

# 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

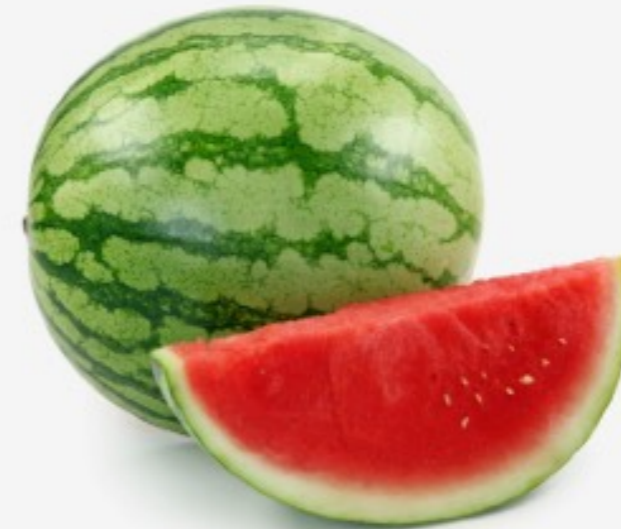
## Wild watermelon

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.



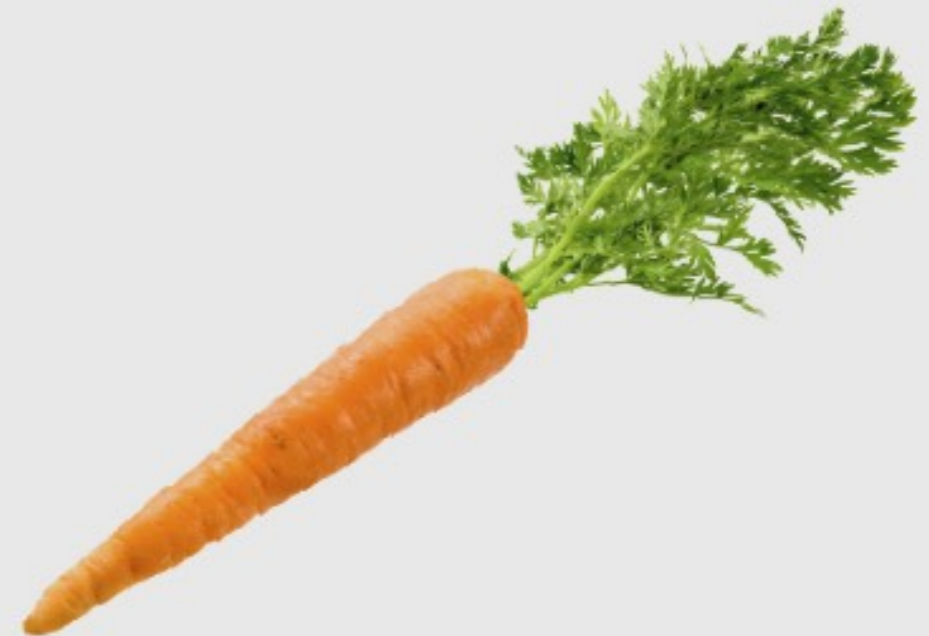
## Wild carrot

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?



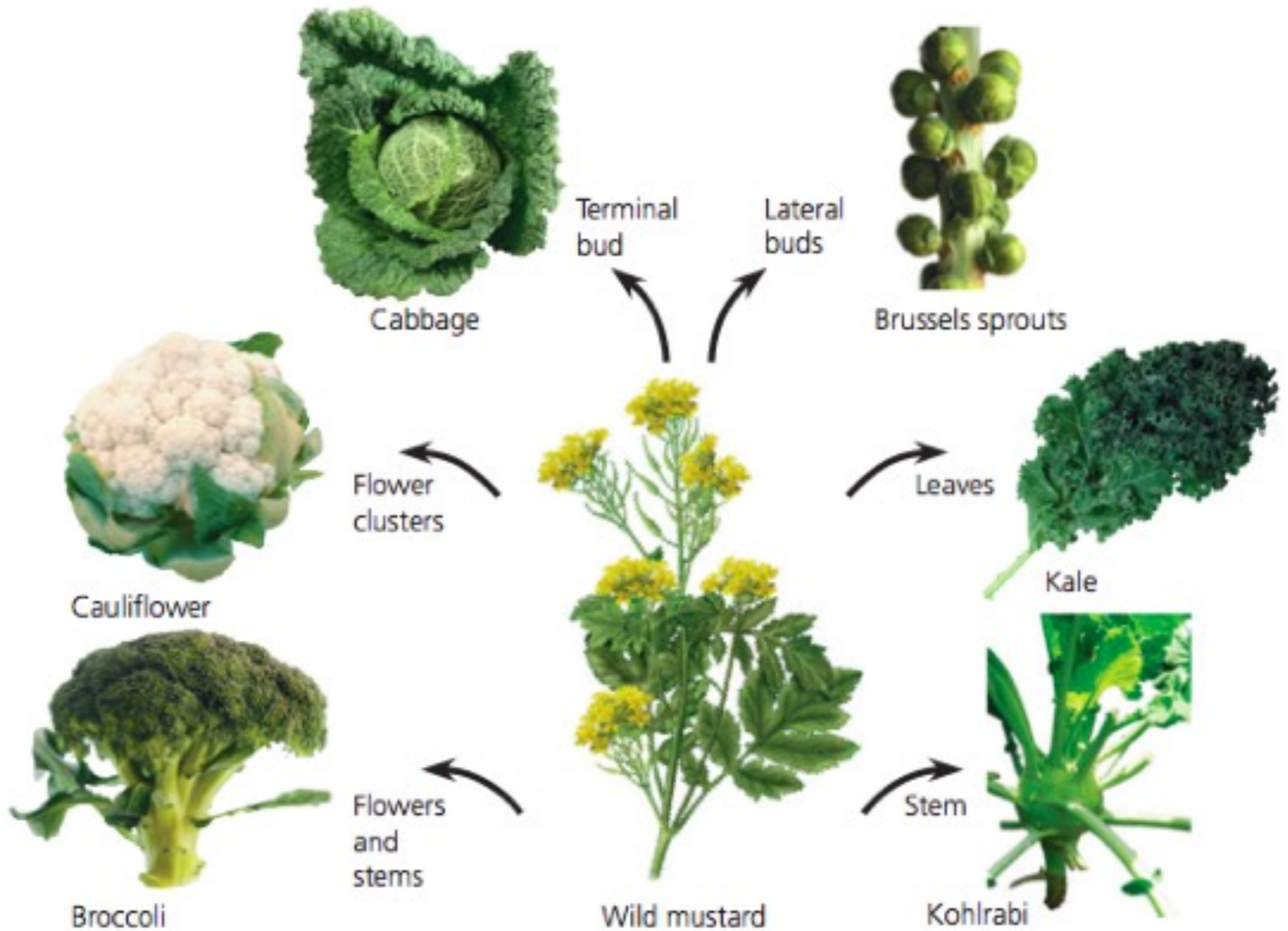
Kale



Broccoli

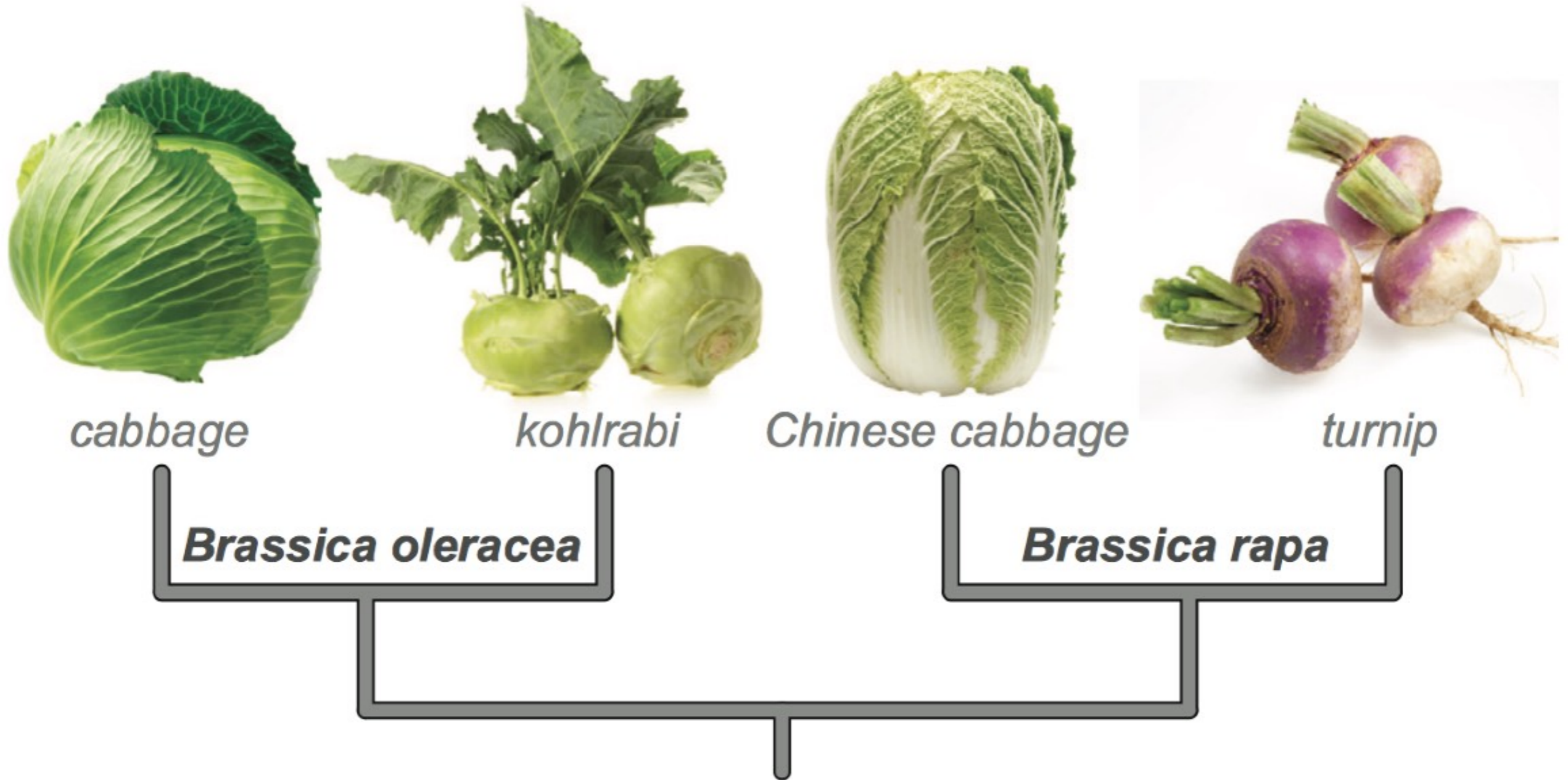


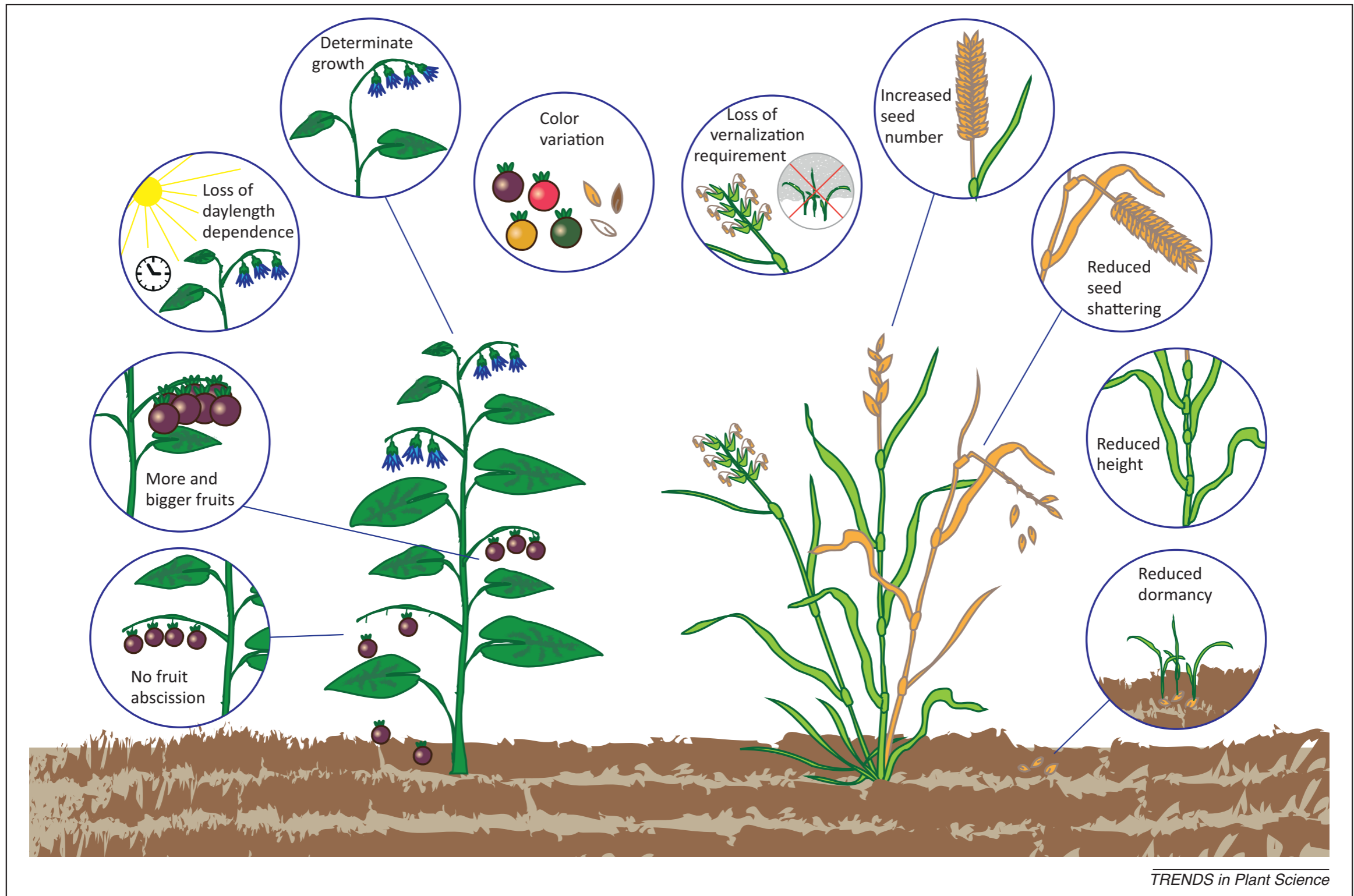
Kohlrabi



**Crops derived from wild mustard (*Brassica oleracea*)**

# Convergent phenotypic changes during domestication





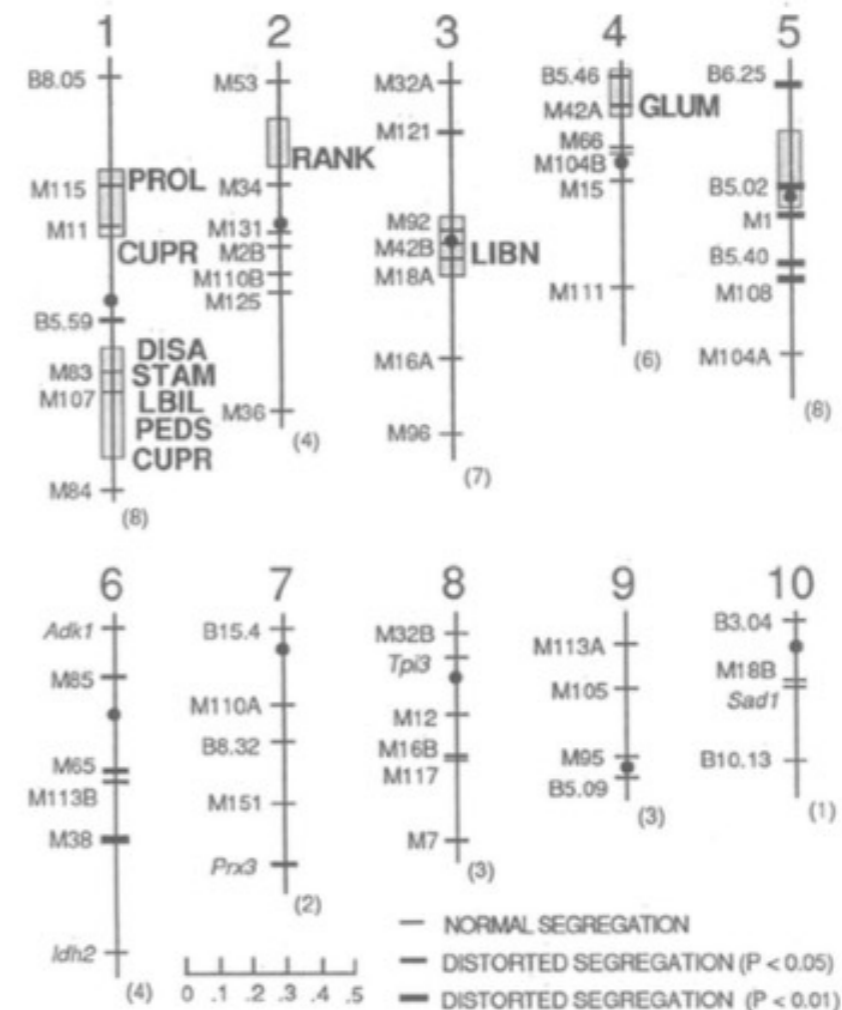
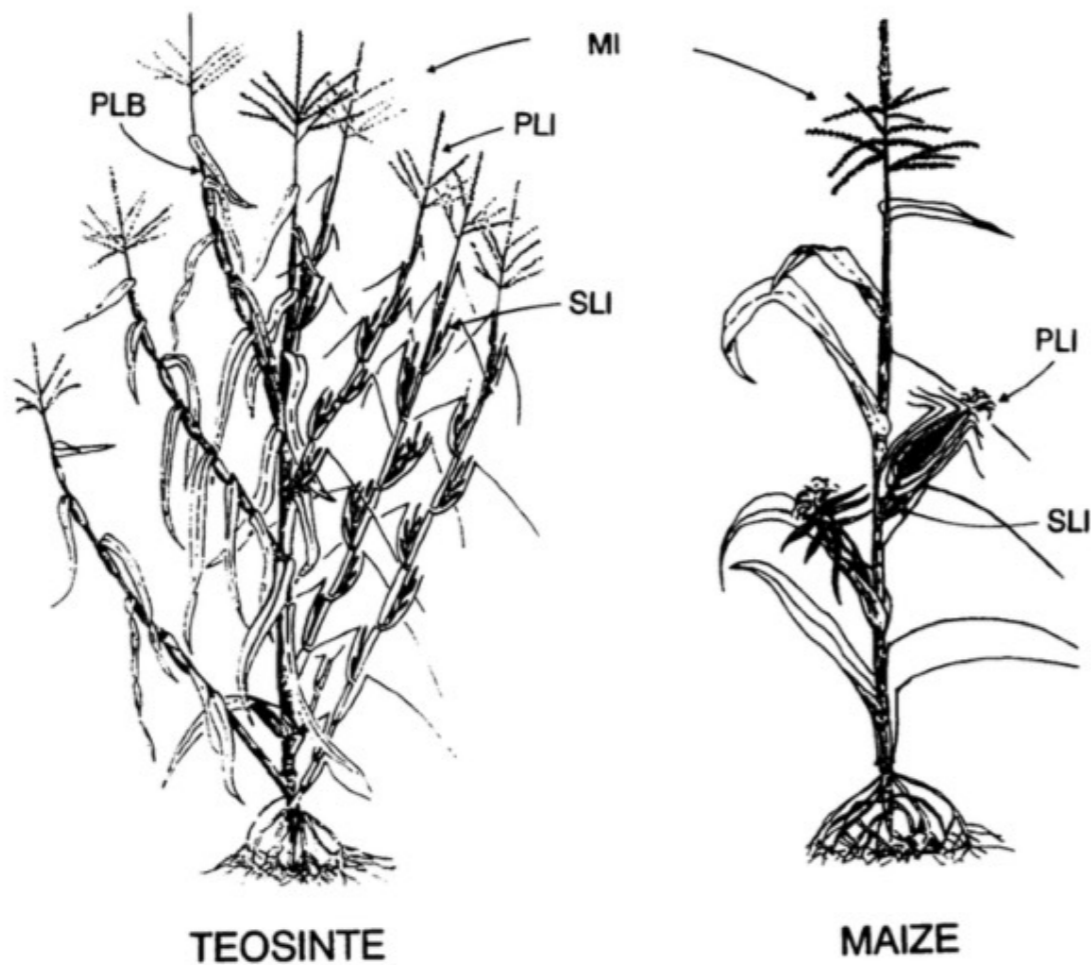
**Figure 1.** Convergent domestication. Convergent phenotypic changes are frequently observed in many different crops because systematic human cultivation often brings about similar demands. Attempts to maximize yield cause selective pressure for an increase in size and number of edible plant parts on 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.

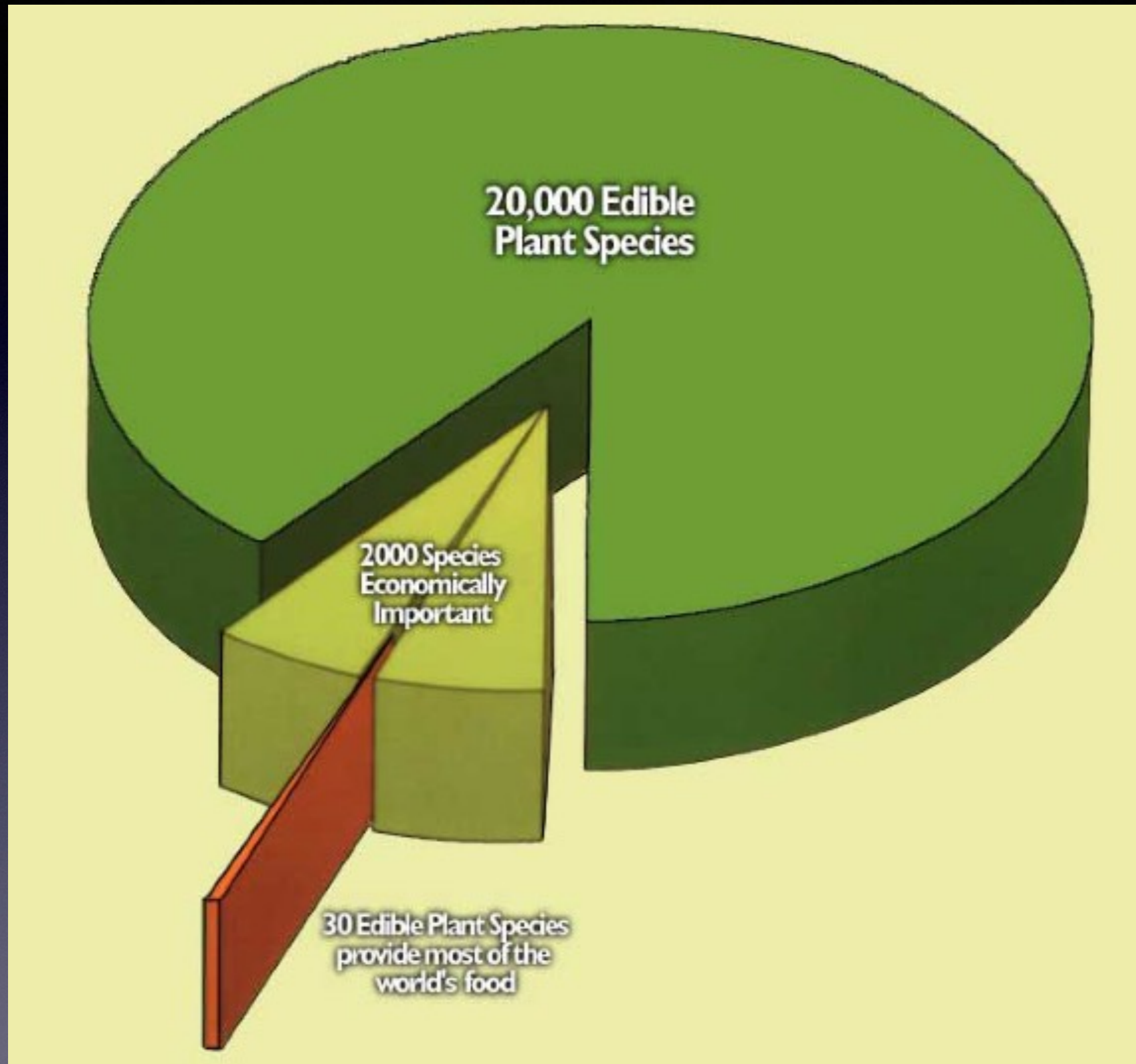


# Major differences between maize and teosinte map to few loci

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

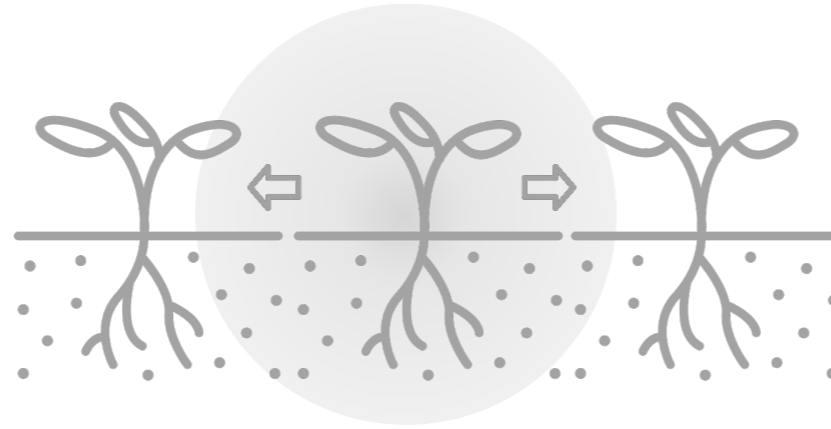
**~400,000 plant species** (<http://www.theplantlist.org>)



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**



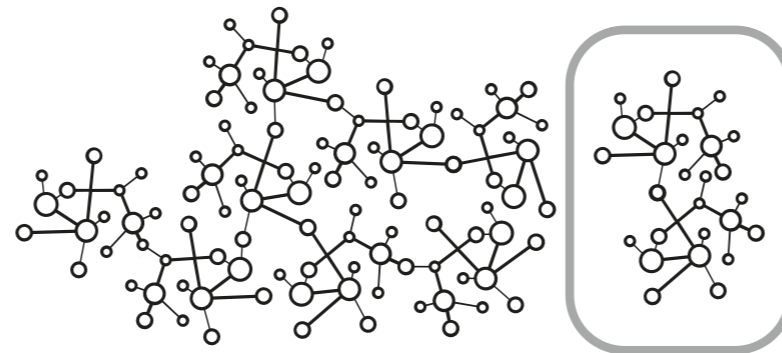
**plant cellular forms**

**DNA code**



**regulatory networks**

**chemistry**

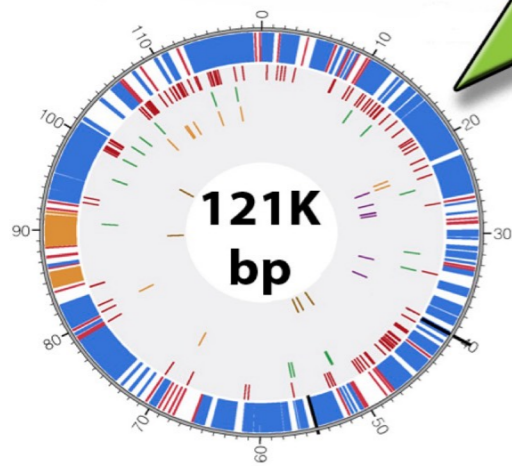


**engineered metabolism**

## Chloroplast

0.5  $\mu\text{m}$

ENERGY



METABOLISM

## Chloroplasts: platform for bioproduction in plants

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

- **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**

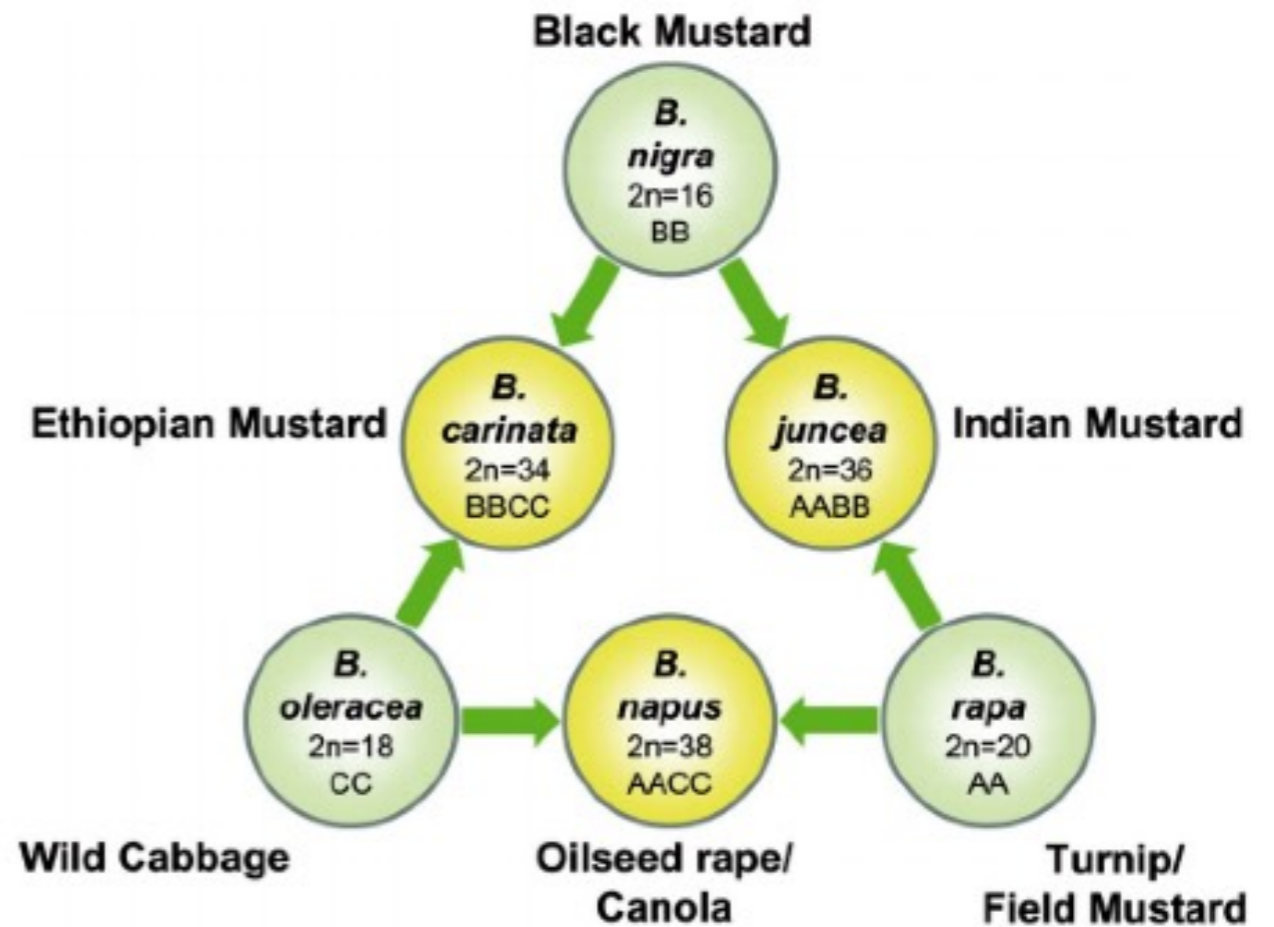


## **Targeted loss-of-gene-function:**

correction of pod-shatter through modified cell differentiation and tissue architecture



# *Brassica napus*



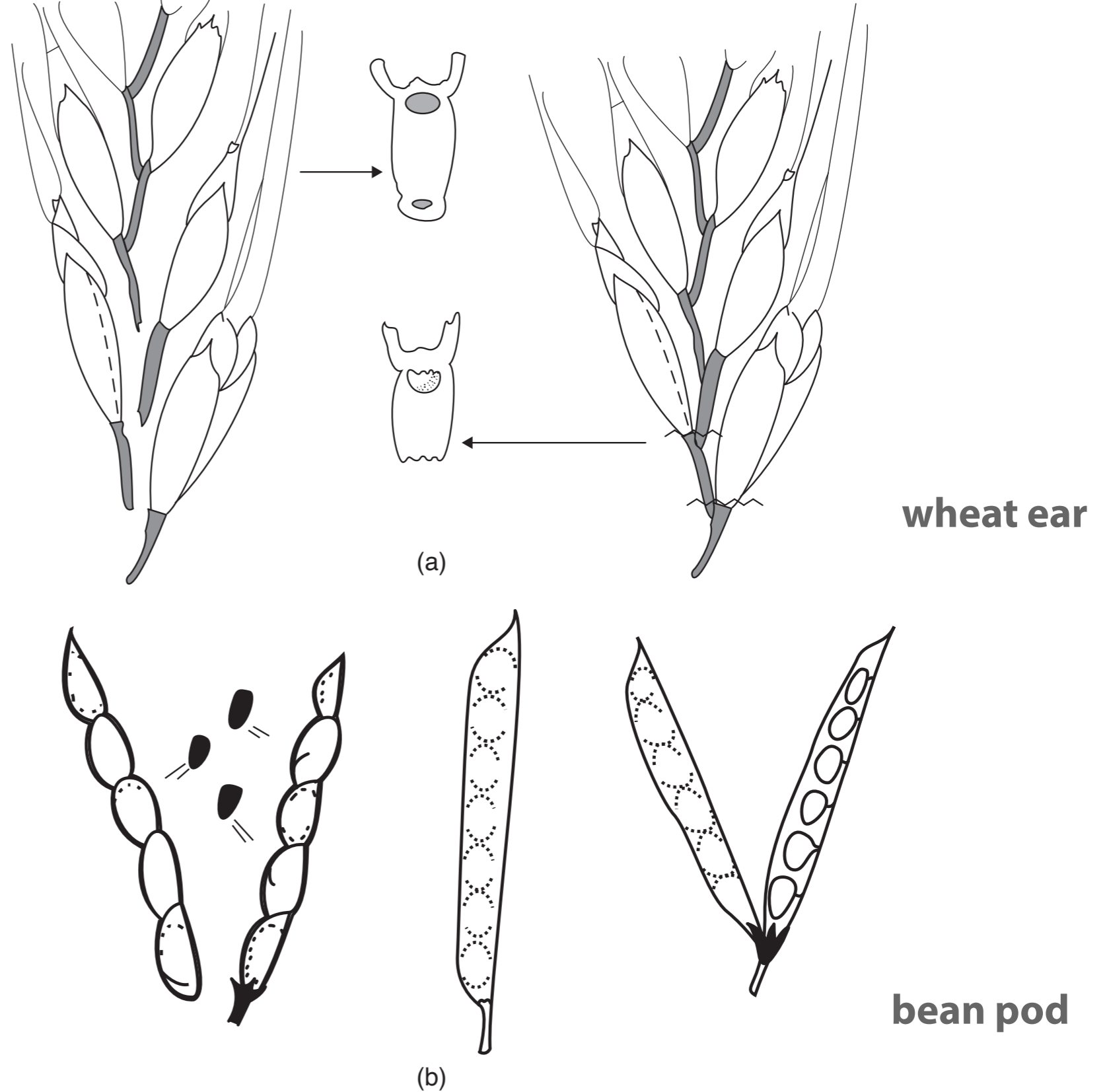
Oilseed rape and Canola are derived from a cross between *Brassica oleracea* and *Brassica rapa*



***Brassica napus* seed have a 45% oil content**

# Crop domestication

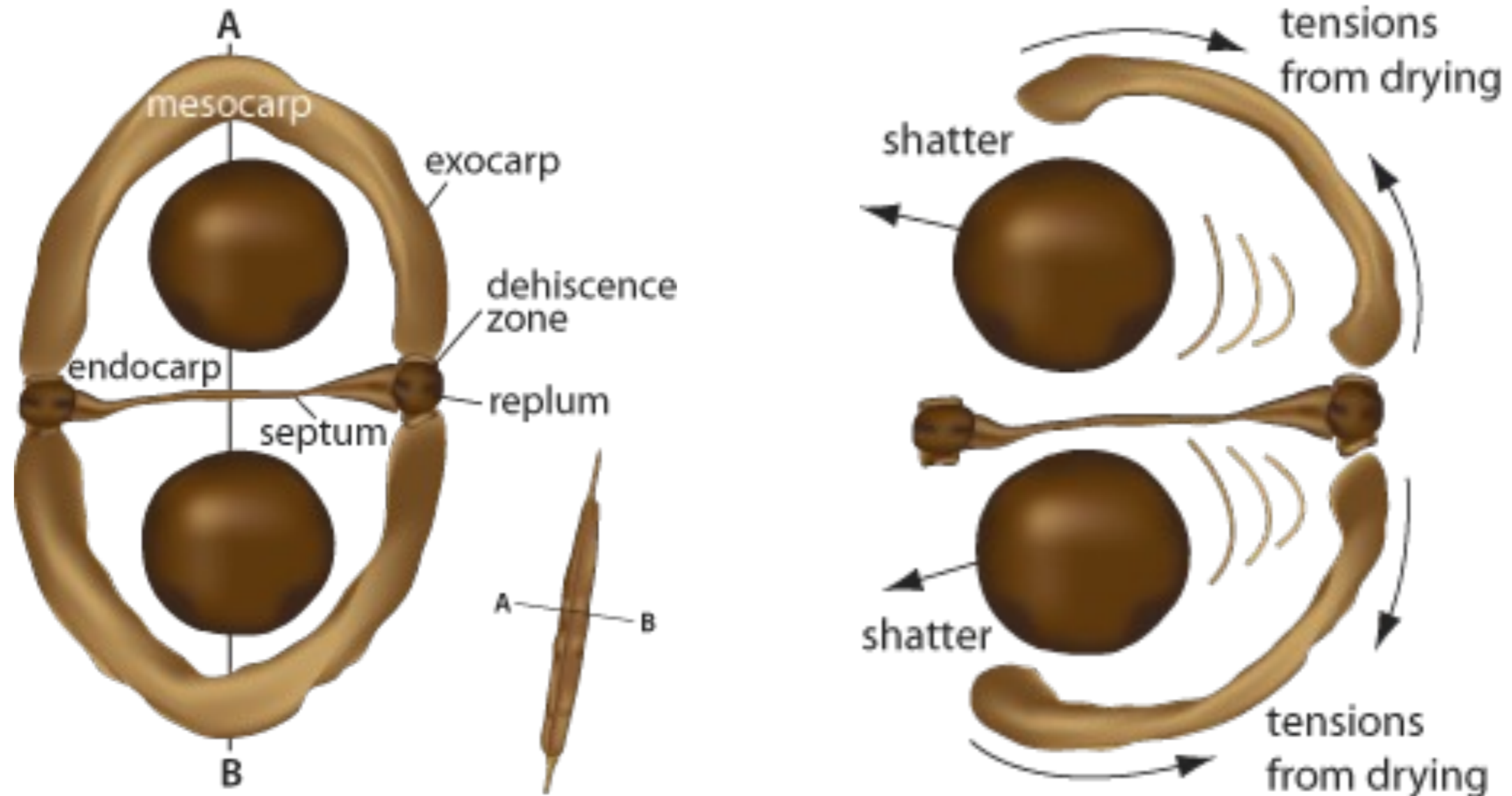
An example of a multicellular trait: reduction of seed shatter and improved yield at harvest



**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)

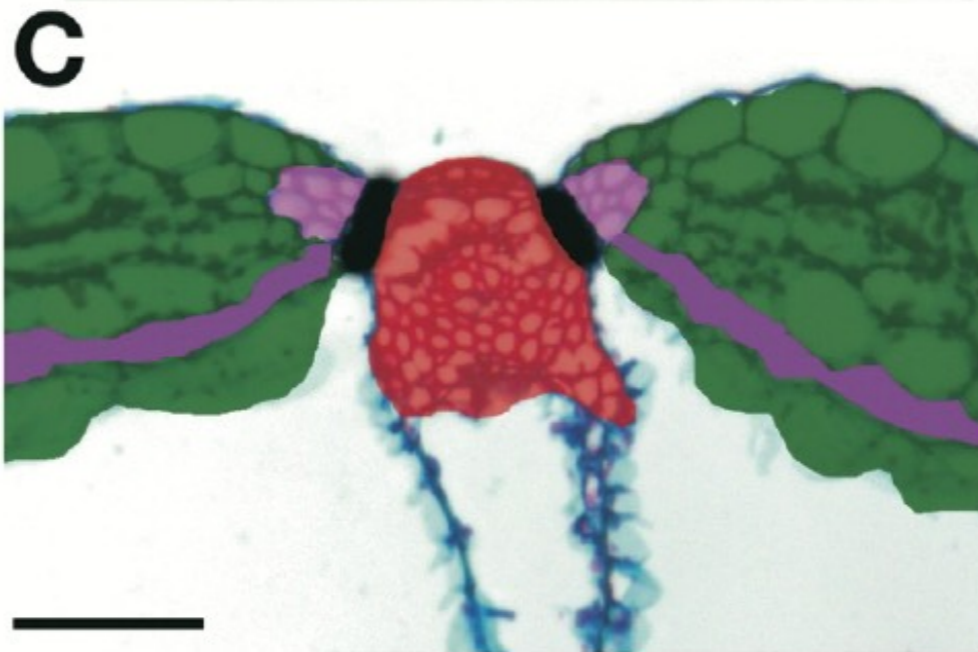
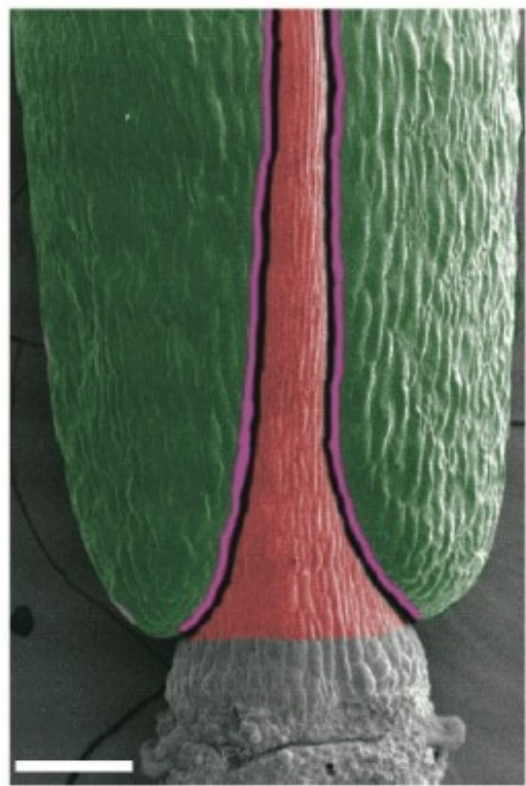
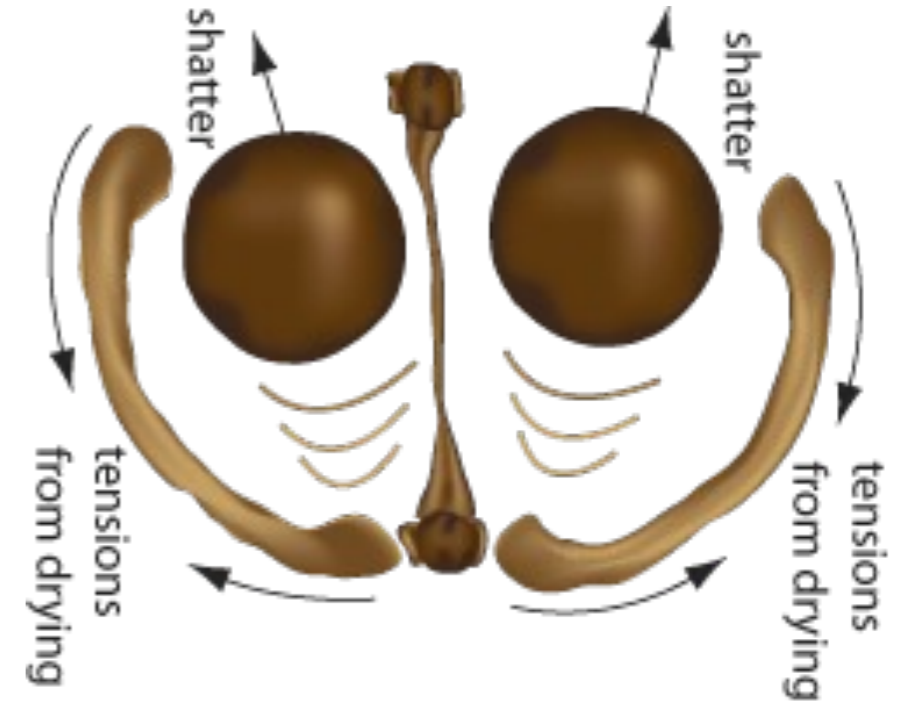
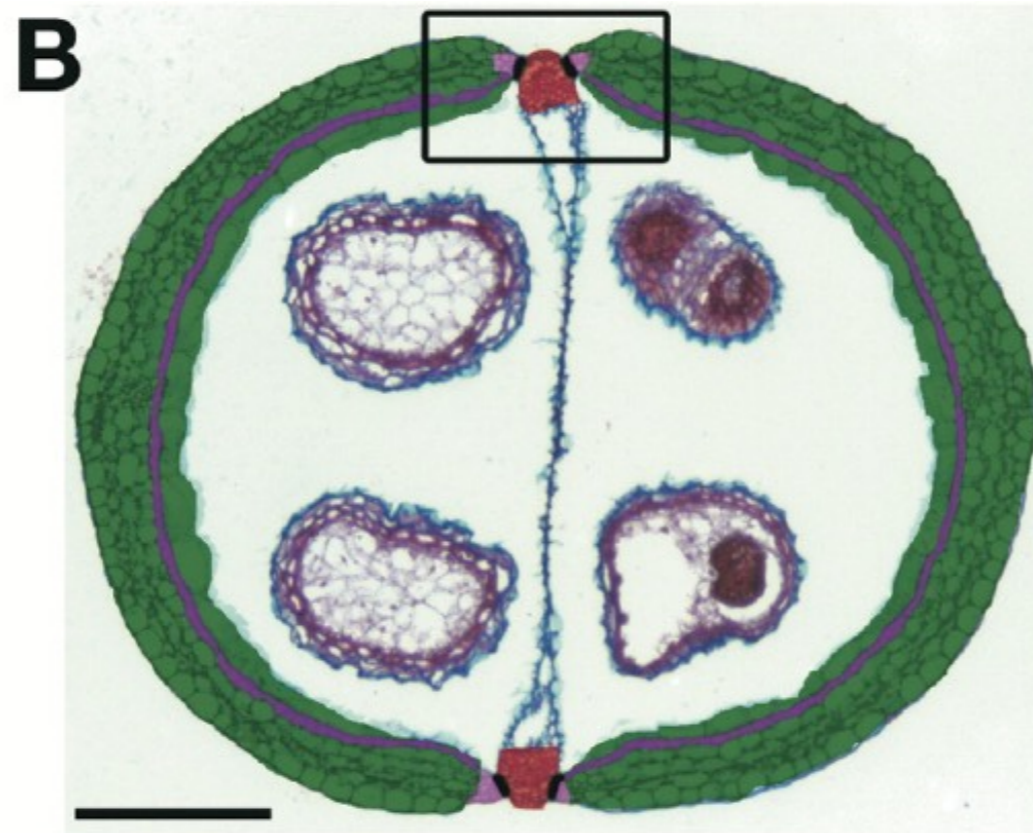
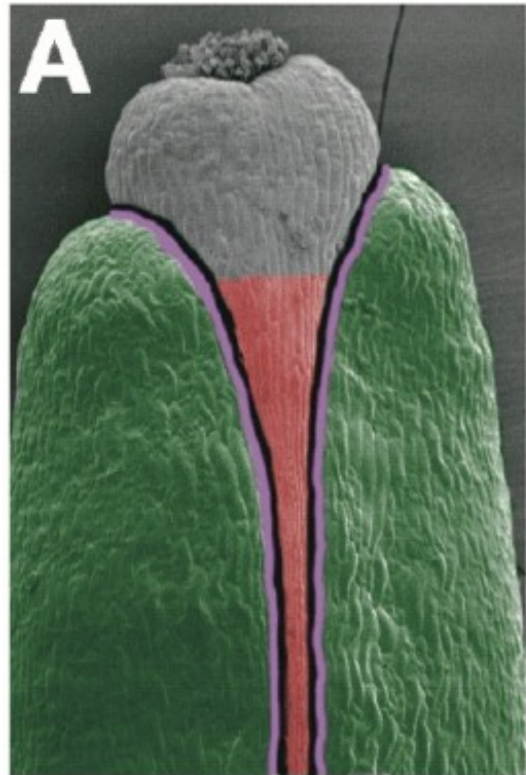
Seed pods are often fragile in the weeks leading up to harvest. During this stage seed pods go through a process of dehiscence (splitting open), commonly known as pod shatter.

This process can result in:

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

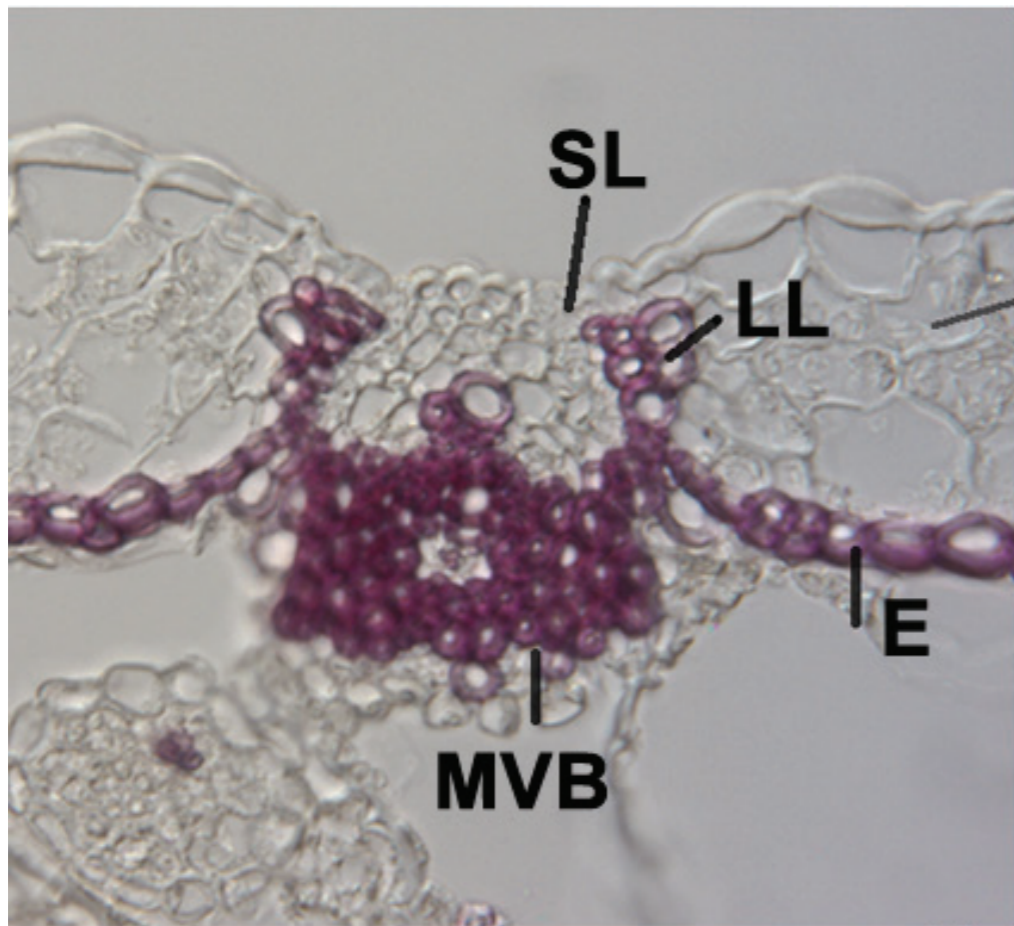
In adverse conditions prior to harvest the potential loss can be as high as 50%

# Specialised cells and valve dehiscence in Arabidopsis

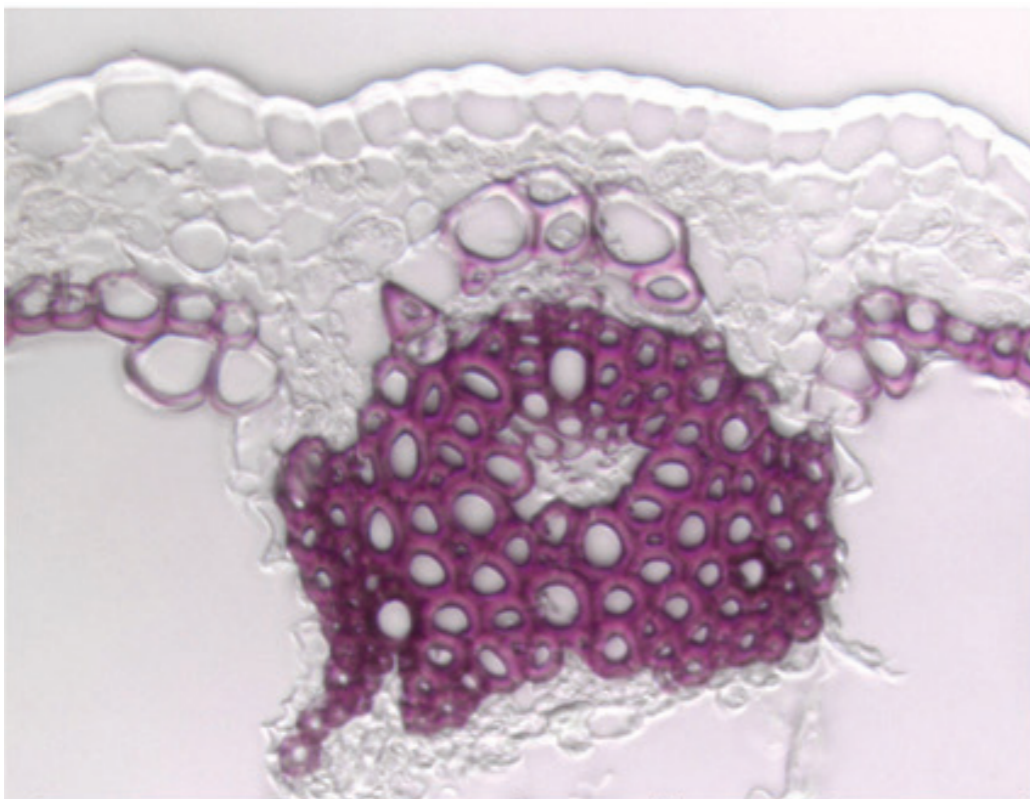


■ valve  
■ replum

■ lignified valve layer  
■ lignified margin layer  
■ separation layer



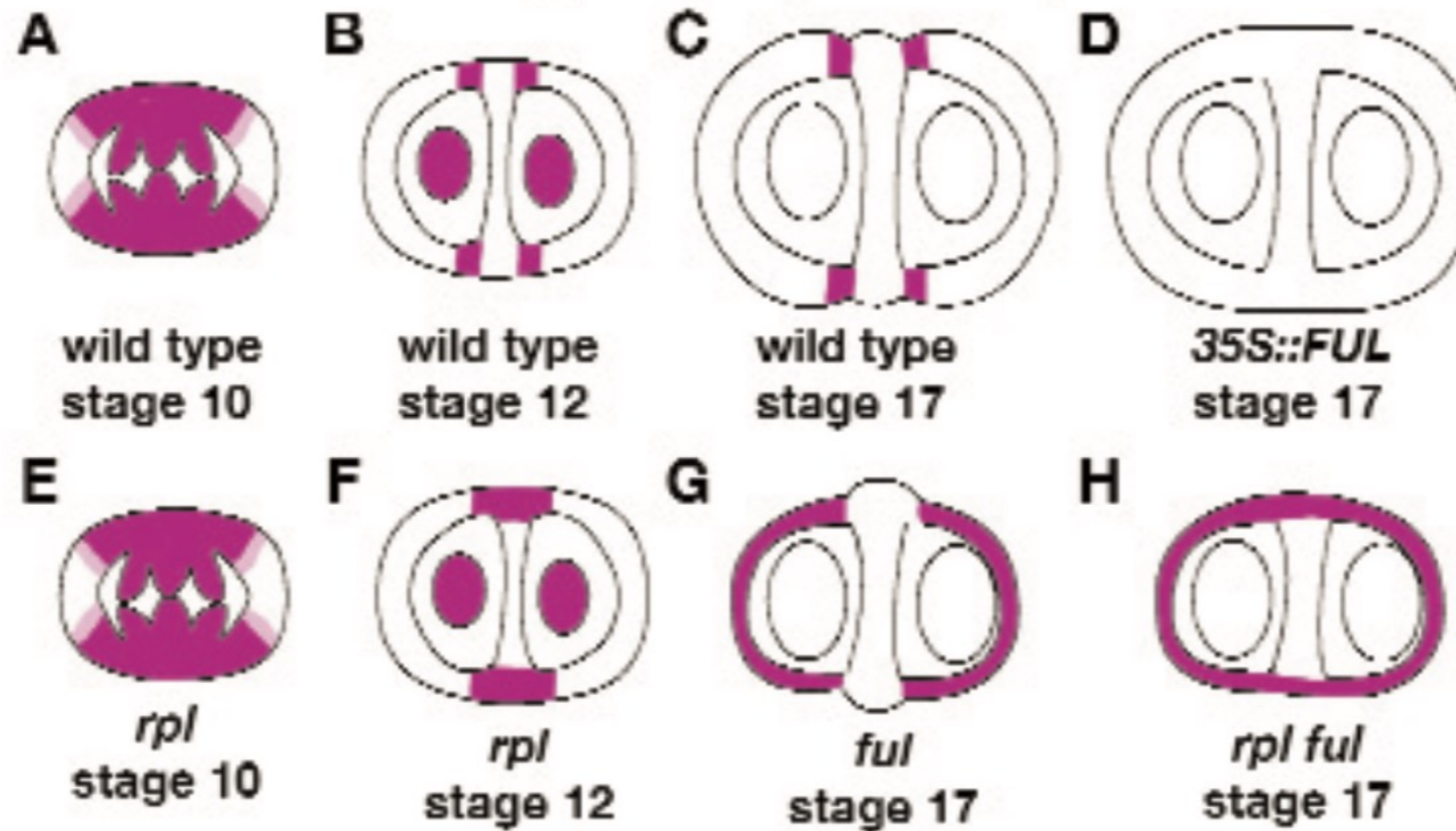
**wildtype**



***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.**

## **SHATTERPROOF (SHP) expression pattern**



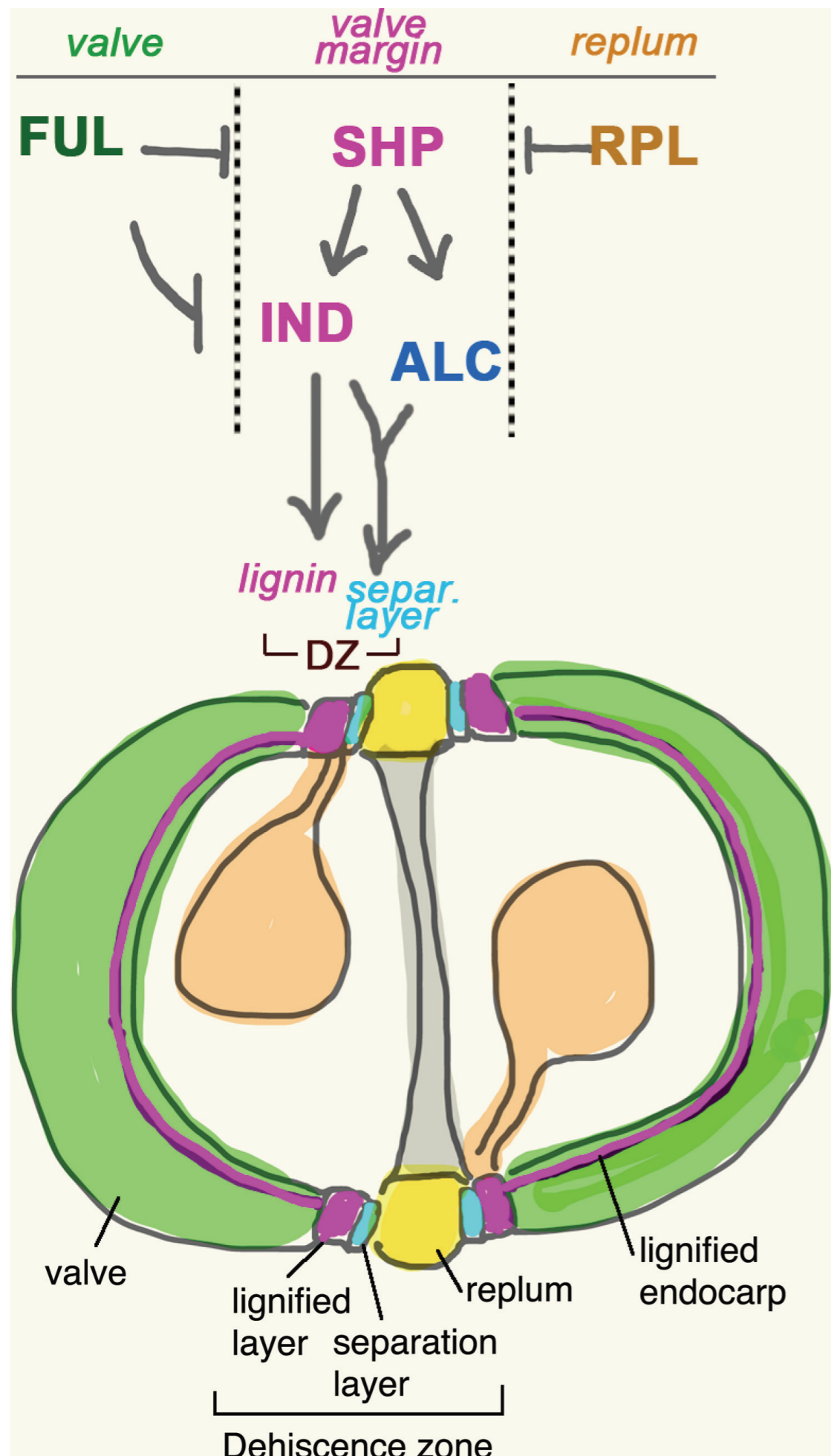
**Figure 15.** *SHP* expression is negatively regulated by *FUL* and *RPL*. (A) Early in development the *SHP* genes are broadly expressed in the gynoecium. At stage 10, their expression extends throughout the valve margins, replum, septum, and developing ovules. Weak expression is also seen extending 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.

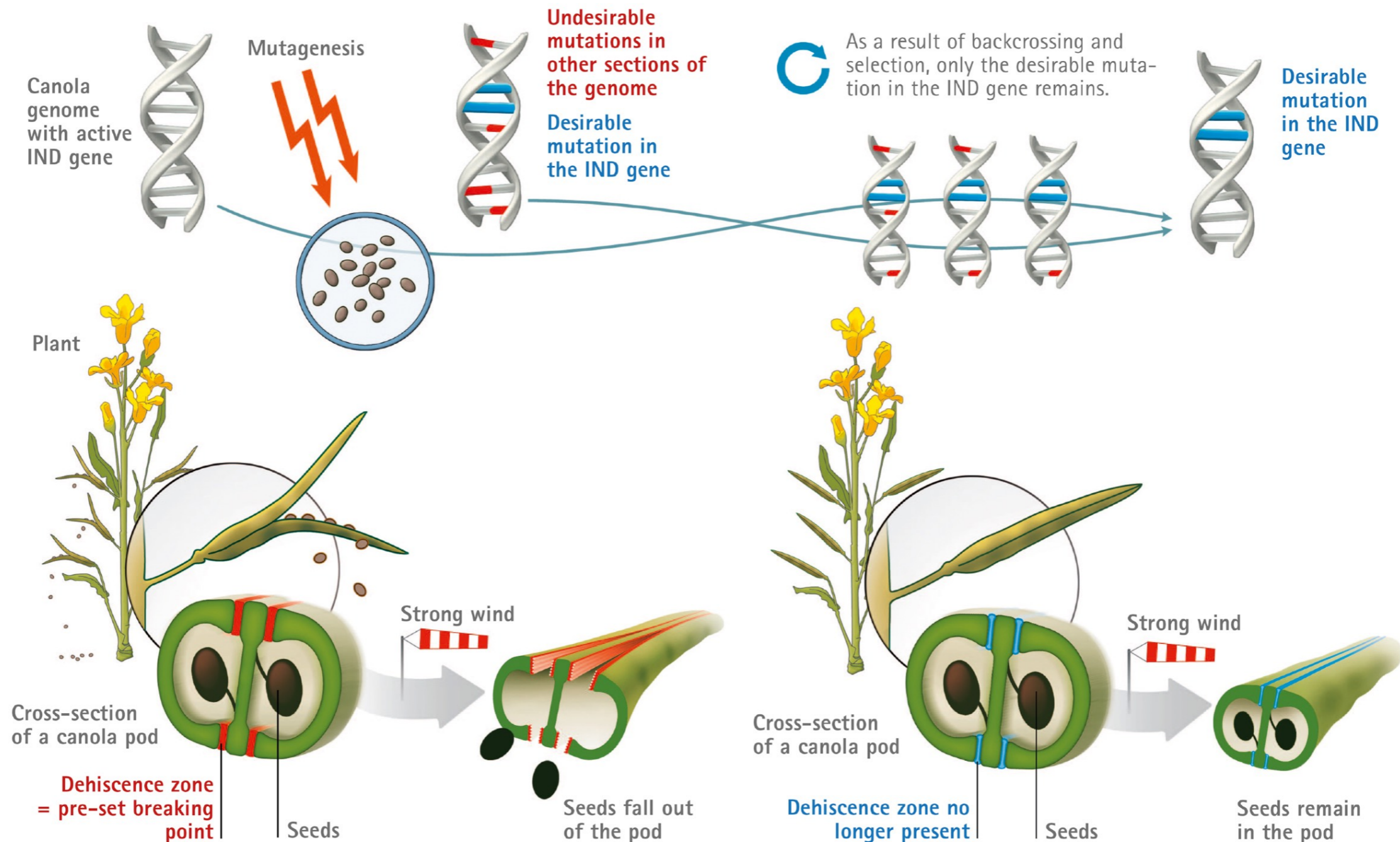
Fruitful (FUL) and Replumless (REP) limit action of Shatterproof (SHP) to the valve margin. SHP induces Indehiscent (IND) and Alcatraz (ALC) to trigger formation of lignified cells and the separation layer in the dehiscence zone.

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.





**InVigor**

**InVigor® L140P**

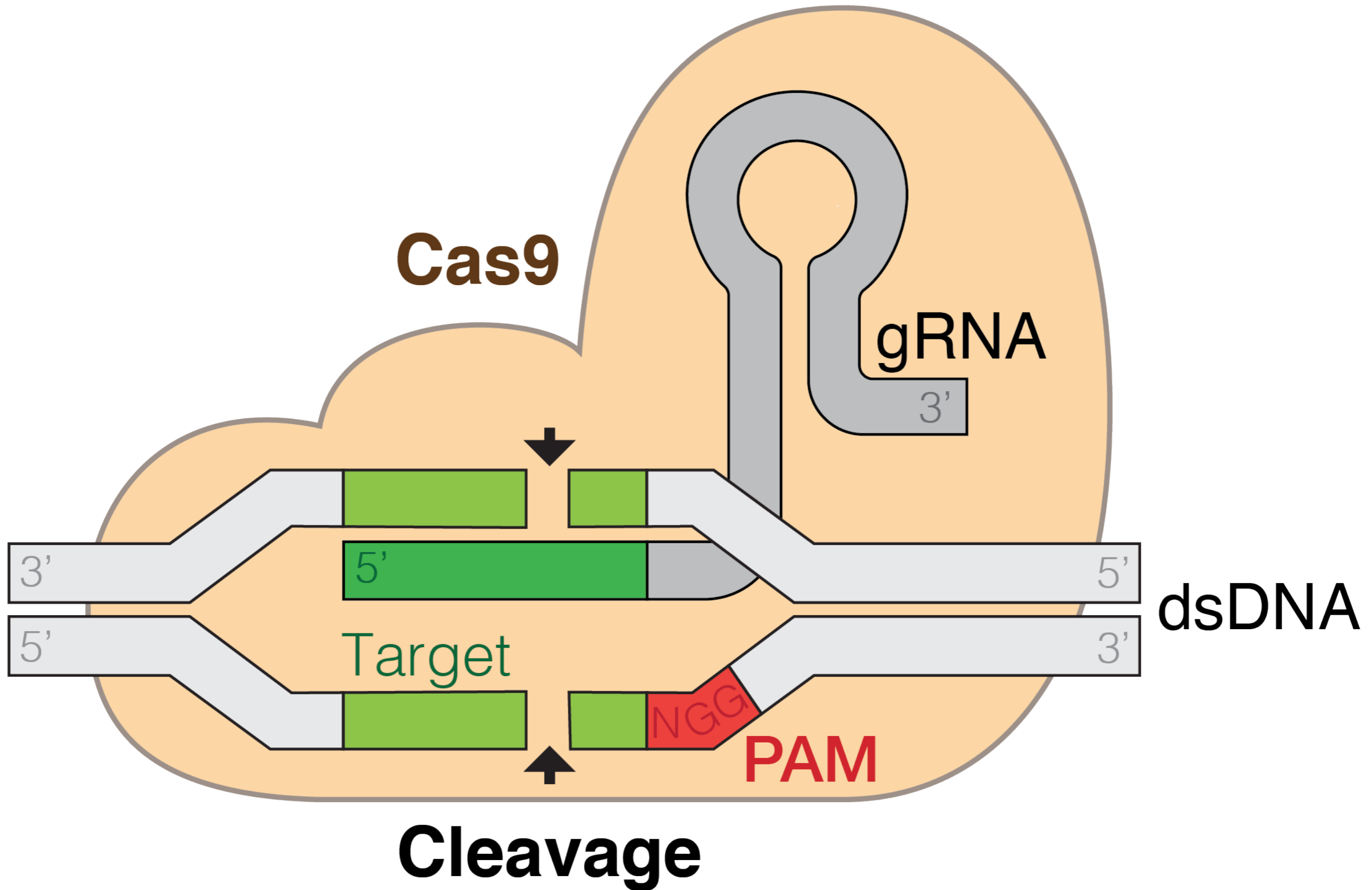
<b>Yield</b>	100% of the checks (InVigor 5440 & Pioneer® 45H29) in 2011/2012 WCC/RRC Co-op trials
<b>Days to Maturity</b>	0.5 days earlier than the average of the checks
<b>Growing Zones</b>	All
<b>Lodging Resistance</b>	Strong
<b>Height</b>	Short–Medium
<b>Blackleg Rating</b>	R (Resistant)
<b>Agronomic Trait</b>	LibertyLink®, Pod Shatter Reduction
<b>Overall Comment</b>	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.

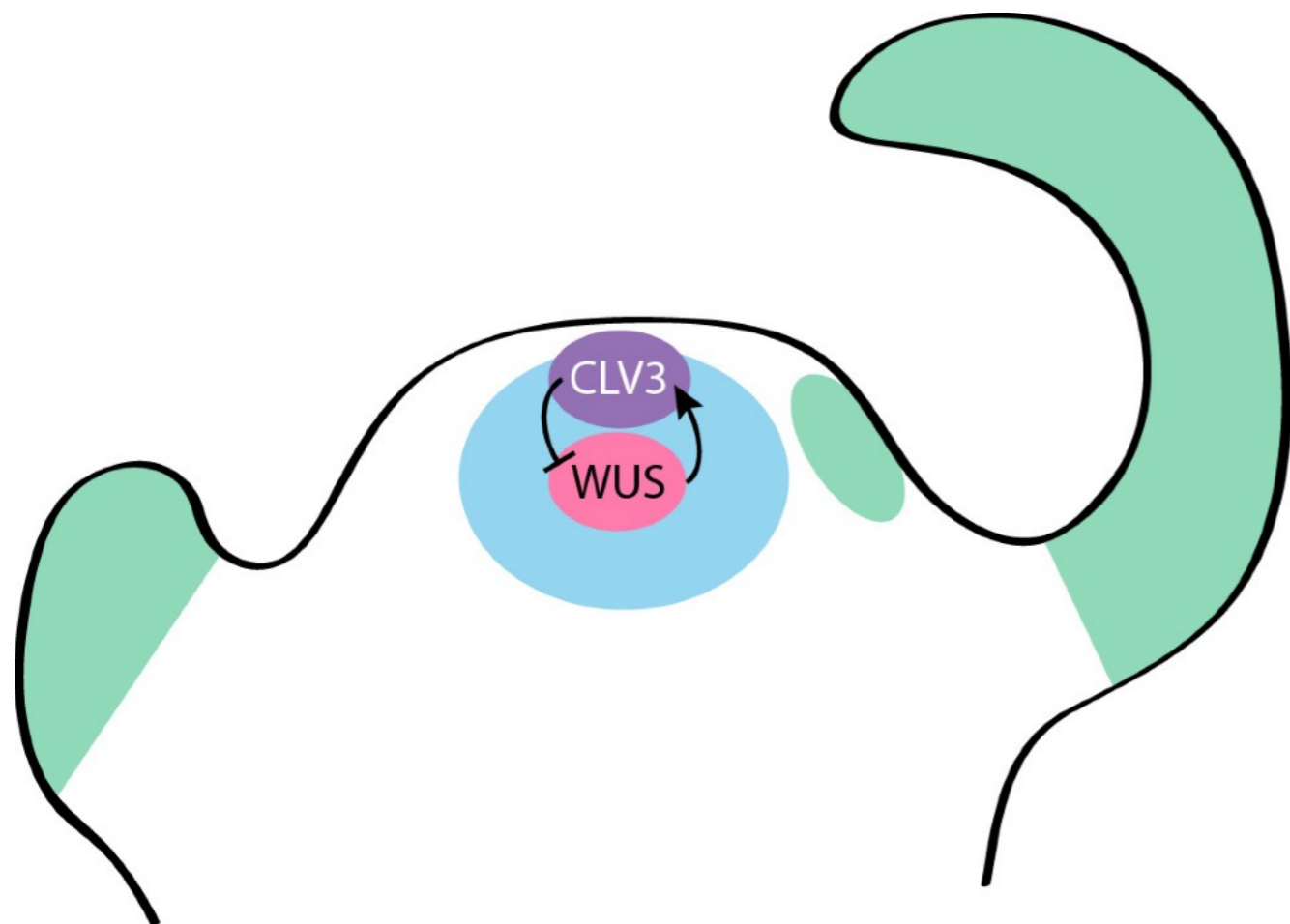


**InVigor**

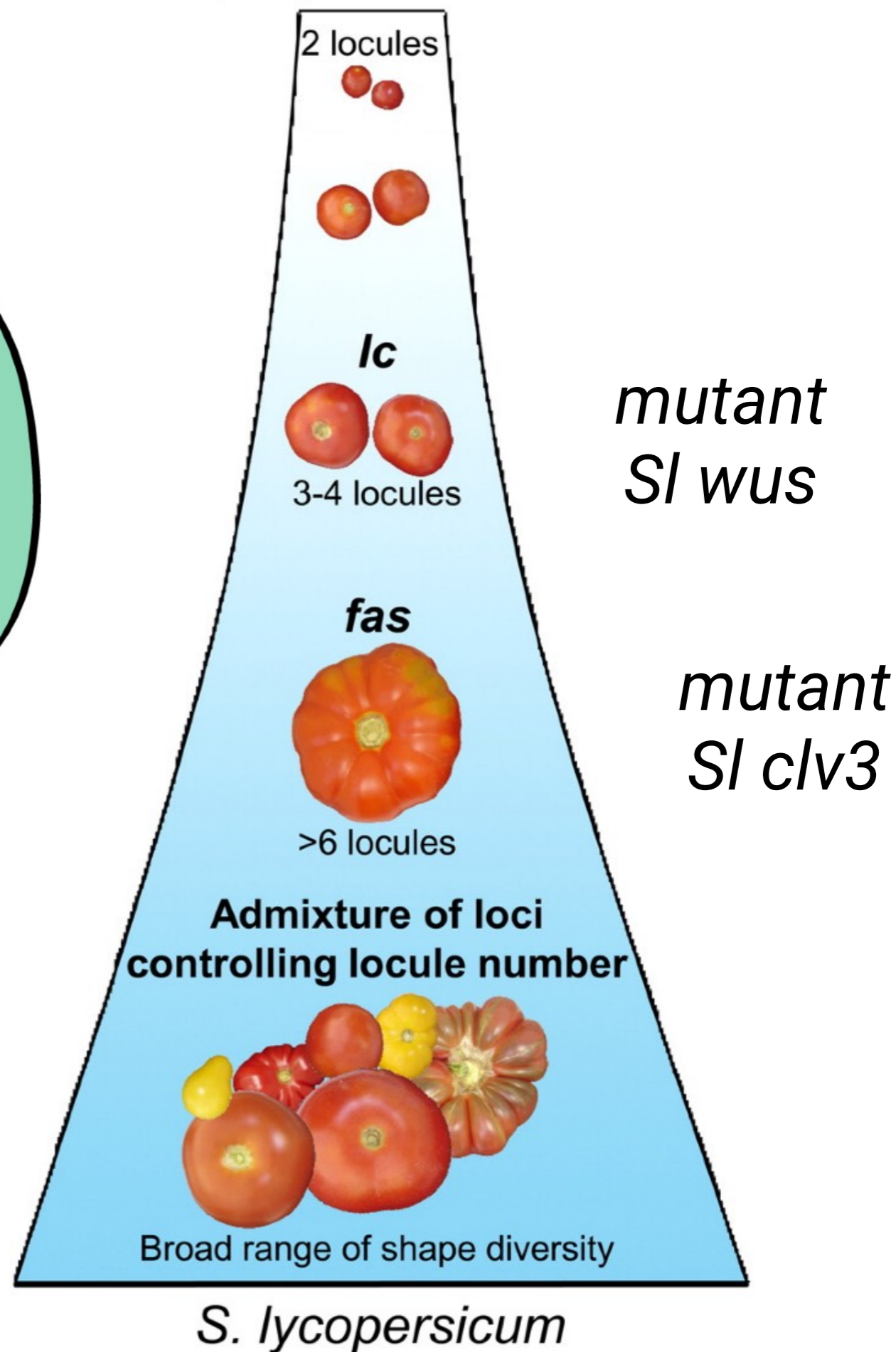
**Editing endogenous regulatory interactions:**  
reprogramming mechanisms for control of  
meristem growth in tomato and related  
plants

# CRISPR-mediated genome editing

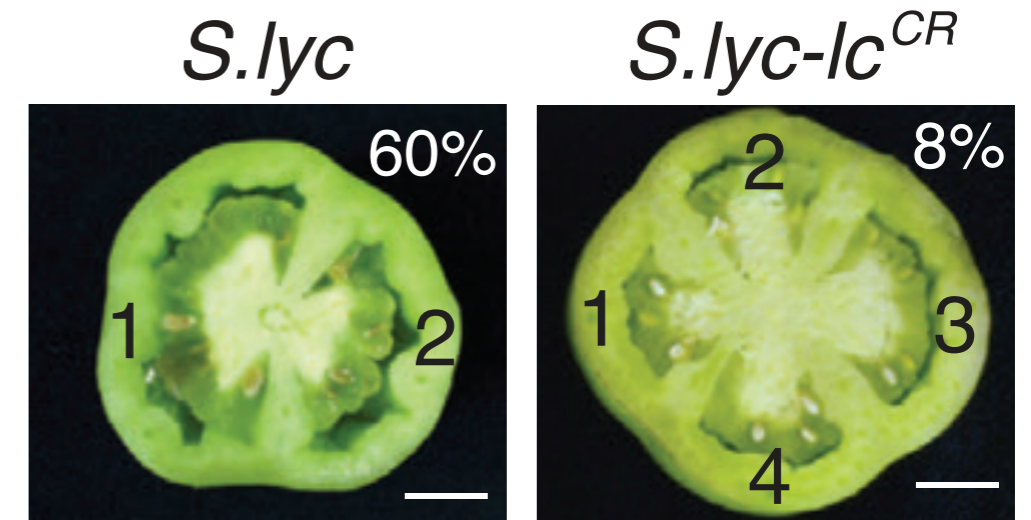
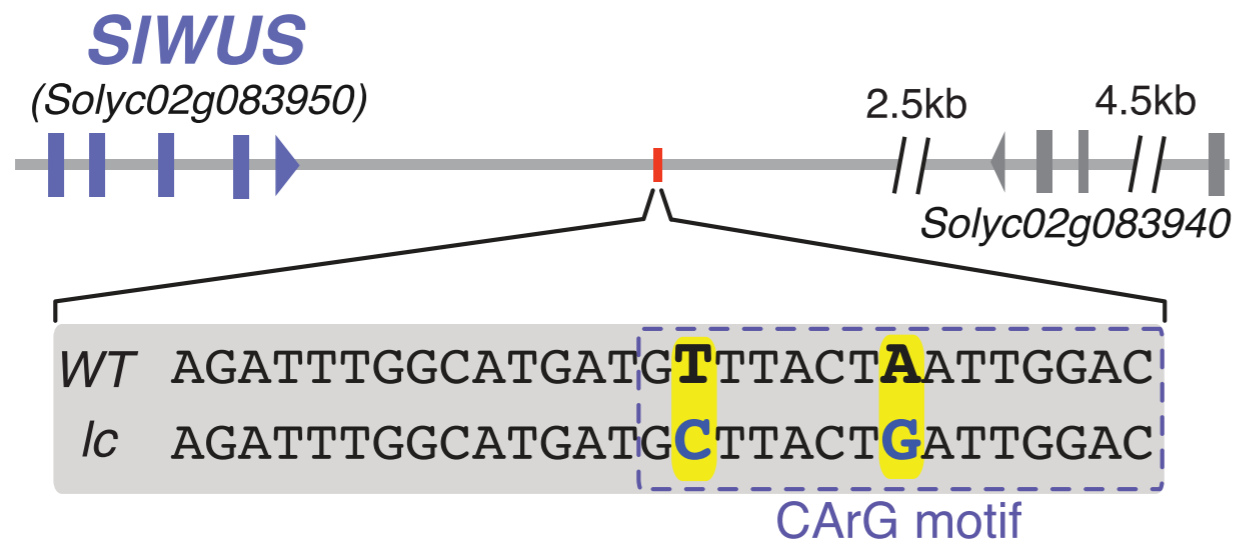
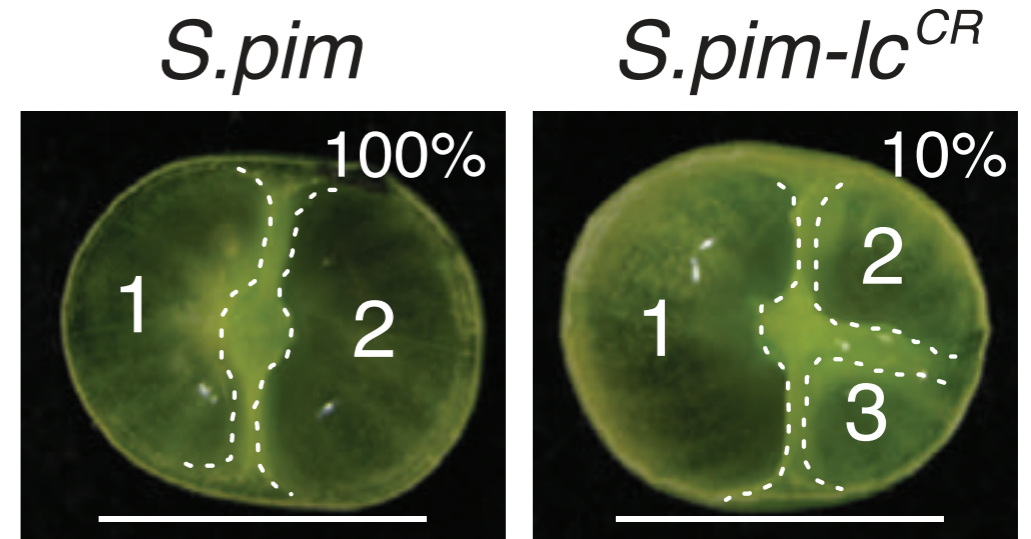
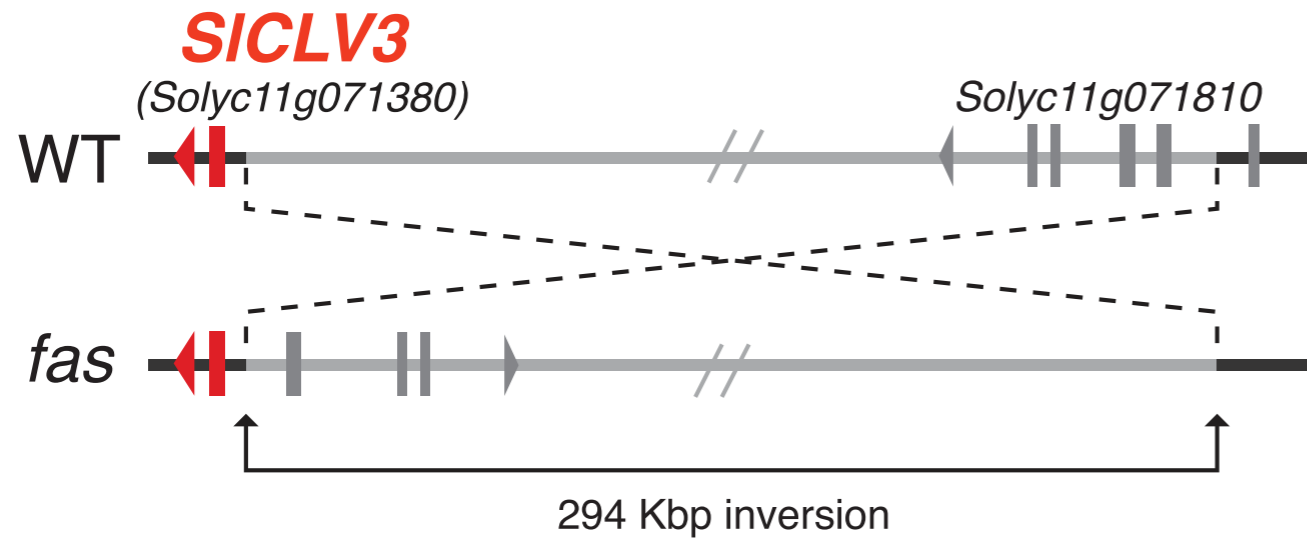




*S. pimpinellifolium*



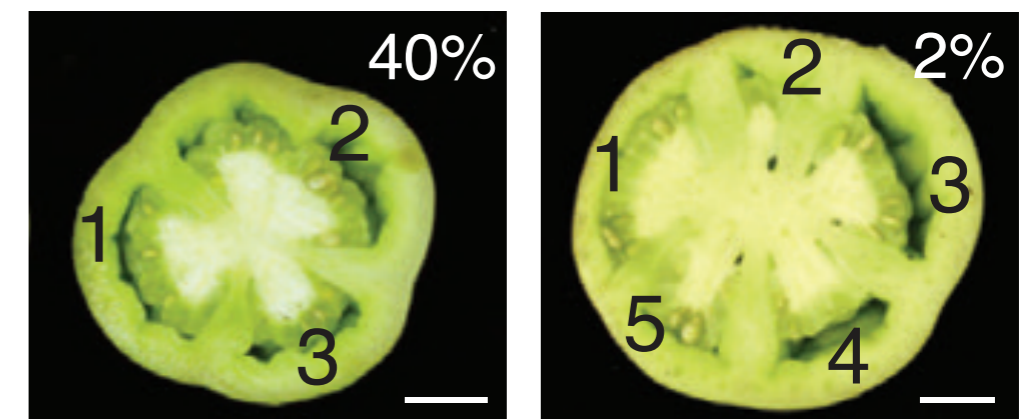
# Recreating known fruit size QTLs in tomato with CRISPR-Cas9



*gRNA* AGATTGGCATGATGTTTACTAATTGGAC  
PAM

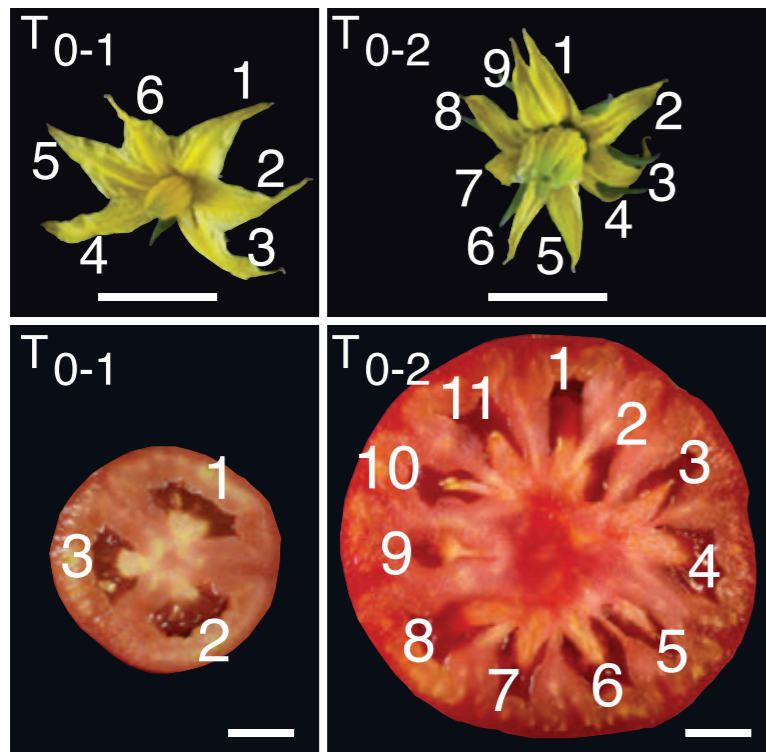
*S.pim-lc<sup>CR</sup>* AGATTGGCATGATGTT---AATTGGAC

*S.lyc-lc<sup>CR</sup>* AGATTGGCATGATGT---AATTGGAC

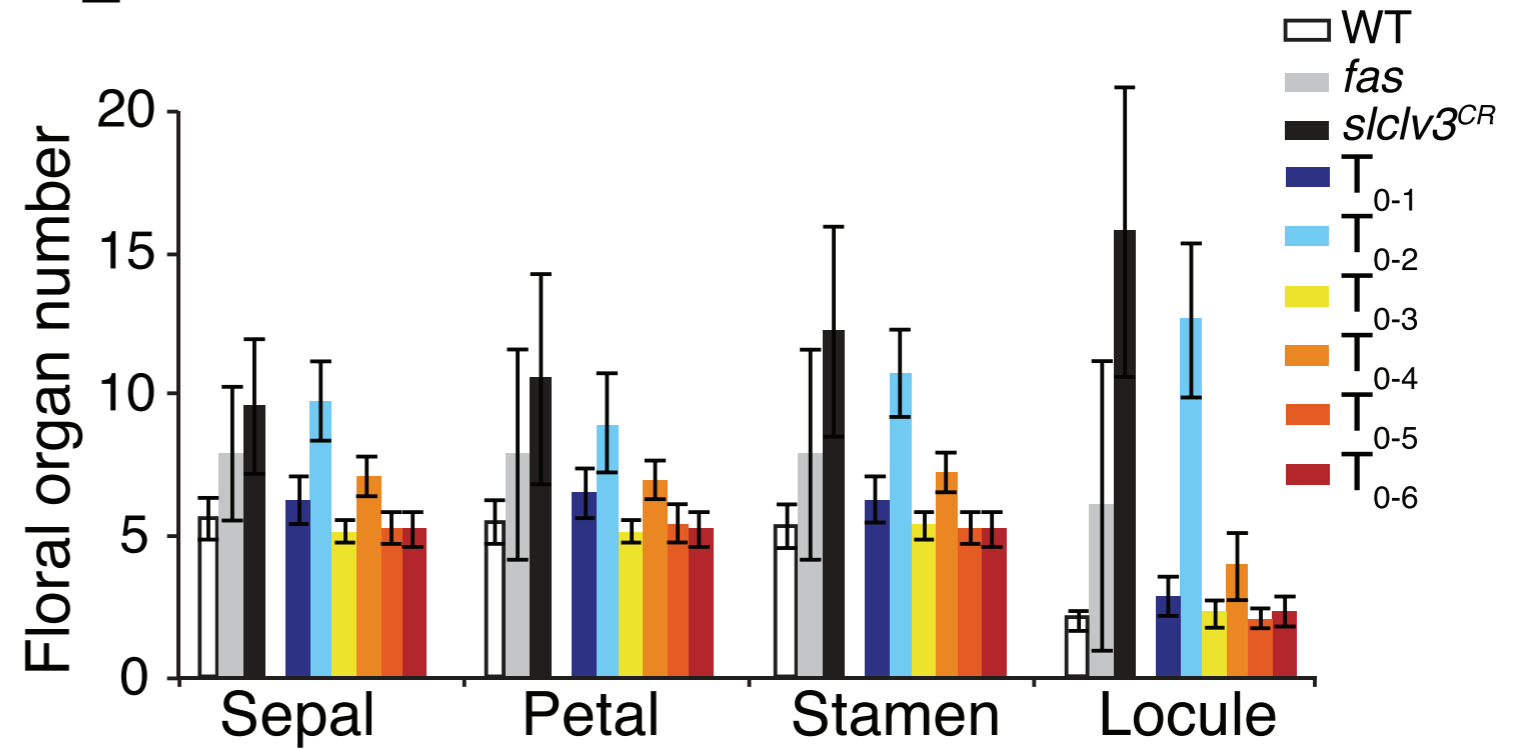


# *SlCLV3/fas* allelic effects on flower and fruit morphology

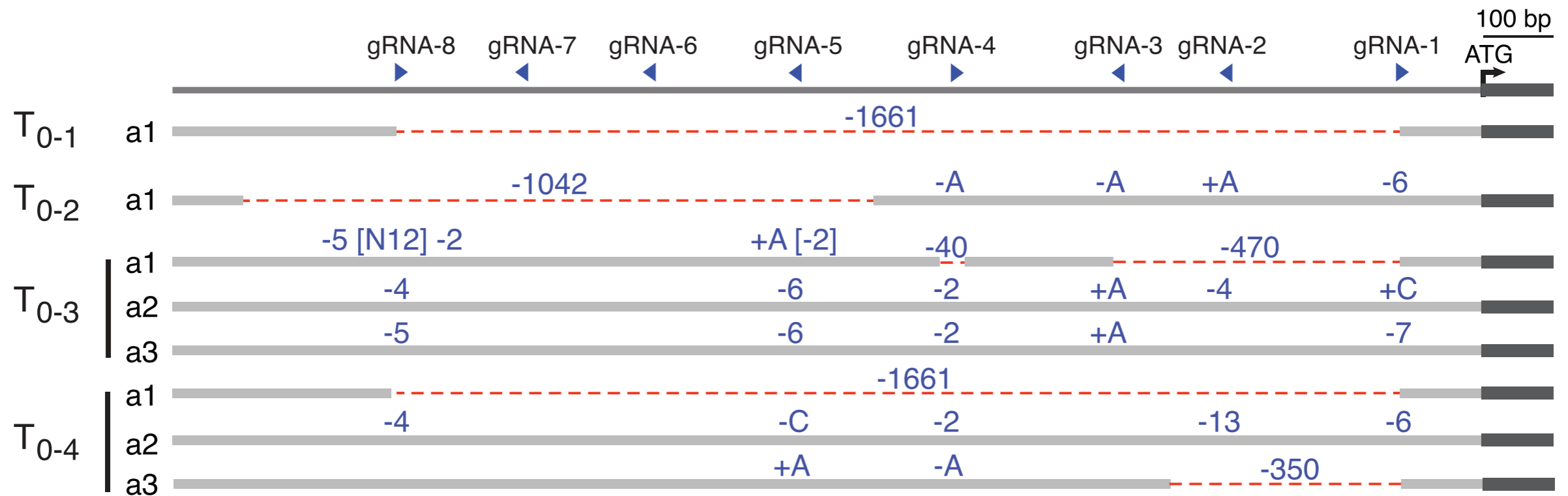
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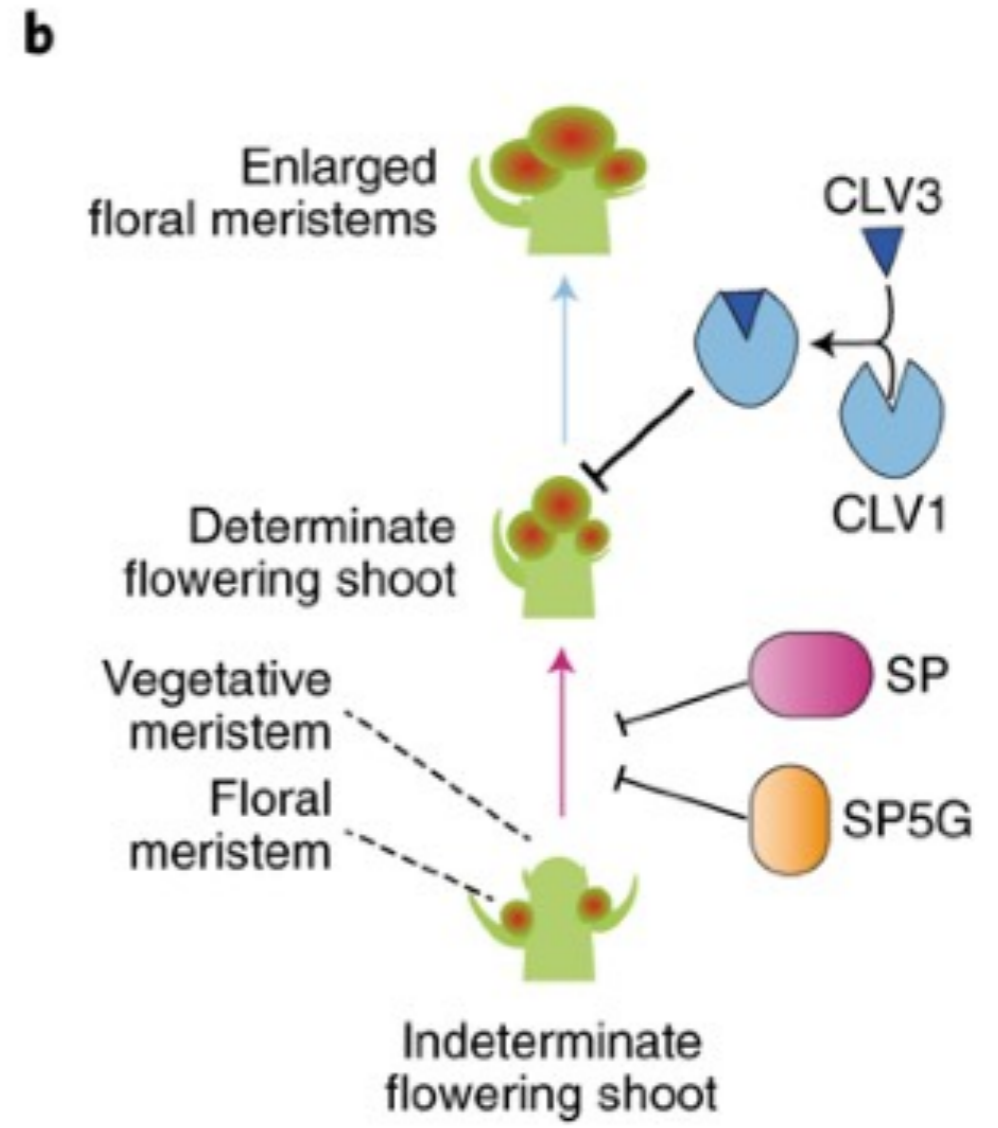
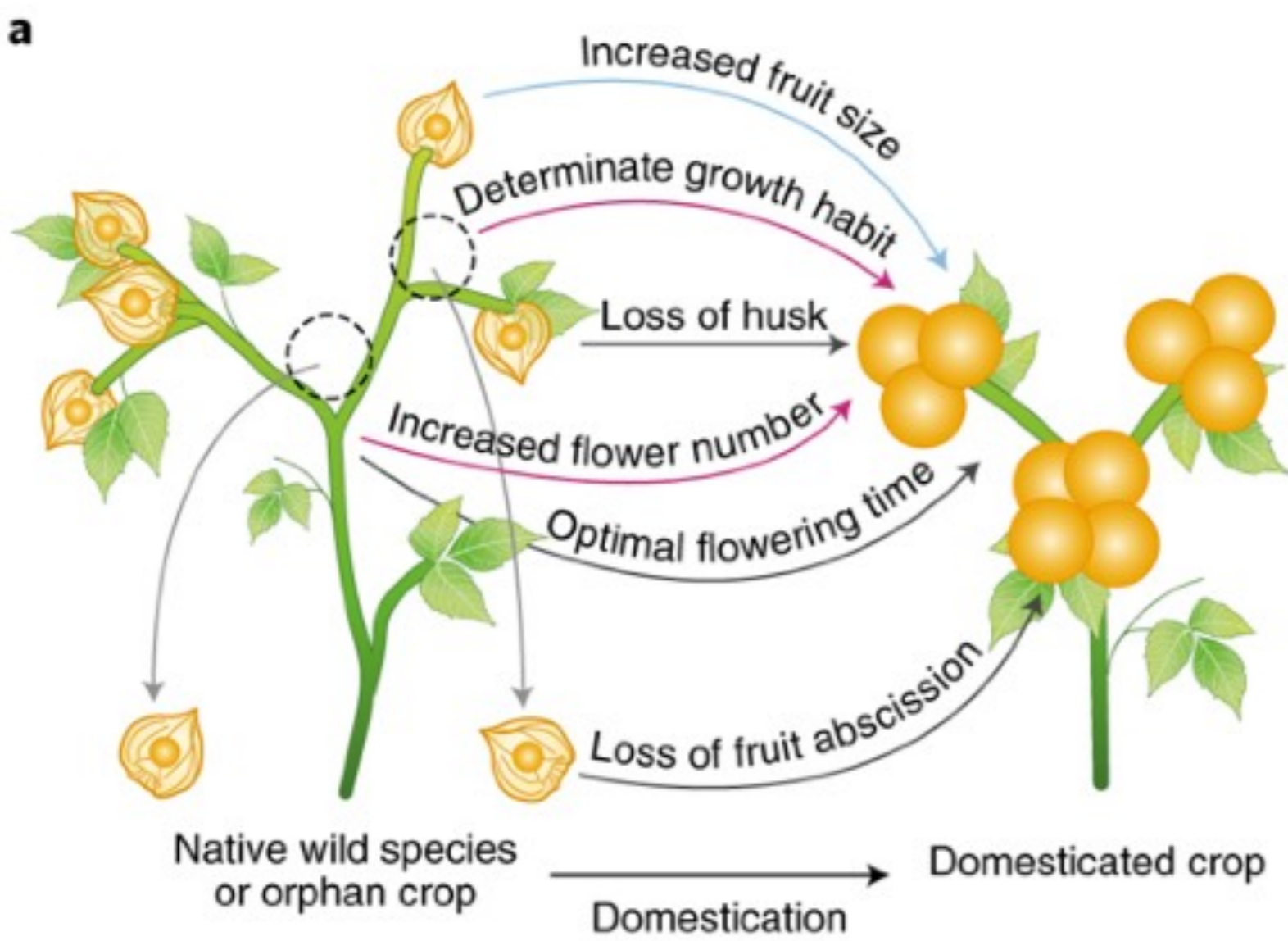
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A Flagship Pioneering Company





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

Zachary H. Lemmon<sup>1</sup>, Nathan T. Reem<sup>2,3,4</sup>, Justin Dalrymple<sup>1,5</sup>, Sebastian Soyk<sup>1,6</sup>, Kerry E. Swartwood<sup>1</sup>, Daniel Rodriguez-Leal<sup>1</sup>, Joyce Van Eck<sup>2,3\*</sup> and Zachary B. Lippman<sup>1,4\*</sup>

Genome editing holds great promise for increasing crop productivity, and there is particular interest in advancing breeding in orphan crops, which are often burdened by undesirable characteristics resembling wild relatives. We developed genomic resources and efficient transformation in the orphan Solanaceae crop *Physalis pruinosa* (groundcherry) and used clustered regularly interspaced short palindromic repeats (CRISPR-Cas9)-associated genome editing (CRISPR-Cas9) to mutate orthologues of tomato domestication and improvement genes. We improved *Physalis pruinosa* flower production and fruit size, thereby improving these major productivity traits. Thus, translating knowledge from model crops enables rapid creation of targeted allelic diversity and novel breeding capabilities in distantly related orphan crops. There has been extensive discussion on leveraging genome editing technologies to improve staple crops, yet their application to regionally important plants grown for subsistence purposes is still lacking, especially in developing countries. Such orphan crops are relatively unknown and typically have not experienced intensive selection for domestication and improvement. Thus, orphan crops are low production, unsuitable at larger agricultural scales, and benefit less from basic research. Genome editing technologies, such as the broadly successful clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 associated genome editing (CRISPR-Cas9), provide opportunities to address these deficiencies, with primary goals to increase quality and yield, improve adaptation and expand genetic diversity of cultivation. The Solanaceae family contains many orphan crops including several wild-cherry and model crops, such as the tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*) and pepper (*Capiscum annuum*). This strong foundation of genetic, developmental and genomic knowledge makes the Solanaceae an excellent platform for translating genome editing to orphan crops. We focused on the orphan crop *Physalis pruinosa* (groundcherry), a wild Solanaceae that is more distantly related to the tomato than the pepper and which is grown in Central and South America for its mildly sweet berries<sup>1</sup>. Barriers to higher productivity and wider cultivation include a wild sprawling growth habit and small ~1 g fruits that drop to the ground due to strong stem abscission (Fig. 1a–c). These undesirable characteristics preclude the wild ancestor of the tomato, *Solanum peruvianum*, for which selection allowed major improvements in shoot architecture, flower production and fruit size<sup>2</sup> (Fig. 1b–d). Although groundcherry and related *Physalis* species have the same chromosome number as related *Solanum* ( $n=12$ ), several challenges remain

**The taming of the shrub**

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

Luca Comai

The world's food supply depends on a few crop species, such as rice, wheat, maize, soy and potato. The availability of genomic information and efficient genome editing tools represents a novel opportunity for crop domestication and improvement<sup>1</sup>. Wild species and unimproved orphan crops can now, in theory, be modified rapidly and in a targeted manner to provide novel and improved crops. Consider groundcherry (*Physalis pruinosa*, Solanaceae) to explore domestication of this species. Specifically, they modify genes whose orthologues control domestication traits in the close relative, tomato. The authors' results demonstrate both the power of this approach and the importance of identifying mechanisms and gene targets. Thanks to genetic domestication of this species, groundcherry can be grown on an agricultural scale because of wild characteristics such as sprawling habit, small, husked fruit and strong fruit abscission. The growth habit and production of small fruits unsuited for agricultural rearing are the characteristics of the wild ancestor *Solanum peruvianum*, which was domesticated to become tomato. Lemmon and co-workers see an opportunity: would modification of the known gene targets of tomato domestication allow corresponding gains in this sister species? Through genome editing, they targeted orthologues of the *SELF-PRENDING* locus in groundcherry, or *SELF-PRENDING* in tomato, a gene that controls indeterminate versus determinate growth in tomato, was too severe to be useful, resulting in extreme dwarfism. Knockout of another *SELF-PRENDING* orthologue, *SP5G*, resulted in increased auxiliary flowering, although caused no change to the primary shoot, nonribbed, fruit density increased. The authors next targeted the *CLAVATA* pathway, which regulates shoot apical meristem size. The interaction of a small peptide, *CLV3*, with its receptors *CLV1* and others. Knockout of *CLV1* resulted in increased flower meristem size, additional flower organs and conversion from two-locule to a larger, three-locule fruit. These manipulations produced variants better suited to, although well short of, full agricultural exploitation and constitute an impressive demonstration of what is possible through a combination

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

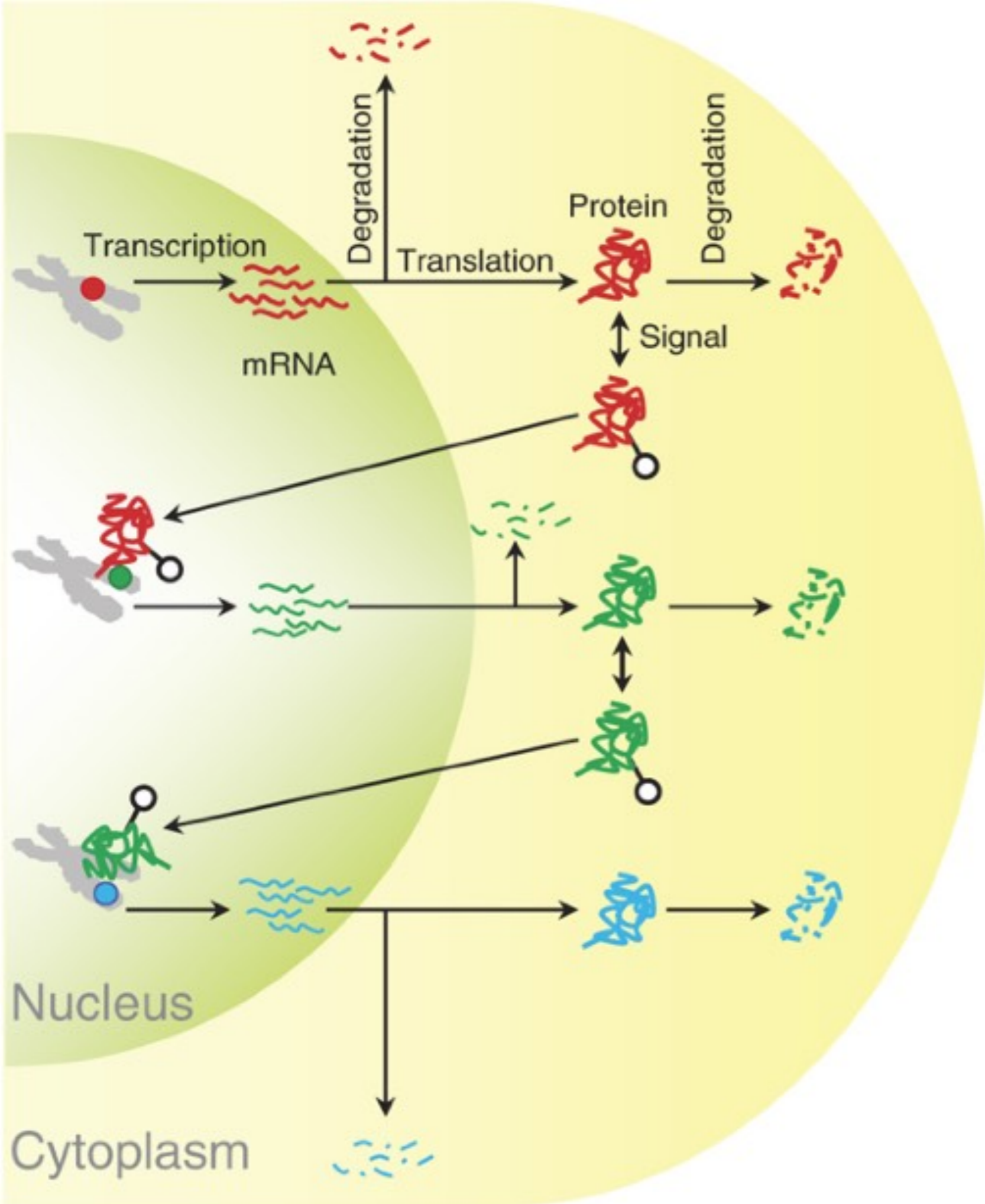
Zachary H. Lemmon, Nathan T. Reem, Justin Dalrymple, Sebastian Soyk, Kerry E. Swartwood, Daniel Rodriguez-Leal, Joyce Van Eck & Zachary B. Lippman.

Nature Plants 4: 766–770 (2018)

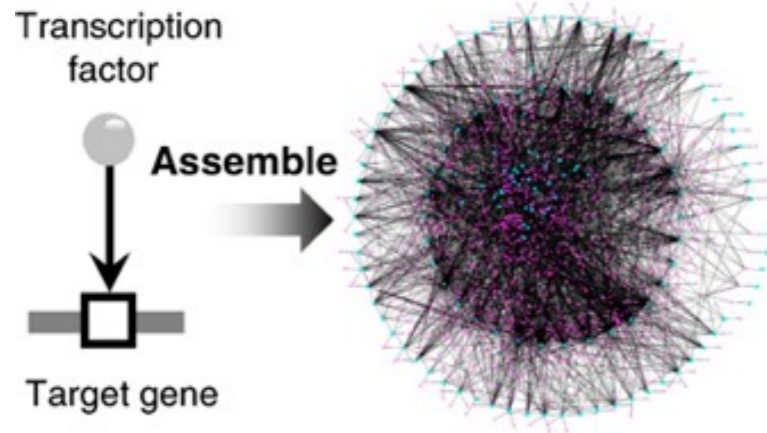
<sup>1</sup> Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA; <sup>2</sup> The Boyce Thompson Institute, Ithaca, NY, USA; <sup>3</sup> Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA; <sup>4</sup> Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA; <sup>5</sup> These authors contributed equally: Nathan T. Reem, Justin Dalrymple, Sebastian Soyk; <sup>6</sup> \*Senior author; <sup>7</sup> Correspondence: jvaneck@cornell.edu

<sup>1</sup> The world's food supply depends on a few crop species, such as rice, wheat, maize, soy and potato. The availability of genomic information and efficient genome editing tools represents a novel opportunity for crop domestication and improvement<sup>1</sup>. Wild species and unimproved orphan crops can now, in theory, be modified rapidly and in a targeted manner to provide novel and improved crops. Consider groundcherry (*Physalis pruinosa*, Solanaceae) to explore domestication of this species. Specifically, they modify genes whose orthologues control domestication traits in the close relative, tomato. The authors' results demonstrate both the power of this approach and the importance of identifying mechanisms and gene targets. Thanks to genetic domestication of this species, groundcherry can be grown on an agricultural scale because of wild characteristics such as sprawling habit, small, husked fruit and strong fruit abscission. The growth habit and production of small fruits unsuited for agricultural rearing are the characteristics of the wild ancestor *Solanum peruvianum*, which was domesticated to become tomato. Lemmon and co-workers see an opportunity: would modification of the known gene targets of tomato domestication allow corresponding gains in this sister species? Through genome editing, they targeted orthologues of the *SELF-PRENDING* locus in groundcherry, or *SELF-PRENDING* in tomato, a gene that controls indeterminate versus determinate growth in tomato, was too severe to be useful, resulting in extreme dwarfism. Knockout of another *SELF-PRENDING* orthologue, *SP5G*, resulted in increased auxiliary flowering, although caused no change to the primary shoot, nonribbed, fruit density increased. The authors next targeted the *CLAVATA* pathway, which regulates shoot apical meristem size. The interaction of a small peptide, *CLV3*, with its receptors *CLV1* and others. Knockout of *CLV1* resulted in increased flower meristem size, additional flower organs and conversion from two-locule to a larger, three-locule fruit. These manipulations produced variants better suited to, although well short of, full agricultural exploitation and constitute an impressive demonstration of what is possible through a combination

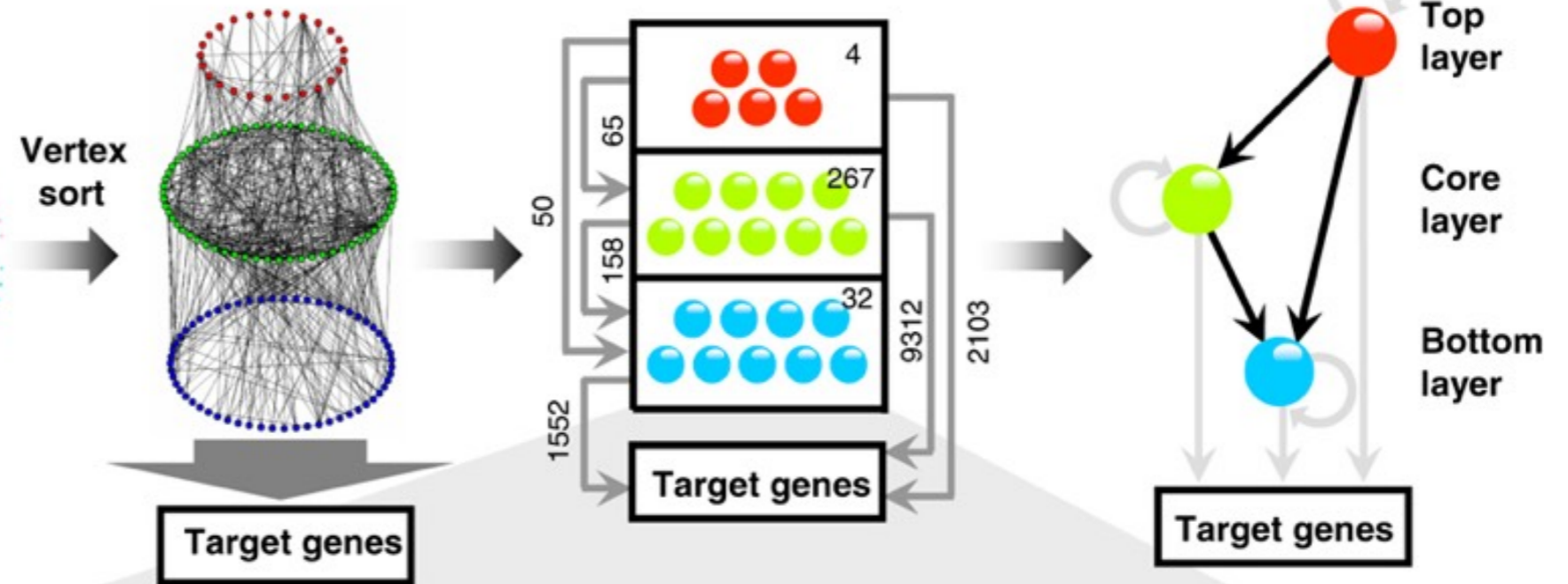
**Pathway engineering in plants:**  
introducing ectopic positive-feedback  
regulatory loops for hyper-expression of  
existing pathways



### A Transcription network



### B Hierarchical organization

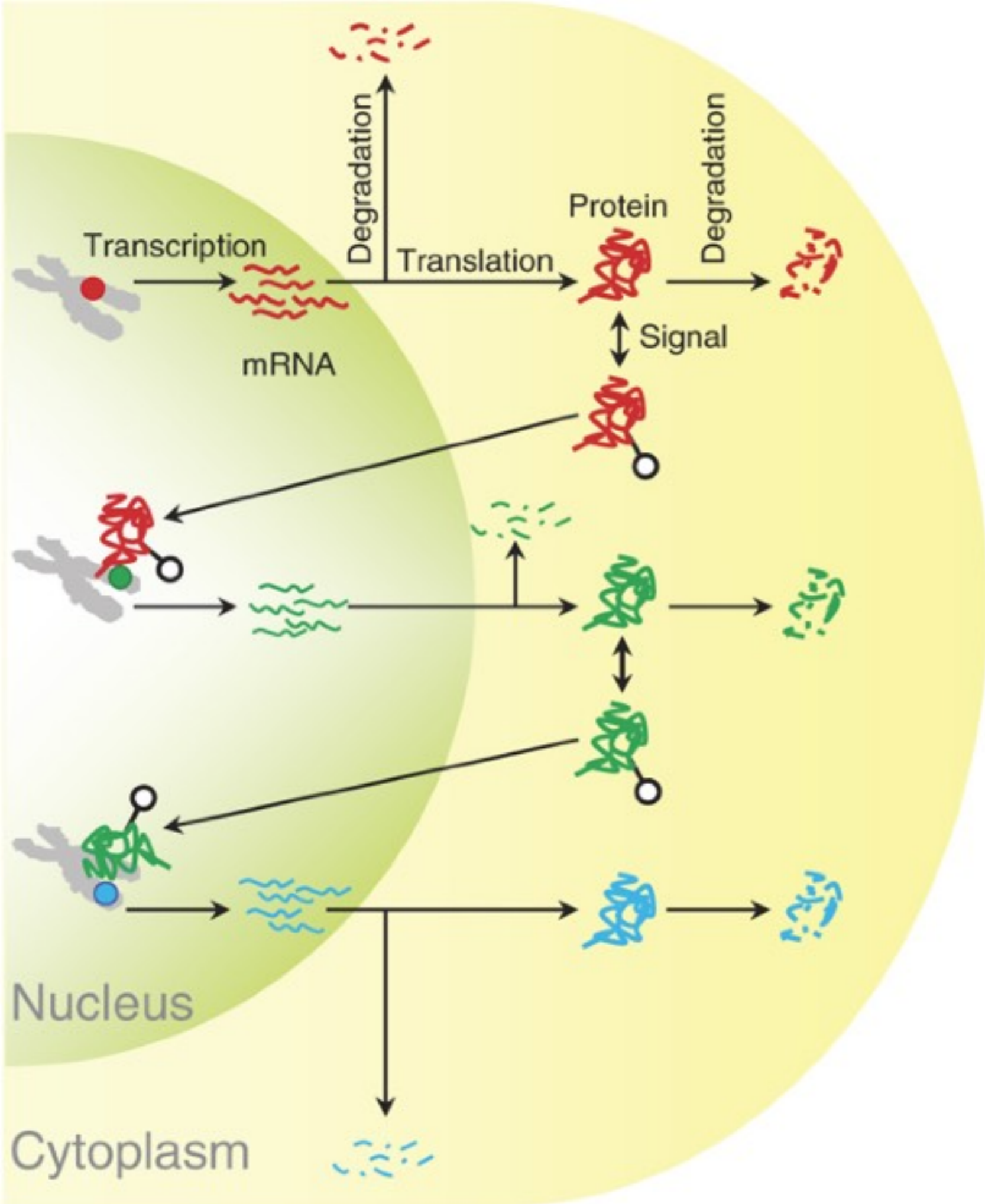


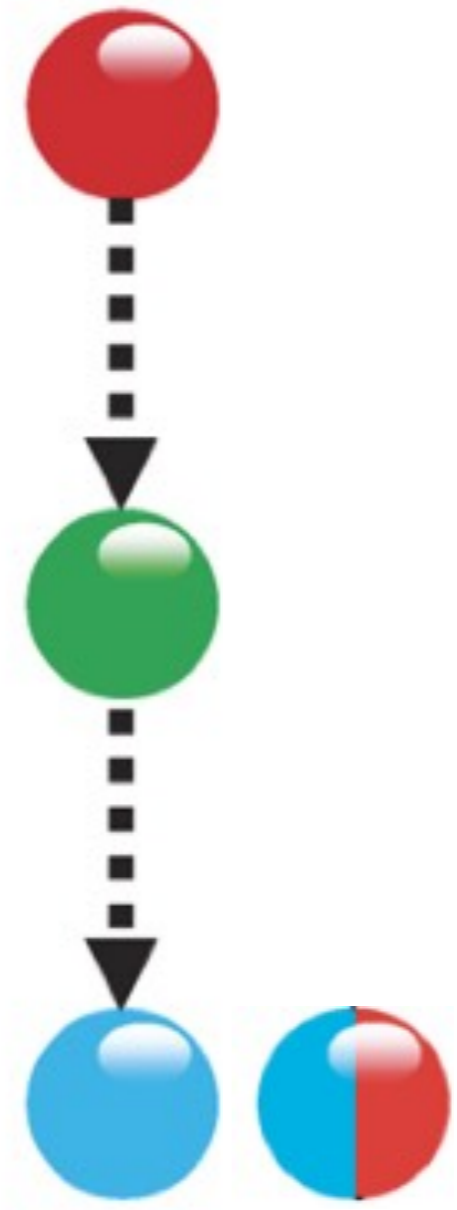
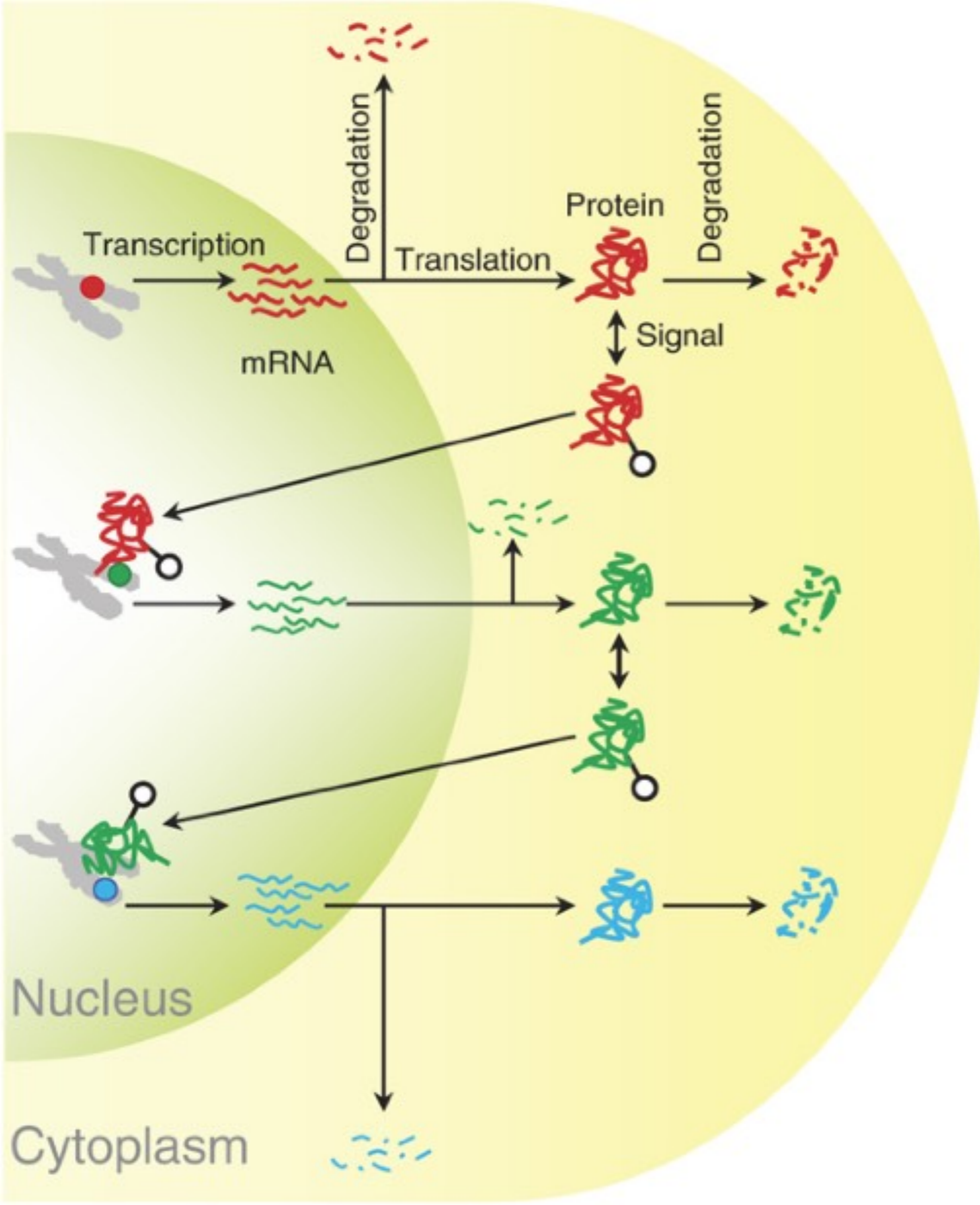
### C Linear ordering

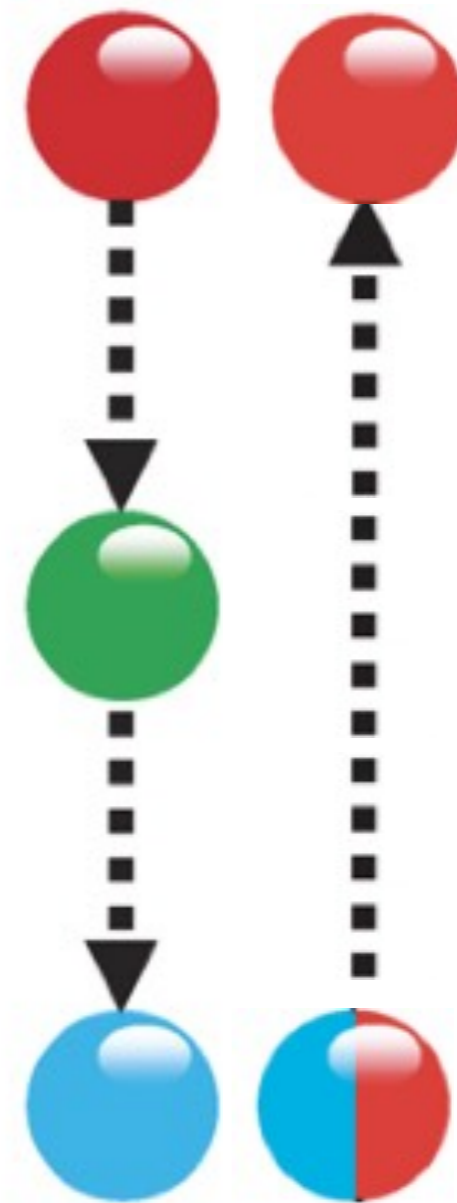
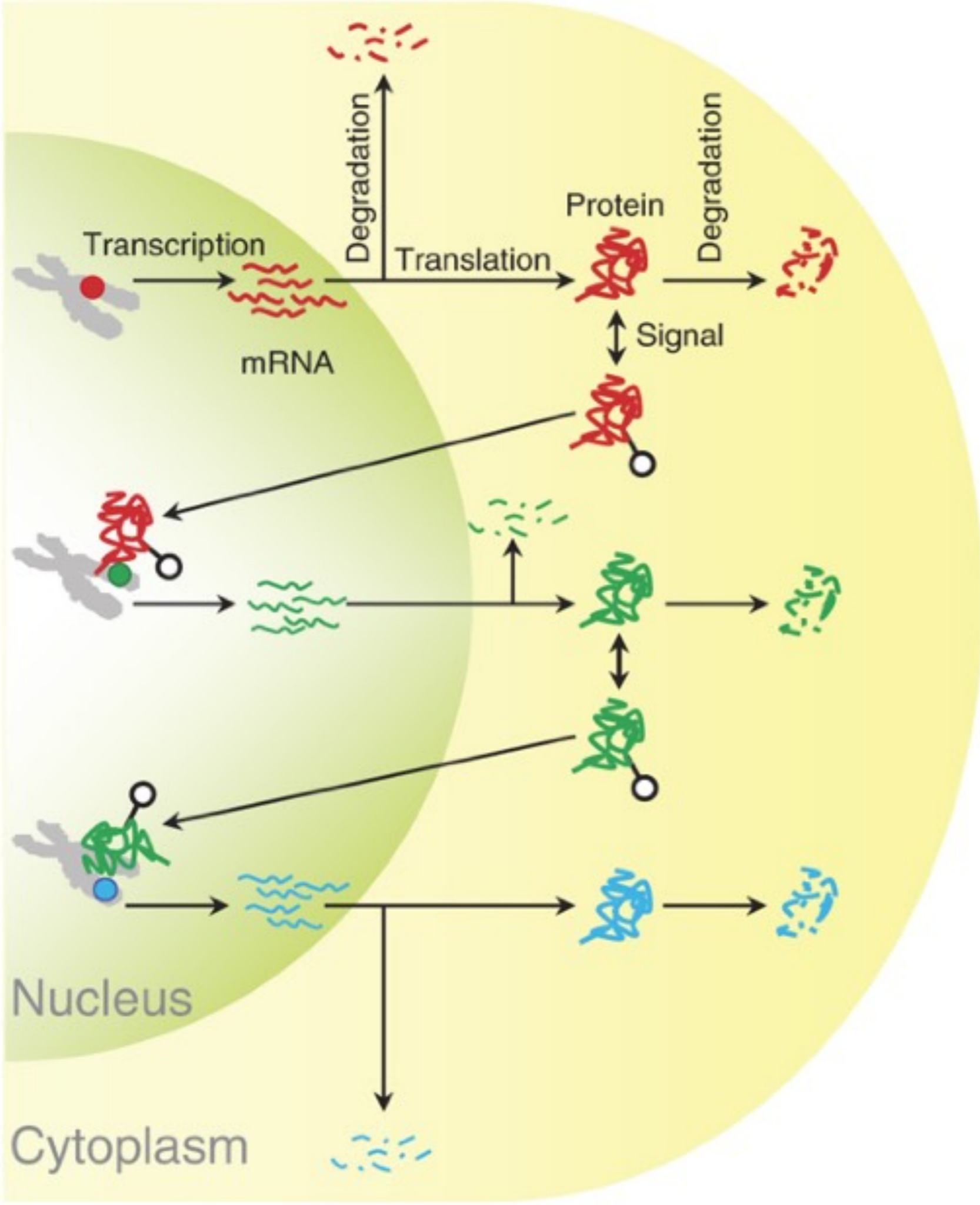
Top (25)	Level 7	OAF1	MAC1	MBP1*	SKN7*	ARG80	ARO80	AZF1	CHA4		RDR1 RLM1 THI2 UPC2 YBL054W YDR266C YJL206C YKR064W YRM1	
	Level 6	ABF1*◀	MAL33	MCM1◀	NRG1*	DAL82	FLO8*	HAL9	MET31			
	Core (64)	Level 5	ACE2	ADR1	AFT1*	AFT2*	ARG81	ASH1	CBF1*			CIN5*
			CUP9	DAL80	DAL81	FHL1*◀	FKH1	FKH2*	GAL4			GAT1
			GAT3	GCN4*	GLN3	GTS1	GZF3	HAP1	HAP4			HCM1*
			HMLALPHA2	HMS2	HSF1*◀	INO2	INO4	IXR1	LEU3			MGA1*
			MIG1	MIG2	MSN2*	MSN4*	NDT80	PHD1*	PLM2*			PUT3
	RAP1*◀	REB1*◀	RIM101	ROX1	RPN4	SKO1	SMP1	SOK2*				
	STE12*	SUT1	SWI4*	SWI5	TEC1*	TOS4*	TOS8*	TYE7				
	XBP1	YAP1	YAP5*	YAP6*	YAP7*	YHP1	YOX1*	UME6*				
Bottom (59)	Level 4	RTG3		CAT8	HMS1	FZF1	HAC1	MET4◀	ACA1			
	Level 3	HMRA1	HMRA2	SPT23	STP1	PDR1	PHO4	CRZ1	CAD1	CUP2		
	Level 2	RME1	SIP4		RGT1	SUM1	UGA3	CST6	ECM22	LYS14	MAL13	
			STP2	BAS1	YAP3	YDR026C		GCR1◀	MOT3	MSN1	MSS11	
Level 1	IME1		HMLALPHA1	MET28	MIG3	PDR3	PDR8	PPR1	RDS1	RGM1	SFP1	
	SFL1		MATALPHA1	STB4	STB5	YDR049W	YRR1	STP4	WAR1	URC2	USV1	
	SRD1							YER130C	YER184C	YML081W	YPR196W	

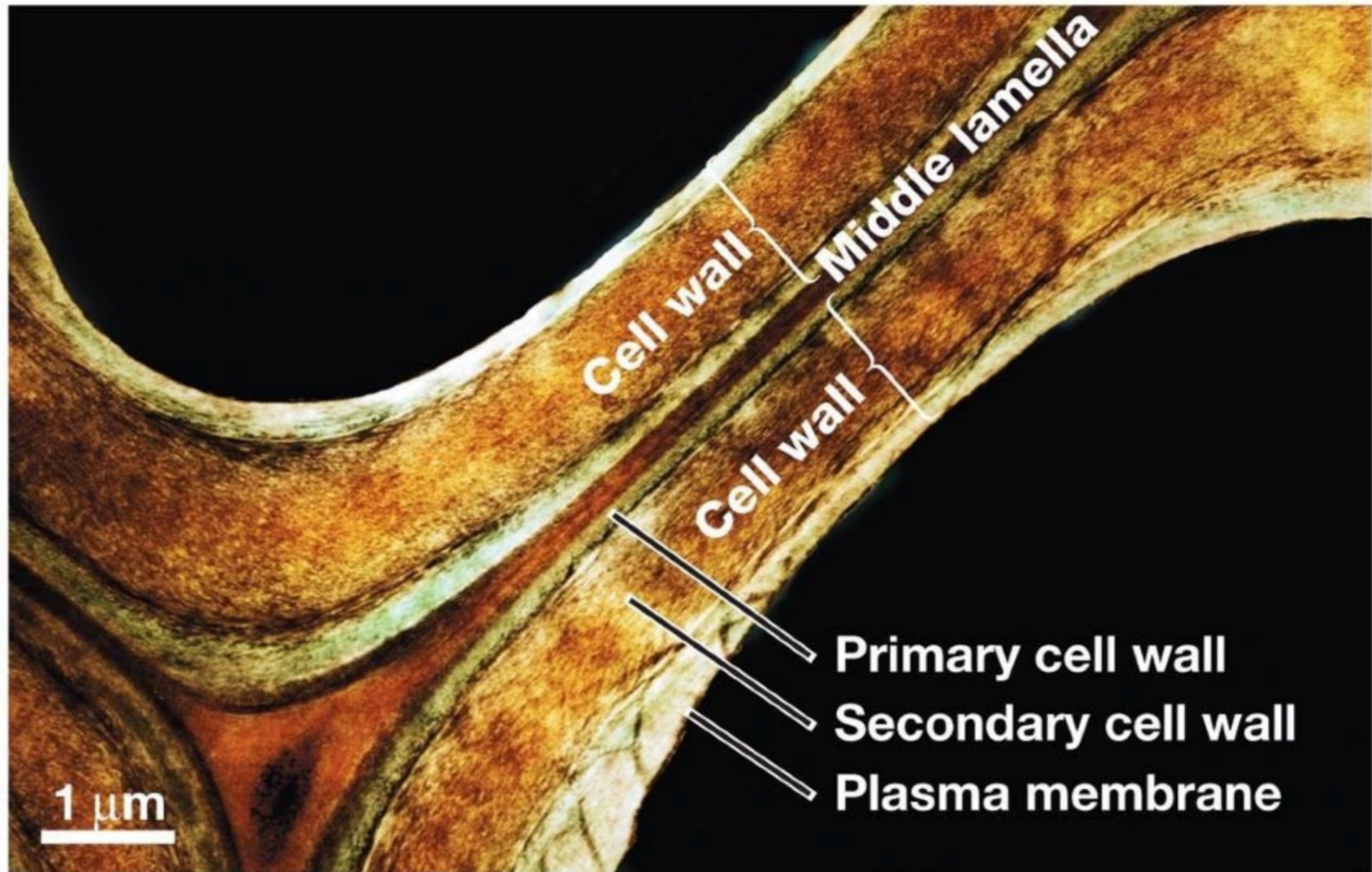
\* Regulatory hubs

◀ Essential genes









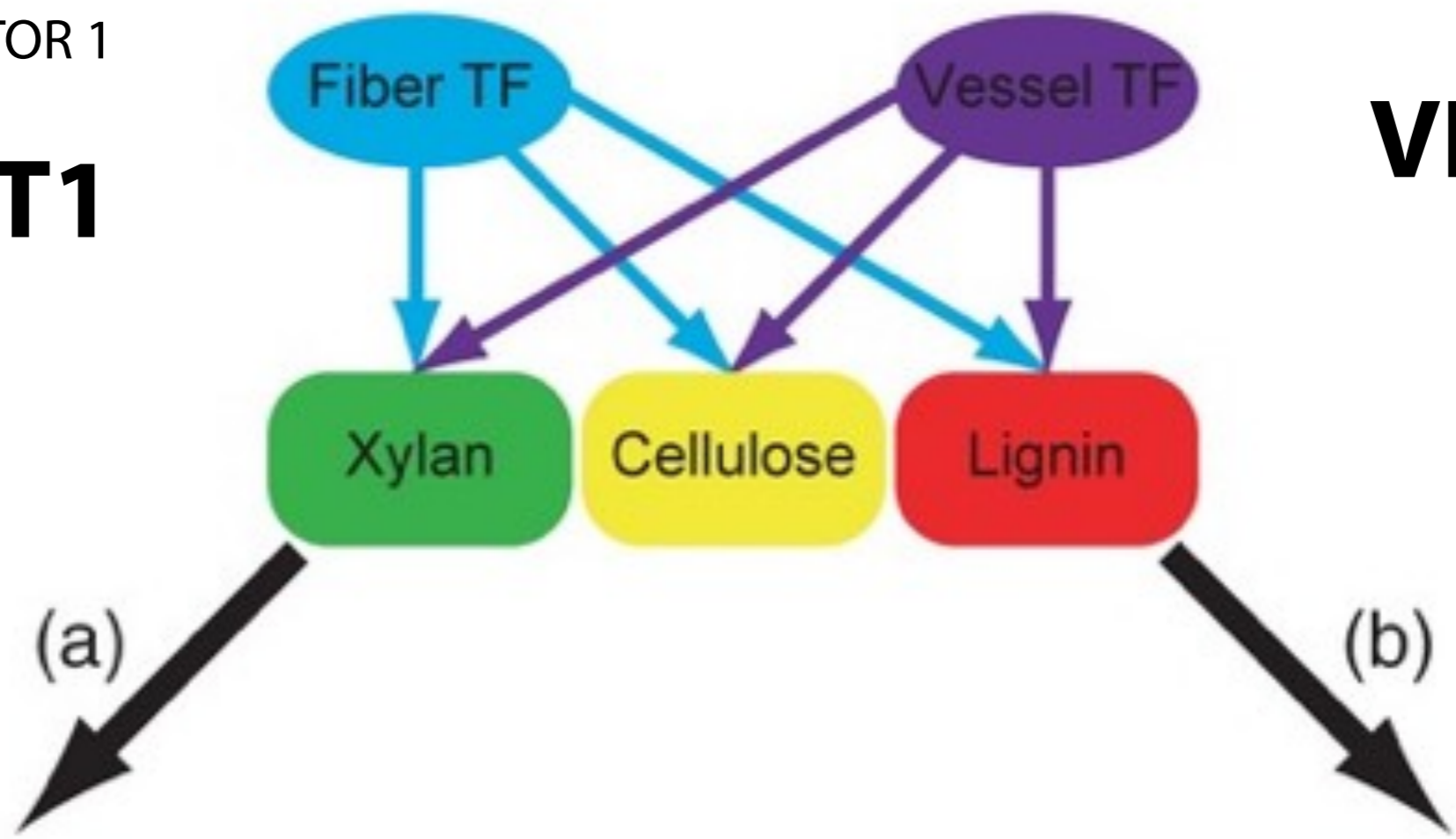
NAC SECONDARY WALL THICKENING PROMOTING FACTOR 1

**NST1**

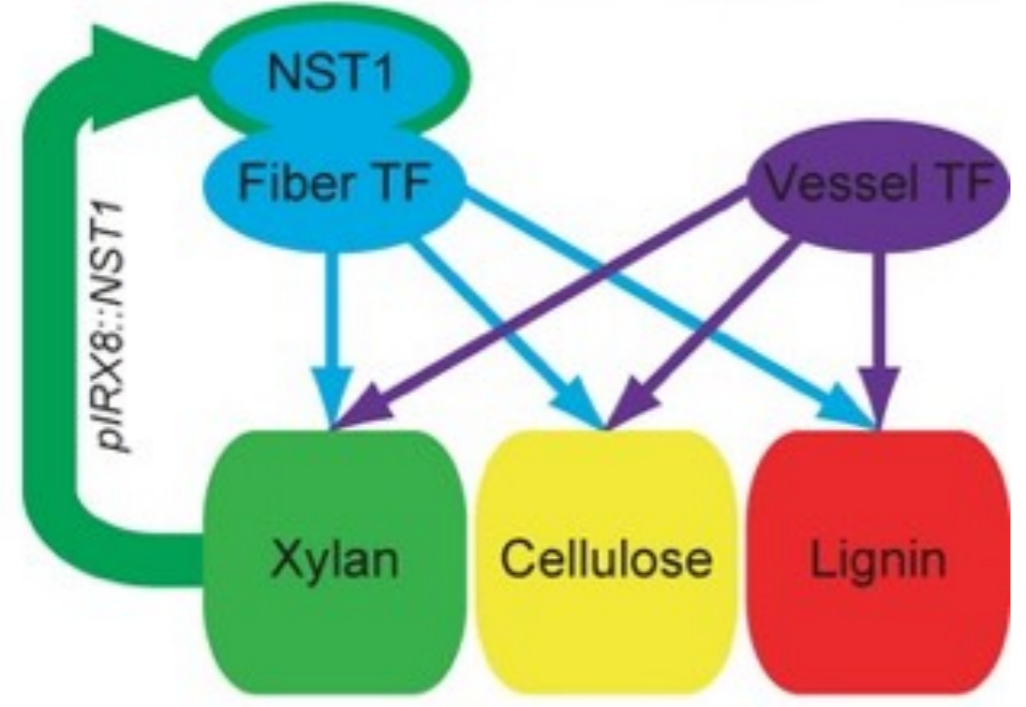
Wildtype

VASCULAR RELATED NAC DOMAIN 6

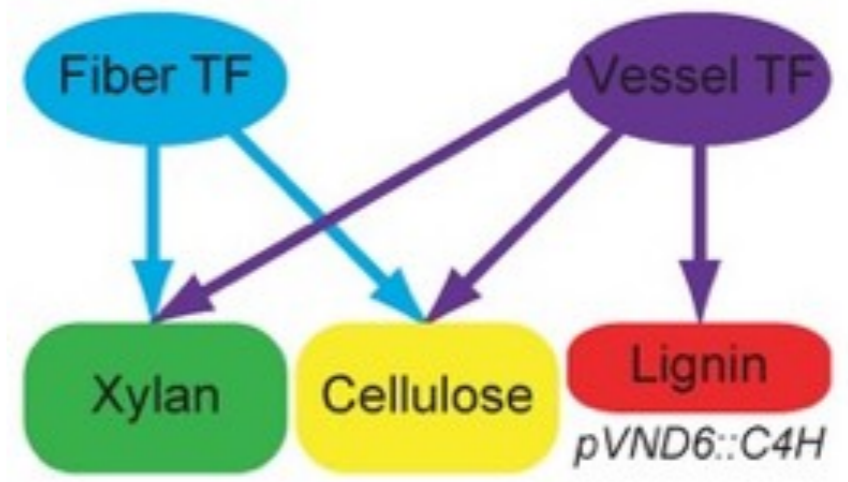
**VND6**



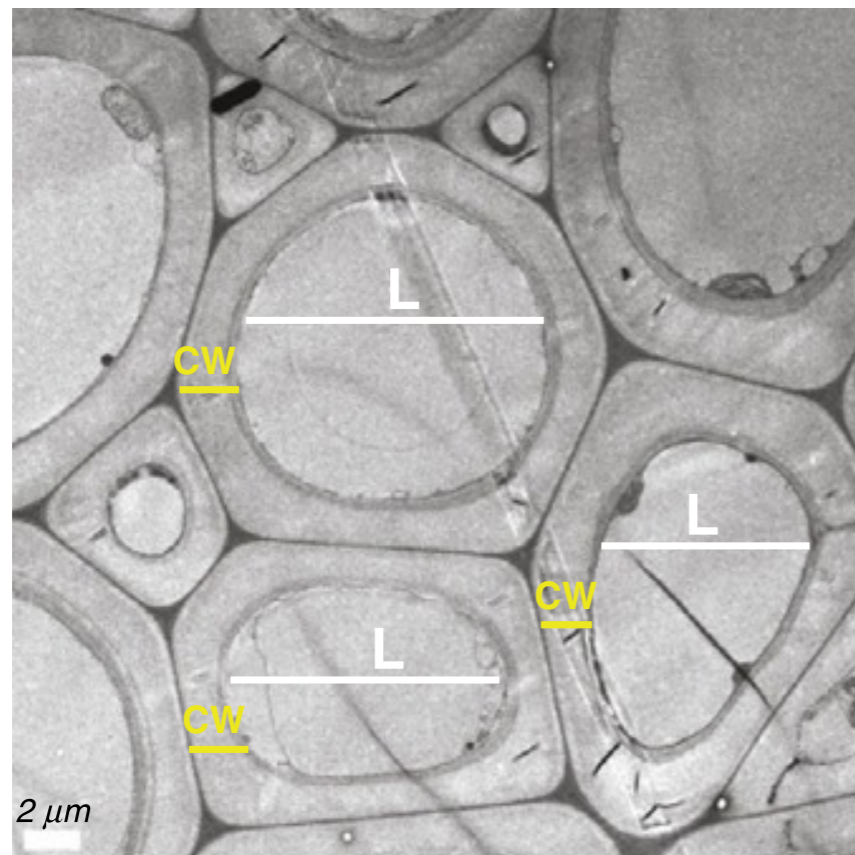
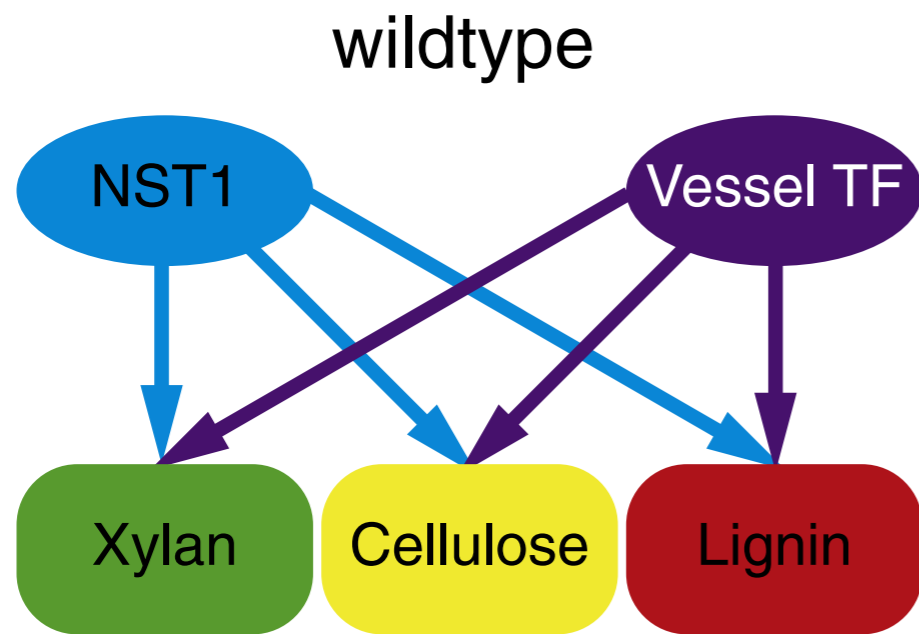
Cell wall deposition engineering



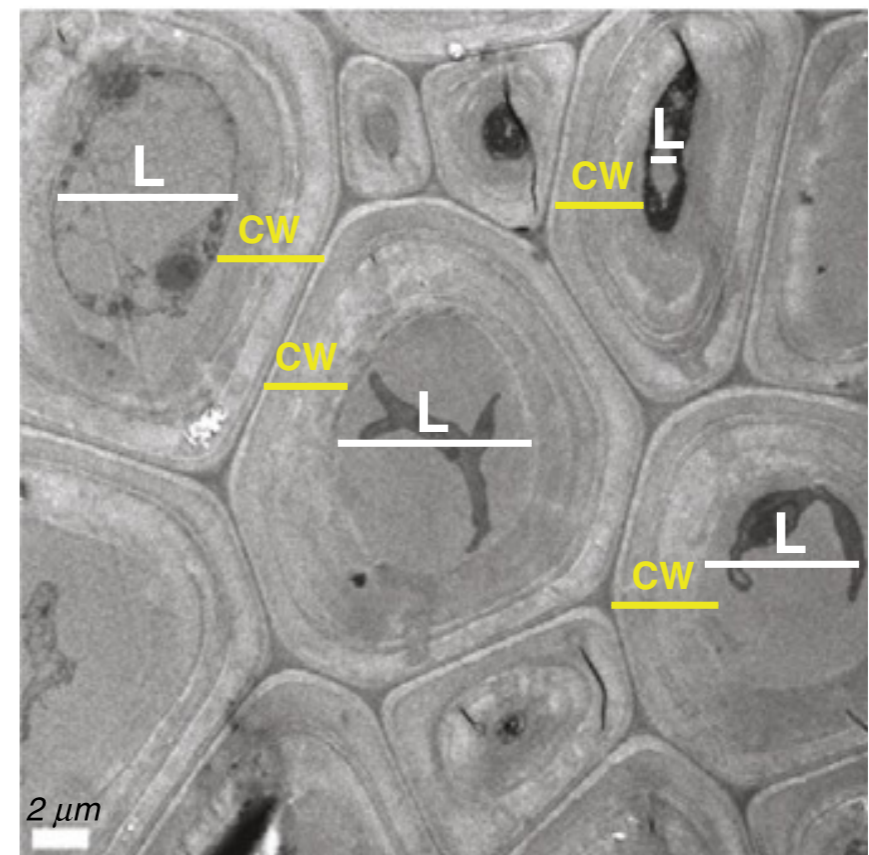
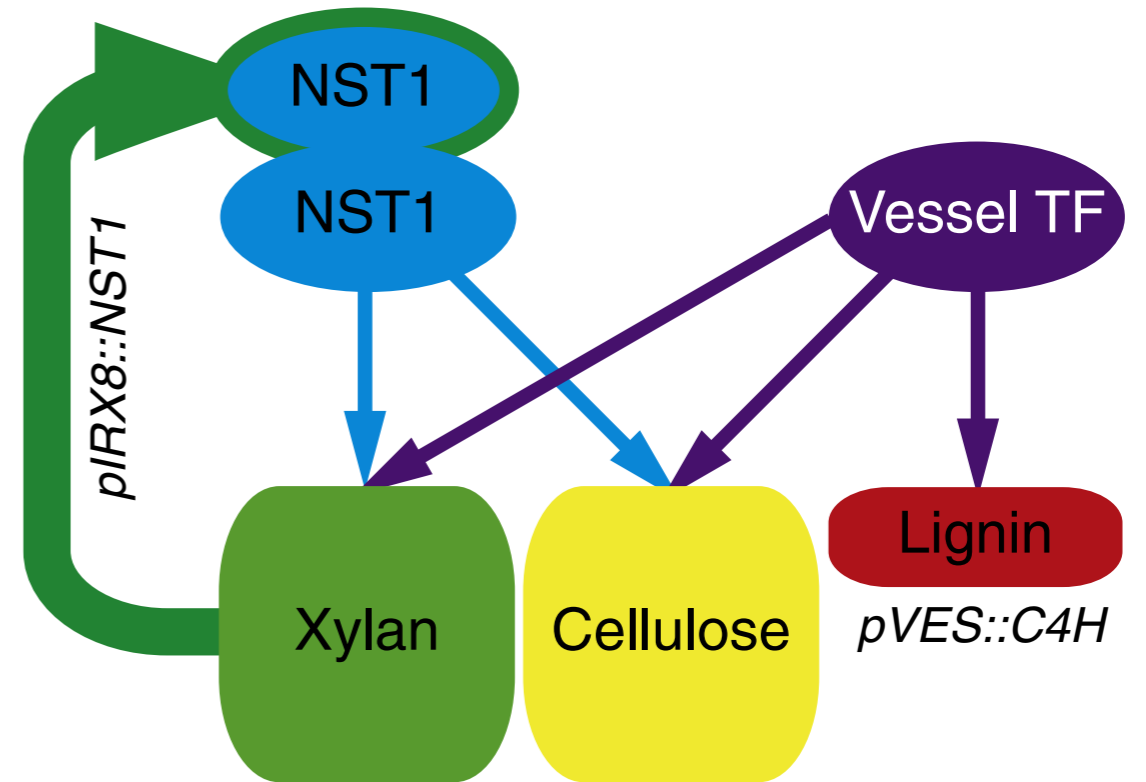
Lignin deposition engineering



(a)



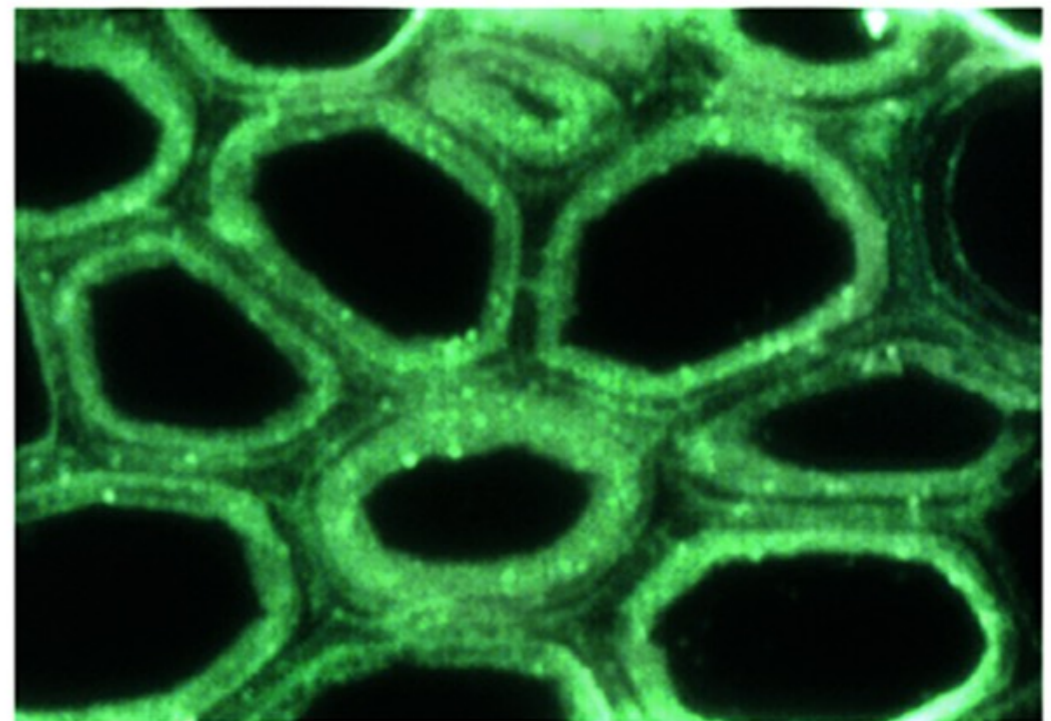
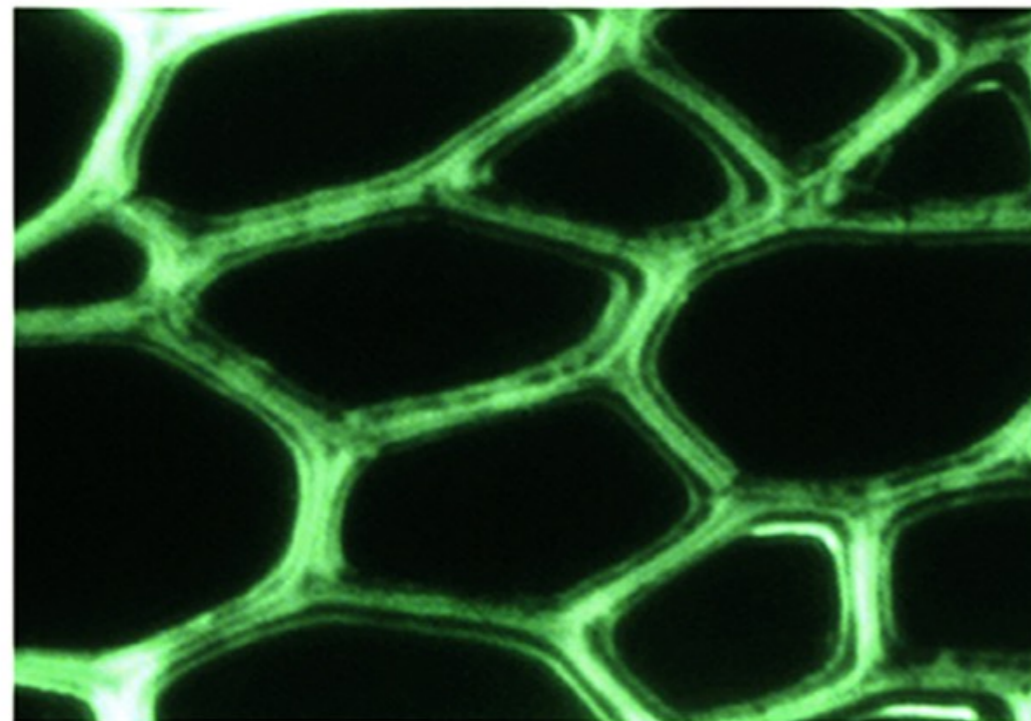
(b) Cell wall deposition engineering



WT



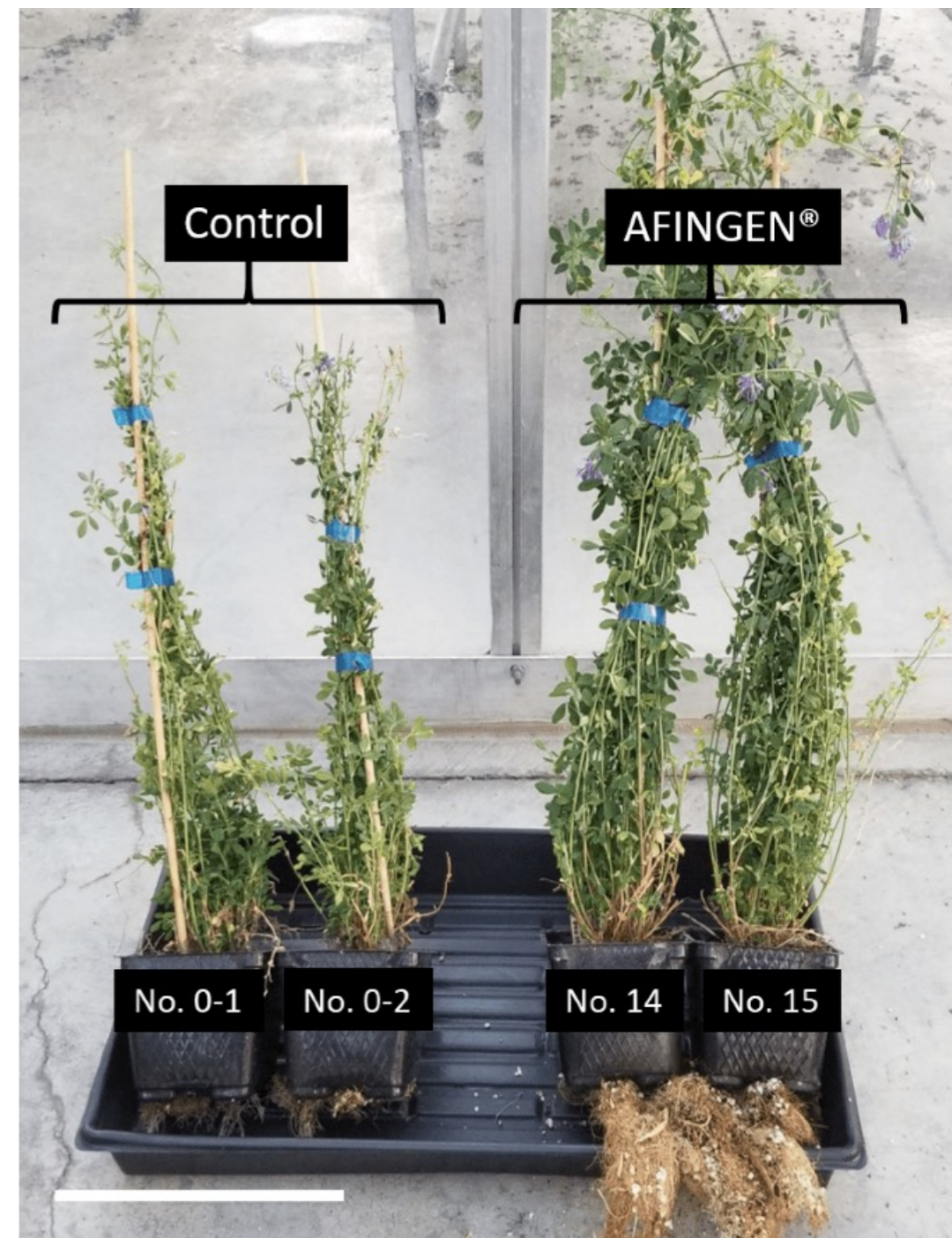
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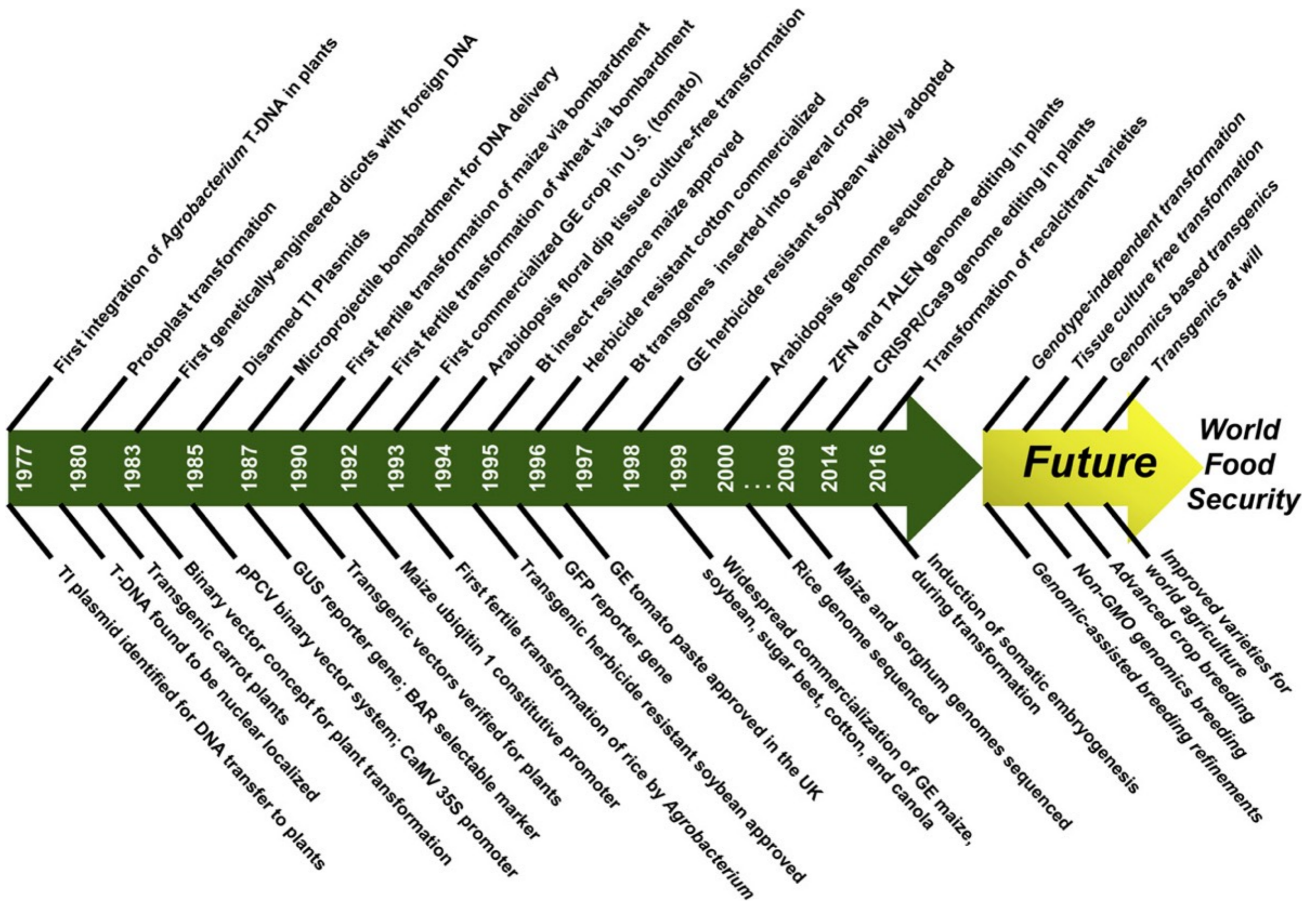
# AFINGEN



**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.