Transcription factors in abiotic stress response

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ITQB

Outline

1 - Plant responses to adverse environmental conditions
2 - Transcription factors and transcriptional regulation
3 - Transcription factors involved in abiotic stress responses
4 - TFs and photosynthetic responses to abiotic stress
5 - Identification and characterization of novel TFs
Plants and the environmental conditions

Environment altered beyond its normal range of variation to adversely affect the individual physiology of the organism in a significant way

Plant stress

Plant response to abiotic stress

ABiotic Stress

50% crop loss worldwide

Signal sensing, perception and transduction

Osmosensor, second messengers, MAPks, Ca^{2+} sensors, CDPks

Transcription control

TFs: CBF/DREB, bZIP, MYC/MYB...

Gene activation

Osmoprotection, water and ion movement, detoxification, and chaperone functions

Stress tolerance

Recovery of cellular homeostasis, functional and structural protection of proteins and membranes

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Osmoprotection, water and ion movement, detoxification, and chaperone functions

Stress tolerance

Recovery of cellular homeostasis, functional and structural protection of proteins and membranes
Abiotic stress tolerance is a multigenic trait

Environmental stimuli or stress

\[\text{TF}\]

\[\text{Stress responsive genes}\]

\[\text{STRESS TOLERANCE}\]

Transcription Factors

Transcription factors (TFs) - proteins that show sequence-specific DNA-binding and that are capable of activating or repressing gene transcription.

Transcription coregulators (coactivators/corepressors), chromatin remodelers, histone acetylases, kinases, and methylases play crucial roles in gene regulation, but lack DNA binding domains and therefore are not classified as TFs.
Transcription in Eukaryotes

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Schematic diagram of a prototypical transcription factor

TFs contain DNA-binding domain (DBD), signal sensing domain (SSD), and a transactivation domain (TAD)

The order of placement and the number of domains may differ in various types of TFs.

The transactivation and signal sensing functions are frequently contained within the same domain.

Transcription Factor Families

DNA binding domain

FAMILY

DNA binding domain(s) + (protein-protein interaction domains)

Subfamily
Arabidopsis Transcription Factors

Relationships and domain shuffling among the different Arabidopsis transcription factor families

Riechmann (2000) Science 290, 2105

Eukaryotic Transcriptional Regulators

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Plant TF families and their function

**AP2/ERF** (144)  Development (flower/seed/root); metabolic pathways; stress response; hormone response (ABA/C2H4)  

**bHLH** (139)  Development (trichome/root/carpel) abiotic stress; secondary metabolism; light responses;  

**MYB** (190)  Development; secondary metabolism; defence response; abiotic stress; hormone response (ABA/GA3); cell cycle; light  

**C2H2(Zn)** (112)  Flower/seed development; abiotic stress; light  

**NAC** (109)  Development (meristem); auxin-response; virus resistance;  

**HB** (90)  Development (several); sucrose signalling; cell death;  

**MADS** (82)  Reproductive organs development; flowering time/abscission;  

**bZIP** (77)  Flower/leaf/photomorphogenic development; seed-storage; defence response; hormone response/biosynthesis;  

**WRKY** (72)  Defence response;  

**C2C2(Zn)** (104)  Seed development/metabolism; flowering time; circadian rhythm; * Abiotic stress*  


Transcriptional regulatory network

Environmental stimuli or stress  
Developmental signals

Induction  
Modification  

Basic machinery

Promoter

mRNA

Gene
**Abiotic stress transcriptional network**

Drought, High salinity  
Biotic stress and wounding  
Jasmonic acid  
ABA  
ABA-independent  
Cold  
Signal perception  

**Manipulation of TFs to improve abiotic stress tolerance**

Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor

Nature Biotechnology 1999 17, 287
**DREB1A driven by the 35 S CaMV vs stress inducible rd29A promoter**

Both 35S:DREB1A and rd29A:DREB1A show enhanced stress tolerance

Nature Biotechnology 1999 17, 287

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DREB1A target genes are strongly expressed under control conditions

OX mimics acclimation

Higher stress tolerance

Nature Biotechnology 1999 17, 287

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Overexpression of *HvCBF4* in rice enhances drought tolerance

12 days drought
1 week recovering

Survival rate:
- Plants overexpressing *HvCBF4* - 90%
- Non transformed plants – 19%

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Overexpression of *HvCBF4* in rice enhances drought tolerance

At*DREB1A* overexpressed in chrysanthemum enhances tolerance to heat stress

Survival rate:

- 3SS:*DREB1A*: 70.8%
- WT: 16.3%

36h at 45°C
3 weeks 22°C
Higher photosynthetic capacity and elevated activity of Rubisco

TF overexpression can improve photosynthetic performance under abiotic stress

<table>
<thead>
<tr>
<th>Over-expressed TF</th>
<th>TF family</th>
<th>Transgene</th>
<th>Stress tolerance</th>
<th>Effect on photosynthesis under stress conditions</th>
<th>Reference</th>
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<td>AP2/EREF1</td>
<td>Tobacco</td>
<td>High yield</td>
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<td>Park et al. (2011)</td>
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Photosynthetic responses to abiotic stress

ABIOITIC STRESS

Stomatal control of CO₂ diffusion
Photosystem II repair
Electron transport
Rubisco activity
Scavenging of ROS
Photorespiration

Photosynthetic efficiency is greatly decreased

Are the photosynthetic responses to abiotic stress modulated at transcriptional level?
Gene expression in rice plants under drought stress

Drought stress causes down-regulation of rice genes coding for proteins involved in the photosynthetic light reactions.

Plant Mol Biol 2009 69, 133

TFs involved in the photosynthetic response to abiotic stress

Fast responses

Long term responses

Annals Botany 2009 103, 609
TFs involved in the photosynthetic response to abiotic stress

Abiotic stress

LIGHT

Non-stomatal

Chloroplast

Calvin cycle

Mesophyll cell

Chloroplast genes

Non-stomatal

Annals Botany 2009 103, 609

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NOVEL TRANSCRIPTION FACTORS REGULATING ABIOTIC STRESS TOLERANCE IN RICE (ORYZA SATIVA L.)
Rice in Portugal

Consumption: 17 kg/capita/year
Production: 60%

PRESENT PROBLEMS:
- Pests and diseases
- Low production
- Infestants
- Cold
- Salinity

What makes rice an ideal model organism?

- Important crop: main source of energy for 2/3 of the world population
- Rice genome: fully sequenced ~ 400 Mb, (maize ~ 2500 Mb, barley ~ 5000 Mb, wheat ~ 16000 Mb)
- High-efficiency genetic transformation
- Genetic and physical maps of high density
- High degree of synteny among genes in cereal genomes
- Insertion knockout mutants available
- Microarrays: for the whole genome ~50.000 transcripts

RICE - AN IDEAL MODEL ORGANISM FOR MONOCOTS AND CEREAL CROPS
Low temperature signalling pathway

COLD

[Ca^{2+}]_{cyt}

ICE1

ICE1-like

Kinases

SIZ1

HOS1

MYB15

DREB1A/ CBF3

DREB1B/ CBF1

DREB1C/ CBF2

ICE1

Proteolysis

Proteolysis

COR genes

ACCLIMATION

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Yeast one-hybrid screening to isolate TFs or other DNA-binding proteins

Transformation

Yeast reporter strain (leu2, his3)

Transcription

if hybrid protein interacts with bait sequence

pACT II

GAL4-AD

cDNA library

pINT1 vector

GAL4-AD

library protein

TATA

HIS3 selection gene

Selection for growth on histidine-lacking medium


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TFs controlling the expression of OsHOS1

![Diagram of TFs controlling the expression of OsHOS1]

ERF1 is down-regulated at low temperature (5°C)
ERF2 is not regulated at transcriptional level

Low temperature signalling pathway

![Diagram of Low temperature signalling pathway]

ADONIS, 6th March 2009
**OsDREB1B gene is induced by cold and drought**

<table>
<thead>
<tr>
<th>Shoot</th>
<th>Root</th>
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<tbody>
<tr>
<td>Mock</td>
<td>0 10' 20' 40' 1h 2h 5h 10h 24h</td>
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<tr>
<td>Control 5°C</td>
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<tr>
<td>Control 10°C</td>
<td></td>
</tr>
<tr>
<td>Control Drought</td>
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</tbody>
</table>

TFs controlling OsDREB1B expression:

GFP::TF

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**OsSTZ (C2H2) binds to OsDREB1B promoter**

OsSTZ is induced by cold, salt, and drought

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<td>Mock</td>
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<td>5°C</td>
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<tr>
<td>10°C ABA NaCl Drought</td>
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</table>

**Internal control**

ADONIS, 6th March 2009
**OsPIF (bHLH) binds to **OsDREB1B** promoter

The control of **OsPIF** expression by cold involves alternative splicing.

<table>
<thead>
<tr>
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<td>24h</td>
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- **Mock**
- **5°C**
- **10°C**
- **ABA**
- **NaCl**
- **Drought**

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<td>24h</td>
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</table>

- **Mock**
- **5°C**
- **10°C**
- **ABA**
- **NaCl**
- **Drought**

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**A C2H2 that binds to **OsDREB1B** promoter**

**C2H2** transcription is induced at a higher level in the roots.

<table>
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- **Mock**
- **5°C**
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- **NaCl**
- **Drought**

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- **Mock**
- **5°C**
- **10°C**
- **ABA**
- **NaCl**
- **Drought**

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ADONIS, 6th March 2009
Low temperature signalling pathway

COLD

[Sa^{2+}]_{cyt}

ICE1

SALINITY

DROUGHT

Kinases

ICE1-like

HOS1

MYB15

ICE1

DREB1A/CBF3

DREB1B/CBF1

DREB1C/CBF2

STZ

bHLH

C2H2

CRT/DRE

ACCLIMATION

OsDREB1A gene is induced by cold

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<th>30'</th>
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<th>2h</th>
<th>5h</th>
<th>7h</th>
<th>12h</th>
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OsActin1

Control

Control

Control

OsDREB1A

bZIP

ATG

OsbZIP

Known to be involved in biotic stress responses

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Low temperature signalling pathway

COLD

[Ca^{2+}]_cyt

Kinases

ICE1-like

SIZ1

DDEB1B/ CBF1

DREB1A/ CBF2

SALINITY

DROUGHT

STZ

bHLH

C2H2

HOS1

ICE1

ICE1

ICE1

P

S

US

MYB15

ICE1

ICE1

ICE1

ERF1

Proteolysis

ACCLIMATION

COR genes

Plant cell responses to salinity

RMC – Root Meander Curling

- perception / response biotic stress

NHX1 - Na^+/H^+ antiport –

tonoplast - response to salt stress

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The expression of *OsRMC* and *OsNHX1* is induced by NaCl

<table>
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<th>5h</th>
<th>12h</th>
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Transcriptional regulation of *OsRMC*

- **ERF3** Homologue to the Arabidopsis ABR1, a negative regulator of ABA responses
- **ERF4** Phosphorylated *in vitro* by MPK12/BWMK1

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Salt signalling pathway

Salt signalling pathway

Preliminary conclusions

- The genes analysed are controlled by several TFs, differentially regulated by different stresses

  Transcriptional Post-transcriptional
  (and post-translational ?)

- Transcriptional regulation of *OsDREB1A* and *OsRMC*
  cold and salinity cross talk with biotic stress signalling, light sensing
ACKNOWLEDGEMENTS

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ADONIS, 6th March 2009