



IIT ROORKEE



NPTEL ONLINE
CERTIFICATION COURSE

Molecular Genetics of Plant Development-IV

Dr. Shri Ram Yadav

Department of Biotechnology

Indian Institute of Technology, Roorkee



Approaches to the study of plant development

- **Reverse Genetics:** An approach to study function(s) of gene(s)
(Genotype to Phenotype)
 - *Identifying & isolation of gene for functional study (gene of interest)*
 - *Analyzing expression pattern of the gene (differential, spatial & temporal)*
 - *Functional characterization of the gene (gain or loss-of-function)*
 - *Gene interaction study (genetic, physical or regulatory interaction)*



Identifying gene of interest for functional study

➤ ***Global genes (Genomics): Identifying genome-wide genes for a developmental process***

- Functional Genomics:

- Monitoring gene expression
- Functional characterization
- Molecular mechanism



Identifying gene of interest for functional study

➤ **Monitoring gene expression:**

- Organ-specific expression pattern (Differential expression pattern)
- Tissue-specific gene expression (Spatial expression pattern)
- Developmental stage-specific gene expression (Temporal expression pattern)
- Promoter trapping (expression pattern)



Monitoring gene expression: Global gene expression analysis

- **Organ-specific or wild-type vs. mutants expression pattern (Differential expression pattern)**

Serial analysis of gene expression (SAGE): Obtaining short sequence tags from large numbers of cDNA clones and finally sequencing cDNA fragments

Differential Display RT-PCR: Compare and identify changes in gene expression

Subtractive Hybridization: PCR-based amplification of only cDNA fragments that differ between a control (driver)

Microarray: Microarrays are microscope chips that are printed with a known DNA sequence

RNA sequencing: Next-generation sequencing to reveal the presence and quantity of RNA in a biological sample



Monitoring gene expression: Global gene expression analysis

- Organ-specific or wild-type vs. mutants expression pattern (Differential expression pattern)

Serial analysis of gene expression (SAGE): obtaining short sequence tags from large numbers of cDNA clones and finally sequencing cDNA fragments.

The Plant Journal (1999) 20(6), 719–726

TECHNICAL ADVANCE

Transcript profiling in rice (*Oryza sativa* L.) seedlings using serial analysis of gene expression (SAGE)

Hideo Matsumura*, Shizuko Nirasawa and
Ryohei Terauchi



Monitoring gene expression: Global gene expression analysis

- Organ-specific or wild-type vs. mutants expression pattern (Differential expression pattern)

Differential Display RT-PCR (DD-RT-PCR): compare and identify changes in gene expression

The Plant Journal (2005) 43, 915–928

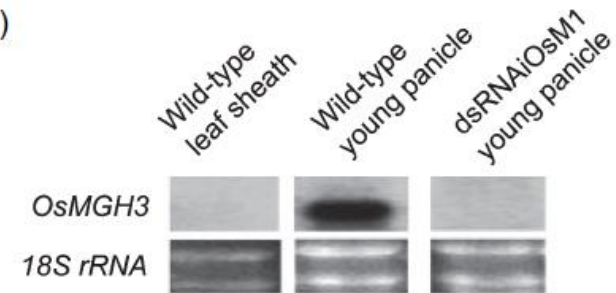
doi: 10.1111/j.1365-313X.2005.02504.x

***OsMADS1*, a rice MADS-box factor, controls differentiation of specific cell types in the lemma and palea and is an early-acting regulator of inner floral organs**

(a)

Kalika Prasad[†], Sriram Parameswaran[†] and Usha Vijayraghavan^{*}

Flower specific target of *OsMADS1*, *OsMGH3* was identified
By DD-RT-PCR



Monitoring gene expression: Global gene expression analysis

- Organ-specific or wild-type vs. mutants expression pattern (Differential expression pattern)

Subtractive Hybridization: PCR-based amplification of only cDNA fragments that differ between a control (driver)

Comparative transcript profiling of gene expression between seedless Ponkan mandarin and its seedy wild type during floral organ development by suppression subtractive hybridization and cDNA microarray

Wen-Ming Qiu, An-Dan Zhu, Yao Wang, Li-Jun Chai, Xiao-Xia Ge, Xiu-Xin Deng and Wen-Wu Guo*

Qiu et al. *BMC Genomics* 2012, **13**:397



Monitoring gene expression: Global gene expression analysis

- Organ-specific or wild-type vs. mutants expression pattern (Differential expression pattern)

Microarray: Microarrays are microscope chips that are printed with a known DNA sequence

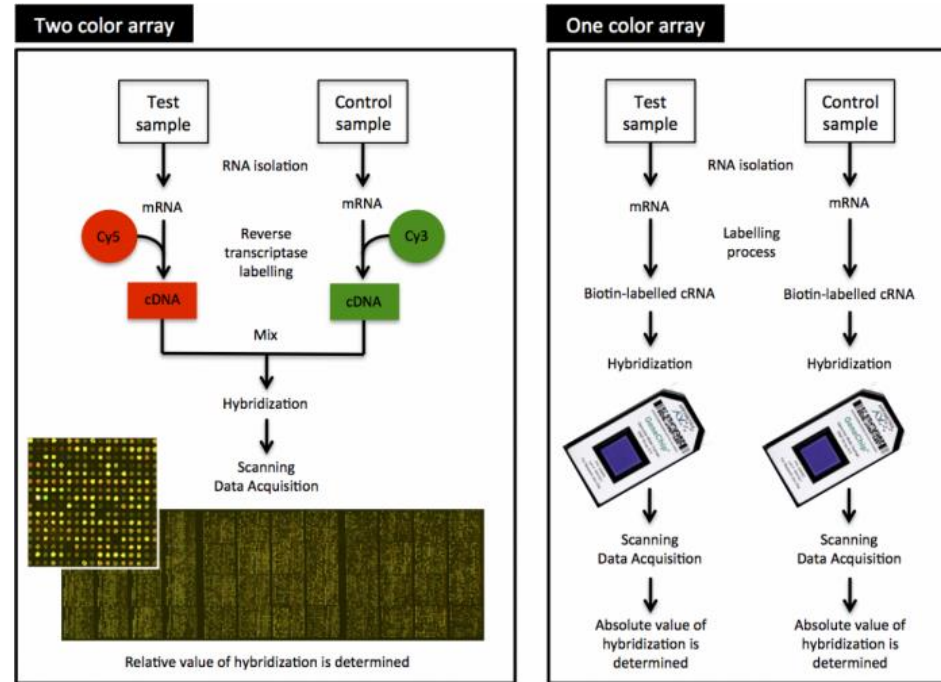


Image source:
<https://www.ebi.ac.uk/>

Monitoring gene expression: Global gene expression analysis

The Plant Journal (2006) 46, 503–511

doi: 10.1111/j.1365-313X.2006.02703.x

Genome-wide analysis of spatial and temporal gene expression in rice panicle development

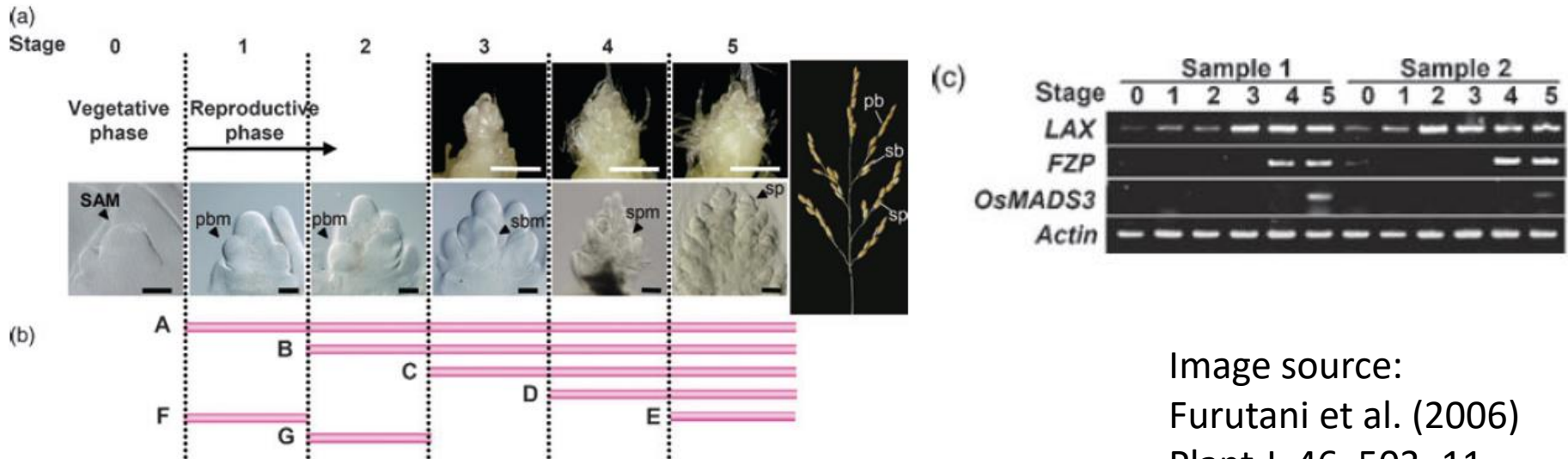


Image source:
Furutani et al. (2006)
Plant J. 46, 503–11.

Monitoring gene expression: Global gene expression analysis

MADS-box gene family in rice: genome-wide identification, organization and expression profiling during reproductive development and stress

Table 2: Panicle and seed developmental stages as well as stress treatments used in this study (DAP, Days After Pollination).

S. No.	Symbols Used	Developmental stages analyzed
1	ML	Mature Leaf (collected before pollination)
2	R	Roots of 7-day old seedling
3	SD	7-day old seedling
4	P1	0–3 cm panicle
5	P2	3–5 cm panicle
6	P3	5–10 cm panicle
7	P4	10–15 cm panicle
8	P5	15–22 cm panicle
9	P6	22–30 cm panicle
10	S1	0–2 DAP
11	S2	3–4 DAP
12	S3	5–10 DAP
13	S4	11–20 DAP
14	S5	21–29 DAP
15	CS	Cold Stress
16	DS	Dehydration Stress
17	SS	Salt Stress

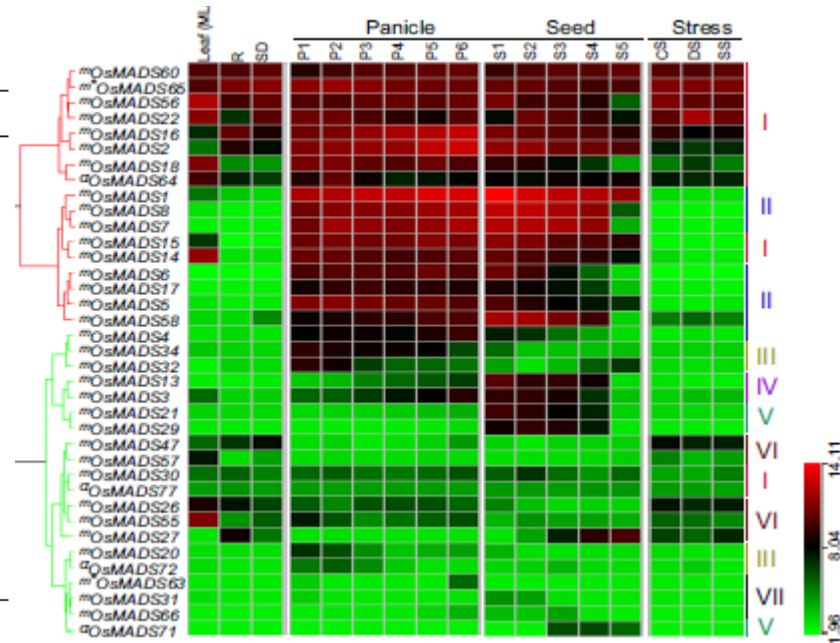
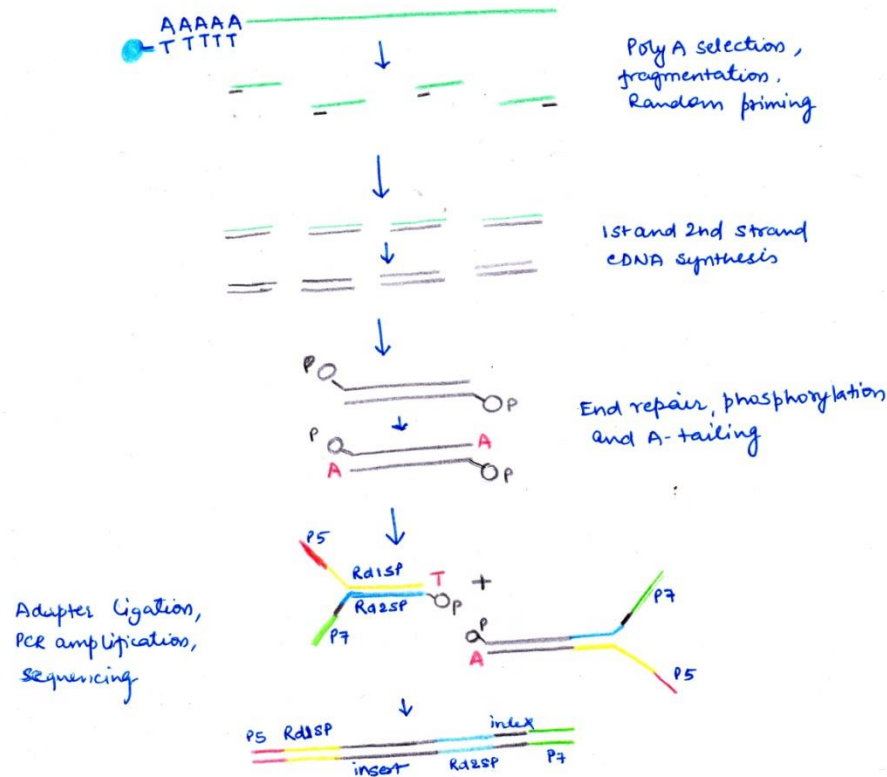


Image source:
Arora et al. (2007)
BMC Genomics 8, 242.

Global gene expression analysis: RNA Sequencing



The complex jujube genome provides insights into fruit tree biology

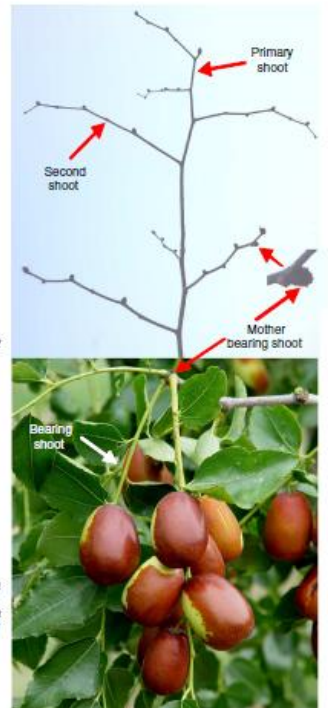
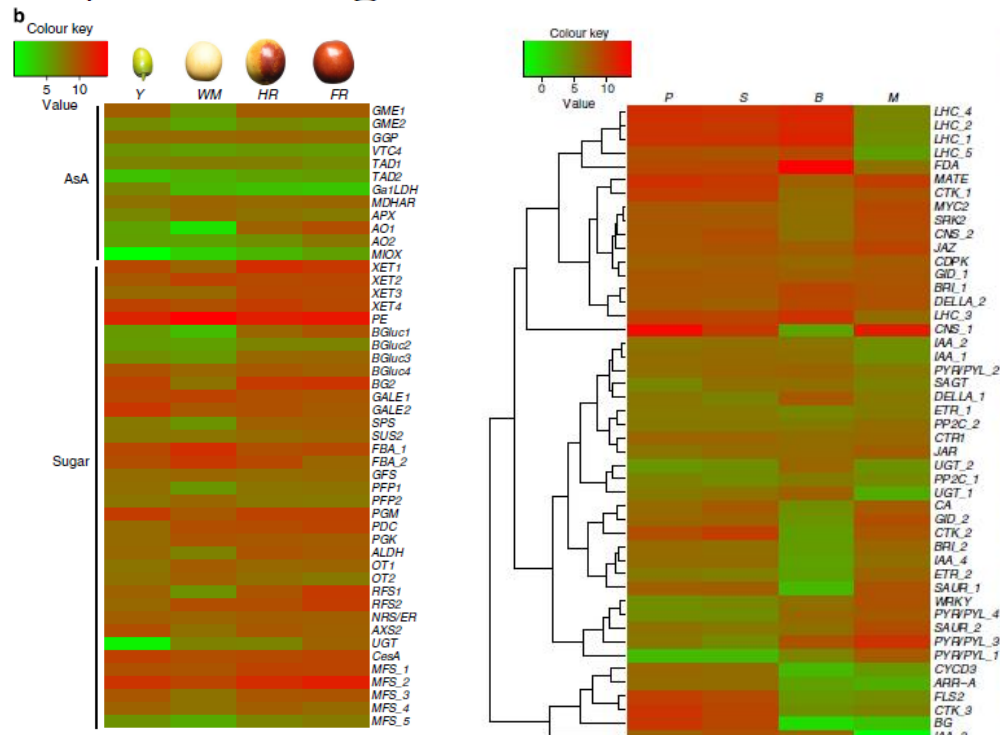


Image source:
Liu et al. (2014)
Nat. Comm. 5, 5315.

Genes involved in sugar and AsA metabolism Heat map of the normalized RNA-Seq data for genes involved in shoot development

Comparative RNA-seq analysis of transcriptome dynamics during petal development in *Rosa chinensis*

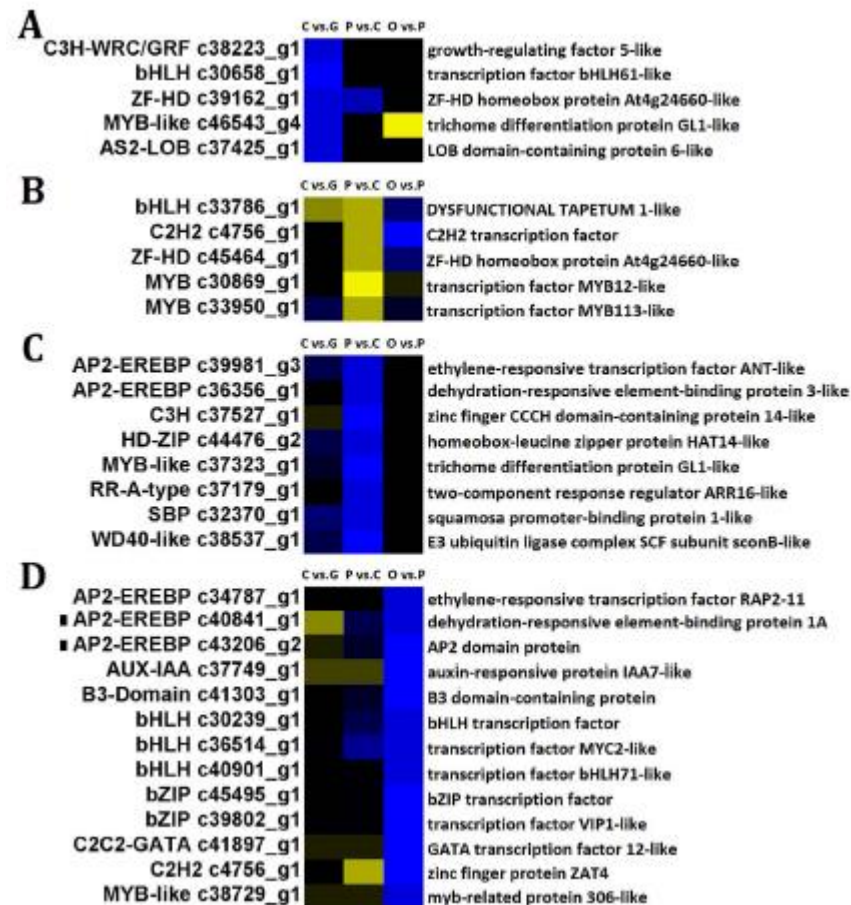
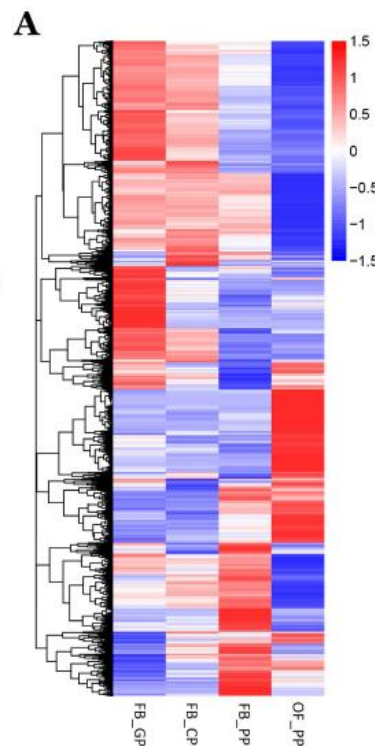


Image source:
Han et al. (2017)
Scientific Reports. 7, 43382.

Approaches to the study of plant development

- **Reverse Genetics:** An approach to study function(s) of gene(s)
(Genotype to Phenotype)
 - *Identifying & isolation of gene for functional study (gene of interest)*
 - *Analyzing expression pattern of the gene (differential, spatial & temporal)*
 - *Functional characterization of the gene (gain or loss-of-function)*
 - *Gene interaction study (genetic, physical or regulatory interaction)*