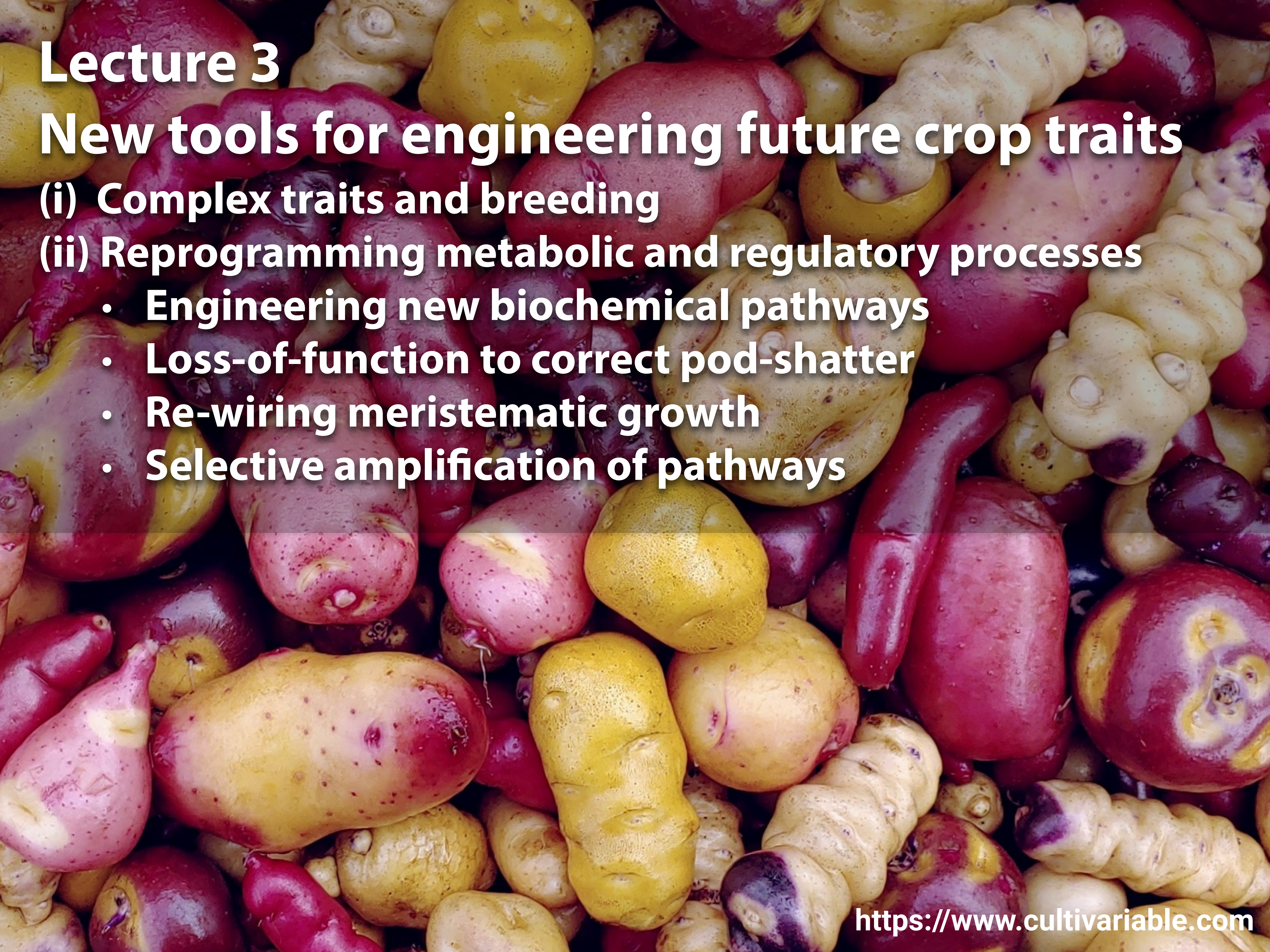


Lecture 3

New tools for engineering future crop traits

(i) Complex traits and breeding

(ii) Reprogramming metabolic and regulatory processes

- Engineering new biochemical pathways
- Loss-of-function to correct pod-shatter
- Re-wiring meristematic growth
- Selective amplification of pathways

https://www.cultivariable.com

Wild watermelon

Originated in North Africa, used as a primitive water carrier. Selection for sweeter taste was linked to pink colour of the flesh.

Modern watermelon

Over time, humans have bred watermelons to have a **bright red**, juicy interior. The **seeds are often removed** by preventing the plants from being fertilized by pollination.

Wild banana

The first bananas may have been cultivated at least **7,000 years ago** in what is now Papua New Guinea, and were **stocky and hard,** with large, tough **seeds** throughout the fruit's interior.

Modern banana

Today's tastier bananas are **hybrids** of two wild banana varieties, **Musa acuminata** and **Musa balbisiana.**

Wild eggplant

Eggplants once came in a wide array of shapes and colors, from **blue to yellow,** and some were **round** rather than oblong. Primitive eggplant varieties had a **spine** where the modern plant's stem connects to its flowers.

Modern eggplant

Selective breeding has made the **spine disappear** and left us with the **oblong purple** vegetable we're familiar with.

Wild carrot

The first carrots were likely cultivated around the 10th century in Asia Minor and were either **white or purple** with thin, forked roots and a **strong flavor.**

Modern carrot

Carrots today are large, **bright orange,** and tasty.

Cabbage

Brussels sprouts

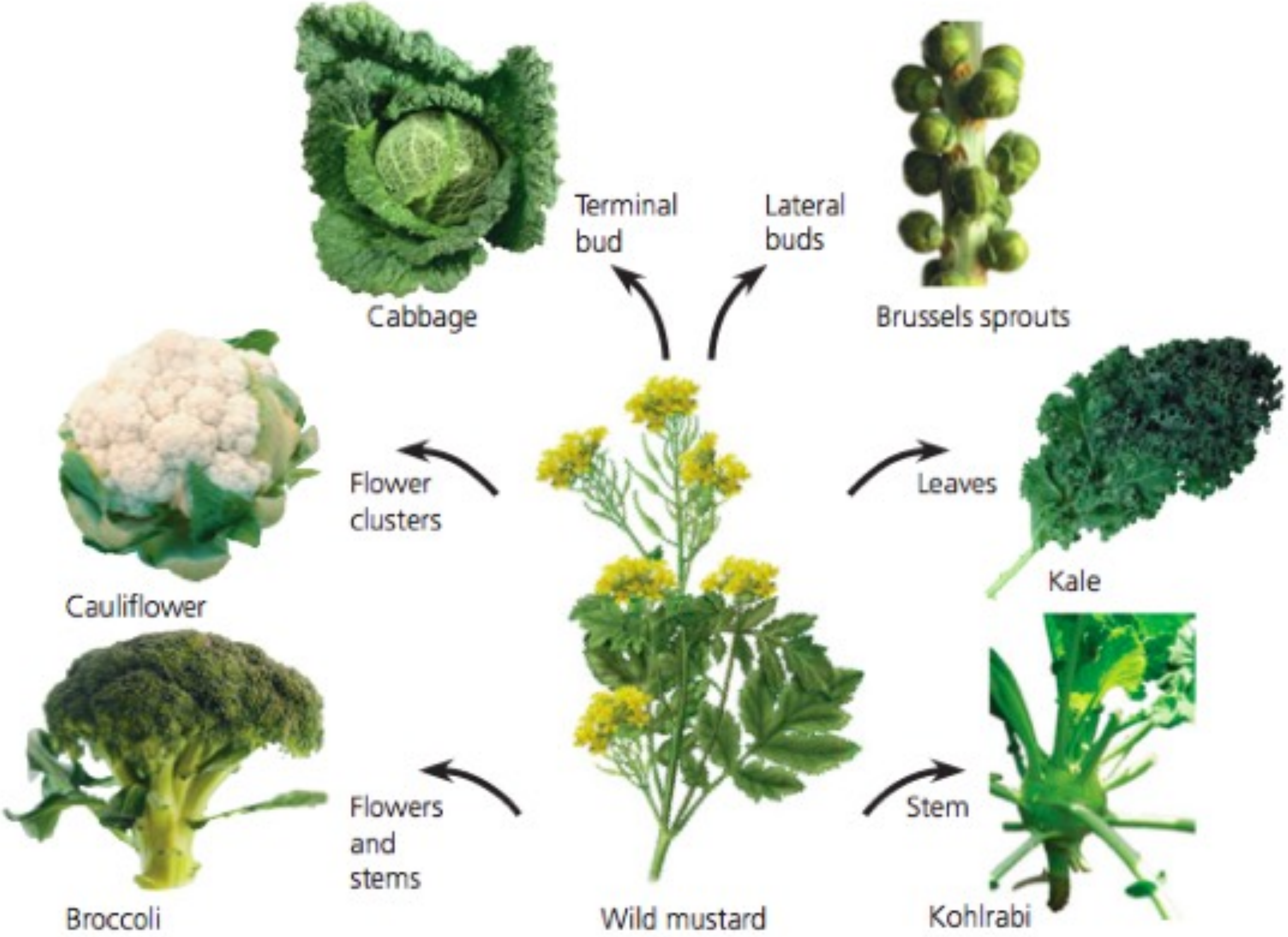
Cauliflower

Are these plants related?

Kale

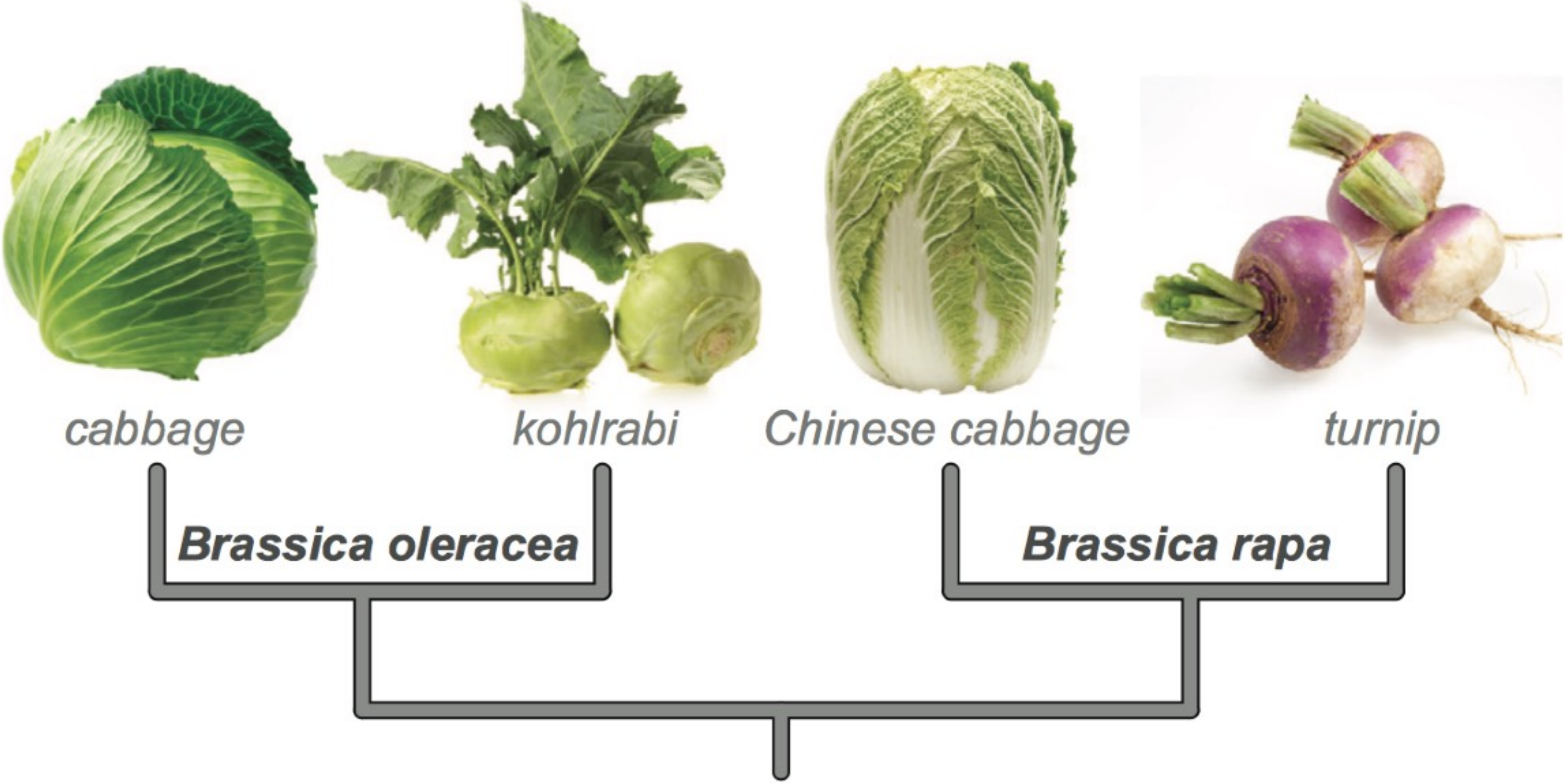
Broccoli

Kohlrabi



Crops derived from wild mustard (*Brassica oleracea*)

Convergent phenotypic changes during domestication



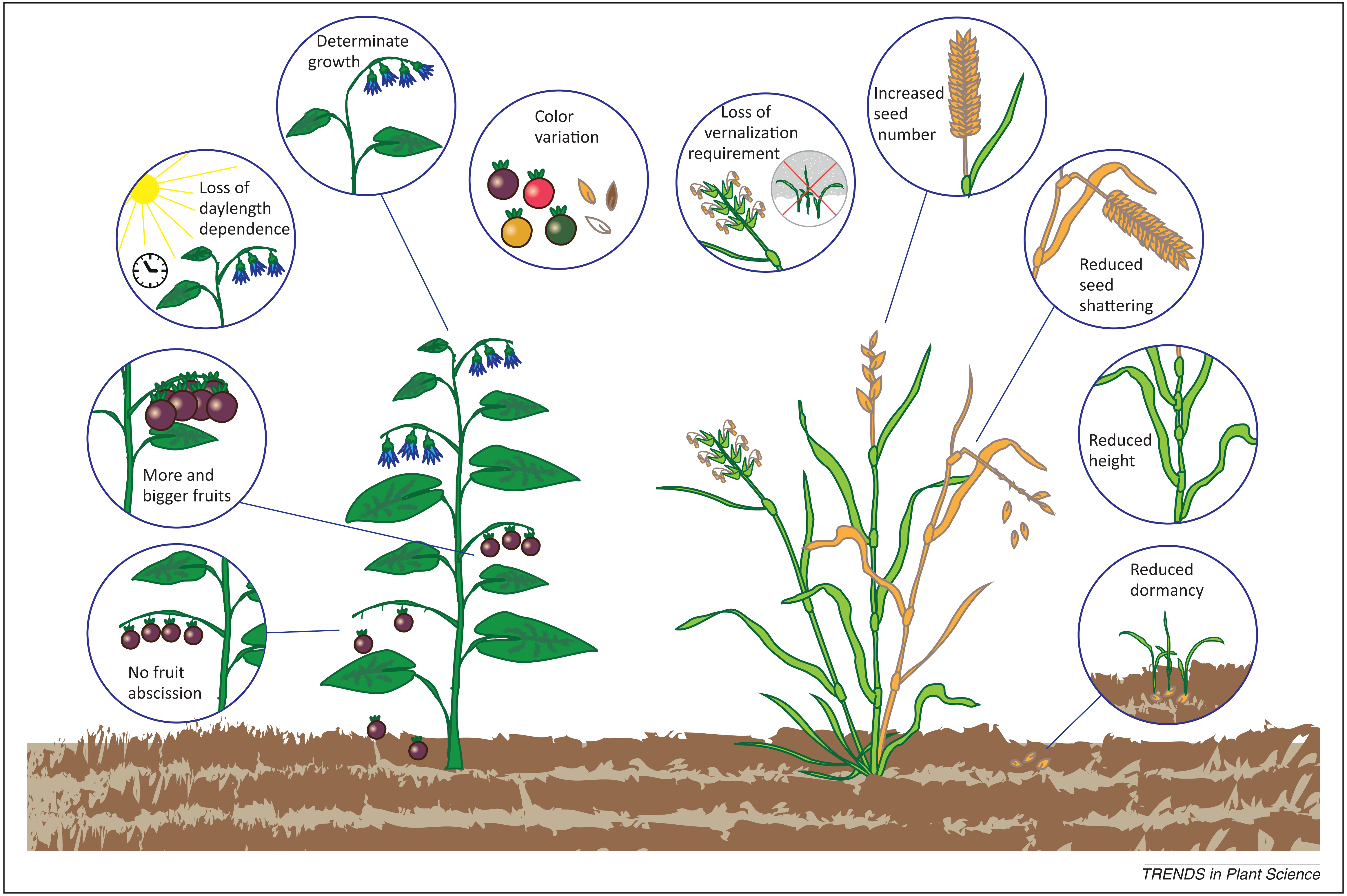


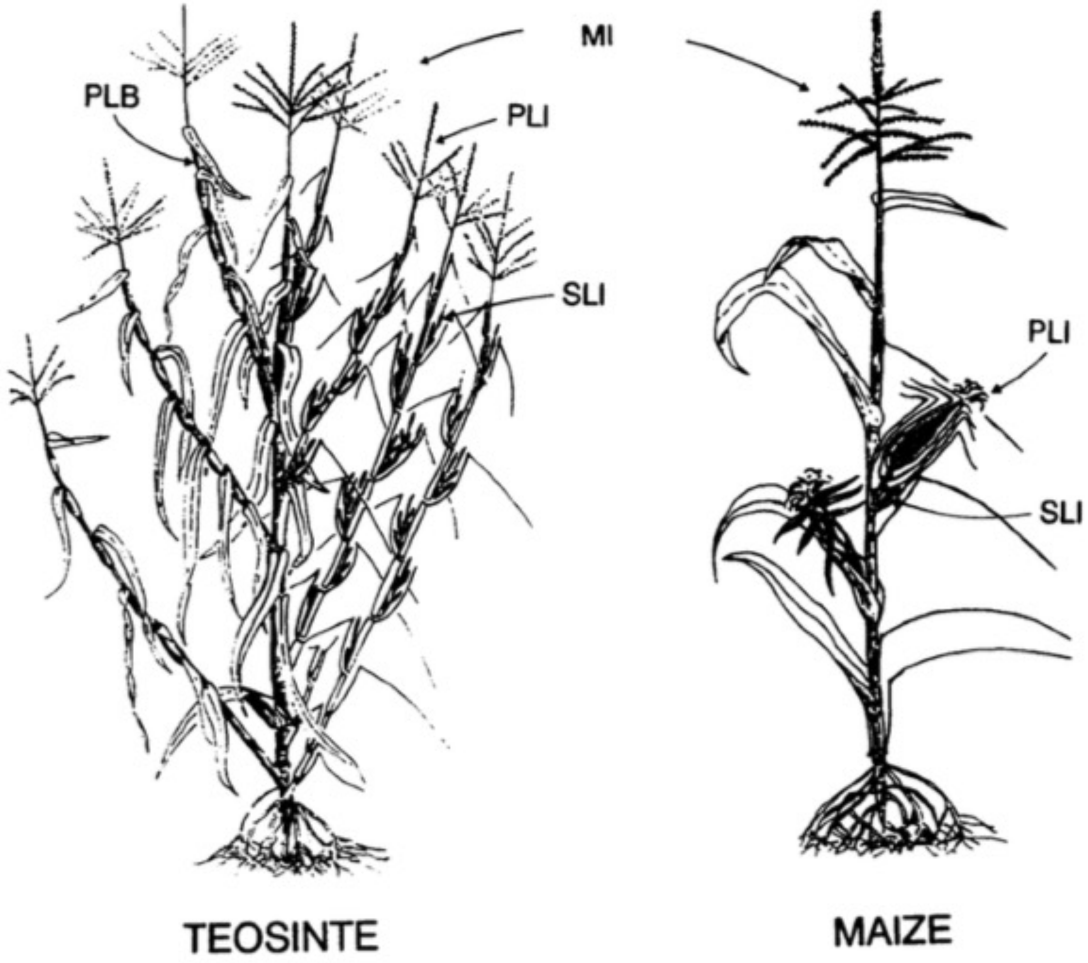
Figure 1. Convergent domestication. Convergent phenotypic changes are frequently observed in many different crops because systematic human cultivation often brings about similar demands. Attempts to maximize yield cause selective pressure for an increase in size and number of edible plant parts on the one hand and for a decrease in natural seed and fruit dispersal mechanisms to reduce yield loss on the other hand. Shifts in cultivation area often require changes in day length dependence or in the vernalization requirement and a reduction in seed dormancy is needed for synchronous germination. Small plants with a determinate growth habit are often selected because they are more robust, have a better yield to overall biomass ratio, and are better suited to mechanical harvesting methods. Finally, satisfying esthetic preferences often drives convergent adaptations, a prominent example being changes in color. Stylized examples of the major angiosperm plant lineages from which current crops originated are shown (eudicot, left; monocot, right) featuring traits of typical wild species. Characters that convergently evolved in various domesticated crops are depicted in circles.

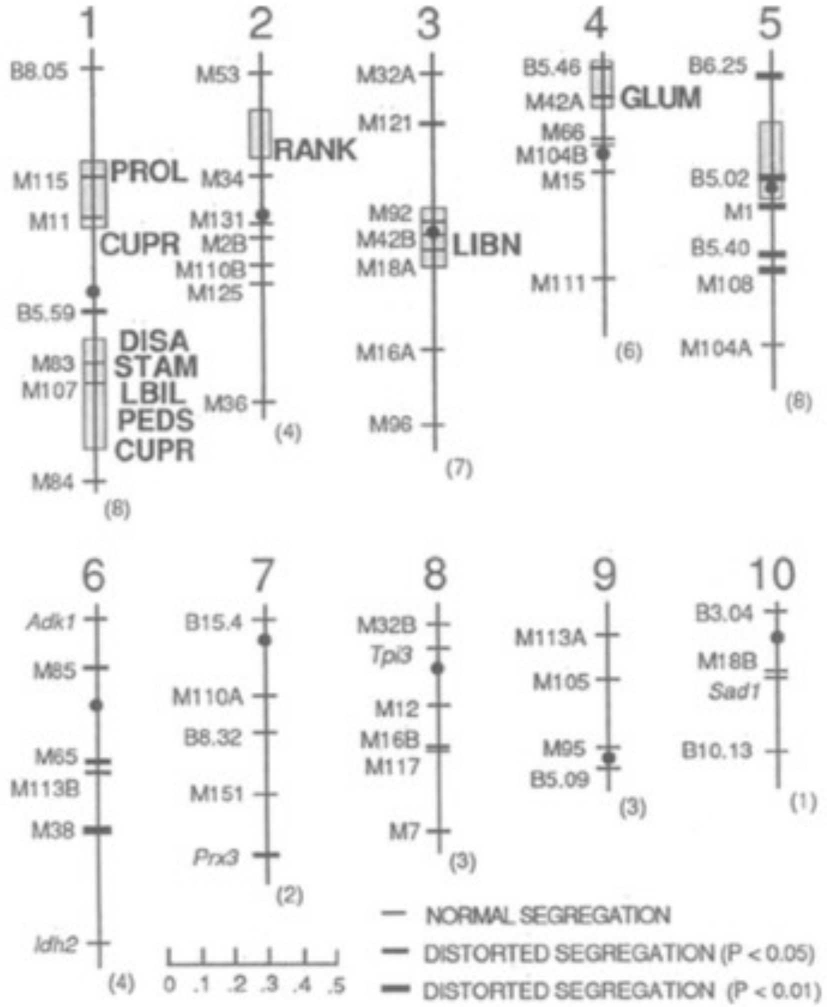
Crop traits

Traits that have been selected for by humans include:

- Determinate growth habit (flowering occurs at the top of the plant, preventing further growth)
- Synchronous ripening, shorter maturity
- Lower content of bitter tasting and harmful compounds
- Reduced sprouting (higher seed dormancy)
- Improved harvest index (the proportion of the plant which is used); larger seed or fruit size
- Elimination of seeds, such as in banana
- Retention of mature seed on the plant (loss of grain shattering)

Many of these traits are multigenic and affect the shape and function of plant tissues and organs. If we want to engineer new crop traits in the future, we will need to understand the way DNA code is able to regulate plant growth and form.





Major differences between maize and teosinte map to few loci

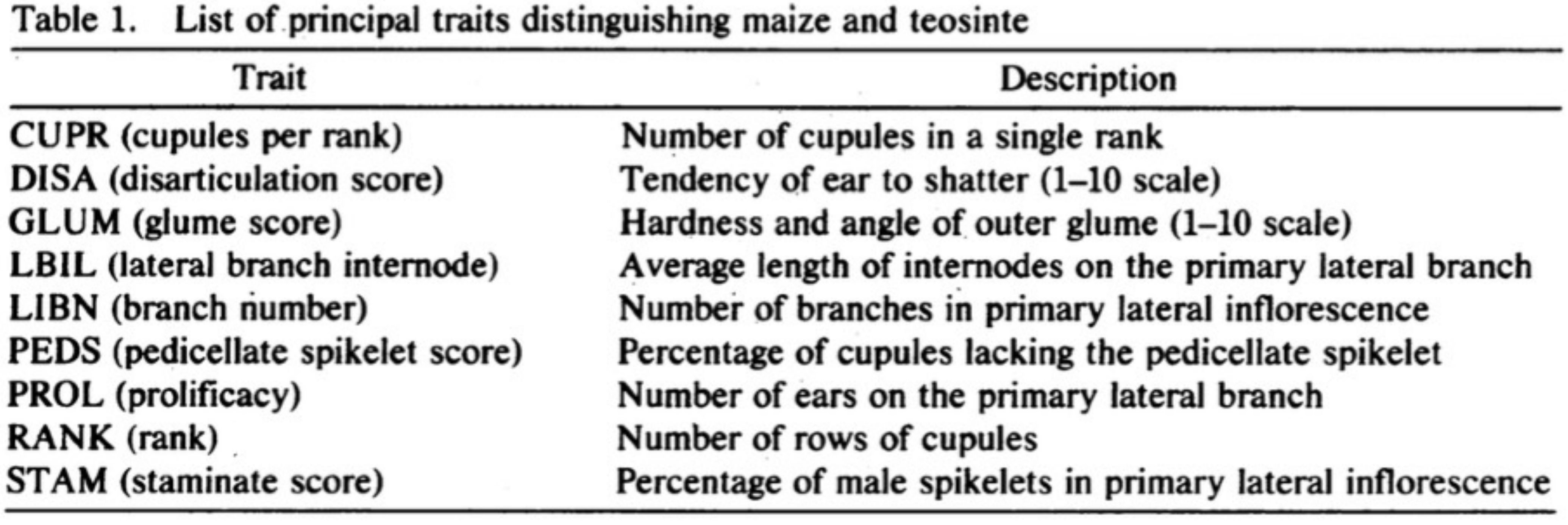
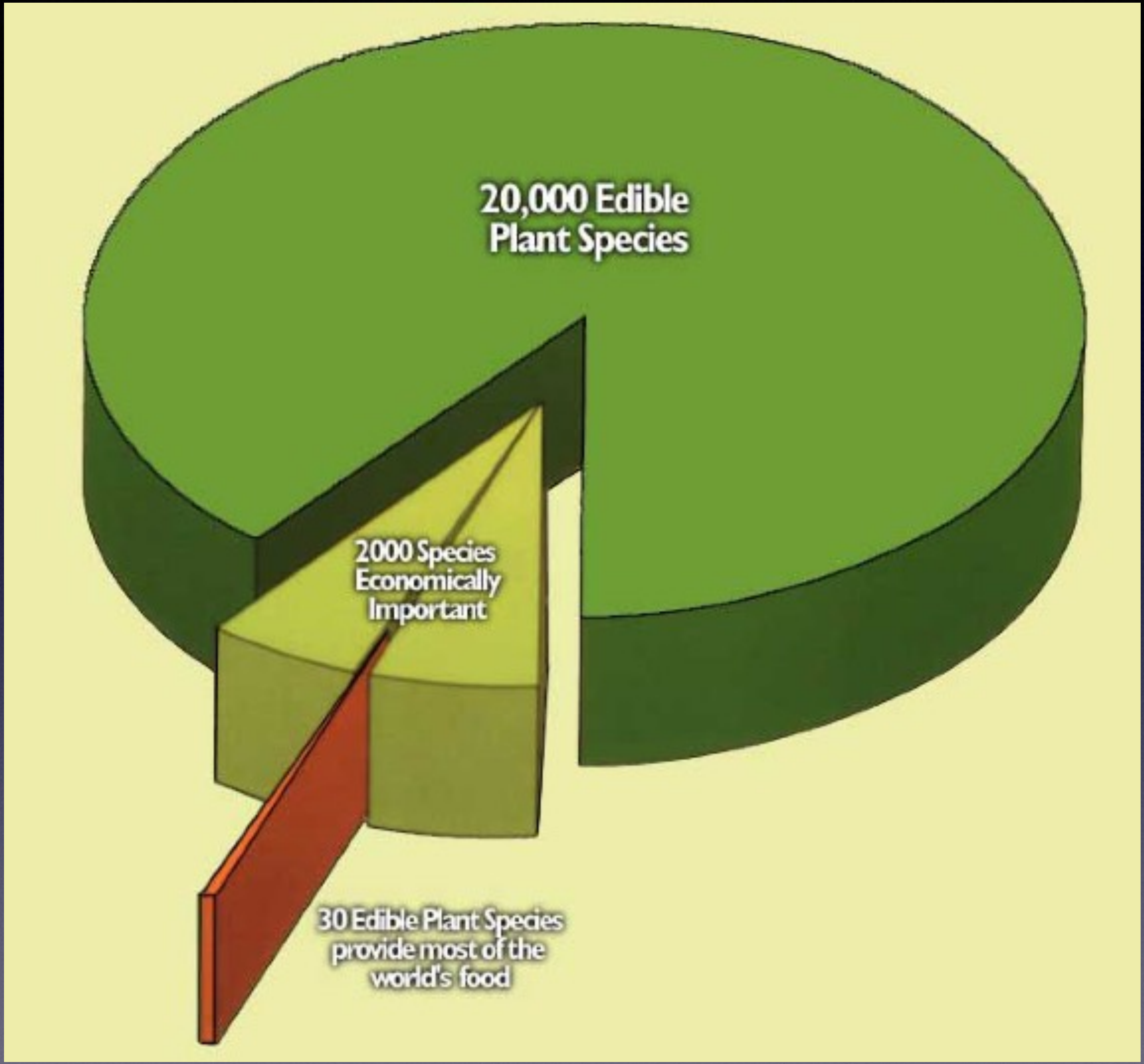


Table 1. List of principal traits distinguishing maize and teosinte

Trait	Description
CUPR (cupules per rank)	Number of cupules in a single rank
DISA (disarticulation score)	Tendency of ear to shatter (1–10 scale)
GLUM (glume score)	Hardness and angle of outer glume (1–10 scale)
LBIL (lateral branch internode)	Average length of internodes on the primary lateral branch
LIBN (branch number)	Number of branches in primary lateral inflorescence
PEDS (pedicellate spikelet score)	Percentage of cupules lacking the pedicellate spikelet
PROL (prolificacy)	Number of ears on the primary lateral branch
RANK (rank)	Number of rows of cupules
STAM (staminate score)	Percentage of male spikelets in primary lateral inflorescence

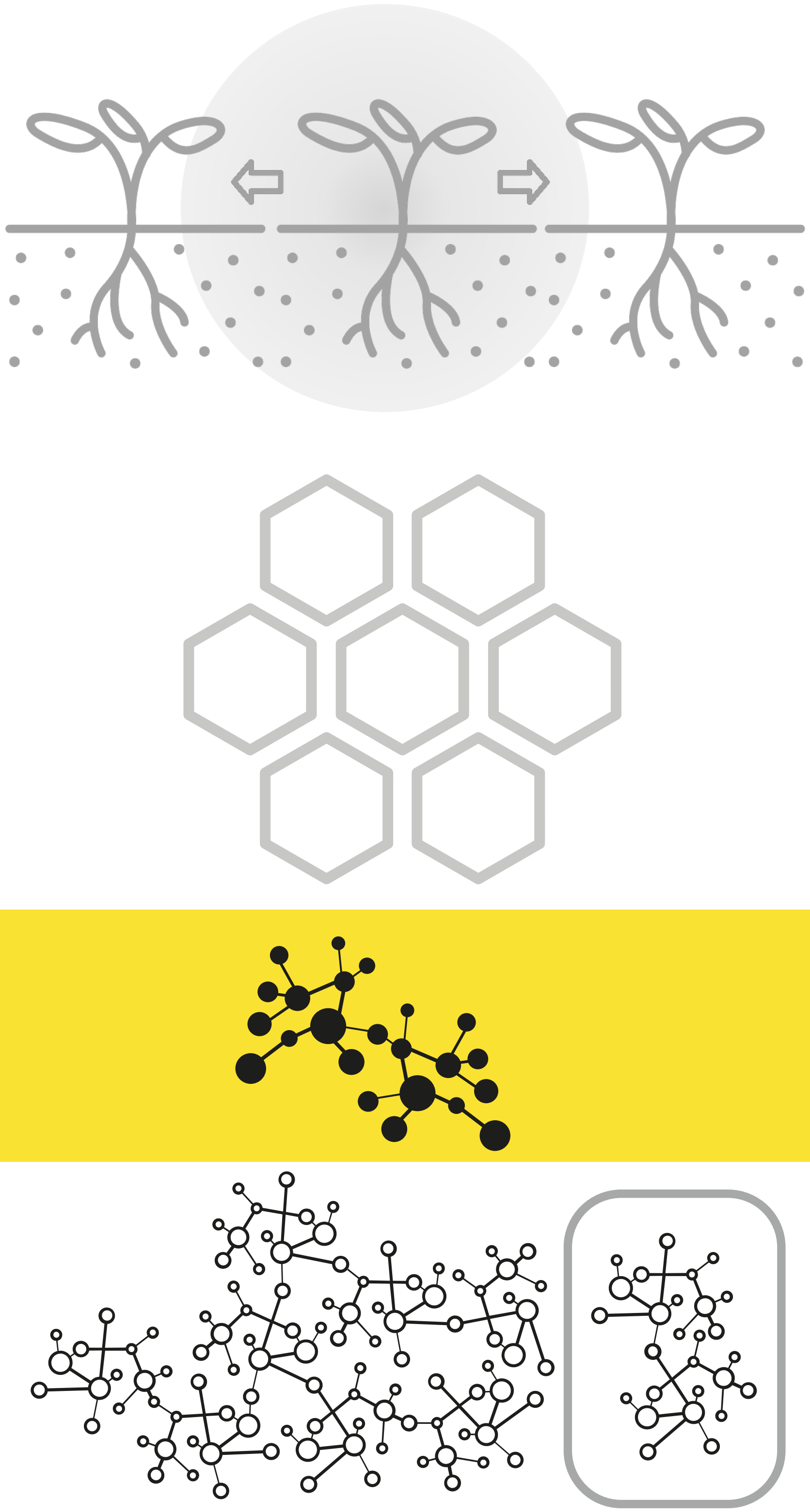
Doebley *et al.*, PNAS (USA) 87: 9888-9892 (1990)

~400,000 plant species (http://www.theplantlist.org)



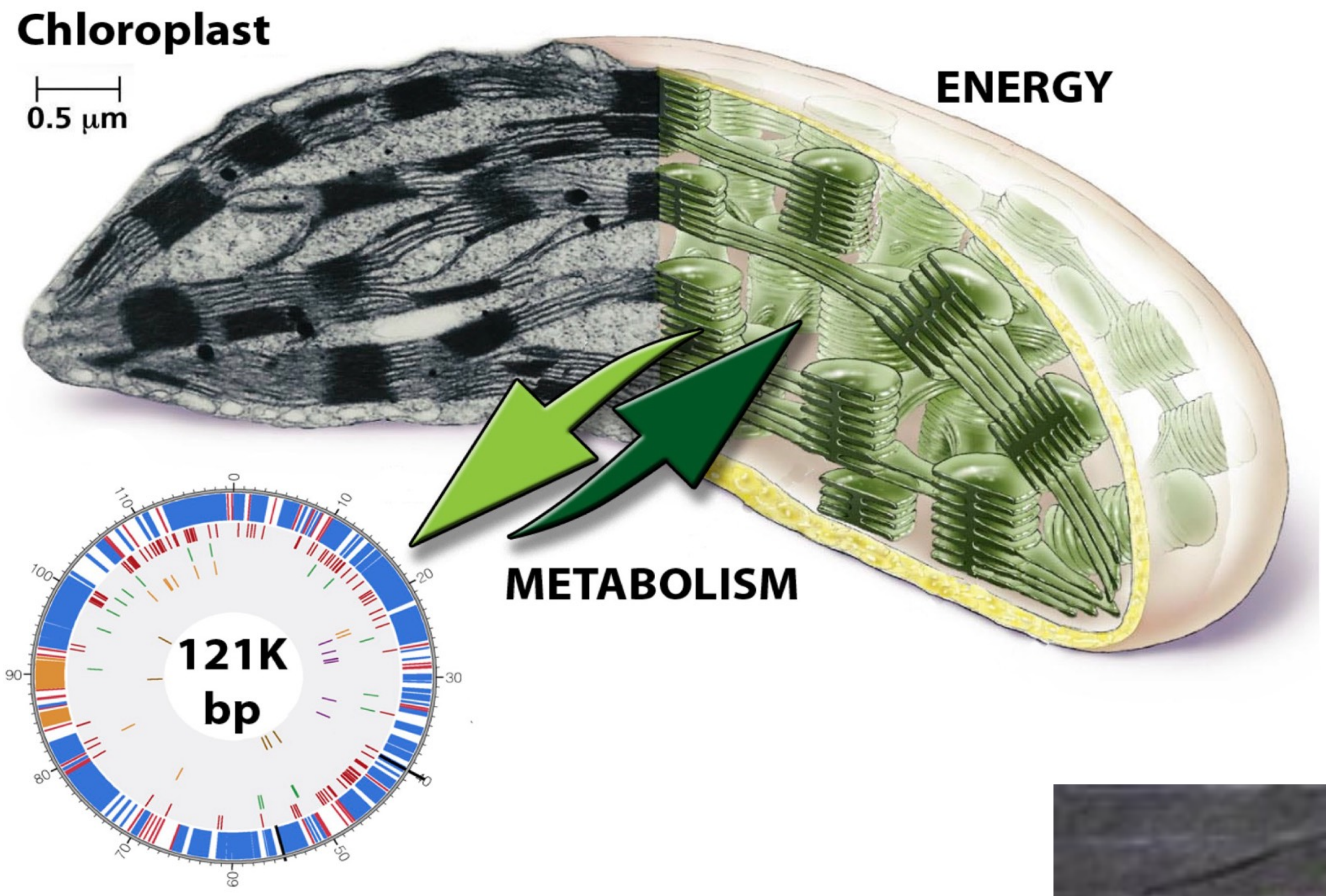
3 crop species (rice, wheat and maize) provide 60% of all calories and 54% of all protein in human food

120 cultivated plant species



biotic interactions	**synthetic ecologies**
organismal physics	**plant cellular forms**
DNA code	**regulatory networks**
chemistry	**engineered metabolism**

Chloroplasts: platform for bioproduction in plants

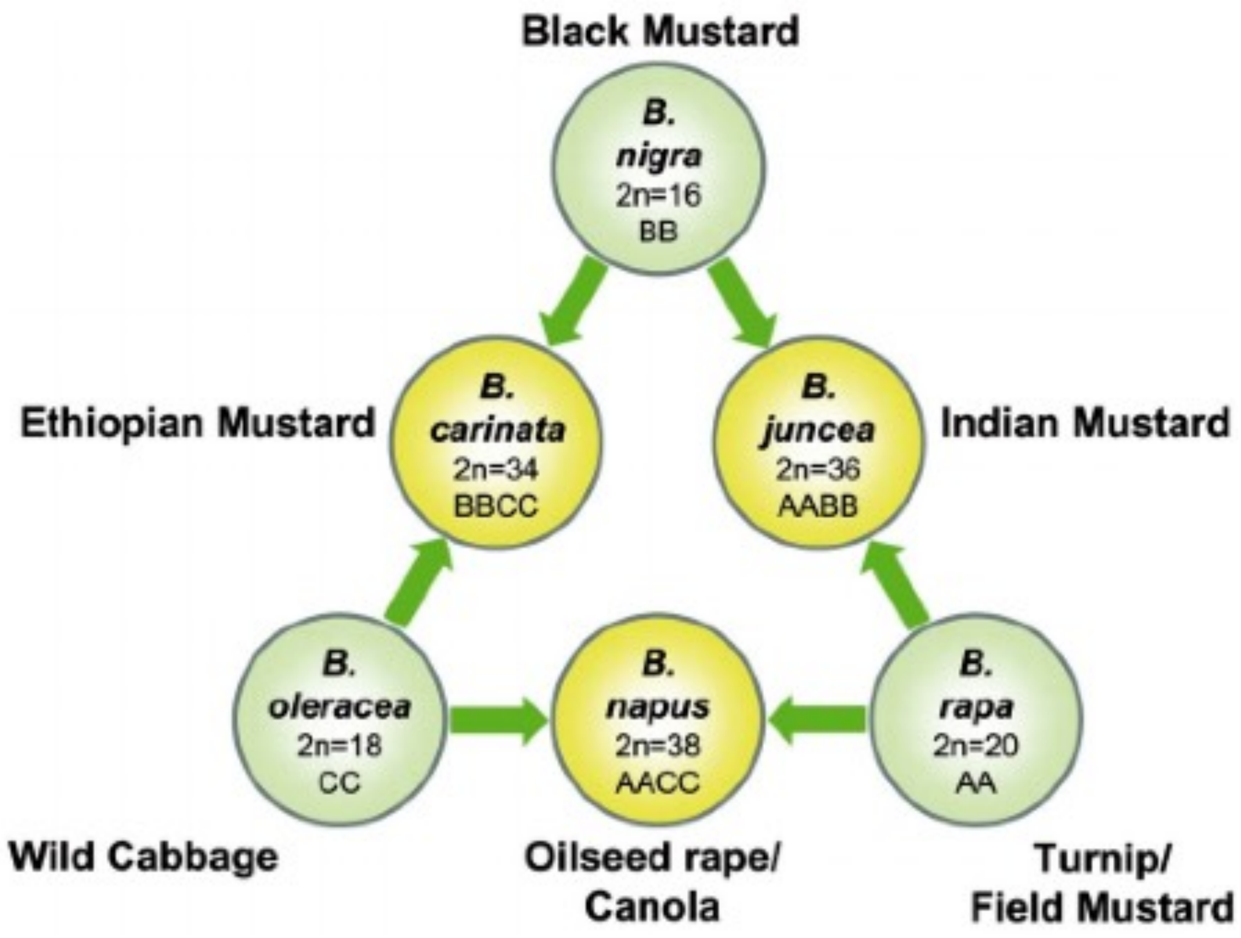


- **Bacteria-like control of gene expression**
- **No gene silencing**
- **High ploidy (~1000 genome copies per cell)**
- **Capable of producing 10-50% of soluble protein from a single gene**
- **Chloroplast genomes highly conserved across the plant**

Chloroplast-based expression of the astaxanthin pathway (Bock lab)

Targeted loss-of-gene-function: correction of pod-shatter through modified cell differentiation and tissue architecture

Brassica napus



Black Mustard

B. nigra
2n=16
BB

Ethiopian Mustard

B. carinata
2n=34
BBCC

B. juncea
2n=36
AABB

Indian Mustard

B. oleracea
2n=18
CC

B. napus
2n=38
AACC

B. rapa
2n=20
AA

Wild Cabbage

Oilseed rape/
Canola

Turnip/
Field Mustard

Oilseed rape and Canola are derived from a cross between *Brassica oleracea* and *Brassica rapa*

Brassica napus seed have a 45% oil content

Crop domestication

An example of a multicellular trait: reduction of seed shatter and improved yield at harvest

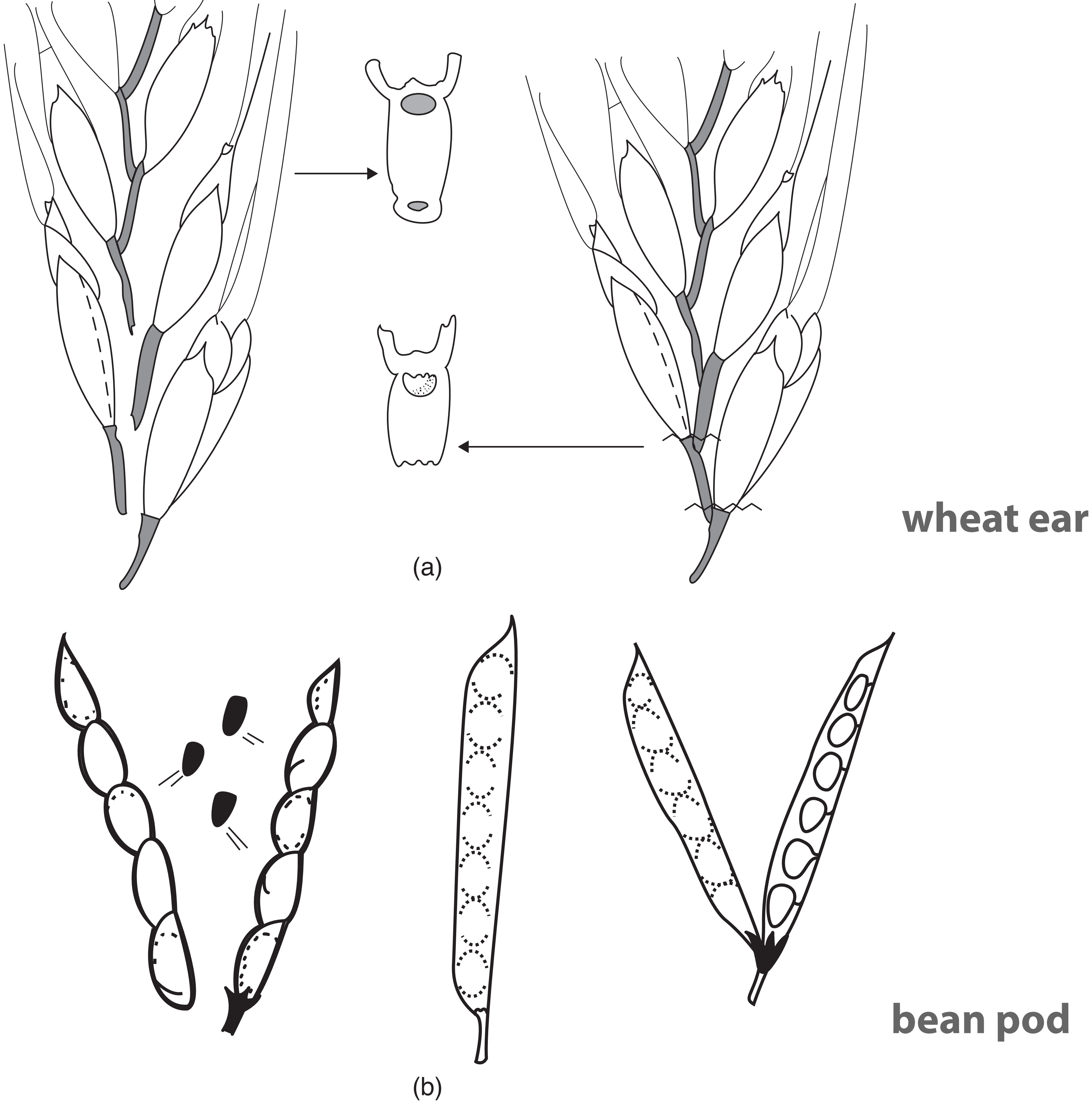
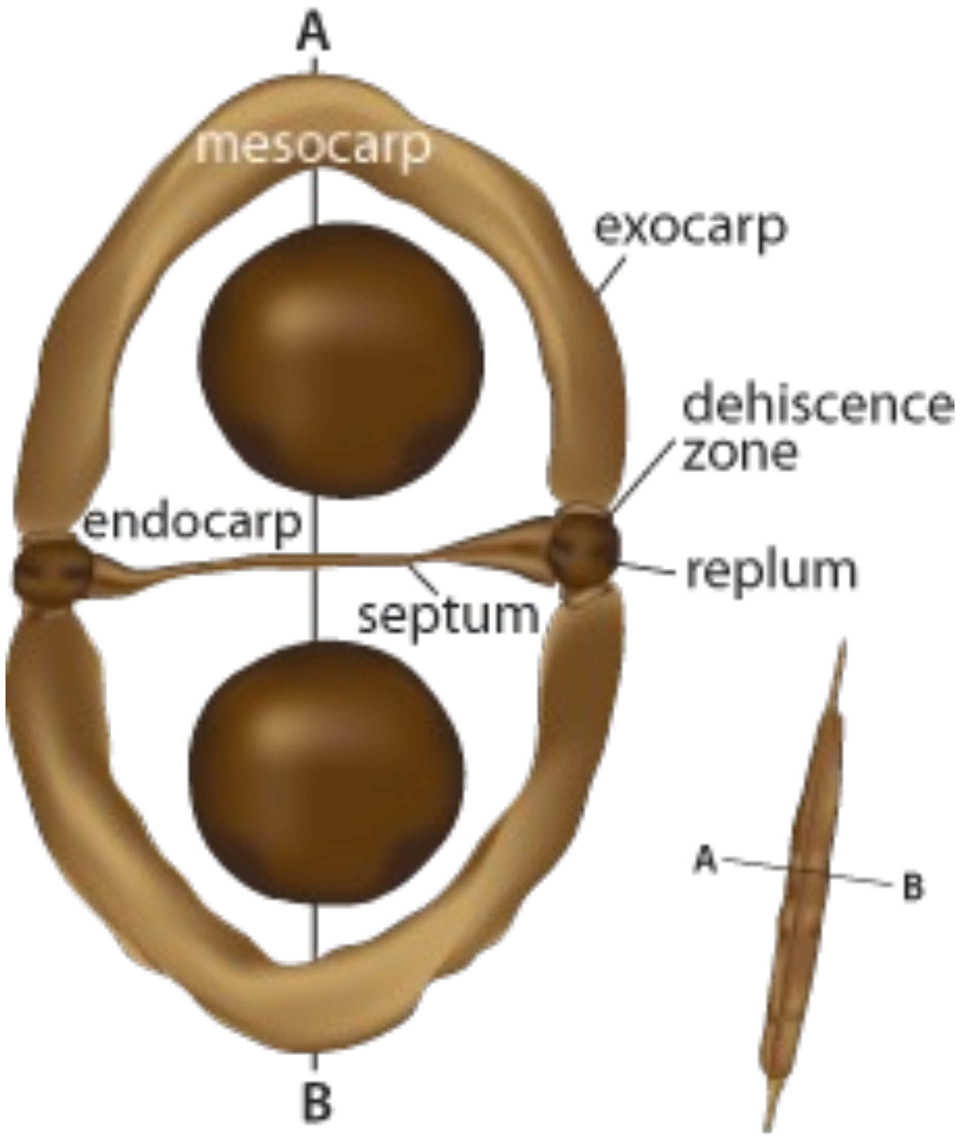
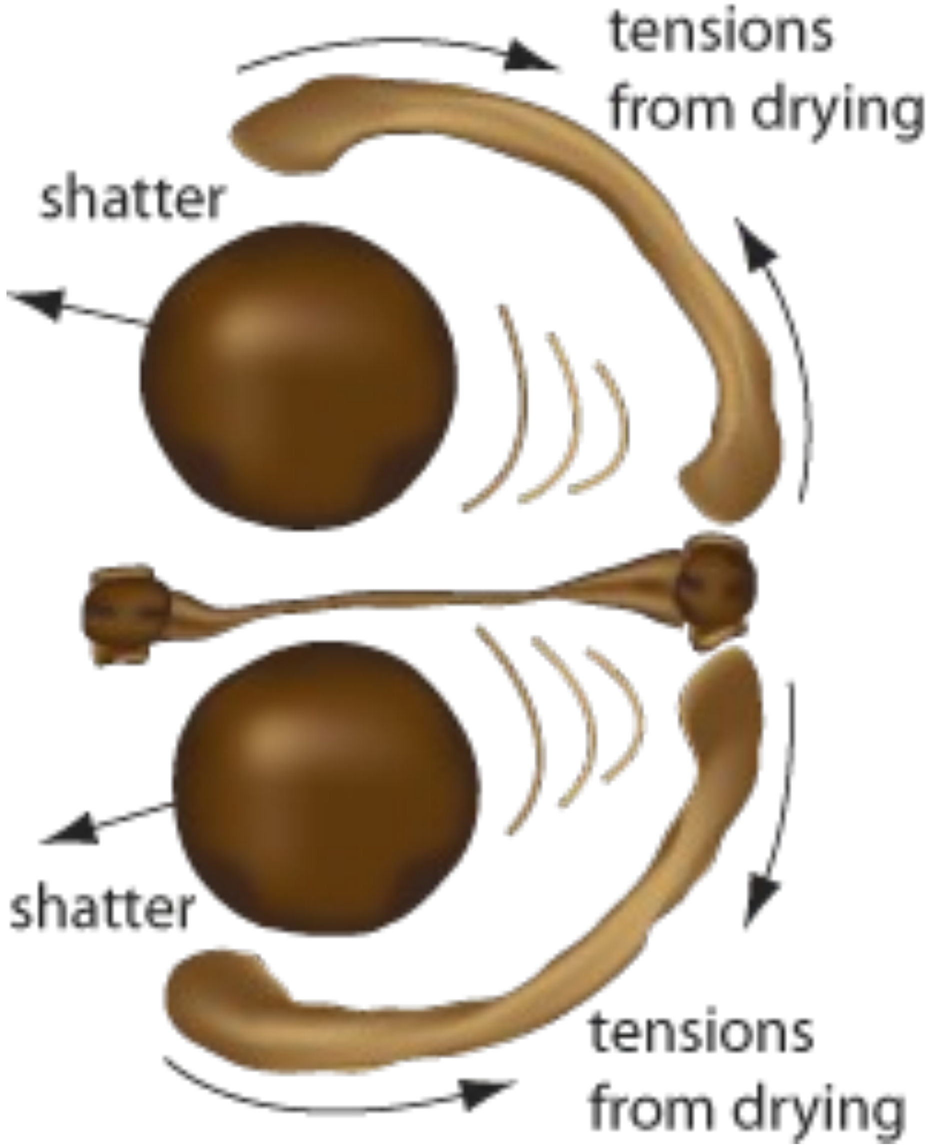


Figure 7.1 Comparisons between wild and domesticated plants in terms of seed dispersal. (a) Comparison between a wild shattering wheat ear (left) and domestic wheat ear with a tough rachis, which requires pounding to break apart (right). The form of rachis segments that can be recovered archaeologically is shown in the middle. (b) Generalized wild bean with pod that twists and opens, dispersing seeds (left) compared with a domestic pod that remains closed (middle) and must be split open by human force (right).

Pod Shatter can result in substantial losses of yield (25-50%)





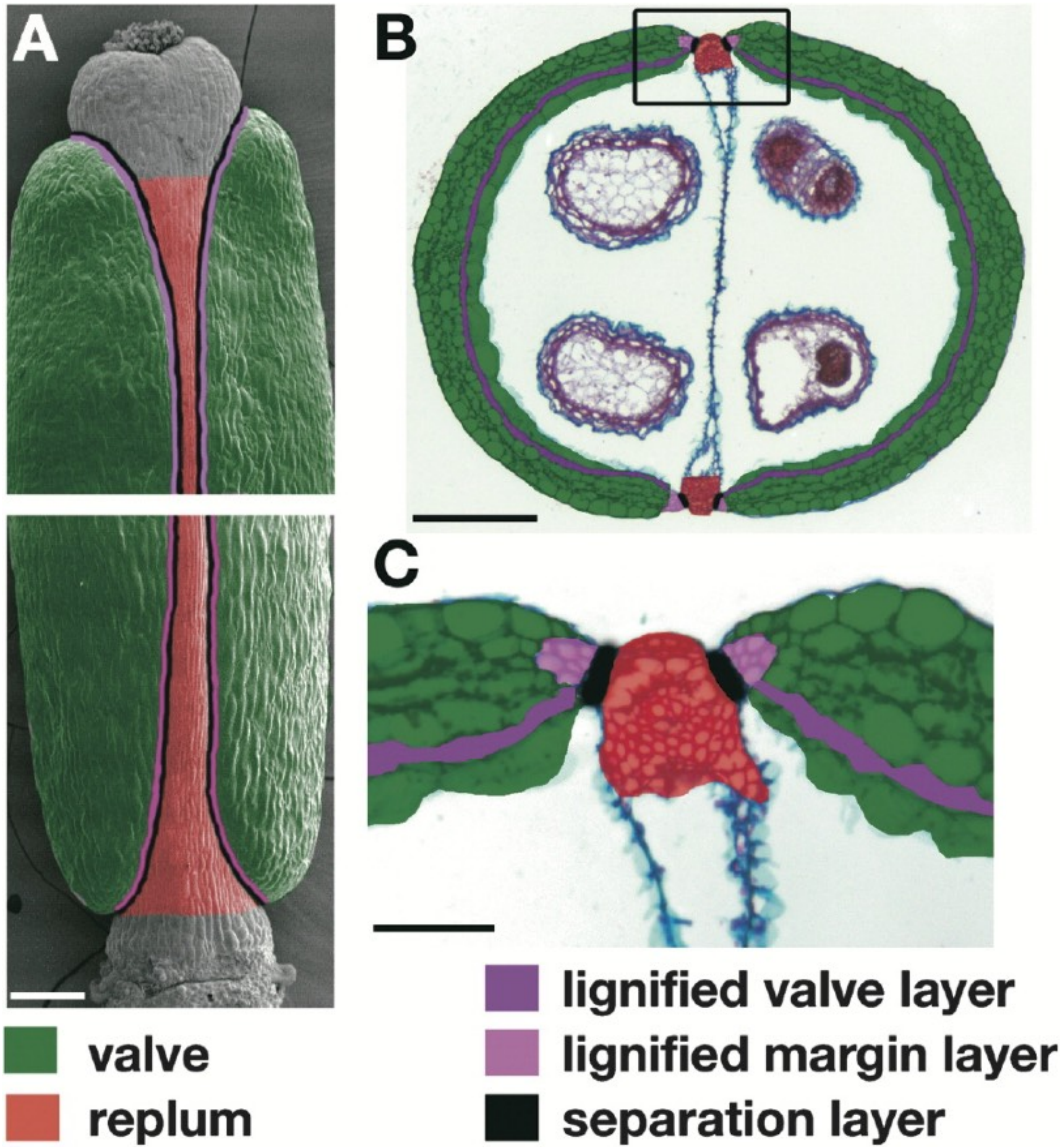
Pod Shatter at harvest of *Brassica rapa* (rapeseed)

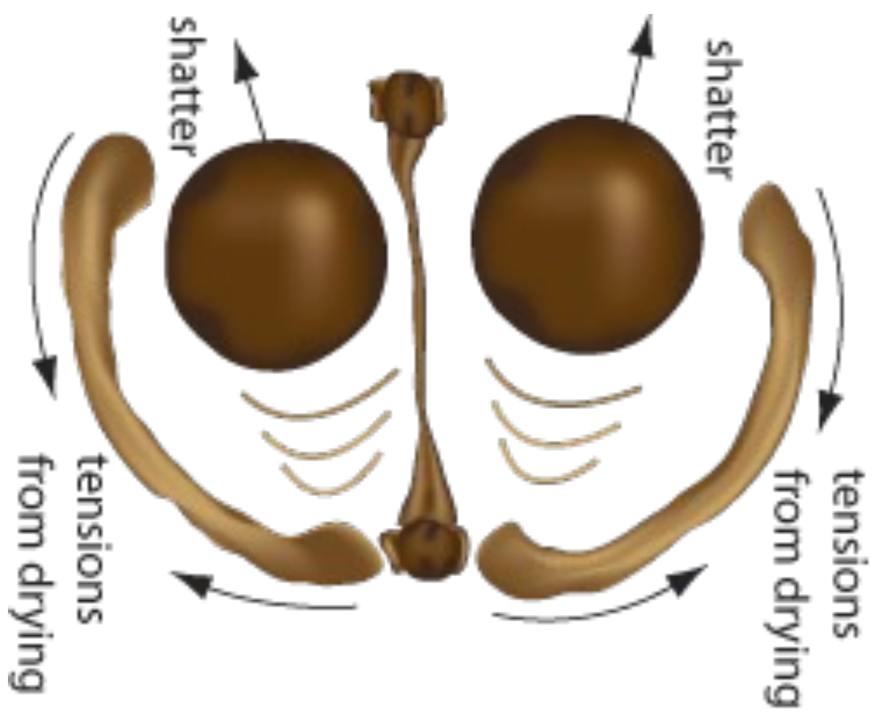
Seed pods are often fragile in the weeks leading up to harvest. During this stage seed pods go through a process of dehiscence (splitting open), commonly known as pod shatter.
This process can result in:

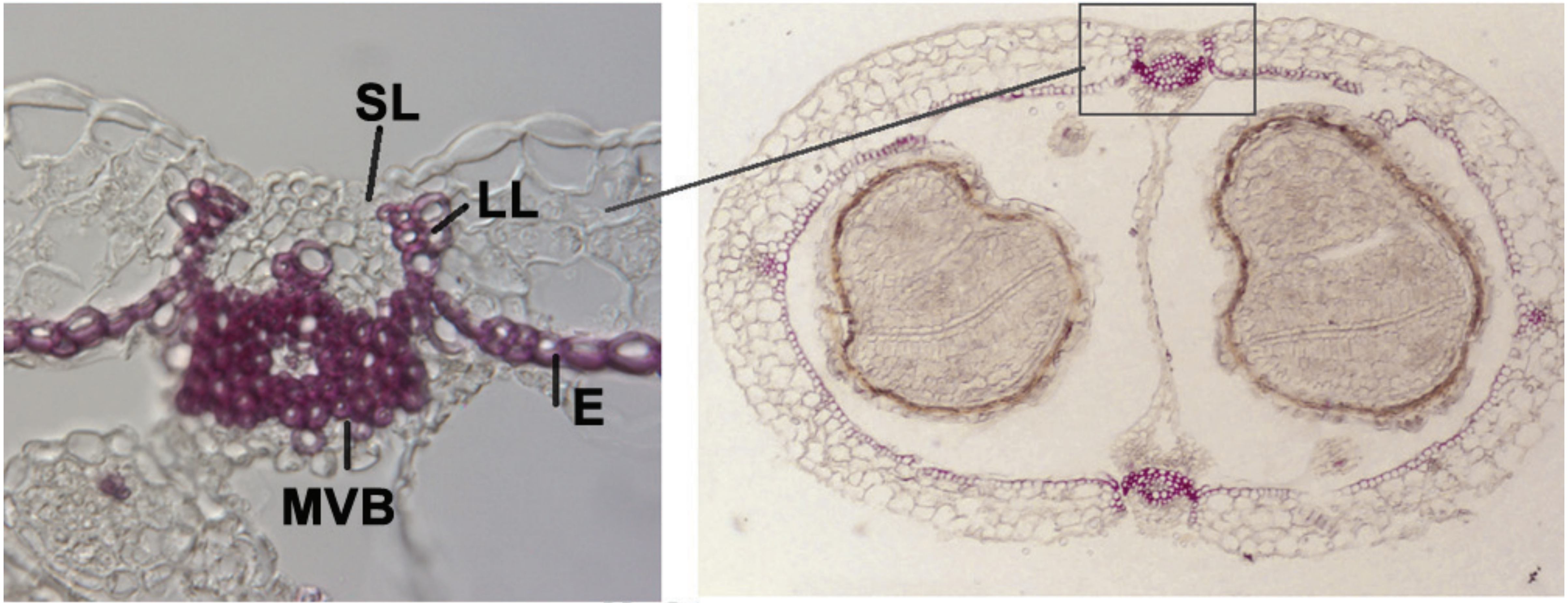
- substantial seed loss
- decrease in yield;
- greater number of volunteers in next season's crop.

In adverse conditions prior to harvest the potential loss can be as high as 50%

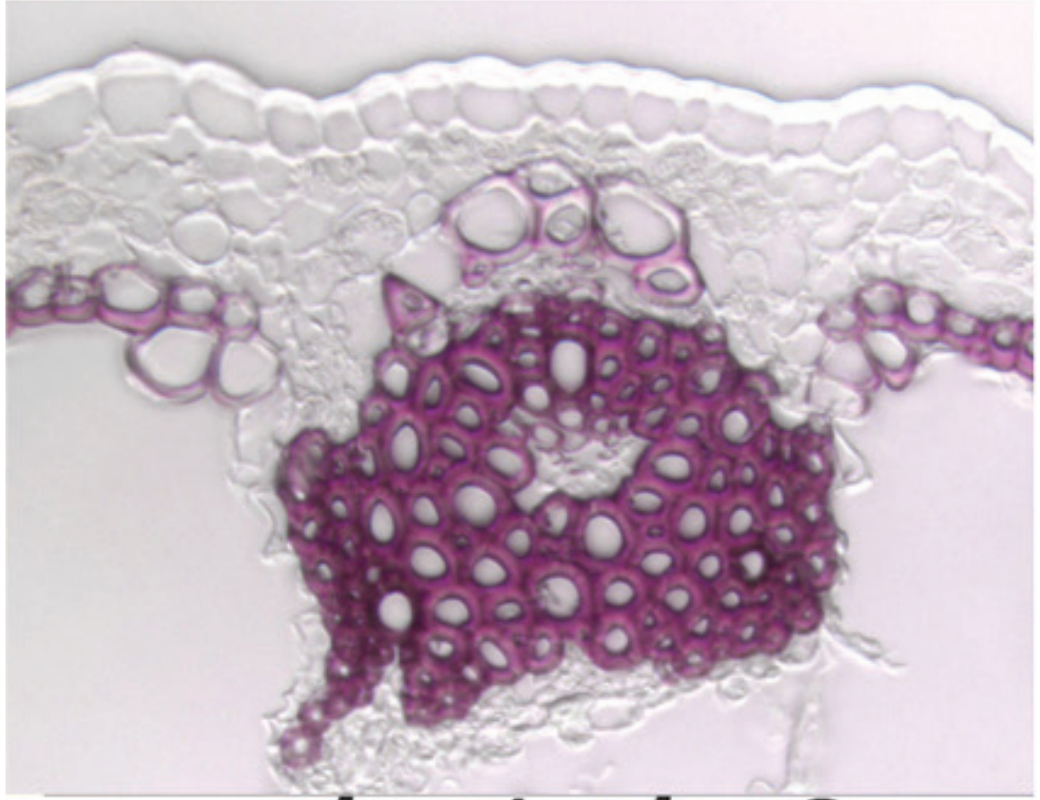
Specialised cells and valve dehiscence in Arabidopsis







wildtype



shp1 shp2

Mutation of *shatterproof1* and *shatterproof2* results in loss of the lignified layer (LL) and separation layer(SL) within the dehiscence zone, and produces a shatterproof silique.

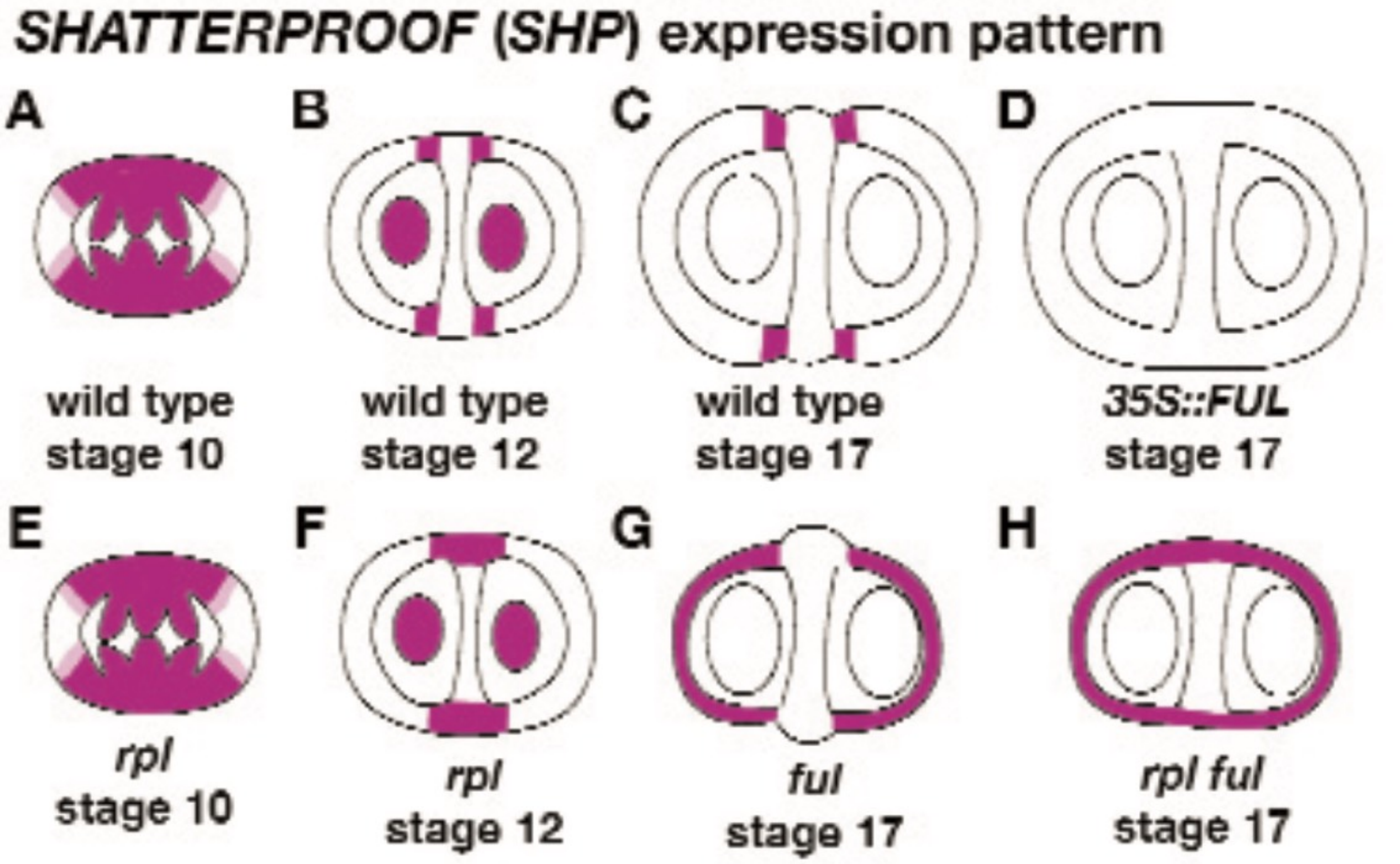
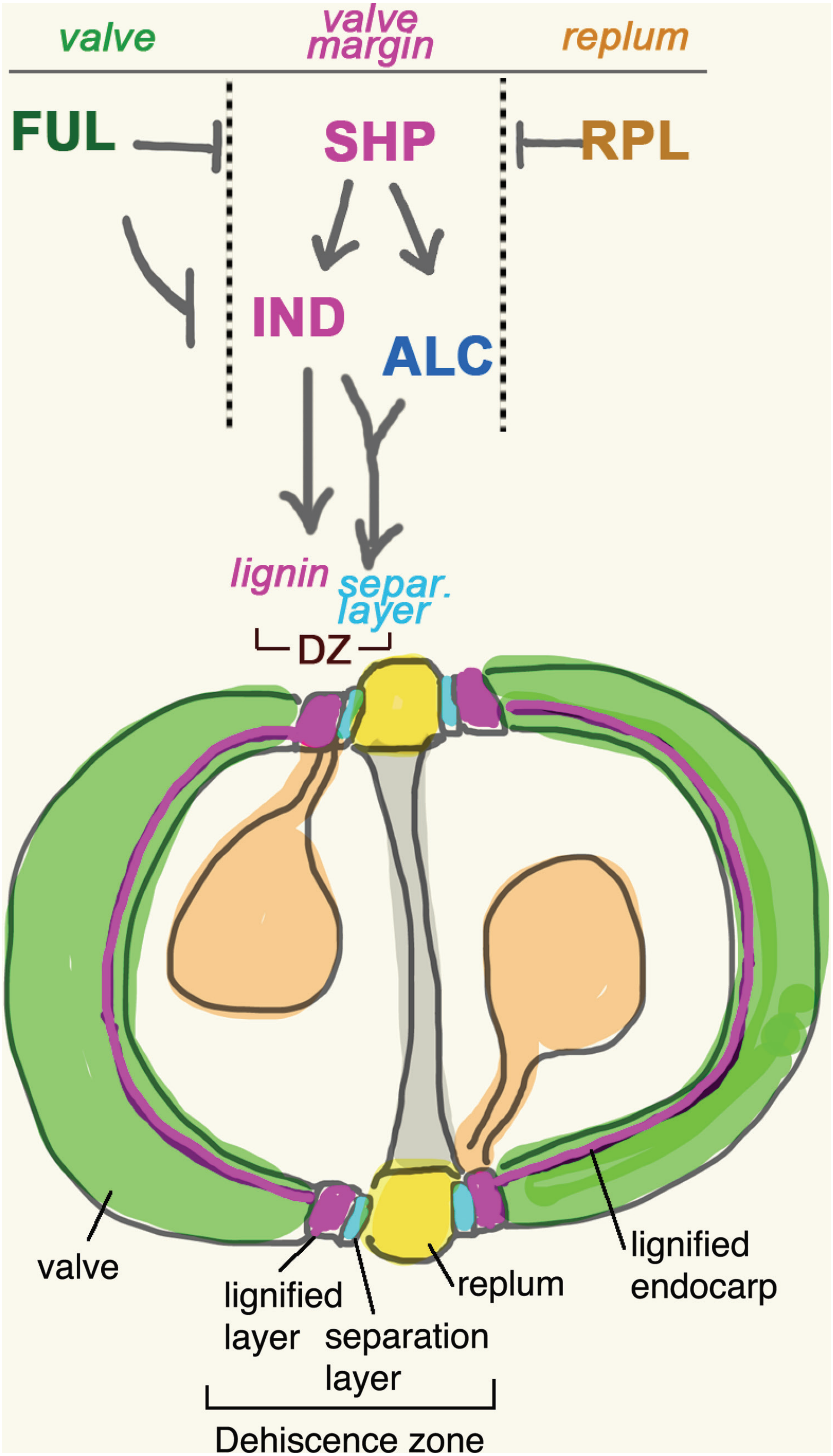


Figure 15. *SHP* expression is negatively regulated by *FUL* and *RPL.* (A) Early in development the *SHP* genes are broadly expressed in the gynoecium. At stage 10, their expression extends throughout the valve margins, replum, septum, and developing ovules. Weak expression is also seen extending into the edges of the valves. (B) At stage 12, *SHP* expression is limited specifically to the valve margins. *SHP* also continues to be expressed in the developing ovules. (C) *SHP* continues to be expressed in the valve margins through stage 17. (D) Ectopic expression of *FUL* in *35S::FUL* fruit is sufficient to block expression of *SHP* in the valve margins. (E) In *rpl* mutants, *SHP* expression is similar to wild type in early stages. (F) At stage 12 in *rpl* mutants, *SHP* continues to be expressed in the replum indicating that *RPL* is required to negatively regulate *SHP* expression in the replum. *SHP* is ectopically expressed in the replum region of *rpl* mutants through stage 17 (not shown). (G) In *ful* mutants, *SHP* is ectopically expressed throughout the valves indicating that *FUL* is required to negatively regulate *SHP* expression in the valves. (H) In *rpl ful* double mutants *SHP* expression completely surrounds the fruit.



Simplified genetic model for the development of the dehiscence zone in Arabidopsis.

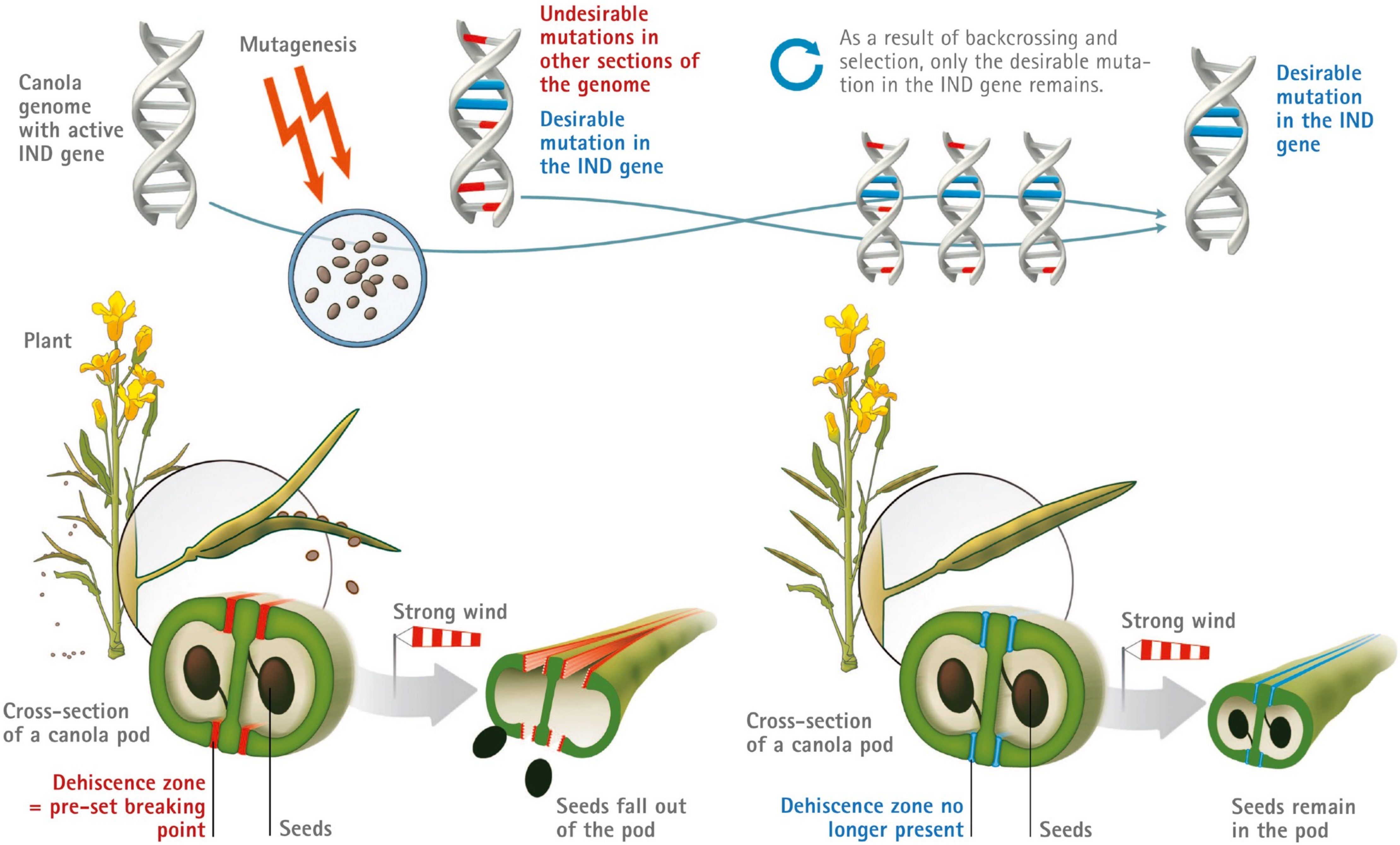
The diagram shows a transverse section across a silique. Valves are shown green, lignified zones: pink, separation layer: blue and replum: yellow.

Fruitful (FUL) and Replumless (REP) limit action of Shatterproof (SHP) to the valve margin. SHP induces Indehiscent (IND) and Alcatraz (ALC) to trigger formation of lignified cells and the separation layer in the dehiscent zone.

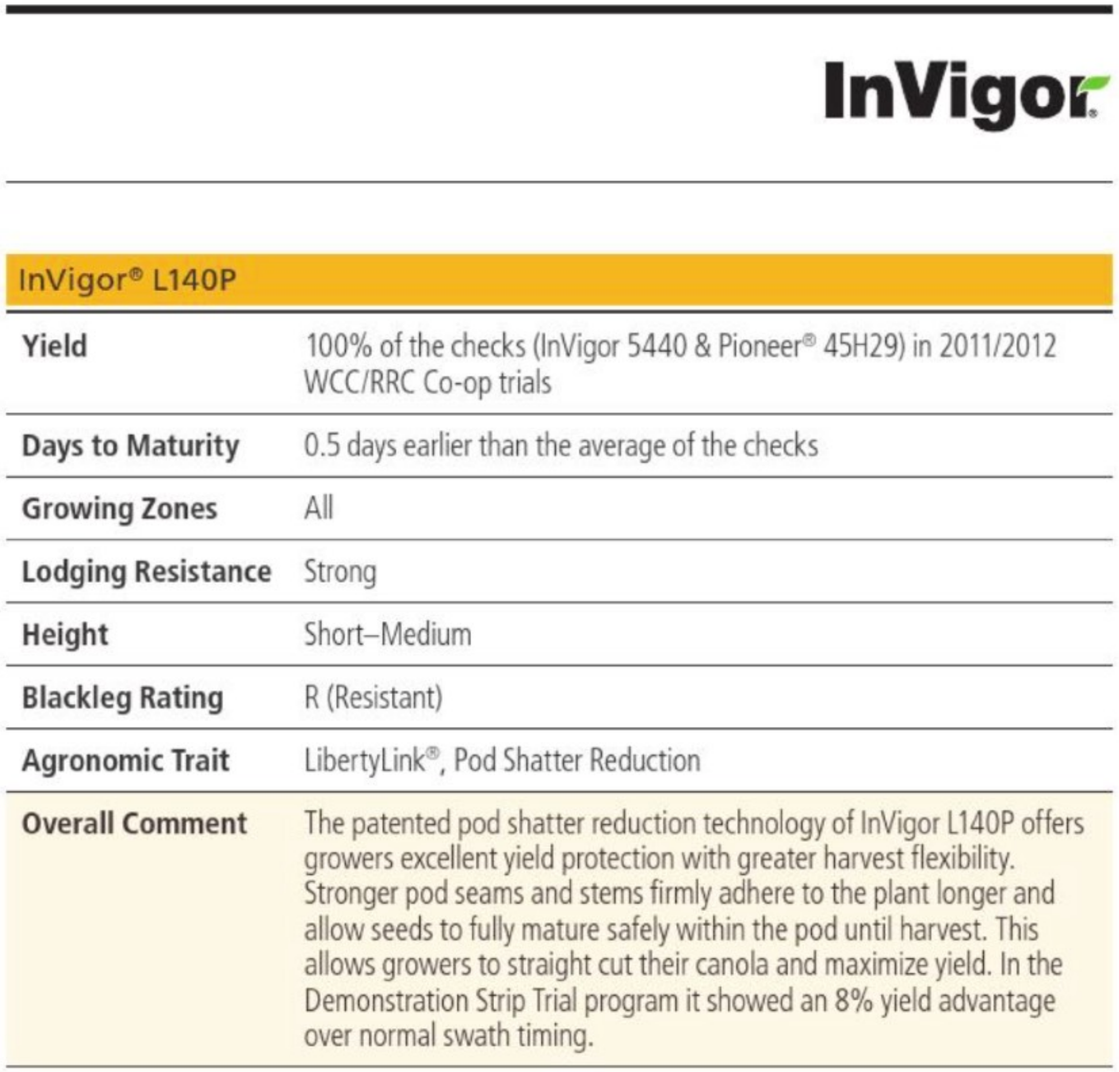
Cristina Ferrándiz and Chloé Fourquin, *Journal of Experimental Botany*, Vol. 65, No. 16, pp. 4505–4513, 2014

Strategy for shatter-resistant pods

The stability of the canola pods can be adjusted using reverse genetics. Researchers generate chemical changes (mutations) in the genotype. The candidates with an IND mutation are backcrossed with the original plant. The canola plants that result from this cross have stronger seed pods. The seeds stay in the pod and do not fall out when buffeted by the wind.



Source: *research* – the Bayer Scientific Magazine



InVigor

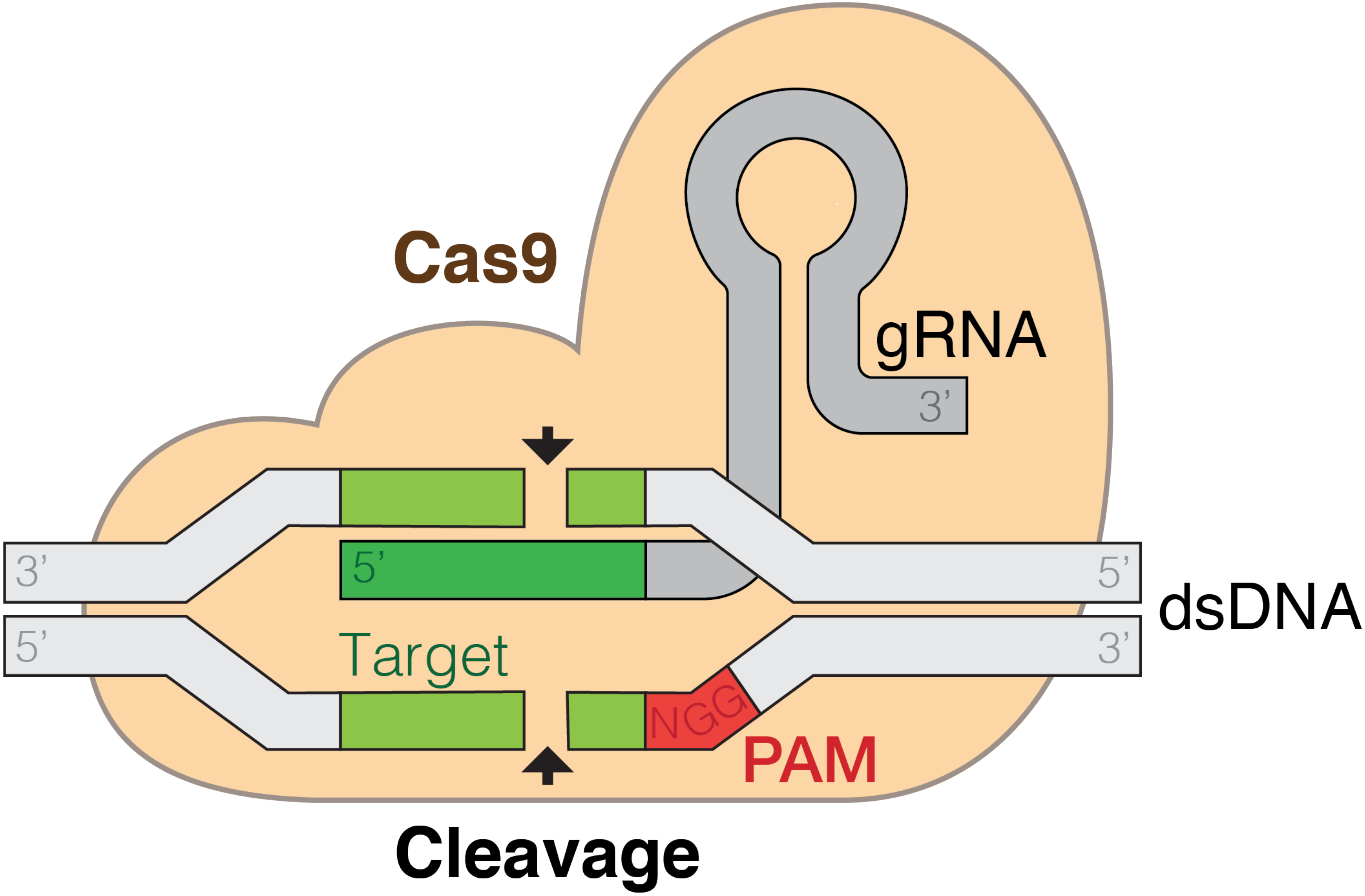
InVigor® L140P	
Yield	100% of the checks (InVigor 5440 & Pioneer® 45H29) in 2011/2012 WCC/RRC Co-op trials
Days to Maturity	0.5 days earlier than the average of the checks
Growing Zones	All
Lodging Resistance	Strong
Height	Short–Medium
Blackleg Rating	R (Resistant)
Agronomic Trait	LibertyLink®, Pod Shatter Reduction
Overall Comment	The patented pod shatter reduction technology of InVigor L140P offers growers excellent yield protection with greater harvest flexibility. Stronger pod seams and stems firmly adhere to the plant longer and allow seeds to fully mature safely within the pod until harvest. This allows growers to straight cut their canola and maximize yield. In the Demonstration Strip Trial program it showed an 8% yield advantage over normal swath timing.

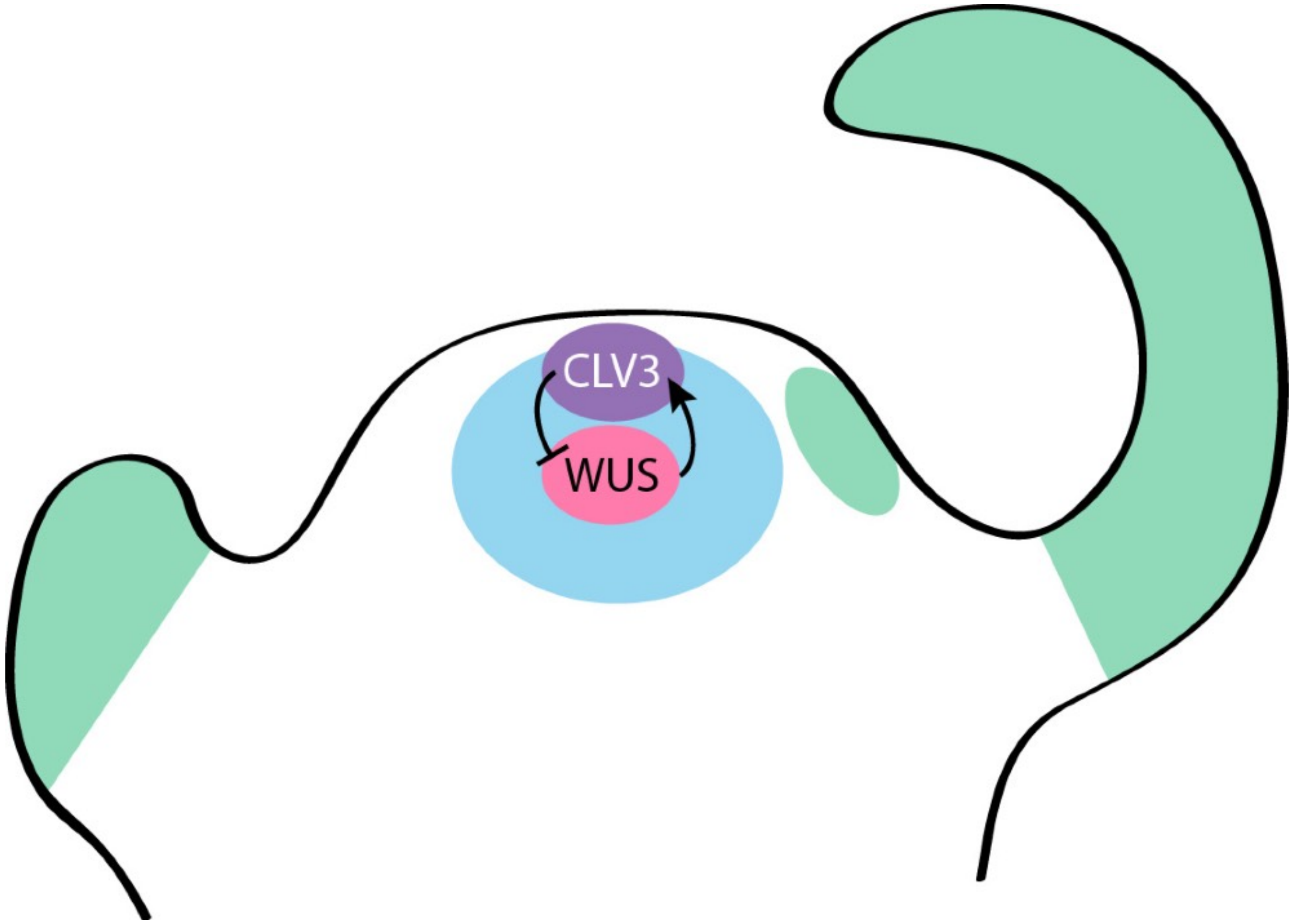
BAYER

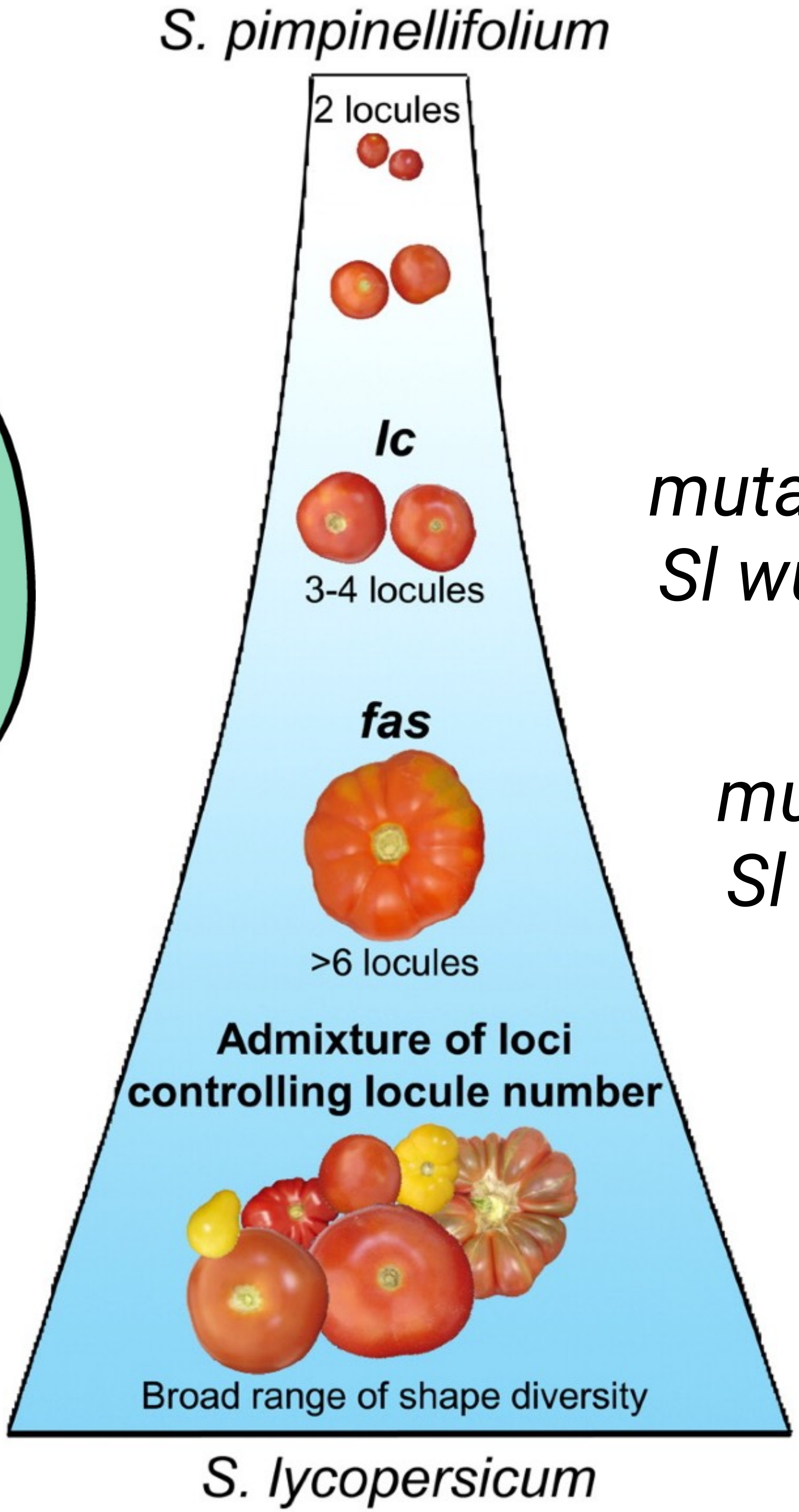
InVigor®

Editing endogenous regulatory interactions: reprogramming mechanisms for control of meristem growth in tomato and related plants

CRISPR-mediated genome editing



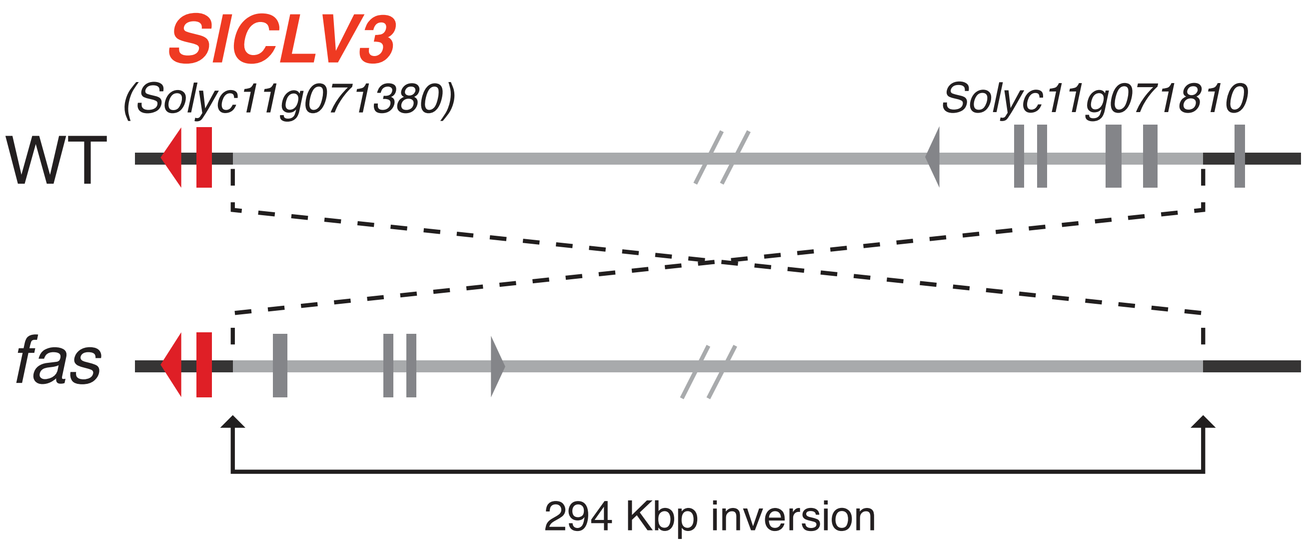


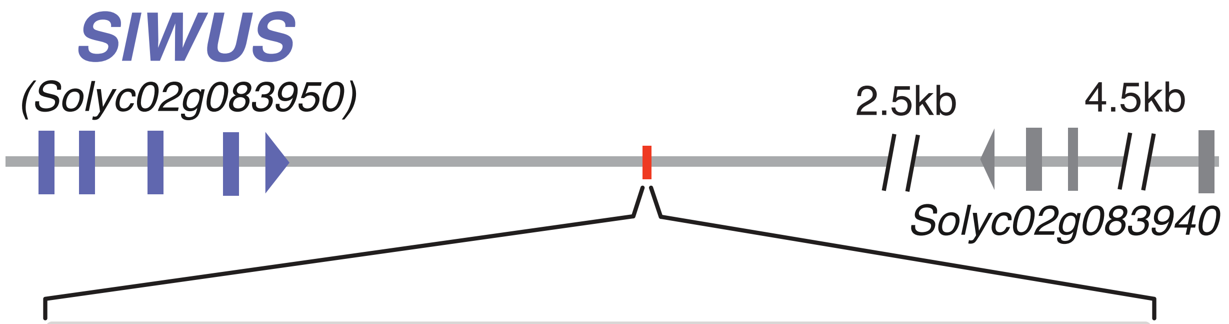


mutant Sl wus

mutant Sl clv3

Recreating known fruit size QTLs in tomato with CRISPR-Cas9

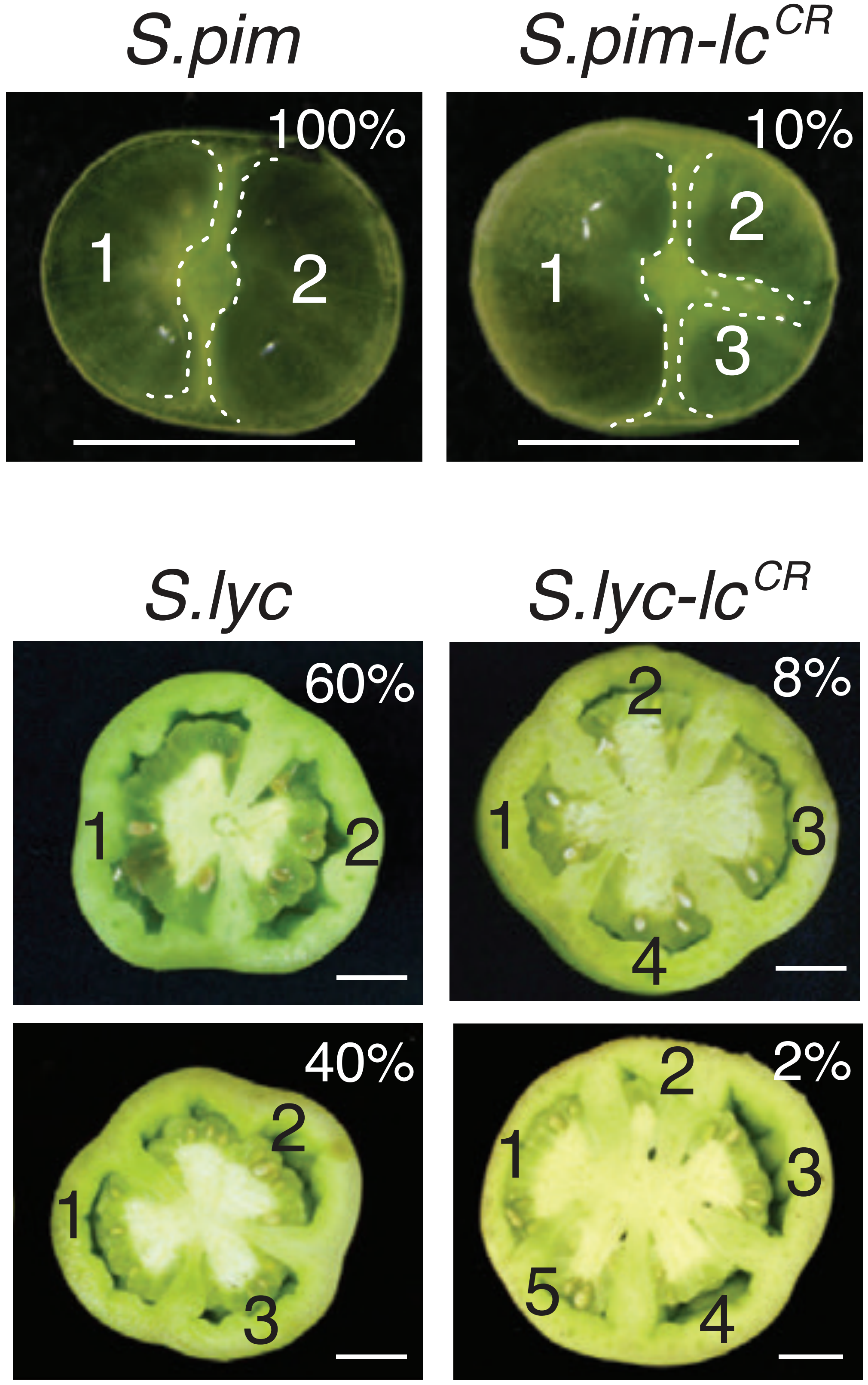




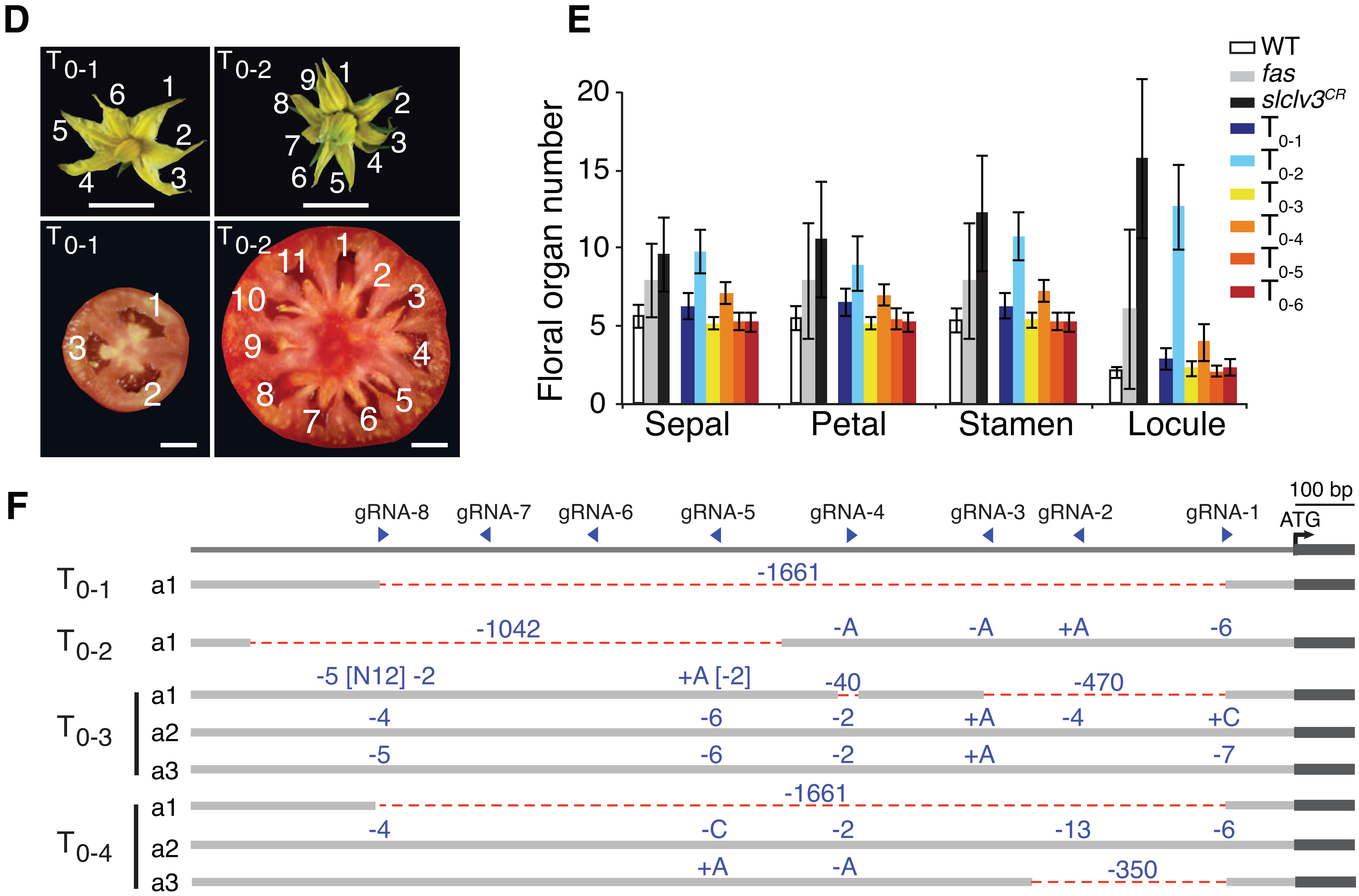
```
WT  AGATTTGGCATGATGTTTACTAATTGGAC
lc  AGATTTGGCATGATGCTTACTGATTGGAC
```

CArG motif

```
gRNA         AGATTTGGCATGATGTTTACTAATTGGAC
                                    PAM
S.pim-lcCR   AGATTTGGCATGATGTT----AATTGGAC
S.lyc-lcCR   AGATTTGGCATGATGT-----AATTGGAC
```

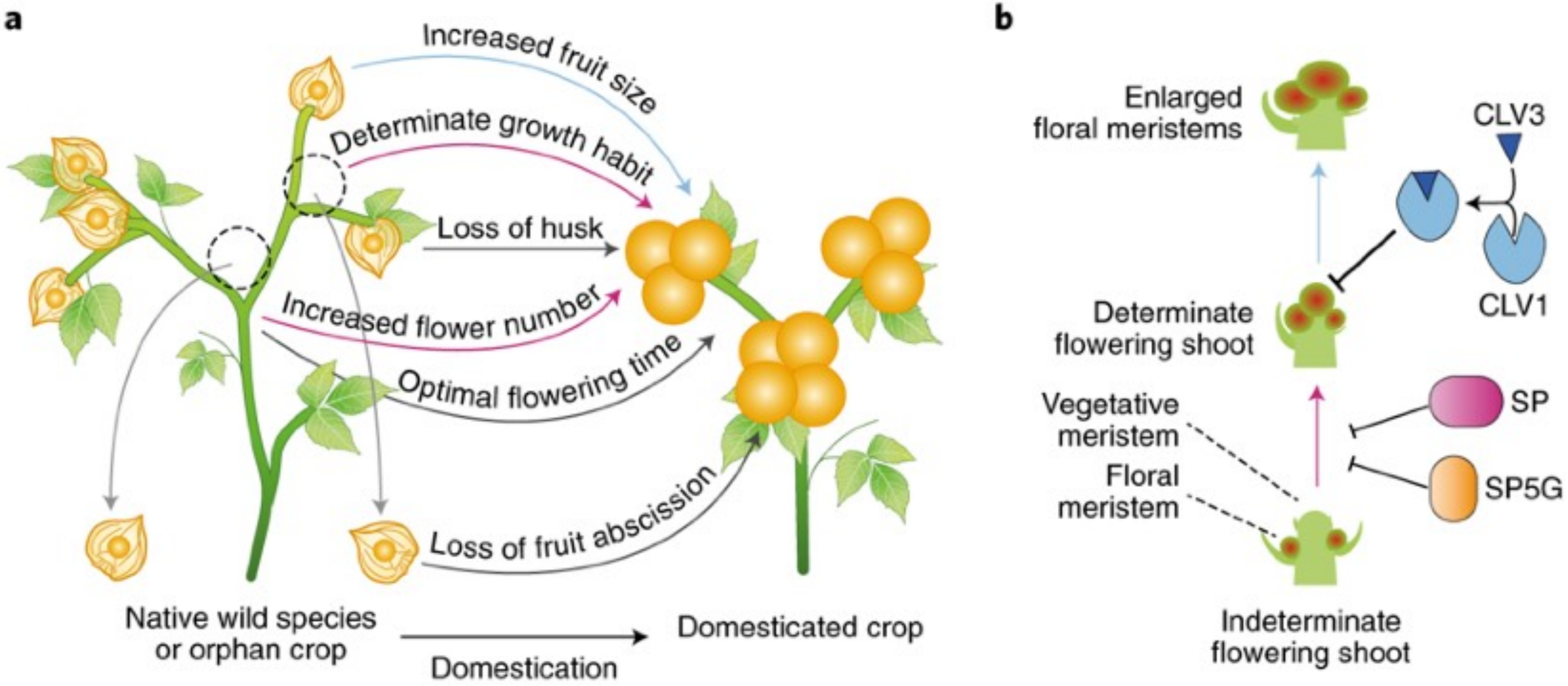


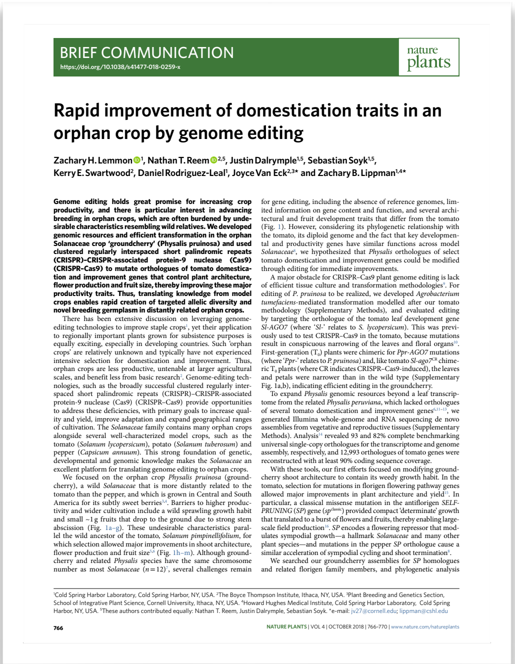
SlCLV3/fas allelic effects on flower and fruit morphology



INARI

A Flagship Pioneering Company




BRIEF COMMUNICATION

nature plants

Rapid improvement of domestication traits in an orphan crop by genome editing

Zachary H. Lemmon, Nathan T. Reem, Justin Dalrymple, Sebastian Soyk, Kerry E. Swartwood, Daniel Rodriguez-Leal, Joyce Van Eck and Zachary B. Lippman


news & views

CROP ENGINEERING

The taming of the shrub

Can genomics, functional analysis and genome editing help build the bridge between orphan crops and modern agriculture?

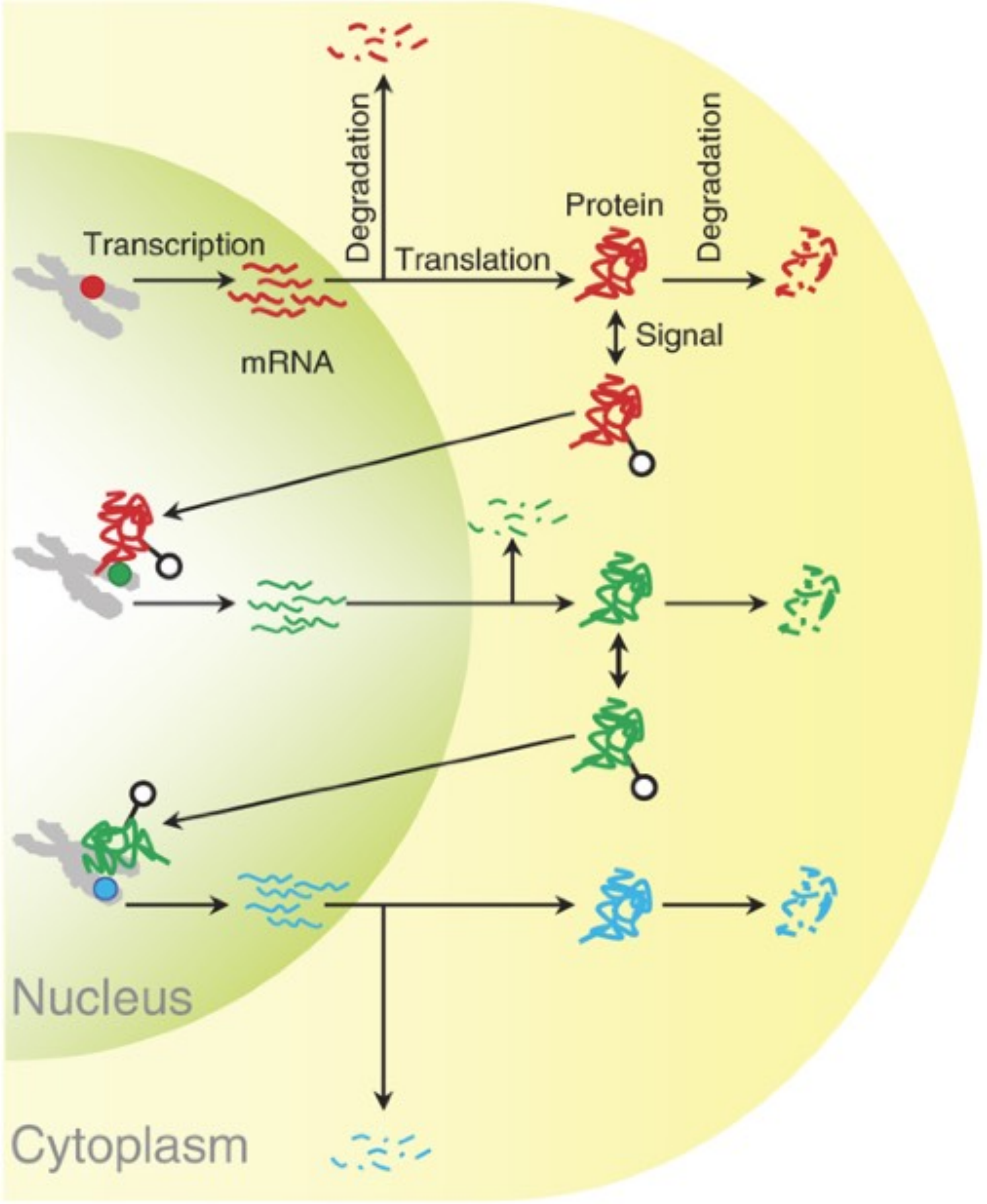
Luca Comai

Rapid improvement of domestication traits in an orphan crop by genome editing.

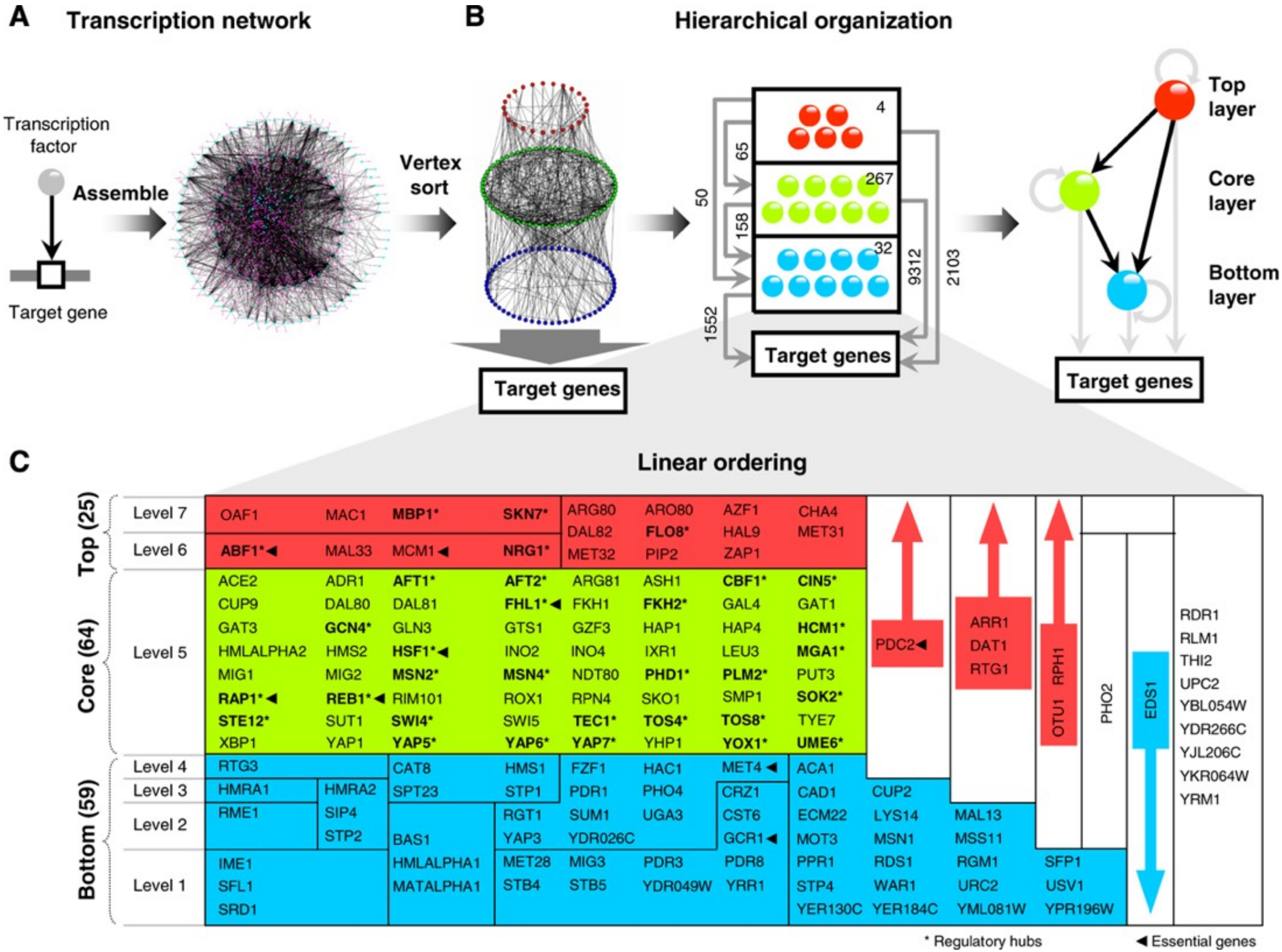
Zachary H. Lemmon, Nathan T. Reem, Justin Dalrymple, Sebastian Soyk, Kerry E. Swartwood, Daniel Rodriguez-Leal, Joyce Van Eck & Zachary B. Lippman.

Nature Plants 4: 766–770 (2018)

Pathway engineering in plants: introducing ectopic positive-feedback regulatory loops for hyper-expression of existing pathways



Molecular Systems Biology 5; Article number 294; doi:10.1038/msb.2009.52



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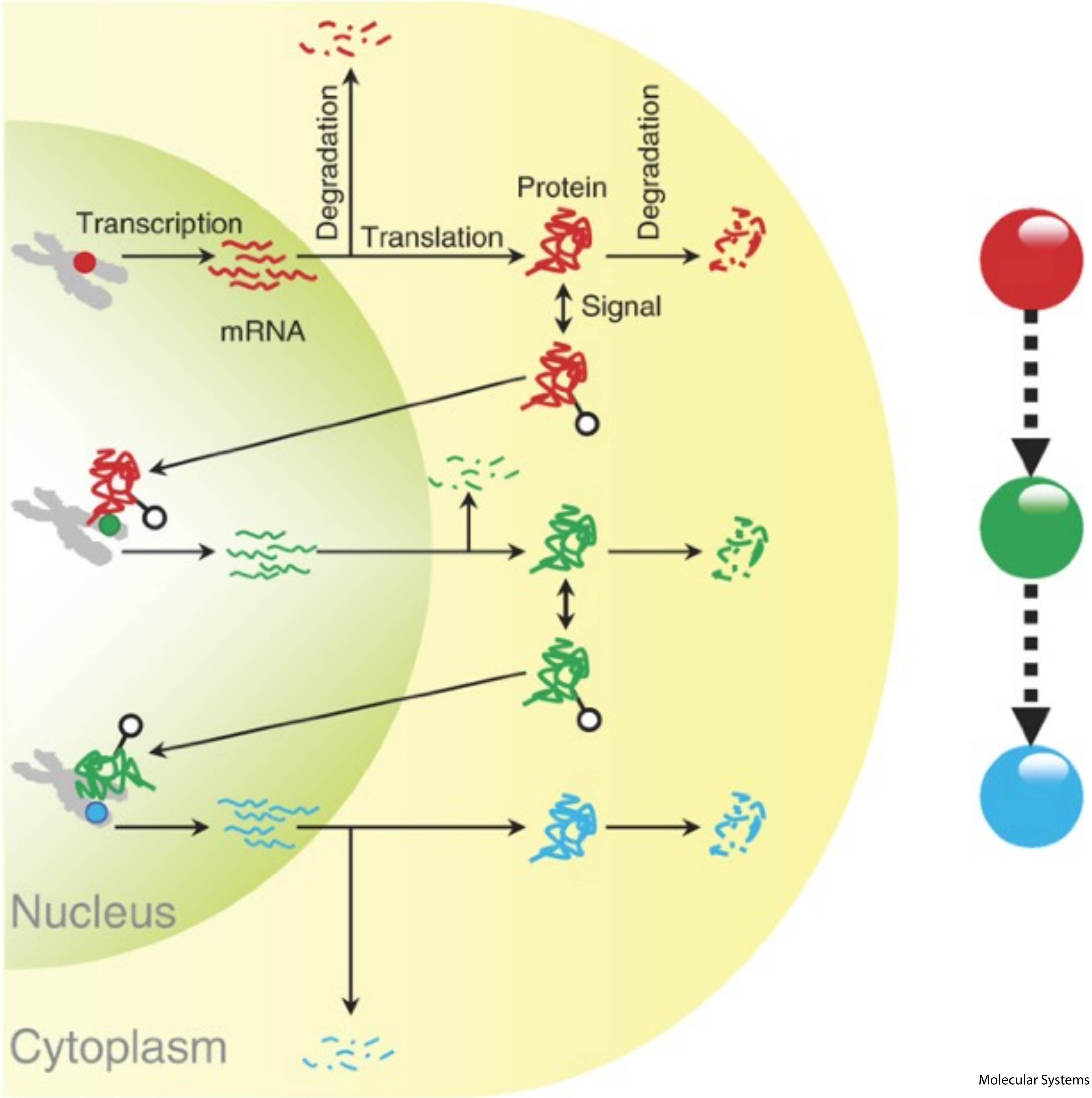

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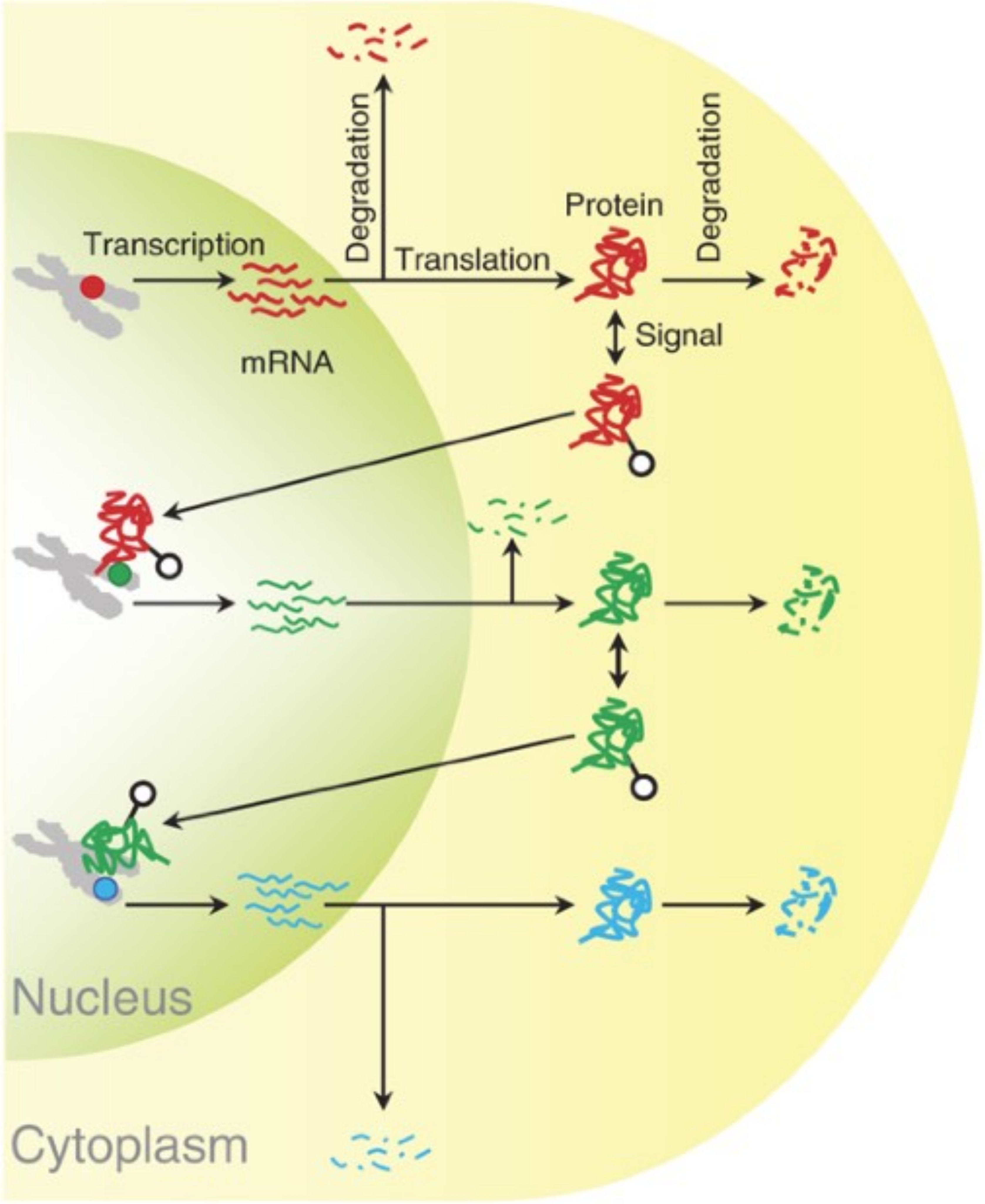

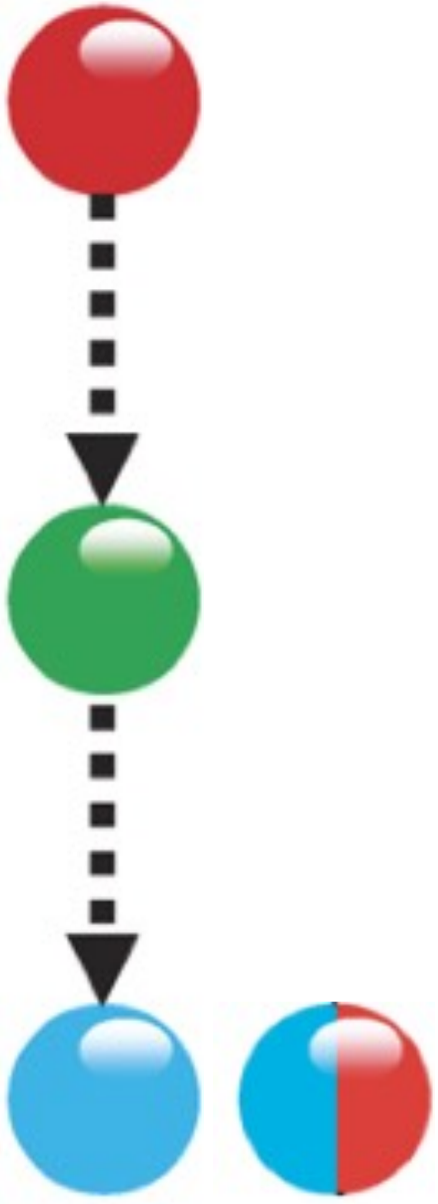

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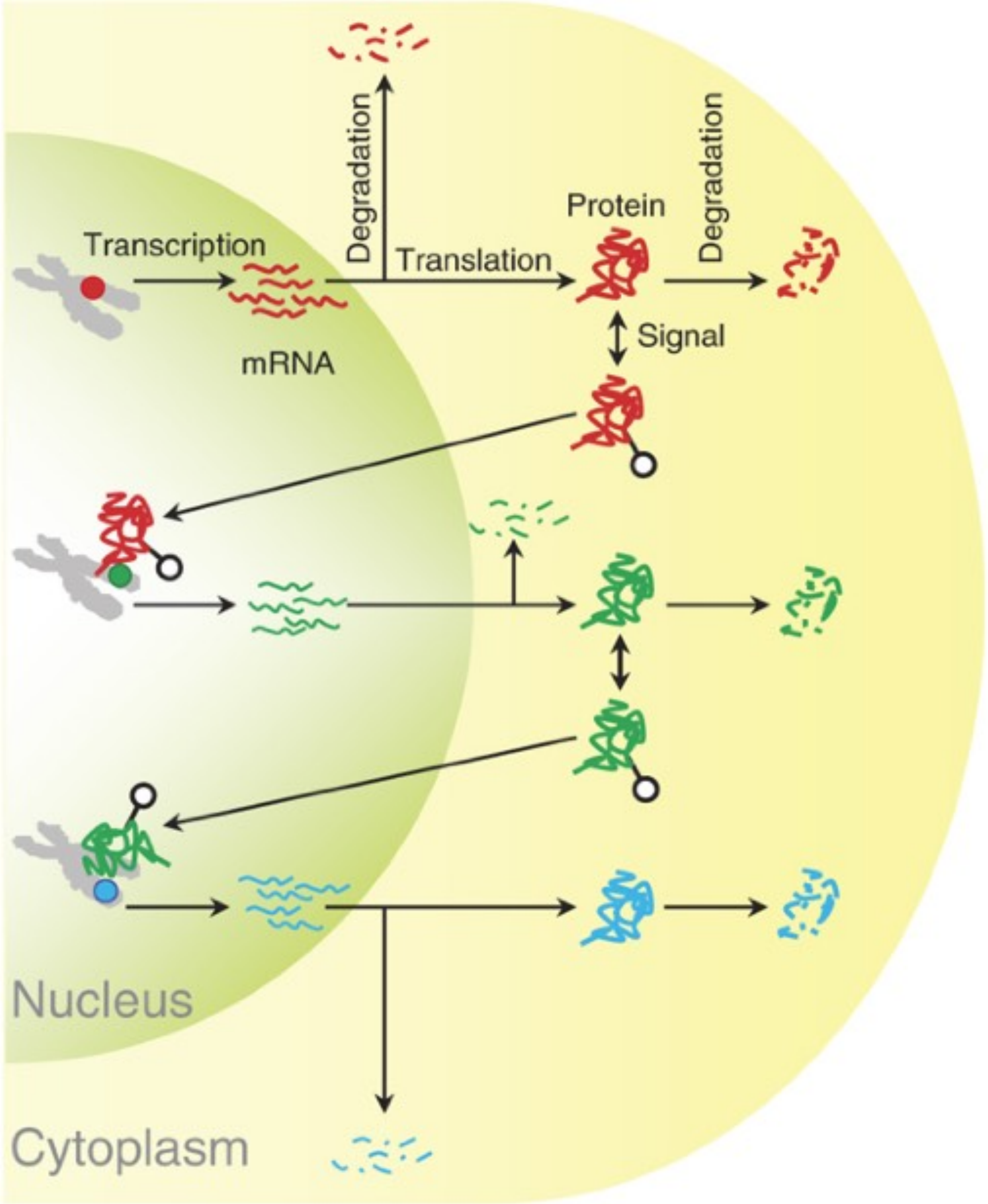
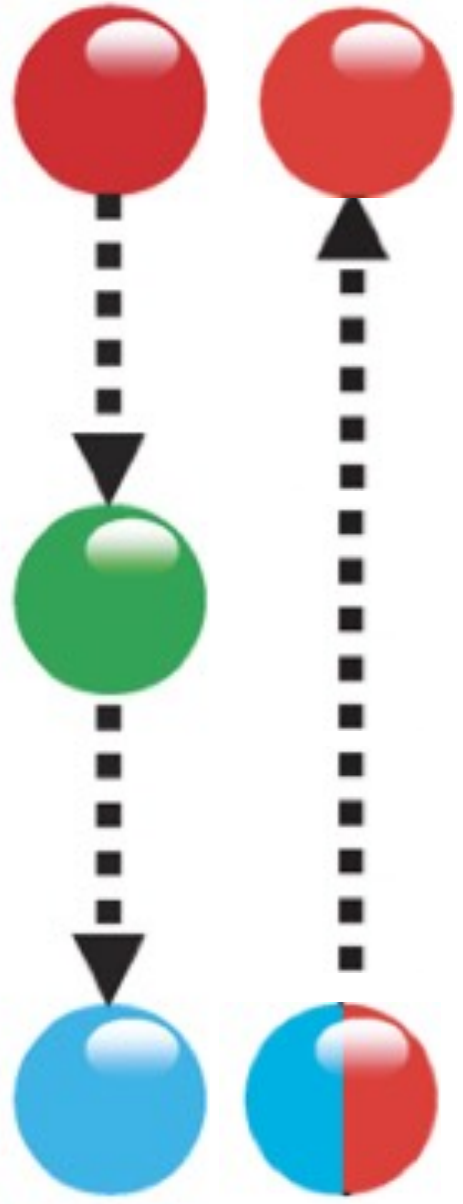
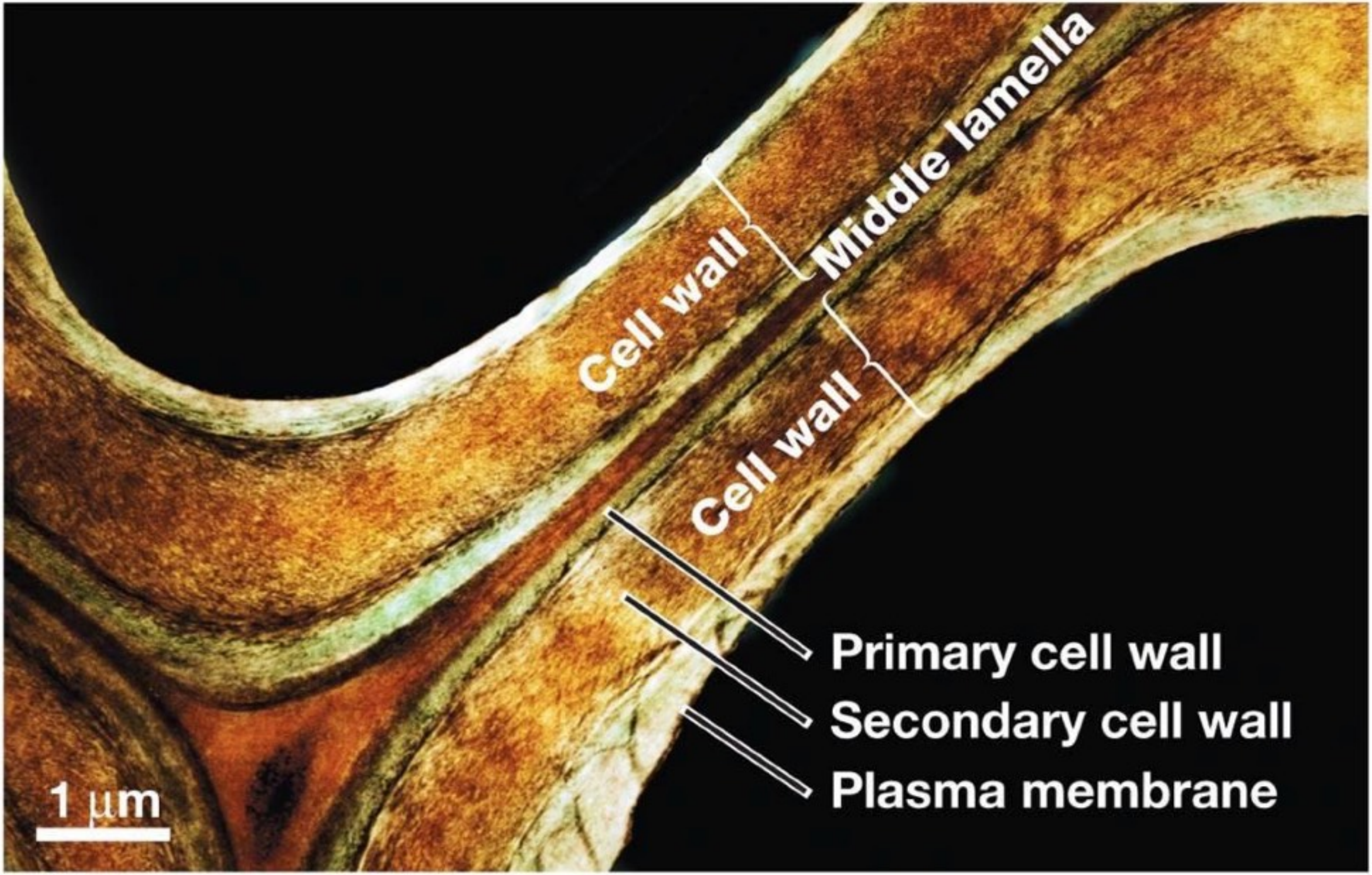




Molecular Systems Biology 5; Article number 294; doi:10.1038/msb.2009.52

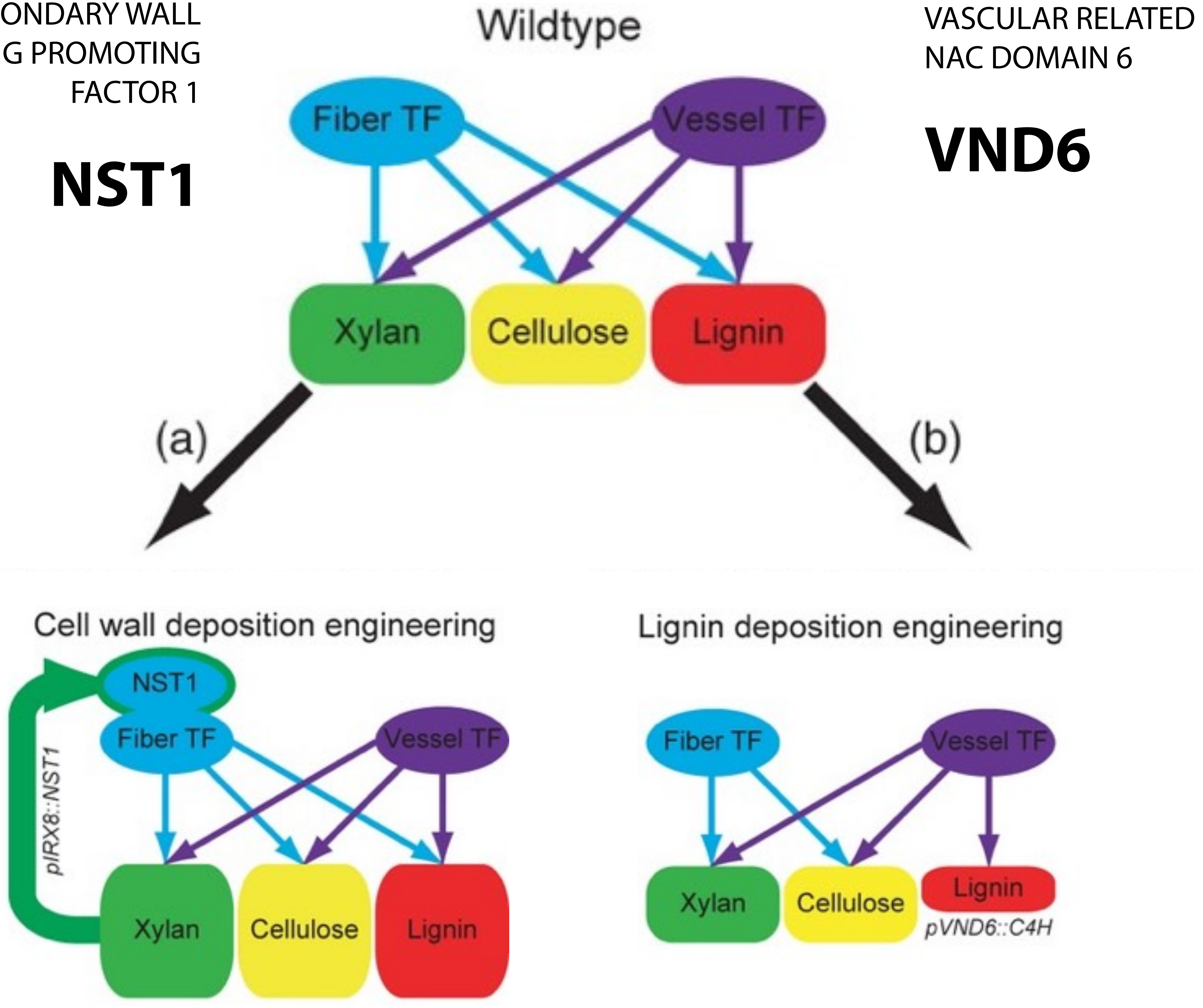

Middle lamella
Cell wall
Cell wall
Primary cell wall
Secondary cell wall
Plasma membrane
1 µm

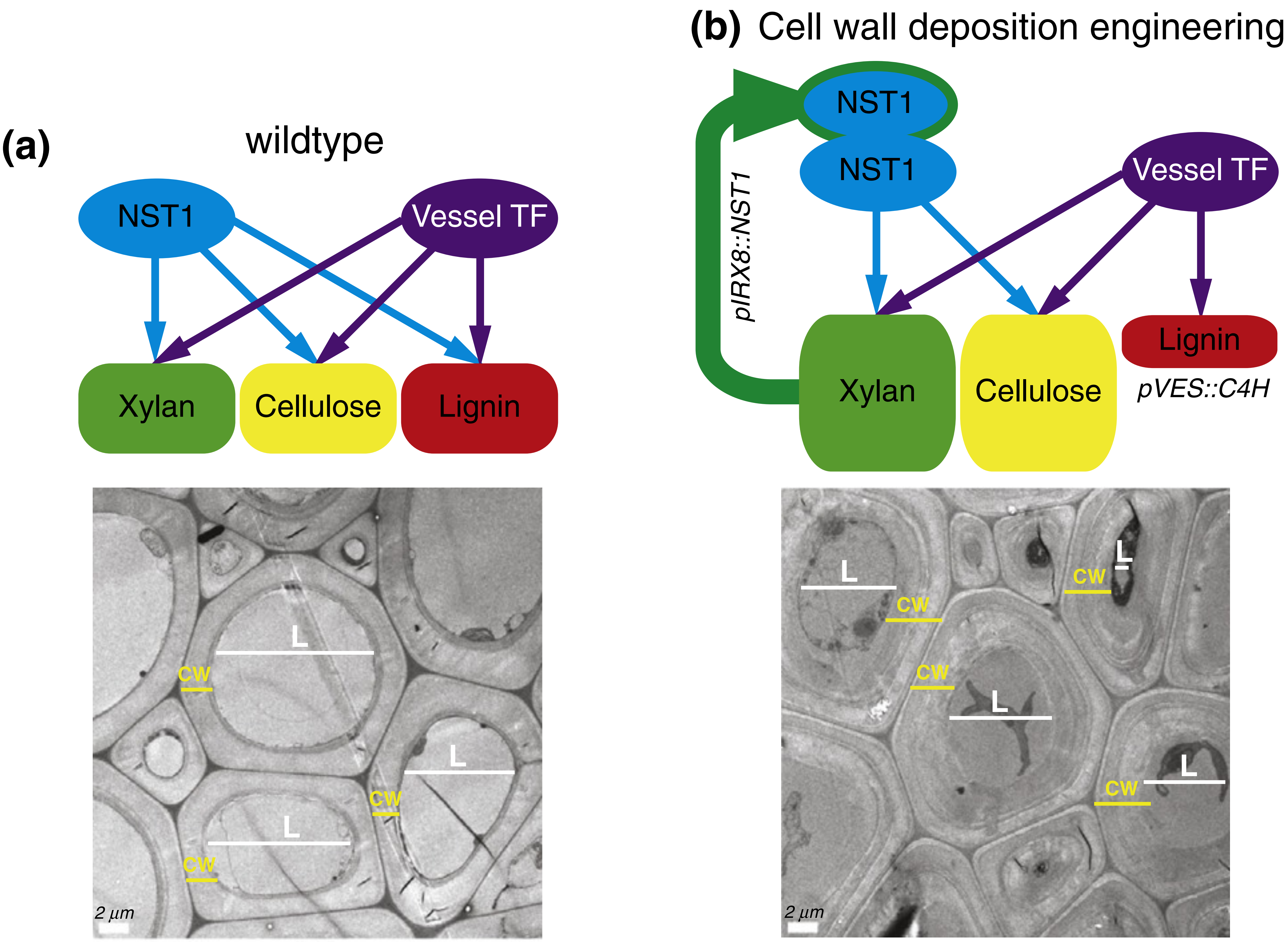
NAC SECONDARY WALL
THICKENING PROMOTING
FACTOR 1

NST1

VASCULAR RELATED
NAC DOMAIN 6

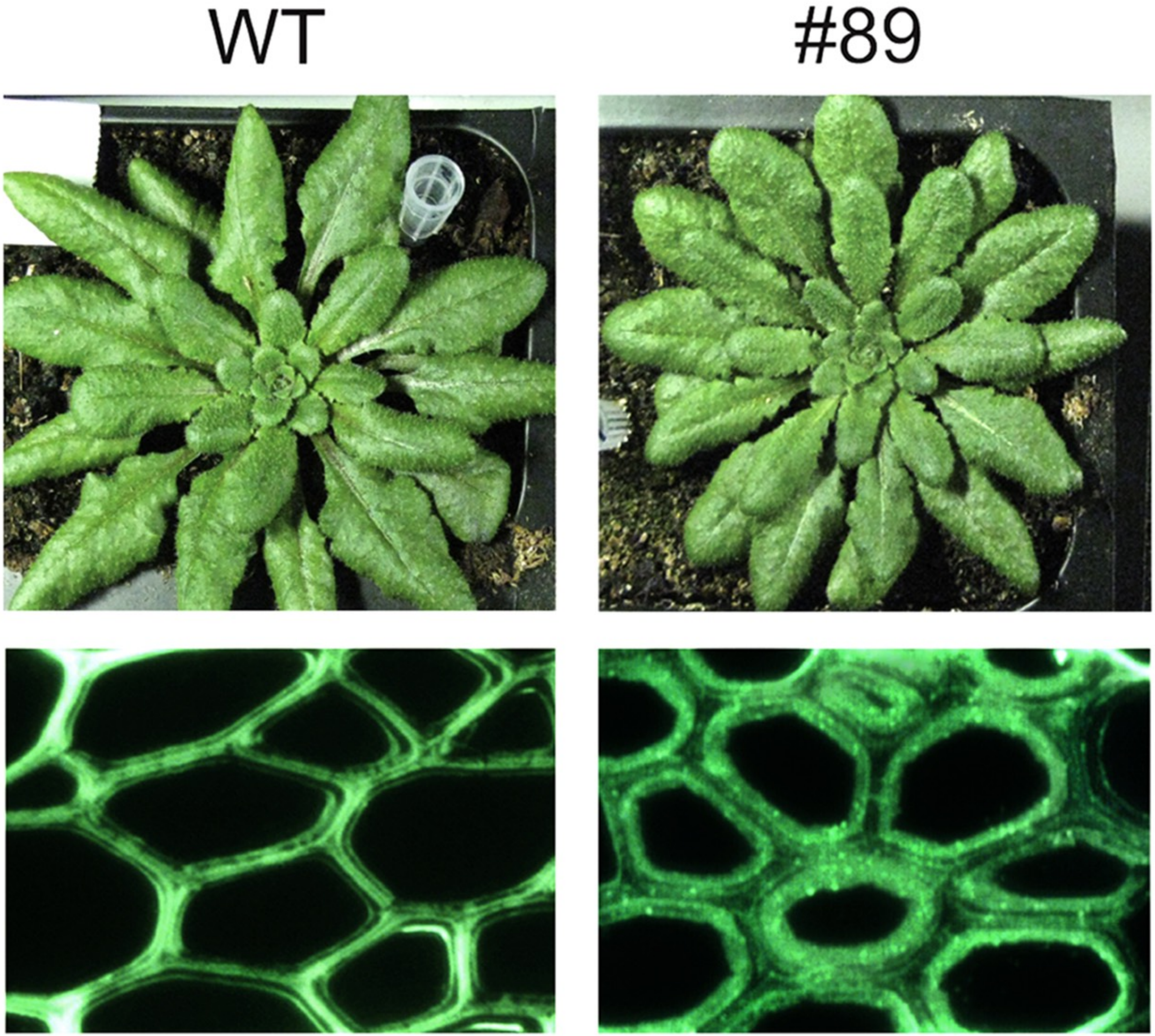
VND6





(a) wildtype

(b) Cell wall deposition engineering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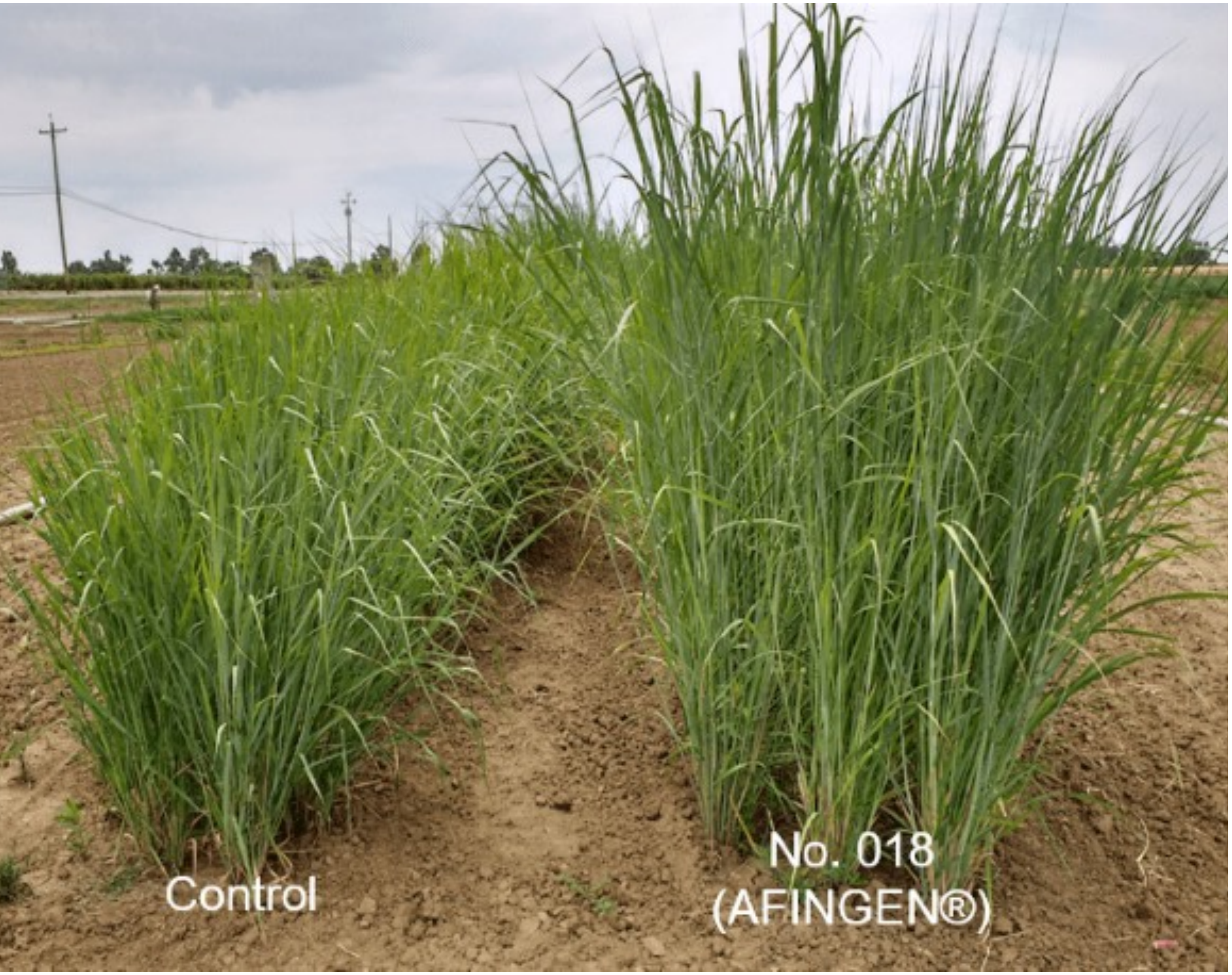
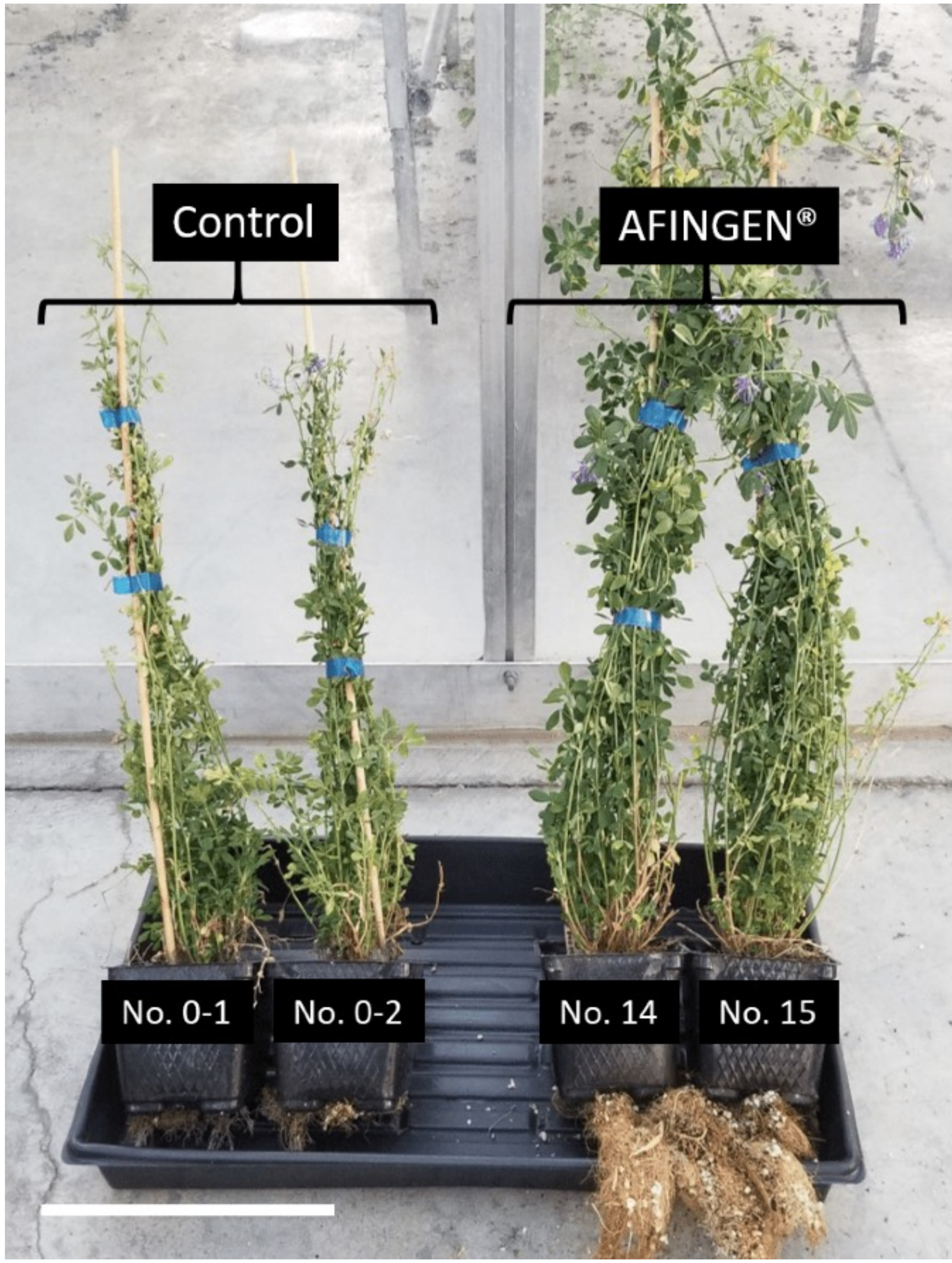


Switchgrass
enhanced xylem formation



Medicago
enhanced root growth

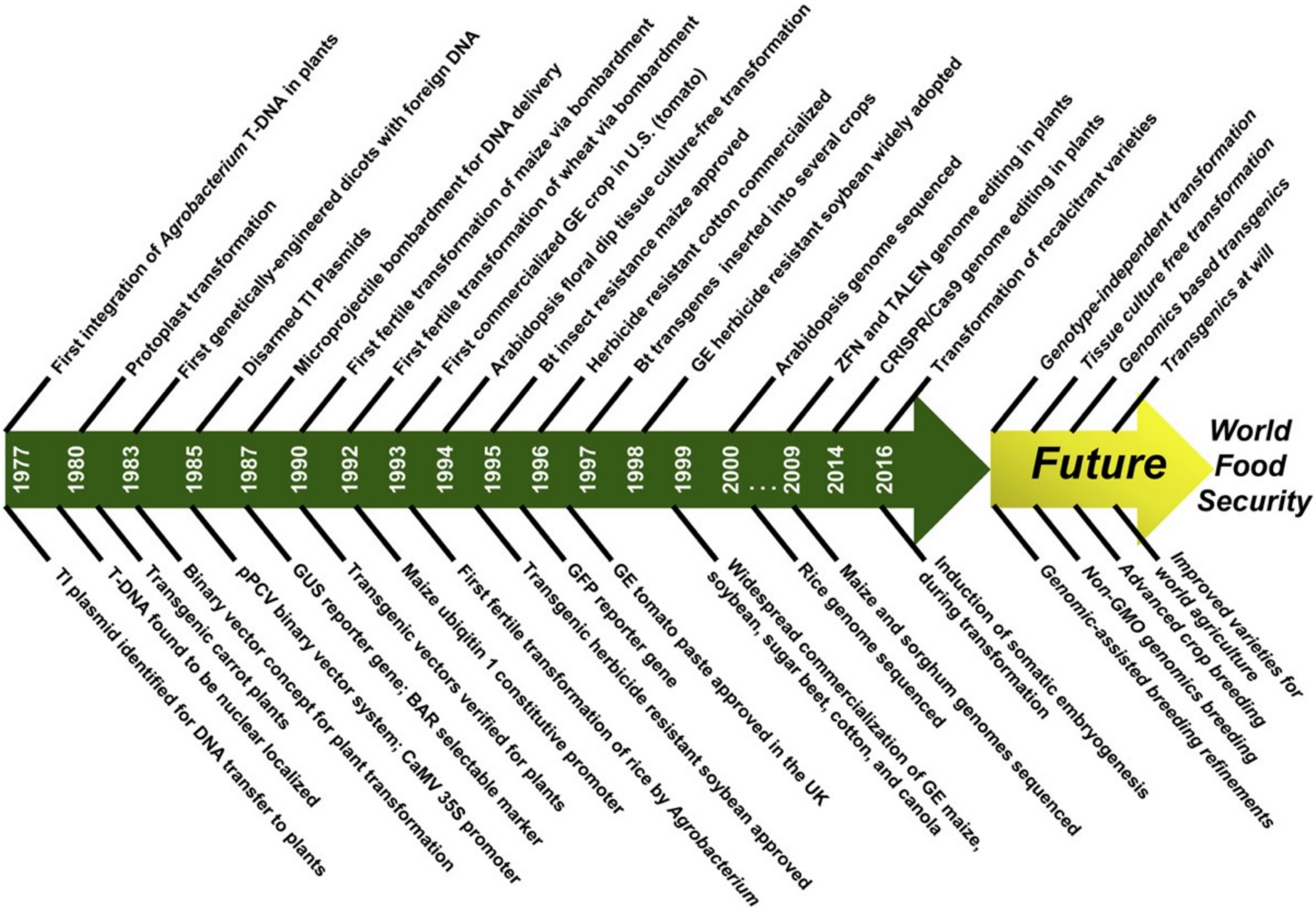


Figure 3. Important Historical Milestones in Plant Transformation.

Since its beginning in 1977, the pace of crop transformation technology development has not been linear. In recent years, the genome editing revolution begs for crop transformation improvements to enable greater food security.