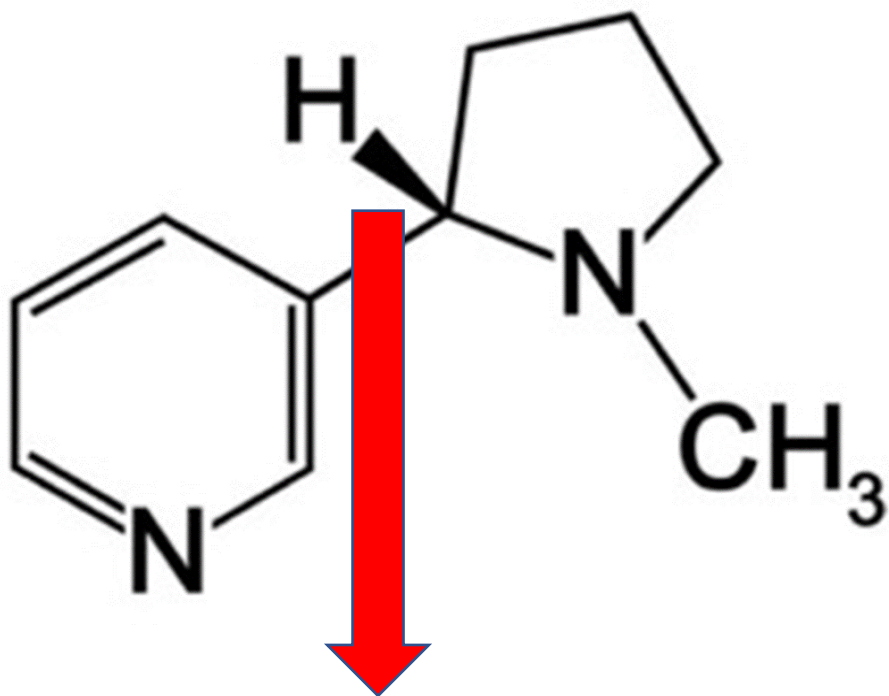


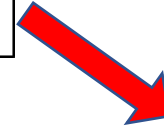
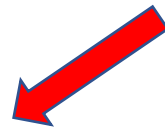
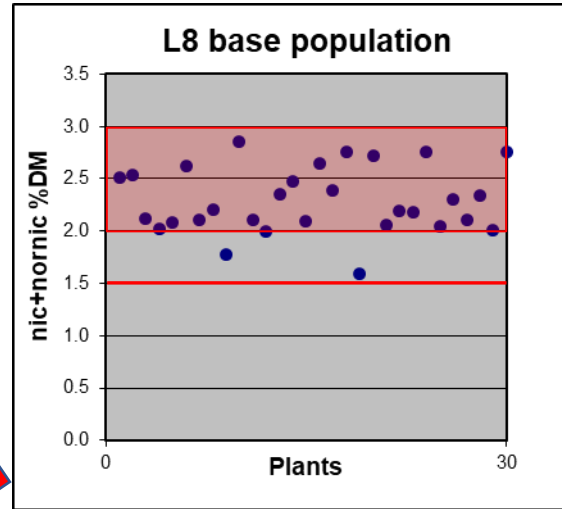
A NOVEL LOW ALKALOID GENE

Anne Fisher, Barunava Patra, Xia Wu, Sanjay Singh, Colin Fisher, Huihua Ji
University of Kentucky, USA

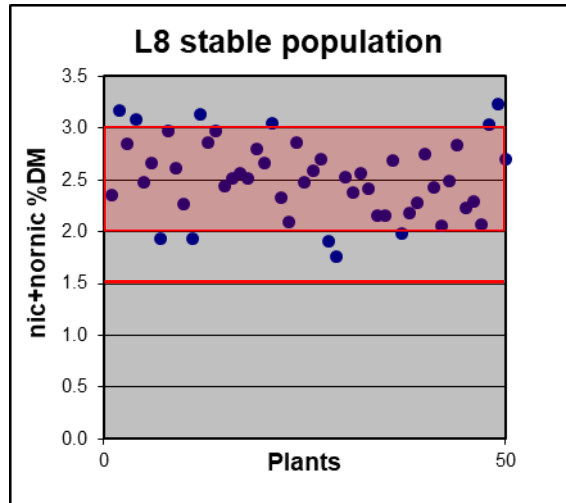


Background – L8 Populations

Single Plant Selection for Conversion

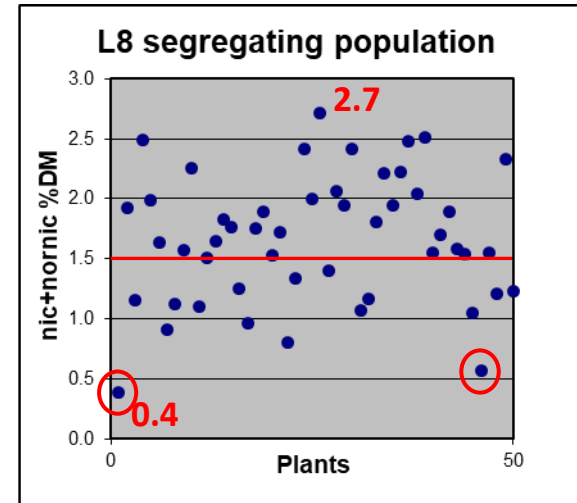


After several generations
single plant selection:



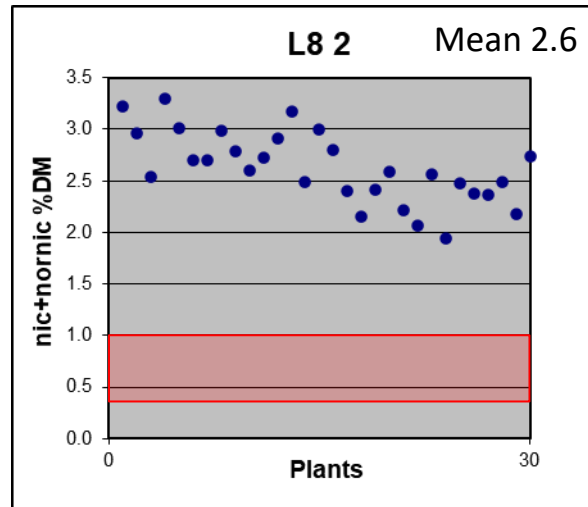
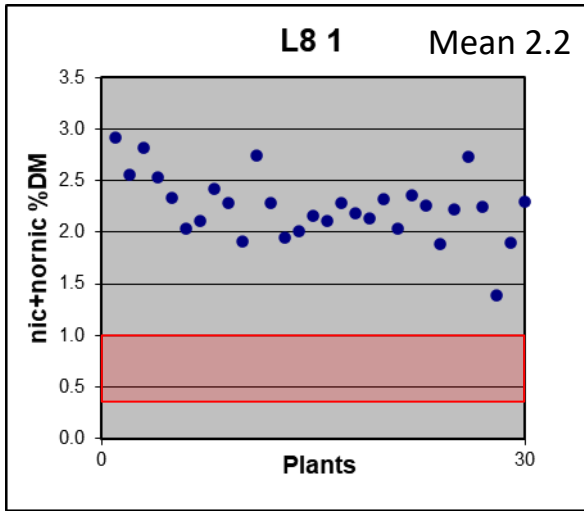
Mean 2.5

Mean 1.7



High & Low Alkaloid L8 Families

2 Families Selected in Parallel from Same Base Population

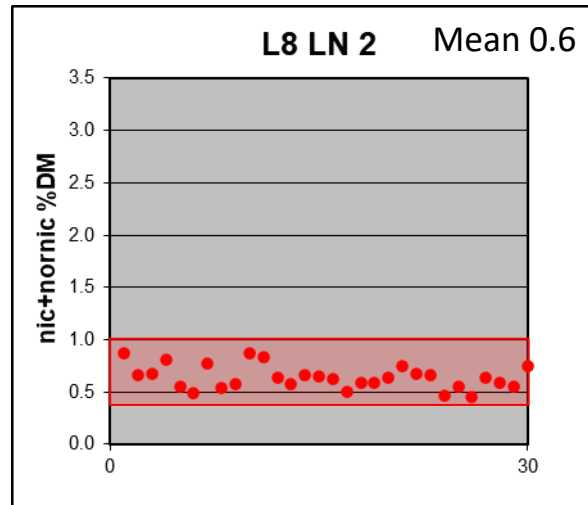
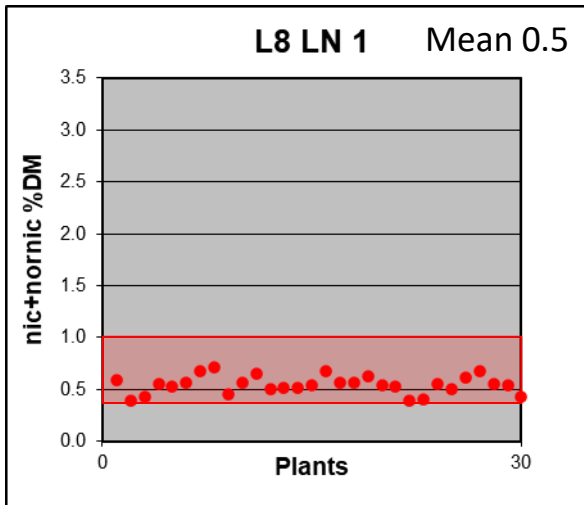


Normal nicotine



- No overlap
- LA *nic1 nic2* mutants?

- Unlikely – lower nicotine family 20-25% of sister family, LA 10-15%
- Designated LN



Lower nicotine

Nic1, Nic2 Markers – L8 Lines & Checks

Line	Nic1 Marker	Nic2 Marker	Nic + Nornic (% DM)	Nic + Nornic Mean (% DM)	Designation
TN 90LC HA check	AA	BB	2.99	3.2	HA
	AA	BB	3.57		
L8 1	AA	bb	2.53	2.3	HI
	AA	bb	2.11		
	AA	bb	2.29		
L8 2	AA	bb	3.23	2.6	HI
	AA	bb	3.30		
	AA	bb	3.30		
	AA	bb	3.01		
	AA	bb	2.71		
L8 LN 1	AA	bb	0.53	0.5	HI ?
	AA	bb	0.53		
	AA	bb	0.54		
	AA	bb	0.43		
	AA	bb	0.50		
L8 LN 2	AA	bb	0.81	0.6	HI ?
	AA	bb	0.49		
	AA	bb	0.58		
LA Bu 21 LA check	aa	bb		~ 0.3	LA
	aa	bb	n/a		
	aa	bb			

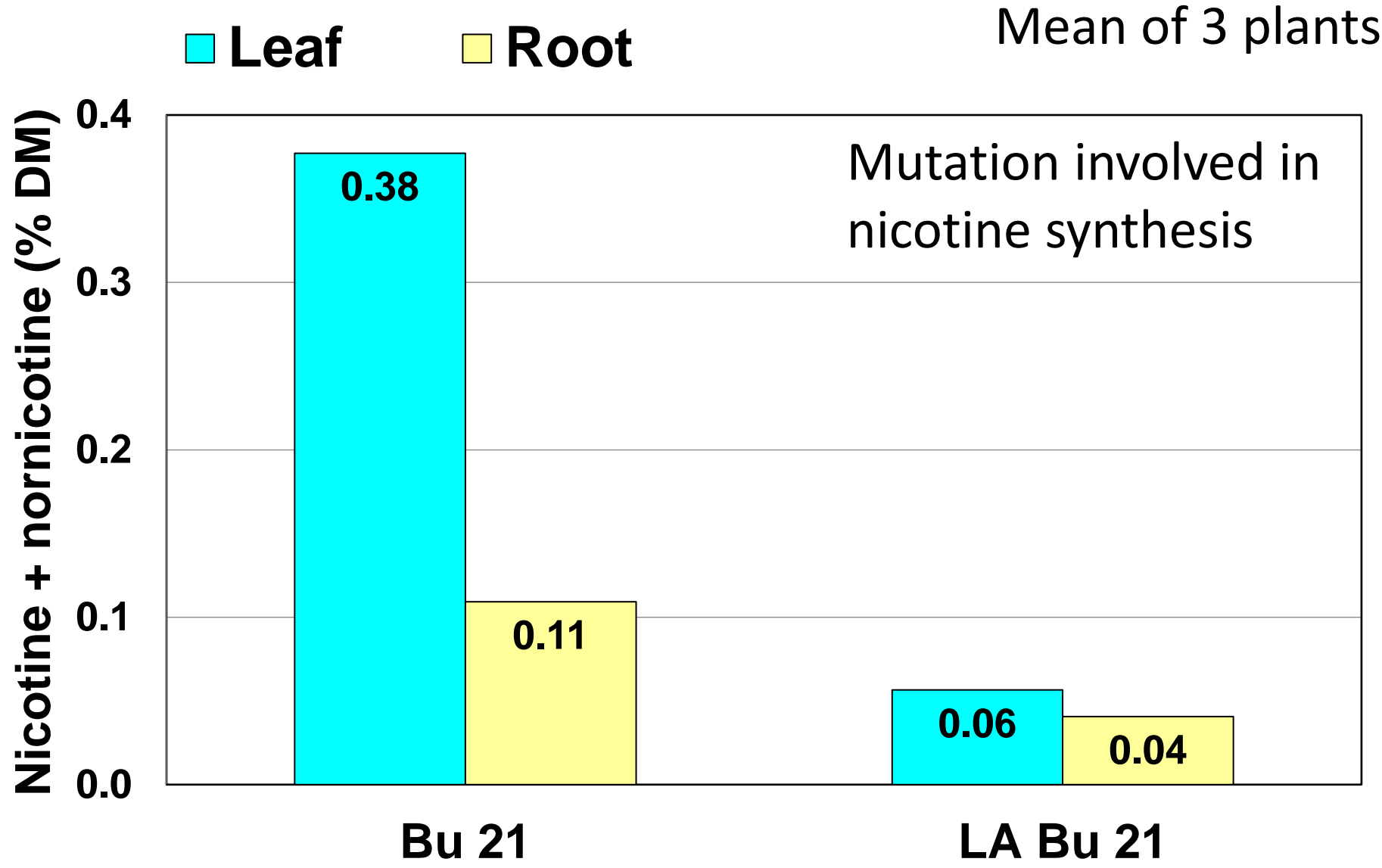
Sampled Leaves & Roots of L8 Lines

L8 (higher nicotine) & L8 LN (lower nicotine) families

Alkaloid analysis



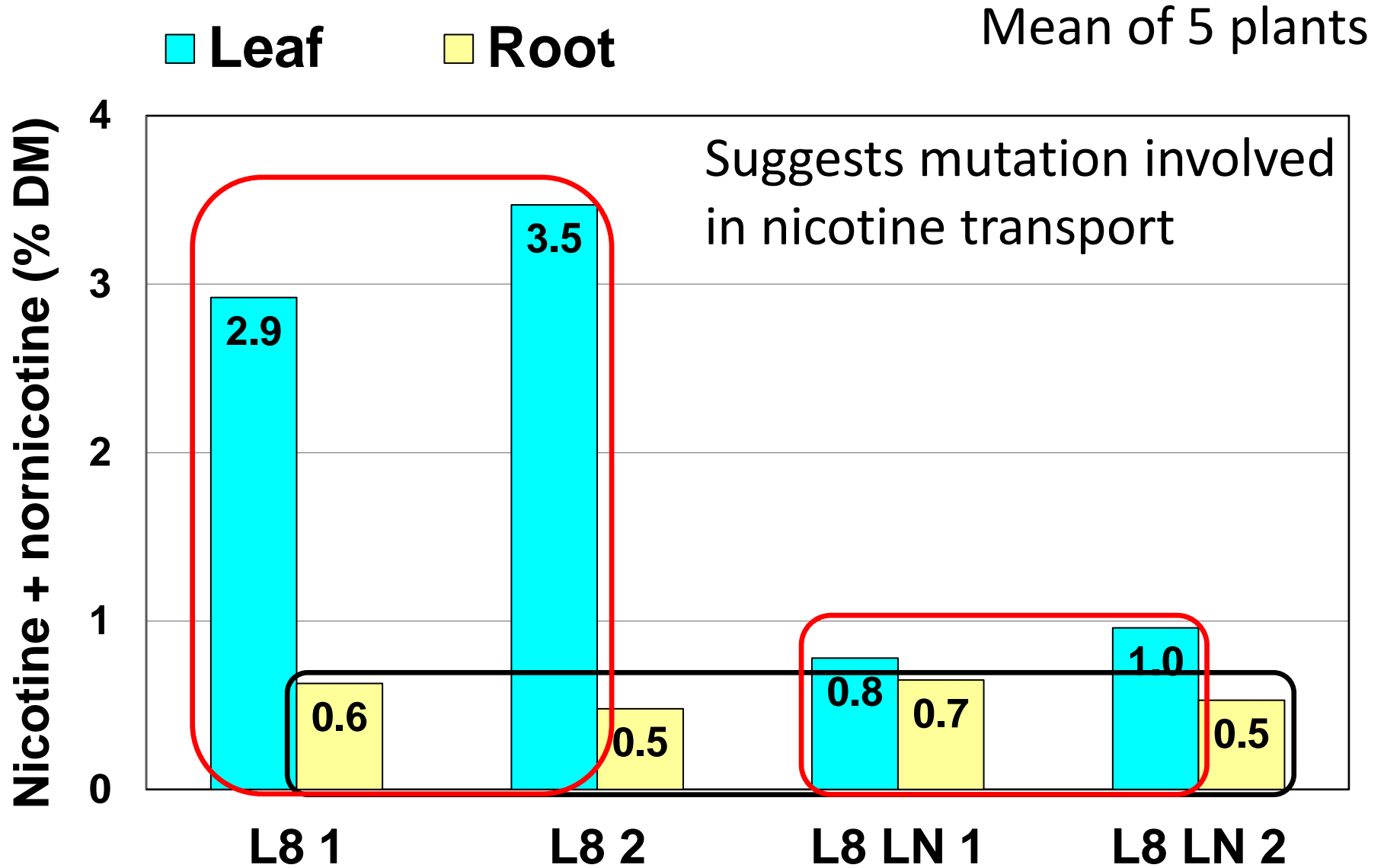
Nicotine + Nornicotine in Leaf and Root – HA vs LA



Young plants in hydroponics

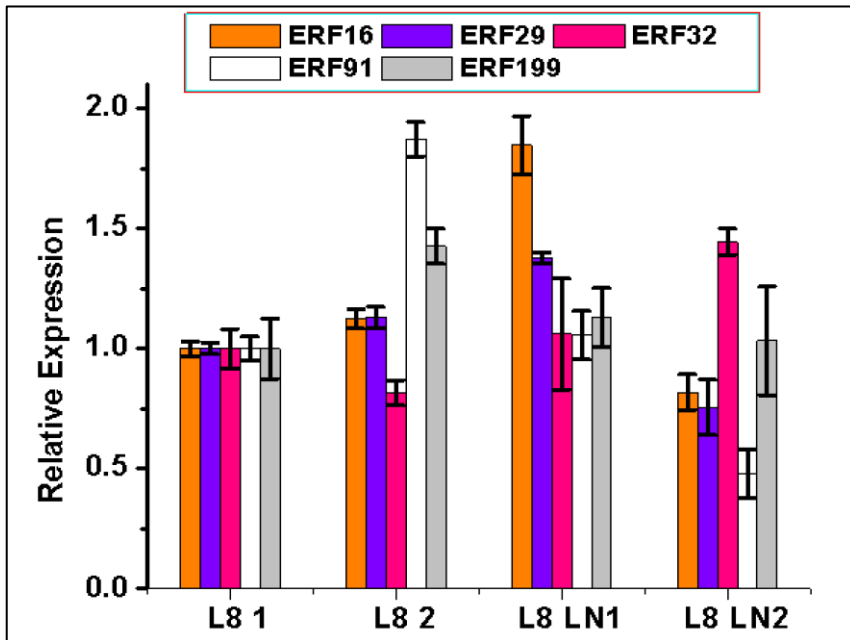
Barunava Patra & Sanjay Singh

Nicotine + Nornicotine in Leaf and Root – L8 Lines

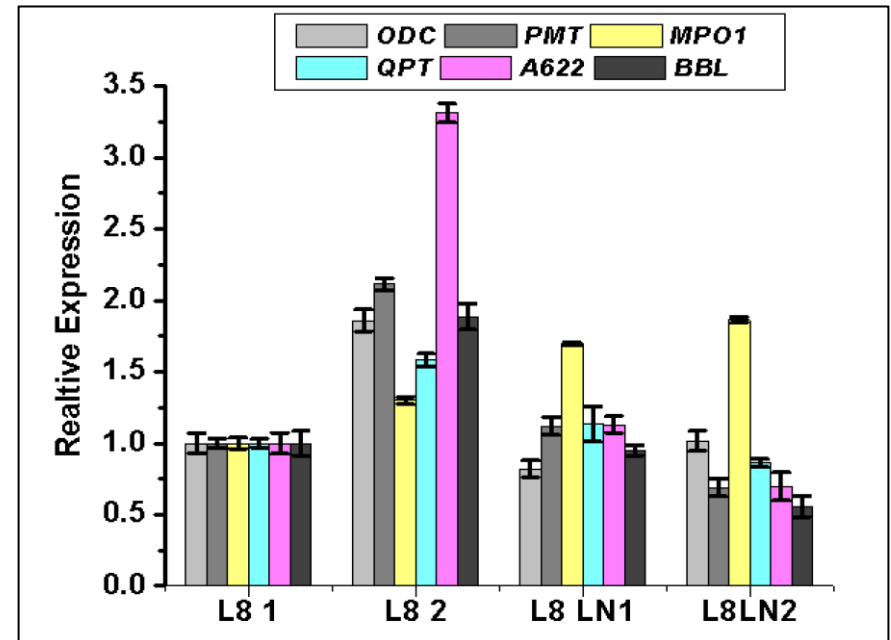


Relative Expression Analysis of Nicotine Pathway Genes & Regulators - Hypotheses

- Lower nicotine in L8 LN associated with regulators not present in the *Nic2* locus, and probably not the *Nic1* locus
 - Nic2* locus deleted in both L8 families, *Nic1* locus present in both (HI)
 - Looked at expression of *Nic1* transcription factors and nicotine biosynthesis genes
- Lower nicotine in L8 LN leaves possibly result of loss/downregulation of transporter/s and/or potential new regulators

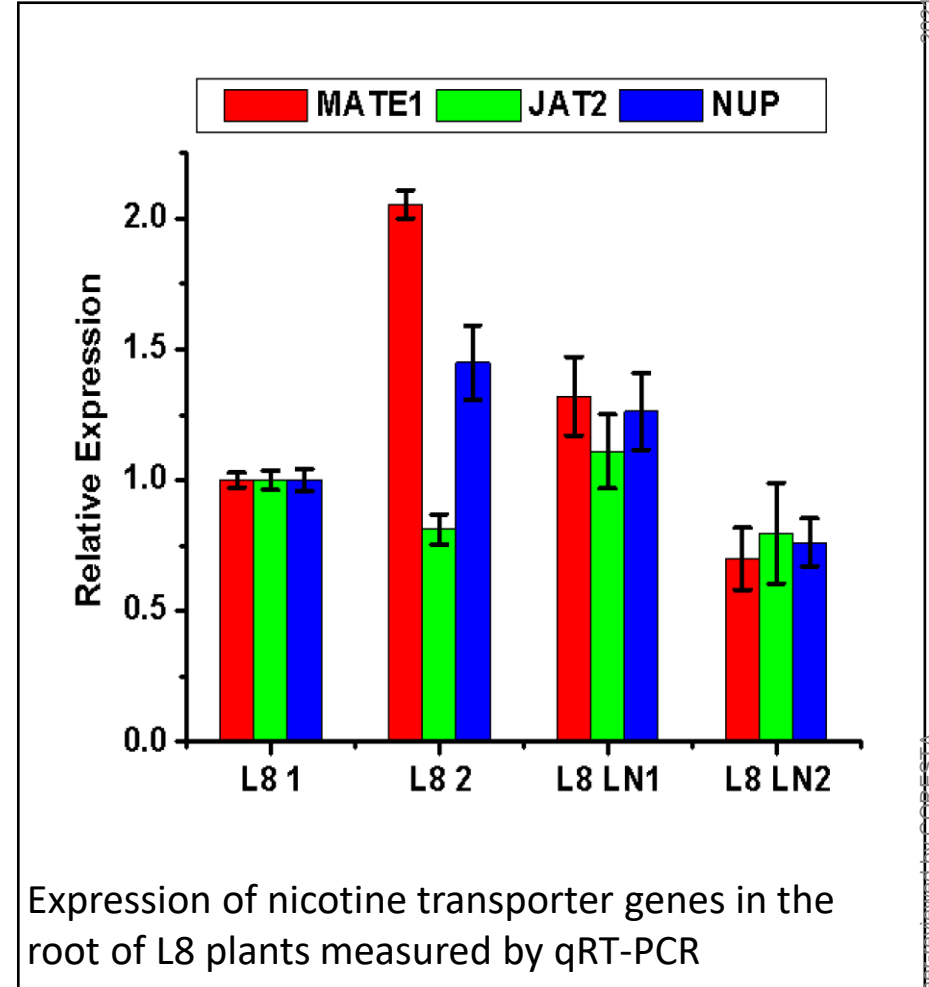
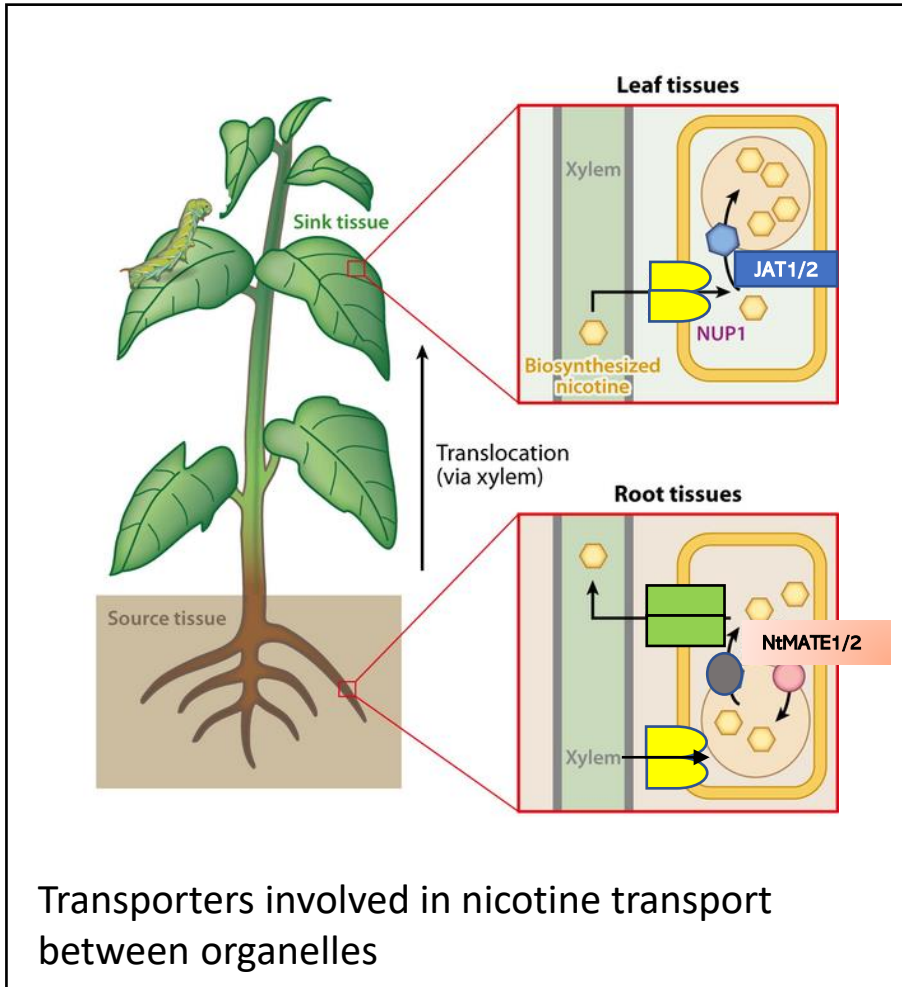


Expression of *Nic1* ERF transcription factors in the root of L8 and L8 LN plants measured by qRT-PCR



Expression of nicotine biosynthesis genes in the root of L8 and L8 LN plants measured by qRT-PCR

Nicotine Transporters



Conclusions

- L8 LN lines carry a low alkaloid gene in addition to *nic2*
 - This gene almost certainly not allelic to *nic1*
- Leaf / root alkaloid data suggest:
 - Nicotine reduction function of nicotine transport, not synthesis
 - Alkaloids in L8 LN reduced in leaf, in root same as L8
 - But – not any known transporter
- The same is not true of *nic1 nic2* mutants
 - Alkaloids in LA lines reduced in both leaf and root
 - Mutation involved in synthesis

Implications

- If allelic to *nic1*, this gene would be of no value
 - Less reduction in nicotine than *nic1*
- Not allelic to *nic1*, as it seems:
 - Of some value if the novel gene reduces nicotine more than *nic2*
 - [*nic1* + recessive novel gene] may give lower nicotine than *nic1 nic 2*
 - Of great value if stacking all three genes reduces nicotine dramatically below levels in LA (*nic1 nic 2*)
- Indication of nicotine transport rather than synthesis as a mode of action important

Future Plans

- Growing F_2 s (novel gene – C):
 - L8 LN x L8
AA**bbcc** AA**bbCC**
 - L8 LN x LA Bu 21 ($1/16$ aabbcc)
AA**bbcc** a**abbCC**
 - L8 x LA Bu 21
AA**bbCC** a**abbCC**
 - L8 LN x TKF 2002 (HA)
AA**bbcc** AA**BBCC**
 - L8 x TKF 2002 (HA)
AA**bbCC** AA**BBCC**
- Alkaloid data
- Leaf samples for possible future marker work
- Identify triple mutant
- Barun pursuing nicotine transport work