Molecular background of stress tolerance: lessons from plant systems

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Extreme environments

- Drought
- Heat
- Cold
- Saline soils
Effect of drought and salinity on plants

Arabidopsis thaliana in stress conditions

- Well watered
- Salinity
- Drought

- Ion toxicity
- Osmotic stress
- Oxidative stress
Physiological effects of drought and salt stress

**Physiological consequences of stress**
- Growth arrest, reduced cell proliferation
- Photosynthesis is reduced (e.g., quantum yield of PSII, carbon fixation)
- Stomatal closure (drought)
- Changes in ion homeostasis (salt stress, e.g., Na+/K+ ratio)
- Changes in hormone content (ABA, ethylene)
- Changes in gene expression patterns, protein profiles
- Accumulation of protective proteins (HSP, LEA, dehydrins)
- Metabolic changes: sugar, N metabolism, TCA cycle, ATP pool, organic acids, amino acids (proline), osmoprotectants (betains)
- Accumulation of reactive oxygen species (ROS), oxidative damage
- Cell death
Effect of cold, drought and salinity on photosynthesis-related metabolism

Cellular ion homeostasis and salt stress

- Ion transport: 
  Na exclusion (SOS1, HKT1), compartmentalization (NHX),

- Osmotic adjustment: 
  osmolyte accumulation (proline, betaines)

- Protection of macromolecules: 
  chaperones (HSP),

- Detoxification: 
  enzymatic (SOD, CAT, APX, GST) 
  non-enzymatic (glutathion, ascorbate, etc.)
Reactive oxygen species during stress

**Generation of H$_2$O$_2$**
- Chloroplast (PSI, PSI)
- Mitochondria
- Apoplast
- Peroxisome
- Glyoxisomes

**H$_2$O$_2$ signalling**
- Cytoplasm, Nucleus
  - (hormones, Ca$^{2+}$, NO, lipid messengers, MAPK)

**H$_2$O$_2$ detoxification**
- Chloroplast
- Peroxisome
- Vacuole

Arabidopsis: aprox. 300 genes involved in ROS production and detoxification.

Plant defences

Defences to reduce damages of drought and salt stress

- Stomatal closure to reduce evaporation and water loss (drought)
- Osmotic adjustment (salt, drought)
- Adjustments in ion transport (salt, ion uptake, transport, sequestration)
- Accumulation of protective proteins (HSP, LEA, dehydrins)
- Biosynthesis of osmoprotectant metabolites (proline, betains)
- Increase in ROS scavenging activity (enzymatic, non-enzymatic)
- Developmental adaptation (e.g. deep root, salt glands, trichomes, hairs, etc.)
Osmoprotectant compounds

Common osmolytes/osmoprotective compounds involved in either osmotic adjustment or protection of cellular structures during salt stress.

- Glycine betain
- Mannitol
- Proline
- Saccharose
- Ononitol
- Trehalose

Proline accumulation in Arabidopsis

![Proline accumulation graph](graph.png)
Regulation of proline metabolism in plants

stress

biosynthesis

Δ-pyrroline-5-carboxylate synthase (P5CS)

Δ-pyrroline-5-carboxylate reductase (P5CR)

cytosole, chloroplast

mitochondrion

Proline

degradation

Δ-pyrroline-5-carboxylate dehydrogenase (P5CDH)

proline dehydrogenase (ProDH)

stress
Transcription regulation of P5CS and ProDH genes in Arabidopsis

- **P5CS1**: stress, ABA-induced, dependent on light
- **P5CS2**: constitutive, low level expression
- **ProDH**: repressed by stress, enhanced in dark
Reduced proline accumulation in p5cs1 mutants

Limited proline accumulation in salt-treated p5cs1 mutant plants.

Arabidopsis p5cs1 insertion mutants

At2g39800
Consequence of reduced proline accumulation in p5cs1 mutants

Reduced survival of p5cs1 mutants in high salt conditions (150mM NaCl)

Enhanced H$_2$O$_2$ accumulation in p5cs1 mutant

Increased lipid peroxidation

In salt-stressed p5cs1 mutants H$_2$O$_2$ content is higher, lipid damage is enhanced than in wild type plants.
Change of antioxidant activities in p5cs1 mutants

Activities of ROS detoxifying enzymes in p5cs1 mutants

Glutathion/ascorbate cycle is reduced, catalase activity is enhanced in p5cs1 mutants.
Photosynthesis and electron transport

Upon salt stress:

Fv/Fm decreased more in p5cs1 than in the WT.

Photosynthesis (PSII) is more susceptible to stress in the p5cs1 mutant than in WT plants.
Functions of proline in plants

- NAD(P)H/ NAD(P)+
- Metabolism (carbohydrate, amino acids)
- Protein, Lipid damage PCD
- ROS
- Photosynthesis enzymes (GST, CAT, APX)
- Rehydration PDH, P5CDH
- Mitochondrial functions (ROS, PCD)
- Development (embryo, root growth, flowering)
- Proteins (proline rich)
- Signaling
- Translation
- Redox balance
- Osmoprotection

- Development (embryo, root growth, flowering)

- Metabolism (carbohydrate, amino acids)
- Protein, Lipid damage PCD
- Photosynthesis enzymes (GST, CAT, APX)
- Rehydration PDH, P5CDH
- Mitochondrial functions (ROS, PCD)
- Development (embryo, root growth, flowering)
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- Translation
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- Functions of proline in plants
Regulation of stress responses in plants

Environmental stress

Signal perception

Early signal transduction

Phosphorylation signals

Hormonal regulation

Transcriptional regulation

Target gene expression

Metabolic, developmental adaption

Receptor(s)

salinity → lipids, ROS, Ca2+

drought → Kinases, phosphatases

heat → ABA

cold

Transcription factors → Target genes

Osmo protection, detoxification, Water transport

miRNA, siRNA
**Arabidopsis thaliana as model plant**

**Arabidopsis thaliana**

- family. Brassicaceae.
- Diploid, self pollination, 3 month generation time.
- Efficient transformation (Agrobacterium).
- Many mutants, large public collections.
- Smallest known plant genome (125 Mb).
- Sequenced genome since 2000.
- Large public databases
  - TAIR: [http://www.arabidopsis.org](http://www.arabidopsis.org)
- Most plant science uses Arabidopsis
  - (50,000 publications in PubMed)
Transcript profiling: Strategy to identify stress-related and tissue-regulated Arabidopsis genes

Ma et al., 2007 Genom.Biol. 8:R49
Cluster analysis of similarly regulated genes

Treatments:
- stress
- hormones
- pathogens
- light
- chemical

Cluster 12: 2715 stress induced genes

Ma et al., 2007 Genom. Biol. 8:R49
Common stress response pathway genes

Ma et al., 2007 Genom.Biol. 8:R49
The Arabidopsis eFP browser: transcript profiling data on the internet

Plant material: 18 days-old plants grown in growth chambers

Treatments: 0-0,5-1-3-6-12-24 hours
- cold: 4°C
- osmotic: 300mM mannitol
- salt: 150mM NaCl
- drought: 15min dry air
- oxidative: 10mM methyl viologen

Wounding: punctured with pins

Samples: shoot, root

Repetitions: 2 biological replicates

Microarray: Affymetrix AHT1 gene chip

Analysed genes: 22400

Changes in gene expression during osmotic stress

Osmotic stress: 300mM mannitol / public transcript profiling data

- **RD29A**
  - Shoot: 0 Hour, 0.5 Hour, 1 Hour, 3 Hours, 6 Hours, 12 Hours, 24 Hours
  - Root: 0 Hour, 0.5 Hour, 1 Hour, 3 Hours, 6 Hours, 12 Hours, 24 Hours

- **P5CS1**
  - Shoot: 0 Hour, 0.5 Hour, 1 Hour, 3 Hours, 6 Hours, 12 Hours, 24 Hours
  - Root: 0 Hour, 0.5 Hour, 1 Hour, 3 Hours, 6 Hours, 12 Hours, 24 Hours

- **RUBISCO**
  - Shoot: 0 Hour, 0.5 Hour, 1 Hour, 3 Hours, 6 Hours, 12 Hours, 24 Hours
  - Root: 0 Hour, 0.5 Hour, 1 Hour, 3 Hours, 6 Hours, 12 Hours, 24 Hours

- **Vacuolar Ca-binding prot.**
  - Shoot: 0 Hour, 0.5 Hour, 1 Hour, 3 Hours, 6 Hours, 12 Hours, 24 Hours
  - Root: 0 Hour, 0.5 Hour, 1 Hour, 3 Hours, 6 Hours, 12 Hours, 24 Hours

Activation and repression of selected Arabidopsis genes by osmotic stress

Molecular processes controlled by ABA

Drought, salinity

Osmotic stress

ABA biosynthesis

ABA

**Induced genes**
- Regulatory genes:
  - Transcription factors (eg. ABF, ABI5, MYB2)
  - Protein kinases (eg. SnRK2, CDPK)
  - Phospholipid signaling, Specific miRNA
- Target genes:
  - Late embryogenesis abundant (LEA)
  - Specific transporters
  - Enzymes in osmoprotectant synthesis (Pro), specific amino acids
  - Carbohydrate, Fatty acid metabolism

**Repressed genes:**
- Genes involved in growth, development
- Ribosomes
- Chloroplasts, photosynthesis,
- Cell wall
- Plasma membrane
ABA-regulated signal transduction

ABA Receptor complex: PP2C, PYR/PYL proteins

Signalizing, phosphorylation: SnRK2 kinases

Key transcription factors:
ABI5: seed development
AREBs: osmotic stress

Target genes:
with ABRE promoter element
Comparison of stress responses in dicots and monocots

Identification of regulatory genes: mutagenesis

Mutagenesis of an important regulatory gene

Elimination or reduction of a stress signalling pathway.

Reduced stress response

Reduced stress tolerance
A SOS1 (Salt Overly Sensitive 1) ion transporter regulates ion balance

The sos1 mutant is hypersensitive to NaCl

Salt sensitivity of wild-type Arabidopsis, the sos1 mutant, and the 35S-SOS1 overexpressing complemented mutant.

SOS1: plasma membrane Na+/H+ antiporter

Shi et al., (2000) PNAS 97:6896-6901
The SOS signaling system regulates ion homeostasis in Arabidopsis.
The ppr40 mutant is stress hypersensitive

**ppr40-1 germination in stress conditions**

<table>
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<tr>
<th>Condition</th>
<th>Wild type</th>
<th>ppr40-1 mutant</th>
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**Sensitivity to oxidative agents**

- Control
- 10mM H$_2$O$_2$
- 2μM paraquat

**Lipid damage in salt-stressed plants**

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<th>Time (hours)</th>
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*ppr40-1 was identified in our T-DNA insertion mutant collection as semidwarf, stress and ABA hypersensitive mutant.*

Respiration in ppr40 mutants and PPR40 overexpressing plants

**Oxygen consumption in mitochondria**

**ppr40-1 mutant**
- Oxygen consumption through Complex III is reduced.
- Cytochrome C oxidase (COX) and alternative oxidase (AOX) activities are enhanced.
- Mitochondrial H$_2$O$_2$ levels are increased.
- Ascorbate consumption is enhanced.

**PPR40 overexpressing line**
- Oxygen consumption in stressed PPR40ox plants is higher through Complex III.
- Alternative oxidase (AOX) activity is enhanced during stress, but not altered in PPR40ox.
- Mitochondrial H$_2$O$_2$ levels are decreased.

**PPR40 is a mitochondrial protein**

Respiration of the ppr40-1 mutant:
- Oxygen consumption through Complex III is reduced.
- Cytochrome C oxidase (COX) and alternative oxidase (AOX) activities are enhanced.
- Mitochondrial H$_2$O$_2$ levels are increased.
- Ascorbate consumption is enhanced.
Gene identification via gene activation

Gene activation

Activation of an important regulatory gene

Activation of a stress signalling pathway.

Enhanced stress response

Enhanced stress tolerance
Gene activation in the Conditional Overexpressing System (COS)
Overexpression of heat shock factor HSFA4A can enhance stress tolerance.

Growth under salt stress

Control

100mM NaCl + Estr

HSFA4A identified with the COS trans-activation system.

Domain structure of HSFA4A

Tolerance to oxidative stress

Pérez-Salamó et al., 2014 Plant Physiol 165: 319-334
Transcript profiling: HSFA4A regulates the expression of hundreds of Arabidopsis genes

HSFA4A-induced genes

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<th>AGI</th>
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Pérez-Salamó et al., 2014 Plant Physiol 165: 319-334
HSFA4A interacts with MAP kinases MPK3 and MPK6

Pérez-Salamó et al., 2014 Plant Physiol 165: 319-334
MAPK signaling in plants

**Effectors: FLG22**

Salt, ozone, UV, high light, heat, heavy metals

Stress-induced gene expression

PAMP induced gene expression
HSFA4A phosphorylated by MAPK3/6

In-gel kinase assay

LER Wt.  IP-MPK3  IP-MPK6
- MPK6
- MPK3

HSFA4A phosphorylation sites determined by Mass spectrometry:
Ser-198
Thr-238, Ser-239
Ser-309
Thr-396

In-vitro kinase assay

HSFA4A phosphorylation is abolished in Ser/Thr mutant

Pérez-Salamó et al., 2014 Plant Physiol 165: 319-334
Reactive oxygen species during stress
Extremophyle plants: gene sources for stress tolerance
Salt tolerance of several Cruciferae species

Leaf area on 150mM NaCl

50% lethality on salt

Large variability exist in salt tolerance among related plant species

Comparison of drought and salt tolerance

Growth and survival on saline soil

Plant survival after drought and revatering

Proline accumulation

Arabidopsis thaliana
Thellungiella halophila
Lepidium crassifolium

Control
Salt stress

Proline concentration

0 0.1 0.2 0.5 0.75 1.0 2.0 mg/ml
Na+ and K+ content in A. thaliana, Thellungiella salsuginea and T. parvula

K+ content is less affected by salt and Na accumulation is lower in Thellungiella than in Arabidopsis.

Several genes that function in Na+ excretion, compartmentalisation or diffusion are expressed at higher level in Thellungiella than in Arabidopsis.

A Lepidium gene can enhance salt tolerance of Arabidopsis

Growth of salt-stressed S12, R12 and Col-0 plants

Photosynthesis:
Quantum yield is higher in salt-stressed S12 plants than in wild type Col-0

Rosette diameter of salt-stressed plants

- Control
- Salt

Quantum yield is higher in salt-stressed S12 plants than in wild type Col-0.
Transcript profiling approach in monocots: rice, wheat
Osmotic stress of wheat plantlets in hydroponics

0 day (10 days old plantlets)

Untreated

PEG-treated:
100 mOsm
200 mOsm
400 mOsm

2. days
4. days
7. days
9. days
11. days
14. days
Sampling
Rice chip – app. 16 000 unigene

- hybridised with PEG-treated / untreated Kobomugi root samples (day 9)
- color flip repeat
- app. 5300 spots gave measurable data in both case

>2x induction: more than 1100 spots
  >5x induction: 345 spots
>2x repression: more than 400 spots
  >5x repression: 77 spots
Number of induced clones

Plainsman: 174
Kobomugi: 1133
56 overlap

Number of repressed clones versus induced clones

Plainsman: 387
Kobomugi: 1133
103 overlap

Number of repressed clones versus repressed clones

Plainsman: 174
Kobomugi: 439
1 overlap

Plainsman: 387
Kobomugi: 439
4 overlap
Putative function of the 24 clones up regulated by PEG both in roots of Kobomugi and Plainsman

<table>
<thead>
<tr>
<th>Putative Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>alcohol dehydrogenase</td>
</tr>
<tr>
<td>alpha-expansin 3</td>
</tr>
<tr>
<td>CwfJ-like family protein / zinc finger</td>
</tr>
<tr>
<td>DnaJ protein family-like</td>
</tr>
<tr>
<td>Glutathione S-transferase (GST class-zeta)</td>
</tr>
<tr>
<td>glutathione transferase</td>
</tr>
<tr>
<td>glutathione transferase</td>
</tr>
<tr>
<td>glutathione transferase</td>
</tr>
<tr>
<td>glutathione transferase</td>
</tr>
<tr>
<td>glutathione-S-transferase 2</td>
</tr>
<tr>
<td>glutathione-S-transferase Cla47</td>
</tr>
<tr>
<td>LEA3 protein</td>
</tr>
<tr>
<td>leucine-rich repeat transmembrane protein kinase, putative</td>
</tr>
<tr>
<td>oxo-phytodienoic acid reductase</td>
</tr>
<tr>
<td>putative glutathione S-transferase</td>
</tr>
<tr>
<td>putative heavy-metal-associated domain-containing protein</td>
</tr>
<tr>
<td>+ 7 unknown protein</td>
</tr>
</tbody>
</table>
Relative transcript level of four selected genes exhibiting induction during osmotic stress

- **ACC synthase**
- **Unknown**
- **GST**
- **ADH**

**Q-PCR approved**

- Kobomugi
- KoboPEG
- Plainsmann
- PlaPEG
EXPERIMENTAL SYSTEM FOR EXPOSURE OF WHEAT PLANTLETS TO LONG TERM DROUGHT STRESS IN EXPANDED PERLITE

„0 day” (16 day old plantlets)

Normal irrigation

1. week

2. week

3. week

4. week

Sampling

Reduced irrigation (30%)
**KOBO MUGI AND PLAISMANN GENOTYPES DIFFER IN VARIETY OF PHYSIOLOGICAL PARAMETERS UNDER WATER STRESS (50% WATER SUPPLY)**

<table>
<thead>
<tr>
<th></th>
<th>Kobomugi</th>
<th>Plainsmann</th>
</tr>
</thead>
<tbody>
<tr>
<td>grain yield (% of control)</td>
<td>68.97</td>
<td>86.74</td>
</tr>
<tr>
<td>stomata conductance (mmol H$_2$O m$^{-2}$ s$^{-1}$)</td>
<td>13.2</td>
<td>16.2</td>
</tr>
<tr>
<td>$^{14}$CO$_2$ fixation (cpmx10$^{-5}$)</td>
<td>59.8</td>
<td>87.4</td>
</tr>
<tr>
<td>fructose accumulation (nmol g$^{-1}$)</td>
<td>37.4</td>
<td>206.5</td>
</tr>
<tr>
<td>lipid peroxidation (MDA pmol cm$^{-2}$)</td>
<td>127.9</td>
<td>105.6</td>
</tr>
<tr>
<td>CuZn SOD (% of control)</td>
<td>160.0</td>
<td>98.0</td>
</tr>
<tr>
<td>Increase in root weight (g/plant)</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

É. Sárvári et al.
Growth rate of two genotypes under water limitation (30% water supply)

**Kobomugi**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1w</th>
<th>2w</th>
<th>3w</th>
<th>4w</th>
<th>4w control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shoot</strong></td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Root</strong></td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Plainsman V**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1w</th>
<th>2w</th>
<th>3w</th>
<th>4w</th>
<th>4w control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shoot</strong></td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Root</strong></td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
</tr>
</tbody>
</table>
**Figure 4.** Relative transcript levels of P5CS mRNAs in roots of the four genotypes. Two to five fold increase could be observed in treated samples (orange) in all four cases compared to their controls (yellow). Maximum level was reached on the third week in three genotypes, however, further increase was displayed in Plainsman.
Figure 5. Relative transcript levels of P5CS mRNAs in shoots of the four genotypes. Four to fourteen-fold increase could be observed in treated samples (dark green) in all four cases compared to their controls (light green). Maximum level was reached on the third week in all four genotypes.
Transcript level changes in wheat roots during drought adaptation measured on barley macroarray

<table>
<thead>
<tr>
<th></th>
<th>Plainsmann (adapting, having good yield)</th>
<th>Kobomugi (fast responding „survivor”)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>During 4 weeks</strong></td>
<td><strong>not changed</strong></td>
<td><strong>73</strong></td>
</tr>
<tr>
<td><strong>Change in 1-2 weeks</strong></td>
<td><strong>up</strong></td>
<td><strong>down</strong></td>
</tr>
<tr>
<td><strong>Change in 3-4 weeks</strong></td>
<td><strong>up</strong></td>
<td><strong>down</strong></td>
</tr>
</tbody>
</table>

(percentage of 10 500 clones)
Cluster analysis

Kobomugi
Plainsman

Kobomugi
Plainsman
Cluster analysis

### Kobomugi
- putative cyclin-dependent kinase B1-1
- expansin EXPB2
- calmodulin-binding heat-shock protein
- xyloglucan endotransglycosylase

### Plainsman

### Kobomugi
- Xet3 protein
- caffeic acid O-methyltransferase
- putative cellulose synthase catalytic subunit
- betaine aldehyde dehydrogenase
Functional classification of genes upregulated in one genotype only

Kobomugi:
- Stress and defense: 19%
- Protein synthesis: 6%
- Protein degradation: 6%
- Gene expression: 29%
- Signal transduction: 9%
- Transport: 11%
- Cytoskeleton and cell wall: 12%
- Cell growth and division: 34%

Plainsman:
- Stress and defense: 29%
- Protein synthesis: 6%
- Protein degradation: 6%
- Gene expression: 19%
- Signal transduction: 9%
- Transport: 11%
- Cytoskeleton and cell wall: 12%
- Cell growth and division: 34%
Conclusions

Divergent drought adaptation strategies of the two genotypes are reflected in their transcript profiles.

Long term adaptation is dependent on moderate changes in the expression of large set of genes in a coordinated manner.

Transient gene activation is characteristic to Kobomugi, while genes of the more adaptive Plainsmann genotype exhibit prolonged upregulation.

Based on the yield performance and photosynthetic activity, Kobomugi represents escaper strategy while Plainsmann cultivar is capable to maintain physiological functions in harmony with gene expression reprogramming.
Effect of water-limitation on root development of wheat genotypes
# Growth parameters of wheat genotypes under optimal condition and water limitation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plainsman 80%</th>
<th>Capelle 80%</th>
<th>Mv Emese 80%</th>
<th>GK Élet (80%)</th>
<th>Kobomugi 80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root fresh weight (g)</td>
<td>1,59 ± 0,52</td>
<td>1,65 ± 0,19</td>
<td>1,06 ± 0,11</td>
<td>0,93 ± 0,12</td>
<td>0,84 ± 0,26</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>40,10 ± 9,98</td>
<td>39,78 ± 10,80</td>
<td>45,14 ± 12,58</td>
<td>51,42 ± 8,86</td>
<td>46,93 ± 7,34</td>
</tr>
<tr>
<td>Shoot fresh weight (g)</td>
<td>1,31 ± 0,38</td>
<td>1,44 ± 0,16</td>
<td>1,40 ± 0,38</td>
<td>1,48 ± 0,22</td>
<td>1,22 ± 0,36</td>
</tr>
<tr>
<td>Shoot length (cm)</td>
<td>30,15 ± 6,08</td>
<td>36,02 ± 2,71</td>
<td>33,40 ± 2,71</td>
<td>40,35 ± 4,81</td>
<td>44,67 ± 2,61</td>
</tr>
<tr>
<td>No. of shoots</td>
<td>3,33 ± 0,82</td>
<td>3,00 ± 0,00</td>
<td>2,60 ± 0,55</td>
<td>2,67 ± 0,52</td>
<td>1,83 ± 0,98</td>
</tr>
<tr>
<td>Root dry weight (g)</td>
<td>0,14 ± 0,04</td>
<td>0,18 ± 0,06</td>
<td>0,14 ± 0,02</td>
<td>0,08 ± 0,01</td>
<td>0,09 ± 0,03</td>
</tr>
<tr>
<td>Shoot dry weight (g)</td>
<td>0,20 ± 0,06</td>
<td>0,21 ± 0,03</td>
<td>0,19 ± 0,05</td>
<td>0,19 ± 0,03</td>
<td>0,17 ± 0,05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plainsman 40%</th>
<th>Capelle 40%</th>
<th>Mv Emese 40%</th>
<th>GK Élet (40%)</th>
<th>Kobomugi 40%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root fresh weight (g)</td>
<td>0,64 ± 0,13</td>
<td>0,35 ± 0,23</td>
<td>0,69 ± 0,18</td>
<td>0,54 ± 0,13</td>
<td>0,40 ± 0,18</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>40,74 ± 6,56</td>
<td>33,75 ± 11,76</td>
<td>57,13 ± 4,91</td>
<td>48,00 ± 11,14</td>
<td>40,77 ± 12,33</td>
</tr>
<tr>
<td>Shoot fresh weight (g)</td>
<td>0,35 ± 0,10</td>
<td>0,23 ± 0,10</td>
<td>0,52 ± 0,09</td>
<td>0,38 ± 0,13</td>
<td>0,33 ± 0,15</td>
</tr>
<tr>
<td>Shoot length (cm)</td>
<td>25,08 ± 4,89</td>
<td>20,45 ± 4,40</td>
<td>30,43 ± 1,19</td>
<td>28,86 ± 5,86</td>
<td>29,35 ± 7,48</td>
</tr>
<tr>
<td>No. of shoots</td>
<td>1,20 ± 0,45</td>
<td>1,00 ± 0,00</td>
<td>1,00 ± 0,00</td>
<td>1,00 ± 0,00</td>
<td>1,00 ± 0,00</td>
</tr>
<tr>
<td>Root dry weight (g)</td>
<td>0,08 ± 0,02</td>
<td>0,04 ± 0,03</td>
<td>0,08 ± 0,03</td>
<td>0,07 ± 0,04</td>
<td>0,05 ± 0,02</td>
</tr>
<tr>
<td>Shoot dry weight (g)</td>
<td>0,06 ± 0,01</td>
<td>0,04 ± 0,02</td>
<td>0,08 ± 0,02</td>
<td>0,06 ± 0,01</td>
<td>0,05 ± 0,02</td>
</tr>
</tbody>
</table>
FIGURE 4 | Seasonal root growth of fully and deficit irrigated maize and sunflower in two soil depths. Root growth across the season at two soil depths for Z. mays (A,C) and H. annuus (B,D). Each bar represents root growth averaged among four mini-rhizotron tubes per treatment. Arrows indicate the beginning of the critical reproductive phase for each crop (R1 in maize, July 23; R3 in sunflower, July 20).

Daily change in transcript profile during water limitation in roots of rice

- cv. Sandora
- control (100%), stressed (20%) water capacity
- 3-3 samples (at 8, 14, 18)
- 22 k rice oligo-chip
Genes induced during the day in rice roots under water limitation

<table>
<thead>
<tr>
<th>Info 1</th>
<th>Info 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-box domain, putative</td>
<td>11682.m04419</td>
</tr>
<tr>
<td>oxidoreductase, short chain dehydrogenase/reductase family</td>
<td>11670.m04026</td>
</tr>
<tr>
<td>AT5g64600/MUB3_12</td>
<td>11686.m00842</td>
</tr>
<tr>
<td>jmjC domain, putative</td>
<td>11668.m04545</td>
</tr>
<tr>
<td>putative chelatase subunit</td>
<td>11669.m03601</td>
</tr>
<tr>
<td>Protein kinase domain, putative</td>
<td>11686.m00607</td>
</tr>
<tr>
<td>Similar to C-x8-C-x5-C-x3-H type Zinc finger protein, putative</td>
<td>11669.m01922</td>
</tr>
</tbody>
</table>

And 7 genes with unknown function
Genes repressed during the day in rice roots under water limitation

<table>
<thead>
<tr>
<th>Info 1</th>
<th>Info 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hpt domain, putative</td>
<td>11674.m04480</td>
</tr>
<tr>
<td>Af10-protein</td>
<td>11667.m05271</td>
</tr>
<tr>
<td>putative reductase</td>
<td>11669.m05442</td>
</tr>
<tr>
<td>O-methyltransferase, putative</td>
<td>11670.m00047</td>
</tr>
<tr>
<td>UDP-glucoronosyl and UDP-glucosyl transferase</td>
<td>11673.m03060</td>
</tr>
<tr>
<td>Similar to GMFP5</td>
<td>11676.m02628</td>
</tr>
<tr>
<td>profilin a</td>
<td>11676.m01466</td>
</tr>
<tr>
<td>hypothetical protein, (thylakoid membrane phosphoprotein 14 kda, chloroplast precursor, putative, expressed )</td>
<td>11667.m05484</td>
</tr>
<tr>
<td>expressed protein, (putrescine-binding periplasmic protein, putative, expressed )</td>
<td>11670.m05010</td>
</tr>
<tr>
<td>putative transport protein particle component</td>
<td>11669.m02901</td>
</tr>
<tr>
<td>pectate lyase precursor (ec 4.2.2.2)</td>
<td>11668.m01124</td>
</tr>
<tr>
<td>putative oxidase</td>
<td>11669.m03600</td>
</tr>
<tr>
<td>receptor-like protein kinase, putative</td>
<td>11674.m01711</td>
</tr>
</tbody>
</table>
Reactive oxygen species

- Ozone
- Drought
- Pathogens
- Herbicides
- Wounding
- Intense light
- Heat and cold
- Heavy metals
- Root nodulation

Oxidative stress
<table>
<thead>
<tr>
<th>Compound</th>
<th>Shorthand notation(s)</th>
<th>Structural representation(s)</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular oxygen (triplet ground state)</td>
<td>$^3\Sigma$</td>
<td>$\ce{O==O}$; $1s^22s^2(\sigma_v)^2(\sigma_u)^2(\pi_v)^2(\pi_u)^2(\pi_y)^1(\pi_x)^1}$</td>
<td>Most common form of dioxygen gas</td>
</tr>
<tr>
<td>Singlet oxygen (first excited singlet state)</td>
<td>$^1\Sigma$</td>
<td>$\ce{O==O}$; $1s^22s^2(\sigma_v)^2(\sigma_u)^2(\pi_v)^2(\pi_u)^2(\pi_y)^2$</td>
<td>UV irradiation, photoinhibition, photosystem II $e^-$ transfer reactions (chloroplasts)</td>
</tr>
<tr>
<td>Superoxide anion</td>
<td>$O_2^-$</td>
<td>$\ce{[O==O]}^-$</td>
<td>Mitochondrial $e^-$ transfer reactions, Mehler reaction in chloroplasts (reduction of $O_2$ by iron–sulfur center $F_X$ of Photosystem I), glyoxysomal photorespiration, peroxisome activity, plasma membrane, oxidation of paraquat, nitrogen fixation, defense against pathogens, reaction of $O_2$ and $OH^-$ in apoplastic space</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>$H_2O_2$</td>
<td>$\ce{H-O-O-O-H}$</td>
<td>Photorespiration, $\beta$-oxidation, proton-induced decomposition of $O_2^-$, defense against pathogens</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
<td>$OH^-$</td>
<td>$\ce{O==H}$</td>
<td>Decomposition of $O_3$ in presence of protons in apoplastic space, defense against pathogens</td>
</tr>
<tr>
<td>Perhydroxyl radical</td>
<td>$O_2H^+$</td>
<td>$\ce{O==O}^-$</td>
<td>Reaction of $O_3$ and $OH^-$ in apoplastic space</td>
</tr>
<tr>
<td>Ozone</td>
<td>$O_3$</td>
<td></td>
<td>Electrical discharge or UV radiation in stratosphere, reactions involving combustion products of fossil fuels and UV radiation in troposphere</td>
</tr>
</tbody>
</table>
Ascorbate-glutathione cycle

Ascorbate oxidation in vivo

Plainsman V

Cappelle Desprez
Ascorbate peroxidases of wheat (APX)

- tAPX
- sAPX1
- sAPX2
- cAPX1
- cAPX2
- mAPX

Mono-dehydro-ascorbate reductase (MDAR)

- cMDAR1
- sMDAR
- mMDAR
- cMDAR2
Expression changes during water shortage - APX

- Cappelle Desprez
- Plainsman V
**Summary**

<table>
<thead>
<tr>
<th></th>
<th>Plainsman V</th>
<th>Cappelle Desprez</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAPX1</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>cAPX2</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>tAPX</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>sAPX2</td>
<td>↔</td>
<td>↑</td>
</tr>
<tr>
<td>cMDAR2</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>sDHAR</td>
<td>↔</td>
<td>↑</td>
</tr>
</tbody>
</table>

Plainsman V: initial transcript level is higher in general.

Plainsman V: increased cytosolic APXs expression followed by cytosolic MDAR; Cappelle Desprez: these increases do not take place, but stroma APX and DHAR mRNA levels increase.
Summary
Regulation of salt and drought tolerance in plants

Thank you for your attention!

This work is supported by the European Union, co-financed by the European Social Fund, within the framework of "Practice-oriented, student-friendly modernization of the biomedical education for strengthening the international competitiveness of the rural Hungarian universities"

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