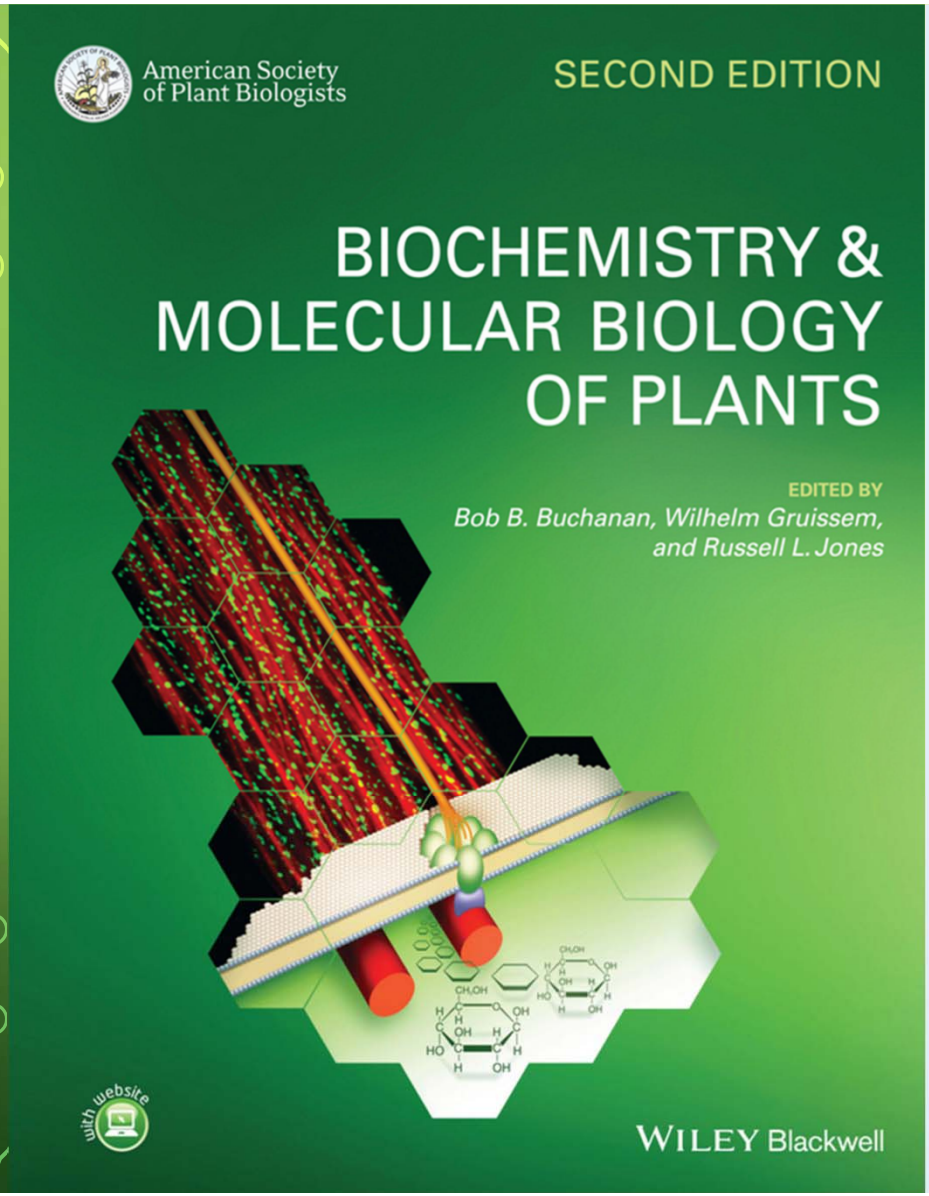

In the name of
God

PHYSIOLOGY OF GROWTH AND DEVELOPMENT

Mahdiyeh Gholami, Ph.D



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Cover image: The illustration on the cover shows a fluorescence image of an *Arabidopsis* epidermal cell depicting the localization of cellulose synthase (CESA, green) and microtubules (red). The overlying graphic shows how the synthesis of a cellulose microfibril (yellow) is related to the CESA complex, portrayed as a rosette of six light green particles embedded in the plasma membrane that are attached to a microtubule by a purple linker protein (CSI1). Fluorescent image courtesy of Chris Somerville and Trevor Yeats, Energy Biosciences Institute, University of California, Berkeley.
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BRIEF CONTENTS

I COMPARTMENTS

1 Membrane Structure and Membranous Organelles 2
2 The Cell Wall 45
3 Membrane Transport 111
4 Protein Sorting and Vesicle Traffic 151
5 The Cytoskeleton 191

II CELL REPRODUCTION

6 Nucleic Acids 240
7 Amino Acids 289
8 Lipids 337
9 Genome Structure and Organization 401
10 Protein Synthesis, Folding, and Degradation 438
11 Cell Division 476

III ENERGY FLOW

12 Photosynthesis 508
13 Carbohydrate Metabolism 567
14 Respiration and Photorespiration 610

IV METABOLIC AND DEVELOPMENTAL INTEGRATION

15 Long-Distance Transport 658
16 Nitrogen and Sulfur 711
17 Biosynthesis of Hormones 769
18 Signal Transduction 834
19 Molecular Regulation of Reproductive Development 872
20 Senescence and Cell Death 925

V PLANT ENVIRONMENT AND AGRICULTURE

21 Responses to Plant Pathogens 984
22 Responses to Abiotic Stress 1051
23 Mineral Nutrient Acquisition, Transport, and Utilization 1101
24 Natural Products 1132

IV METABOLIC AND DEVELOPMENTAL INTEGRATION

15 Long-Distance Transport 658

Introduction 658

15.1 Selection pressures and long-distance transport systems 658

15.2 Cell biology of transport modules 664

15.3 Short-distance transport events between xylem and nonvascular cells 668

15.4 Short-distance transport events between phloem and nonvascular cells 673

15.5 Whole-plant organization of xylem transport 691

15.6 Whole-plant organization of phloem transport 696

15.7 Communication and regulation controlling phloem transport events 705

Summary 710

16 Nitrogen and Sulfur 711

Introduction 711

16.1 Overview of nitrogen in the biosphere and in plants 711

16.2 Overview of biological nitrogen fixation 715

16.3 Enzymology of nitrogen fixation 715

16.4 Symbiotic nitrogen fixation 718

16.5 Ammonia uptake and transport 735

16.6 Nitrate uptake and transport 735

16.7 Nitrate reduction 739

16.8 Nitrite reduction 744

16.9 Nitrate signaling 745

16.10 Interaction between nitrate assimilation and carbon metabolism 745

16.11 Overview of sulfur in the biosphere and plants 746

16.12 Sulfur chemistry and function 747

16.13 Sulfate uptake and transport 750

16.14 The reductive sulfate assimilation pathway 752

16.15 Cysteine synthesis 755

16.16 Synthesis and function of glutathione and its derivatives 758

16.17 Sulfated compounds 763

16.18 Regulation of sulfate assimilation and interaction with nitrogen and carbon metabolism 764

Summary 767

17 Biosynthesis of Hormones 769

Introduction 769

17.1 Gibberellins 769

17.2 Abscisic acid 777

17.3 Cytokinins 785

17.4 Auxins 795

17.5 Ethylene 806

17.6 Brassinosteroids 810

17.7 Polyamines 818

17.8 Jasmonic acid 821

17.9 Salicylic acid 826

17.10 Strigolactones 830

Summary 833

18 Signal Transduction 834

Introduction 834

18.1 Characteristics of signal perception, transduction, and integration in plants 834

18.2 Overview of signal perception at the plasma membrane 838

18.3 Intracellular signal transduction, amplification, and integration via second messengers and MAPK cascades 843

18.4 Ethylene signal transduction 847

18.5 Cytokinin signal transduction 850

18.6 Integration of auxin signaling and transport 852

18.7 Signal transduction from phytochromes 857

18.8 Gibberellin signal transduction and its integration with phytochrome signaling during seedling development 861

18.9 Integration of light, ABA, and CO_2 signals in the regulation of stomatal aperture 866

18.10 Prospects 870

Summary 870

19 Molecular Regulation of Reproductive Development 872

Introduction 872

19.1 The transition from vegetative to reproductive development 872

19.2 The molecular basis of flower development 881

19.3 The formation of male gametes 889

19.4 The formation of female gametes 897

19.5 Pollination and fertilization 902

19.6 The molecular basis of self-incompatibility 908

19.7 Seed development 913

Summary 923

20 Senescence and Cell Death 925

Introduction 925

20.1 Types of cell death 925

20.2 PCD during seed development and germination 930

20.3 Cell death during the development of secretory bodies, defensive structures and organ shapes 932

20.4 PCD during reproductive development 937

20.5 Senescence and PCD in the terminal development of leaves and other lateral organs 940

20.6 Pigment metabolism in senescence 948

20.7 Macromolecule breakdown and salvage of nutrients in senescence 951

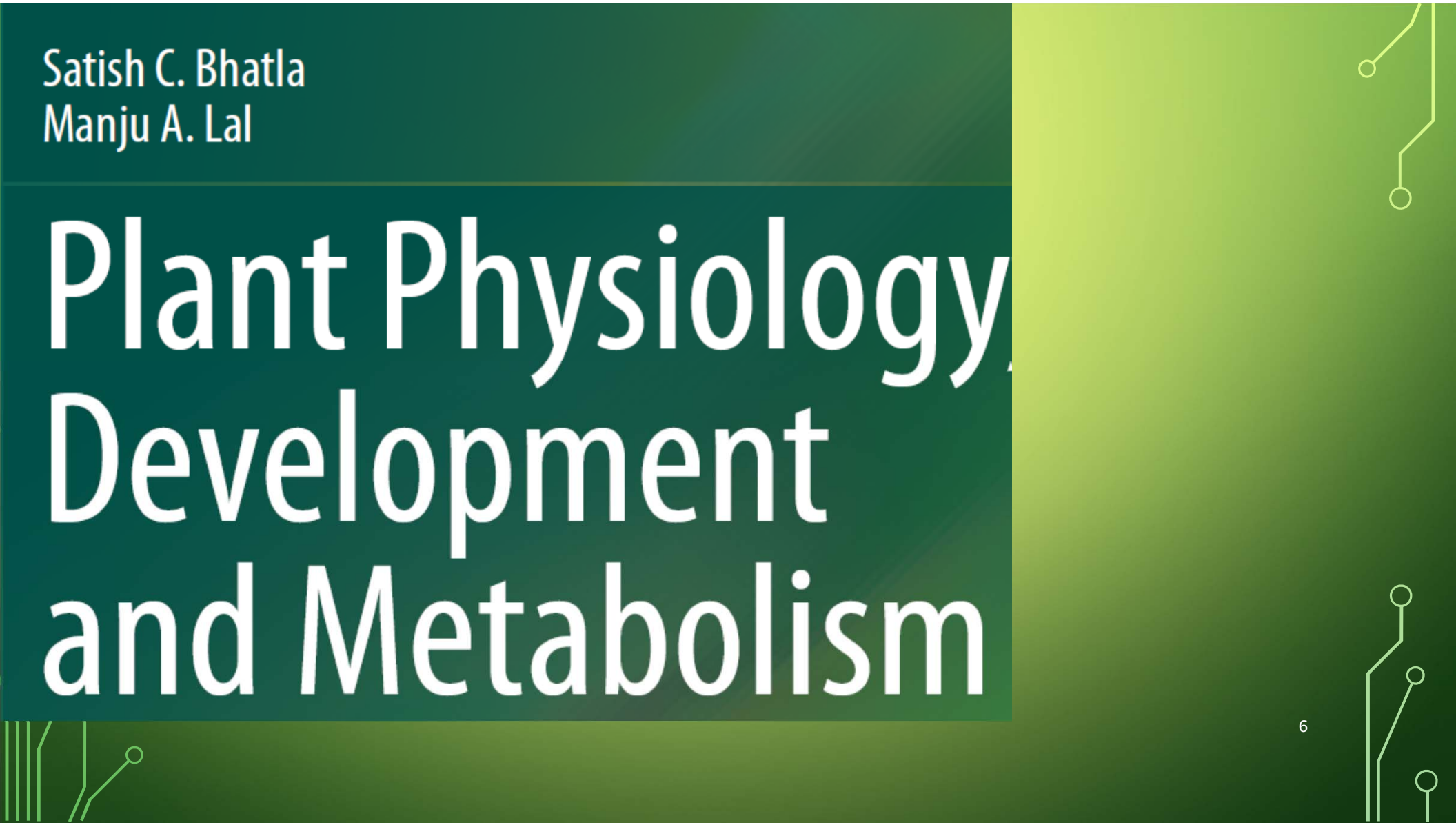
20.8 Energy and oxidative metabolism during senescence 957

20.9 Environmental influences on senescence and cell death I: Abiotic interactions 961

20.10 Environmental influences on senescence and cell death II: PCD responses to pathogen attack 964

20.11 Plant hormones in senescence and defense-related PCD 974

Summary 982


Satish C. Bhatla
Manju A. Lal
Plant Physiology,
Development
and Metabolism

Part III Development

13 Light Perception and Transduction 363
13.1 Light Absorption by Pigment Molecules 364
13.1.1 Quantitative Requirement for Pigment Excitation 365
13.2 The Nature of Light 366
13.3 Absorption and Action Spectra 368
13.4 Light Parameters That Influence Plant Responses 368
13.5 Light Absorption Depends on Leaf Anatomy and Canopy Structure 369
13.6 Photoreceptors 370

14 Plant Growth Regulators: An Overview 391
14.1 Plant Growth Regulators (PGRs) 391
14.2 Experimental Approaches to Understand Perception and Transmission of Hormone Action 392
14.3 Estimation and Imaging of Hormones in Plant Tissues 395
14.3.1 Nanosensors for *In Planta* Rapid Detection of Plant Hormones 396
14.3.2 Fluorescent Plant Hormone Biosensors 396
14.4 Summary 397
Further Reading 398

15 Auxins 399
15.1 Discovery of Auxin 399

17

Biosynthesis of Hormones

Gerard Bishop, Hitoshi Sakakibara, Mitsunori Seo, and Shinjiro Yamaguchi



Introduction

The Meaning of a Plant Hormone

Plant hormones are *a group of naturally occurring, organic substances which influence physiological processes at low concentrations.*

A number of biological processes in plants are affected by internal and external stimuli through the involvement of plant hormones.

Biosynthesis of plant hormones can occur at different morphological and cytological locations, and their actions may be observed at their sites of production, or they may get translocated to some distant target tissues to evoke a response.

Generally, plant hormones are produced in meristems, leaves, and developing fruits.

Different plant hormones can cause opposite effects on a particular developmental process at varying concentrations.

Antagonistic and synergistic interactions among various plant hormones further add to the complexity of hormonal actions in higher plants.

Different hormones are able to influence the biosynthesis of some other hormones or may interfere with their signaling mechanisms.

A hormone can evoke different responses in different tissues or at different times of development in the same tissue.

Different tissues require varying amounts of hormones of specific kinds for their development. Such differences are referred to as differences in sensitivity.

Plant Growth Regulators (PGRs)

PGRs are a broader category of compounds which include plant hormones, their synthetic analogs, inhibitors of hormone biosynthesis, and blockers of hormone receptors. Precisely, plant growth regulators (PGRs) may be defined as naturally occurring or synthetic compounds which affect metabolic and developmental processes in higher plants, mostly at low concentrations. PGRs do not possess any nutritive or phytotoxic value.

"True" PGRs are compounds which directly and specifically interfere with the actions of naturally occurring hormonal systems in higher plants. "Atypical" PGRs, however, act by bringing about a local or transient phytotoxic effect.

Principal PGRs modulating plant development can be grouped into four categories

PGRs derived from **isoprenoid** pathway, starting from isopentenyl diphosphate. This includes cytokinins, gibberellins, brassinosteroids, abscisic acid, and strigolactones.

PGRs derived from amino acids, viz., indole-3-acetic acid (IAA), serotonin and melatonin, derived from tryptophan; ethylene derived from methionine, and polyamines derived from **arginine** or **ornithine**; nitric oxide derived from arginine, salicylic acid (SA), .

PGRs derived from lipids, e.g., jasmonic acid derived from α-linolenic acid.

Others (of varied nature). It includes hydrogen sulfide (H_2S), nitric oxide, carbon monoxide (CO), karrikins, and systemins.

REGULATORY FACTORS IN HORMONE ACTION:

LEVEL, LOCATION AND SIGNAL TRANSDUCTION

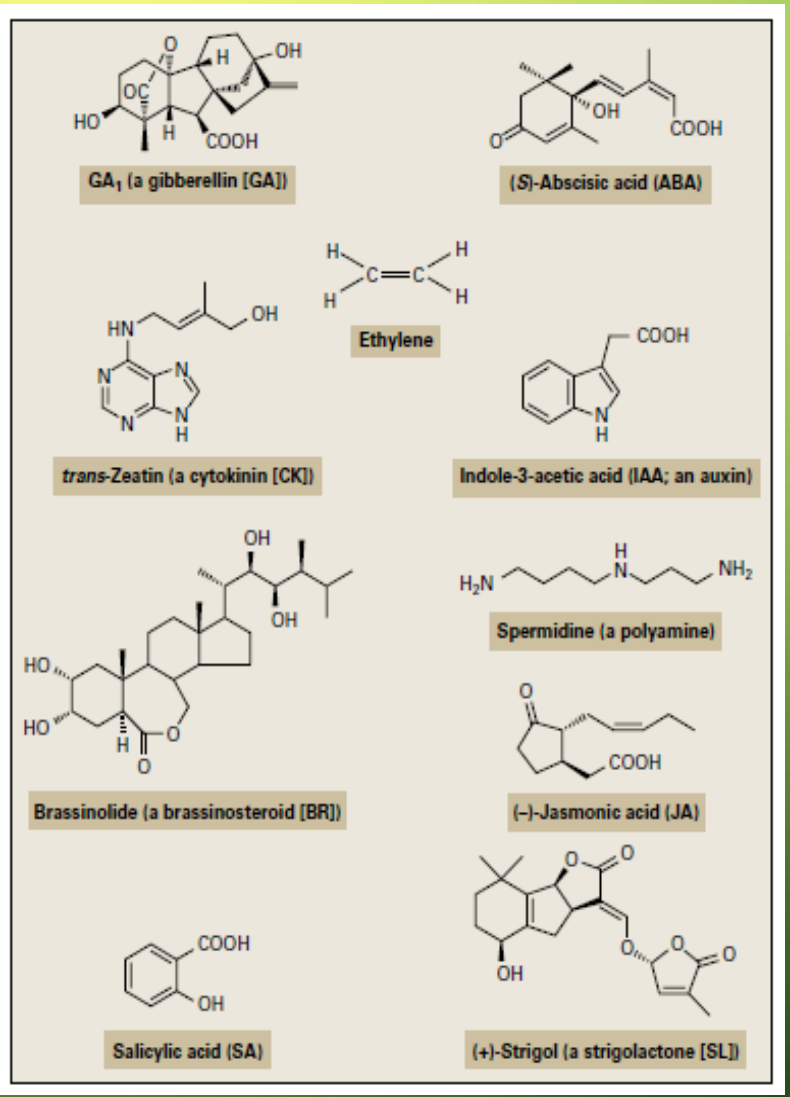
The way in which a plant hormone influences growth and development depends on:

1) The amount present: this is regulated by biosynthesis, degradation and conjugation.

2) The location of the hormone: this is affected by movement or transport.

3) The sensitivity (or responsiveness) of the tissue: this involves the presence of receptors and signal-transduction chain components.

FIGURE 17.1 *Structures of representatives from the 10 types of plant hormones discussed in this chapter.*



17.1 Gibberellins

Gibberellins (GAs) were first isolated from the fungus *Gibberella fujikuroi* in 1926 by the Japanese scientist Eiichi Kurosawa, who was investigating the causative agent of *bakanae*, the "foolish seedling" disease of *Oryza sativa*, which was frequently responsible for major reductions in grain yield.



FIGURE 17.2 O. sativa *seedling infected with* Bakanae *(foolish seedling) disease caused by the fungal pathogen,* Gibberella fujikuroi.

The compounds proved to be identical, and the structure of gibberellic acid, now known as GA3, was elucidated in 1956. Shortly thereafter, GAs were shown to be endogenous components of plants, and it became apparent that they were not merely an interesting group of fungal metabolites but endogenous regulators of many aspects of higher plant growth and development.

1.1 Among more than 100 GAs identified from plants, only a few act as bioactive hormones

- Among more than 100 GAs identified from plants, only a few function as bioactive hormones.
- Many nonbioactive GAs present in plants are biosynthetic precursors of bioactive forms or are inactivated metabolites.
- The most commonly found bioactive GAs in plants are GA1 and GA4.

GA1 and GA4, both have a hydroxyl group on c-3β, a carboxyl group on c-6, and a γ-lactone between c-4 and c-10.

The recent identification of a soluble GA receptor, GIBBERELLIN INSENSITIVE DWARF 1 (GID1), from *O. sativa* and its homologs in *Arabidopsis* and *Hordeum vulgare* has demonstrated that these structural requirements for bioactive GAs are reflected in their affinity for the GID1 receptor.

GA3 and GA7, 1,2-double bond analogs of GA1 and GA4, respectively, are also active per se in plants.

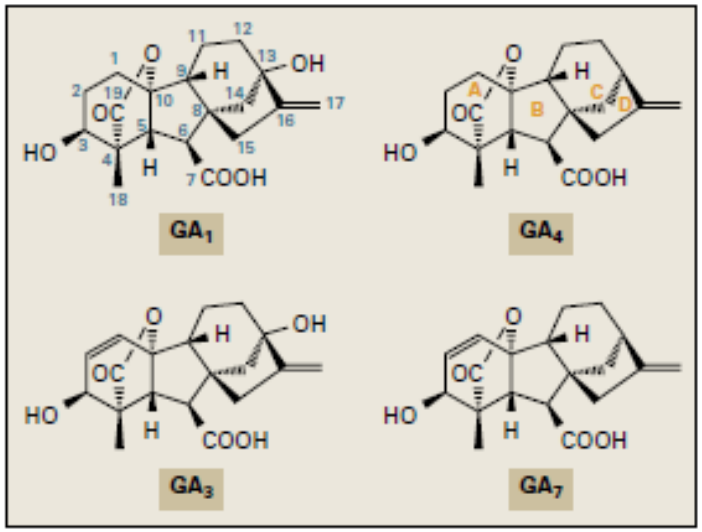


FIGURE 17.3 *Structures of bioactive GAs in plants.*

1.2 Bioactive GAs affect many aspects of plant growth and development

- GAs are best known for their influence on:

stem elongation and leaf growth



FIGURE 17.4 *Effect of GA_3 on stem elongation of dwarf* P. sativum *seedlings: (left) control plants, (right) plants seven days after treatment with 5 μg of GA_3.*
Source: A. Crozier, University of Glasgow, UK; previously unpublished.

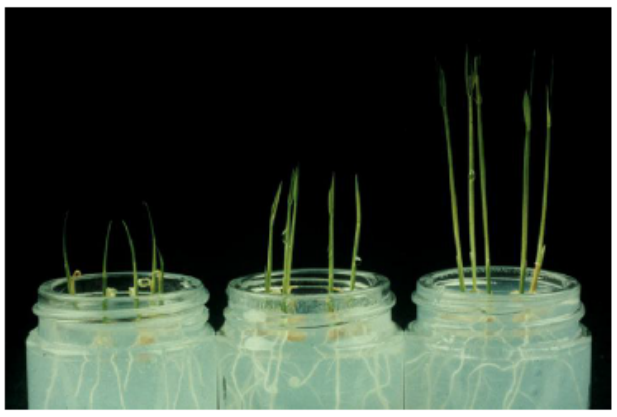


FIGURE 17.5 *Promotion of Tanginbozu dwarf* O. sativa (d35) *leaf sheath elongation 3 days after treatment with GA_3: (left) control; (center) 100 pg of GA_3 per seedling; (right) 1 ng of GA_3 per seedling.*
Source: A. Crozier, University of Glasgow, UK; previously unpublished.

Young rice plants growing in deep water show pronounced effect of gibberellin on internode elongation so that its foliage may remain above water in the field. Striking growth rates, as much as 25 cm per day, have been observed in rice plants under flooding conditions.

Partial submergence of rice plants is believed to reduce partial pressure of O_2 which triggers the biosynthesis of ethylene precursor ACC. ACC transported to the aerial parts gets converted to ethylene. Ethylene, in turn, reduces the level of ABA which acts as an antagonist to GA. Submerged rice tissue thus becomes more responsive (enhanced sensitivity) to endogenous GA, resulting in marked elongation of internodes.

- *Effects*

➢Stem growth

➢Bolting in long day plants

➢Induction of seed germination

➢Enzyme production during germination

➢Fruit setting and growth

➢Induction of maleness in dioecious flowers

Reproductive Development

- ✓ Gibberellins can substitute for long day or low temperature requirement for flowering in many plants. Thus, it is a component of flowering stimulus.
- ✓ In plants where flowers are unisexual, sex determination is regulated genetically as well as by environment and nutritional status. For example, in maize plants staminate flowers are observed in tassel. Exposure to short days and cool nights increases endogenous gibberellin levels and causes feminization of tassel flowers. On the other hand, in dicots, like cucumber and spinach, gibberellins have an opposite effect, i.e., they promote formation of staminate flowers.
- ✓ Further, stamens and/or flower receptacles have been identified as two potential sites for bioactive GA synthesis. It is suggested that GAs are transported from these organs to promote petal growth.
- ✓ GAs also play a very important role in pollen germination and pollen tube growth. Pollens in GA deficient mutants do not germinate unless treated with exogenous GA.

XYLOGENESIS AND CELLULOSE PRODUCTION

GA promotes cambial activity and xylogenesis in trees through the induction of cellulose synthase genes and phenylalanine ammonia lyase (PAL) activity involved in xylem development. The xylogenesis-promoting effect of GA is prevalent both in woody and nonwoody (e.g., carrot, celery) species.

TRICHROME EMERGENCE

The development of trichomes, which are modified single cell epidermal protrusions, is modulated by GA. Activity of GA is also required for cotton fiber elongation.

1.3 GA biosynthesis starts in the plastid

- The initial steps in biosynthesis of GAs involve formation of isopentenyl diphosphate and its conversion to geranylgeranyl diphosphate (GGDP), a common precursor for various terpenoids, such as carotenoids and phytol.

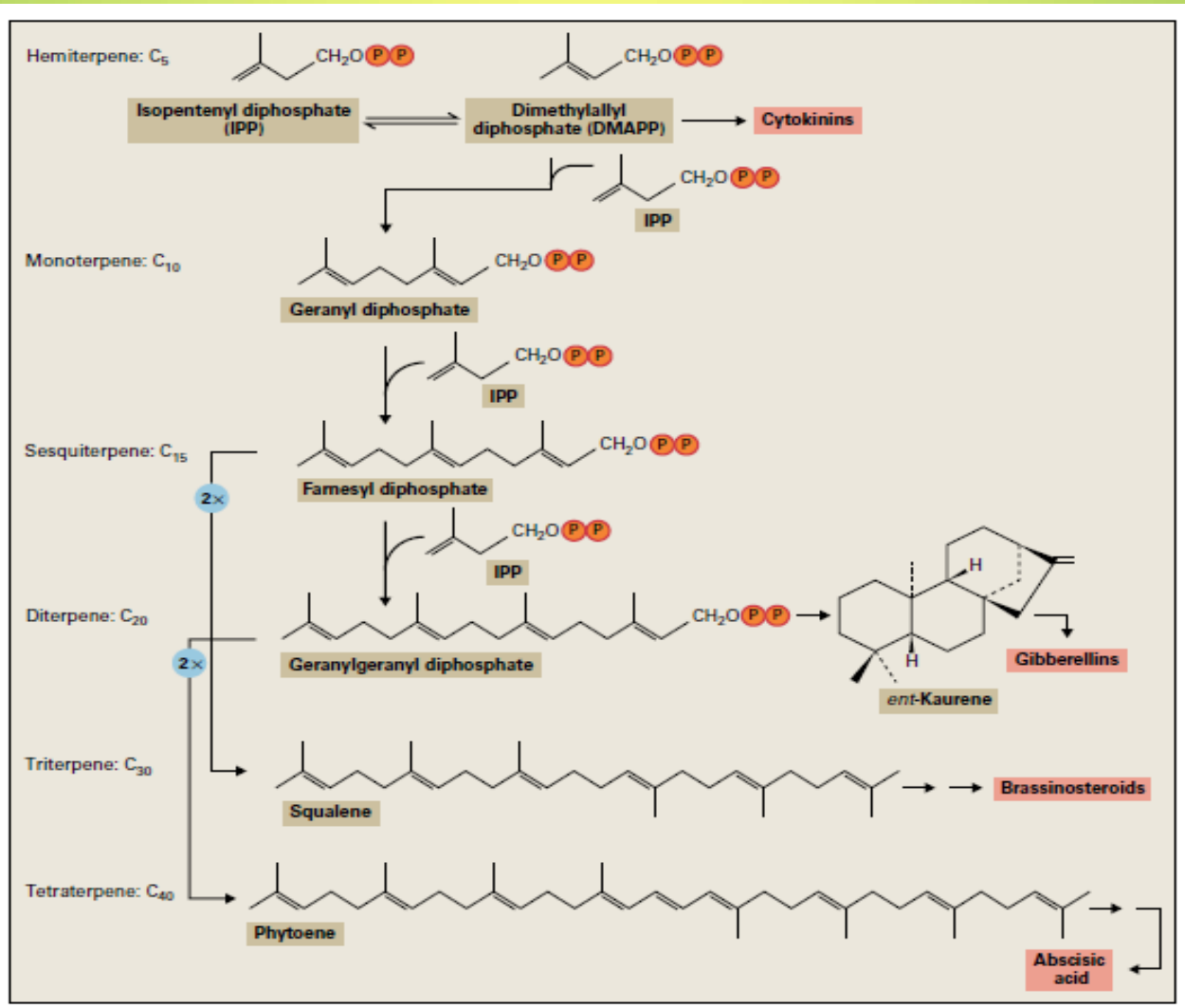
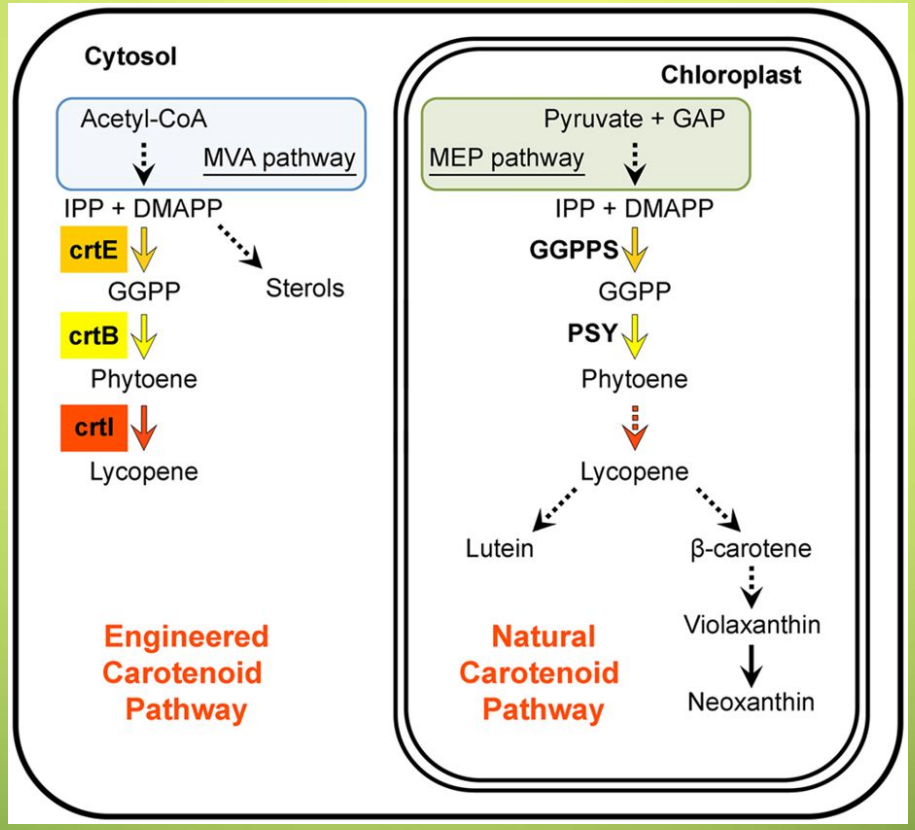


FIGURE 17.6 *Terpenoid biosynthesis pathway, showing biosynthetic origins of GAs as well as CKs, BRs, and ABA.*



The methylerythritol 4-phosphate (MEP- The non-mevalonate pathway) pathway is the recently discovered source of isoprenoid precursors isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) in most bacteria, some eukaryotic parasites, and the plastids of plant cells. The precursors lead to the formation of various isoprenoids having diverse roles in different biological processes. Some isoprenoids have important commercial uses. the glyceraldehyde 3-phosphate (GAP)-pyruvate pathway are alternative routes for the biosynthesis of the central isoprenoid precursor, isopentenyl diphosphate.

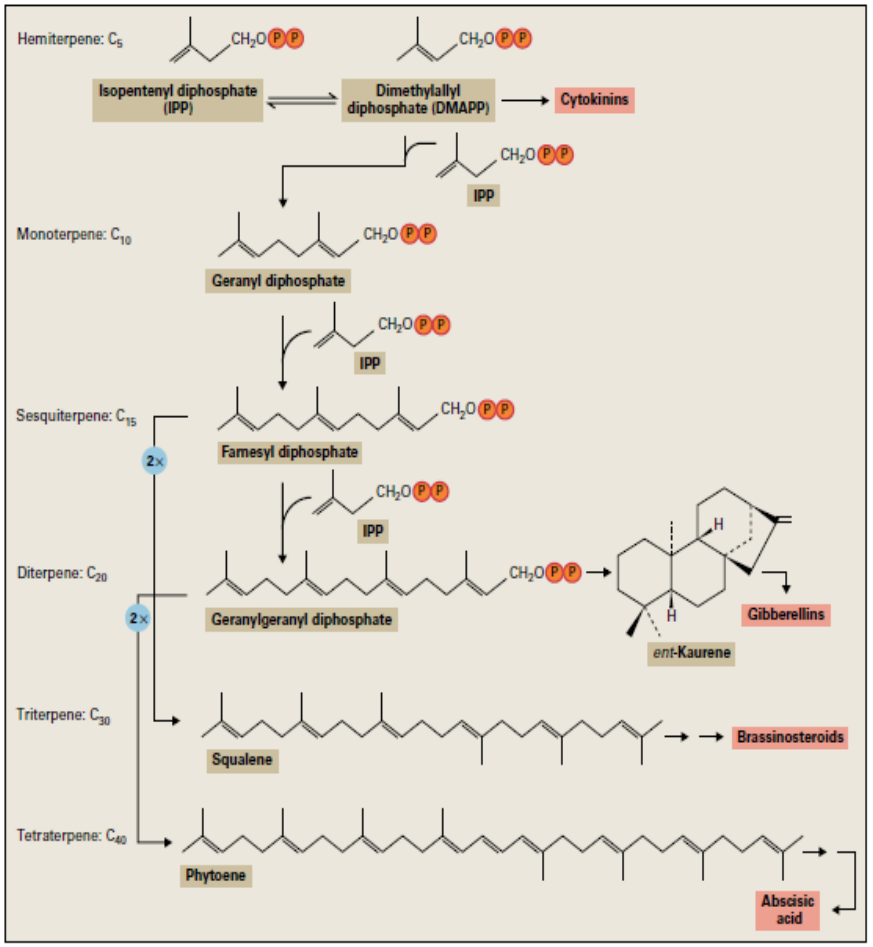
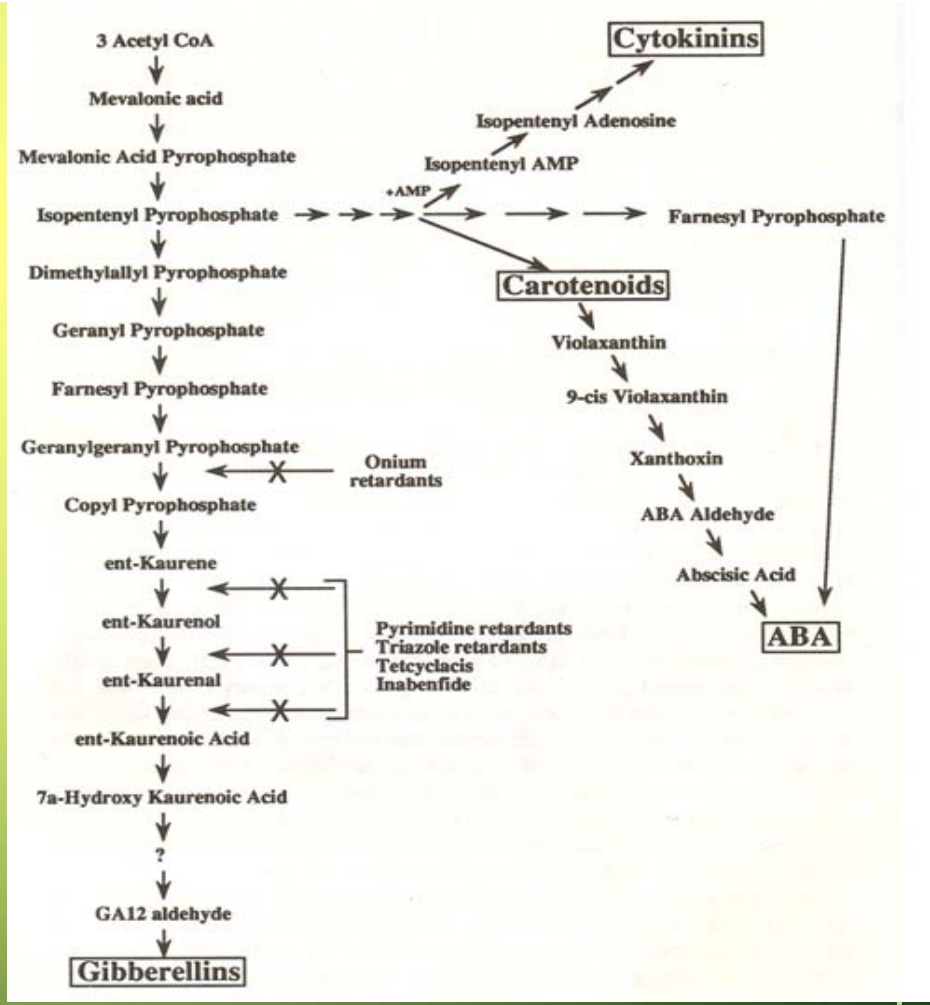


FIGURE 17.6 *Terpenoid biosynthesis pathway, showing biosynthetic origins of GAs as well as CKs, BRs, and ABA.*



methylerythritol phosphate (MEP) pathway in plastids provides the majority of isopentenyl diphosphate to GAs, whereas only a minor contribution is from the cytosolic mevalonate pathway.

1.4 In plants, the synthesis of *ent*-kaurene from geranylgeranyl diphosphate is catalyzed by two distinct enzymes

1.5 Cytochrome P450 monooxygenases convert *ent*-kaurene to GA12

ent-Kaurene is oxidized to GA12 by NADPH-dependent, membrane-bound CYP450 monooxygenases: *ent*-kaurene oxidase (KO, CYP701A) and *ent*-kaurenoic acid oxidase (KAO, CYP88A).

- KO catalyzes the three-step oxidation on C-19 to synthesize ent-kaurenoic acid.

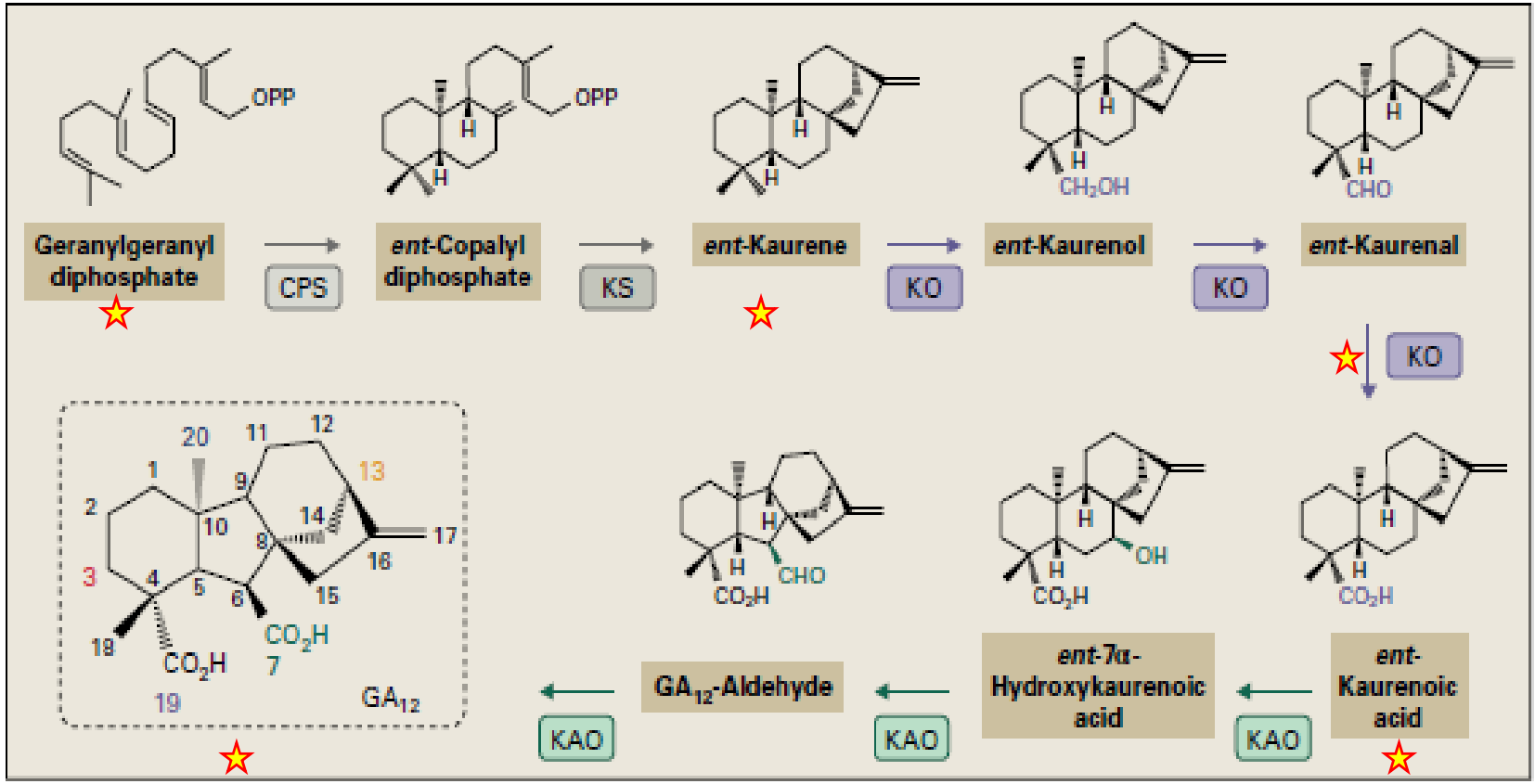


FIGURE 17.7 *Early stages of plant GA biosynthesis. CPS, ent-copalyl diphosphate synthase; KS, ent-kaurene synthase; KO, ent-kaurene oxidase; KAO, ent-kaurenoic acid oxidase. Colored carbon atoms on GA_{12} are oxidized for the synthesis of GA_1 (see also Fig. 17.9).*

The nitrogen-containing heterocyclic compounds

paclobutrazol, uniconazole, tetcyclasis, and ancymidol

retard plant growth by inhibiting KO.

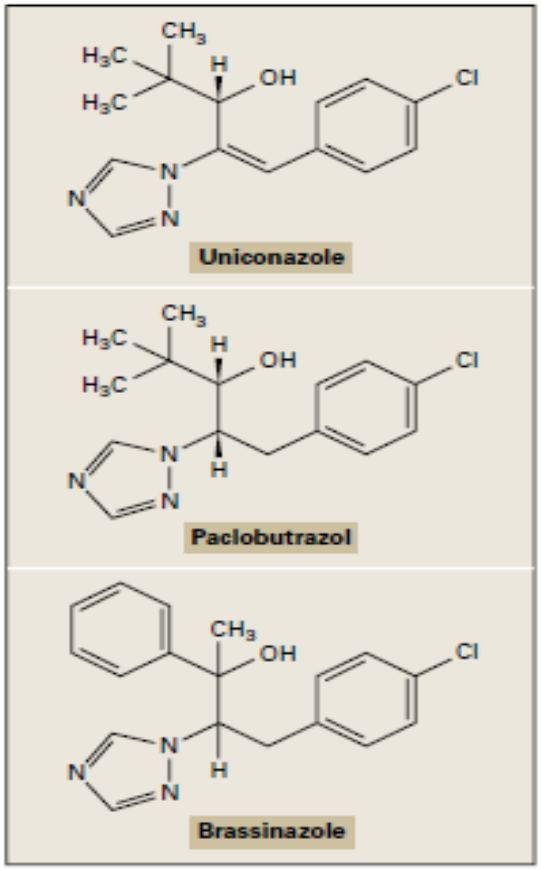
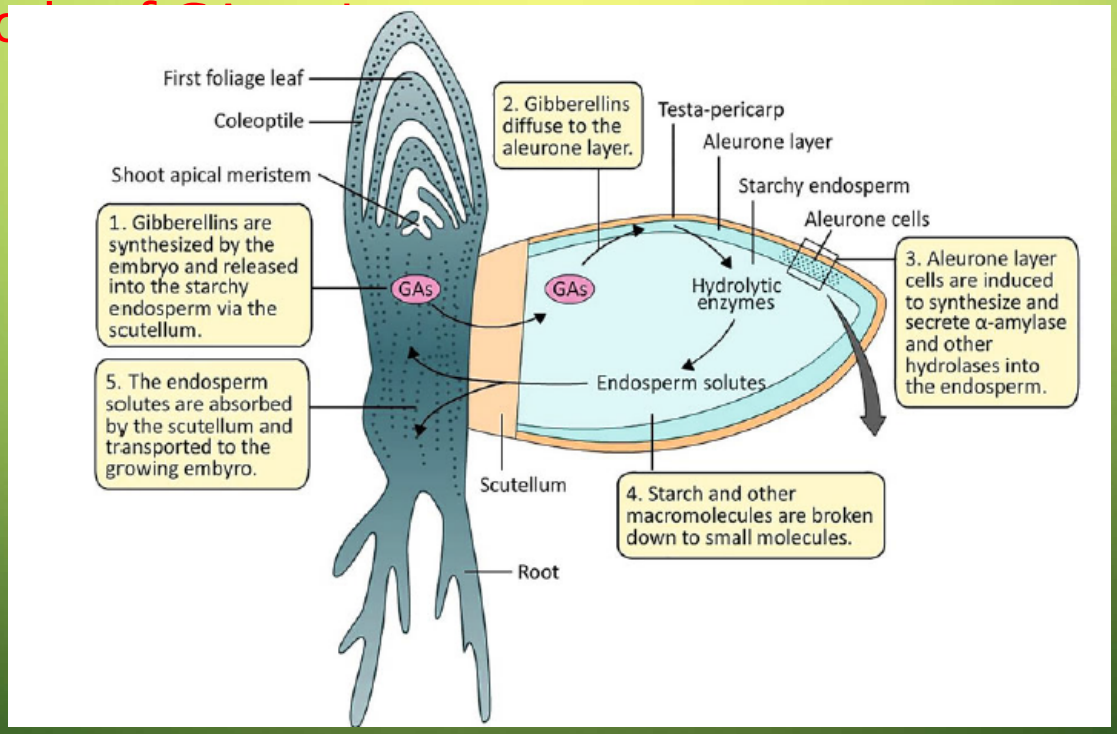


FIGURE 17.8 *Structures of uniconazole, paclobutrazol, and brassinazole. Uniconazole and paclobutrazol are triazoles that inhibit KO (see Fig. 17.7). However, uniconazole is not specific and also suppresses BR biosynthesis. The structurally related compound, brassinazole, is a strong BR inhibitor, blocking at least one CYP450 mediated step in the BR biosynthesis pathway (see Fig. 17.58). Its effect on GA biosynthesis is undetermined.*

MODE OF ACTION

- Gibberellin signal transduction with reference to stimulation of α-amylase synthesis in cereal aleurone layers has been extensively worked out to understand mo



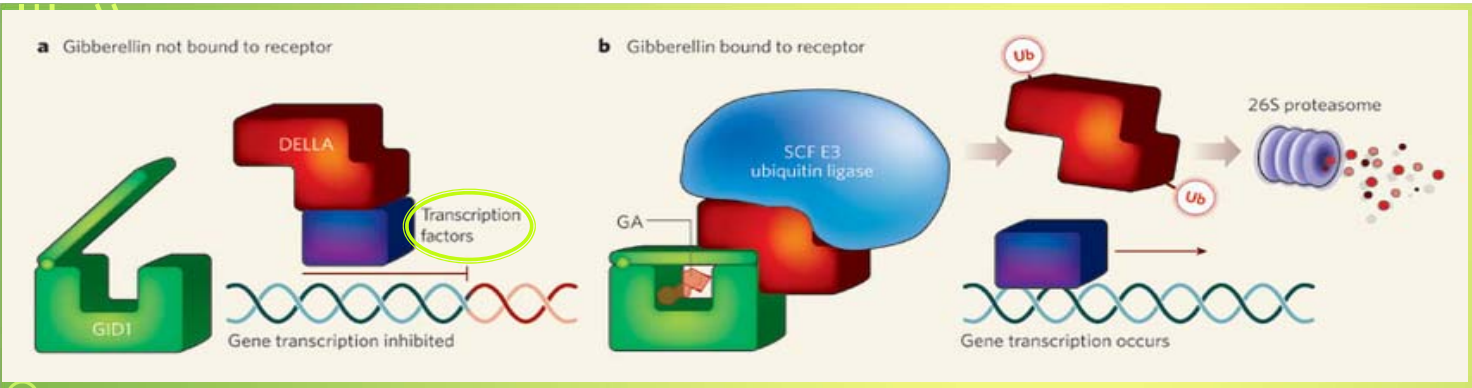
During seed germination, food stored in the endosperm is broken down and transported to the growing embryo by the activity of α and β amylases.

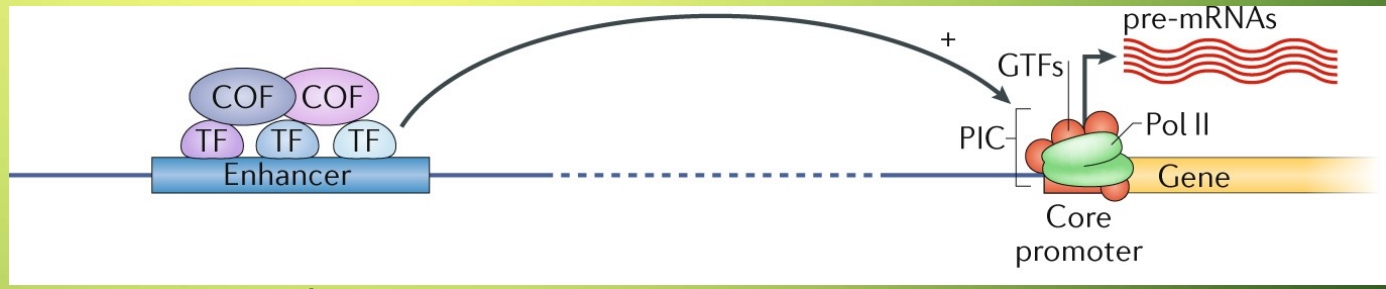
Main function of the aleurone layer is the synthesis and release of hydrolytic enzymes. Cells of the aleurone layer undergo programmed cell death thereafter.

When de-embryonated half seeds of barley are kept close to the excised embryo, they trigger the release of α-amylase, and starch is digested. GA_3 is known to substitute for embryos in stimulating starch degradation. It exerts its effect by enhancing the transcription of α-amylase mRNA.

Radioactive isotope-labeling studies have demonstrated that the stimulation of α-amylase activity by gibberellin involves de novo synthesis of the enzyme from amino acids rather than activation of pre-existing enzymes.

The stimulation of α-amylase gene expression by gibberellins is mediated by a special transcription factor which binds to the promoter region of α-amylase gene. Certain regulatory DNA sequences (gibberellin response elements) have been identified in the promoters that are involved in binding the protein.



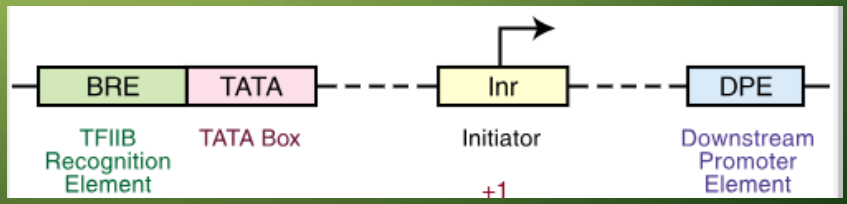


Basal promoter elements

Response modulus

TATA+initiator

Transcription



Synthesis of GA-MγB mRNA in the aleurone cells increases within 3 h of GA_3 application.

This happens to be several hours before the increase in α-amylase mRNA level.

Protein synthesis inhibitors have no effect on MγB mRNA production.

Thus, GA-MγB is a **primary response gene** or **early gene**.

α-amylase gene is a **secondary response gene** or **late gene,** as its transcription is blocked by protein synthesis inhibitors, like cycloheximide (CHI).

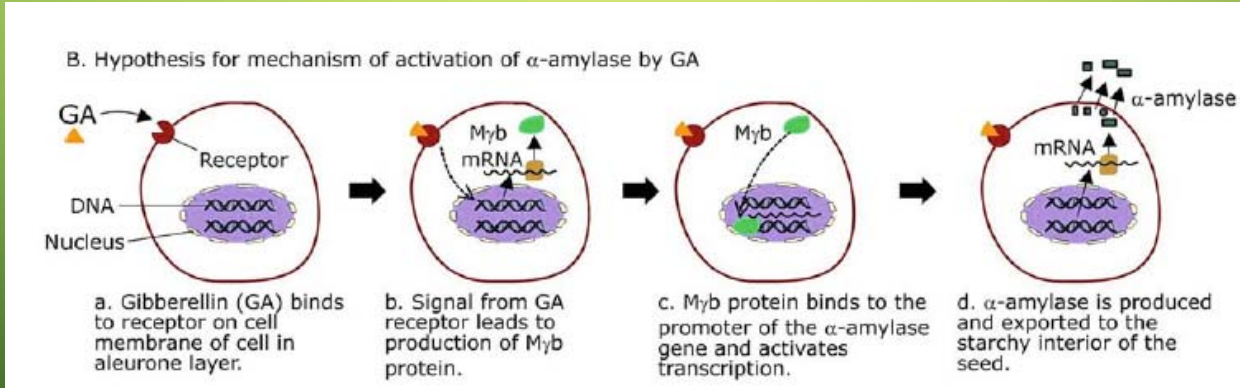


Fig. 17.6 Sequence of events leading to α-amylase biosynthesis in aleurone layer of cereal grains in response to GA

GA signaling is mediated through the DELLA protein (nuclear protein) which functions as negative regulators of growth. GA perceived by a soluble receptor protein GID1 (GA-insensitive dwarf 1). Binding of GA with GID1 proteins enhances its interaction with DELLA which in turn enhances its interaction with F-box protein (SLY1). This leads to ubiquitination of DELLA protein followed by its degradation by 26S proteosome pathway. Removal of DELLA proteins facilitates the transcription of GA-regulated genes

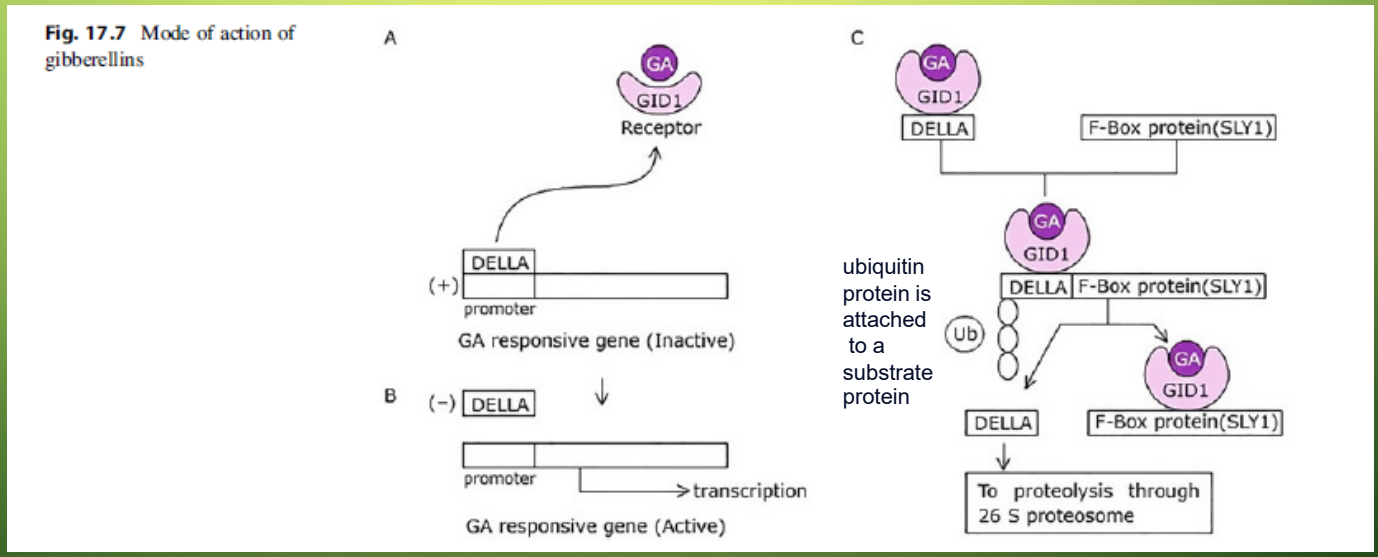


Fig. 17.7 Mode of action of gibberellins

1.6 Conversion of GA12 to bioactive GAs involves two pathways: one in which c-13 is hydroxylated early, and one in which c-13 is not hydroxylated

1.7 Bioactive GAs are rendered inactive via multiple metabolic routes in plants

- The best-characterized inactivation reaction is oxidation on C-2, catalyzed by GA 2-oxidase.
- The *Eui* gene encodes a CYP450 monooxygenase, CYP714D1, which inactivates GAs by epoxidation of the 16,17-double bond of various GAs, including GA4, GA9, and GA12.
- methylation of GAs is a common inactivation reaction in other plants.
- GAs can be converted into conjugates in plants. Conjugation of GAs to glucose

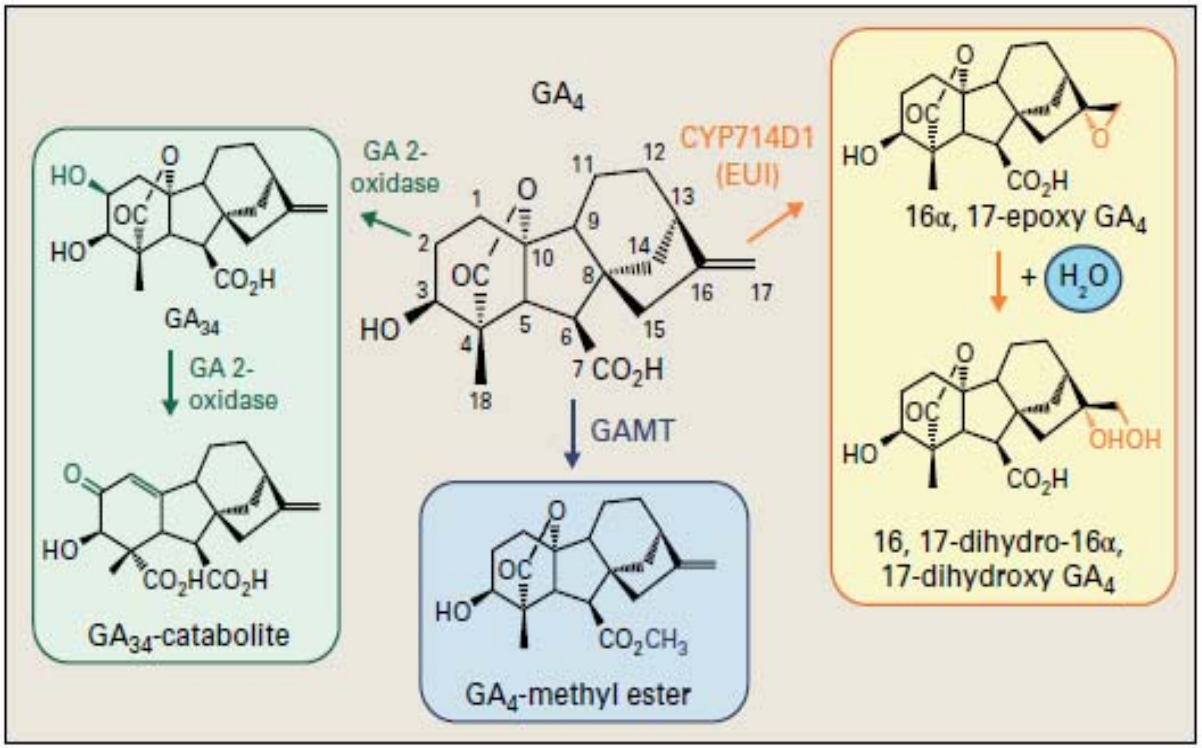


FIGURE 17.10 *GA_4 inactivation pathways. Inactivation by GA 2-oxidase introduces a 2β-hydroxyl group. In some cases, further metabolism occurs to form GA-catabolites, in which C-2 is oxidized to a ketone and the lactone is opened with the formation of a double bond. 16α,17-epoxy GAs produced by CYP714D1 (EUI) are hydrolyzed to give 16,17-dihydrodiols either in plants or during purification. GAMT catalyzes methylation of the C-6 carboxyl group. GAs are also converted into various glucose conjugates in plants (not shown).*

1.8 There are remarkable differences in GA biosynthesis pathways and enzymes between plants and fungi

1.9 Some enzymes that participate in GA biosynthesis are feedback-regulated

- The concentration of bioactive GAs in plants is under homeostatic control via feedback regulation.
- The effect of GA concentration is targeted to late biosynthesis enzymes, GA 20-oxidase and GA 3-oxidase, and the GA inactivating enzyme, GA 2-oxidase.

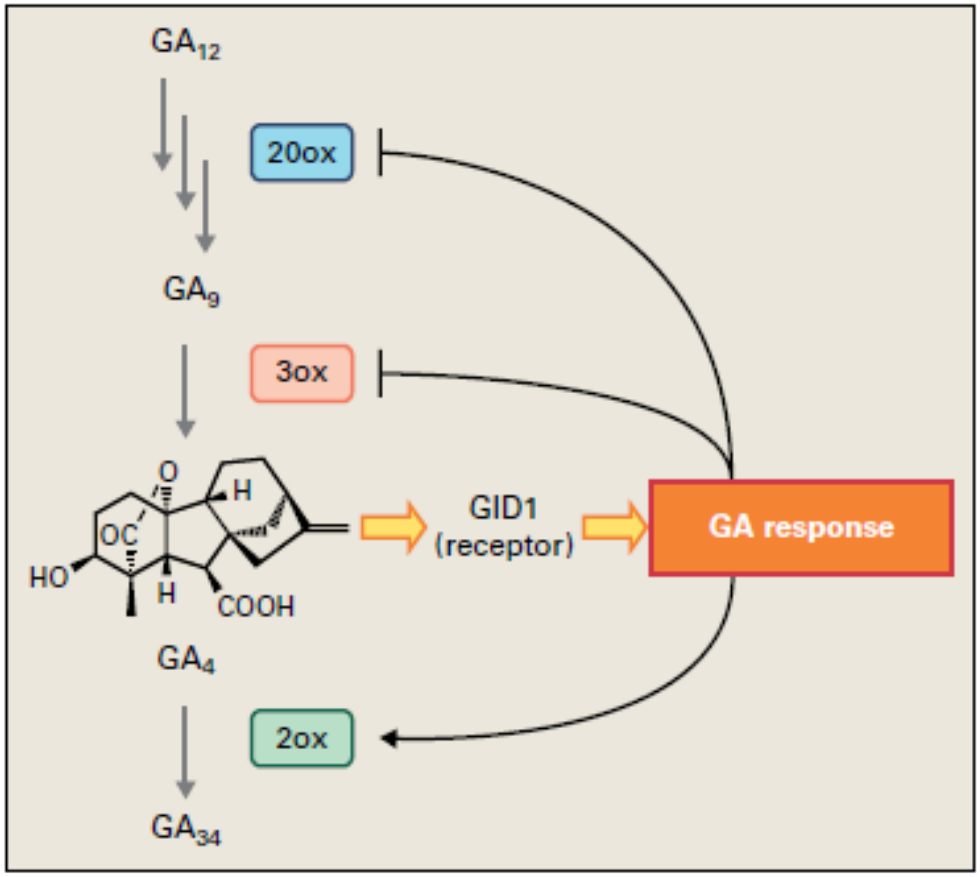


FIGURE 17.11 *Feedback regulation of GA biosynthesis and inactivation pathways. T-bars and black arrow depict negative and positive regulation, respectively. Gray arrows indicate metabolic conversions. 20ox, GA 20-oxidase; 3ox, GA 3-oxidase; 2ox, GA 2-oxidase.*

1.10 GA Mediates light-induced germination of lettuce seeds

- Evidence suggests that in some cases, bioactive GAs function as key mediators between perception of environmental signals And the resulting growth responses.
- A clear link between GA metabolism and phytochrome has been established in the case of red light (RL)-induced germination.
- Following a pulse of RL, germination is preceded by elevated GA 3-oxidase expression and an increase in the GA1 content.

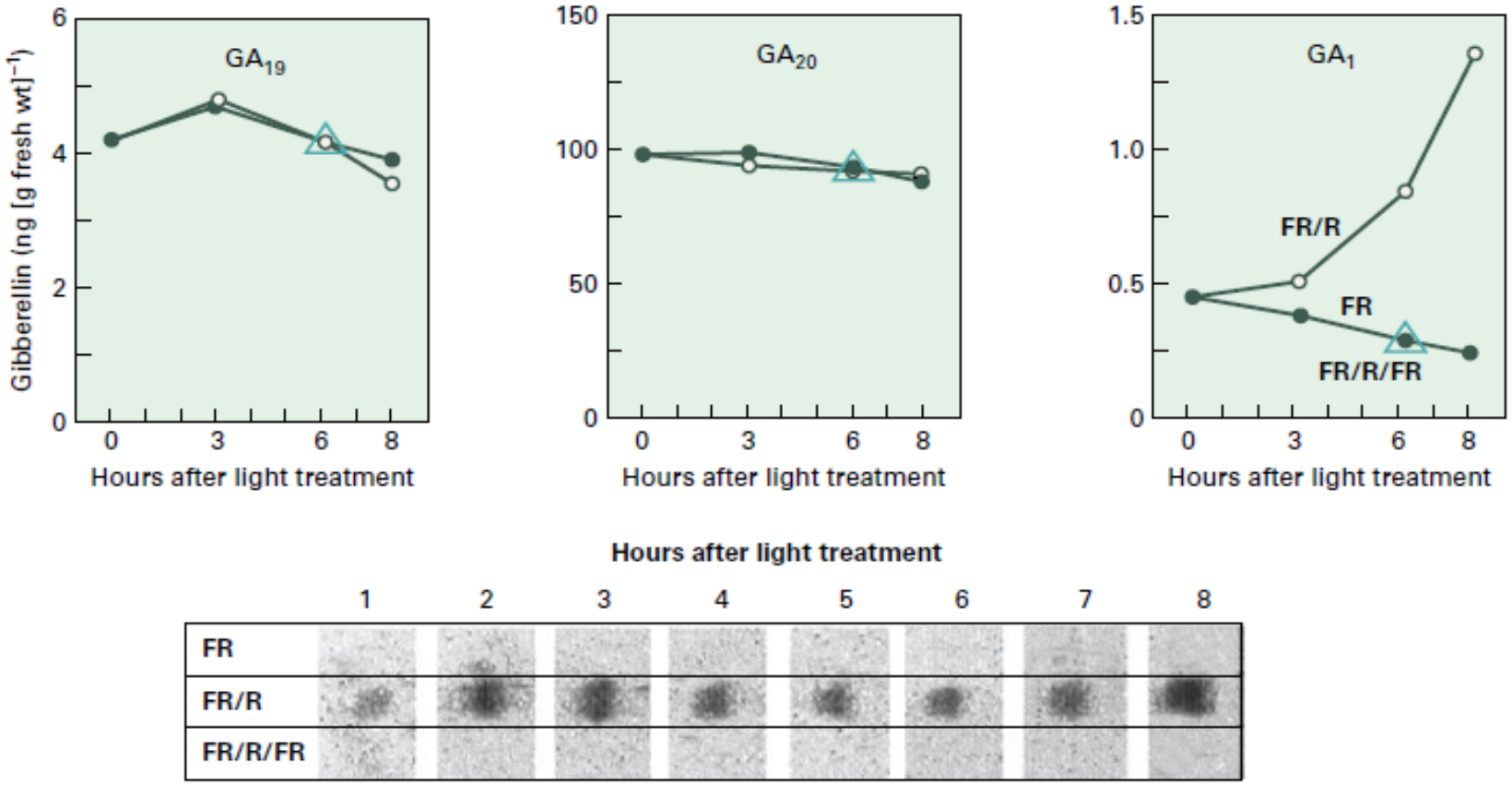


FIGURE 17.12 *Regulation of GA in Grand Rapids* L. sativa *by red (R) and far-red (FR) light. (Upper panels) Effects of R and FR light on GA levels. Note that phytochrome-mediated, R light induced seed germination is associated with increased GA_1 content. (Lower panels) Hybridization of a GA 3-oxidase cDNA clone to northern blots containing 50 µg of total RNA extracted from* L. sativa *seed after treatment with R and FR light. Source*: Electrophoretograms: Toyomasu et al. (1998). Plant Physiol. 118: 1517–1523.

1.11 Mutations in the GA pathway led to major advances in agriculture

- In the 1960s, the introduction of semi-dwarf cultivars of cereal crops, including *O. sativa* and *Triticum* spp., in combination with increased fertilizer use, led to a dramatic increase in food production worldwide. The impact of these advances in agriculture was termed the "green revolution".



FIGURE 17.13 *A semi-dwarf* O. sativa *cultivar carrying the* sd-1 *gene (dee-geo-woo-gen, left) and its tall isogenic line (woo-gen, right).*

17.2 Abscisic acid

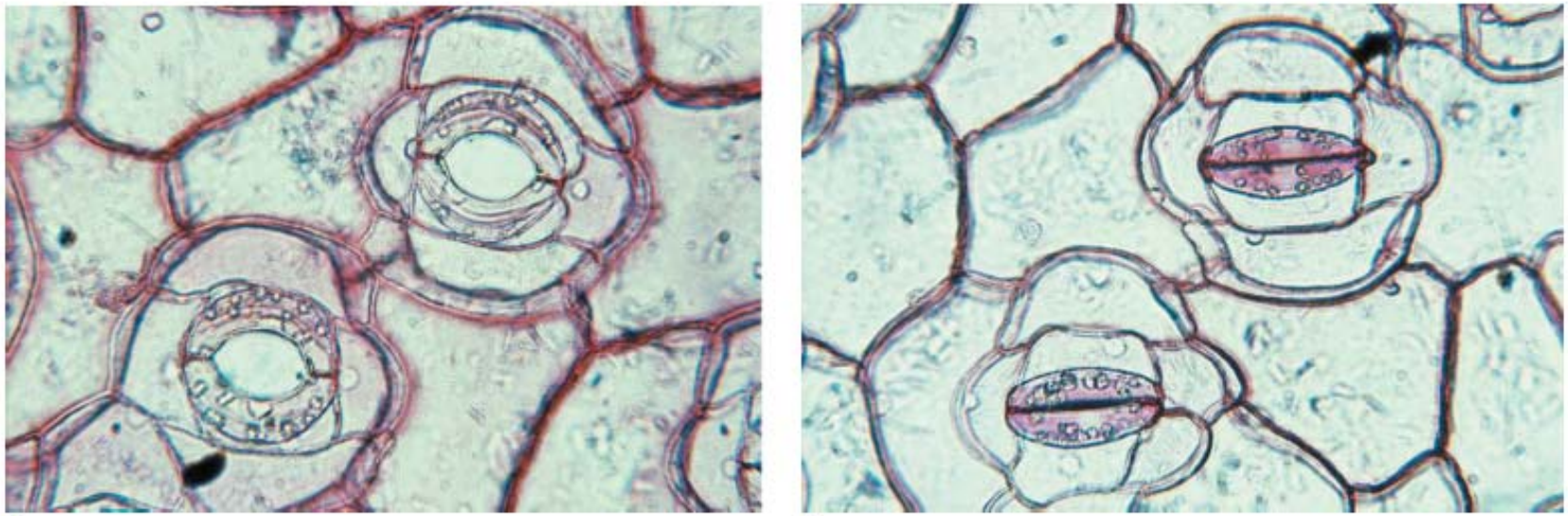
- Growth-inhibiting compounds were isolated from plants in the early 1950s.
- Experiments detected compounds that inhibited coleoptile elongation, referred to as the β-inhibitor complex.
- In the early 1960s, US scientists isolated an abscission-accelerating compound called "abscisin II" from young *Gossypium hirsutum* fruits and simultaneously researchers in the UK isolated a dormancy-inducing factor called "dormin."
- The structure of abscisin II, later called **abscisic acid** (ABA), was determined in 1965 and dormin was subsequently shown to be ABA.

2.1 Contrary to its name, ABA does not induce abscission

- As its name indicates, ABA was originally thought to induce abscission, however, abscission is regulated by ethylene rather than ABA.
- Increased internal concentrations of ABA are unlikely to impose dormancy of *S. tuberosum* tubers or resting buds of deciduous trees.
- No support for conjecture that asymmetric distribution of ABA is involved in the negative geotropic response of roots.



FIGURE 17.15 *Precocious germination (vivipary) of immature* Z. mays *seeds homozygous for* vp1. *The color-less* vp1 *kernels lack anthocyanins in the aleurone layer, and germinate before maturity because of ABA insensitivity. The seed is viable if transplanted directly from immature ears.*
Source: S. McCormick, University of California, Berkeley; previously unpublished.



A B

FIGURE 17.16 *ABA-induced stomatal closure. Epidermal strips of* Commelina communis *L. incubated in buffer (10 mM Pipes, pH 6.8) containing 50 mM KCl and supplied with CO_2-free air. The stomata are open after two to three hours (A). When transferred to the same solution plus 10 μM ABA, the pores close within 10 to 30 minutes (B).*
Source: J. Weyers, University of Dundee, UK; previously unpublished.

Effects

- Stomatal closure
- Inhibits shoot growth
- Protein synthesis
- Counteracts the effect of gibberellin
- Some aspects of dormancy
- Response to wounding

DESICCATION TOLERANCE

The water content of seeds gradually increases during early phase of tissue differentiation and cell expansion. It subsequently decreases during the phase of reserve accumulation and seed maturation. Accompanying maturation, seeds undergo desiccation tolerance and dormancy. A variety of protectants, such as sugars, antioxidants late embryogenesis abundant (LEA) proteins accumulate during the period of desiccation tolerance. LEA proteins interact with sugars resulting in the formation of a glassy state which suspends the metabolic activities of the seeds and minimize membrane damage. Gene expression associated with LEA and HSP is modulated via ABA signaling mechanisms.

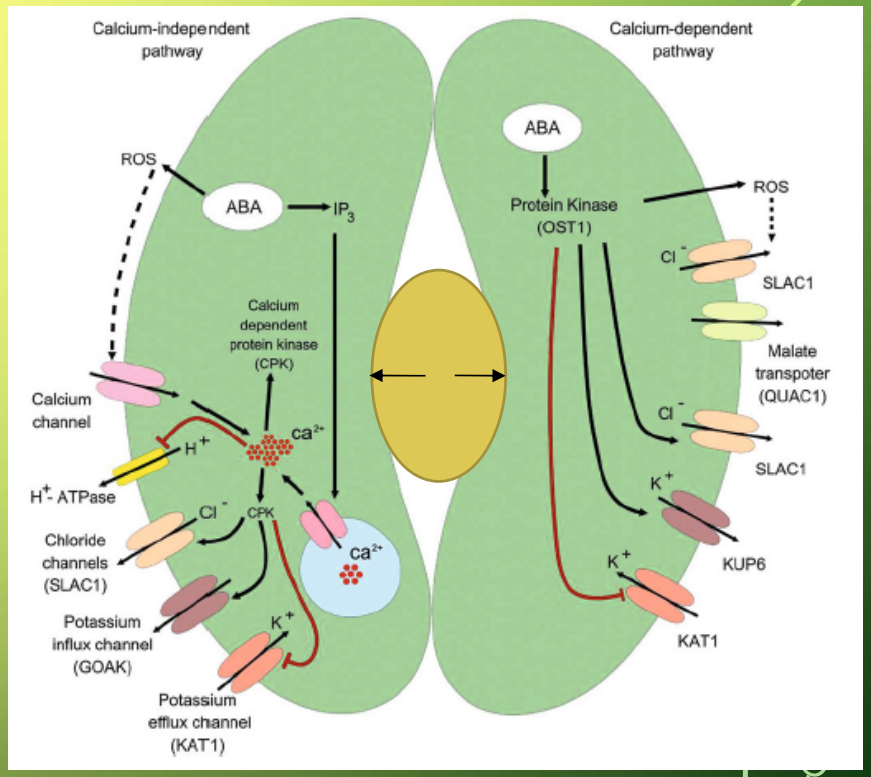
Table 18.1 ABA-induced proteins under various abiotic stress conditions

Crops	Proteins	Stress conditions
Rice	RAB 16A, 16B	Salt and desiccation
Wheat	EM, RAB 15	Drought
Barley	Dehydrins B8, B9, B17, B18	Desiccation
Maize	pMAH9	Drought and wounding
Tobacco	Osmotin	Salt
Tomato	TAS 14	Salt and drought
Potato	Pt II	Wounding

Under water-deficit conditions:

- Several LEA genes which are ABA-responsive marker genes for protection of plants from dehydration damage are up-regulated.
- ABA also induces genes involved in the synthesis of wax to minimize water loss from leaves.
- During osmotic stress, ABA speeds up conversion of starch to soluble sugars by transcriptional control of the expression of genes for alpha and beta amylase through several ABFs (ABRE-binding factors).
- Likewise, through the transcriptional regulation of ABFs, ABA also regulates chlorophyll biosynthesis.
- ABA is also involved in the regulation of several processes accompanying flower development and their abscission, fruit growth and ripening.
- ABA accelerates leaf senescence through transcriptional regulation. The induction of two marker genes for leaf senescence, ORESAR1 (ORE1) and SENESCENCE-ASSOCIATED GENE12 (SAG12), is transcriptionally activated during senescence by ABA signaling, involving ABFs.
- ABA plays a very significant role in the closing of stomata by regulating ion fluxes across guard cell membrane.

1. activation of anion channels brings about anion efflux and depolarization of guard cell membrane.
2. Membrane depolarization activates depolarization-activated potassium efflux channels.
3. Since both potassium efflux channels and anion channels can remain activated for several minutes in the guard cells, this facilitates significant release of solutes from guard cells, leading to the closure of stomatal aperture.



2.2 Many fungi, including fungal pathogens of the genera *Cercospora* and *Botrytis*, synthesize ABA from farnesyl diphosphate

By the early 1970s, two pathways leading to ABA had been proposed. The "direct C_{15}" route envisaged ABA production from the C_{15} precursor farnesyl diphosphate, while the "indirect C_{40}" route postulated oxidative cleavage of a putative C_{40} intermediate, such as violaxanthin, to yield a C_{15} intermediate of ABA.

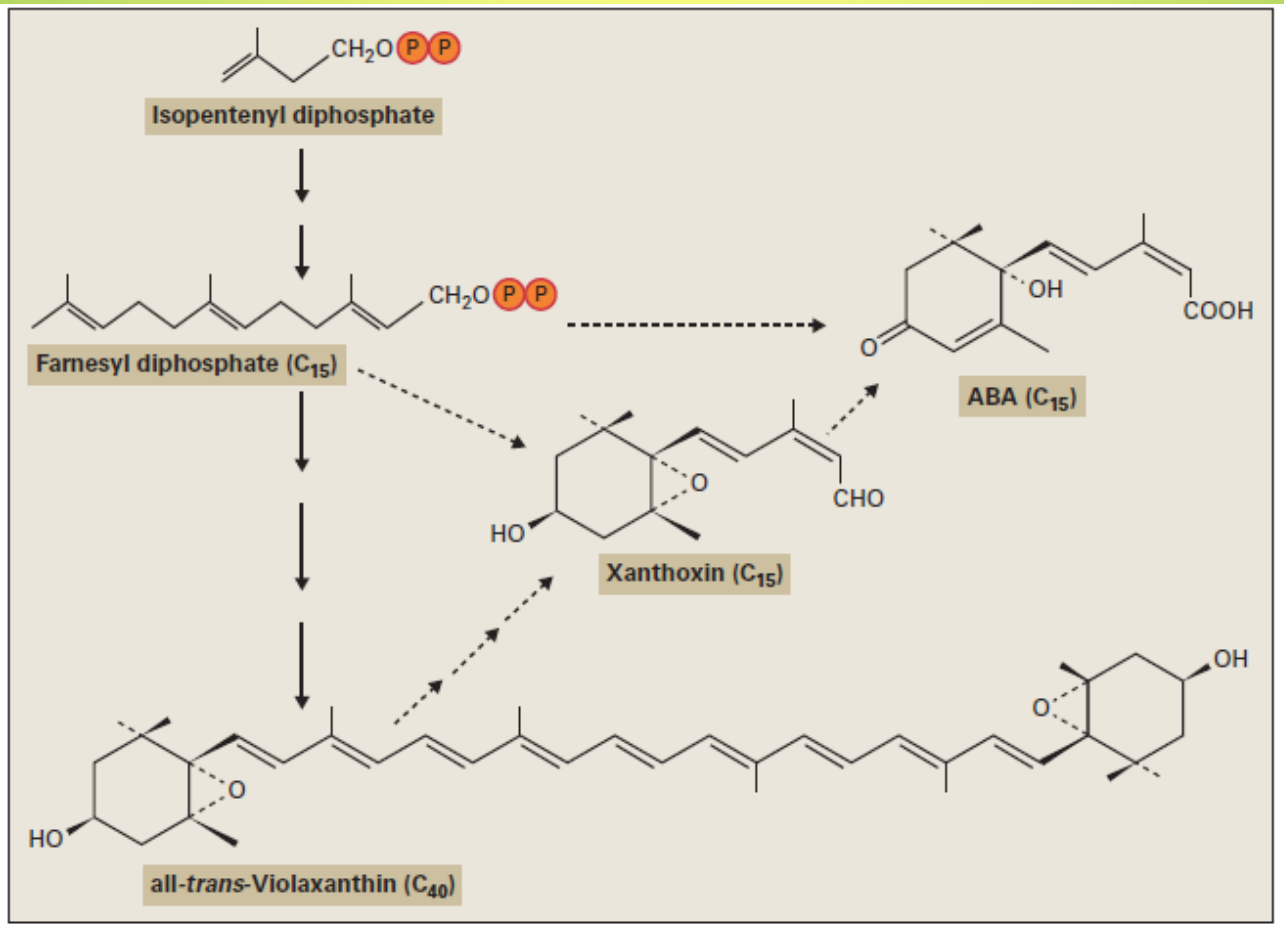


FIGURE 17.17 *Summary of two possible ABA biosynthetic routes. In the direct C_{15} pathway, farnesyl diphosphate is modified to yield ABA. Alternatively, in the indirect C_{40} pathway, a carotenoid, 9-cis-violaxanthin, is cleaved to form a C_{15} ABA precursor, xanthoxin.*

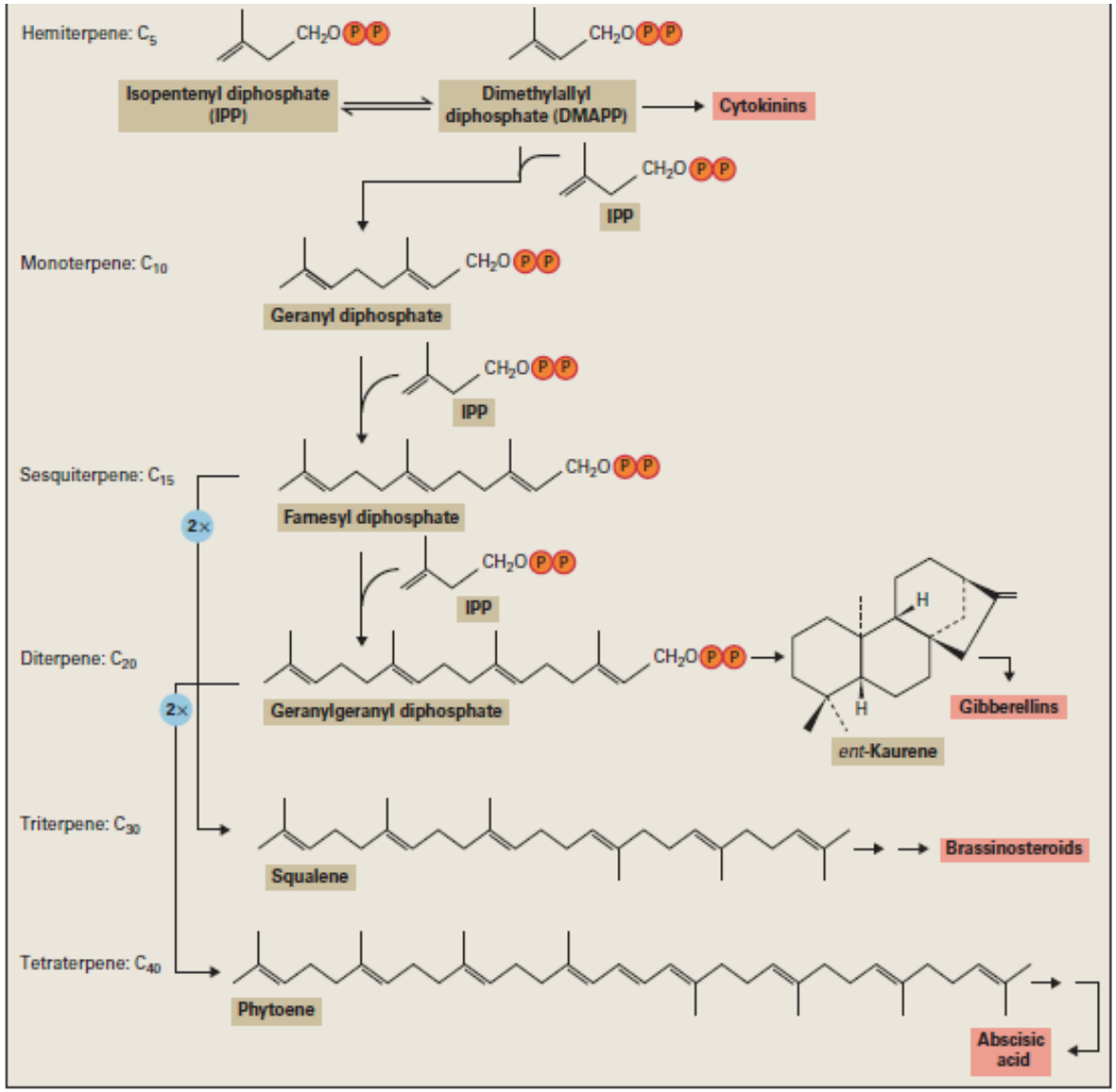


FIGURE 17.6 *Terpenoid biosynthesis pathway, showing biosynthetic origins of GAs as well as CKs, BRs, and ABA.*

2.3 Gas chromatography–mass spectrometry showed that plants synthesize ABA from a C_{40} precursor

Experiments with mutants and with inhibitors of carotene synthesis were keys to understanding ABA biosynthesis.

Firm evidence that C_{40} xanthophylls are intermediates in ABA biosynthesis was obtained in 1984.

1′-deoxy-ABA was not the immediate ABA precursor in water-stressed leaves.

ABA was synthesized from a preformed precursor with oxygen atoms at the 1′ and 4′ positions.

This was consistent with a C_{40} pathway to ABA, with $^{18}O_2$ cleaving violaxanthin to form xanthoxin labeled at the 1-CHO group, followed by oxidation of the labeled xanthoxin to yield ABA with one ^{18}O atom in the carboxyl group.

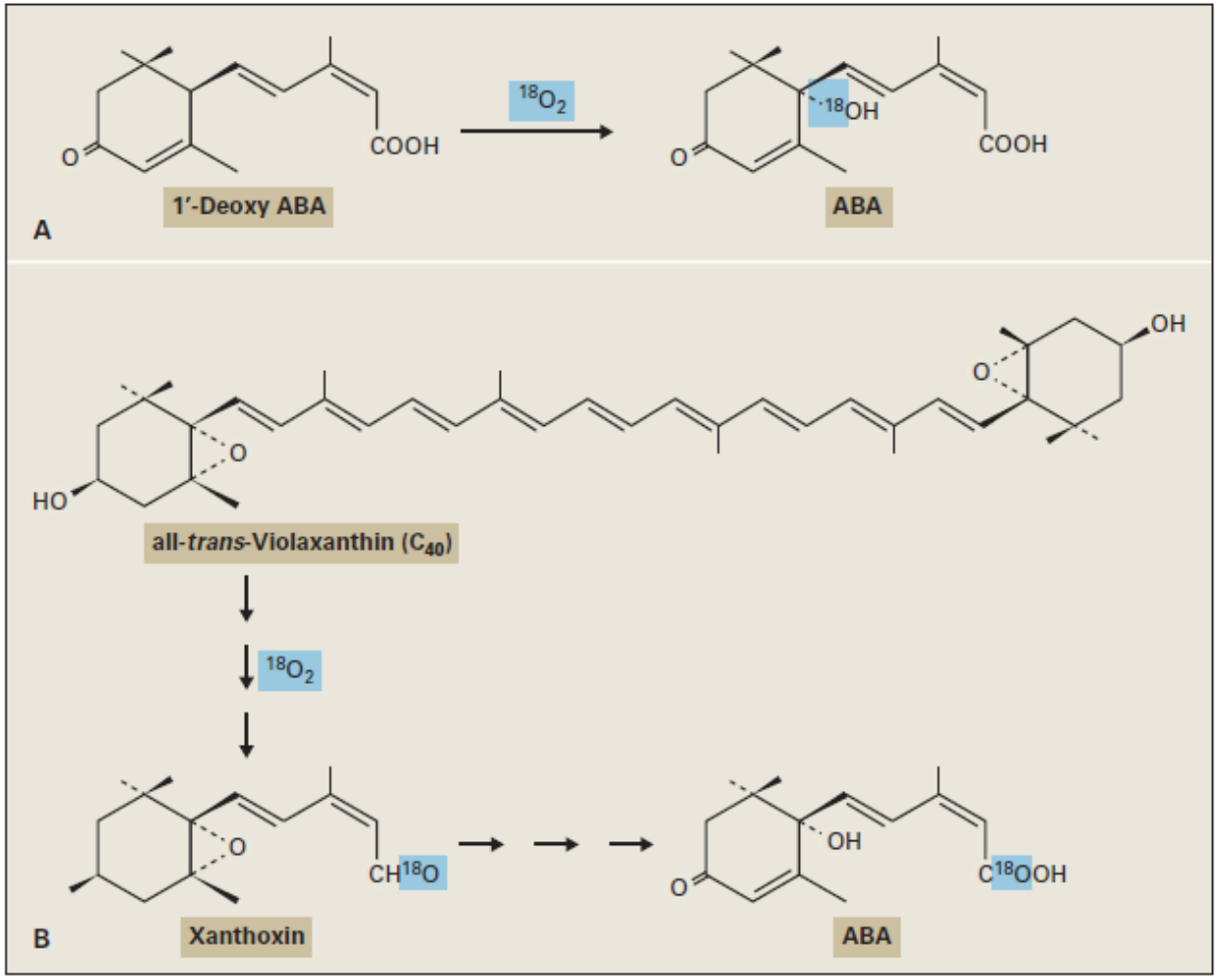


FIGURE 17.20 *Isotopic labeling experiments confirmed the existence of an indirect C_{40} plant ABA biosynthetic pathway. GC-MS revealed that ABA synthesized by plant tissues in the presence of $^{18}O_2$ was not labeled at the 1′-hydroxyl group, as would be expected if ABA were generated directly from 1′-deoxy-ABA (A). However, the appearance of label in the ABA carboxyl group was consistent with oxidative cleavage of all-*trans*-violaxanthin and subsequent conversion of xanthoxin to ABA (B).*

2.4 ABA synthesis is regulated by a cleavage reaction that generates the first C_{15} intermediate

Many ABA-deficient viviparous (*vp*) mutants of *Z. mays* are blocked at various points in the terpenoid and carotenoid biosynthesis pathways.

GGDP synthase, a key enzyme in the terpenoid pathway, catalyzes the three successive condensations with isopentenyl diphosphate that convert dimethylallyl diphosphate to the C_{20} compound GGDP.

As a consequence of GGDP deficiency, seedlings of the *vp*12 mutant have low chlorophyll content and reduced capacity for synthesis of carotenoids and ABA.



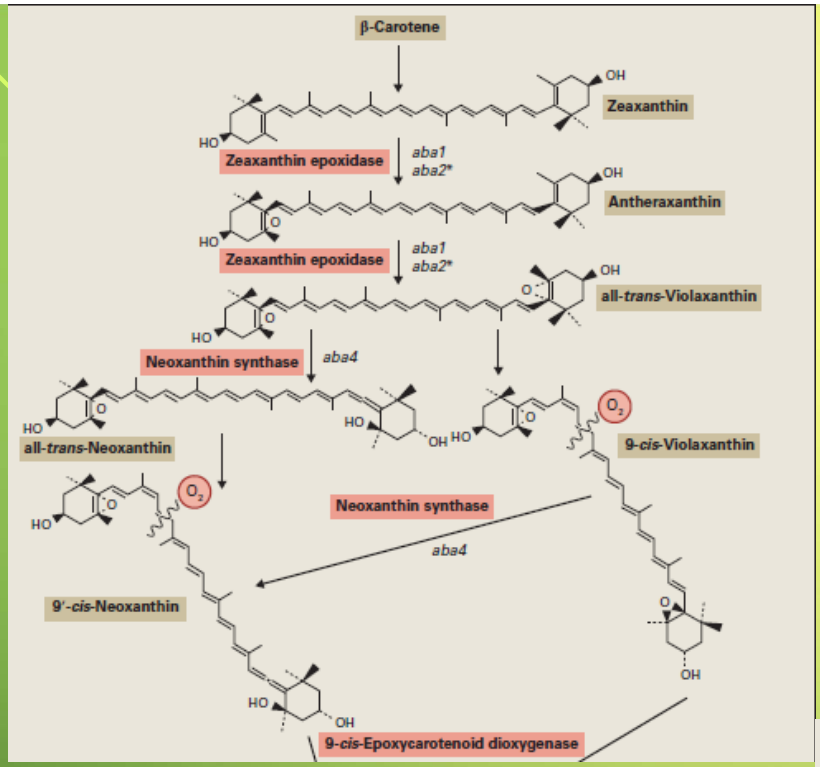
FIGURE 17.19 *Early stages in the indirect C_{40} ABA biosynthesis pathway: production of geranylgeranyl diphosphate (GGDP) and synthesis of β-carotene. Enzymes deficient in Z. mays vp mutants are indicated. The chemical inhibitors fluridone and norflurazon block conversion of phytoene to phytofluene (not shown).*

The first committed step in the ABA biosynthesis pathway, oxidative cleavage of 9′-*cis*-neoxanthin and/or -*cis*-violaxanthin, yields the first C15 intermediate, xanthoxin.

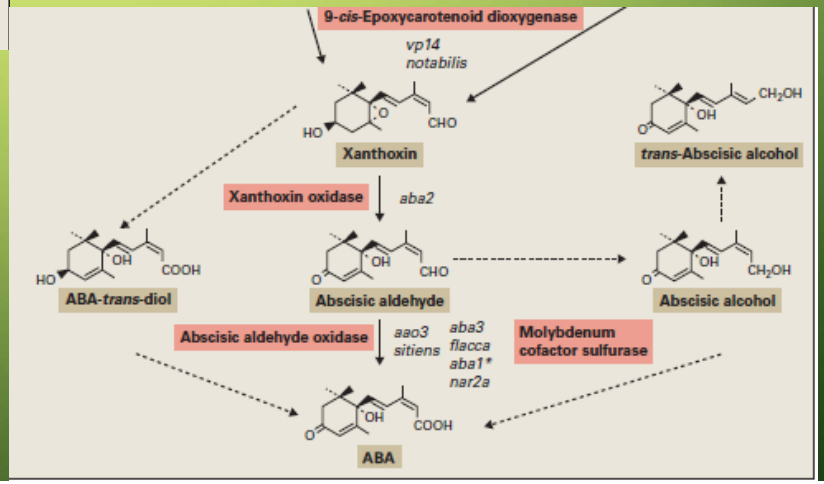
9-*cis*-epoxycarotenoid dioxygenase that catalyzes this conversion, is encoded by a multigene family.

- 9-*cis*-epoxycarotenoid dioxygenase encoded by *AtNCED3* gene is responsible for ABA production upon water stress,
- while *AtNCED6* and *AtNCED9* gene products are involved in ABA production in seeds.

This conversion appears to be a rate-limiting step in ABA biosynthesis;



- FIGURE 17.21 Later stages in the indirect C40 ABA biosynthesis pathway: β-carotene to ABA. Biosynthetic steps blocked in the following mutants are indicated. *Arabidopsis mutants*: aao3, aba1, aba2, aba3, aba4; *H. vulgare:* nar2a; *S. lycopersicum mutants*: flacca, notabilis, sitiens; *N. plumbaginifolia mutants*: aba1*, aba2*; *Z. mays mutant*: vp14



The penultimate step of the ABA biosynthesis pathway converts xanthoxin to abscisic aldehyde.

Oxidation of abscisic aldehyde, catalyzed by abscisic aldehyde oxidase, leads to formation of ABA.

- There are many mutants defective in conversion of abscisic aldehyde to ABA.
- The activity to produce ABA from xanthoxin via abscisic aldehyde is not increased following the onset of water stress

2.5 ABA is metabolized to several compounds, including phaseic acid, dihydrophaseic acid, and glucose conjugates

- The main route involves hydroxylation of the 8′ carbon, spontaneous rearrangement of the resulting 8′-hydroxy-ABA to form phaseic acid (PA), and reduction to dihydrophaseic acid and *epi*-dihydrophaseic acid.
- The alternative routes in ABA metabolism involve conversion to 7′- and 9′-
- hydroxy-ABA.
- conjugation to form ABA-β-glucosyl ester and ABA-1′-*O*-glucoside.

ABA 8′-hydroxylase is a membrane bound CYP450 monooxygenase classified as *CYP707A*. Single or multiple mutants defective in these genes contain elevated endogenous ABA levels.

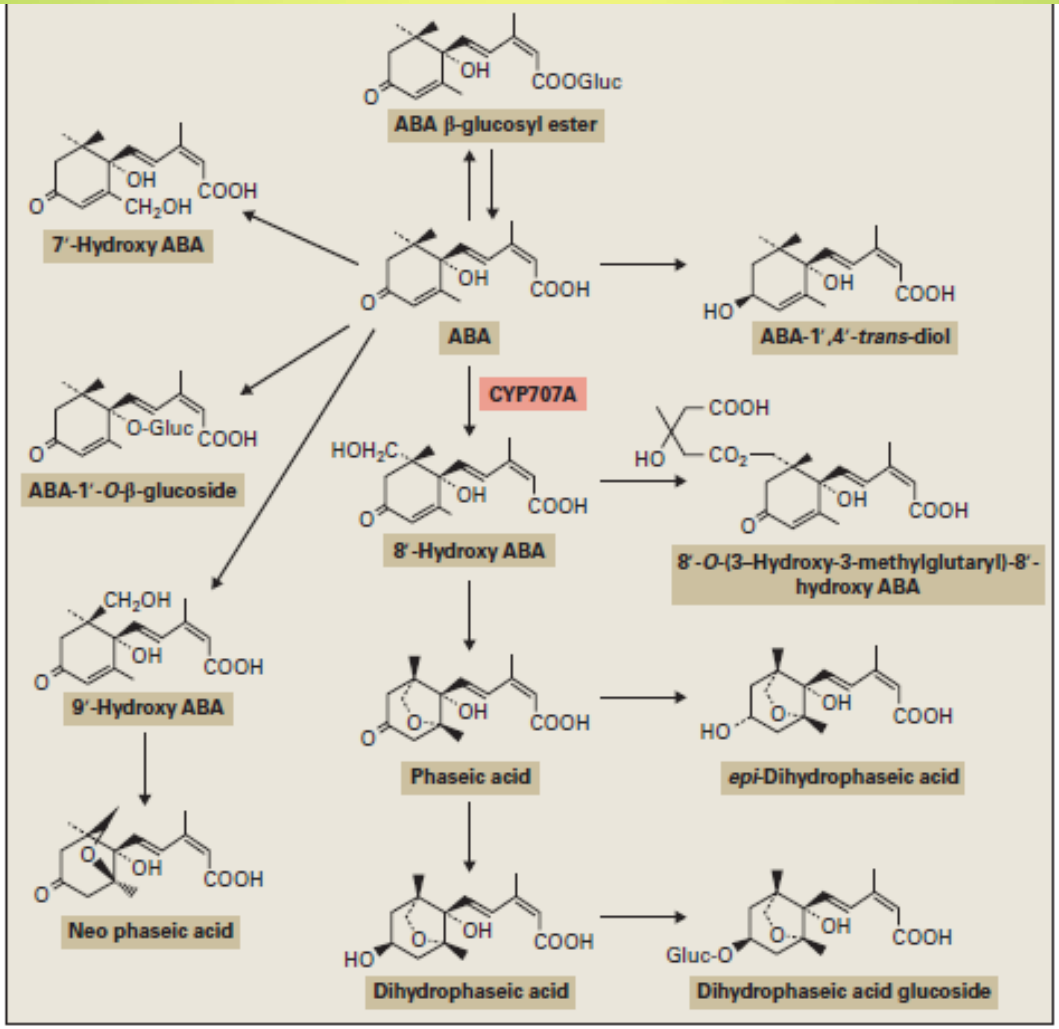


FIGURE 17.22 *ABA metabolism pathways. The major route proceeds by way of 8′-hydroxy ABA, which is rapidly converted to phaseic acid; this in turn, is reduced to* epi*-dihydrophaseic acid and dihydrophaseic acid. Dihydrophaseic acid undergoes conjugation to yield dihydrophaseic acid 4′-O-glucoside. ABA can also be conjugated, forming ABA β-glucosyl ester and ABA-1′-O-β-glucoside.*

17.3 Cytokinins

The discovery of CKs has its roots in the 1930s quest for chemical factors that would allow the culturing of plant tissues in synthetic media.

In the 1950s, a substance that strongly stimulated cell proliferation in *Nicotiana tabacum* tissue culture was first purified and crystallized from autoclaved herring sperm DNA extracts. This growth-stimulating compound *N*6-furfuryl aminopurine was named kinetin.

Kinetin has not been found in living plants and is believed to be an artificial by-product of DNA breakdown.

Kinetin, in combination with auxin, was found to promote the initiation and maintenance of cell division in cultured *N. tabacum* parenchyma.

A naturally occurring kinetin-like substance was first isolated from immature *Z. mays* endosperm in the early 1960s and named zeatin (*trans*-zeatin, tZ).

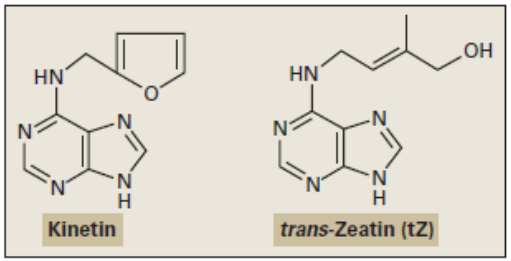


FIGURE 17.23 *CK structures. The first CK identified was the synthetic compound Kinetin. The first plant CK isolated was* trans-Zeatin.

- Usage of the term “cytokinins” for kinetin-like compounds was defined as a “generic name for substances which promote cell division and exert other growth regulatory functions in the same manner as kinetin.”

FIGURE 17.24 Arabidopsis *callus production is induced by auxin (IBA) and CK (tZ). Callus subcultured on auxin medium produces only roots (left); but on medium containing a high ratio of CK to auxin produces shoots (right).*
Source: T. Kakimoto, Osaka University, Japan; previously unpublished.



- *Effects*

✓Cell division

✓Morphogenesis

✓Growth of lateral buds

✓Leaf expansion

✓Senescence

✓Stomatal opening

✓Chloroplast development



Source–sink relationships: a fundamental framework

SOURCE

Mature photosynthetic leaves
Produce and export assimilates, primarily carbohydrates.

Phloem transport

SINK

Fruits, roots, young leaves
Import assimilates for growth, respiration, or storage.

Sink size

Physical capacity related to cell number and cell size.

Sink activity

Physiological capacity for import, metabolism, and storage.

Sink strength

Capacity to attract, import, and use or store assimilates.

Key message: productivity depends on both assimilate supply and sink capacity.

How source–sink relationships regulate plant growth

1 **Assimilate production**
Photosynthesis in source leaves

2 **Phloem transport**
Loading, translocation, and unloading

3 **Sink metabolism**
Import, utilization, respiration, and storage

4 **Growth and yield**
Biomass accumulation and organ development

Three interacting determinants

- Assimilate production by source organs
- Competition among sink organs
- Sink capacity for import and utilization

The source–sink ratio alone does not fully describe sink strength.

Carbohydrate import: from sucrose to hexose transport



Evidence from the tomato study

- Fruit thinning increased starch, sucrose, and hexose contents.
- Expression of the examined LeHT genes increased as fruit load decreased.
- LeHT1 and LeHT2 showed prominent expression at the onset of ripening.

Coordinated CWI activity and LeHT expression suggest an association, not proof of direct causation.

Phytohormonal regulation of fruit growth

Auxin

- Participates in fruit development and growth
- Associated with cellular processes involved in fruit expansion
- Concentration increased as fruit load decreased

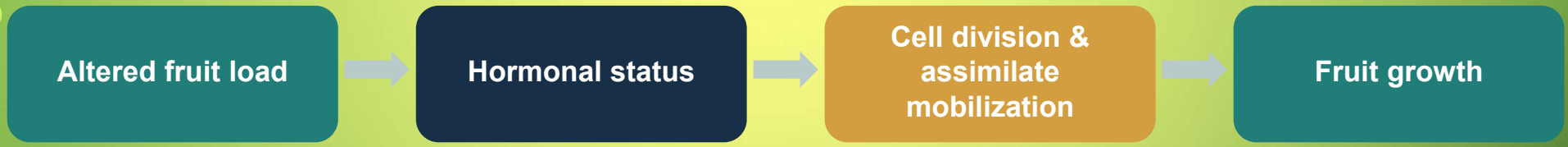
Cytokinin

- Regulates cell division and aspects of sink development
- May contribute to assimilate mobilization toward sinks

Interpretation

Hormonal status is an important component of the relationship between sink demand, cellular growth, and assimilate use.

Why is cytokinin a key component of the framework?



Study-supported observations

- Lower fruit load was associated with higher cytokinin concentration and faster relative fruit growth.

The results support associations; they do not prove that cytokinin alone caused the observed responses.

1. Cytokinins can influence source activity by affecting photosynthesis and carbohydrate supply.

2. Cytokinin signaling can regulate genes involved in cell-cycle progression, DNA replication, and the processes required for cell division.

3. Cytokinins can affect sink strength and the allocation of nutrients and assimilates among plant organs.

4. Cytokinin-mediated regulation of cell division during early fruit development can influence cell number and, consequently, the potential final size of the fruit.

5. Sink capacity depends on several factors, including cell number, cell size, and the capacity of tissues to import and utilize carbon and nutrients.

6. Cytokinins may regulate the expression of genes encoding sugar transporters, including SWEET, SUT/SUC, and hexose transporter (HT) families.

7. Cytokinins can increase cell-wall invertase activity, promoting sucrose hydrolysis into glucose and fructose and facilitating carbon uptake by sink tissues.

8. Cytokinins may link the growth capacity of sink organs with their ability to acquire and utilize assimilates

3.1 Structural variations occur in the side chains of CKs

- Isoprenoid CKs have an isoprene-derived side chain and the common active forms include isopentenyladenine (iP; *N6*-(Δ2-isopentenyl)adenine), *trans*-zeatin (tZ), *cis*-zeatin (cZ), and dihydrozeatin (DZ).
- In general, iP and tZ are active, and their derivatives are abundant, but they are susceptible to degradation by CK oxidase (CKX).
- cZ is less active and relatively more stable than tZ and iP because of its low affinity for CKX.
- DZ appears to be biologically stable because of its resistance to CKX and is generally found in small quantities except for some legumes.

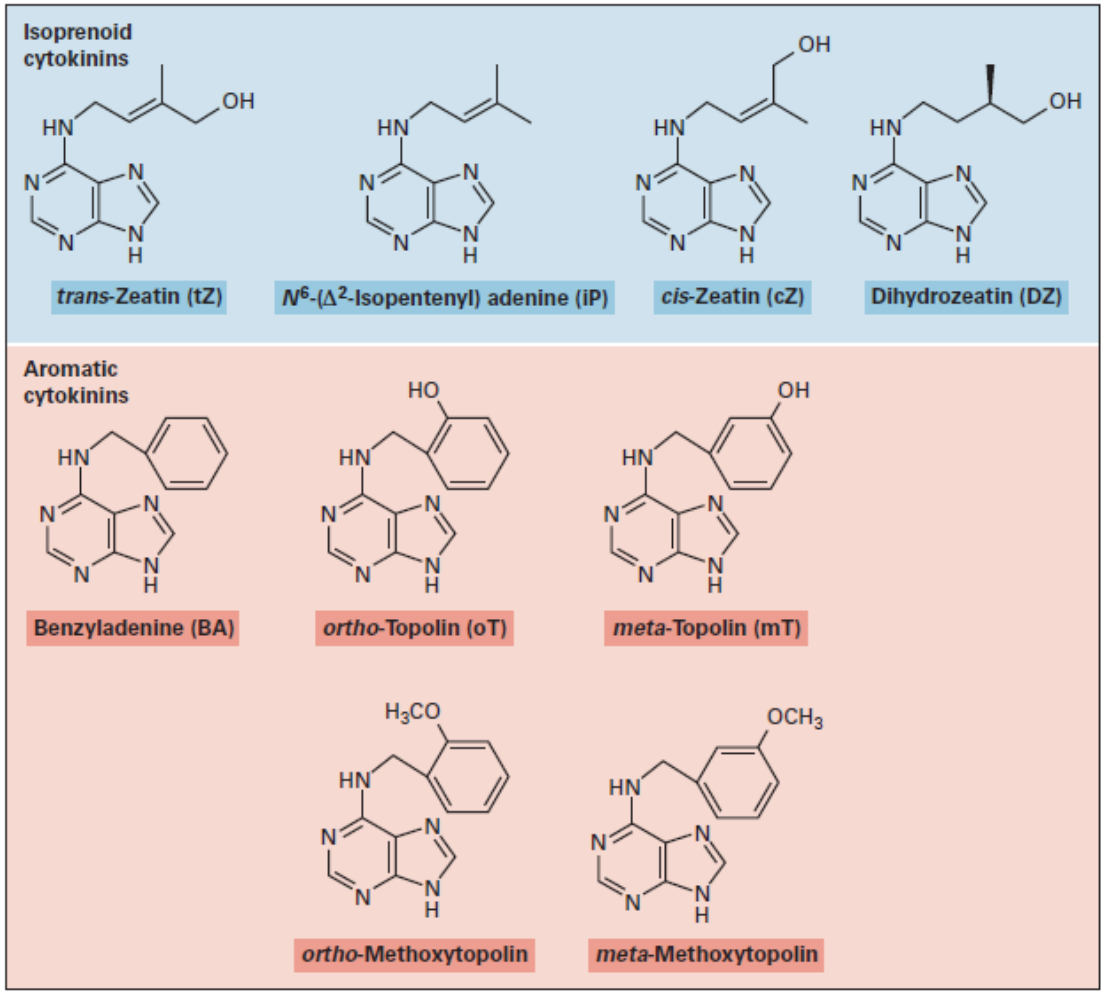


FIGURE 17.25 *Structures of naturally occurring CKs. In general, isoprenoid CKs are in greater abundance than aromatic CKs. Aromatic CKs have been described in only some plant species. Benzyladenine is found in* P. patens.

3.2 CKs are present as conjugates

- CKs are present in plants as nucleoside, nucleotide, and glycosidic conjugates.

- Glucosylation of CK occurs at the *N3, N7,* or *N9*-positions of the purine moiety to form *N*-glucosides.
- Amino acid conjugates of CK have also been isolated from some organisms. Lupinic acid, which was first found in lupine seedling, is an alanine onjugate of tZ.

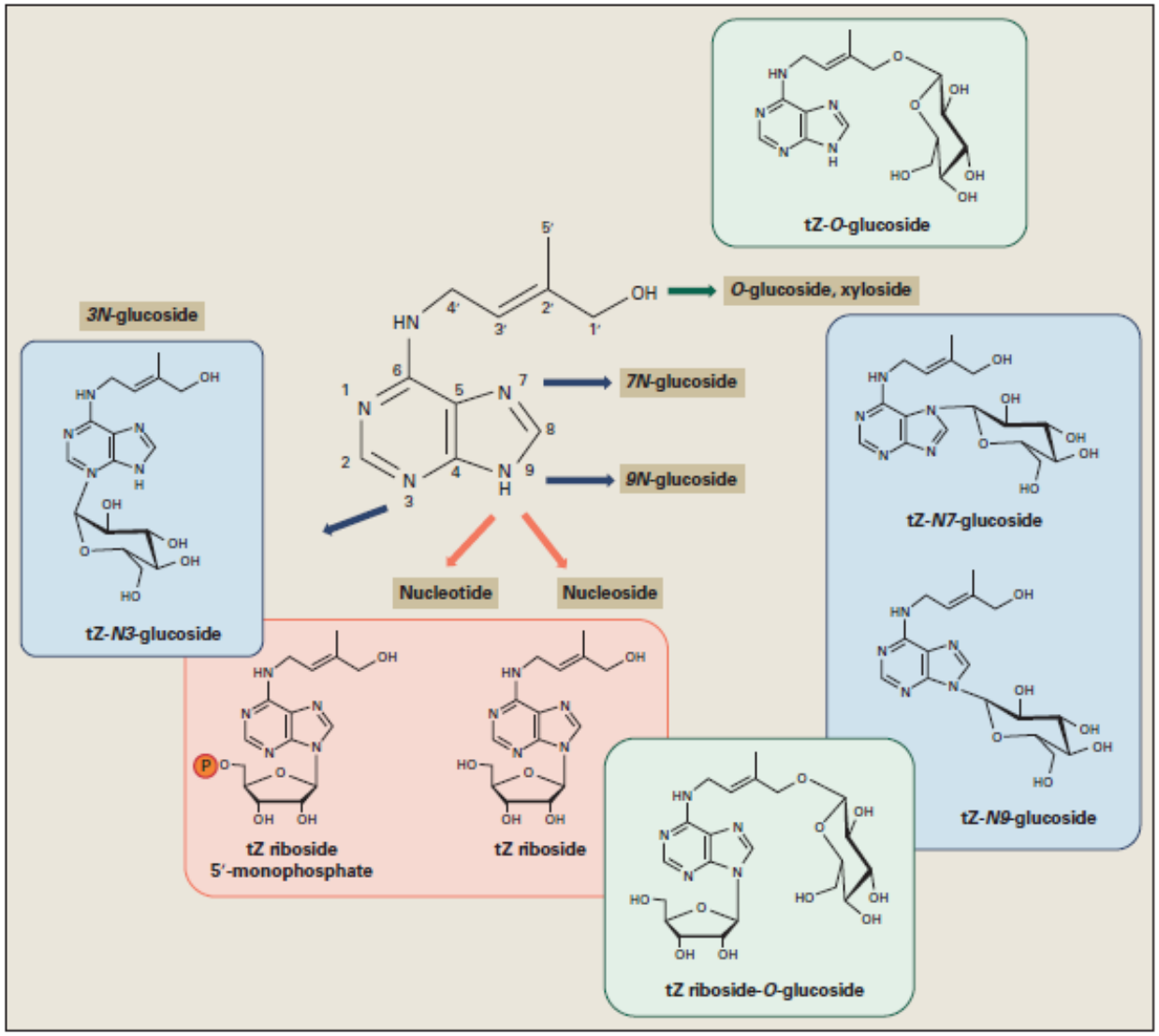


FIGURE 17.26 *Conjugates of tZ. Only representative structures are shown. Conjugation of iP, cZ, and DZ occurs in the same manner, but iP conjugates do not include O-glycosides because they lack a hydroxyl group at the end of the side chain. P represents a phosphate group.*

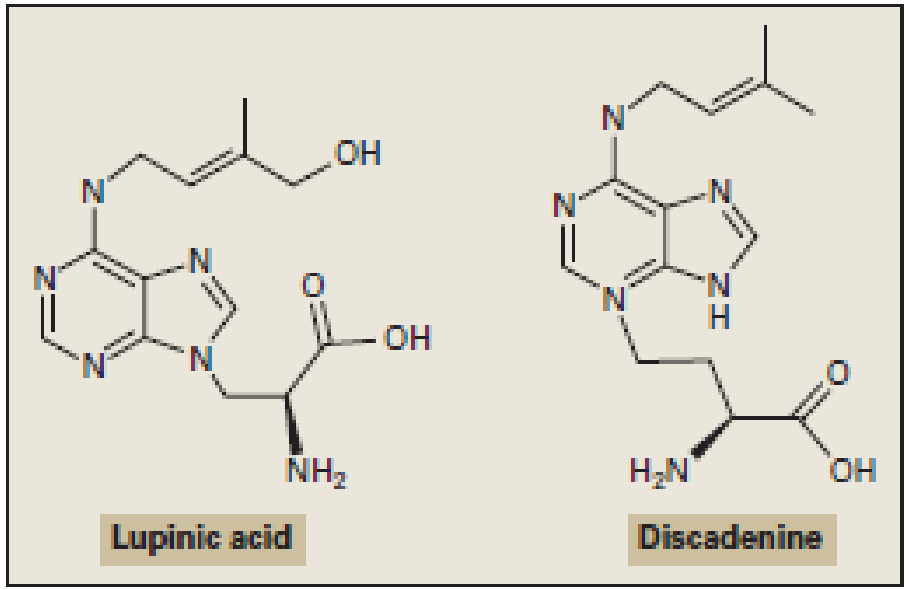


FIGURE 17.27 *Structures of amino acid CK conjugates. Discadenine is only found in slime molds.*

3.3 The initial step of isoprenoid CK biosynthesis is catalyzed by:

adenosine phosphate-isopentenyltransferase (IPT)

The major isoprenoid CK biosynthesis step in plants is conjugation of adenine nucleotide and the prenyl-moiety of dimethylallyl diphosphate (DMAPP), catalyzed by adenosine phosphate-isopentenyltransferase (IPT) (Fig. 17.28). This reaction requires a divalent metal ion, such as Mg^{2+}. Plant IPTs predominantly use ATP or ADP rather than AMP as a prenyl-acceptor.

Prenylation is the covalent attachment of a lipid consisting of either three (farnesyl) or four (geranylgeranyl) isoprene units to a free thiol of a cysteine side chain at or near the C-terminus of a protein.
Prenylation is the addition of hydrophobic molecules to a protein or chemical compound. It is usually assumed that prenyl groups facilitate attachment to cell membranes.

3.4 *trans*-Hydroxylation in tZ biosynthesis is catalyzed by a CYP450 monooxygenase

In *Arabidopsis* the *trans*-hydroxylation of the prenyl side chain in tZ biosynthesis is catalyzed by CYP450 monooxygenases CYP735A1 and CYP735A2.

- Expression of *CYP735A1* and *CYP735A2* is upregulated by CK itself and downregulated by auxin or ABA.

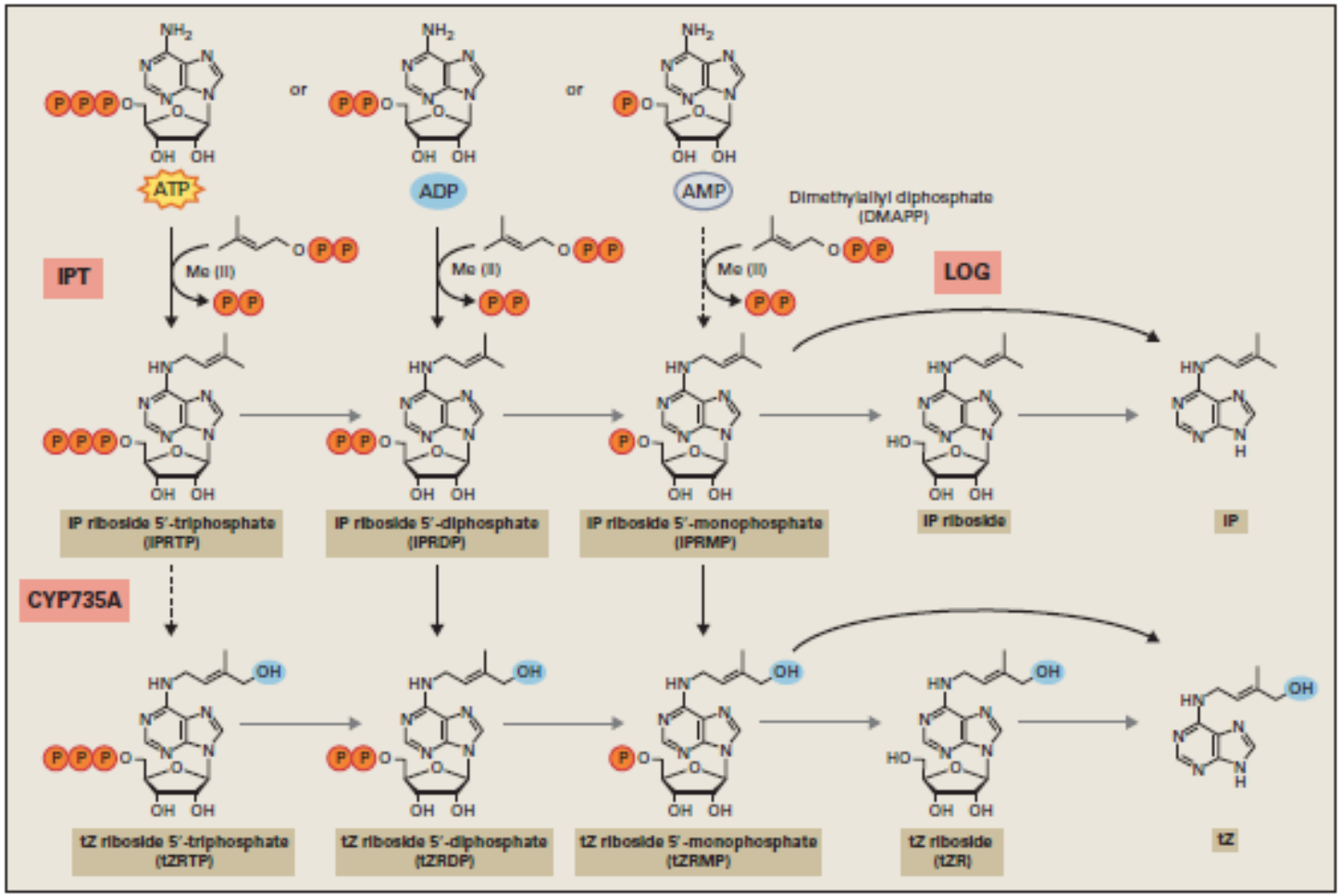


FIGURE 17.28 *Plant CK de novo biosynthesis pathway. Plant IPTs preferably utilize ATP or ADP as isoprenoid acceptors to form iPRTP and iPRDP, respectively. CYP735A preferentially utilizes iPRMP and iPRDP as substrate. Synthetic route indicated by gray arrows are not well characterized at the genetic level. For enzymes involved in the two-step pathway, see Fig. 17.33. P, phosphate group. Me (II), a divalent metal ion.*

3.5 There are two pathways for formation of active CKs

3.6 LONELY GUY catalyzes the direct activation pathway

3.7 *Agrobacterium* IPT has distinct substrate specificity



FIGURE 17.29 *Three-month-old crown gall tumor on a* Rosa *spp. stem inoculated with wild-type Agrobacterium tumefaciens.*

Some plant pathogenic bacteria utilize CKs to influence plant growth. *Agrobacterium tumefaciens* infects eudicots as well as some monocots and induces the formation of crown galls.

- *A. tumefaciens* integrats the T-DNA (Transfer-DNA) region of the Ti-plasmid into the plant nuclear genome.
- Ti-plasmids commonly contain an *IPT* (*Tmr*) within the T-DNA.
- Ti- plasmids contain another *IPT* gene (*Tzs*) in a region that is not transferred to the host plant.
- Tmr and Tzs are structurally related to higher plant IPTs.
- Tmr and Tzs can utilize substrates to produce iP and tZ.
- *Agrobacterium* IPTs can synthesize tZ nucleotide directly in the absence of host plant CYP735A when HMBDP is the substrate.

3.8 *Agrobacterium* IPT creates a bypass of direct tZ-type CK synthesis in host plastids

In *Agrobacterium*-infected plant cells, Tmr and Tms, encoded on the T-DNA, overproduce CK and auxin, respectively, resulting in hypertrophic and hyperplastic cell growth.

- *Tmr*-overexpressing transgenic plants almost exclusively contain tZ-type CKs.
- Tmr is targeted to and functions in plastids of infected host plant cells.
- In plastid stroma, Tmr allows the cell to synthesize CK from HMBDP without CYP735A-mediated hydroxylation.
- This bypass enables *A. tumefaciens* to produce large amounts of tZ in order to induce gall formation.

3.9 Structural studies reveal the molecular mechanism of the initial step in CK biosynthesis

3.10 CKs are also produced through degradation of tRNA

In addition to prenylation of adenine nucleotide by IPT, plants can produce CK through degradation of tRNA.

Organisms such as mammals and bacteria, in addition to plants, contain prenylated adenine in a subset of tRNA species.

Several CK species derived from tRNA-degradation have been reported, such as iP riboside (iPR), cis-zeatin riboside (cZR), tZR and their 2-methylthio derivatives.

The first step of modification is catalyzed by tRNA-IPT.

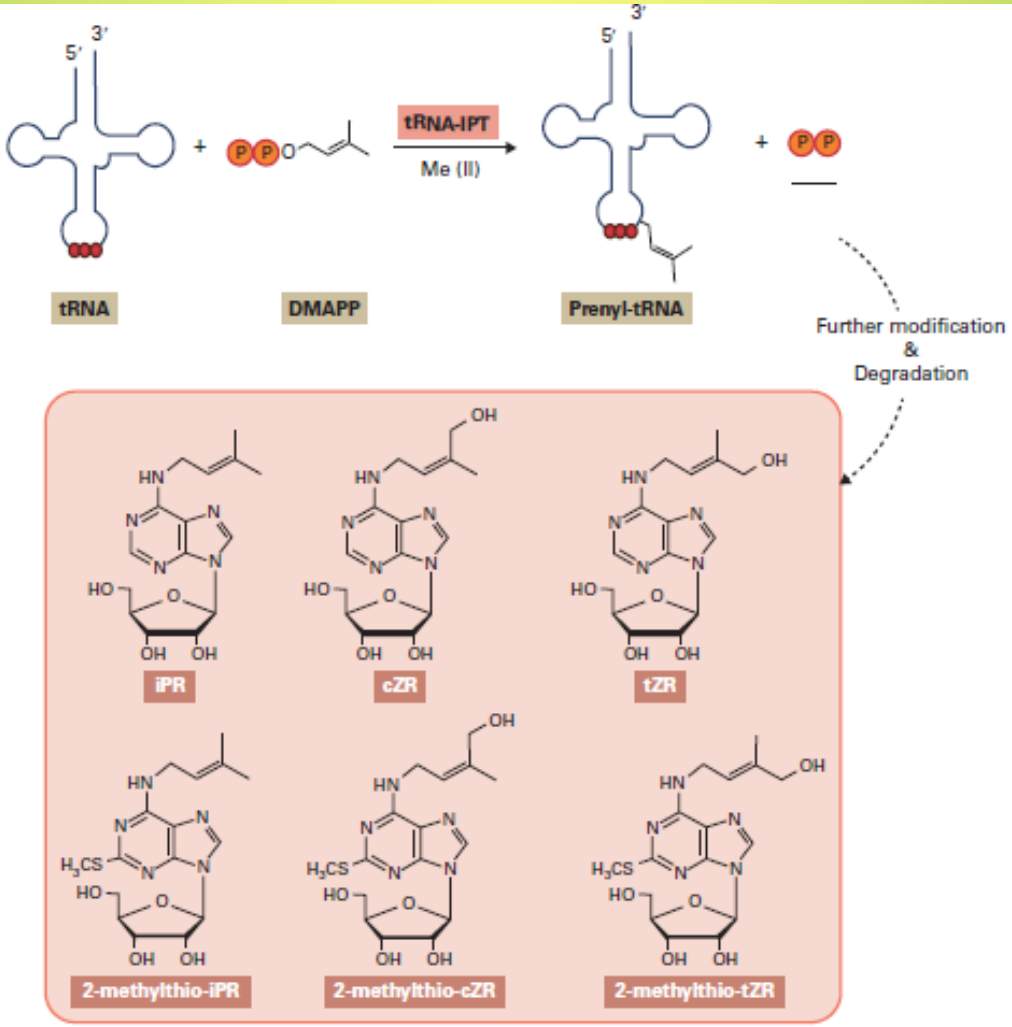


FIGURE 17.32 *CK production by tRNA degradation. tRNA-IPT conjugates DMAPP to adenine in a subset of tRNA species. Further modification and degradation results in the release of CKs. Structures of CKs derived from tRNAs are shown.*

3.11 The initial step of CK biosynthesis occurs in multiple subcellular compartments using DMAPP from different origins

DMAPP is produced through the MEP (The non-mevalonate pathway) and mevalonate (MVA) pathways.

In general, the MEP pathway is found in bacteria and plastids, and the MVA pathway in the cytosol of eukaryotes.

Application of lovastatin, an inhibitor of the MVA pathway, leads to a decrease in tZ-type CK accumulation in tobacco BY-2 cell cultures, suggesting that the MVA pathway can be the predominant origin of tZ-type CKs.

3.12 tZ can be converted to DZ and cZ

- The double bond of the tZ side chain can be enzymatically reduced to DZ by a zeatin reductase.
- In addition to degradation of tRNA, cZ-type CKs can be formed by isomerization of tZ.

3.13 Interconversion of CK nucleobase, nucleoside and nucleotide are partially shared with the purine salvage pathway

3.14 Degradation by CKX plays a key role in regulating cytokinin activity

In addition to biosynthesis and activation, deactivation is an important step in controlling active CK levels.

CKX mediates irreversible CK degradation by cleaving the side chain of an unsaturated isoprenoid side chain.

Among the naturally occurring CKs, iP, iPR, tZ, tZR, cZ, cZR, *N*-glucosides, and *N*-alanyl conjugates can be in vitro substrates, but generally CKX has a higher affinity for iP, tZ, and their nucleosides than others.

Aromatic CKs are resistant to CKX.

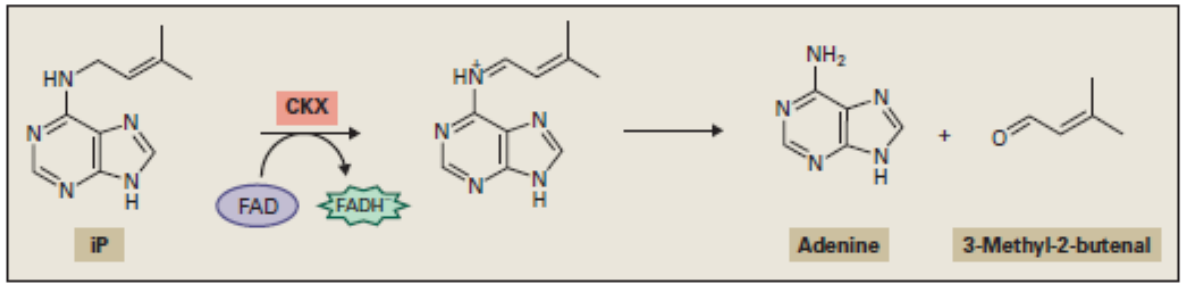
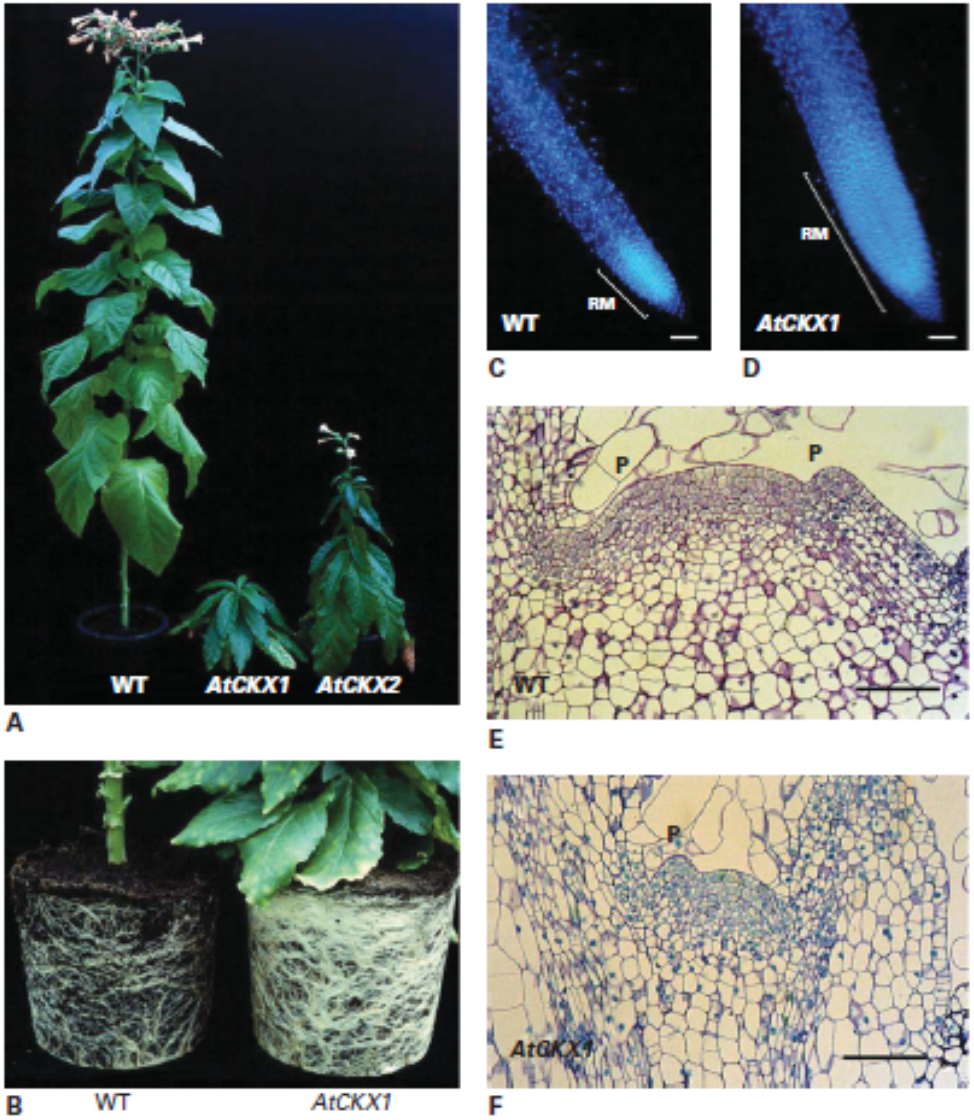


FIGURE 17.34 *CKX removes the side chain from CK molecules. CKX catalyzes the oxidation of the secondary amine group on the side-chain of the adenine ring. The resulting imine product is nonenzymatically hydrolyzed, producing adenine and an aldehyde.*

FIGURE 17.35 *Opposite phenotypes resulting from reduction of CKs in* N. tabacum *shoot and root. (A) Shoot phenotype of wild-type (WT) and transgenic lines overexpressing two different constructs of the* Arabidopsis *CKX genes:* AtCKX1 *and* AtCKX2. *(B) Root phenotype of WT and* AtCKX1. *(C), (D) Root apices of WT and* AtCKX1 *stained with DAPI. (E, F) Longitudinal section through shoot apical meristem (SAM) of WT and* AtCKX1. *The SAM is reduced in CK-deficient plants (*AtCKX1*). RM, root meristem; P, leaf primordia. Bar represents 100 μm.*



Reduction of CKX2 expression by natural variation increases CK levels in *O. sativa* (Fig. 17.36), indicating that control of CK activity in shoot apical meristems at the degradation step is mportant for agricultural productivity.



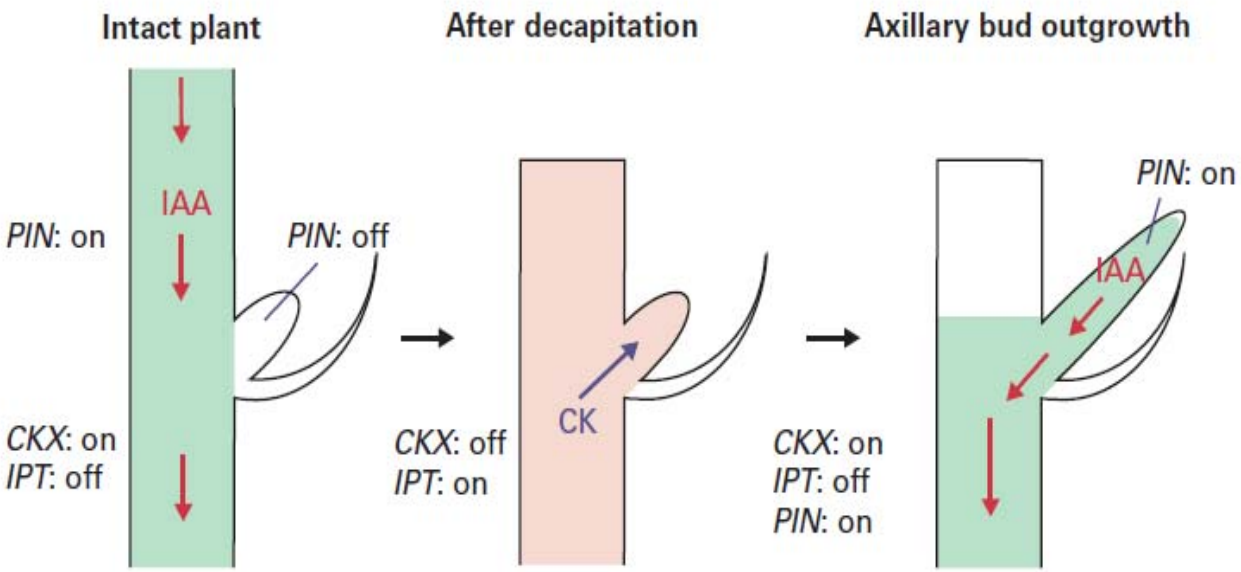
FIGURE 17.36 O. sativa CKX *gene mutation increases grain number. Panicles of Koshihikari (left) and Habataki (right). Natural variations within the Habataki* CKX2 *gene reduce expression levels in shoot meristems, resulting in increased grain numbers.*

3.15 Local CK metabolism plays an important role for regulating apical dominance

The mutual regulation of auxin and CKs plays a central role in the control of axillary bud outgrowth and dormancy (Fig. 17.37).

This regulatory system consists of the auxin-dependent expression of CKX and PIN, which is a component of auxin polar transport, and repression of IPT by auxin.

3.16 Glucosylation of the side chain hydroxyl group is catalyzed by zeatin O-glucosyltransferase



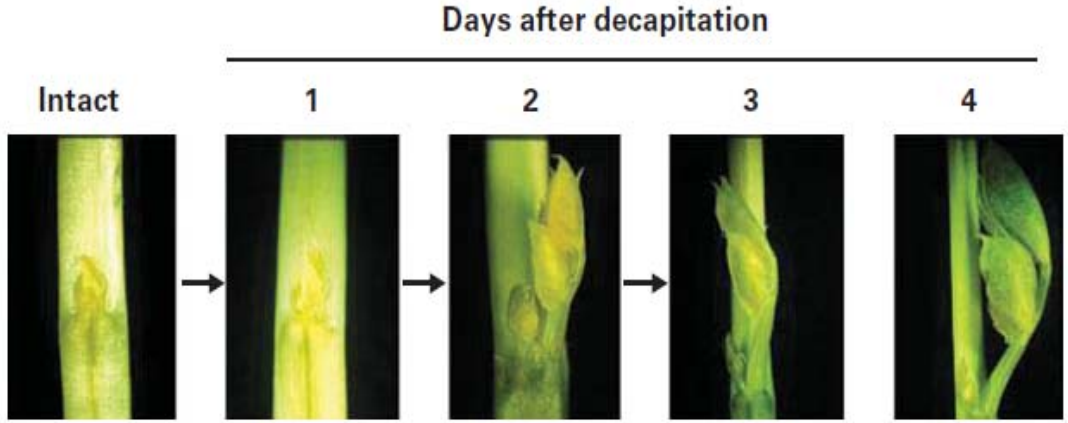


FIGURE 17.37 *A model of the interactions between auxin and CKs in controlling apical dominance. In an intact plant, auxin derived from the shoot apex maintains* PIN1 *and* CKX *expression and represses* IPT *expression. Since CK level is kept lower, axillary bud outgrowth does not occur. After decapitation (1 day after decapitation in photo), the auxin level in the stem decreases, resulting in the release of repression of* IPT *expression and down-regulation of* CKX *and* PIN1 *expression. The de novo synthesized CK in the stem is transported into dormant axillary buds and initiates their outgrowth. After axillary bud outgrowth (2 day and after in photo), de novo synthesized IAA from the new shoot apex again represses* IPT *expression and induces* CKX *and* PIN1.

.4 Auxins

- ✓During the 19th century, Theophili Ciesielski studied the geotropic responses of plants.
- ✓Charles Darwin and his son Francis investigated phototropism as well as geotropism.
- ✓These investigations laid the groundwork for Fritz Went, who in 1926 obtained from oat coleoptiles a diffusible growth- promoting factor subsequently named "auxin."
- ✓ The primary auxin present in most plants was eventually identified as indole-3-acetic acid (IAA).
- ✓Indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid, and phenylacetic acid, which are also known as naturally occurring auxins.
- ✓Synthetic auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthalene-1-acetic acid (NAA) are used extensively in horticulture to induce rooting and to promote the set and development of fruit. At high concentrations the synthetic auxins are effective herbicides against broad-leaved plants.

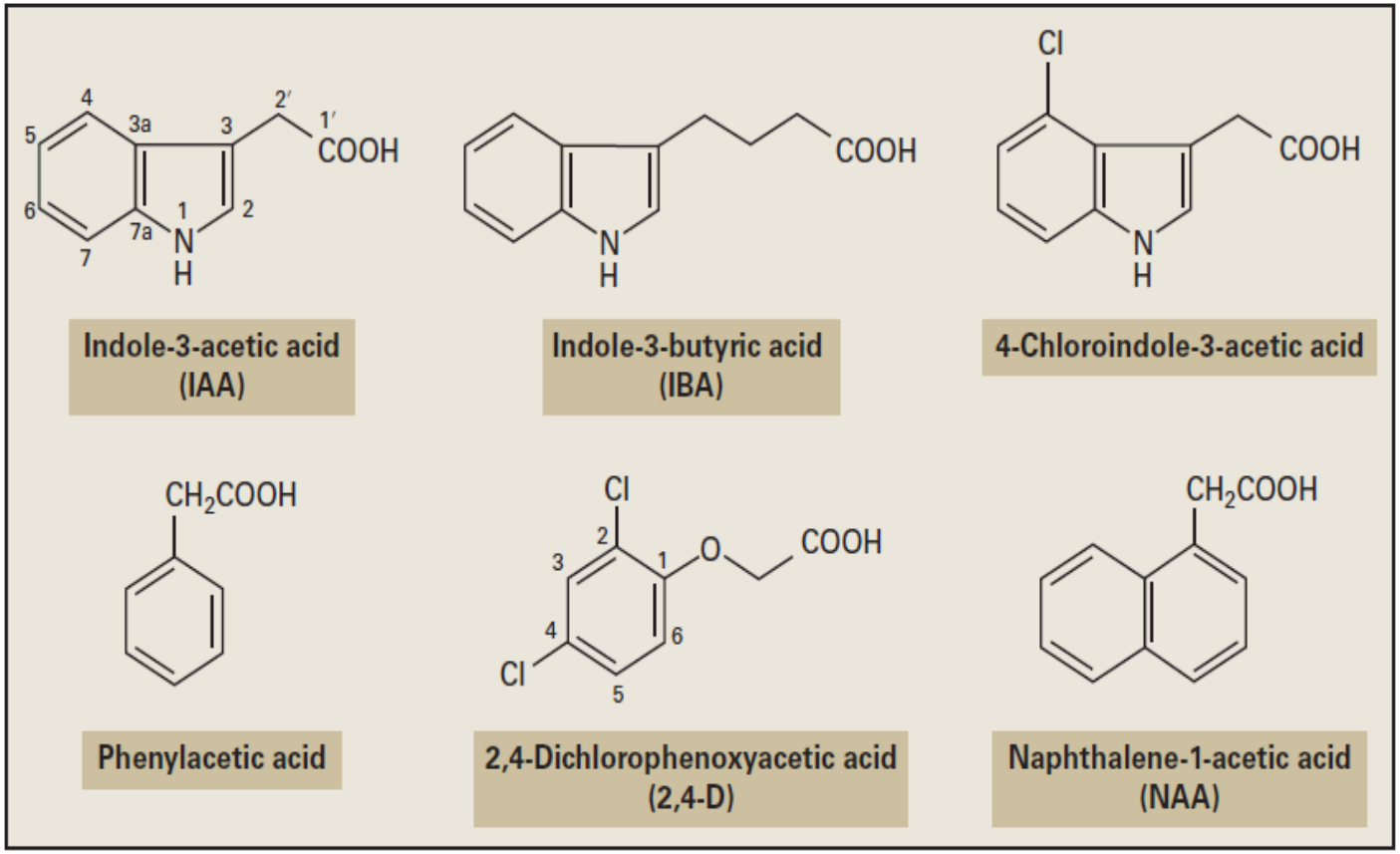


FIGURE 17.38 *Structures of auxins. IAA is the most widely distributed plant auxin. IBA, 4-chloroindole-3-acetic acid, and phenylacetic acid also naturally occur, but are less prevalent. 2,4-D and NAA are synthetic auxins.*

- *Effects*

❖Cell enlargement

❖Cell division

❖Vascular tissue

❖ Root initiation

❖Tropistic responses

❖Apical dominance

❖Leaf senescence

❖Leaf and fruit abscission

❖Fruit setting and growth

❖Assimilate partitioning

❖Fruit ripening

❖Flowering

❖Growth of flower parts

❖Promotes femaleness in dioecious flowers

The auxin activity in plants is primarily regulated by control of IAA content via several processes:

- de novo biosynthesis,
- inactivation by various conjugation
- catabolic pathways.

There are two de novo IAA biosynthesis pathways:

- one of which is dependent on the amino acid precursor l-tryptophan
- l-Trp-independent,

Hydrolysis of IAA conjugates also releases active auxin.

IAA content in individual tissues can also be influenced by basipetal polar transport that results in the downward movement of IAA from apical meristems and young leaves towards the root system.

4.1 Multiple routes are employed in the L-Trp-dependent auxin Biosynthesis

In plants, multiple routes have been identified in l-Trp dependent IAA biosynthesis:

their first products are:

- indole-3- pyruvic acid
- tryptamine.

Indole-3-pyruvic acid is formed by Trp aminotransferase, followed by conversion to IAA by YUCCA, (a Flavin-containing Monooxygenase).

- Tryptamine is produced by decarboxylation of l-Trp, catalyzed by Trp decarboxylase. Tryptamine is then converted to indole-3- acetoaldehyde, and then to IAA.

Indole-3-ethanol and its conjugates are produced in a side shunt from indole-3-acetaldehyde. These compounds may have a storage role, given that they can be rapidly reconverted to indole-3-acetaldehyde and used as a substrate for IAA biosynthesis.

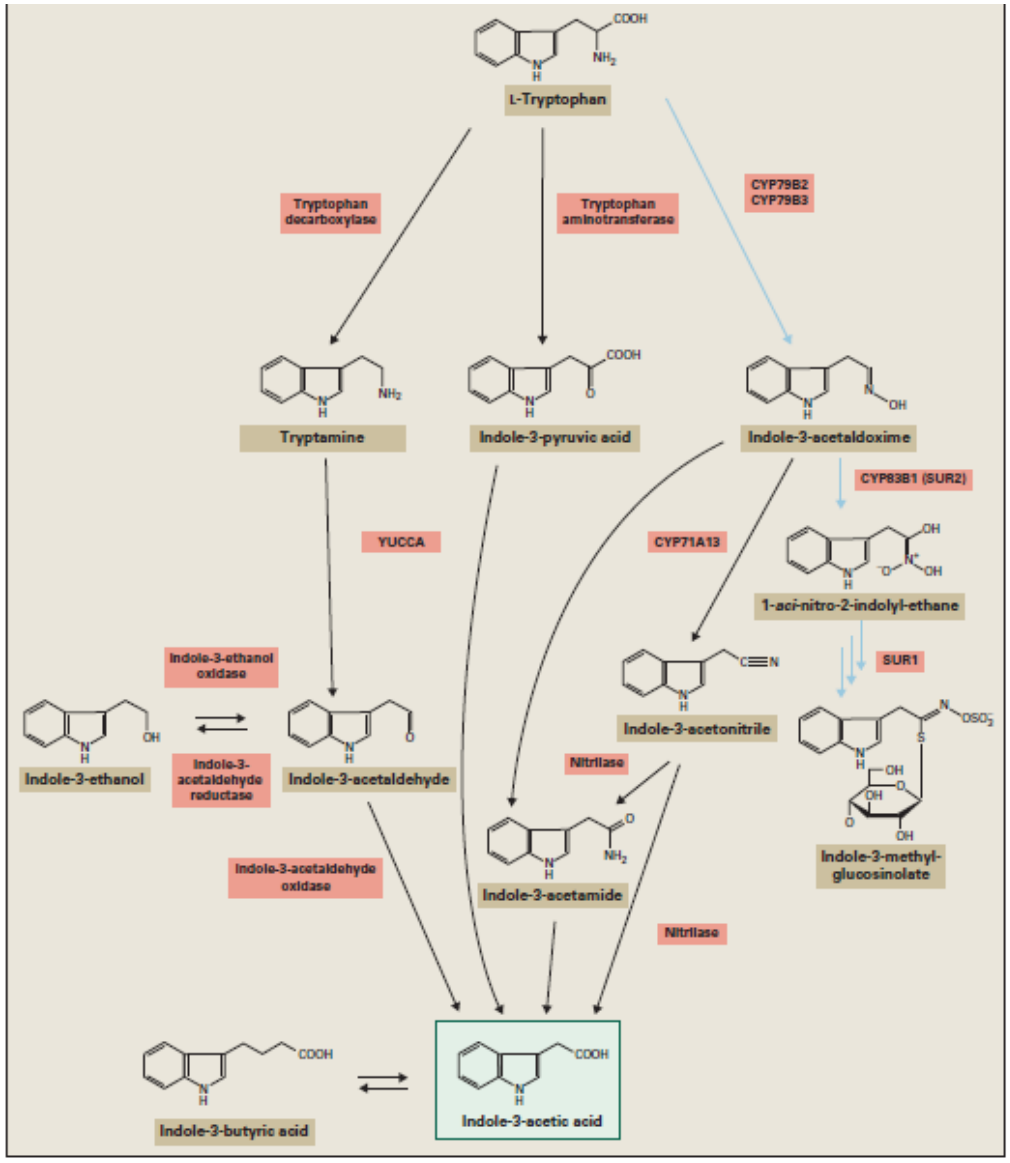


FIGURE 17.39 *L-Trp-dependent auxin biosynthesis pathway. Blue arrows indicate metabolic pathway shared with glucosinolate biosynthesis.*

- IBA has been found in a number of plants. It has auxin activity and is used to induce root formation on cuttings.
- IAA is converted to IBA in *Z. mays* and *Arabidopsis.*
- IBA synthase uses acetyl-CoA and ATP as cofactors.
- Exogenous IBA is conjugated rapidly by plants.
- Conversion of IBA to IAA has also been reported.

4.2 YUCCAs play a key role in auxin biosynthesis and plant development

- several lines of evidence from studies on gain of function and loss-of-function mutants demonstrates a key role for YUCCAs in auxin biosynthesis.
- *YUCCA* is encoded by a small multigene family, members of which are differentially expressed according to tissue.
- The expression of *YUCCAs* is temporally and spatially restricted and they are expressed in shoot and inflorescence apices, cotyledon tips in mature embryos, stamen, pollen, and stipules.
- de novo IAA biosynthesis mediated by YUCCA has a crucial role in the formation of floral organs and vascular tissues.

4.3 IAA biosynthesis pathway is shared with glucosinolate biosynthesis pathway in a few plant families

4.4 TAA1s also play a role in auxin biosynthesis and plant development and are required for shade avoidance

- Identification of genes for Trp aminotransferase (TAA1) in *Arabidopsis* revealed that auxin biosynthesis via indole-3- pyruvic acid is involved in shade avoidance responses and in ethylene–auxin interactions.
- Multiple mutants of *TAA1* family of genes show severe auxin related phenotypes, such as defects in root gravitropism, vasculature organization, and shoot and flower development.

4.5 Tryptophan-independent IAA biosynthesis was demonstrated, but the biological importance remains unclear

Evidence for IAA biosynthesis independent of l-Trp has been obtained with the orange pericarp (*orp*) mutant of *Z. mays.*

Seeds of the *orp* mutant contain increased concentrations of two l-Trp precursors, anthranilate and indole.

Despite their diminished capacity for l-Trp synthesis, *orp* mutants are rich in IAA.

Although *orp* seedlings contain roughly one-seventh the l-Trp present in wild-type *Z. mays*, IAA contents are increased 50-fold.

FIGURE 17.40 Z. mays *ear segregating kernels for the two-gene recessive trait orange pericarp* (orp)*; the orange kernels are homozygous for both mutant genes.*
Source: J. Cohen, University of Minnesota, MN, and A.D. Wright, University of Missouri, Columbia; previously unpublished.



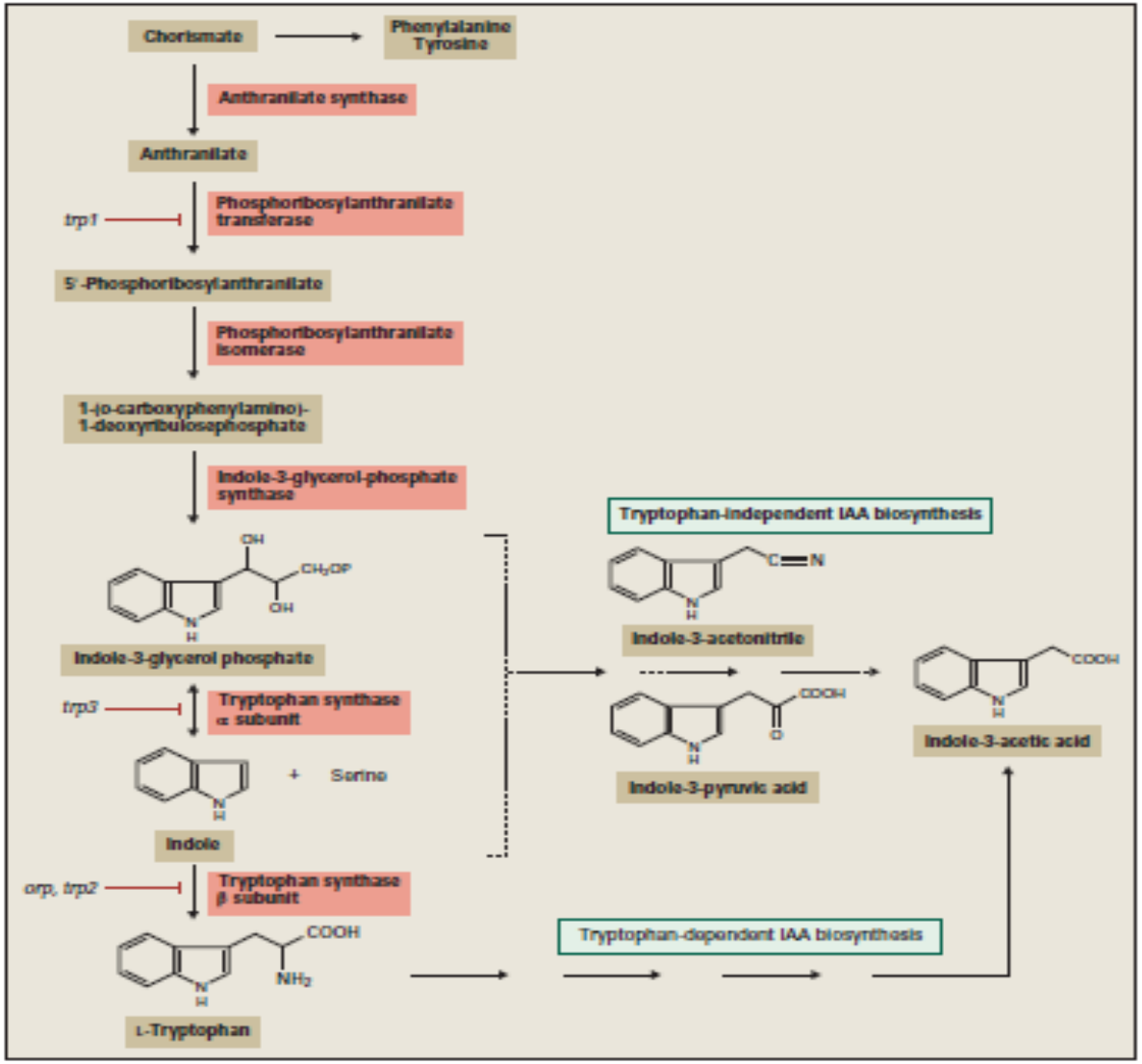


FIGURE 17.41 *Biosynthesis of L-Trp and L-Trp dependent and independent IAA biosynthesis. The enzymes affected in Z. mays orp and Arabidopsis trp1, trp2, and trp3 mutants are indicated.*

4.6 Several pathways for IAA conjugation and catabolism have been elucidated

IAA catabolism results in loss of auxin activity and irreversibly decreases the size of the IAA pool. Catabolism can proceed by:

decarboxylative or

nondecarboxylative pathways,

and in either case can involve oxidation of the indole ring.

As with CKs, catabolism of IAA sometimes involves conjugation reactions.

For many years, the decarboxylative catabolism was thought to represent the major IAA degradation pathway in plant tissues. However, evidence obtained with *Z. mays*, tomato and pea indicates that peroxidases have only a minor role in the regulation of endogenous IAA pools. IAA–amino acid conjugates that were once perceived as storage products have been identified as intermediates in nondecarboxylative catabolic pathways that deactivate IAA irreversibly.

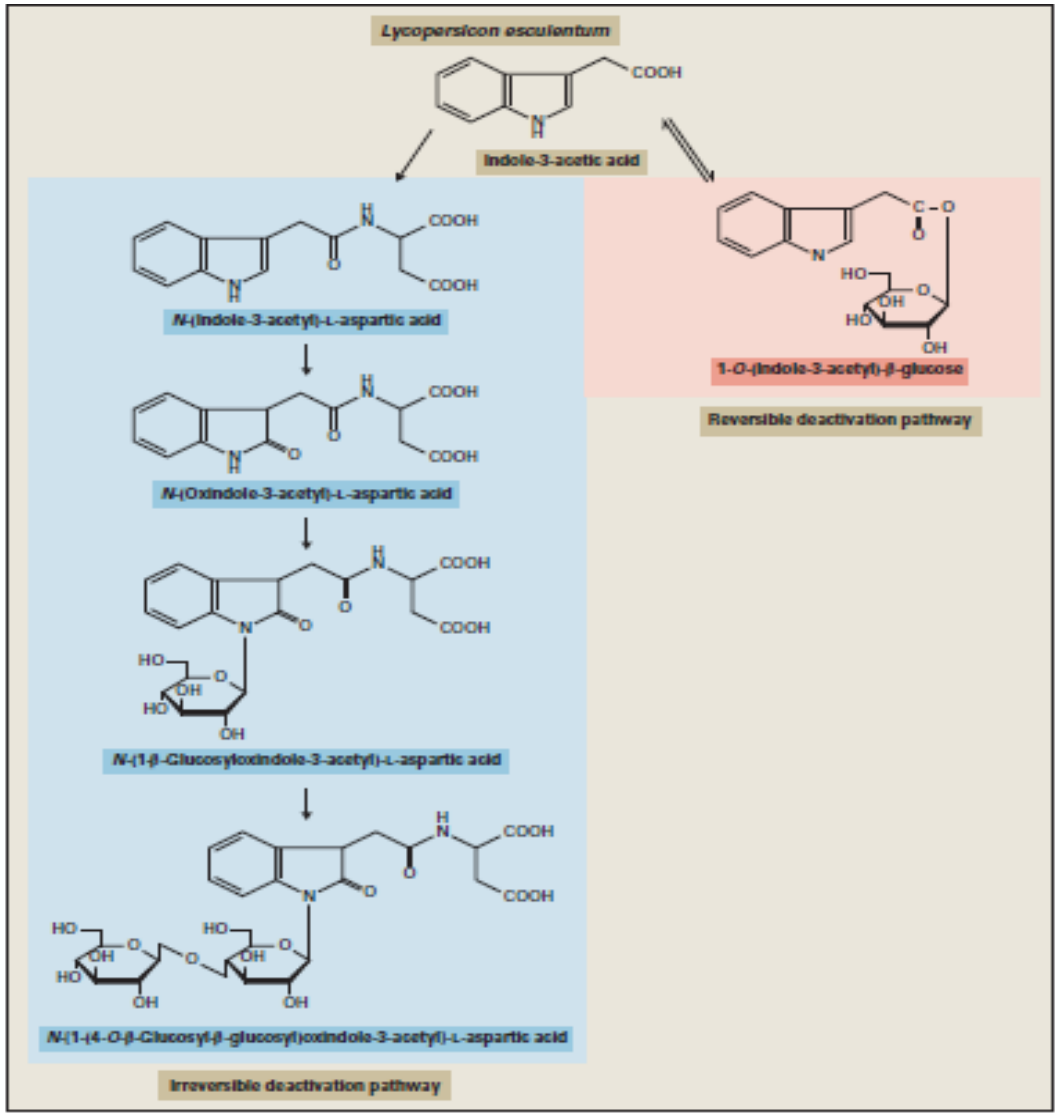


FIGURE 17.42 *Nondecarboxylative catabolism and conjugation of IAA in S. lycopersicum pericarp discs. N-(1-β-Glucosyloxindole-3-acetyl)-L-aspartic acid and N-[1-(4-O-β-glucosyl-β-glucosyl)oxindole-3-acetyl]-L-aspartic acid are permanently deactivated IAA conjugates formed in both green and red S. lycopersicum fruits, whereas the 1-O-(Indole-3-acetyl)-β-glucose formed by red pericarp tissues can be converted back to IAA.*

4.7 IAA ester conjugates serve as storage products in *Z. mays* seeds

The conjugation reactions, including aspartylation or glutamylation of the 1′ carboxyl, *N*-glycosylation of the indole ring, and glycosylation of either the 3 or 7 hydroxyl groups, appear to permanently deactivate IAA.

O-glycosylation of the 1′ carboxyl is typically reversible, so IAA–ester conjugates may function as storage products.

❖During the first days of germination, the *Z. mays* embryo derives most of its IAA from these three conjugates. However, the supply of hydrolyzable IAA conjugates declines as the seedling grows, and the young plant rapidly develops the capacity to synthesize IAA.

4.8 Amino acid conjugation of IAA is catalyzed by enzymes of the GH3 family and together with IAA-amido hydrolases are involved in IAA homeostasis

A subset of GH3 family proteins as IAA-amido synthetase, which catalyzes amino acid conjugation of IAA in the presence of ATP and $Mg2+$.

GH3.2 to GH3.6 and GH3.17 conjugate various l-amino acids, such as l-Ala l-Asp, l-Glu, l-Met, and l-Tyr to IAA in vitro, and can utilize indole-3-pyruvic acid, indole-3-butyric acid, phenyl acetic acid, and naphthalene-1-acetic acid as substrates.

- Most of the IAA-amido synthetase genes are responsive to auxin treatment, suggesting that plants use feedback regulation to control the amounts of active auxin

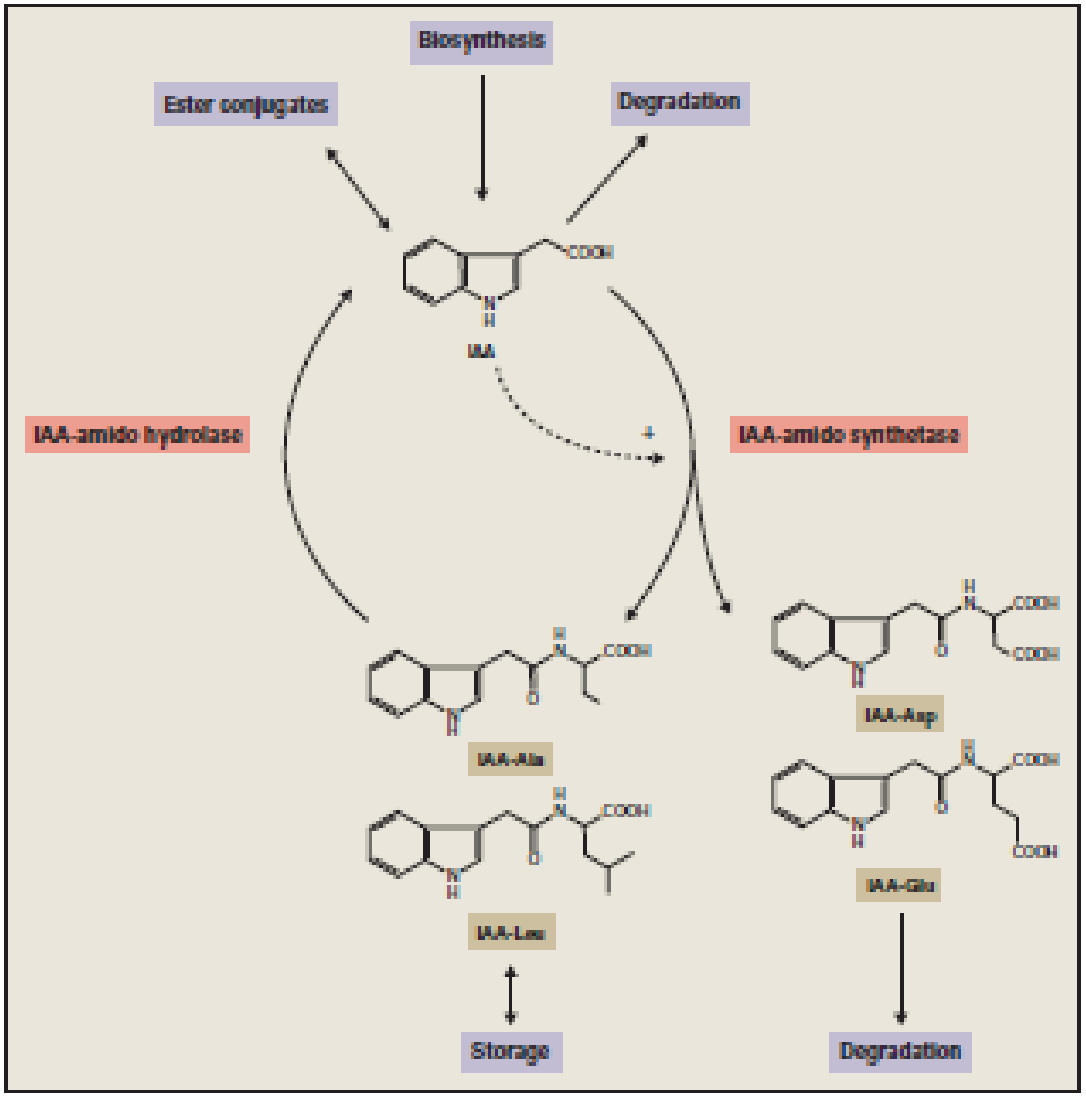


FIGURE 17.45 *Metabolic homeostasis regulating IAA concentration by IAA-amido synthetases and hydrolases. Dotted arrow shows transcriptional activation for several IAA-amido synthetase genes.*

4.9 Some bacterial pathogens encode novel IAA synthesis and conjugation pathways

some bacterial enzymes catalyze the production of plant hormones. The enhanced synthesis of IAA in *A. tumefaciens*-induced galls and tumors results from expression of two bacterial genes that are transferred to the plant when the T-DNA integrates into the host genome. These genes are associated with a unique two-step Trp-dependent pathway to IAA.

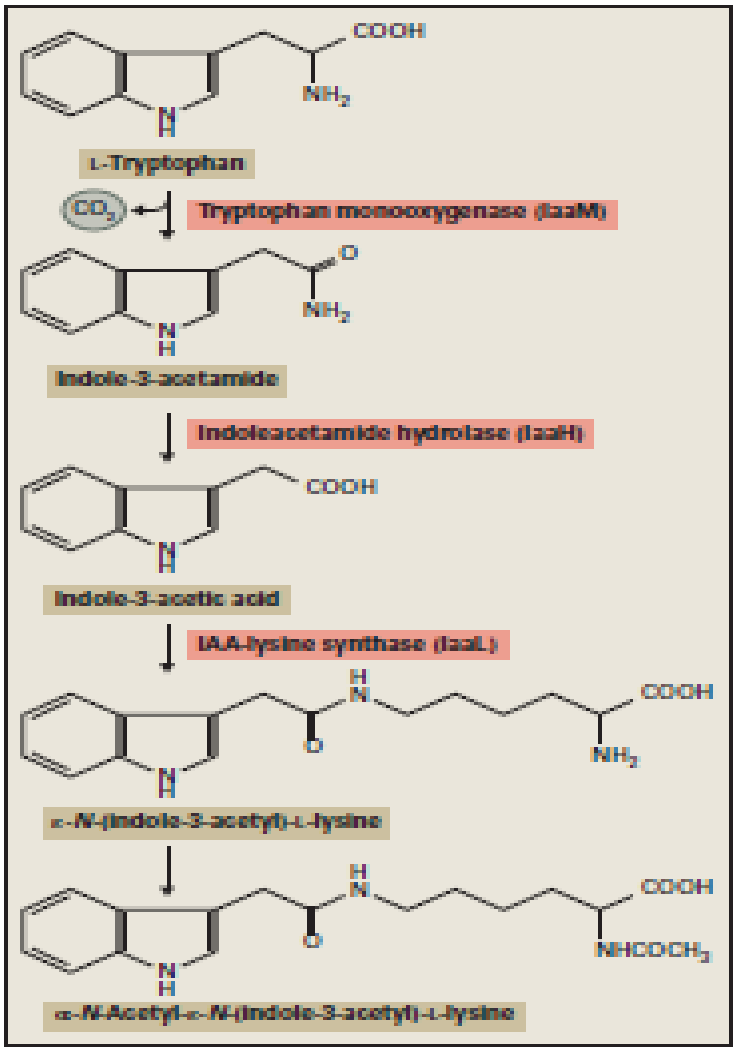


FIGURE 17.45 *IAA biosynthesis and conjugation pathways in* A. tumefaciens *and* Pseudomonas savastanoi. *iaaL does not occur in* A. tumefaciens.

4.10 Transgenic plants expressing IAA biosynthesis genes have been used to study the effects of excess amounts of endogenous IAA

- when IAA production is enhanced, conjugation appears to play a key role in regulating the size of the endogenous IAA pool and maintaining an almost normal phenotype.

High concentrations of IAA are typically accompanied by an increased rate of ethylene biosynthesis, and it was not initially possible to determine whether the phenotypic effects of IAA overproduction were a direct result of IAA or a consequence of increased ethylene levels.

major phenotypic effects, including:

- pronounced apical dominance,
- dwarfism,
- excess adventitious root formation,
- increased phloem and xylem formation,
- excess lignification,
- leaf epinasty,
- and abnormal flower production



FIGURE 17.47 *Eight-week-old tobacco plants,* N. tabacum *cv. Petit Havana SR1: wild-type plant (left); IAA-overproducing plant expressing* A. tumefaciens iaaH *and* iaaM *genes under the control of the CaMV 35S promoter (right). Note the severe stunting associated with production of IAA at about 500% of wild-type concentrations. Source:* Adapted from Nilsson et al. (1993). Plant J. 3:681–689.

The phenotype of the Samsun double-transformants, in which IAA overproduction is not accompanied by increased ethylene biosynthesis, shows that apical dominance and leaf epinasty are controlled primarily by IAA, whereas reduced stem elongation is an indirect consequence of high ethylene concentrations

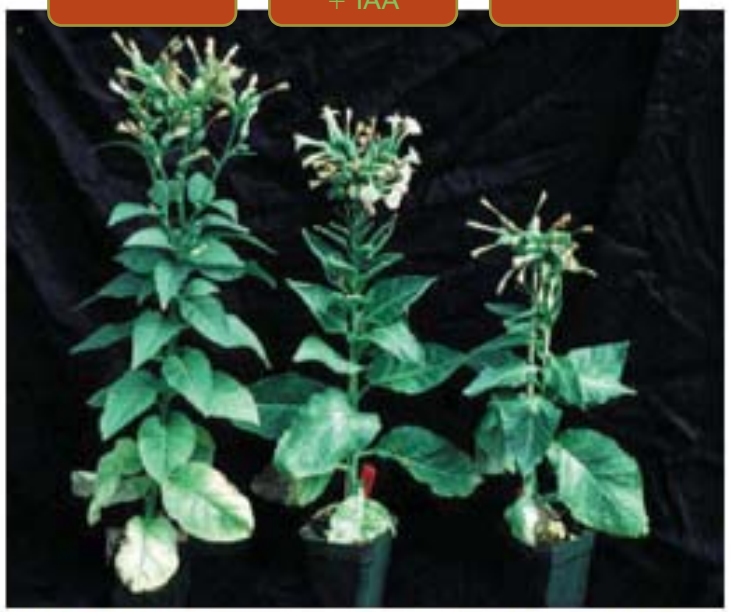


FIGURE 17.48 *Uncoupling of auxin and ethylene effects in eight-week-old transgenic* N. tabacum *cv. Samsun. (Left) An ethylene-deficient plant expressing a* Pseudomonas *ACC deaminase gene under the control of the figwort mosaic virus 19S promoter. The phenotype is indistinguishable from wild-type plants. (Middle) A double transformant with increased IAA content and decreased ethylene production. (Right) An IAA-overproducer expressing the* A. tumefaciens iaaM *gene under the control of the CaMV 35S promoter. The phenotype indicates that apical dominance and leaf epinasty are primarily controlled by IAA, whereas ethylene is partially responsible for the inhibition of stem elongation observed in IAA-overproducing plants.* Source: Romano et al. (1993). Plant Cell 5:181–189.

4.11 GAs increase IAA pools, whereas CKs may down-regulate IAA synthesis and turnover

Application of GA3 to Little Marvel dwarf pea seedlings enhances shoot growth with a concomitant eightfold increase in IAA content of elongating tissues. Conversely, the size of the endogenous IAA pool is reduced in the Alaska pea seedlings dwarfed by treatment with uniconazole.

In contrast to GA3, CK reduce the size of endogenous IAA pools. Transgenic tobacco plants that express the A. tumefaciens ipt gene overproduce CKs. Compared with wild-type plants, these transgenics contain significantly lower concentrations of free IAA and in most cases IAA conjugates. Rates of IAA biosynthesis also are reduced.

17.5 Ethylene

In 1886, while a graduate student in St. Petersburg, Dimitry Nikolayevich Neljubow noticed that etiolated pea seedlings grew horizontally in laboratory air, and vertically in air from outside the laboratory.

After an extensive study to exclude cultural practices, light, and temperature as causative agents, he showed that **ethylene**, in the gas used for lighting, induced this abnormal growth.

Many of ethylene's physiological effects on plant growth and development, including its impact on seed germination, root and shoot growth, flower development, senescence and abscission of flowers and leaves, and the ripening of fruit, were discovered prior to 1940. Subsequent work has since shown that ethylene also participates in the modulation of plant responses to a range of biotic and abiotic stresses.

Effects

- The so called *triple response* (a decrease in stem elongation, a thickening of the stem and a transition to lateral growth)
- Maintenance of the apical hook in seedlings (*apical hook—a structure of dicotyledonous plants shaped by the bended hypocotyl that eases the penetration through the covering soil*).
- Stimulation of numerous defense responses in response to injury or disease.
- Release from dormancy
- Shoot and root growth and differentiation
- Adventitious root formation
- Leaf and fruit abscission
- Flower induction in some plants
- Induction of femaleness in dioecious flowers
- Flower opening
- Flower and leaf senescence
- Fruit ripening

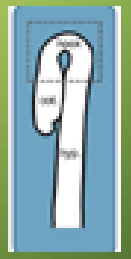




FIGURE 17.49 *The triple response to ethylene of six-day-old etiolated* P. sativum *seedlings and four-day-old etiolated* Vigna radiata *bean seedlings. (A) Untreated control* P. sativum *seedlings (0) and* P. sativum *seedlings grown for two days in air supplemented with ethylene at 0.1, 1.0, and 10 µl/ml. Note the concentration-dependent effects of ethylene on diageotropism, inhibition of epicotyl elongation, and lateral enlargement of the epicotyl. (B) Control* V. radiata *seedlings (0) and* V. radiata *seedlings grown for two days in air supplemented with 1 and 10 µl/ ml ethylene, which induces a concentration-dependent inhibition of hypocotyl elongation, lateral enlargement of the hypocotyl, and extreme bending of the apical hook. (C) Magnification of ethylene-treated etiolated* V. radiata *seedlings.*
Source: (A–C) H. Mori, Nagoya University, Japan; previously unpublished.

5.1 Ethylene is synthesized from *S*-adenosyl-l-methionine via the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC)

- Synthesis of ethylene from its immediate precursor 1- aminocyclopropane -1-carboxylic acid (ACC) is catalyzed by ACC oxidase (ACO).
- ACC is produced from SAM in a reaction catalyzed by ACC synthase (ACS).

These reactions are part of the methionine cycle or Yang cycle named after S. F. Yang, who carried out the early work in elucidating the pathway

In addition to its role in ethylene biosynthesis, SAM is involved in the biosynthesis of polyamines (PA) and a wide range of methylation reactions.

ACS has been isolated from a number of plant tissues following induction by factors that include exogenous IAA, wounding, lithium chloride stress, and climacteric fruit ripening.

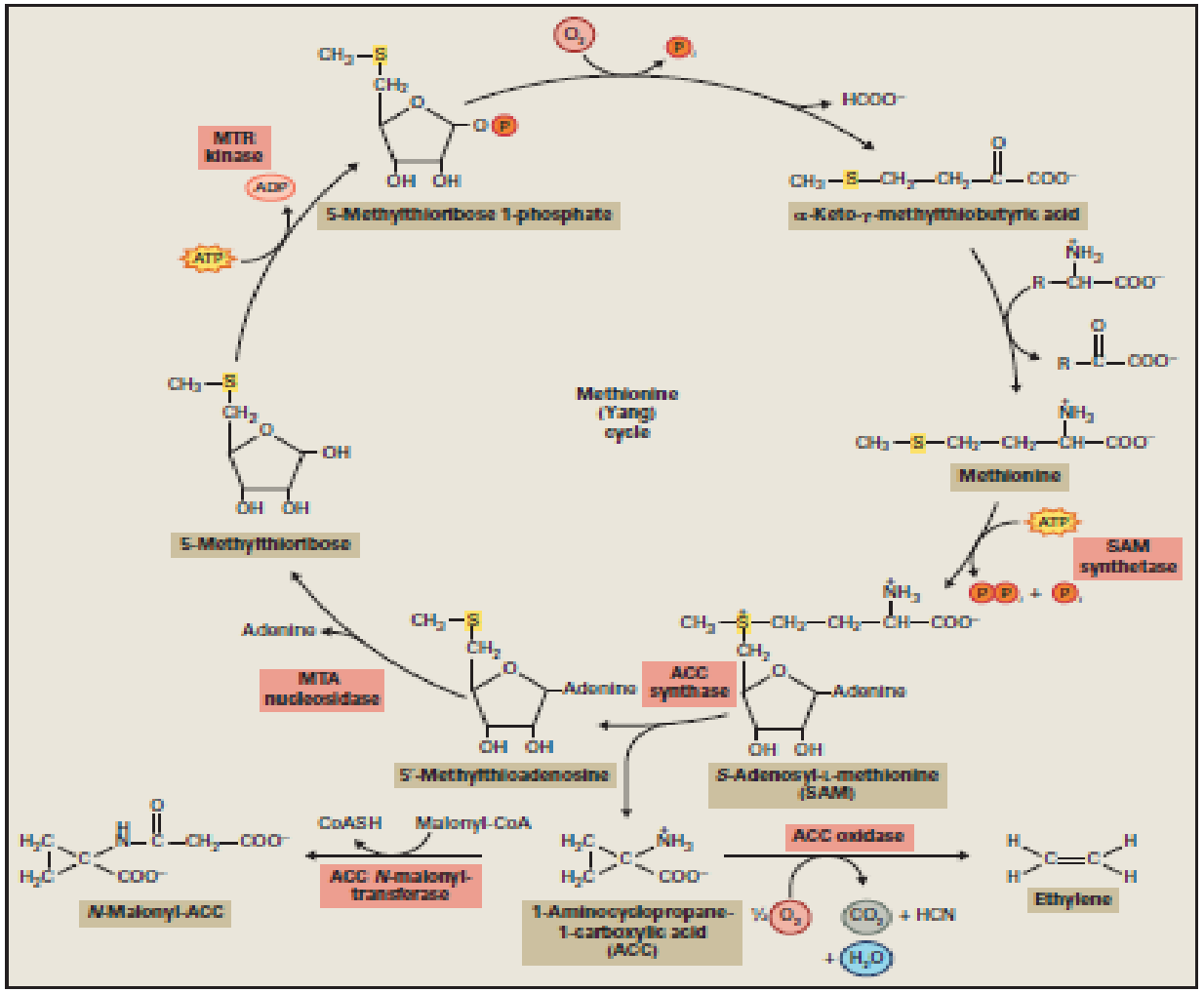


FIGURE 17.50 *The Met cycle and ethylene biosynthesis. Ethylene is synthesized from Met by way of SAM and ACC. The enzymes that catalyze these three steps are SAM synthase, ACC synthase (ACS), and ACC oxidase (ACO). 5′-Methylthioadenine, a product of the ACO reaction, is salvaged for the resynthesis of Met through the methionine cycle (see Chapter 7). If the methylthio group from SAM were not recycled, Met availability and ethylene biosynthesis would probably be restricted by sulfur availability. By converting ACC to N-malonyl-ACC instead of to ethylene, plants can deplete the ACC pool and thereby reduce the rate of ethylene production.*

5.2 ACSs are major regulators of ethylene biosynthesis

ACSs catalyze the rate-limiting step in ethylene biosynthesis.

ACSs levels are controlled by:

transcription

and

protein stability.

Increased ethylene production, associated with germination, ripening, flooding, and chilling, is **invariably** accompanied by increased ACC production due to induction or activation of ACS.

ACSs requires pyridoxal phosphate for activity and is sensitive to inhibitors of pyridoxal phosphate, especially aminoethoxy-vinyl glycine and amino-oxy acetic acid. These inhibitors allow investigators to distinguish between the effects of ACS and ACO.

The naturally occurring isomer of SAM, (–)-*S*-adenosyl-lmethionine, is the preferred substrate for ACS, while (+)-SAM is an effective inhibitor.

However, incubating the enzyme with **high** concentrations of **(–)-SAM** can irreversibly modify and **inhibit ACS**.

This "suicide inactivation" involves covalent linkage of a fragment of the SAM molecule to the active site of the enzyme. This substrate-dependent inactivation may be a contributory factor in the rapid turnover of ACS in plant tissues.

ACSs levels are controlled by: transcription and protein stability.

Transcriptional control

Ethylene biosynthesis rates are influenced by other plant hormones and by ethylene itself.

Auxins promote ethylene synthesis by enhancing the rate of ACC production. Transcript analysis showed that auxin application results in increased levels of certain ACS mRNAs, indicating transcriptional control.

The respective ACS genes have been shown to have *cis* acting auxin-response elements.

Unripe developing fruits have auto inhibitory ethylene production and certain ACS genes are transcribed. During ripening ethylene can promote (autocatalyze) its production and different ACS genes are transcribed.

Control via protein turnover

Early observations indicated that ACS stability in *S. lycopersicum* fruits varied between ripe and unripe fruits.

More recent experiments have shown that ACS turnover plays an important role in regulating ethylene production and that the C-terminal regions of the ACS protein act in regulating this turnover.

The stability of ACS proteins is influenced by their phosphorylation status at C-terminal regions with the phosphorylated forms being more stable.

Type 1 ACS proteins are phosphorylated by a MAPK in response to stress, for example, pathogen and wounding, and by a CDPK. Phosphorylation increases protein stability whereas unphosphorylated proteins are degraded by an undefined mechanism. Type 2 ACS proteins are phosphorylated by a CDPK and this prevents binding of the ETO (**E**thylene **o**verproducing) gene product. ETO1 can catalyze the addition of ubiquitin to ACS. Once ACS is ubiquitinated it is targeted for proteolysis.

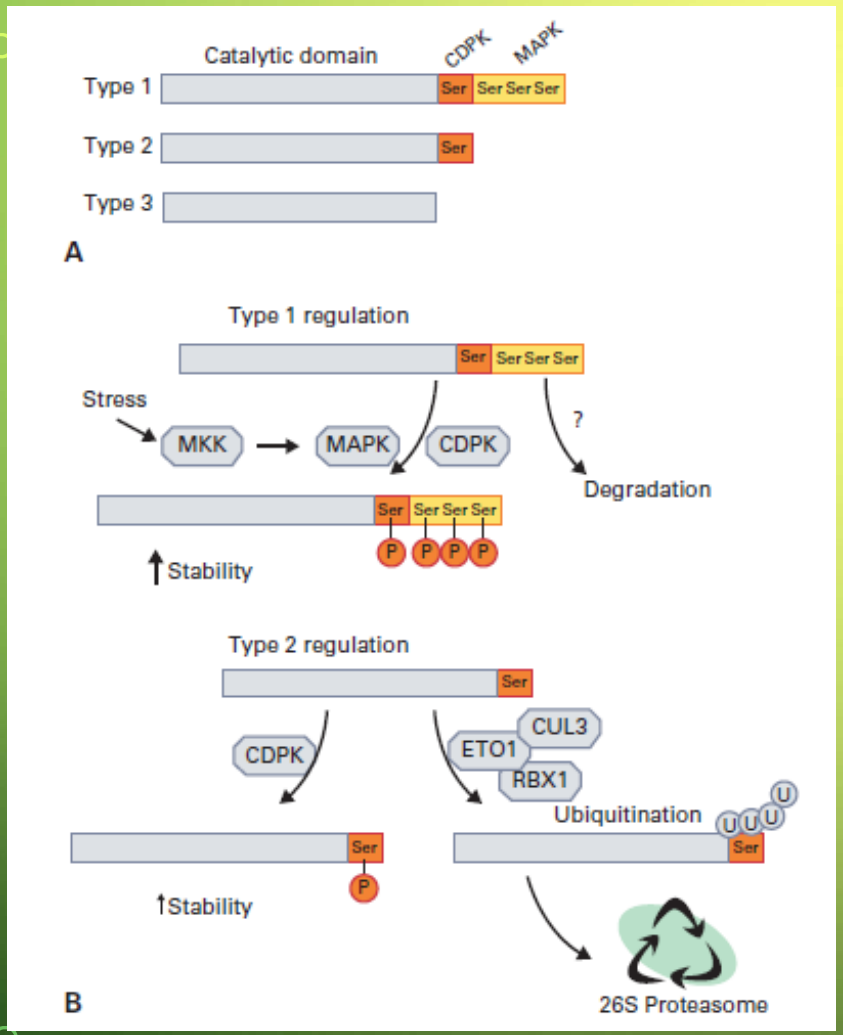


FIGURE 17.51 *ACS types and mechanisms of protein regulation. (A) ACS proteins fall into three major classes according to their C-terminal sequences. Type 1 have three conserved Ser residues (yellow) that can be phosphorylated by a MAPK and a single conserved Ser (red) that can be phosphorylated by a CDPK. Type 2 are slightly truncated and have a Ser that can be phosphorylated by a CDPK. Type 3 lack any conserved C-terminal Ser residues. (B) Type 1 regulation: CDPK and MAPK phosphorylation of C-terminal region increases ACS stability. The MAP kinase (MKK), induces MAPK activity when stimulated by stress e.g. wounding or pathogen attack. Unphosphorylated protein is removed by a yet to be defined mechanism. Type 2 regulation: CDPK phosphorylation of C-terminal Ser increases ACS stability by preventing binding of the ETO protein. Unphosphorylated ACS undergoes ETO binding and this facilitates the formation of a RING E ligase complex that includes a Cullin3 (CUL3) protein and a RING Box 1 protein (RBX1). The E3 ligase adds ubiquitin (U) moieties to the ACS protein thereby targeting it for degradation by the 26S proteasome.*

5.3 ACC oxidase resisted biochemical characterization and was cloned using molecular techniques

The conversion of ACC to ethylene is catalyzed by ACO, previously referred to as "ethylene-forming enzyme".

- The ACO reaction can be summarized in:

$$ACC + O_2 + \text{ascorbate} \xrightarrow{Fe^{2+}} C_2H_2 + CO_2 + \text{dehydroascorbate} + HCN + 2H_2O$$

ACO is activated by one of its products, carbon dioxide. The cyanide generated by the reaction is detoxified by conversion to β-cyanoalanine, which is further metabolized to Asn or γ-glutamyl-β-cyanoalanine.

All plant tissues appear to contain ACO, as measured by the rate of ethylene evolution in the presence of a saturating concentration of ACC. Under stress conditions, in response to ethylene, and at selected stages of development (e.g., fruit ripening), ACO activity increases markedly. Both senescence and ripening-induced increases in ACO activity are a result of increased transcription.



FIGURE 17.52 *Effect of antisense ACO genes on ripening and spoilage of* S. lycopersicum *cultivar Ailsa Craig fruit picked three weeks after onset of ripening and stored at room temperature for three weeks. (Left) Fruits from the descendants of the original TOM13-antisense plants, which generate about 5% of the normal amount of ethylene. They ripen fully but do not overripen and deteriorate. (Right) Fruits from wild-type plants grown and stored under identical conditions. They produce normal amounts of ethylene and consequently exhibit severe signs of over-ripening.*

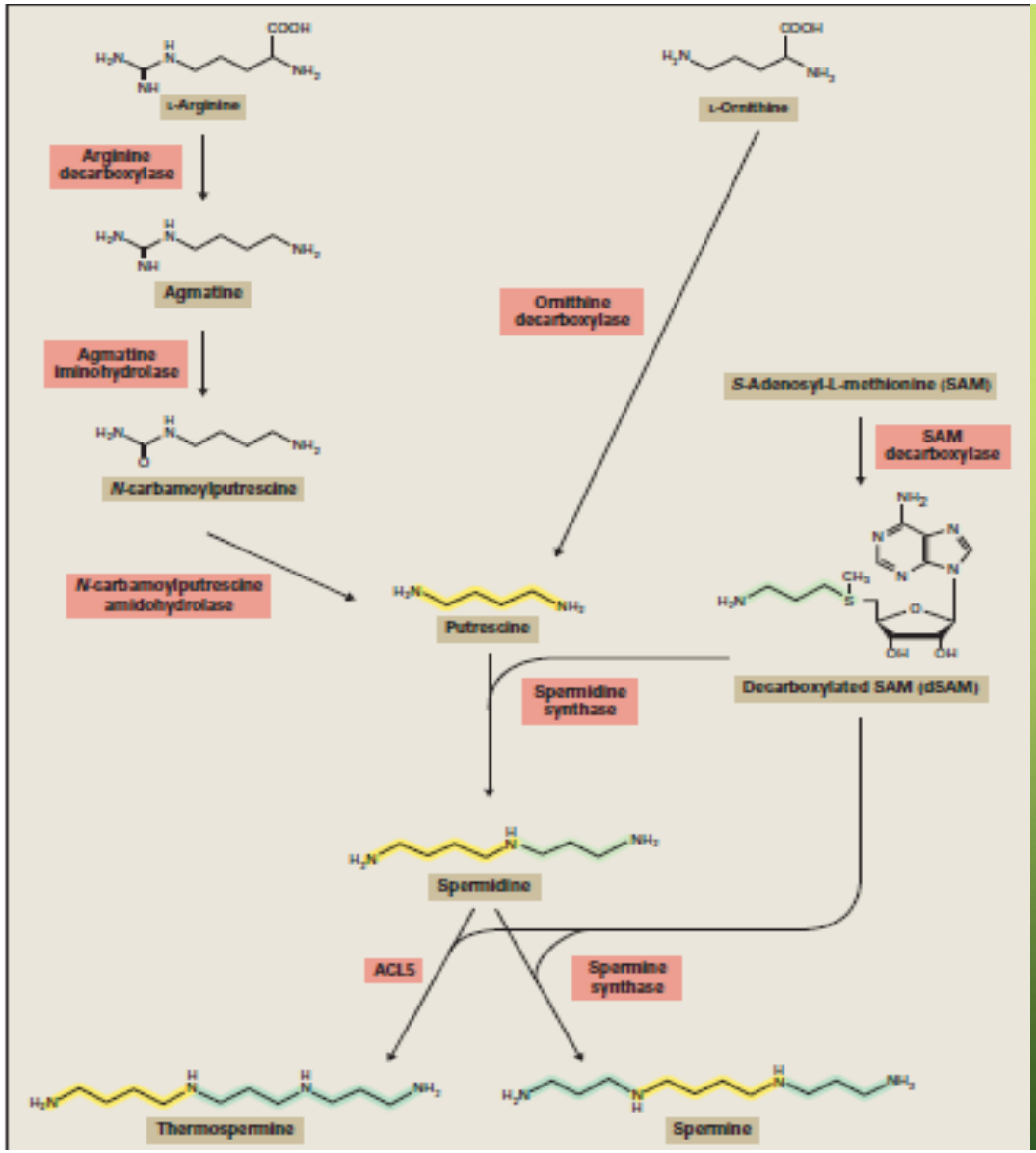
Source: D. Grierson, University of Nottingham, UK; previously unpublished.

5.4 When supply of available SAM is low, ethylene and polyamine biosynthetic pathways may compete for this shared substrate

- Biosynthesis of both ACC and PAs involves the incorporation of the aminopropyl group from SAM.

Under certain conditions, competition for SAM may restrict rates of ethylene or PA production. Inhibition of ACC synthesis by aminooxyacetic acid results in increased PA production. Conversely, inhibition of PA biosynthesis leads to increased concentrations of ACC and ethylene.

This implies that one SAM-dependent pathway is stimulated when the other is blocked. When competition for the available SAM is circumvented by low demand for PAs, or when ACC levels are increased by upregulation of 5′-methylthioribose-recycling enzymes, ethylene and PA production will not directly interact.


L-Arginine
Arginine decarboxylase
Agmatine
Agmatine iminohydrolase
N-carbamoylputrescine
N-carbamoylputrescine amidohydrolase
L-Ornithine
Ornithine decarboxylase
S-Adenosyl-L-methionine (SAM)
SAM decarboxylase
Decarboxylated SAM (dSAM)
Putrescine
Spermidine synthase
Spermidine
ACL5
Spermine synthase
Thermospermine
Spermine

5.5 Most hormones must be catabolized, but volatile ethylene can be released as a gas

Prior to 1975, ethylene metabolism by plants was considered to be an artifact, caused by bacterial contamination.

There is now evidence from plants grown in sterile conditions that [^{14}C]ethylene is oxidized to [^{14}C]CO2 or converted to [^{14}C]ethylene oxide and [^{14}C]ethylene glycol.

Ethylene metabolism exhibits a very high K_M indicative of a chemical reaction rather than a physiological process.

In peas, the concentration of ethylene yielding a half-maximal rate of ethylene metabolism is ≈1,000 times the concentration required for half-maximal response in the pea growth test.

It is likely that ethylene metabolism is largely a consequence of artificially elevated ethylene levels.

The major route by which plant tissues lose ethylene is probably diffusion to the surrounding atmosphere.

5.6 Repression of ethylene biosynthesis can delay over-ripening in fruit, and represents an important field of biotechnological research

Two different biotechnological strategies have been employed to generate transgenic tomato fruit that resist over-ripening.

1: the overexpression of a *Pseudomonas* gene encoding ACC deaminase, reduces ethylene levels in fruits by catalyzing the conversion of ACC to α-ketobutyric acid and NH3.

2: limiting ethylene biosynthesis involves use of antisense gene constructs against either ACO or ACS.

The phenotype of transgenic tomato fruit expressing antisense ACO:

- ethylene production is inhibited by about 95% during ripening.
- fruits grow normally and begin to change color, losing chlorophyll and accumulating lycopene, at the
- same stage of development as nontransformed fruit.
- exhibit reduced reddening and an increased resistance to over-ripening and shriveling when stored at
- room temperature for prolonged periods.
- do not soften as readily and can be left on the plant longer to ripen more fully.

6 Brassinosteroids

- ✓ In the early 1960s, researchers hypothesized that the rapid germination and growth of pollen grains was associated with the presence of a growth promoter.
- ✓ A crude extract of pollen from *Brassica napus* (rape) induced rapid elongation of *P. vulgaris* (pinto bean) internodes distinct from GA-mediated stem elongation.
- ✓ This early work led to the isolation and identification of brassinolide (BL) the first steroidal plant growth regulator.
- ✓ A second plant steroid, castasterone (CS) was isolated from insect galls of chestnut (*Castanea*).
- ✓ Today, a number of related steroidal compounds have been isolated from a variety of plant sources, and collectively called **brassinosteroids** (BRs).
- ✓ More than 40 BRs have been identified and, structurally, they are C27-, C28-, and C29-steroids with different functional groups on the A- and B-rings and side chain.
- ✓ Brassinolide, a C28-BR, has the highest biological activity of the BRs and is distributed widely in the plant kingdom, along with biosynthetically related compounds.

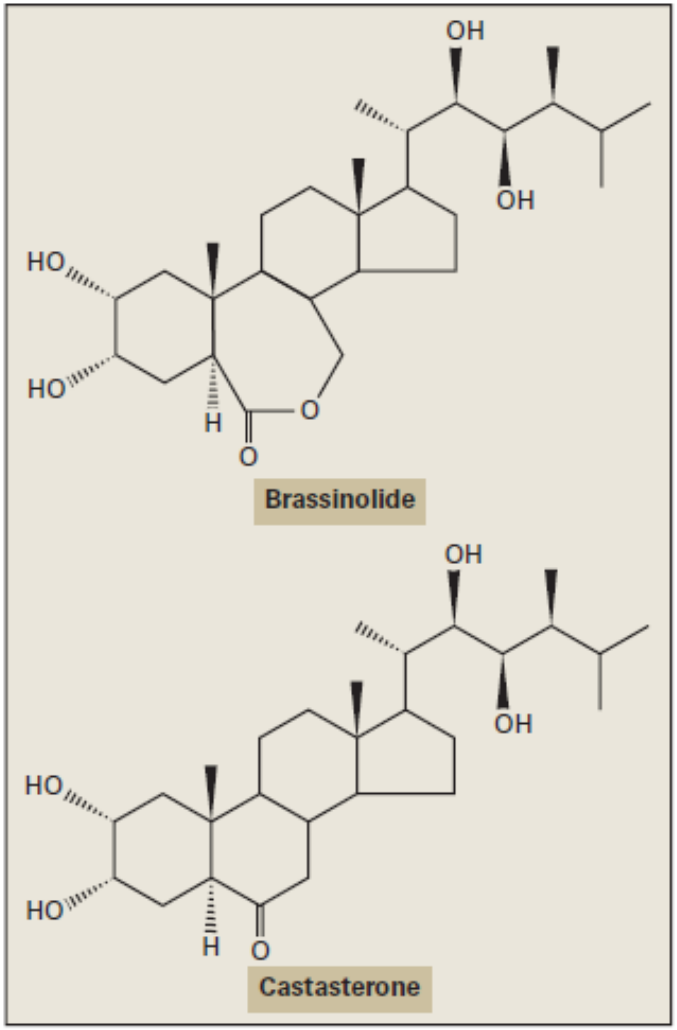


FIGURE 17.53 *Structures of brassinolide and castasterone.*

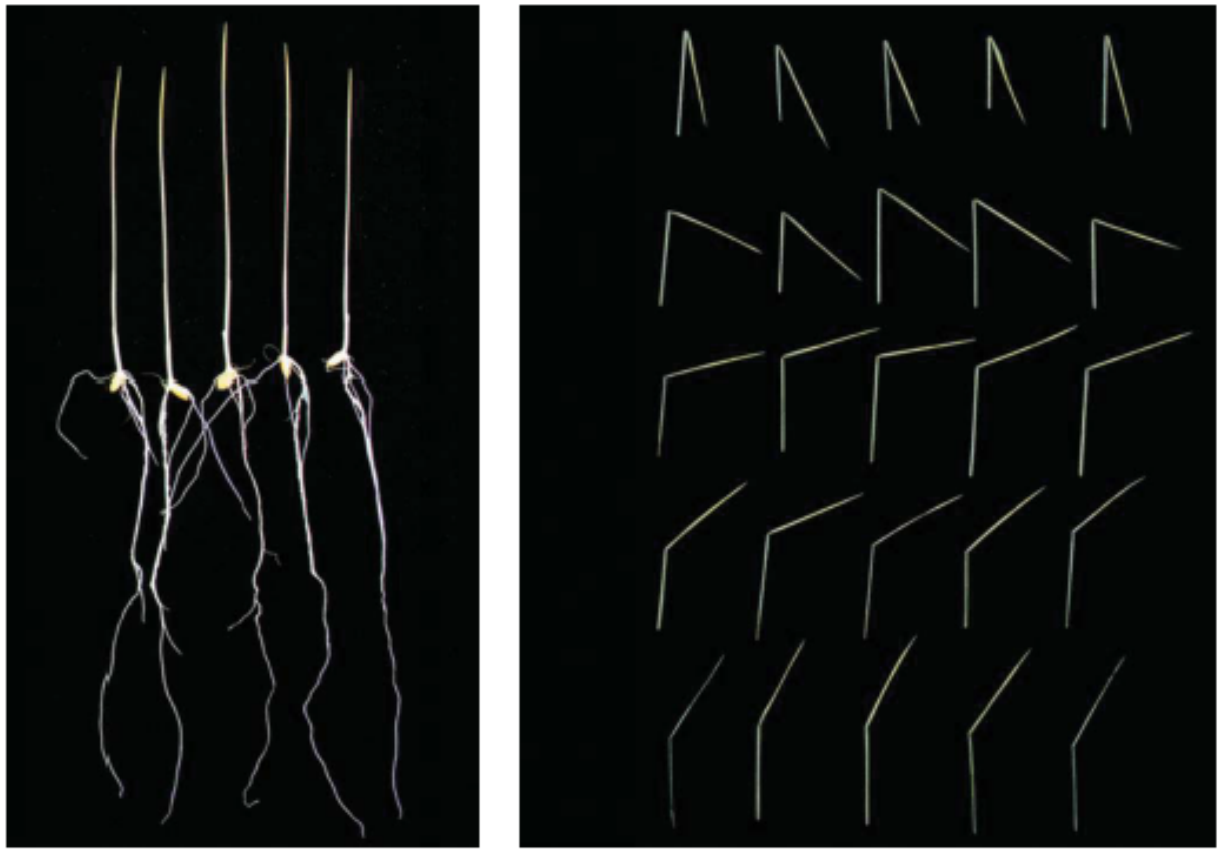


FIGURE 17.54 *Etiolated* O. sativa *seedlings used to obtain excised leaf lamina (left) and the effects of increasing concentrations of BL on the rice lamina inclination bioassay (right). BL and other BRs induce a dose-dependent swelling of the adaxial cells of the joint between the leaf's blade and sheath. This bioassay is very sensitive and is used in studies on BR structure–activity relationships as well as for screening fractionated plant extracts for BR activity.*

Source: T. Yokota, Teikyo University Utsunomiya, Japan; previously unpublished.

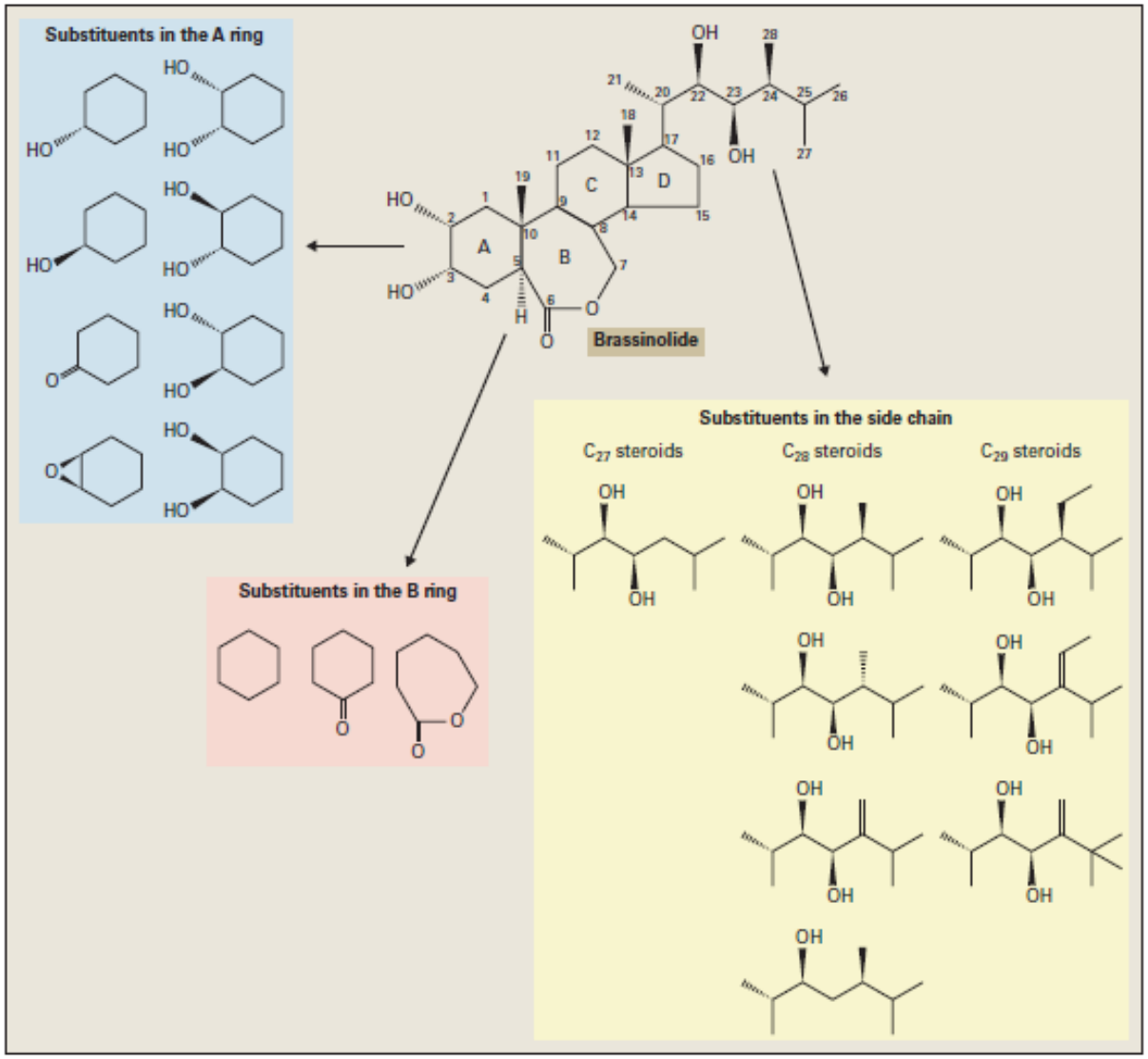


FIGURE 17.55 *Functional groups in the A ring, B ring, and side chain of natural BRs. BL is the most biologically active BR.*

6.1 BRs affect a range of morphological characteristics

Application of BRs induces a broad spectrum of responses, including:

- increased stem elongation,
- pollen tube growth,
- unrolling of grass leaves,
- bending of grass leaves at the sheath/blade joints,
- proton pump activation,
- reorientation of cellulose microfibrils,
- xylogenesis,
- and enhanced ethylene production.

The existence of dwarf mutants of *Arabidopsis*, *P. sativum*, *O. sativa* and *S. lycopersicum* defective in BR biosynthesis or signaling indicates that BRs promote the growth or elongation of plant cells.



FIGURE 17.56 *Sixteen-day-old* Arabidopsis *seedlings: wild-type (left), BL-deficient det2 mutant (center), BL-deficient dim mutant (right). Source:* T. Yokota, Teikyo University, Utsunomiya, Japan; previously unpublished.

- *Arabidopsis* mutants grown in light have dark green leaves with reduced cell size, reduced apical dominance and diminished male fertility.
- Curiously, when grown in dark, these mutants do not etiolate and share some characteristics with light grown plants.
- When BL is applied to BR biosynthesis mutants these phenotypic abnormalities are reversed.

6.2 The plant sterol campesterol is a precursor of brassinolide

All plant sterols are synthesized from cycloartenol.

A characteristic feature of plant sterols is alkylation at C-24.

- Cholesterol, however, a widely distributed minor sterol in plants, is not alkylated at C-24.
- Common sterols found in plants include:

sitosterol (C29), stigmasterol (C29), campesterol (C28), 24-epicampesterol (or 22-dihydrobrassicasterol) (C28), and cholesterol (C26).

- Except for stigmasterol, all these compounds are thought to be BR precursors.
- Sitosterol is the most abundant sterol and comprises 50–80% of the total sterol content of many plants, but it appears not to be a preferred precursor for BR biosynthesis.
- In many plants, BL and biosynthetically related compounds are the major BRs, indicating that campesterol is utilized preferentially for biosynthesis.

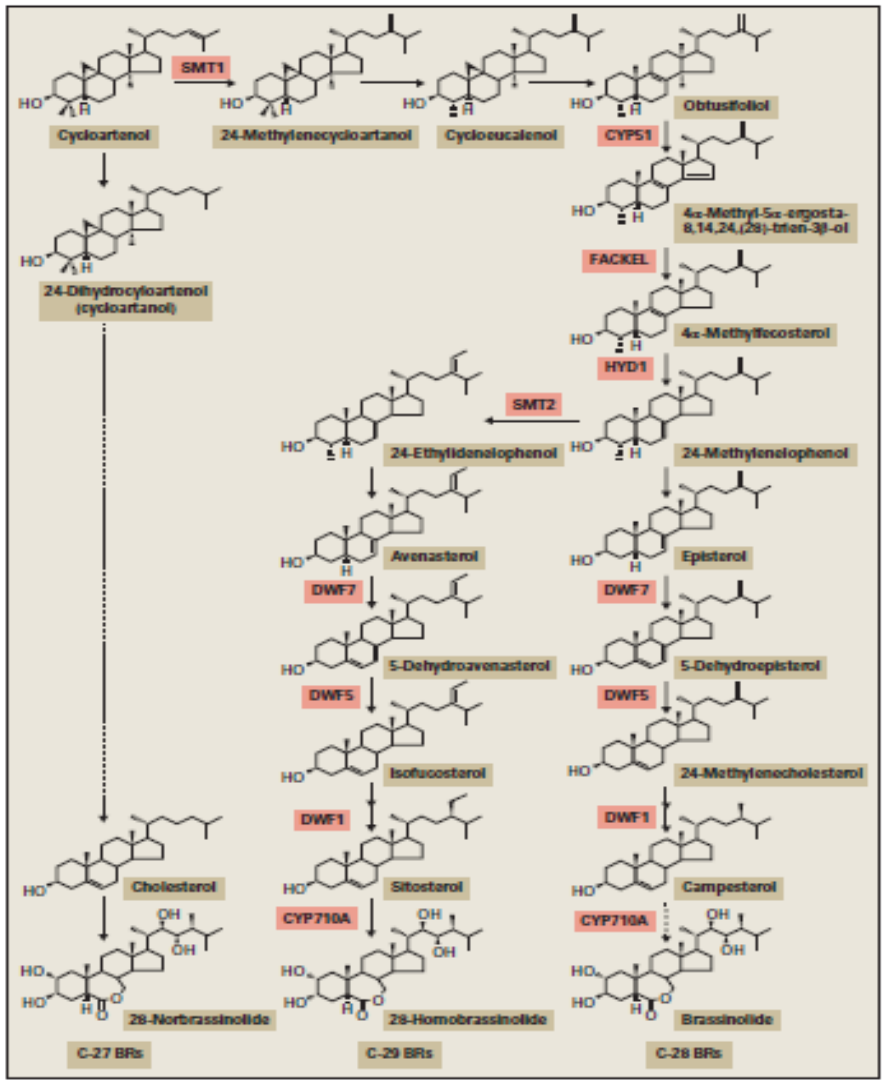


FIGURE 17.57 *Biosynthesis of sterols in vascular plants and biosynthetic lesions in* Arabidopsis *mutants. The biosynthesis of sterols in vascular plants is not fully understood, especially the synthesis of cholesterol. Broken lines indicate multiple steps. Sites where* Arabidopsis *gene products act on the pathway are indicated: CYP51; CYP710A; DWF1, DWARF1; DWF5, DWARF5; DWF7, DWARF7, FACKEL; HYD1, HYDRA1; SMT1, STEROL METHYLTRANSFERASE1; SMT2, STEROL METHYLTRANSFERASE2.*

6.3 The synthesis of BL from campesterol

6.4 Chemical inhibitors have been used to dissect pathways of BR biosynthesis

- Triazoles, such as uniconazole and paclobutrazol, inhibit *ent*-kaurene oxidase, a CYP450 involved in GA biosynthesis.
- Modification of uniconazole in which the *tert*-butyl group is replaced by a phenyl group generated brassinazole, a strong inhibitor of BR synthesis.
- Treatment of plants with brassinazole generates phenotypes similar to BR-synthesis mutants.

6.5 BR metabolism routes have been identified

- Molecular genetic techniques have also revealed the importance of glycosylation and sulfonation in BR metabolism.

Analysis of UDP-glycosyltransferases (UGTs) has shown that plant steroids can act as substrates, and in *Arabidopsis* 23-*O*glucosylation of BL and CS is observed. Transgenic plants overexpressing UGT73C5 contained reduced amounts of BRs and displayed BR-deficient phenotypes that can be rescued by exogenous application of BRs.

- Epimerization of the 2- and 3-hydroxyls, followed by glucosylation or esterification
- Hydroxylation of C-20 and successive side chain cleavage
- Glucosylation of C-23 hydroxyl group
- Hydroxylation of C-25 and C-26 and subsequent glucosylation

7 Polyamines

8 Jasmonic acid

- Jasmonic acid (JA) and related compounds, often referred to as jasmonates collectively, were initially isolated as inhibitors of plant growth.
- Later, they have been shown to regulate various physiological processes in plants, including development of flowers and defense responses against herbivores and fungal pathogens.
- Jasmonates are similar structurally to prostaglandins, autacoidal hormones that exhibit various biological activities in mammals. Both jasmonates and prostaglandins are derived from fatty acids.

- Methyl jasmonate is well known in the perfume industry as a fragrant component of the essential oils of jasmine (*Jasminum grandiflorum*).
- In 1971, JA was isolated from culture filtrates of a fungus, *Botryodiplodi theobromae*, as a plant growth inhibitor.
- Cucurbic acid was isolated from immature pumpkin (*Cucurbita* sp.) seeds in 1977 and identified as a new type of growth inhibitor, different from ABA.
- In the early 1980s, JA and methyl jasmonate were detected as senescence promoting or growth-retarding substances in many plant species.
- In 1989, the 12-*O*-β-glucoside of tuberonic acid (12-hydroxy-(+)-7-isojasmonic acid) was characterized as a potato tuber-inducing factor.
- (–)-JA and methyl (–)-jasmonate are the major jasmonates isolated from plant tissues. Their naturally occurring stereoisomers, (+)-7-isojasmonic acid and methyl (+)-7-isojasmonate, also appear to be active agents in planta

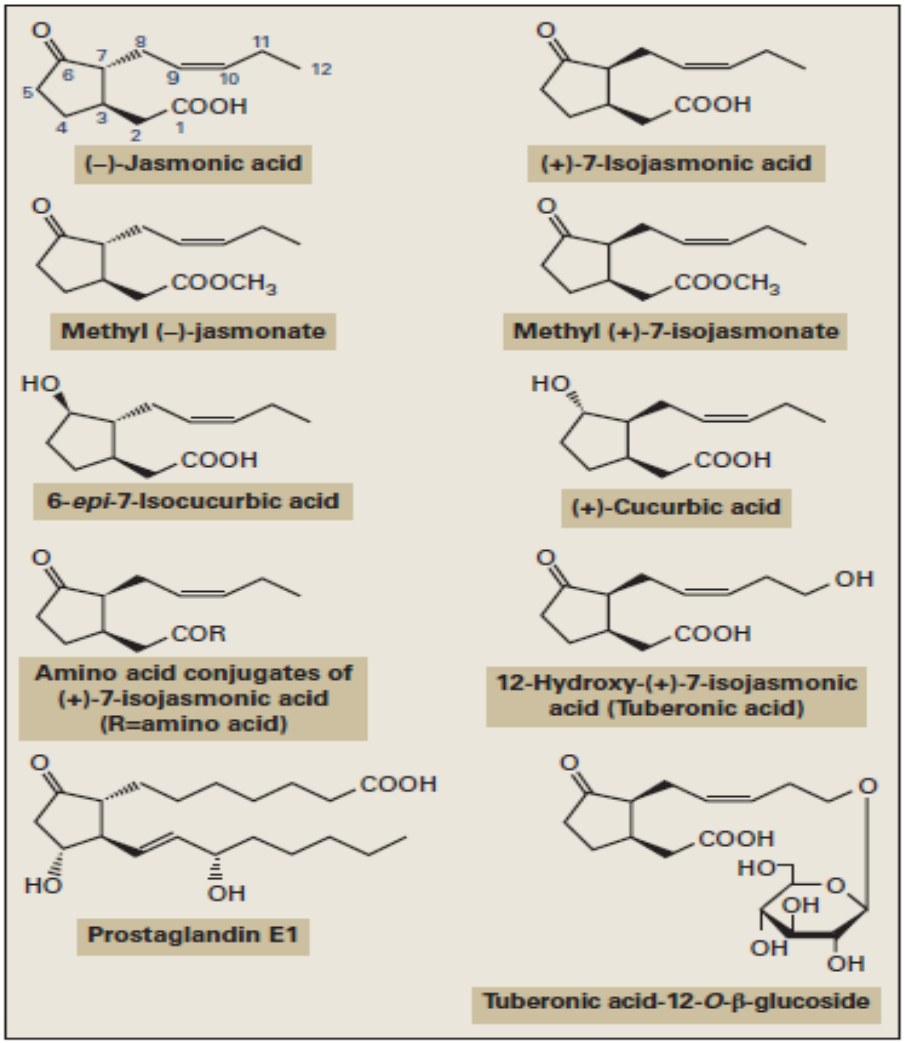


FIGURE 17.66 *Structures of JA and its derivatives. The structure of prostaglandin E1, a mammalian autacoidal hormone, is also shown.*

8.1 Jasmonates inhibit growth processes in many tissues and are essential for reproductive development and pathogen resistance

- Exogenous JA was first reported to inhibit the growth of *O. sativa*, *T. aestivum*, and *L. sativa* seedlings.
- It was subsequently shown to inhibit seed and pollen germination, retard root growth, and promote the curling of tendrils.
- JA content is high in vegetative sink tissues, and the compound regulates accumulation of vegetative storage proteins during seed development in *Glycine max* and *Arabidopsis*.

Arabidopsis mutants severely defective in JA biosynthesis or response are male sterile. Fertility of such JA synthesis mutants can be rescued by exogenous JA treatment. In Arabidopsis JA-deficient mutants, anther filaments do not elongate sufficiently to a position above the stigma, suggesting that:

- JA plays a role in coordinating the timing of growth and development of floral organs.
- JA plays an important role in plant resistance to insects and necrotrophic pathogens. Plants accumulate JA and its derivatives when they are wounded or treated with pathogen-derived elicitors (oligosaccharides).

Exogenous treatment with JA activates expression of hundreds of defense-related genes. Changes in gene expression result in upregulation of synthesis of defense-related proteins and metabolites, including antifungal proteins, antifeedants, phytoalexins, and volatiles that attract predatory insects. JA treatment also downregulates expression of genes encoding enzymes involved in growth and primary metabolism, including photosynthesis, so that resources can be used for defense.

8.2 Jasmonates are synthesized from α-linolenic acid

The first step of JA biosynthesis is release of α-linolenic acid (a C18 poly-unsaturated fatty acid) from plastid membrane lipid by lipase.

α-Linolenic acid is converted to 13-hydroperoxylinolenic acid, and then 12,13(S)-epoxylinolenic acid.

AOS that catalyzes the formation of 12,13*(S)*-epoxylinolenic, is a plastid-localized CYP450 enzyme with several unusual characteristics. Unlike the majority of CYP450 enzymes which act as monooxygenases, AOS does not require oxygen and is therefore not an oxidase, nor does it require NADPH. AOC also is a plastid-localized enzyme.

The second stage of JA biosynthesis occurs in the peroxisome.

8.3 Jasmonoyl-isoleucine is an active hormone in the jasmonate signaling pathway

- JA is subjected to an amide-linked conjugation to isoleucine (Ile) and other amino acids to yield jasmonoyl-isoleucine (JA-Ile) and other jasmonoyl-amino acid conjugates, respectively.
- In Arabidopsis, these conjugation reactions are catalyzed by an enzyme encoded by the *JAR1 (JASMONATE RESISTANT 1*) locus. Similar reactions are catalyzed by a subset of GH3 family proteins for synthesis of amino acid conjugates of IAA.

jar1 mutants were isolated because of their resistance to exogenous JA in a root growth inhibition assay. Evidence suggests that JA-Ile synthesis is under positive feedback regulation. Many genes that encode JA-Ile biosynthesis enzymes are positively regulated by JA treatment.

8.4 Coronatine is a bacterial toxin that activates jasmonate signaling

Coronatine (CO) is a phytotoxin produced by some plant pathogenic bacteria. There is evidence that CO exerts its virulence effects by activating JA signaling in host plants. The structural similarity of CO to JA-Ile supports the idea that this toxin acts as a molecular mimic of JA-Ile.

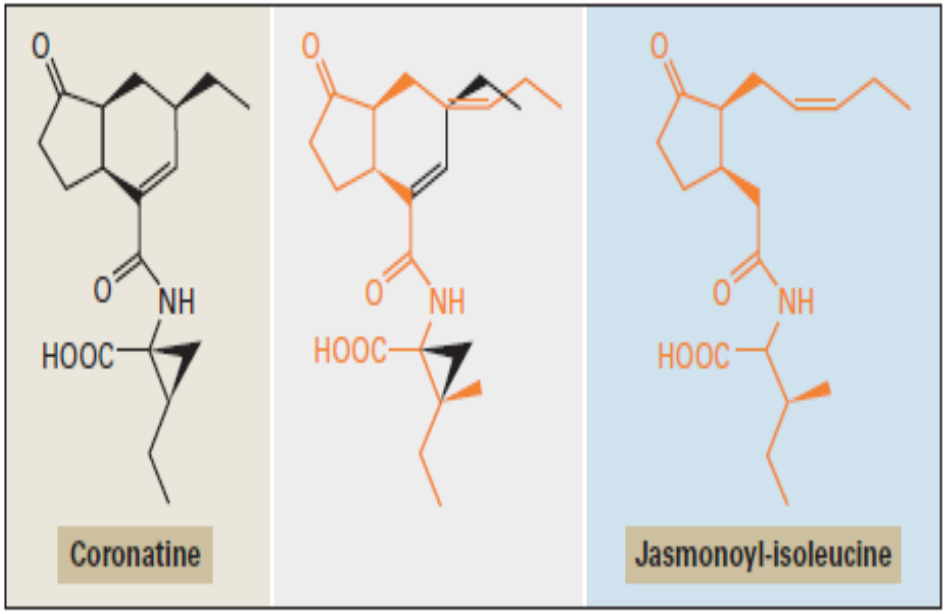


FIGURE 17.68 *Structural similarity between coronatine, a bacterial toxin (left) and jasmonoyl-isoleucine (right). To highlight the similarity, these structures are superimposed (center).*

8.5 JA is metabolized to various products via multiple pathways

- Besides being converted to amino acid conjugates, JA can undergo several other modification reactions.
- JA is converted to volatile methyl jasmonate by an SAM-dependent carboxyl methyltransferase. Methyl jasmonate is thought to act as an airborne signal in interplant communication, being released in response to wounding and other abiotic stresses.
- Another volatile compound, *cis*-jasmone, is synthesized from JA by decarboxylation, but the enzyme responsible for this reaction has not been clarified.
- The carboxyl group of JA can also be modified through conjugation with glucose to give jasmonoyl-1-β-glucose.
- 12-Hydroxy-(+)-7-isojasmonic acid is known as tuberonic acid because of its tuber-inducing activity in potato.
- **12-hydroxylation of JA and subsequent glucosylation and sulfonation appears to be a widespread JA metabolic route in plant.**

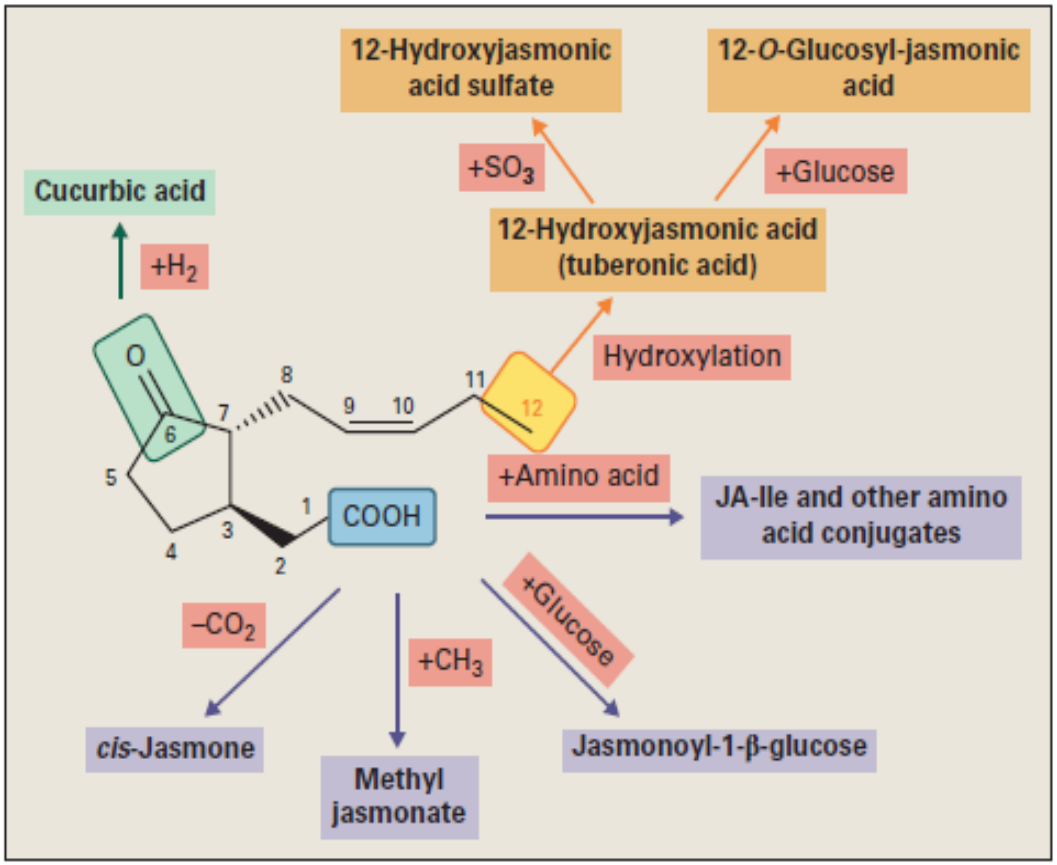


FIGURE 17.69 *Metabolism of JA in plants. JA is metabolized through modification of the carboxyl group (blue), C-12 oxidation (orange) or the reduction of C-6 ketone (green). 12-Hydroxy-(+)-7-isojasmonic acid is further metabolized to conjugates.*

9 Salicylic acid

Salicylic acid (SA) is far better known for its medicinal properties than for its regulatory roles in plants. Although many physiological effects of SA application have been reported, only a few have been shown to be physiologically important in plants, for example, the regulatory role of SA in thermogenesis of various species and in the response of plants to pathogens.

9.1 Salicylic acid can retard senescence of petals and induce flowering

- Soluble aspirin tablets disintegrate rapidly in water, and the acetylsalicylic acid is converted to SA.

- Adding aspirin to a vase of water containing cut flowers will retard senescence of petals and make flowers last longer. This is probably a consequence of reduced rates of ethylene biosynthesis, in light of reports that SA blocks the conversion of ACC to ethylene.

- SA also induces flowering in the duckweeds *Lemna gibba*, *Spirodela polyrrhiza*, and *Wolffia microscopia* when these long-day plants are grown under a noninductive short day Photoperiod.This effect is not specific, however, and related phenolics also can stimulate flowering in these and other species. Furthermore, **because SA concentrations are similar in vegetative and induced tissues, the compound is unlikely to be an endogenous flower-inducing signal**.

9.2 Salicylic acid regulates thermogenesis in voodoo lily

studies on thermogenesis in flowers of voodoo lily (*Sauromatum guttatum*):

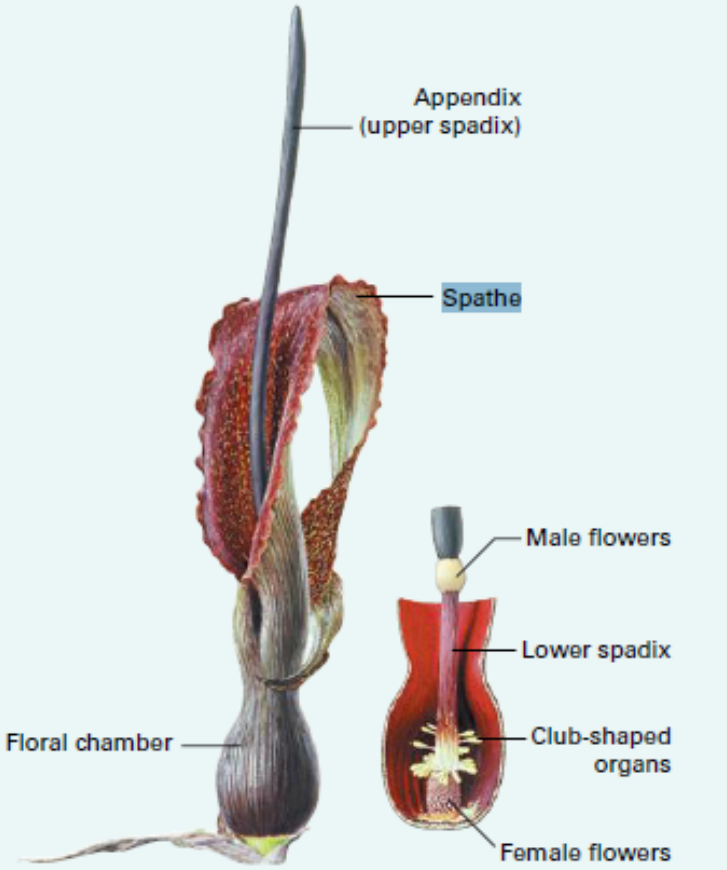
During thermogenesis, much of the electron flow in mitochondria is diverted from the cytochrome respiration pathway to the cyanide-insensitive nonphosphorylating electron transport pathway unique to plant mitochondria. Also, the glycolytic pathways and Krebs cycle enzymes, which provide substrates for alternative oxidase, are activated. Energy released by electron flow through this alternative respiratory pathway is not conserved as chemical energy but is released as heat.

In 1937 it was proposed that thermogenesis in *S. guttatum* was initiated by "calorigen," a water-soluble substance produced in the staminate flower primordia positioned between the upper and lower spadix.

In 1987, calorigen was identified as SA when it was shown that treating immature spadix sections with as little as 0.12 µg of SA per gram fresh weight caused their temperatures to rise by as much as 12°C. Large increases in endogenous concentrations of SA occur immediately prior to onset of thermogenicity in both upper and lower spadix.

The AOX and uncoupling proteins participate in thermogenesis during flowering in a few plants, mostly members of the family Araceae. These plants, which include skunk cabbage and voodoo lily, produce a club-like structure (called an appendix) on the developing floral apex. The outer layers of the appendix contain more mitochondria than most plant tissues. During anthesis (pollen release), mitochondria in the appendix use the AOX and uncoupling proteins to respire at very high rates. The free energy released as heat raises tissue temperatures 10°C to 25°C above ambient conditions and volatilizes odorous compounds that attract pollinators. In some cases, this mechanism can mimic the scent of rotting flesh and is used to deceive insects that lay their eggs.

It is unlikely that the AOX and uncoupling proteins promote thermogenesis in other plant tissues, because plants are not generally thermogenic. Observed rates of respiration in most plant organs are simply too low to generate significant amounts of heat, even if all electron flow were to be shunted through the AOX and even if uncoupling proteins completely prevented limitation of the electron transfer rate by dissipating the mitochondrial membrane potential.



Source: Benstead, Fife, Scotland, UK; previously unpublished.

All plant species examined to date possess genes for the AOX; however, AOX activity in any given tissue varies, depending on the level of gene expression and the activation status of the oxidase (see below). Often, AOX expression is greatly enhanced in response to an environmental stress. AOX is insensitive to classic inhibitors of cytochrome *c* oxidase (CN^-, N_3^-, CO, and NO) and of complex III (antimycin-A, myxothiazol); however, the AOX can be specifically inhibited by salicylhydroxamic acid (SHAM) and *n*-propylgallate.

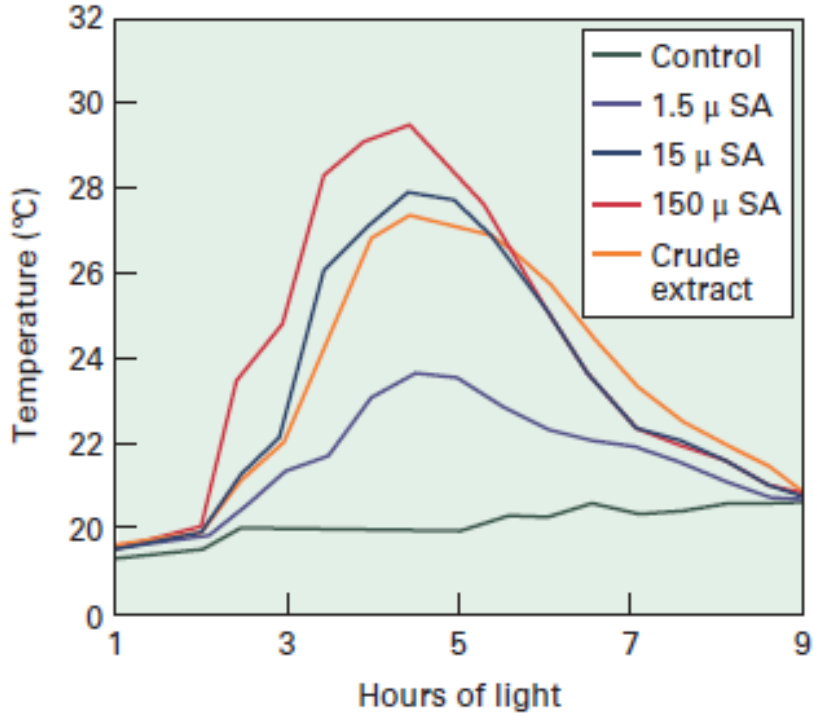


FIGURE 17.70 *The response of the* Sauromatum guttatum *appendix (upper spadix) to application of SA or a crude extract of male flowers of the same species. Both exogenous treatments result in a marked increase in appendix temperature.*

9.3 Salicylic acid production is associated with disease resistance

- More widespread interest in SA was generated when it was closely linked to the hypersensitive response.



FIGURE 17.71 *Necrotic lesions on the leaf of resistant* Nicotiana tabacum *formed in response to infection with TMV.*
Source: J. Draper, University of Wales, Aberystwyth, UK; previously unpublished.

- Increased resistance, which develops over a period of several days to a week following initial pathogen invasion, is referred to as systemic acquired resistance (SAR). Associated with hypersensitive response and SAR is synthesis of five or more families of serologically distinct, pathenogenesis-related (PR) proteins. Two of these families encode the hydrolytic β-1,3-glucanases and chitinases.
- The indication that SA was involved in plant defense against disease came with the finding that SA treatment of the leaves of Tobacco Mosaic Virus (TMV)-susceptible tobacco (*N. tabacum* cv. Xanthi *n*) induced the accumulation of PR proteins and enhanced resistance to TMV infection.

9.4 Two distinct pathways exist for the biosynthesis of salicylic acid in plants

In 1993, SA biosynthesis in *N. tabacum* was proposed to involve a side branch of the phenylpropanoid pathway, which converts *trans*-cinnamic acid to benzoic acid (BA), the latter undergoing 2-hydroxylation to form SA.

After TMV-inoculation, a large pool of benzoic conjugate (100 μg/g fresh weight) is hydrolyzed, releasing BA, which activates BA 2-hydroxylase (BA2H), responsible for catalyzing the conversion of BA to SA.

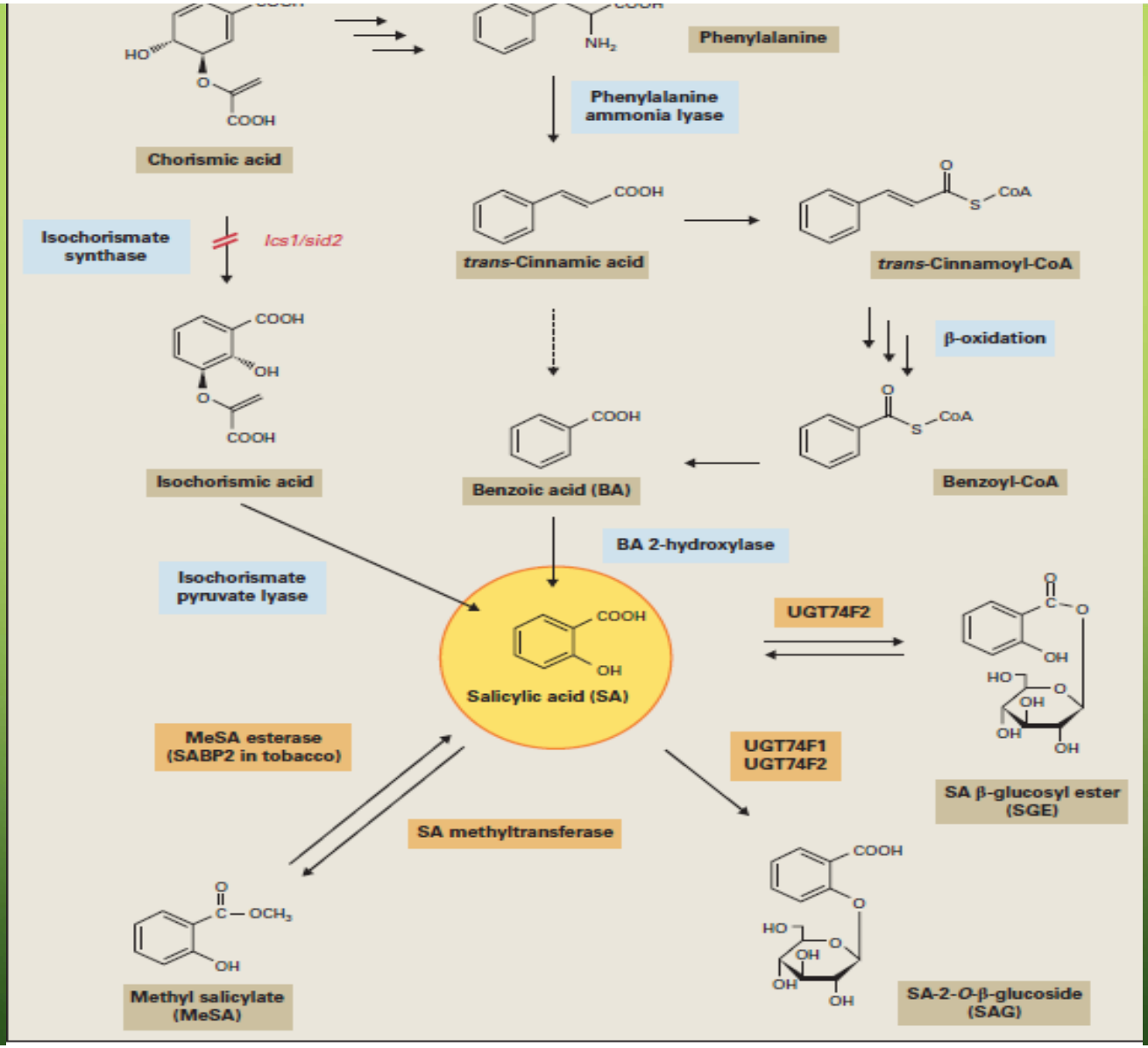
The rate-limiting step in SA biosynthesis is the conversion of *trans*-cinnamic acid to BA.

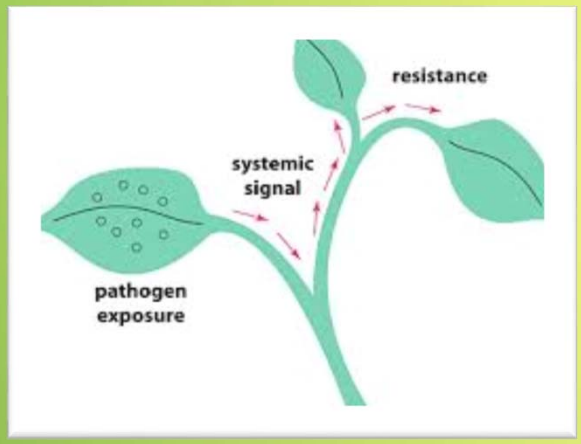
9.5 Methyl salicylate may play a role as a mobile signal in systemic acquired resistance

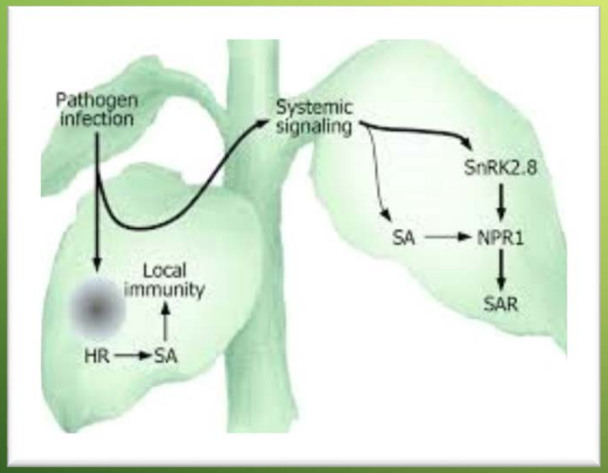
In both healthy and infected tobacco leaves, the main SA metabolites are SA 2-*O*-β-glucoside (SAG) and SA β-glucosyl ester (SGE), with the latter occurring transiently and in smaller quantities than the former.

The carboxyl group of SA is methylated to form methyl salicylate (MeSA) by a carboxyl methyltransferase that uses SAM as methyl donor. MeSA is converted back to SA by MeSA esterase.

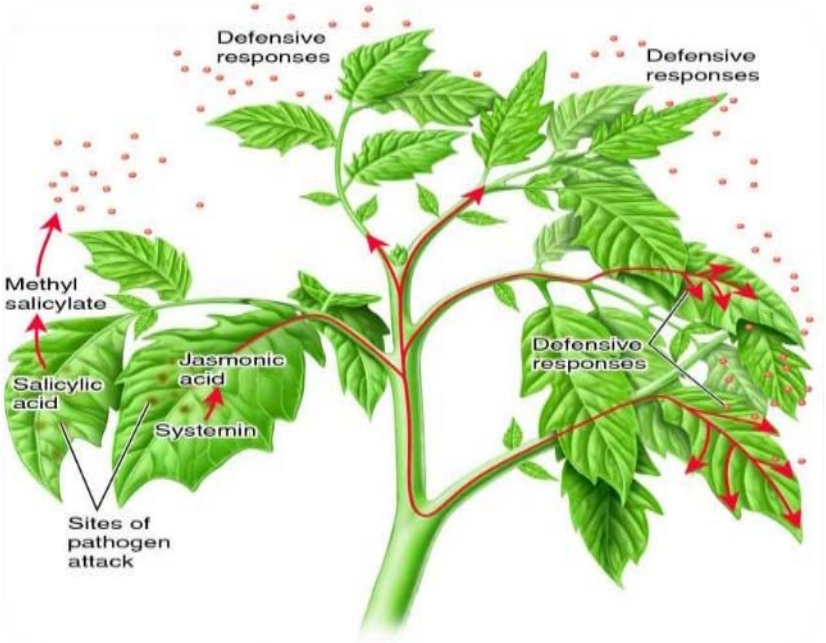
MeSA esterase activity is required for SAR signaling in systemic *N. tabacum* leaves. In transgenic *N. tabacum* plants with elevated MeSA esterase activity in infected leaves, MeSA concentrations do not increase in infected leaves, phloem exudates and systemic leaves.


Chorismic acid
Phenylalanine
Phenylalanine ammonia lyase
Isochorismate synthase
Ics1/sid2
trans-Cinnamic acid
trans-Cinnamoyl-CoA
β-oxidation
Isochorismic acid
Benzoic acid (BA)
Benzoyl-CoA
BA 2-hydroxylase
Isochorismate pyruvate lyase
Salicylic acid (SA)
UGT74F2
MeSA esterase (SABP2 in tobacco)
UGT74F1 UGT74F2
SA β-glucosyl ester (SGE)
SA methyltransferase
Methyl salicylate (MeSA)
SA-2-O-β-glucoside (SAG)





SYSTEMIC ACQUIRED RESISTANCE



Submitted to : Dr. KP Singh
Department of Plant Pathology

Sudha Nandini Sharma
Ph.D (Plant Pathology)
Id : 43970

10 Strigolactones

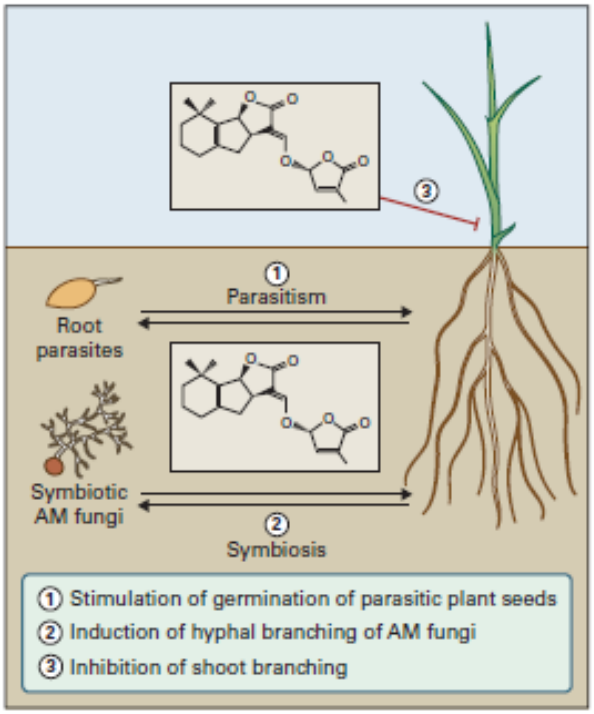


FIGURE 17.75 *SLs are released from roots and act as chemical signals in communication with root parasite seeds and arbuscular mycorrhizal (AM) fungi in the rhizosphere. They also act as endogenous regulators of shoot branching in plants.*

Parasitic plants of the *Striga*, *Orobanche*, and *Alectra* genera (Orobanchaceae) are root parasites that cause losses in crop yields in many parts of the world. Control of these parasitic plants is difficult because they produce a huge number of seeds that remain viable in soil for many years until they detect germination stimulants released from host roots. A pure crystalline germination stimulant of *Striga* seeds was isolated in 1966 from cotton root exudates. This stimulant, termed strigol, has a characteristic chemical structure, including two lactone rings linked via an enol ether bridge (Fig. 17.74). Later, diverse strigol-related compounds, collectively called **strigolactones (SLs)**, were isolated as germination stimulants of parasite seeds in root exudates of various plant species (Fig. 17.74).

Until recently, a biological function of SLs in host plants was unknown. It was discovered in 2005 that SLs are plant-derived signals that induce hyphal branching of arbuscular mycorrhizal fungi (AM fungi), a phenomenon that is observed at the initial stage of colonization (Fig. 17.75). This symbiotic interaction is observed in more than 80% of terrestrial plants. Since AM fungi facilitate the uptake of water and mineral nutrients such as phosphate and nitrate by host plants, it is considered that plants evolved to produce SLs to enable AM fungi to colonize their roots. Parasitic plants are thought to abuse these chemical signals to recognize the vicinity of a potential host plant (Fig. 17.75). Studies using various SLs and their synthetic analogs suggest that the structure of the CD rings of SLs is crucial for their biological activity.

10.1 Genetic studies suggested a novel carotenoid-derived signal inhibits shoot branching

- The participation of auxin and CK in the regulation of axillary bud activity has long been known. Besides these two hormones, studies with excessive shoot branching mutants suggested the involvement of a novel hormone-like compound in inhibiting outgrowth of axillary buds.
- Grafting experiments suggest that some of these mutations affect genes required for synthesis of this hormone, while others are required for perception or transduction of the signal.

Excessive shoot branching phenotype of *max1*, *max3*, and *max4* mutants is rescued when grafted onto wild type rootstock, while the *max2* scion on wild-type rootstock still produces excessive branching.

These results suggest that *MAX1*, *MAX3*, and *MAX4* are required for the synthesis of a root-derived graft-transmissible signal that inhibits axillary bud outgrowth, while *MAX2* is involved in perception or transduction of this signal in shoots.

Since a *max4* rootstock does not cause excessive branching in wild type scion, the shoot branching inhibitor synthesized in the scion (shoot) is also sufficient to prevent excessive shoot branching.

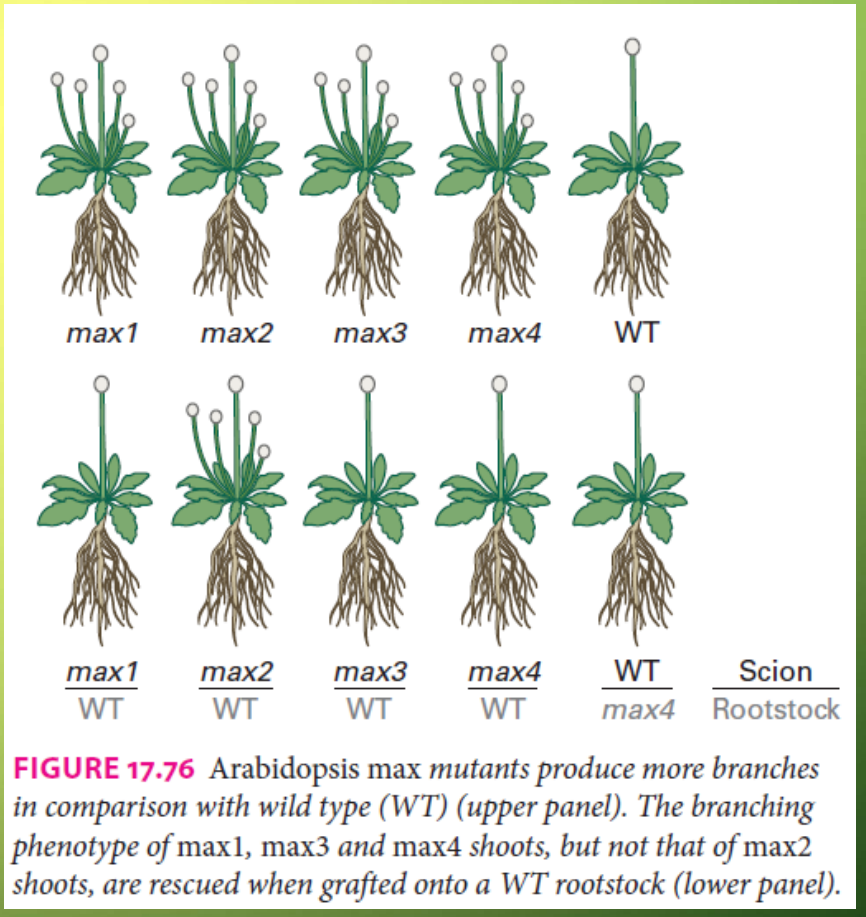


FIGURE 17.76 Arabidopsis max *mutants produce more branches in comparison with wild type (WT) (upper panel). The branching phenotype of* max1, max3 *and* max4 *shoots, but not that of* max2 *shoots, are rescued when grafted onto a WT rootstock (lower panel).*

MAX genes encode carotenoid cleavage dioxygenase 7 (CCD7).

MAX1 encodes a protein in the CYP450 monooxygenase superfamily, of which members are typically involved in metabolism of lipophilic small molecules, as discussed earlier.

These findings are consistent with the idea that MAX1, MAX3 and MAX4 participate in biosynthesis of a carotenoid-derived hormone-like compound.

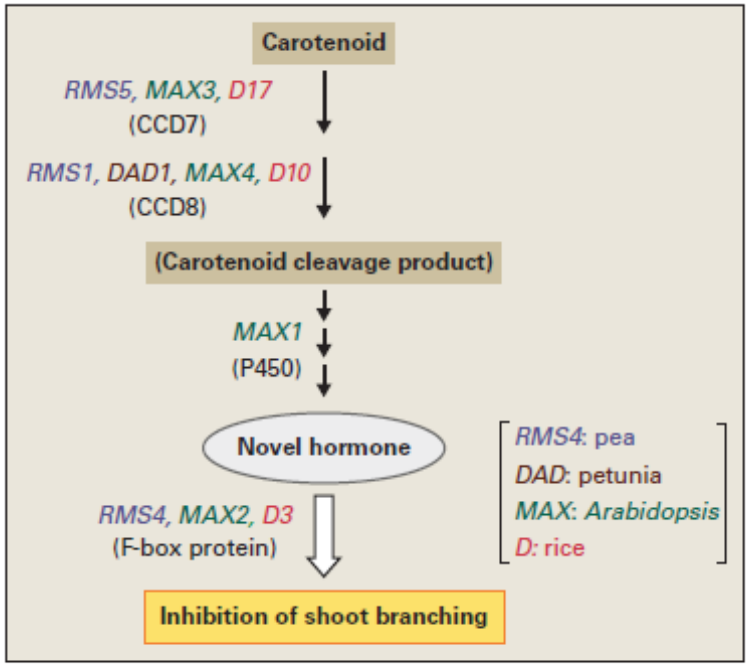
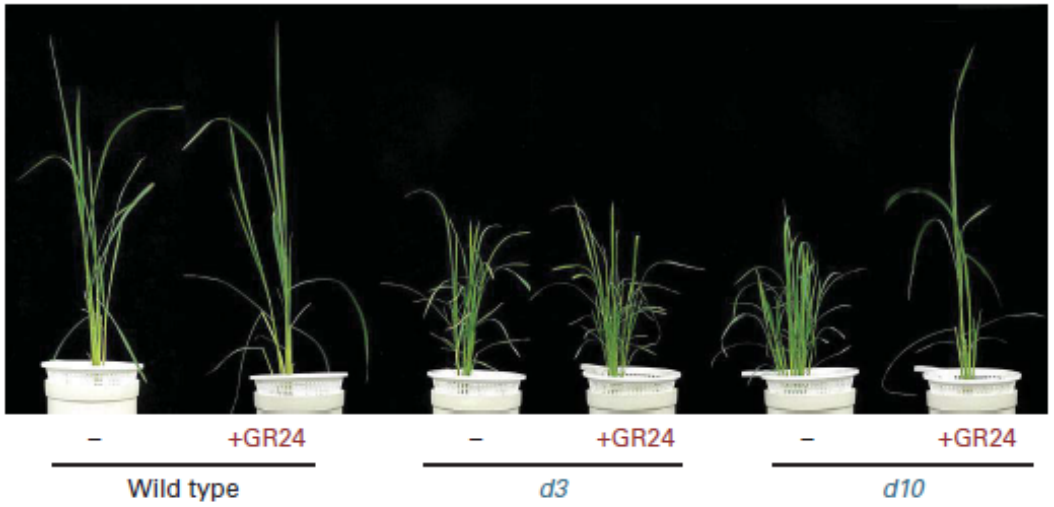


FIGURE 17.77 *A hypothetical pathway for the novel shoot branching inhibitor hormone. Carotenoid cleavage dioxygenases (CCDs) and a CYP450 are required for synthesis, whereas an F-box protein is probably involved in perception or transduction of the hormonal signal.*

10.2 Strigolactone inhibits shoot branching

Recently, SLs have been shown to inhibit shoot branching.

FIGURE 17.78 *The excessive branching (tillering) and semi-dwarf phenotypes of the* O. sativa *d10 mutant are fully complemented by exogenous GR24, a synthetic SL analog (supplied at 1 μM in the hydroponic culture media). In contrast, the d3 mutant is insensitive to GR24.*



- SL concentrations in root exudates and in roots are significantly decreased in the *ccd7* and *ccd8* mutants of *P. sativum* and *O. sativa*.
- Axillary bud outgrowth in *ccd7* and *ccd8* mutants of *P. sativum*, *O. sativa* and *Arabidopsis* is inhibited by exogenous SL.

Current data indicate that SLs act as a new class of **branch inhibiting hormones** or their biosynthetic precursors

10.3 Strigolactone biosynthesis pathway remains to be elucidated

Until recently, the biosynthetic origin of SLs was unknown, although their chemical structures suggested that they are terpenoids.

In recent studies using chemical inhibitors and mutants of *S. lycopersicum* and *Z. mays,* SLs were shown to be derived from carotenoids. This is consistent with the discovery that carotenoid cleavage dioxygenases, CCD7 and CCD8, participate in SL biosynthesis.

10.4 Strigolactone biosynthesis is under feedback regulation

In *O. sativa, P. sativum*, and *Petunia*, genes encoding CCD8 are highly upregulated in the SL-deficient and -insensitive mutant backgrounds, suggesting feedback regulation of CCD8 expression.

Consistent with this, *CCD8/D10* gene expression is downregulated by SL treatment in *O. sativa*.

Furthermore, SL levels are substantially increased in the SL-insensitive *O. sativa d3* mutant in comparison with wild type.

These results indicate that there is a link between SL action and biosynthesis in these plant species, as previously recognized for other plant hormones.

10.5 Strigolactone production is elevated under mineral nutrient deficiency

The amounts of SLs released from roots are drastically increased under deficiency of phosphate or nitrate in hosts of AM fungi.

This is thought to be an adaptive response of plants under nutrient deficiency in order to maximize the symbiotic interaction with AM fungi, which assist the uptake of mineral nutrients by the host plant.

This idea is supported by the observation that *Lupinus albus* (white lupin), a nonhost of AM fungi, does not increase SL production in response to phosphate deficiency, unlike the mycotrophic *Trifolium pratense*.

These results indicate that SL production is closely related to the strategy of plants for mineral nutrient acquisition.

SUMMARY

A battery of endogenous hormones regulate plant growth and responses to environmental stimuli, including GAs, ABA, CKs, IAA, ethylene, BRs, PAs, JA, SA, and SLs. Most of these compounds are present in plant tissues at low concentrations (nanogram or sub-nanogram per gram fresh weight) and pool sizes are tightly controlled by various biosynthetic, catabolic, and conjugation pathways. To date, the major biosynthesis routes for these hormones have been elucidated and most of the genes encoding enzymes in these pathways have been identified. Combinations of different experimental approaches, including chemistry, biochemistry, molecular biology, and genetics, have contributed to these achievements. The use of hormone-deficient and -overproduction mutants has been particularly powerful in the isolation of new genes, as well as in understanding the biochemical function of gene products.

Although these hormones are diverse in their chemical structures, there are some common features in their biosynthetic and catabolic pathways. Members of the CYP450 monooxygenase superfamily are frequently involved in the biosynthesis and/or deactivation of various hormones, including GAs, ABA, CKs, IAA, BRs, JA, and SLs. Methylation of a carboxyl group by SAM-dependent methyl transferases also participates in homeostasis of multiple hormones discussed in this chapter. With the exception of ethylene, conjugation with glucose or an amino acid commonly occurs in many hormone metabolism pathways. However, in all cases, hormone conjugation is only one of many routes for deactivation. At least in some cases, hormone conjugates are thought to function as a storage form, from which a free, bioactive hormone can be released upon hydrolysis. It has long been assumed that hormone conjugates are not bioactive per se in plants. However, a recent finding that JA-Ile (and some other amino acid conjugates of JA) might act as an active signal in the JA response pathway provides a new view regarding the role of hormone conjugation.

Studies have shown that the major part of hormone biosynthetic and catabolic pathways are conserved in diverse plant species, but in some cases, species-specific biosynthesis routes have been found, including one of the IAA pathways in *Arabidopsis*, which relates to the production of indole glucosinolates as secondary metabolites in this species. Increasing evidence suggests that many hormones, if not all, function as intermediates between the perception of environmental signals and resulting alterations in growth and developmental patterns. Examples include light-regulation of GA_1 concentrations in lettuce seeds, an increase in ABA concentrations under water stress, and JA accumulation in response to wounding. In addition, hormone concentrations are regulated by the action of other hormones.

There is emerging evidence suggesting that hormone concentrations in plants are determined as a consequence of multiple layers of regulation. For example, in both GA and BR pathways, negative feedback regulation involves a change in accumulation of particular mRNAs encoding biosynthesis and inactivating enzymes. However, as shown for ACS in ethylene biosynthesis, hormone homeostasis can also be controlled by turnover of the enzyme through protein modification. Advances in hormone biosynthesis research have started to expand our understanding of how plants utilize multiple classes of hormones to regulate their growth and development under changing environmental conditions.