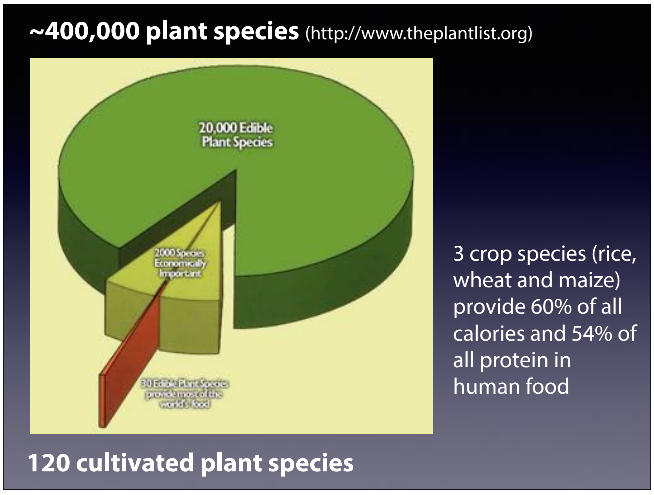
Lecture 3: Crop traits

NST PMS 1B

Following the discussion of single gene traits, DNA parts and assembly in Lecture 2 - this lecture describes examples of more complicated agronomic traits. In particular, it focuses on the cellular basis for growth of plant tissues and organs, and the implications for future engineering of new traits.

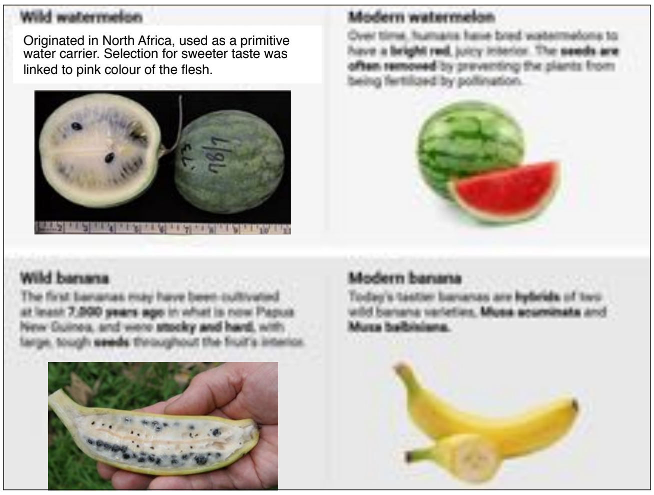


~400,000 plant species (http://www.theplantlist.org)

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

120 cultivated plant species

Crop plants sample a tiny fraction of total plant diversity. It estimated that there are around 400,000 plant species on Earth. Only around 20,000 of these have ever been used by humans as food, and only 2000 plant species have any economic importance as food crops. 30 species provide most of the world's food. Three species - rice, wheat and maize, provide 60% of calories and over half of the protein in human food. A vast potential reservoir of biological diversity remains untapped.



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.

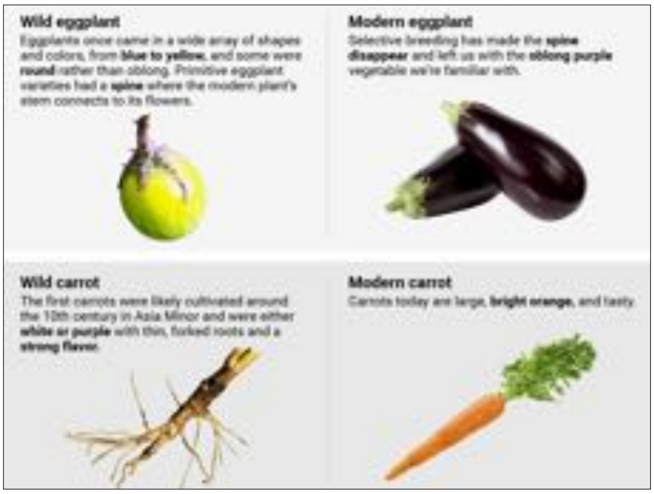
Wild banana

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.**

Ancient species are provided raw material for domestication of crop plants. Domestication has occurred over millennia, and often accompanied by substantial changes in phenotype. For example, melons were thought to have been originally used in prehistoric times as natural water carriers in northern Africa. The wild melons have a high water content but are bitter. The selection for sweeter tasting melons unintentionally produced pink flesh, as the genetic loci for colour and sweetness are closely positioned. In addition, bananas were first domesticated in Papua New Guinea. These were diploid and contained seeds. Modern bananas are triploid, sterile and seedless…and genetically homogeneous.



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.**

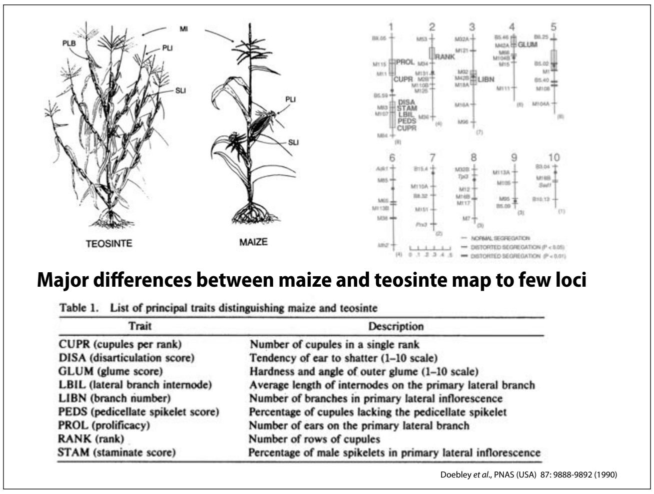
Modern carrot

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

Eggplants, or aubergine, have been grown in southern and eastern Asia since prehistory. A relative of the nightshade family, domestication has led to changes in size, colour, alkaloid content and loss of spines.

Carrot was cultivated and used as a storage root similar to modern carrots in Central Asia beginning in the 10th century. The first domesticated carrot roots were purple and yellow, arriving in Western Europe and finally in England between the 11th and 15th centuries. Orange carrots were not well documented until the 15th and 16th centuries in Europe, indicating that orange carotenoid accumulation may have resulted from a secondary domestication event.

In each of these cases, centuries or even millennia of domestication was required to produce the productive and more palatable crop plants that we recognise today.



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)

As we saw in Lecture 1, work from John Doebley's lab has mapped the genetic differences between teosinte and maize. Genetic studies identified the relatively few gene loci account for around 90% of the difference in form between teosinte and maize. These cause differences in traits like vegetative branching, morphology and floral architecture.

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)

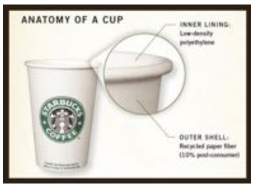
Many of these traits are multigenic and affect the shape and function of plant tissues and organs. If we want to engineer new crop traits in the future, we will need to understand the way DNA code is able to regulate plant growth and form.

Many, if not most, of the important traits introduced during domestication are the result of coordinated changes in plant growth and form. While there may be simple genetic triggers for these changes, the modified traits are the result of programmed alterations in complex developmental and metabolic pathways. What underpins programmed plant growth? Can these elements be easily reconfigured by human engineers?

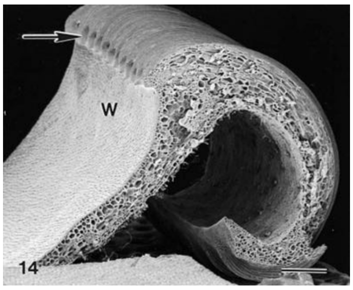

BBC Natural History Unit

This time-lapse video from the BBC Natural History unit in Bristol shows the growth of a pitcher plant (*Nepenthes sp*.). It first emerges as an extension of a leaf. The stolon elongates, and a small nub of tissue at the tip expands to form the body of the pitcher. The hollow structure contains a lid, which eventually pops open.

The pitcher plant is functionally similar to a paper coffee cup. Both form sealed vessels that will eventually fill with liquid. Both are largely composed of cellulose.



paper cup



pitcher plant

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Both cup-like structures possess curved lips, one to prevent spillage, the other to prevent egress of insects. Both are lined, one with polyethylene the other with epicuticular wax. Despite these similarities the plant pitcher and coffee cup are built in very different ways. Around 200 billion paper cups are produced per annum, worldwide. They are all made in broadly similar fashion. Raw materials are harvested, processed and assembled by high-throughput machines according to a particular fixed blueprint. The biological cup is built by cellular growth. Single progenitor cells proliferate and differentiate, and create the cup-like structure by a process of self organisation.

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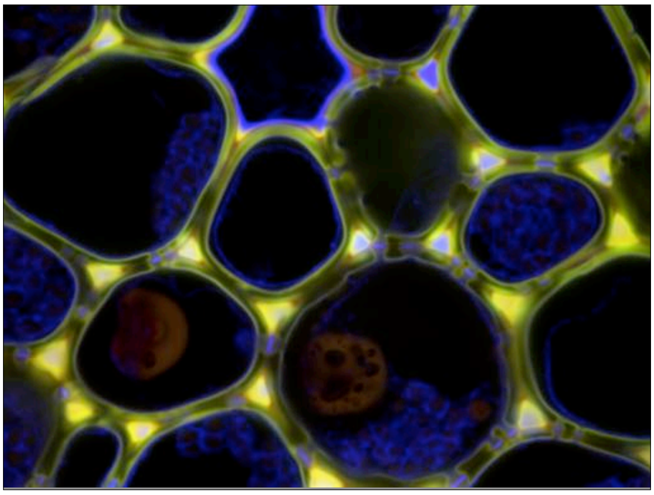
Changes in DNA-based instructions can result in reprogramming the overall architecture and structure of the plant organ. Compared to its human-made counterpart, the construction of the biological cup is more robust, and the design more flexible. What are the basic principles at work?



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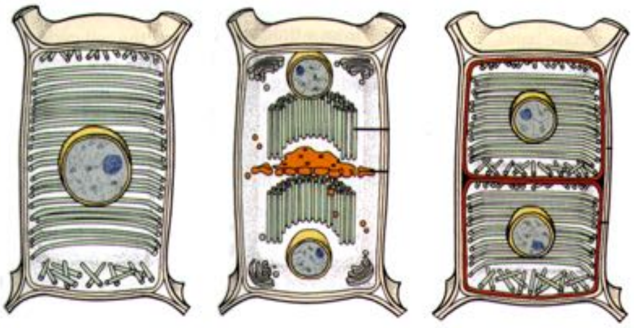
The growth of a plant organ is due to the collective activity of individual cells. Each cell in the organism contains a copy of the genome, and is to some degree an independent agent. Cells adopt different fates through developmental communication and self organisation. Cells may be programmed to divide and proliferate or to differentiate.

Plant cells are immobilised.
Morphogenesis is driven by cell division and elongation.



Cells are the common unit for gene expression

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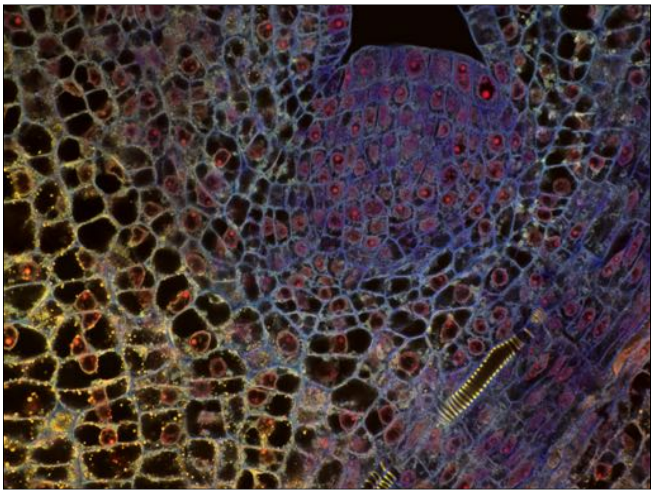
Cells are the functional unit for gene expression. In plants, cells are encased in cell walls, which act as a semirigid matrix. Cells are immobilised with respect to each other. Plant cells grow by a process of cell wall softening and deposition of new wall material, while expansion is driven by hydrostatic pressure inside the cell. After cell enlargement, nuclear duplication and cytokinesis, the formation of new cell walls takes place within existing cells. A phragmoplast structure of fused membrane material (orange) is formed, and this acts as a template for formation of the new wall.



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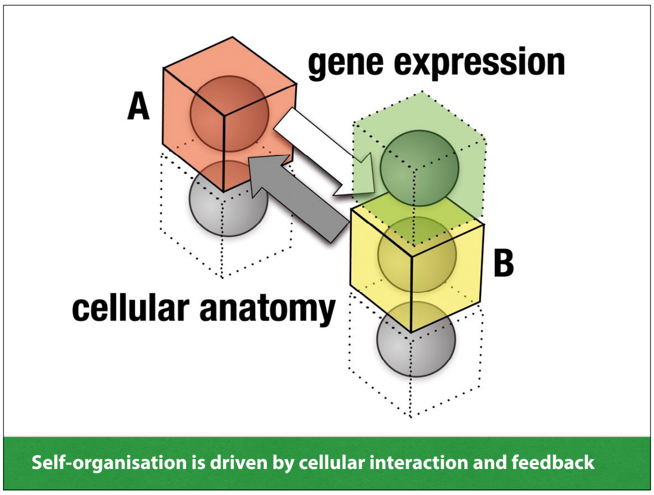
Cell-cell signalling results in the formation of cohorts of cells that act as organised tissues to regulate tissue growth in the formation of specialised structures during organogenesis.



gene expression

cellular anatomy

Self-organisation is driven by cellular interaction and feedback

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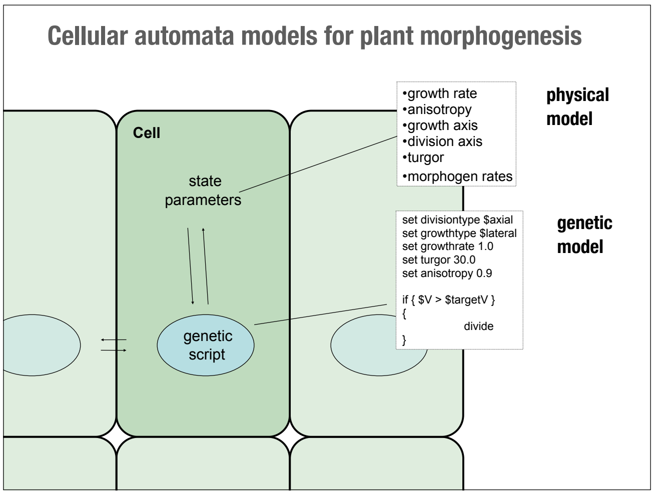
Studies of plant development indicate that cells primarily adopt their particular fate due to local genetic interactions. A hypothetical cell might be cued to divide in a plant tissue, and create two daughter cells. The two daughter cells will have different neighbouring environments, and be positioned to communicate with different cells, and bootstrap increased asymmetry. There is a very close relationship between local cellular anatomy and patterns of gene expression. In a structure like that of the developing pitcher plant, these interactions are expanded million fold and occurring simultaneously. The structure of a pitcher plant is not determined by a genome-encoded blueprint, rather it is determined by a myriad of simultaneous interactions within the growing population of cells - where DNA code regulates the behaviour of each cell during this process. The construction of an ordered biological structure is highly social, and bears much resemblance to self-organisation in human systems, such as financial markets, politics, etc.



Cellular automata models for plant morphogenesis

Cell

state parameters

genetic script

- growth rate
- anisotropy
- growth axis
- division axis
- turgor
- morphogen rates

physical model

```
set divisiontype $axial
set growthtype $lateral
set growthrate 1.0
set turgor 30.0
set anisotropy 0.9

if { $V > $targetV }
{
                divide
}
```

genetic model

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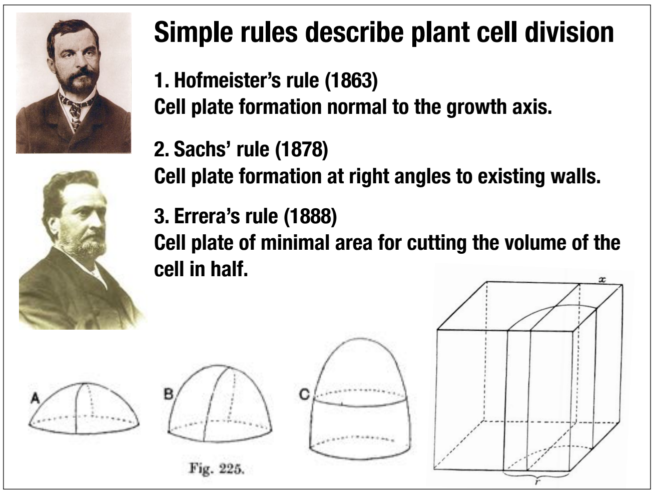
In order to rationally design and engineer (rather than select) the kind of traits have proved necessary for domestication of existing crop plants, it will be necessary to better understand the relationship between the genome and the cellular dynamics of plant development. Computer models provide an insight into how relatively simple genetic and physical processes can combine to produce organised behaviour by emergence, rather than top-down control.
Here is an example, where cells are described as automata - each with genetic script, state parameters and physical properties.



Simple rules describe plant cell division

1. Hofmeister's rule (1863)
Cell plate formation normal to the growth axis.

2. Sachs' rule (1878)
Cell plate formation at right angles to existing walls.

3. Errera's rule (1888)
Cell plate of minimal area for cutting the volume of the cell in half.

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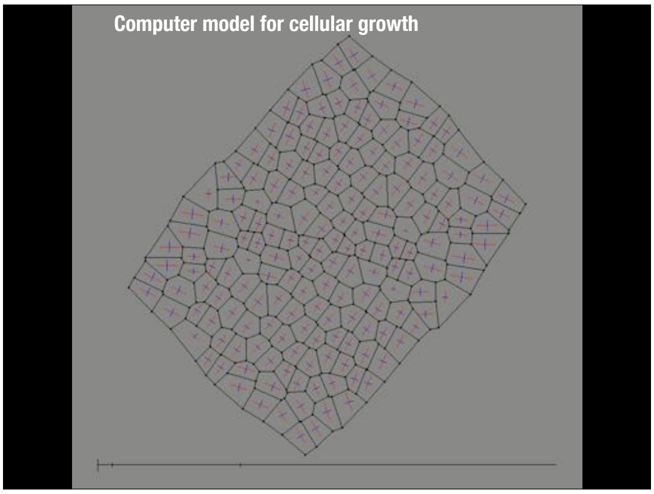
Symmetric cell divisions in plants are governed by rules observed in the 19th century. (i) The new cell wall cuts across the long axis of the cell. (ii) The new cell wall formed at right angles to the existing walls. (iii) The size (or area) of the new cell wall is minimised. Microtubules and other elements of the cytoskeleton act to mediate these dynamic processes. The rules can be used in simple computer models.



Computer model for cellular growth

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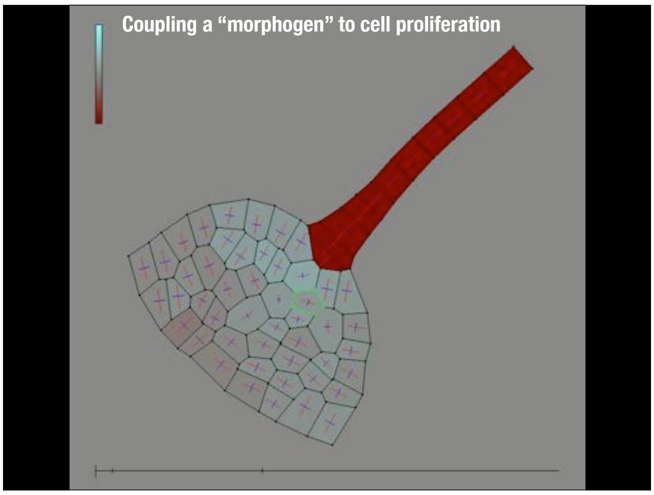
In this simple case, cells are programmed to elongate in one direction and to divide once the cell has reached twice its original size. After division, the axis of elongation is switched by 90°. A single cell is programmed to divide and it forms a sheet of cells in this 2D model. Physical interactions between the cells result in formation of zig-zag patterns of cell walls, due to energy minimisation, similar to soap bubble foams.



Coupling a “morphogen” to cell proliferation

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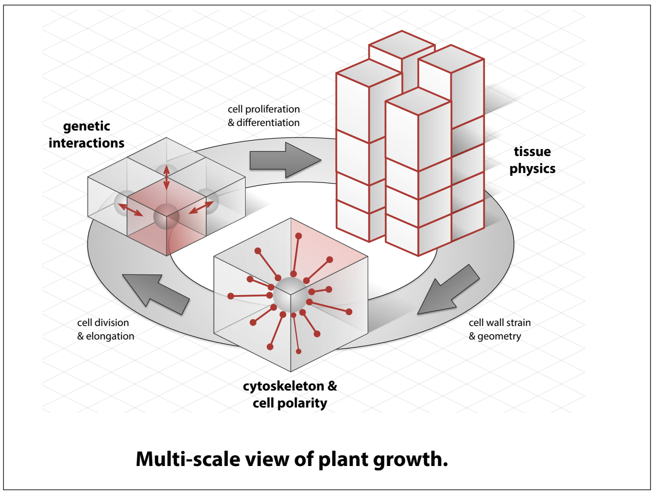
In this second model, two different cell states are introduced. Red coloured cells are capable of only growth and division in one direction, to produce a column of cells. The cells coloured cyan are programmed to behave the same as the previous model. However the physical constraints due to attachment to the other cell type results in a “wine-glass” like shape. This morphology is not explicitly programmed, but emerges from the simple system.



genetic interactions

cell proliferation & differentiation

tissue physics

cell division & elongation

cell wall strain & geometry

cytoskeleton & cell polarity

Multi-scale view of plant growth.

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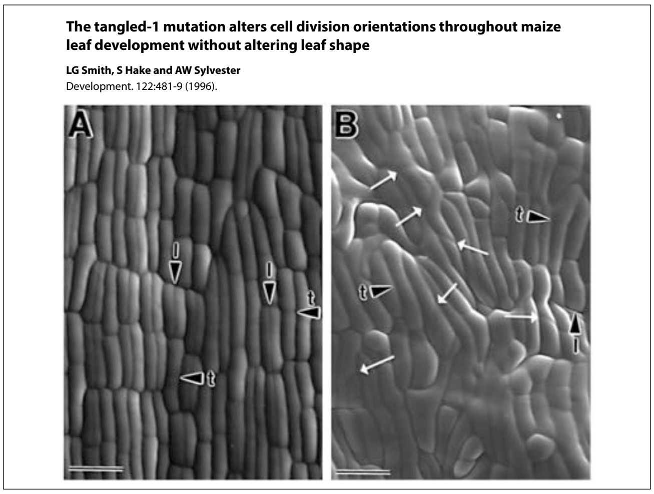
There are multiple levels of interaction and feedback between subcellular organisation, cellular interactions and tissue-wide physics during growth.

(i) Interaction between cytoskeletal elements and local cell wall determinants (such as strain or geometry) regulates the polarity of cell division and elongation.
(ii) Genetic interactions between neighbouring cells trigger gene expression, cell proliferation and differentiation.
(iii) Cellular growth results in physical strains that are transmitted across tissues and constrain cell growth.
(iv) Physical constraints on cell size and shape regulate timing and orientation of individual cell divisions and guide morphogenesis.



The tangled-1 mutation alters cell division orientations throughout maize leaf development without altering leaf shape

LG Smith, S Hake and AW Sylvester

Development. 122:481-9 (1996).

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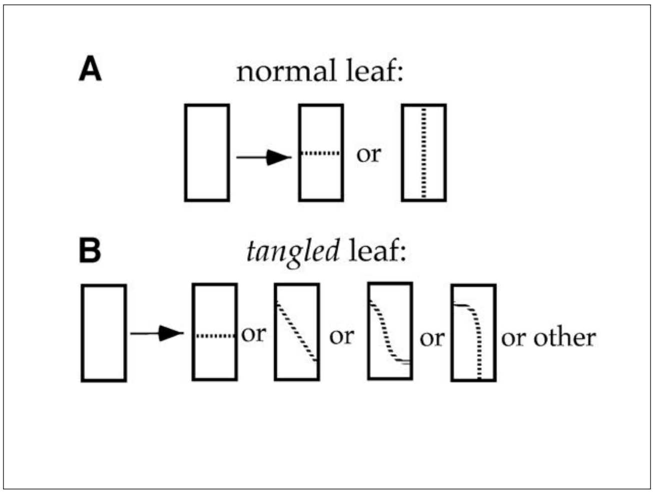
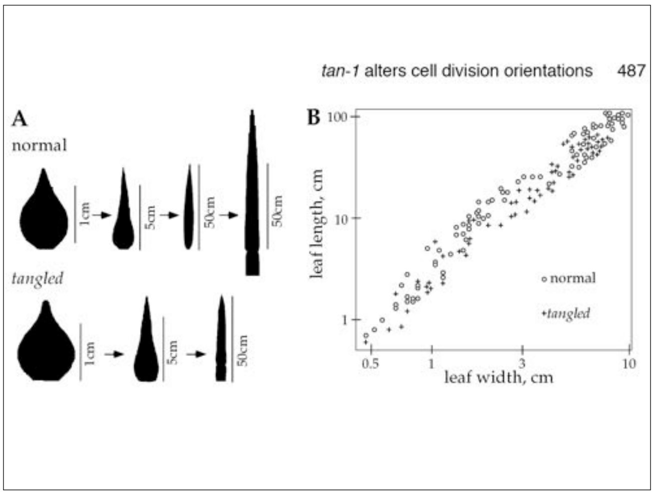
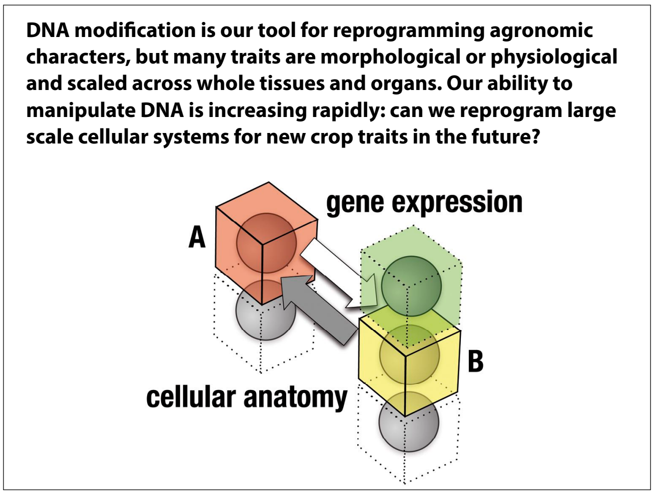
A real world example of emergence: self-organisation during growth of maize leaves. Monocot leaves grow from their base with a series of highly regular cell divisions that produce the strap-like leaf. The maize *tangled-1* mutation causes a defect in microtubule organisation, and patterns of cell division are highly deranged.



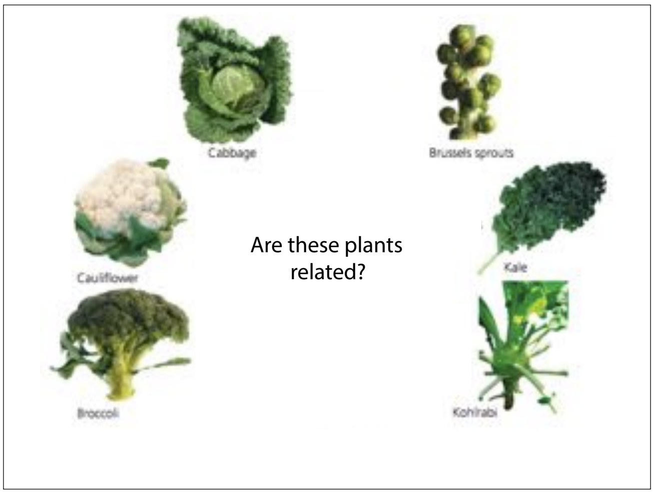
In a normal maize leaf patterns of cell division are either parallel or normal to the axis of the leaf blade. In the tangled one mutant many other patterns of cell division are seen.


tan-1 alters cell division orientations 487

Despite highly disordered patterns of cell division, leaves of the *tangled-1* mutant maintain a shape similar to wild-type. Dynamic interactions within the proliferating leaf tissue result in compensation for highly disorganised patterns of division at the cellular level, and contribute robustness to the system.


DNA modification is our tool for reprogramming agronomic characters, but many traits are morphological or physiological and scaled across whole tissues and organs. Our ability to manipulate DNA is increasing rapidly: can we reprogram large scale cellular systems for new crop traits in the future?

A major scientific challenge in the plant field is to better understand the dynamic interactions that give rise to precise developmental outcomes. In other words, to understand how one dimensional DNA code can be translated into four dimensional outputs. Success in this task will allow new approaches to the design and reprogramming of agronomically relevant traits in plants.


Are these plants related?

An introduction to examples of efforts in this area, looking at historic and new trait development in relatives of the Brassica family. Many of these plants might be taken to be different species, however observed morphological differences are often due to selective breeding.

Crops derived from wild mustard (*Brassica oleracea*)

For example, all of these recognisably different vegetables are derived from the same ancestor species, *Brassica oleracea* or wild mustard. Breeding has led to the enhancement or exaggeration of particular features. For example the appearance of cauliflower is due to over-proliferation of shoot meristems, broccoli has a proliferation of floral buds, cabbage and Brussels sprouts have exaggerated vegetative meristems, and kohlrabi has a swollen stem.

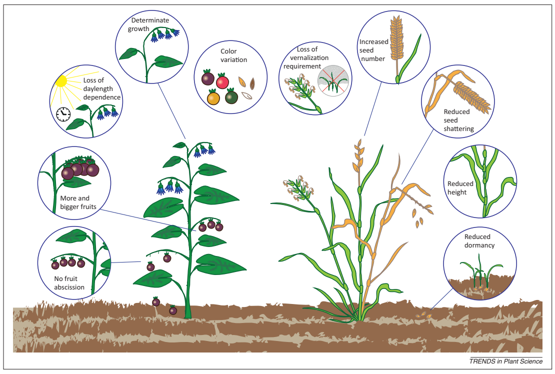
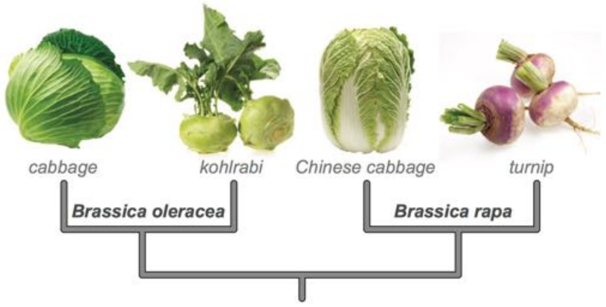


Figure 1. Convergent domestication. Convergent phenotypic changes are frequently observed in many different crops because systematic human cultivation often brings about similar demands. Attempts to maximize yield cause selective pressure for an increase in size and number of edible plant parts on the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

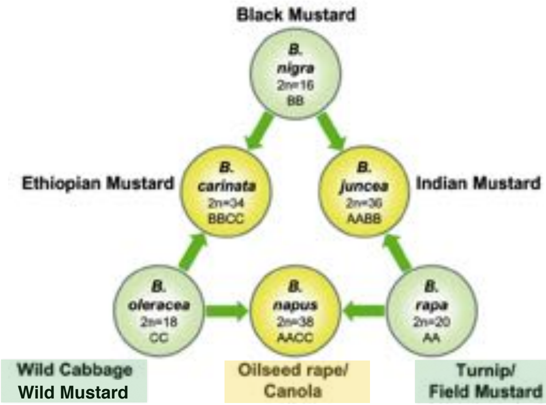
Certain agronomic traits have common benefits in different crops. As a result, domestication has seen the parallel and convergent acquisition of traits in different species. For example, this diagram shows the benefits of similar traits in hypothetical dicot and monocot species - such as determinant growth, larger fruiting bodies and reduced fruit or seed loss.

Convergent phenotypic changes during domestication



An example of convergent trait development in two brassica species. For both *Brassica oleracea* and *Brassica rapa,* genetic variants have been selected independently for (i) indeterminate vegetative meristems and proliferation of leaves, and (ii) hyper proliferation of tissues at the base of the stem.

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



Brassica napus is derived from a cross between *Brassica oleracea* and *Brassica rapa*, and is thought to be a relatively new species, since the earliest reliable record appears only 500 years ago. Although feral populations are common, no truly wild populations have been recorded. Both *B. rapa* and *B. oleracea* have wide geographic ranges and geographically distinct centres of diversity. Molecular studies suggest that the maternal parent of *B. napus* was likely to be *B. oleracea*, due to similarities in restriction patterns of their chloroplast genomes.

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Brassica napus

In a final example, we will look at *Brassica napus*, which has given rise to the oilseed rape crop, also known as canola.

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

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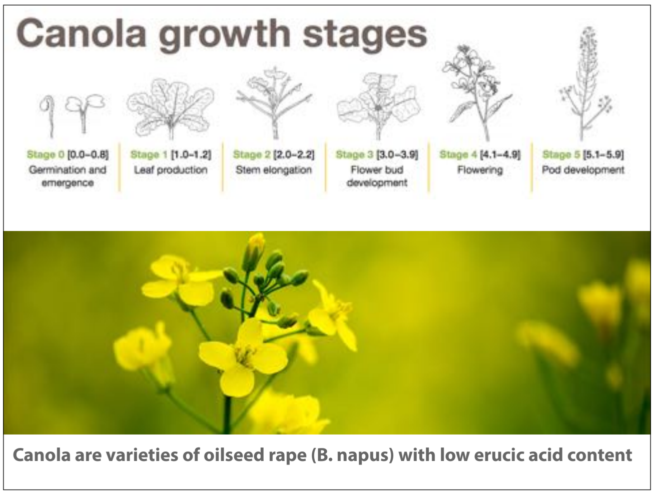
Canola growth stages

Canola are varieties of oilseed rape (B. napus) with low erucic acid content

Canola is an oilseed crop. After planting and subsequent vegetative growth, the plants flower and set seed. The seed is harvested at the end of the growing season and pressed to extract oil.

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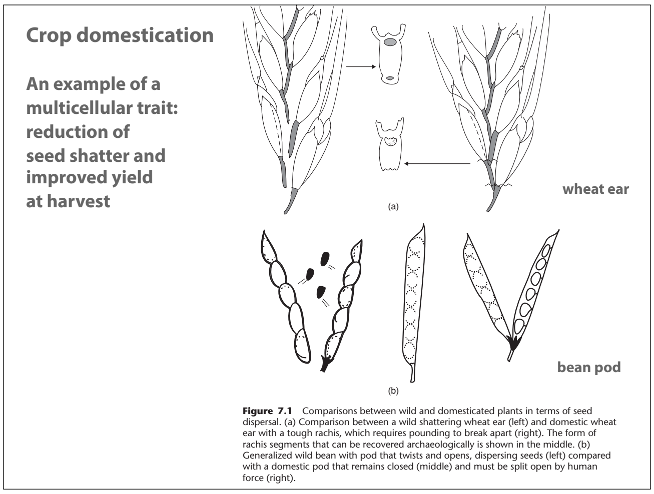

Crop domestication

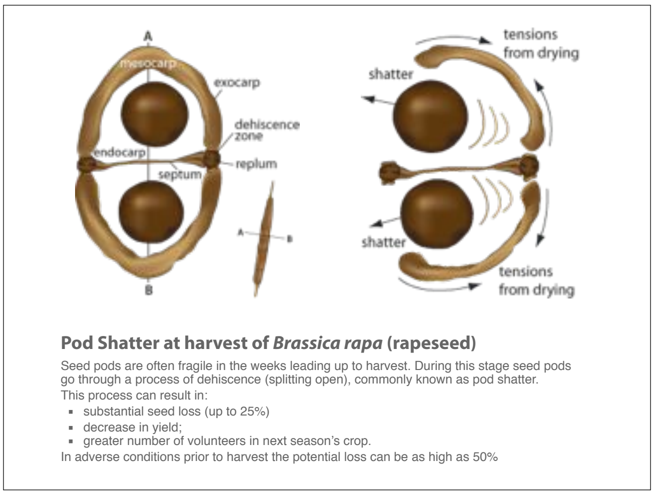
An example of a multicellular trait: reduction of seed shatter and improved yield at harvest

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).

Wild plants rely on seed dispersal to maintain their population. In an agricultural context, this corresponds to seed shatter and losses in yield. A feature of the domestication of many seed crops is the selection for mutants that reduce seed shatter. Wheat seed are held in an ear with a central axis, or rachis. The rachis of wild type wheat plants contains abscission layers that result in breakage of the rachis and seed dispersal. Domesticated wheat have been selected for toughened rachis that allow retention of seed for harvesting. Similarly, domesticated crops with pod-borne seed are generally modified for reduced pod shatter and seed retention.

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

Pod shatter can result in substantial losses of yield (25-50%) for Canola and rapeseed oil crops.



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:

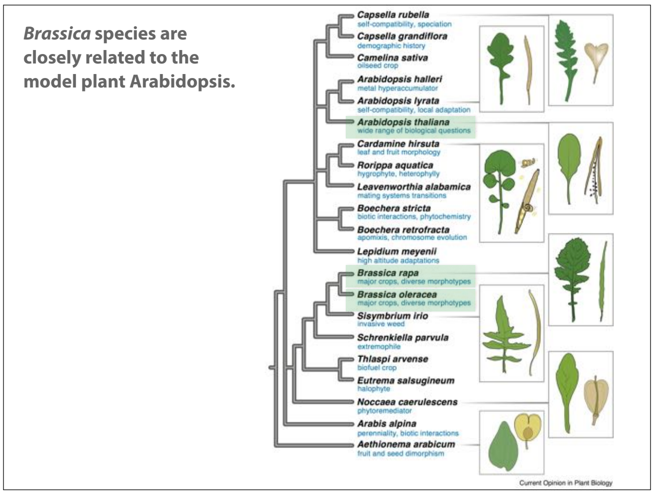
- substantial seed loss (up to 25%)
- decrease in yield;
- greater number of volunteers in next season's crop.

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

Oilseed rape is a relatively recently domesticated crop. 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 (up to 25%)
- decrease in yield;
- greater number of volunteers in next

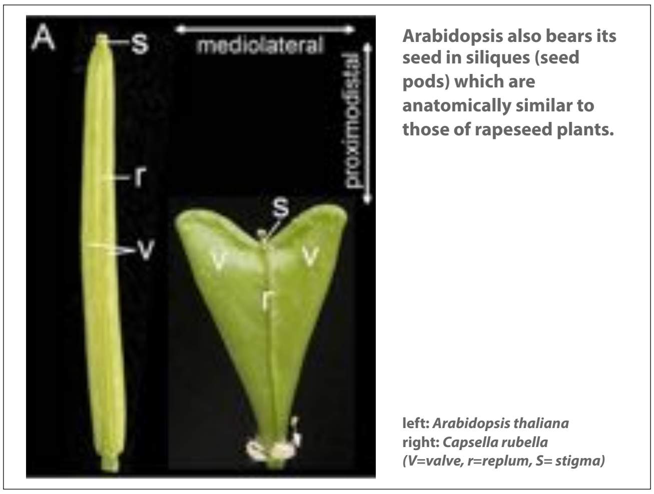
season's crop.
In adverse conditions (such as high winds) prior to harvest the potential loss can be as high as 50%



***Brassica* species are closely related to the model plant Arabidopsis.**

Current Opinion in Plant Biology

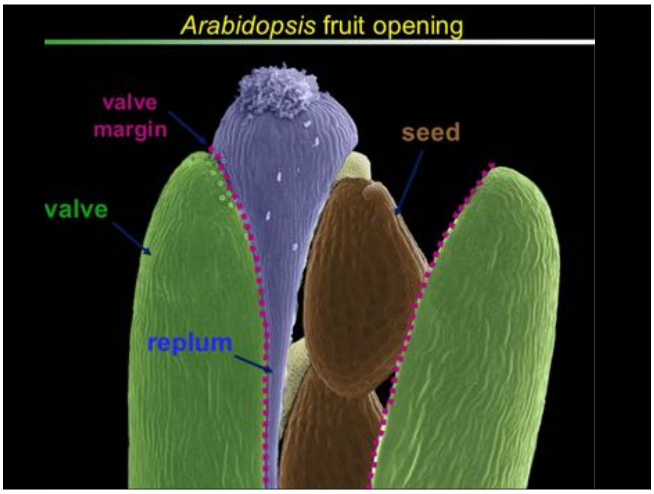
Plants within the Brassicaceae family share many common features. The chart shows overall leaf and fruit structure across the family. The seed pods of *Brassica oleracea* and *Brassica rapa* are similar to the model plant *Arabdopsis thaliana* - the world's best genetically characterised plant.



Arabidopsis also bears its seed in siliques (seed pods) which are anatomically similar to those of rapeseed plants.

left: ***Arabidopsis thaliana***
right: ***Capsella rubella***
(V=valve, r=replum, S= stigma)

Arabidopsis seed are carried in siliques (pods) that are formed late in flower development and expand after fertilisation and seed growth. They are formed by fusion of two carpels, to create joined chambers that contain multiple ovules - that after fertilisation will each form mature seed. S = stigma, the pollen receptive tissue at the apex of the female floral structure. R = replum, support structure at the point of contact for the two valves (V). Analogous structures are found in *Arabidopsis*, *Capsella* and *Brassica spp*.



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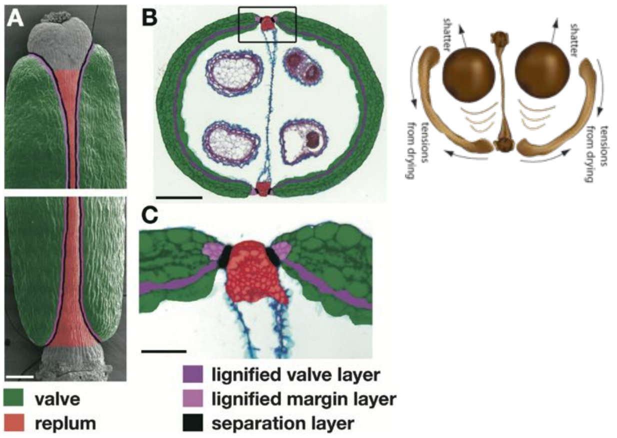
Coloured scanning electron micrograph of opening of an Arabidopsis silique (fruit). At maturity, the silique and seeds undergo desiccation. This causes a build up of physical tension within the walls of the fruit. The junction between the valves and replum is inherently weak(dehiscence zone), and eventually the valves tear apart from the replum at this junction at valve margins.

Specialised cells and valve dehiscence in Arabidopsis



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The differentiation of specialised cells in the valve margins ensures that valve separation (dehiscence) occurs efficiently. In Arabidopsis, we see the presence of strong, lignified cells (i) as a layer within each valve, and connected to this, (ii) a strengthened layer at the valve margin. Desiccation causes tissue shrinkage and build up of tension in each valve. The lignified layers within the valves ensure that these forces are transmitted efficiently to the margins. Eventually, the cellular connections between valve and replum must give way, and the seed pod shatters, releasing the seed.

Genetically identified regulators of seed shatter

Species	Gene(s)	Gene category	Molecular function	Phenotypic effect
Arabidopsis thaliana	*SHATTERPROOF1/2*	Transcription factor	Transcriptional regulator (MADS)	Indehiscent pod
	INDEHISCENT	Transcription factor	Transcriptional regulator (bHLH)	Indehiscent pod
	ALCATRAZ	Transcription factor	Transcriptional regulator (bHLH)	Partially indehiscent pod
	FRUITFULL	Transcription factor	Transcriptional regulator (MADS)	Premature bursting pod
	REPLUMLESS	Transcription factor	Transcriptional regulator (homeodomain)	Partially indehiscent pod
	NST1/3	Transcription factor	Transcriptional regulator (NAC)	Indehiscent pod
	ADPG1/2	Endo-polygalacturonase	Degrade cell wall matrix	Indehiscent pod
	GA3ox1	Catalytic enzyme	GA biosynthesis	Partially indehiscent pod
Glycine max	*SHATTERING1-5*	Transcription factor	Transcriptional regulator (NAC)	Indehiscent pod
	PDH1	Dirigent-like protein	Lignin biosynthesis	Indehiscent pod
Solanum lycopersicum	*JOINTLESS*	Transcription factor	Transcriptional regulator (MADS)	Non-shedding fruit
	MACROCALYX	Transcription factor	Transcriptional regulator (MADS)	Non-shedding fruit
	SLMBP21	Transcription factor	Transcriptional regulator (MADS)	Non-shedding fruit
	LATERAL SUPPRESSOR	Transcription factor	Transcriptional regulator (GARS)	Non-shedding fruit
Oryza sativa	*Shattering4*	Transcription factor	Transcriptional regulator (Myb)	Non-shattering seed
	qSH1	Transcription factor	Transcriptional regulator (homeodomain)	Non-shattering seed
	SH5	Transcription factor	Transcriptional regulator (homeodomain)	Non-shattering seed
	SHATTERING ABORTION1	Transcription factor	Transcriptional regulator (AP2)	Non-shattering seed
	Shattering1	Transcription factor	Transcriptional regulator (YABBY)	Non-shattering seed?
Sorghum bicolor	*Shattering1*	Transcription factor	Transcriptional regulator (YABBY)	Non-shattering seed
Sorghum propinquum	*SpWRKY*	Transcription factor	Transcriptional regulator (WRKY)	Non-shattering seed
Zea mays	*Shattering1*	Transcription factor	Transcriptional regulator (YABBY)	Non-shattering seed?
Triticum aestivum	*Q*	Transcription factor	Transcriptional regulator (AP2/ERF)	Free-threshing character

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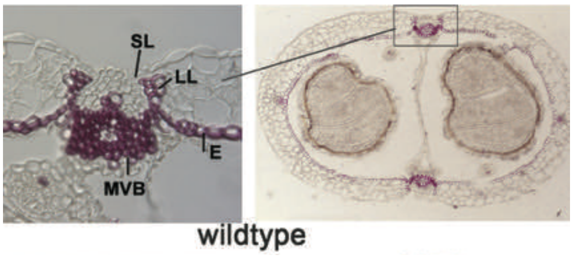
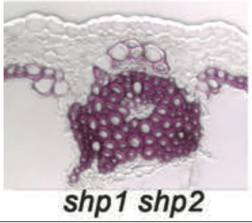
Genetic analysis of mutant plants, where seed shatter is defective, has allowed identification of key gene regulators.





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.

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Notably, there are two MADS box transcription factors in *Arabidopsis* that play a redundant role in precisely specifying the lignified cells at the valve margins. If both genes are disrupted, these few cells at the junction of the valve and replum tissue are not specified properly. This precise and minor defect results in siliques that do not shatter normally, and the genes have been named Shatterproof 1 and 2.

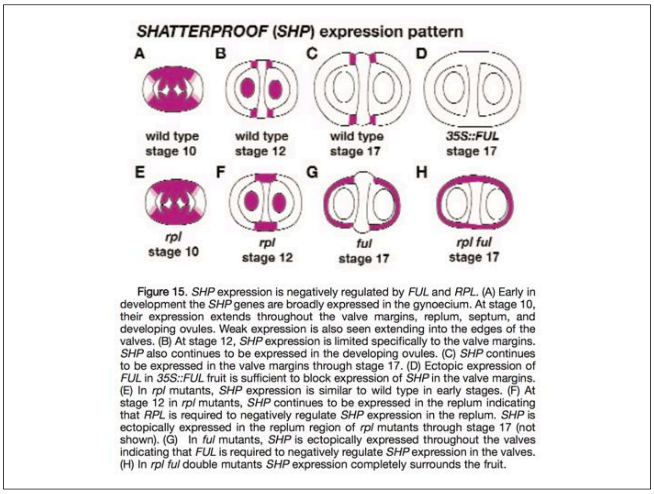
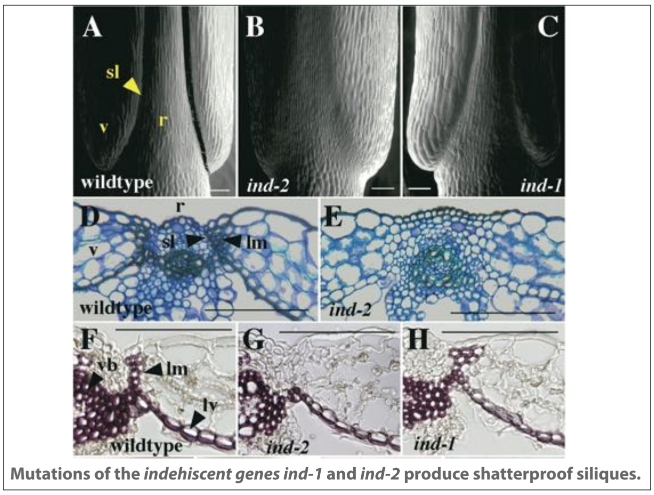


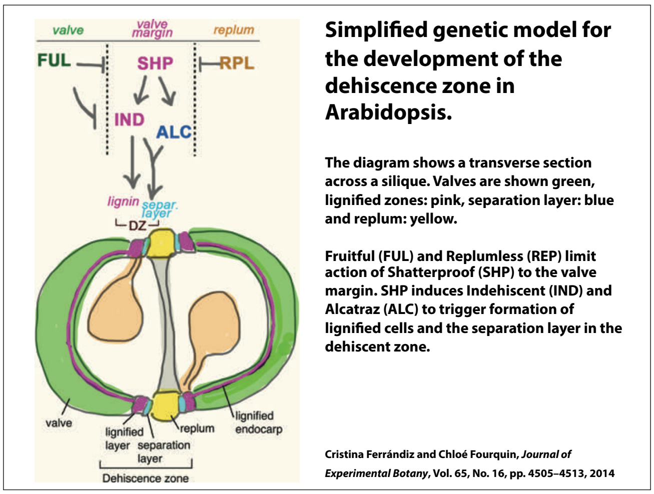
Figure 15. *SHP* expression is negatively regulated by *FUL* and *RPL*. (A) Early in development the *SHP* genes are broadly expressed in the gynoecium. At stage 10, their expression extends throughout the valve margins, replum, septum, and developing ovules. Weak expression is also seen extending into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

1. There are regulatory genes expressed in the valve and replum that limit *Shatterproof* expression to the valve margin. These are the MADS box protein encoding gene Fruitfull (*Ful*) expressed in the valve, and the homeodomain protein encoding gene Replumless (*Rpl*), which is expressed in the replum. Shatterproof gene expression is normally limited to the valve margin (C) in mature siliques. However, loss of Ful gene function results in expansion of SHP expression into the valve (G). Loss of Rpl gene function results in expansion of SHP expression into the replum (F).



Mutations of the *indehiscent genes ind-1* and *ind-2* produce shatterproof siliques.

2. There are genes downstream of Shatterproof 1 and 2 that are also required for formation of the lignified valve margin cells and separation layer. Examples of these are bHLH-class transcription factors, Indehiscent and Alcatraz. Strong mutant alleles of Indehiscent (e.g. *ind-2*) cause marked disruption of of the valve margin - with loss of lignified cells.



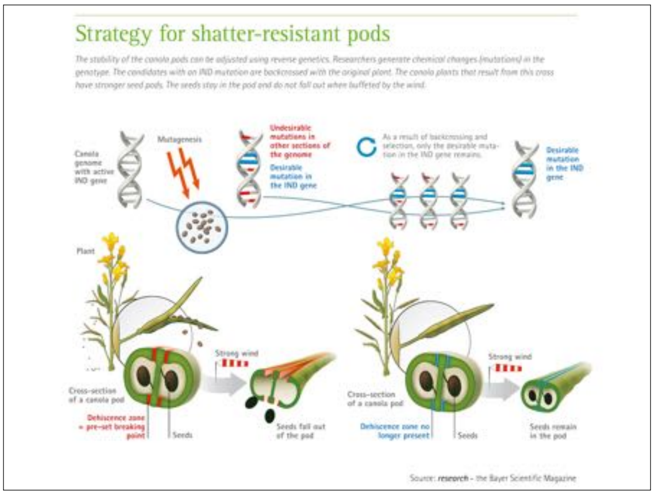
Simplified genetic model for the development of the dehiscence zone in Arabidopsis.

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

REPLUMLESS and FRUITFULL are expressed either side of the valve margin, and they act in concert to limit the domain of expression of the SHATTERPROOF proteins. In turn, SHATTERPROOF 1&2 regulate downstream functions required for specification of the lignified cell layer and separation zones in the valve margin.


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

Understanding of the genetic and cellular processes involved in establishing dehiscence zones in Arabidopsis has led to the development of engineering strategies for reducing pod shatter in rapeseed varieties. In this example from Bayer, Canola lines have been selected with defects in the IND genes. In addition, Canola lines with reduced pod shatter have been produced through expression of antisense genes and use of CRISPR/Cas9 induced gene knockouts.

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.

Field trial of modified Canola with the "Pod Shatter Reduction" trait from Bayer. Trait engineering requires the careful balance of reduced pod shatter with the need for ease of seed separation during harvesting. Further, engineering of the *Brassica napus* genome can be complicated by its teraploid (AACC) nature, and this is being aided by highly efficient CRISPR/Cas9 techniques for targeted mutagenesis.

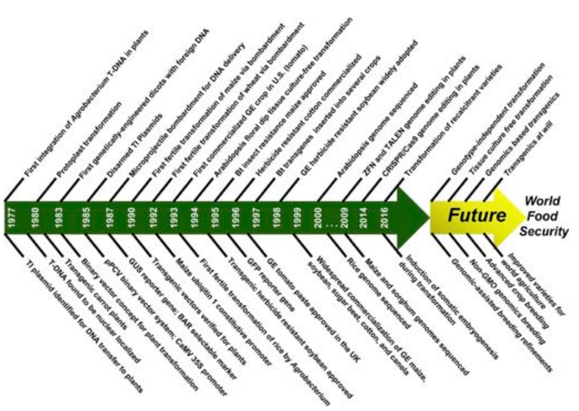


Figure 3. Important Historical Milestones in Plant Transformation.

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.

The history of crop domestication has demonstrated the genetic plasticity of plants, and the benefits of manipulation of complex traits (e.g. microarchitecture of plant organs to reduce pod shatter). As our our ability to manipulate plant genomes improves, along with our understanding of plant development and growth - new possibilities for the rational design of plant improvements become feasible.

This is very timely, as there is continued pressure to increase crop yields, due to constraints on the availability fertile land and water, and pressure from population growth and demand for improved food quality.