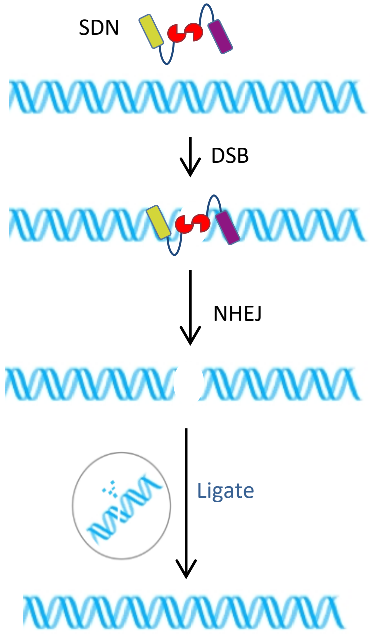
🟧 Transgene (antisense against GBSS in this case)

Amylose free potato via SDN1



🟩 Target gene (GBSS in this case)

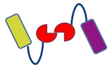
🟥 Transgene (expression of the TALEN raised against GBSS in this case)



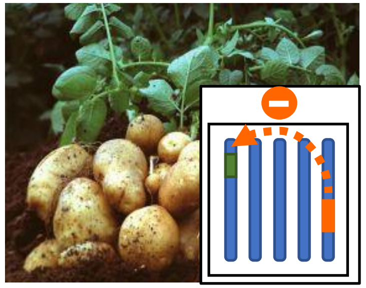
Random repair with
gain or loss of base
pairs
SDN1
Gene off

Use of SDN in plant breeding

SDN1 strategy

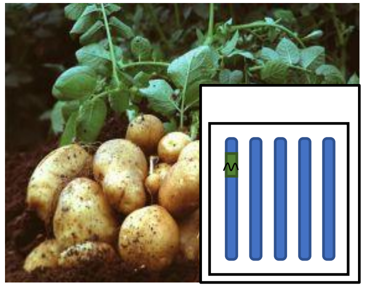


Amflora potato from BASF



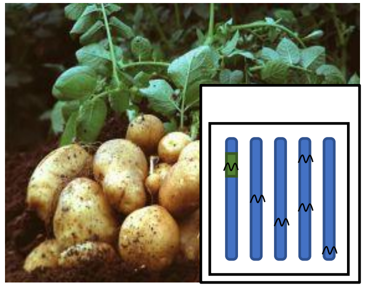
Classical transgenesis

Amylose free potato via SDN1



New technology

Amylose free potato via EMS



Classical breeding

QUESTIONS:			
Was this plant a transgenic?	YES	NO	NO
Is this plant a transgenic?	YES	NO	NO
Is this plant different from the “mother” plant?	Could be YES Depend on transgene side effects	Could be YES Depend on OTA	Could be YES Depend on mutagenesis
Subjected to EFSA analysis	YES	?	NO
Can I detect the origin of this plant?	YES	NO	NO

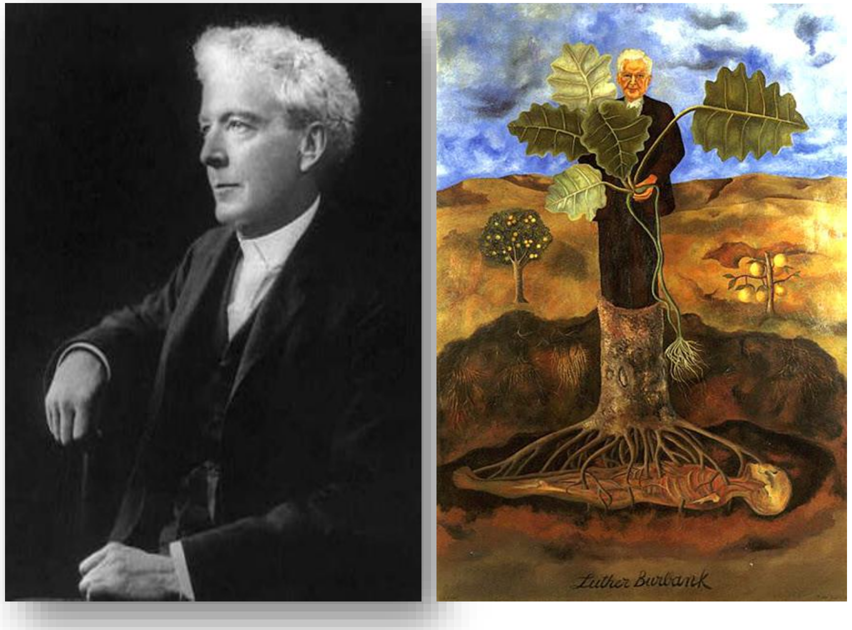
Conclusions

1. Molecular characterization (MC) contributes to hazard and risk identification, but must be complemented by biological evidence.
2. Both intended and unintended effects must be addressed.
3. New molecular techniques are emerging for the characterization of GMPs.
4. New breeding techniques are emerging for the genetic modification of plants, challenging the current risk assessment approach. Their status is still not clear ...

“Conventional” *vs.* “new” breeding ...

- *“We have recently advanced our knowledge of genetics to the point where we can manipulate life in a way never intended by nature.”*
- *“We must proceed with utmost caution in the application of this new found knowledge.”*

Luther Burbank, 1906



Pioneer in agricultural science

Trends in
Plant Science

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Special Issue: Feeding the World: The Future of Plant Breeding

Opinion

Europe's Farm to Fork Strategy and Its Commitment to Biotechnology and Organic Farming: Conflicting or Complementary Goals?

Kai P. Purnhagen,[1,*,@] Stephan Clemens,[2] Dennis Eriksson,[3,@]
Louise O. Fresco,[4,@] Jale Tosun,[5] Matin Qaim,[6,@] Richard G.F. Visser,[7]
Andreas P.M. Weber,[8] Justus H.H. Wesseler,[9,@] and David Zilberman[10]

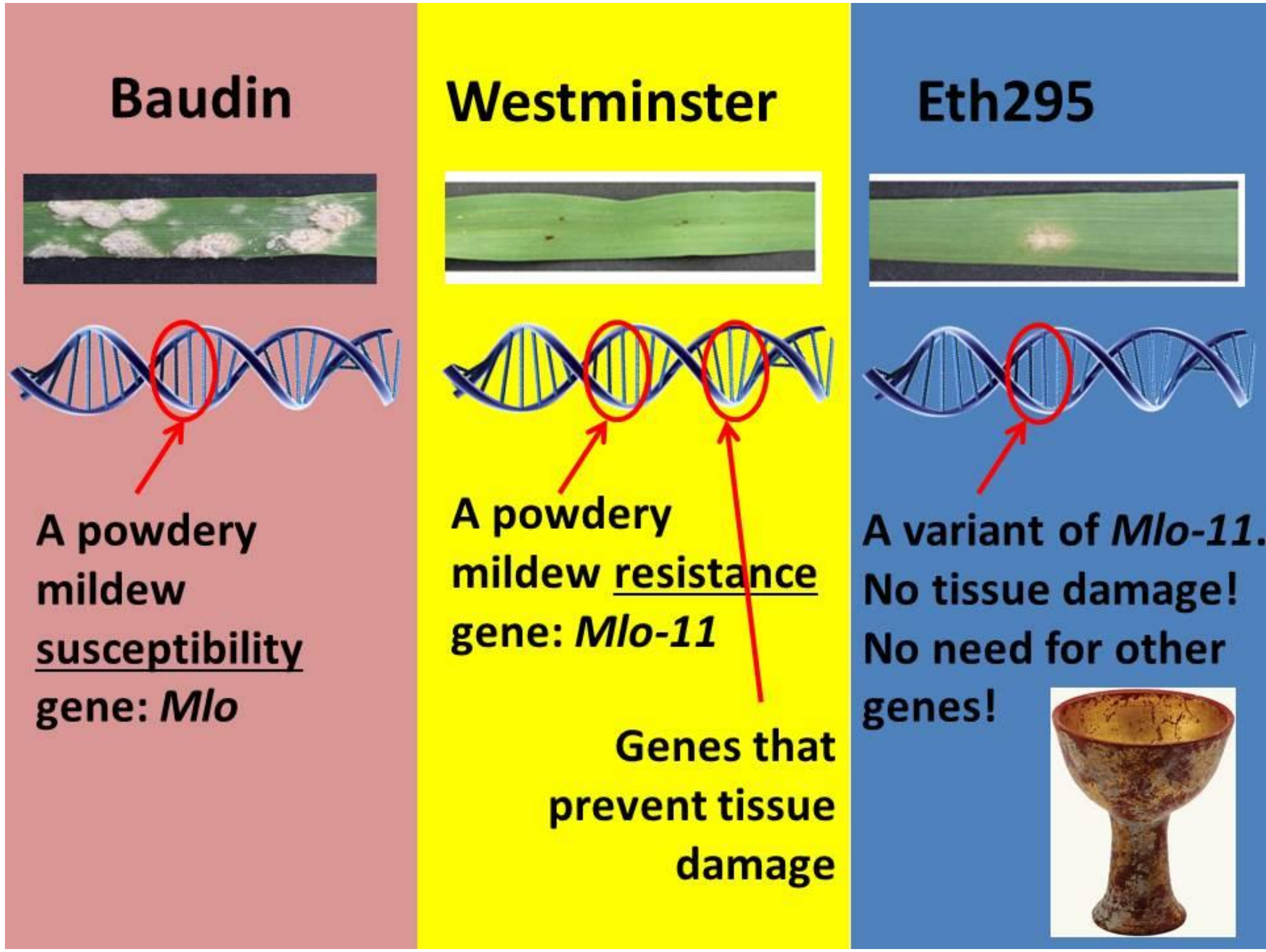
e.g. Potato

- Beyond the yield gap, there are further environmental problems jeopardizing SDG 15 caused by organic farming. Especially in organic potato and horticultural production, **toxic copper-based pesticides are widely used to control fungal diseases**. Furthermore, a few relevant insect pests in organic farming can only be controlled with certain broad-spectrum biological insecticides that are known to also harm honeybees and other nontarget organisms.

Can you grow organic potatoes without copper?

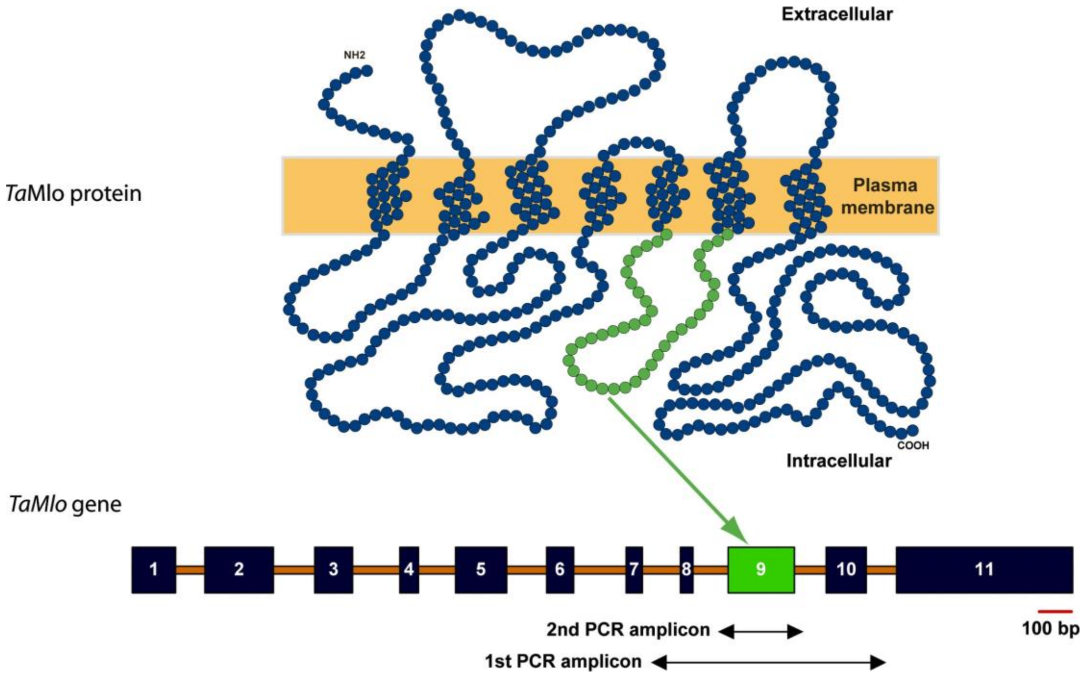
Mlo allele

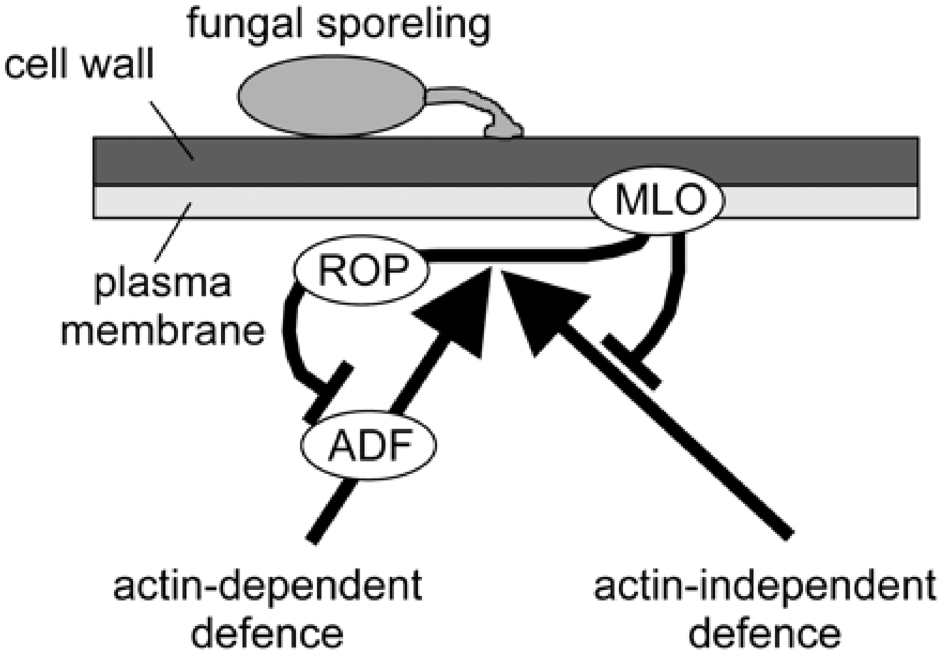
- An example is the Mlo gene, which confers durable resistance to powdery mildew in barley. The recessive resistance allele mlo is a loss-of-function variant discovered decades ago in a landrace and has been widely used in barley breeding ever since . Generating corresponding mlo alleles with genome editing techniques in species such as wheat, tomato, grape, and other crops achieves comparable disease resistance



Mlo allele

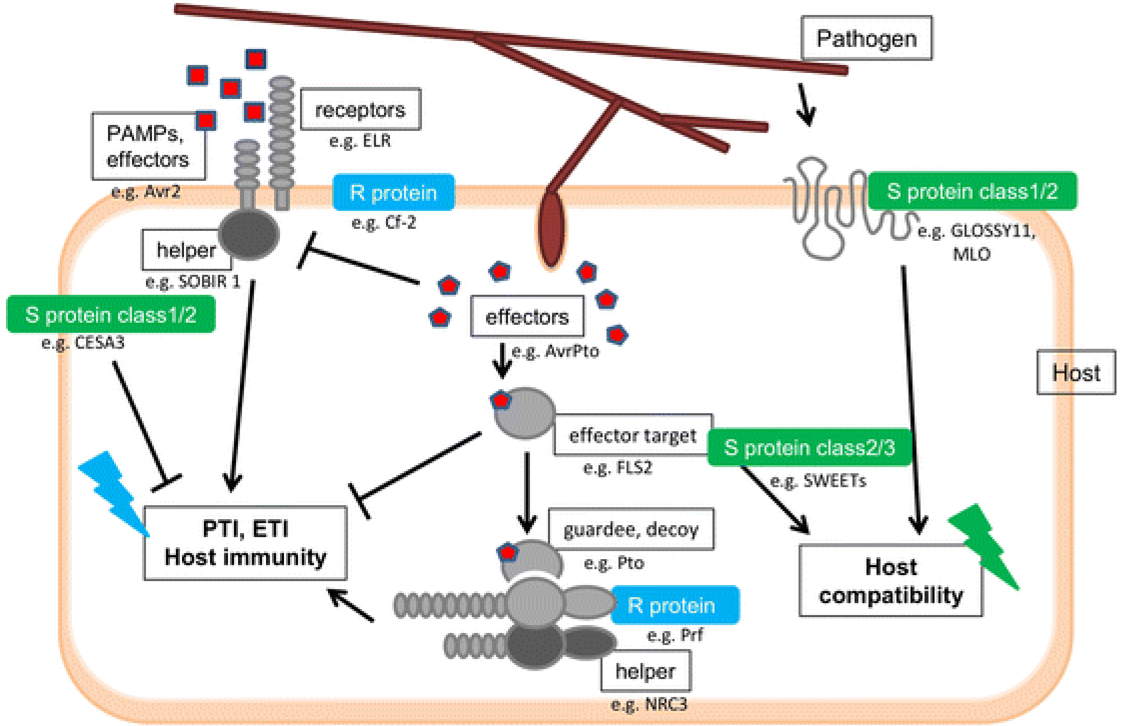
- Barley (Hordeum vulgare) HvMlo and Arabidopsis AtMLO2 encode members of a family of plant-specific integral membrane proteins with seven membrane-spanning domains
- HvMLO and AtMLO2 are potentially targeted for pathogenesis by family members of the Erysiphales, common ascomycete pathogens that represent the causal agents of the powdery mildew disease in plants



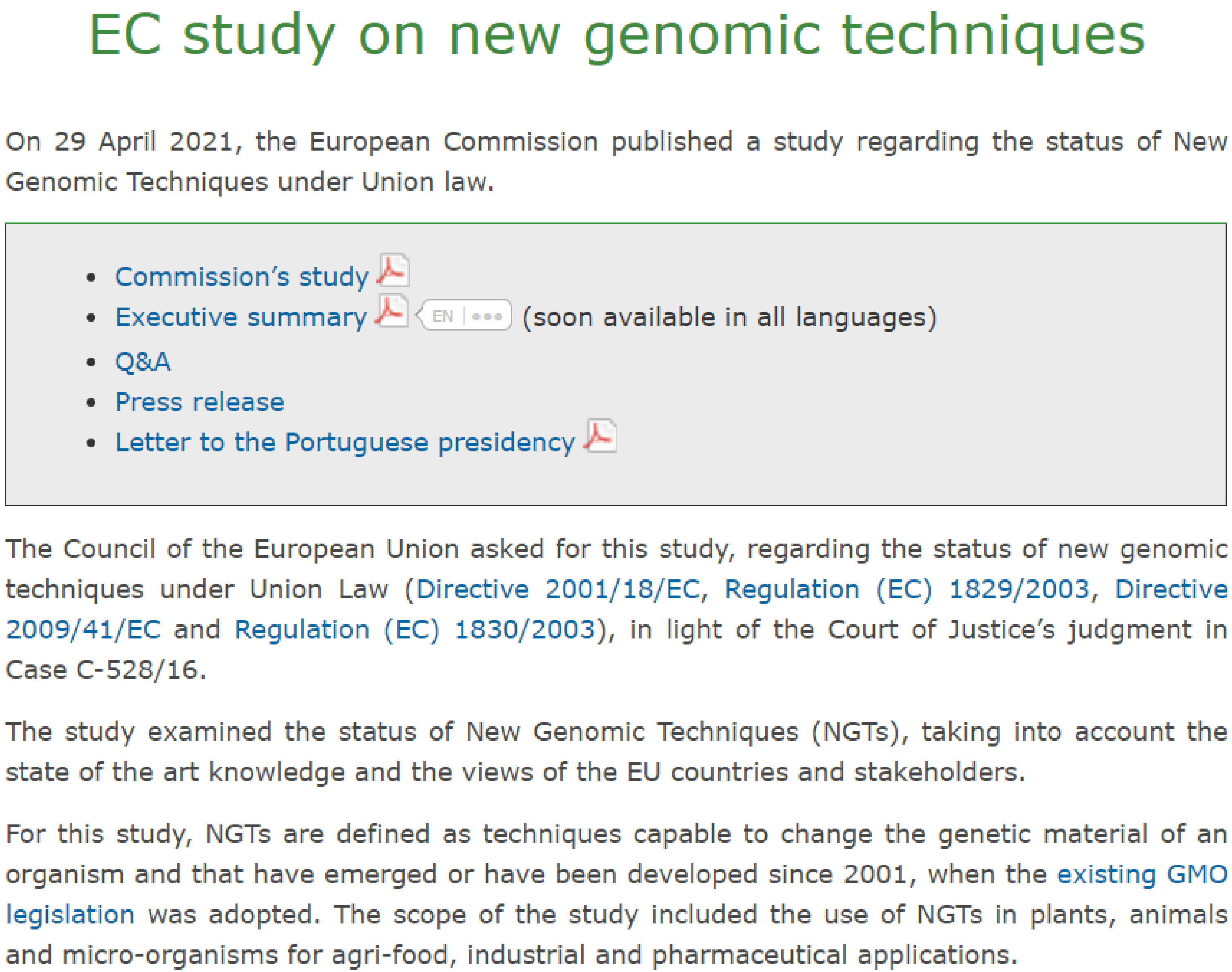


SWEET suger exporters

- Similarly, broad-spectrum resistance to bacterial blight in rice, an important disease in Asian and African countries, was successfully engineered by changing only a few bases in the promoters of genes encoding SWEET proteins. The pathogen can no longer activate expression of these sugar exporters and thus lacks the extracellular nutrient supply essential for its virulence. Many more examples of pest and disease resistance through gene editing exist



https://ec.europa.eu/food/plant/gmo/modern_biotech/new-genomic-techniques_en



EC study on new genomic techniques

On 29 April 2021, the European Commission published a study regarding the status of New Genomic Techniques under Union law.

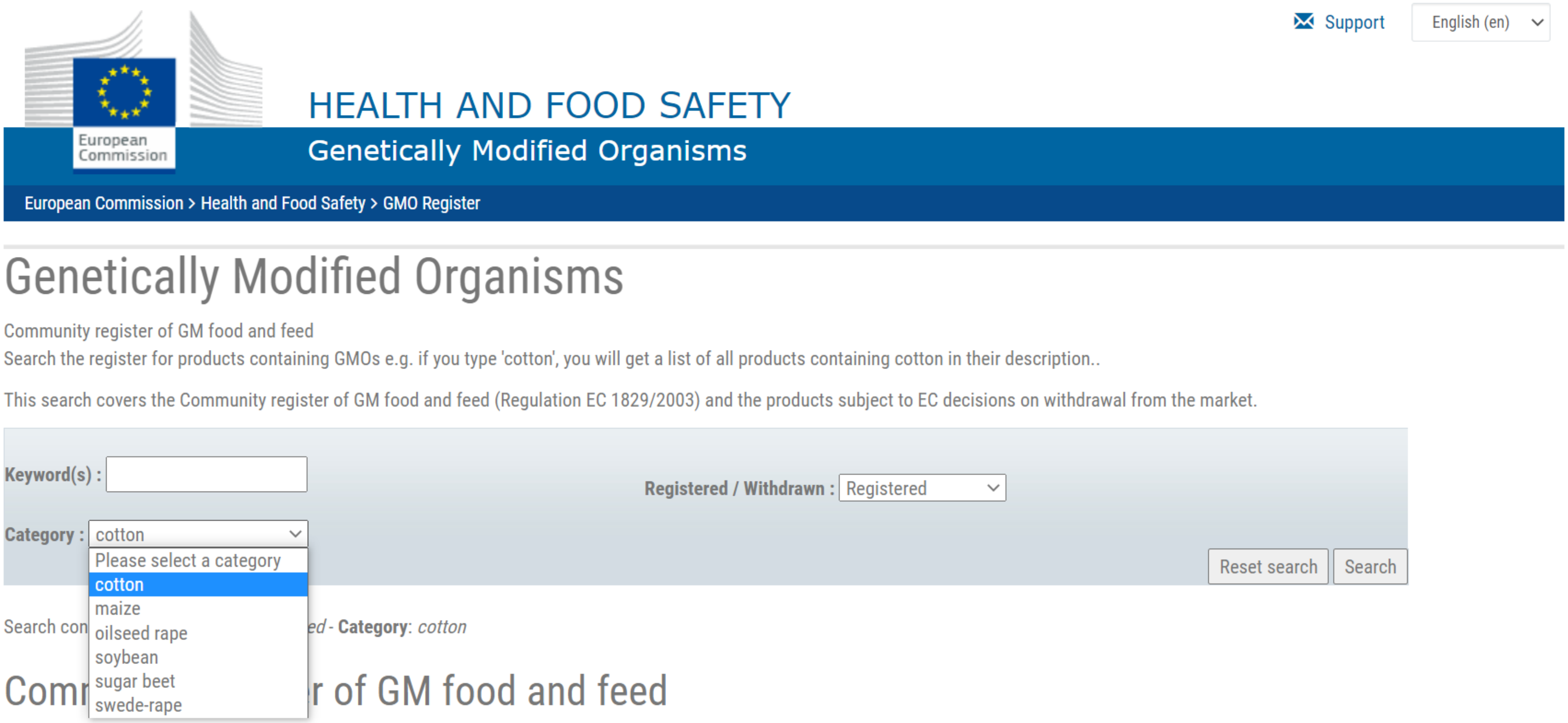
- Commission's study
- Executive summary (soon available in all languages)
- Q&A
- Press release
- Letter to the Portuguese presidency

The Council of the European Union asked for this study, regarding the status of new genomic techniques under Union Law (Directive 2001/18/EC, Regulation (EC) 1829/2003, Directive 2009/41/EC and Regulation (EC) 1830/2003), in light of the Court of Justice's judgment in Case C-528/16.

The study examined the status of New Genomic Techniques (NGTs), taking into account the state of the art knowledge and the views of the EU countries and stakeholders.

For this study, NGTs are defined as techniques capable to change the genetic material of an organism and that have emerged or have been developed since 2001, when the existing GMO legislation was adopted. The scope of the study included the use of NGTs in plants, animals and micro-organisms for agri-food, industrial and pharmaceutical applications.

https://webgate.ec.europa.eu/dyna/gm_register/index_en.cfm



Genetically Modified Organisms

Community register of GM food and feed
Search the register for products containing GMOs e.g. if you type 'cotton', you will get a list of all products containing cotton in their description..

This search covers the Community register of GM food and feed (Regulation EC 1829/2003) and the products subject to EC decisions on withdrawal from the market.

Keyword(s) :

Registered / Withdrawn : Registered

Category : cotton

Search con ed - **Category**: *cotton*

Com r of GM food and feed

EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

EXECUTIVE SUMMARY

COMMISSION STAFF WORKING DOCUMENT

Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16

SWD(2021) 92

The Council of the European Union[1] asked the Commission to submit, by 30 April 2021, *a study in light of the Court of Justice's judgment in Case C-528/16 regarding the status of* ***new genomic techniques*** *under Union law.* It also asked the Commission to submit a proposal accompanied by an impact assessment, if appropriate in view of the outcomes of the study, or otherwise to inform it of other measures required as a follow-up to the study.

For this study, 'new genomic techniques' (NGTs) are defined as techniques that are capable of altering the genetic material of an organism and that have emerged or have been developed since 2001, when the current legislation on genetically modified organisms (GMOs) was adopted. Information and views on the status and use of new genomic techniques in plants, animals and micro-organisms for agri-food, industrial and pharmaceutical applications were gathered from Member States and EU-level stakeholders via a targeted consultation. The study was further supported by expert contributions[2] on specific aspects regarding safety, testing methods and technological and market developments.

The study makes it clear that organisms obtained through new genomic techniques are subject to the GMO legislation. However, developments in biotechnology, combined with a lack of definitions (or clarity as to the meaning) of key terms, are still giving rise to ambiguity in the interpretation of some concepts, potentially leading to regulatory uncertainty.

nature food

PERSPECTIVE

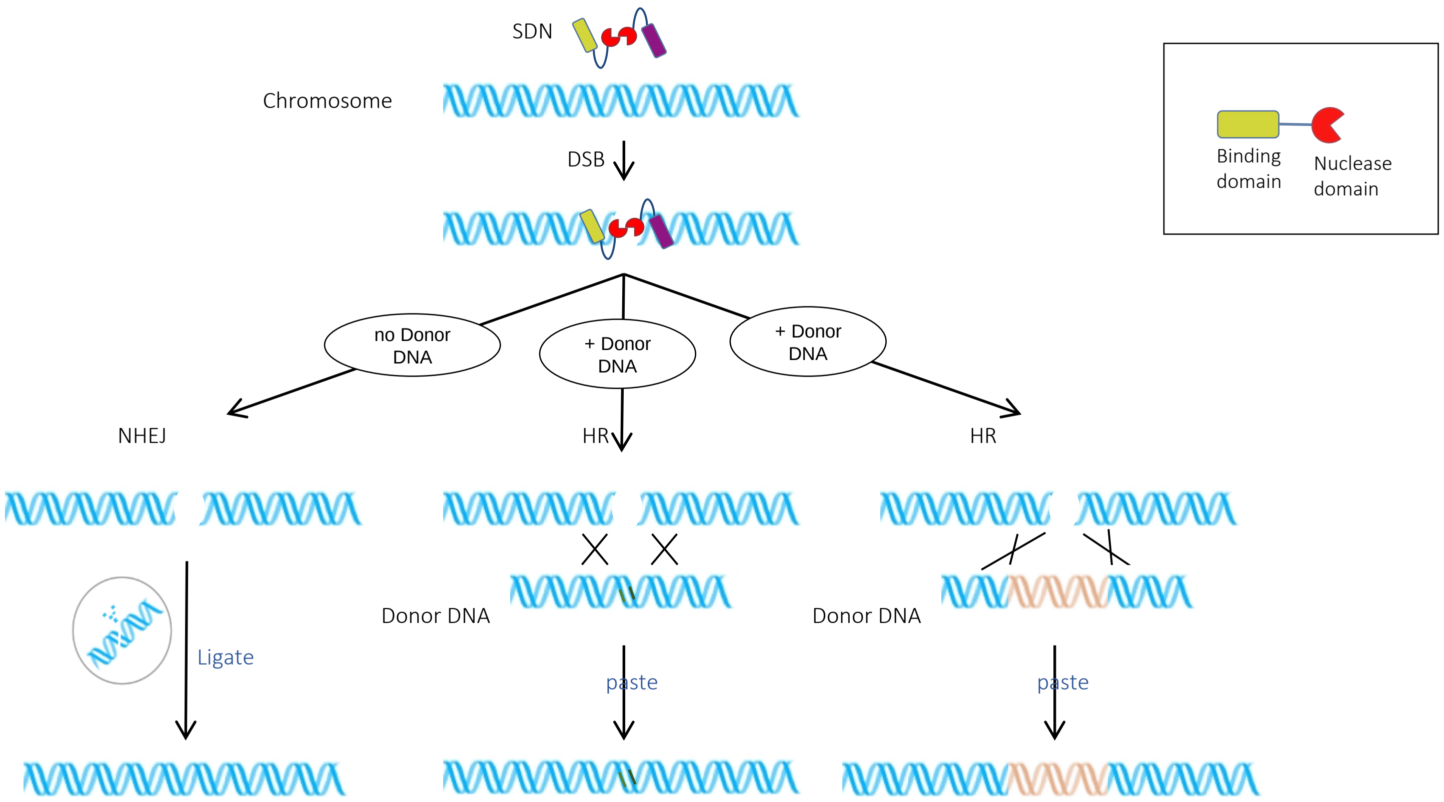
https://doi.org/10.1038/s43016-020-0051-8

A CRISPR way for accelerating improvement of food crops

Yi Zhang[1], Mathias Pribil[1], Michael Palmgren[2] ✉ and Caixia Gao[3,4] ✉

CRISPR technology, which is widely used for plant genome editing, will accelerate the breeding of food crops beyond what was imaginable before its development. Here we provide a brief overview of CRISPR technology, its most important applications for crop improvement and several technological breakthroughs. We also make predictions of the applications of CRISPR technology to food crops, which we believe would provide the potential for synthetic biology and domestication of crops. We also discuss the implications of regulatory policy for deployment of the technology in the developing world.

What about site directed nucleases?



Random repair with gain or loss of base pairs
SDN1
Gene off

Gene modification at one or more positions
SDN2
Gene edit

DNA insertion
SDN3
Add gene

regulations covering GMOs[11]. Early in 2017, the USDA proposed a rule for regulating gene-edited crops: products that contain deletions of any size (SDN-1), or single base-pair substitutions (SDN-2) would be exempt from regulation[44].

The European Union likewise has a process-based regulation and following a decision by the European Court of Justice on 25 July 2018: any use of CRISPR technology to modify a plant will result in a product being classified as a GMO[49]. This ruling was anticipated as nucleic acid sgRNA molecules will always be required when using CRISPR. A new political decision by the European Commission will be required before genome-edited crops can be exempted from being classified as GMOs in the European Union.

Argentina also employs product-based regulation and offers a good example of national legislation on plant breeding innovations. In 2015, the country issued a regulation for products of 'New Breeding Techniques' and provided regulatory criteria for gene-edited crops[45]. In 2018, Argentina established a regulatory classification for gene-edited crops: products generated by SDN-1 are not GMO; no regulatory criteria were issued for those generated by SDN-2; crops modified by SDN-3 were classified as GMOs[46].