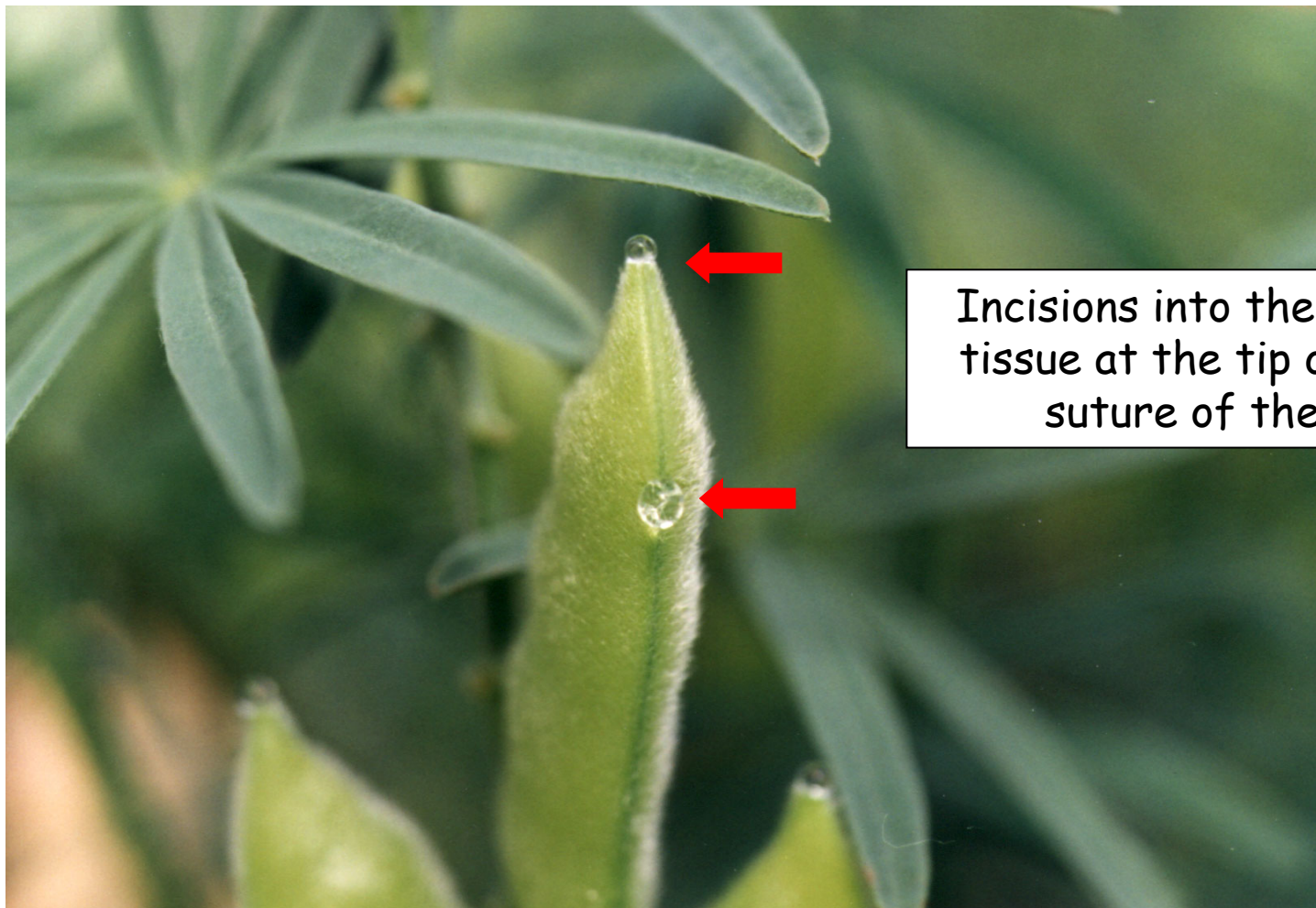


School of Plant Biology, UWA and
School of Biological Sciences,
University of Sydney

Craig Atkins, Penny Smith and Caren Rodriguez

**Lupin- a Model Grain Legume Species for
Studies of Translocation and Signaling**



Incisions into the vascular tissue at the tip or on the suture of the pod

Lupins are compulsive haemophiliacs

Occlusion of sieve tubes at the wound occurs but it is just a bit slow

What is in lupin phloem?

- Water (ca 75% by wt)
- Lots of sucrose (20-30% by wt)
- N- mainly as asparagine (ca 5% by wt)
- All the mineral nutrients required for growth and development
- At least 13 quinolizidine alkaloids
 - Lots of other things

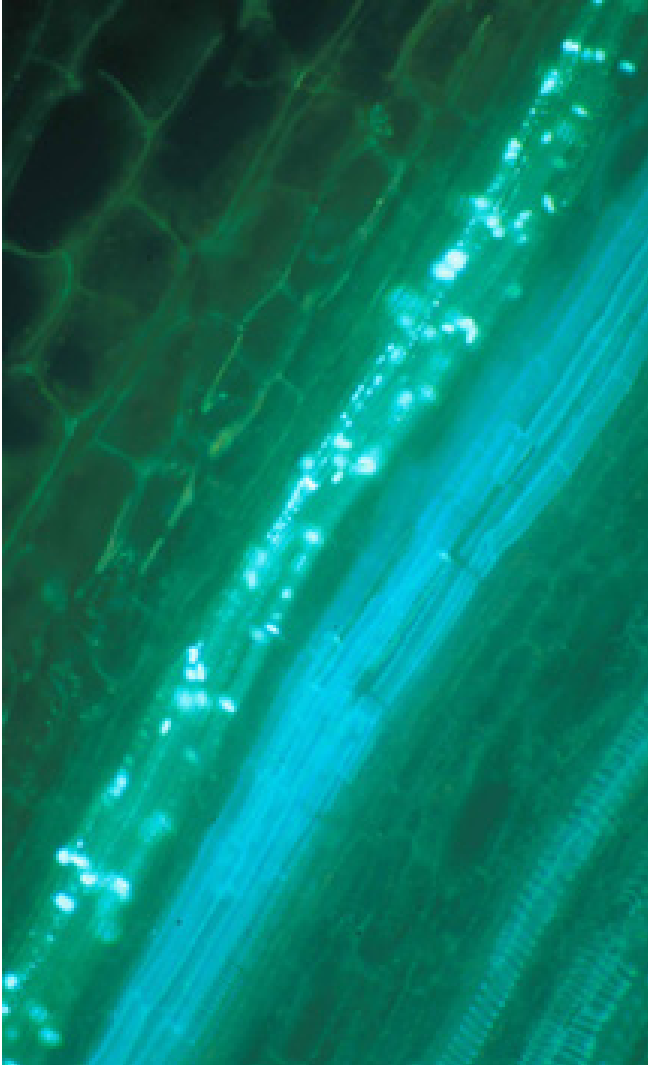
Other things ?

- all the known plant growth regulators or their precursors
- low MW peptides (bioactive?)
- more than 200 proteins
- mRNA
- siRNA
- miRNA
- unknown translocated signals



both these classes of small RNAs are extremely powerful regulators of gene expression

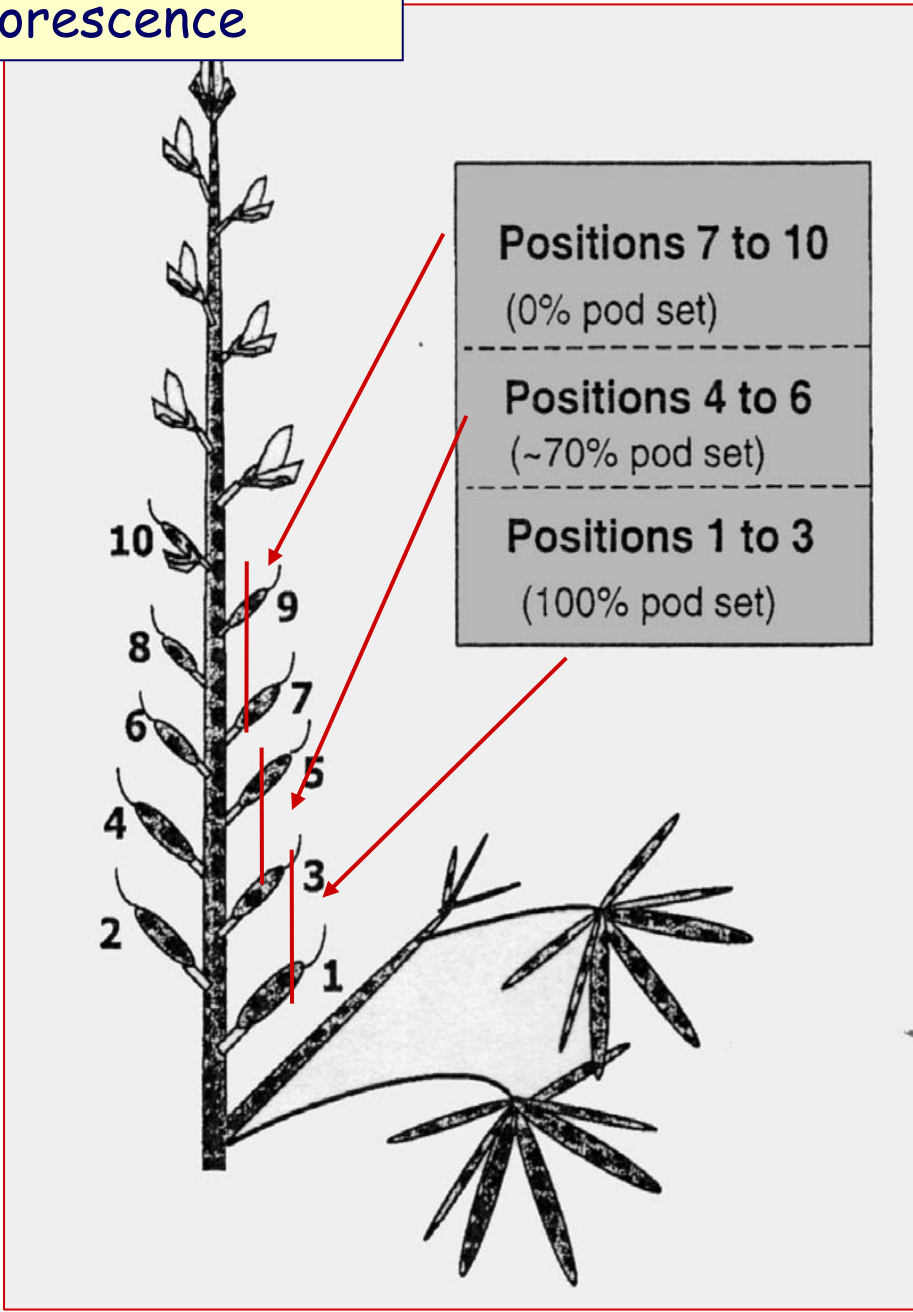
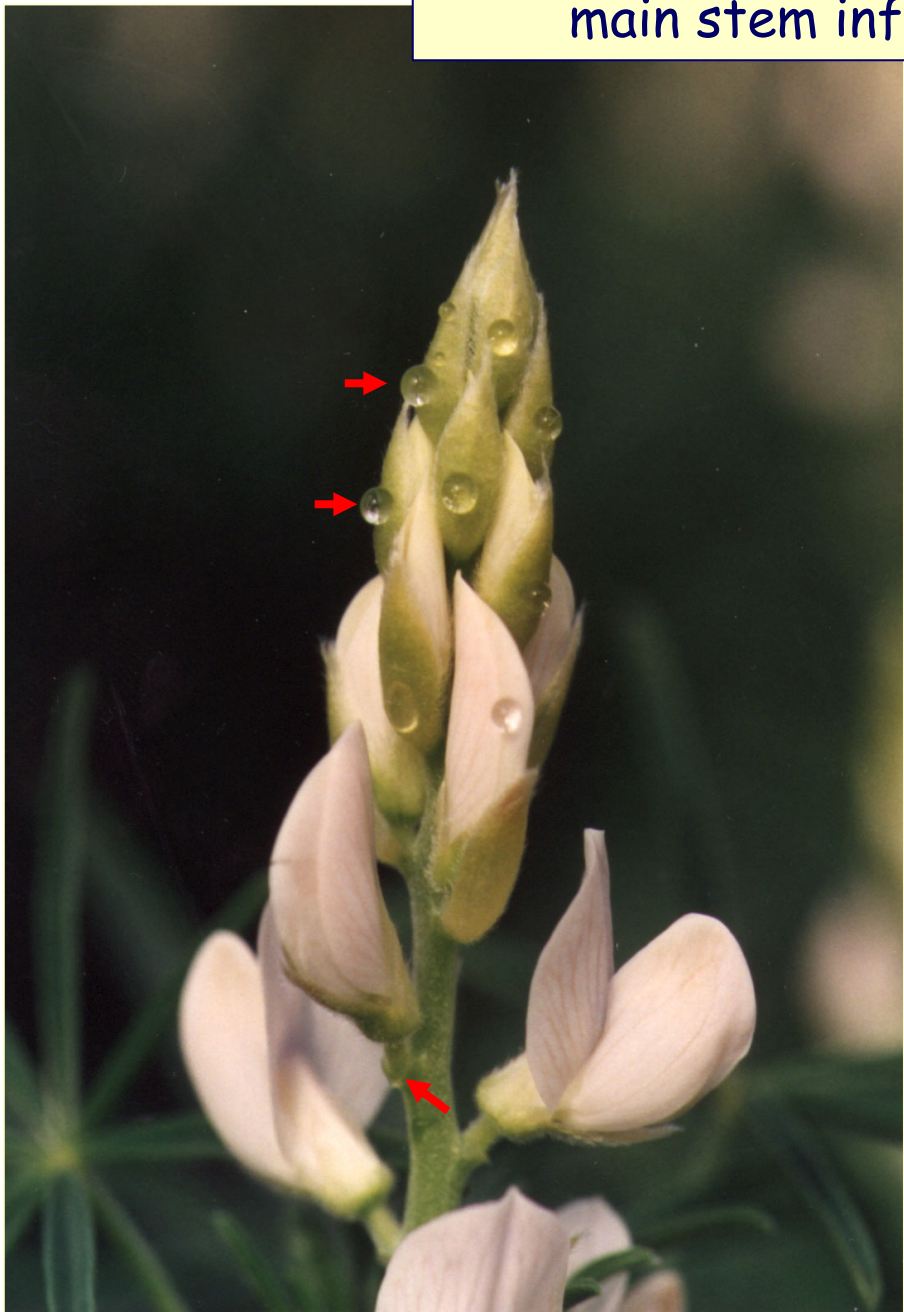
Phloem acts as a long distance communication network



- floral induction
- lateral/cluster root formation
- root development in response to soil N
- autoregulation of nodulation
- Pi accumulation
- establishment of leaf polarity?
- probably lots of other things

(pedicel- *Lupinus mutabilis*- aniline blue- UV)

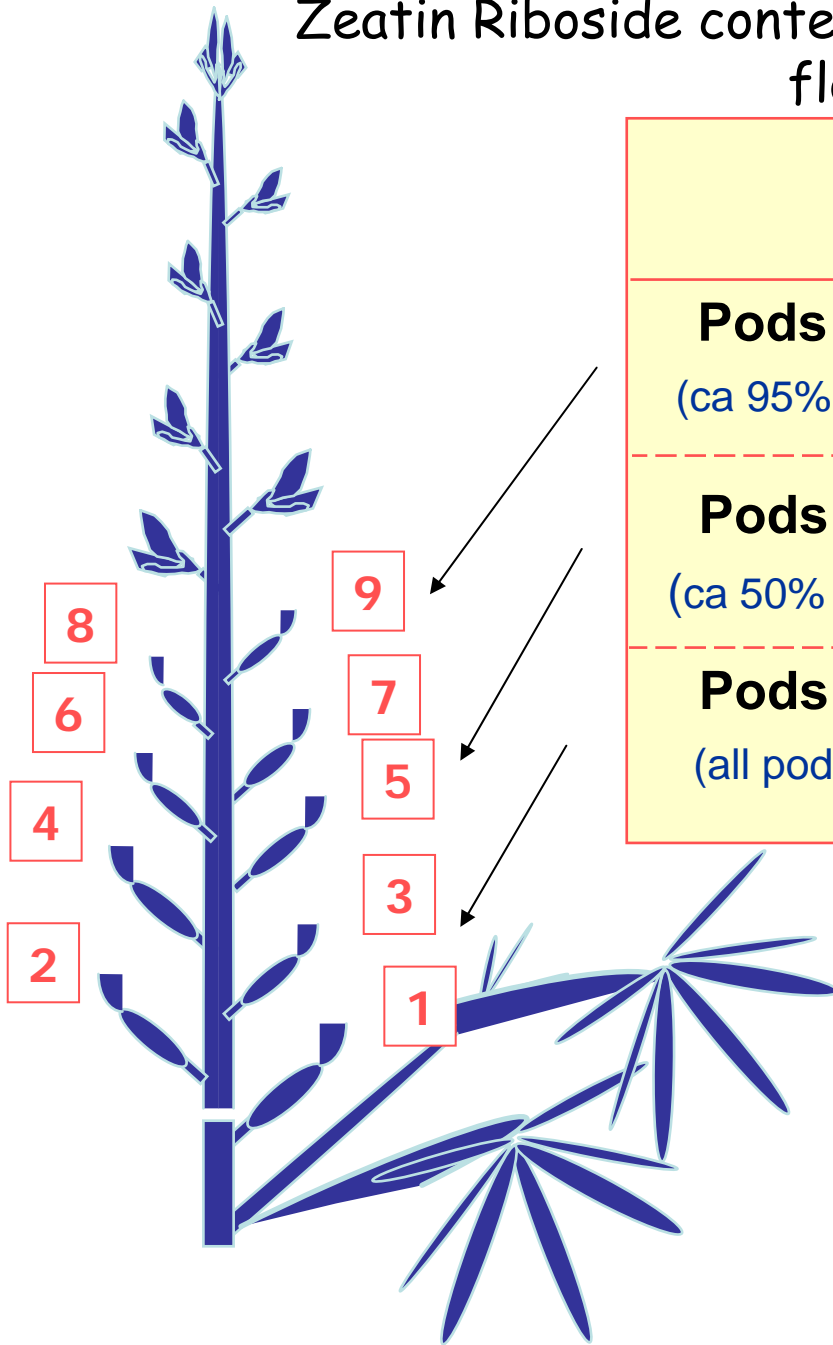
main stem inflorescence



Podset is an important agronomic trait

- in all our grain crops the rate of abortion determines yield potential
 - clearly heritable as HI
- impacted by environmental 'stress'
- legumes, including lupins, are especially prone to excessive flower abortion
 - **i.e. they set up a very variable sink**
 - to say the least this feature frustrates farmers

Zeatin Riboside content 4 days after anthesis at each floret position



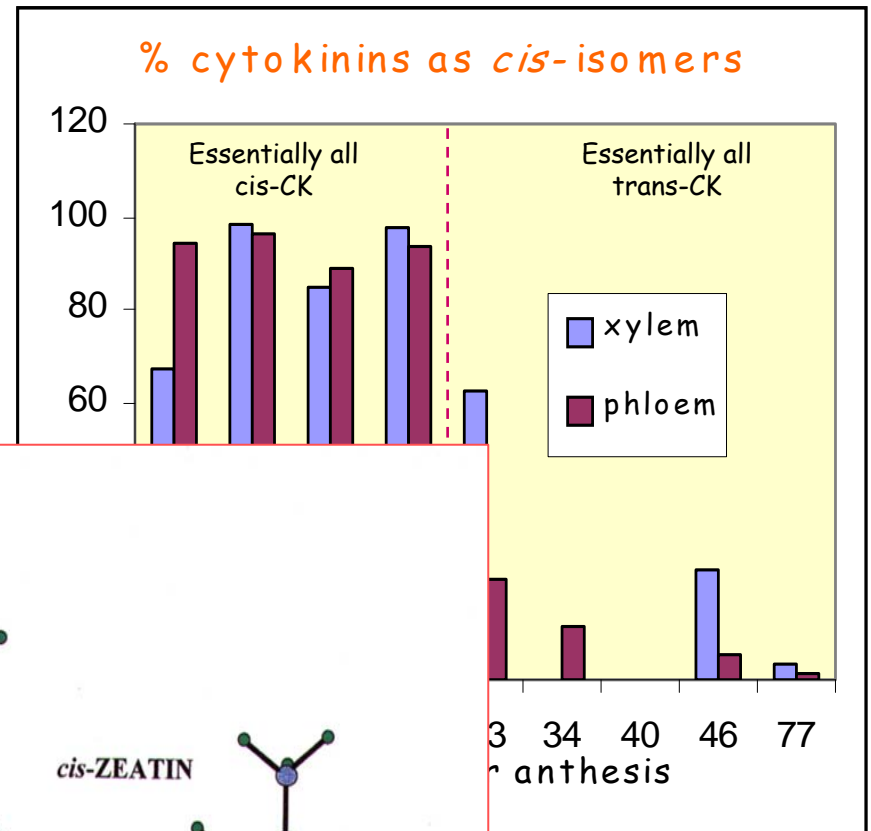
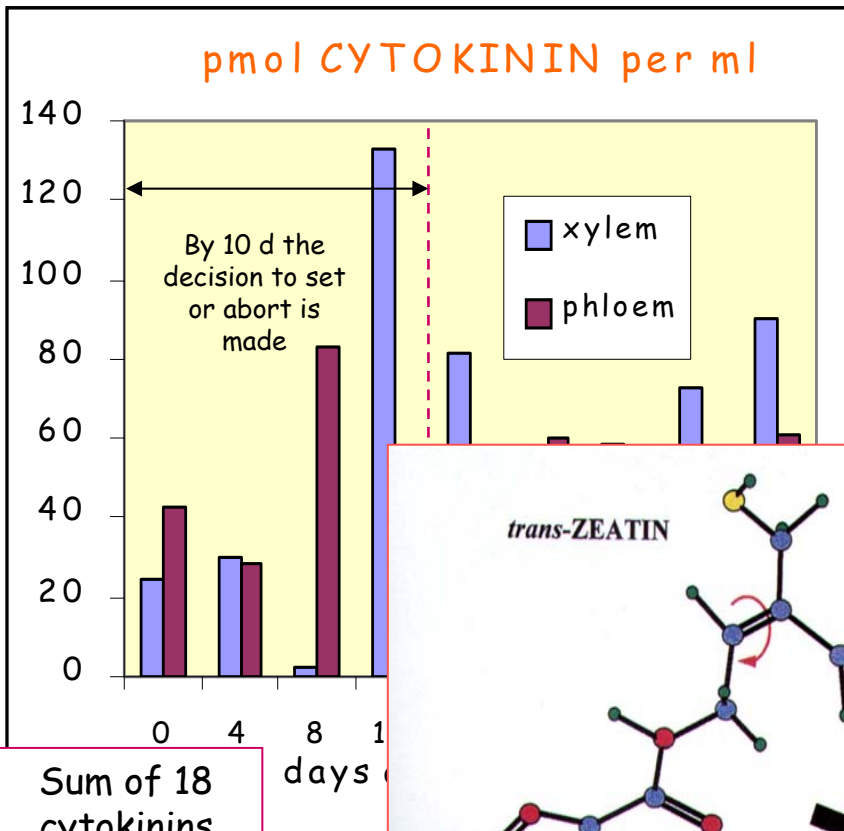
	<i>cis</i> -ZR (ng/gFwt)	<i>trans</i> -ZR (ng/gFwt)
Pods 7 to 9 (ca 95% abortion)	110	0
Pods 4 to 6 (ca 50% abortion)	80	20
Pods 1 to 3 (all pods set)	21	35

inactive?

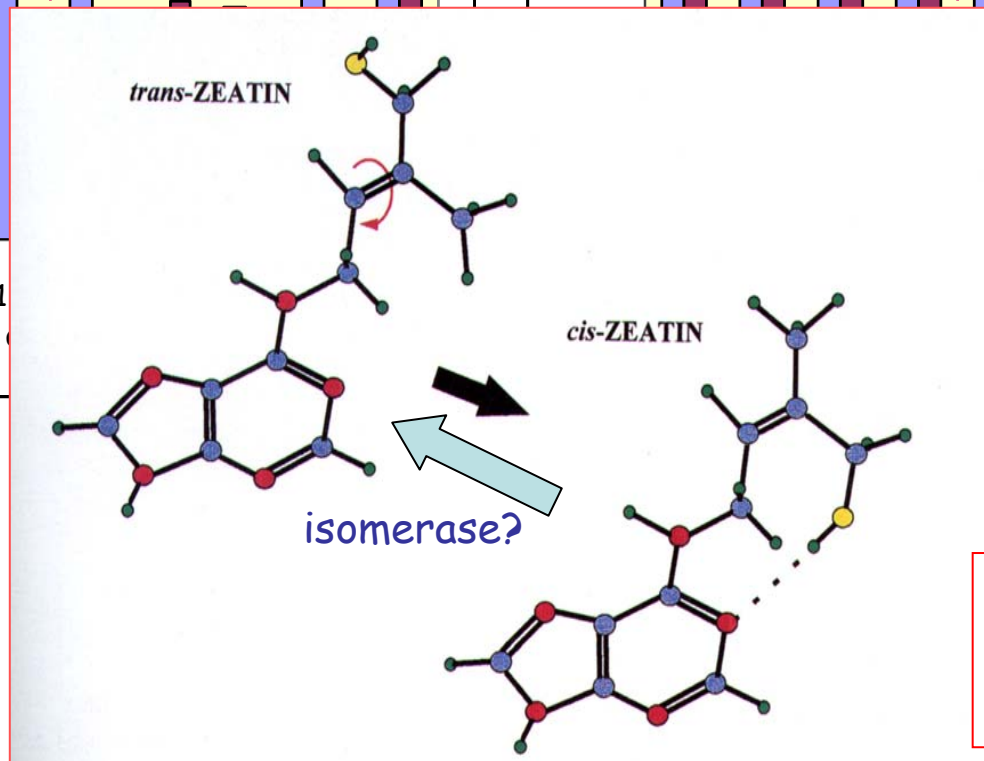
active?

given the 'role' of CK in regulating cell cycle activity and cell division, having a lot of inactive isomer around is not likely to be positive

Along with assimilates translocation also delivers **cytokinins**



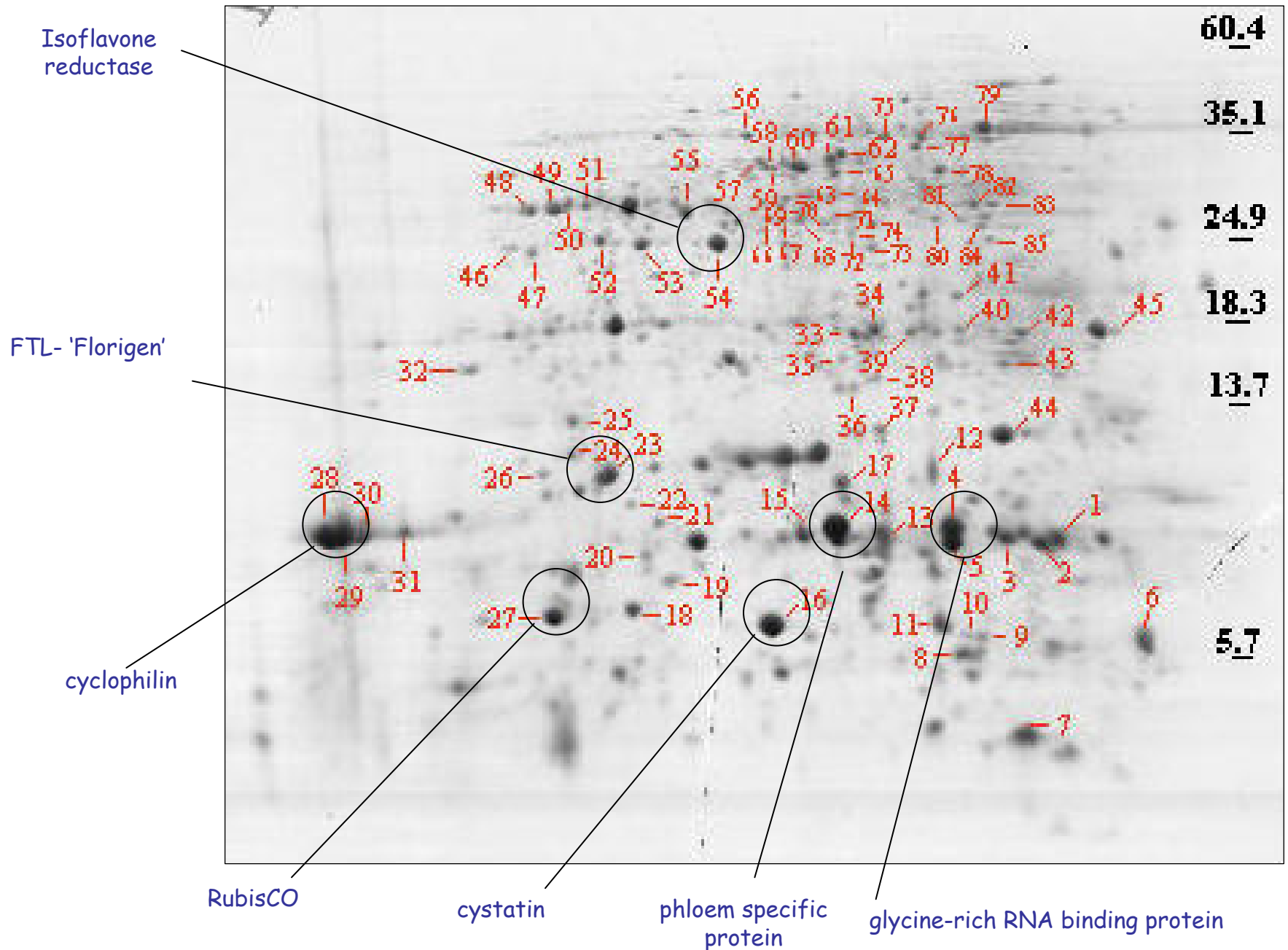
Sum of 18 cytokinins measured by GC-MS using standards labelled with stable isotopes



as yet no enzyme and no gene

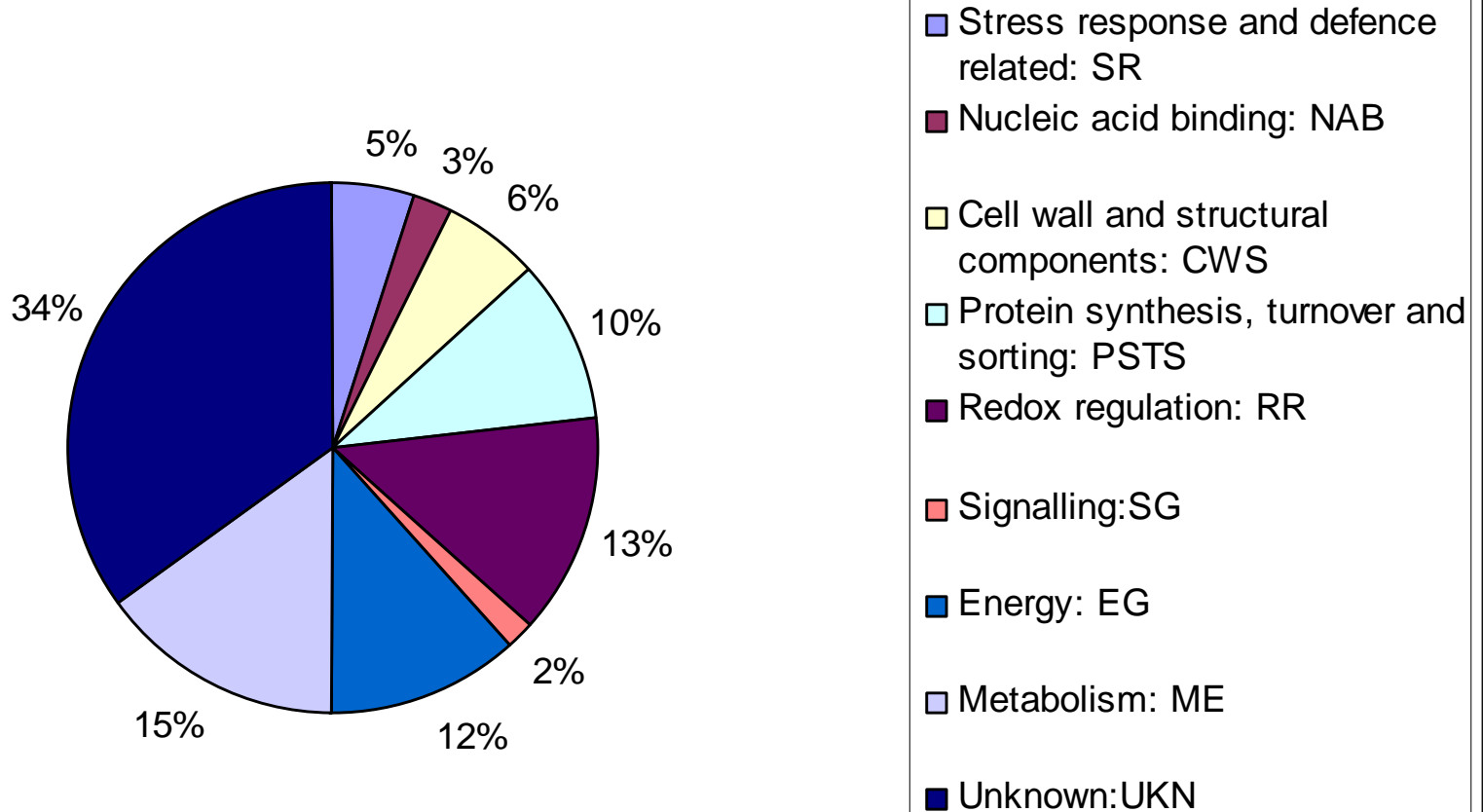
2D-Gel Electrophoresis of proteins from 10 ml phloem from *L. albus*

kDa



PROTEOMIC ANALYSIS

Peptide Sequences for 120 proteins in *L. albus* phloem exudate



Transcriptomic analysis of *L. albus* phloem exudate

- Sequenced ca 1000 ESTs
- ca 800 matches from the databases but many unnamed or unknown
- 20 or more viral protein transcripts (BYMV)
- Proteins associated with energy and carbohydrate metabolism, N metabolism
- Proteins related to stress responses, oxidative stress etc
- Proteins like tubulin associated with cell wall formation etc
- Many, many more- the detailed analysis is in train

MicroRNAs

- small RNAs (19-24nt)
 - non-coding
- act at post-transcriptional level to silence target genes
- mutants in miRNA biogenesis have severe developmental abnormalities

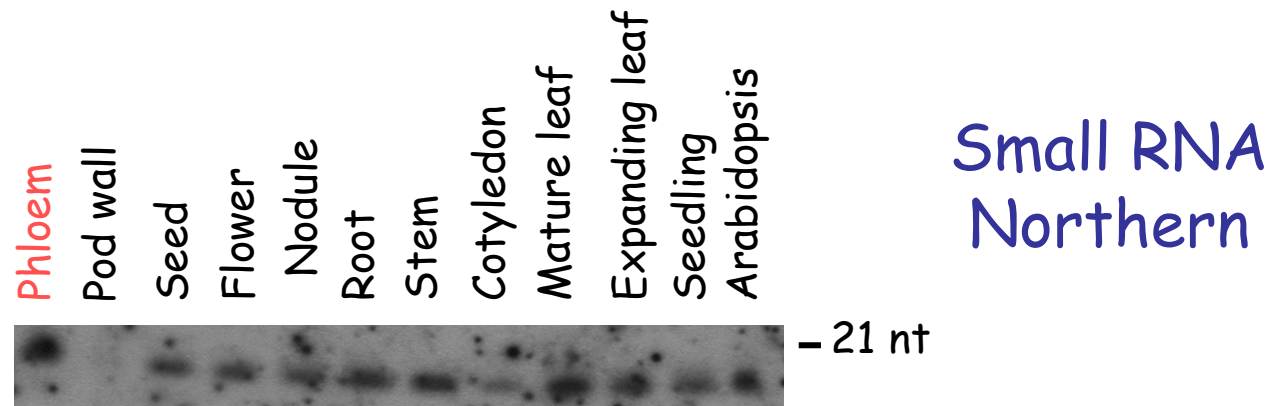
Could miRNAs act as signaling molecules transported in phloem?

- miRNAs act in a similar manner to small interfering RNAs (siRNAs) which act systemically to silence transgenes
 - miRNA target genes have roles in developmental processes
- some miRNAs are regulated in response to environmental conditions (stress or nutrient availability)
 - this makes them ideal candidates as transported signaling molecules

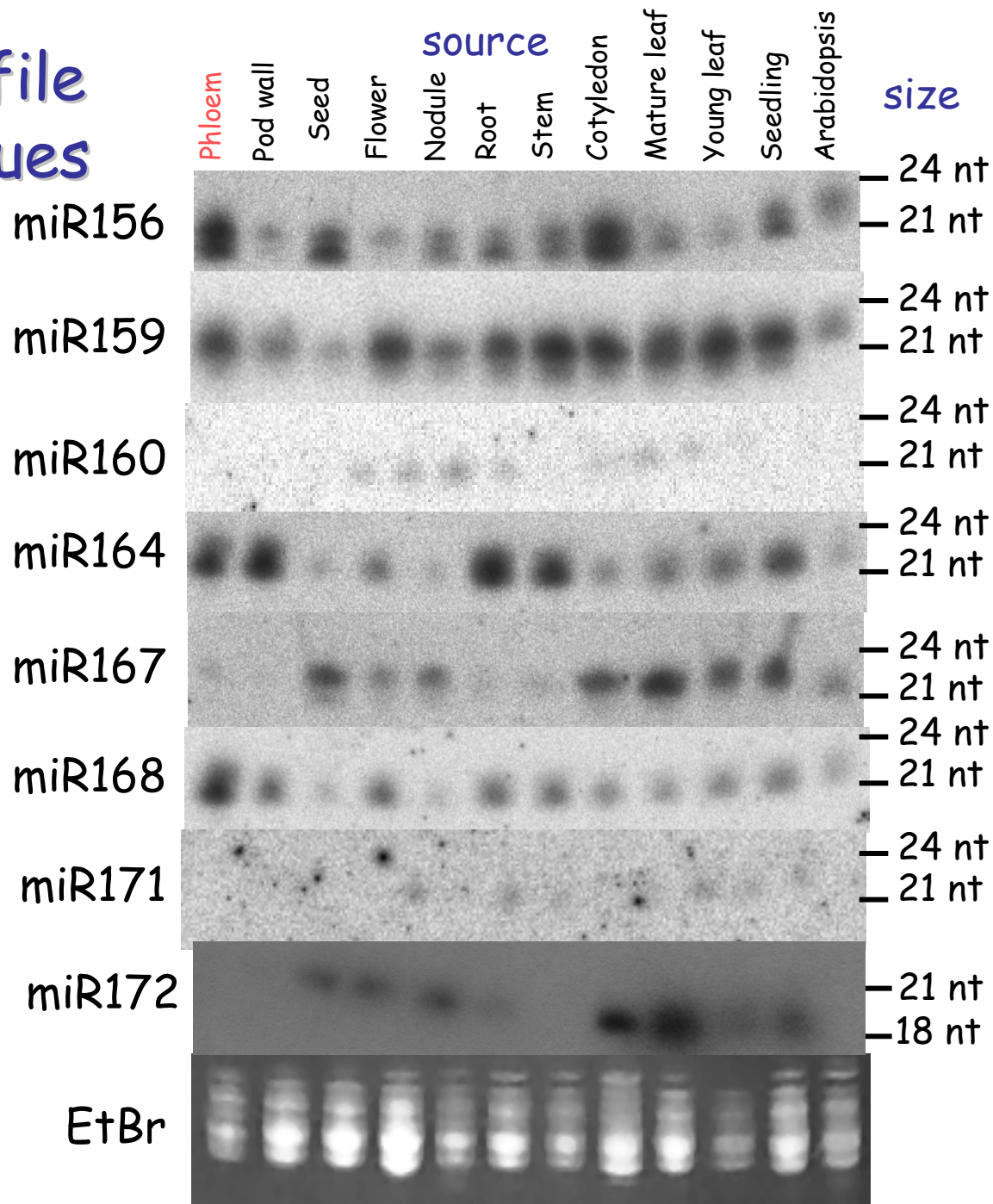
miR166 in white lupin (*L. albus*)

- miRNA is conserved in rice and Arabidopsis
 - used complementary sequence as probe
- ³²P-labelled oligonucleotide probe for small RNA gel blot

takes 1-2 ml of phloem to isolate 2 μ g of small RNA

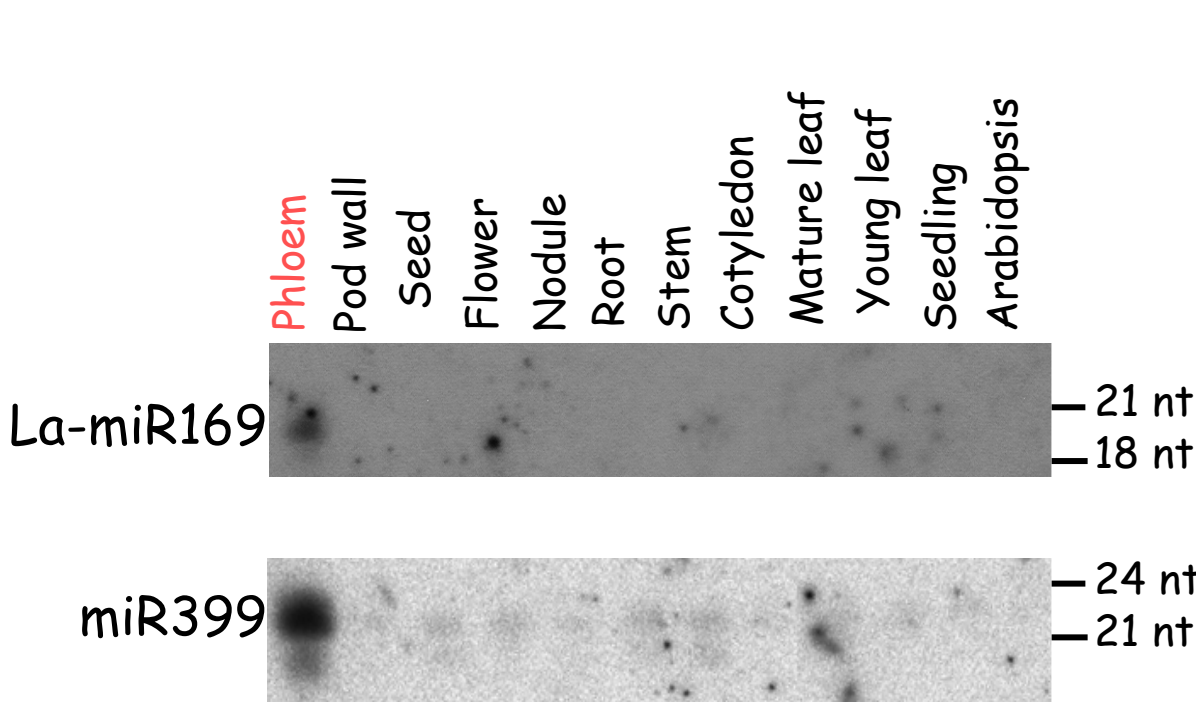


miRNA profile in lupin tissues



~2 μ g small
RNA / lane

miRNAs with more restricted distribution



Arabidopsis targets

CCAAT-binding factor-
HAP2-like proteins
involved in specification of
cotyledon identity &
embryo maturation

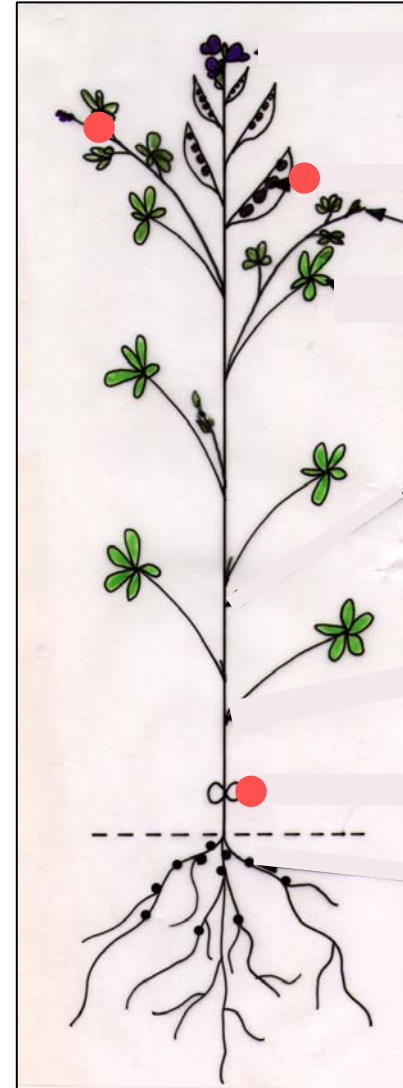
ubiquitin conjugating
enzyme

With advent of locked nucleic acid probes we can confirm localisation
via *in situ* hybridisation

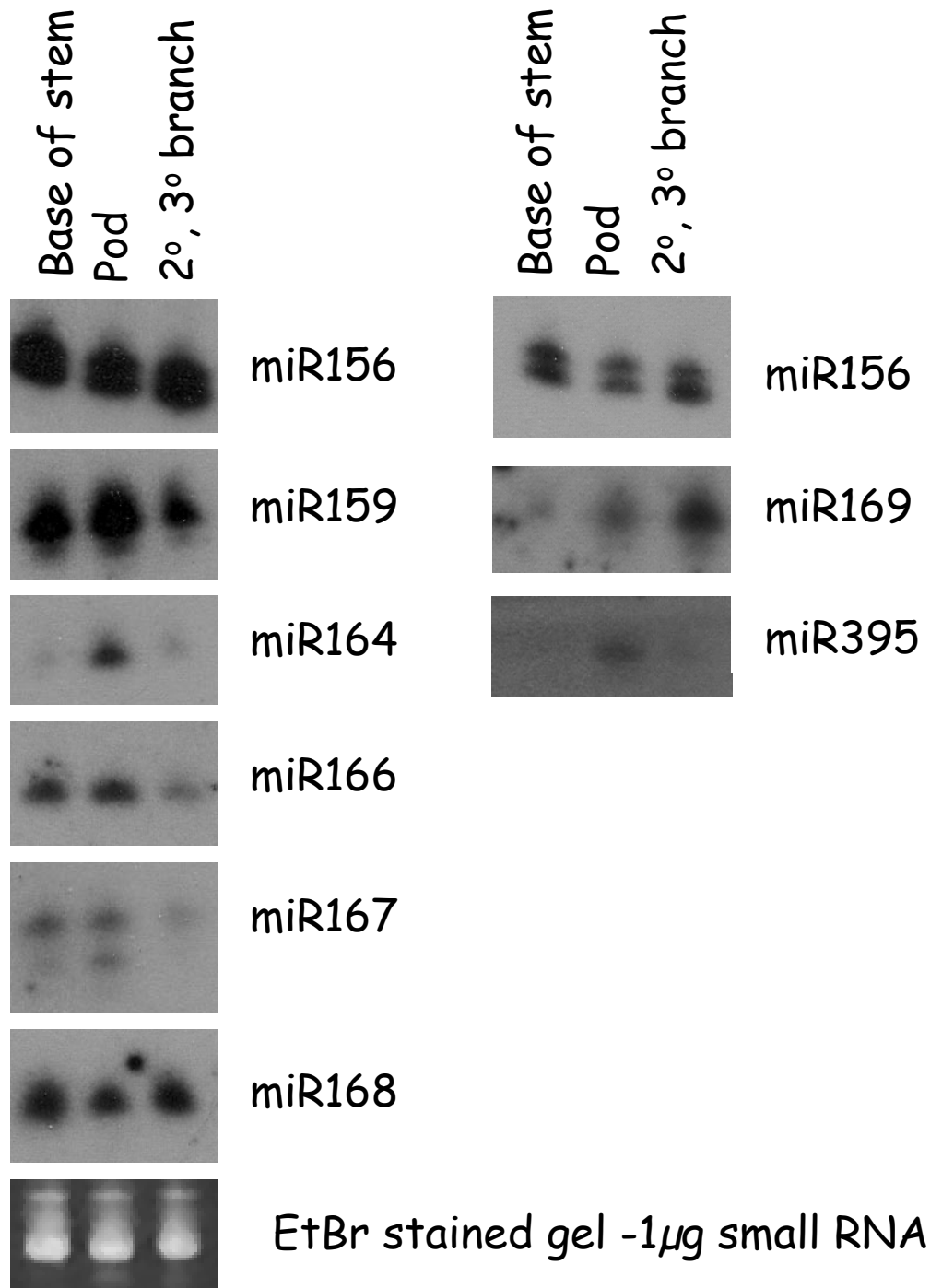
miRNA cloned from <i>L. albus</i>	Library	Predicted target families in Arabidopsis	Function of target gene/s
miR159*	phloem	MYB (8), TCP (5) transcription factors	cell proliferation & differentiation
miR164*	phloem	NAC domain proteins / CUC1, CUC2, NAC1, +2	SAM formation, auxin-induced lateral root growth
miR165/166*	phloem	HD-Zip transcription factors	Vascular & leaf development, leaf polarity
miR168*	phloem	ARGONAUTE	gene silencing
miR169*	phloem	CCAAT-binding factor-HAP2-like proteins	specification of cotyledon identity, embryo maturation
miR172*	seedling	AP2-like transcription factors	floral organ identity, time of flowering, seed mass and yield
miR393	seedling	F-box proteins, bHLH transcription factors	
miR395*	phloem		ATP sulphurylases, sulphate transporters
miR398	phloem		copper superoxide dismutase, cytochrome C oxidase
miR399*	phloem		Ubiquitin conjugating enzyme, phosphate transporter
miR408	seedling		peptide release factor, plantacyanin

Translocation of miRNAs?

- if translocated is there specificity in loading and unloading?
- does miRNA profile differ in phloem from different parts of the plant?



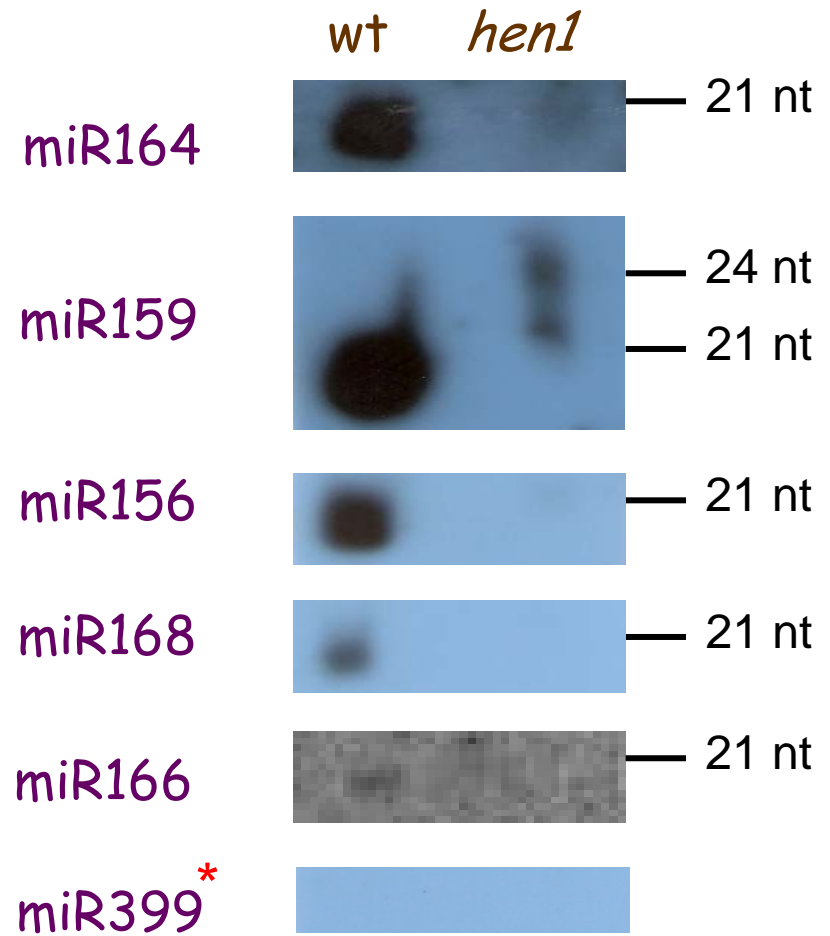
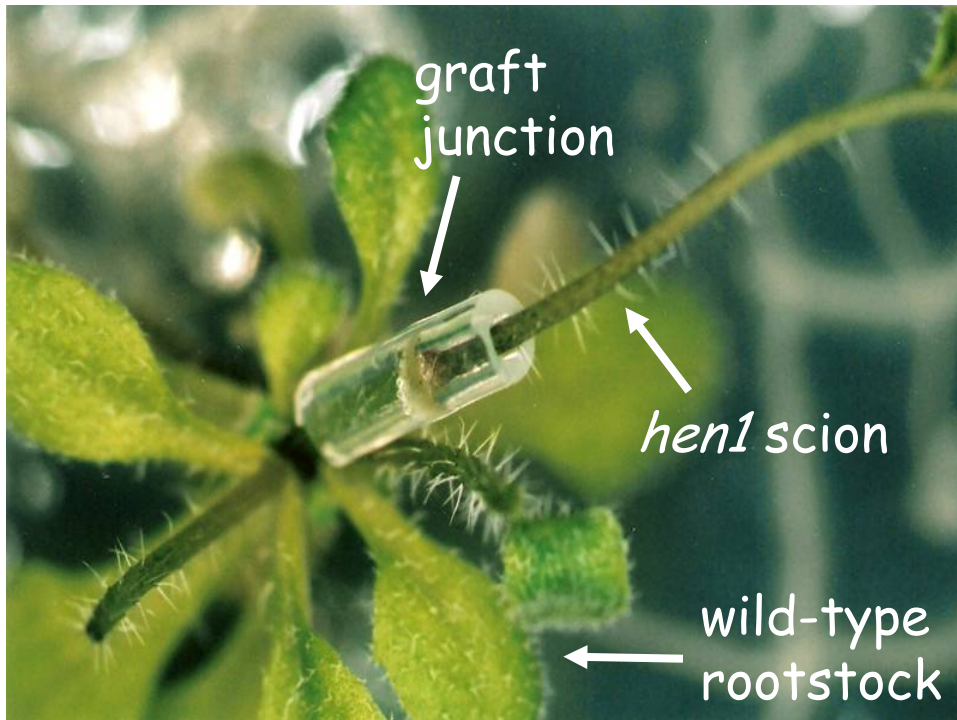
MiRNA profile
is different in
phloem from
different
sites in the
plant



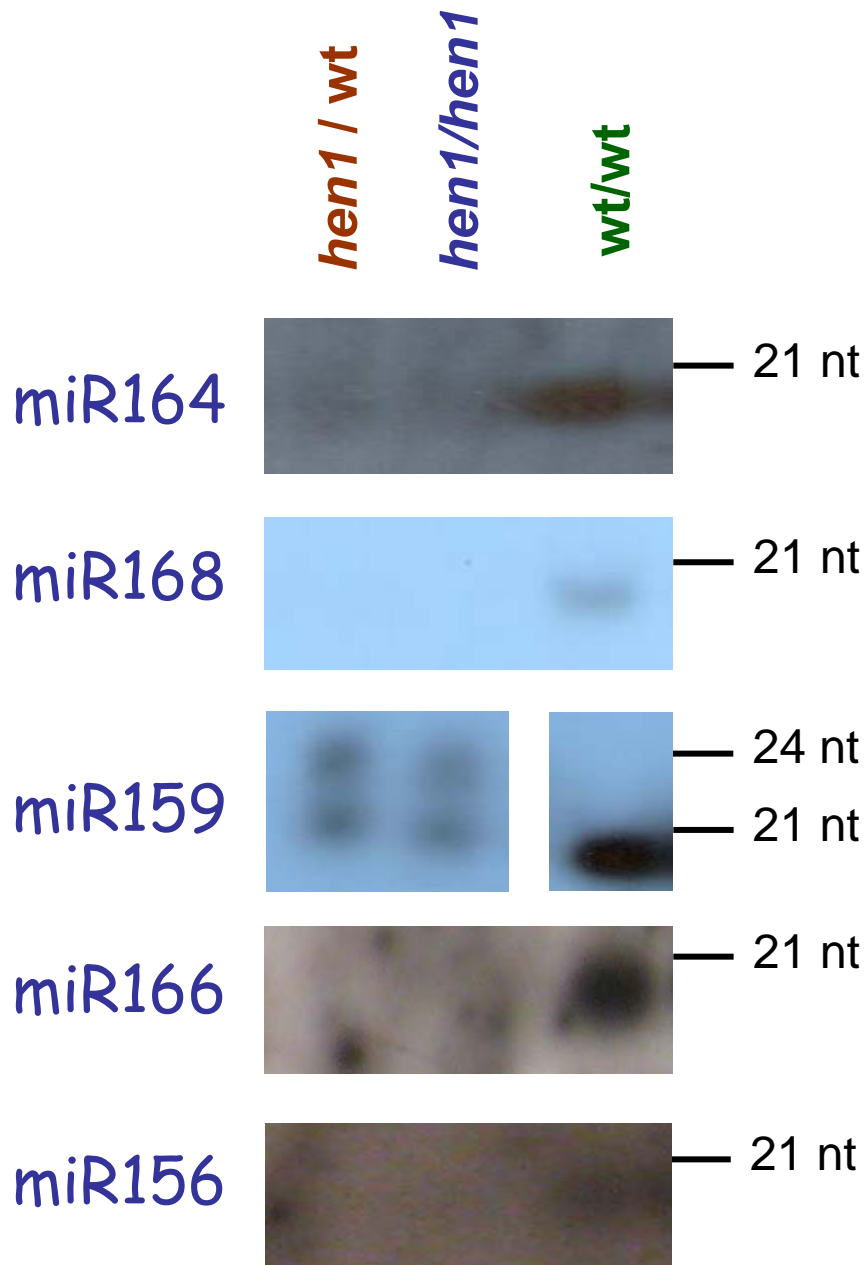
Are micro RNAs phloem mobile?

◆ HEN1 protein methylates miRNAs

◆ *hen1* mutant exhibits a number of developmental defects



* only expresses in response to Pi deprivation



No change in *hen1* scion phenotype was observed



No evidence of miRNA translocation across the graft junction was observed

- There is no evidence of miRNA translocation in phloem.
- miRNAs expression could be cell autonomous.
- miRNAs could have limited cell to cell movement.
 - Could trans-acting siRNAs (ta-siRNAs) be the phloem mobile signal?
 - early days

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