Importància del metabolisme de poliamines en els mecanismes de tolerància de les plantes a l'estrès abiótic
Average yields and record yields of 8 major crops. Effect of biotic and abiotic stresses

<table>
<thead>
<tr>
<th>Crop</th>
<th>Record yield (RY)</th>
<th>Average yield</th>
<th>Biotic losses</th>
<th>Abiotic losses (AL)</th>
<th>AL as a % of RY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayze</td>
<td>19,300*</td>
<td>4,600</td>
<td>1,952</td>
<td>12,700</td>
<td>65.8</td>
</tr>
<tr>
<td>Wheat</td>
<td>14,500</td>
<td>1,880</td>
<td>726</td>
<td>11,900</td>
<td>82.1</td>
</tr>
<tr>
<td>Soybean</td>
<td>7,390</td>
<td>1,660</td>
<td>666</td>
<td>5,120</td>
<td>69.3</td>
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<tr>
<td>Sorghum</td>
<td>20,100</td>
<td>2,830</td>
<td>1,051</td>
<td>16,200</td>
<td>80.6</td>
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<td>Oat</td>
<td>10,600</td>
<td>1,720</td>
<td>924</td>
<td>7,960</td>
<td>75.1</td>
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<td>Barley</td>
<td>11,400</td>
<td>2,050</td>
<td>765</td>
<td>8,590</td>
<td>75.4</td>
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<tr>
<td>Potato</td>
<td>94,100</td>
<td>28,300</td>
<td>17,775</td>
<td>50,900</td>
<td>54.1</td>
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<tr>
<td>Sugar beet</td>
<td>121,000</td>
<td>42,600</td>
<td>17,100</td>
<td>61,300</td>
<td>50.7</td>
</tr>
</tbody>
</table>

* Kg per hectare

Buchanan et al. 2000. Biochemistry & Molecular Biology of Plants. ASPP. Maryland
Enhanced Greenhouse Effect and Climate Change
Flooding by Iceberg Defrosting

Artic circle

Perito Moreno
Global Water Scarcity in Southern Regions

- **SUSQUEDA RESERVOIR-2008 (25%)**
- **SAU RESERVOIR-2004**
- **SAU RESERVOIR-2008 (14%)**

![World Map showing water scarcity](image1)

**CATALUNYA**

Source: International Water Management Institute
Increasing Population versus Arable Land Surface

Urgent Need to Improve Plant Tolerance to Stress
INTERNATIONAL NETWORK IN PLANT ABIOTIC STRESS (INPAS)

Largest abiotic stress international network in the world

More than 76 group leaders from 38 countries

Participations

- 200 publications
- 3 Books of Abstracts
- PI Sci special issue
- PSB special issue
- 3 joined patents
- 6 EU proposals
- web: http://cost-inpas.org/
- Satellite Meetings
- TV, radio, newspapers

Dissemination

Scientific Exchanges

- 25 STSM

Argentina, Australia, Egypt, India, Israel, Japan, South Africa, Tunisia

Organized by H. Kollist and U. Niinemets

Cyprus 18-19 November 2011

index.php?id=181&action_number=FA0605
Approaches to Improve Abiotic Stress Tolerance

Stress-associated metabolites

- Osmolytes, Osmo-protectants
- Polyamines
- Carbon Metabolism

Stress tolerance improvement

Genetic Engineering in crops

Marker-Assisted Crop Breeding

Stress-associated genes and proteins

- Signalling Components
- Chaperones
- LEA proteins

UNIVERSITAT DE BARCELONA
POLYAMINES

Putrescine

\[ \text{H}_2\text{N} - \text{C} - \text{C} - \text{C} - \text{C} - \text{N} - \text{H} \text{H}_2 \]

Spermidine

\[ \text{H}_2\text{N} - \text{C} - \text{C} - \text{C} - \text{C} - \text{N} - \text{H} \text{H}_2 \]

Spermine

\[ \text{H}_2\text{N} - \text{C} - \text{C} - \text{C} - \text{C} - \text{N} - \text{N} - \text{H} \text{H}_2 \]
POLYAMINES INCREASE IN RESPONSE TO ABIOTIC STRESSES

Abiotic Stress

Increased PA levels (Put, Spd or Spm)

Function

Mechanism(s) of action
MODEL PLANT AND TECHNIQUES

Genome, Transcriptome, Proteome

Genome (all genes): What could happen
Transcriptome (all mRNAs): What might be happening
Proteome (all proteins): What is happening

Metabolome

A. 2.8 mM glucose
- A. 2.8 mM glucose
- A. 2.8 mM glucose

B. 16.7 mM glucose
- B. 16.7 mM glucose
- B. 16.7 mM glucose

Genetic manipulation
(overexpressors, mutants)

Arabidopsis thaliana
POLYAMINE BIOSYNTHESIS IN ARABIDOPSIS

**Arabidopsis thaliana**

- ARG \(\rightarrow\) AGM \(\rightarrow\) NCP \(\rightarrow\) Putrescine
- SAM \(\rightarrow\) dSAM
- SAMDC1, SAMDC2, ADC1, ADC2
- SPDS1, SPDS2
- SPMS
- Spermidine \(\rightarrow\) Spermine
1) Correlation between $ADC1/ADC2$ expression with PUT increase
2) No correlation between $SAMDC1$ and $SAMDC2$ expression with SPM decrease
CHARACTERIZATION OF T-DNA INSERTIONAL MUTANTS

ADC knock-outs

PUT levels under COLD

PUT levels are reduced in adc mutants in response to cold
**RESPONSES TO FREEZING**

14-21 day-old plants grown on soil

**Acclimated plants**

Days          1        2        3        4         5        6        7

20°C

Days          1        2        3        4        5        6        7        8       9       10      14

**Non-acclimated plants**

6h freezing

% survival

*adc2* mutants are also more sensitive to freezing
COMPLEMENTATION OF FREEZING SENSITIVITY BY PUT

PUT complements freezing sensitivity of *adc* mutants

PUT complements freezing sensitivity of *adc* mutants

PUT improves freezing freezing tolerance of WT plants

OVEREXPRESSION OF ADC1 IN TRANSGENIC PLANTS

Non-acclimated plants after 6h at -5°C

Overexpression of ADC1 improves freezing tolerance

EXPRESSION OF CBFs IN RESPONSE TO COLD

With exception of CBF3, no significant changes are observed in the CBF modulon.
The CBF signalling pathway is not significantly affected in adc mutants.
Cold-repressed genes in *adc1-1* mutants in relation to wt plants

Electronic Northern by using the botany array resource database Canada
(http://bbc.botany.utoronto.ca/)
Among the cold repressed genes in *adc1* mutants there is a key group of ABA-responsive genes.
PUT is essential for a proper acclimatation of plants to cold due, at least in part, to a regulation of *NCED* expression and ABA levels.
GENE EXPRESSION AND PA LEVELS IN RESPONSE TO DROUGHT

1) Correlation between ADC2 higher expression with PUT increase
2) No correlation between SPDS1 and SPMS expression with SPD and SPM levels
DROGH SENSITIVITY OF ADC1/ADC2-OVEREXPRESSORS

3 weeks 14 days 2 weeks

Overexpression of ADC2 (no ADC1) confers drought tolerance
RELATIVE WATER CONTENT AND TRANSPIRATION RATE

wt                      2.1                   3.6                     9.12                 7.2

2.1

3.6

RWC

Transpiration rate

wt  7.2  9.12  3.6  2.1

mmol H₂O m⁻² s⁻¹

dehydration days
STOMATAL APERTURE AND ABA LEVELS

- Reduced stomata aperture


No significant changes in ABA levels
Las sobreexpresoras ADC2 tienen fenotipo similar al de mutates afectados en la ruta de señalización a ABA.

Ello sugiere que el mecanismo de tolerancia a la sequía está relacionado con la respuesta a ABA y no a su biosíntesis.
DIAMINE AND POLYAMINE CATABOLISM IN PLANTS

Putrescine → Spermidine → Spermine

1. Diamine oxidation:
   - CuAO
   - O₂ + H₂O
   - NH₃
   - H₂O₂
   - 4-Aminobutanal

2. Terminal oxidation:
   - PAO
   - O₂ + H₂O
   - H₂O₂
   - 4-Aminobutanal

3. Other reactions:
   - 1-Pyrroline → GABA
   - 1,3-Diaminopropane (DAP)
   - β-Alanine
   - N-(3-aminopropyl)-4-aminobutanal
PAOs in Arabidopsis and the back-conversion pathway

- Putrescine
- Spermidine
- Spermine
- \text{AtPAO3}/\text{PAO2}
- \text{AtPAO5} ?
- Back-conversion?
- Terminal oxidation ?
- \text{AtPAO1}
- \text{AtPAO4}
EXPRESSION OF PAO GENES UNDER SALT STRESS

PAO folds relative to 0h

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>PAO1</th>
<th>PAO2</th>
<th>PAO3</th>
<th>PAO4</th>
<th>PAO5</th>
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<tbody>
<tr>
<td>0</td>
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<td>96</td>
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</tbody>
</table>
CHARACTERIZATION OF *pao5* INSERTIONAL MUTANTS

*PAO5* (At4g29720)

No expression of *PAO5* in mutants: knock-outs

Zarza et al. In preparation
**pao5 MUTANTS ARE MORE TOLERANT TO SALT**

**Day**

<table>
<thead>
<tr>
<th>NaCl</th>
<th>0</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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<td>100mM</td>
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</tbody>
</table>

**Water content**

**Chl levels**

**4 week-old plants**

**Samples 50/100/150mM:**
8h, 1h, 3h, 8h, 24h, 48h, 96h

**flood 4h**
PA LEVELS IN MUTANTS DURING SALT TREATMENT

PUT

SPD

SPM levels are higher in *pao5* than WT at 0h and during salt treatment.

**Is PAO5 involved in terminal oxidation?**
The accumulation of Na⁺ vs K⁺ is delayed in the mutants vs WT

Is there a possible interaction of SPM/SPD with ion channels?

Kusano T et al. (2007) Plant Signal Behav. 2:251-252
El mutante pao5 tiene un mayor número de genes sobreexpresados o reprimidos que el wt tanto a 0h como después de 3h de estrés salino.
INDUCTION OF GENES IMPLICATED IN HISTONE DEMETHYLATION

mRNA expression levels. Results in log2. Green colour means genes repressed in pao5.1 mutant at time 0h (without salt treatment) respect to Wt at 0h (without salt treatment); red colour means genes overexpressed.

Histone methyltransferase activity
Histone demethylase activity (LDL1, JMJD5)
Histone acetyltransferase activity
Histone deacetylase activity

Histone demethylases containing Jumonj C (JmjC) domain [H3K4me3 demethylation]
Amino oxidase domain-containing demethylases. [H3K4me1-2 demethylation]

Estos genes están implicados en la regulación epigenética de la expresión génica

H: histona
K: Lisina
Me: metilo
PAO5 could act as a histone demethylase in Arabidopsis

Ello permitiría explicar la gran cantidad de genes cuya expresión está alterada en pao5 con respecto al wt
**Metabolomics**

Metabolite changes in mutant vs WT (0 time)

**WT**

- Salt
- No salt

**OSMOPROTECTANTS:**
- Sucrose
- Ethanolamine (choline & Gly-betaine)
- Melibiose
- Galactinol
- Proline

**pao5.1**

- Salt
- No salt
Results in folds respect to wt time 0. Mean ± se
INTERACCIONS OF PA WITH OTHER SIGNALS/MECHANISMS

Abiotic stress

ABA

IP₃

[Ca²⁺]cyt

Ion channels

Polyamines

ROS (H₂O₂)

NO

ABA

PAO5: Histone demethylase?

Biosynthesis

Response

Stomatal regulation – Na⁺/K⁺ homeostasis

Abiotic stress tolerance

INPAS: Scientific Challenge
INPAS: Scientific Challenge

Multidisciplinary Consortium of Scientist Working in Plant Stress Biology