

## **SP2\_13: Crop gene expression profiles and stress-gene arrays**

### **A case study**

**Comparative gene expression analysis in rice and wheat  
during vegetative stage under drought condition**

**Tiegang LU**

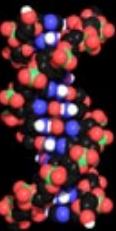
# Rationale

- **Rice is a model cereal crop to study the stress response at molecular level due to the availability of whole genome information and other molecular tools.**
- **Wheat is one of the most drought-tolerant cereals, providing a useful platform to understand tolerance mechanisms.**
- **Rice and wheat genome has high synteny and it is feasible to use rice chips to do hybridization with wheat RNAs.**

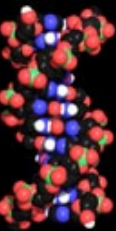


# Objectives

- 1. Through collaborative experiments using existing array platforms for cross-species analyses to identify stress responsive genes and mechanisms.**
- 2. Explore the use of orthology data to produce subarrays of stress genes for broad usage in multiple crops.**



**This work is to make a comparative study of drought responsiveness in rice and wheat by taking advantage of greater degree of drought tolerance of wheat on one side and availability of whole genome level information from rice on the other side.**



# Work Planned

- 1. Identification of common stress phenotype across monocots under drought.**
- 2. Monocot cross-species hybridization using rice whole genome and oligo arrays**
- 3. Data mining to identify common orthologs from cross-species array data.**
- 4. Stress database to elucidate molecular components for plant drought tolerance**
- 5. Validation of gene expression**



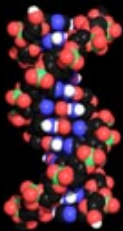
# **Experiments I**

**Comparative expression analysis of rice and wheat  
under drought condition**

**IRRI, BGI and NIAS**

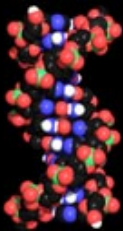
# Plant Materials

- 1. Rice: IR64 (highly drought susceptible *indica* variety); Apo (drought tolerant upland *indica* variety)**
  - 2. Wheat: Weebil ( drought tolerant spring wheat); Seri (drought susceptible spring wheat)**
- Rice plants were grown in greenhouse and wheat plants were grown in Phytotran.**
  - Water stress was imposed after 33 DAS by withholding further watering.**



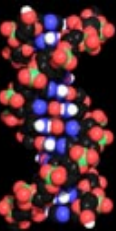
# Plant Materials -stress phenotype

- 1. Rice cultivars showed leaf rolling symptom on the 4th day after stress.**
- 2. Wheat plants took 10-13 days to show leaf rolling symptoms when the available soil moisture reaches 25-30%.**
- 3. At this level of stress, the Relative Water Content of leaves reached around 50% in rice, whereas the wheat plants maintained around 65% RWC.**
- 4. Sampling was done based on the development of leaf rolling symptoms.**



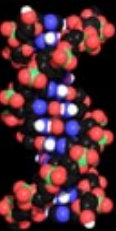
# Gene expression analysis

1. **Microarray hybridizations were done using rice - 60K Oligos array at Beijing Genomics Institute, Beijing, China.**
2. **In all the genotypes, the control samples were hybridized against the water stressed samples.**
3. **Completed two biological replications.**
4. **Spot identification and quantitative analysis were performed using GenePix software. Array data normalization, data transformation and statistical tests were performed using TIGR – TMeV tool.**

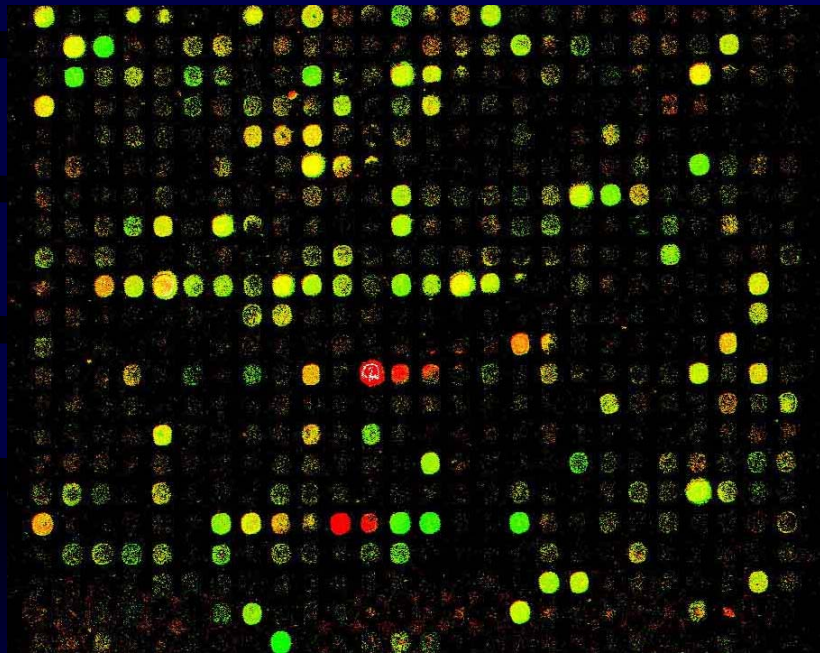


# Efficiency of cross-species hybridization

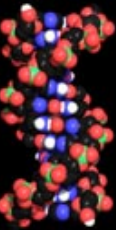
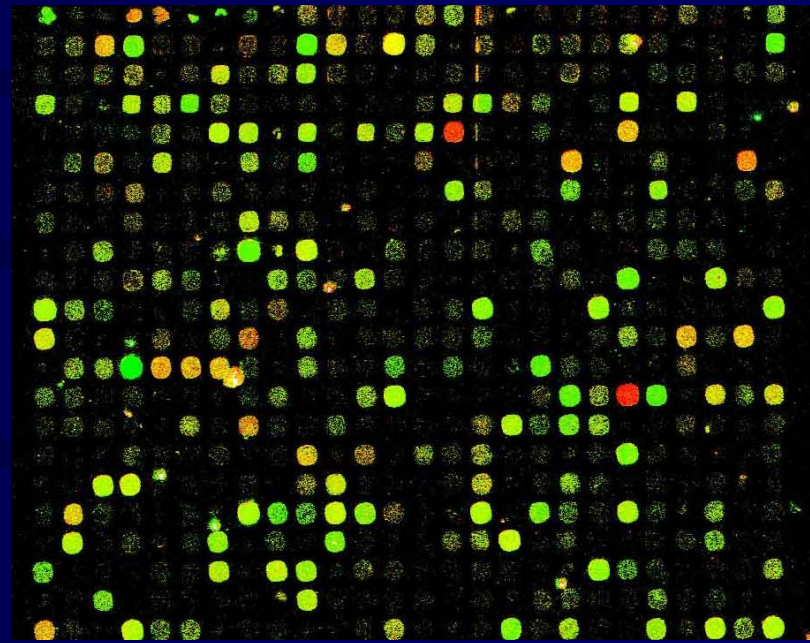
- 1. We detected around 29494 signals (number of significantly hybridized signals) in rice-rice hybridization. Interestingly in the wheat-rice (wheat RNA hybridized against rice chip) hybridization, we detected around 19053 signals.**
- 2. This cross species hybridization accounted for more than 60% when compared with the homologous (rice-rice) hybridization.**



## Rice vs rice hybridization



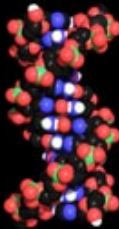
## Wheat vs rice hybridization



# Classification of drought-inducible genes in rice

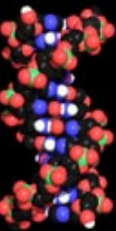
Significant gene Expression	APO	IR 64	Common to both APO and IR64	APO specific	IR64 specific
Number of genes Up regulated during drought	233	106	77	156	29
Number of genes Down regulated during drought	422	293	153	269	140

Results shown are based on two biological replications at  $p=0.05$ .



# Drought responsive gene expression in wheat

- 1. Around 52 genes were found to be drought responsive in the wheat variety “Weebil”.**
- 2. The lesser number genes might be due to the low hybridization signal due to low homology between orthologous genes.**
- 3. Only twelve genes showed common expression pattern in both rice and wheat.**
- 4. Five genes (Putative arginase, ubiquitin, metallothionin, serine/threonine protein kinase etc.,) showed contrasting pattern of expression in rice and wheat. They were upregulated in wheat and down-regulated in rice.**

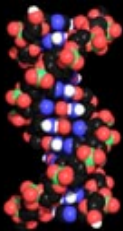


# **Experiments II**

**Comparative expression analysis of rice and wheat  
under drought condition**

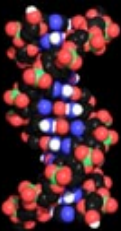
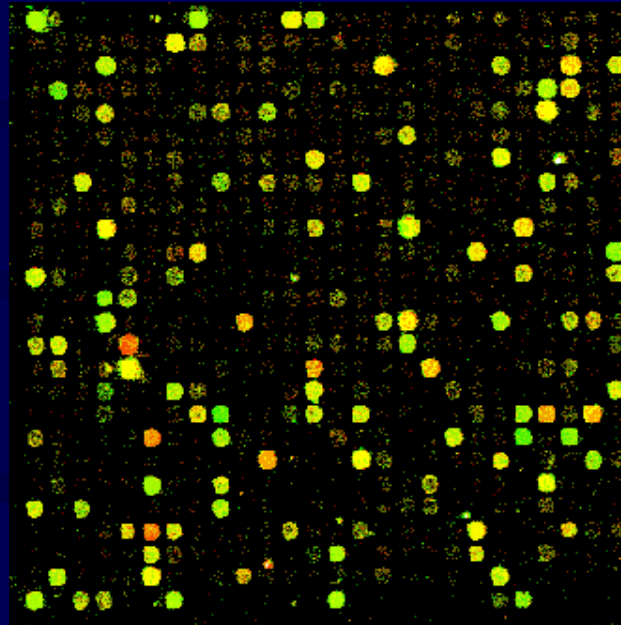
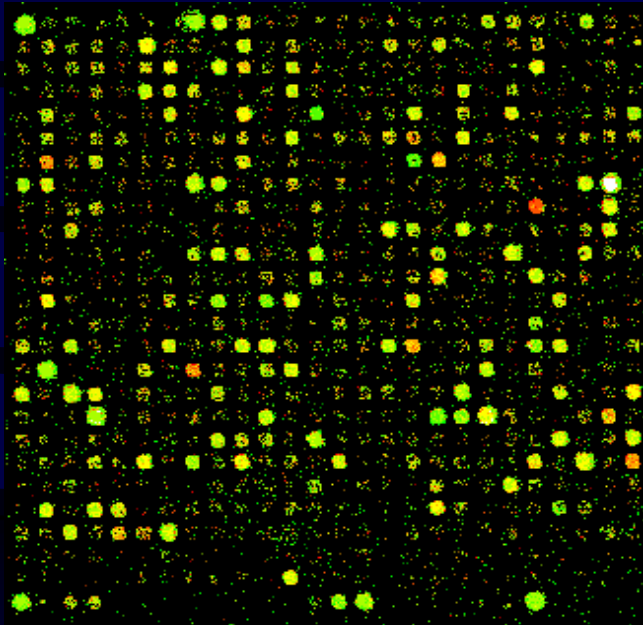
# Plant Materials

- 1. Wheat: Hanxuan No.10 ( the most drought tolerant winter wheat selected from hundreds of Chinese varieties).**
- 2. Seeds were germinated and plants were grown in the incubator at 20°C in 12hr light/12hr dark condition.**
- 3. Seedlings 15 days after germination were collected and treated with PEG-6000 ( -0.5MPa) for 0hr, 1hr, 6hrs, 24hrs.**



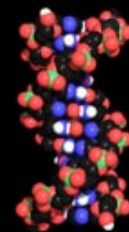
# Preliminary Data

## 0 vs. 24 hr PEG-6000 treatment



# Differentially expressed genes (766)

ID	Name	log2(KH1/KH4)	TIGR description
Os054364 01	OsIFCC022557	6.03	cdNA Photosystem II 10 kDa polypeptide(756)
Os002390 01	OsIFCC022557	5.7	cdNA Photosystem II 10 kDa polypeptide(756)
Os002434 01	OsIRUA001029	4.41	cdNA At5g49760(2694)
Os002458 01	OsIFCC041903	4.2	cdNA probable dehydrogenase F23N20.17(843)
Os026301 02	OsIRUA006907	2.67	cdNA probable dormancy-associated protein(1186)
Os029018 01	OsIFCC039486	2.54	cdNA hypothetical protein(471)
Os009053 01	OsJRFA059621	2.54	cdNA New cDNA-based Gene(2481)
Os006571 01	OsIFCC019376	2.51	cdNA retrotransposon protein, putative, (2685)
Os054271 01	OsJRUA000705	2.5	cdNA probable dormancy-associated protein(1186)
Os054389 01	OsJRUA000751	2.35	cdNA retrotransposon protein, putative, (815)
Os032194 01	OsIFCC043434	2.32	cdNA F-box protein interaction domain, (1173)
Os003251 01	OsIRUA001502	2.25	cdNA BAG domain, putative(1281)
Os032984 01	OsIFSB000853	2.19	cdNA hypothetical protein(483)
Os034247 01	OsIFCC034401	2.13	cdNA hypothetical protein(549)
Os028725 01	OsIFCC045246	2.07	cdNA hypothetical protein(213)
Os035646 01	OsIFCC013384	2.07	cdNA retrotransposon protein, putative, (2058)
Os025656 01	OsJRFA110485	2.04	cdNA hypothetical protein(255)
Os037049 01	OsIFCC026013	2.03	cdNA Similar to PSII 47 kDa protein(285)
Os001463 01	OsIFCC014124	1.97	cdNA expressed protein(1233)
Os019244 01	OsJRFA100776	1.95	cdNA brix domain containing protein 1(1366)
Os027887 01	OsIFCC028369	1.93	cdNA hypothetical protein(555)
Os002596 01	OsIFCC023890	1.91	cdNA Chalcone and stilbene synthases, (1200)
Os009832 01	OsJRFA061599	1.91	cdNA expressed protein(2803)
Os036348 01	OsIFCC032595	1.91	cdNA hypothetical protein(486)
Os017123 01	OsJRFA072515	1.91	cdNA Similar to lob domain protein 1(1097)
Os011874 01	OsJRFA065235	1.87	cdNA putative thiamin biosynthesis protein(2119)
Os054366 01	OsIFCC032704	1.87	cdNA Ribulose biphosphate carboxylase, (1066)
Os017718 01	OsJRFA073356	1.86	cdNA universal stress protein family(1012)
Os035755 01	OsIFSC048073	1.85	cdNA hypothetical protein(474)
Os027722 01	OsIFCC000225	1.83	cdNA Bowman-Birk serine protease(306)
Os016588 01	OsJRFA071719	1.83	cdNA hypothetical protein(1575)
Os037401 01	OsIFCC000521	1.82	cdNA hypothetical protein(486)



Os004022_01	OsIRUA001976	cDNA Glycosyl hydrolases family 17, putative(2173)	-2.02
Os034861_01	OsIFCC035396	cDNA Chalcone and stilbene synthases,(1224)	-2.07
Os026223_01	OsJRFA111180	-	-2.07
Os009883_01	OsJRFA061968	cDNA geranylgeranyl reductase(1750)	-2.08
Os037626_01	OsIFCC016817	cDNA NB-ARC domain, putative(2883)	-2.08
Os014758_01	OsJRFA069192	cDNA MIP family channel proteins(960)	-2.09
Os011926_01	OsJRFA065309	cDNA Oxidoreductase NAD-binding domain,(1478)	-2.13
Os010723_01	OsJRFA063486	cDNA myb-like DNA-binding domain, SHAQKYF(2077)	-2.14
Os001365_01	OsIRUA000546	cDNA Similar to cellulose synthase-like(3648)	-2.16
Os054660_01	OsIFCC018041	cDNA salt tolerance-like protein.(690)	-2.16
Os054319_01	OsIFCC021360	cDNA fructose-1,6-bisphosphatase(1474)	-2.16
Os035261_01	OsIFCC010096	cDNA calcium binding EF-hand protein,(198)	-2.18
Os002029_01	OsIRUA000817	cDNA ATPase, AAA family, putative(1839)	-2.19
Os049905_01	OsIFSB005174	cDNA hypothetical protein(402)	-2.2
Os040245_01	OsIFCC043471	cDNA Psd, putative(909)	-2.22
Os029679_01	OsIFCC042303	cDNA Glycosyl hydrolases family 18(894)	-2.26
Os014118_01	OsJRFA068329	cDNA SOUL heme-binding protein(657)	-2.27
Os052159_01	OsIFCC039118	cDNA ethylene-responsive transcriptional(816)	-2.28
Os051899_01	OsIFCC003213	cDNA glycine dehydrogenase(3551)	-2.3
Os012339_01	OsJRFA065834	cDNA U-box domain, putative(4065)	-2.38
Os042449_01	OsIFCC021321	cDNA UDP-glucuronosyl and UDP-glucosyl(1443)	-2.47
Os054418_01	OsIFCC036105	cDNA ATPase, AAA family, putative(1839)	-2.47
Os010113_01	OsJRFA062559	cDNA aldehyde dehydrogenase (NAD) family(1533)	-2.51
Os002320_01	OsIRUA000963	cDNA Chlorophyll A-B binding protein,(807)	-2.51
Os008686_01	OsJRFA058624	cDNA ABC1 family, putative(2745)	-2.52
Os026853_01	OsIRUA005168	cDNA glycine dehydrogenase(3551)	-2.58
Os002379_01	OsIFCC011448	cDNA glycine dehydrogenase(3551)	-2.61
Os001615_01	OsIRUA000650	cDNA glycine dehydrogenase(3551)	-2.69
Os057565_02	OsIFCC001808	cDNA Cyclin, N-terminal domain, putative(1843)	-2.71
Os056448_01	OsIFCC000373	cDNA F-box domain, putative(1248)	-2.88
Os016919_01	OsJRFA072245	cDNA vacuolar acid invertase(2442)	-2.98
Os033867_01	OsIFCC035025	cDNA probable dehydrin - rice(890)	-3
Os002146_01	OsIRUA000889	cDNA Chlorophyll A-B binding protein,(1173)	-3.54
Os011001_01	OsJRFA063984	cDNA LEA protein - rice(973)	-3.59

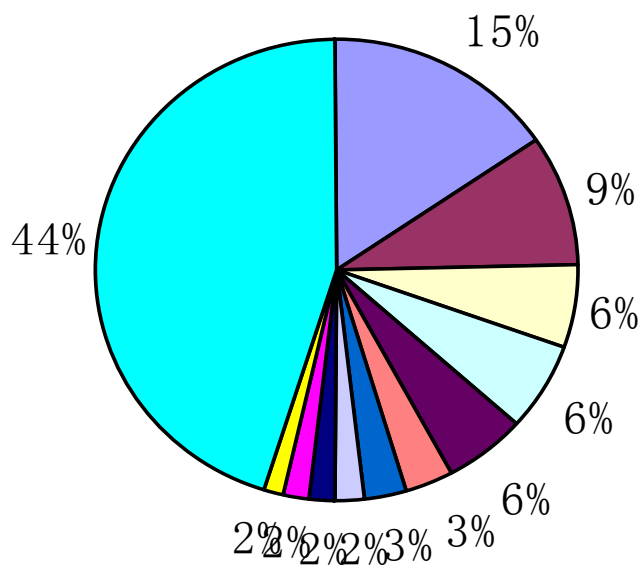
## Differentially expressed genes (766)

- 1. We detected around 30331 signals (number of significantly hybridized signals) in wheat-rice hybridization (0 vs. 24 hr treated wheat RNA hybridized against rice chip), about 50% of the total rice sequences.**
- 2. Of them, 446 are up-regulated and 320 are down regulated.**

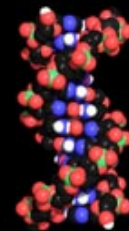


# GO classification of 446 up-regulated genes

耐旱24小时处理上调基因的GO分类

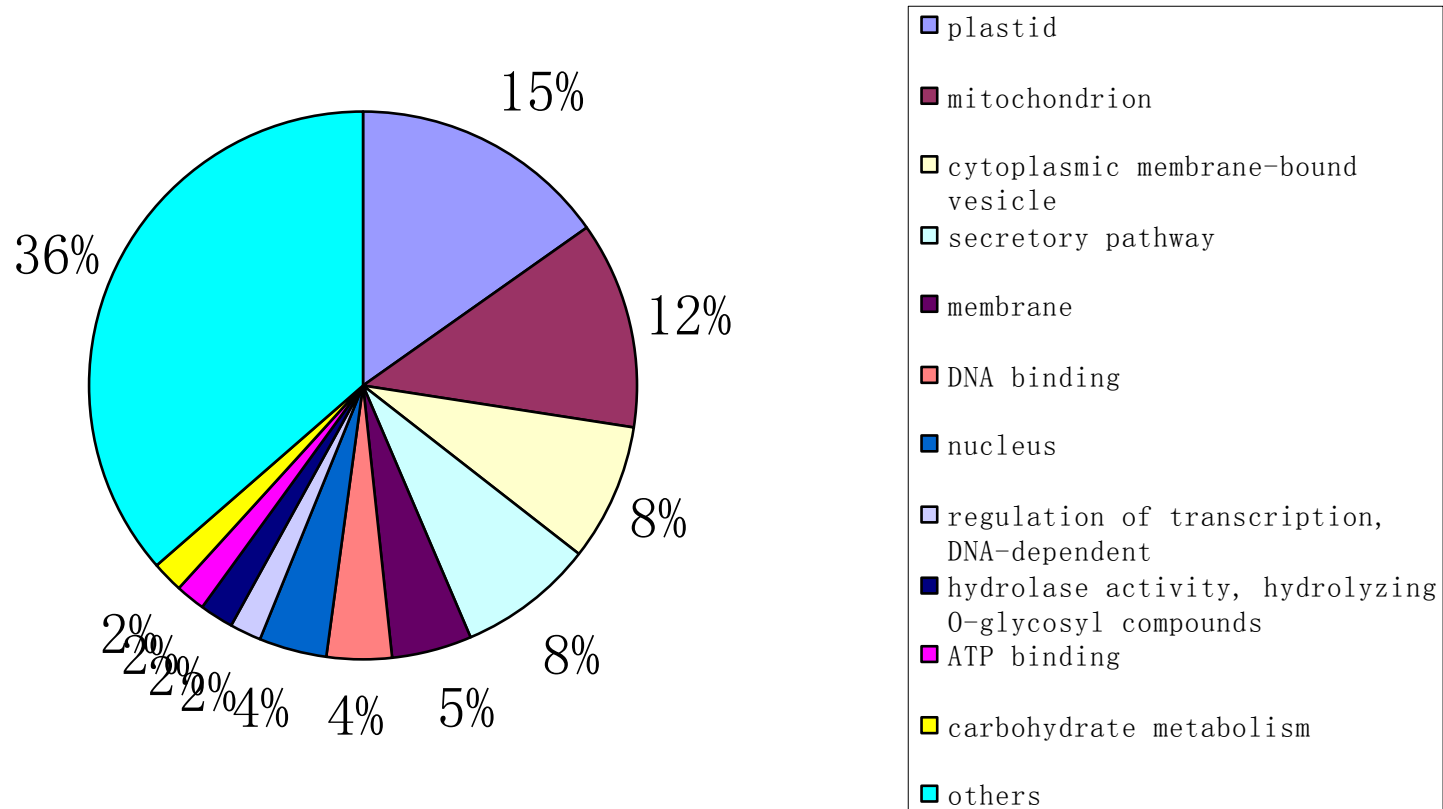


- plastid
- mitochondrion
- cytoplasmic membrane-bound vesicle
- secretory pathway
- membrane
- ATP binding
- electron transport
- DNA binding
- nucleus
- metabolism
- oxidoreductase activity
- others



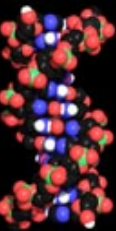
# GO classification of 320 down-regulated genes

耐旱24小时处理下调基因的GO分类



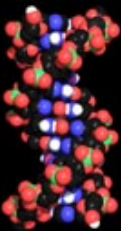
# Experiments III

**Comparative expression analysis of rice and wheat  
under salinity condition**



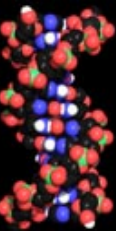
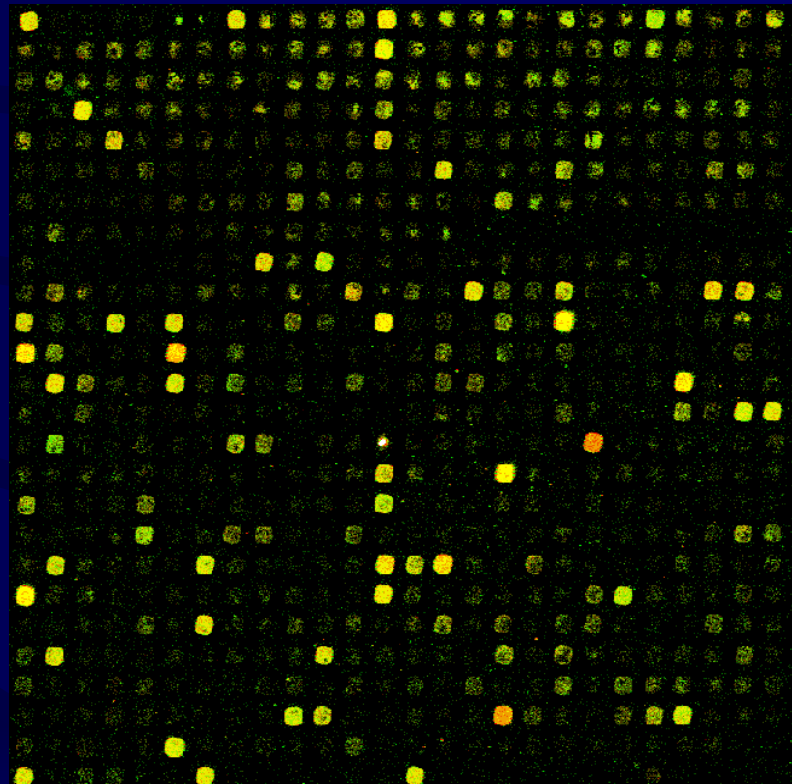
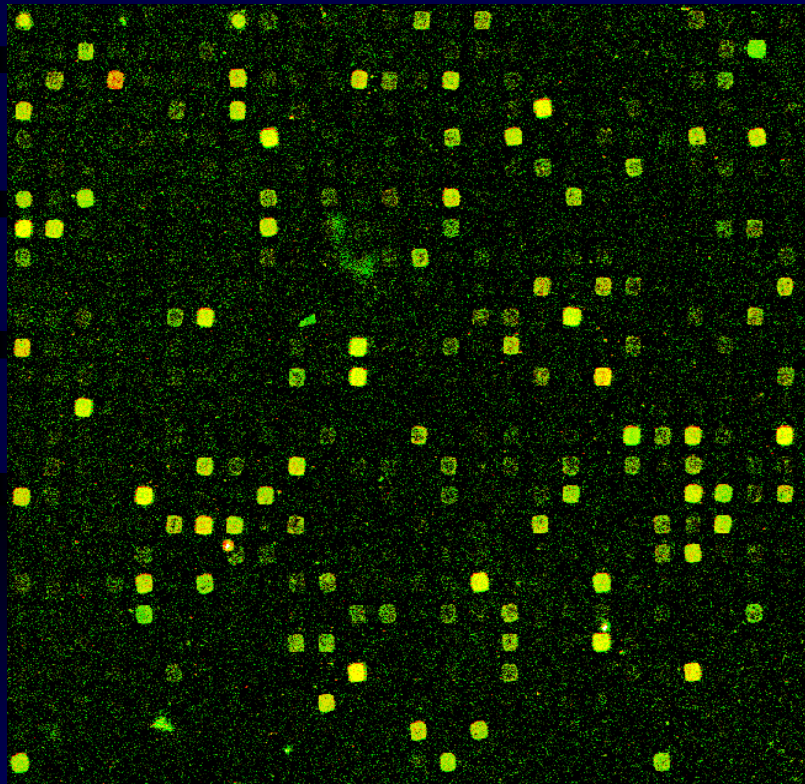
# Plant Materials

- 1. Wheat: 98160 (has strong tolerance to salinity, could grown well in medium or soil containing 1% sodium chloride).**
- 2. Seeds were germinated and plants were grown in the incubator at 20°C in 12hr light/12hr dark condition.**
- 3. Seedlings 15days after germination were collected and treated with 1.0% sodium chloride for 0hr, 2hrs, 6hrs.**



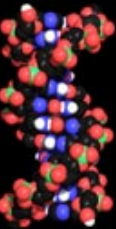
# Preliminary Data

0 vs. 6hr 1.0% sodium chloride treatment



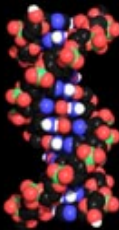
# Common Differential expressed genes in two biological replicates (31)

ID	Name	TIGR_description	log2(6H1/CK1)
Os027722 01	OsIFCC000225	cDNA Bowman-Birk serine protease(306)	1.89
Os022642 02	OsIRUA006831	cDNA Male sterility protein, putative(1506)	1.75
Os044122 01	OsIFSB003299	-	1.75
Os037099 01	OsIFCC040878	cDNA hypothetical protein(366)	1.6
Os009246 01	OsJRFA060173	cDNA AT3g03310/T21P5 27(1695)	1.53
Os023181 02	OsIRUA006848	cDNA nonspecific lipid-transfer protein 2(919)	1.37
Os035641 01	OsIFCC032297	cDNA MtN3/saliva family, putative(723)	1.35
Os037246 01	OsIFCC043528	-	1.3
Os042727 01	OsIFCC033952	cDNA expressed protein(723)	1.26
Os027686 01	OsIFCC009368	cDNA hypothetical protein(498)	1.21
Os000666 01	OsIFCC022925	cDNA Raffinose synthase or seed imbibition(2843)	1.21
Os052542 01	OsIFCC004580	cDNA expressed protein(1449)	1.13
Os055323 01	OsIFCC009335	cDNA hypothetical protein(459)	1.09
Os027641 01	OsIFCC037450	cDNA Similar to probable lipid transfer(598)	1.03
Os005036 01	OsIFCC009631	cDNA imbibition protein homolog, putative(2790)	1.02
Os020360 01	OsJRFA102352	cDNA expressed protein(1445)	1.02
Os053057 01	OsIFSC048191	cDNA expressed protein(466)	1.02
Os042821 01	OsIFCC031761	cDNA hypothetical protein(1362)	0.953
Os034390 01	OsIFSB001102	cDNA retrotransposon protein, putative, (1704)	0.922
Os055248 01	OsJRUA000901	-	-1.06
Os056867 01	OsJRUA001487	cDNA Similar to ndhB(633)	-1.09
Os051998 01	OsIFCC032344	cDNA photosystem i reaction centre subunit(829)	-1.1
Os003797 01	OsIRUA001841	cDNA Rieske Fe-S precursor protein(1124)	-1.13
Os002371 01	OsIRUA000992	cDNA chlorophyll a/b binding protein 1. (1364)	-1.19
Os032279 01	OsIFCC037013	cDNA Chlorophyll A-B binding protein, (1173)	-1.25
Os052019 02	OsIFCC038501	cDNA plastocyanin, chloroplast precursor(771)	-1.64
Os004384 01	OsIRUA007230	-	-1.68
Os002029 01	OsIRUA000817	cDNA ATPase, AAA family, putative(1839)	-1.91
Os058312 02	OsIFCC017481	cDNA Similar to thioredoxin peroxidase(504)	-1.92
Os054418 01	OsIFCC036105	cDNA ATPase, AAA family, putative(1839)	-2.28
Os026599 01	OsIRUA004921	cDNA putative dehydrogenase(2943)	-2.52



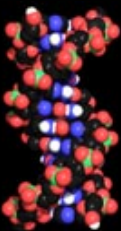
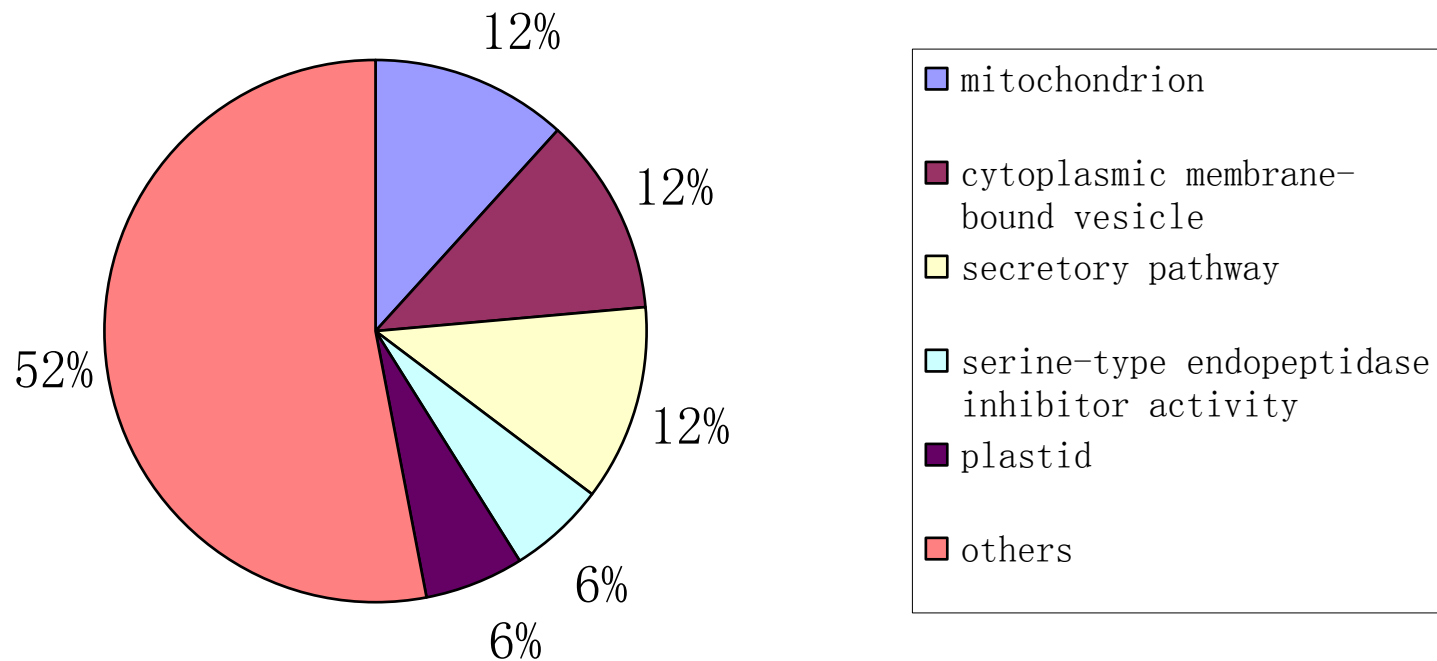
## **Common Differentially expressed genes (31)**

- 1. We detected 22680 and 14293 signals (number of significantly hybridized signals) in wheat-rice hybridization (0 vs. 6hr treated wheat RNA hybridized against rice chip), about 37% and 24% of the total rice sequences, respectively in two biological replicates.**
- 2. Of them, 31 have common expression patterns (19 are up-regulated and 12 are down regulated).**



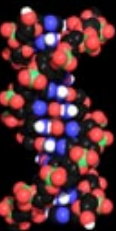
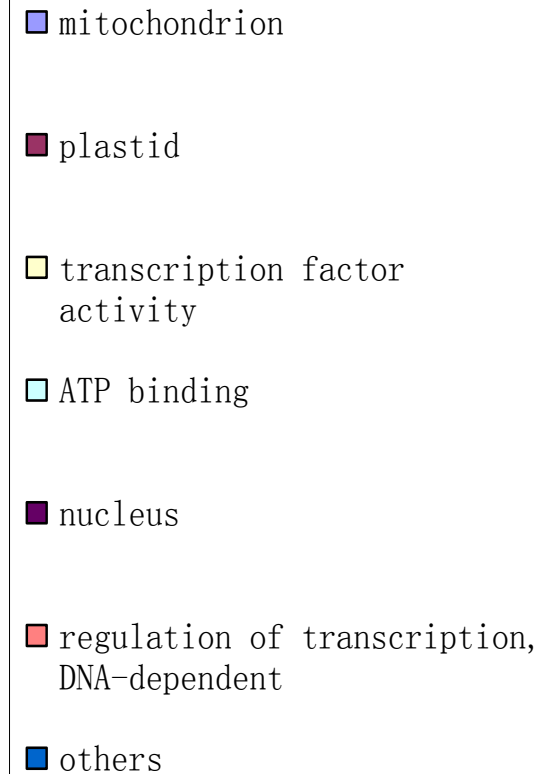
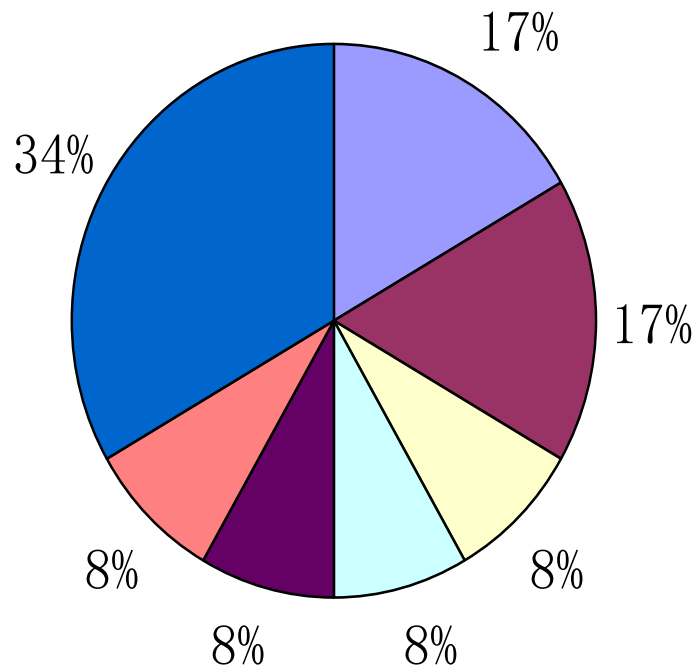
# GO classification of 19 up-regulated genes

耐盐6小时处理上调基因的GO分类



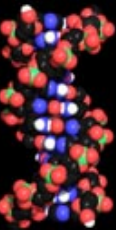
# GO classification of 12 down-regulated genes

耐盐6小时处理下调基因的GO分类



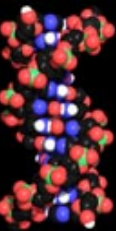
# Summary of the Data

- 1. Wheat-rice comparative gene expression using cross species hybridization is feasible. About 50% of rice 60K sequences could be successfully hybridized with wheat mRNAs at the best.**
- 2. Wheat stress responsive genes have been identified by using wheat-rice cross species hybridization.**



# Future Plan

- **Completing of remaining biological replications and data analysis**
- **Validation of microarray results by using another rice-chip platform from Agilent Technologies.**
- **Validation of microarray results by qRT-PCR.**
- **Validating the feasibility of studying drought responsiveness in wheat by using rice chips.**
- **Identifying potential markers linked with drought tolerance by using this gene expression data.**



# Thanks to

Xuehui Sun, Ruilian Jing, Xueyong Zhang, Tiegang Lu

**The Chinese Academy of Agricultural Sciences**

Raveendran Muthurajan, Manavalan Laxmi Praba, Lafitte Renee, Leung Hei

**International Rice Research Institute**

Kouji Satoh, Shoshi Kikuchi

**National Institute of Agrobiological Sciences**

Qiang Gan, Guozhen Liu

**Beijing Genomics Institute**