

Plants are some of nature's best chemists

Outline: Alkaloids: Function and biosynthesis

Secondary metabolites of plants are naturally occurring products that appear to have no direct function to growth and development.

Physiological Role: They are used by organism (a) for defense against herbivores and pathogens, and (b) as attractants of pollinators.

Three principle groups of secondary products are

1. Nitrogen containing compounds-incl. Alkaloids, made from amino acids
2. Terpenoids: acetyl CoA
3. Phenolic compounds: PEP, erythrose 4C sugar

They are synthesized from primary metabolites.

History and Uses of alkaloids

Biosynthetic pathway of opiates in opium poppy:

Regulation of alkaloid biosynthesis

Where and how are the alkaloids synthesized? From amino acids, tyr

What regulates the synthesis and the enzyme activities?

Recent research progress from Facchini et al.

Production of alkaloids by biotechnology

Three principle groups of secondary products:

Examples: see table 12-3 from Chrispeels and Sadava, 1994.

1. Nitrogen containing cpds

Alkaloids
Amines
Cyanogenic glucosides

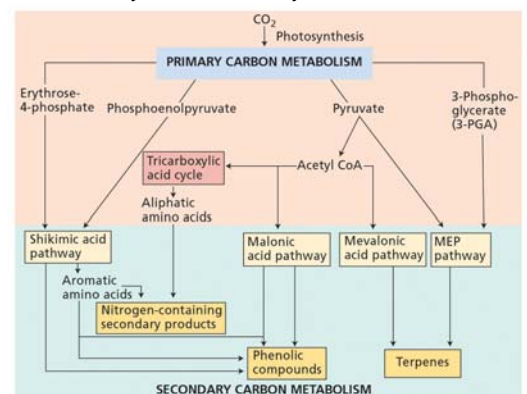
2. Terpenoids: synthesized from 5C isoprene units

Carotenoids
Monoterpenes (10C)
Sesquiterpenes 15 C
Diterpenes 20 C

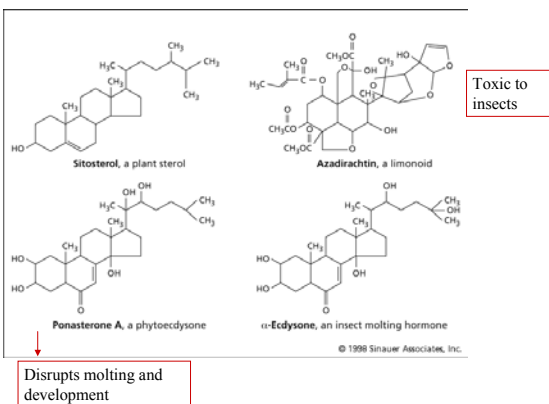
3. Phenolic cpds

Flavonoids- pigments of flowers; defense; attracting Rhizobium in legumes

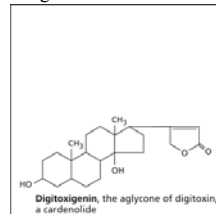
13-4 Taiz. biosynthesis of secondary metabolites



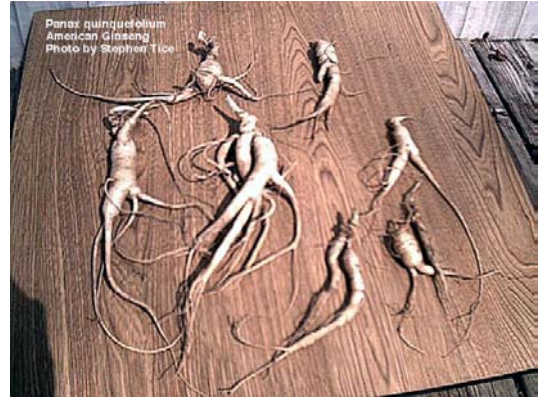
13-12 Taiz Triterpenes



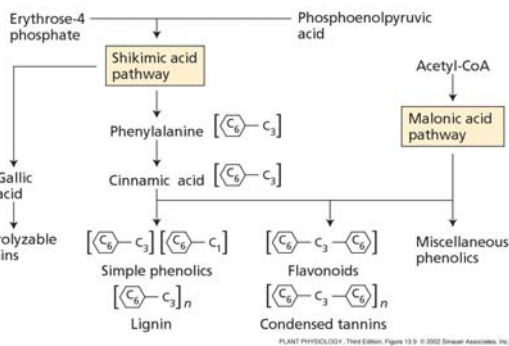
Digitalis- foxglove source of digitoxin



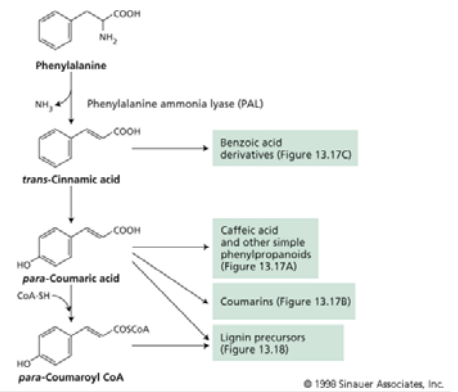
Ginseng contains triterpene saponins



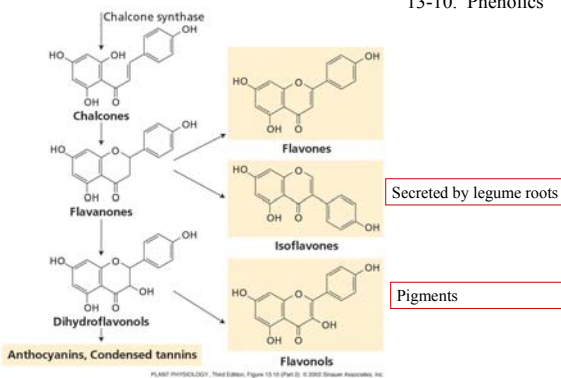
13-9. Plant Phenolics



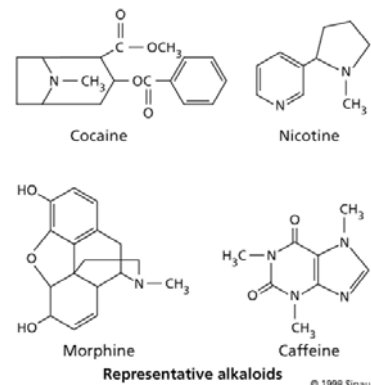
13-16 Taiz: Phenolic biosynthesis from Phe



13-10. Phenolics



13-17. Taiz. Alkaloids contain N.



N-containing compounds are synthesized from amino acids

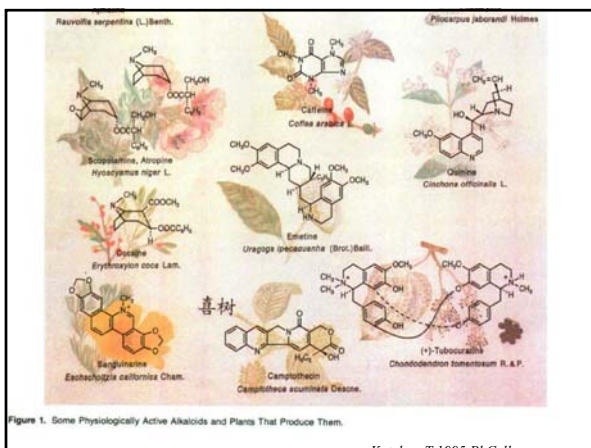
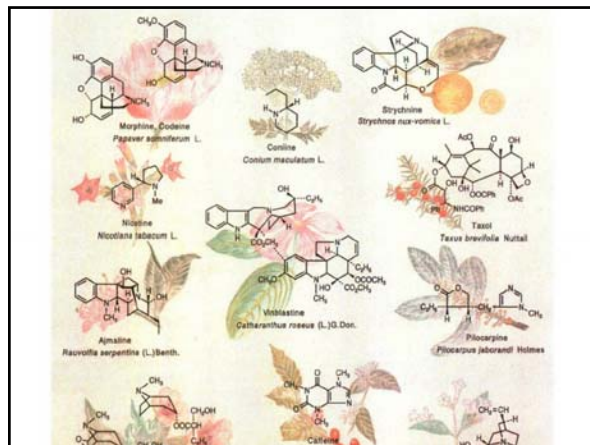
b. Tyrosine ----> ---> ---> ---> morphine

How?

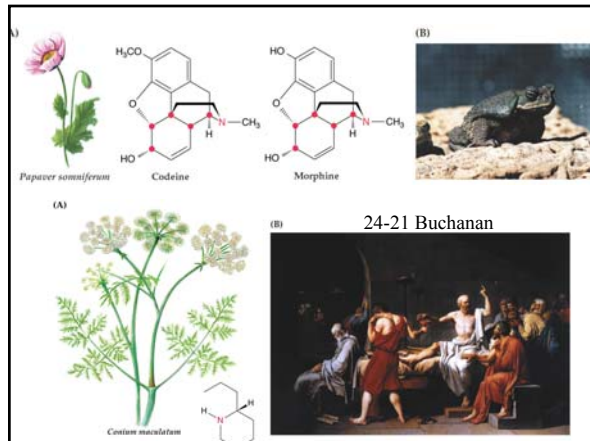

Where?

What regulates its synthesis?

Is synthesis induced by pathogens or herbivores?

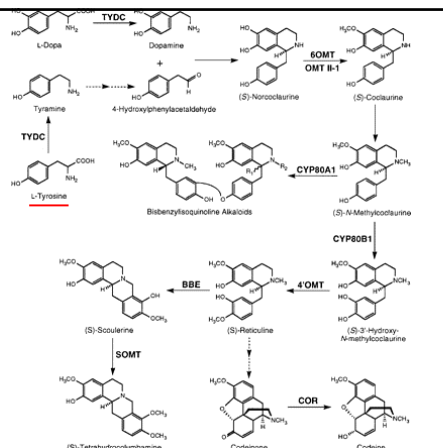


Opium poppy
Papaver somniferum



From Facchini
2001 ARPP

Synthesis of
Codeine



TYDC is the first enzyme of the pathway

Early steps in the biosynthetic pathways leading to benzylisoquinoline alkaloids and hydroxycinnamic acid amides of tyramine showing the sites of action of key gene products.

NS, (S)-Norcoclaurine synthase;

THT, tyramine

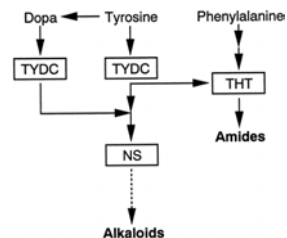
hydroxycinnamoylCoA:tyramine

hydroxycinnamoyltransferase;

Dopa, dihydroxyphenylalanine.

Many (15) genes encode

TYDC

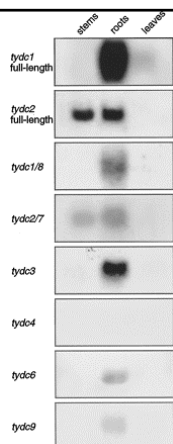


Where is the alkaloid synthesized?

Separate organs.
Extract total RNA.
Probe for the TYDC mRNA.

Approach: RNA gel-blot hybridization analysis for various members of the tydc gene family in mature opium poppy organs.

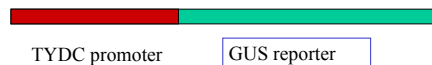
Facchini 1998 PP



How do you determine where and when a gene is expressed in plants?

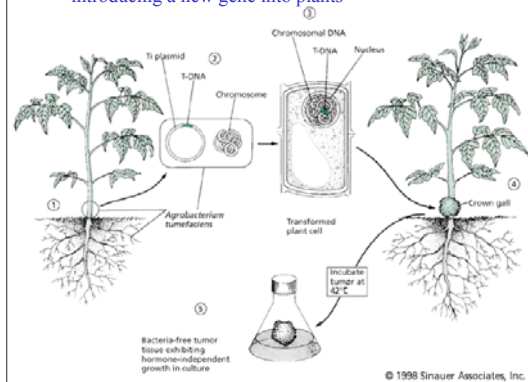
Method-2: Promoter driven reporter gene (GUS) activity in transgenic plants

a. Make a DNA construct using the desired promoter fused with reporter gene



b. Introduce this DNA construct into plants using a special bacterium.

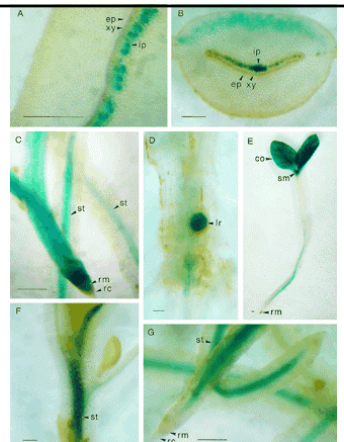
21-4 Taiz. Agrobacterium-mediated transformation-introducing a new gene into plants



Histochemical localization of GUS activity in transgenic tobacco expressing tydc promoter-GUS constructs.

- A. Phloem of stem
- B.
- C. Root- meristem
- D.
- E. Seedling
- F Vascular
- G

Facchini et al 98.



Capsule contains hi morphine but low TYDC.

Show RNA blot data.

Interpret.

Q: Why are the alkaloids made in the plant?

Hypothesis: Defense

How do you test this?

Propose an experiment

Method:

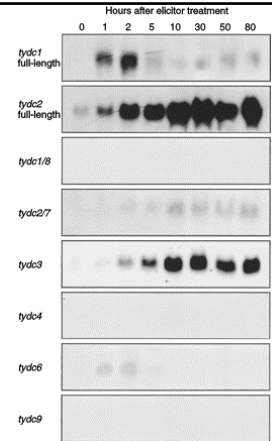
-Expose plant to fungal elicitor for 0 to 80 h.

-isolate RNA

-Test for levels of TYDC RNA expression.

Result: RNA gel-blot hybridization analysis for various members of the tydc gene family in elicitor-treated opium poppy cell-suspension cultures. (Fungal elicitor)

Conclusion?



How do you produce secondary products in quantity and at low cost?

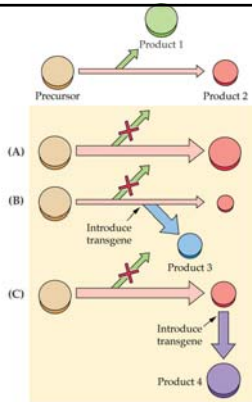
Genetic engineering of secondary products in plants or tissue culture

A. Block a pathway

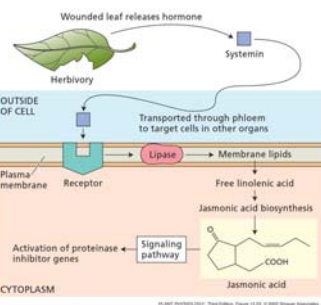
B. Introduce a gene

C. Introduce a new gene

From: 24-40 Buchanan



Taiz 13-23. Jasmonic acid, a stress hormone, activates defense responses



-Wounding by herbivores
-Stimulates increase in JA
-JA induces expression of genes involved in plant defense.

e.g. alkaloid biosynthetic genes

Sanguinaria canadensis flower



Synthesis of Scopolamine, Tropine

Facchini 2001 ARPP

