PLNT2530 (2025)
Unit 10a

Applications of Plant Biotechnology in Agriculture: Herbicide Resistant Crops

Plant Biotechnology

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Chapters 5-10

Herbicides

Ideal herbicide should: (-Critical Reviews in Plant Sci 9:1-15, 1990)

- Be effective against a wide range of “weeds”
- Have no effect on animals, birds or insects
- Be rapidly immobilized in the soil (to prevent water contamination)
- Be short-lived in the soil (rapidly degraded)

Herbicide mode of action

Designed to target essential biochemical pathways

Mechanisms of tolerance used by resistant or engineered plants

- Detoxification (degradation, modification, conjugation)
- Target site insensitivity
- Overproduction of the target site
- Active ingredient sequestration away from target site

Example: Glyphosate (Roundup) resistance

Targets Shikimic acid pathway which leads to many secondary metabolites including:

- aromatic amino acids
- auxins
- phytoalexins
- lignin
- folic acid
- plastiquinones

Over 30% of the carbon fixed by the plant passes through this pathway.

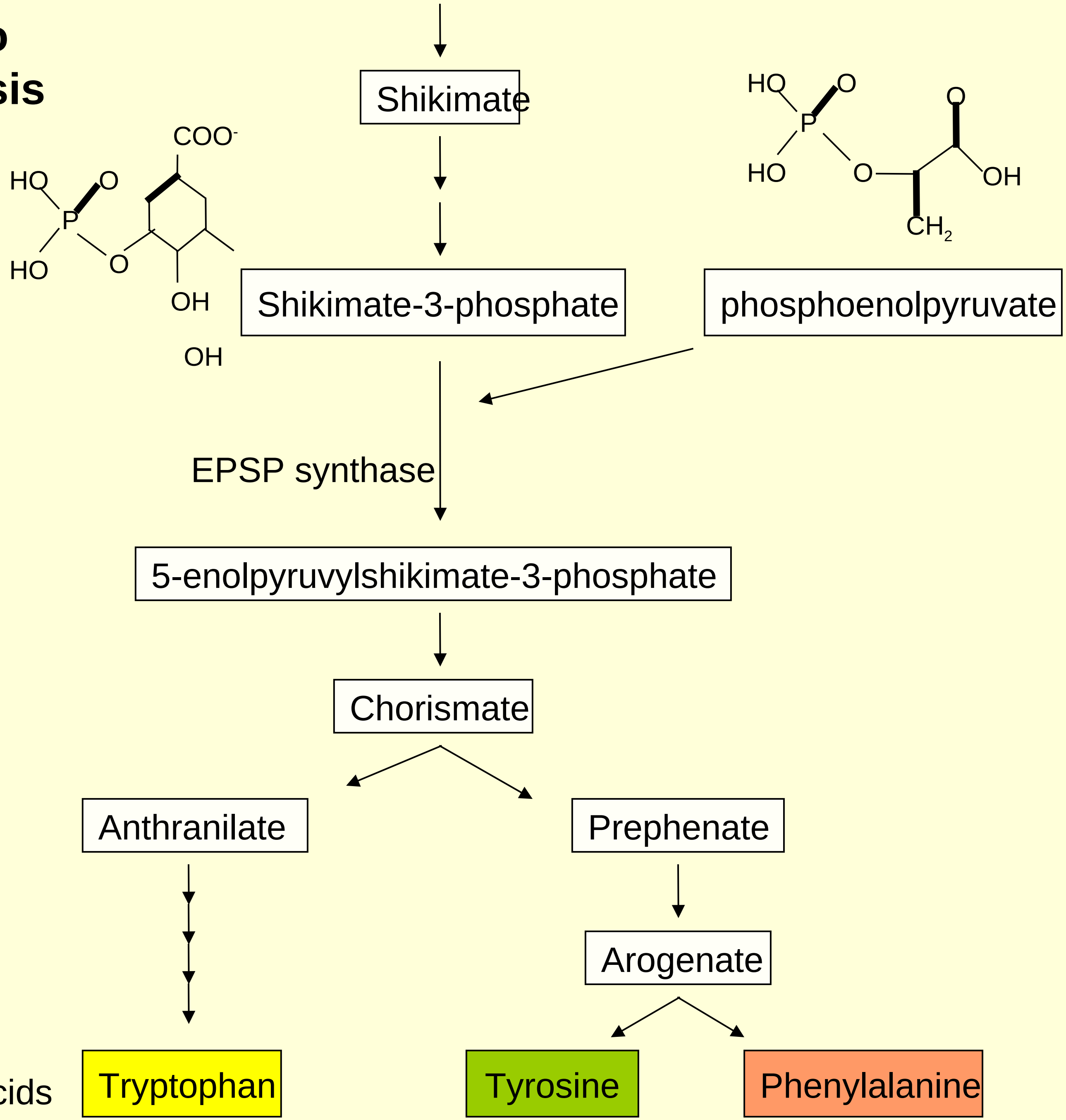
The Shikimic acid pathway occurs in chloroplasts. It also occurs in bacteria, but is not found in animals or in archaea.

Glyphosate is effective against a wide range of broad leafed plants.

Amino acid structures and codes
http://www.neb.com/tools-and-resources/usage-guidelines/amino-acid-structures

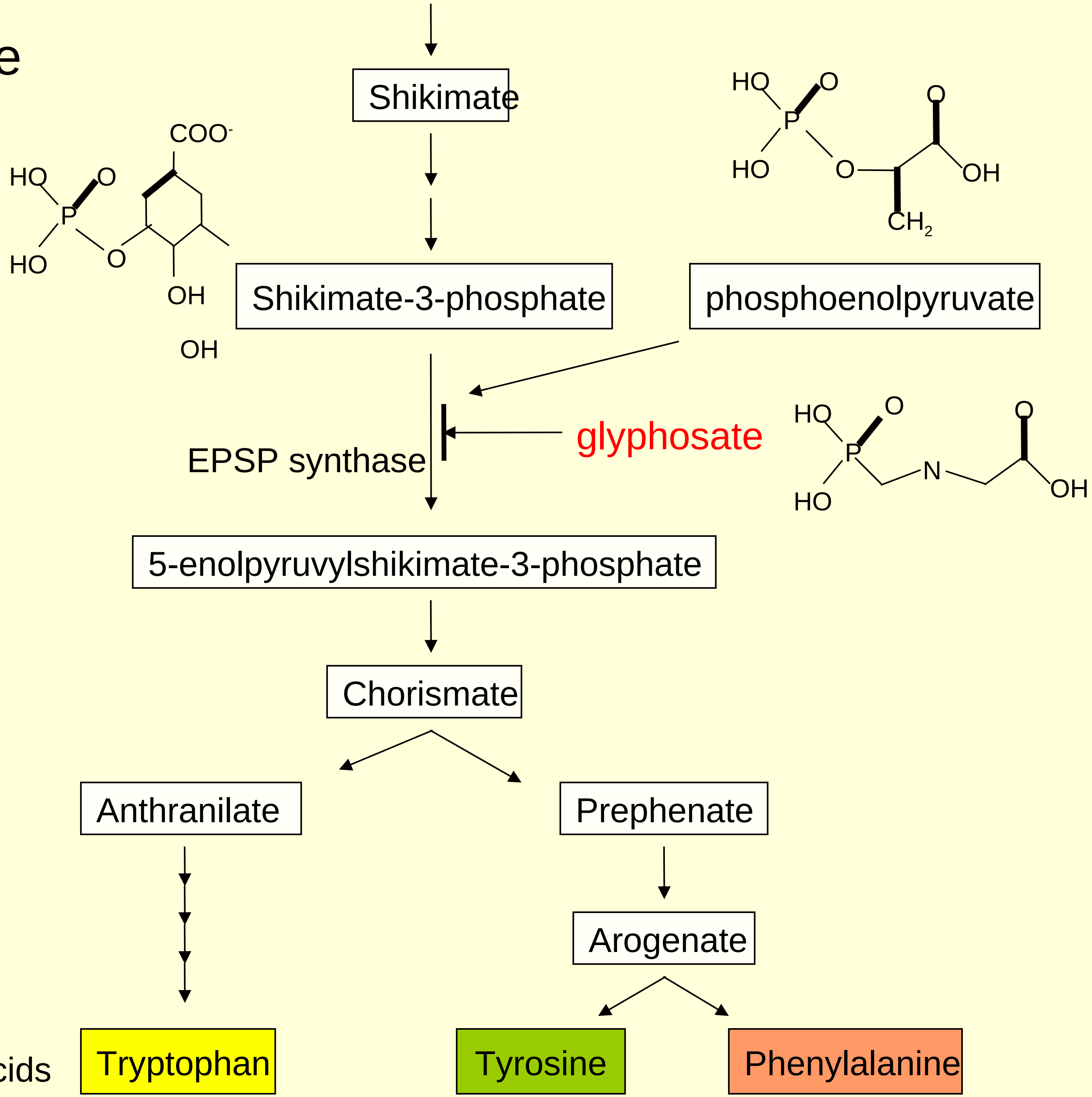
Aromatic amino acid biosynthesis

Pathway occurs in the chloroplast



Essential amino acids

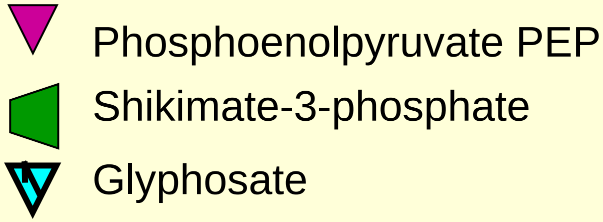
Glyphosate

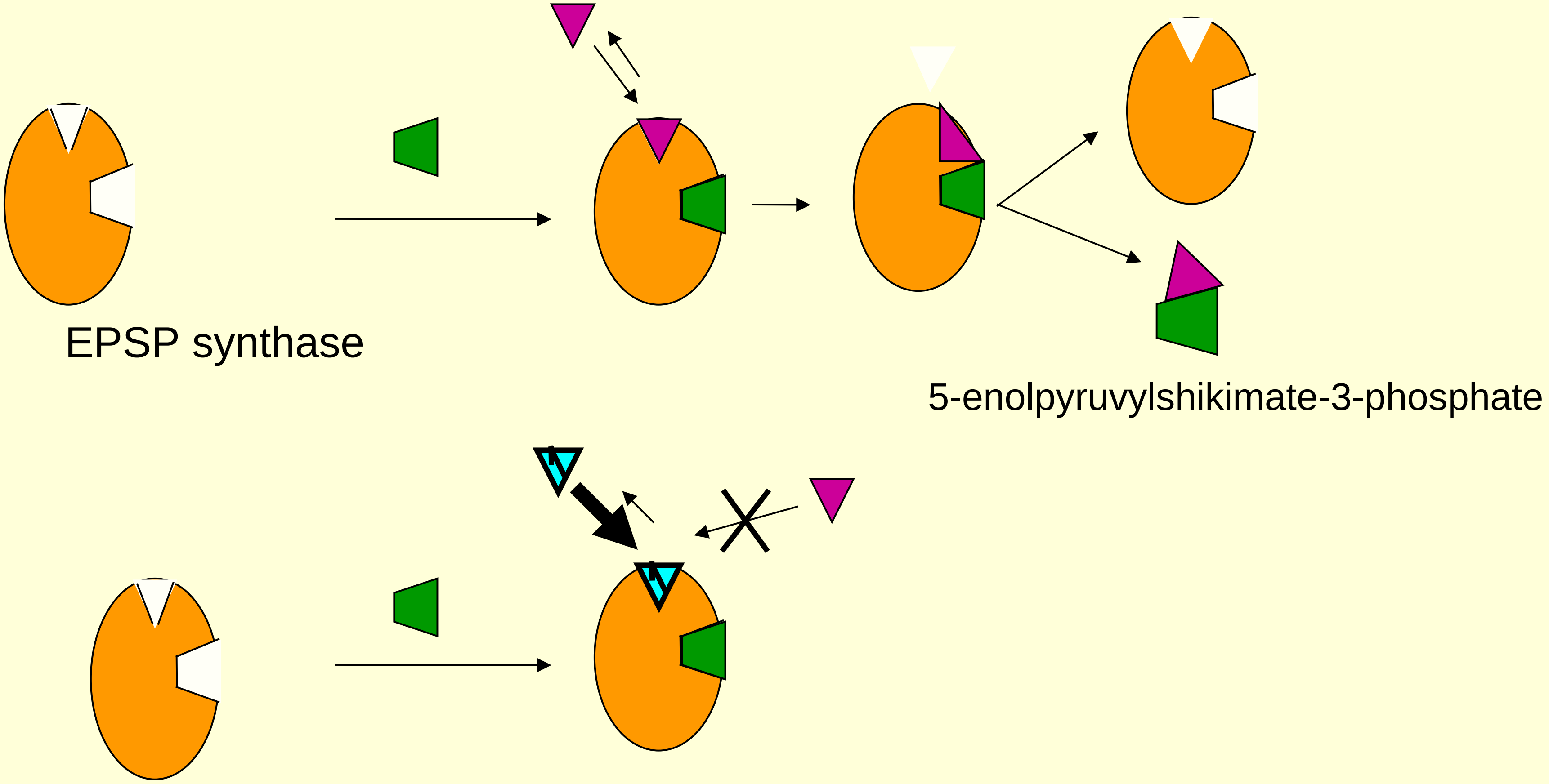


Pathway occurs in the chloroplast

Essential amino acids

EPSP synthase





Strategies for achieving resistance/tolerance to glyphosate

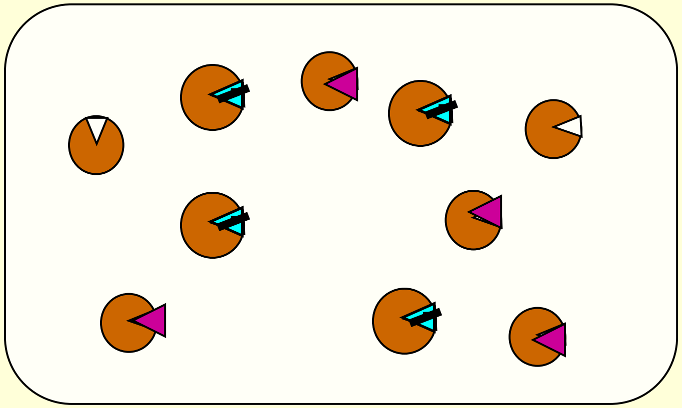
1. Over-expression of plant's EPSPS gene

Testing for resistance in Petunia

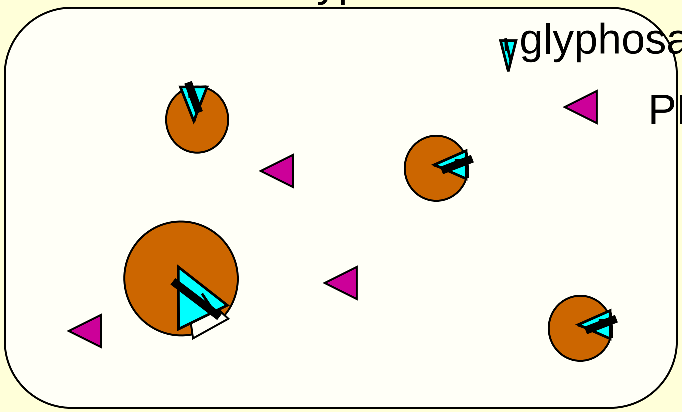
Isolated the petunia cDNA for EPSPS gene (nuclear encoded gene, targeted to chloroplast)

Re-introduced under control of CaMV 35S promoter -resulted in 40X higher EPSPS activity and a 2-4 fold higher tolerance to glyphosate than the untransformed wild-type

Over production of EPSPS



Wild-type



Over-production allows a certain level of glyphosate to be bound while still allowing some PEP to be bound by the excess EPSPS. Weeds would tolerate less than the transformed crop.

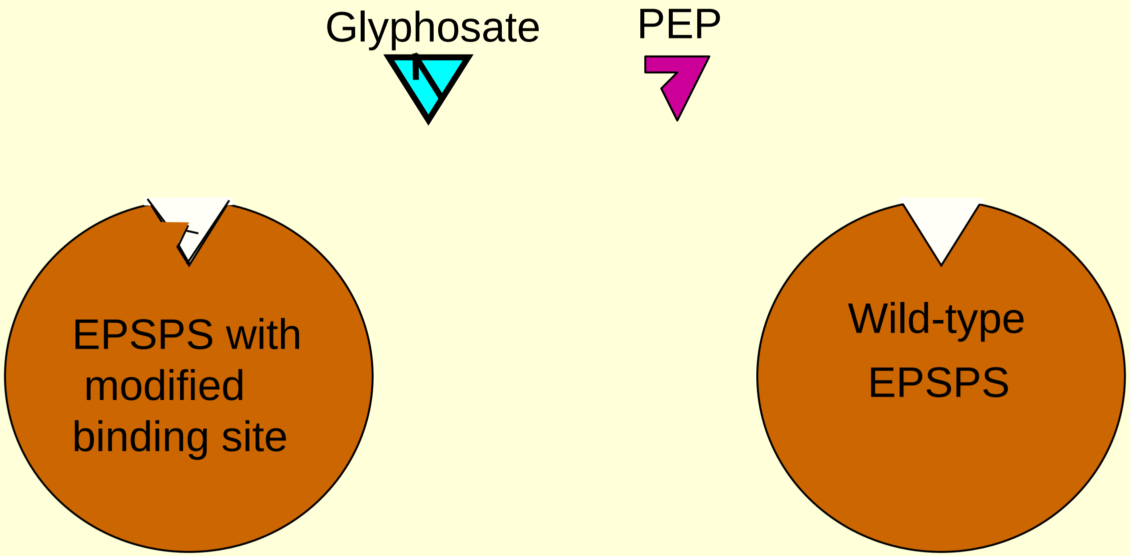
Strategies

2. Modified binding site in EPSPS

-obtain from tolerant organisms or

-mutated forms which are more tolerant than the wild type

-essential that the modification doesn’t effect enzyme activity



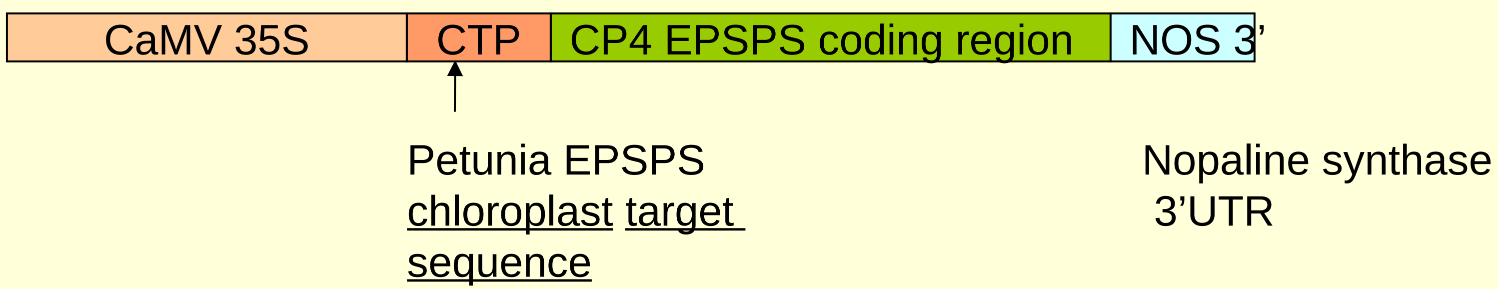
Roundup Ready™ gene from Monsanto works in most dicot crops – (glyphosate tolerant)

- EPSP synthase comes from a glyphosate tolerant Agrobacterium strain CP4. (tolerance is due to modified binding site)

- Gene is driven by a CaMV 35S promoter which works in most tissues (constitutive promoter)

- EPSP synthase reaction occurs in the chloroplast (nuclear transformation requires a chloroplast targeting peptide sequence for the protein

- Codon changes are sometimes necessary for good expression of prokaryotic genes in higher eukaryotes



1. The Standard Code (transl_table=1)

By default all transl_table in GenBank flatfiles are equ
is not equal to id 1, it is shown as a qualifier on the CI

TTT F Phe	TCT S Ser	TAT Y Tyr	TGT C Cys
TTC F Phe	TCC S Ser	TAC Y Tyr	TGC C Cys
TTA L Leu	TCA S Ser	TAA * Ter	TGA * Ter
TTG L Leu i	TCG S Ser	TAG * Ter	TGG W Trp
CTT L Leu	CCT P Pro	CAT H His	CGT R Arg
CTC L Leu	CCC P Pro	CAC H His	CGC R Arg
CTA L Leu	CCA P Pro	CAA Q Gln	CGA R Arg
CTG L Leu i	CCG P Pro	CAG Q Gln	CGG R Arg
ATT I Ile	ACT T Thr	AAT N Asn	AGT S Ser
ATC I Ile	ACC T Thr	AAC N Asn	AGC S Ser
ATA I Ile	ACA T Thr	AAA K Lys	AGA R Arg
ATG M Met i	ACG T Thr	AAG K Lys	AGG R Arg
GTT V Val	GCT A Ala	GAT D Asp	GGT G Gly
GTC V Val	GCC A Ala	GAC D Asp	GGC G Gly
GTA V Val	GCA A Ala	GAA E Glu	GGA G Gly
GTG V Val	GCG A Ala	GAG E Glu	GGG G Gly

i - has been observed as an initiation codon

http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=tgencodes#SG1

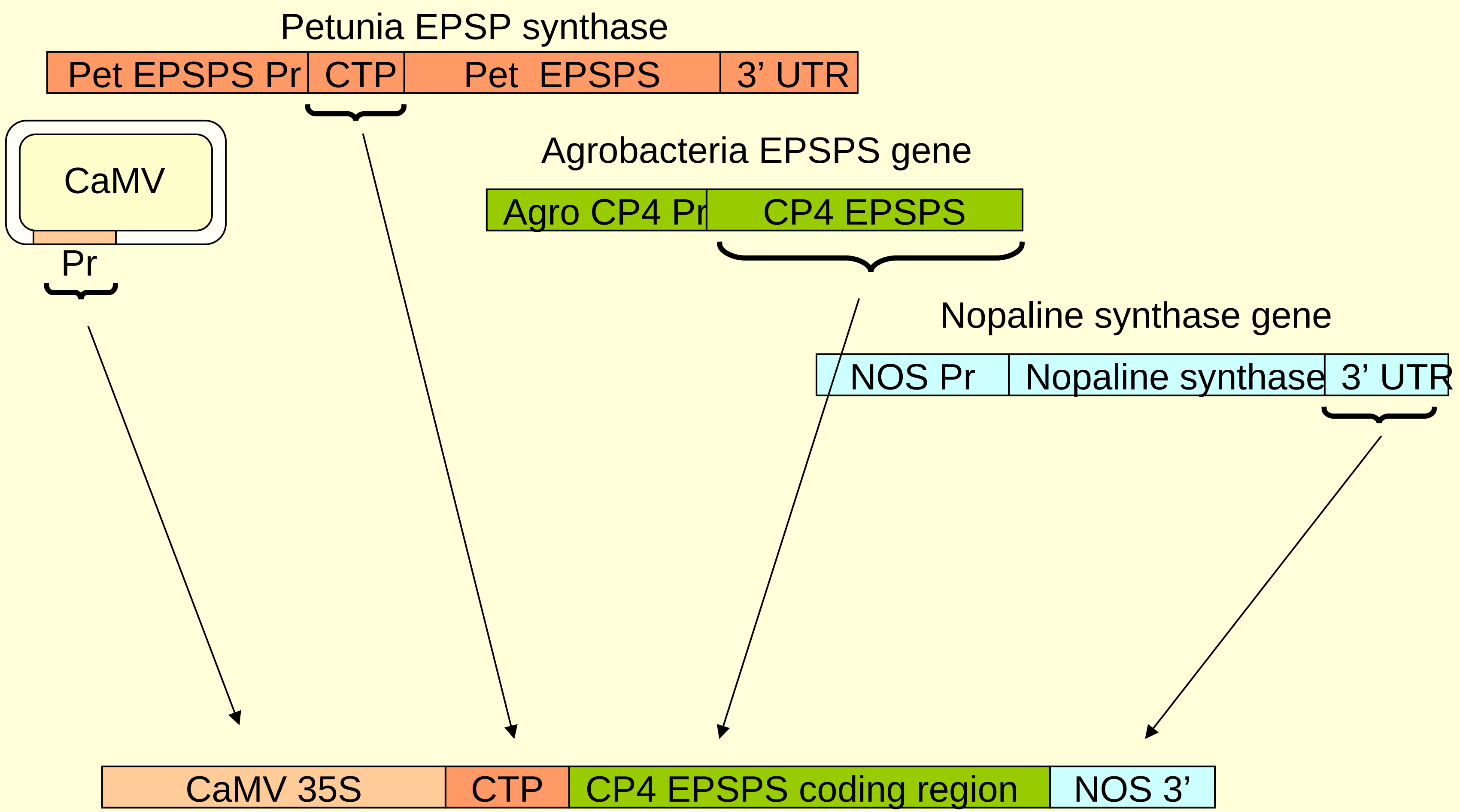
11. The Bacterial, Archaeal and Plant Plastid Code (transl_table=11)

```
TTT F Phe      TCT S Ser      TAT Y Tyr      TGT C Cys
TTC F Phe      TCC S Ser      TAC Y Tyr      TGC C Cys
TTA L Leu      TCA S Ser      TAA * Ter      TGA * Ter
TTG L Leu i    TCG S Ser      TAG * Ter      TGG W Trp

CTT L Leu      CCT P Pro      CAT H His      CGT R Arg
CTC L Leu      CCC P Pro      CAC H His      CGC R Arg
CTA L Leu      CCA P Pro      CAA Q Gln      CGA R Arg
CTG L Leu i    CCG P Pro      CAG Q Gln      CGG R Arg

ATT I Ile i    ACT T Thr      AAT N Asn      AGT S Ser
ATC I Ile i    ACC T Thr      AAC N Asn      AGC S Ser
ATA I Ile i    ACA T Thr      AAA K Lys      AGA R Arg
ATG M Met i    ACG T Thr      AAG K Lys      AGG R Arg

GTT V Val      GCT A Ala      GAT D Asp      GGT G Gly
GTC V Val      GCC A Ala      GAC D Asp      GGC G Gly
GTA V Val      GCA A Ala      GAA E Glu      GGA G Gly
GTG V Val i    GCG A Ala      GAG E Glu      GGG G Gly
```

i - has been observed as an initiation codon

http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=tgencodes#SG11

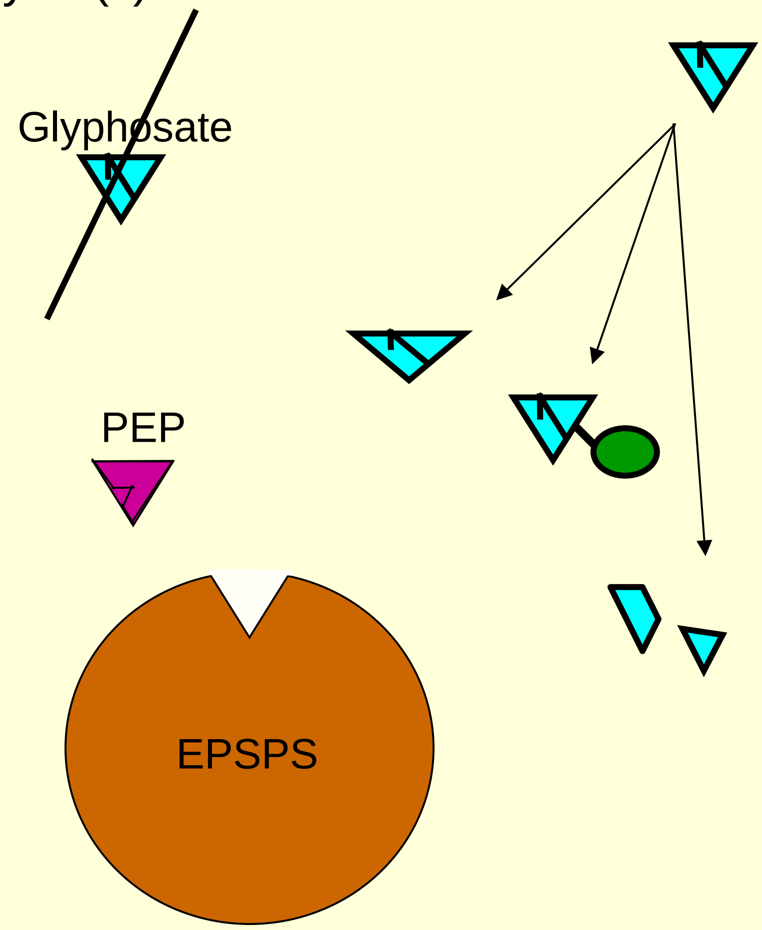
Assembling one of the glyphosate tolerance genes



Strategies

3. <u>Detoxification</u> degrade, conjugate or otherwise modify the glyphosate so that it no longer can bind to EPSPS

- requires addition of gene(s) that encodes the necessary enzyme(s)

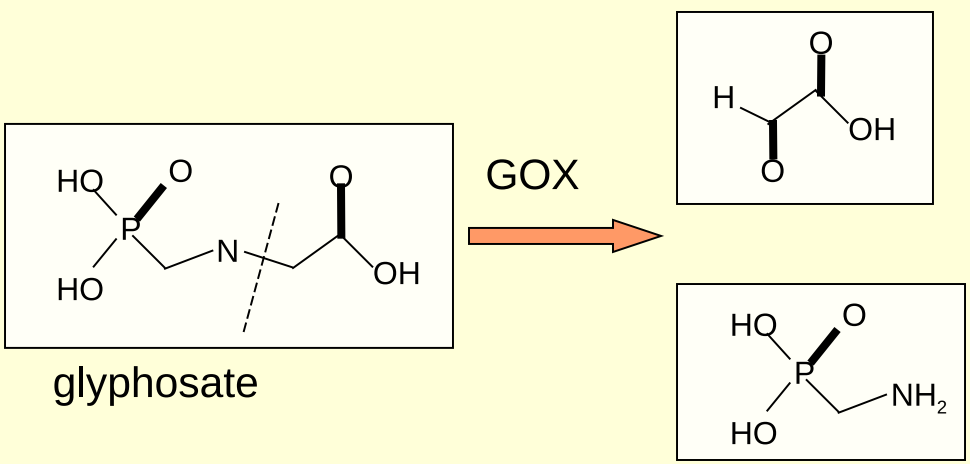


Roundup Ready genes

- In canola the previous gene construct is not enough. Glyphosate is translocated throughout the plant but accumulates in the meristem regions to concentrations above the tolerant levels.

- A second gene was added which oxidized the glyphosate into two easily metabolized products.

- *Ochrobactum anthropi* (soil bacteria) degrades glyphosate with an enzyme designated GOX for glyphosate oxidoreductase.



glyphosate

Glyoxylate

(Normal plant metabolite)

Aminomethylphosphonic acid

(Can be converted to glycine by plant)

Roundup Ready Canola

- Possess CP4 insensitive EPSPS gene
- Also has the GOX gene
  - Detoxifies the glyphosate which could accumulate in the meristem cells and might otherwise become toxic
  - Like the CP4 gene this bacterial GOX gene needed to have codons changes from the original sequence to codons which work efficiently in the plant system.

Issues with glyphosate and engineered resistance to it

- Overuse could lead to resistance in non-crop species
- Outcrossing of resistance gene from crop to weedy relatives
- Introduction into many crop species results in previous Roundup Ready crop becoming a less controllable weed in next crop
- Differential sensitivity to glyphosate among microorganism may mean soils microflora may change where the herbicide is heavily used.
  - Concern that beneficial bacteria and fungi could be suppressed relative to pathogens
- Relatively safe herbicide for animals, birds, people since pathway does not exist in animals.

Herbicide-resistant crops in North America

Herbicides	Crop	Year	Resistance mechanism
Bromoxynil	Cotton	1995	Enhanced degradation
Sethoxydim	Maize	1996	Altered target site
Glufosinate	Maize Canola	1997 1997	Altered target site
Glyphosate	Soybean	1996	Altered target site
	Canola	1997	Altered target site and enhanced degradation
	Cotton	1997	Altered target site
	Maize	1998	Altered target site
Imidazolinones[a]	Maize Canola	1993 1997	Altered target site
Sulfonylureas	Soybean	1994	Altered target site
Triazines[a]	Canola	1984	Altered target site

[a] Not transgenic

Duke, Stephen O., (2011) Herbicide Resistant Crops
http://ddr.nal.usda.gov/bitstream/10113/46272/1/IND44427308.pdf

Stephen Duke from the USDA points out that over the last 20 years, no new classes of herbicide, with respect of modes of action, have entered the market. He cites several possible reasons:

- A small number of companies with proprietary herbicides dominate the market
- The existing herbicides still work well
- New herbicides must pass rigorous tests for environmental health and safety, making the costs of development high
- Maybe all the good target sites have already been discovered
- Ongoing proprietary research is secret, so we really don't know what is in the pipeline

But.... we can't ignore the evolution of herbicide resistance in weeds. Resistance to most herbicides has already been seen in weed populations.

Roundup-Ready Turfgrass

- The product: Scotts Miracle-Gro Roundup-Ready Kentucky Bluegrass
  - glyphosate resistance gene
  - no other foreign genes (eg. antibiotic resistance)
  - delivered by biolistics
  - avoids APHIS* regulatory restrictions on GMOs with pest-derived sequences (eg. 35S promoter is from plant virus)
  - presumed market is golf courses and lawns
- 2011 - Approved by USDA
- 2014 - lawn trials begin at homes of employees
- As of Apriil 2021, has not been released for retail sale. In part this can be attributed to the accidental release of another roundup-ready grass (creeping bentgrass, used for golf courses) which became a pest in Oregon.
- If marketed, this would be the first GM plant sold directly to the consumer

*APHIS - USDA Animal and Plant Health Inspection Service

References

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Duke, S.O. 2012. Why are there no new herbicide modes of action in recent years. Pest Management Science. 68:505-512.

In Major Shift, USDA Clears Way for Modified Bluegrass New York Times July 6, 2011.
http://www.nytimes.com/gwire/2011/07/06/06greenwire-in-major-shift-usda-clears-way-for-modified-bl-51693.html

After nearly two decades of research and some controversy, Scotts Miracle-Gro in Marysville is preparing to test a genetically modified grass seed in the family lawns of a small number of employees this growing season. Columbus Dispatch, Jan. 31, 2014.
http://www.dispatch.com/content/stories/business/2014/01/31/scotts-tests-modified-grass-seed-at-homes.html

Roundup Ready Kentucky Bluegrass: Benefits and Risks, Turfgrass Science, Univ. Minn., April 28, 2014
http://turf.umn.edu/news/roundup-ready-kentucky-bluegrass-benefits-and-risks

USDA Responds to Regulation Requests Regarding Kentucky Bluegrass
July 2011
https://www.aphis.usda.gov/aphis/newsroom/news/!ut/p/z0/fYzLDoIwEEW_hqWZgfhYg1EjIWBiTKCbZiANVLCFtqD8vcgHuDsn9wEMcmCKJlmTk1pRt3jB9jzJtkc_umNyOd9OGD6yOLqmGGC6gxjY_8LyIJ_DwEJglVZOfBzk1DfS8lWV450sDZnZQ0tcibc1Wr88_NGGTNXISdg1C9D3V8CDh5Xj7TIeq3bmZTeK2pC10Les-AJ