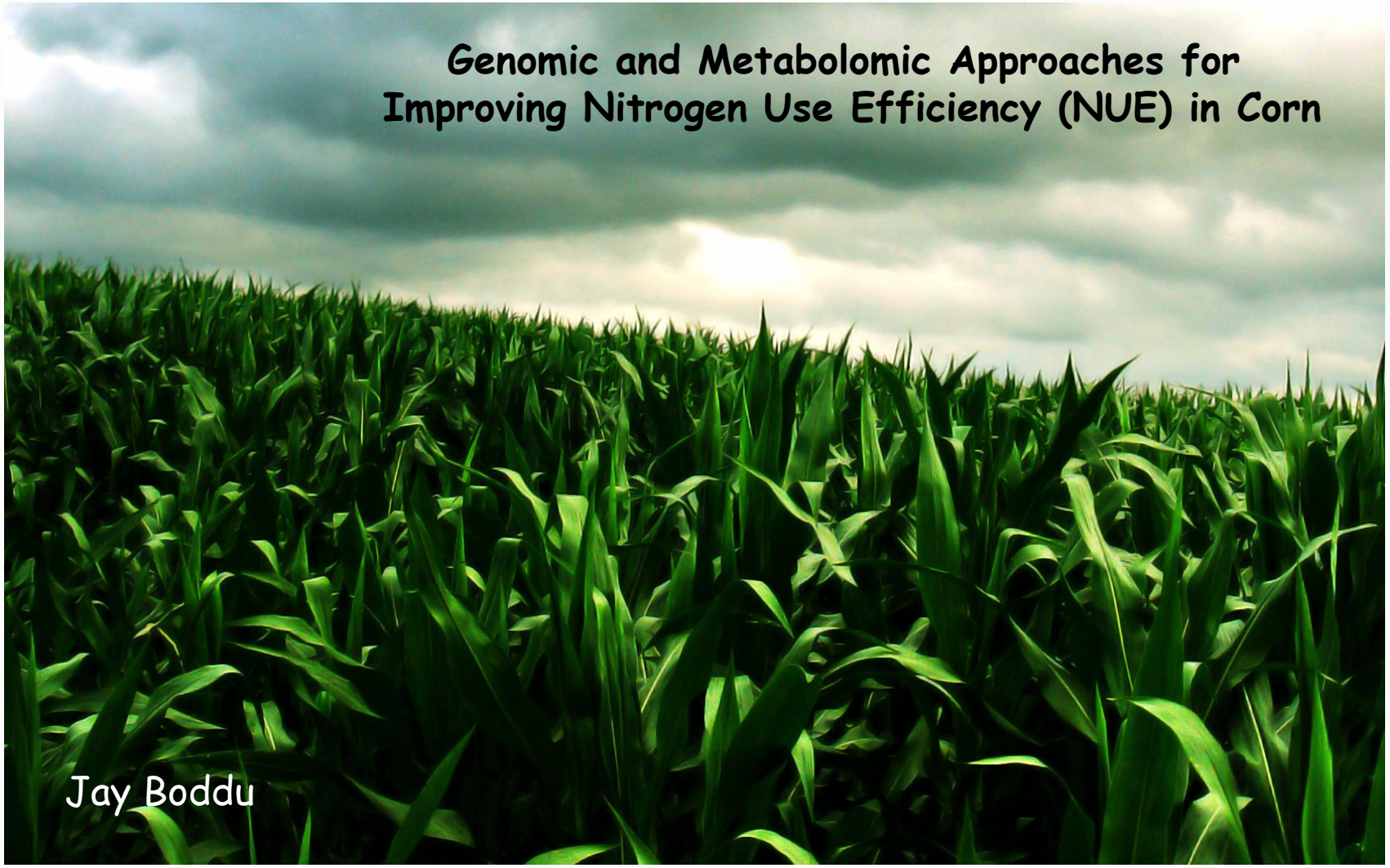
Genomic and Metabolomic Approaches for Improving Nitrogen Use Efficiency (NUE) in Corn



Jay Boddu

University of Illinois at Urbana-Champaign

Genomic and Metabolomic Approaches for Studying Nitrogen Use Efficiency (NUE) in Corn

PAST & PRESENT

QTL identification
- NUE, NutE, NupE, Grain yield, Biomass etc.

Long-term selection
- Illinois Protein and Oil lines

Identification of pathways involved in N metabolism
- Microarrays, MPSS, Kernel culture and Deep sequencing

Development of functional markers
- Expression QTL (eQTL), metabolic QTL (mQTL)s and SNPs

Effect of the fixed alleles on select phenotypes
- Allelic fixation and heterotic vigor

Evaluation of source-sink relationships
- N uptake, utilization and remobilization

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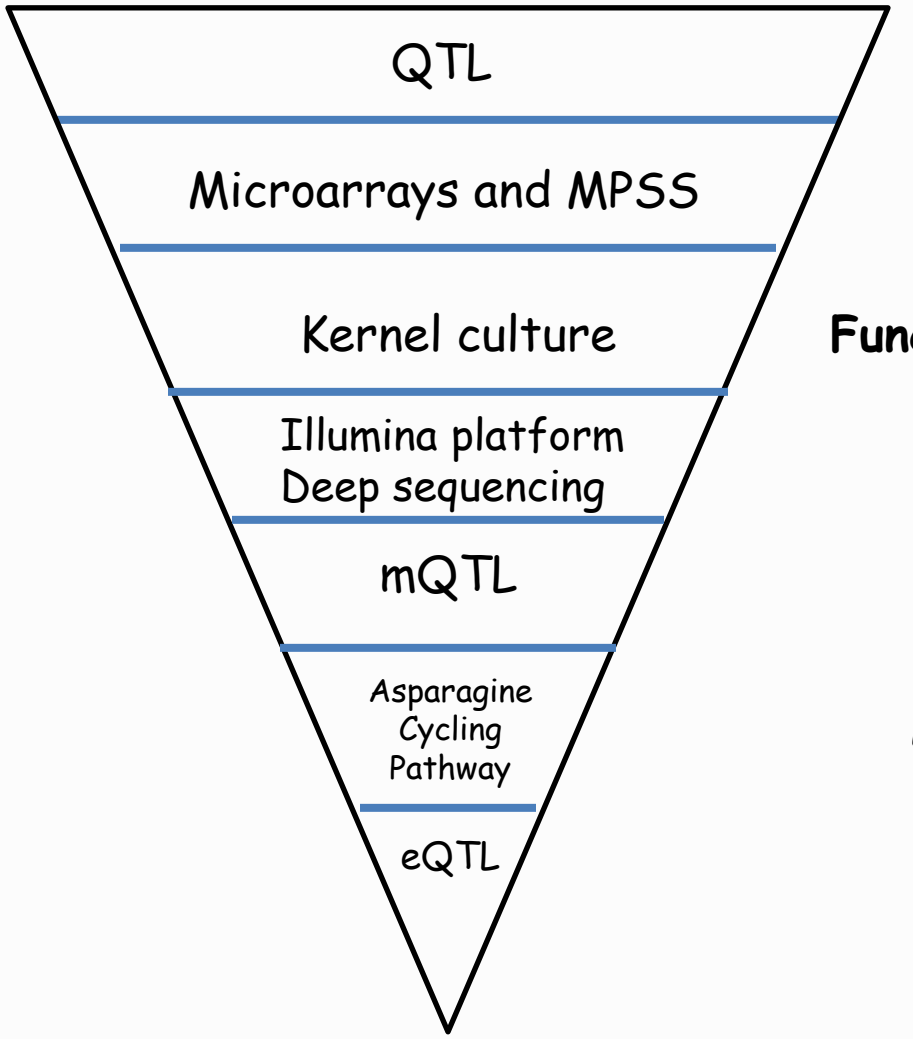
Work flow of the NUE project management



Genetics

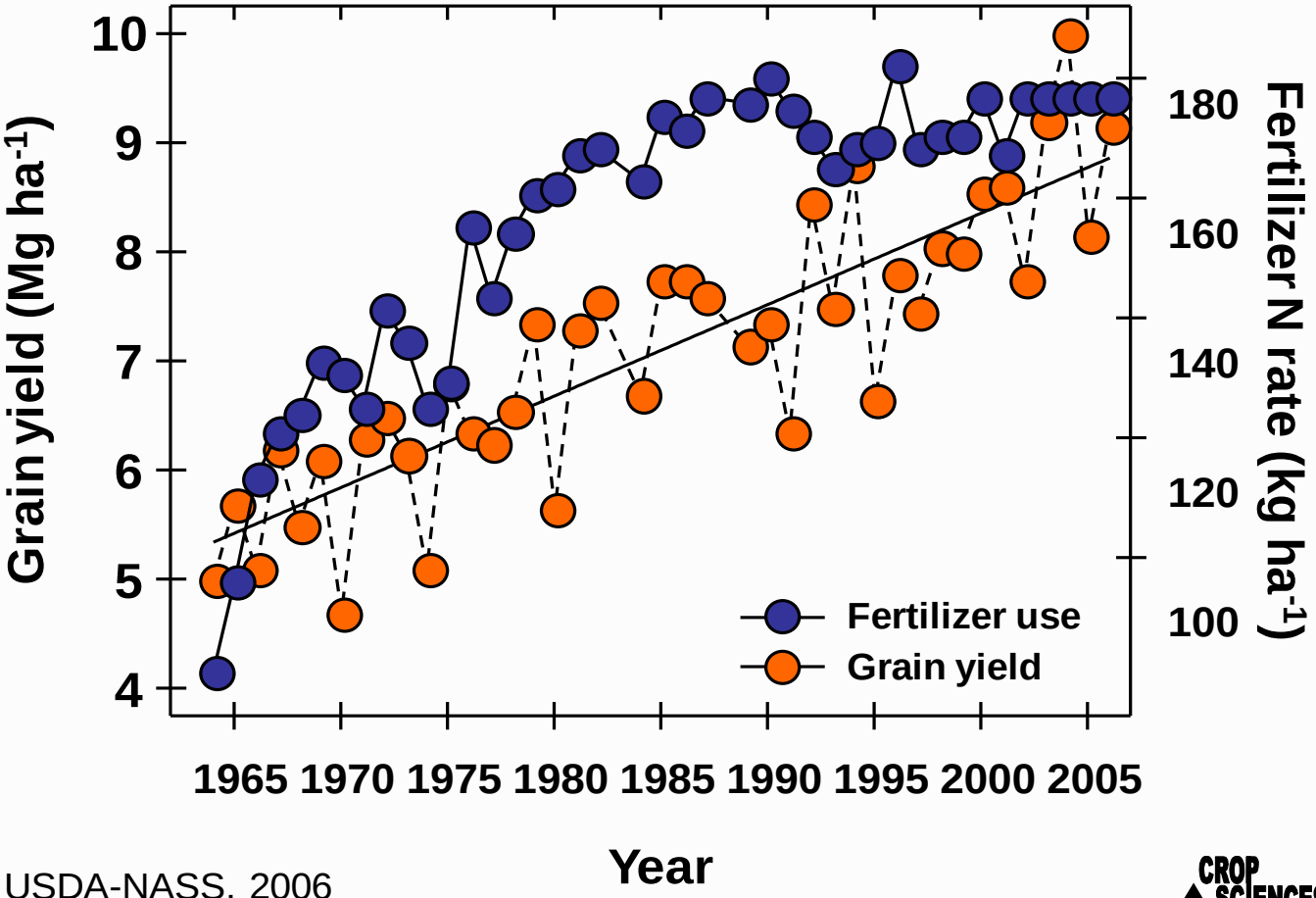
Functional Genomics

Genomics

Metabolomics

Asparaginase, **Asparagine synthetase** and their regulation

Historical Increases in Maize N Utilization



Data USDA-NASS, 2006

CROP SCIENCES
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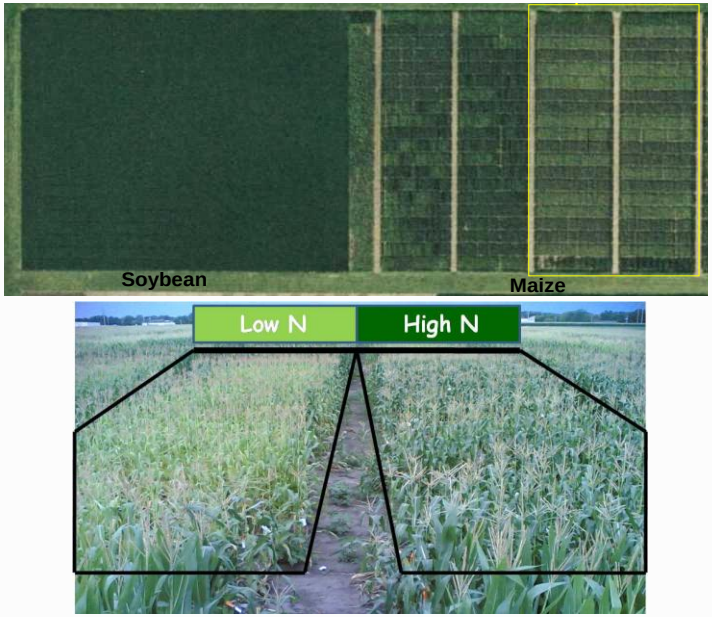
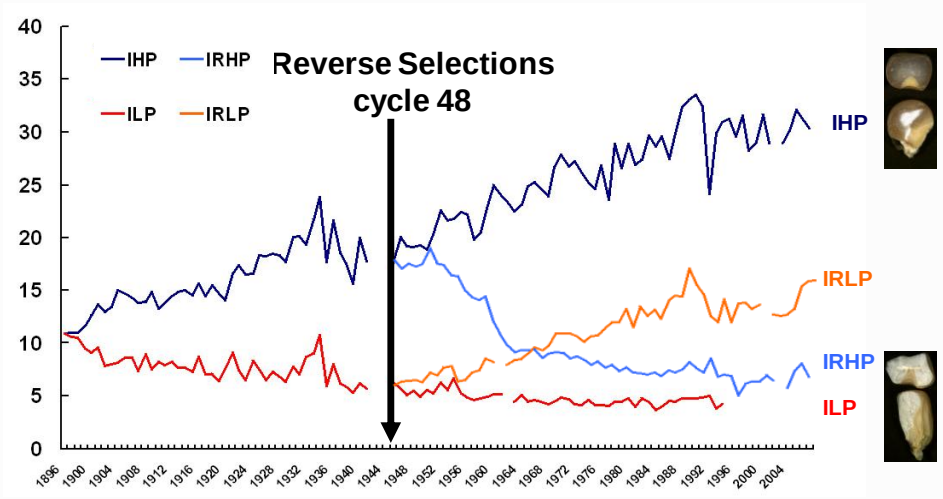
Nitrogen Responsive Nursery



Illinois Protein Lines



Nitrogen Use Efficiency (NUE) :

- Grain yield increase per unit fertilizer applied

Nitrogen Utilization Efficiency (NUtE) :

- Ratio of grain yield to the total plant N

Nitrogen Uptake Efficiency (NUpE) :

- Percentage of fertilizer-applied N found in the plant

Genetic material predominantly used in Nitrogenes project

1. IBMRIL population for linkage analyses

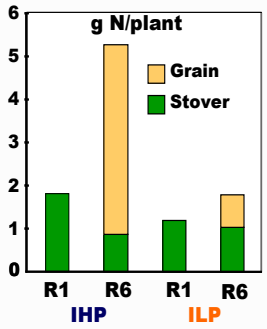
2. Nested Association Mapping (NAM) founder lines

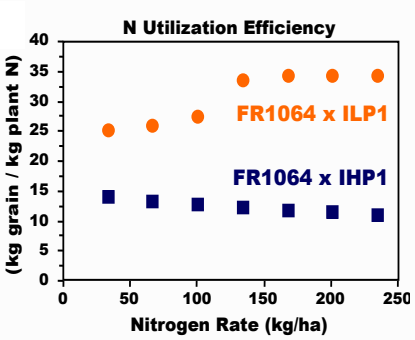
Tzi8	Tropical	Ky21	Temperate
CML333	Tropical	MS71	Temperate
CML277	Tropical	Oh43	Temperate
Ki11	Tropical	Oh7B	Temperate
NC358	Tropical	M162W	Temperate
CML247	Tropical	B97	Temperate
CML52	Tropical	HP301	Sweet & Pop
CML228	Tropical	IL14H	Sweet & Pop
Ki3	Tropical	P39	Sweet & Pop
NC350	Tropical		
CML69	Tropical		
CML103	Tropical		
CML322	Tropical		
Mo18W	Mixed		
M37W	Mixed		
Tx303	Mixed		

Photoperiod Insensitive

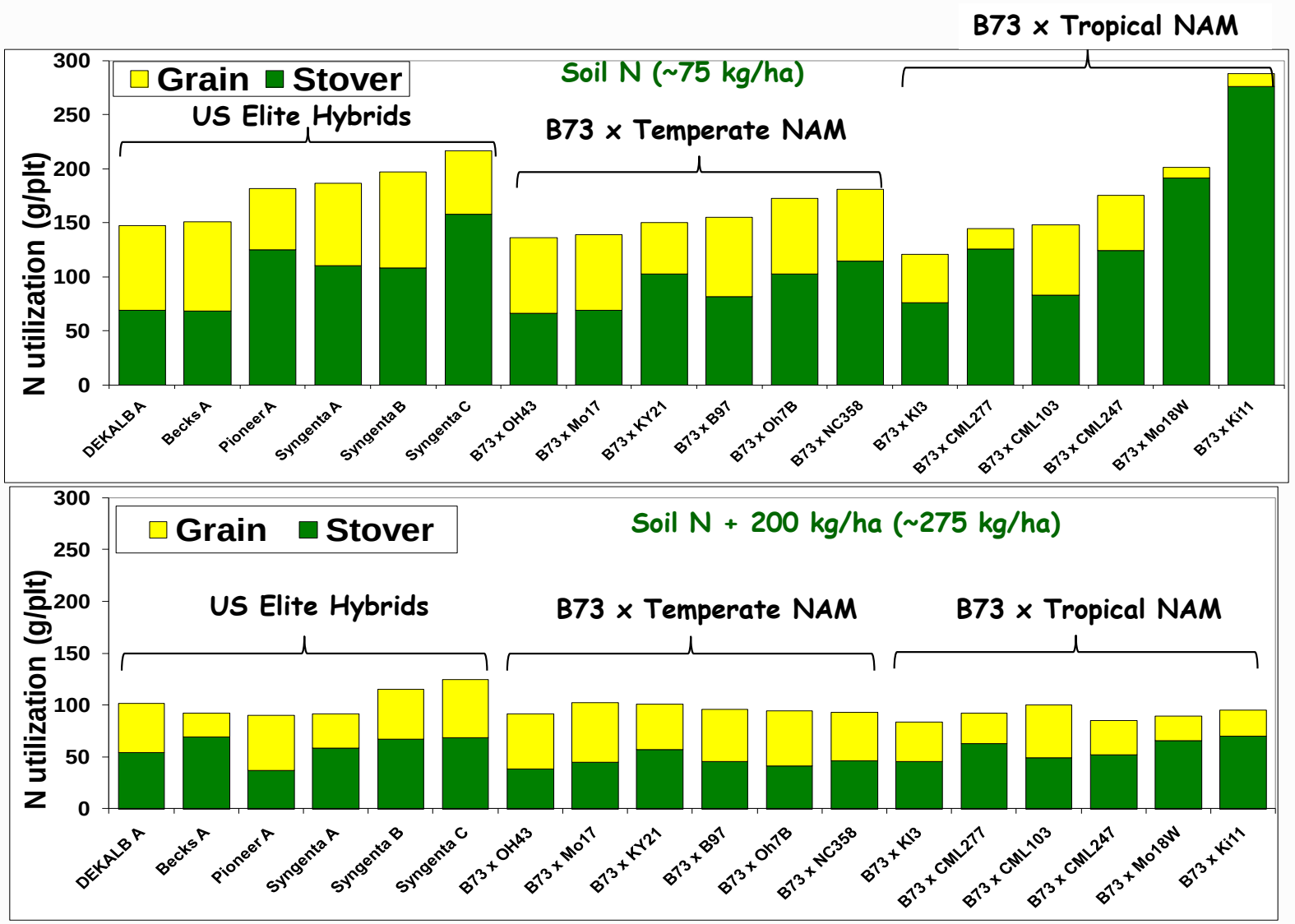
Photoperiod Sensitive

3. The Illinois protein lines differ in the N utilization efficiency

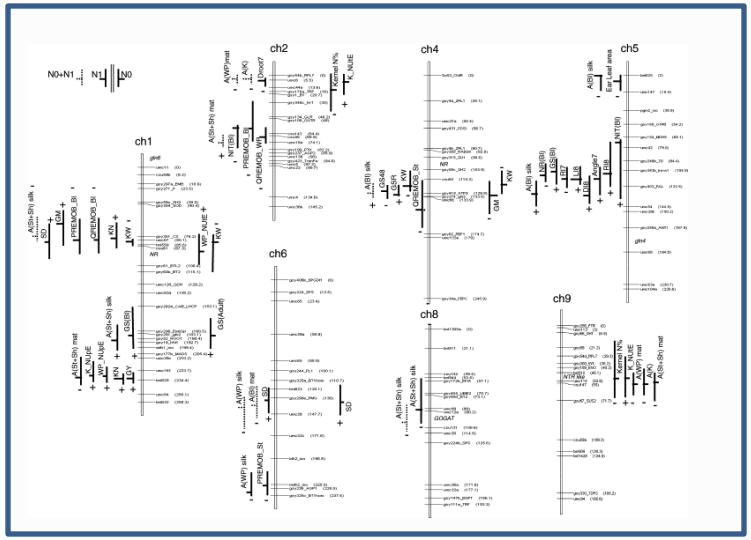




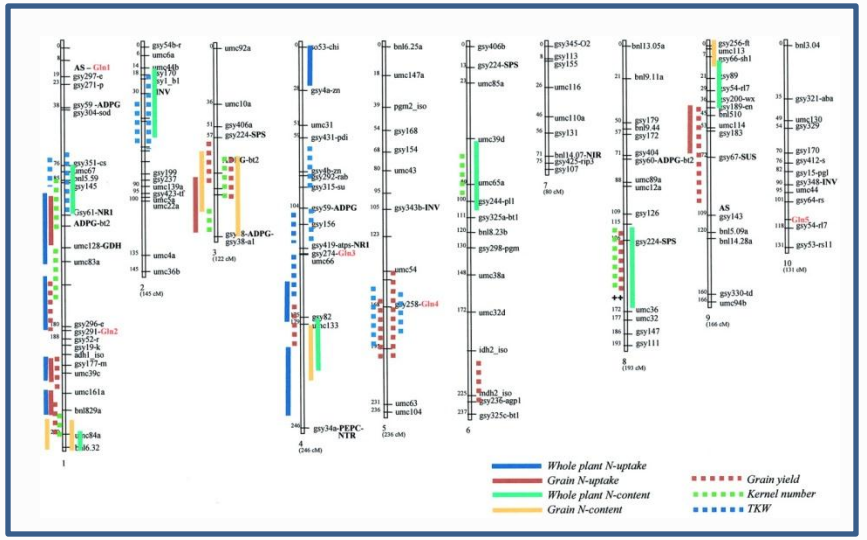
Genetic Variation for N Utilization in Maize Hybrids



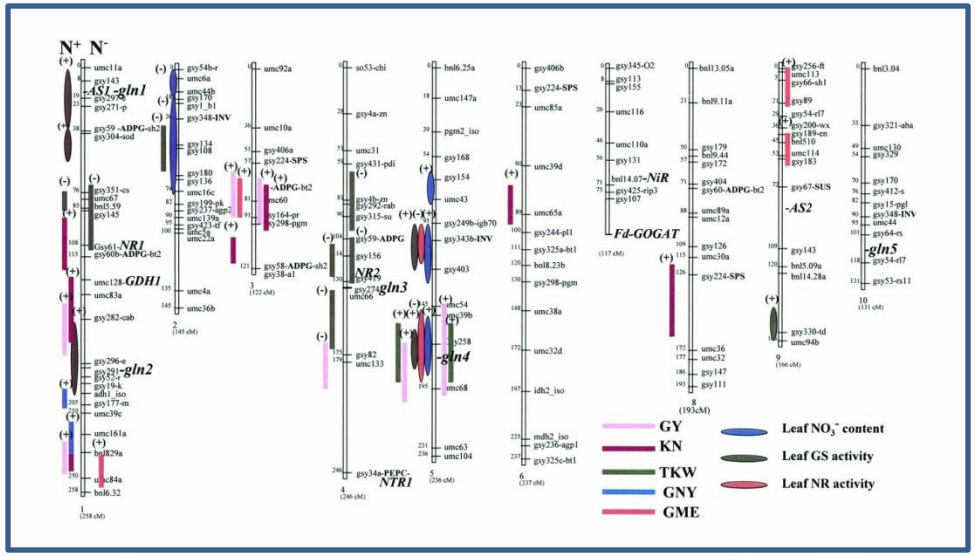
NUE-related QTL in maize



Coque et al., 2006

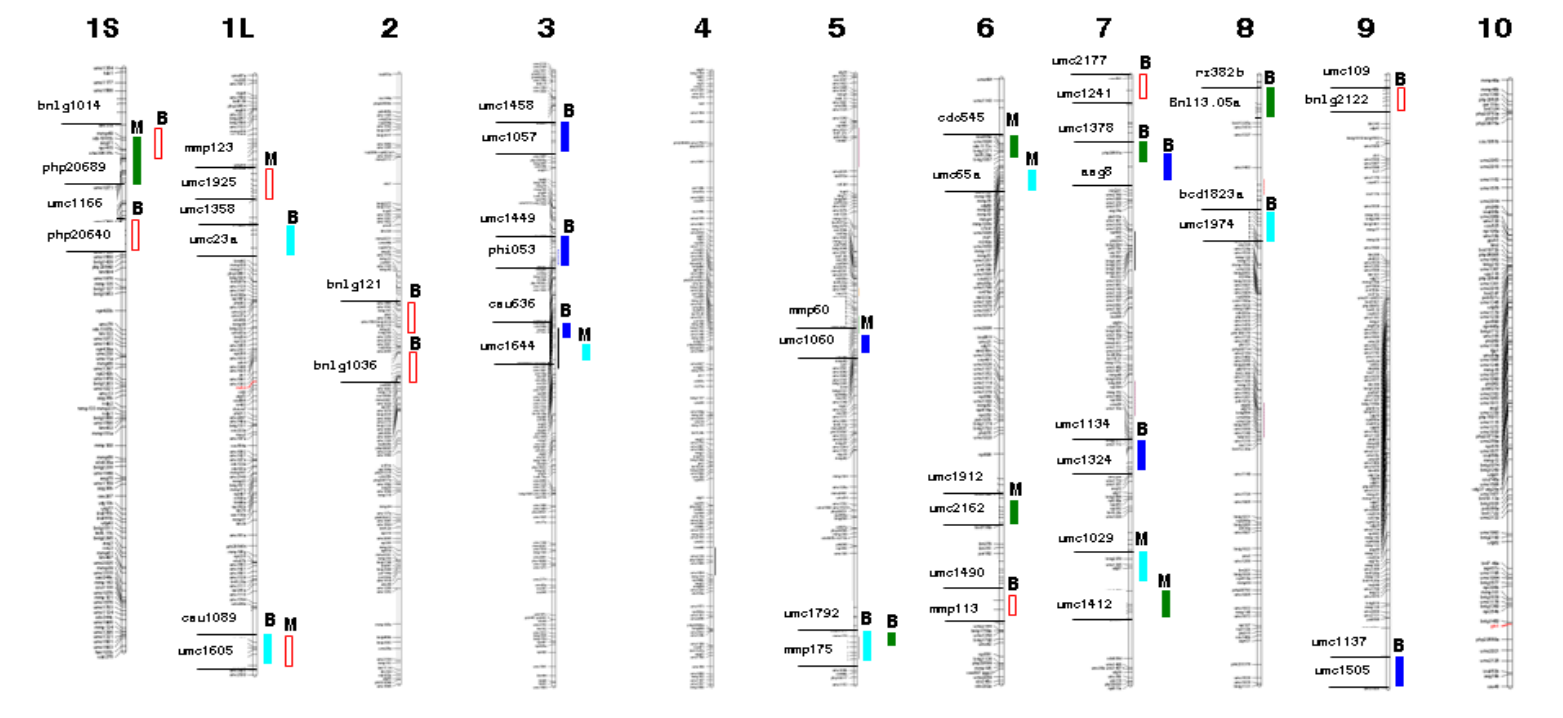


Gallais and Hirel., 2004



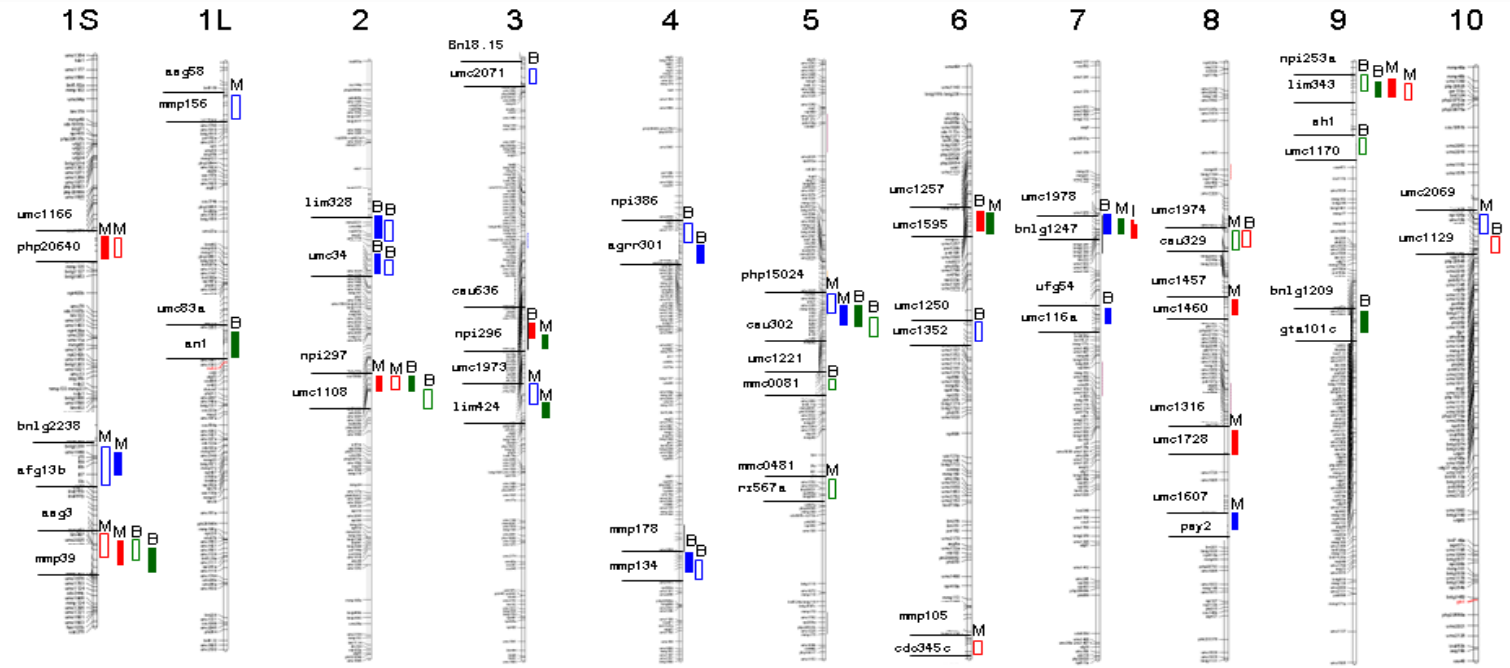
Hirel et al., 2001

Genetic Utilization (GU), NUE, NUtE and NUpE QTL in maize



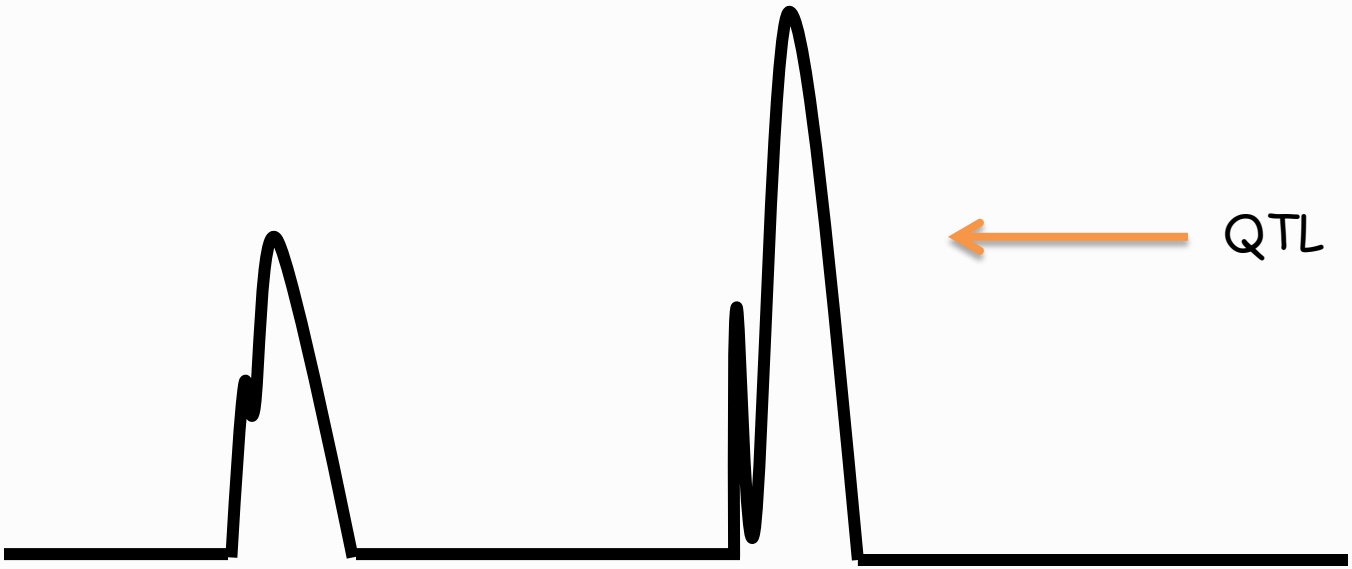
Genetic map of IBMRIL population showing QTL detected for genetic utilization (red boxes), nitrogen (N) use efficiency (green boxes), N uptake efficiency (dark blue boxes), and N utilization efficiency (light blue boxes). B = B73 allele increases trait value, M = Mo17 increases trait value. Open boxes are QTL at 0 N, filled boxes are QTL at high N. The markers flanking the interval for each QTL or cluster of QTL are indicated

Grain composition QTL

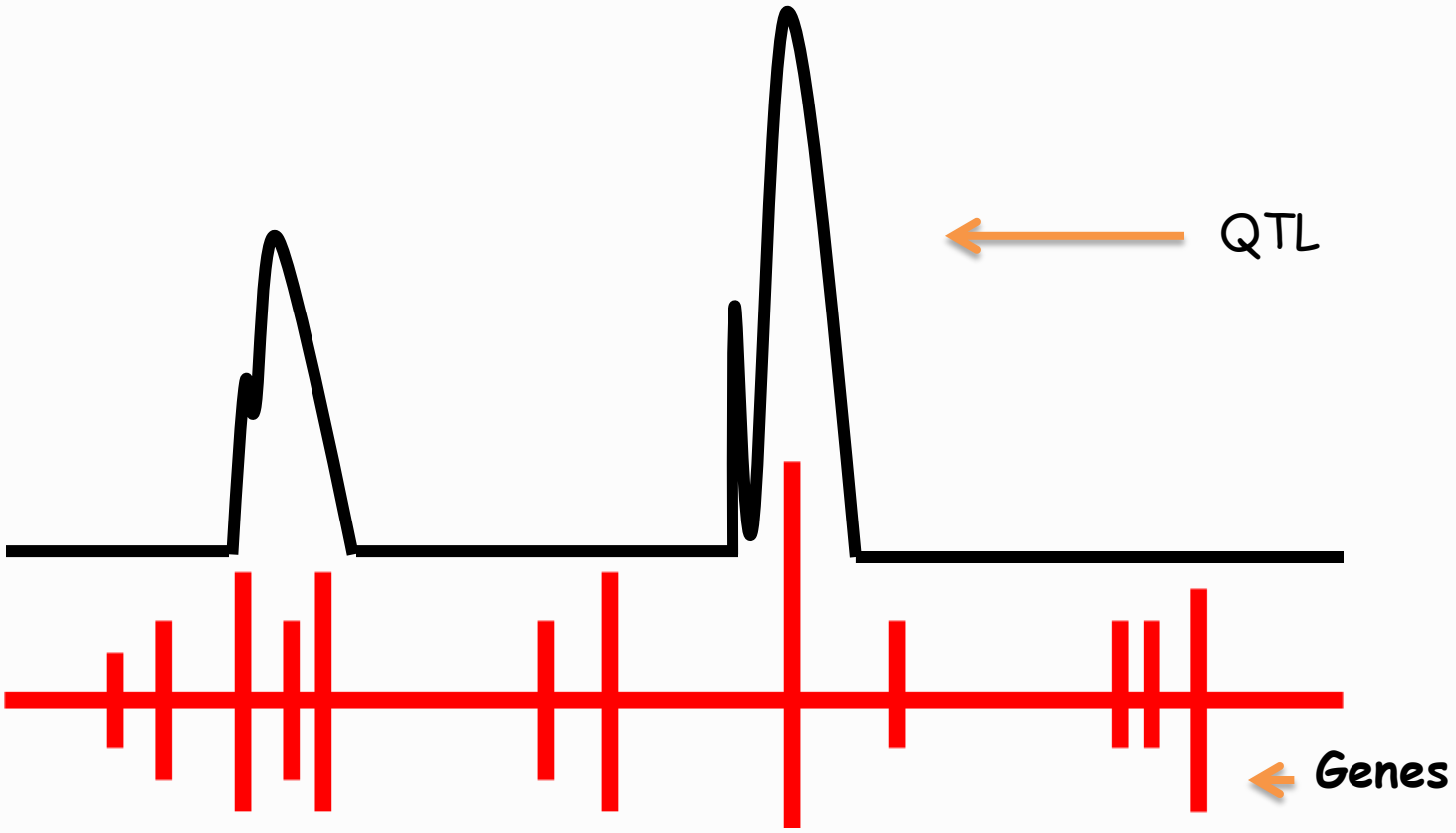


Genetic map of IBMRIL population showing QTL detected for kernel protein concentration (red boxes), starch concentration (green boxes), and oil concentration (dark blue boxes). B = B73 allele increases trait value, M = Mo17 increases trait value. Open boxes are QTL at 0 N, filled boxes are QTL at high N. The flanking markers for each QTL or cluster of QTL are indicated

Functional genomics to identify genes behind the observed QTL



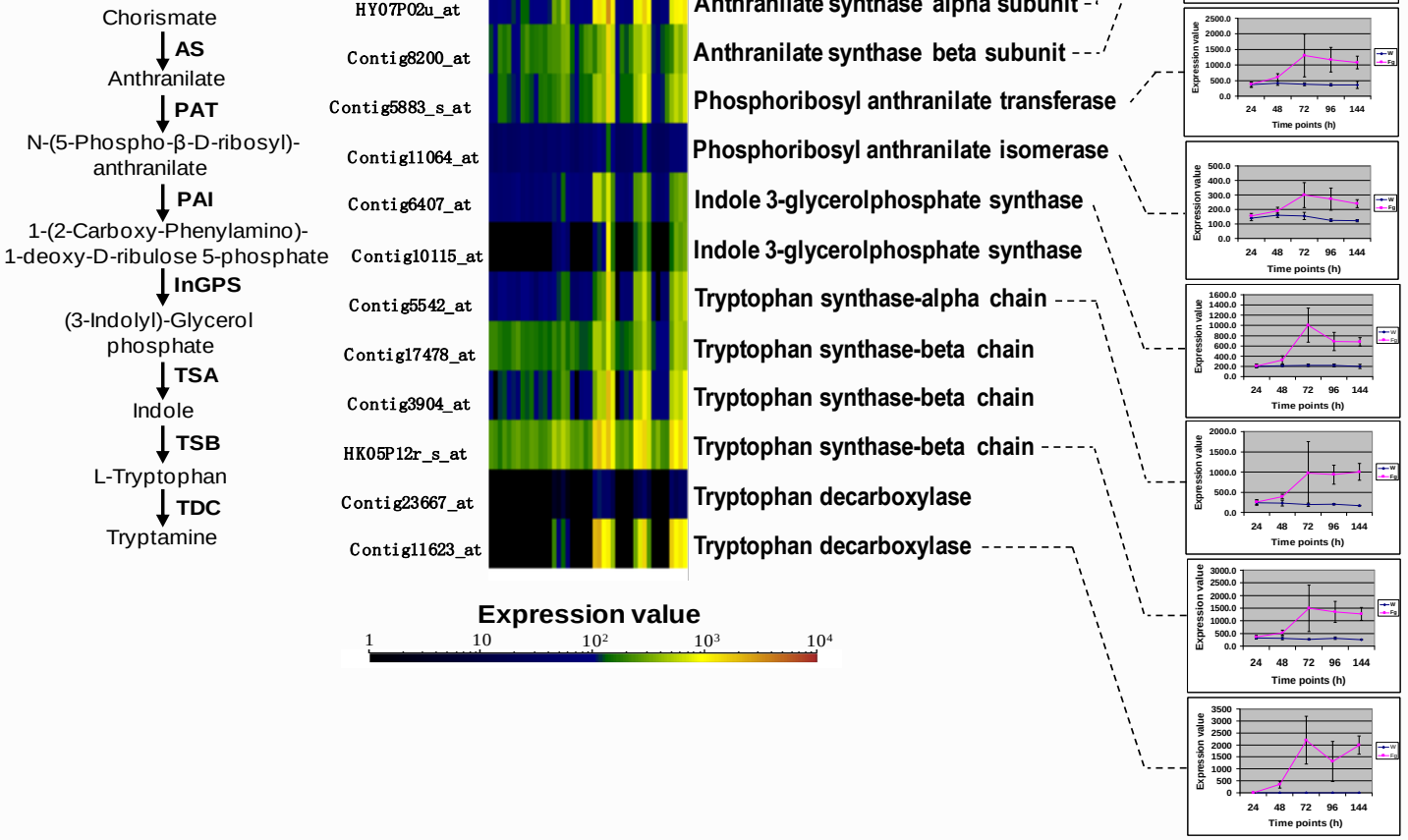
Functional genomics to identify genes behind the observed QTL



NITROGEN RESPONSIVE TRANSCRIPTOME ANALYSIS USING MICROARRAYS

Question	Tissue types	Genotypes	Stages	N treatment
N Uptake	Root	B73	Seedling	No
		IHP		
Remobilization	Leaves	B73	V10 & V16	No
		IHP		
		ILP		
		Mo17		
Source behavior	Leaves	B73	V8	Yes
		IHP		
		ILP		
		IRHP		
		IRLP		
Sink development	Seeds	B73	8 DAP & 16 DAP	No
		IHP		
		ILP		
		IRHP		
		IRLP		
Sink development	Earshoot	B73	Earshoot at anthesis	Yes
		B73 X Mo17		
		High yielding IBMRIs		
		Low yielding IBMRIs		
		Tropical genotypes		

Can we observe something like this ?



Boddu et al., MPMI, 2006

Genotype, Tissue, Development and N treatment specific differentially accumulating maize transcriptome

Plant organ/Tissue	Comparison	Up- regulated in	
16 DAP seeds	IHP vs ILP	IHP	921
		ILP	469
	IHP vs IRHP	IHP	726
		IRHP	647
	ILP vs IRLP	ILP	1114
		IRLP	1394
Ear shoot	BMO (High N) vs B73 (Low N)	BMO (High N)	1087
		B73 (Low N)	725
	BMO (Low N) vs B73 (Low N)	BMO (Low N)	1146
		B73 (Low N)	961
	IBMHY (High N) vs IBMHY (Low N)	IBMHY (High N)	20
		IBMHY (Low N)	17
	IBMLY (High N) vs IBMLY (Low N)	IBMLY (High N)	52
		IBMLY (Low N)	24
8 DAP Leaf	ILP vs IHP	ILP	1757
		IHP	1554
	ILP vs IRHP	ILP	309
		IRHP	284
	ILP vs IRLP	ILP	256
		IRLP	191
V16 Leaf	B73 vs IHP	B73	957
		IHP	888
	B73 vs ILP	B73	1014
		ILP	813
V10 Leaf	B73 vs IHP	B73	954
		IHP	698
	B73 vs ILP	B73	916
		ILP	667
V8 Leaf	B73 (High N) vs IHP(High N)	B73 (High N)	1123
		IHP (High N)	715
	B73 (High N) vs IHP(Low N)	B73 (High N)	1085
		IHP (Low N)	854
	B73 (High N) vs B73(Low N)	High N	320
		Low N	221
	IHP (High N) vs IHP(Low N)	High N	537
		Low N	170
ROOT	B73 vs IHP	B73	667
		IHP	506

Important conclusions from microarray experiments

A baseline dataset was derived

Genotype and organ/tissue specific patterns were observed

Distinct nitrogen-specific responses were recorded
in IBMRILs that differed in their yield response

Photosynthesis specific transcriptional machinery was induced
by high N condition in juvenile earshoot

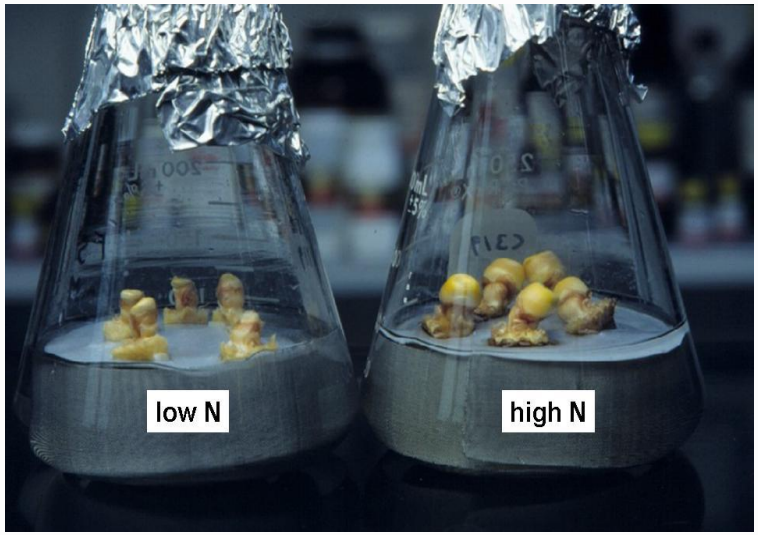
**Amino acid metabolism transcripts (eg. AS, ASNase, GS, AspAT etc.)
that participate in asparagine cycling pathway
showed prominent N-response**

Coordinated expression of asparagine synthetase
and asparaginase was found in protein lines

The Kernel Culture: A Controlled Environment Approach

Trait	GY_0 correlation	GY_{max} correlation	N Response
Grain yield (GY)			1.88
Kernel number	0.93	0.90	1.58
Kernel weight	0.19	-0.33	1.07
N use efficiency		0.31	0.32
N uptake efficiency		0.04	0.59
N utilization efficiency		0.25	0.53
V8 stover biomass	0.04	0.03	1.00
V8 stover N concentration	-0.24	-0.31	1.15
R1 stover biomass	-0.53	-0.14	1.12
R1 stover N concentration	0.61	0.26	1.84
Total biomass	-0.10	0.31	1.33
Total plant N concentration	0.38	0.20	1.82
Harvest index	0.86	0.51	1.32
Grain protein concentration	-0.77	-0.632	1.31

Kernel abortion



Kernel Culture

Transcriptomics

Illumina Genome Analyser

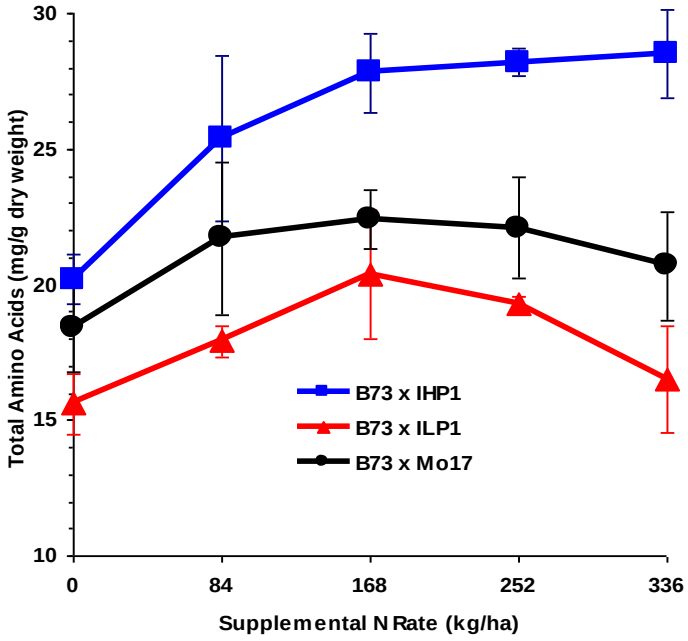
Metabolomics

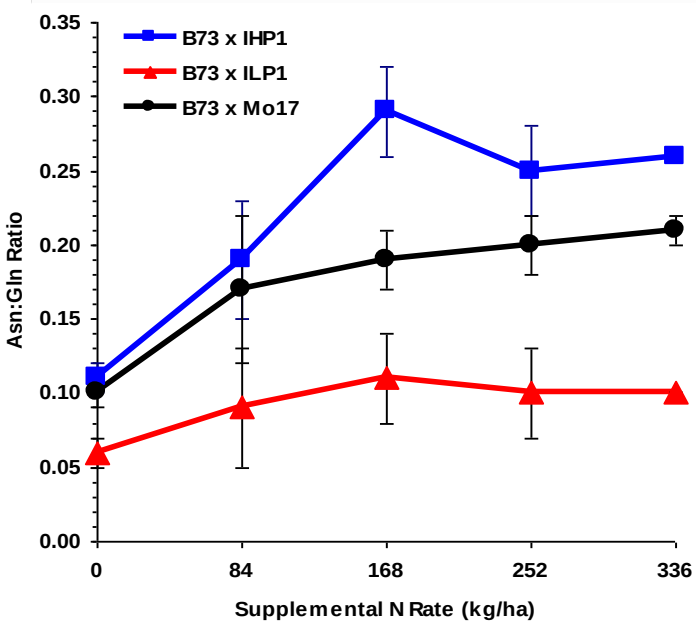
Agilent 1200 series rapid resolution HPLC system

Amino Acid Metabolism in Maize Earshoots. Implications for Assimilate Preconditioning and Nitrogen Signaling

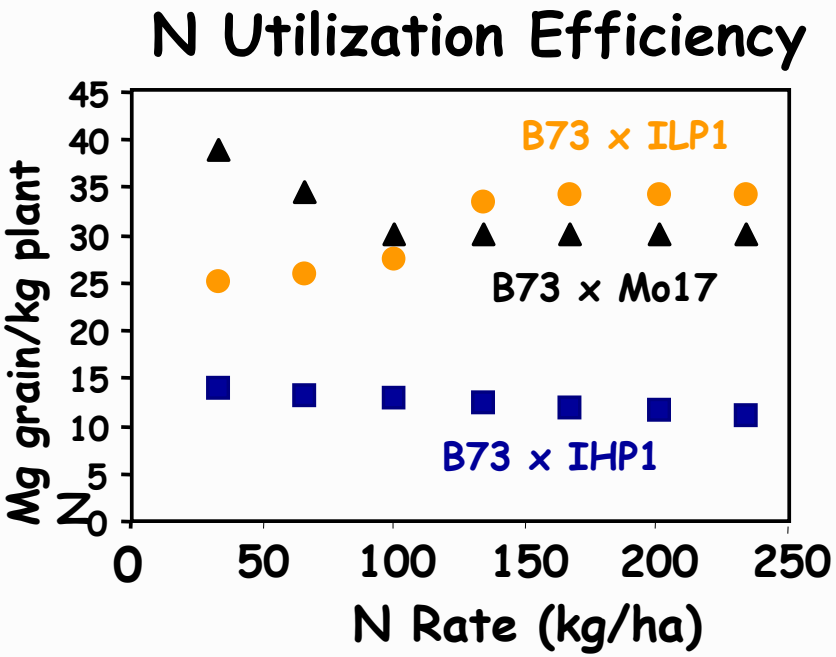
Seebauer et al., 2004. Plant Physiol.

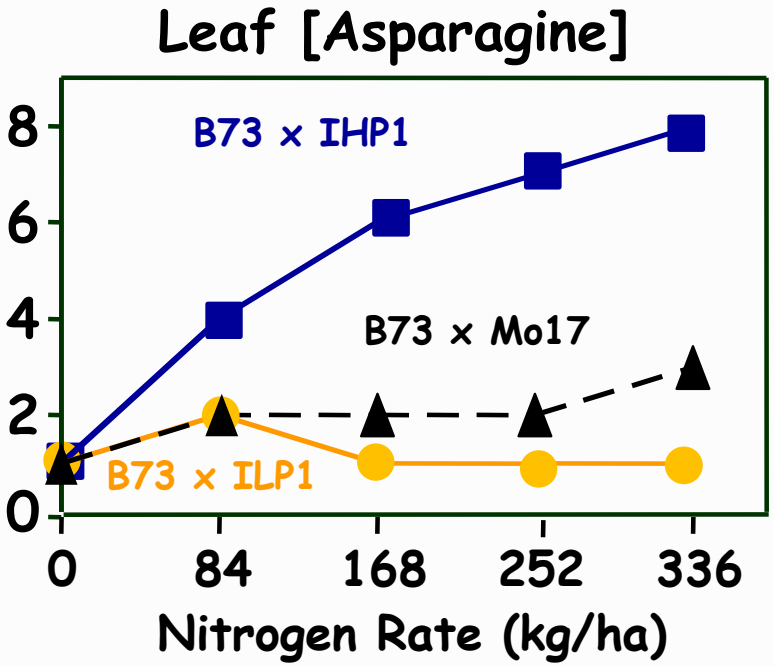
Genotype specific free amino acid concentrations





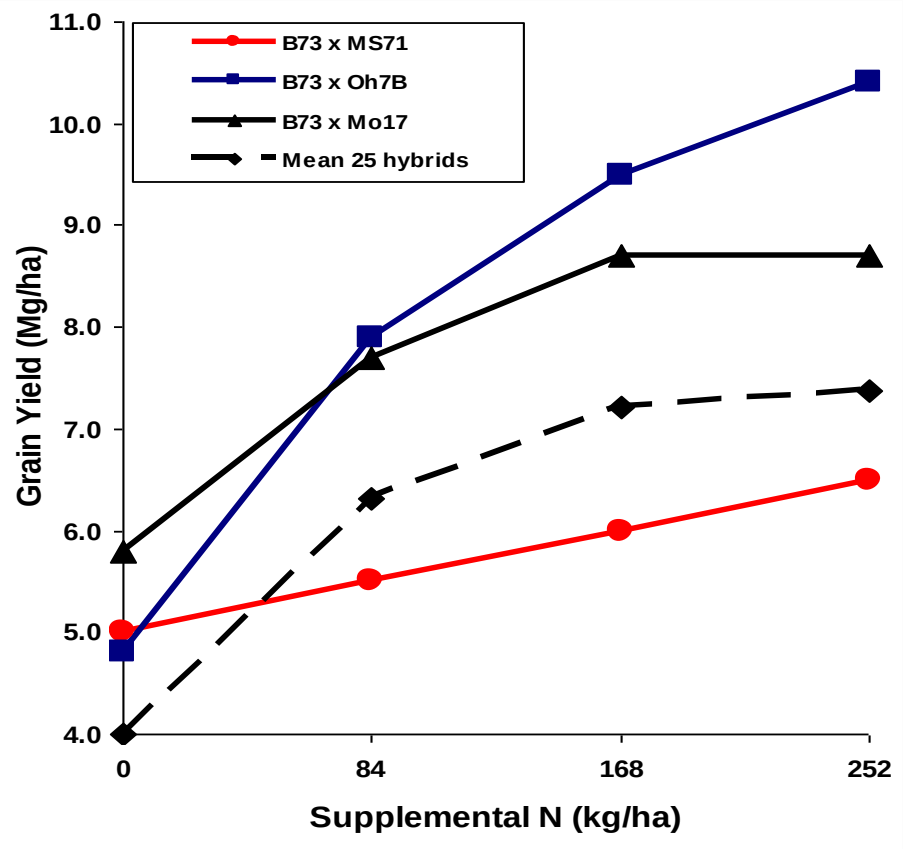
N Utilization Differences in B73 x IPS Hybrids





- High N utilization is associated with low leaf Asn.
- Greater Asn accumulation in IHP hybrid is correlated with high grain protein and low N utilization in IHP.

Grain yield responses to N of 25 Diversity hybrids



Genotypes selected for kernel culture experiment

B73 X MS71: Lowest N response

B73 X Mo17: Medium N response

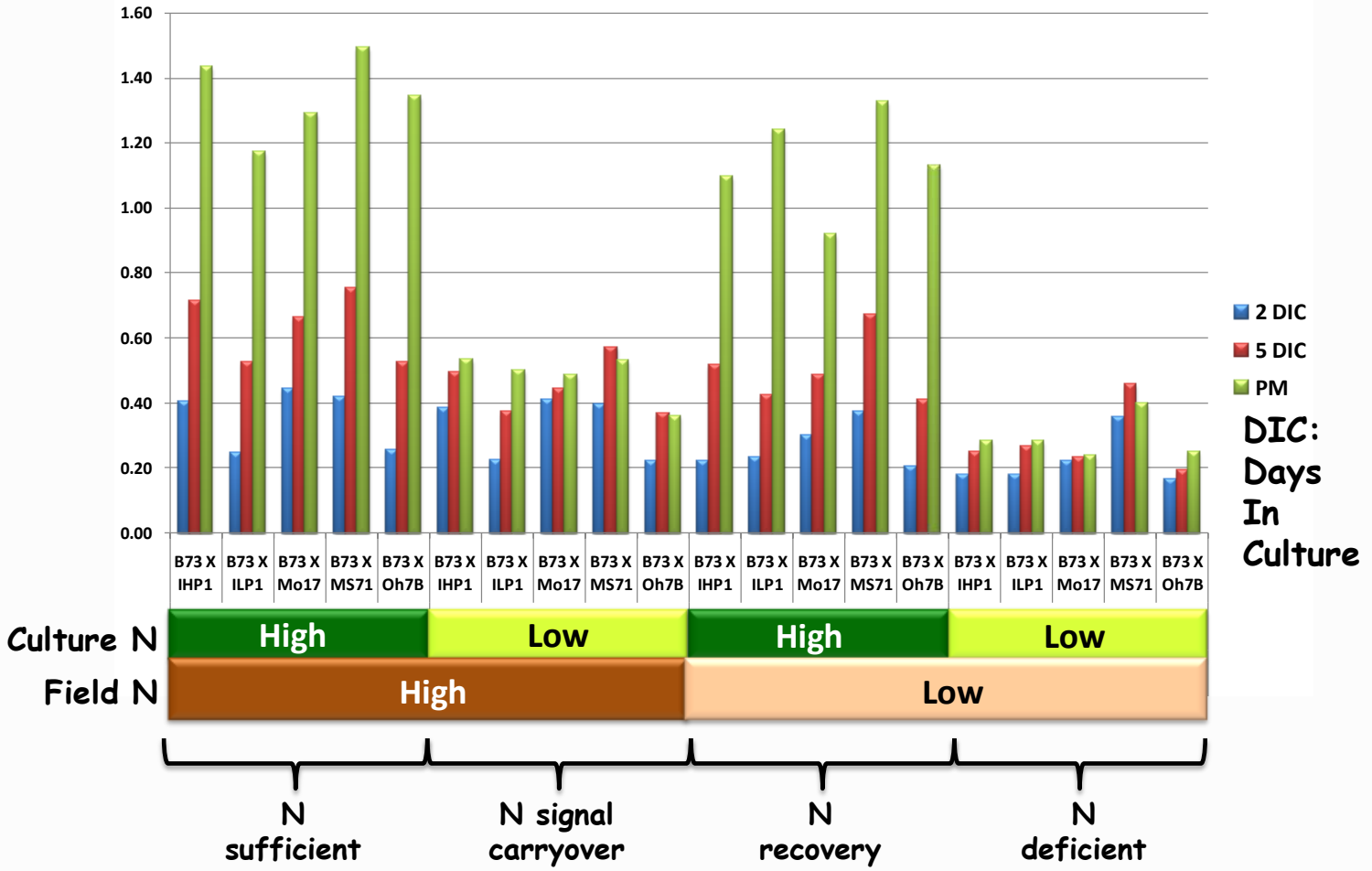
B73 X Oh7B: Highest N response

B73 X IHP1

B73 X ILP1

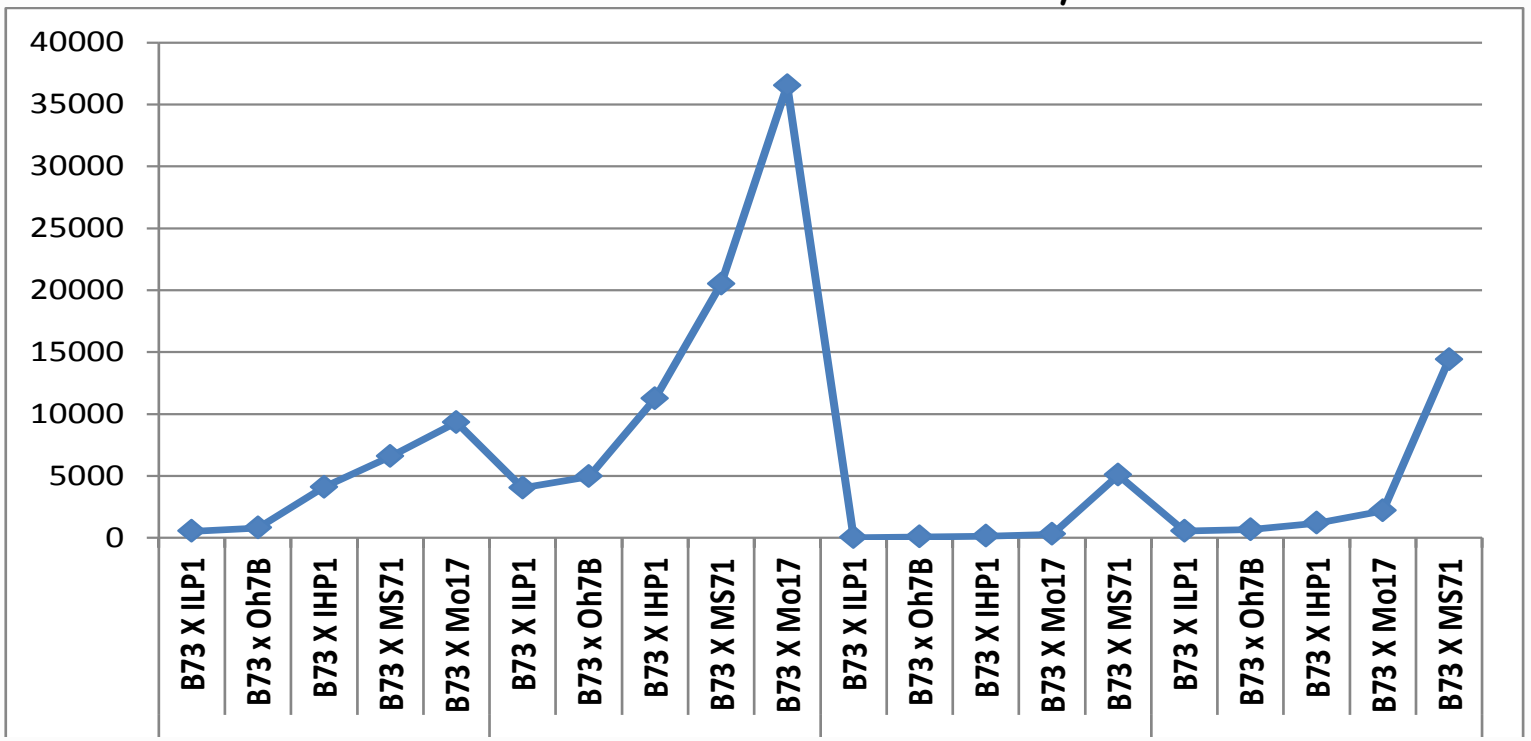
} Physiological extremes for N response

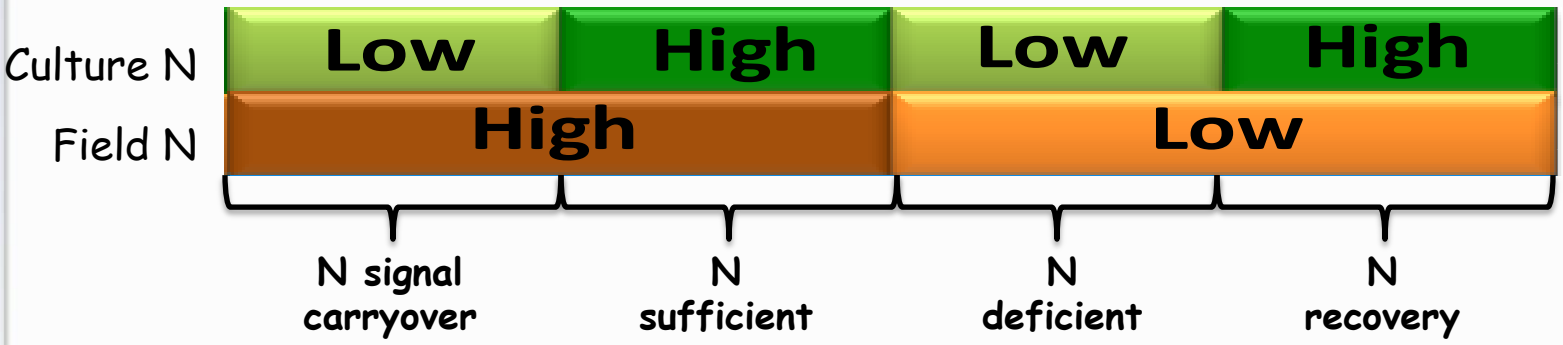
Genotype dependent grain filling in kernel culture as inferred by kernel weights



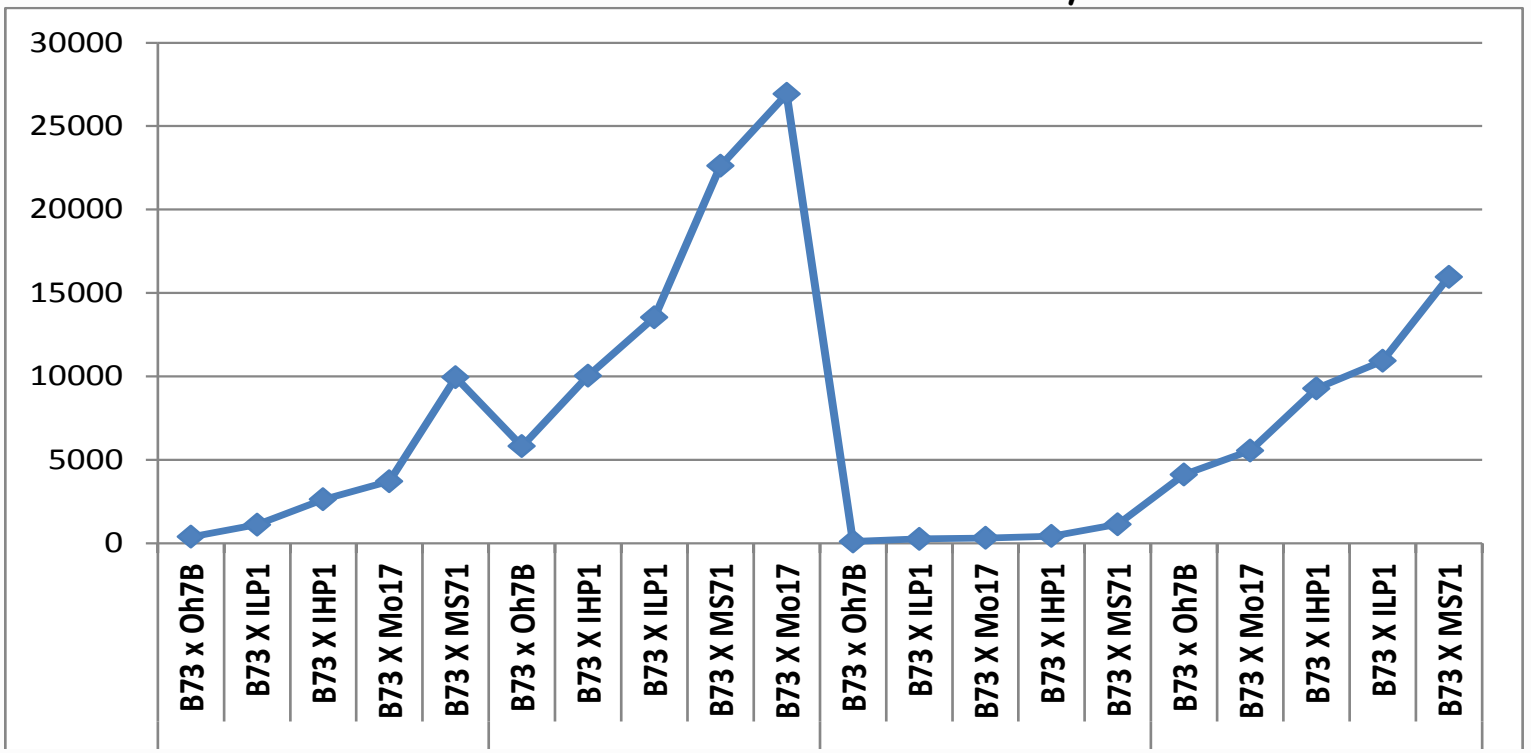
B73 X Oh7B showed highest N-recovery from 2 DIC to 5 DIC
Indicating its high N-utilization efficiency

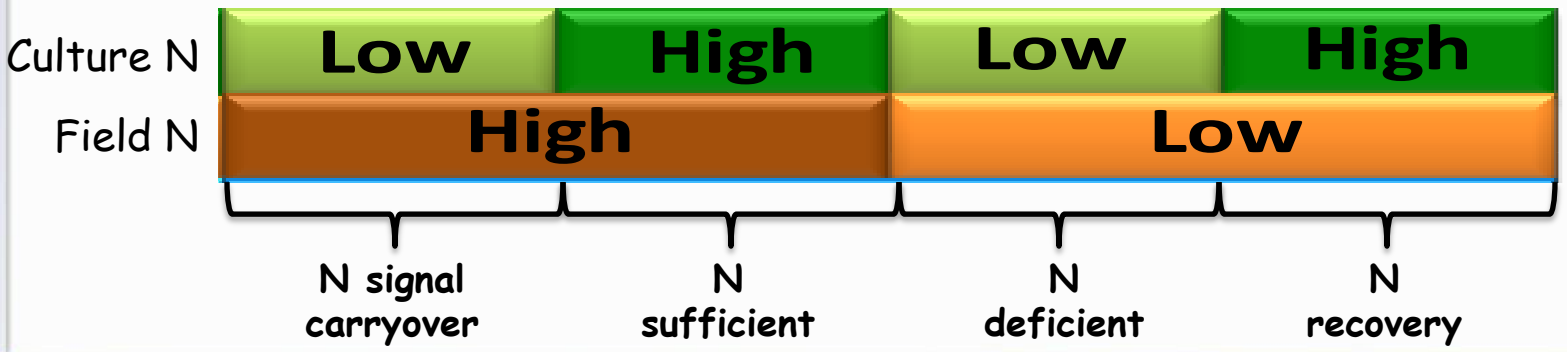
Genotype and N-treatment specific asparagine accumulation in cultured kernels at 2 days in culture



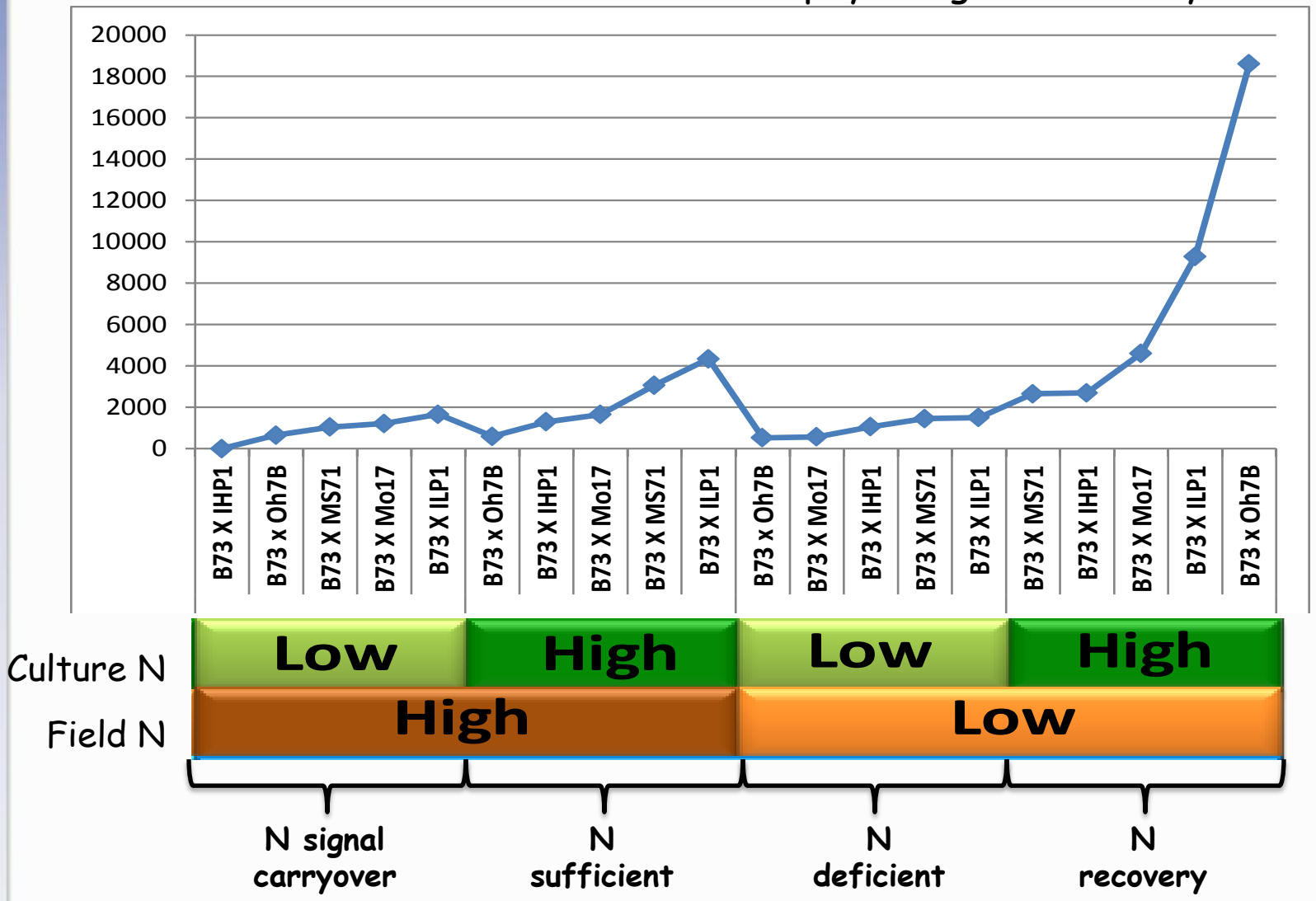


Genotype and N-treatment specific asparagine accumulation in cultured kernels at 5 days in culture





Genotype and N-treatment specific asparagine accumulation in cultured kernels at physiological maturity



In majority of the tested parameters such as N-deficiency, N-sufficiency and N-recovery, the cultured kernels of B73 X Oh7B hybrid showed lowest asparagine levels in agreement with the previously observed fact that the asparagine levels negatively correlates with N-utilization

Diverse genotypes to test correlations between select agronomic traits with immature earshoot free amino acids

DIVERSITY PHOTOPERIOD SENSITIVE	DIVERSITY PHOTOPERIOD INSENSITIVE	EXPIRED PATENT VARETY PROTECTION LINES	OLD INBRED LINES
CML69	B97	LH1	IHP
CML228	CML103	LH123	ILP
CML247	HP301	LH82	D1
CML277	IL14H	Mo17	D3
CML322	Ky21	PH207	D2
CML333	Ms71	PHG35	D9
Ki3	Oh43	PHG39	D4
Ki11	Oh7B	PHG47	FR1064
M37W	P39	PHG84	D5
Mo18W		PHJ40	M162W
Tx303		PHZ51	Va85
Tzi8			

These inbreds were crossed to IHP and ILP and were grown at High and Low N conditions

Correlations between NUE related traits with earshoot free amino acids in diverse genoypes

High N

Hybrids

	Alanine	Arginine	Asparagine	Aspartic acid	Glutamic acid	Glutamine	Lysine	Threonine
Grain Weight	0.00	0.00	-0.13	0.02	0.01	-0.06	0.00	-0.08
Cob Weight	-0.15	-0.09	-0.19	-0.02	-0.11	-0.04	-0.05	-0.22
Stover Biomass	-0.13	-0.01	-0.05	-0.15	-0.10	-0.14	-0.14	-0.07
Total N	-0.13	-0.07	-0.12	-0.18	-0.09	-0.21	-0.17	-0.14
Grain Moisture	0.01	-0.20	-0.22	-0.19	-0.10	-0.18	-0.08	-0.21
Starch	0.03	-0.21	-0.31	-0.18	-0.01	-0.29	-0.10	-0.30
Oil	-0.04	0.06	0.18	0.30	0.18	0.30	0.27	0.15
Protein	-0.03	0.20	0.31	0.16	0.00	0.25	0.07	0.30

Inbreds

	Alanine	Arginine	Asparagine	Aspartic acid	Glutamic acid	Glutamine	Lysine	Threonine
Grain Weight	-0.50	-0.39	-0.32	0.01	-0.07	-0.32	-0.13	-0.50
Cob Weight	-0.32	-0.10	-0.34	0.04	0.09	-0.31	-0.38	-0.28
Stover Biomass	0.31	0.22	0.17	0.12	0.28	-0.13	-0.31	0.51
Total N	0.26	0.30	0.13	0.12	0.30	0.00	-0.16	0.49
Grain Moisture	0.08	-0.04	-0.02	-0.23	-0.09	-0.06	0.12	-0.17
Starch	-0.45	-0.55	-0.28	0.03	-0.16	-0.45	-0.05	-0.44
Oil	-0.01	0.01	0.05	0.15	0.00	0.25	0.15	0.20
Protein	0.44	0.58	0.26	-0.03	0.20	0.34	-0.10	0.46

Low N

Hybrids

	Alanine	Arginine	Asparagine	Aspartic acid	Glutamic acid	Glutamine	Lysine	Threonine
Grain Weight	0.30	0.03	0.01	0.27	0.41	-0.05	0.11	0.42
Cob Weight	0.06	-0.01	0.02	0.02	0.07	-0.24	-0.11	0.06
Stover Biomass	0.00	0.06	-0.03	-0.12	-0.17	0.02	-0.06	-0.15
Total N	-0.02	0.07	-0.08	-0.14	-0.10	-0.09	-0.05	-0.12
Grain Moisture	0.13	-0.15	-0.22	-0.01	0.15	-0.09	0.19	0.19
Starch	0.16	-0.03	-0.14	0.08	0.28	-0.23	0.14	0.17
Oil	-0.15	0.17	0.08	-0.09	-0.17	0.17	-0.25	-0.19
Protein	-0.11	0.08	0.15	-0.02	-0.20	0.21	-0.10	-0.12

Inbreds

	Alanine	Arginine	Asparagine	Aspartic acid	Glutamic acid	Glutamine	Lysine	Threonine
Grain Weight	0.33	0.34	0.38	-0.23	-0.43	0.33	0.42	-0.21
Cob Weight	0.02	-0.01	-0.04	0.05	-0.15	0.05	0.03	-0.07
Stover Biomass	-0.17	-0.27	-0.49	0.66	0.65	-0.16	-0.57	0.21
Total N	-0.15	-0.25	-0.35	0.48	0.46	-0.19	-0.44	0.22
Grain Moisture	-0.09	0.33	0.11	0.01	-0.02	0.23	0.22	0.05
Starch	0.15	0.46	0.28	-0.39	-0.52	0.26	0.50	-0.38
Oil	0.04	-0.05	0.12	-0.12	-0.25	-0.24	0.12	0.09
Protein	-0.18	-0.53	-0.38	0.47	0.65	-0.25	-0.61	0.43

Hybridization of diverse genotypes with IHP/ILP alleles decreased the predictive ability of earshoot free amino acids

For some amino acids especially Asn and Gln the correlations reversed with N treatment

-1 to -.4
-.39 to -.2
.2 to .39
.4 to 1

Correlations between NUE related traits with earshoot free amino acids in IBMRIL population

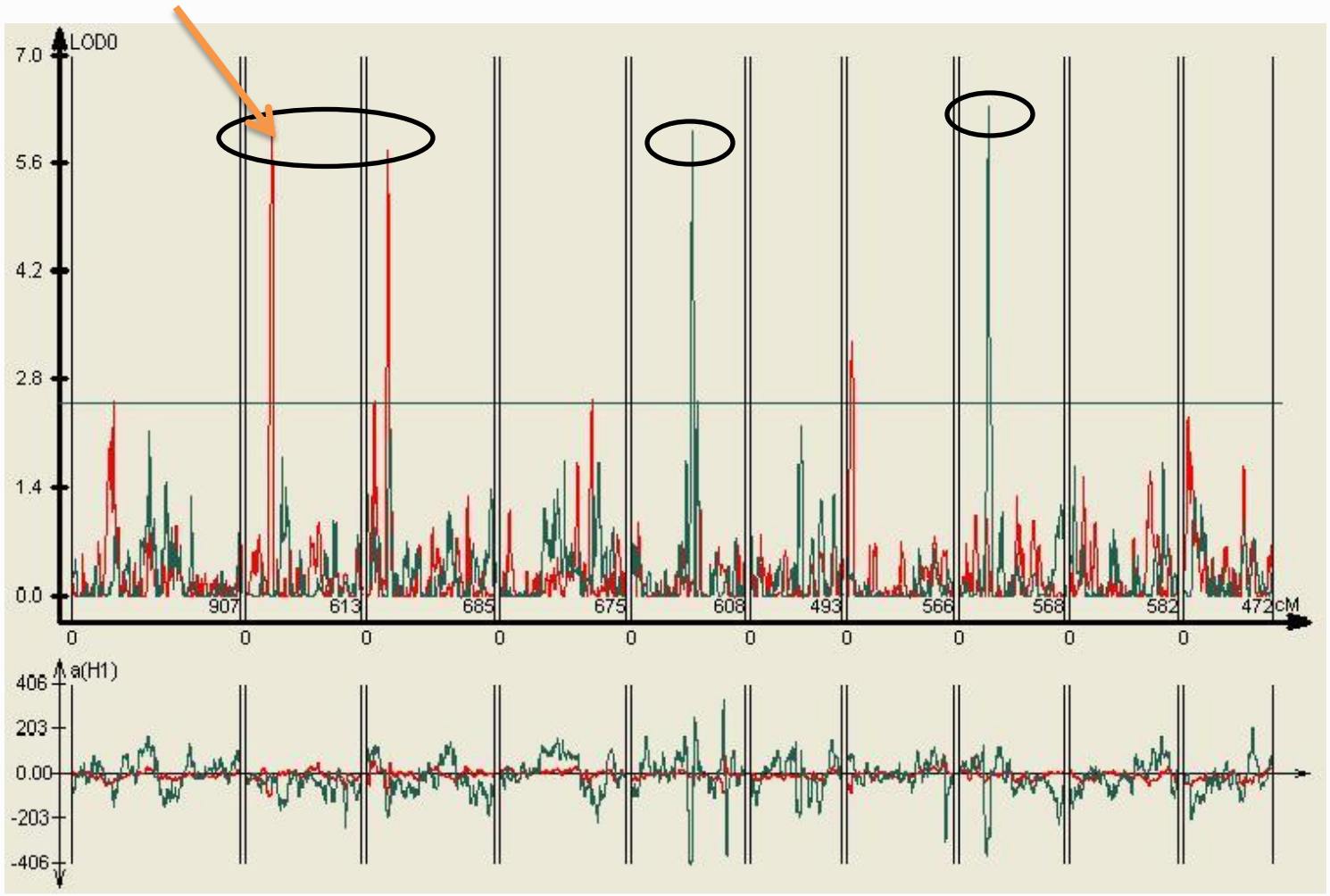
High N

trait	starch	oil	protein	biomass	yield	HI	Knum	stovN	NHI
ala	0.16	-0.03	-0.15	-0.18	0.09	0.2	0.09	-0.25	0.24
arg	0.03	-0.13	0	-0.21	-0.16	0.07	-0.11	-0.28	0.19
Asparagine	**0.13**	**-0.02**	**-0.12**	**-0.29**	**-0.06**	**0.19**	**-0.04**	**-0.33**	**0.24**
asp	-0.15	0	0.16	0.04	-0.28	-0.19	-0.21	-0.12	0.04
glu	-0.07	0.1	0.06	-0.06	-0.2*	-0.06	-0.13	-0.23	0.15
Glutamine	**0.08**	**-0.05**	**-0.07**	**-0.21**	**-0.11**	**0.1**	**-0.05**	**-0.27**	**0.18**

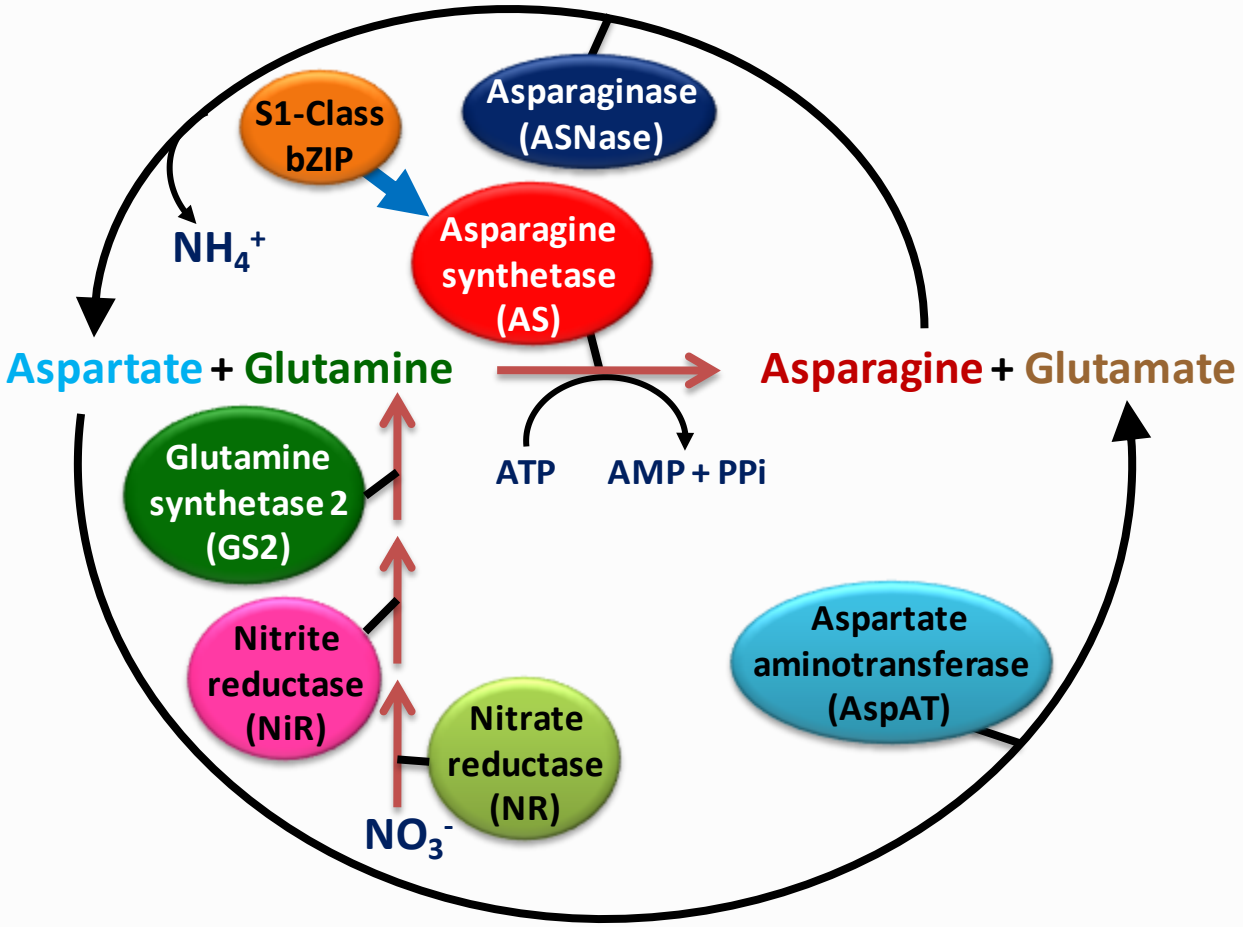
Low N

Trait	Starch	Protein	RS	Biomass	Yield	HI	Knum	StovN	NHI
ala	0.26	-0.26	0.22	-0.3	0.25	0.29	0.21	-0.22	0.23
arg	0.27	-0.26	0.27	-0.41	0.3	0.37	0.3	-0.31	0.29
Asparagine	**0.3**	**-0.32**	**0.34**	**-0.46**	**0.37**	**0.44**	**0.32**	**-0.35**	**0.36**
asp	-0.1	0.08	0.04	-0.04	-0.05	-0.02	0.04	0.12	-0.11
glu	-0.05	0.02	0.05	-0.09	-0.01	0.03	0.05	0.15	-0.12
Glutamine	**0.14**	**-0.15**	**0.16**	**-0.33**	**0.16**	**0.25**	**0.16**	**-0.2**	**0.18**

Metabolite QTL (mQTL) identification using earshoot asparagine and glutamine concentrations



The Asparagine Cycling Pathway



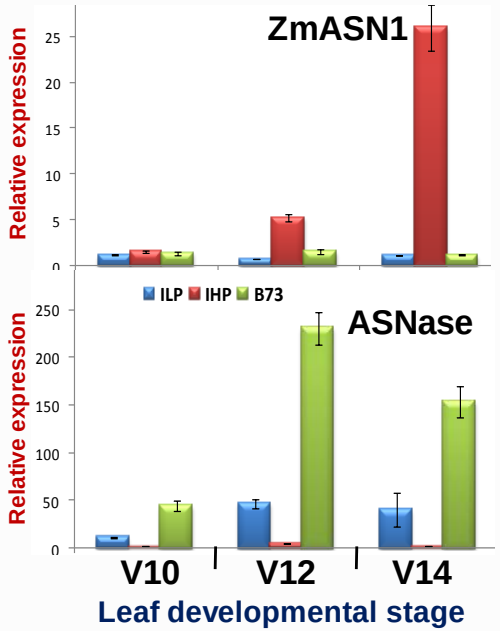
Genes selected for expression QTL (eQTL) analysis

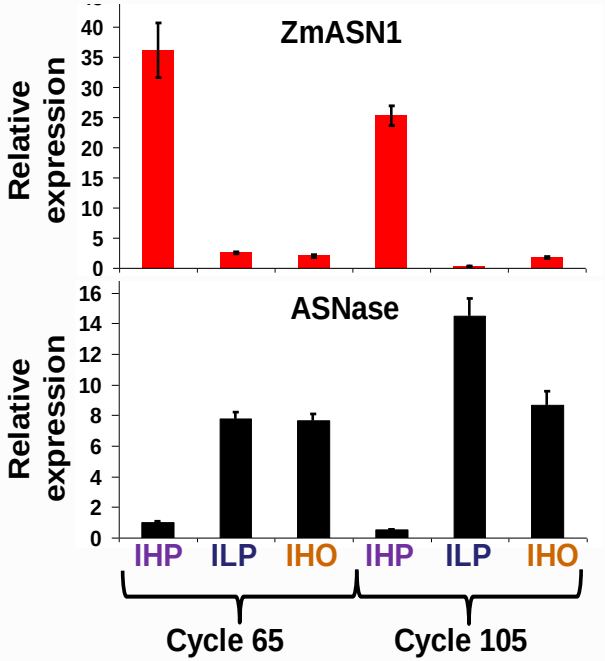
Gene	Forward Primer Sequence	Reverse Primer Sequence	Clone	Bin
Tubulin Beta 7	AGCAGATGTGGGACTCCAAGAACAT	GTTCTTGTTCTGCACGTTCAGCATT	NA	NA
Glutamine Synthetase 2	CGCCCTACACCGACAAGGTCAT	GCTTCTCCTGTGCTAGATCCATCGTA	NA	10.07
Nitrite Reductase 2	CCCTACACCAACCTTCTCTCCTCCTA	GCATGTACGCGAGGTCGTTGAT	AC183914.2	4.07
Nitrite Reductase 6	GGCCATCCATTCTTGAGCGTGTA	GATGAAGCTTGTGACAAAGGCGAA	AC193463.3	6.02
Malate Dehydrogenase	CTTCTGCACCAGGTACGACCTC	TCTTGTCCGAAAACCTCACCA	AC206527.1	1.08
Aspartate Aminotransferase	TTGGAGGAGTTAACCAGGCGAGAA	AGGCCTCAGAAACCCCAAGAATT	AC196774.3	5.08
Asparagine Synthetase 2	CGGAGCCCTCCCCGGACAGCACCATCTTCACG	GTCGTCGGCCTCGAGGACGGCATCGACGCGAT	AC208436.1	1.03
Asparaginase	CCCGCCATCTAAACACAAATCAAA	CAATCTCCATGCAATCGCTACTACTAGTG	AC206980.1	2.02
PEP Carboxylase	CCTTGAGCCACTTGAGCTGTGCTA	GGATGTCCAGCTTCACCAGGGA	NA	9.03

Expression QTL (eQTL) identification for selected genes using V8 leaf of IBMRI population

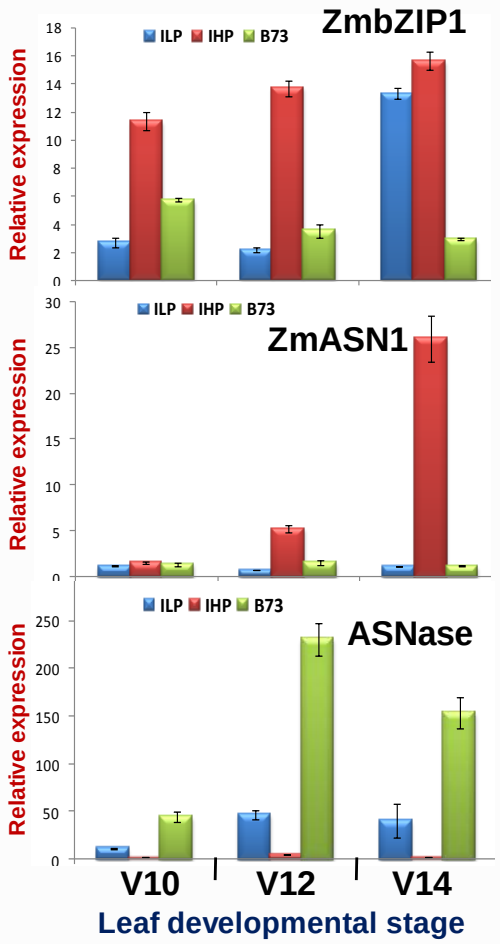
Gene	Chromosome	Position (cM)	Flanking Markers	cis/trans	LOD	R2	Additive Effect
GS2	6	106.6-127.6	rz242a-psb108	trans	6.78	0.25	0.49
	8	258.2-265.3	php20714-umc1460	trans	3.75	0.11	0.26
	10	120.6-130.7	umc130-lim2	trans	3.29	0.10	-0.23
NiR2	6	108.6-132.4	rz242a-umc1595	trans	3.95	0.13	0.19
	6	270.4-288.5	mmp62-umc1020	trans	5.04	0.17	0.22
NiR6	1	573.5-585.7	csu374b-bnlg1556	trans	2.89	0.09	-0.57
	3	304.8-310.6	umc26a-csu636	trans	2.77	0.08	0.56
	5	112.2-125.6	rz630f-psr922a	trans	3.57	0.1	0.6
	7	285.1-315.4	npi394-umc56	trans	10.8	0.26	-1.28
MDH	2	266.7-273.2	bnlg108-umc1861	trans	3.02	0.1	0.45
	4	325.4-332.5	umc1945-mmp97	trans	2.67	0.08	0.39
	6	246.6-256.4	umc1352-umc2141	trans	25.87	0.55	3.24
AspAT	5	580.9-586.7	umc1225-umc104b	cis	2.69	0.09	-0.21
	6	94.6-119.4	mmp10-mmp51	trans	6.89	0.22	0.3
	10	115.9-123.8	isu085b-npi105a	trans	4.17	0.14	0.27
	10	193.2-196.7	umc64a-umc1246	trans	2.56	0.08	-0.2
AS2	5	5.1-15.3	csu1087-umc1445	trans	3.54	0.13	-0.33
	6	107.0-129.2	rz242a-psb108	trans	4.99	0.16	0.37
	8	95.9-104.8	umc1483-bnlg1194	trans	2.98	0.12	0.31
	8	218.5-225.1	mmp195f-umc1415	trans	3.88	0.11	-0.33
	10	170.9-173.9	umc1239-umc155	trans	2.91	0.08	0.25
Asnase	2	43.1-71.3	umc1165-umc1265	cis	19.94	0.56	1.26
	2	189.0-203.1	umc1776-mmp42	trans	3.69	0.07	0.51
	5	117.3-137.1	rz630f-mmp130	trans	3.62	0.06	-0.43
	5	407.5-412.6	mmp104-mmc0481	trans	2.54	0.05	-0.42
	9	262.4-284.4	bnlg1209-npi580a	trans	3.48	0.06	-0.43
PEPC	1	54.3-75.8	cdo1081b-bnlg1014	trans	3.86	0.12	-0.17
	2	270.6-275.1	umc1259-umc2088	trans	2.79	0.09	0.14
	6	139.1-169.6	csu923-npi223a	trans	7.92	0.29	0.26

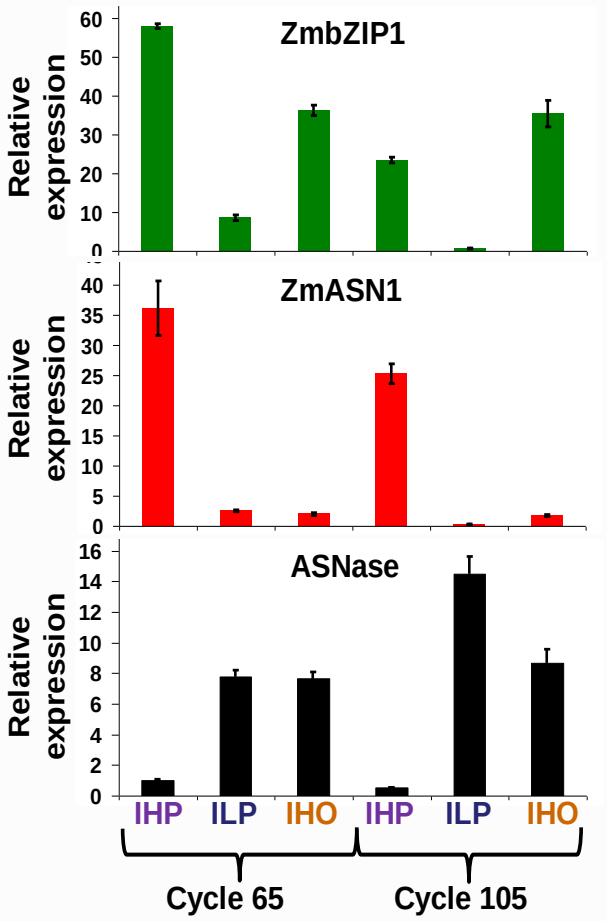
Coordinated expression of Asparaginase and Asparagine synthetase genes



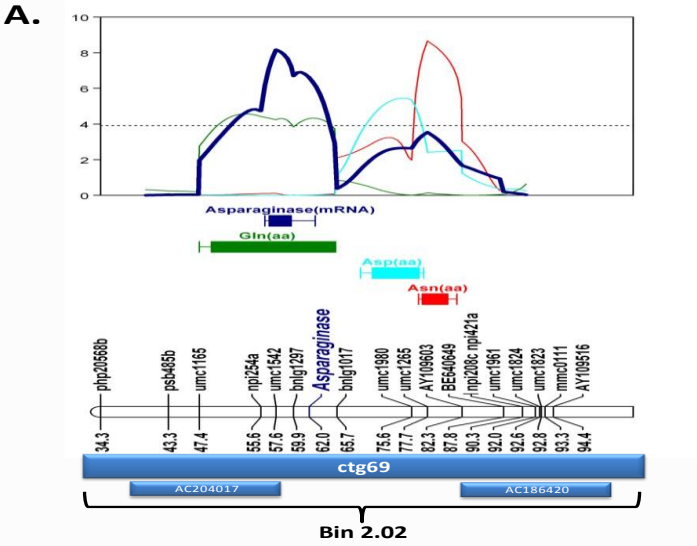


Coordinated expression of Asparaginase and Asparagine synthetase genes





QTL controlling RNA expression of Asn pathway cycling genes, free amino acids, and agronomic N utilization (NutE) in the IBM population



What next…

Find gene groups using the improved coverage obtained from analysis of kernel culture mRNAseq data

Improve confidence on the identified gene groups by comparing overlaps between mRNAseq data and pre-existing profiling data

Obtain genomic locations of differentially accumulating transcrips and connect the dots between the NUE QTL and the genes

Design reciprocal crosses using diverse genotypes with IHP, ILP B73 and Mo17 to improve and reaffirm the predictability of NUE using earshoot free amino acid profiles

AND

Try to improve N U E…

… as much as WE CAN

Jeff Church

Devin Nichols

The Stephen Moose Corn Functional Genomics Lab at Illinois

Abe

Wess

Fred Below

Collaborator

& His Lab

Matt Hudson

Collaborator

& His Lab

Ed Buckler

Collaborator

& His Lab

University of Illinois at Urbana-Champaign