APPLICATIONS

Tissue specific Promoters

Male sterility and Hybrid vigor

Most commercial varieties of vegetable and field crops are F1 hybrids that perform stably over a wide range of environments. Incorporating male sterility reduces the labour required for hybrid seed production and ensures high varietal purity.

Plant male sterility is a condition where the plant fails to produce functional pollen. This failure could be due to defects in anther specification and differentiation, abnormal microsporogenesis and pollen development, non-dehiscent anthers, or the inability of pollen to germinate and fertilize the female gametes. Male-sterile female lines form the foundation of large-scale hybrid seed production in various crop species.

Such hybrids with superior agronomic traits such as higher yield, enhanced disease resistance and stress tolerance have greatly contributed to food, feed and nutritional security.

Heterosis is a phenomenon whereby heterozygous hybrid progeny are superior to both homozygous parents.

Male sterility and Hybrid vigor

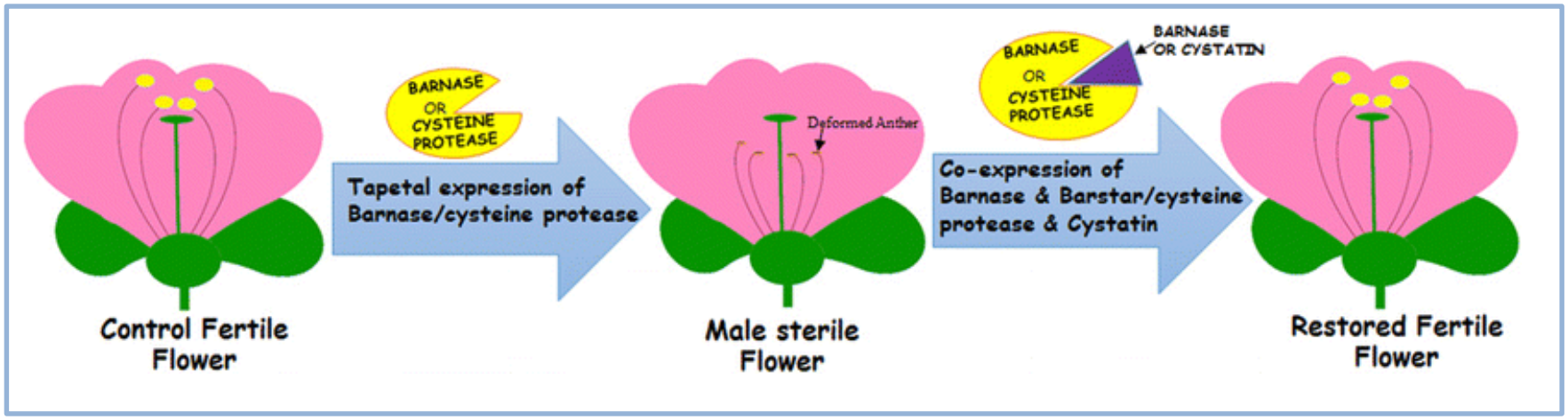
Pollination control system described as a procedure in plant breeding adapted by a plant breeder in order to obtain desired hybrid cultivars.

In these systems, male floral parts of one parent line are removed (termed as the process of emasculation) making them male sterile to avoid self-pollination and to ensure cross pollination by the desired parent line. The objective is to develop a system for pollination control to facilitate hybrid crop varieties that **exhibit heterosis in specific combinations** resulting in wider adaptation to the environment over the years with the stability of performance.

Removal of male flower or floral parts through manual emasculation is the most widely used method to avoid self-pollination. However, **it is a tedious and costly procedure as the most crops have small, bisexual flowers**.

Other pollination control systems include the **use of male sterility in plants**. Male sterility is defined as the failure of a plant to generate viable pollen grains retaining female fertility unaltered.

Male sterility and Hybrid vigor



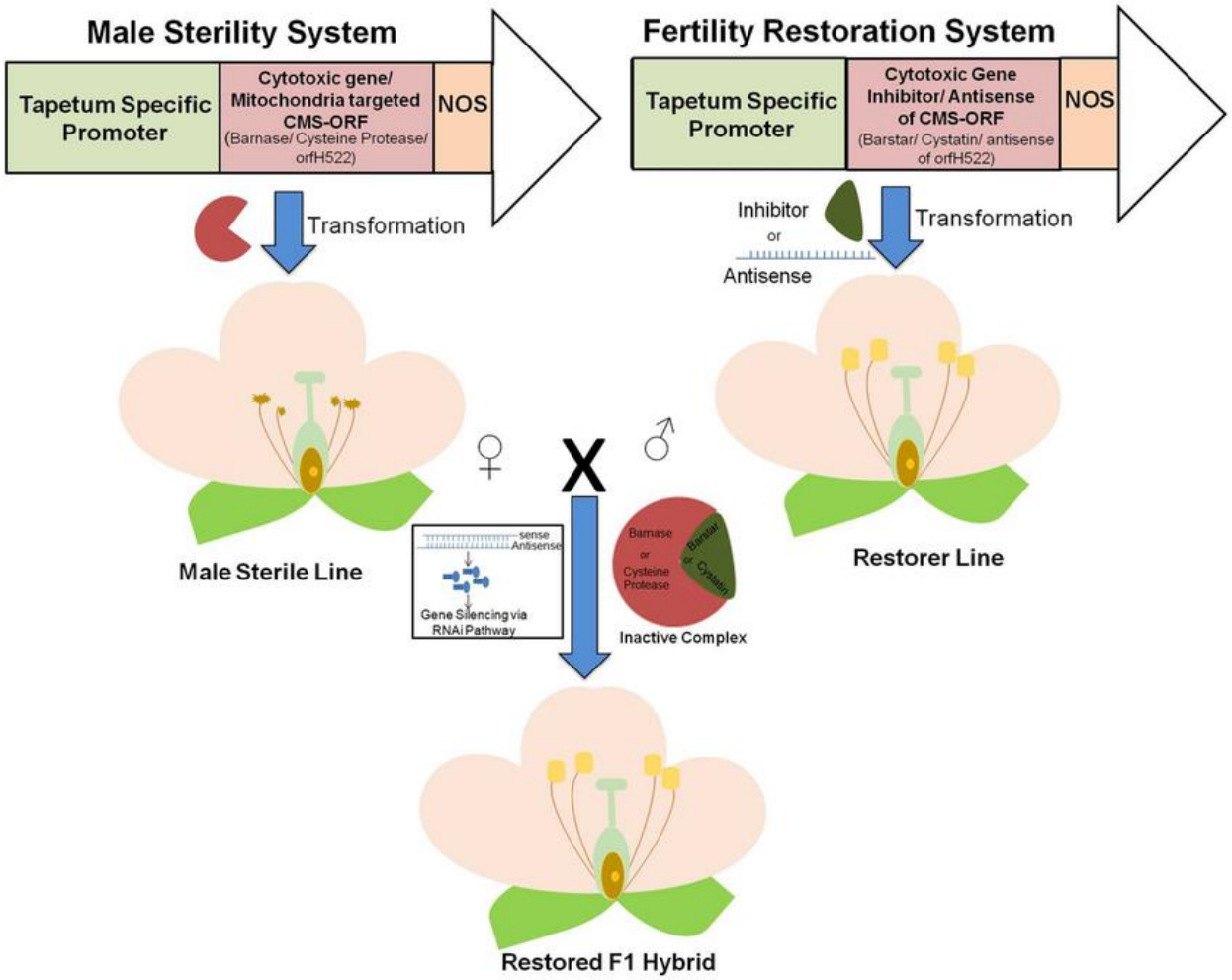
Tapetal expression of barnase/cysteine protease resulted in **male sterility** and coexpression of barstar/cystatin with barnase/cysteine protease **restored the male fertility**

Barnase is extracellular RNase; **barstar** is inhibitor of barnase (both from bacterium **Bacillus amylo liquefaciens** which uses barnase for protection from microbial predators and barstar to protect itself from barnase.

Fuse the barnase and barstar genes to **TA29 promoter** (**TA29 is a plant gene that has tapetum specific expression**). Brassica napus cv. Darkar containing the TA29–barnase construct are male sterile; those with TA29–barstar are not affected by the transgene (Male fertile).

Cross male sterile (barnase) with male fertile (barstar) to get hybrid seed, which now has both barnase and barstar expressed in tapetum and, hence, is fully fertile. barstar is dominant over barnase)

Barnase and Barstar genes for male sterility and restoration



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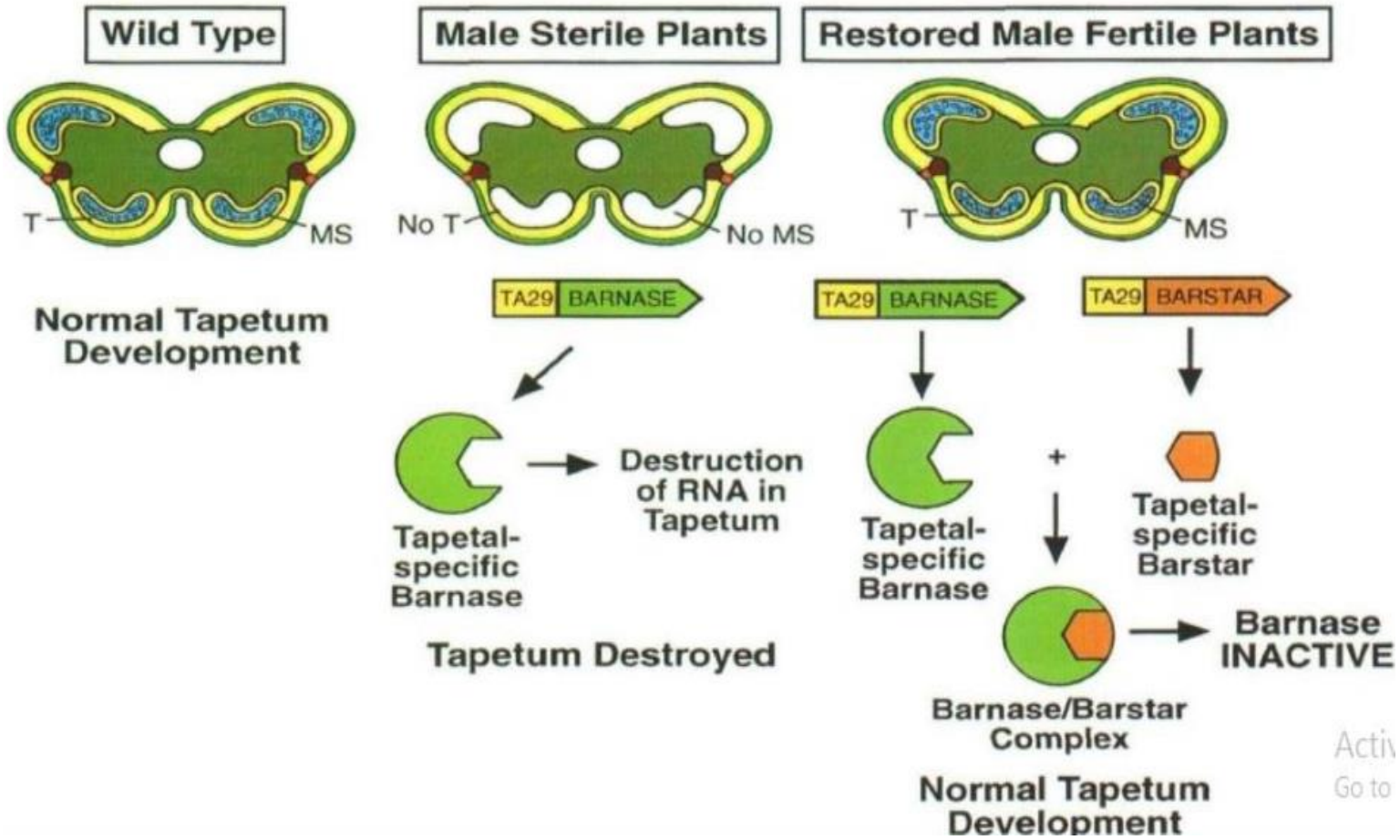
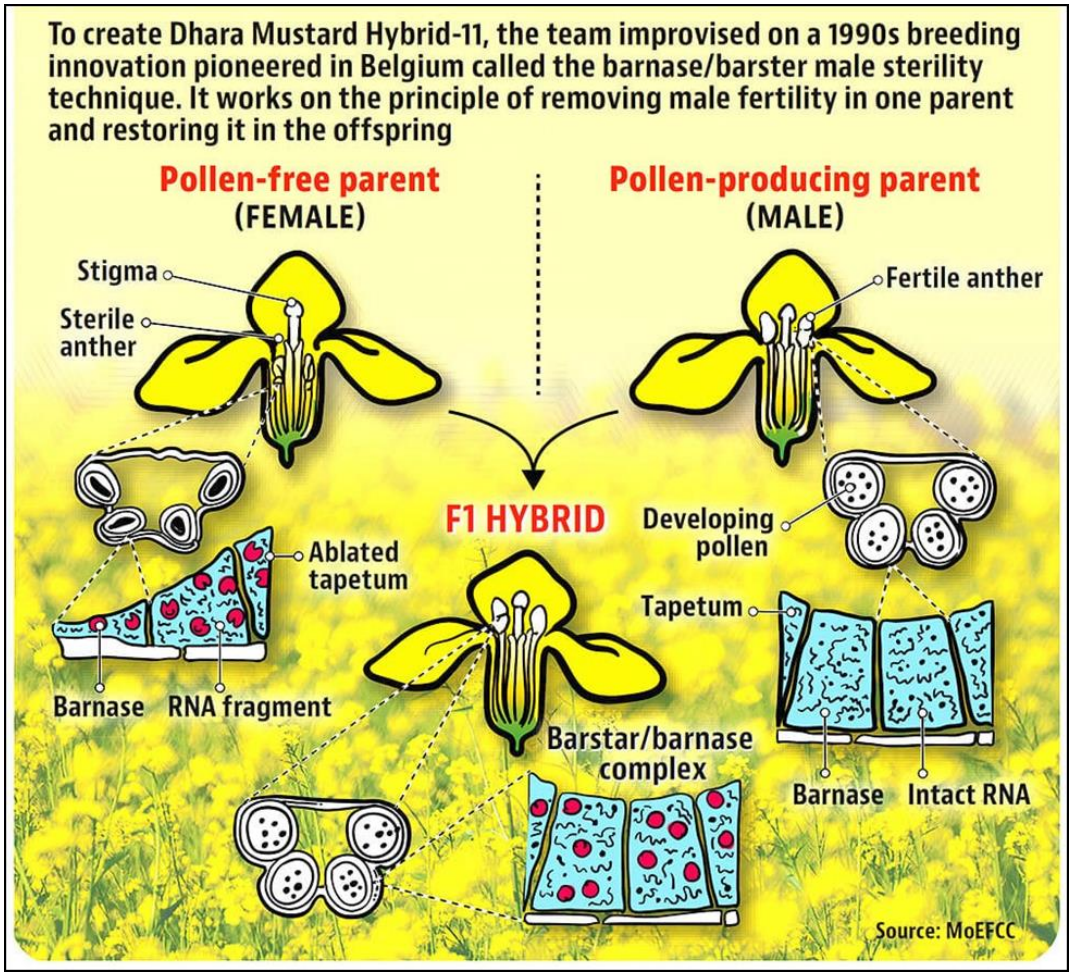
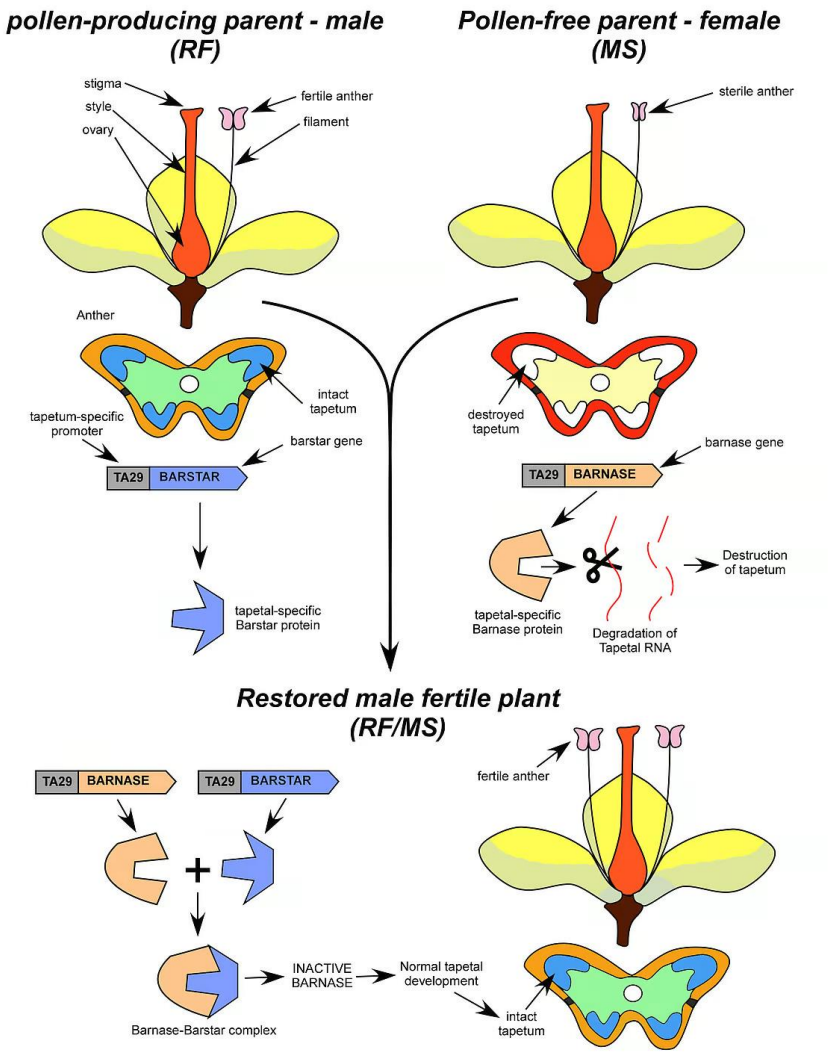


Tapetum-specific promoter for Hybrid vigor





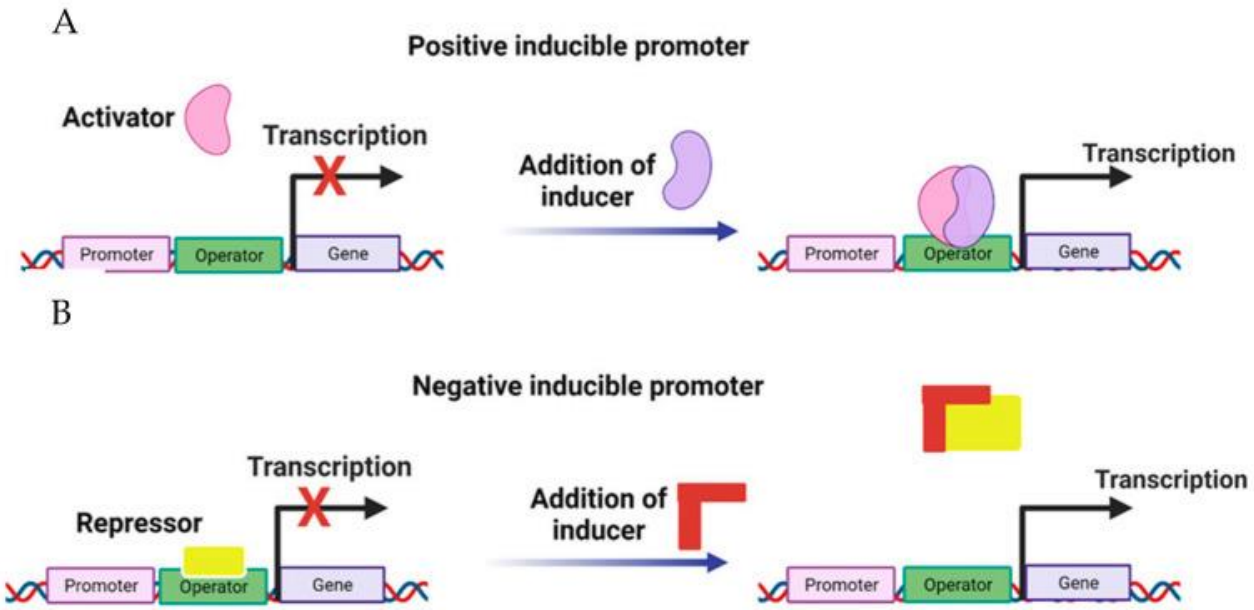
some of the most **substantial gains in crop productivity**, breeding barriers have fundamentally limited **soybean** from reaping the benefits of **hybrid vigour**.

Soybean flowers self-pollinate prior to opening and thus are not readily amenable to outcrossing. The **barnase/barstar male sterility/rescue system** can be used in soybean to produce hybrid seeds.

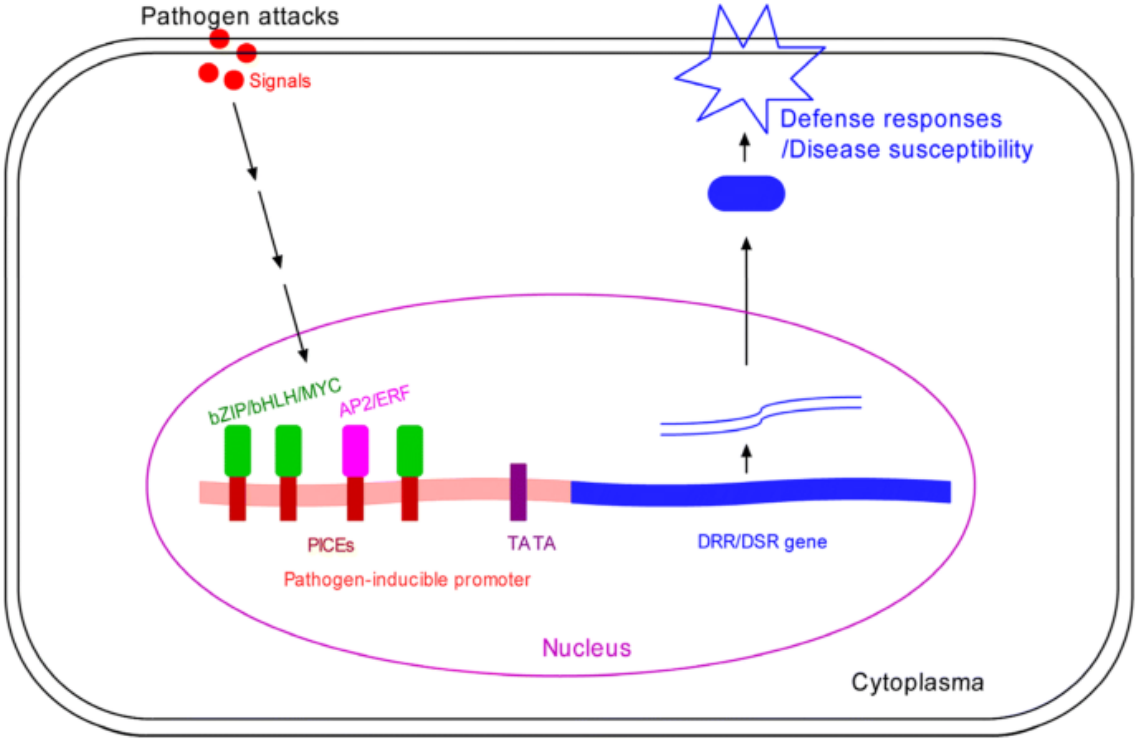
By expressing the cytotoxic ribonuclease, barnase, under a tapetum-specific promoter in soybean anthers, pollen maturation completely blocked, creating male sterile plants. We show that fertility can be rescued in the F1 generation of these barnase-expressing lines when they are crossed with pollen from plants that express the barnase inhibitor, barstar.

Inducible Promoters

Inducible promoters are **activated by hormones, chemicals, environmental conditions**, and **biotic or abiotic stresses**; and they contain a cis-acting element that **could bind different transcription factors involved in the stimuli**. In turn, the performance of inducible promoters is not usually affected by endogenous factors.



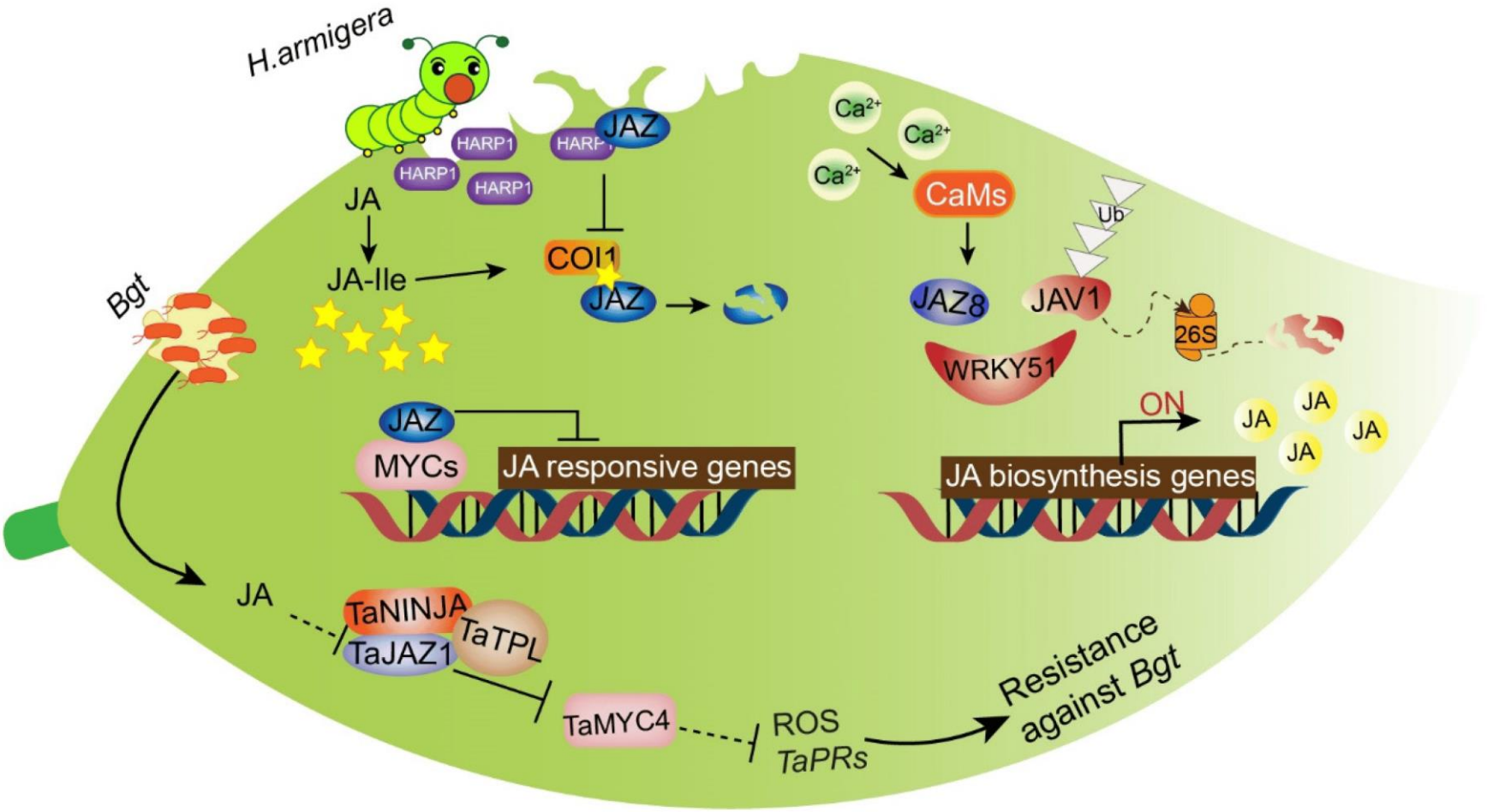
Pathogen Inducible Promoter



Pathogen-inducible promoters have been devised by **engineering several pathogen-inducible genes** and act as excellent platforms for regulated transcription control in plants under pathogen attack.

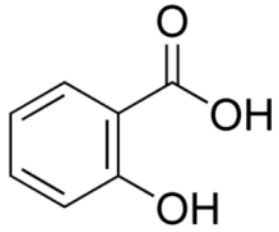
In this context, the **GCC-like elements** and the **W boxes** have been well established as potent pathogen elicitors. **Minor modifications in the core *cis* sequences can lead to multiple stress-elicitor functions**. For instance, **JERE (AGACCGCC), which is a modified GCC-like element**, **imparts both jasmonate- and elicitor-responsive expression**. **DRE (TACCGAC)** confers cold, salt-, and dehydration-responsive expression, and box S (AGCCACC) is linked to fungal elicitors.

Pathogen Inducible Promoter

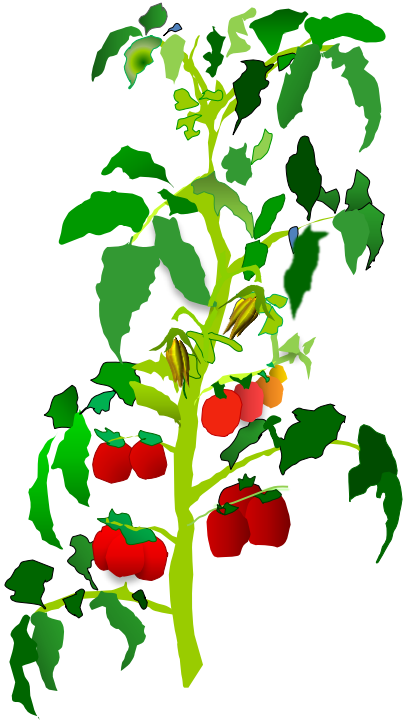


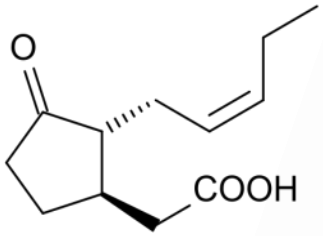
Hormonal responses to biotic stress

Bacteria, fungi, viruses – Biotrophic organisms



Salicylic Acid

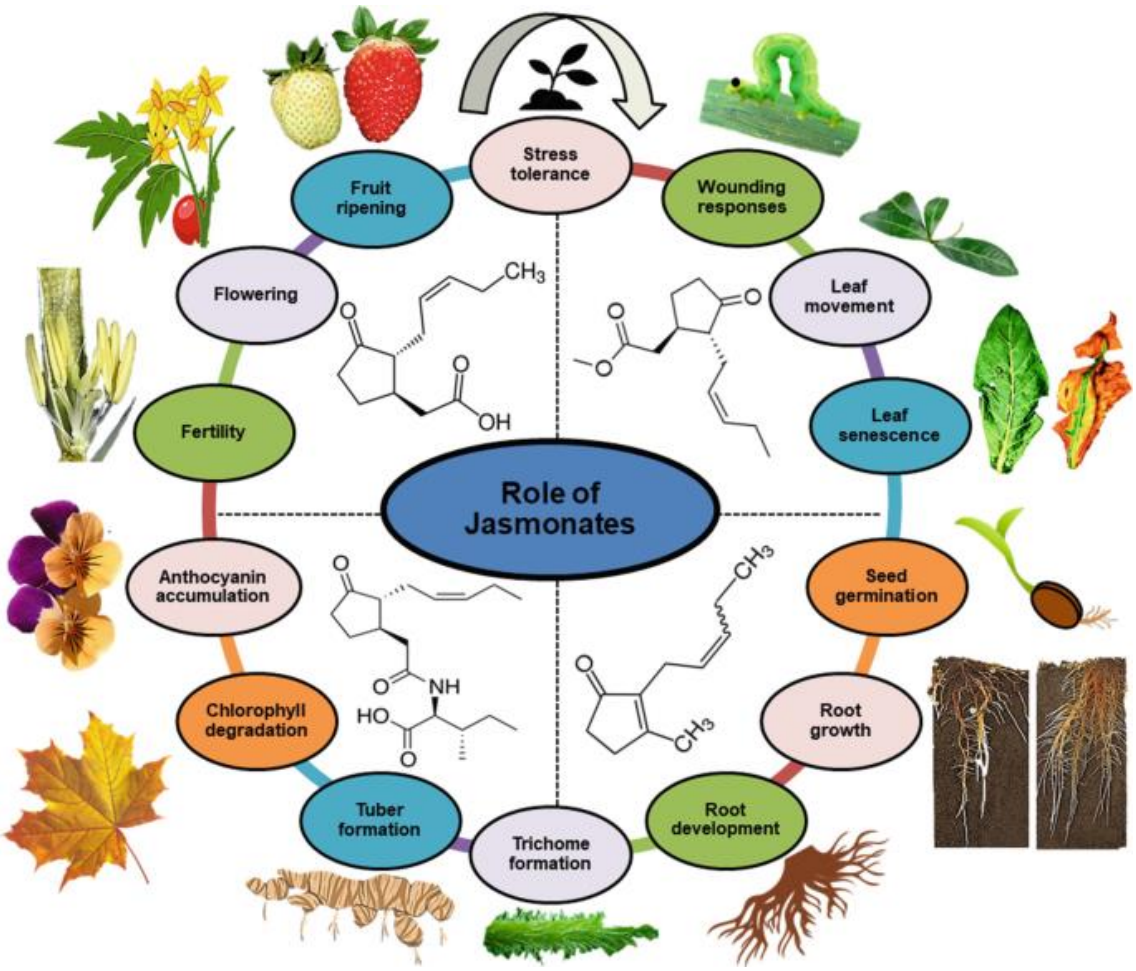




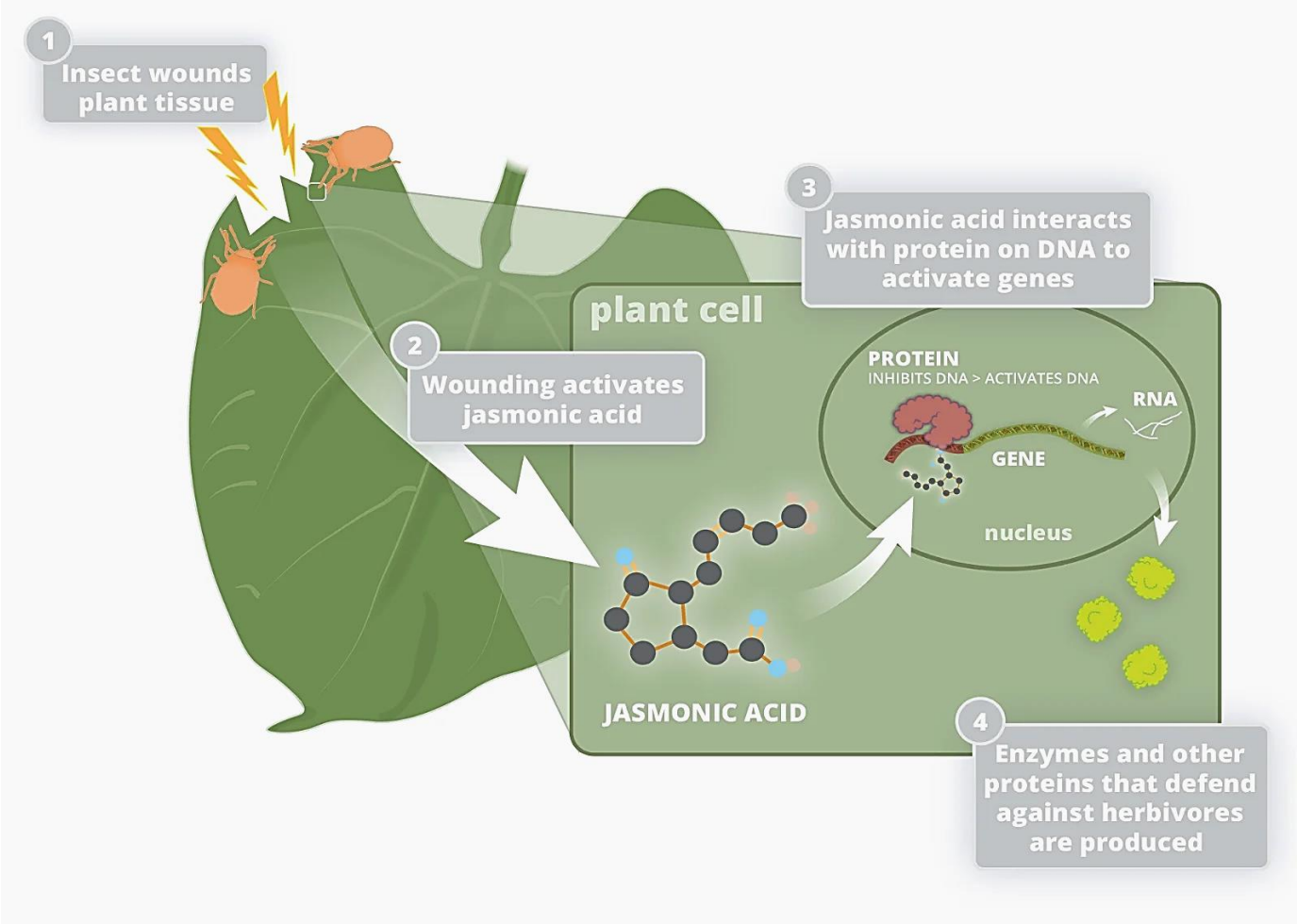
Jasmonates

Herbivores – insects, other animals, fungi – Necrotrophic organisms

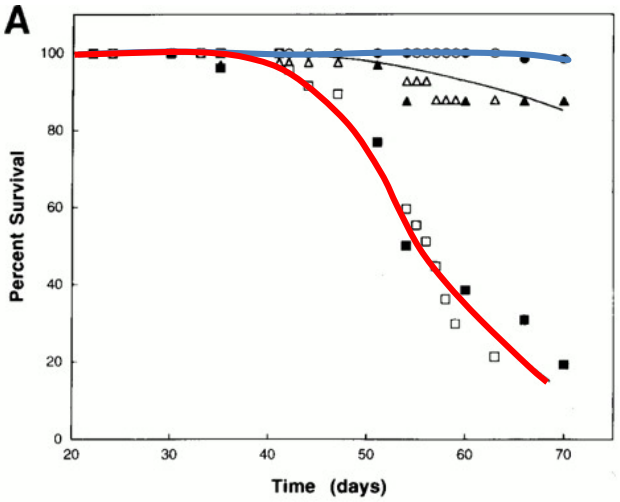
Jasmonate induces many plant processes



Jasmonate induces plant defence



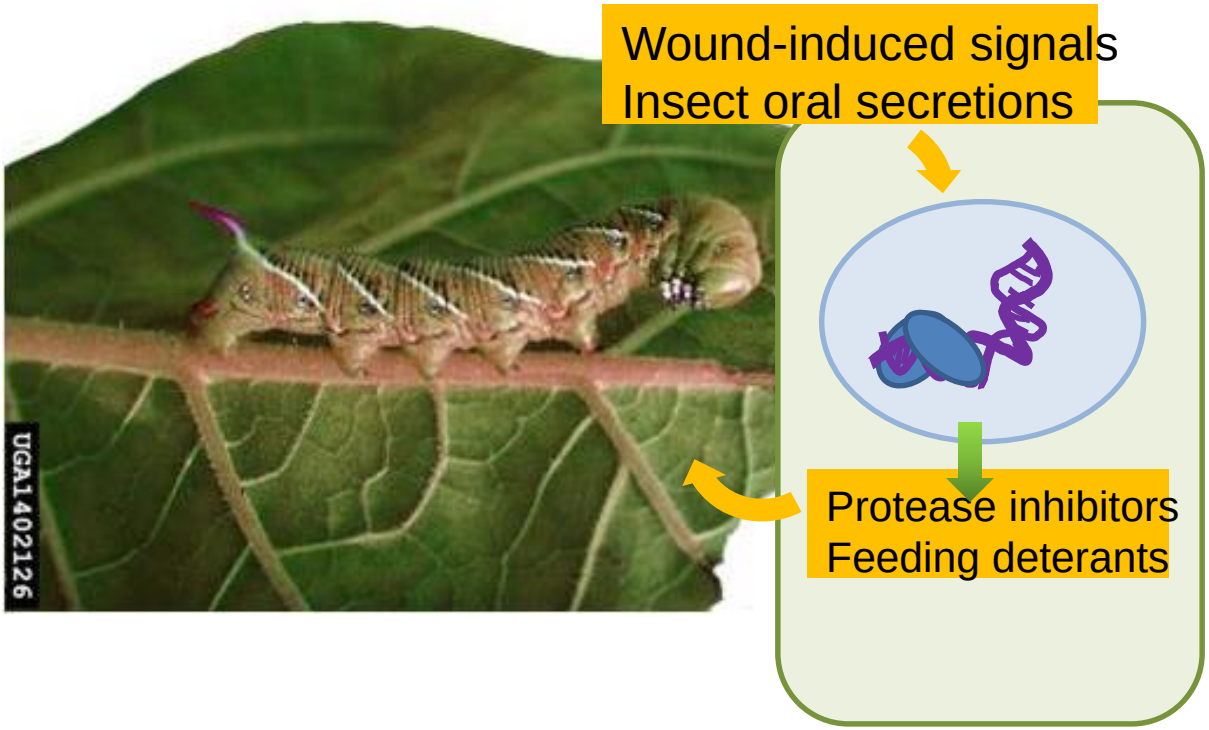
Jasmonate signaling contributes to defense against herbivory



When exposed to hungry fly larvae, plants unable to produce JA have low rates of survival.

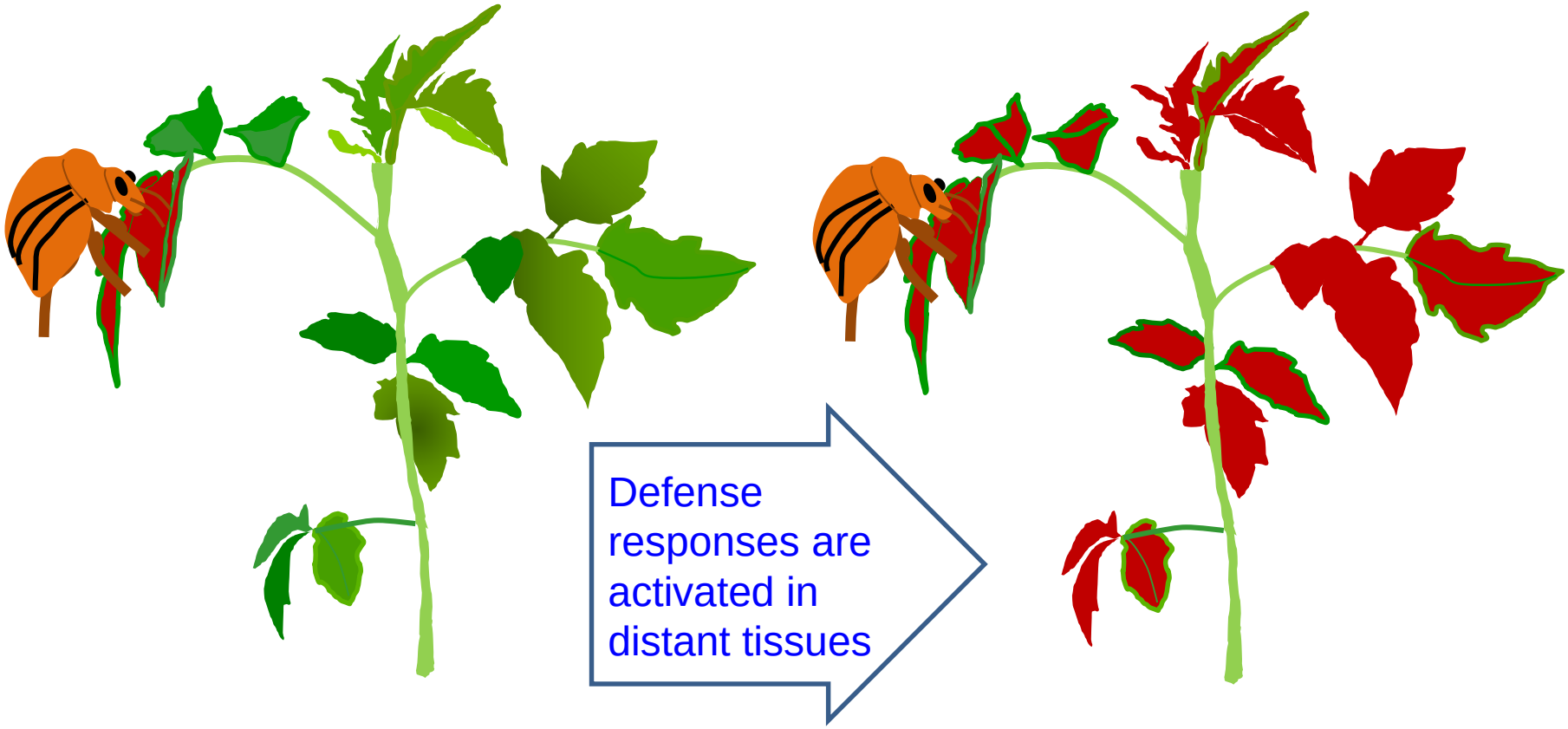
WT Mutant without JA

Jasmonates induce the expression of anti-herbivory chemicals

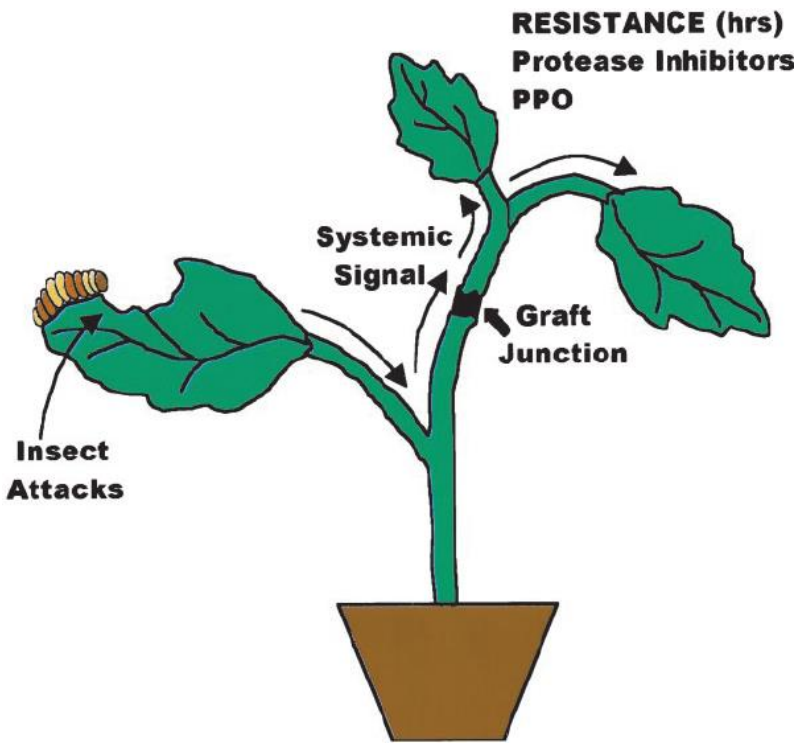


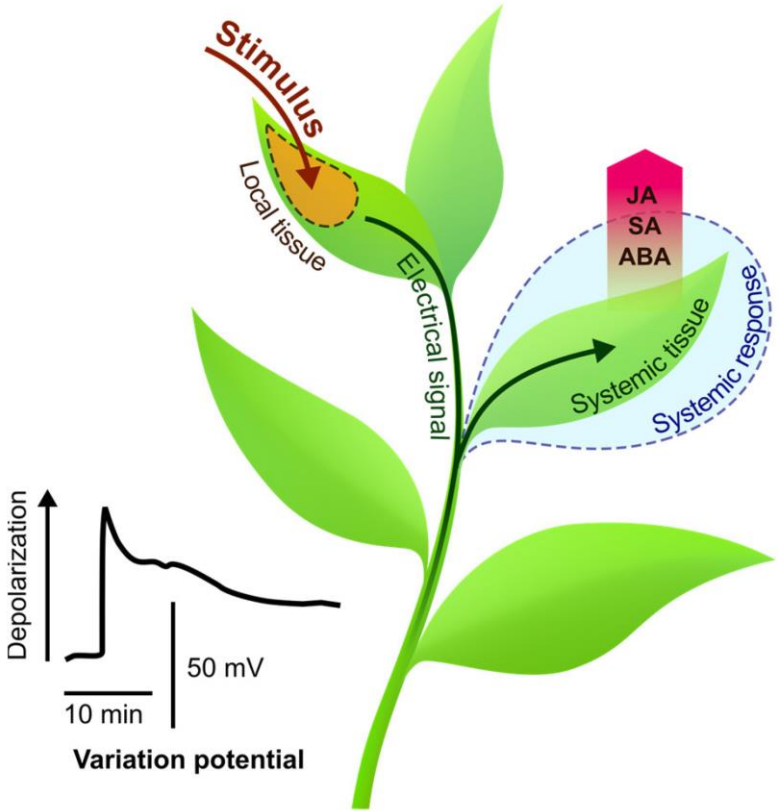
When a plant leaf is wounded, it triggers a complex cascade of signals, including electrical, chemical (like jasmonic acid and ethylene), and hydraulic signals, that lead to both local and systemic defense responses, including tissue repair and regeneration

Jasmonates contribute to systemic defense responses



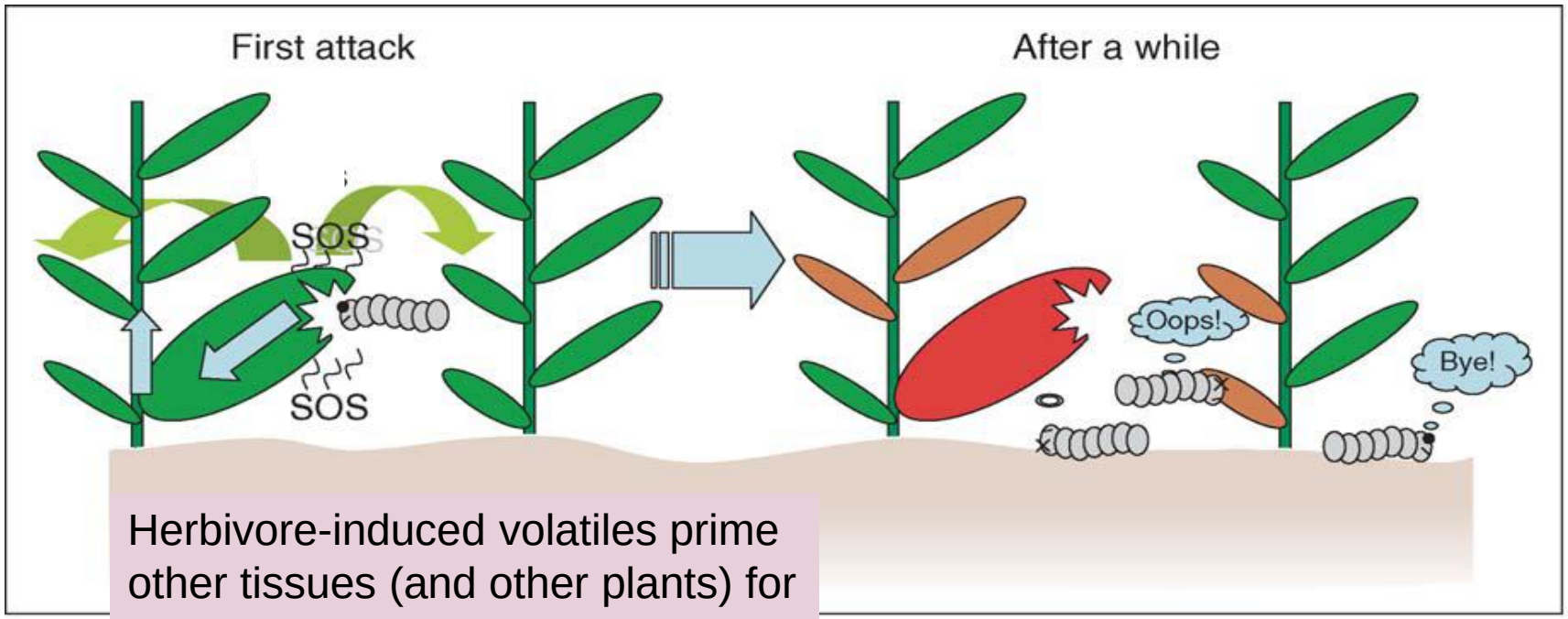
Systemic Resistance





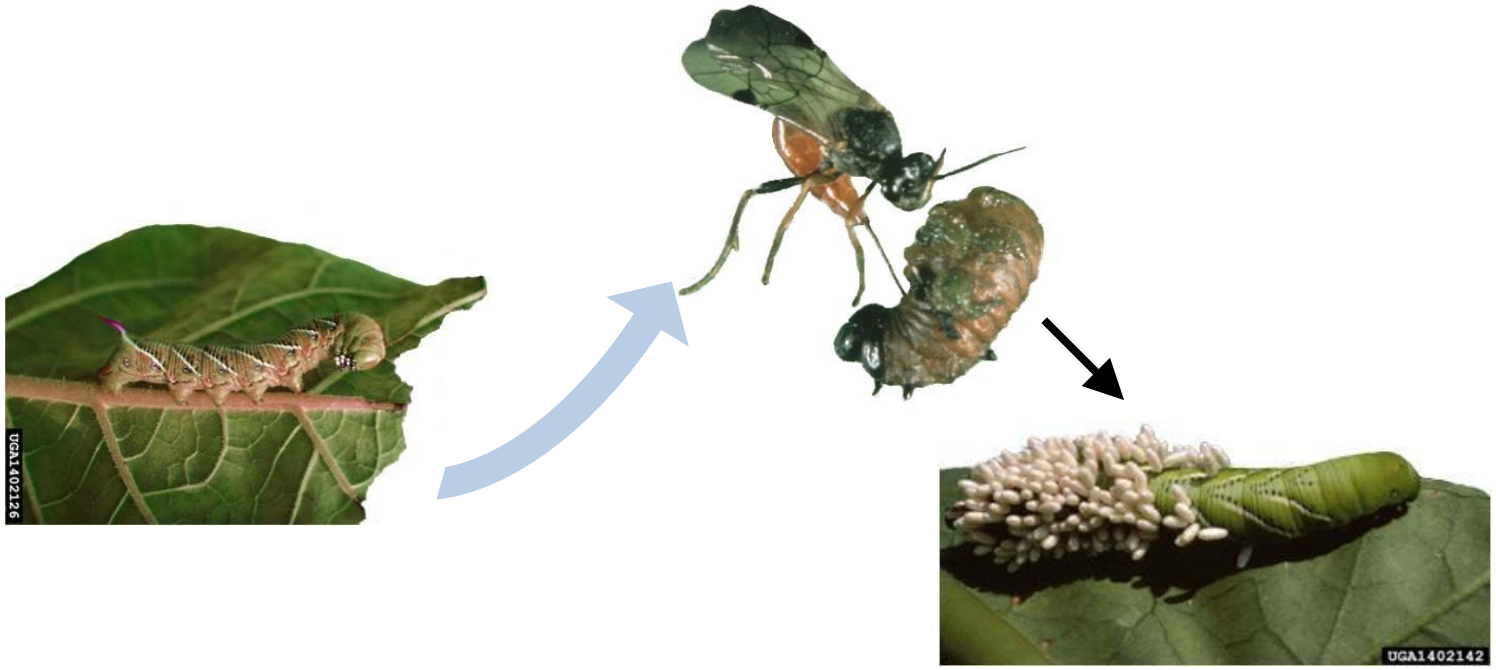
Integration of Electrical Signals and Phytohormones in the Control of Systemic Response

Jasomonates stimulate production of volatile signaling compounds

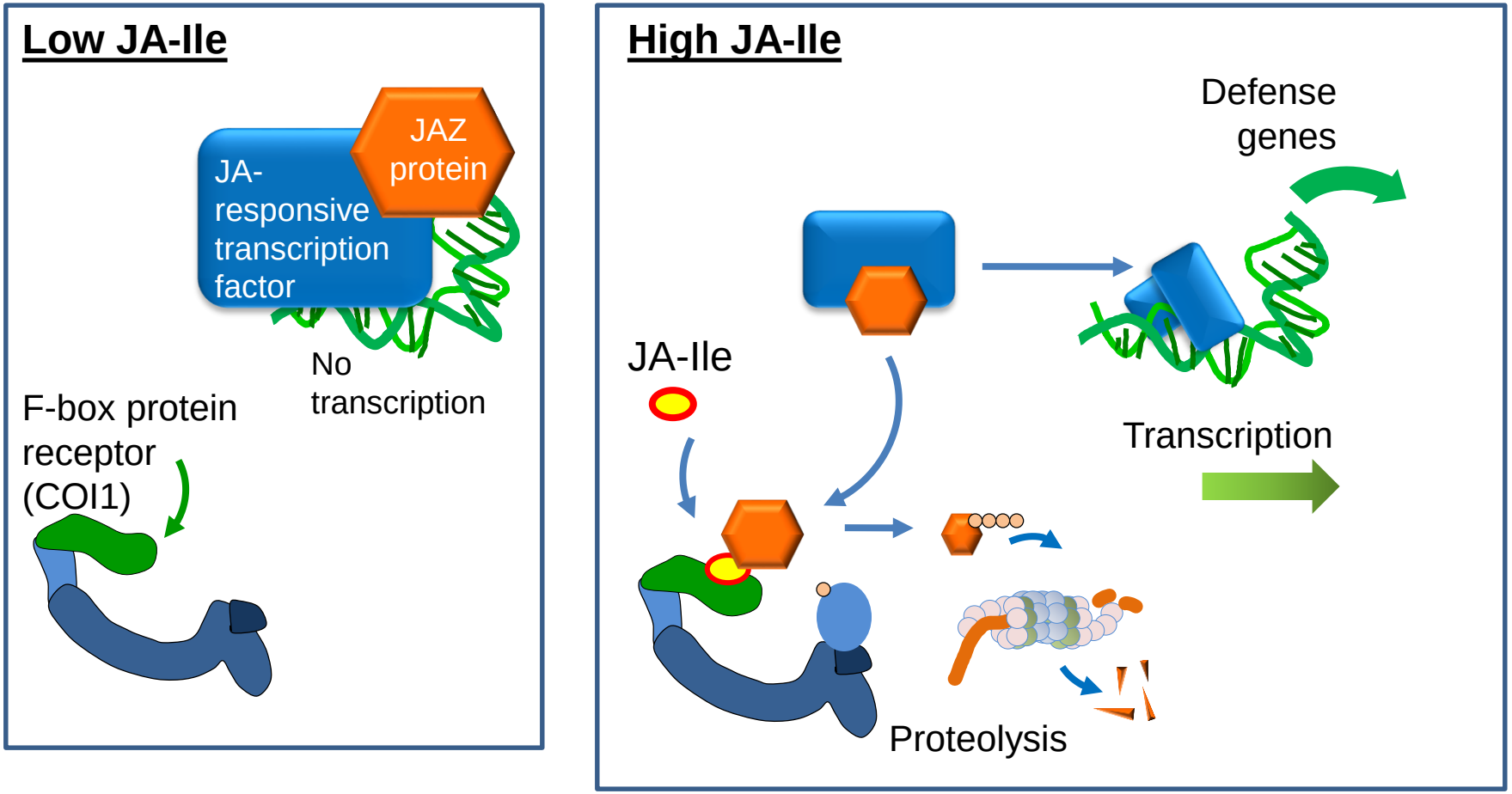


Herbivore-induced volatiles prime other tissues (and other plants) for attack making them unpalatable (indicated in red).

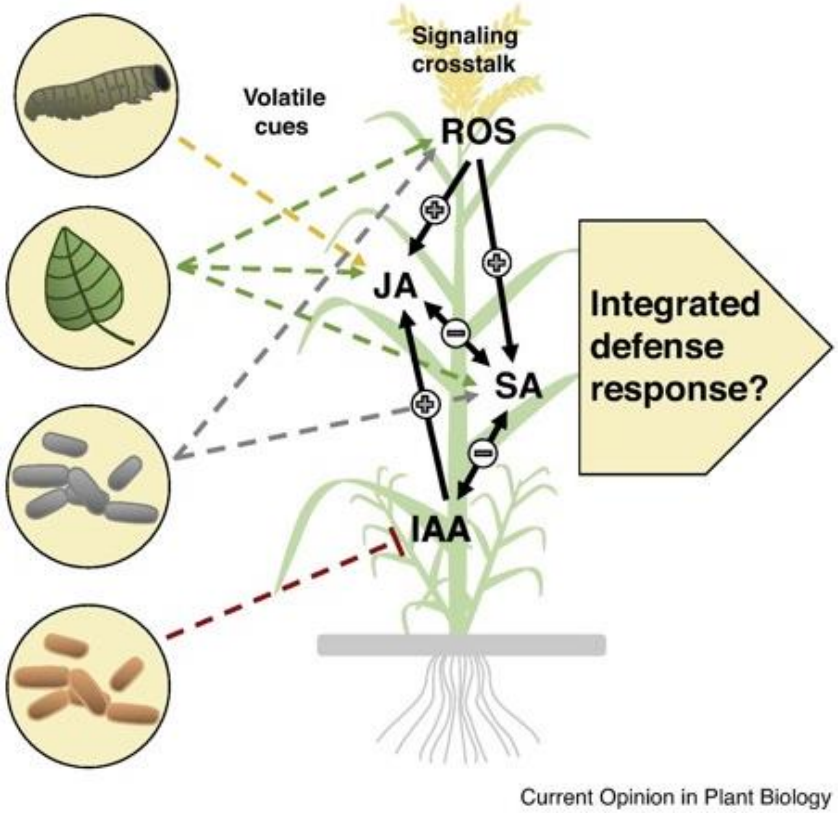
Herbivore-induced volatiles are recognized by carnivorous and parasitoid insects



JA-induced changes in gene expression



Volatiles as inducers and suppressors of plant defense and immunity — perception and signaling

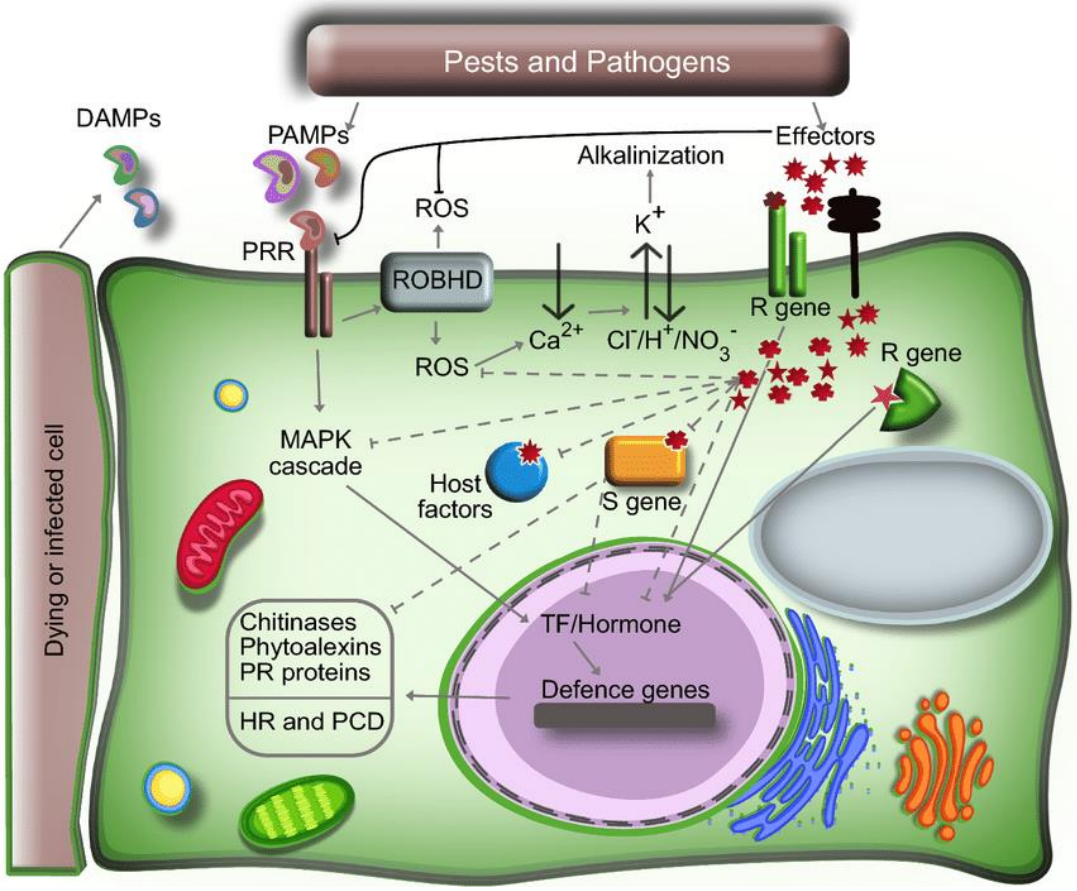


When plants are under attack by herbivores and microbes, **running away is not an option**.

As a defence, **plants produce volatile organic compounds** (VOCs) that repel herbivores, attract enemies of the herbivores, or alarm surrounding plants;

VOCs mostly viewed as positive regulators in the **plant defence system**. They are cues for hormonal pathways, including jasmonate (JA), salicylic acid (SA) and auxin (IAA) signaling, and **trigger plant signalling cascades**.

Plant pathogen interaction



Plant disease resistance responses are induced upon **recognition of PAMPs and/or effectors** from pests and pathogens **by plant PRR proteins**. This recognition leads to the **reprograming of transcriptional regulation of defence genes and of plant hormonal responses**. Some effectors specifically bind to, induce and/or decrease gene expression of target genes or protein activity. Pathogens can also negatively impact plant growth and developmental-associated processes (e.g. transcriptional expression of genes and negative regulation of signalling pathways)