

# QUINOLIZIDINE ALKALOID BIOSYNTHESIS: INSIGHTS FROM TRANSCRIPTOME ANALYSES (RNASeq)



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**L.albus**



**L.angustifolius**

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**L.luteus**



**L.mutabilis**

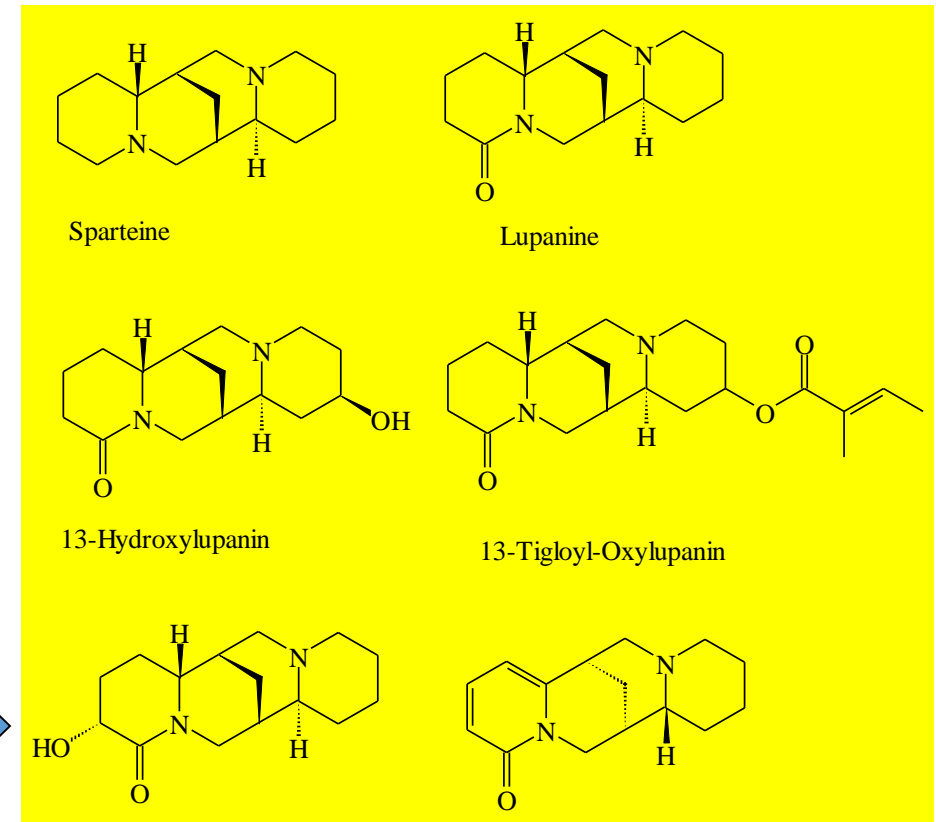
# Non-nutritional Factors (NNFs)

## Heat-labile NNFs

- Protease-Inhibitors

## Heat-stable NNFs

- Oligosaccharides
- Saponins
- Phytic acid
- Isoflavones, flavonoids
- Quinolizidine alkaloids (QA)



High structural variability → 200 QA

Flower: 4%  
Carpels: 3.3%  
Petals: 1%  
Pollen: 4.7%  
Seeds: 4-8%  
Fruit: 3.9%

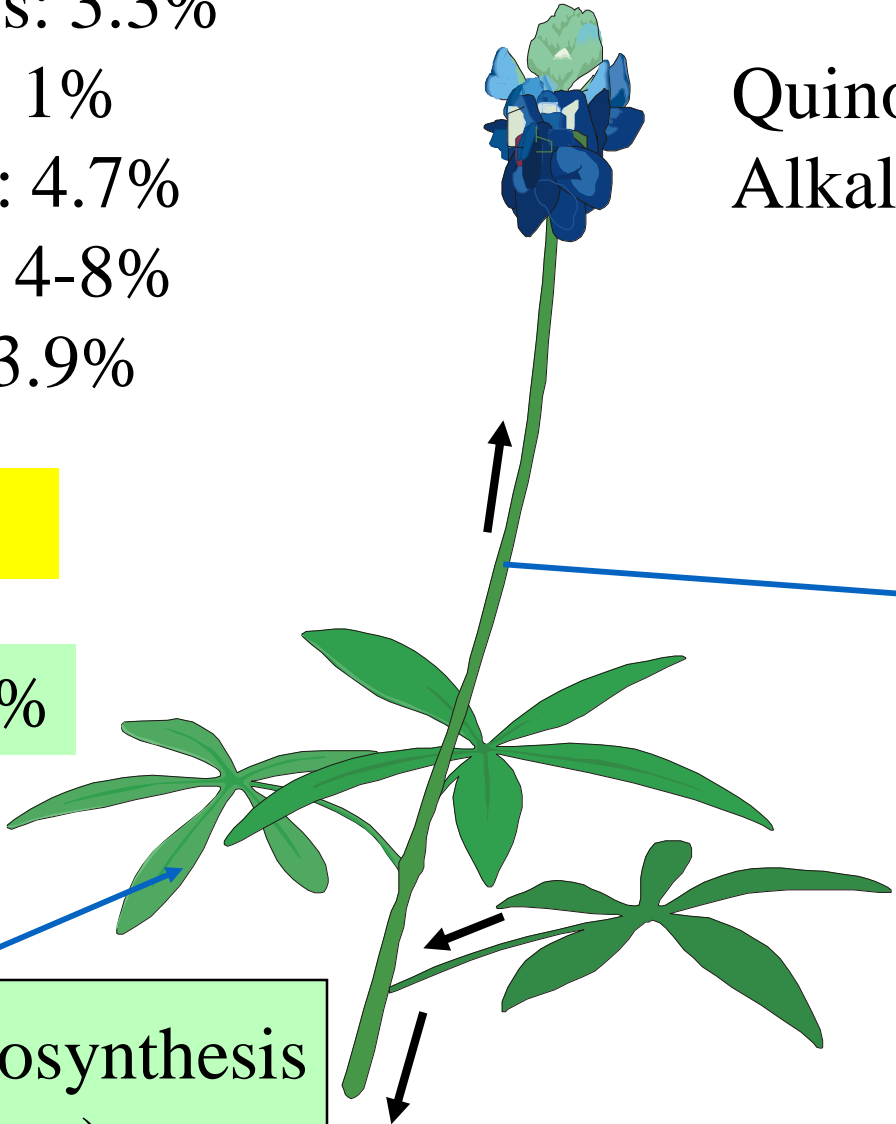
# *Lupinus*

Quinolizidine  
Alkaloid concentrations

Storage in vacuoles

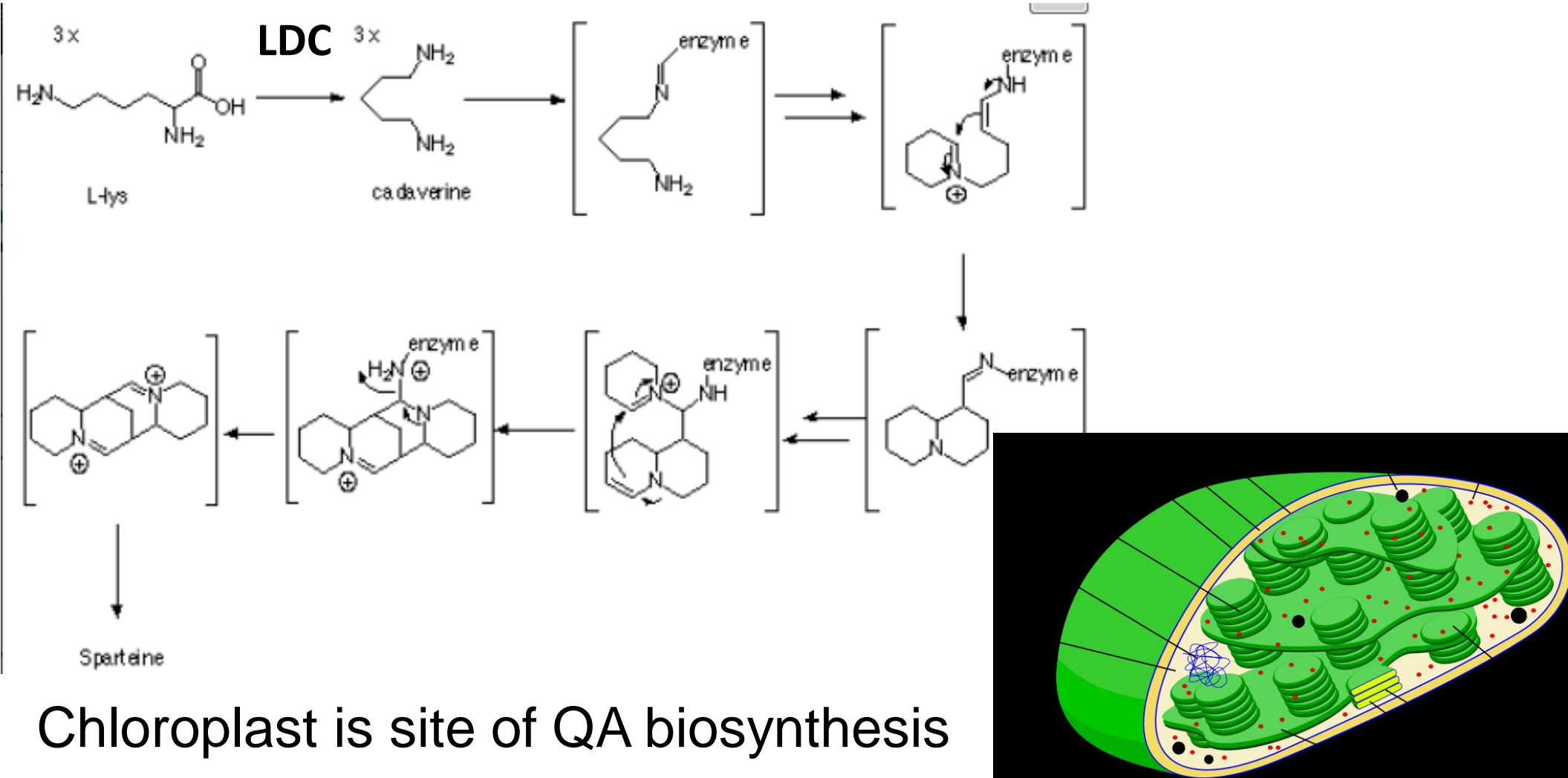
Leaf: <4%

Site of biosynthesis  
(chloroplast)



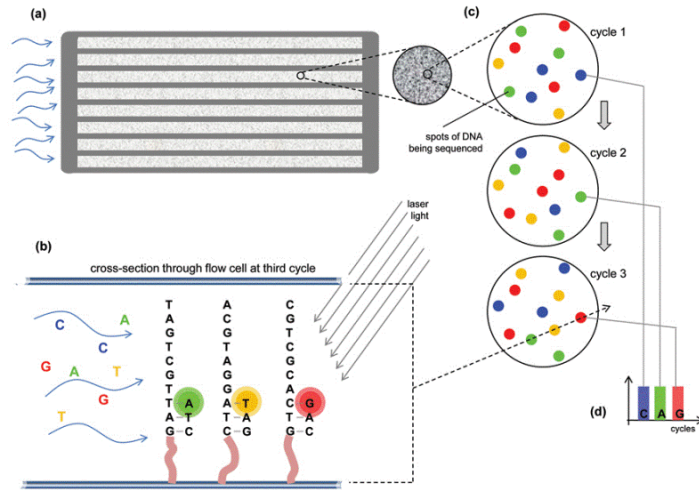
Epidermis (6%)  
Phloem <5 mg/ml  
Xylem <0.05 mg/ml

# Biosynthesis and Physiology of Quinolizidine Alkaloids (QA)



# NEXT GENERATION SEQUENCING

## Sequencing by synthesis



Illumina HiSeq2500

## Sequencing

First Generation: Sanger-methodology

- Capillary-Electrophoresis

Next Generation Sequencing - NGS:

- Pyrosequencing (454)

- **Sequencing by synthesis (Illumina)**

- Ion semiconductor sequencing

- Single-molecule real time sequencing (PacBio)

- Nanopore

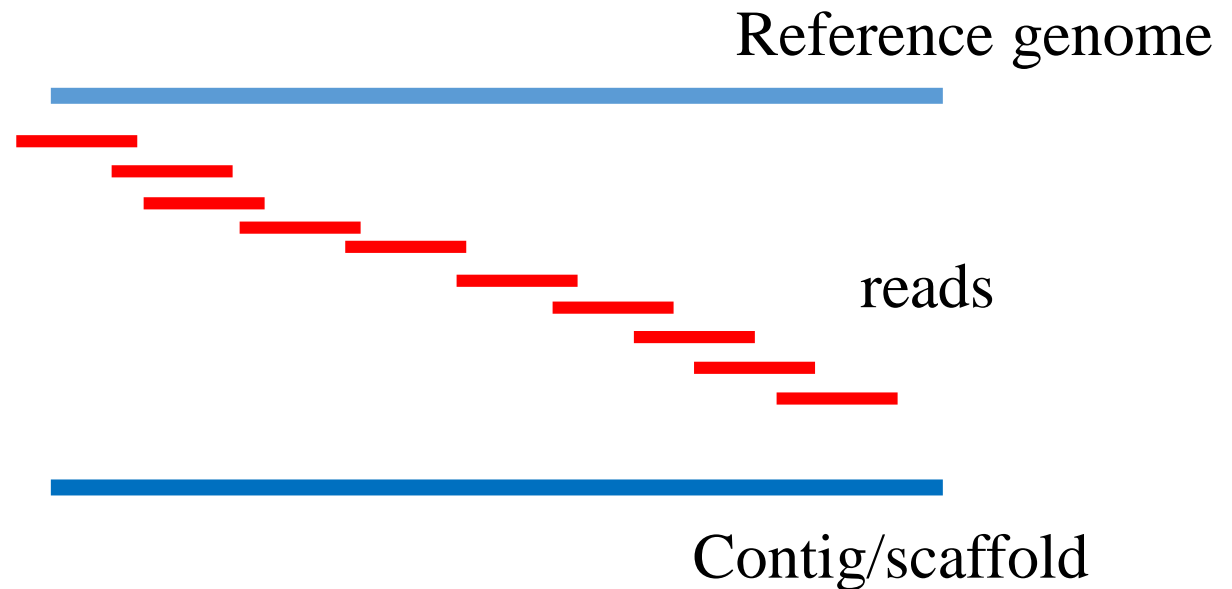
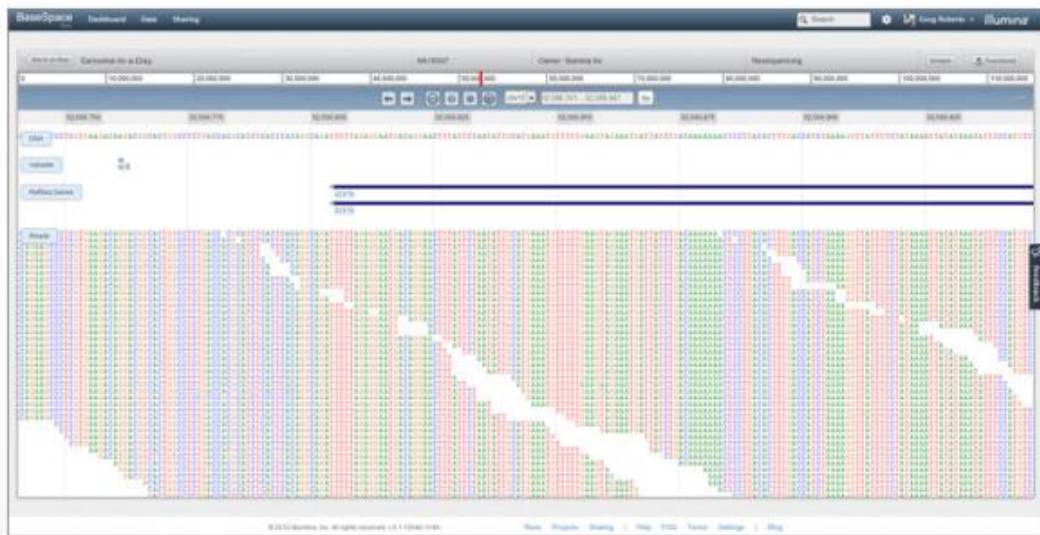


# BIOINFORMATICS

- Over 250 million reads/lane
- Alignment of reads with reference genome or reference transcriptome
- Assembly of contigs/scaffolds
- Annotation of contigs to known genes
- Transcriptome analyses:
- Quantification of transcript numbers

- Reference genome /transcriptome
- de novo assembly

## Genome-in-a-day dataset



# RNASeq- Pipeline

RNASeq data



TRINITY



Mapping and Quantification

bwa mem, samtools view, sort, index  
idx stats  
BUSCO, Blastn, blastx



Count matrix, annotation, BUSCO scores



Filter low expressed transcripts:R

Remove redundant transcripts: CD-Hit



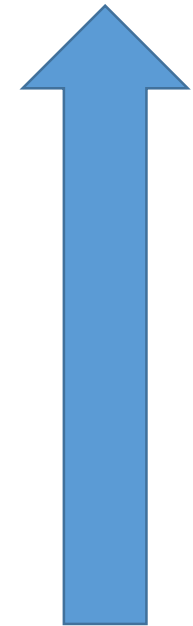
**filtered transcriptome**

De novo transcriptome

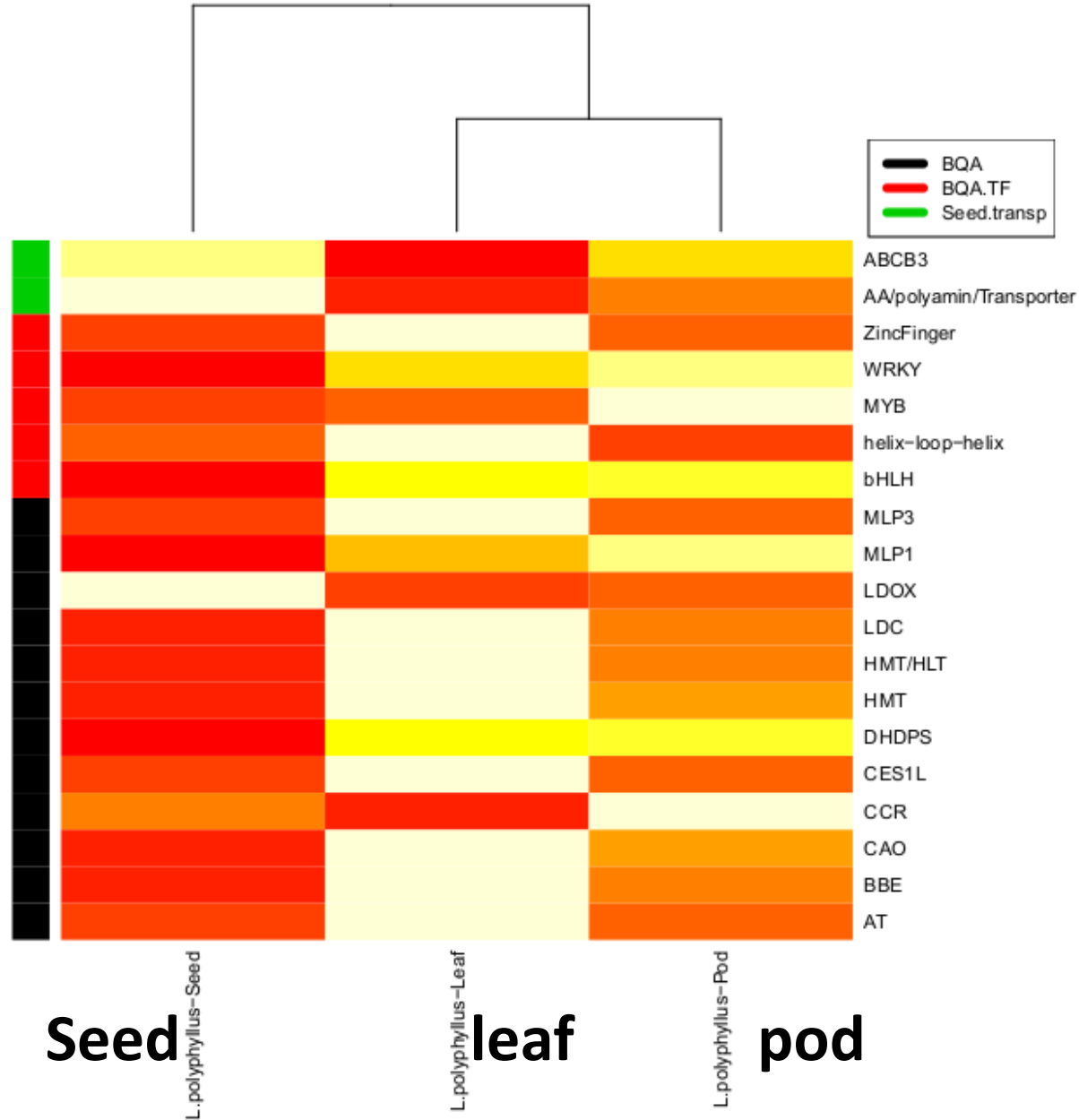
Quality metrics

Functional annotation

Quantification of expressed transcripts



# Heat map: *L. polyphyllus* QA related genes



**Lysine**



**Lysine decarboxylase (LDC)**

**Cadaverine**



**Oxidase / transaminase**

**Tetracyclic alkaloid**



**Estertransferase**

**Esteralkaloids**

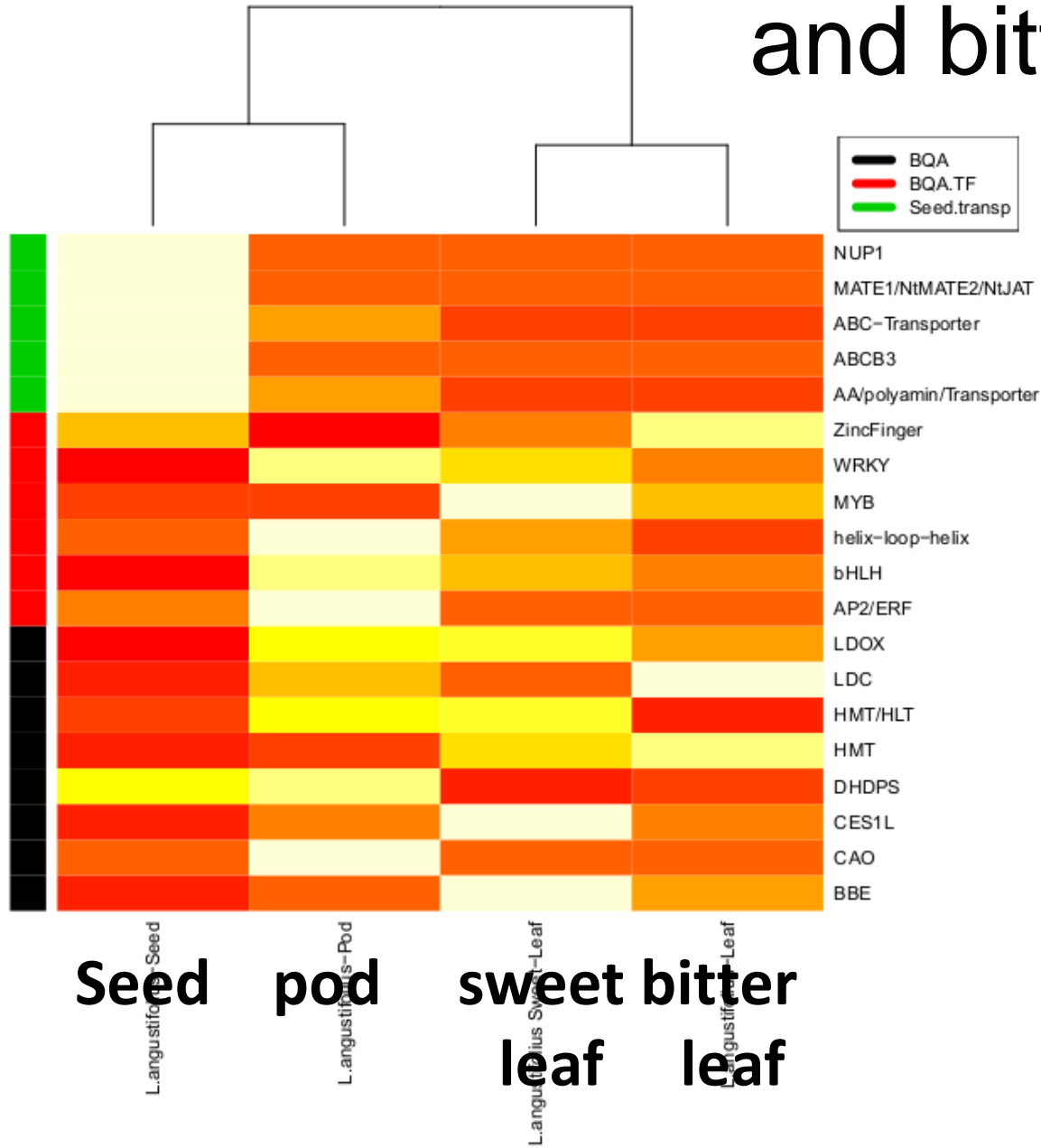
**Seed transporter**

First set of candidate genes

Frick et al Front. Plant Sci., 31 January 2017



# *L.angustifolius* QA related genes: sweet and bitter lupins



Lysine



Lysine decarboxylase (LDC)

Cadaverine



Oxidase / transaminase

Tetracyclic alkaloid

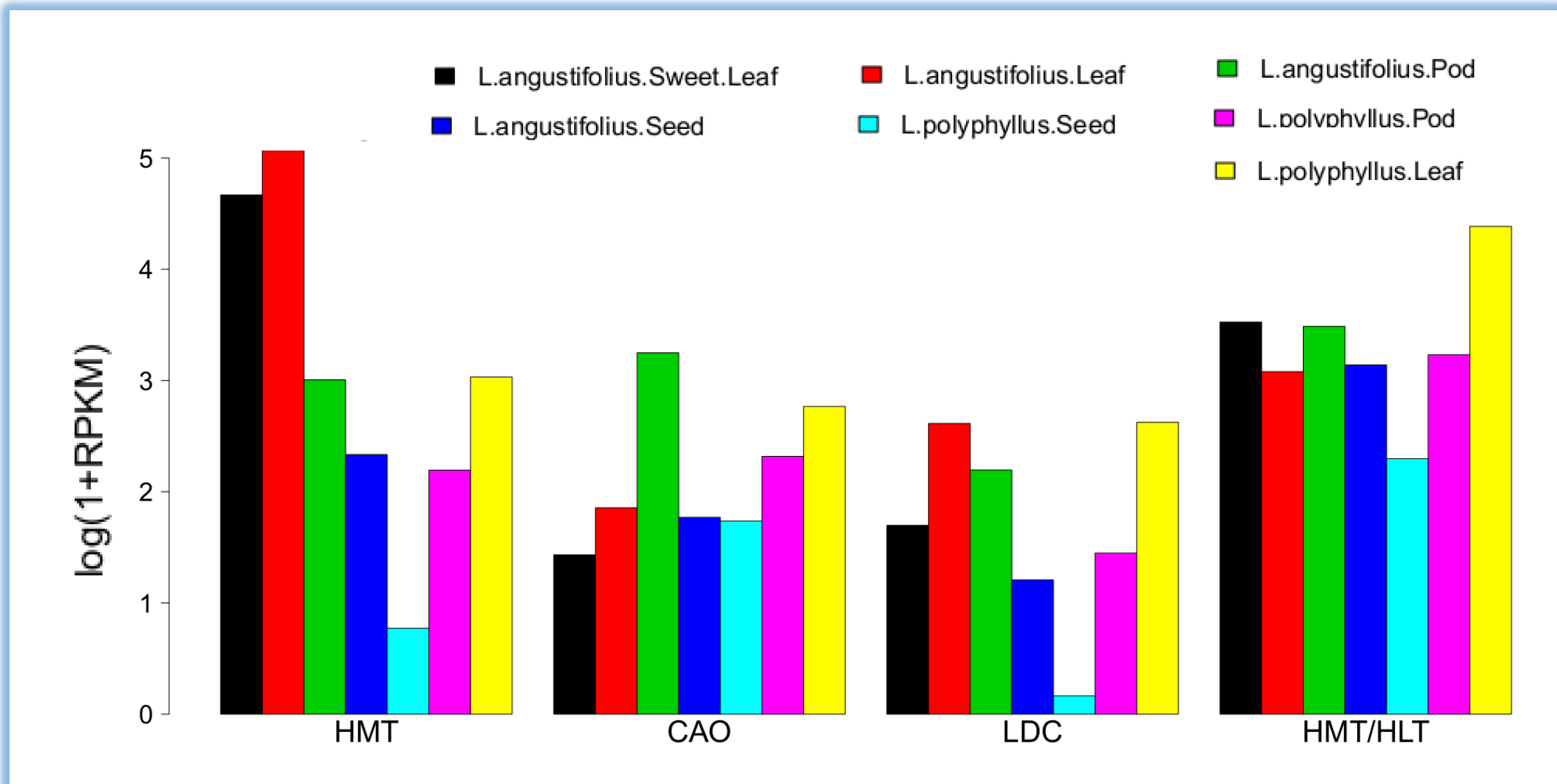


Estertransferase

Esteralkaloids

Seed transporter

# Some candidate QA biosynthesis genes



*Lupinus polyphyllus*

Lysine



Lysine decarboxylase (LDC)

Cadaverine



Oxidase / transaminase

Tetracyclic alkaloid



Estertransferase

Esteralkaloids

Seed transporter



chloroplastic gamma aminobutyrate transaminase

chloroplastic gamma aminobutyrate transaminase

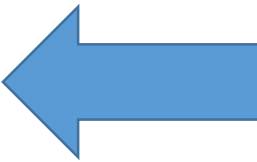
**aminotransferase**

ornithine aminotransferase

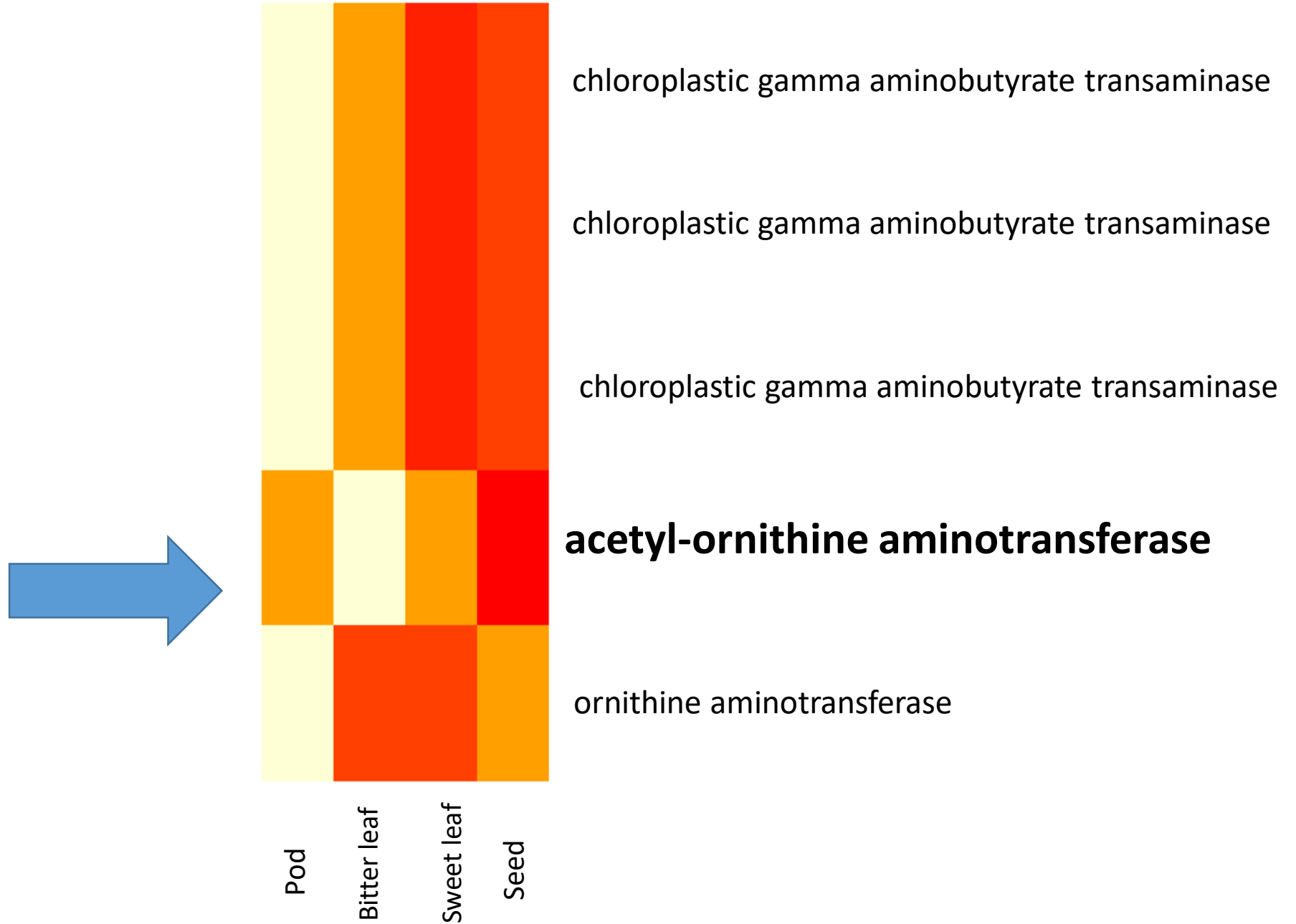
Pod

Leaf

Seed



# Lupinus angustifolius



# Some Candidate Seed transporters

Lysine



Lysine decarboxylase (LDC)

Cadaverine



Oxidase / transaminase

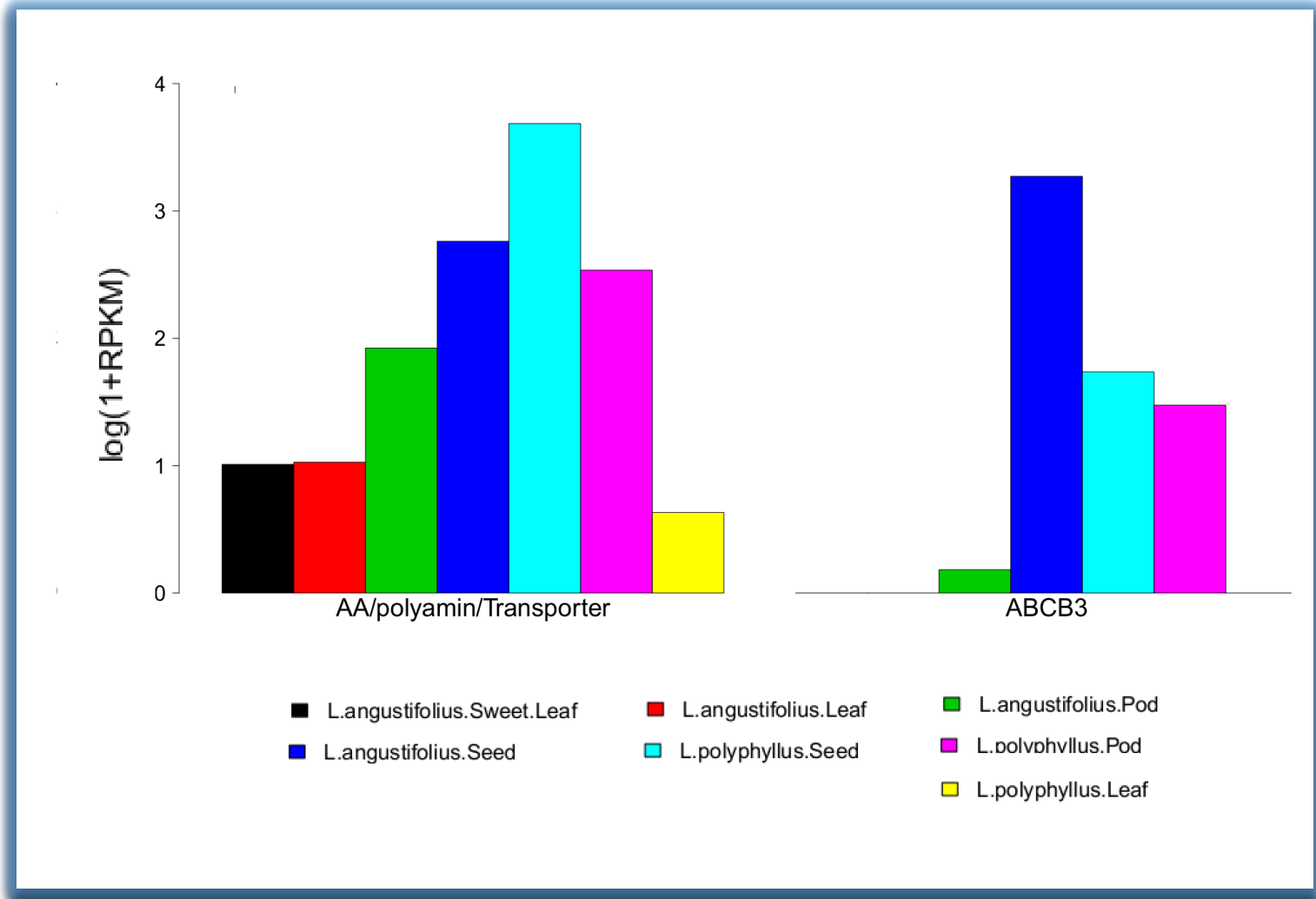
Tetracyclic alkaloid



Estertransferase

Esteralkaloids

Seed transporter



## Conclusions

- **RNASeq is a powerful tool**
- **Identification of candidate genes**
  - **LDC**
  - **Oxidase/ aminotransferase**
  - **Estertransferase**
  - **Seed transporter**
  - **Transcription factors**
- **Deeper analyses**
- **Knock out mutants (CRISPR/Cas)**
- **Translation to lupin breeding & selection**

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**Thanks for your attention**