

ISA

Genética Quantitativa e  
Melhoramento de Plantas

Epigenetics in Plant Breeding  
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ULISBOA

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AGRONOMIA

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# ISA

## *2nd session*

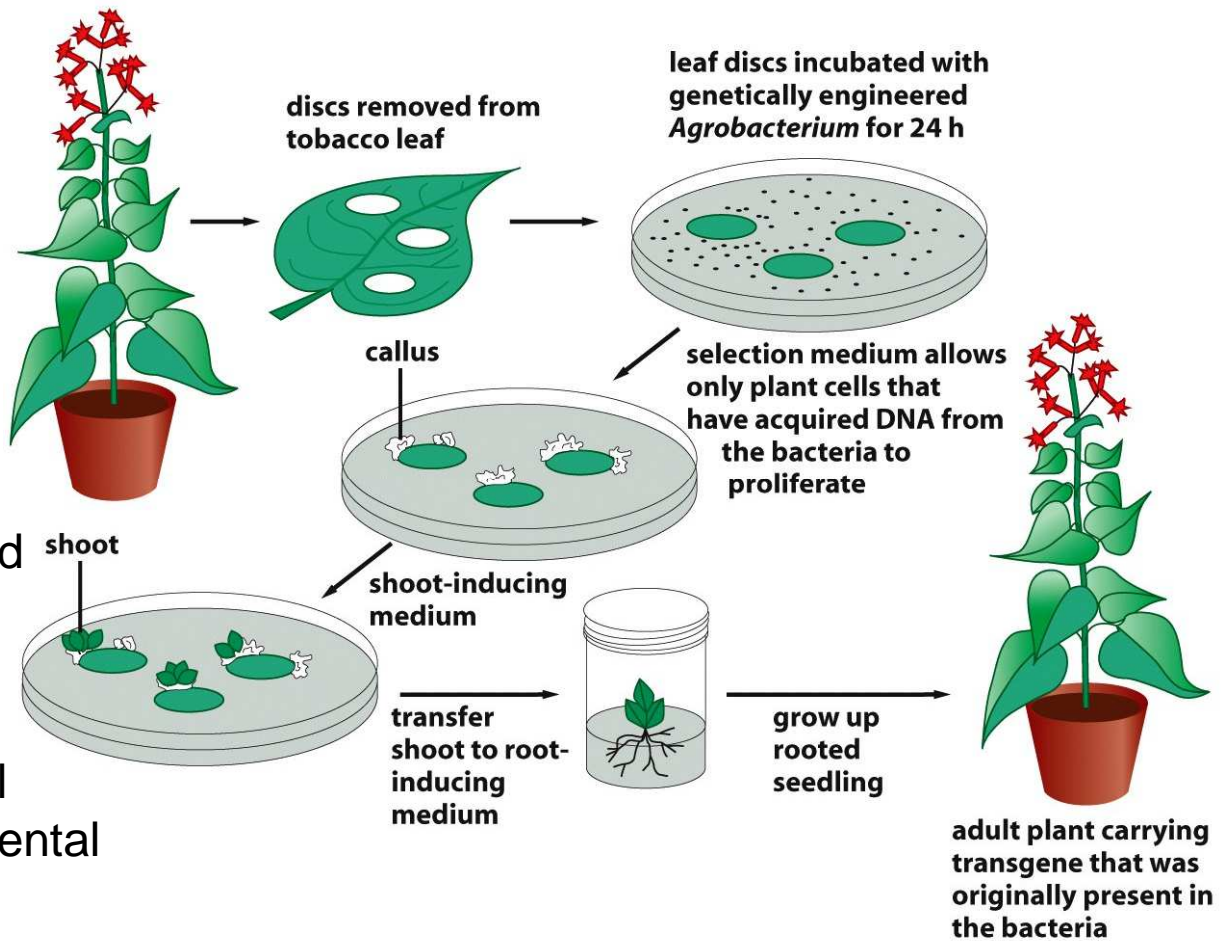
- Spontaneous or induced epialleles
- Environmental stress and plant adaptation
- Epigenetically breeding systems
- Epigenetic changes driving genetic mutations

In most cases, plant breeding efforts have focused on harnessing natural variation through **genetic crosses** and the evaluation of progeny. This is often followed by the creation of stable inbred or hybrid varieties that can be sold as seeds for agricultural production.

However, for other species, breeders have been forced to use **clonal propagation** to maintain ideal varieties. Many fruits are the result of clonal propagation and are based on a single genetic variety.

In these species, there is the potential for epigenetic variation among different 'sports' or clonal propagants, and **epigenetic variation that arises among cells or tissues can be passed on if it is stable through scions or clonal propagation either by grafting or tissue culture.**

In plant micropropagation as well as in vegetatively (clonally) propagated plants usually occurs reprogramming of previous methylation patterns which in addition to removing deleterious alleles, may also be opportunities to unleash epigenetic information and generate new beneficial alleles in species

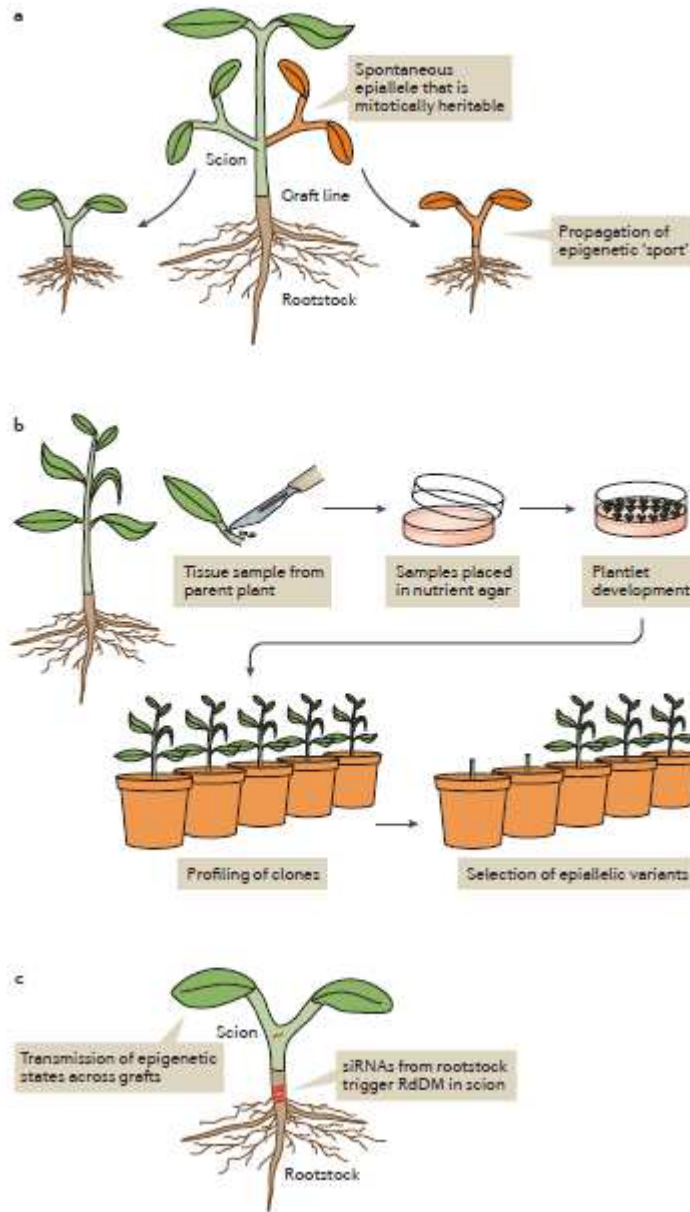


In many agricultural species, tissue culture is a necessary part of plant transformation and clonal propagation.

Tissue culture represents an extreme form of environmental stress and a unique developmental trajectory



## Epigenetic variation and clonal propagation



a) The vegetative propagation of plants creates the opportunity to generate and harness epigenetic variation. Whether epigenetic variants arise spontaneously or are induced, they can be stably maintained and propagated through grafting or tissue culture if the epialleles are stably maintained through mitosis

b) For plants that are generated by tissue culture, the process of tissue culture can induce a number of epigenetic changes.

**Profiling of chromatin marks may therefore be necessary to identify beneficial or deleterious epialleles.**

a) In grafted plants, the scion tissue may have targeted epigenetic variation owing to the expression of mobile signals such as small interfering RNAs (siRNAs) in the rootstock.

## Somaclonal variation

Somaclonal variation arises in plants and animals when differentiated somatic cells are induced into a pluripotent state, but the resulting clones differ from each other and from their parents.

In agriculture, somaclonal variation has hindered the micropropagation of elite hybrids and genetically modified crops, but the mechanism responsible was unknown for a long period.

## Study of oil palm points to one avenue for epigenetic information being used for agricultural improvement in clonally propagated species.

- Most oil palms are hybrids derived from a cross between two subspecies.
- Once a high-performing cross is identified, it is widely deployed across many plantations by using tissue culture to develop many clones.
- Unfortunately, a subset of the clones that have passed through tissue culture exhibits a 'mantled' phenotype that destroys the productivity of the tree



Normal (a) and fertile mantled (b) fruit shown as whole fruit (top), longitudinal sectioned (middle) and cross sectioned (bottom). Black arrows denote pseudocarpels; white arrows denote kernel

### **The mantled trait is not apparent until maturity, years after planting.**

The analysis of genomic DNA methylation has identified a mark that is associated with the mantled trait. Changes in DNA methylation of the *EgDEF1* gene result in aberrant transcripts for a floral identity gene that lead to undesirable morphology changes. Understanding the epigenetic basis for this trait provide avenues for using DNA methylation profiling to identify the defective clones.

# identification of epialleles induced in tissue culture in clonally propagated oil palms

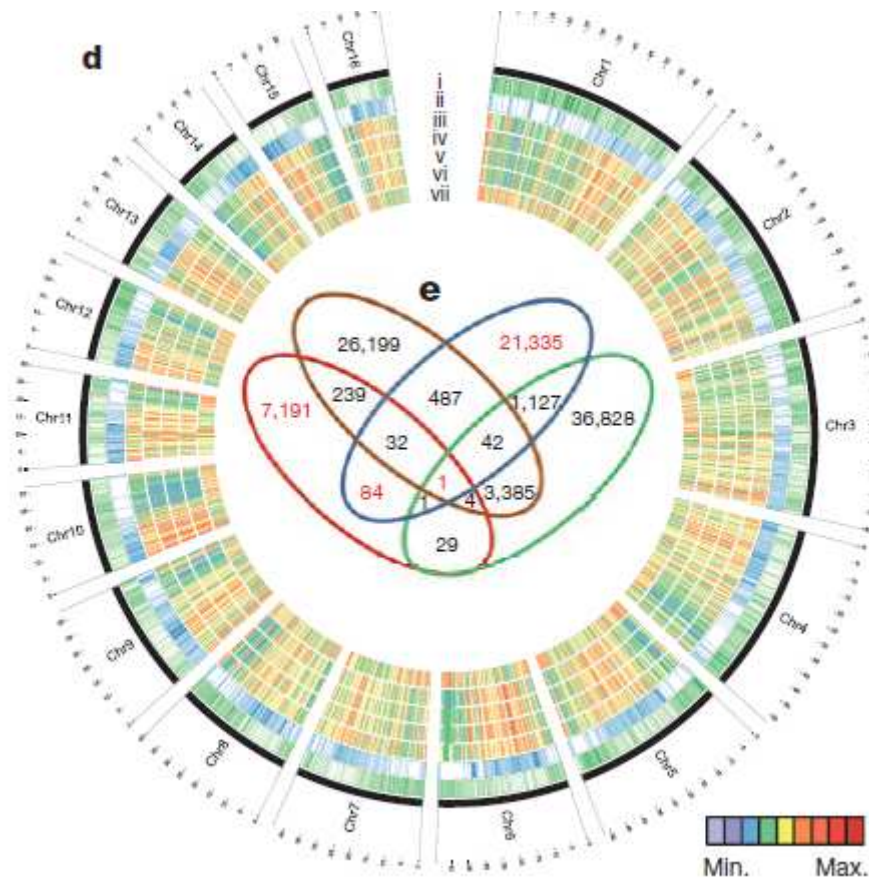


Loss of *Karma* methylation and of small RNA in tissue culture contributes to the origin of mantled, while restoration in spontaneous revertants accounts for non-Mendelian inheritance.

**The ability to predict and cull mantling at the plantlet stage will facilitate the introduction of higher performing clones and optimize environmentally sensitive land resources**

Circos plot of oil palm chromosomes.

- i gene density
- ii repeat density
- iii cytosine methylation density (whole-genome bisulfite sequencing)
- iv cytosine methylation densities (microarray)
- v normal
- vi mantled
- vii differential cytosine methylation of normal minus mantled



Venn diagram of microarray features differentially methylated between leaves from mantled and normal



## Examples of stable epialleles in different plant species

Species	Gene/Locus	Epigenetic Variation	Phenotypic Traits
<i>Arabidopsis thaliana</i>	<i>SUP</i> ( <i>SUPERMAN</i> )	Mutagen induced	Increased numbers of stamens and carpels
	<i>FWA</i> ( <i>Flowering Wageningen</i> )	Mutagen induced	Late flowering
	<i>PAI2</i> ( <i>Phosphoribosyl Anthranilate Isomerise</i> )	Trans-acting (small RNAs)	Only gene expression affected; no specific phenotype
	<i>BAL1</i>	Mutagen induced	Dwarfing and elevated disease resistance
	<i>AG</i> ( <i>AGAMOUS</i> )	Mutagen induced	Affect flower structure
	<i>BNS</i> ( <i>BONSAI</i> )	<i>adm1</i> -induced syndrome	Stunted growth
	<i>FOLT1</i> ( <i>folate transporter 1</i> )	Trans-acting (small RNAs)	Reduced fertility
	<i>QQS</i> ( <i>Qua-Quine Starch</i> )	Spontaneous	Higher starch accumulation
	<i>PPH</i> ( <i>Pheophytin Pheophorbide Hydrolase</i> )	Spontaneous	Inhibits leaf senescence
	<i>HISN6B</i> ( <i>Histidinol-phosphate aminotransferase 1</i> )	Spontaneous	Hybrid incompatibility
<i>Zea Mays</i>	<i>r1</i> ( <i>red1</i> )	Spontaneous	Reduced pigmentation
	<i>b1</i> ( <i>booster 1</i> )	Spontaneous	Reduced pigmentation
	<i>pl1</i> ( <i>purple plant 1</i> )	Spontaneous	Reduced pigmentation
	<i>p1</i> ( <i>pericarp color 1</i> )	Spontaneous	Reduced pigmentation
	<i>lpa1</i> ( <i>low phytic acid1</i> )	Paramutagenic	High inorganic phosphate in seeds
<i>Linaria vulgaris</i>	<i>Lcyc</i> ( <i>Linaria cycliodes</i> )	Spontaneous	Floral symmetry; dorsiventral flower axis
<i>Solanum lycopersicum</i>	<i>CNR</i> ( <i>Colorless non-ripening</i> )	Spontaneous	Normal fruit ripening
	<i>VTE3</i> ( <i>Vitamin E</i> )	Spontaneous	Tocopherol accumulation in fruit
<i>Oryza sativa</i>	<i>DI</i> ( <i>Drawf1</i> )	Spontaneous	Dwarf
	<i>SPL14</i> ( <i>Squamosa Promoter binding protein-Like</i> )	Spontaneous	Panicle branching and higher grain yield
	<i>FIE1</i> ( <i>Fertilization-Independent Endosperm 1</i> )	Spontaneous	Dwarf
	<i>RAV6</i> [ <i>Related to Abscisic Acid Insensitive 3 (ABI3)/Viviparous1 (VPI) 6</i> ]	Spontaneous	Larger lamina inclination and smaller grain size
	<i>AK1</i> ( <i>Adenylate Kinase 1</i> )	Spontaneous	Defects in photosynthetic capacity
	<i>ESP</i> ( <i>Epigenetic Short Panicle</i> )	Spontaneous	Short panicle
<i>Elaeis guineensis</i>	<i>DEF1</i> ( <i>DEFICIENS</i> )	Spontaneous	Mantled fruit
<i>Brassica rapa</i>	<i>SP11/SCR</i> ( <i>S locus protein 11/S locus cystein rich</i> )	Trans-acting (small RNAs)	Self-incompatibility
<i>Cucumis melo</i>	<i>CmWIP1</i> ( <i>WASP/N-WASP-interacting protein 1</i> )	Transposon Insertion	Sex determination

Not all epigenetic marks are erased through meiosis being transgenerationally heritable and opening the door to a new approach of utilizing epigenetic variation as a breeding tool for inheritance in crops

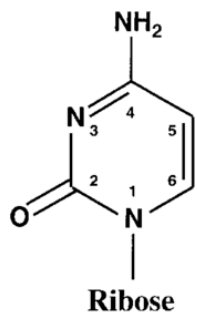
Various stable epialleles affecting floral morphology, flowering time, disease resistance, pigmentation, and leaf senescence have been reported in different plant species.

epigenetic quantitative trait loci (epiQTL) controlling flowering time and primary root length were identified and showed high heritability (up to 90%)

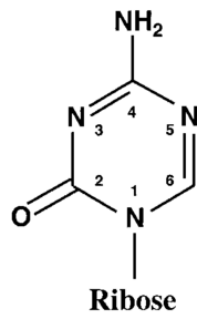
Since epiQTLs are stably inherited and show phenotypic diversity, they are good targets for natural/artificial selection for crop improvement.

Phenotypic variants were Initially generated through treatments with the methylation inhibitor 5-azacytidine which erase previous methylation patterns.

Structures of cytidine and its 5-aza-analog.



**Cytidine**



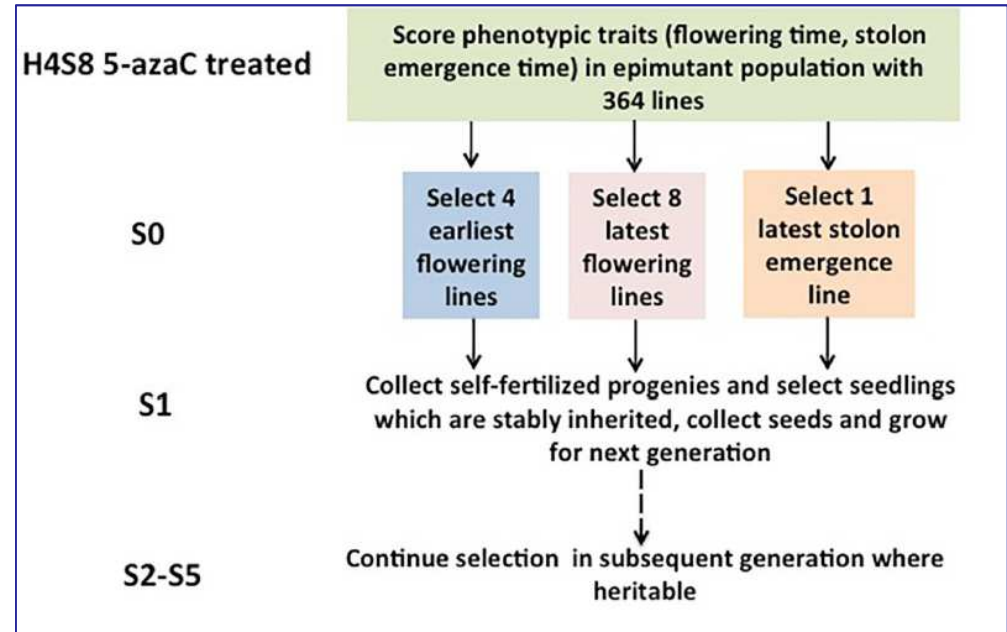
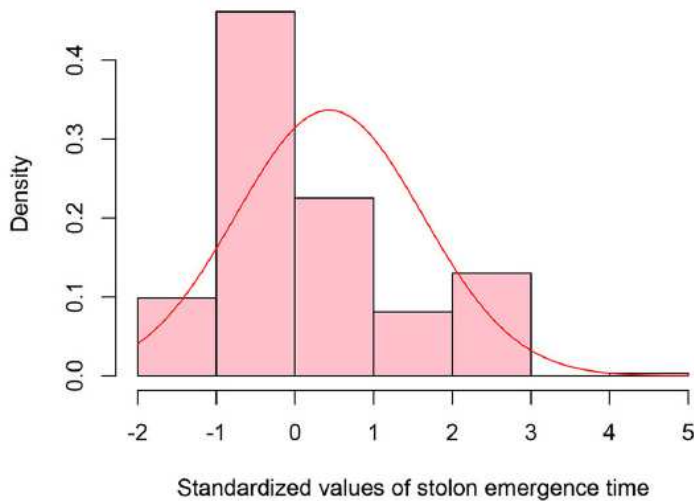
**5-Azacytidine  
(5-Aza-CR)**



**Evaluation of epigenetic marks  
erasure through  
mitosis or meiosis**

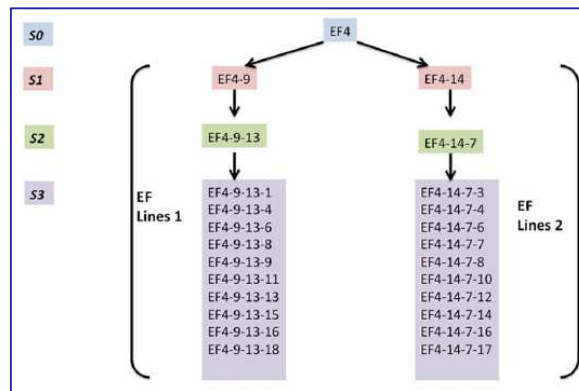
Induced hypomethylated epi-mutant population of genetically identical individuals of *Fragaria vesca*, resulting from repeated selection of extreme phenotypic traits (such as early flowering), show heritable phenotypes

The selection started with an **hypomethylated seedling population** with genetically identical background derived from 5-azacytidine (5-azaC) treated seeds



Distribution of standardized values for stolon emergence time observed in the epimutangenized population relative to control population mean.

Early flowering time is an important characteristic in strawberry breeding and production, being flowering time genes often targets of selection to increase fitness in order to better adapt to the environment



The overview of four generations of two early flowering lines (lines 1 and 2) selected from a hypomethylated population treated with 5-azaC

The percentage of early flowering individuals in the early flowering epimutant lines (54.4%) was nearly **three times** the percentage of control lines (20.0%) showing a significant difference between the early flowering families and control lines

TABLE 4 | Composition of the early flowering lines in each generation in epimutant and control lines.

Generation	Early flowering epimutant			Control			$\chi^2$	p-Value
	Number of early flowering lines	Number of lines	%	Number of early flowering lines	Number of lines	%		
S0	65	293	22.2	14	59	23.7	0.07	0.80
S1	23	69	33.3	5	36	13.9	4.57	0.03
S2	14	61	22.9	4	30	13.6	1.17	0.28
S3	70	94	74.5	6	45	13.3	45.90	<0.001
S4	43	79	54.4	4	20	20	7.59	0.01
S5	18	36	50	1	24	4.2	13.98	<0.001

Early flowering lines were determined by the number of lines with flowering time less than the mean of the control minus one standard deviation (mean - SD).

Early flowering time is an important characteristic in strawberry breeding and production, being flowering time genes often targets of selection to increase fitness in order to better adapt to the environment

**Flowering time in S5 in early flowering lines through meiosis and mitosis.**

Meiosis				Mitosis			
Families	Size	Mean	SD	Families	Size	Mean	SD
EF4-9-13-15-18	12	75.9*	2.6	EF4-9-13-15'	15	17.3***	4.0
EF4-14-7-6-12	12	76.2*	2.4	EF4-14-7-6'	15	20.9*	4.4
EF4-14-7-14-12	12	74.3***	2.3	EF4-14-7-14'	15	21.3*	5.2
Control	24	78.5	2.8	Control'	15	27.1	7.1

In the clonally propagated generation through mitosis, flowering time in early flowering families was around 6–10 days earlier than in control lines.

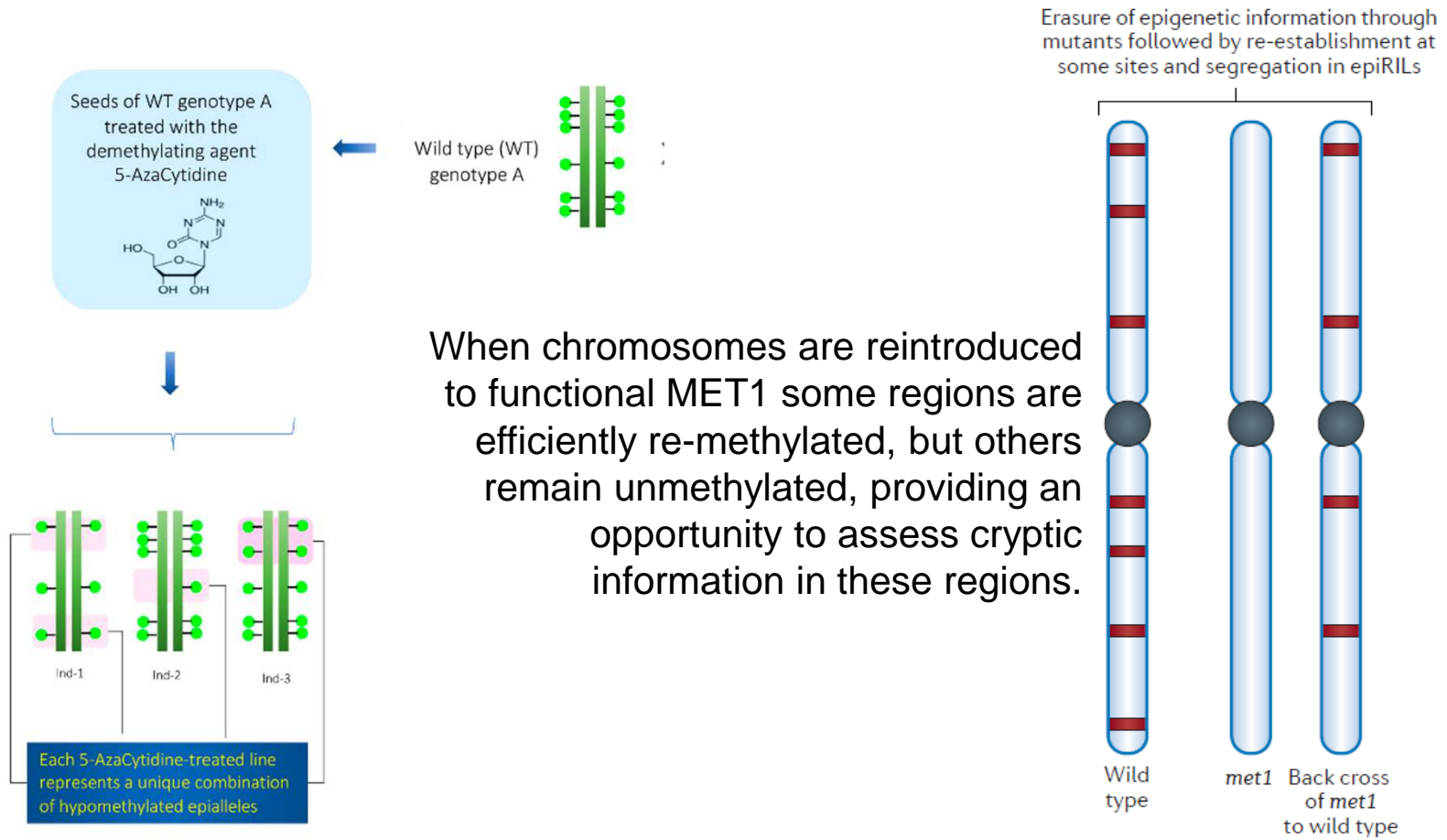
**Vegetatively propagated early flowering plants were on average advanced 5 days earlier than the lines propagated through seed**



- Compared with epigenetic reprogramming in mammals, erasure and re-establishment of plant epigenome is not very efficient, and therefore some epimutations are very stably inherited for generations in plants.
- In addition, the germline develops in terminally differentiated somatic tissues in plants, which allows transmission of epigenetic information from the chromatin of somatic tissues to the next generation.

To understand the contribution of epiallele formation to phenotypic variations and their transgenerational inheritance to offspring, Arabidopsis inbreeding lines of mosaic epigenome called **epigenetic recombinant inbred lines (epiRILs)** have been generated

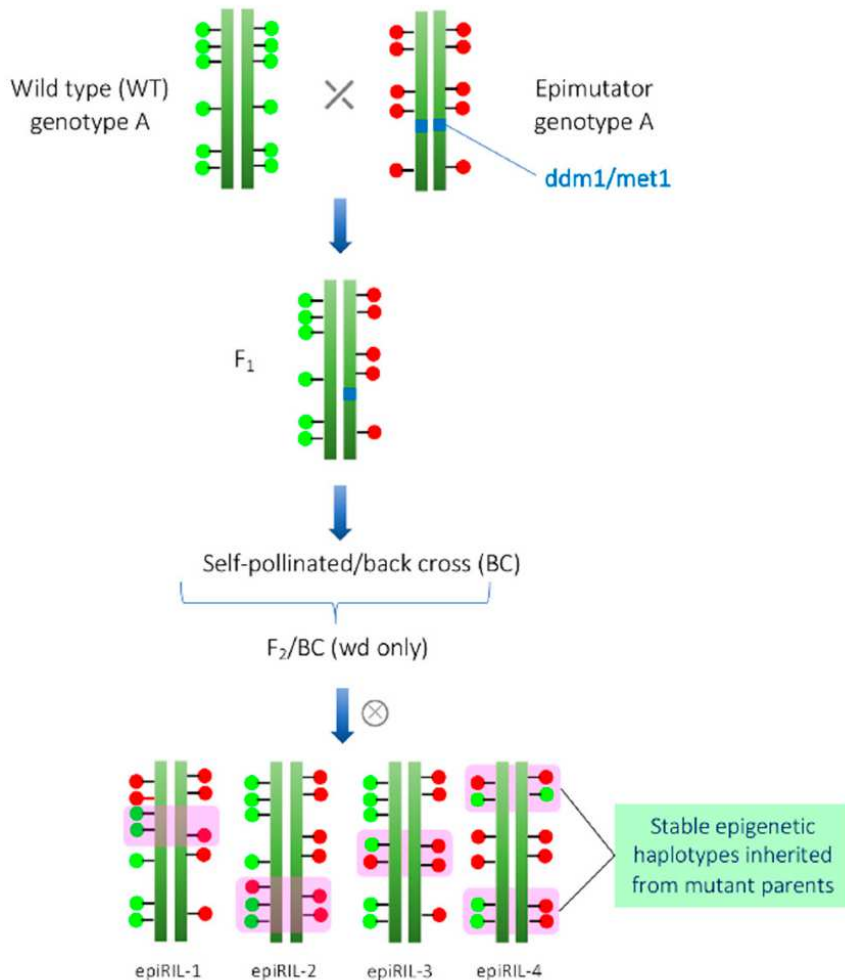
# Epialleles can be generated by 5-azaC or by the passage of a chromosome through a mutant background such as METHYLTRANSFERASE 1 (*met1*), which erases CG methylation



Changes in epigenetic modifications often result in formation of “epialleles” or “epimutations,” *loci* showing variable epigenetic states that are often stably inherited.

## Epigenetic recombinant inbred line (epiRIL)

Quasi-homozygous lines that are almost identical at the genetic level but segregate at the DNA methylation level are produced from an initial cross between two individuals with few DNA sequence differences but with contrasting DNA methylation profiles, followed by 6–8 generations of self-pollination.



Construction of epiRILs with stable inheritance by crossing of two parents (wild-type and epimutator parents (*met1* or *ddm1*) with different epigenetic states.

The green and red circles that overlay the genome sequence illustrates the different epigenetic states of the two parents.

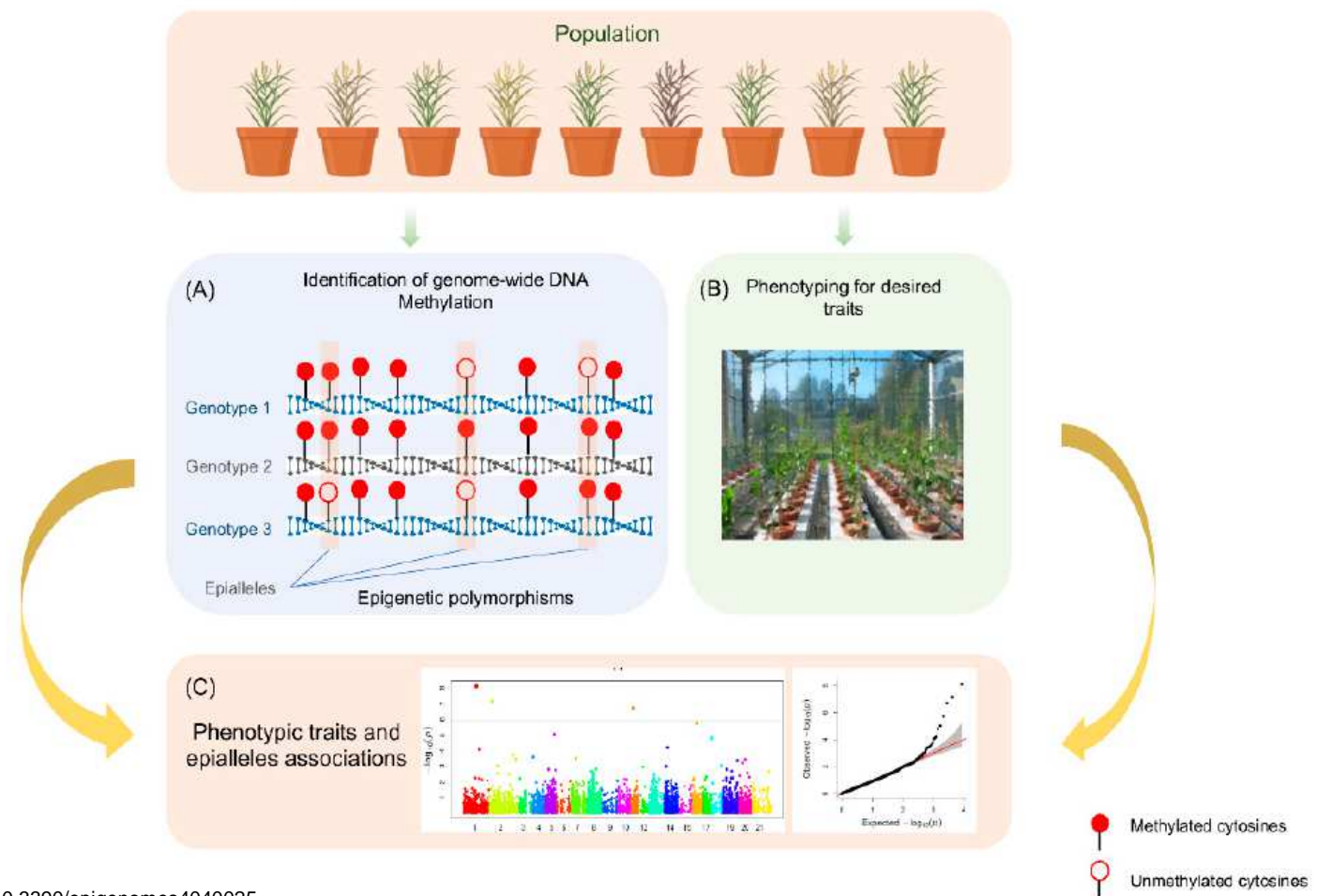
**The stability of phenotypic characters in met1 and ddm1 epiRILs is however quite different.**

- Phenotypes in met1 derived epiRILs are very unstable, and several lines were unable to advance to F8 generation due to abnormal development and infertility.
- Unlike, ddm1 derived epiRILs were found highly stable and more than 99% lines advanced to F8 generations without any abnormality .

In met1 derived epiRILs de-novo methylations may be a possible reason for the phenotypic and epigenetic instability across generations

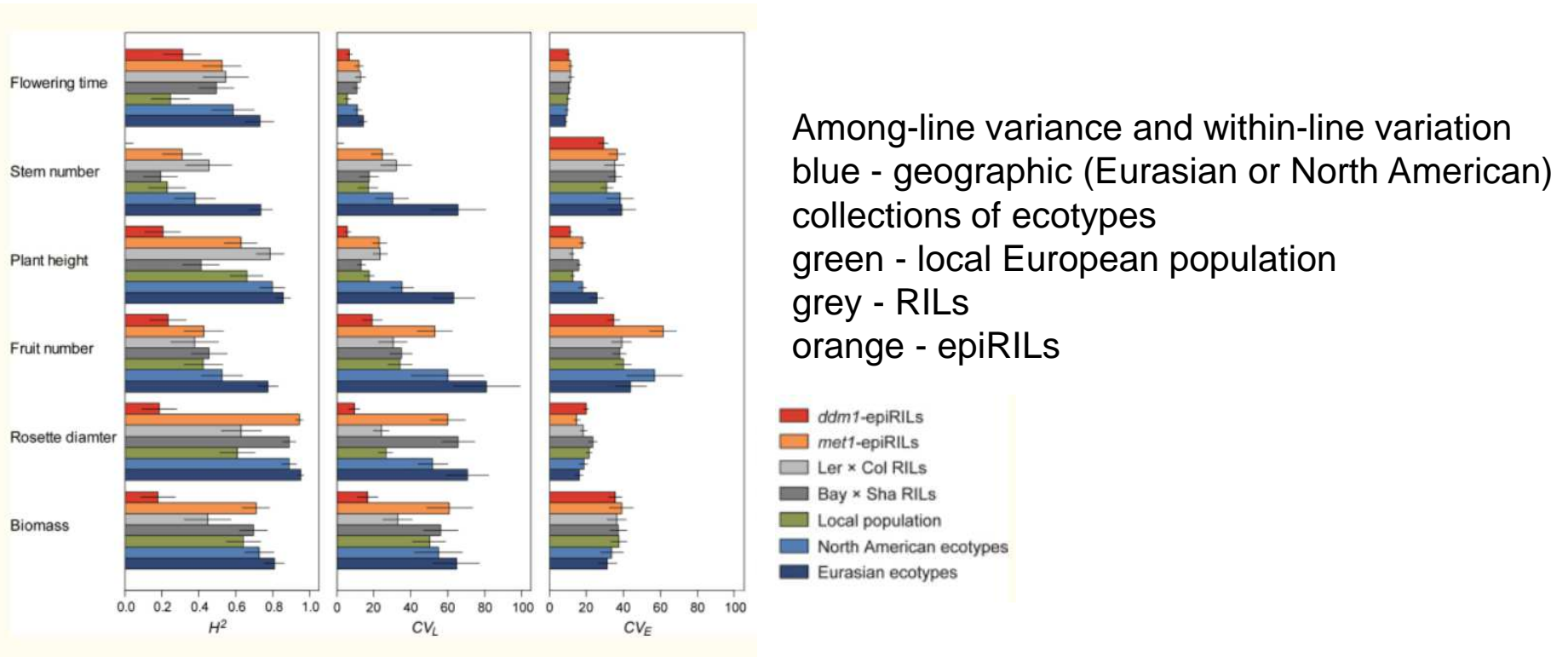
Schematic representation of epigenome-wide association mapping (EWAS) involves three major steps:

- (A) epigenotyping to explore different epialleles,
- (B) precise phenotyping of diverse germplasm,
- (C) statistical analysis to identify epiQTLs.



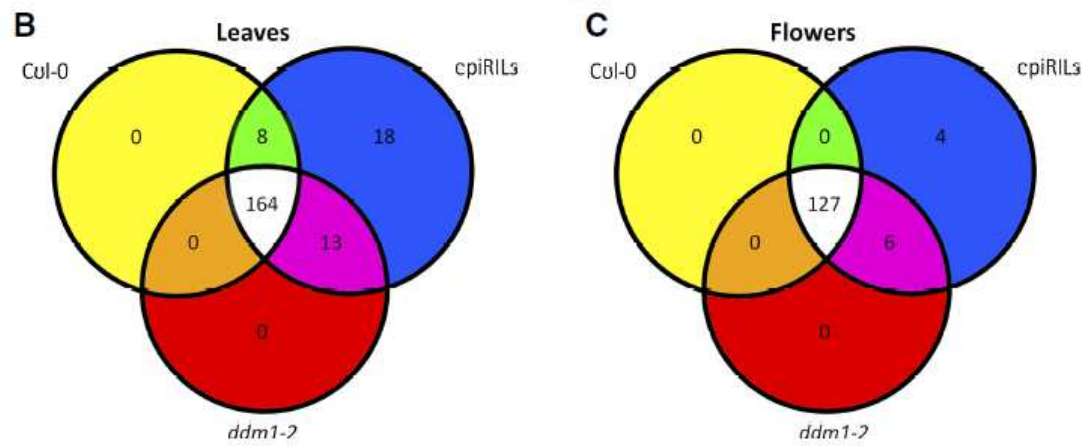


## Estimates of among-line and within-line phenotypic variation for the analysed groups of *A. thaliana* lines



Since the phenotypic variation in epiRILs is mainly caused by epigenetic differences, whereas in RILs and natural ecotypes it is mainly driven by sequence variation, results indicate that variation in DNA methylation create heritable phenotypic variation that is substantial and of a similar order of magnitude as that observed in natural and more DNA sequence-based systems.

## The impact of epigenetic variation on leaves and flowers metabolic composition of a panel of *Arabidopsis thaliana* epigenetic recombinant inbred lines (epiRILs)



(B) Number of metabolites detected in the leaves of the parents Col-0 and ddm1-2, and the epiRILs.

(C) Number of metabolites detected in the flowers of the parents Col-0 and ddm1-2, and the epiRILs.

Results indicate that epi-allelic variation can impact metabolic diversity in a quantitative and qualitative manner in both flowers and leaves, possibly via small RNAs

The ability to easily generate transgenic plants has greatly benefitted epigenetic research. This has been facilitated by insertion mutants or the use of transgene-induced RNAi to knockout or knockdown the expression of candidate genes, such as genes homologous to epigenetic regulators identified in other organisms

**Methylation changes mediated by knockout of specific genes**  
Investigating the amenability of the MSH1 system for inducing agronomically valuable epigenetic variation in soybean

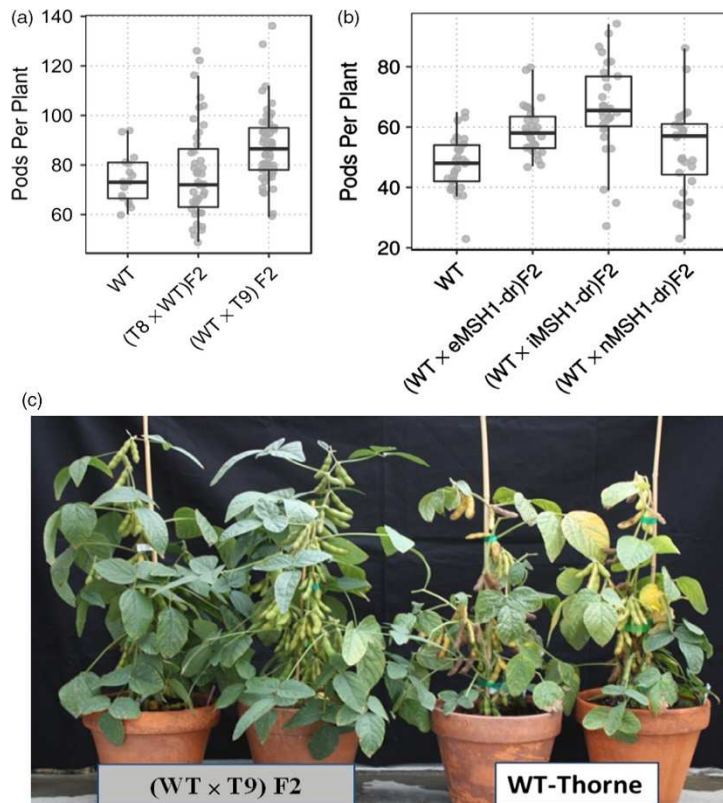


RNAi suppression of *MSH1*, a plant-specific gene present in multiple plant species and which produces a range of developmental changes accompanied by modulation of defence, phytohormone and abiotic stress response pathways along with methylome repatterning.

***msh1*-conditioned developmental reprogramming is retained independent of transgene segregation, giving rise to transgene-null 'memory' effects**

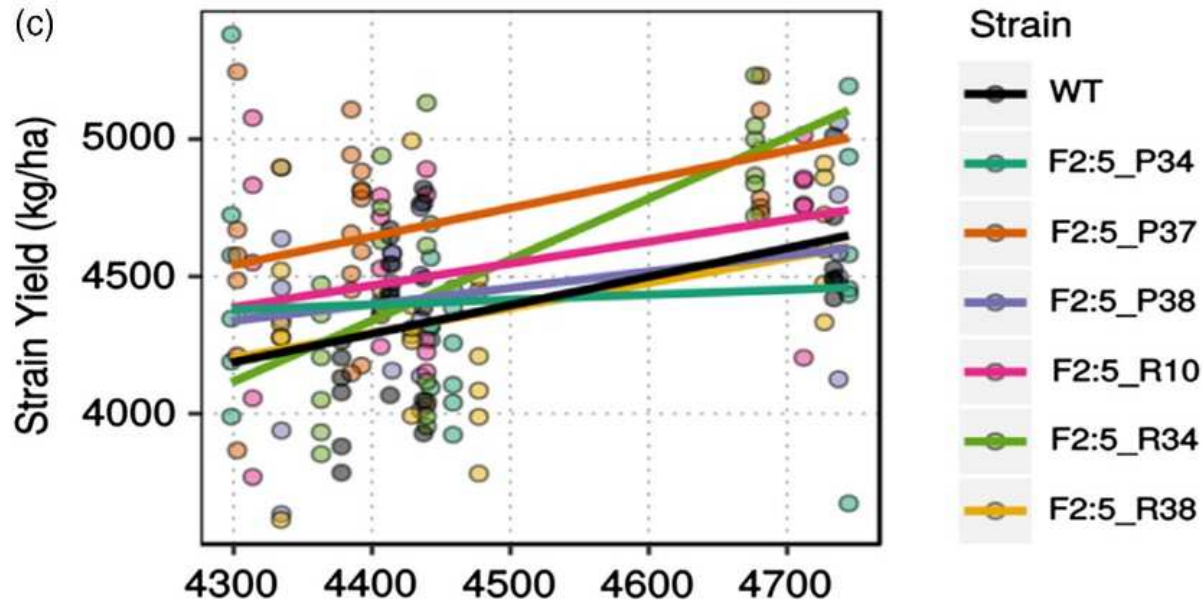
# An epigenetic breeding system in soybean for increased yield and stability

Increased variation for number of pods per plant in different epi-F<sub>2</sub> populations in the glasshouse.



- (a) Enhanced variation for pods per plant in two epi-F<sub>2</sub> populations compared to wild type grown under glasshouse conditions.
- (b) Variation in F<sub>2</sub> performance for number of pods per plant in the glasshouse for populations derived from a range of *msh1* memory phenotypes.
- (c) WT x T9 epi-F<sub>2</sub> lines showing increase in number of pods per plant and delayed maturity compared to wild type.

## Selected MSH1 epi-lines show increased yield compared to wild type in multi-year field trials



Enhanced growth measured as total seed weight in kg/ha normalized to 13% moisture for selected epi- $F_{2:4}$  lines in field experiments.

Yield data pooled from three replicates each from four locations showing superior yield performance of  $F_{2:5}$  P37 across environmental index for yield in kg/ha.

These data support the potential of *MSH1*-derived epigenetic variation in plant breeding for enhanced yield and yield stability.



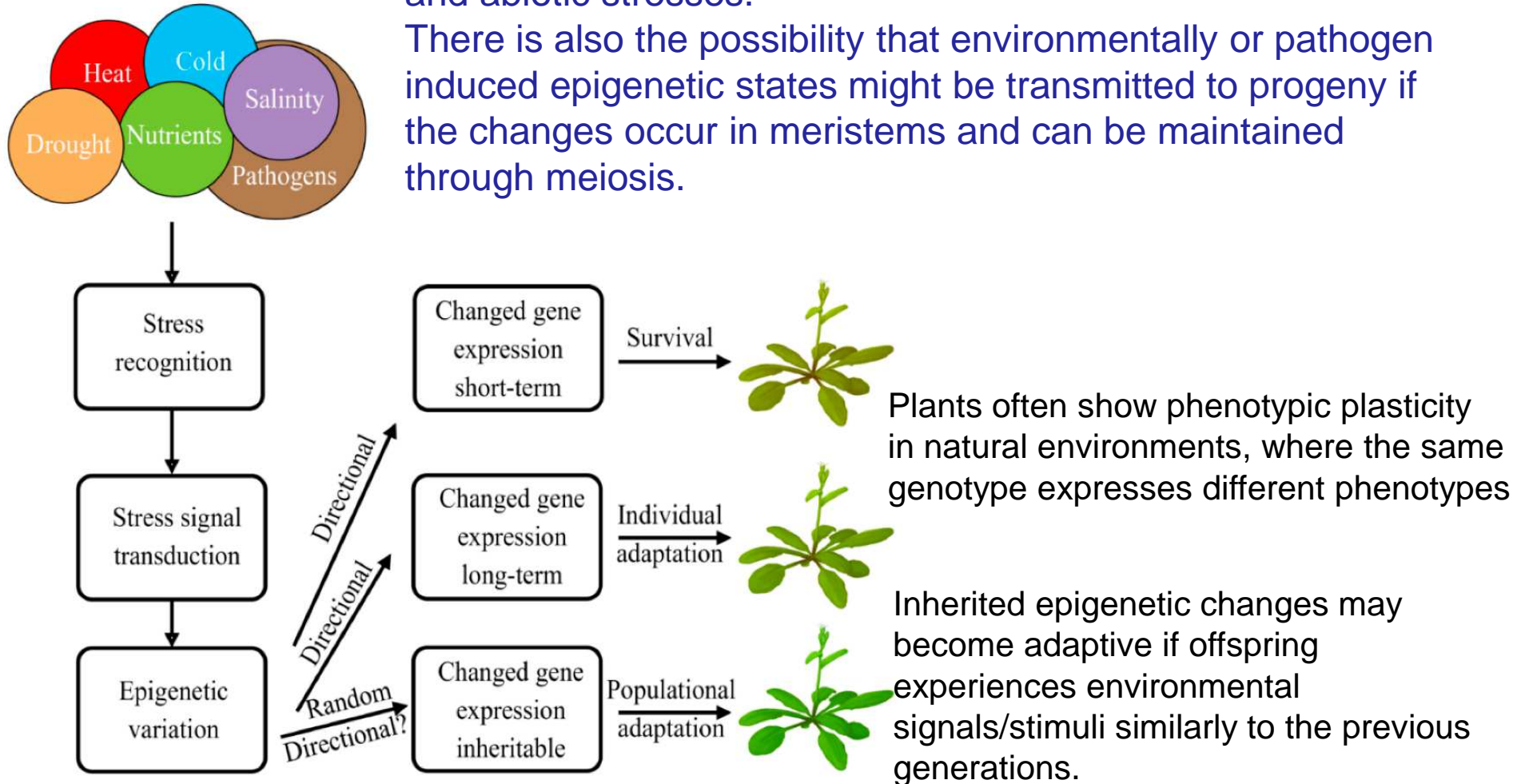
The parental environment may also affect offspring phenotypes by transmitting epigenetic states to developing embryos, which may lead to transmission of phenotypic variations across generations.

- Even though heritability of epigenetic modifications has been well established, it is still under study the degree to which, environmentally induced epigenetic variants are transferred from generation to generation

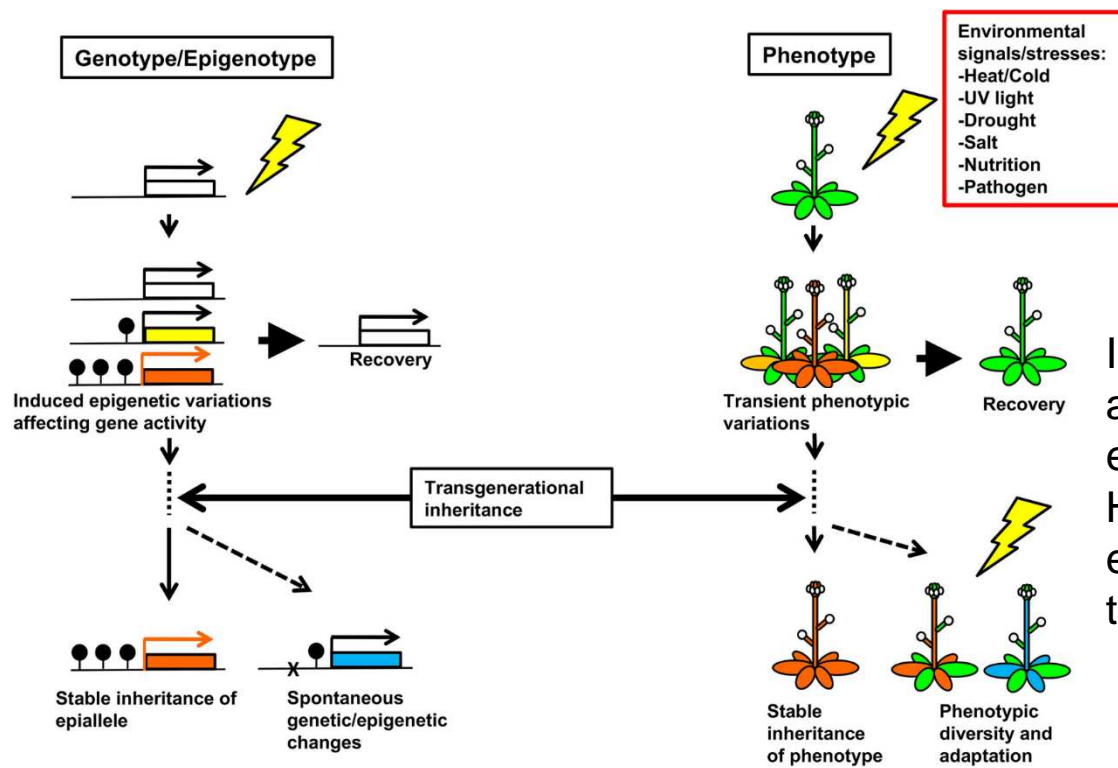
# Short and long-term plant adaptation to environmental stresses

Many examples of plant phenotypic plasticity are regarded as reactions to the environment, for example, responses to biotic and abiotic stresses.

There is also the possibility that environmentally or pathogen induced epigenetic states might be transmitted to progeny if the changes occur in meristems and can be maintained through meiosis.



# Accumulated epigenetic alterations of stressed plants can be transferred to their progeny as epigenetic transgenerational memory

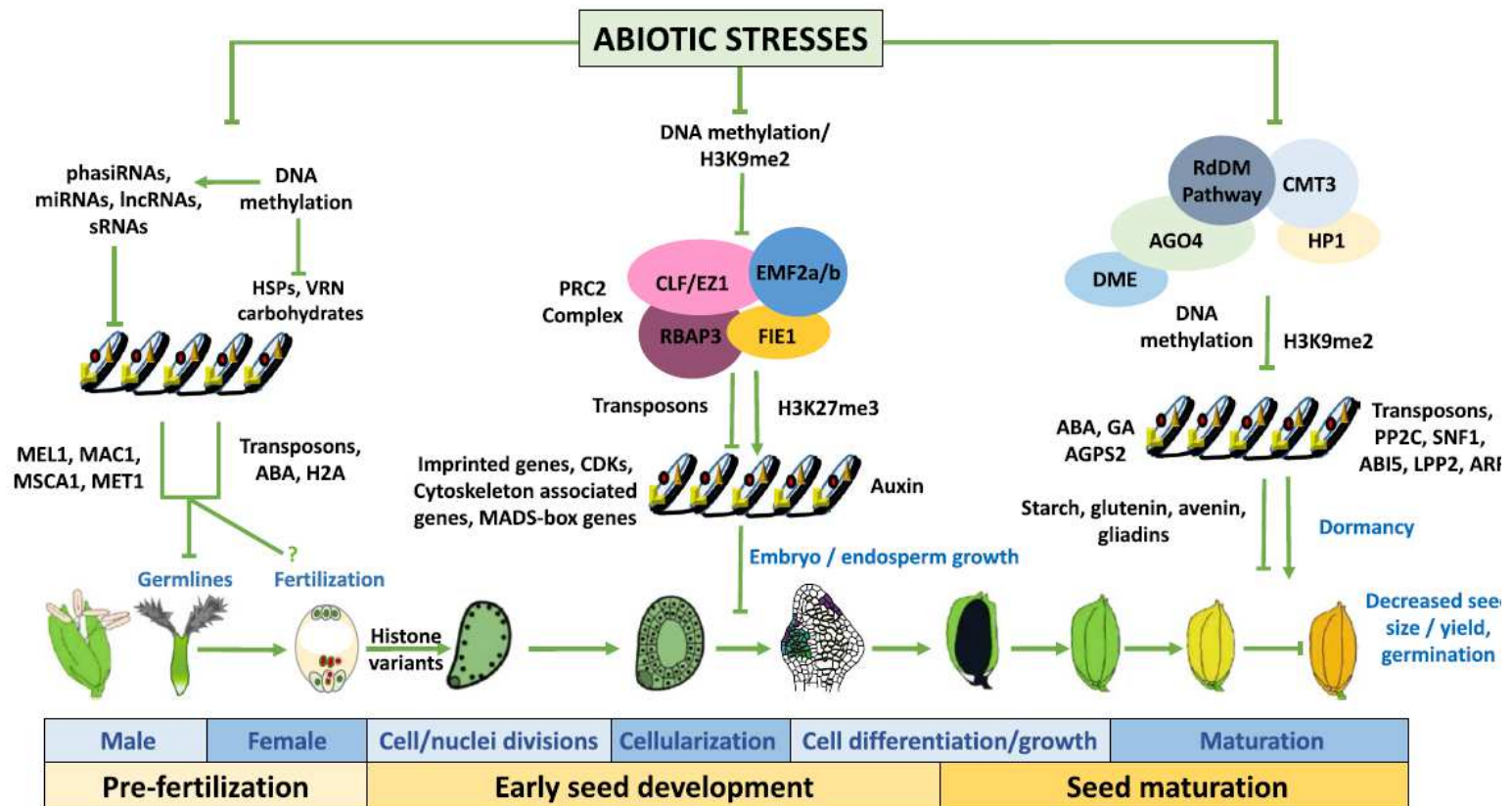


In most cases, epigenetic changes are transient, reverting to the initial epigenetic state. However, in some cases, induced epigenetic changes may have transgenerational transmission

Epimutation rates have been estimated in *A. thaliana* as  $4.5 \times 10^{-4}$  methylation polymorphisms per site per generation, which is much higher than the genetic mutation rate of  $7 \times 10^{-9}$  base substitutions per site per generation in the same population. Epigenetic changes can also revert to the default state.

# Effects of abiotic stresses on the epigenetic status of cereals during reproductive development.

Various stages during development are especially sensitive to environmental stresses



For adaptation, the environment that offspring experience is more important, since the adaptive value of transgenerational epigenetic inheritance of a phenotype caused by environmental factors should depend on the predictability of the environment across generations

## In natural environments, epigenetic variation can contribute to phenotypic divergence in ecologically relevant traits.

Epigenetic mechanism not only represses genes that are no longer needed, it also introduces more plasticity into the expression of genes among individuals that carry the same genome.

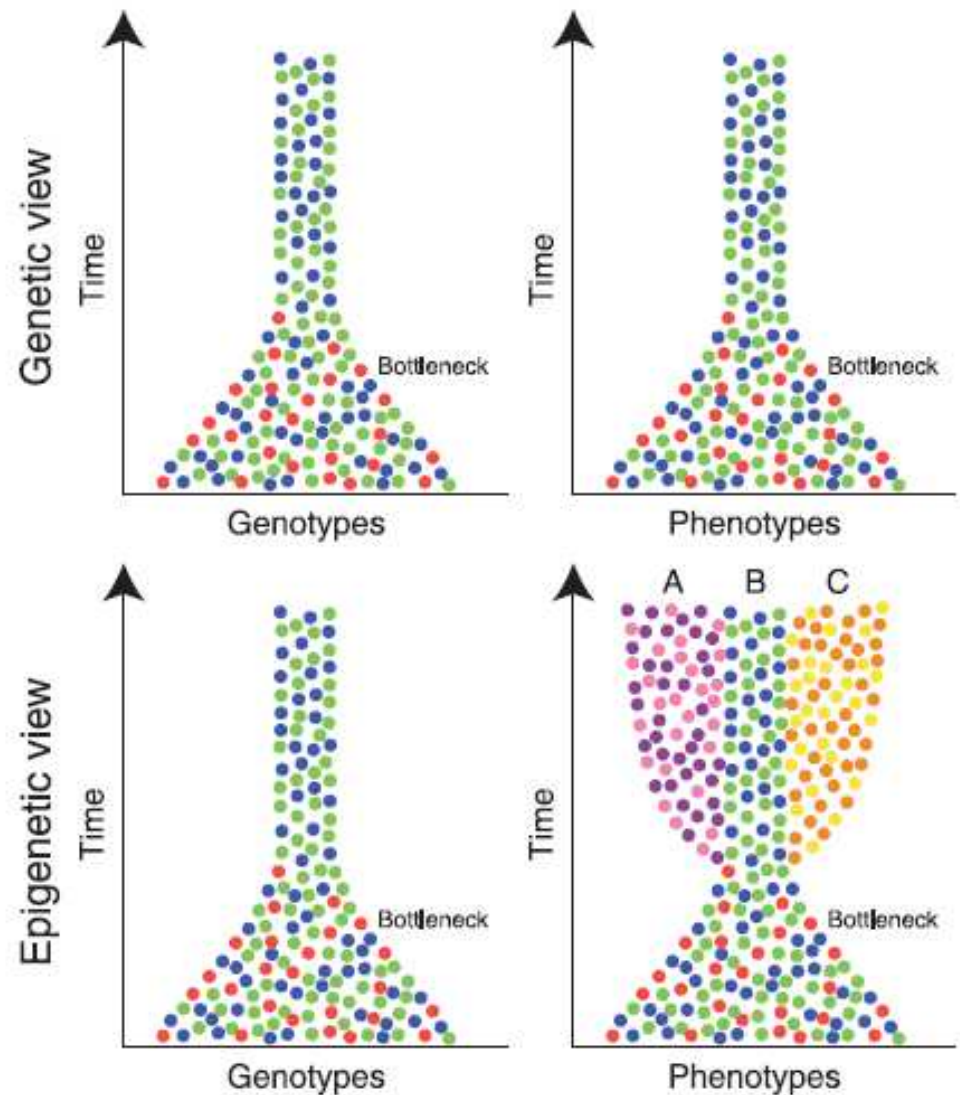
The epigenetic contribution to plastic, and possibly adaptive phenotypic variation, may also be important in rapidly changing environments. Epigenetically-inherited traits can arise simultaneously in many individuals, in contrast to phenotypes linked to genetic mutations.

Epigenetic processes can enable rapid responses of organisms to the environmental changes, which are not possible by slower adaptation through natural selection. In other words, epigenetic mechanisms buffer plants against heterogeneous environment faster than genetic adaptation

## Epigenetically mediated novel phenotypes

Accordingly to Natural Selection, bottleneck winnows genotypes and their corresponding phenotypes resulting in a: **Loss of diversity at both the phenotypic and genotypic levels**

Extreme environments or ecological changes can however induce new evolutionary opportunities through the emergence of novel **epialleles**.





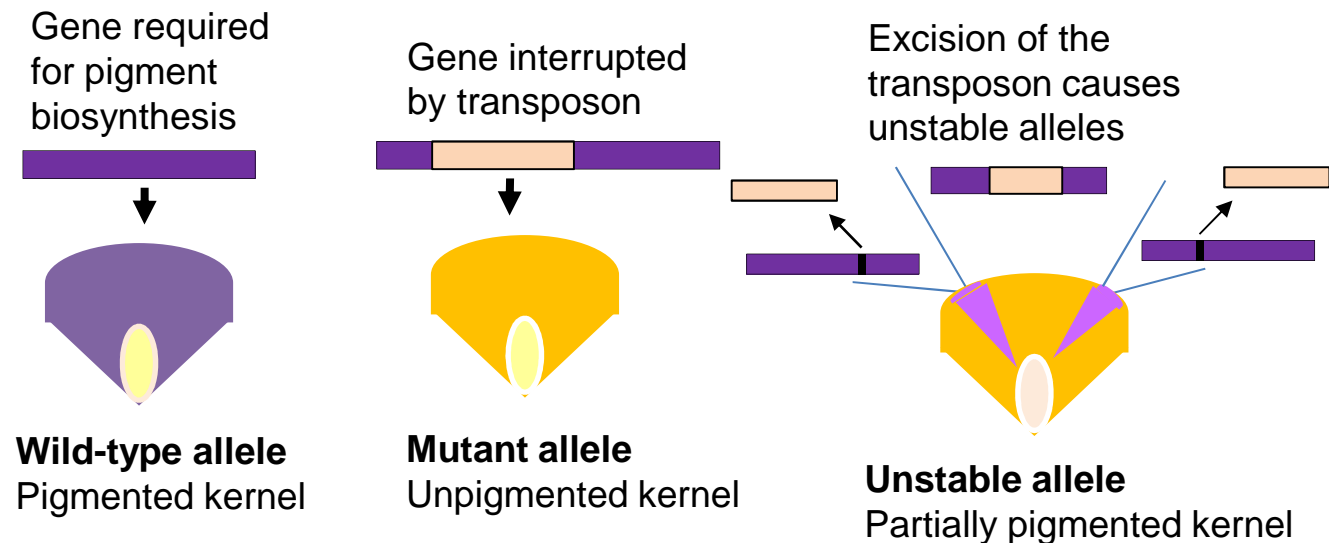
# Epigenetic changes can also drive genetic modifications

**Barbara McClintock** (1902-1992)

Cold Spring Harbor Laboratory, NY  
discovery of mobile elements in corn (*Zea mays*) in  
the 1940's although the Nobel Prize in Physiology  
and Medicine was only attributed in 1983!!!

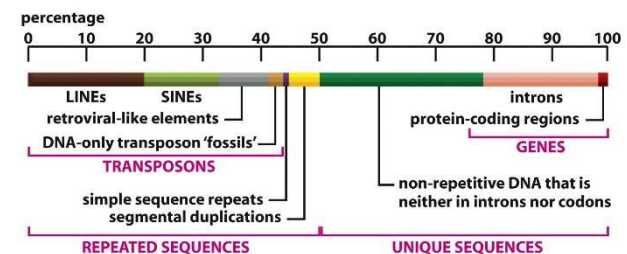


Genes can be altered/mutated due to the  
insertion of transposable elements



## Transposons are abundant in almost all species

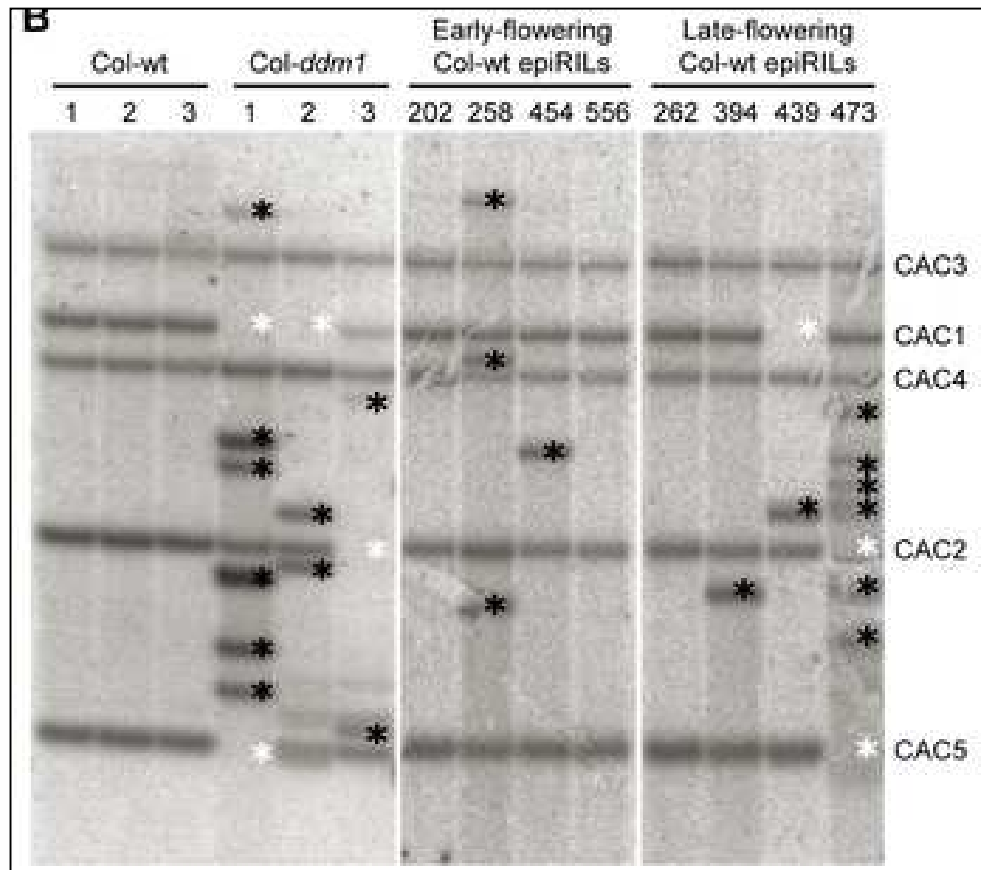
Organism	% of genome derived from transposons
• Yeast - <i>S. cerevisiae</i>	3%
• Nematode - <i>C. elegans</i>	6%
• <i>Arabidopsis thaliana</i>	14%
• Fruitfly - <i>D. melanogaster</i>	15%
• Rice - <i>Oryza sativa</i>	14%
• <i>Homo sapiens</i>	44%
• Corn - <i>Zea mays</i>	60%



- Fragments of DNA that can insert into new chromosomal locations
- Responsible for large scale chromosome rearrangements and single-gene mutagenic events

# Transposable elements are silenced by DNA methylation

## New genomic insertions of Transposable Elements

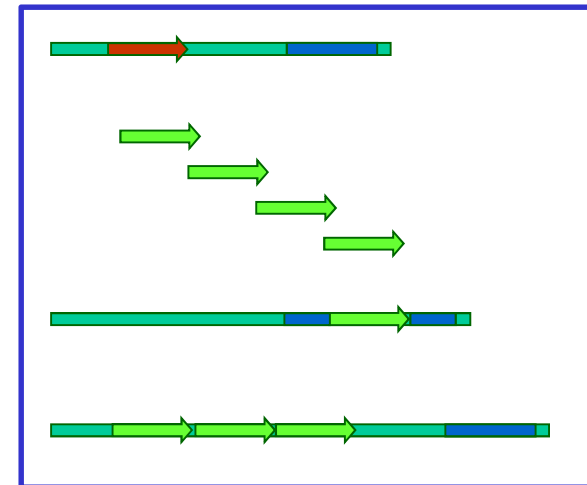


In plants, DNA methylation levels are generally higher at TEs as compared to genic regions. Silencing TEs is required to maintain genome stability

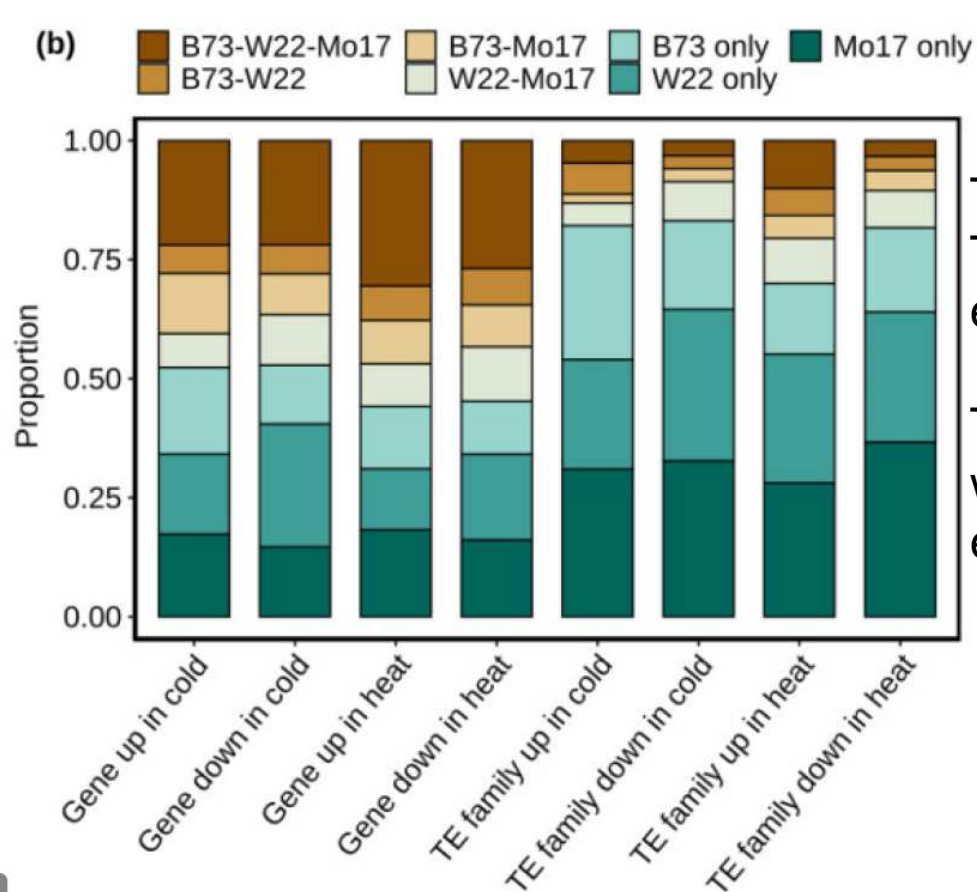
Transposable elements are major components of most genomes (more than 40% of the human genome)

May have the capacity to change location within the genome

Their motility is generally silenced by DNA methylation



## Expression changes for genes and transposable elements (TEs) under cold and heat stress conditions



Three maize (*Zea mays*) genotypes, B73, Mo17, and W22, were studied

There is substantial variation for which TE families exhibit stress-responsive expression in the different genotypes

The focus was on a subset of families where it was possible to monitor the expression of individual elements.

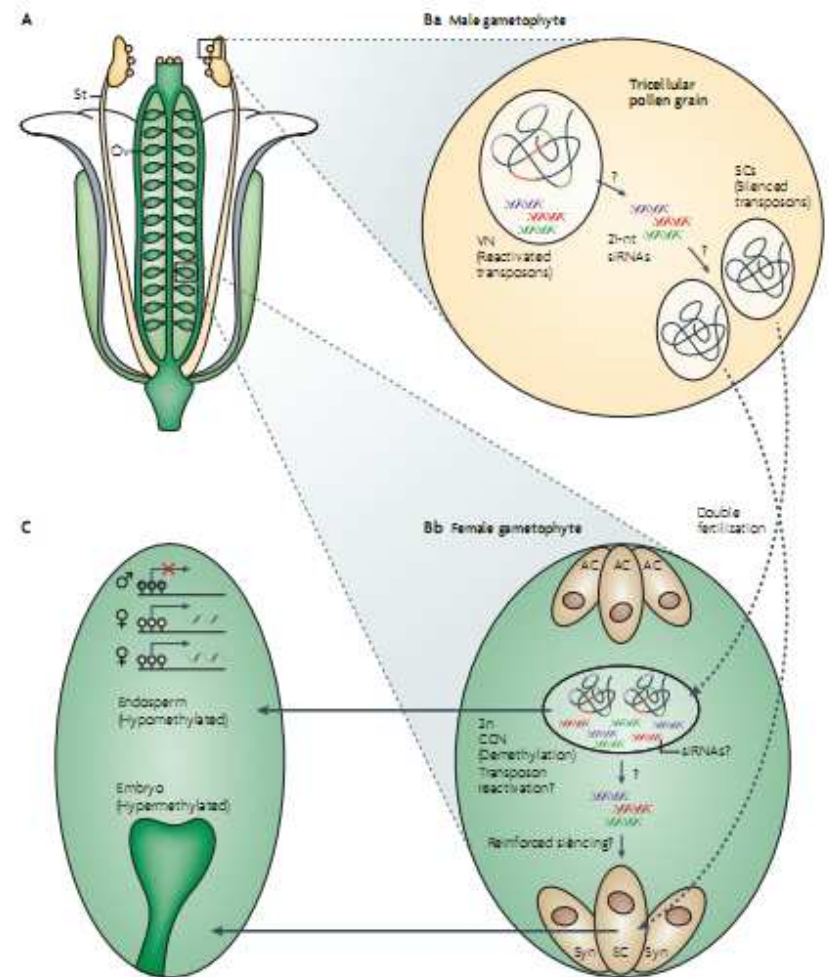
The elements that contained small regions lacking DNA methylation regions showed enriched expression while fully methylated elements were rarely expressed in control or stress conditions.

## Evidence that mobile RNAs are important transmitters of information between plant cells extends to the male and female gametophytes

In pollen, there is evidence that small RNAs produced by the vegetative cell can direct silencing in the two sperm cells

Derepression of transposable elements in the vegetative cell allows for the biogenesis of siRNAs that then move to the sperm cells to reinforce the silencing of the corresponding transposons.

In this way, epigenetic programming in sperm cells, in which transposition would result in frequent deleterious mutations, is guaranteed via the activity of the vegetative cell, which does not contribute genomic information to the next generation



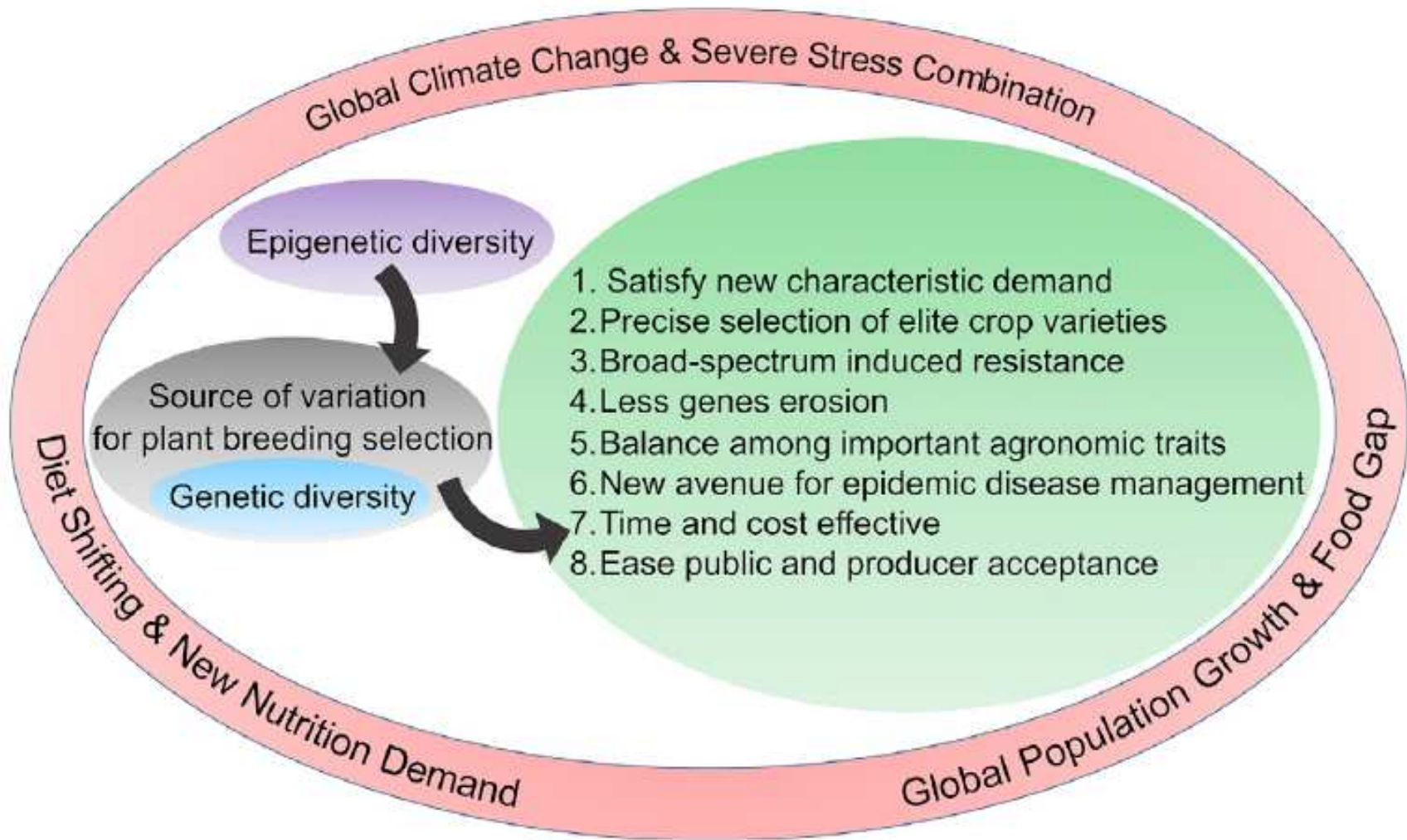
## **Conclusions**

The rapidly increasing global crop demand due to world population growth and changes in diet, combined with the challenges imposed on crop production due to global climate change are highlighting the urgency for improving both crop yield and quality.

Epigenetics can provide novel directions to drive plant breeding strategies and is rapidly gaining its place among modern crop breeding approaches.



# Epigenetic Diversity Contribution to Plant Breeding Schemes





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Agradeço a atenção dispensada  
com votos de desafiante estudos