Specialized Metabolism in Plants

Driving growth and adaptation to nitrogen availability

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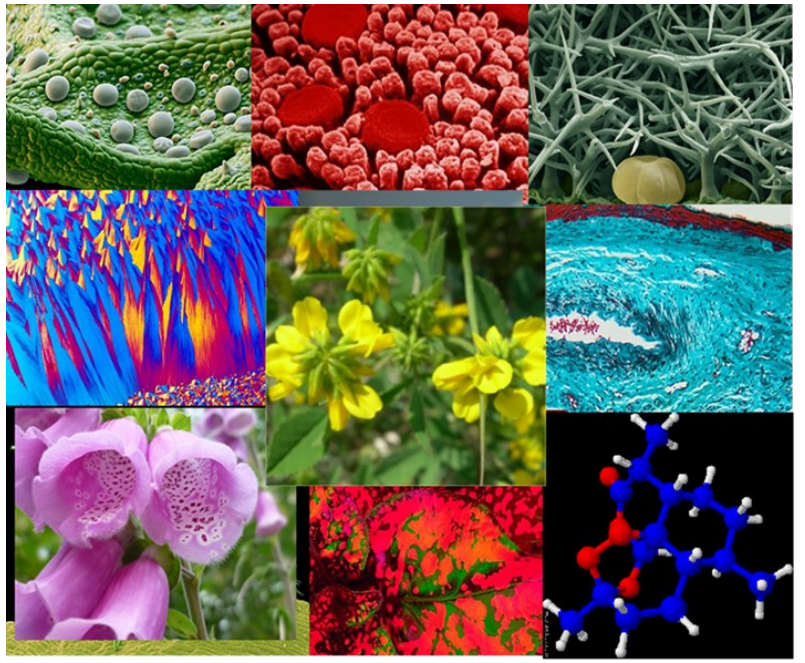

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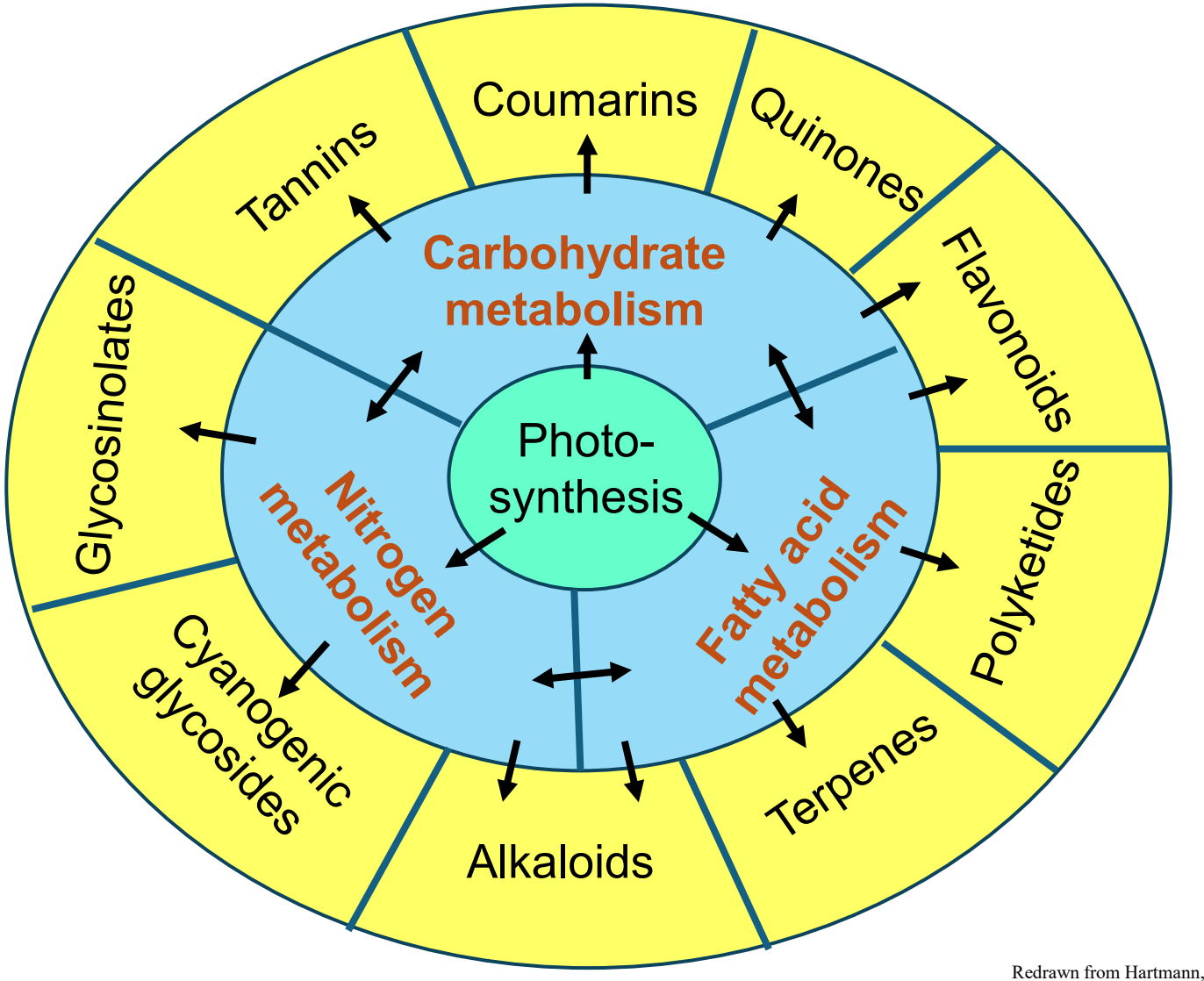
Secondary Metabolites *or* Specialized Metabolites *or* Natural Products

- >200.000 compounds described/ isolated
- deriving from precursors of the primary metabolism
- confined to a specific lineage or species or even cultivar/ tissues/ developmental stage
- not essential to growth and life / required for interaction of plants with their environment and produced in response to stress

TODAY:

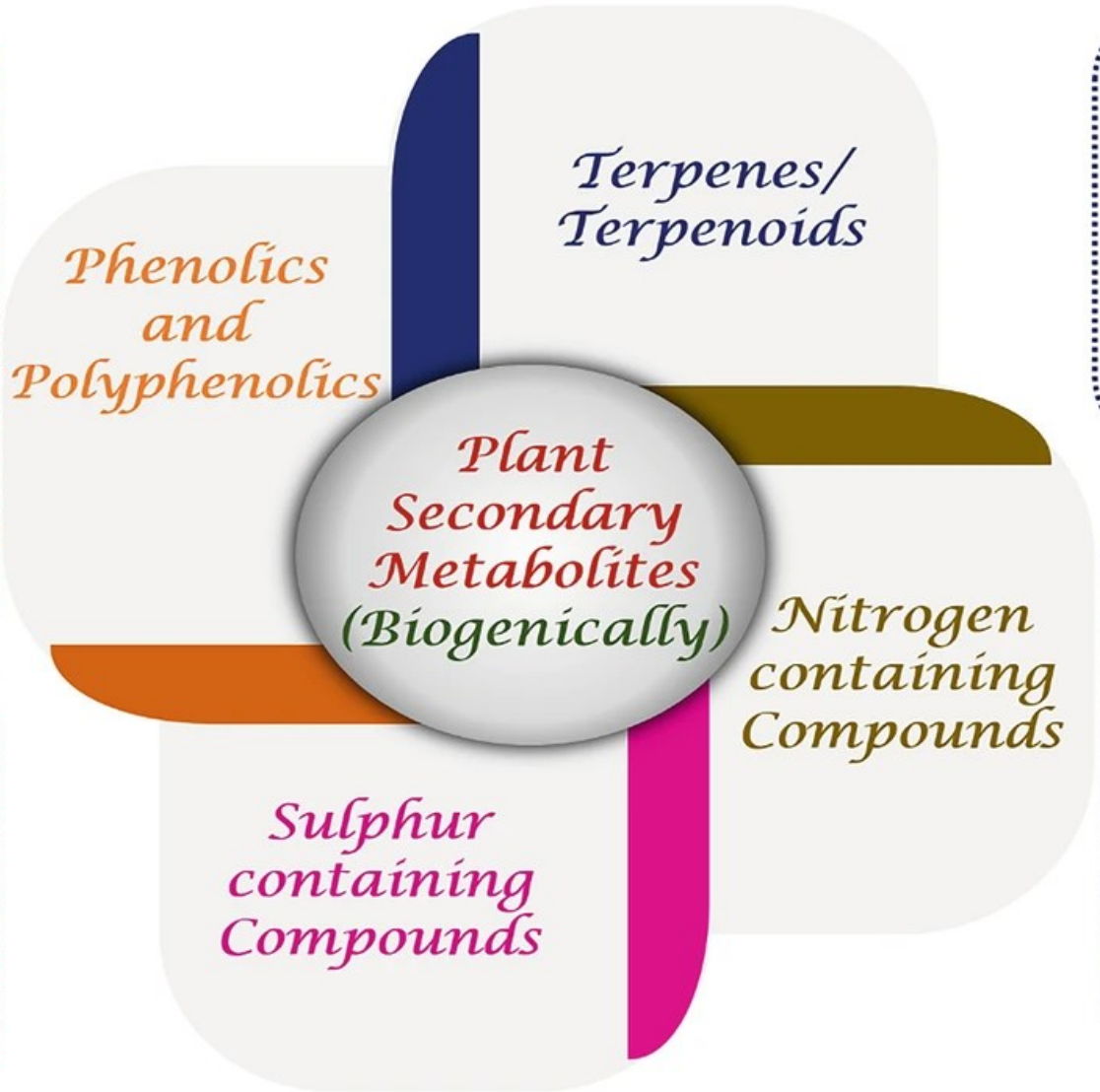
- Structures and basic biosynthetic pathways
- Functional roles in plant development, physiology and defence
- Root exudates
- Identifying novel functional roles
- Altering plant metabolic profiles

Basic structural classes



Redrawn from Hartmann, T. (1996). Diversity and variability of plant secondary metabolism: a mechanistic view. Entomologia Experimentalis et Applicata 80: 177-188.

Basic structural classes



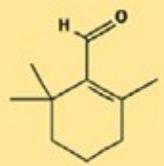
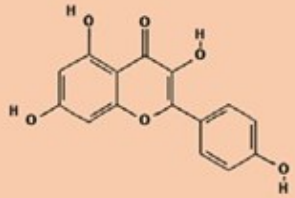
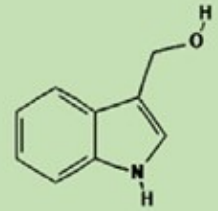
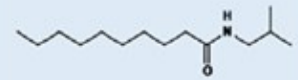
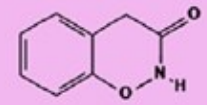
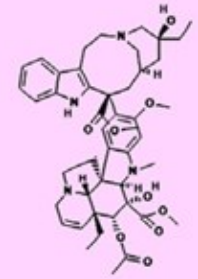
- Phenolic acid
- Flavonoids
- Stilbenes
- Coumarins
- Tannins
- Liginans
- Curcuminoids
- Xanthones
- Napthoquinones
- Acetophenones

- Allinins
- Glucosinolates
- Phytochelatins
- Phytoalexins
- Defensins
- Thionins

- Monoterpenes
- Sesquiterpenes
- Diterpenes
- Triterpenes
- Tetraterpenes
- Polyterpenes

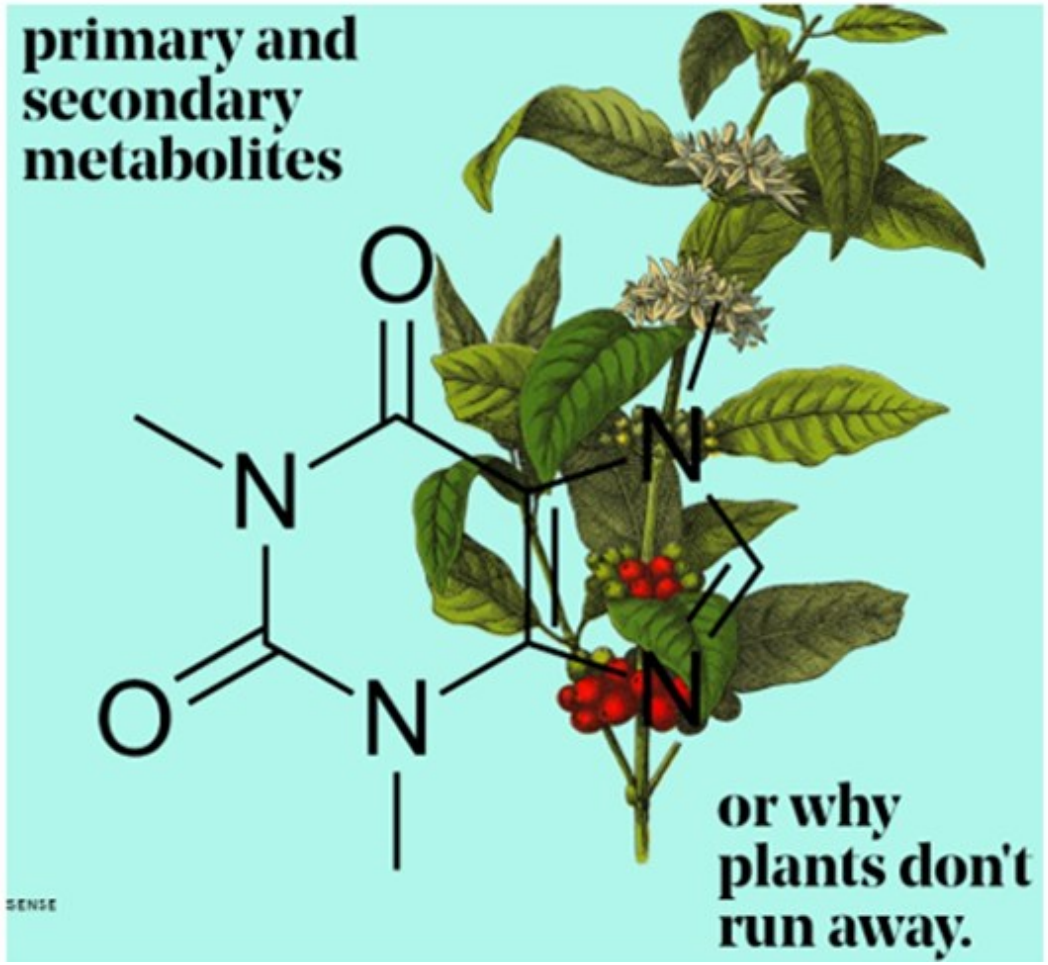
- Free Alkaloids
- Pseudoalkaloids
- Protoalkaloids
- Polyamine-derived alkaloids
- Non-protein amino acid
- Cyanogenic glycosides
- Cyclopeptide alkaloids

Frequently studied classes of specialized metabolites

Class	TERPENOIDS / ISOPRENOIDS	PHENYLPROPANOIDS	GLUCOSINOLATES	ALKAMIDES	BENZOXAZINOIDS	ALKALOIDS
Description	Metabolites produced from isoprene units	Phenolic compounds derived from phenylalanine	Sulfur-containing metabolites	Fatty acid amides	Tryptophan-derived metaboiltes	Nitrogen-containing metabolites derived from aminoacids or nucleotides
Some metabolite groups	Diterpenes, triterpenes, sesquiterpenes, carotenoids, apocarotenoids, sterols	Flavonoids, anthocyanins, lignins, tannins	Aliphatic, aromatic, indolic glucosinolates	Aliphatic, cyclic or aromatic amine residue + C8 to C18 saturated or insaturated, or aromatic chain.	Benzoxazinones and benzoxazolinones	True alkaloids, protoalkaloids, peptide and cyclopeptide alkaloids
Example structure	 β-Cyclocitral	 Kaempferol	 Indole-3-carbinol	 N-isobutyl-decanamide	 Benzoxazinone	 Vinblastine

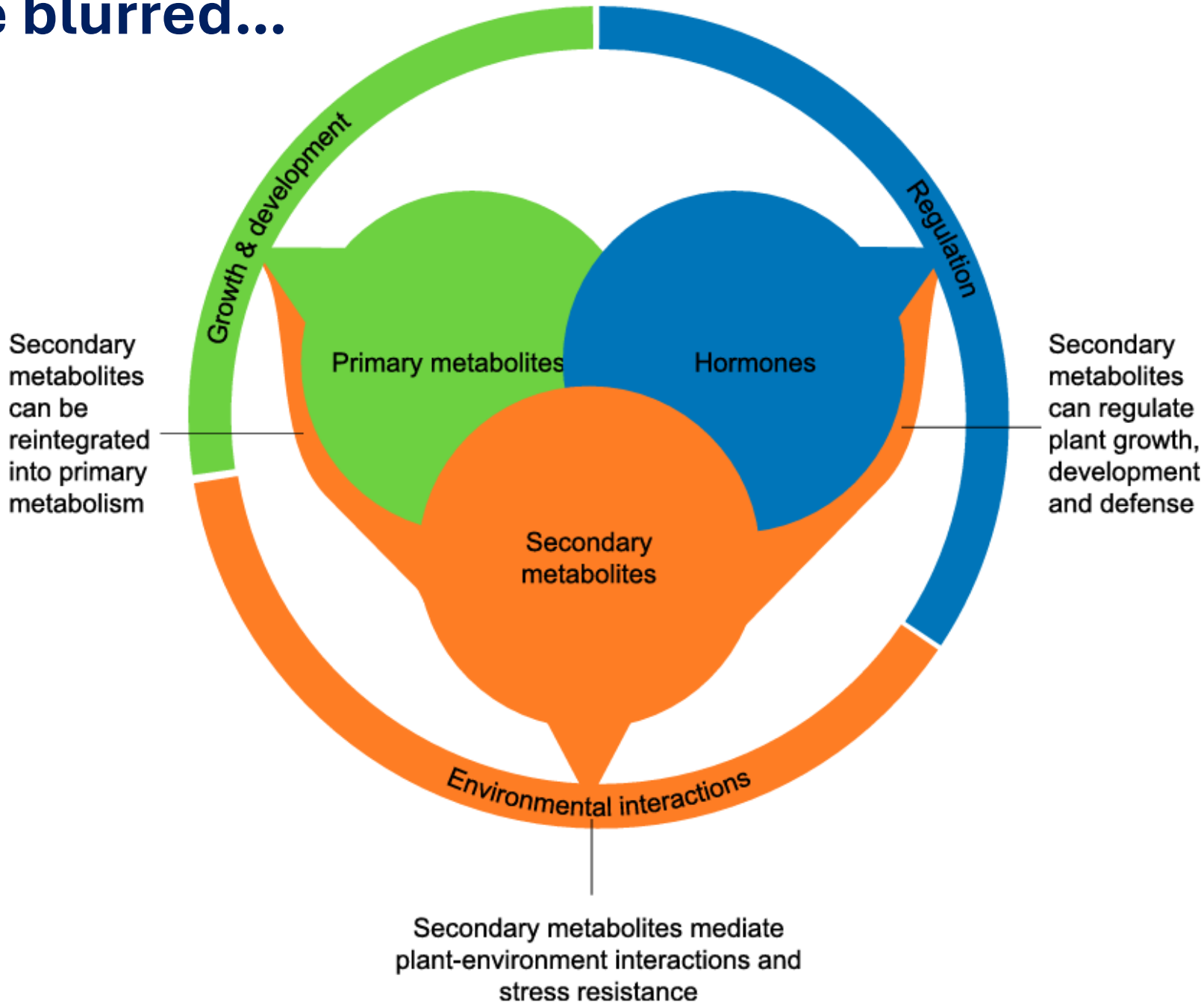
Current Opinion in Plant Biology

Why plants produce SM?

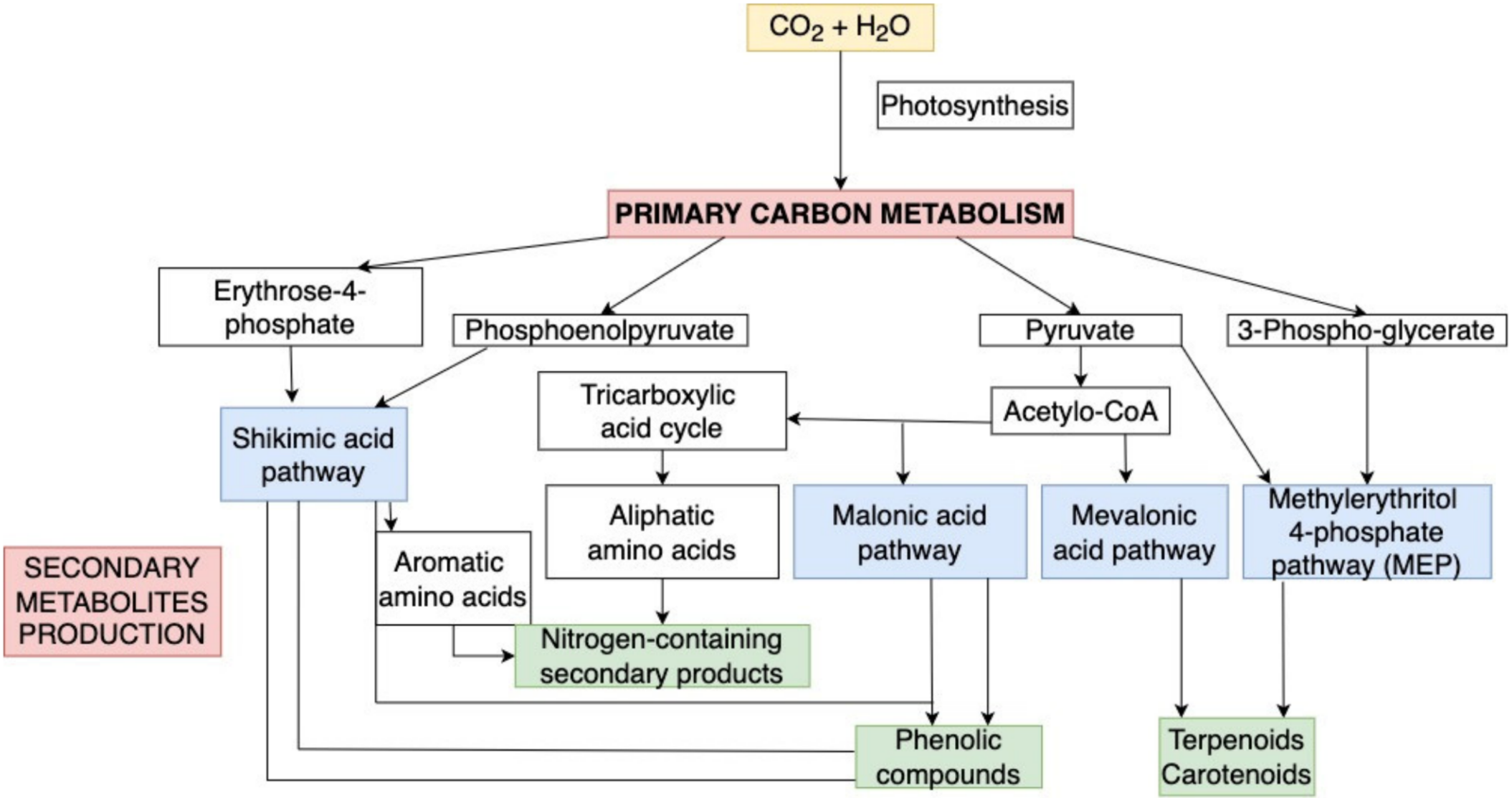


- Defence against herbivores
- Attractants to pollinators
- Allelopathy
- Symbiosis (beneficial microorganisms)
- Tolerance to abiotic stresses

But boundaries are blurred...



Main Biosynthetic Pathways



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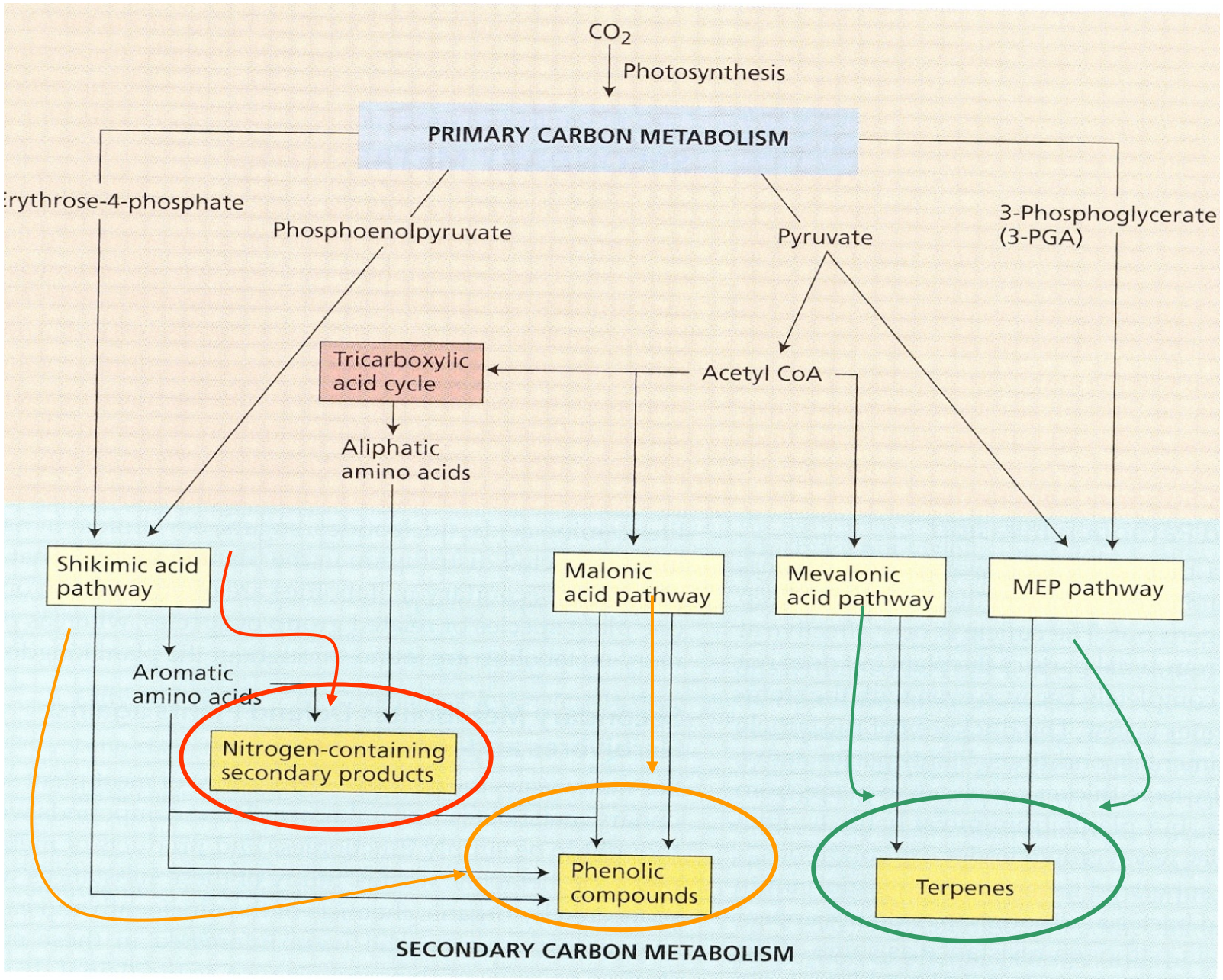
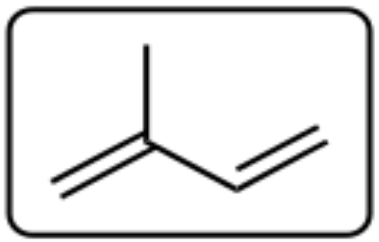


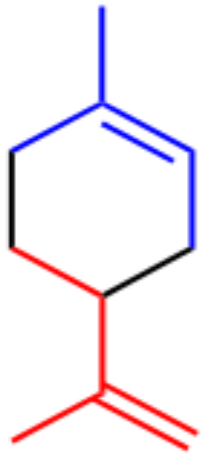
TERPENES

Isoprene

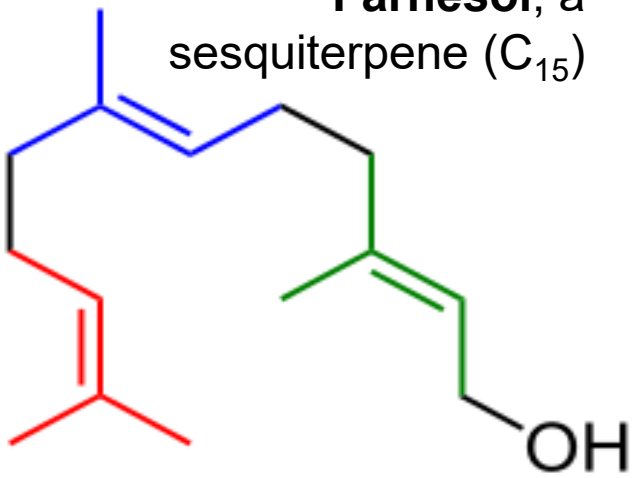


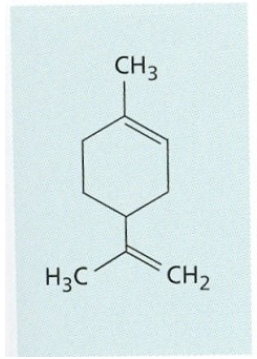
Isoprene (C_5)

Limonene, a monoterpene (C_{10})

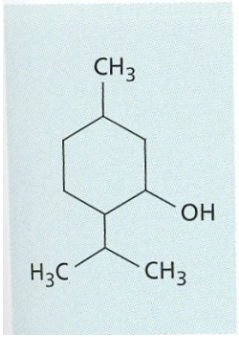


Farnesol, a sesquiterpene (C_{15})

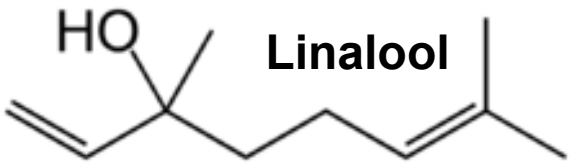




limonene

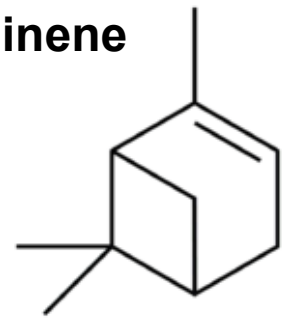


menthol



Linalool

Pinene



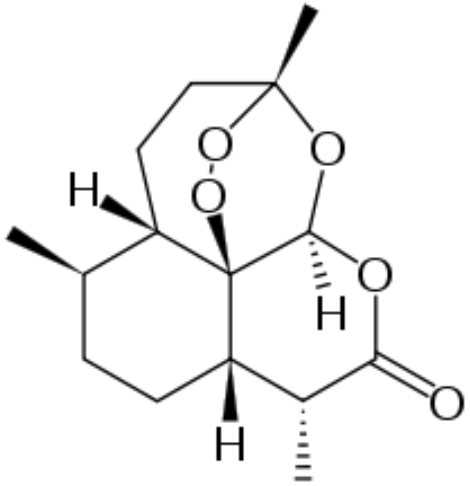
Eucalyptol

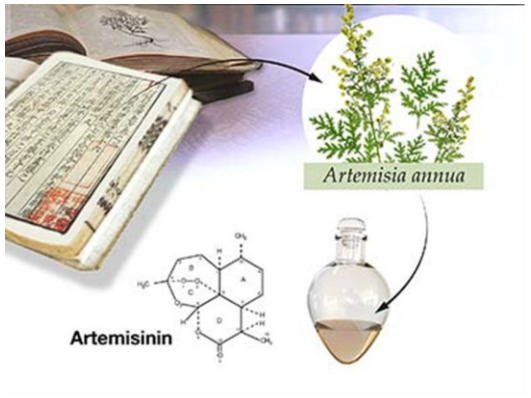
Image sources: Calvero; Wilhelm Thomé; Forest & Kim Starr; Karan A. Rawlins, University of Georgia

Antimalarial from *Artemisia annua*

Artemisia annua

Artemisinin



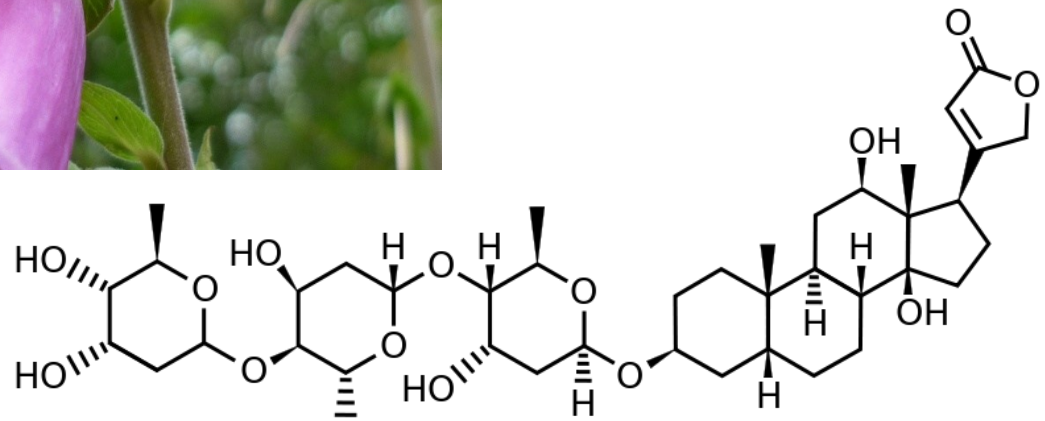


Dr Tu Youyou

Nobel Prize in Physiology or Medicine 2015

1785 "*An Account of the Foxglove, and Some of its Medical Uses*" **William Withering (1741 – 1799)**

Digoxin, cardiac glycosites

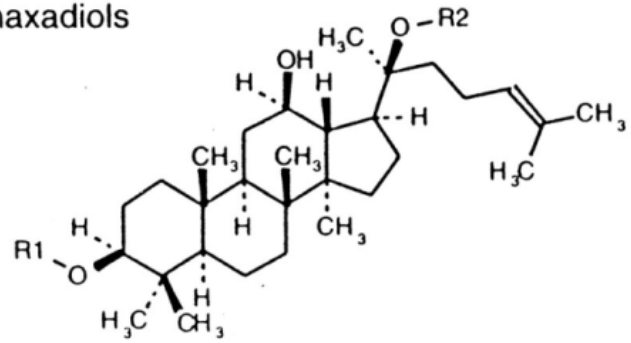


Digitalis purpura

Krikler, D.M. (1985). The foxglove, "the old woman from Shropshire" and William Withering. Journal of the American College of Cardiology. 5: 3A-9A; Lee, M.R. (2001) William Withering (1741–1799): A Birmingham Lunatic. Proc. R. Coll. Physicians Edinb. 31: 77-83. Wilkins, M.R., Kendall, M.J. and Wade, O.L. (1985). William Withering and digitalis, 1785 to 1985. BMJ. 290: 7-8.

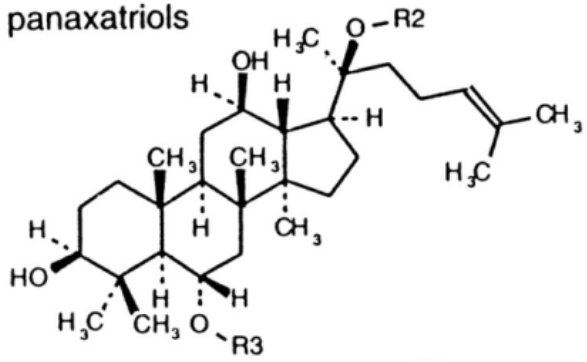
Ginseng (*Panax* spp.)

panaxadiols

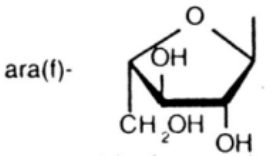


	R1	R2
ginsenoside Rb_1	*O*-glc-(1→2)-glc-	*O*-glc-(1→6)-glc-
ginsenoside Rb_2	*O*-glc-(1→2)-glc-	*O*-ara(p)-(1→6)-glc-
ginsenoside Rc	*O*-glc-(1→2)-glc-	*O*-ara(f)-(1→6)-glc-
ginsenoside Rd	*O*-glc-(1→2)-glc-	glc-

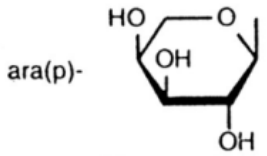
panaxatriols



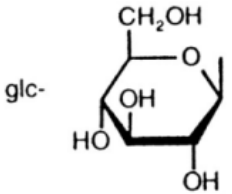
	R2	R3
ginsenoside Re	glc-	*O*-rha-(1→2)-glc-
ginsenoside Rf	H-	*O*-glc-(1→2)-glc-
ginsenoside Rg_1	glc-	glc-
ginsenoside Rg_2	H-	*O*-rha-(1→2)-glc-



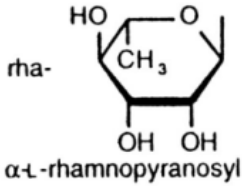
α-L-arabinofuranosyl



α-L-arabinopyranosyl



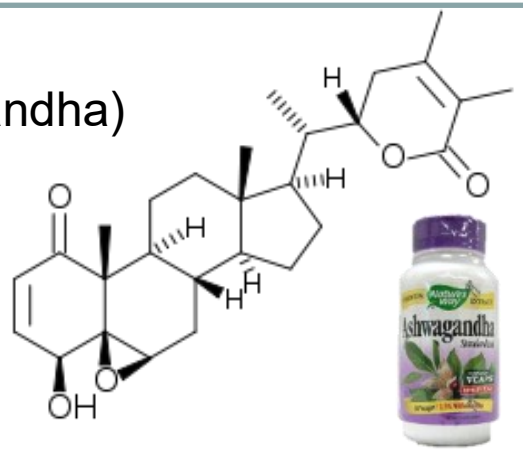
β-D-glucopyranosyl



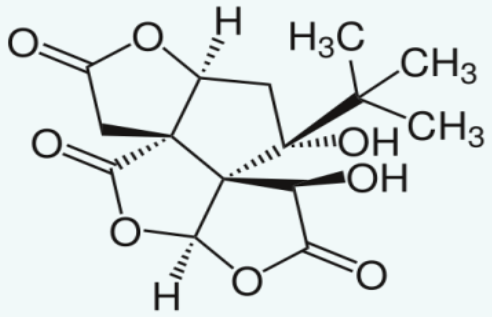
α-L-rhamnopyranosyl

Briskin, D.P. (2000). Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant Physiol. 124: 507-514; William M. Brown Jr., bugwood..org

Withfarin A,,
Withania somnifera (ashwagandha)



Yinxingye *Ginkgo biloba*

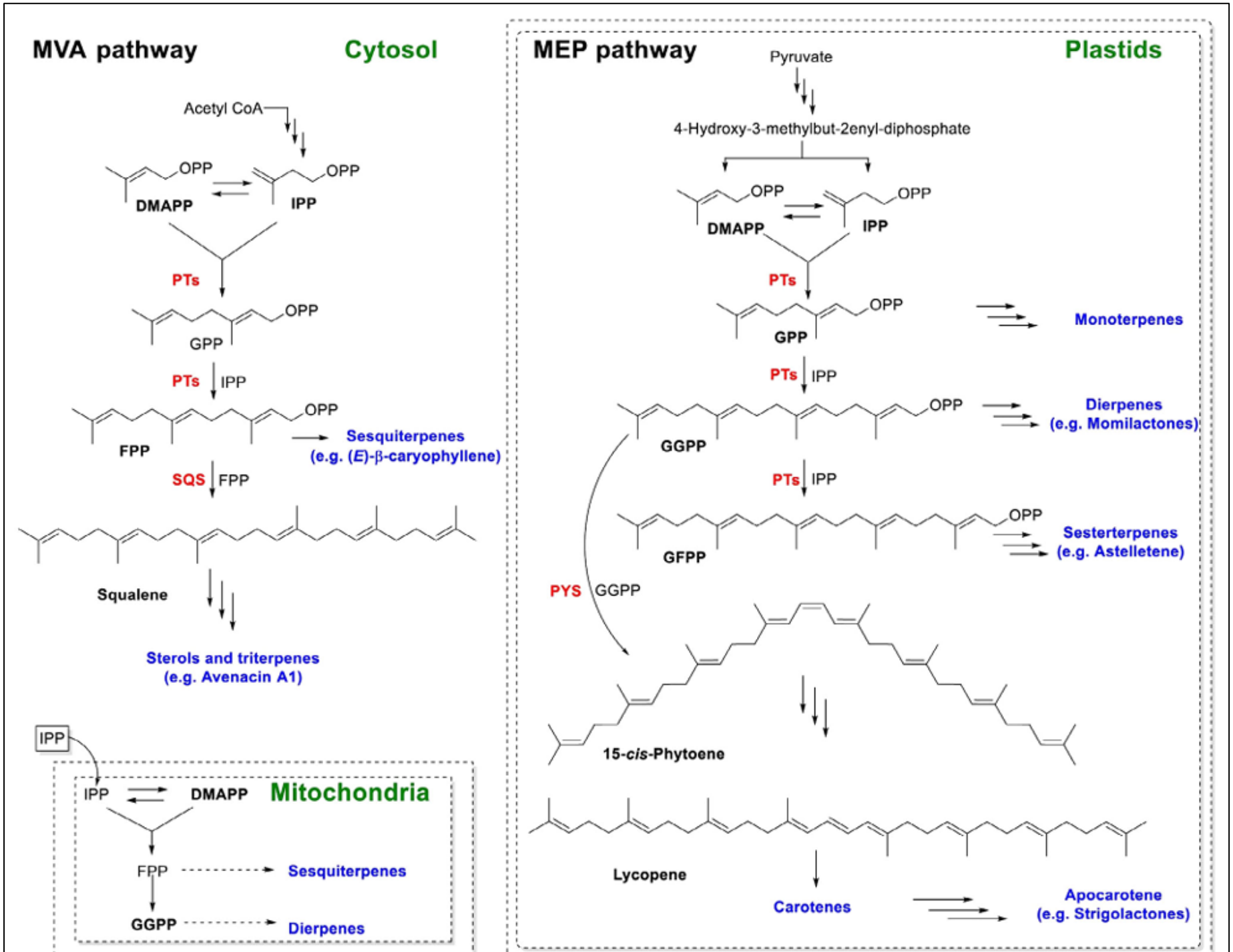


Bilobalide

ginkgolides και bilobalides

Terpene Biosynthesis

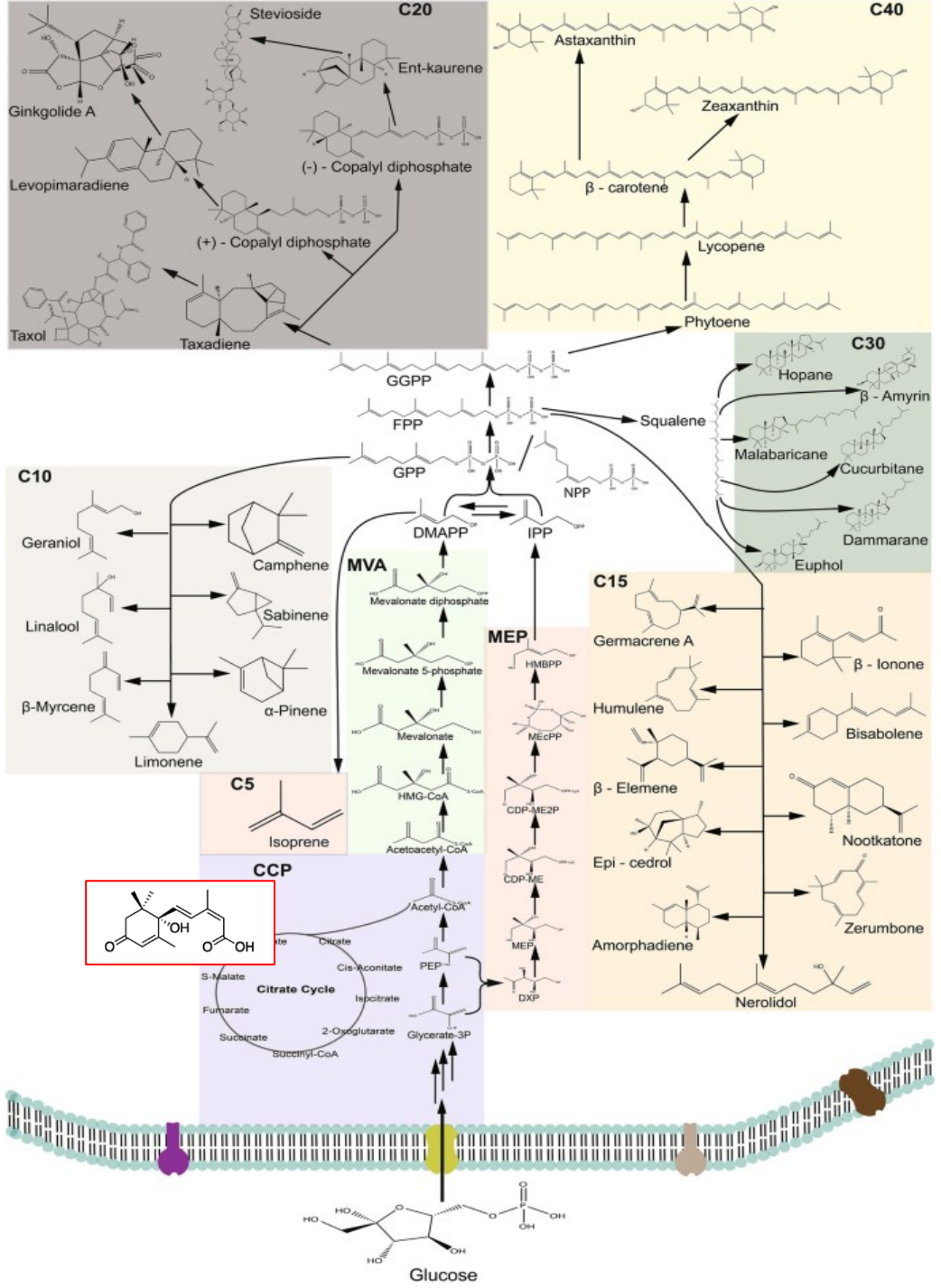
Key enzymes:
Prenyltransferases



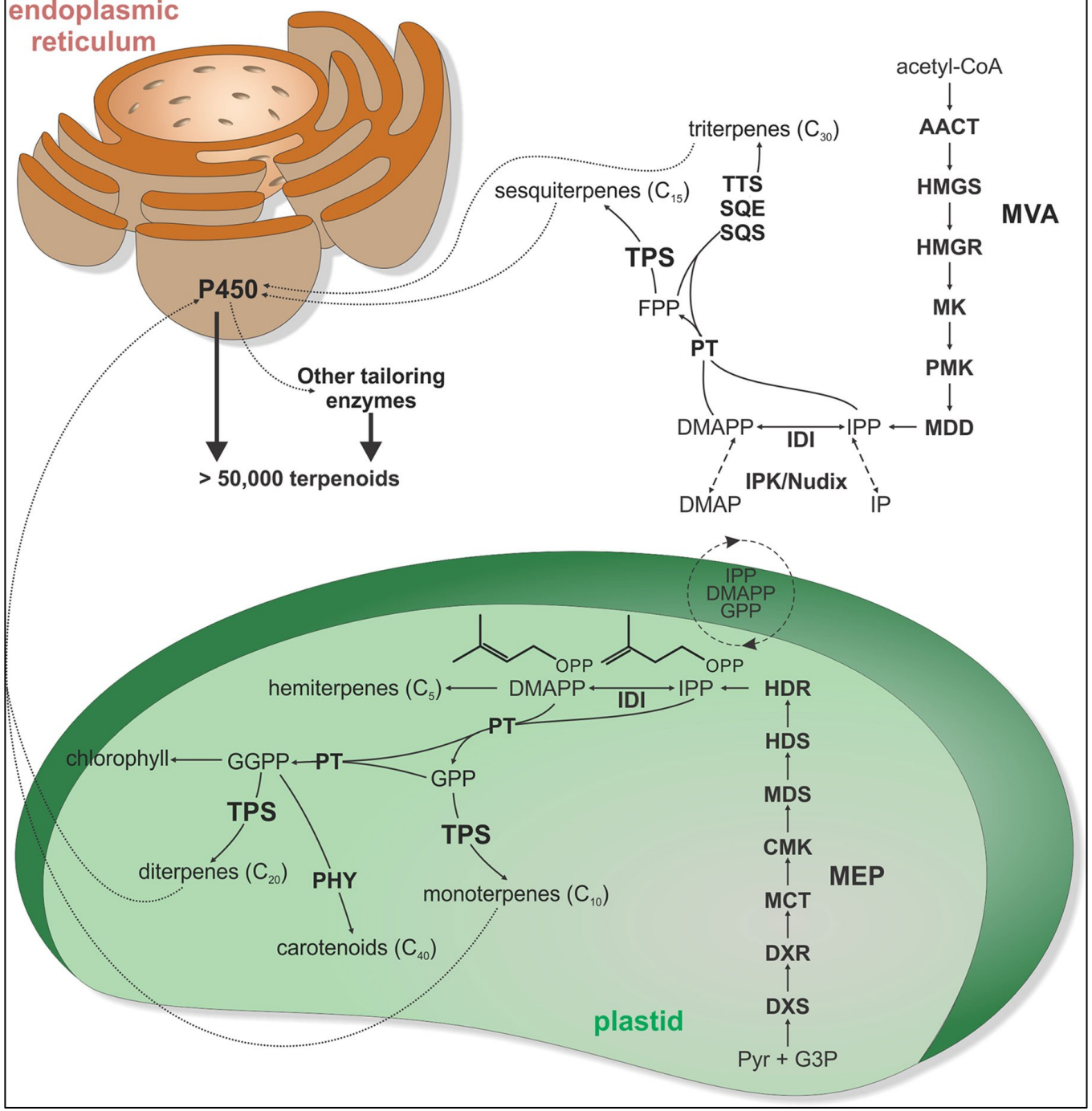
- **Terpene cyclases**

- **Tailoring enzymes**:

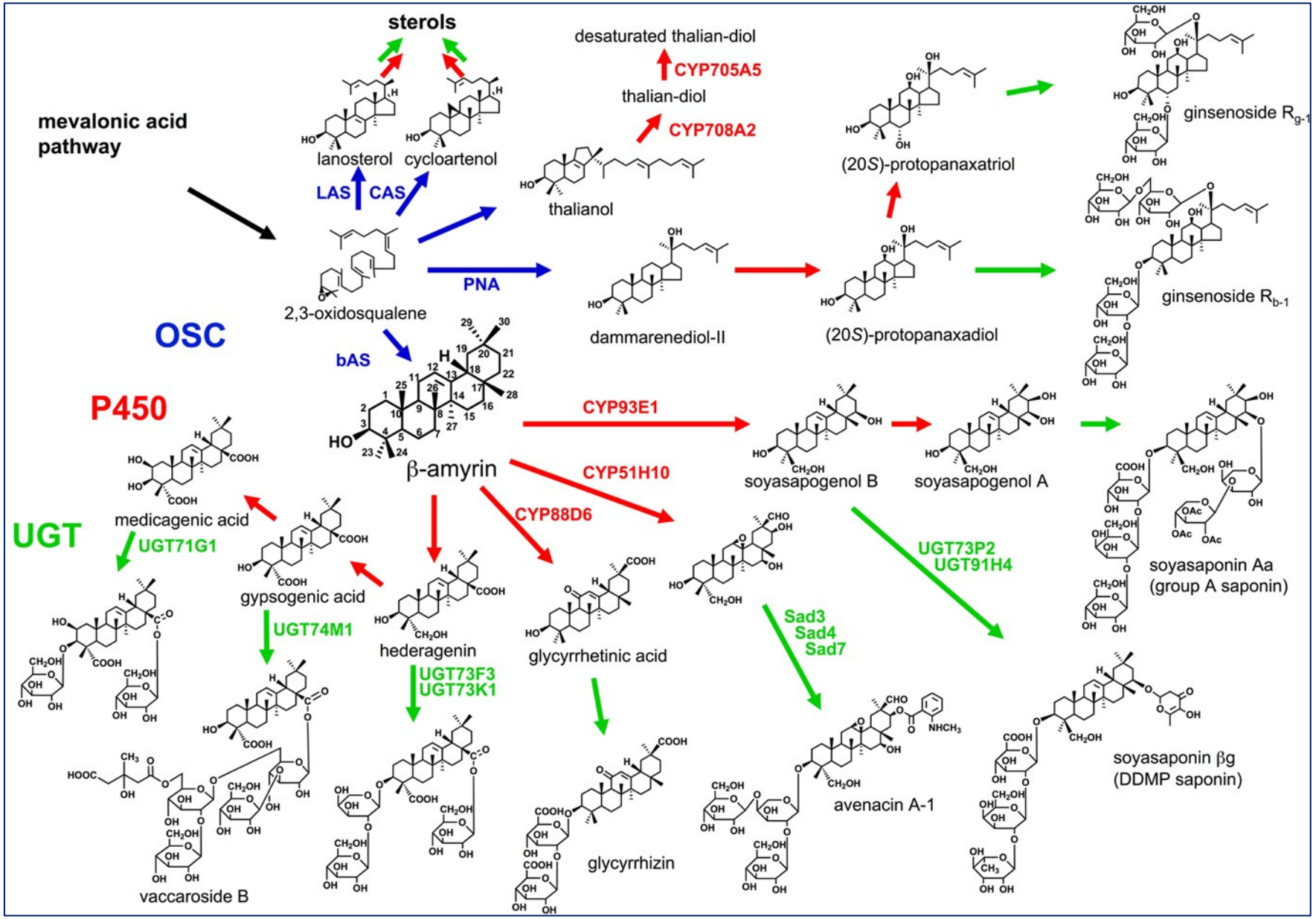
  - P450 oxygenases
  - Glusosyltransferases
  - Alkyltransferases
  - Acyltransferases

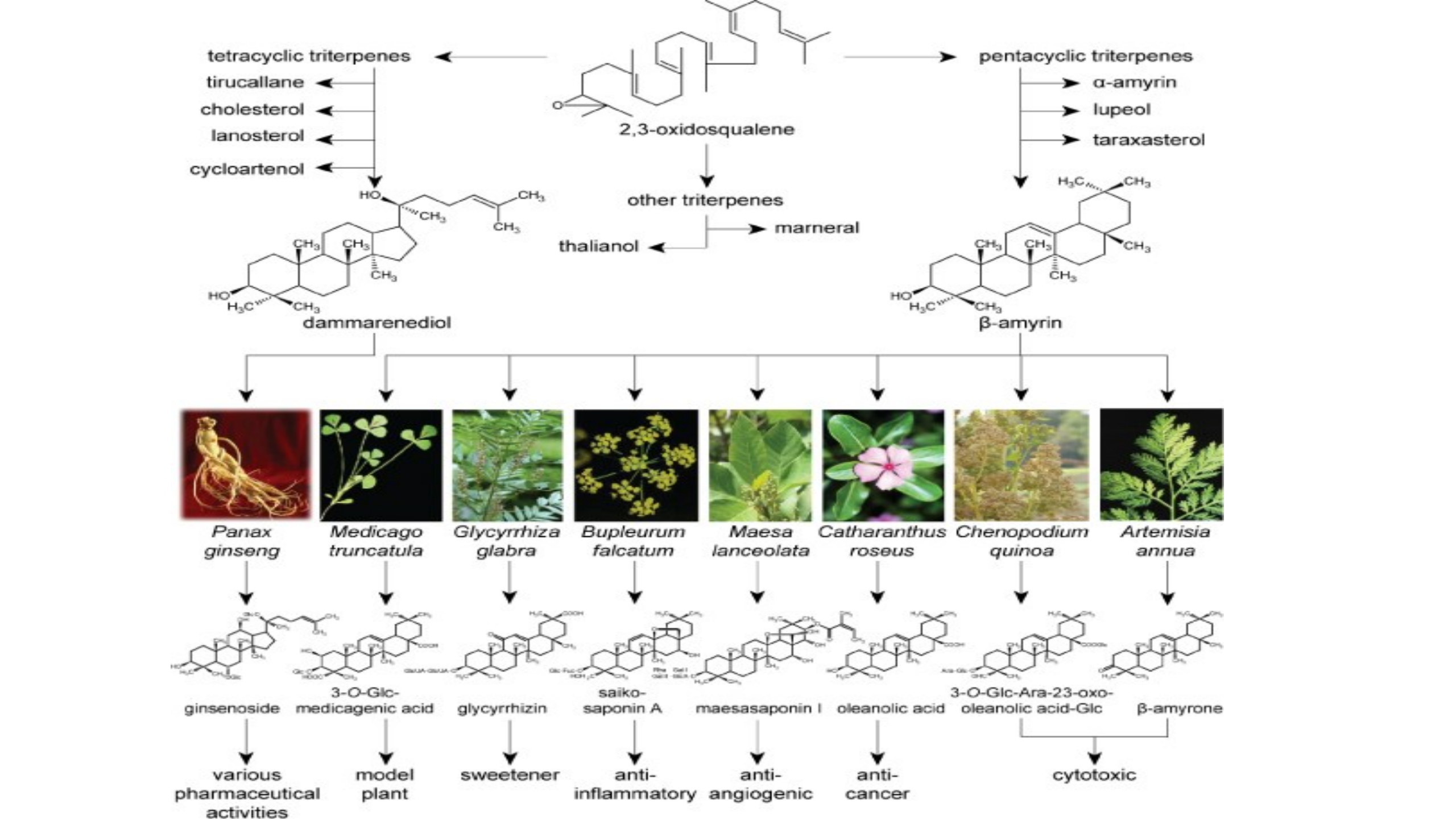


Biosynthesis is compartmentalized



“Signature” and “tailoring” enzymes act on basic backbone molecules and committed intermediates




tetracyclic triterpenes
tirucallane
cholesterol
lanosterol
cycloartenol
2,3-oxidosqualene
other triterpenes
thalianol
marneral
pentacyclic triterpenes
α-amyrin
lupeol
taraxasterol
dammarenediol
β-amyrin
Panax ginseng
Medicago truncatula
Glycyrrhiza glabra
Bupleurum falcatum
Maesa lanceolata
Catharanthus roseus
Chenopodium quinoa
Artemisia annua
ginsenoside
3-O-Glc-medicagenic acid
glycyrrhizin
saiko-saponin A
maesasaponin I
oleanolic acid
3-O-Glc-Ara-23-oxo-oleanolic acid-Glc
β-amyrone
various pharmaceutical activities
model plant
sweetener
anti-inflammatory
anti-angiogenic
anti-cancer
cytotoxic

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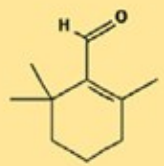
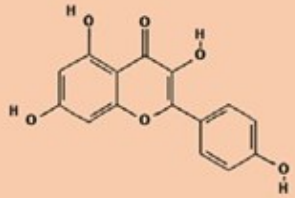
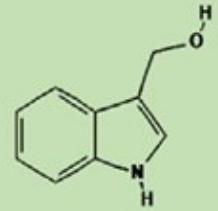
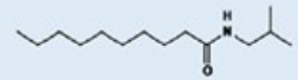
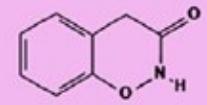
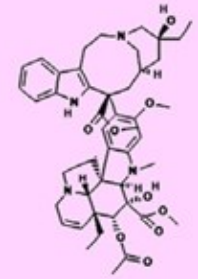
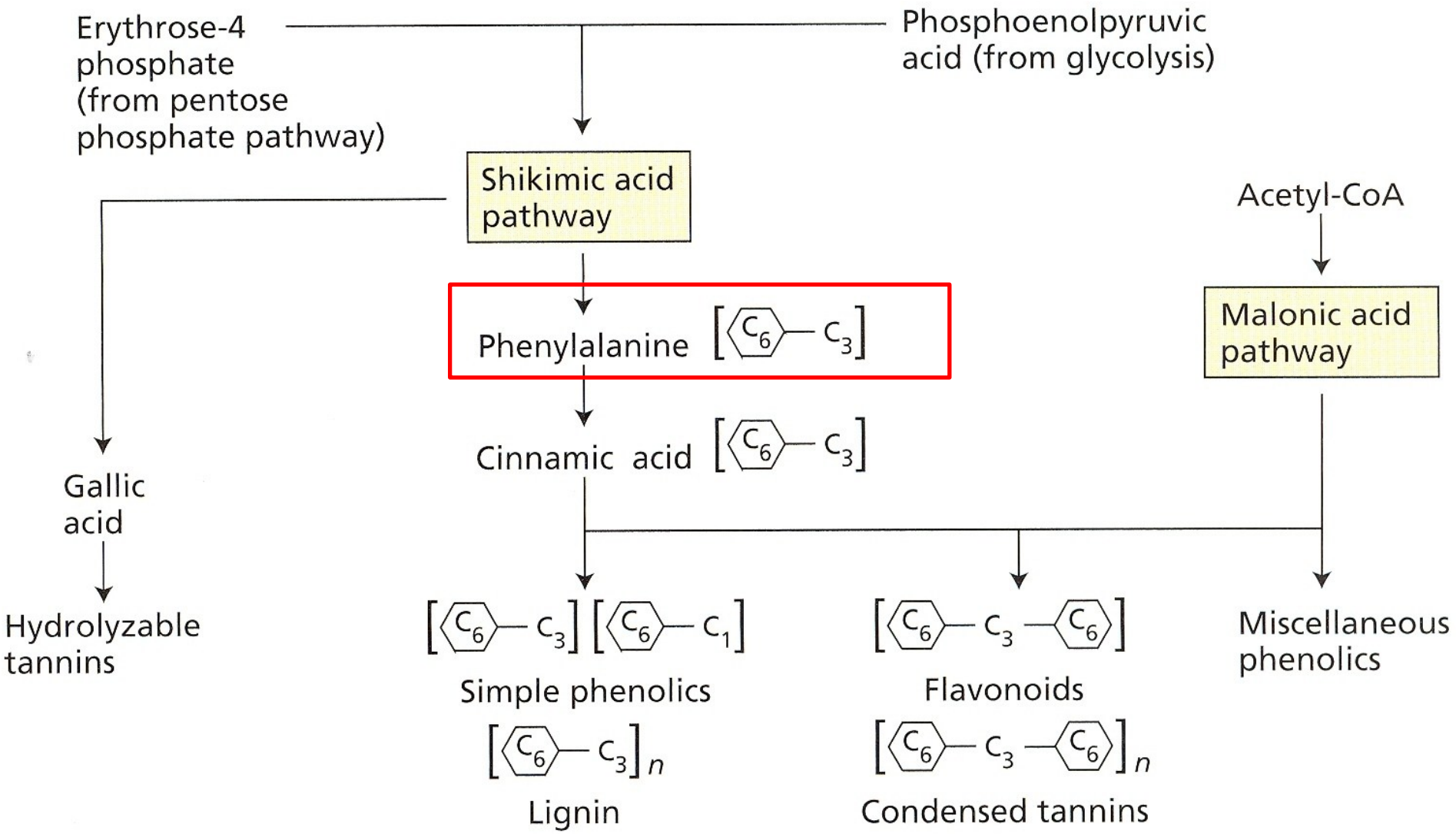
Example structure	 β-Cyclocitral	 Kaempferol	 Indole-3-carbinol	 N-isobutyl-decanamide	 Benzoxazinone	 Vinblastine

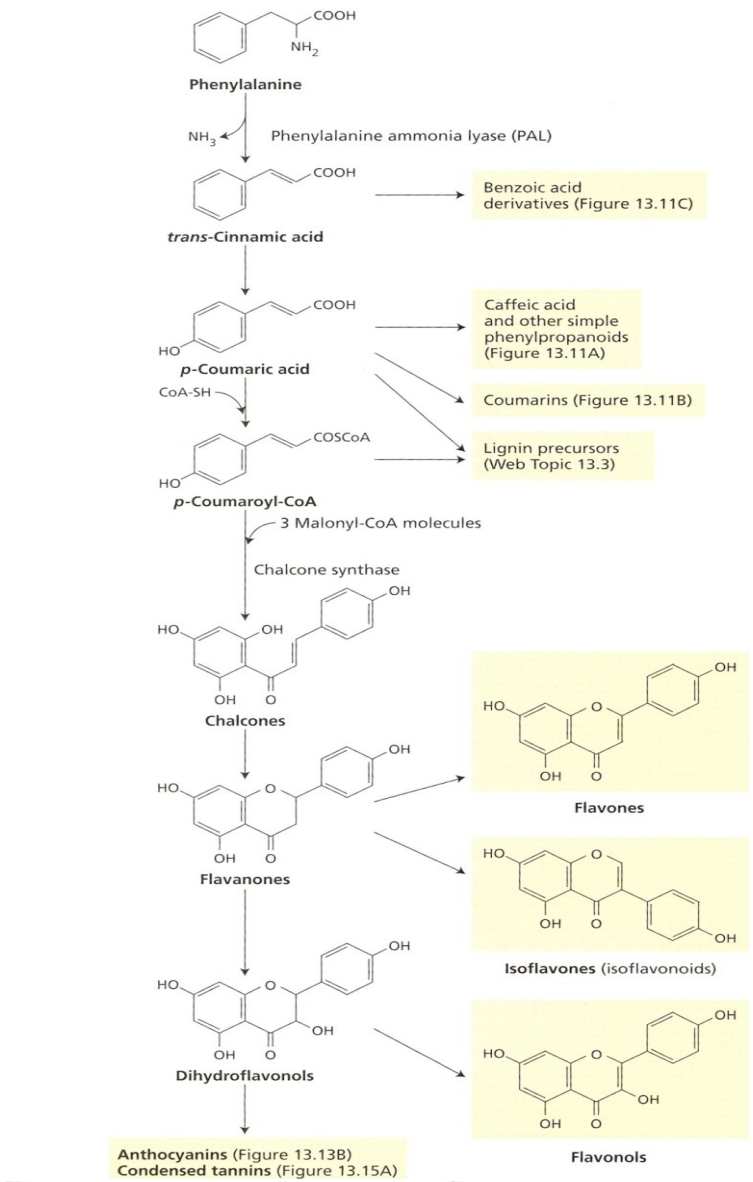
Current Opinion in Plant Biology

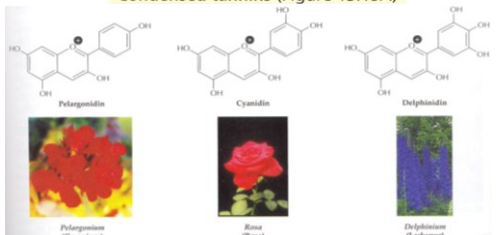
Phenolic compounds

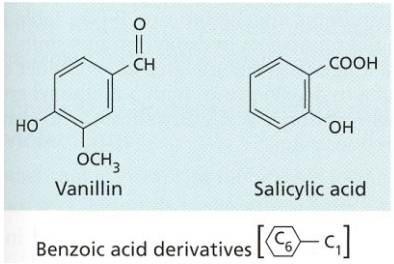
(“old” molecules)

Biosynthetic pathway

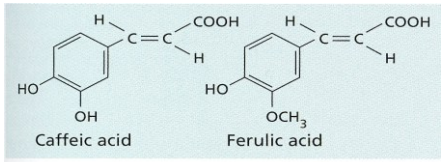




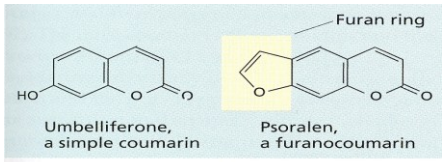




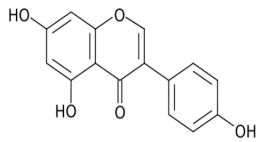
Benzoic acid derivatives $[C_6-C_1]$



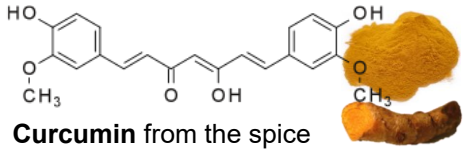
Simple phenylpropanoids $[C_6-C_3]$



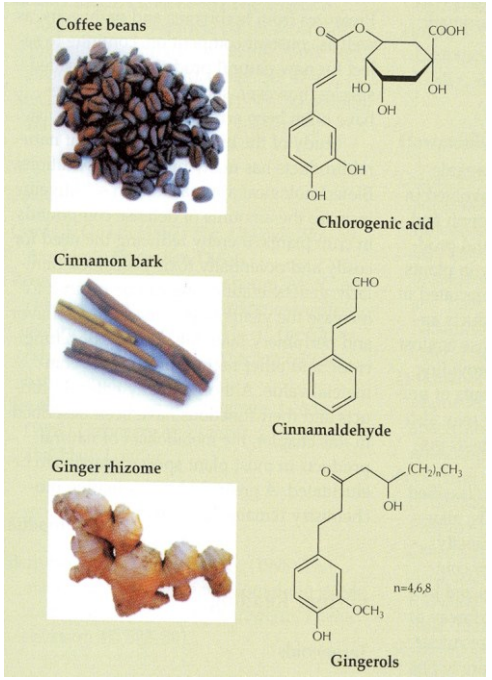
Coumarins $[C_6-C_3]$



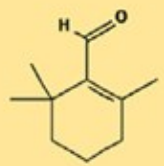
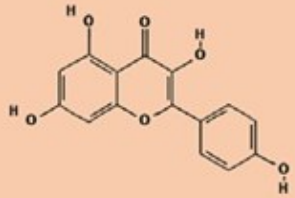
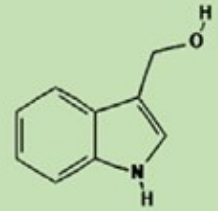
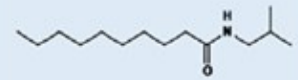
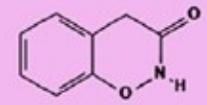
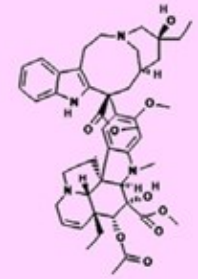
Genistein an isoflavonoid from soy beans



Curcumin from the spice turmeric

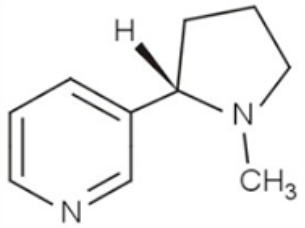


Frequently studied classes of specialized metabolites

Class	TERPENOIDS / ISOPRENOIDS	PHENYLPROPANOIDS	GLUCOSINOLATES	ALKAMIDES	BENZOXAZINOIDS	ALKALOIDS
Description	Metabolites produced from isoprene units	Phenolic compounds derived from phenylalanine	Sulfur-containing metabolites	Fatty acid amides	Tryptophan-derived metaboiltes	Nitrogen-containing metabolites derived from aminoacids or nucleotides
Some metabolite groups	Diterpenes, triterpenes, sesquiterpenes, carotenoids, apocarotenoids, sterols	Flavonoids, anthocyanins, lignins, tannins	Aliphatic, aromatic, indolic glucosinolates	Aliphatic, cyclic or aromatic amine residue + C8 to C18 saturated or insaturated, or aromatic chain.	Benzoxazinones and benzoxazolinones	True alkaloids, protoalkaloids, peptide and cyclopeptide alkaloids
Example structure	 β-Cyclocitral	 Kaempferol	 Indole-3-carbinol	 N-isobutyl-decanamide	 Benzoxazinone	 Vinblastine

Current Opinion in Plant Biology

Nitrogen-containing compounds- Alkaloids



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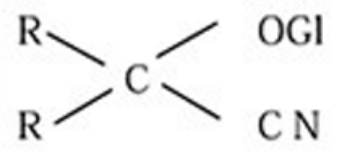
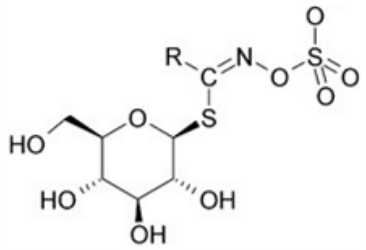
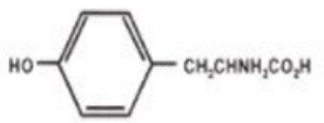
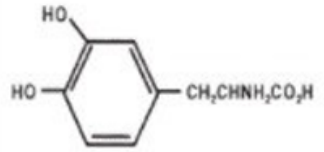




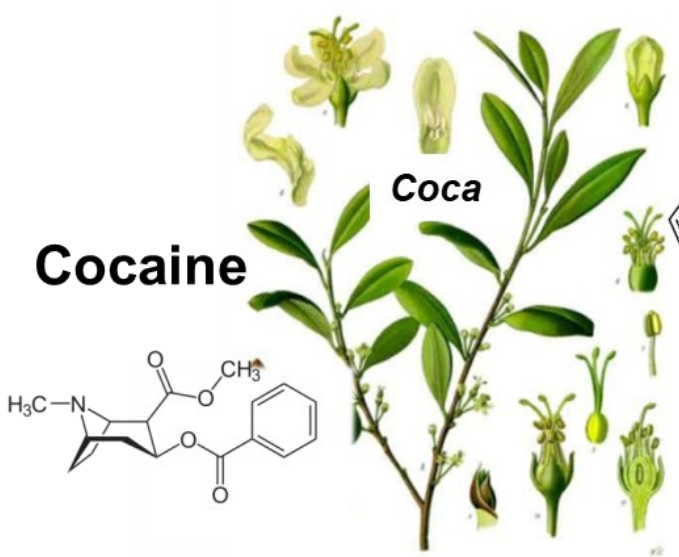


Protein amino acid, tyrosine



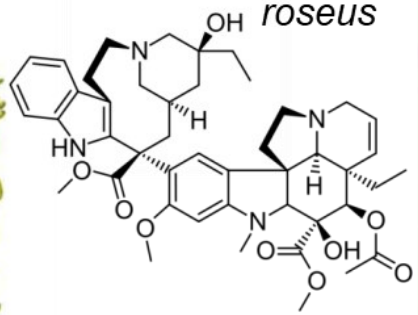
Non-protein amino acid, L-DOPA

Alkaloids include our most important medicinal compounds

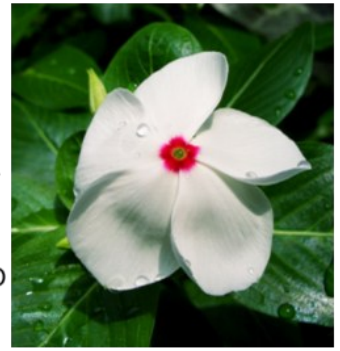


Cocaine

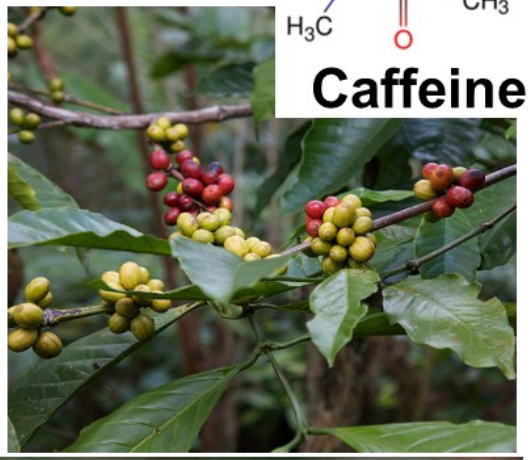
Catharanthus roseus

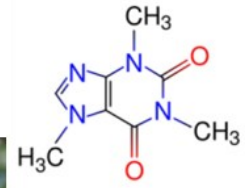


Vincristine



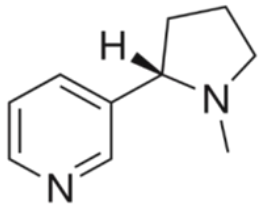
Coffee



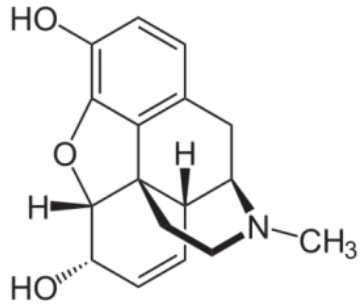


Caffeine

Nicotine



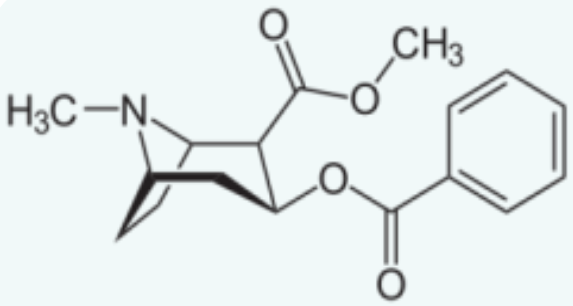
Nicotiana tabacum



Morphine

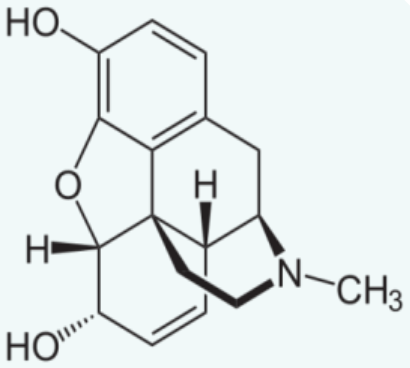
Papaver somniferum

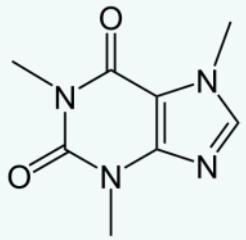
Biosynthesis originates from amino acids



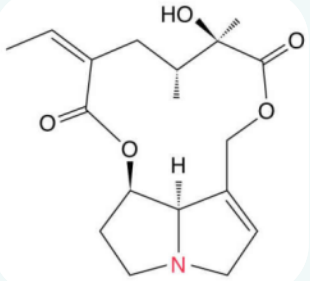
Tropane alkaloids include cocaine

There are approximately 2500 **benzylisoquinoline alkaloids** (BIAs), including morphine

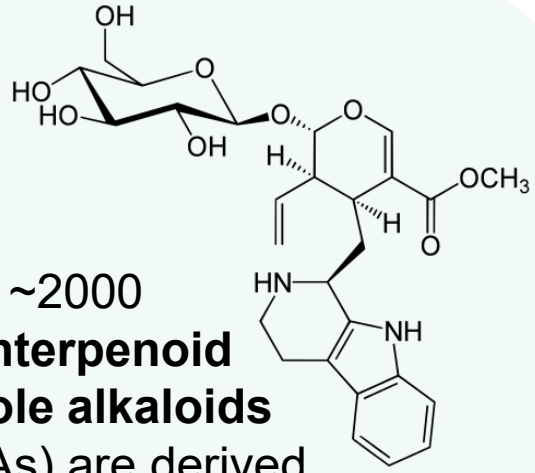




Caffeine is a **purine alkaloid**



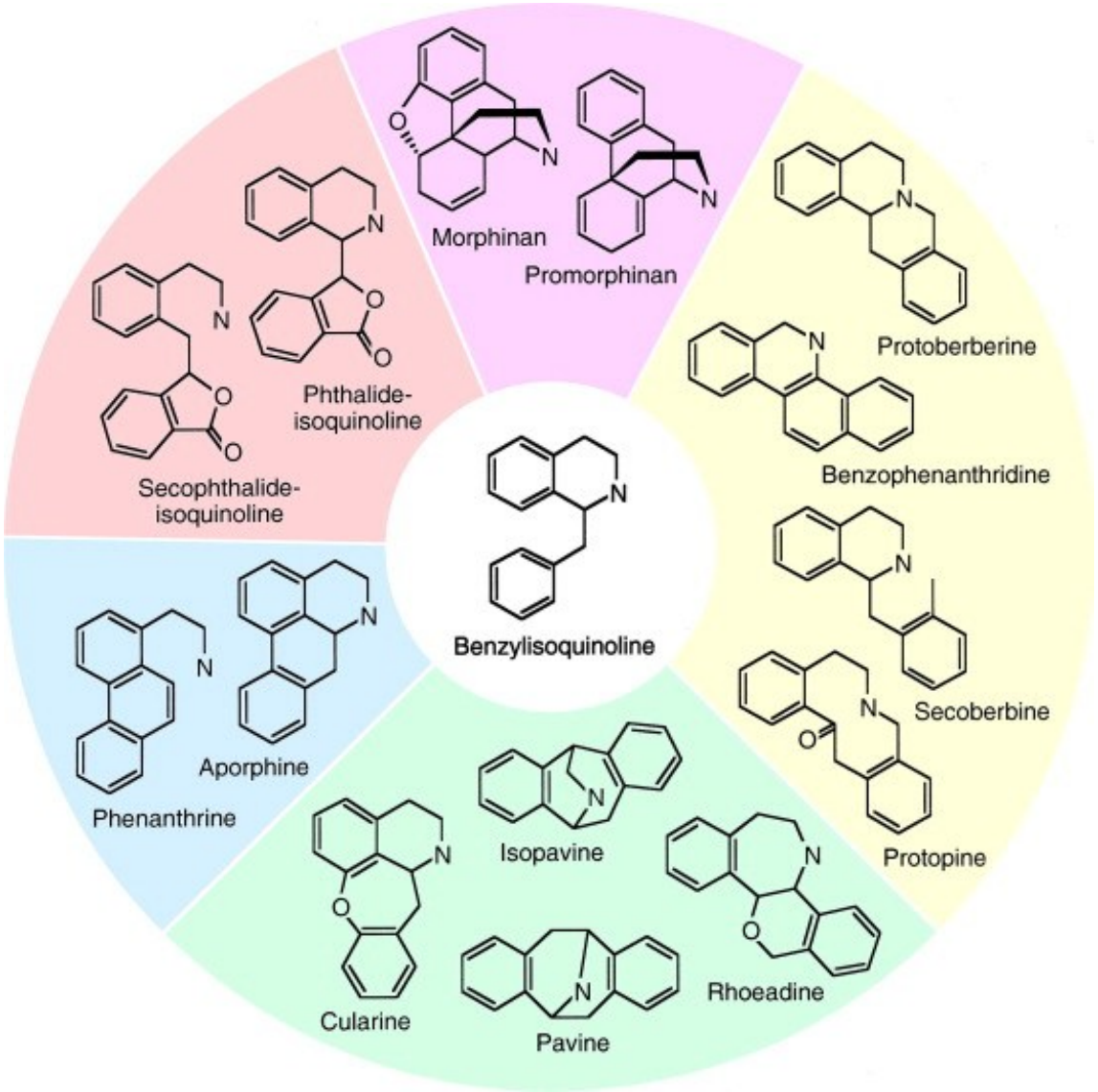
Senecionine is a **pyrrolizidine alkaloid**



The ~2000 **monterpenoid indole alkaloids** (MIAs) are derived from strictosidine

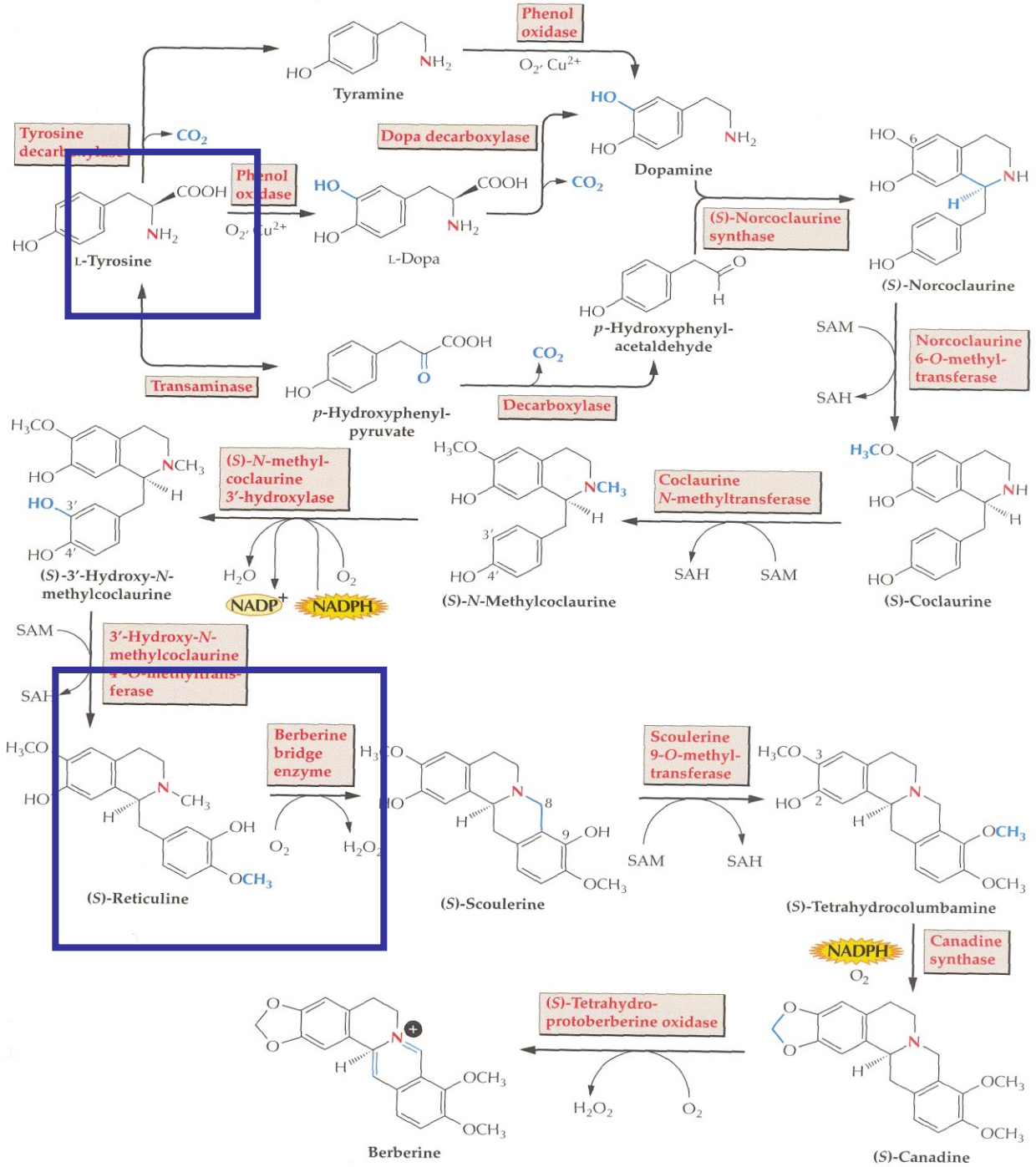
Benzylisoquinoline (BIAs)

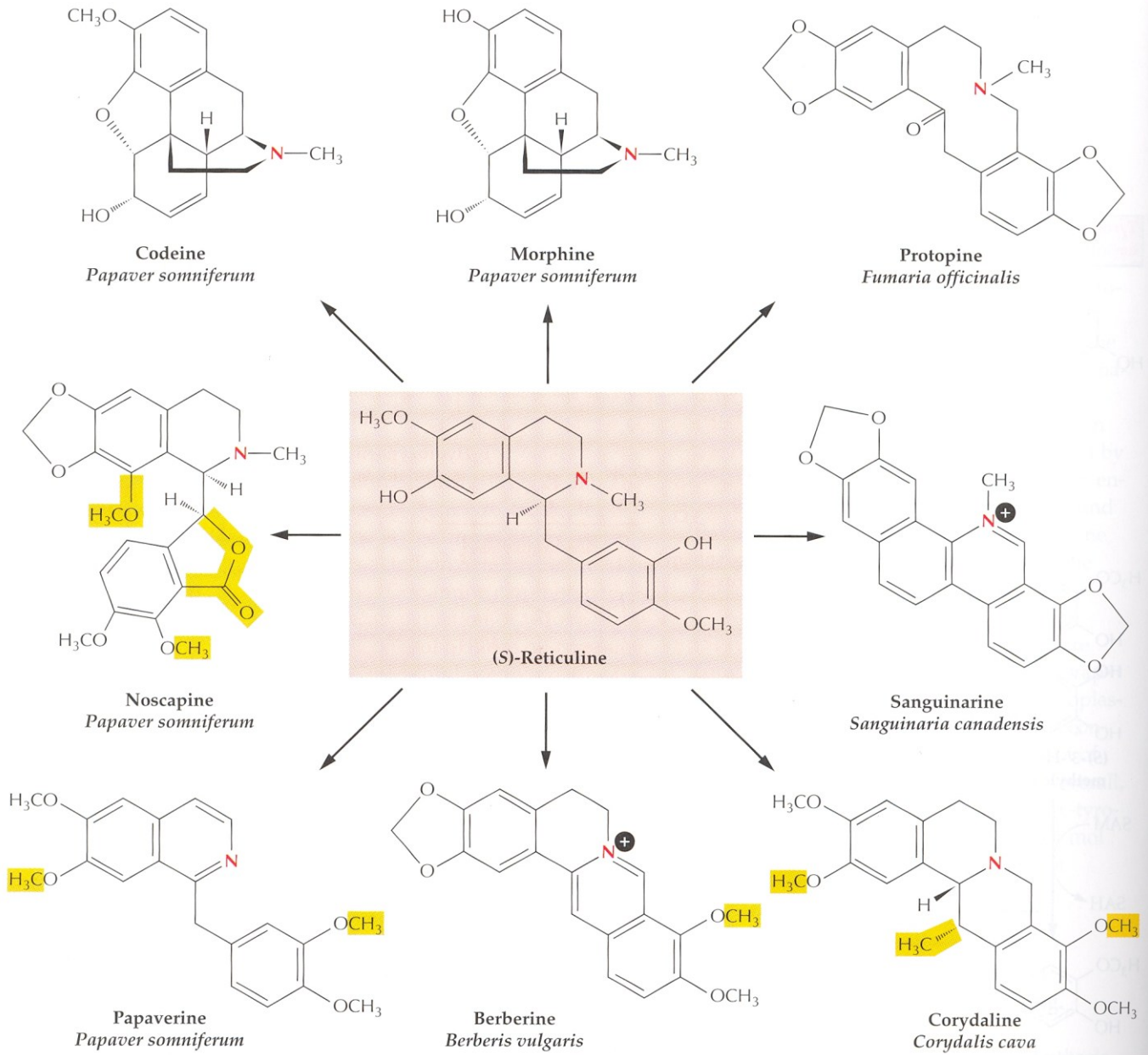
>2500 compounds are derived from one precursor



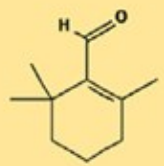
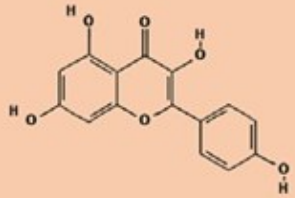
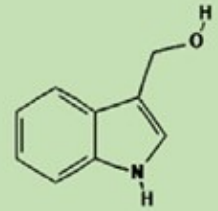
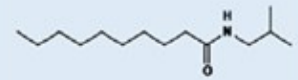
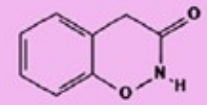
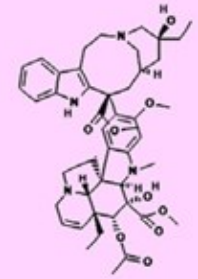
Reprinted from Liscombe, D.K., MacLeod, B.P., Loukanina, N., Nandi, O.I. and Facchini, P.J. (2005). Evidence for the monophyletic evolution of benzylisoquinoline alkaloid biosynthesis in angiosperms. Phytochemistry. 66: 1374-1393 with permission from Elsevier; Richard Old, XID Services, Inc.

An example


Tyramine
Phenol oxidase
O_2, Cu^{2+}
Dopamine
Dopa decarboxylase
CO_2
Tyrosine decarboxylase
L-Tyrosine
Phenol oxidase
L-Dopa
(S)-Norcoclaurine synthase
(S)-Norcoclaurine
p-Hydroxyphenyl-acetaldehyde
SAM
SAH
Norcoclaurine 6-O-methyl-transferase
Transaminase
p-Hydroxyphenyl-pyruvate
Decarboxylase
(S)-N-methyl-coclaurine 3′-hydroxylase
Coclaurine N-methyltransferase
(S)-3′-Hydroxy-N-methylcoclaurine
(S)-N-Methylcoclaurine
(S)-Coclaurine
H_2O
O_2
NADP+
NADPH
3′-Hydroxy-N-methylcoclaurine 4′-O-methyltransferase
Berberine bridge enzyme
Scoulerine 9-O-methyl-transferase
(S)-Reticuline
(S)-Scoulerine
(S)-Tetrahydrocolumbamine
H_2O_2
NADPH
Canadine synthase
(S)-Tetrahydro-protoberberine oxidase
Berberine
(S)-Canadine

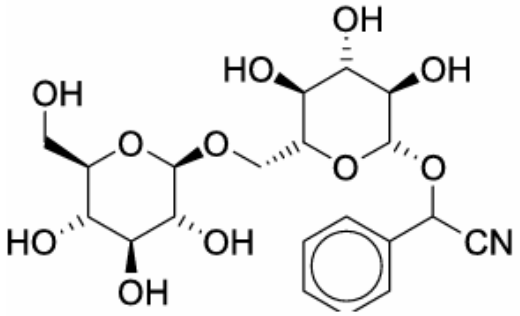

Codeine
Papaver somniferum
Morphine
Papaver somniferum
Protopine
Fumaria officinalis
Noscapine
Papaver somniferum
(S)-Reticuline
Sanguinarine
Sanguinaria canadensis
Papaverine
Papaver somniferum
Berberine
Berberis vulgaris
Corydaline
Corydalis cava

Frequently studied classes of specialized metabolites

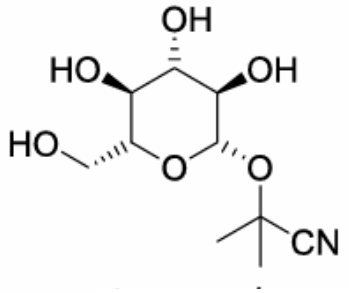
Class	TERPENOIDS / ISOPRENOIDS	PHENYLPROPANOIDS	GLUCOSINOLATES	ALKAMIDES	BENZOXAZINOIDS	ALKALOIDS
Description	Metabolites produced from isoprene units	Phenolic compounds derived from phenylalanine	Sulfur-containing metabolites	Fatty acid amides	Tryptophan-derived metaboiltes	Nitrogen-containing metabolites derived from aminoacids or nucleotides
Some metabolite groups	Diterpenes, triterpenes, sesquiterpenes, carotenoids, apocarotenoids, sterols	Flavonoids, anthocyanins, lignins, tannins	Aliphatic, aromatic, indolic glucosinolates	Aliphatic, cyclic or aromatic amine residue + C8 to C18 saturated or insaturated, or aromatic chain.	Benzoxazinones and benzoxazolinones	True alkaloids, protoalkaloids, peptide and cyclopeptide alkaloids
Example structure	 β-Cyclocitral	 Kaempferol	 Indole-3-carbinol	 N-isobutyl-decanamide	 Benzoxazinone	 Vinblastine

Current Opinion in Plant Biology

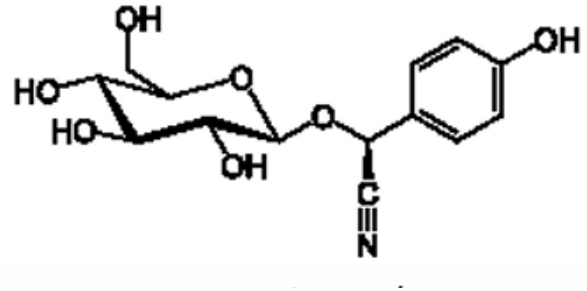
cyanogenic glycosides



Amygdalin



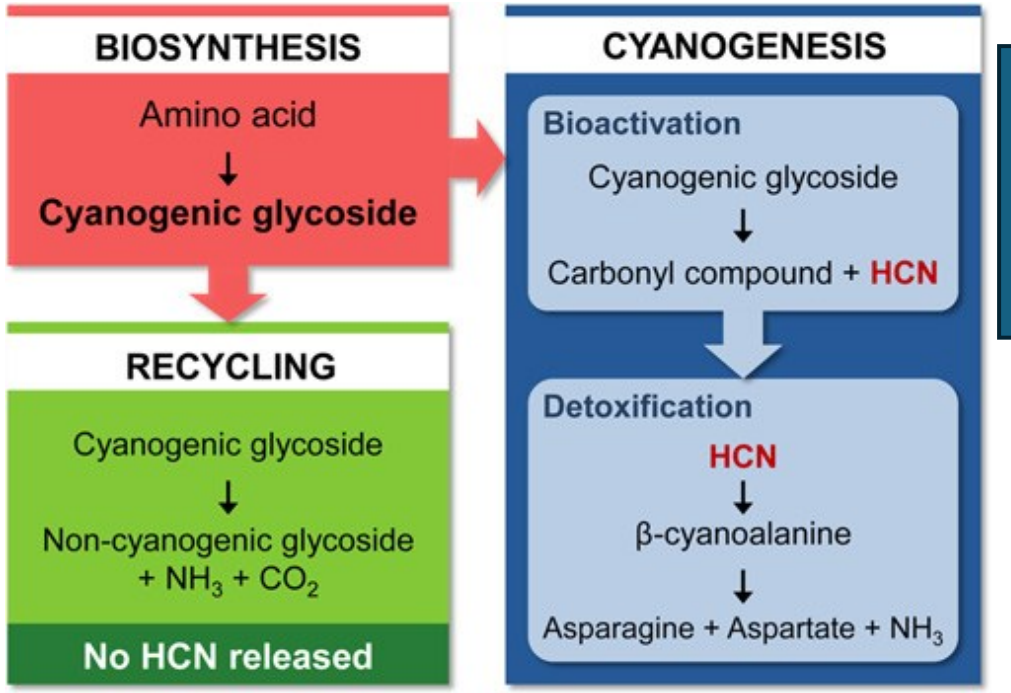
Linamarin

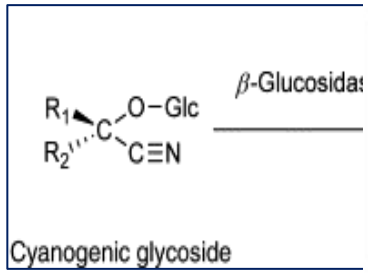


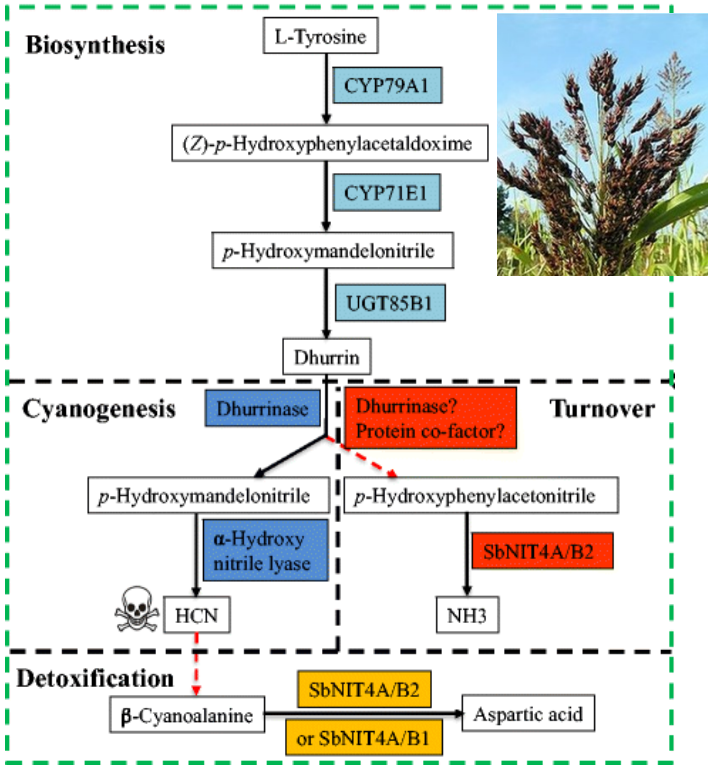
Dhurrin

>3000 species are considered cyanogenic plants

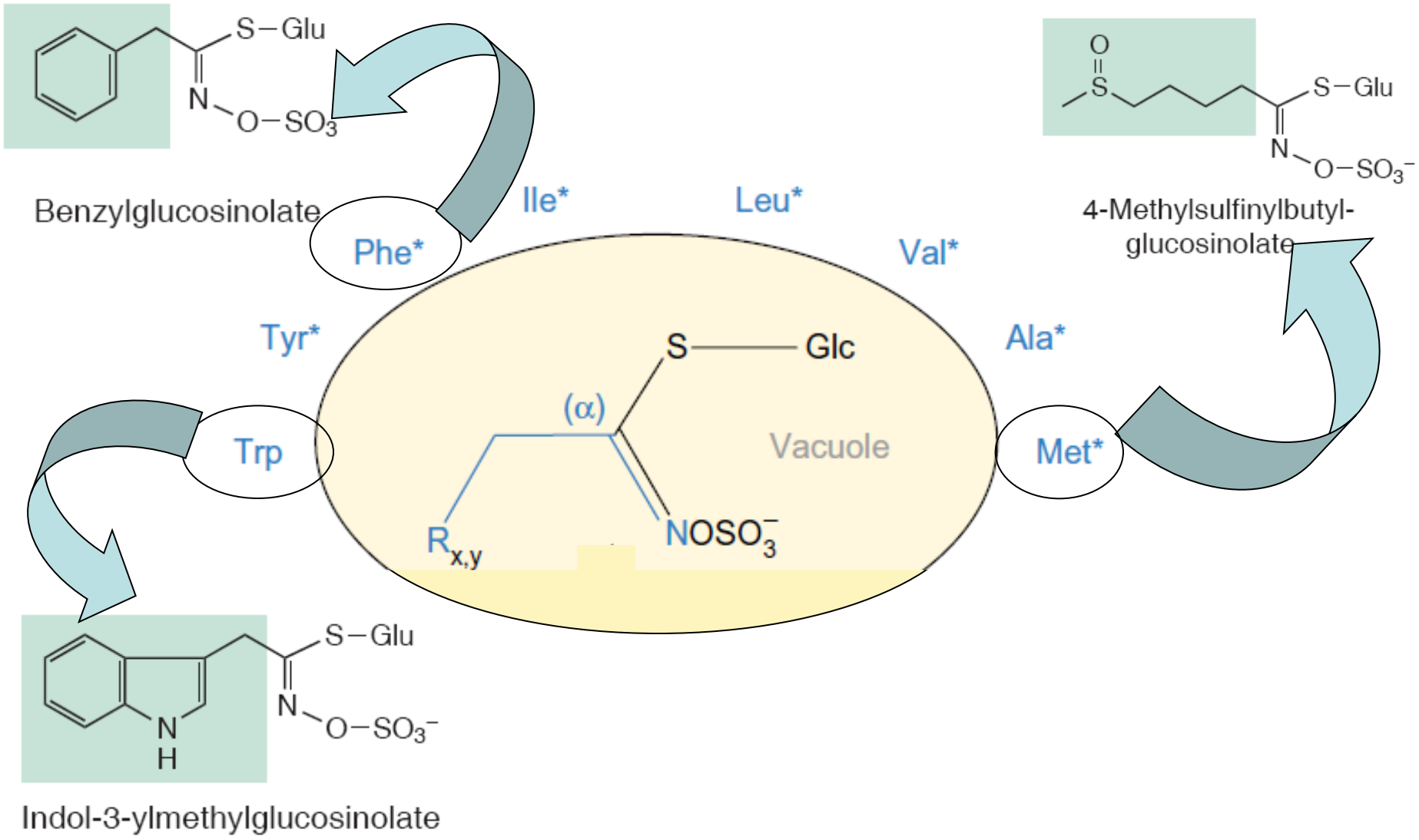
Metabolism of cyanogenic glycosides

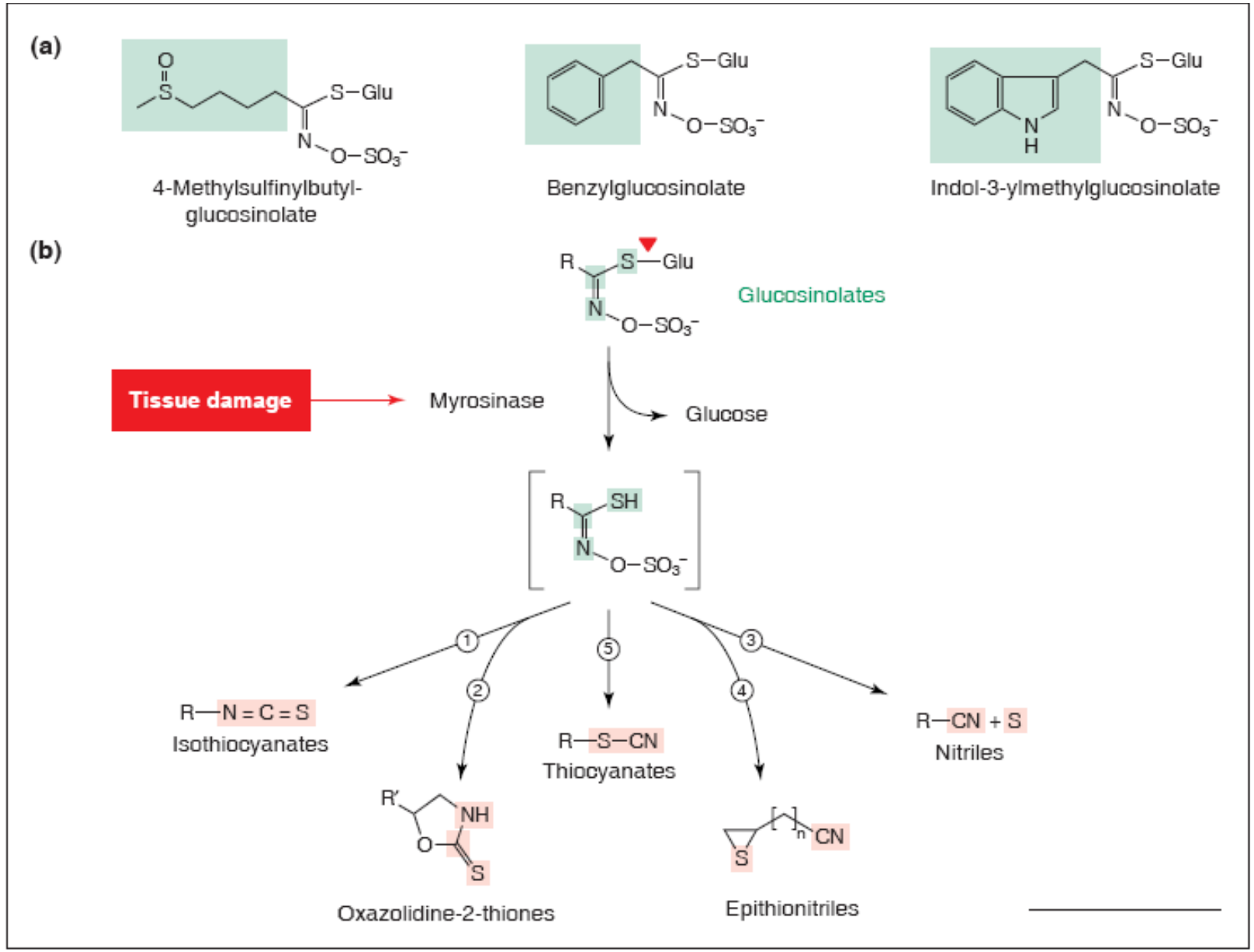




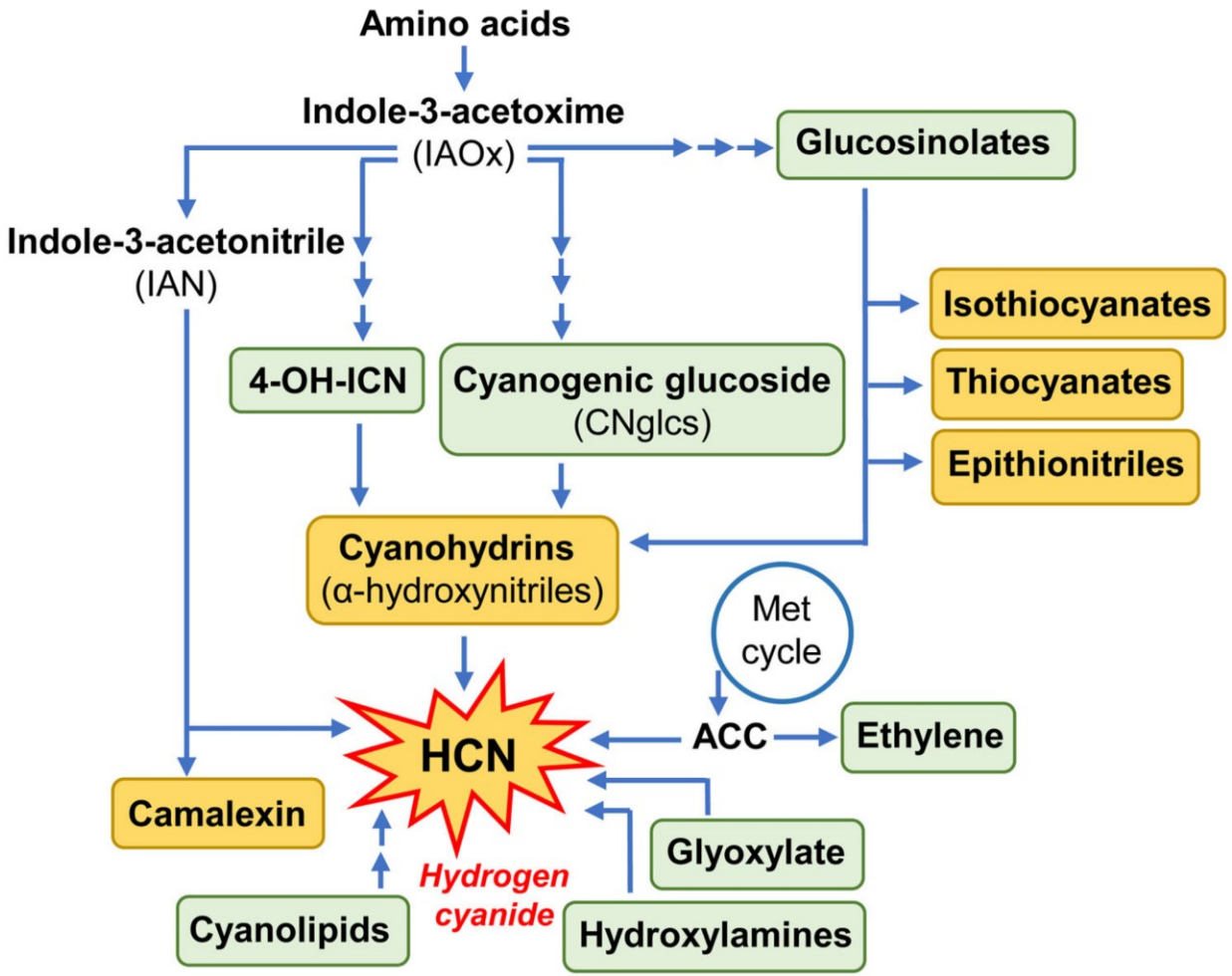


Glucosinolates




(a)
4-Methylsulfinylbutyl-glucosinolate
Benzylglucosinolate
Indol-3-ylmethylglucosinolate
(b)
Glucosinolates
Tissue damage
Myrosinase
Glucose
R—N=C=S
Isothiocyanates
R—S—CN
Thiocyanates
R—CN + S
Nitriles
Oxazolidine-2-thiones
Epithionitriles

metabolic crosstalk of plant pathways involved in HCN production



Martinez & Diaz, 2024

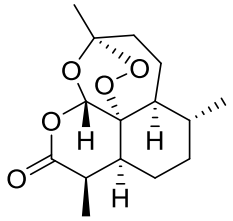
Plants are really good chemists!!

Tea tree oil

Melaleuca alternifolia

ANTI-BACTERIAL/FUNGAL

AGROCHEMICAL

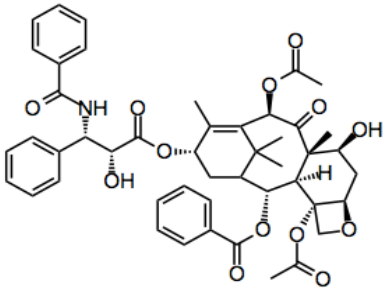


Artemisinin

Wormwood

(*Artemisia annua*)

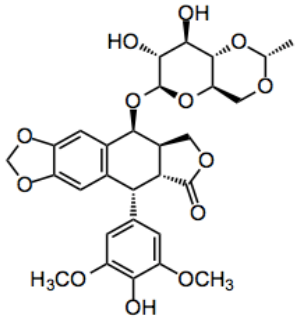
ANTI-MALARIAL



Taxol

Yew (*Taxus baccata*)

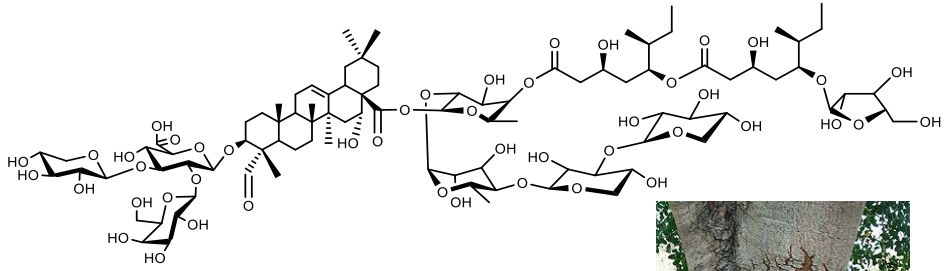
ANTI-CANCER



Etoposide

Mayapple (*Podophyllum)*

ANTI-CANCER

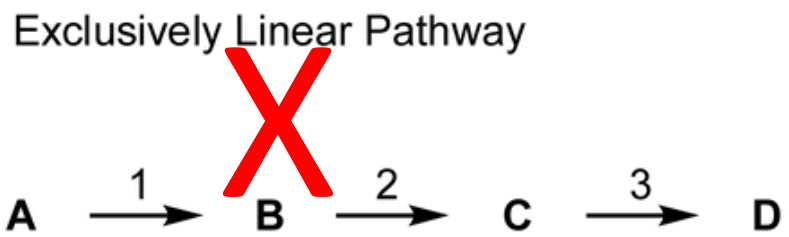


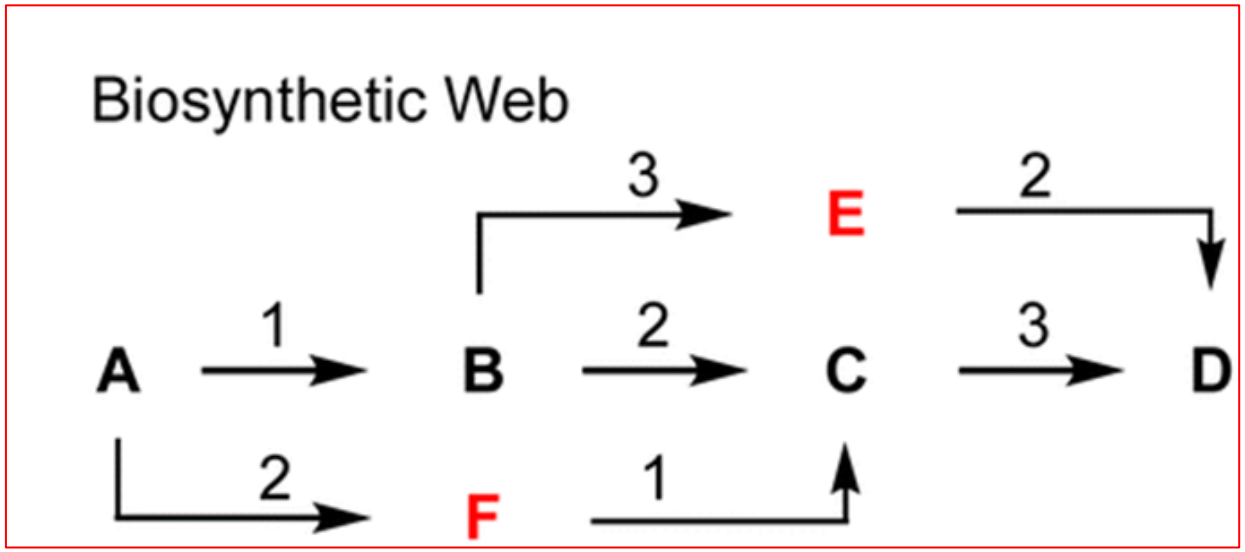
QS-21

Soapbark (*Quillaja saponaria*)

ADJUVANT

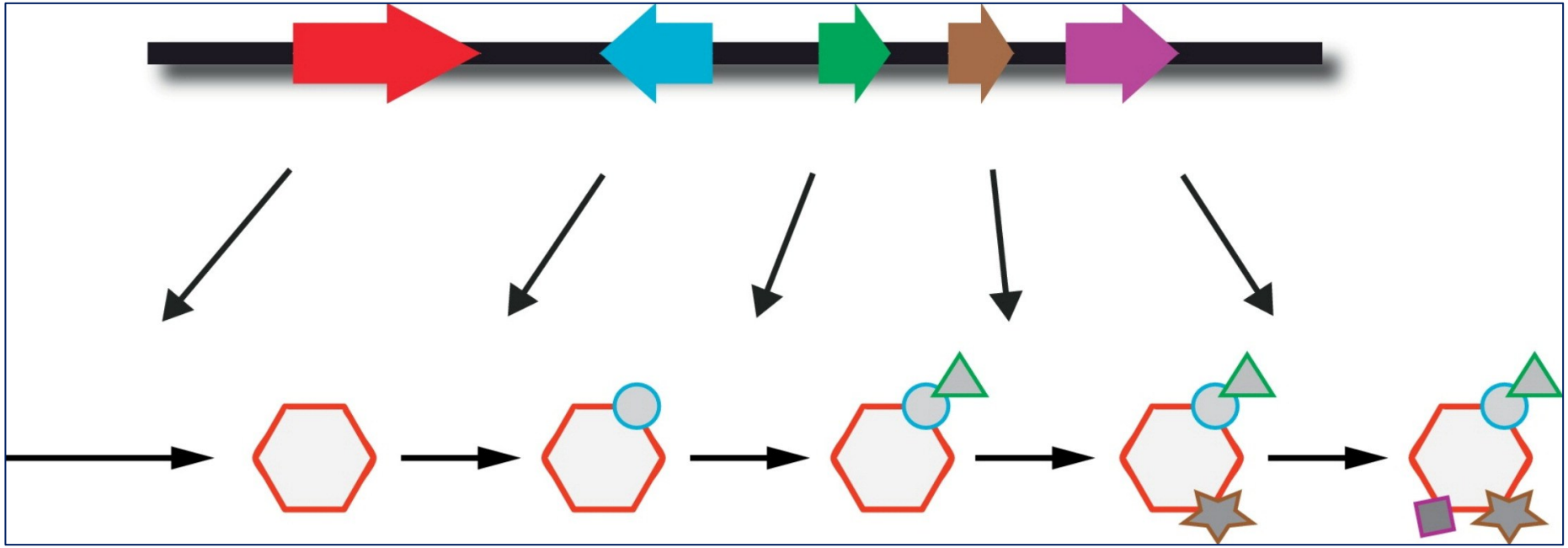
Traits of biosynthetic pathways of secondary metaoblites





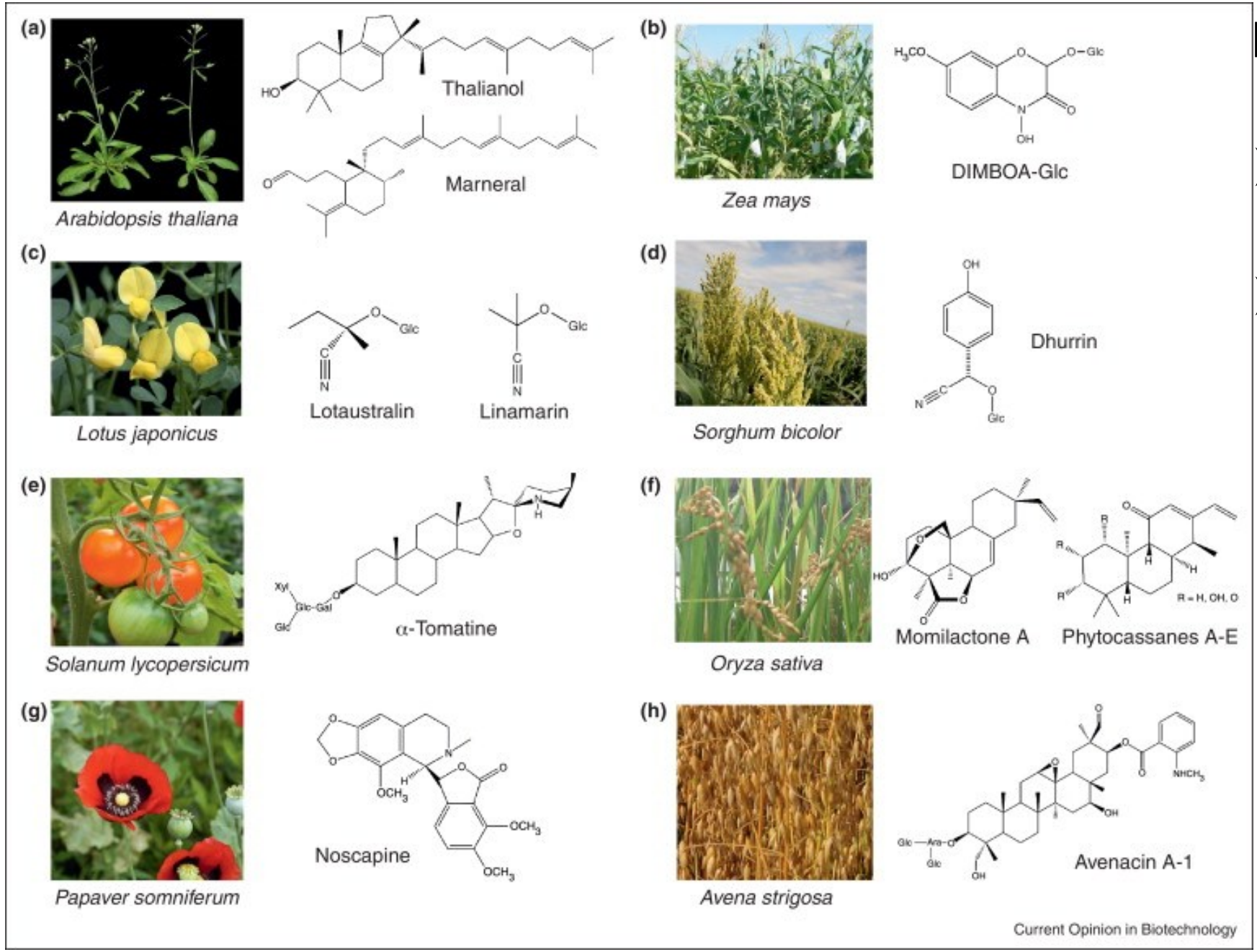
Traits of biosynthetic pathways of secondary metaoblites

- Steps are tightly co-ordinated
- Biosynthetic genes are organized on metabolic gene clusters on plant genomes

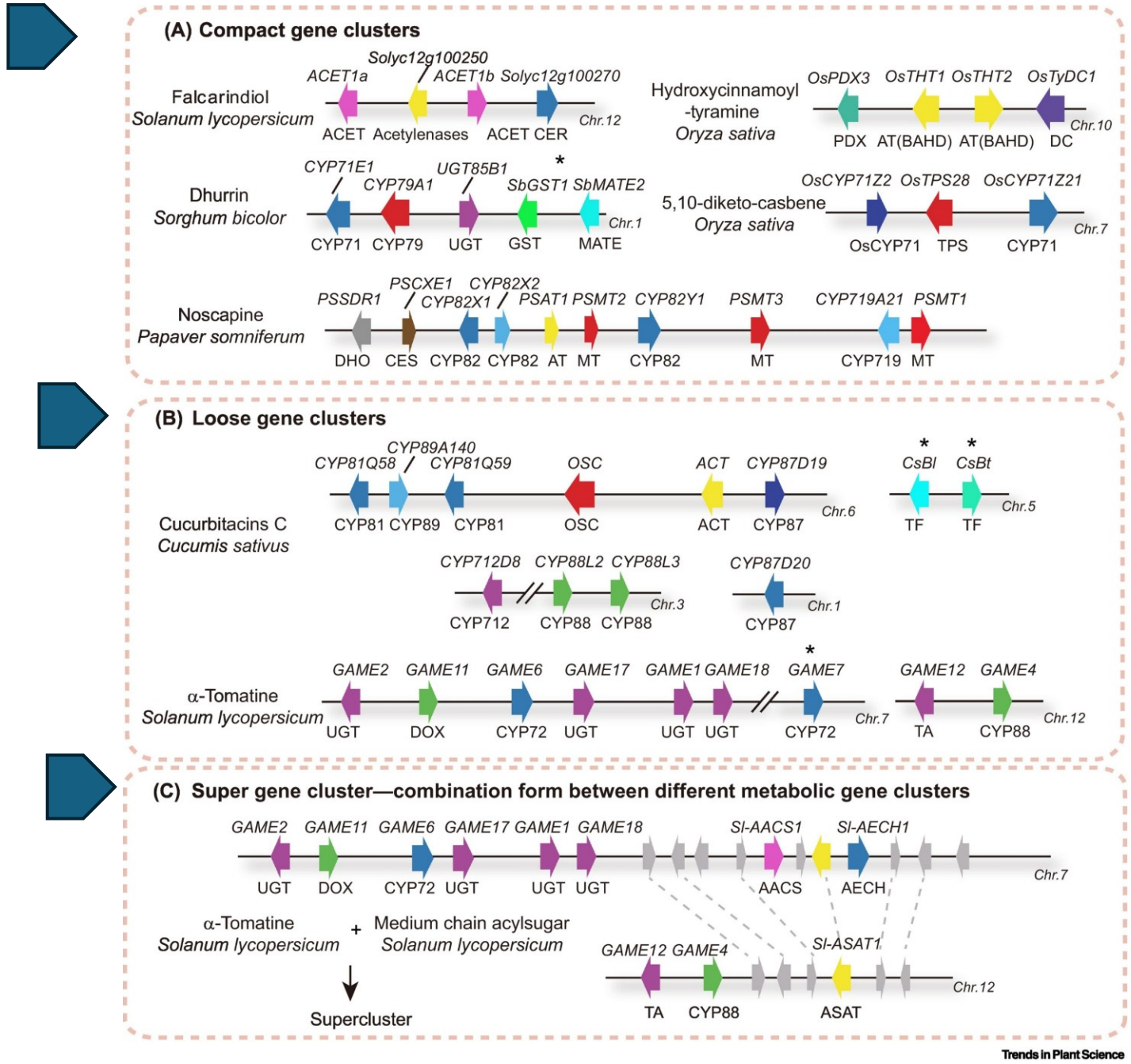


Metabolic clusters

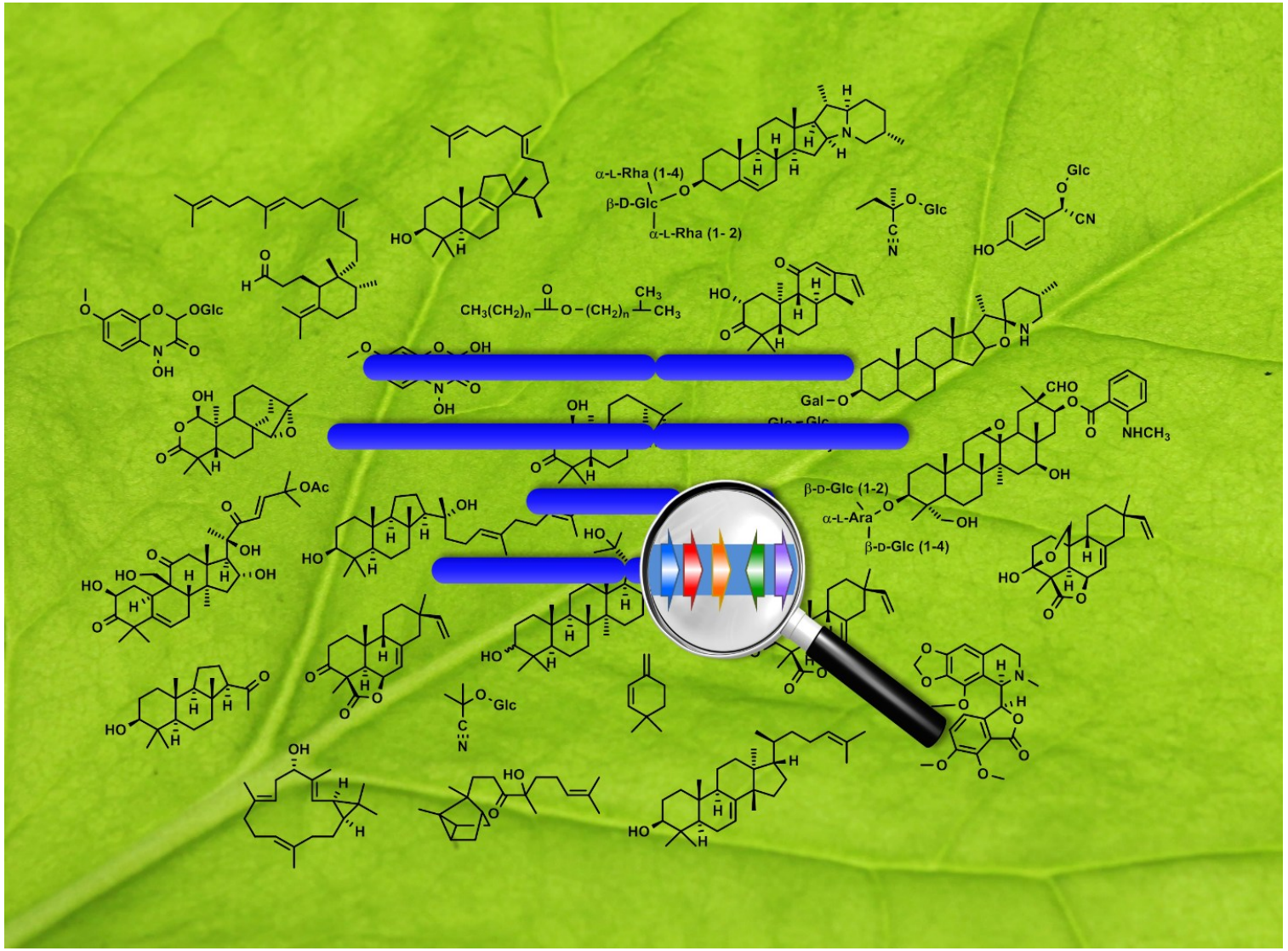
- For different classes
- In many plants



Nützmann & Osbourn,2014


(A) Compact gene clusters
Falcarindiol
Solanum lycopersicum
ACET1a
Solyc12g100250
ACET1b
Solyc12g100270
ACET
Acetylenases
ACET
CER
Chr.12
Hydroxycinnamoyl
-tyramine
Oryza sativa
OsPDX3
OsTHT1
OsTHT2
OsTyDC1
PDX
AT(BAHD)
AT(BAHD)
DC
Chr.10
Dhurrin
Sorghum bicolor
CYP71E1
CYP79A1
UGT85B1
SbGST1
SbMATE2
CYP71
CYP79
UGT
GST
MATE
Chr.1
5,10-diketo-casbene
Oryza sativa
OsCYP71Z2
OsTPS28
OsCYP71Z21
OsCYP71
TPS
CYP71
Chr.7
Noscapine
Papaver somniferum
PSSDR1
PSCXE1
CYP82X1
CYP82X2
PSAT1
PSMT2
CYP82Y1
PSMT3
CYP719A21
PSMT1
DHO
CES
CYP82
CYP82
AT
MT
CYP82
MT
CYP719
MT
(B) Loose gene clusters
Cucurbitacins C
Cucumis sativus
CYP89A140
CYP81Q58
CYP81Q59
OSC
ACT
CYP87D19
CsBI
CsBt
CYP81
CYP89
CYP81
OSC
ACT
CYP87
TF
TF
Chr.6
Chr.5
CYP712D8
CYP88L2
CYP88L3
CYP712
CYP88
CYP88
Chr.3
CYP87D20
CYP87
Chr.1
α-Tomatine
Solanum lycopersicum
GAME2
GAME11
GAME6
GAME17
GAME1
GAME18
GAME7
GAME12
GAME4
UGT
DOX
CYP72
UGT
UGT
UGT
CYP72
TA
CYP88
Chr.7
Chr.12
(C) Super gene cluster—combination form between different metabolic gene clusters
GAME2
GAME11
GAME6
GAME17
GAME1
GAME18
Sl-AACS1
Sl-AECH1
UGT
DOX
CYP72
UGT
UGT
UGT
AACS
AECH
Chr.7
α-Tomatine
Solanum lycopersicum
+
Medium chain acylsugar
Solanum lycopersicum
Supercluster
GAME12
GAME4
Sl-ASAT1
TA
CYP88
ASAT
Chr.12
Trends in Plant Science

Rapid identification of pathway genes in plant genomes using new computational methods



secondary metabolites have multiple multiple functions that incorporate defense but also growth, development and regulation

Plants have both a conserved and a unique, variable, and flexible repertoire of regulators –

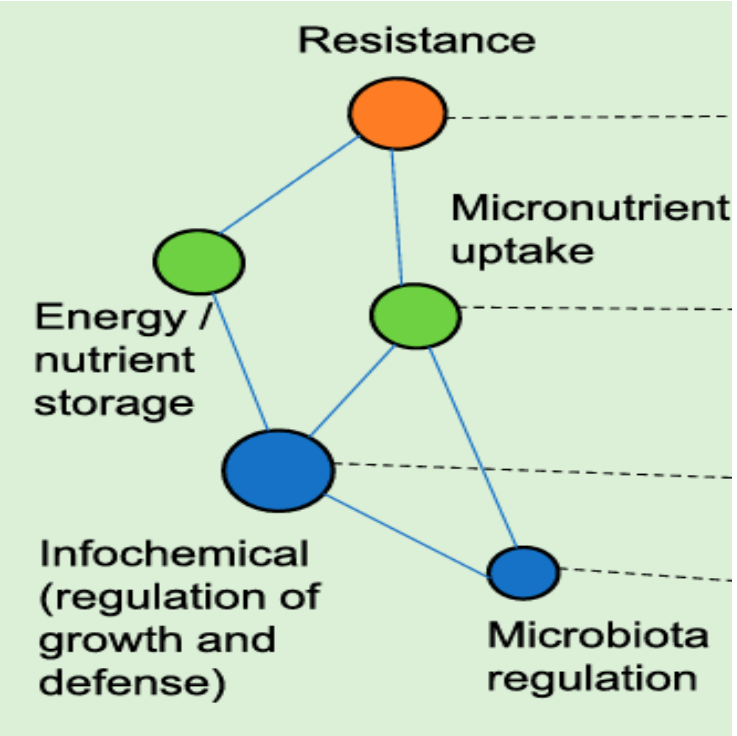
which likely contributes to their potential to colonize variable and challenging habitats

SM affect basic developmental processes in plants

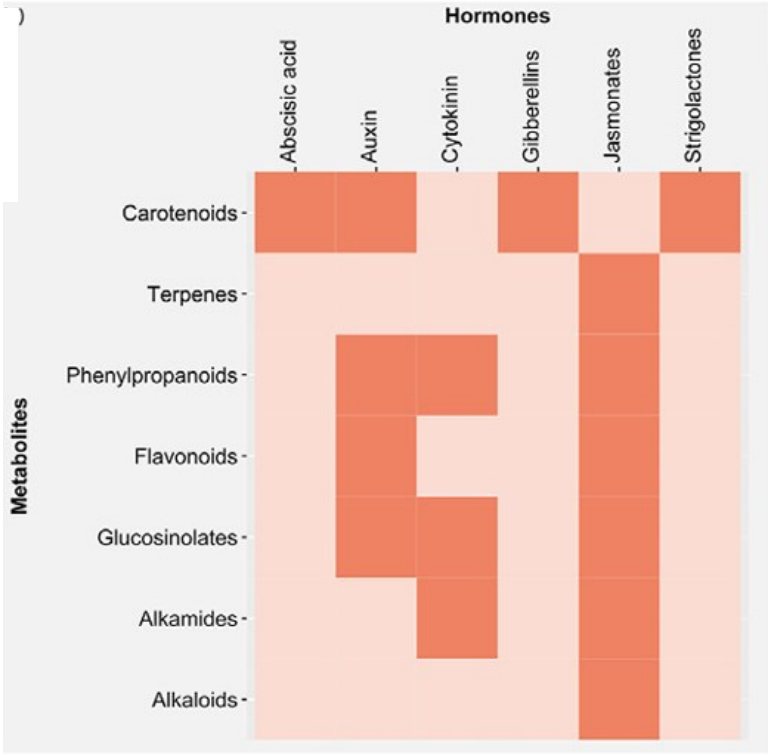
Revisiting the functional partitioning of primary/secondary metabolites and hormones

SM are not end points, but integrated components of **metabolic networks that are dynamically shaped by environmental selection and internal cues**

highly connected plant metabolic networks



Interaction of specialized metabolites with hormonal pathways



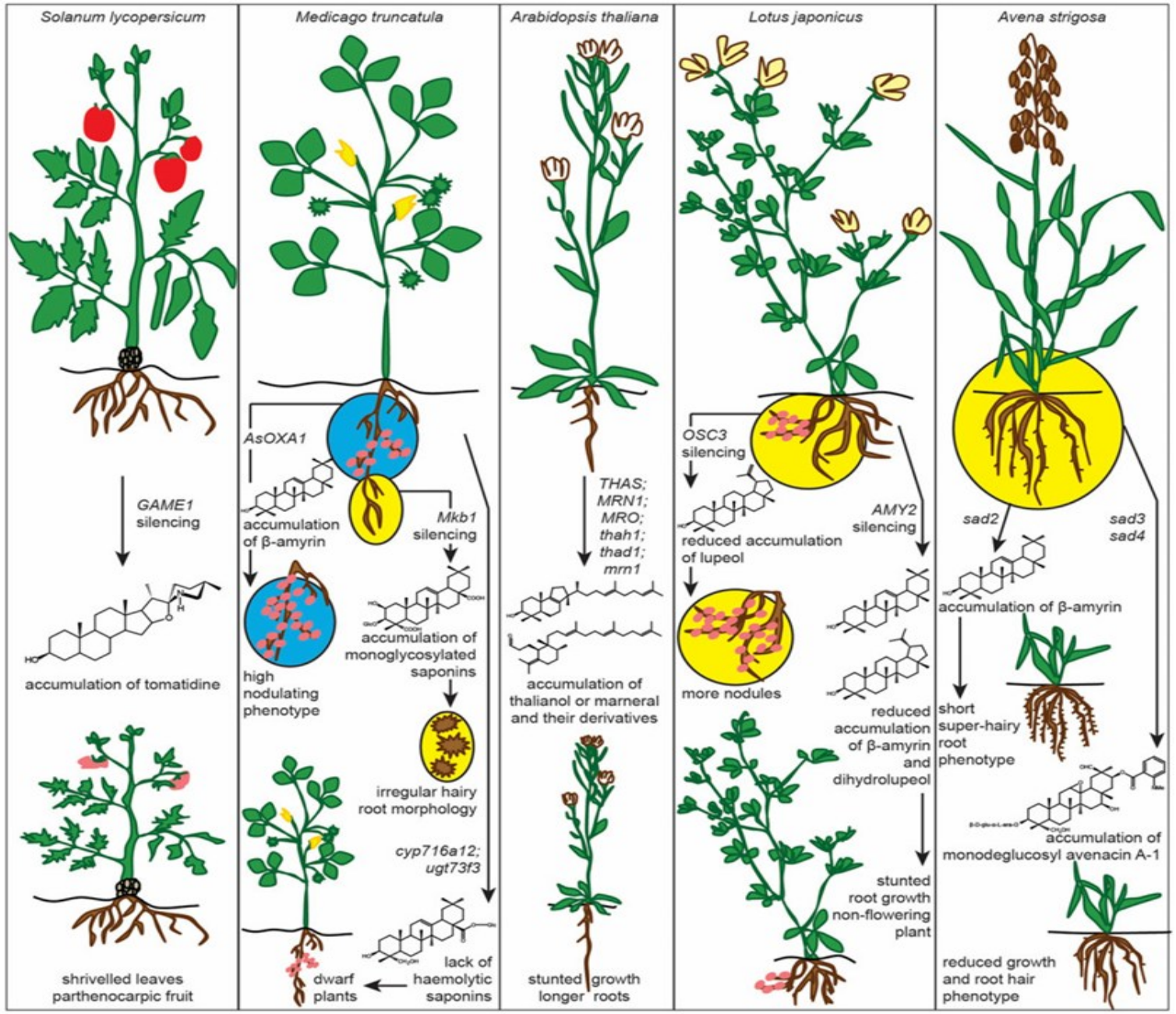
SM can function as potent regulators of plant growth and defense as well as primary metabolites *sensu lato*

Durán-Medina et al, 2021

Some examples

- Kaempferol: is integrated into ubiquinone
- Flavonoids: modulate lateral root development through auxin transport alteration
- Zaxinone(apocarotenoid): triggers the biosynthesisof strigolactones and abscisic acid
- b-cyclocitral (apocarotenoid): Cell divisions in root meristems and lateral root branching
- b-amyrin (triterpene): involved in root hair development
- Cis-cinnamic acid(phenylpropanoid): inhibits auxin efflux
- Glucosinolates: change flowering time dependent on the genetic background and Inflorescence architecture and fecundity (balance of hormonal regulators cytokinins and jasmonates)
- **3-hydroxypropylglucosinolate (3OHPGSL): functions via genes in the TOR pathway**

Plant growth and development effects resulting from altered *in planta* saponin biosynthesis

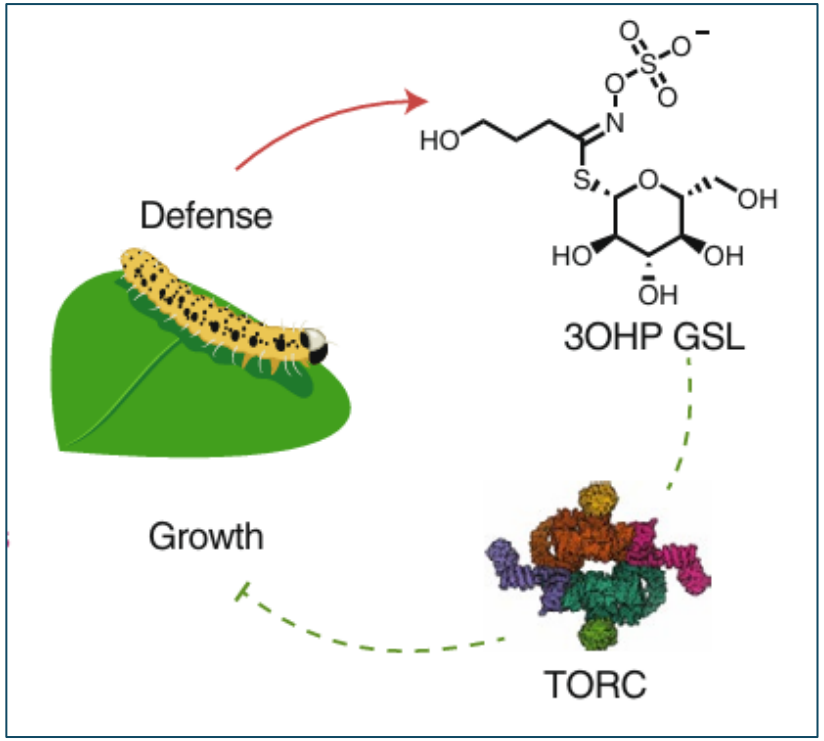


Moses et al, 2014

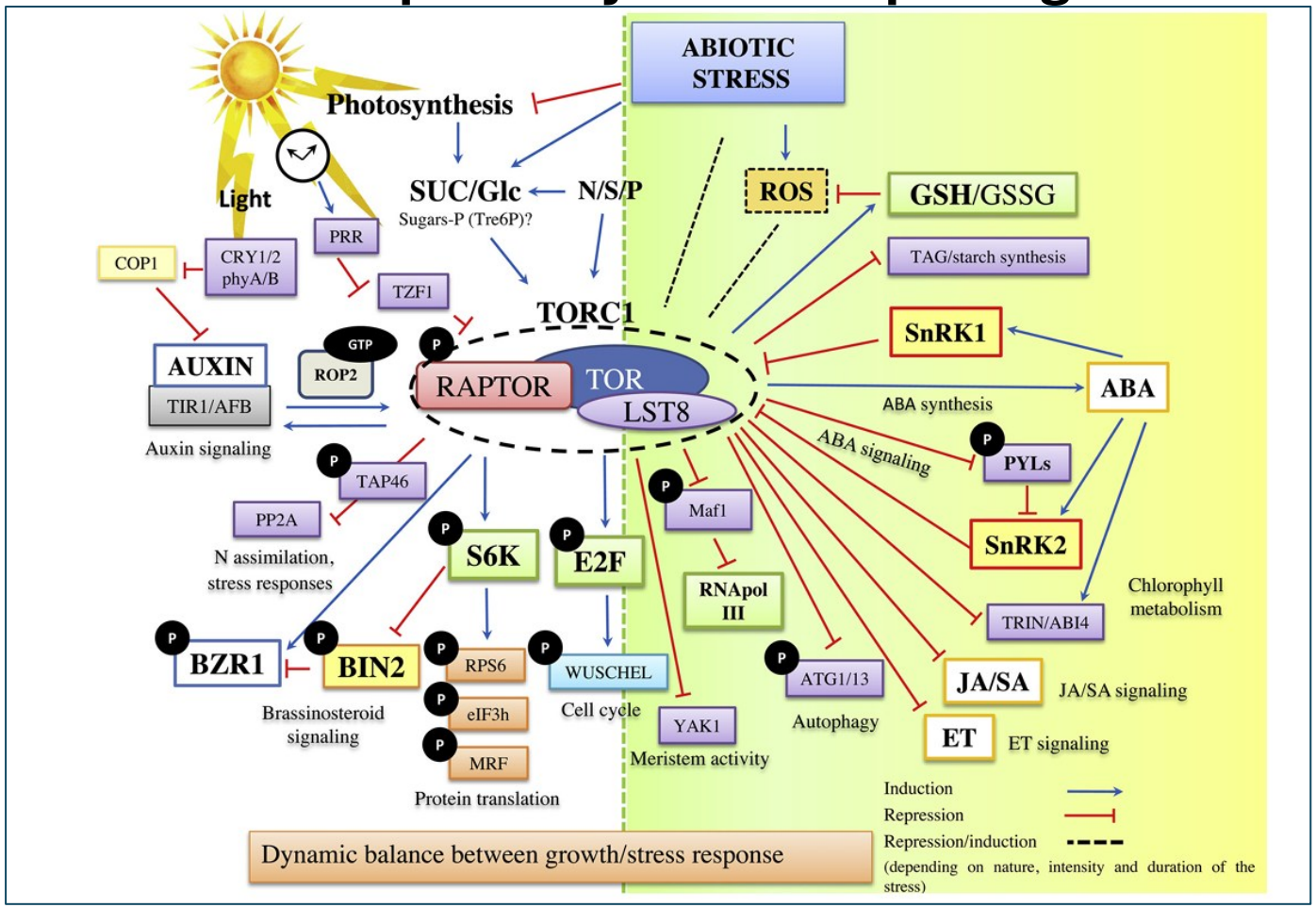
Molecular mode of action??

“young” plant metabolites can influence evolutionarily conserved pathways

3OHPGSL functions via genes in the TOR pathway



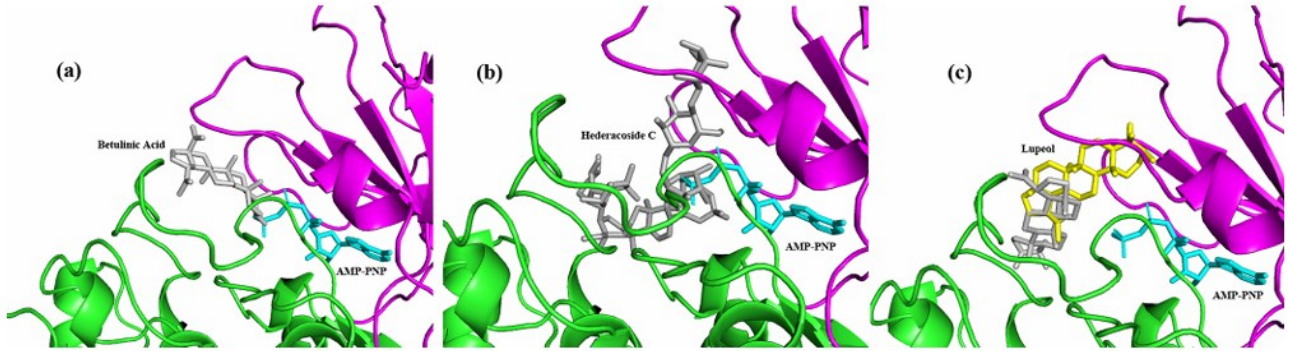
The TOR pathway controls plant growth



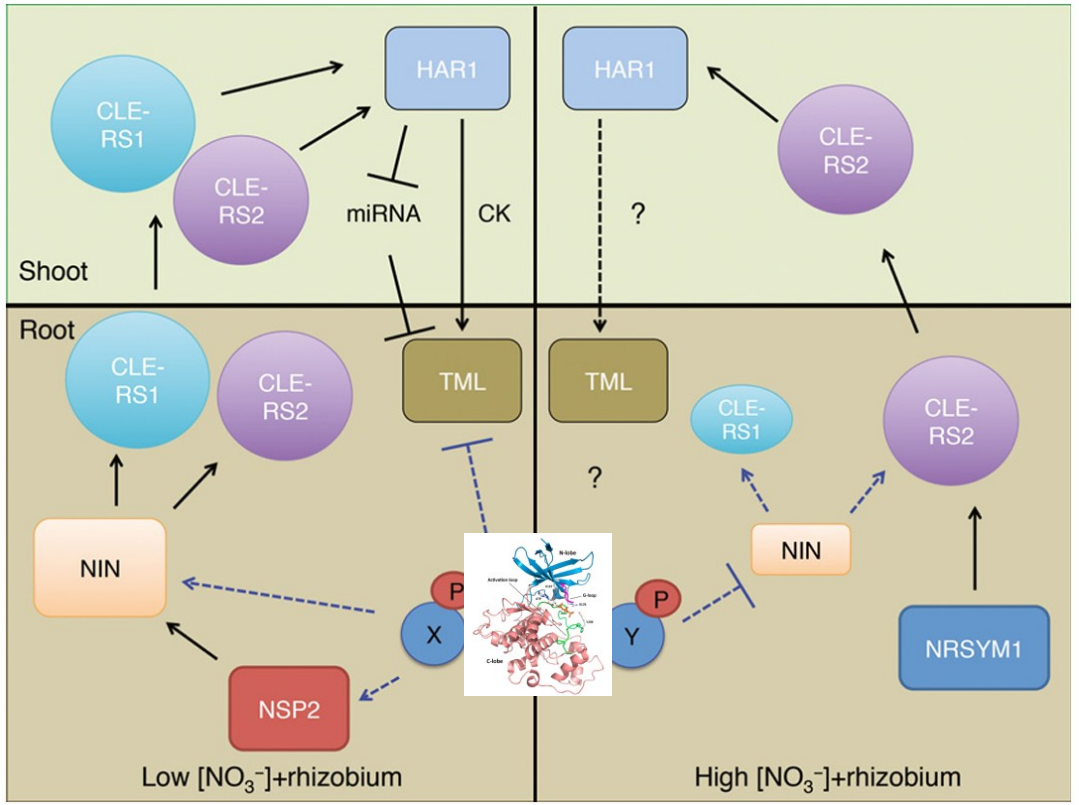
Triterpenes affect the developmental process of nodulation

LSK1 integrates the nitrate-induced suppression of nodulation with rhizobium-induced AON signaling.

Lupeol binds and inhibits to the LsK1 (GSK3β-like)



betulinic acid hederacoside C **lupeol**

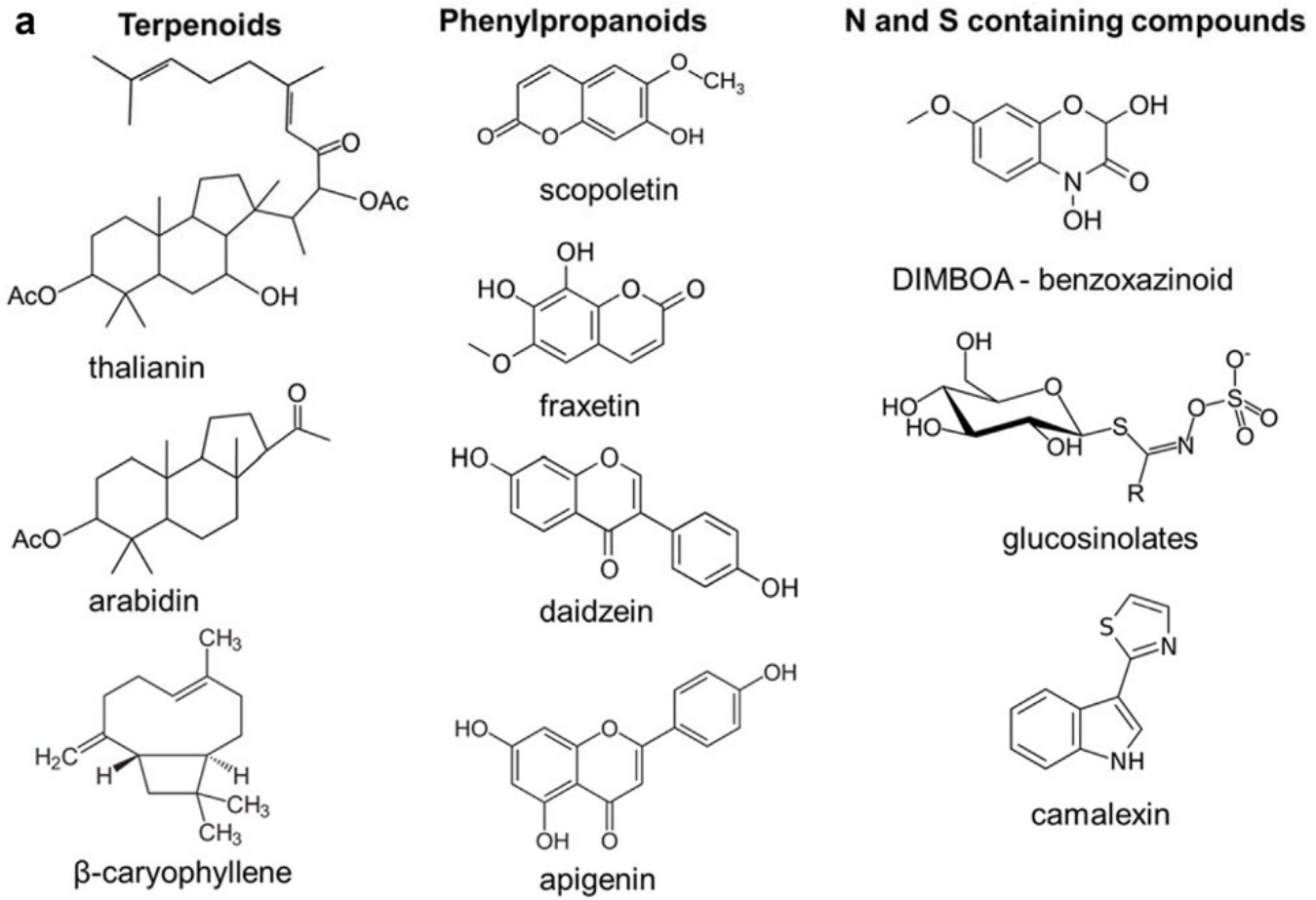


*autoregulation of nodulation (AON) pathway

Garagounis et al, 2019, Solovou et al, 2024

secondary metabolites shape the composition and function of the plant microbiome

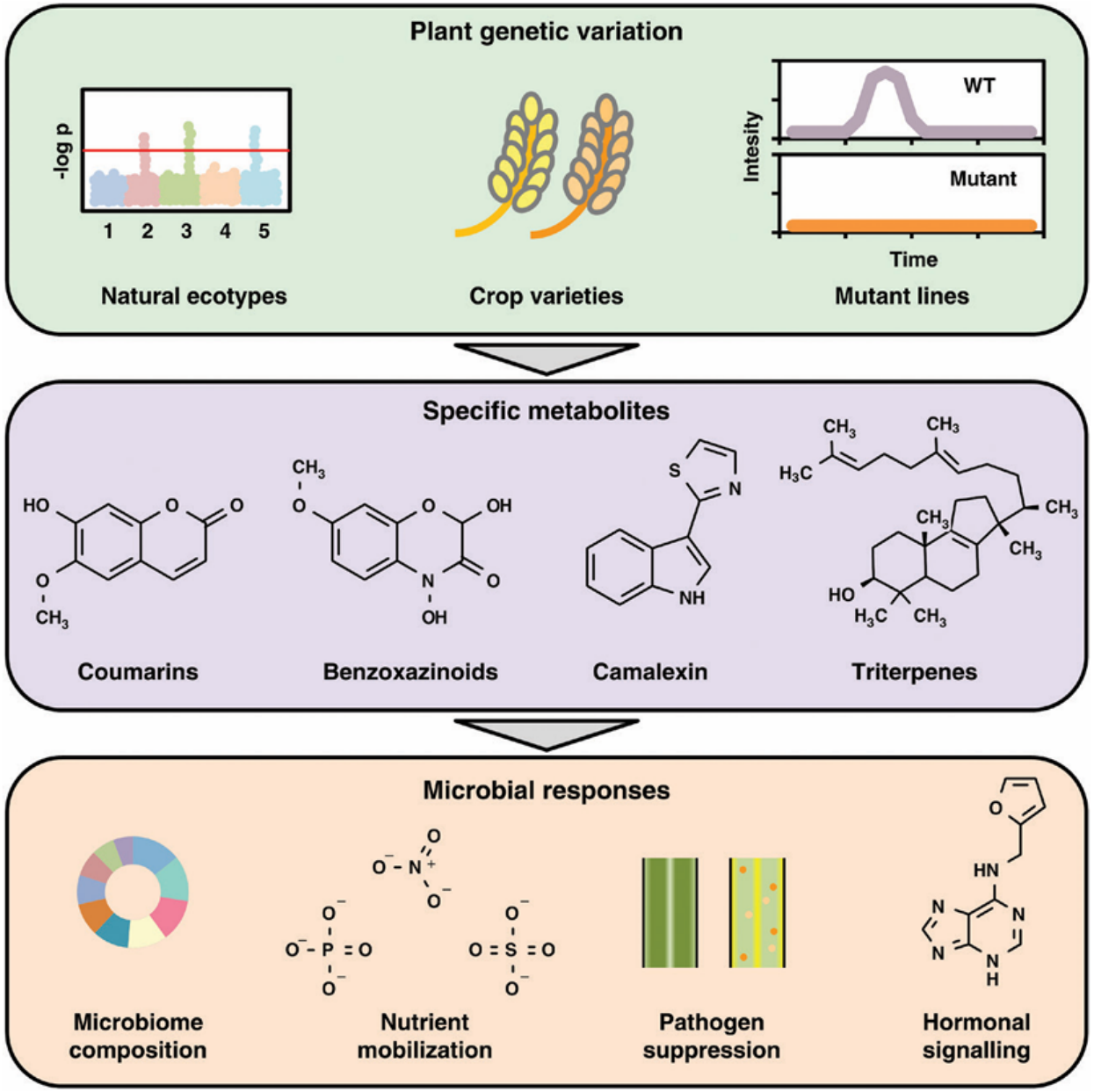
Root exudates shape root microbiome composition



dissected through analyses of root microbiome composition of natural populations or mutants unable to synthesize the compounds and in many cases verified by chemical complementation

Koprivova and Kopriva, 2022

SM: a mechanistic link between plant genetics and microbiome functions

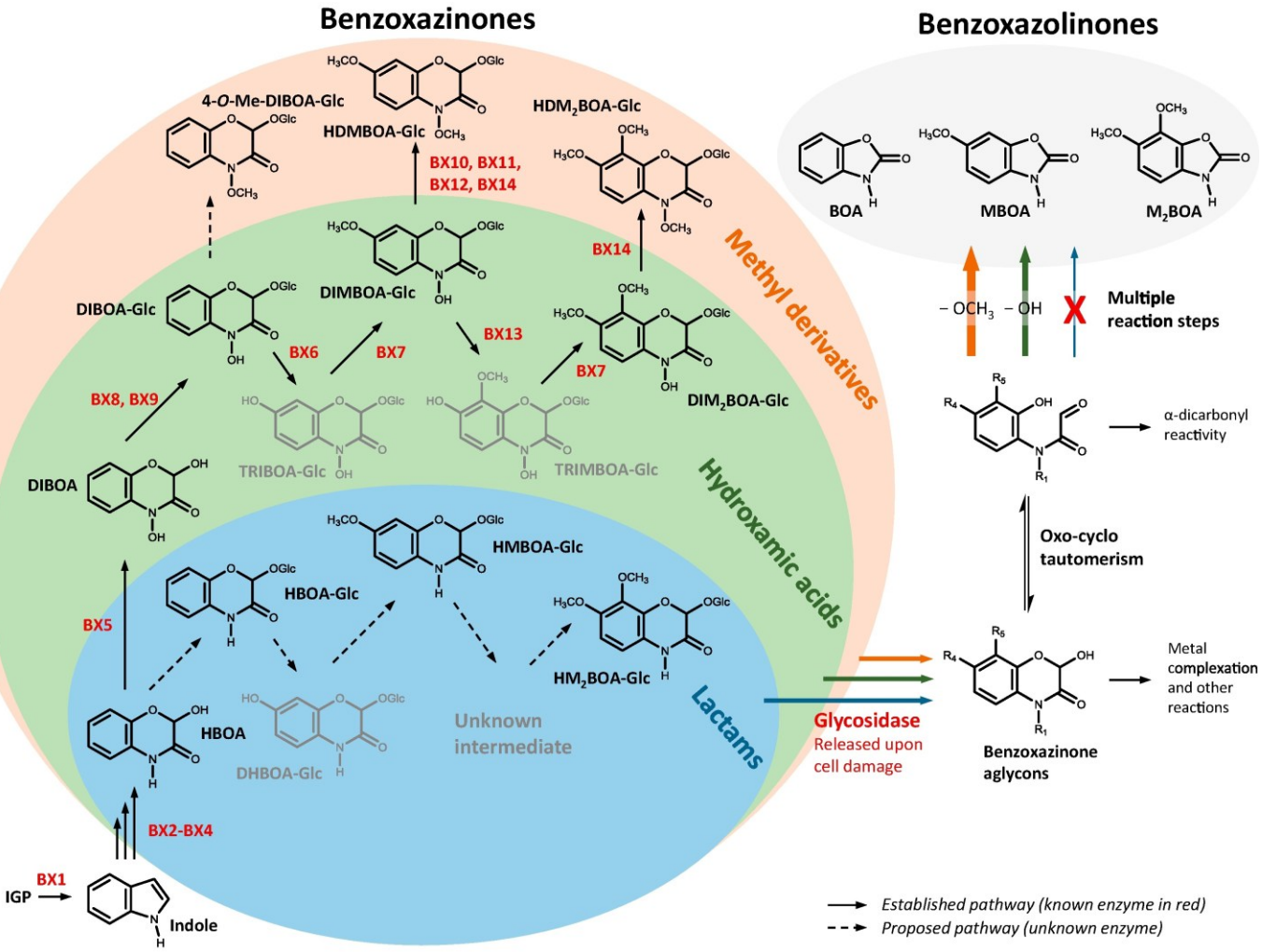


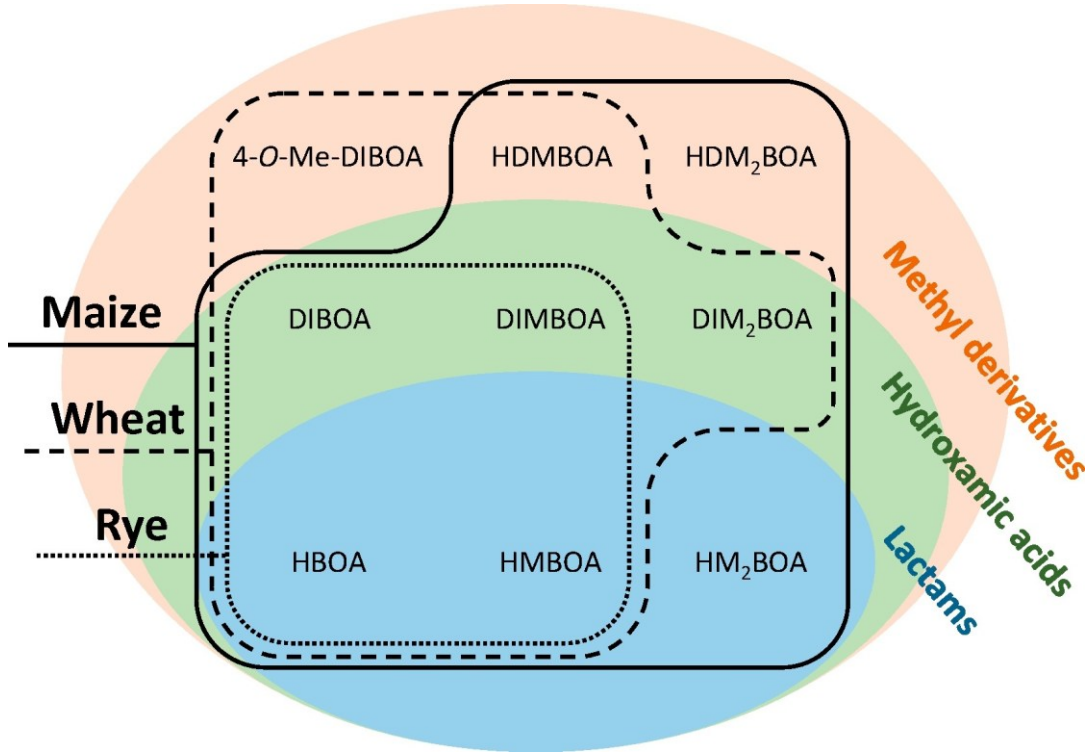
Jacoby et al, 2020

e.g. MBOA was responsible for the selection of a resistance-inducing soil microbiome

Plant–soil feedback

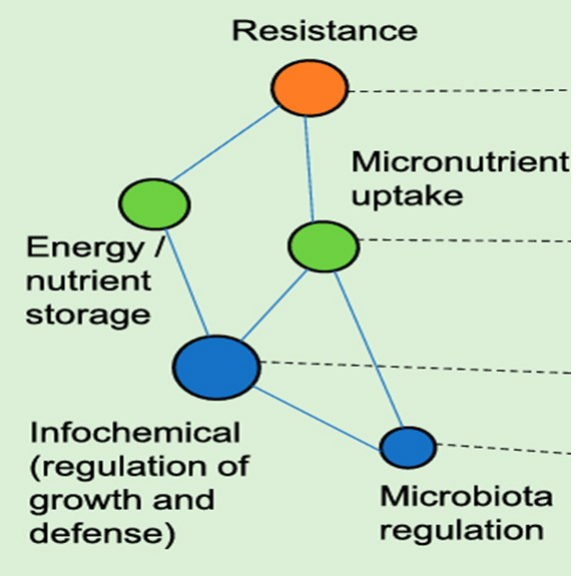
Soil legacy

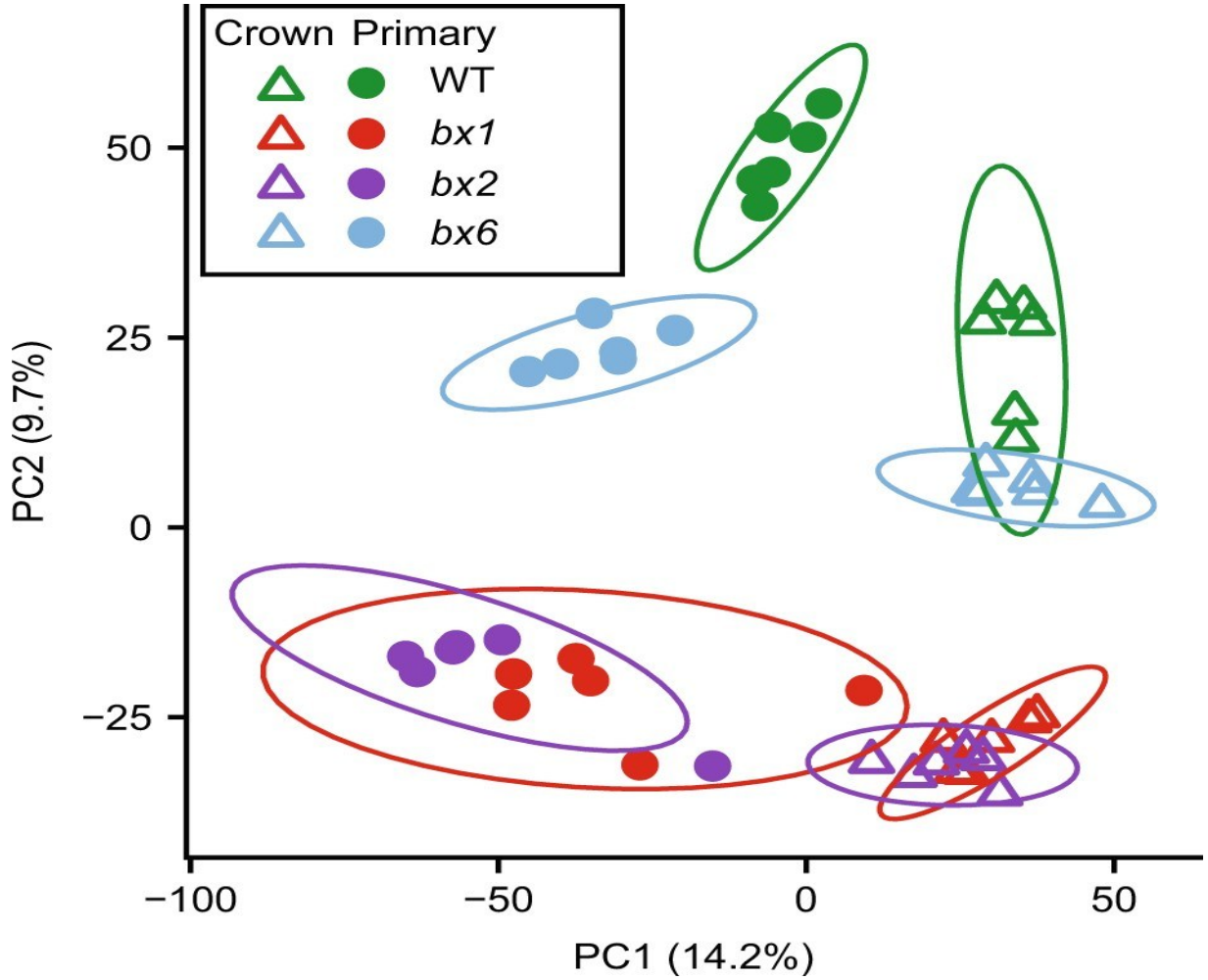




Hu et al, Nature Communications, 2018

Metabolite profile in primary and crown root of of wild-type (WT) and *bx* mutants in maize

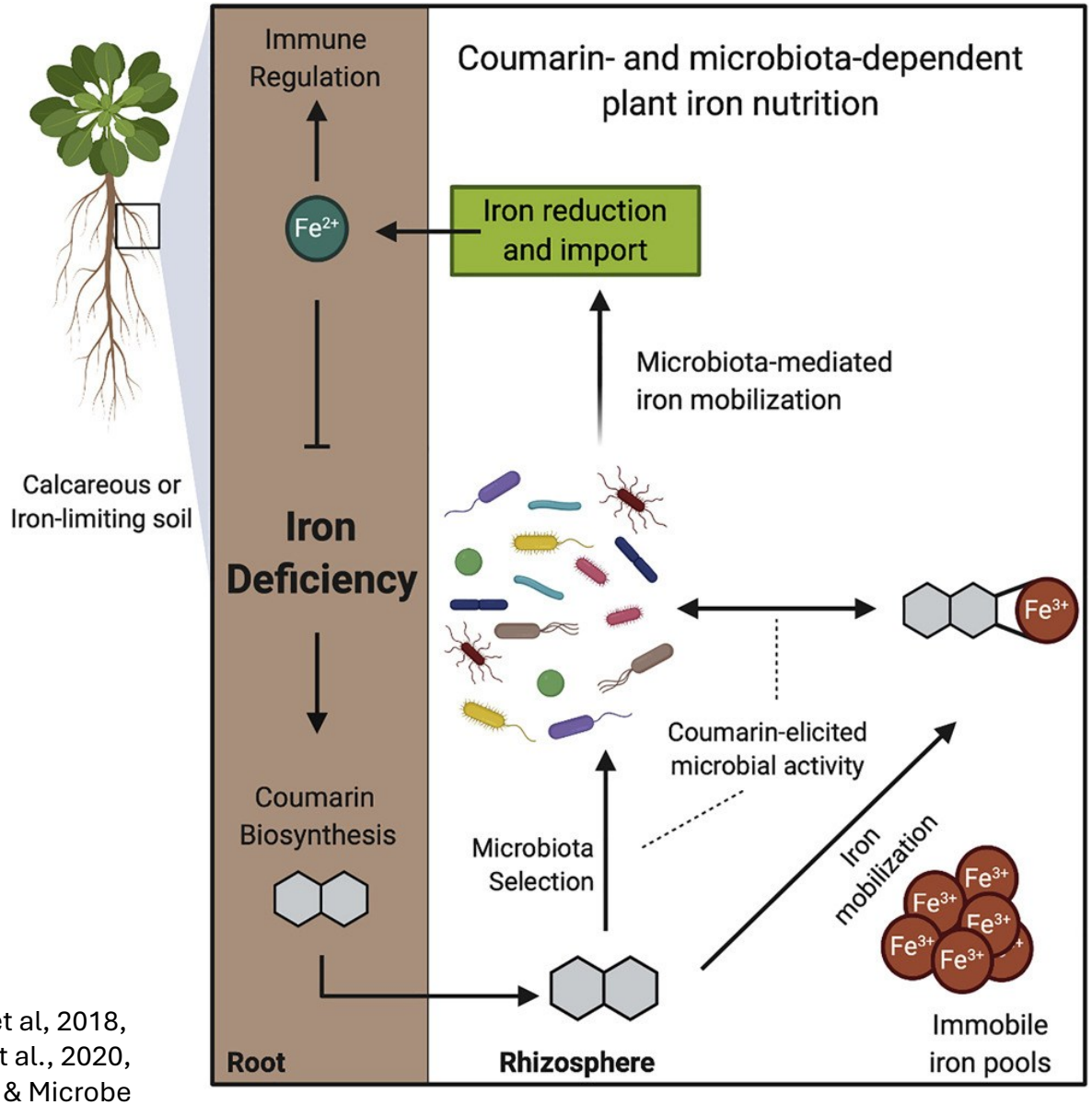




ISME J, Volume 13, Issue 7, July 2019, Pages 1647–1658, https://doi.org/10.1038/s41396-019-0375-2

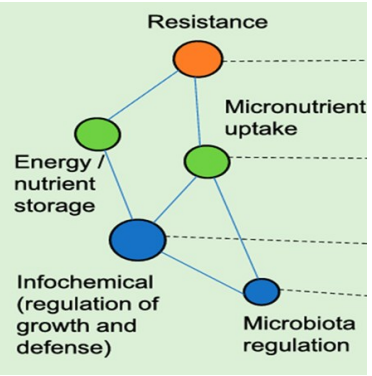
OXFORD UNIVERSITY PRESS

e.g. Coumarin changes in microbiome functions



Stringlis et al, 2018,
Harbort et al., 2020,
Cell Host & Microbe

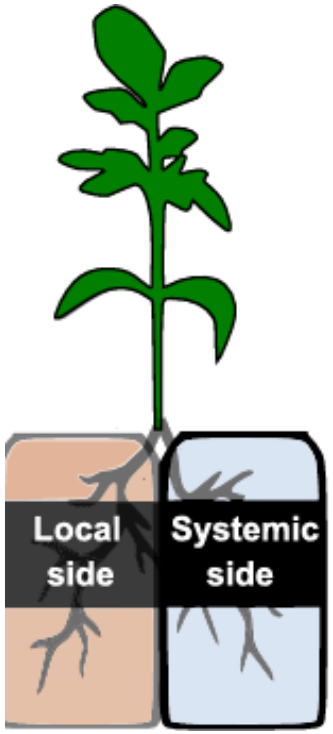
connected to plant mineral nutrition and defence

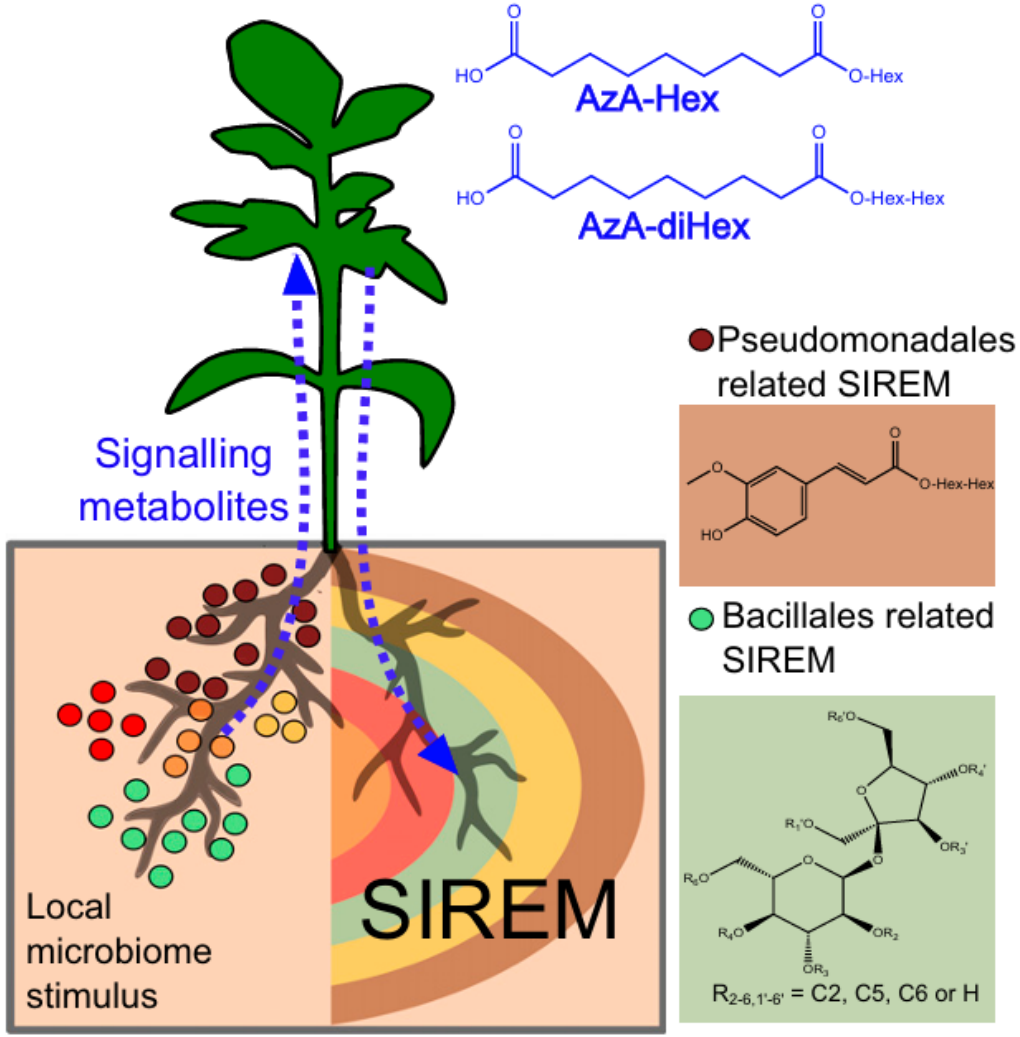


under iron starvation
Plants induce exudation of coumarins that in turn modulate the composition of the microbiome to facilitate iron uptake

microbiota also affect the composition of root exudates

systemically induced root exudation of metabolites (SIREM)





Pseudomonadales related SIREM

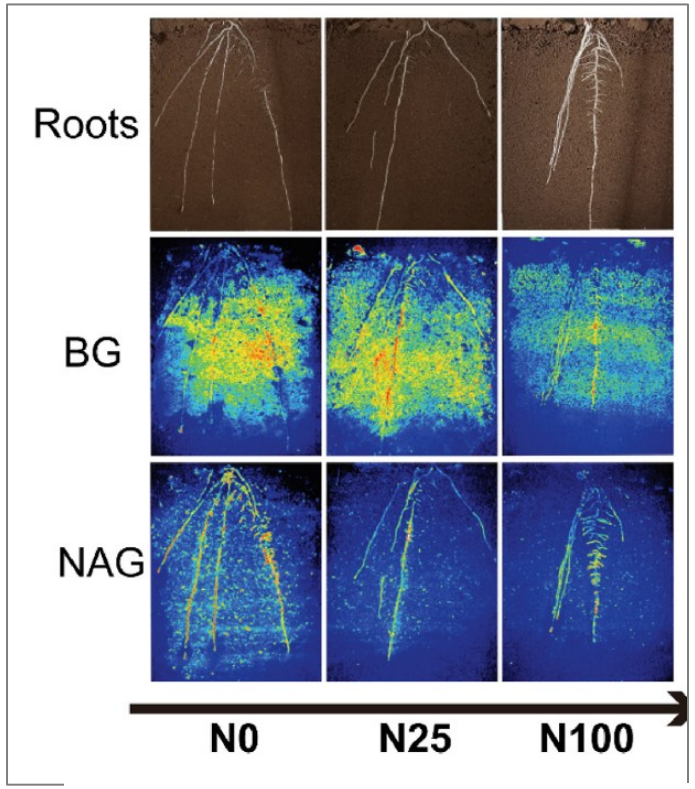
ferulic acid hexose

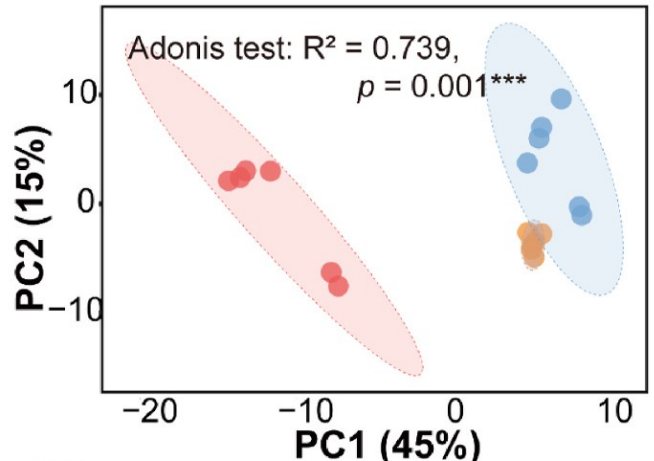
Bacillales related SIREM

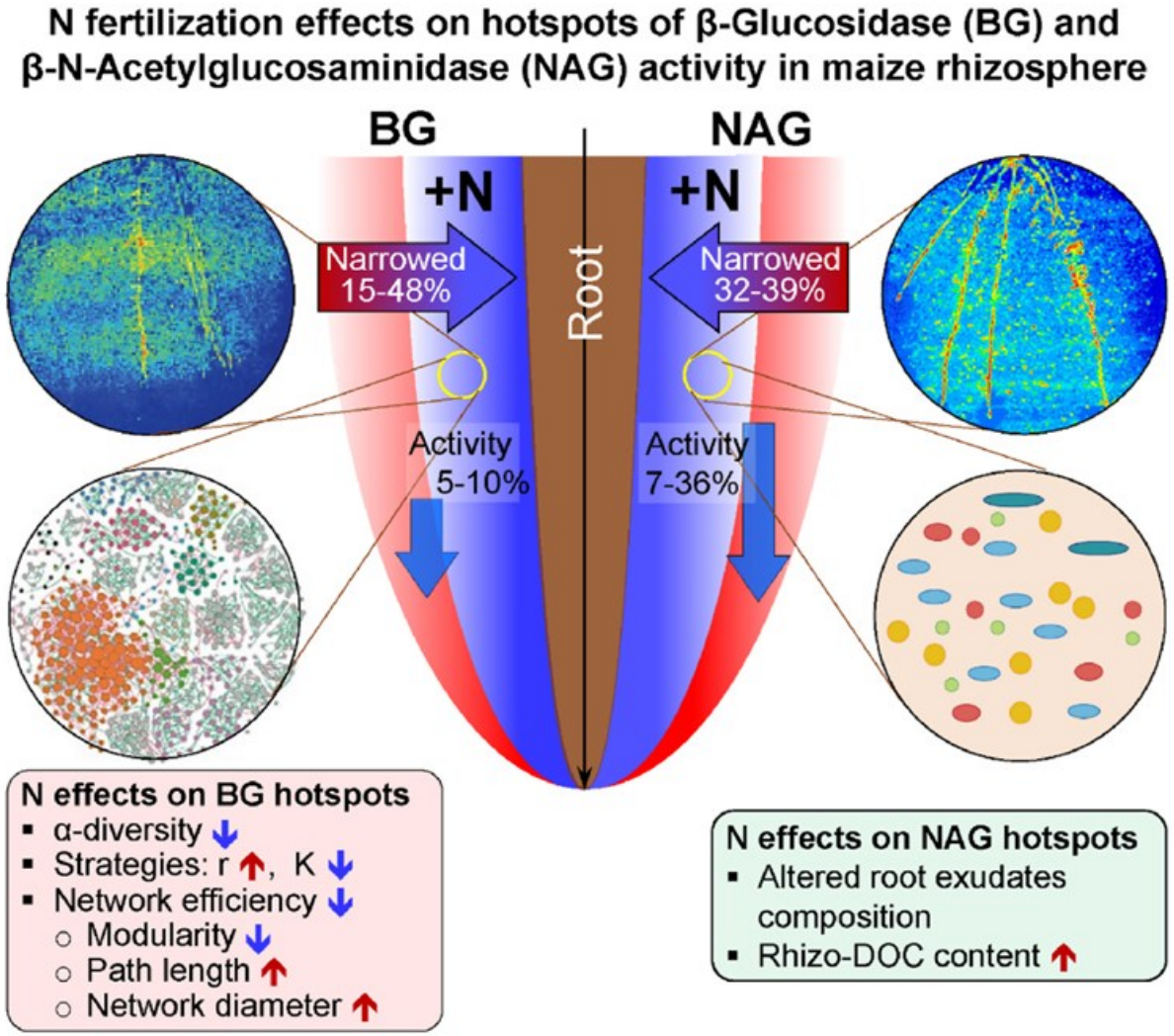
acyl sucrose S4:19

Korenblum. Proc Natl Acad Sci U S A 2020,

Plants “listen” and respond: active modification of root exudates adjust the rhizosphere to the supply of N- metabolic editing







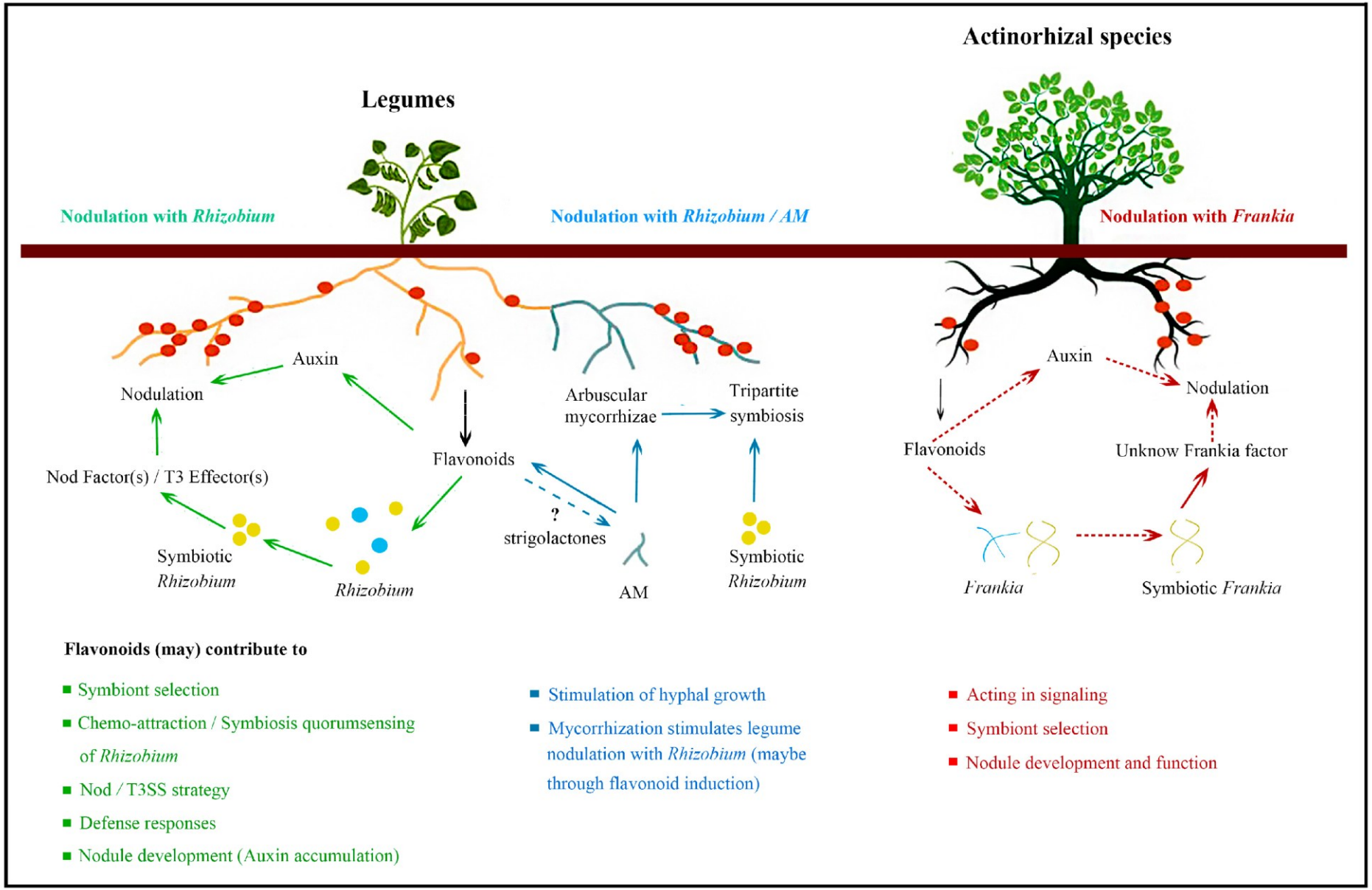
Hao et al., 2022

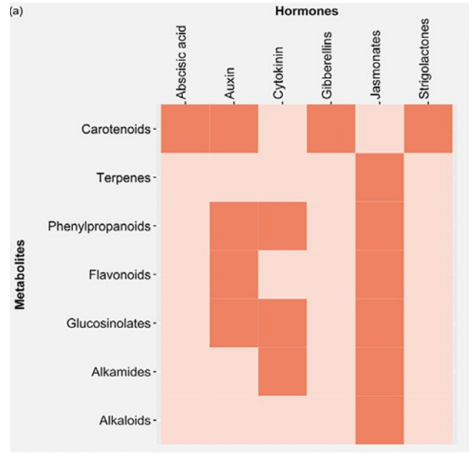
(More) OPEN QUESTIONS about (Secreted) Specialized Metabolites

- Do they act directly or after modification *in situ*?
- What underlies the specificity of the metabolites, for example, between closely related triterpenes or coumarins?
- How do they increase or inhibit growth of the microbes?
- How is the function of the metabolites affected by soil edaphic factors?

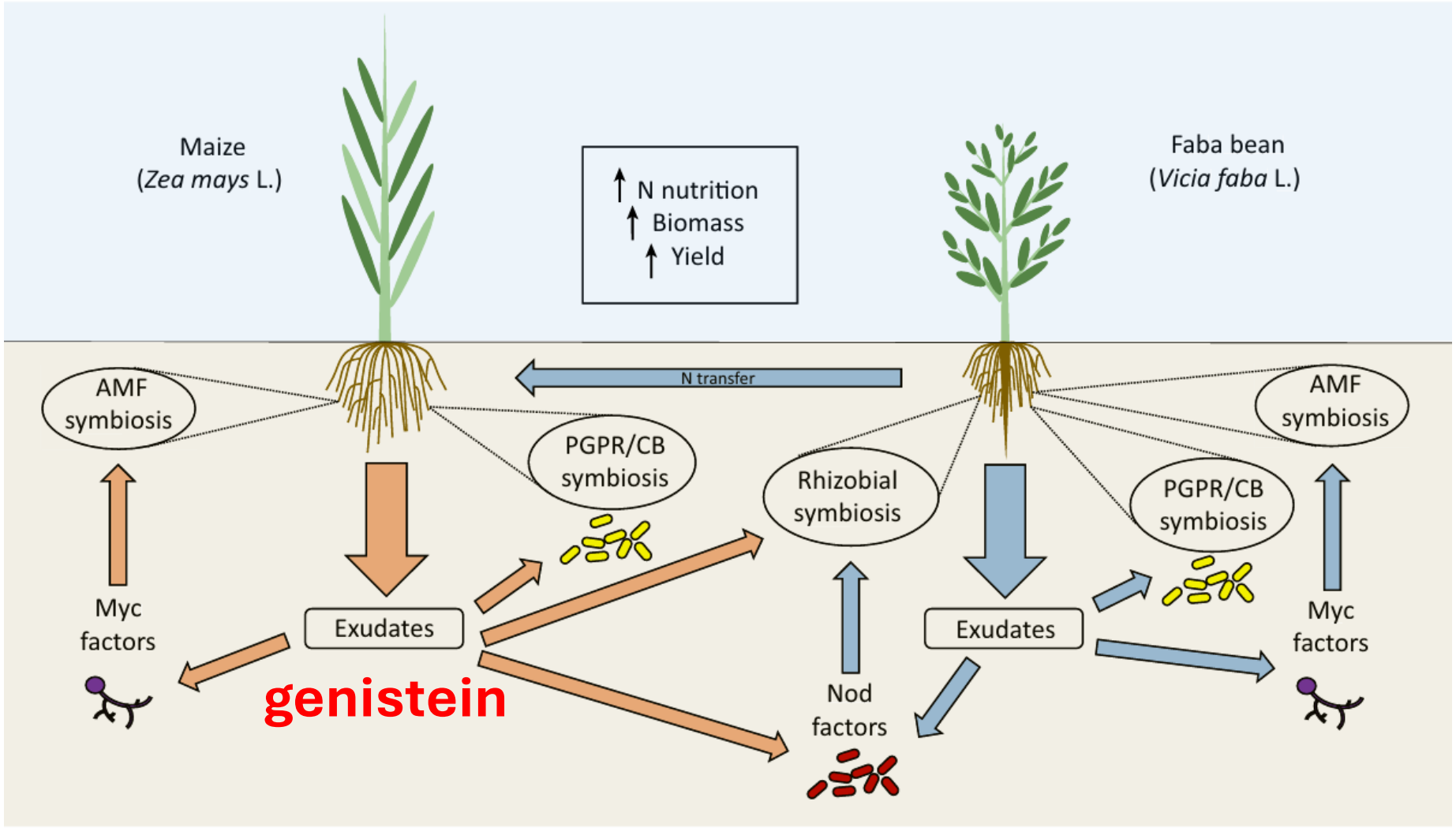
Plant Root Exudates Shape the Nitrogen Cycle

Flavonoids and strigolactones mediate beneficial interactions in the root

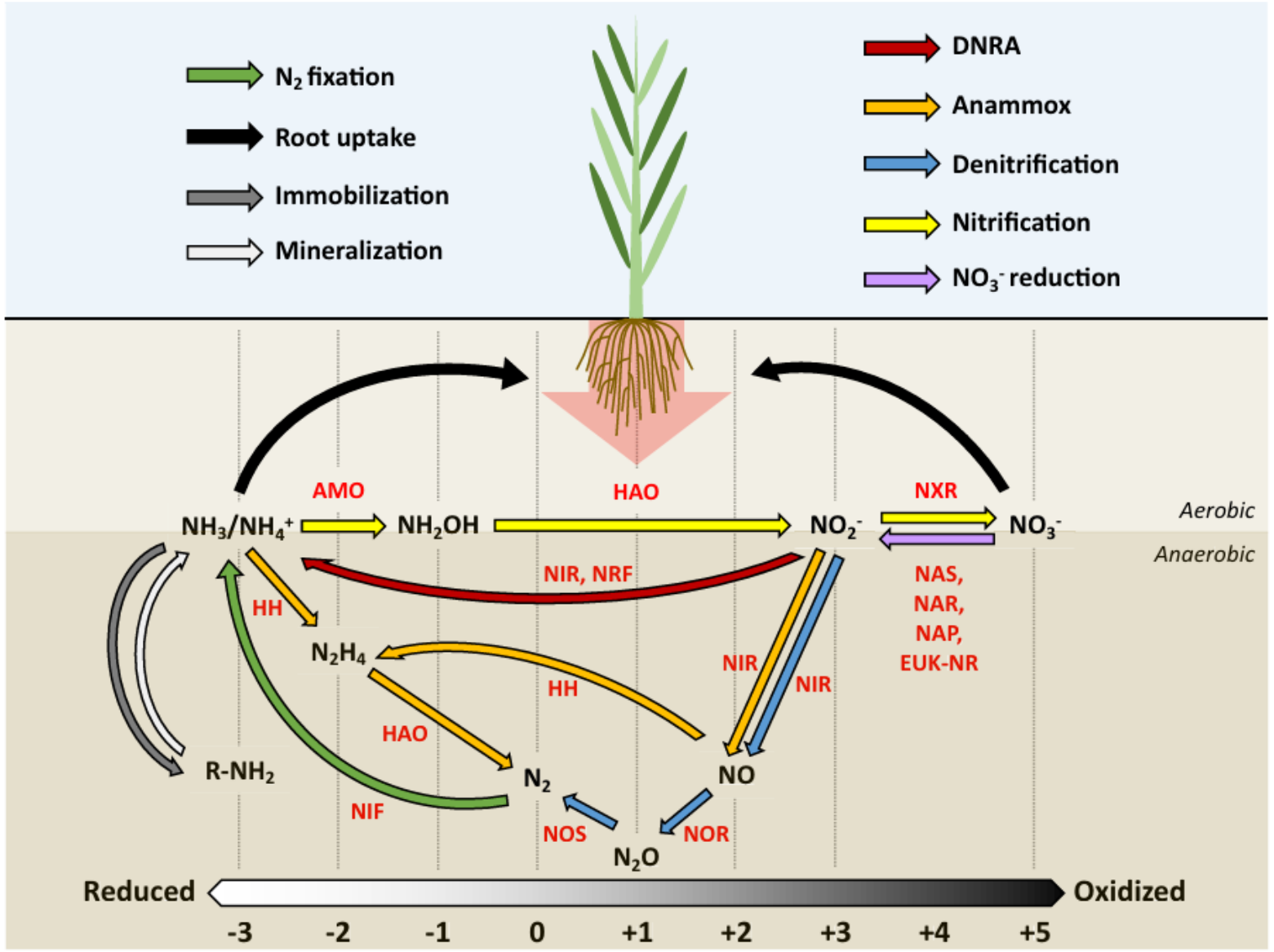




Metabolite cross-talk at a plant community level takes place



Root exudates affect nitrogen cycle in soil

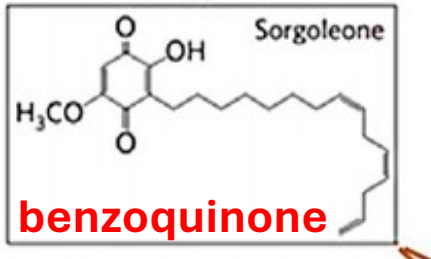


All identified BNIs today are structural diverse AND have multiple functions in plant physiology

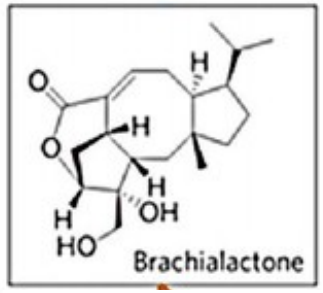
Sorghum
(*Sorghum bicolor*)

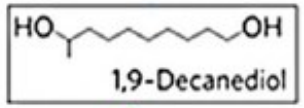
Signalgrass
(*Brachiaria humidicola*)

Rice
(*Oryza sativa*)



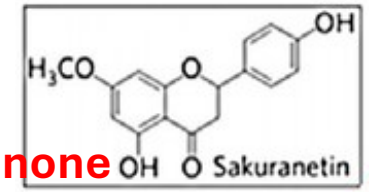
benzoquinone



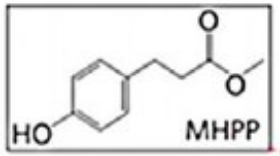


Cyclic diterpene

?



flavanone

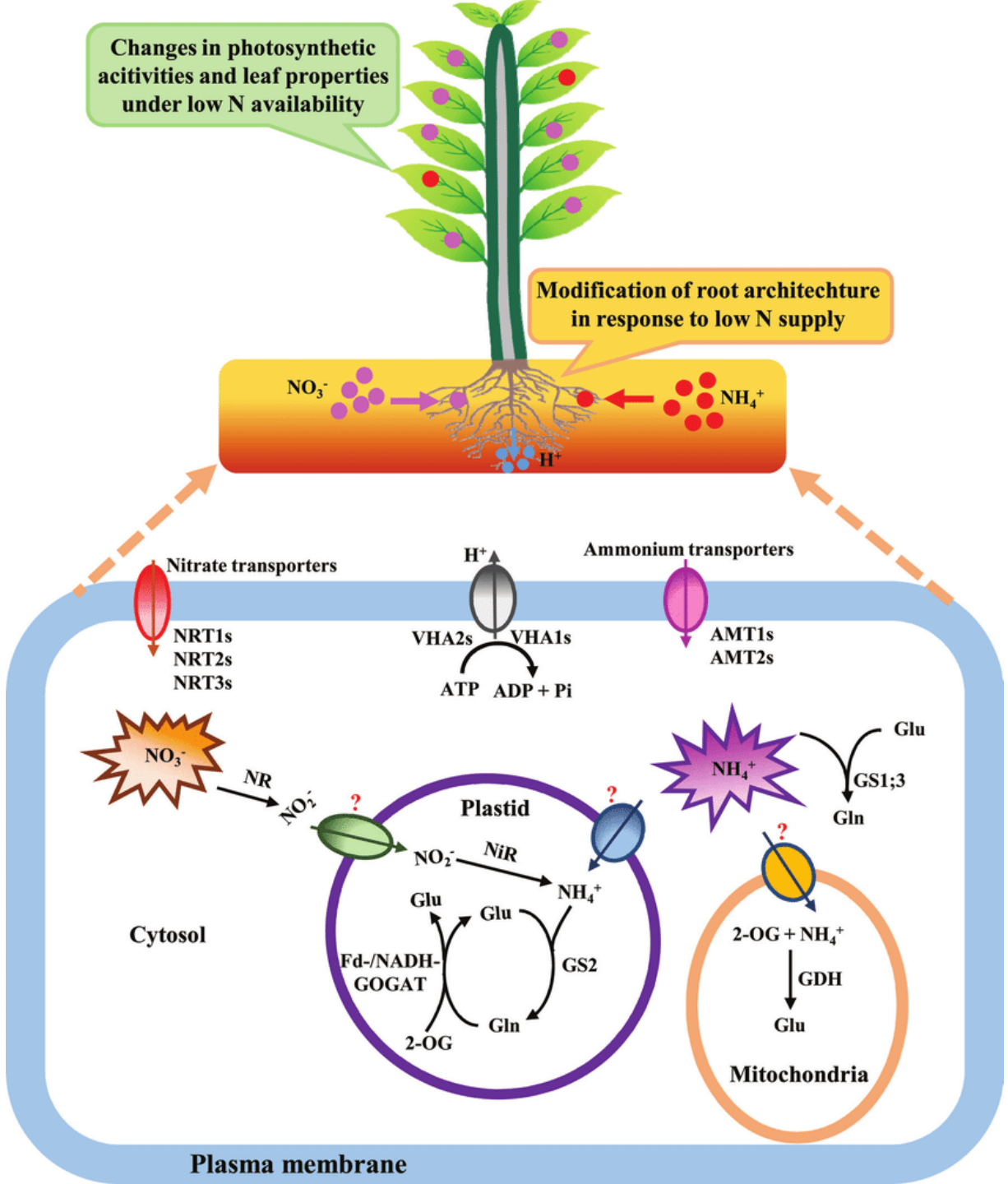


phenylpropanoid

AMO HAO ? NXR

$NH_3/NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^-$

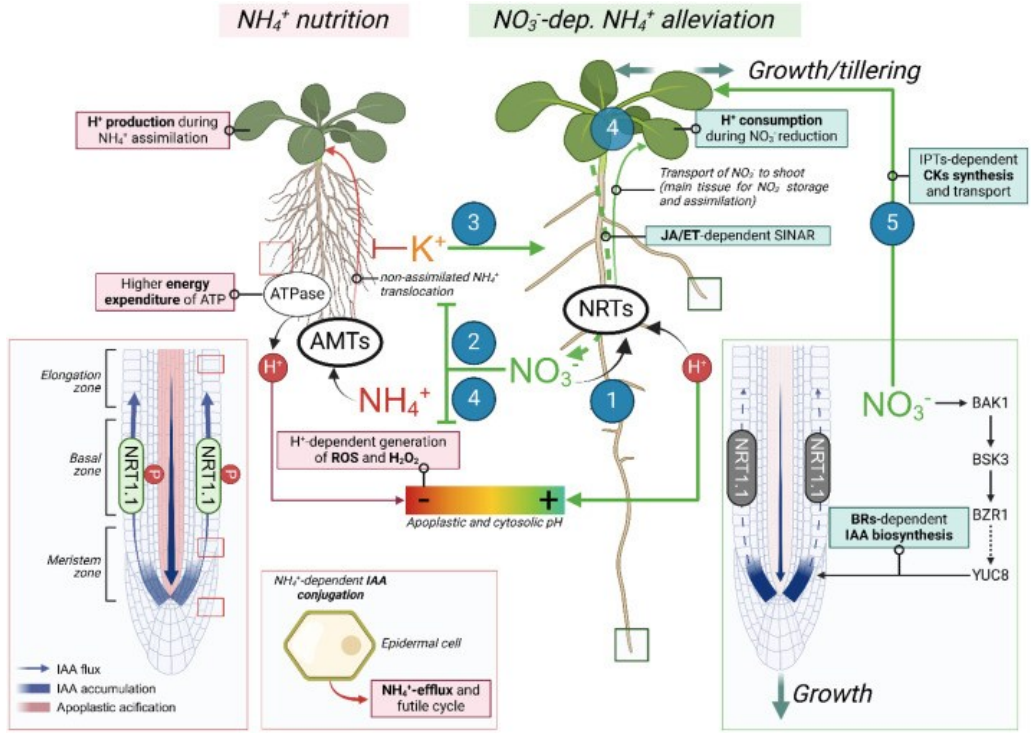
N uptake and metabolism in plants

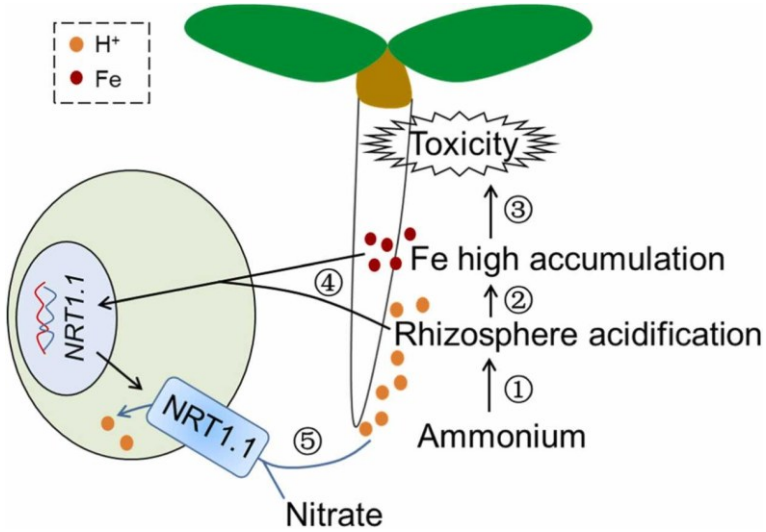


Luo et al, 2013, JExB

N uptake and metabolism in plants intersects with other signalling pathways

- NO_3 acts as a **signaling molecule**
- NO_3^- is linked to P –starvation signalling
- NO_3^- is linked to Fe depletion
- NO_3^-alleviates the toxicity derived from ammonium nutrition





- Ammonium is known to affect rapidly the internal and external pH of roots
- NH4 is linked to Fe toxicity

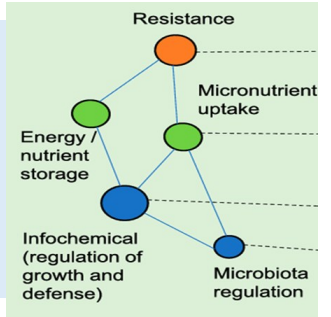
Strategies to further study metabolites, mechanisms and interactions

Strategies to rationally identify novel plant exudate molecules functioning as BNIs ?

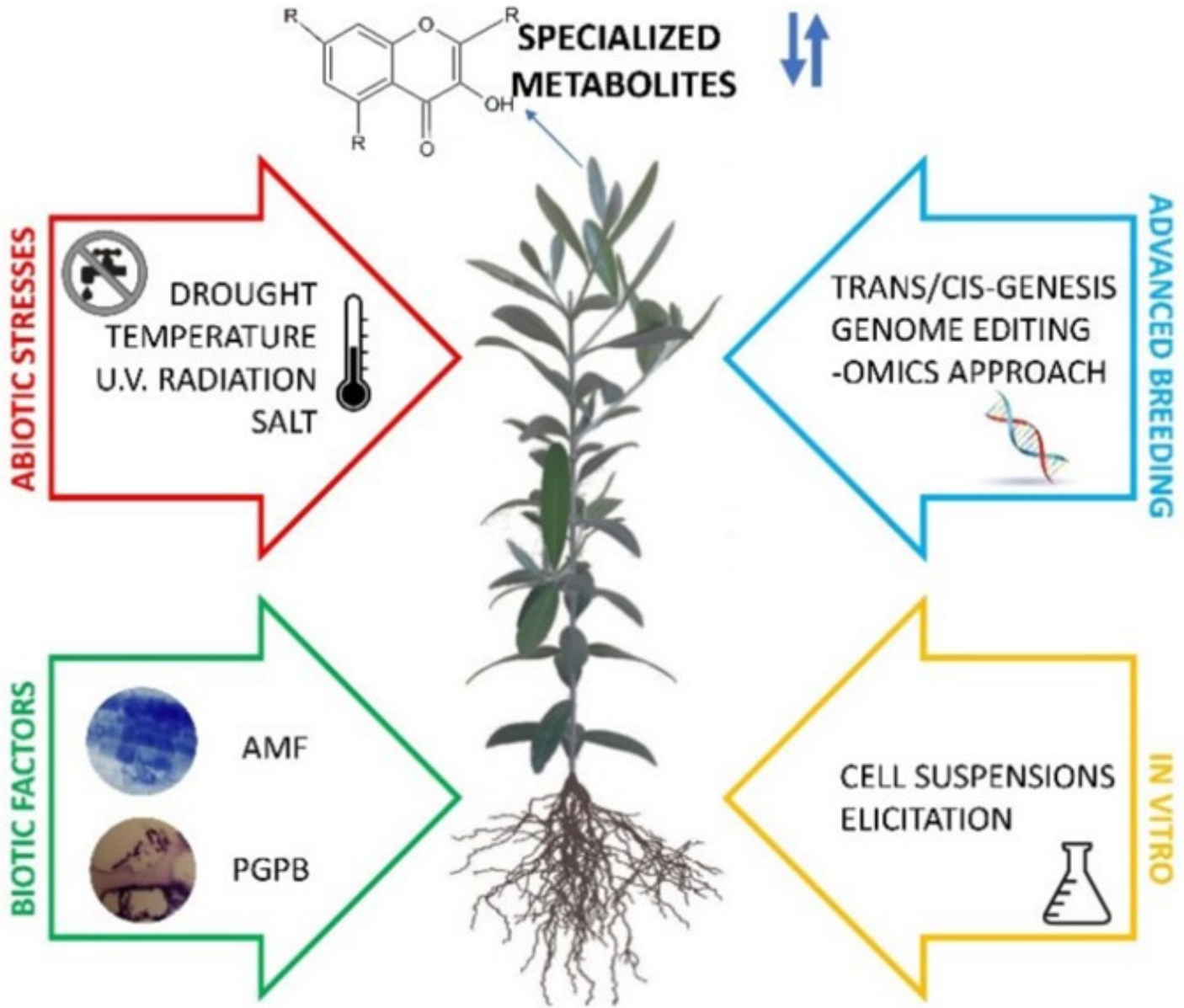
Why and when would a plant exudate molecules like BNIs?

- BNI exudation has a metabolic cost to the plant
- An increase in BNI exudation should be co-selected with improved ammonium uptake
- BNIs are beneficial when ammonium concentrations are large
- Negative feedback to nitrate concentration is necessary
- BNIs are beneficial when the plant is an ammonium-preferred crop (e.g. rice)
- **Optimal ammonium-nitrate ratio (ANR) for different crops**

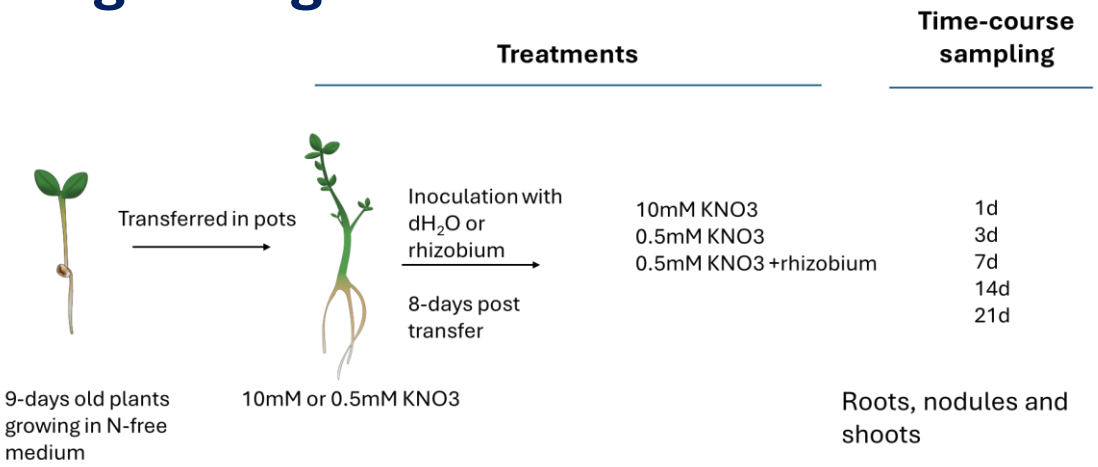
→ **Expand criteria for *in vitro* screening of root exudates**:
determine plant physiological and developmental parameters, abiotic conditions, inducers e.t.c.



Modulate Specialized Metabolism

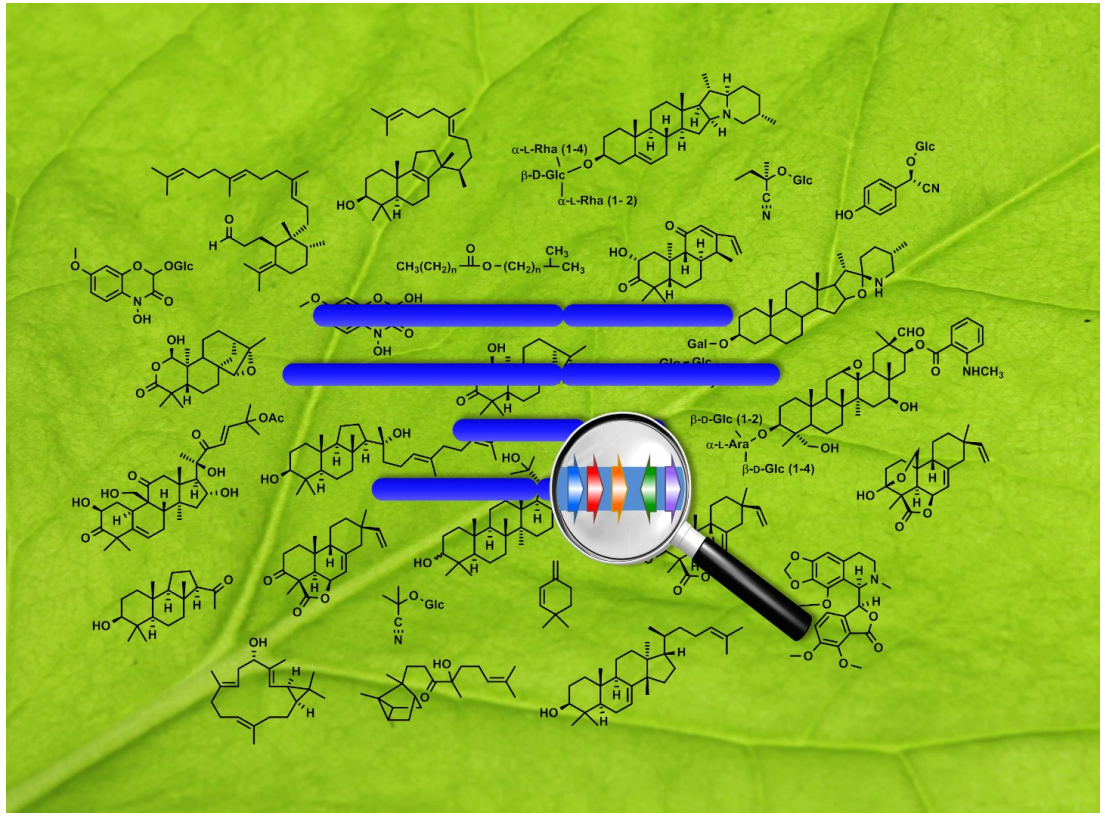


Co-expression network identifies novel candidates in nitrogen signalling



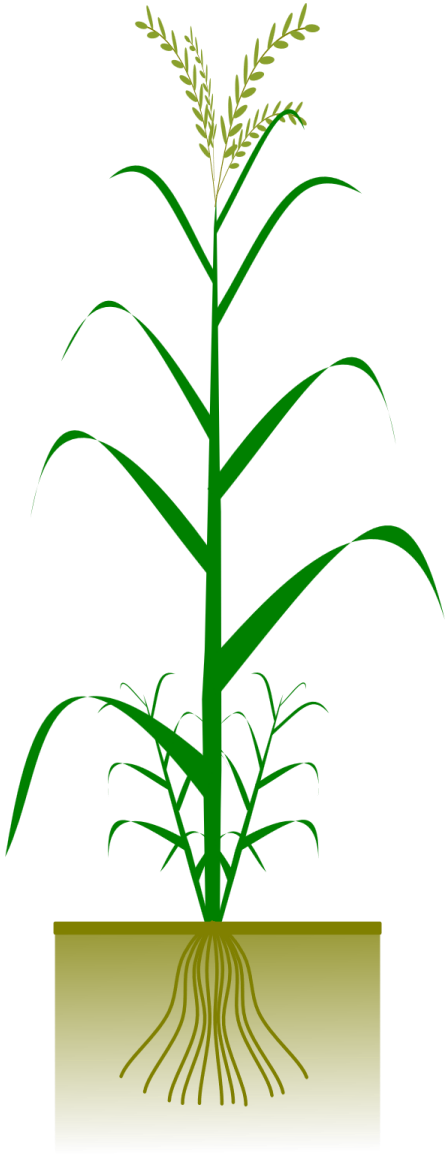
Chemical genetics approaches, combined with modified or synthetic metabolites that overcome genetic redundancy hindrances (e.g. used to identify the receptor of abscisic acid)

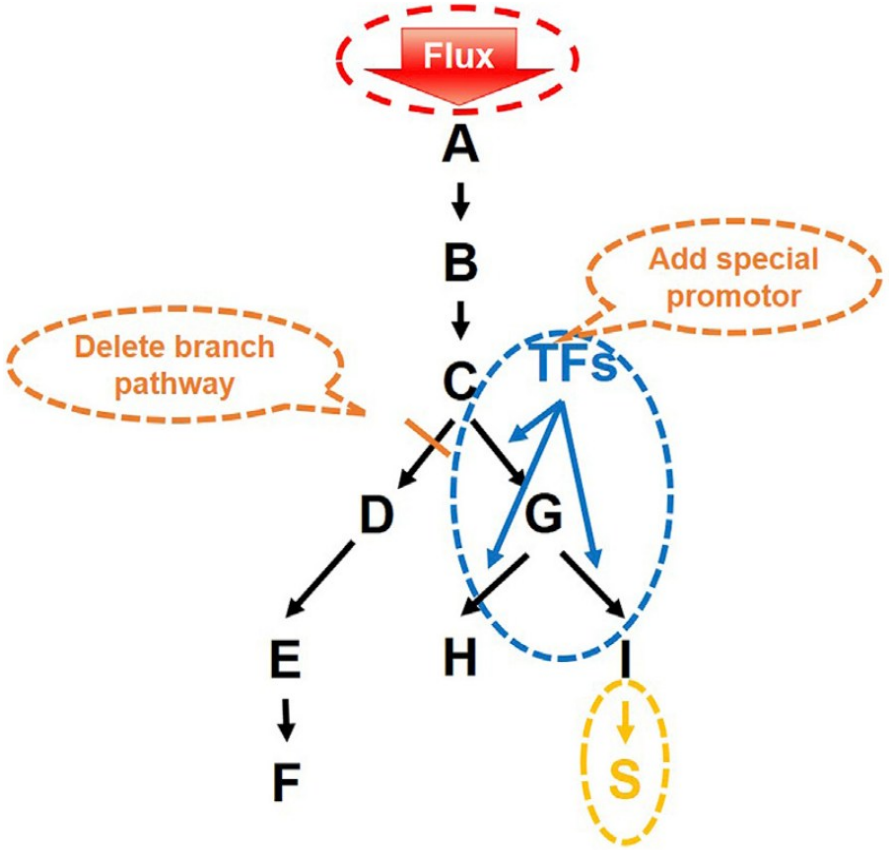
Rapid identification of pathway genes in plant genomes using new computational methods



Strategies to rationally design and produce novel plant exudate molecules functioning as BNIs ?

Various strategies to increase metabolite accumulation

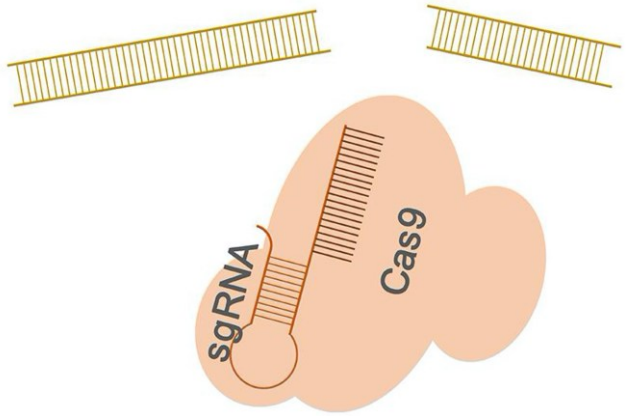




e.g. Shifted metabolic profile AND increased ammonium uptake

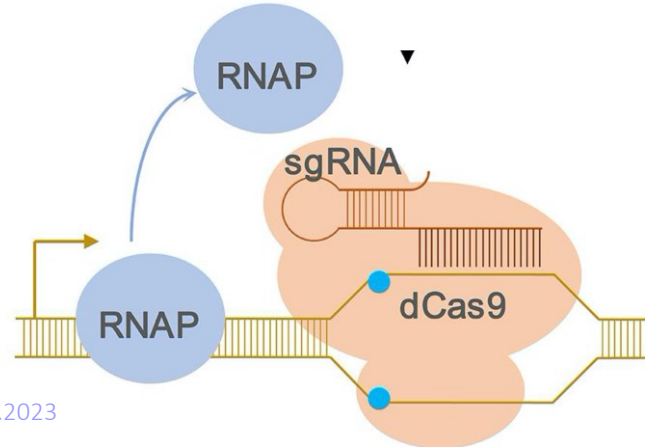
(root architecture and ammonium transporters/ reprogram signaling pathways to improve plant traits)

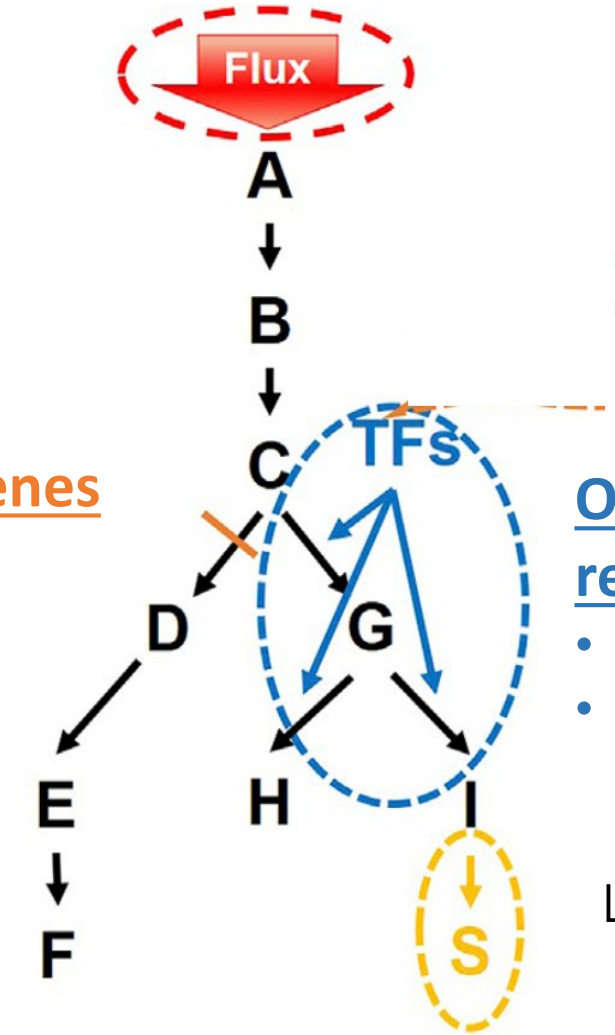
Can synthetic biology help us?

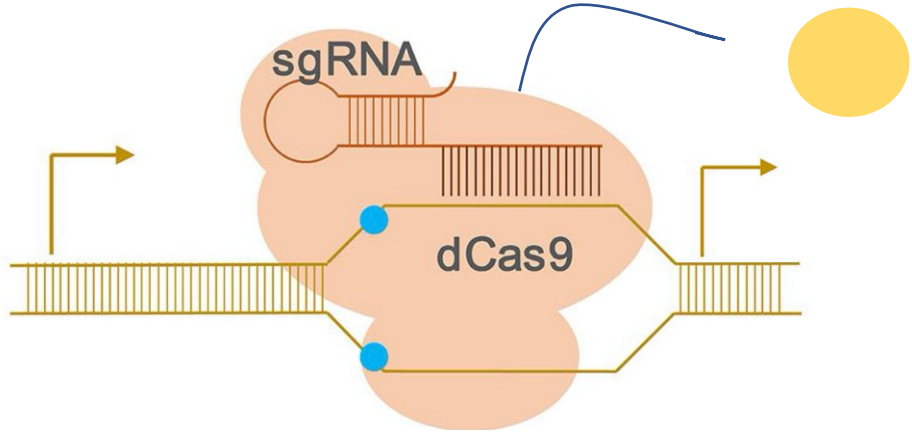


Knock out/down biosynthetic genes

- **CRISPR/Cas9 targeting**
- CRISPRinhibition

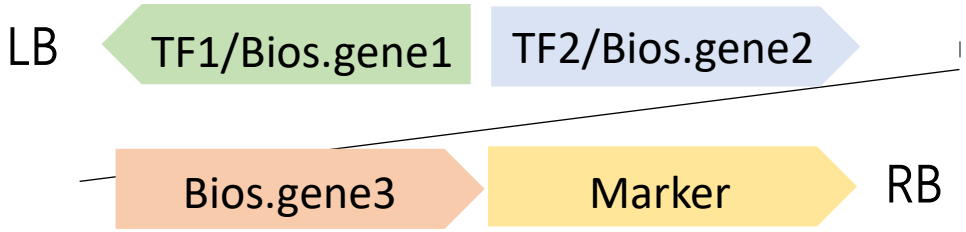


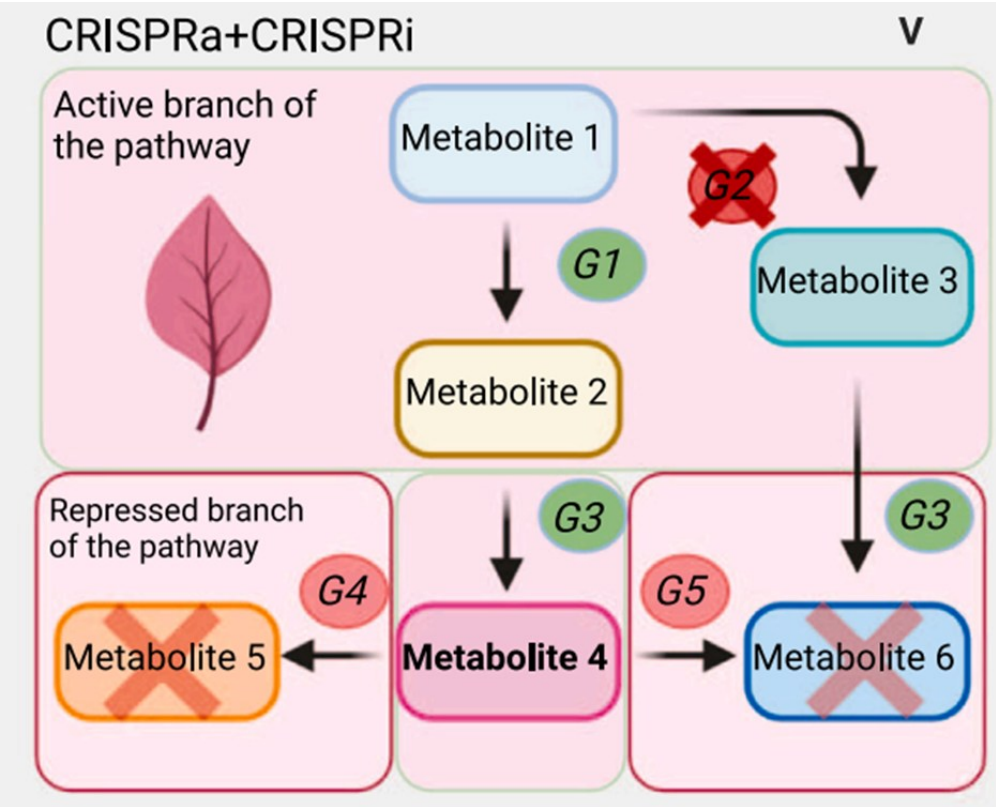


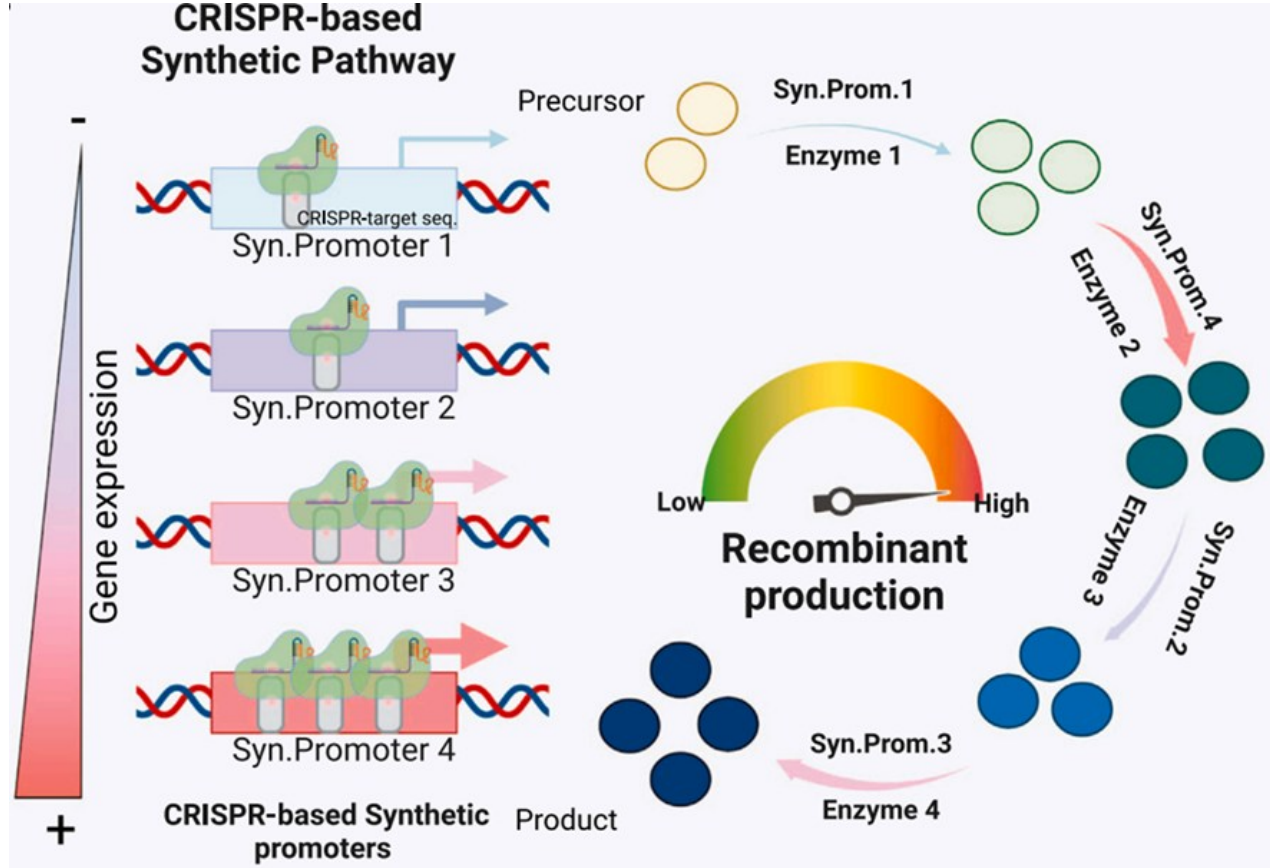


Overexpression of biosynthetic or regulatory genes or TFs

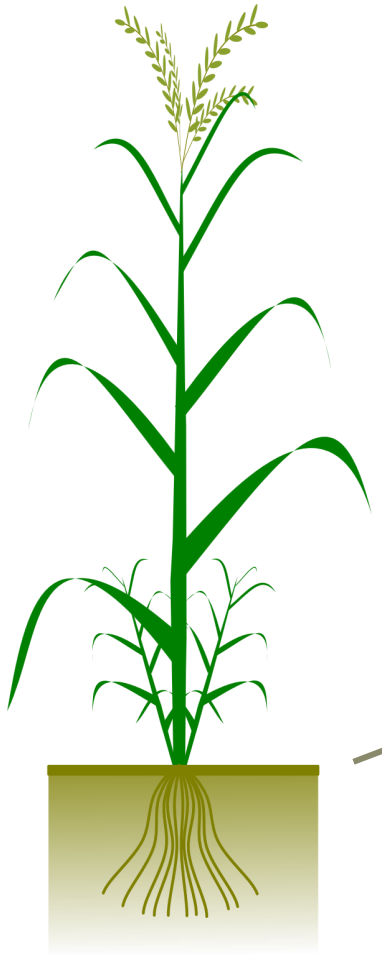
- CRISPRactivation
- **Heterologous expression**

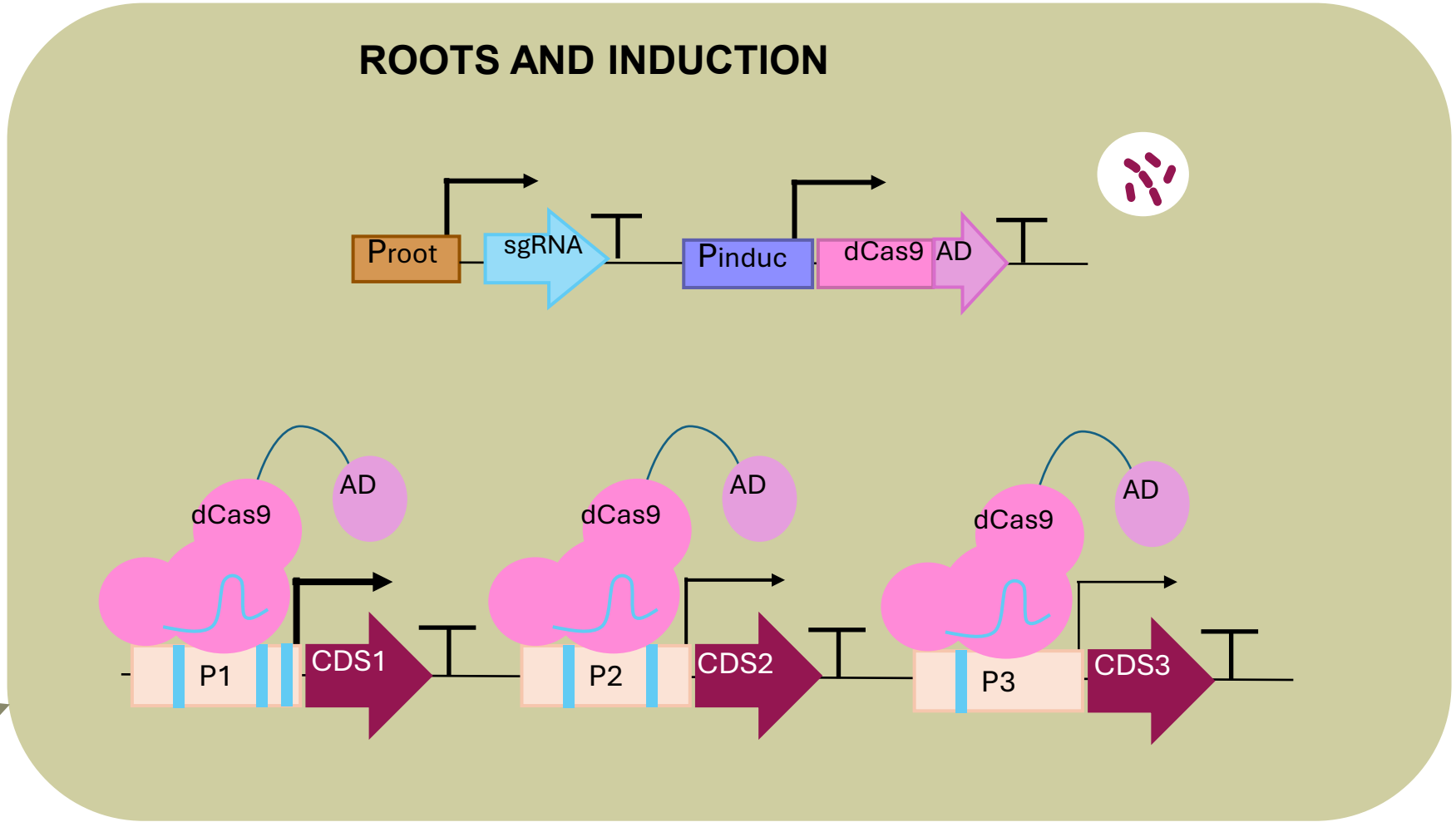



CRISPRa+CRISPRi
Active branch of the pathway
Metabolite 1
G2
G1
Metabolite 3
Metabolite 2
Repressed branch of the pathway
G3
G3
G4
G5
Metabolite 5
Metabolite 4
Metabolite 6


CRISPR-based Synthetic Pathway
Precursor
Syn.Prom.1
Enzyme 1
CRISPR-target seq.
Syn.Promoter 1
Syn.Promoter 2
Syn.Promoter 3
Syn.Promoter 4
Gene expression
Low
High
Recombinant production
Syn.Prom.4
Enzyme 2
Enzyme 3
Syn.Prom.2
Syn.Prom.3
Enzyme 4
Product
CRISPR-based Synthetic promoters

Can synthetic biology help us?





Tissue specific expression

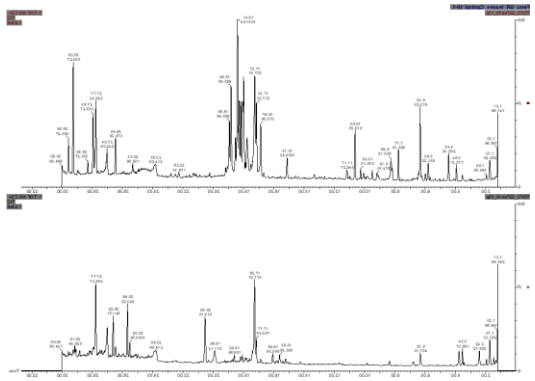
Inducible expression

(Low N?? Microbiota??)

AND

Metabolite production with potential BNI activity

Using synbio DBTL



Modeling

Learn

Design

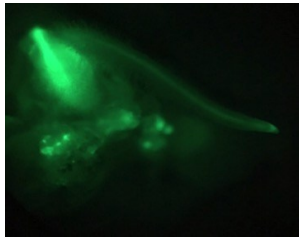
Test

Build

Metabolitic profiles

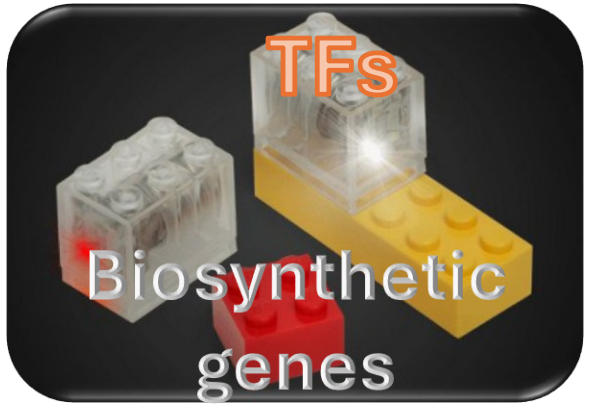
Plant lines

Plant transformation



Scale-proper production

Production of custom-designed metabolites

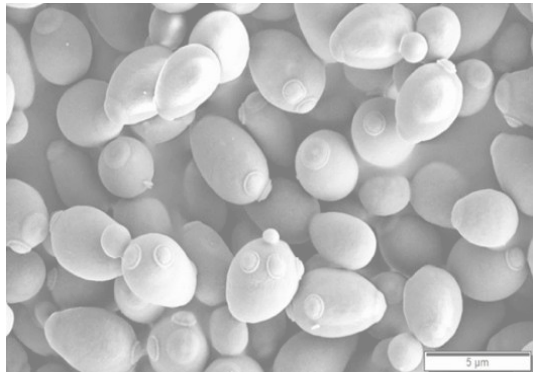


engineer tissues or plants with more specialized cells that produce and accumulate them

Use plants as platforms for large-scale production of BNIs

Instead of…

***S. cerevisiae* bioreactors**



Tobacco leaf transient infiltrations
(small-scale)

Hairy-root tissue cultures
(large-scale)

Thank you for your attention

References

plant secondary metabolism_growth and adaptation.zip