



Cross-Species Network Analysis Uncovers Conserved Nitrogen-Regulated Network Modules in Rice

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Background

1. Nitrogen (N)-regulatory networks is systematic and reasonable in Arabidopsis, a data-rich model species.
2. Improving plant N use efficiency by transgenic technology can diminish the use of N fertilizers.
3. Translating network knowledge from Arabidopsis to enhance the identification of N-regulatory networks in rice, one of the most important crops in the world.



Materials and Methods

- 1.Plant Growth and Treatment Conditions
- 2.RNA Isolation and RT-qPCR Analysis
- 3.Microarray Experiments and Analysis
- 4.Orthology Analysis
- 5.Network Analysis and Data Retrieval
- 6.Network Construction
- 7.Network Visualization and Analysis
- 8.Supernode Network Analysis
- 9.Phylogenetic Analysis

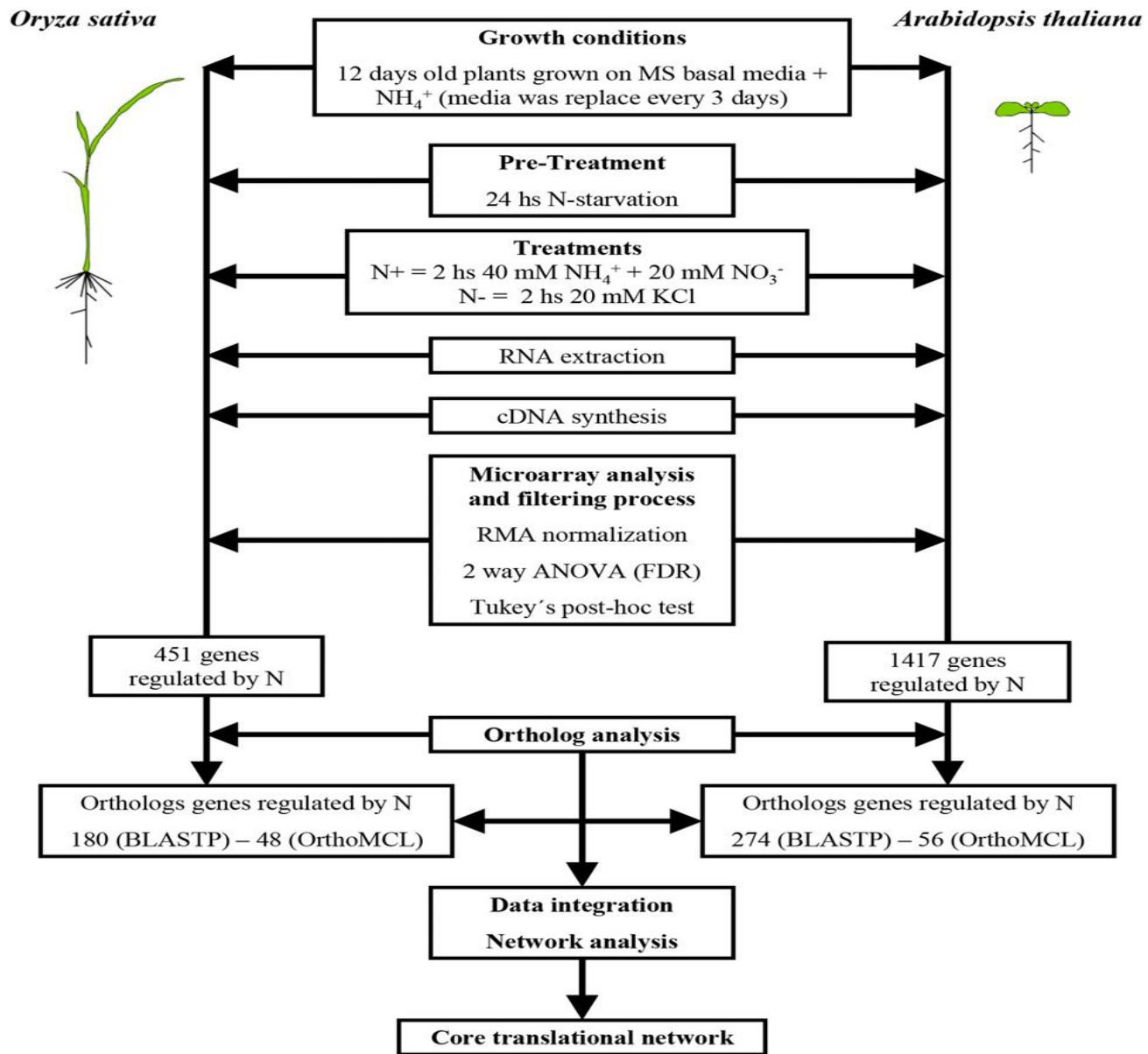


Figure 1. Schematic diagram of the experimental and data-mining approach used in this study.



Results

The Effect of N Treatment on Genome-Wide Expression in Rice

- a.** 451 genes in rice that were significantly regulated in rice by N treatment.
- b.** In rice shoots, 103 genes were N induced, and 39 genes were repressed in response to N treatment. In rice roots, 234 genes were N induced, while 106 genes were repressed in N-treated samples.
- c.** Rice roots appear to have a much larger response in terms of the number of genes.

Table II. Selected rice genes regulated by N in shoots and roots (for details, see “Materials and Methods”)

For the full list of genes, see Supplemental Table S1. The fold change of N-response genes was calculated as the ratio between N and KCl expression values. The *P* value cutoff is 0.05 or less, and the fold change is 1.5-fold or greater (shown are \log_2 values; the fold change cutoff \log_2 for 1.5 = 0.585). NC, No change.

Gene Identifier	Gene Description	Log ₂ Ratio	
		Root	Shoot
Nitrate uptake			
LOC_Os02g02170	High-affinity nitrate transporter, putative, expressed	-0.93	NC
LOC_Os02g38230	Component of high-affinity nitrate transporter, putative, expressed	1.66	NC
Nitrate/nitrite assimilation			
LOC_Os02g53130	Nitrate reductase, putative, expressed	2.15	3.32
LOC_Os01g25484	Ferredoxin-nitrite reductase, chloroplast precursor, putative, expressed	2.37	2.67
Ferredoxin reduction			
LOC_Os01g64120	Ferredoxin6, chloroplast precursor, putative, expressed	3.28	4.22
LOC_Os05g37140	Ferredoxin6	1.33	0.89
LOC_Os03g57120	Ferredoxin-NADP reductase, root isozyme, chloroplast precursor, putative, expressed	1.55	1.95
LOC_Os04g44650	Ferredoxin-thioredoxin reductase	0.67	0.95
Pentose phosphate pathway			
LOC_Os08g08840	Glc-6-P/phosphate translocator2, chloroplast precursor, putative, expressed	0.84	NC
LOC_Os07g22350	Glc-6-P 1-dehydrogenase2, chloroplast precursor, putative, expressed	1.62	1.07
Ammonium assimilation			
LOC_Os01g48960	Glu synthase, chloroplast precursor, putative, expressed	1.17	NC
LOC_Os04g56400	Gln synthetase, chloroplast precursor, putative, expressed	1.09	0.71



Genome-Wide Effects of N Treatment in Arabidopsis

a. 1,417 Arabidopsis genes were identified to be N responsive compared with the control treatment.

b. In Arabidopsis shoots, 166 genes were N induced and 184 genes were repressed in response to N treatments. In Arabidopsis roots, 757 genes were N induced and 424 genes were repressed.



Table III. Selected *Arabidopsis* genes regulated by N in shoot and/or roots (for details, see “Materials and Methods”)

The fold change of N-response genes was calculated as the ratio between the N and KCl expression values. The *P* value cutoff is 0.05 or less, and the fold change is 1.5-fold or greater (shown are \log_2 values; the fold change cutoff \log_2 for 1.5 = 0.585). NC, No change.

Gene Identifier	Gene Description	Log ₂ Ratio	
		Roots	Shoots
Nitrate uptake			
At1g69850	Nitrate transporter1:2; calcium ion binding/transporter (NRT1:2)	-0.62	-1.04
At5g50200	Nitrate transmembrane transporters (NRT3:1)	1.79	2.04
At1g08090	High-affinity nitrate transporter2.1 (NRT2:1)	2.76	2.35
Nitrate/nitrite assimilation			
At1g37130	Nitrate reductase2 (NIA2)	NC	2.65
At1g77760	Nitrate reductase1 (NIA1)	3.69	5.85
At2g15620	Nitrite reductase; ferredoxin-nitrate reductase (NIR1)	3.33	6.33
Ferredoxin reduction			
At2g27510	Ferredoxin3; electron carrier (ATFD3)	1.52	3.16
At4g05390	Root FNR1; oxidoreductase (ATRFNR1)	2.49	3.99
At1g30510	Root FNR2; oxidoreductase (ATRFNR)	2.75	4.43
Pentose phosphate pathway			
At1g24280	Glc-6-P dehydrogenase3 (G6PD3)	3.34	4.78
At5g13110	Glc-6-P dehydrogenase2 (G6PD2)	1.93	2.99
Ammonium assimilation			
At5g35630	Gln synthetase2 (GS-GLN2)	1.14	NC
At5g16570	Gln synthetase1;4 (GLN1;4)	-1.20	NC
At5g53460	NADH-dependent Glu synthase1 (GLT1)	1.38	2.40
Glu biosynthesis/degradation			
At1g51720	Glu dehydrogenase, putative	1.14	NC
At5g07440	Glu dehydrogenase2 (GDH2)	1.76	NC



Table I. *Number of N-regulated genes in rice and Arabidopsis*

Percentages of regulated genes for each type of regulation are in parentheses.

Genes	Roots		Shoots	
	Induced	Repressed	Induced	Repressed
Rice, 451 genes	234 (51.8%)	106 (23.5%)	103 (22.8%)	39 (8.6%)
Arabidopsis, 1,417 genes	757 (53.4%)	424 (29.9%)	166 (11.7%)	184 (12.9%)



whether was the overlap between the rice and Arabidopsis N-responsive genes significant?

A total of 1,417 genes were selected randomly from Arabidopsis genes present on the Affymetrix chip, and 451 rice genes were selected randomly from genes present on the rice Affymetrix chip.

Using BLASTP homology, these results suggest that, despite the difference in the number of responsive genes, rice and Arabidopsis respond very similarly to the N treatments provided.



Network Analysis Identifies Conserved Genes Involved in N Signaling in Rice

1. Creation of a Rice-Arabidopsis N-Regulatory Network

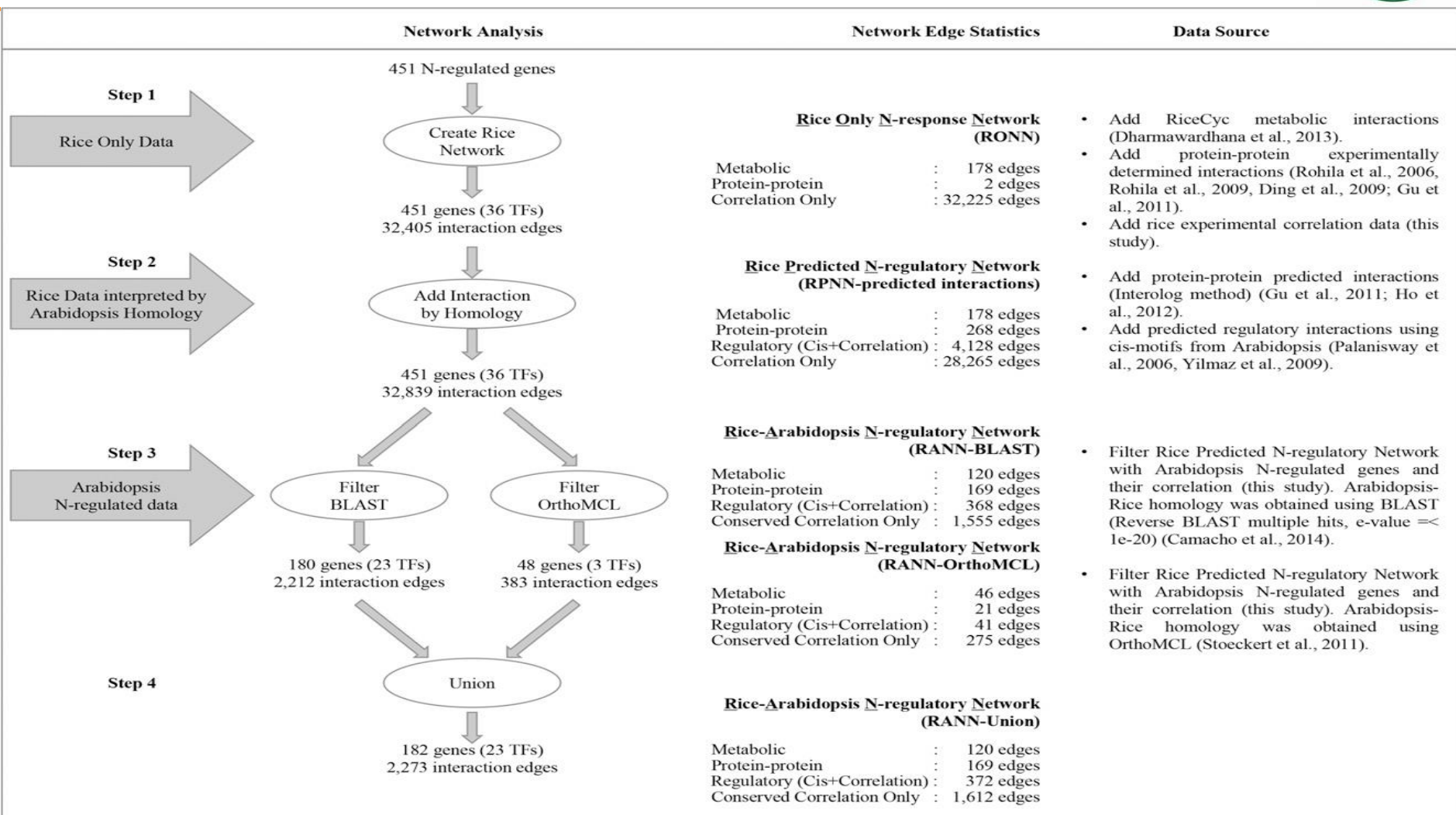


Figure 2. Work flow of the network analysis of N-regulated genes differentially expressed in rice resulting in RANN-Union.

Table IV. TFs in RANN-Union

For each step in network construction (Fig. 2), TFs were rank based on their number of connections in the network.

Rice Gene Locus	Rice Gene Description	No. of Directed Connections			
		RPNN-Predicted Interactions	RANN-BLAST	RANN-OrthoMCL	RANN-Union
LOC_Os03g55590	DNA-binding protein, putative, expressed	180	45	17	46
LOC_Os01g53260	OsWRKY23: superfamily of rice TFs having WRKY and zinc finger domains, expressed	162	41		41
LOC_Os01g64000	Abscisic acid response element-binding factor, putative, expressed	138	35		35
LOC_Os01g06640	DNA-binding protein, putative, expressed	191	31		31
LOC_Os07g02800	Myb-like DNA-binding domain, SHAQKYF class family protein, expressed	150	30	14	30
LOC_Os01g43650	OsWRKY11: superfamily of rice TFs having WRKY and zinc finger domains, expressed	166	26	10	29
LOC_Os11g47870	Chitin-inducible gibberellin-responsive protein2, putative, expressed	163	27		27
LOC_Os09g35030	sbCBF6, putative, expressed	229	16		16
LOC_Os09g25070	OsWRKY62: superfamily of rice TFs having WRKY and zinc finger domains, expressed	170	14		14
LOC_Os01g34060	DNA-binding protein, putative, expressed	99	12		12
LOC_Os04g42950	DNA-binding protein, putative, expressed	96	12		12
LOC_Os09g32260	Ambidopsis NAC079 (ANAC079)/ANAC080, putative, expressed	129	12		12
LOC_Os11g08210	NAC domain-containing protein71, putative, expressed	99	11		11
LOC_Os02g15340	NAC domain-containing protein76, putative, expressed	67	10		10
LOC_Os04g55970	DNA-binding protein, putative, expressed	143	10		10
LOC_Os12g10660	Salt tolerance-like protein, putative, expressed	50	10		10
LOC_Os03g04310	DNA-binding protein, putative, expressed	84	7		7
LOC_Os10g42130	ANAC071, putative, expressed	78	6		6
LOC_Os01g64020	TF Histone Gene Binding Protein-1b, putative, expressed	219	5		5
LOC_Os08g42550	AP2 domain-containing protein, expressed	61	5		5
LOC_Os06g41100	TGA10 TF, putative, expressed	119	2		2
LOC_Os05g37170	TF TGA6, putative, expressed	90	1		1
LOC_Os02g06910	Auxin response factors, putative, expressed	163			
LOC_Os03g21710	WRKY DNA-binding domain-containing protein, expressed	79			
LOC_Os03g47730	Knotted1-interacting protein, putative, expressed	61			
LOC_Os03g55220	Helix-loop-helix DNA-binding protein, putative, expressed	175			
LOC_Os04g56990	Transcription factor, putative, expressed	293			
LOC_Os05g20930	Transcriptional regulator RABBIT EARS, putative, expressed	195			
LOC_Os05g38140	Basic helix-loop-helix (bHLH) TF, putative, expressed	79			
LOC_Os06g07030	Dehydration-responsive element-binding protein, putative, expressed	101			
LOC_Os09g26420	Ethylene response factor, putative, expressed	69			
LOC_Os09g36160	SHORT INTERNODES, putative, expressed	30			



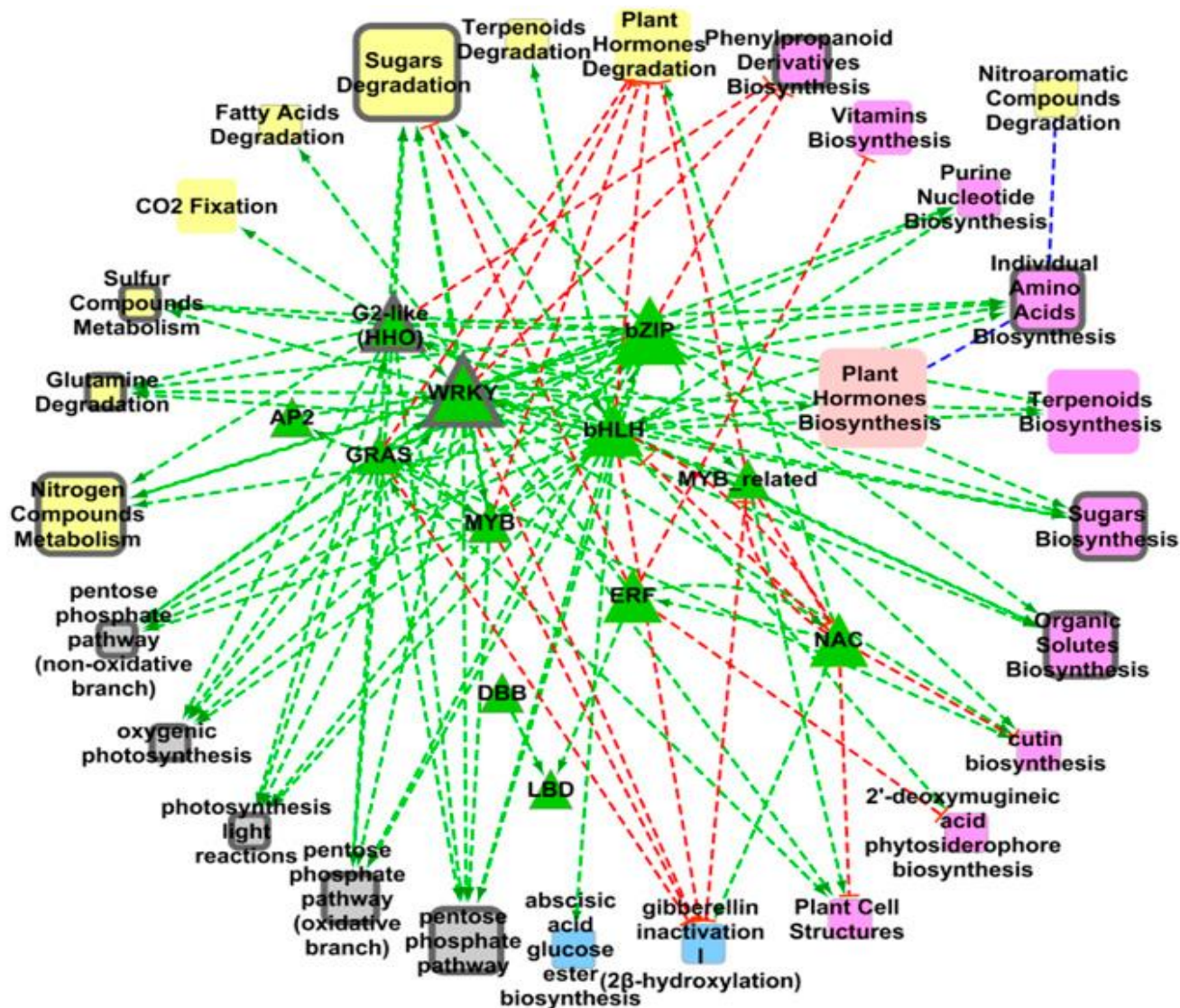
2. Creation of an Arabidopsis-Rice N-Regulatory Network

The resulting Arabidopsis-rice N-regulatory network (ARNN-Union) has 276 genes. By definition, the identities of the genes from ARNN-Union are equal to those of RANN-Union. The number of genes is different, however, because in most cases rice genes have more than one N-regulated ortholog in Arabidopsis. Following this rationale, ARNN-Union contains 34 TFs, while RANN-Union contains only 23 TFs.



Supernode Analysis of RANN-Union

The supernode analysis groups genes with the same biological processes, functional terms, and annotations into a single node whose size is proportional to the number of genes in the supernode.



- Degradation/Utilization/Assimilation**
- Biosynthesis**
- Activation/Inactivation/Interconversion**
- Fatty Acids and Lipids**
- Generation of Precursor Metabolites and Energy**
- Transcription Factor families**



Two Predicted TF Families Conserved in RANN-Union Are Biologically Validated

- a.** two TF families, HHO/HRS1 family, whose roles in N signaling have been experimentally validated.
- b.** TGA family members in Arabidopsis and rice are identified by phylogenetic analysis.



Personal summary

This study provides a novel analysis of N-regulated gene networks conserved across two highly divergent species: rice (a monocot) and Arabidopsis (a dicot).

Despite their large phylogenetic distance, our analysis revealed a set of N-regulated genes, TFs, and network modules conserved in rice and Arabidopsis exposed to the same N-treatment conditions.



本研究创新点：利用数据比对的方式对不同物种保守基因调控网络模块的揭示，尤其是在农作物中占主导地位的水稻的氮营养调控基因网络模块的揭示，此类研究非常新颖。



此研究对我的启发：1、由预测基因提供相关水稻氮高效利用基因 2、文章中提供许多数据库资源

the Gene Expression Omnibus database
(<http://www.ncbi.nlm.nih.gov/geo/>)

The Institute for Genomic Research Rice Genome
Annotation Database
(<http://rice.plantbiology.msu.edu/>)

RAP-DB (<http://rapdb.dna.affrc.go.jp/>)

the PRIN database, the Rice kinase database,
PlantTFDB



此研究可以改进地方：1、实验处理的时候应该在各物种适宜生长的条件下再设置一个对照，取消外界环境对植物生长的胁迫。

2、基因芯片分析方法可以由RNA-seq代替，方便，准确性更高，这样可以减少RT-qPCR的使用，降低实验成本。



THANK YOU!