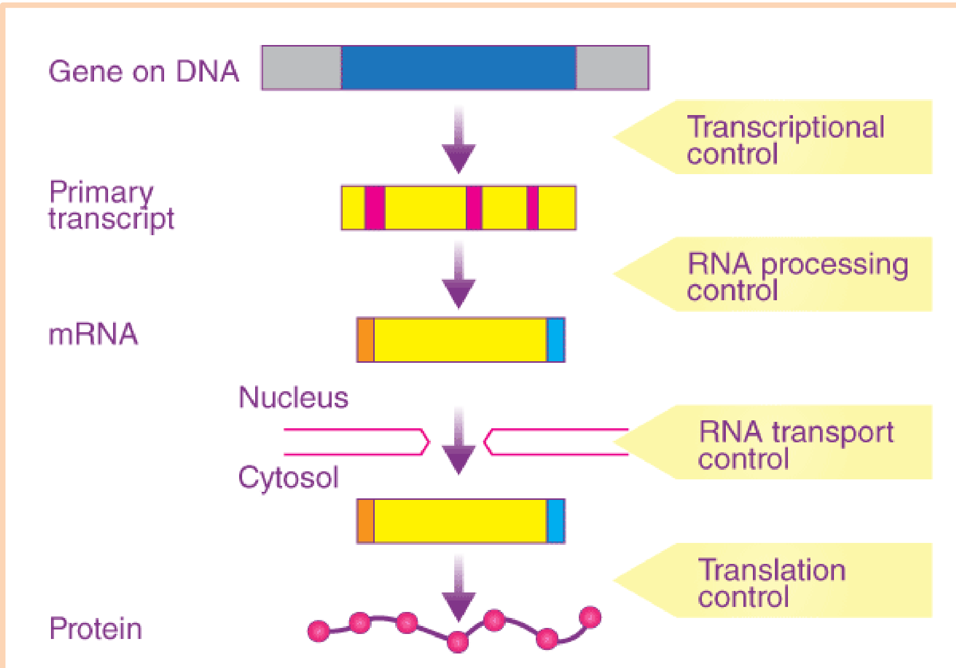
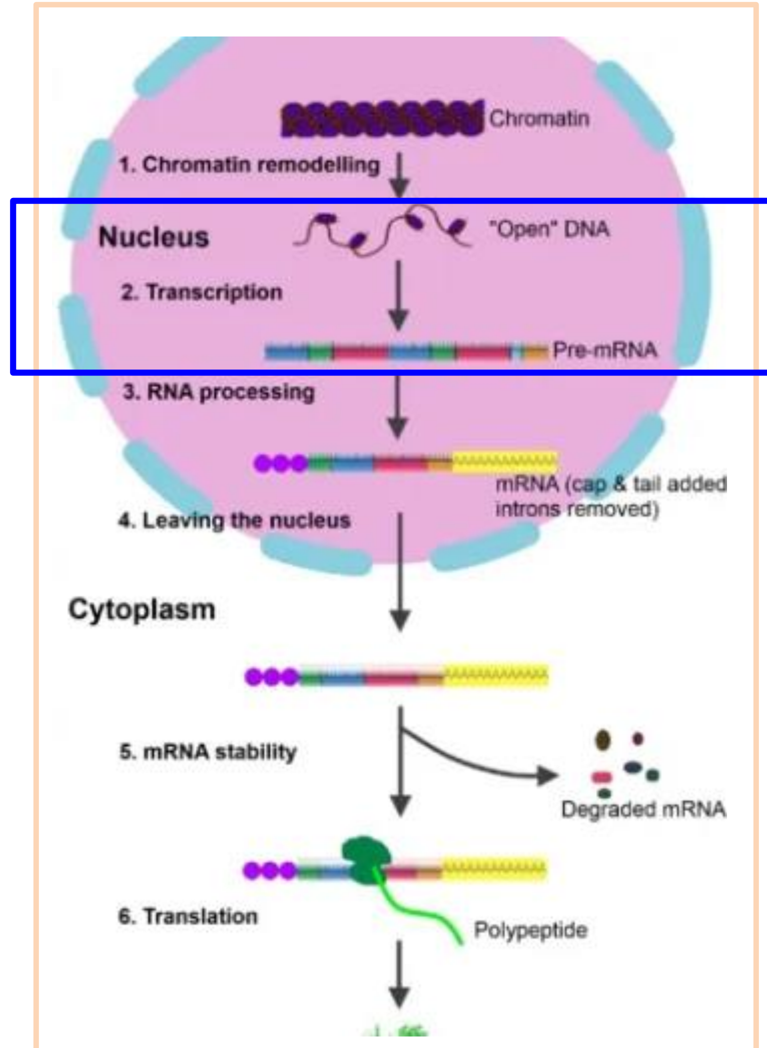
Promoters

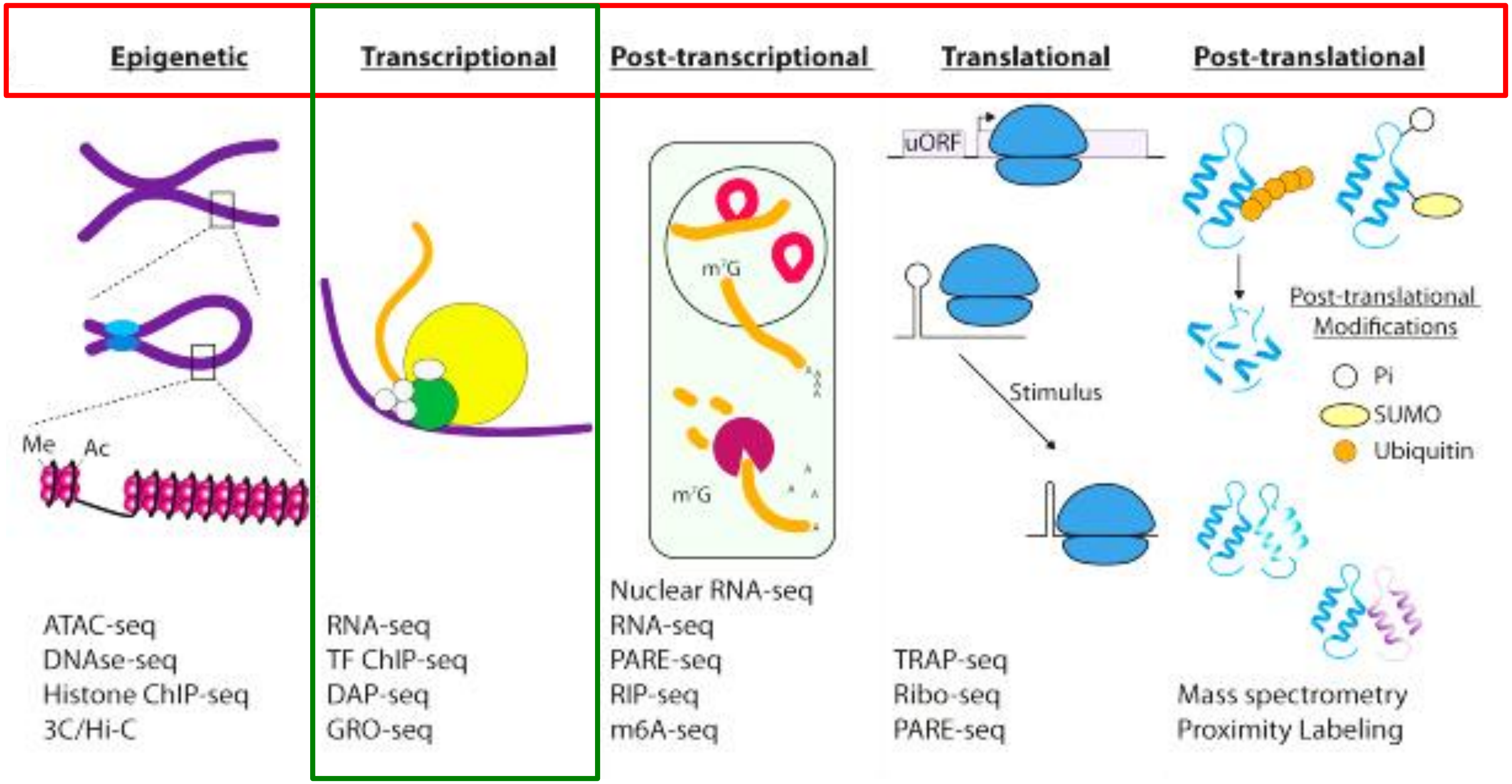
Regulates Plant Gene Expression

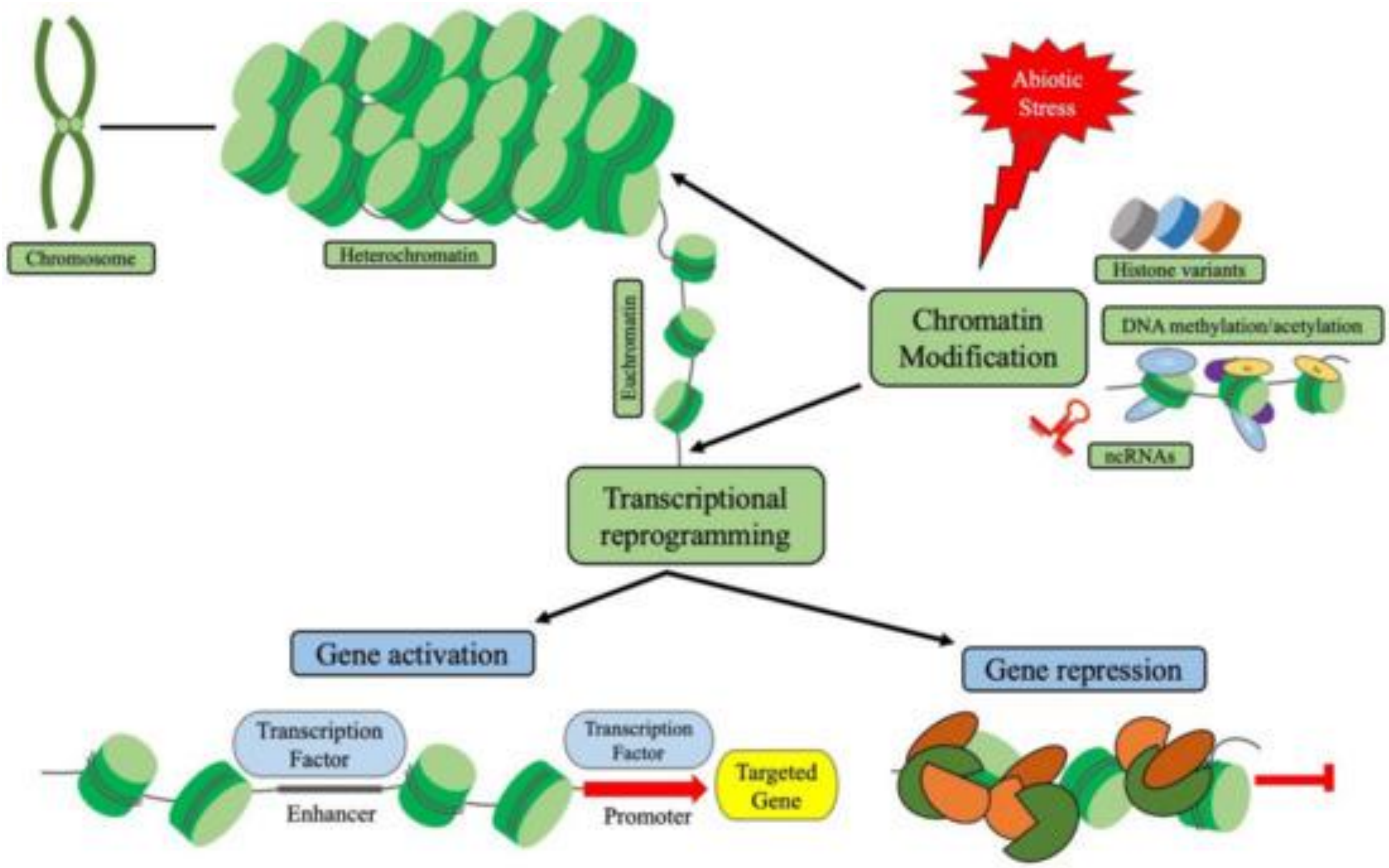
Gene expression controlled at Many Levels





Gene expression controlled at Many Levels





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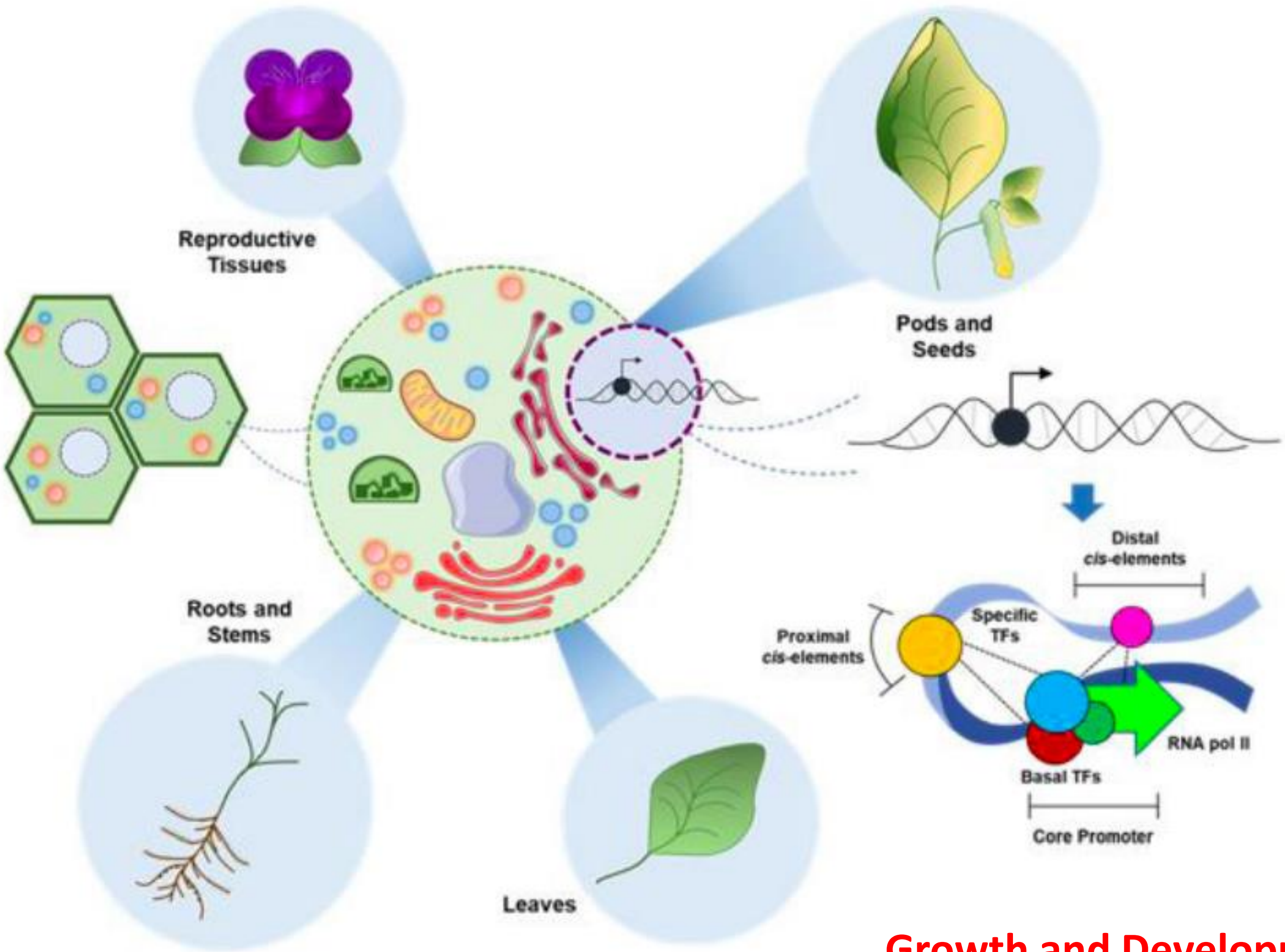
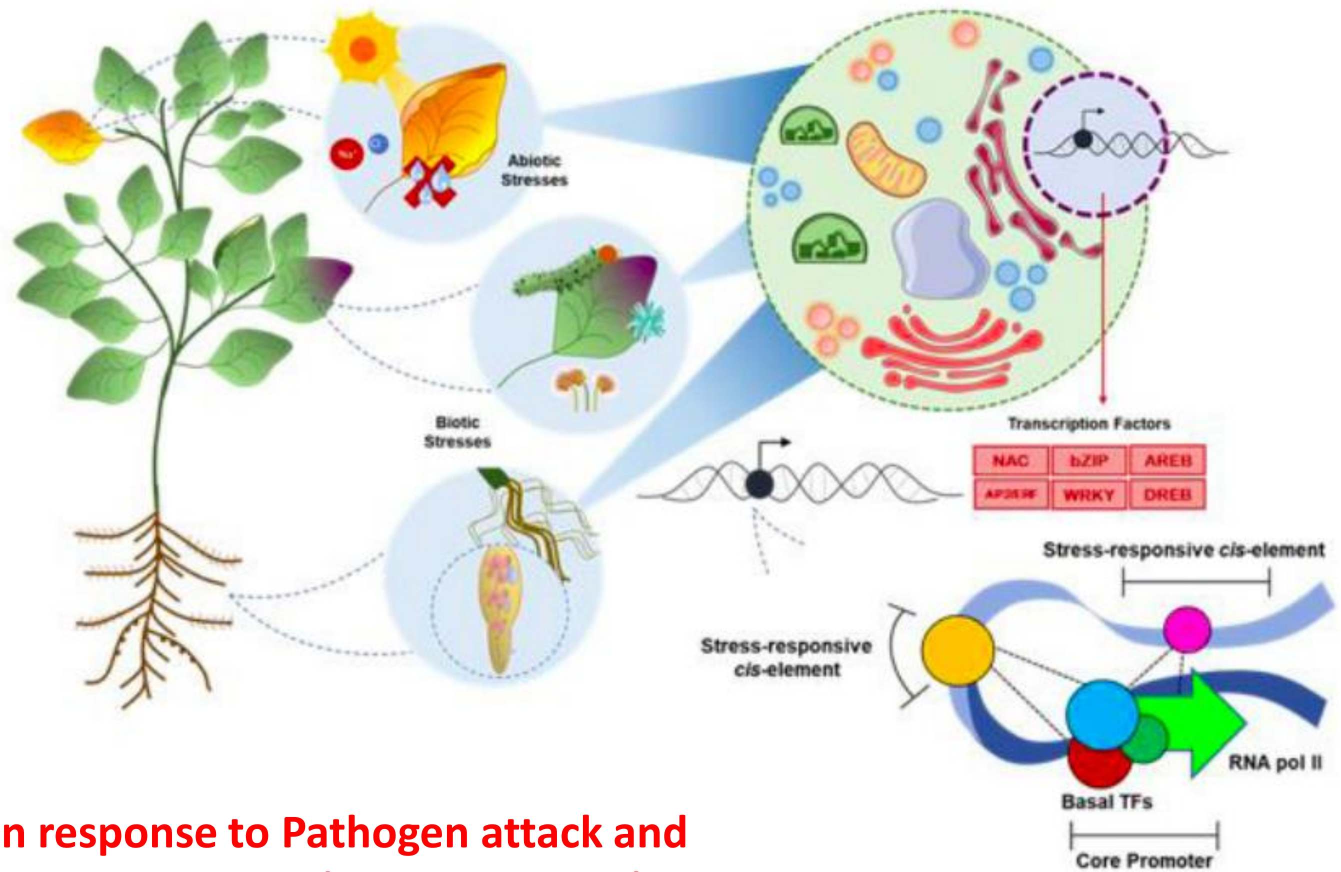
Gene Expression in Plants



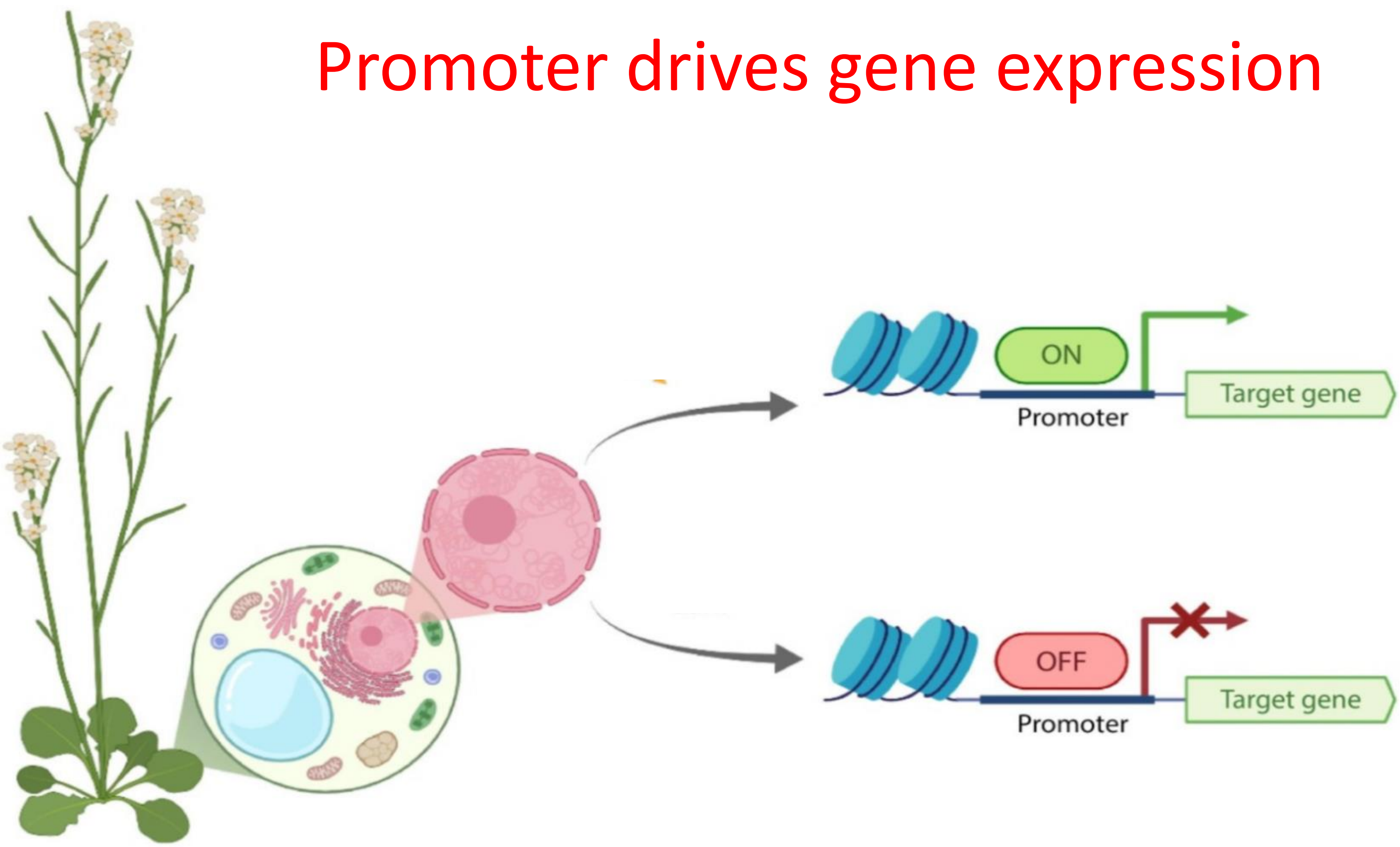
Growth and Development

Gene expression in Plants

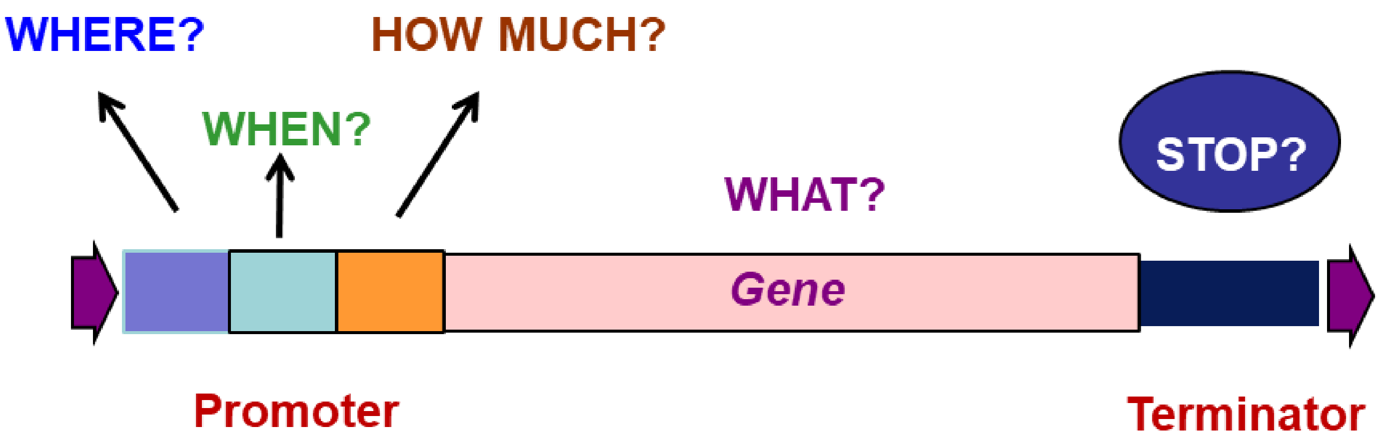


In response to Pathogen attack and External signal (climate change), Nutrition alteration

Promoter drives gene expression

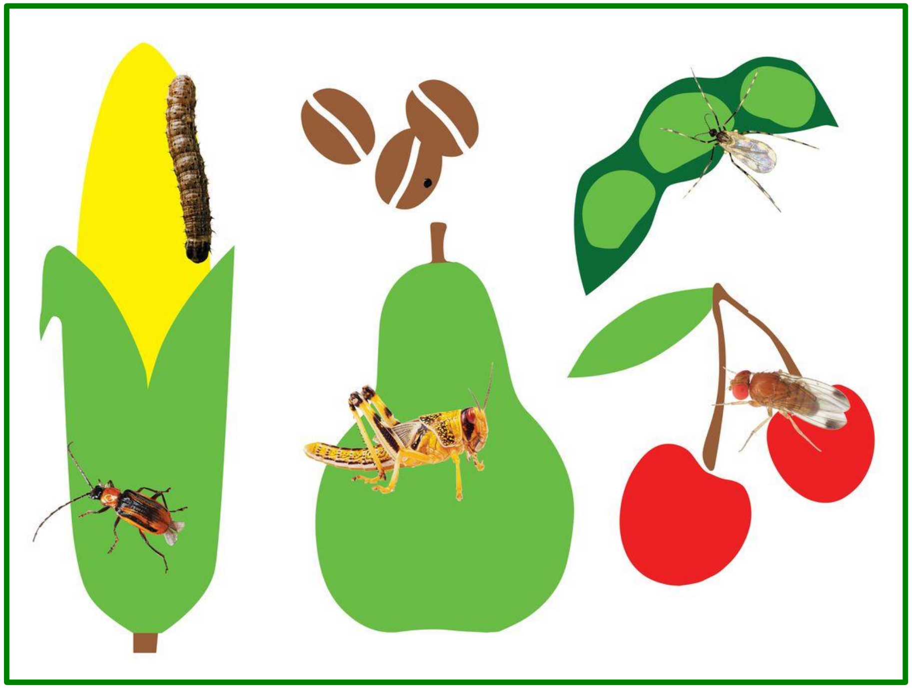


Promoter decides the strength and specificity of gene expression



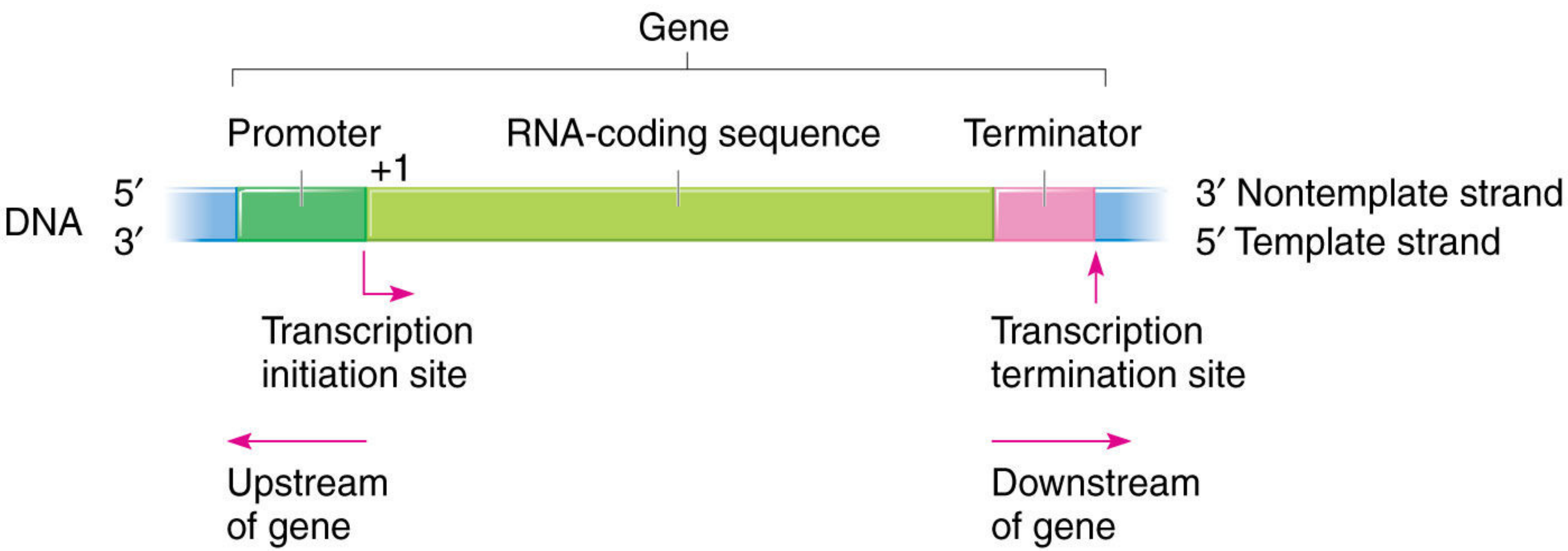
Promoter regulates **level (strength)** and **pattern i.e, the timing (temporal) and tissue type (spatial)** of gene expression

Plant must express Stress Responsive Genes in <u>Specific tissue</u> or under <u>Specific conditions</u>



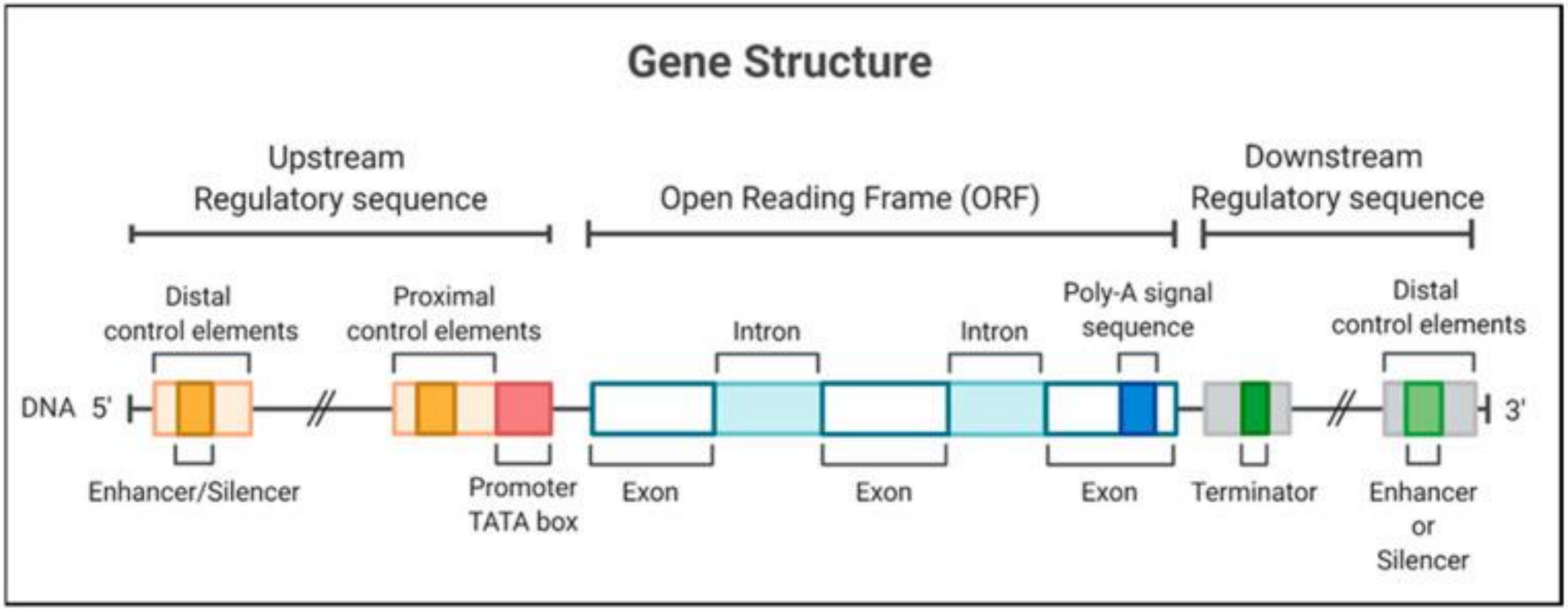
- Promoters **direct gene expression** in a **temporal**, **spatial**, and **controlled** manner
- Great diversity of promoters
- promoters are crucial for biotechnology, ensuring the correct expression of a gene

Promoters are Upstream of gene coding region



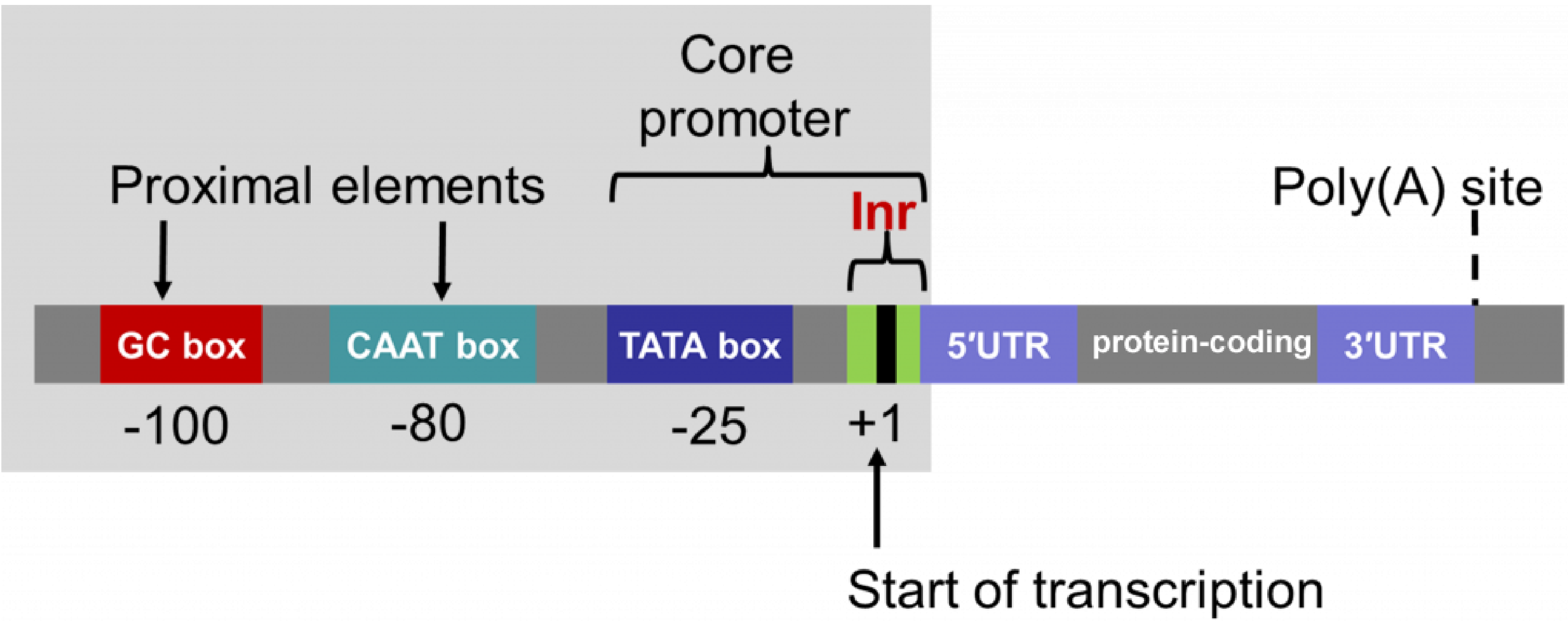
Promoters are DNA sequences located **upstream of gene coding regions** and **contain multiple *cis*-acting elements**, which are **specific binding sites for proteins** involved in the **initiation and regulation of transcription**.

Promoters contains TWO regions



Plant promoters have two regions, **CORE PROMOTER** and **DISTAL REGION** comprising enhancers and silencers. Both these regions contain cis elements, which proteins, known as transcriptional factors may bind to.

Proximal control elements or Minimal region



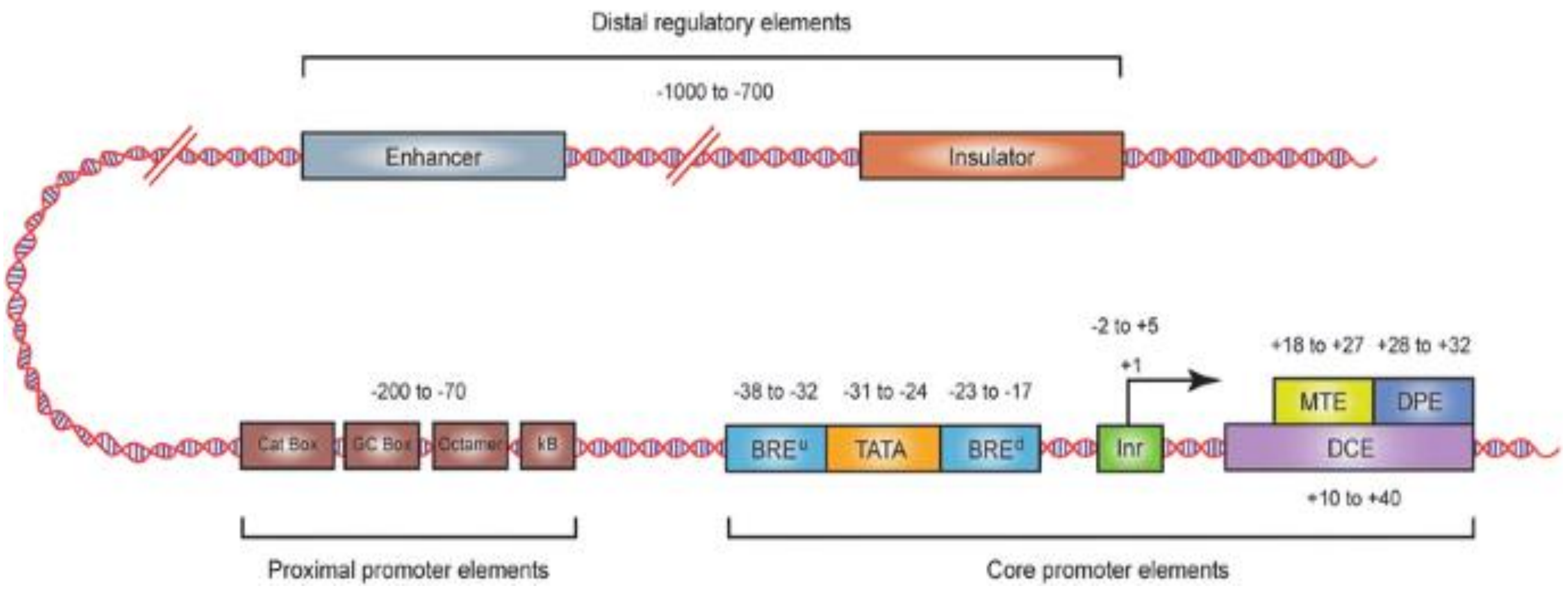
Proximal control elements responsible for the transcription induction

Proximal control elements

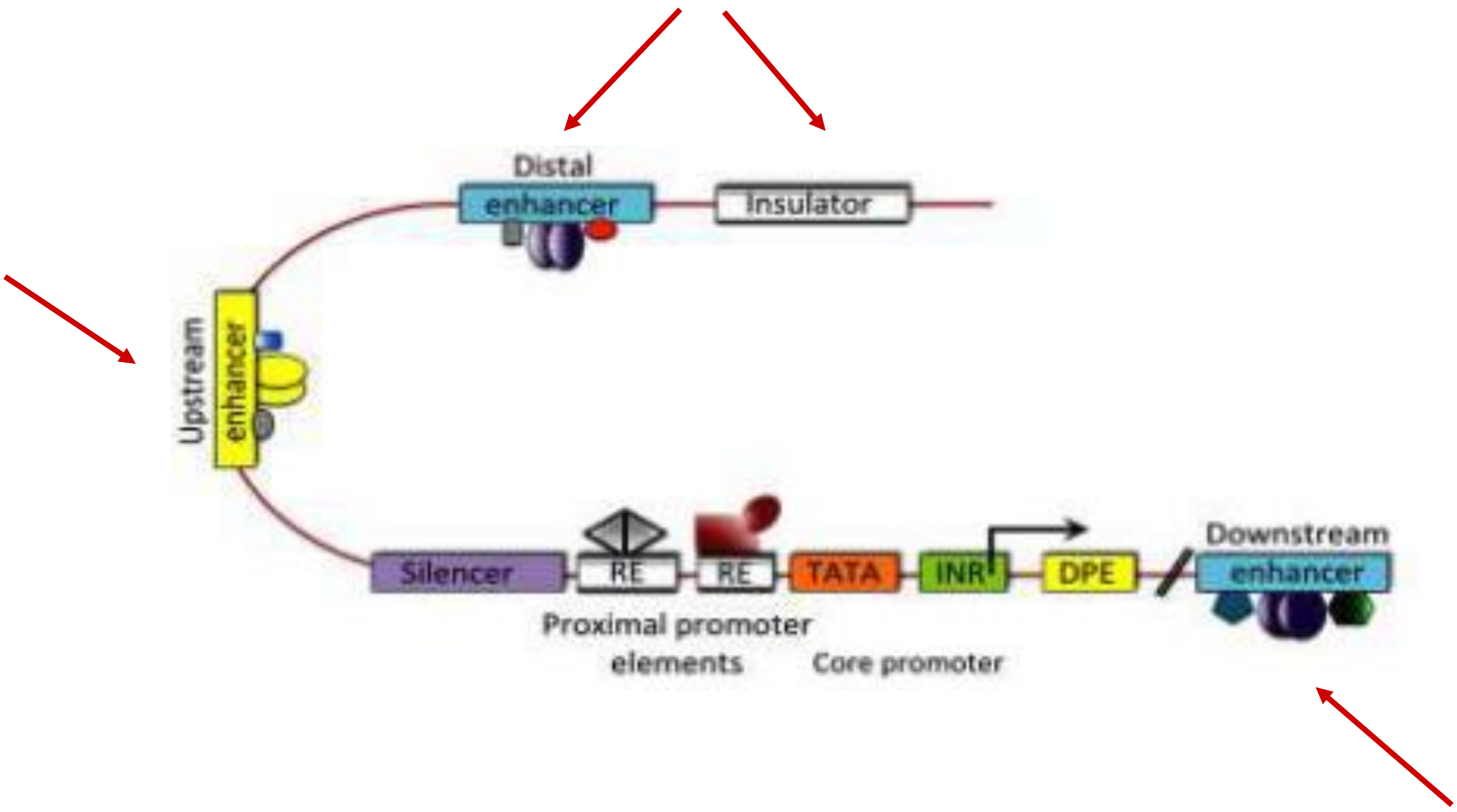
Conserved eukaryotic promoter elements	Consensus sequence
CAAT box	GGCCAATCT
TATA box	**TATAAT**
GC box	GGGCGG
CAP site	TAC

Conserved among all organisms

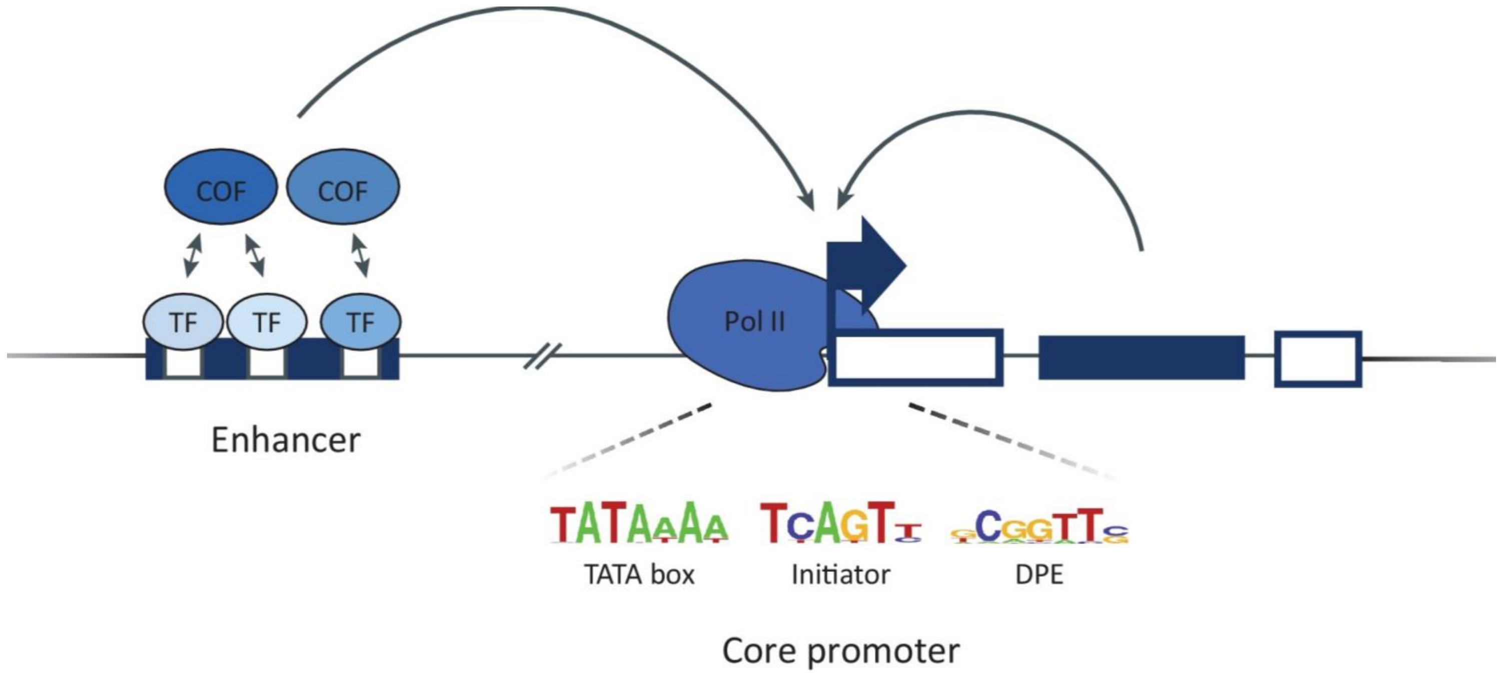
Plant promoters have two regions, **CORE PROMOTER** and **DISTAL REGION**



DISTAL REGION has **enhancer** and **insulator** elements

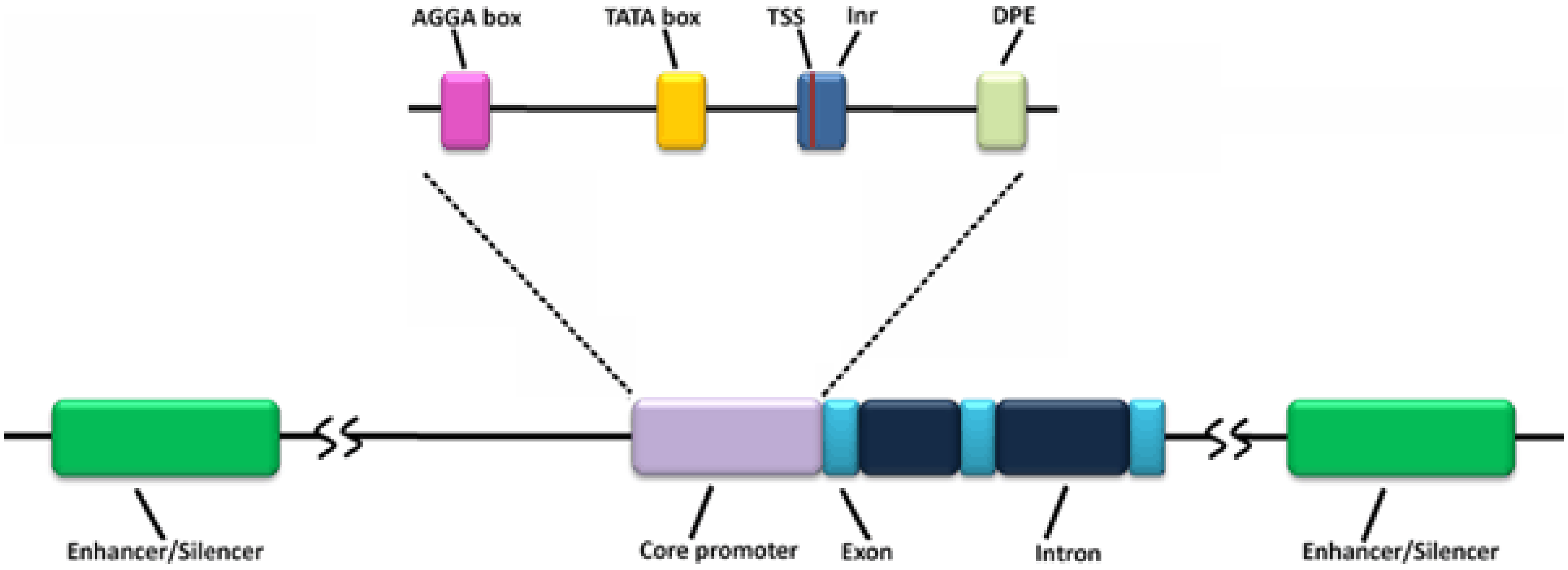


Downstream Promoter Element



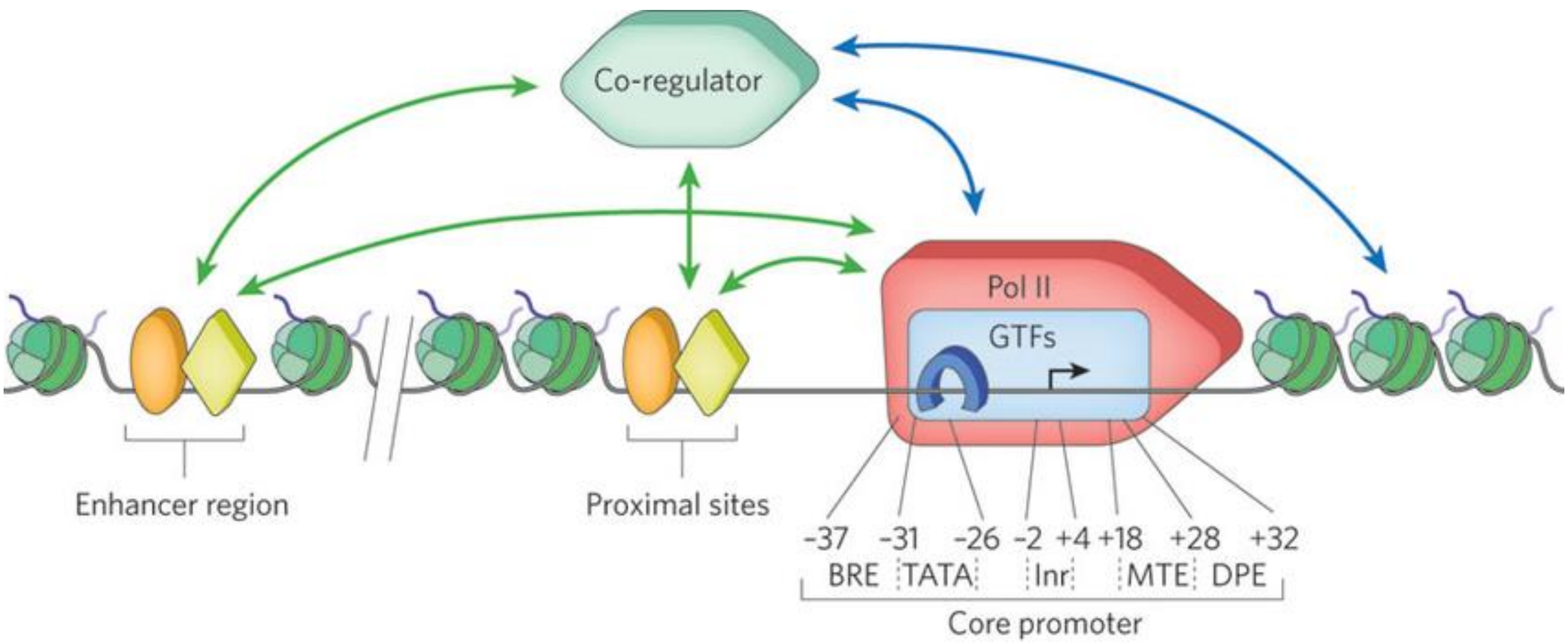
Downstream Promoter Element (DPE) are mostly found TATA less promoters

Role of Plant Enhancer Element

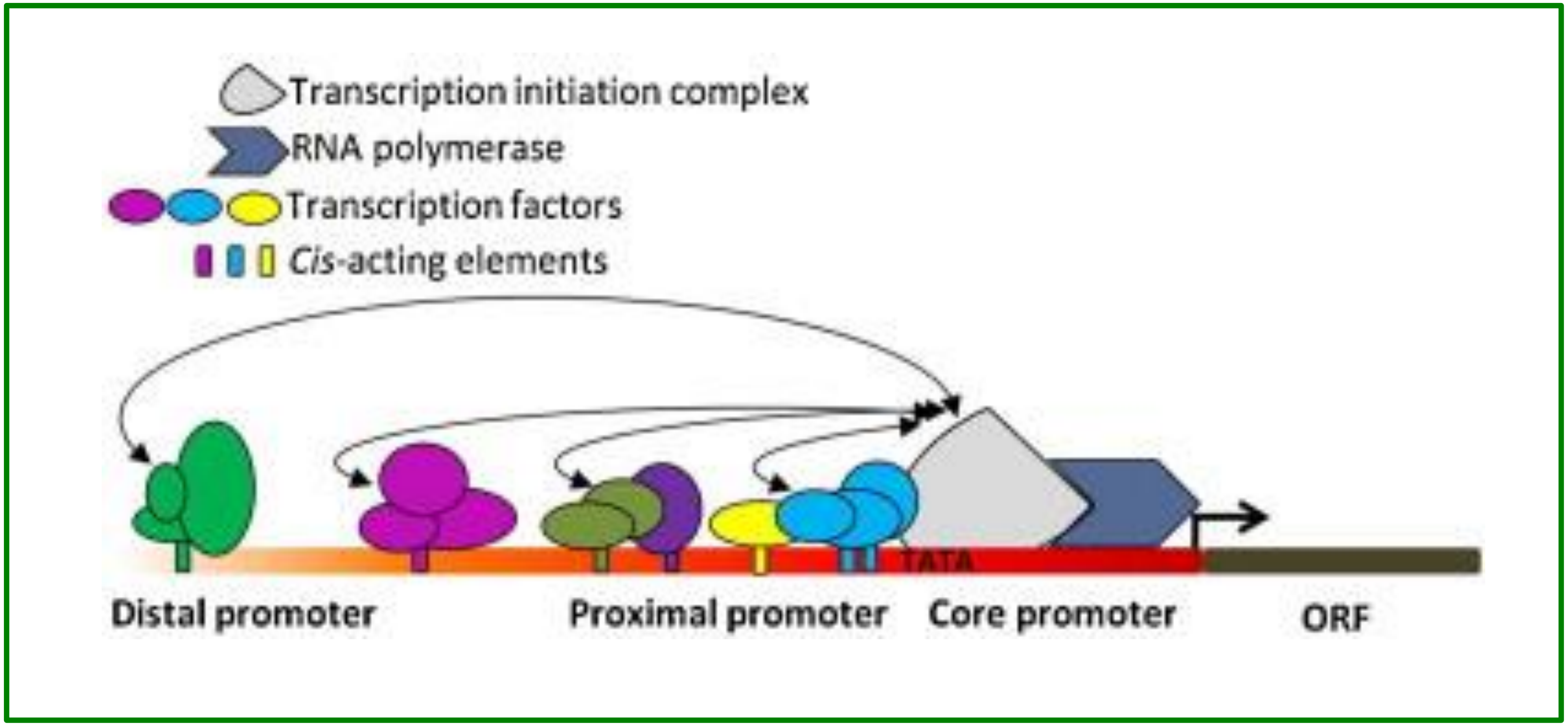


Plant enhancers are **present at distinct positions**, often at a notable distance **from downstream or upstream of the promoter sequence**. They enhance the gene expression by recruiting specific TFs.

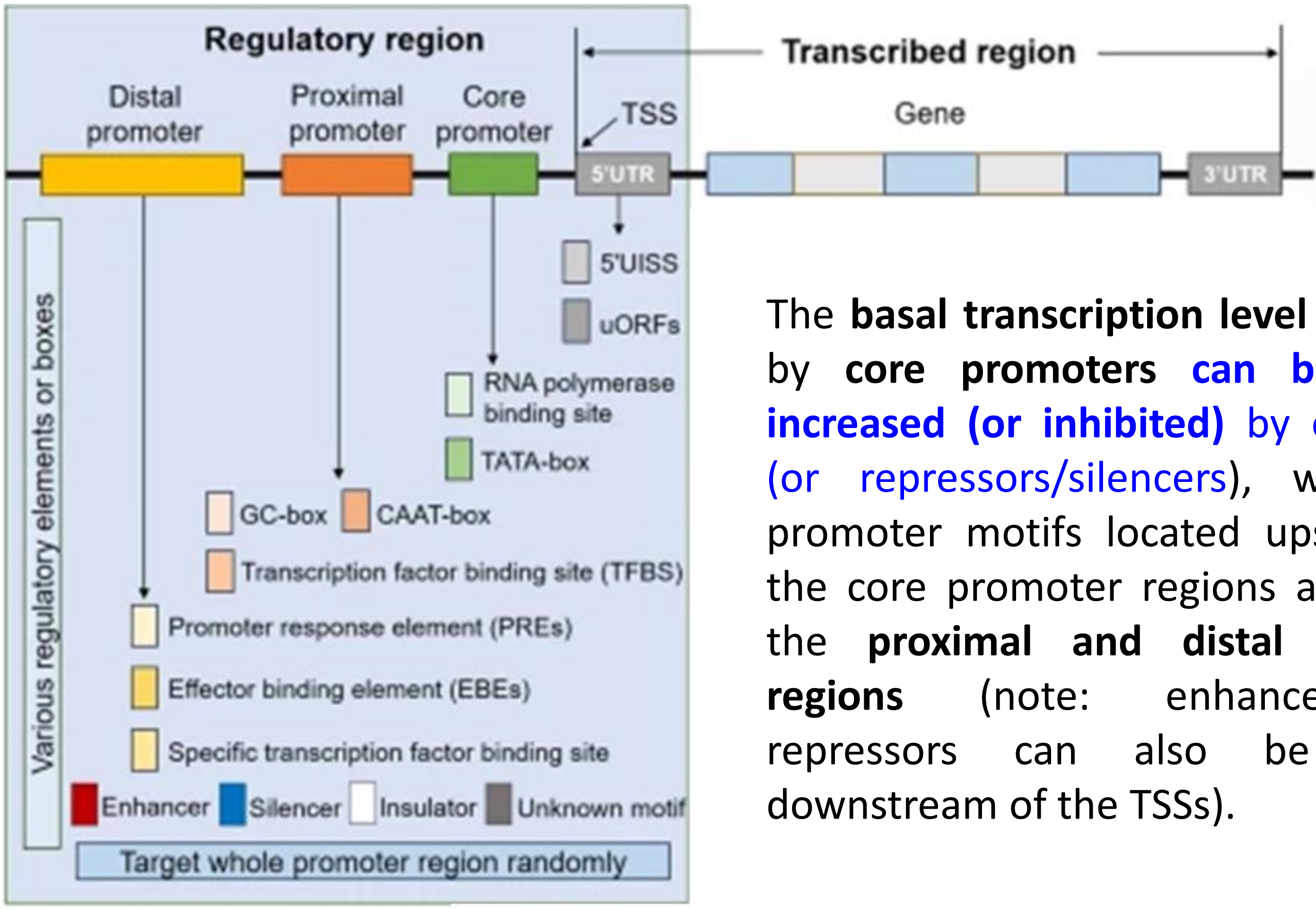
Distal promoter regions



Promoters contain multiple *cis*-acting elements, which are **specific binding sites for proteins** involved in the **Initiation and Regulation of transcription**.

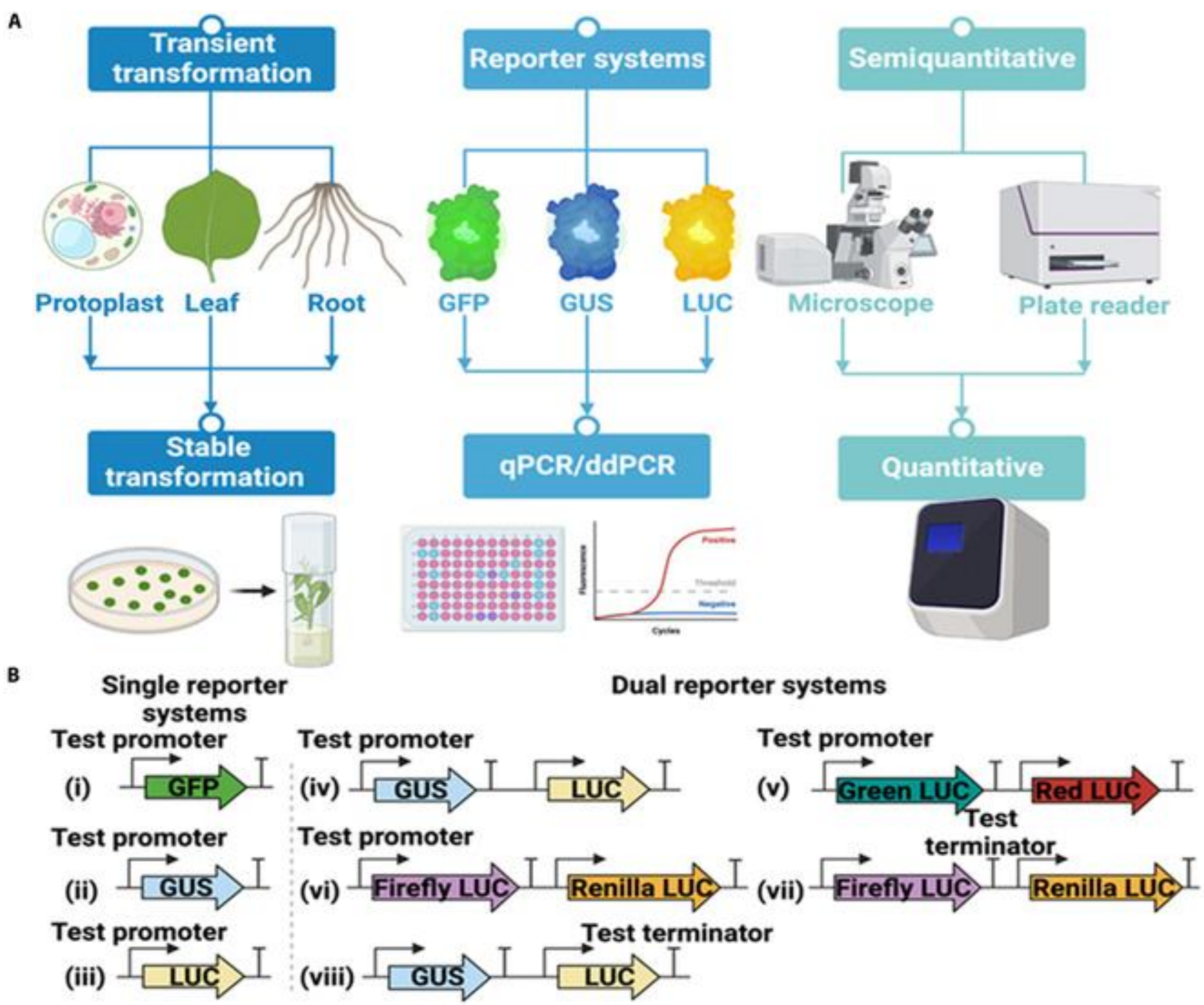


Core promoter is the minimal promoter region responsible for Basal transcription level



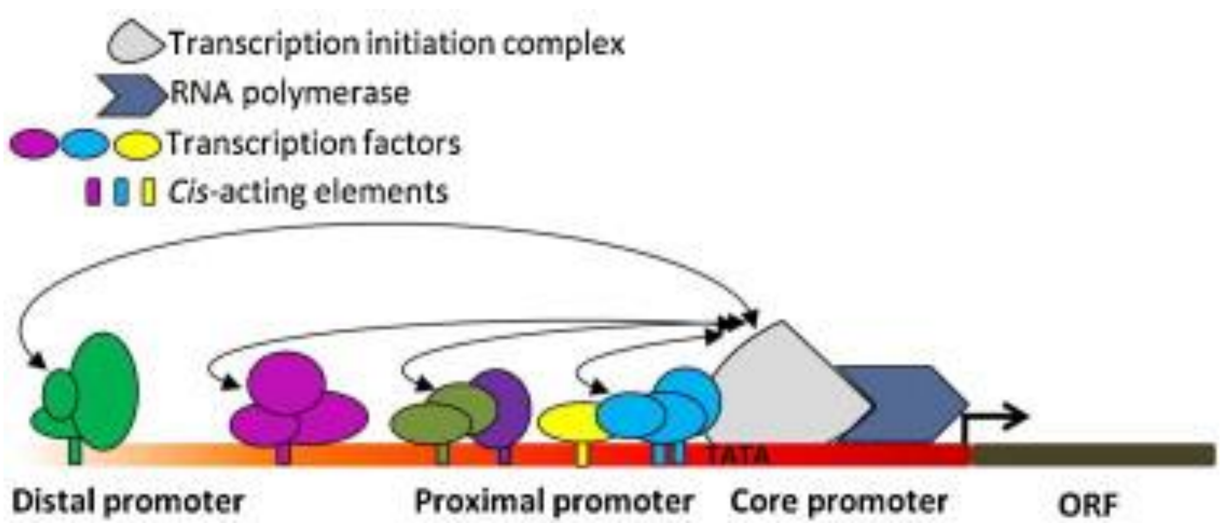
The **basal transcription level** conferred by **core promoters can be greatly increased (or inhibited)** by enhancers (or repressors/silencers), which are promoter motifs located upstream of the core promoter regions and define the **proximal and distal promoter regions** (note: enhancers and repressors can also be located downstream of the TSSs).

Detailed Examination of Promoter Elements

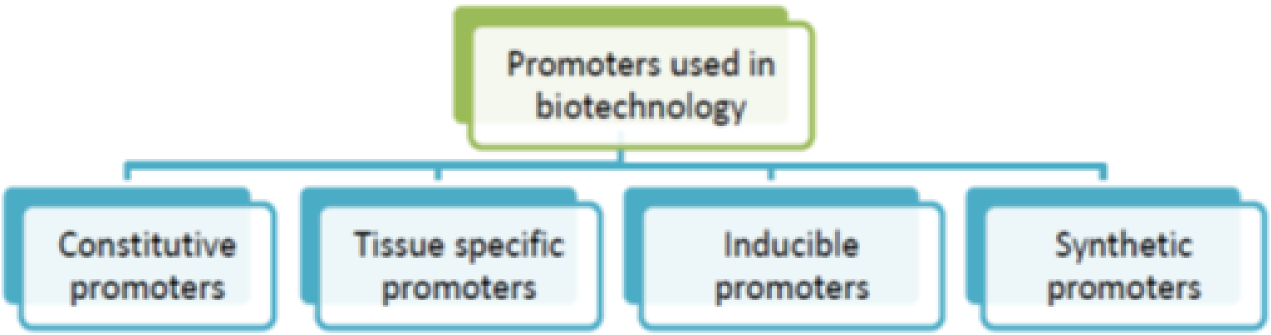


Cis elements in Promoter that decide strength and specificity

***Cis*-regulatory elements interact bidirectionally** and <u>in tandem with</u> TFs, cofactors, and chromatin-remodeling complexes to determine the **strength and the temporal and spatial expression patterns** of a gene during plant growth and development and in response to changing environmental conditions.

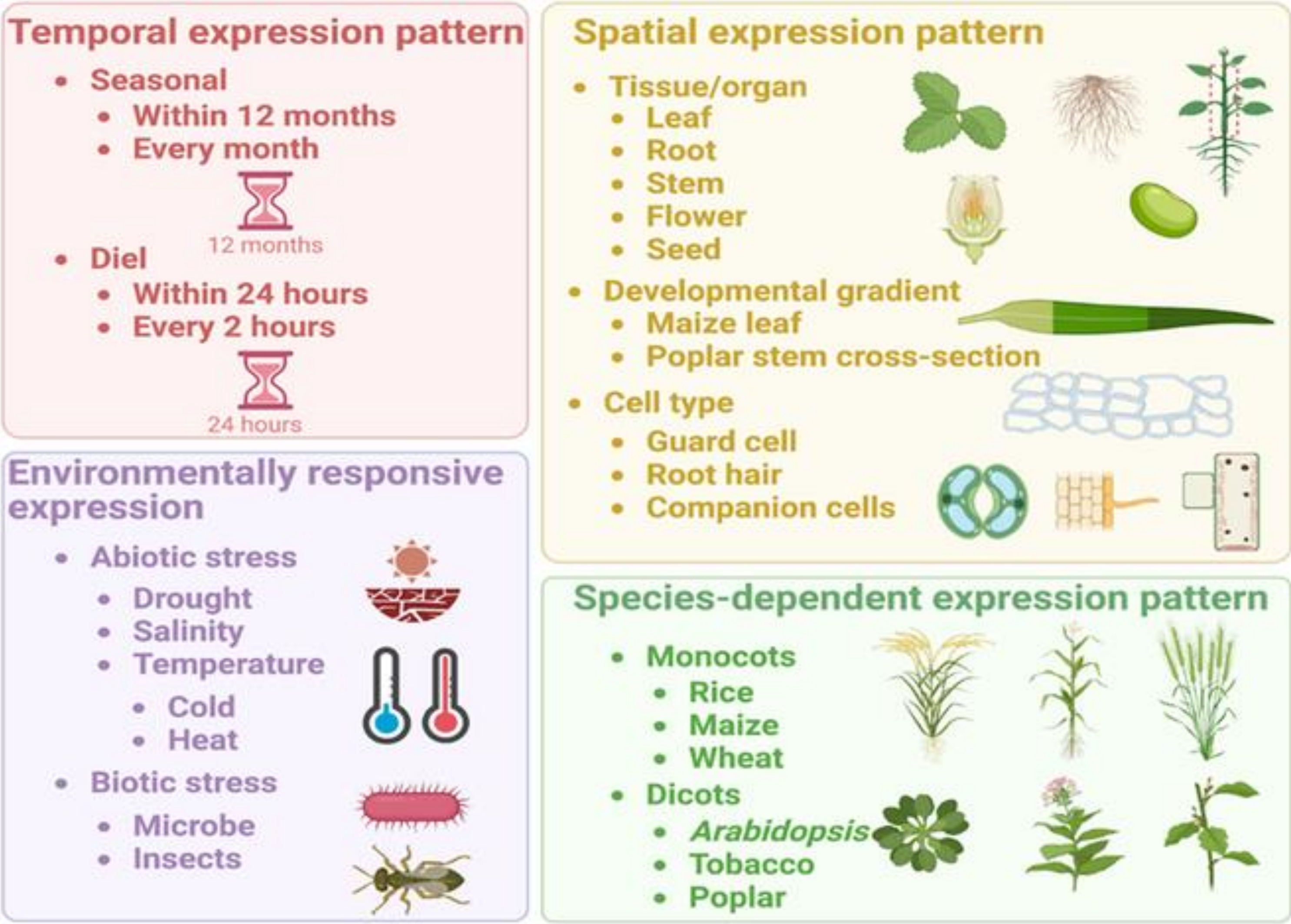


Choice of Plant Promoter



to confer constitutive, spatial and/or temporal transgene expression, is one of the key determinants used in plant biotechnology applications

Gene expression can be **constant or can show temporal or spatial regulation pattern**



Temporal expression pattern
Seasonal
Within 12 months
Every month
12 months
Diel
Within 24 hours
Every 2 hours
24 hours
Spatial expression pattern
Tissue/organ
Leaf
Root
Stem
Flower
Seed
Developmental gradient
Maize leaf
Poplar stem cross-section
Cell type
Guard cell
Root hair
Companion cells
Environmentally responsive expression
Abiotic stress
Drought
Salinity
Temperature
Cold
Heat
Biotic stress
Microbe
Insects
Species-dependent expression pattern
Monocots
Rice
Maize
Wheat
Dicots
Arabidopsis
Tobacco
Poplar

Constitutive Promoter

Constitutive expression of reporter gene (GUS) in Plants

widely expressed in plant tissues and organs, not regulated by specific conditions or **specific transcription factors**

Constitutive plant promoters

Constitutive promoter have **transcriptional regulatory regions** widely expressed in plant tissues and organs, **not regulated by specific conditions** or **specific transcription factors**, universally applied in transgene expression in plants.

Viral promoters, such as cauliflower mosaic virus (pCaMV35S), peanut chlorotic streak virus (pPC1SV), and figwort mosaic virus (pFMV) promoters are the most widely used constitutive promoters.

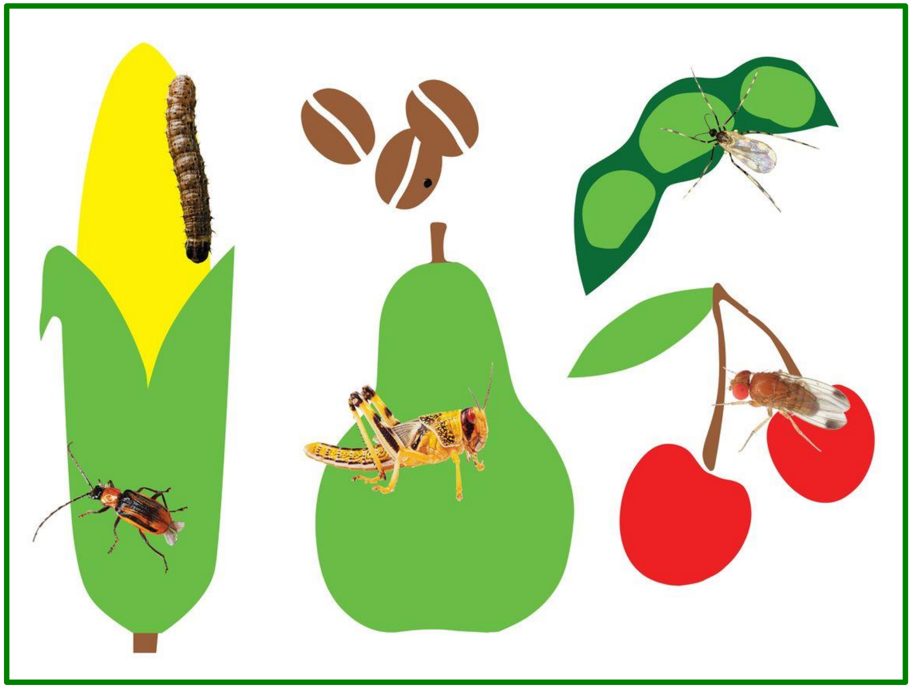
Despite its strong quasi-universal gene expression capacity, **pCaMV35S** has become the **most widely used constitutive viral promoter** in transgenic plants. However, **(1)** its activity is generally low in reproductive tissues, prompting a demand for plant tissue-specific promoters towards the expression of genes whose phenotypical effect is relevant in flower buds, anthers, pollen, and related tissues. In addition, **(2)** excessively high transcript levels generated under viral promoter's control can interplay some pleiotropic effects on transgenic plants. Furthermore, **(3)** simultaneous expression of different transgenes under the control of the same promoter usually triggers post-transcriptional gene silencing mechanisms (Freitas et al., 2019).

Tissue Specific Promoters

Tissue-specific promoter **directs the gene expression** in a **particular tissue** (such as in roots, seeds, or the vascular system), or **only certain cell types**.

Use of a tissue-specific promoter in the expression cassette **can restrict unwanted transgene expression** as well as facilitate persistent transgene expression.

Expression of Stress Responsive Genes in Specific tissue or under Specific conditions is very important



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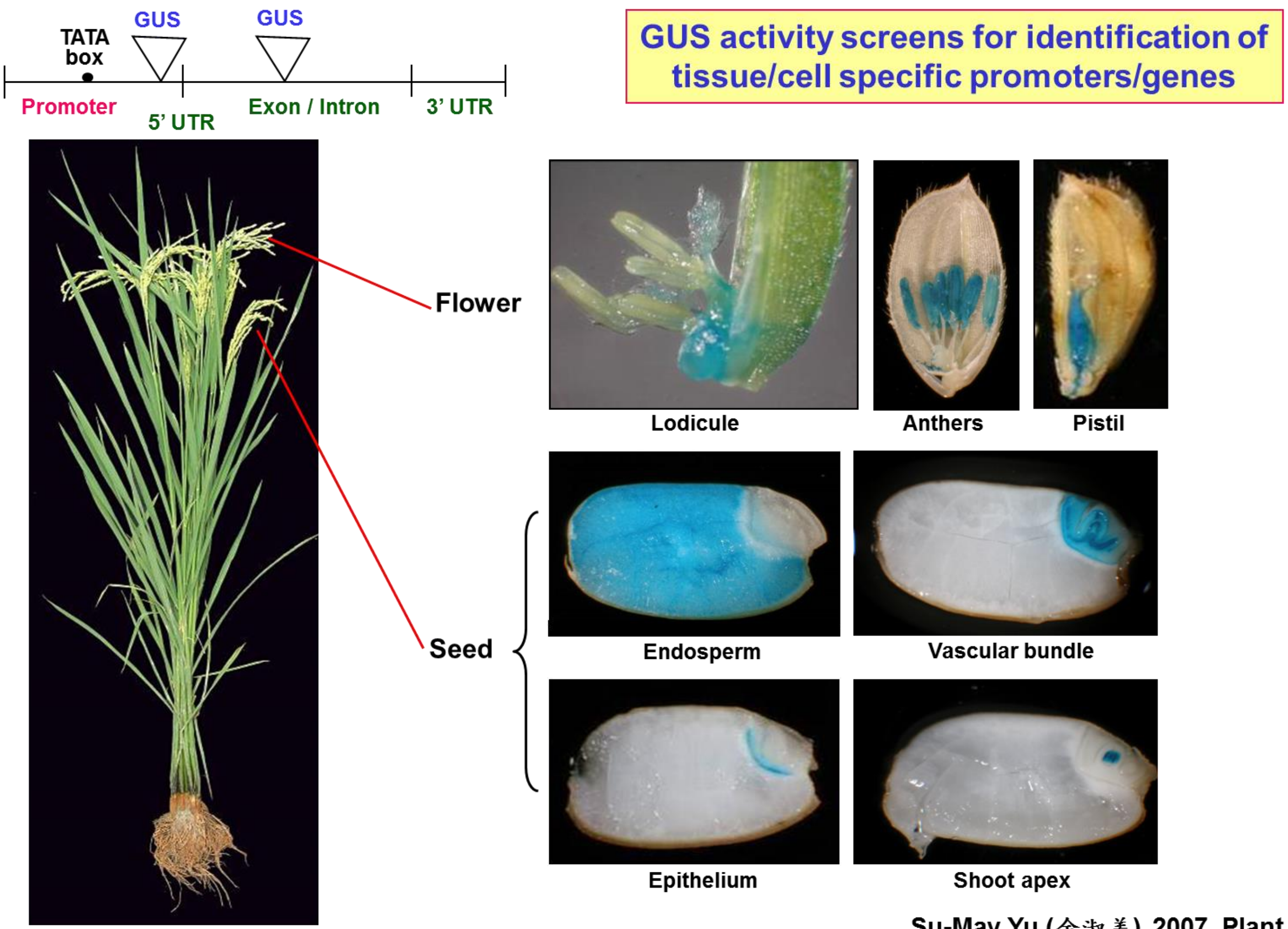
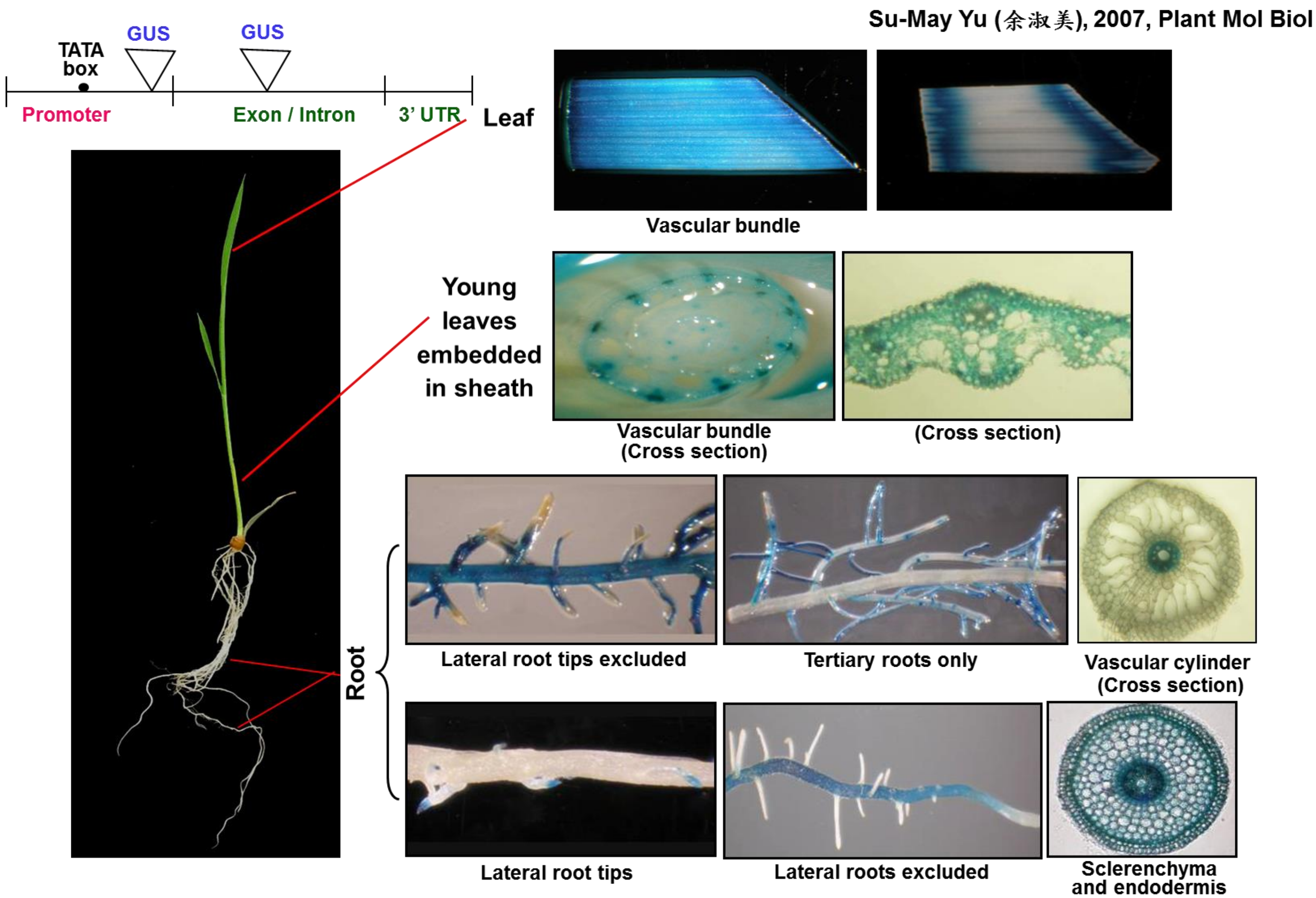
Tissue specific Promoters



Su-May Yu (余淑美), 2007, Plant Mol Biol

Seed-endosperm specific expression of GUS when driven by wheat glutein promoter

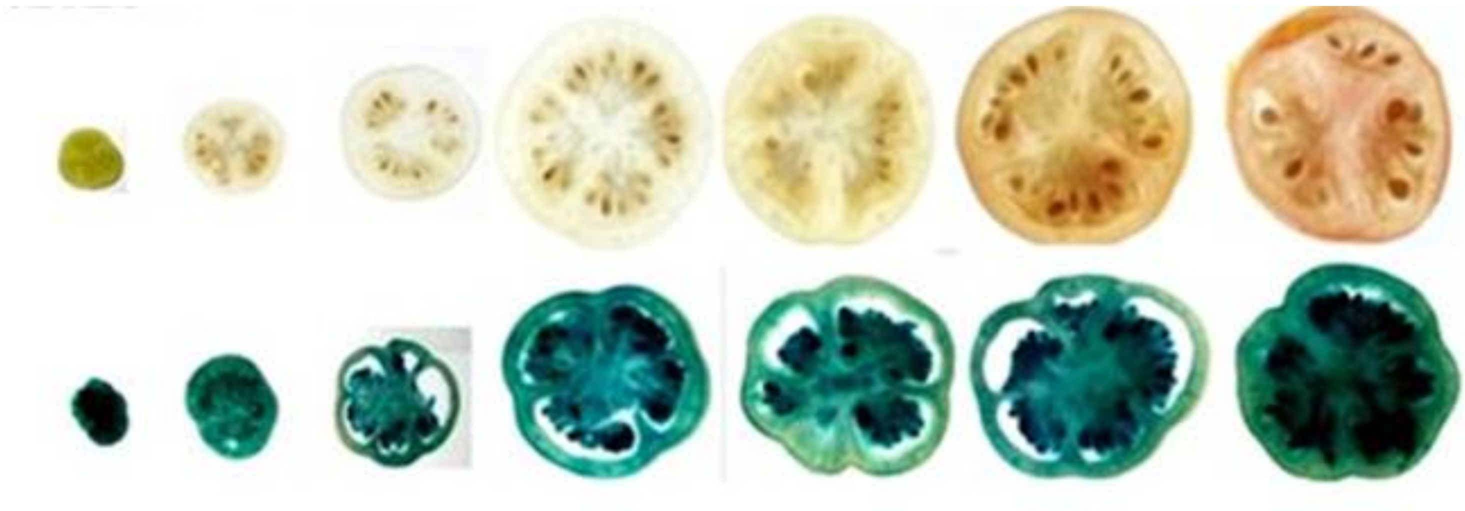
Tissue specific Promoters



GUS expression in seed driven by seed specific β-phaseolin promoter

β-Conglycinin promoter from soy, and α-globulin promoter from cotton are further seed-specific promoters with the peak activity during the middle and late phase of seed maturation. β-phaseolin promoter from bean is another promoter of described activity. β-1,3-glucanase promoter from peas (*PsGNS2*) is an interesting example of seed-specific construct, as it provides transgene expression in the seed coat.

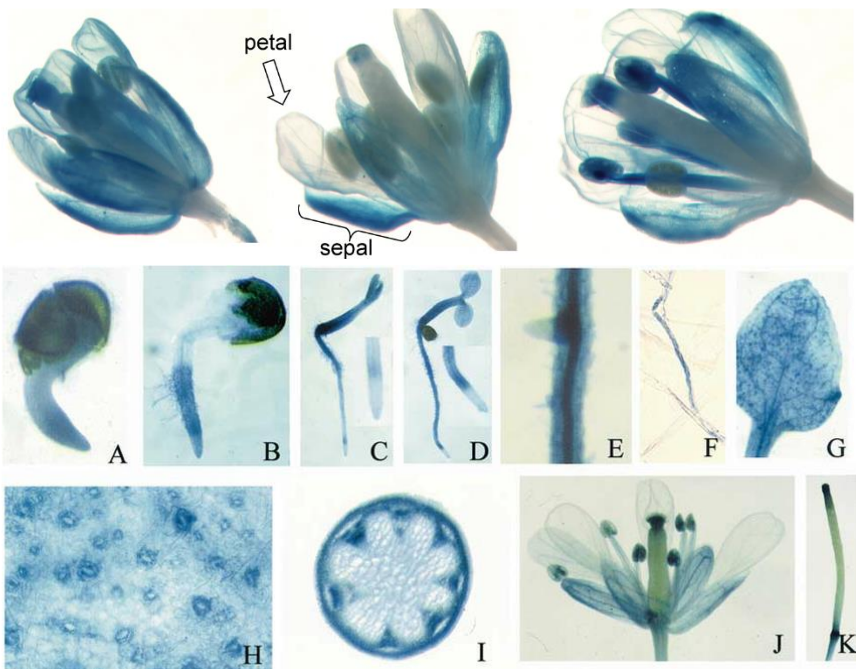
Tissue specific Promoters

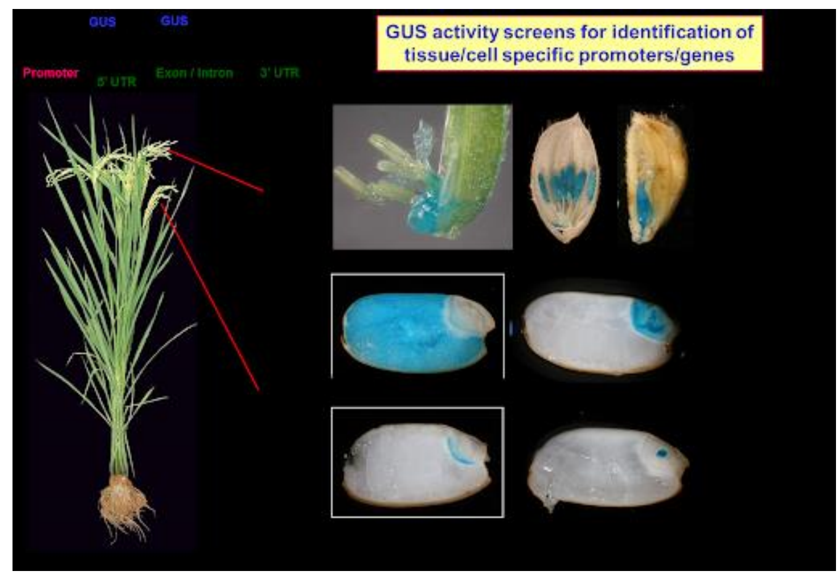


GUS expression in Tomato fruits driven by tomato fruit specific E8 promoter

E8 promoter, isolated from tomato, alike other promoters regulating fruit maturation genes, provides given type of expression.. Further important application of fruit- specific promoters is related to maintainin***E8*** **is used for antigen production in fruits and improving the typical tomato aroma and flavour**g their freshness after harvest.

Tissue specific promoters





ETH zürich

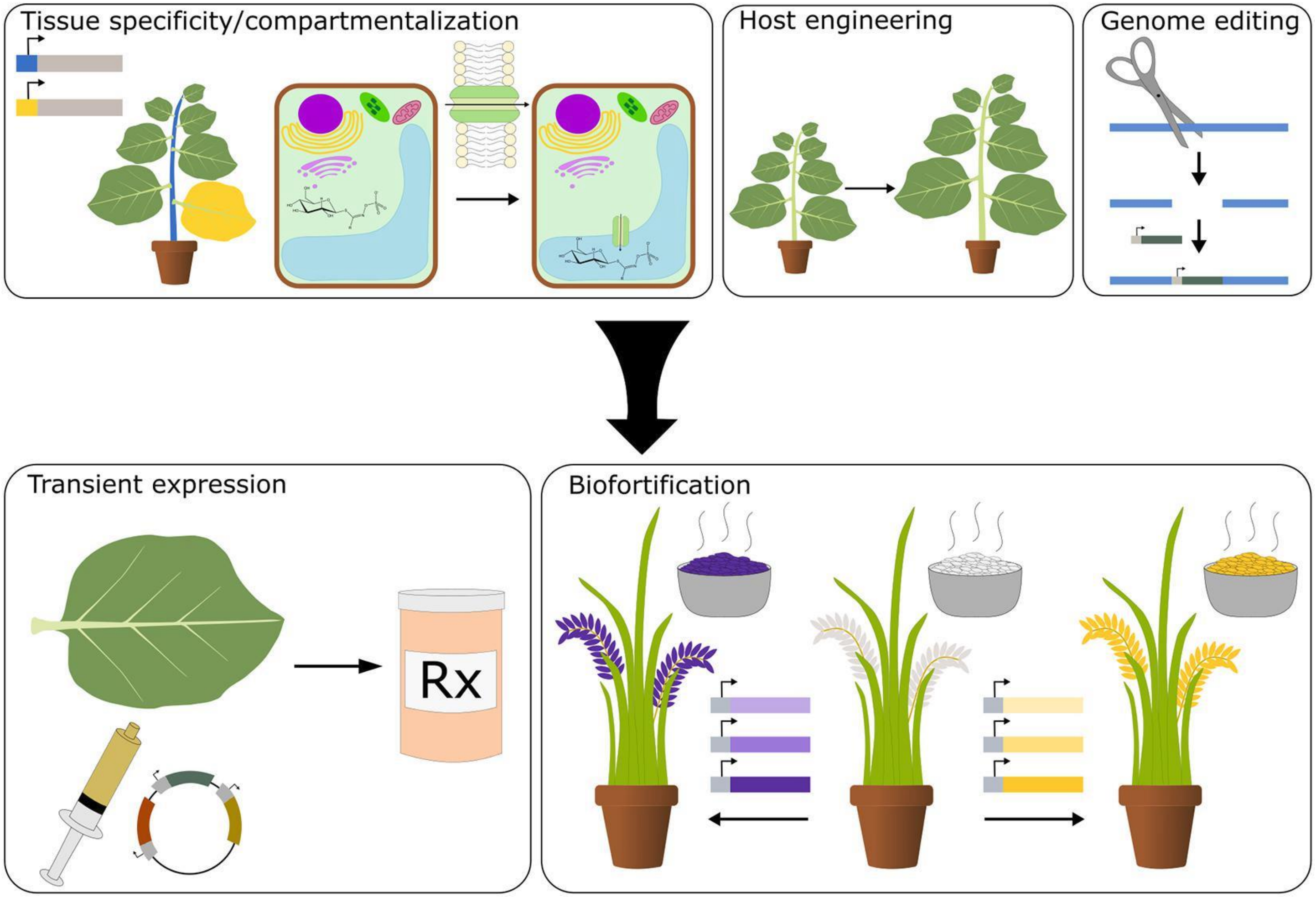
Department of Biology

Institute of Molecular Plant Biology

Tissue-specific promoter library for cassava

Generating a tissue-specific promoter library for cassava.
Images show transgenic cassava with different promoters driving a *GUS* reporter gene (expression stained in blue).

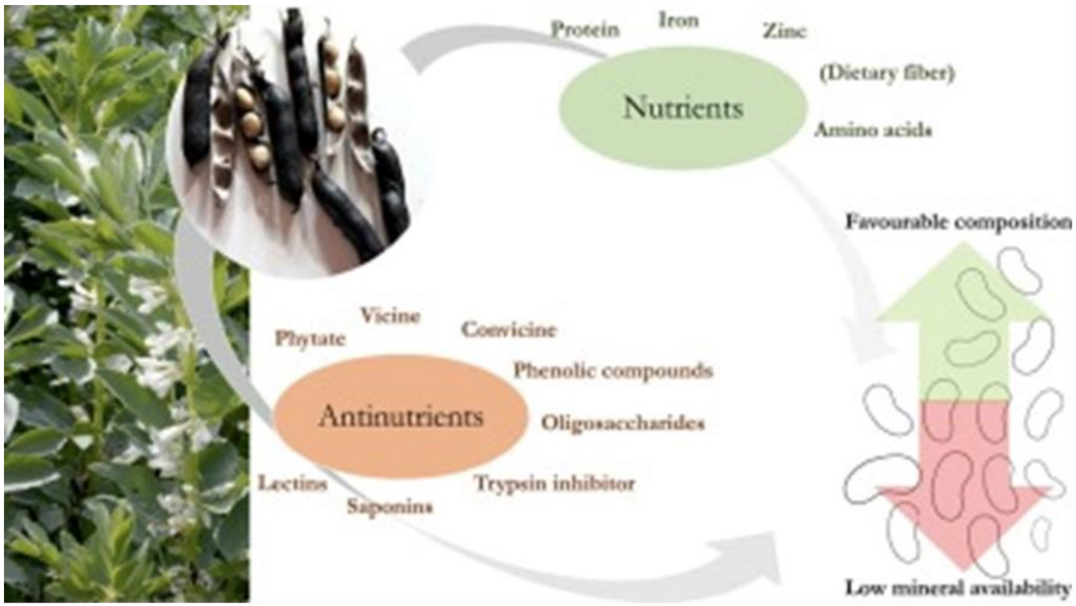
Transcriptional regulation underlying tissue specificity of gene expression



Seed specific Promoter to arrest Storage Pests

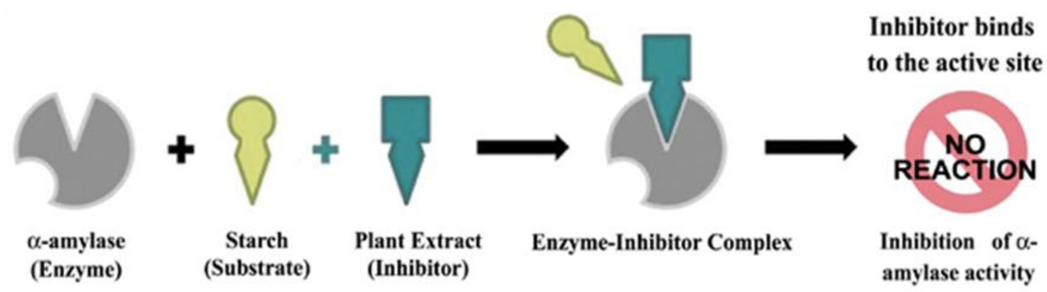
Seed-specific promoters drive gene expression specifically in seed tissues, enabling researchers to **engineer plants with desired traits in seeds**, such as improved yield, quality, or storage characteristics or **<u>seed protection</u>**

Plant Proteins that interfere with insect survival



Protease inhibitor families in plants:

1. Soybean trypsin inhibitor (Kunitz) family
2. Bowman-Birk inhibitor family
3. Barley Trypsin inhibitor family
4. Potato Inhibitor I family
5. Potato Inhibitor II family
6. Squash Inhibitor family
7. Ragi 1-2/Maize bifunctional inhibitor family
8. Carboxypeptidase A. B inhibitor family
9. Cysteine proteinase inhibitor family (cystatins)
10. Aspartyl proteinase inhibitor family



Mechanism of α-amylase inhibition by plant extract.

Class of Proteases:

(1)Serine
(2)Threonine
(3)Cysteine
(4)Aspartic
(5)Metallo

Seed specific Promoter to arrest Storage Pests

Examples of Seed-Specific Promoters:

Phaseolin (phas)-promoter: Derived from genes encoding 7S globulins from Phaseolus vulgaris.

Legumin B4 (leB4)-promoter: Derived from genes encoding 11S globulins from Vicia faba.

usp-promoter: From non-storage protein genes of V. faba.

sbp-promoter: From non-storage protein genes of V. faba.

pBSU1, pAt5g10120, pAt5g54000: Arabidopsis seed-specific promoters.

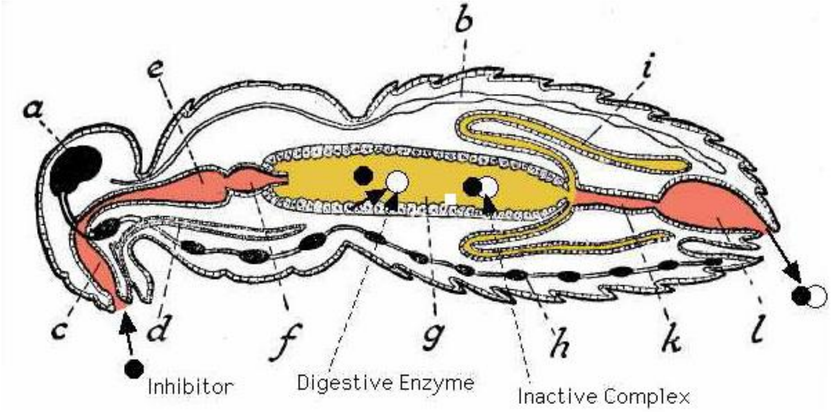
MUM4 0.3Pro: A seed coat-specific promoter fragment.

AtTT10 and AtDP1: Arabidopsis promoters driving seed-coat specific gene expression in pennycress and camelina.

PROLAM26, RAL2, RAL4, and CAPIP: Rice promoters from putative seed-specifically expressed genes

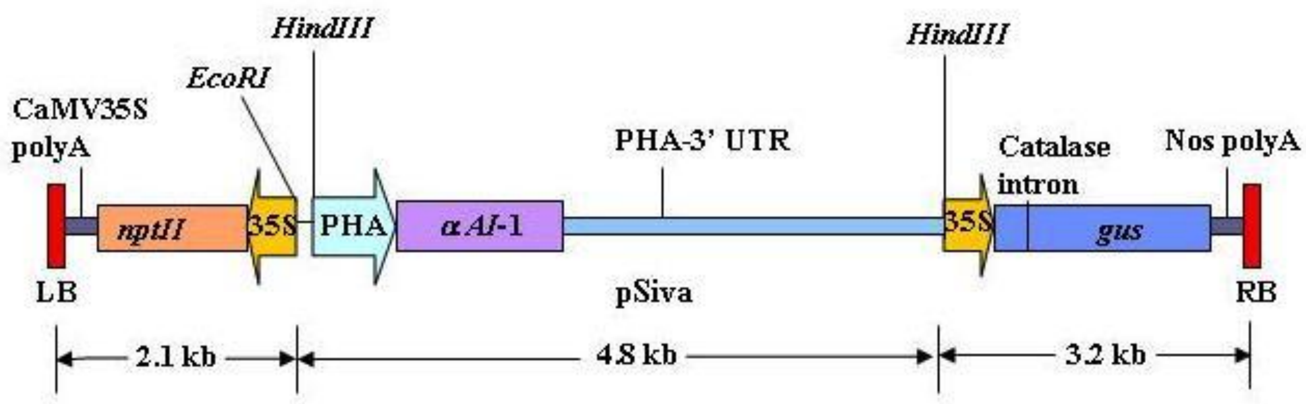
Common Bean Seeds: Source of α–amylase inhibitor

Mechanism of anti-metabolic action of α–amylase inhibitor



- Carbohydrate metabolism is targeted
- Leads to starvation and death of pests

Common bean α–amylase inhibitor gene expressed under a **seed specific promoter**

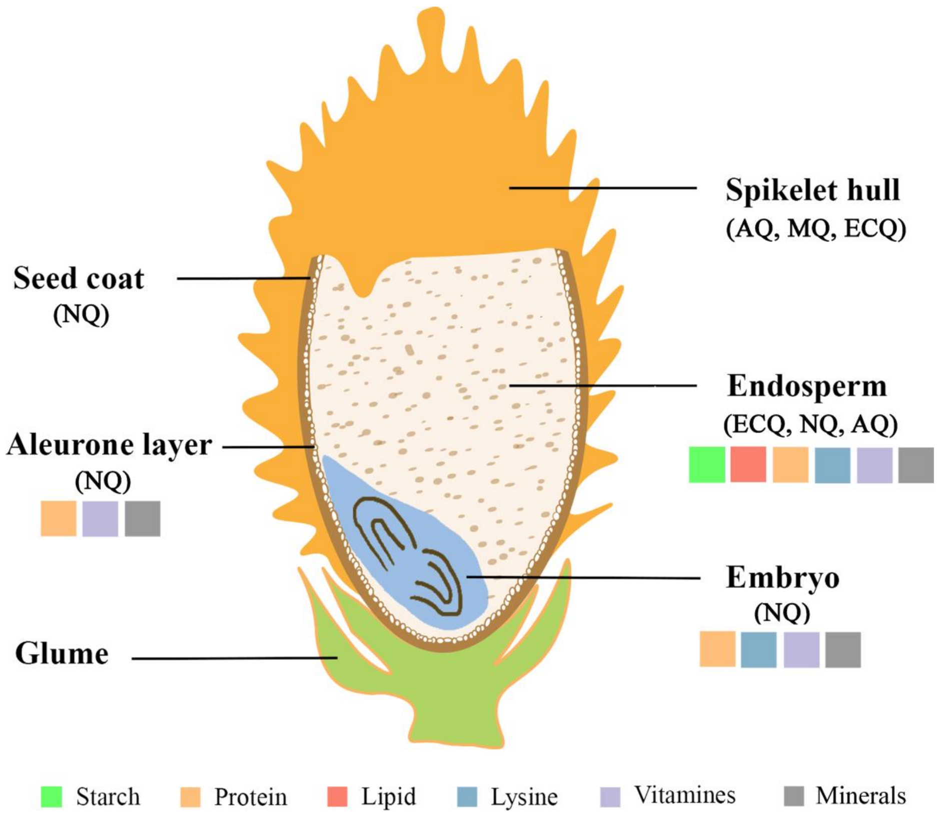


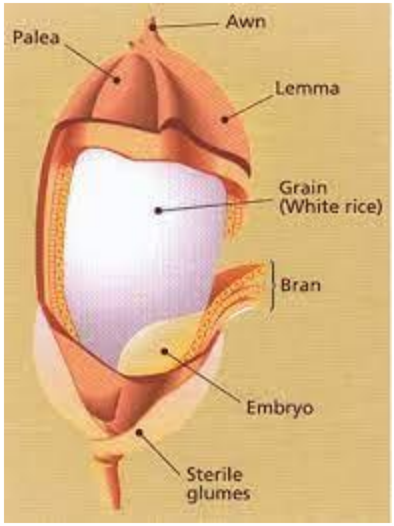
Phytohemagglutinin (PHA) is **a seed storage protein and a type of lectin originally discovered in the common bean (Phaseolus vulgaris) for its blood-agglutinating effect** which can be toxic in raw or undercooked beans.
PHA is insecticidal as well.

Transgenic cowpea expressing common bean α-ai1 gene

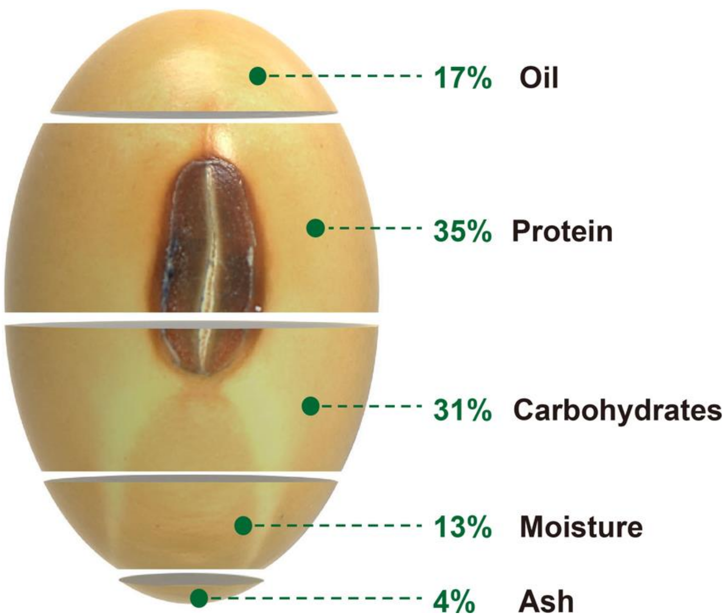
Normal seeds Transgenic seeds

Seed Components

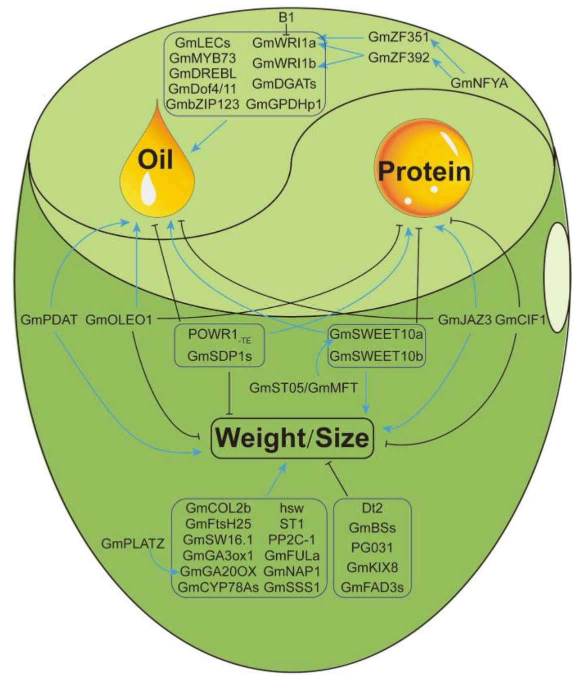




Seed specific Promoter for Oil accumulation in seeds



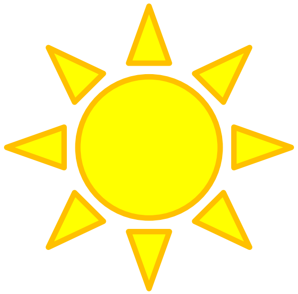
Composition of stored mature soybean seeds



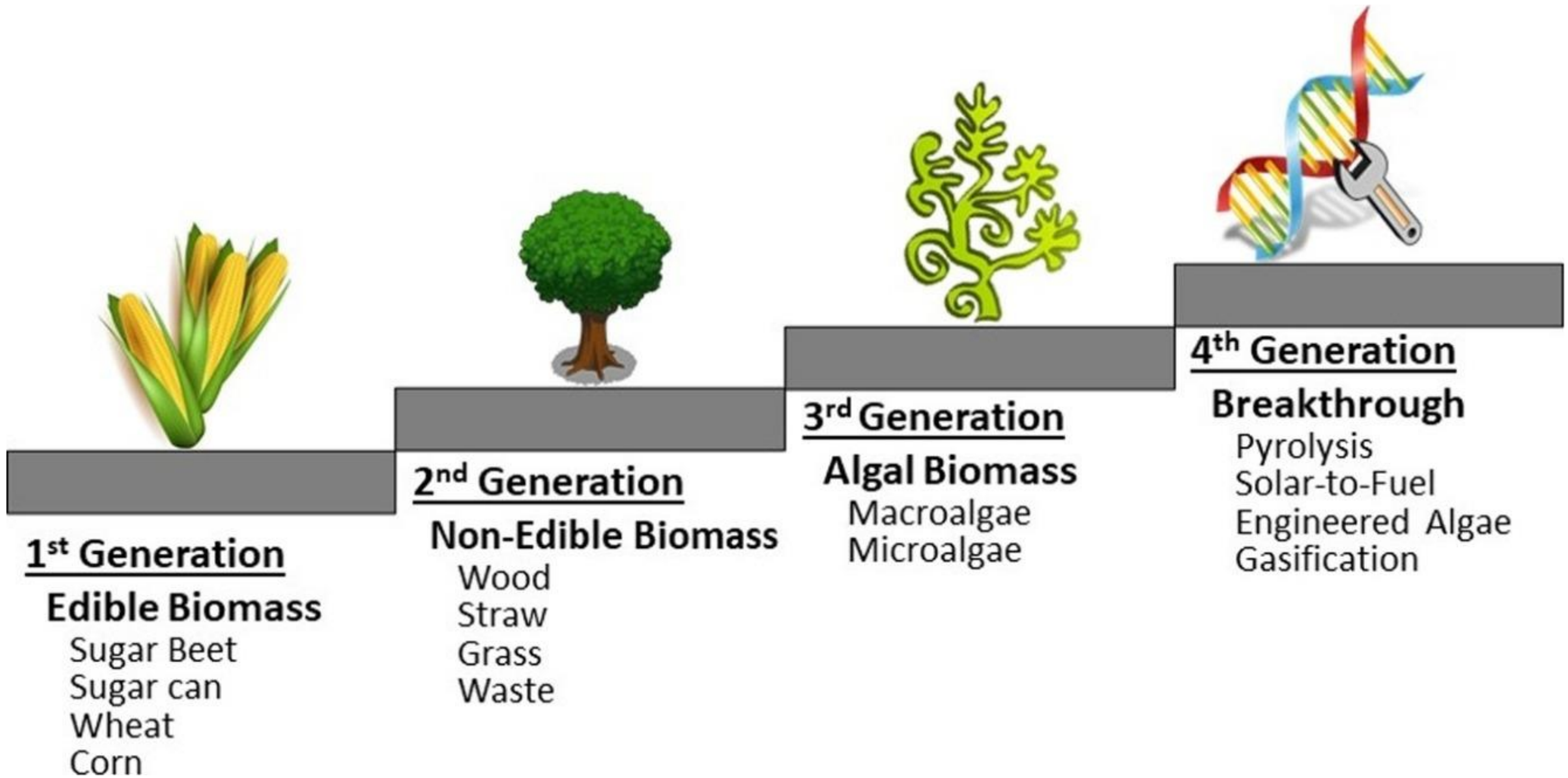
Regulatory genes that influence seed size/weight, oil content and protein content in soybean

Seed weight/size, oil and protein content are the **three major traits** determining seed quality, and seed weight also influences soybean yield.

Plants can be a source of biodiesel

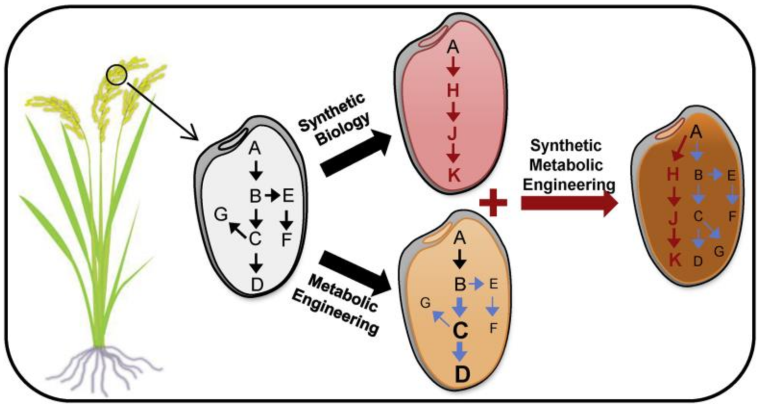


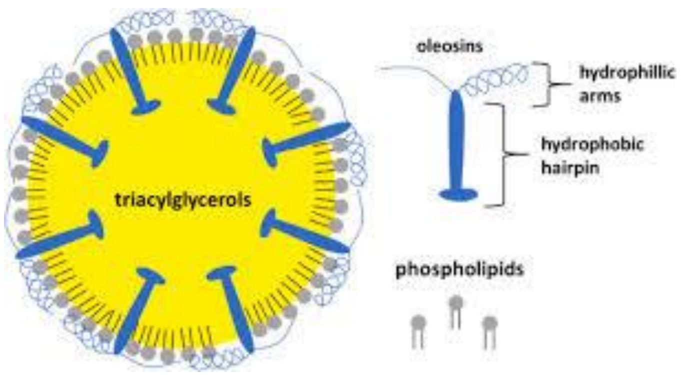
Biodiesel produced from rape, algae and soybeans are replacing petroleum-derived diesel.



1st Generation
Edible Biomass
Sugar Beet
Sugar can
Wheat
Corn
2nd Generation
Non-Edible Biomass
Wood
Straw
Grass
Waste
3rd Generation
Algal Biomass
Macroalgae
Microalgae
4th Generation
Breakthrough
Pyrolysis
Solar-to-Fuel
Engineered Algae
Gasification

Cytosolic triacylglycerols (TAGs) are the major neutral storage lipids in plants





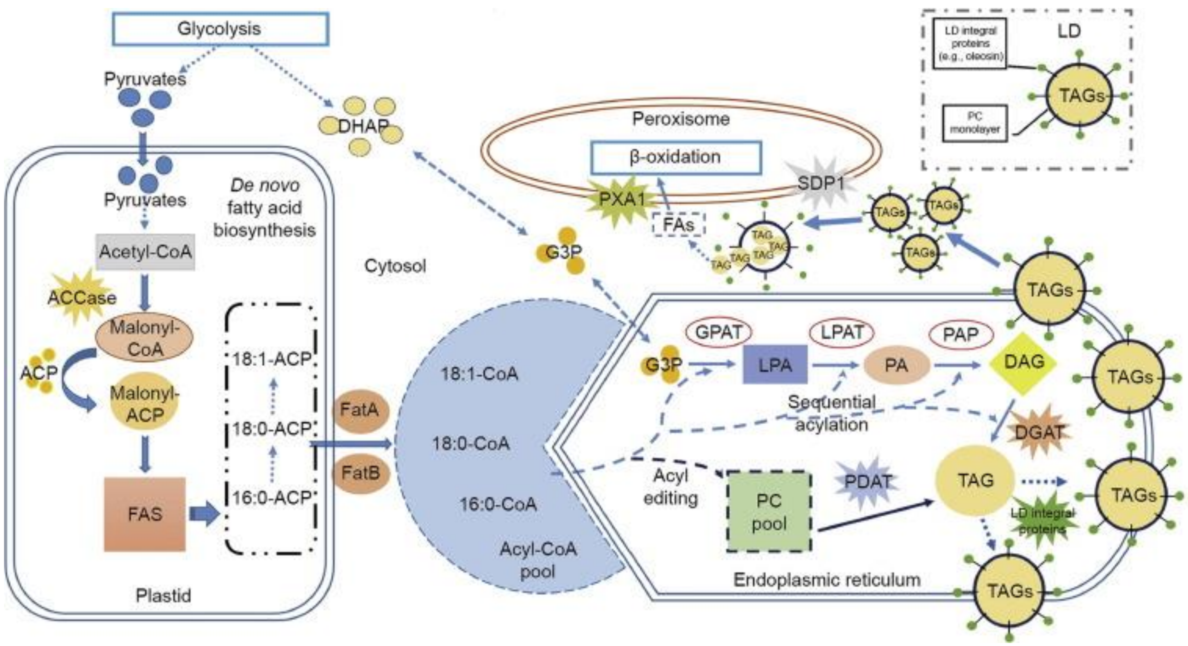
These **energy-rich molecules** are especially **abundant in seeds and oleaginous fruits,** whereas in leaves, TAGs are typically low in abundance and represent < 1% of glycerolipids.

Plant-derived TAGs (oil) are **an important source of calories in human and animal diets**, and they are **suitable feedstocks to produce biodiesel**.

Bioengineering of plant lipid metabolism has become an area of intensive research as **high TAG lines can help to meet the increasing need for sustainable oils** for our rapidly growing population taking into account climate change.

Seed Oil Biosynthesis

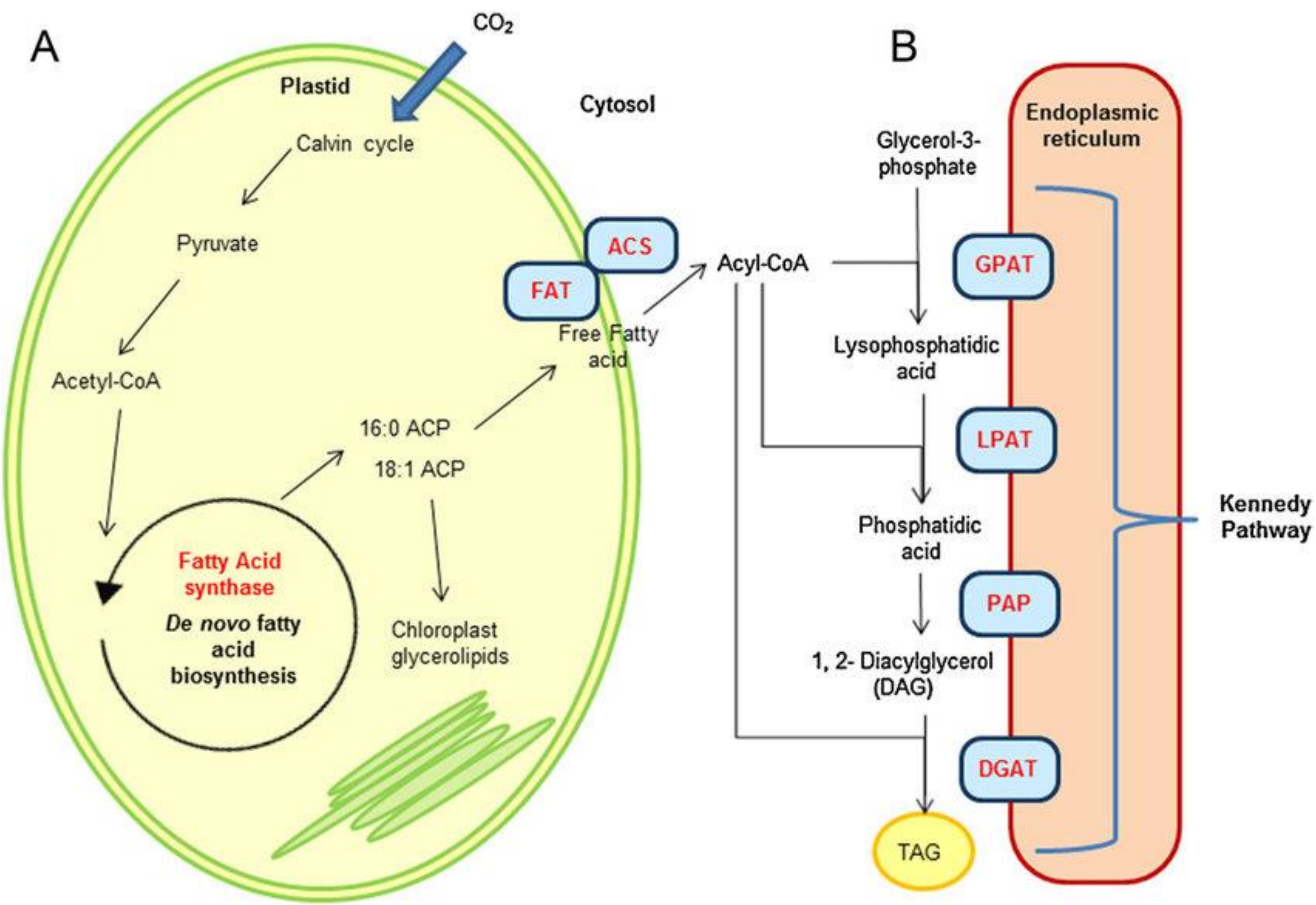
TAGs are assembled from fatty acids **produced in the plastid** and exported in activated form to the **endoplasmic reticulum where the fatty acids are esterified** to a glycerol backbone by a series of acyltransferases.



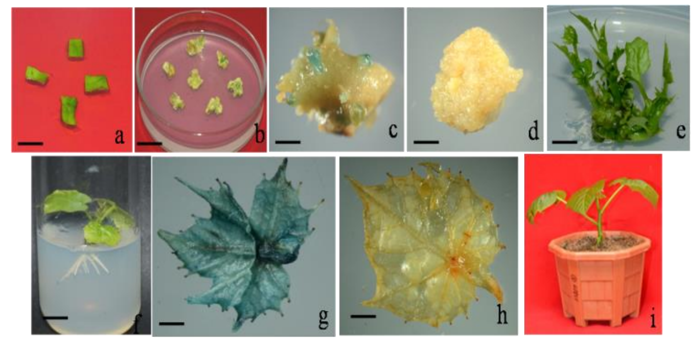
The TAGs are compartmentalized into cytosolic lipid droplets consisting of a hydrophobic core of TAGs that is surrounded by a phospholipid monolayer embedded with various coat proteins. **Oleosins** are the most abundant **lipid droplet coat proteins** in seeds, whereas they are almost entirely absent in vegetative. Anchored in the lipid droplet, oleosins stabilize small cytosolic lipid droplets from 'birth' at the endoplasmic reticulum into the mature stage. After germination, oleosins are subject to proteolysis via the ubiquitin-proteasome system, which facilitates TAG mobilization, providing energy and carbon for seedling growth.

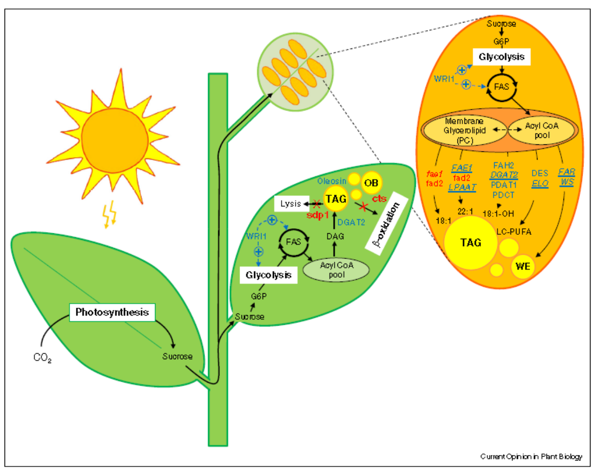
Fatty acid synthesis (Chloroplast), FA modification & Glycerolipid assembly (ER), and TAG (Triacyl glycerol) packaging

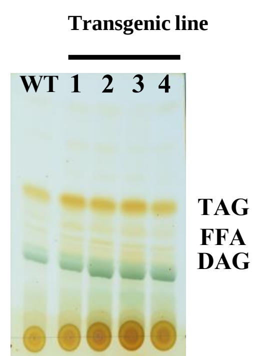
Rate Limiting Steps of TAG Biosynthesis
(Key targets for oil quantity and quality improvement)

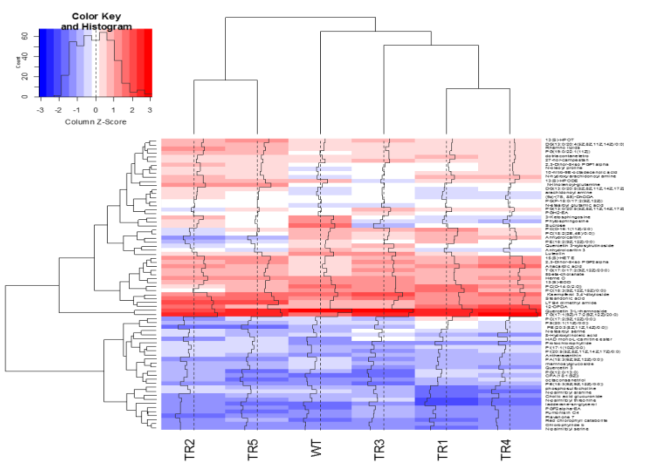


Seed specific overexpression of AtDGAT1 gene in Jatropha enhanced oil accumulation for biofuel application





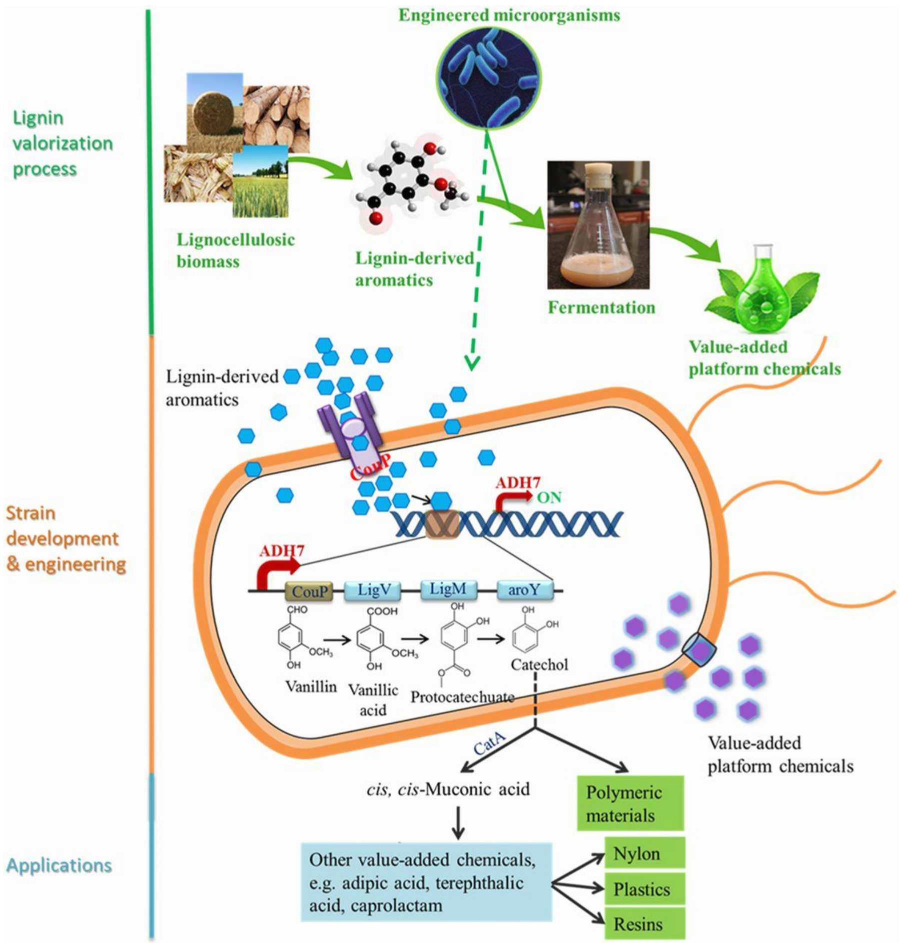




Heatmap of the lipids, lipid derivatives and other secondary metabolites detected in Transgenic *Jatropha curcas* and wild type

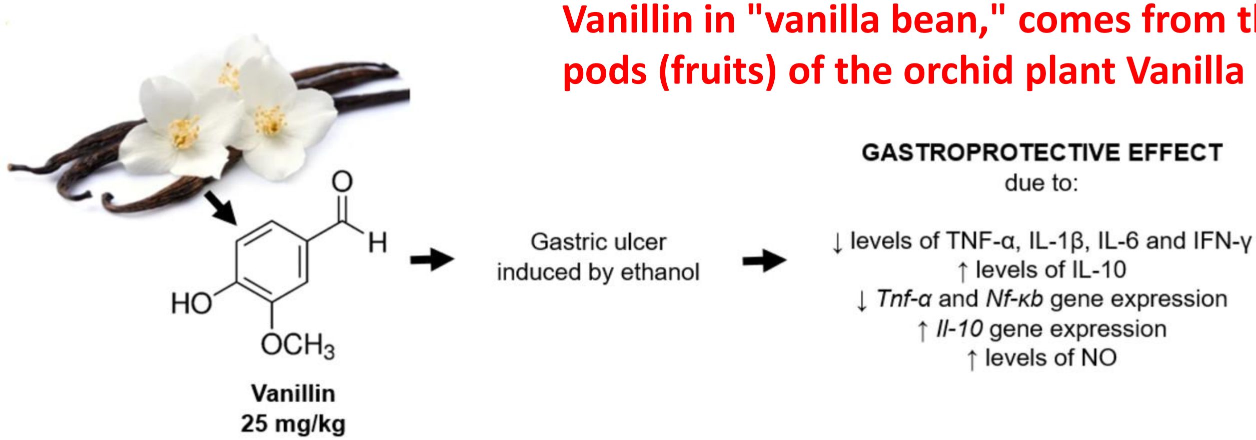
Collaborative research with **Kazusa DNA Research Institute** and **Toyota Biofuel Lab** (Japan)

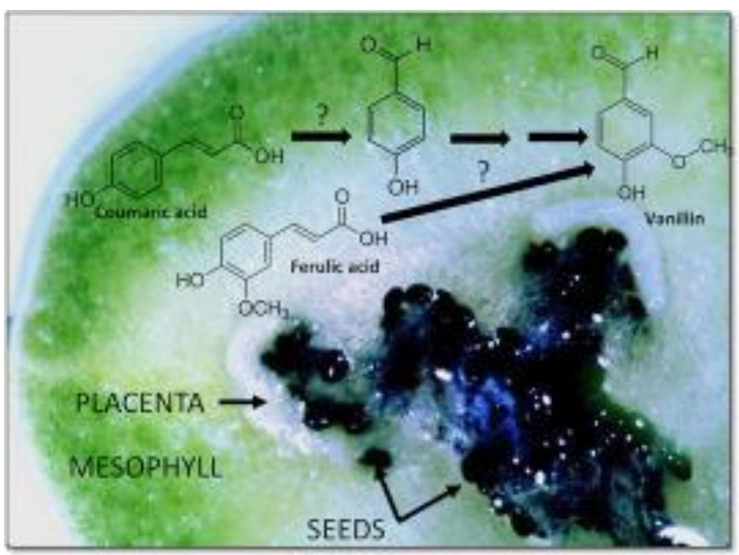
Toward engineering E. coli with an autoregulatory system for lignin valorization



Flavouring compounds accumulate in specific plant parts

Vanillin in "vanilla bean," comes from the cured, dried pods (fruits) of the orchid plant Vanilla planifolia.

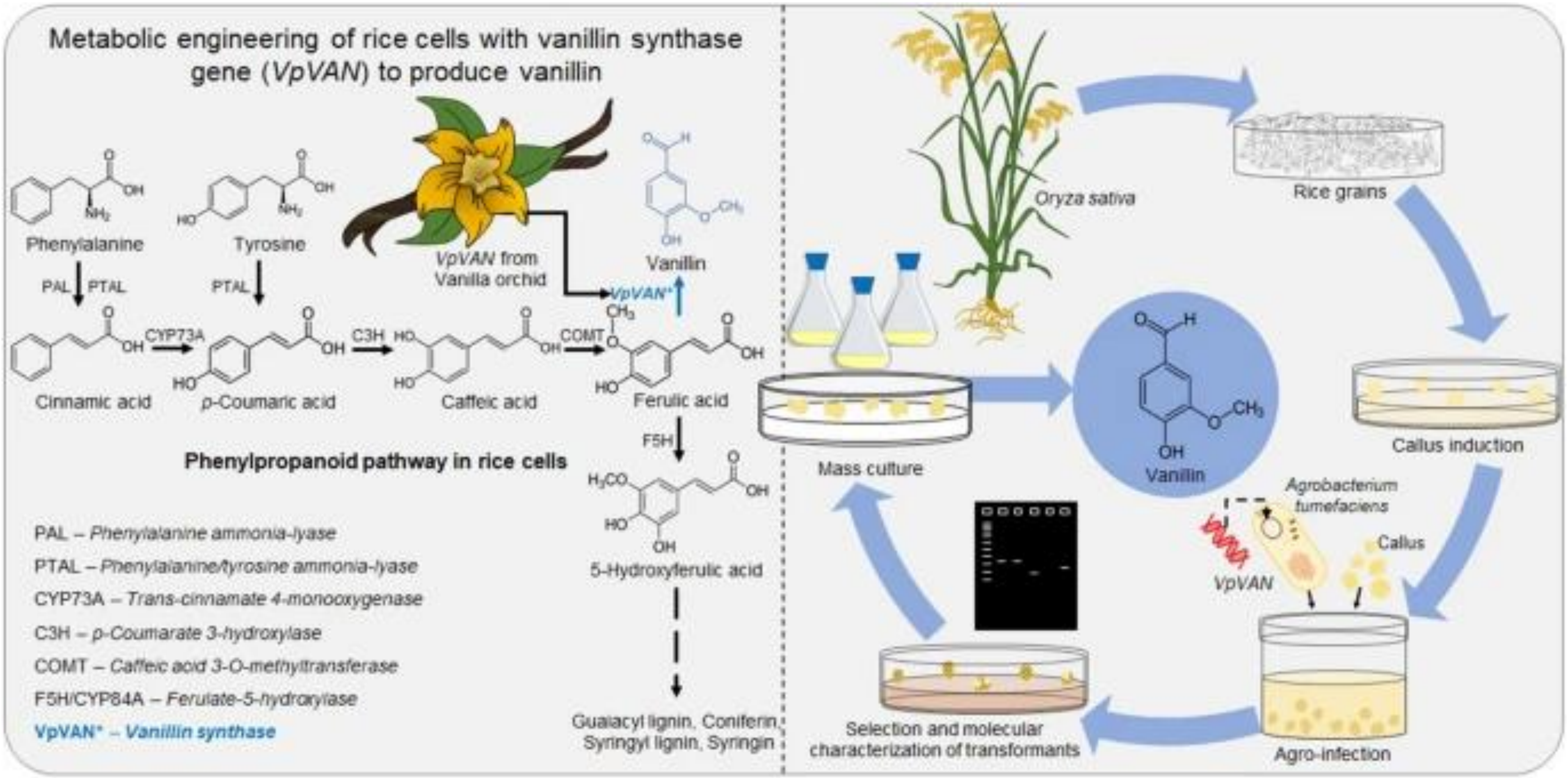


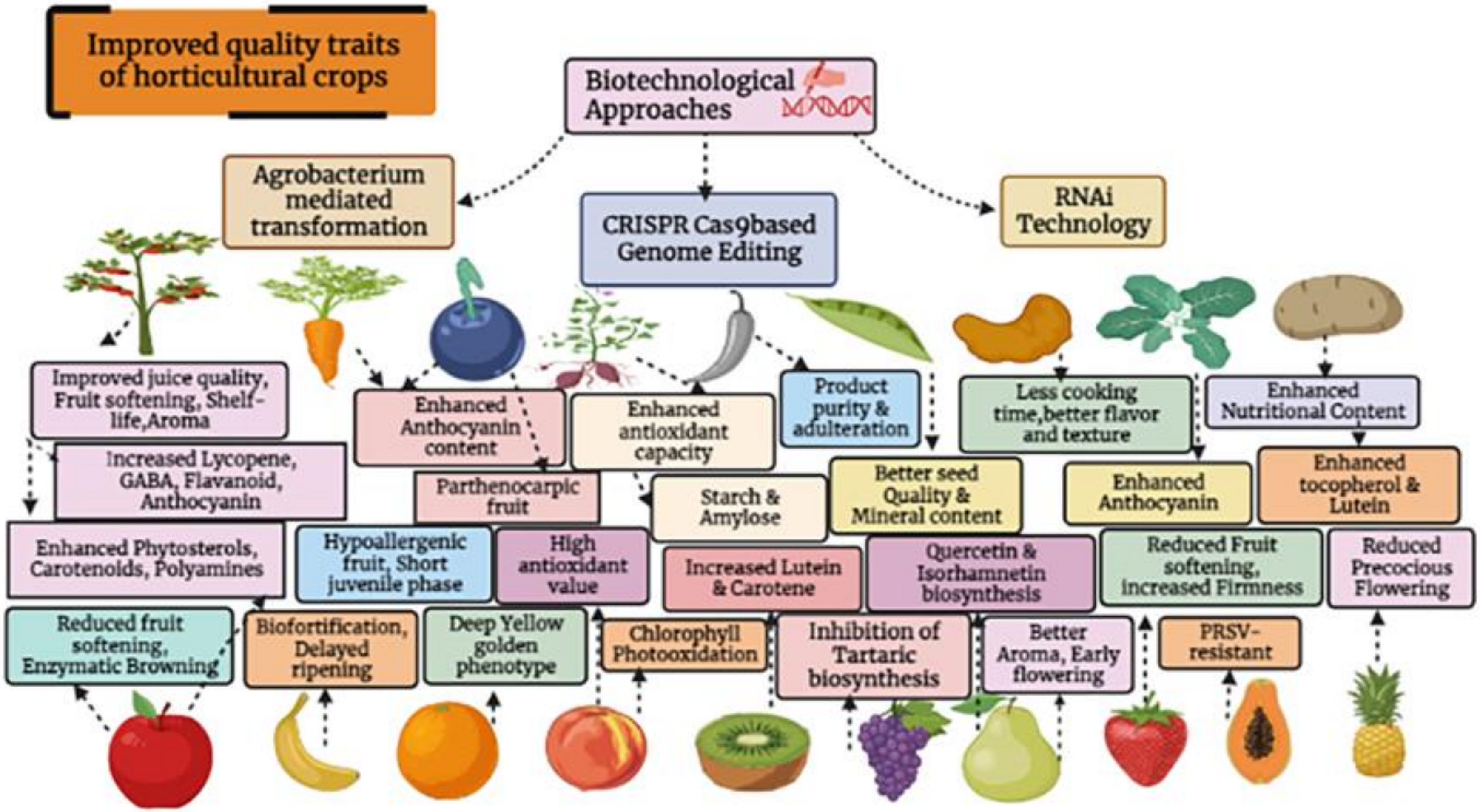


an enzyme from the vanilla orchid Vanilla planifolia with the ability to convert ferulic acid directly to vanillin. This is the **final step in the biosynthesis of vanillin, which is then converted to its storage form, glucovanillin, by glycosylation**.

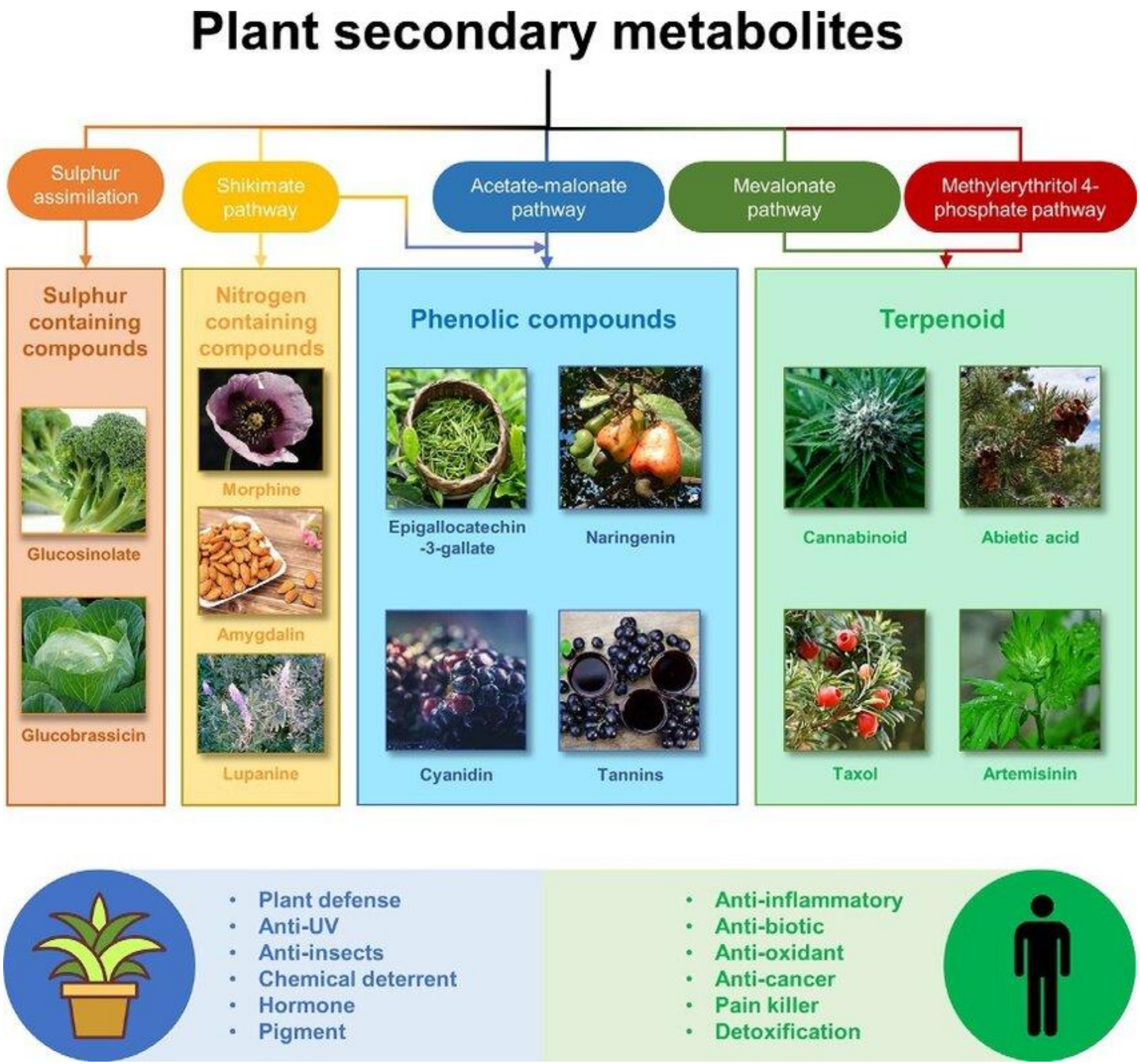
The existence of such a "vanillin synthase" could enable biotechnological **production of vanillin from ferulic acid using a "natural" vanilla enzyme**.

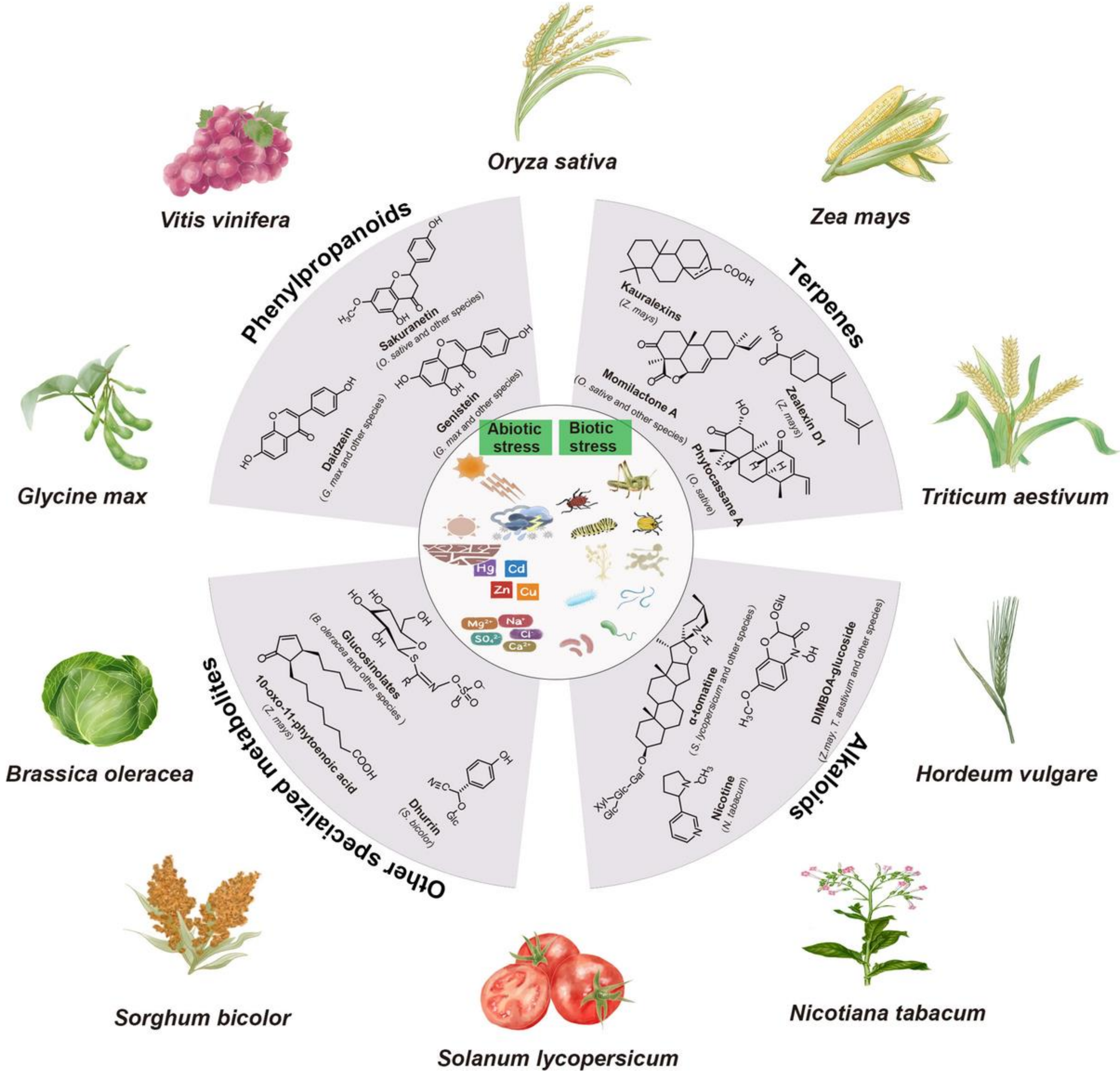
Biotechnological Production of Vanillin



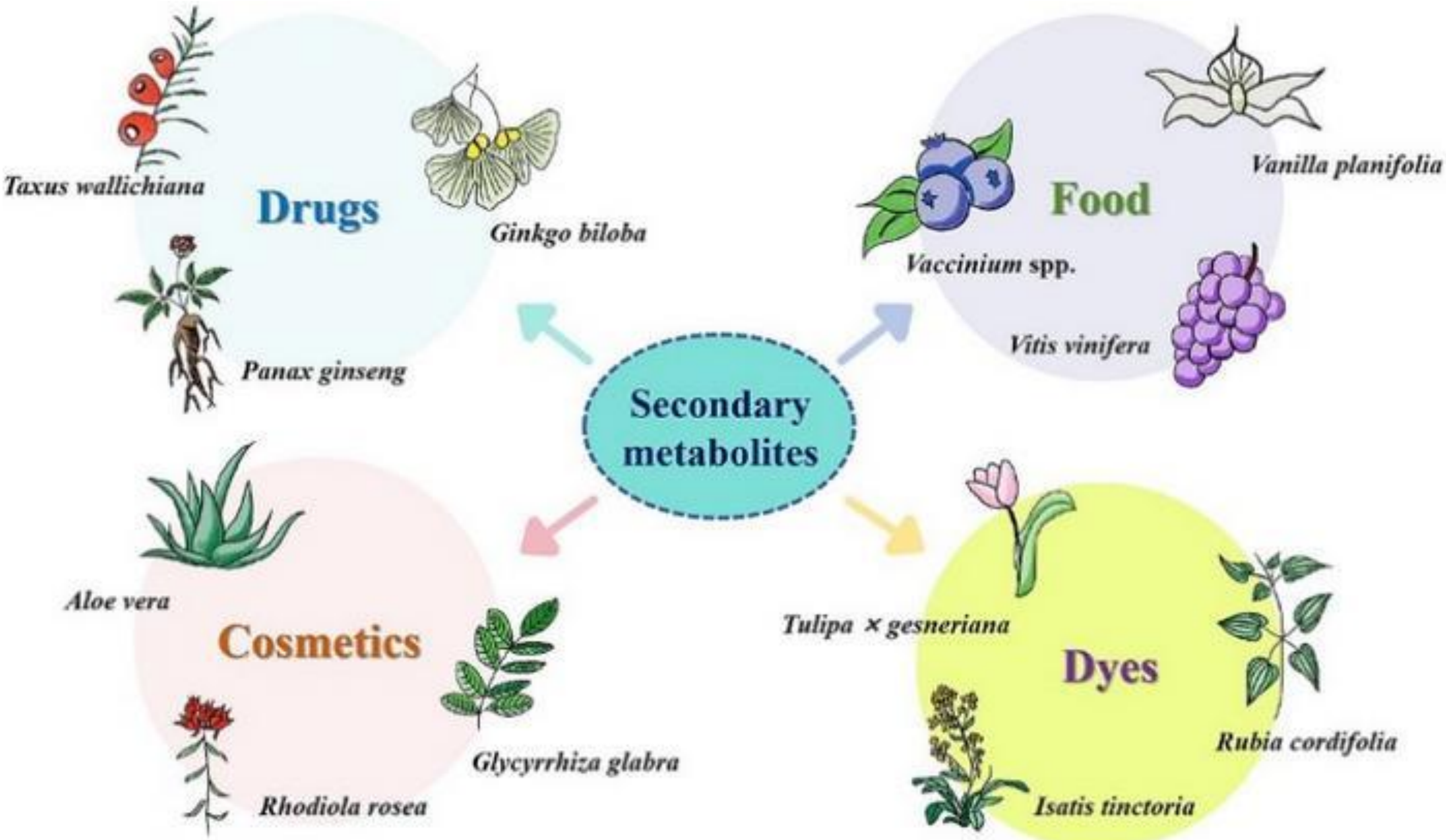


Improved quality traits of horticultural crops
Biotechnological Approaches
Agrobacterium mediated transformation
CRISPR Cas9based Genome Editing
RNAi Technology
Improved juice quality, Fruit softening, Shelf-life,Aroma
Increased Lycopene, GABA, Flavanoid, Anthocyanin
Enhanced Anthocyanin content
Enhanced antioxidant capacity
Product purity & adulteration
Less cooking time,better flavor and texture
Enhanced Nutritional Content
Parthenocarpic fruit
Starch & Amylose
Better seed Quality & Mineral content
Enhanced Anthocyanin
Enhanced tocopherol & Lutein
Enhanced Phytosterols, Carotenoids, Polyamines
Hypoallergenic fruit, Short juvenile phase
High antioxidant value
Increased Lutein & Carotene
Quercetin & Isorhamnetin biosynthesis
Reduced Fruit softening, increased Firmness
Reduced Precocious Flowering
Reduced fruit softening, Enzymatic Browning
Biofortification, Delayed ripening
Deep Yellow golden phenotype
Chlorophyll Photooxidation
Inhibition of Tartaric biosynthesis
Better Aroma, Early flowering
PRSV-resistant


Plant secondary metabolites
Sulphur assimilation
Shikimate pathway
Acetate-malonate pathway
Mevalonate pathway
Methylerythritol 4-phosphate pathway
Sulphur containing compounds
Glucosinolate
Glucobrassicin
Nitrogen containing compounds
Morphine
Amygdalin
Lupanine
Phenolic compounds
Epigallocatechin -3-gallate
Naringenin
Cyanidin
Tannins
Terpenoid
Cannabinoid
Abietic acid
Taxol
Artemisinin
• Plant defense
• Anti-UV
• Anti-insects
• Chemical deterrent
• Hormone
• Pigment
• Anti-inflammatory
• Anti-biotic
• Anti-oxidant
• Anti-cancer
• Pain killer
• Detoxification

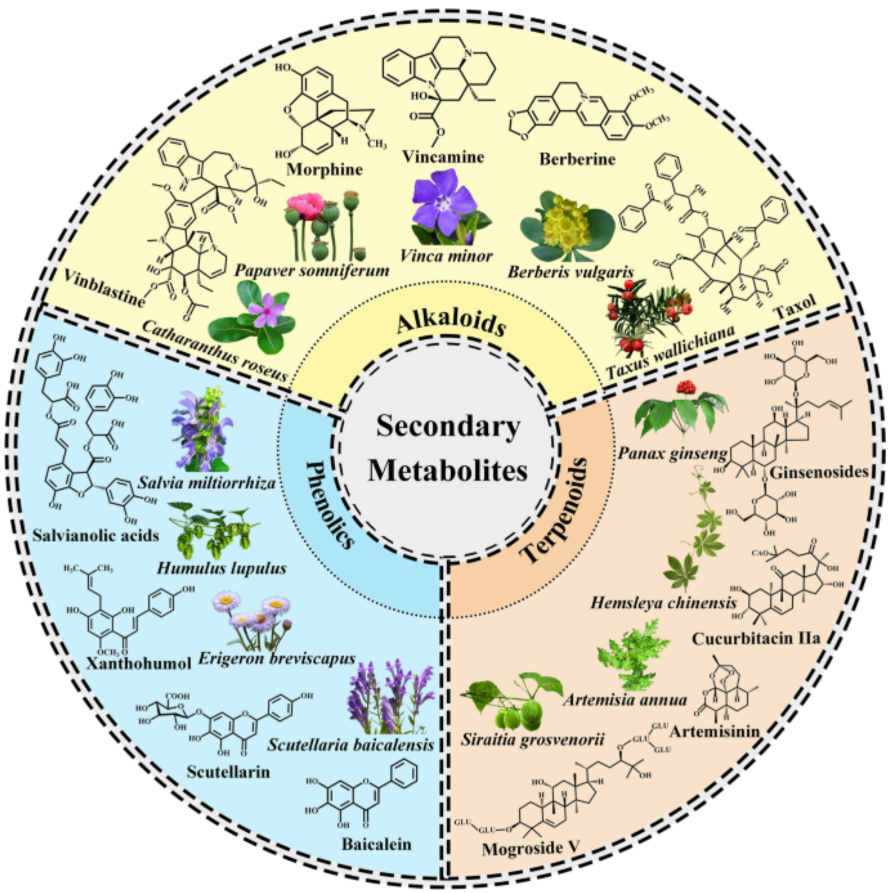


Oryza sativa
Zea mays
Vitis vinifera
Phenylpropanoids
Terpenes
Sakuranetin
(O. sative and other species)
Genistein
(G. max and other species)
Daidzein
(G. max and other species)
Kauralexins
(Z. mays)
Momilactone A
(O. sative and other species)
Zealexin D1
(Z. mays)
Phytocassane A
(O. sative)
Glycine max
Triticum aestivum
Abiotic stress
Biotic stress
Hg
Cd
Zn
Cu
Glucosinolates
(B. oleracea and other species)
10-oxo-11-phytoenoic acid
(Z. mays)
Dhurrin
(S. bicolor)
α-tomatine
(S. lycopersicum and other species)
DIMBOA-glucoside
(Z.may, T. aestivum and other species)
Nicotine
(N. tabacum)
Brassica oleracea
Hordeum vulgare
Other specialized metabolites
Alkaloids
Sorghum bicolor
Nicotiana tabacum
Solanum lycopersicum

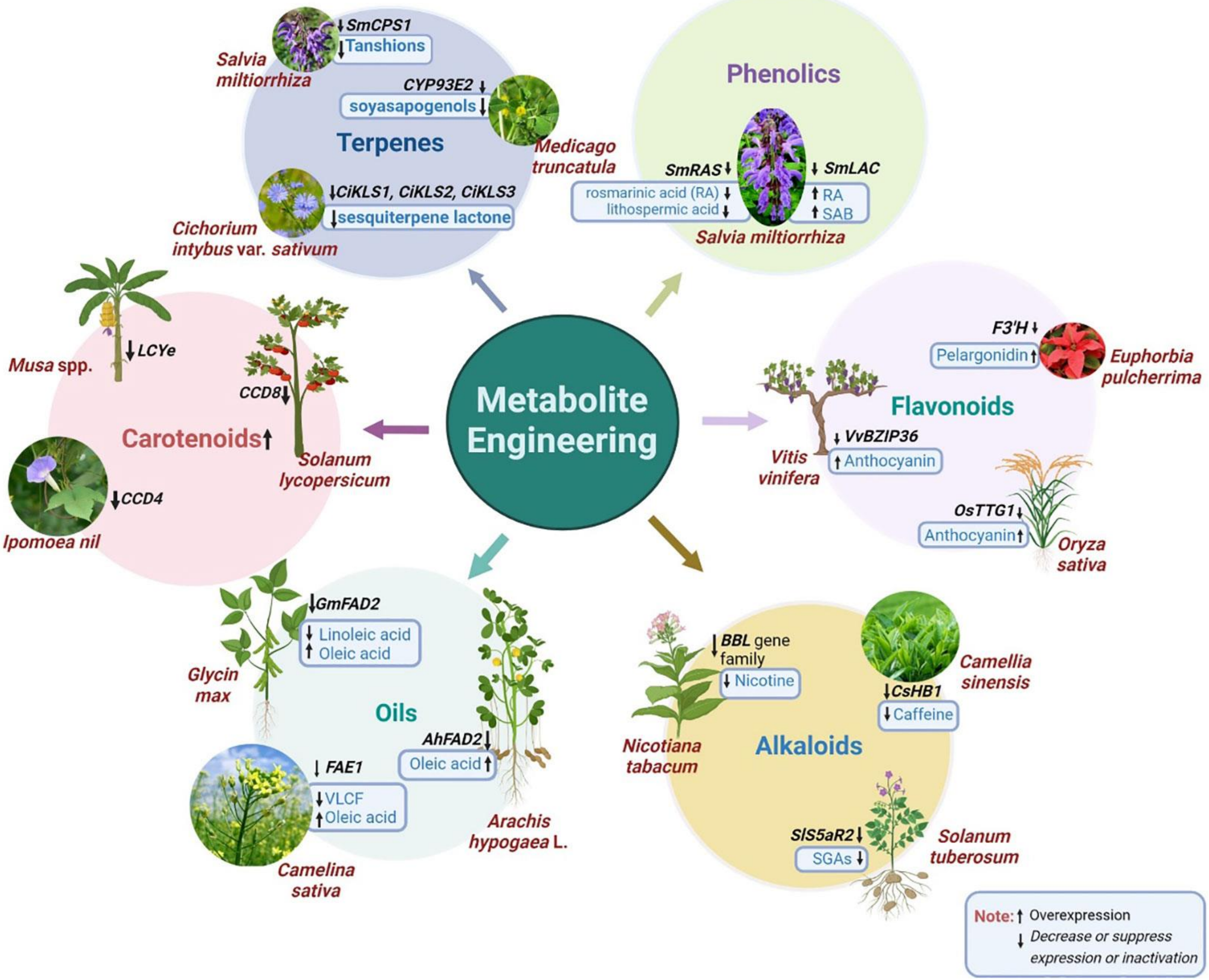


Secondary metabolites
Drugs
Taxus wallichiana
Ginkgo biloba
Panax ginseng
Food
Vanilla planifolia
Vaccinium spp.
Vitis vinifera
Cosmetics
Aloe vera
Glycyrrhiza glabra
Rhodiola rosea
Dyes
Tulipa × gesneriana
Rubia cordifolia
Isatis tinctoria

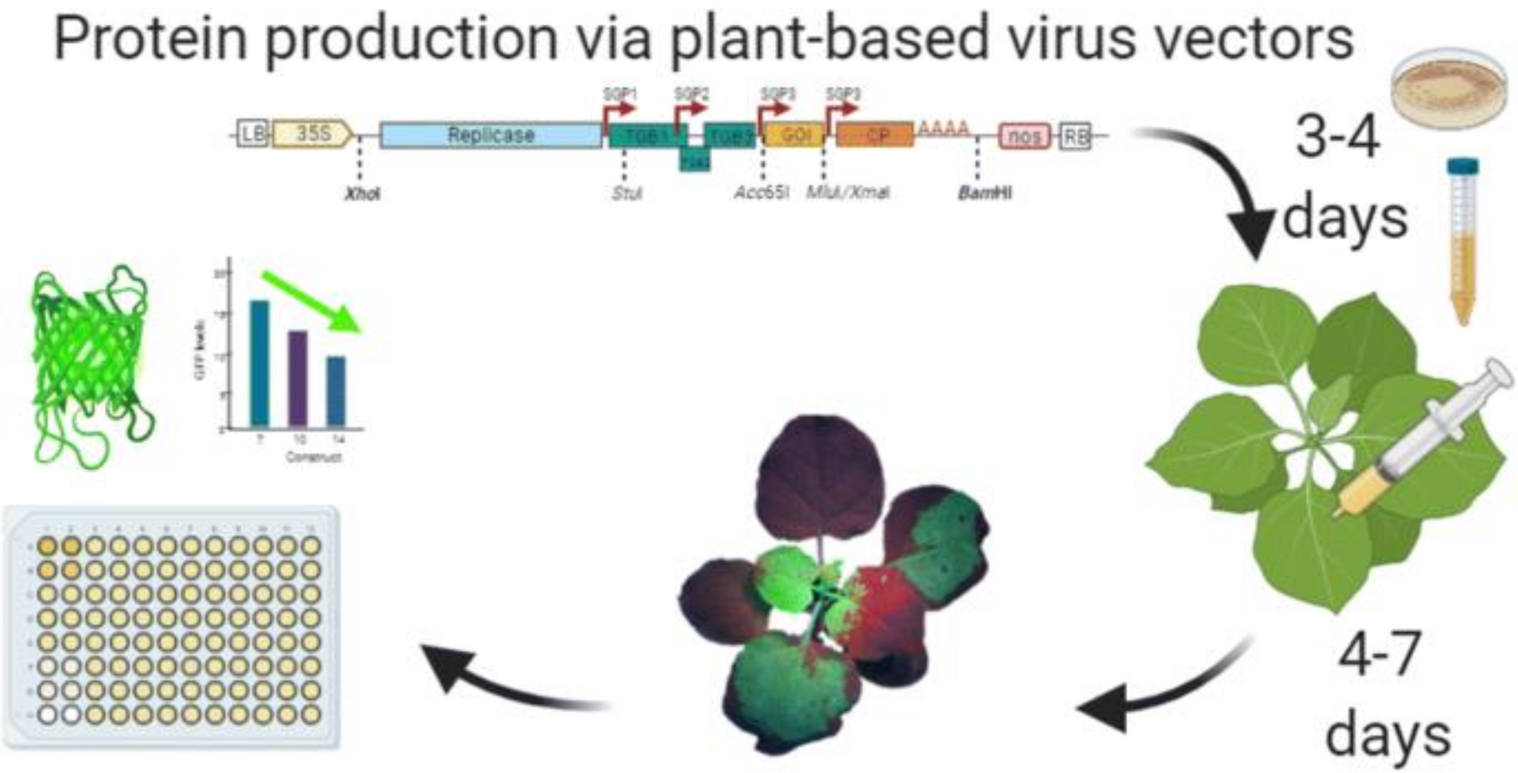
Medicinal plants represent a huge reservoir of secondary metabolites (SMs), substances with significant pharmaceutical and industrial potential



However, obtaining secondary metabolites remains a challenge due to their **low-yield accumulation** in medicinal plants; moreover, these secondary metabolites are **produced through tightly coordinated pathways involving many spatiotemporally and environmentally regulated steps**. The first regulatory layer involves a complex network of transcription factors; a second, more recently discovered layer of complexity in the regulation of SMs is epigenetic modification, such as DNA methylation, histone modification and small RNA-based mechanisms, which can jointly or separately influence secondary metabolites by regulating gene expression.



Metabolite Engineering
Terpenes
SmCPS1
Tanshions
Salvia miltiorrhiza
CYP93E2
soyasapogenols
Medicago truncatula
CiKLS1, CiKLS2, CiKLS3
sesquiterpene lactone
Cichorium intybus var. sativum
Phenolics
SmRAS
SmLAC
rosmarinic acid (RA)
lithospermic acid
RA
SAB
Salvia miltiorrhiza
Flavonoids
F3'H
Pelargonidin
Euphorbia pulcherrima
VvBZIP36
Anthocyanin
Vitis vinifera
OsTTG1
Anthocyanin
Oryza sativa
Alkaloids
BBL gene family
Nicotine
Nicotiana tabacum
CsHB1
Caffeine
Camellia sinensis
SlS5aR2
SGAs
Solanum tuberosum
Oils
GmFAD2
Linoleic acid
Oleic acid
Glycin max
AhFAD2
Oleic acid
Arachis hypogaea L.
FAE1
VLCF
Oleic acid
Camelina sativa
Carotenoids
LCYe
Musa spp.
CCD8
Solanum lycopersicum
CCD4
Ipomoea nil
Note: Overexpression
Decrease or suppress expression or inactivation



Development and optimization of a pepino mosaic virus-based vector for rapid expression of heterologous proteins in plants