Welcome

MBB 604: Advances in crop biotechnology (3+0)

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Transgenic approaches for improvement of plant abiotic stress tolerance

Environmental stress physiology

- Drought stress
- Salt stress
- High temperature
- Cold stress

Introduction

- A variety of abiotic stresses causing crop loss of about >50%
- Due to the declining water availability, by 2025, 30% crop production will be at risk
- World Bank projects that the climate change will reduce crop yield by 20% or more by the year 2050
- So for that reason transgenic plant have to be developed

APPROACHES

1. Engineering of genes for **Osmolyte biosynthesis**
2. Engineering of genes encoding enzymes for **scavenging active oxygen**
3. Engineering of genes encoding **LEA Proteins**
4. Engineering of genes encoding heterologous enzymes with different **temperatures**
5. Genetic engineering of **molecular chaperones**
6. Engineering of genes encoding **transcription factors**
7. Genetic engineering of **cell membranes**

1. Engineering of Genes for Osmolyte biosynthesis

- Tolerance to abiotic stresses has mainly been achieved at cellular levels of osmotically-active solutes

  - Proline, glycine betaine
  - Sugars such as sucrose, trehalose and fructans
  - Sugar alcohols like sorbitol, mannitol, ononitol, pinitol and polyols

- These osmolytes are uniformly neutral with respect to cellular functions

Accumulation of these molecules helps plants to –

- Retain water within cells and protects cellular compartments from injury caused by dehydration
- Maintains turgor pressure during water stress
- Stabilize the structure and function of certain macromolecules
- Signalling functions or induction of adaptive pathways
- Scavenge reactive oxygen species

Proline

- Proline accumulates in many organisms in response to drought and salinity
- Proline is encoded by a nuclear gene ***Pyrroline-5-carboxylate synthetase (P5CS)***
- Proline may serve as a **hydroxyl radical scavenger** reducing the acidity of the cell
- It may also function as an **osmolyte** and **molecular chaperone**
- Protect protein integrity and enhance the activities of different enzymes

proline...

- Glutamate can be achieved by using the *P5CS* gene from Moth bean (*Vigna aconitifolia*)
- Antisense proline dehydrogenase (*PDH*) in transgenic *Arabidopsis*
- However, *P5CS*, limiting enzyme in proline biosynthesis, feedback inhibition by proline
- Removed this feedback inhibition by site-directed mutagenesis and the resulting gene ***P5CSF129A***

Transgenic plants for Proline

Gene	Molecular function	Source	Transformed plant	Performance of transgenic plant	Additional note
P5CS	*D1-pyrroline-5-carboxylate synthase*	*V. aconitifolia*	*O. sativa*	Drought & salt tolerance	Increased biomass (higher fresh shoot & root weight)
P5CS F129 A	*Mutated P5CS*	*V. Aconitifolia*	*N. tabacum*	Oxidative & osmotic stress tolerance	Removal of feedback inhibition resulted in 2-fold high proline than the plants expressing P5CS
PDH	*Proline dehydrogenase*	*A. thaliana*	*G. max*	Drought & heat tolerance	Rapid increase in proline resulted in least water loss under drought stress

Transgenic plants for Proline

Gene	Molecular function	Source	Transformed plant	Performance of transgenic plant	Additional note
P5CSF129A	*Mutated P5CS*	*V. aconitifolia*	*O. sativa*	Salt stress tolerance	Transgenic plants with high proline level showed higher biomass & growth performance under salinity stress
P5CSF129A	*Mutated P5CS*	*V. aconitifolia*	*P. vulgaris*	Drought tolerance	Elevated free proline resulted in better adaptation to water stress
δ-OAT	*Ornithine-daminotransferasea*	*A. thaliana*	*N. Plumbaginifolia*	Osmotic stress tolerance	Transgenic plant showed higher germination and increased biomass

Glycine Betaine

- GB accumulates in the **chloroplasts** and **plastids** and increases the tolerance of plants to various abiotic stresses (drought, salinity and freezing)
- The physiological role of GB in **alleviating osmotic stress**
- It can also **protects proteins** and **enzyme** activities under water deficits and **stabilize membranes** during freezing

- It can help stabilize the protein tertiary structure and prevent or reverse disruption of the tertiary structure

- Accumulation of GB is limited due to **choline** supply

- Transgenic potato plants expressing a bacterial choline oxidase (*betA*) gene leads to high levels of GB under drought stress

Transgenic plants for Glycine betaine

Gene	Molecular function	Source	crop	Function	Additional note
BADH	*Betaine aldehyde dehydrogenase*	*A. Hortensis*	*O. sativa*	Improved growth & salinity tolerance	10% transgenic plants set seed in 0.5% NaCl solution in greenhouse
BADH	*Betaine aldehyde dehydrogenase*	*H. Vulgare*	*N. tabacum*	Salt tolerance	Tolerance was judged by high biomass & photosynthetic
CHO	*Choline dehydrogenase*	*E. coli*	*O. sativa*	Drought & salt tolerance	Higher yield under stresses
betA	*Choline dehydrogenase*	*E. coli*	*Z. mays*	Drought tolerance at seedling stage	Grain yield was significantly higher compared with wild-type

Transgenic plants for Glycine Betaine

Gene	Molecular function	Source	Crop transformed	Performance of transgenic plant	Additional note
betA	*Choline dehydrogenase*	*E. coli*	*G. hirsutum*	Drought tolerance	GB accumulation was positively correlated with drought stress
codA	*Choline oxidase*	*E. coli*	*S. tuberosum*	Oxidative, Drought & salt Stress tolerance	Stress-inducible GB production in non accumulators resulted in tolerance
CMO	*Choline monooxygenase*	*A. hortensis*	*G. hirsutum*	Salt tolerance	Seed cotton yield was significantly higher in transgenic plants

Sugars and Sugar Alcohols

- Accumulation of sugar-related compounds, response to osmotic stress
- These compounds **stabilize** the **membranes** and **proteins** during dehydration
- Sugars can replace the water molecules and stabilize the proteins or membranes in a similar of water molecules
- They can form a glass phase in the dry state of high viscosity have capable of slowing down chemical reactions lead to long-term stability in a living system

Trehalose

- Trehalose, a rare **non-reducing sugar**
- Trehalose protects the biological molecules in response to different stress conditions
- It does not accumulate to high enough levels in most plants, probably because of the presence of trehalase activity

trehalose...

- Trehalose synthesized in two steps from **glucose-6-phosphate** and **uridine diphosphoglucose**, *via* trehalose-6-phosphate

- The first step is catalyzed by **trehalose phosphate synthase** (TPS), and the second by **trehalose-6-phosphatase** (TPP)

Transgenic plants for Trehalose

Gene	Molecular function	Source	Transformed plant	Function	Additional note
TPS1	Trehalose-6-phosphate synthase	*S. cerevisiae*	*N. tabacum*	Enhanced drought tolerance	A few transgenic plants showed phenotypic alteration along with stress tolerance
otsA & otsB	*TPS & TPP* fusion gene	*E. coli*	*O. sativa*	Drought, salt & cold tolerance	Sustained growth, less photo-xidative damage, enhanced photosynthesis under various stresses
TPP1	Trehalose-6-phosphate phosphatase	*O. sativa*	*O. sativa*	Enhanced salt & cold stress Tolerance	Analysis of transgenic plants suggested a possible role of TPP in transcriptional regulation pathways

Transgenic plants for Trehalose

Gene	Molecular function	Source	Transformed plant	Function	Additional note
TPS1	*Trehalose-6-phosphate synthase*	*S. cerevisiae*	*L. esculentum*	Drought, salt & oxidative stress tolerance	Sustained yield under all stress
TP	*Trehalose phosphorylase*	*P. sajor-caju*	*S. officinarum*	Drought tolerance	Transgenic plants did not withered even after 10 days of water deficit & also exhibited no pleiotropic effects
TPS1+ TPS2	*Bifunctional gene*	*S. cerevisiae*	*M. sativa*	Multiple abiotic stress tolerance	Improved growth with significant increase in biomass

Fructans and other Carbohydrates

- Fructan or polyfructose molecules serve as the main **storage carbohydrate** in many plant species

- However, fructans are to be involved in abiotic stress tolerance by virtue of their presence in vacuoles

- Fructans are also thought to protect plants against drought and cold stress evidence of this was derived from transgenic plants

- It act as regulators or signal molecules, thus influencing plant metabolism, as scavengers of ROS

- It promote the process of root branching, thus increasing the root surface and subsequent water uptake

Fructans and other Carbohydrates

Gene	Molecular function	Source	Transformed plant	Function
sacB	Levan sucrase, a fructosyl transferase	B. subtilis	N. tabacum, B. vulgaris	PEG-induced drought tolerance
Imt1	Myo-inositol-o methyl transferase	E. coli	N. tabacum	Drought & salt tolerance
mtlD	Mannitol-1-phosphate dehydrogenase	E. coli	S. melongena	Salt, drought & chilling tolerance
Ipk2β	Inositol polyphosphate 6-/3-kinase	A. thaliana	N. tabacum	Enhanced drought, salinity & freezing tolerance

2. Engineering of genes encoding enzymes for scavenging active oxygen

- Plants suffering from various stresses, which leads to overproduces active oxygen species in cells

- To minimize the damaging effects of active oxygen, plants produces antioxidant to detoxifying harmful oxygen

- Genes encoding enzymes with antioxidant capacity, such as ascorbate peroxidase, superoxide dismutase and glutathione reductase

Example:

- Transgenic alfalfa overproducing **superoxide dismutase (SOD)** showed reduced injury from water deficit and freezing stresses in field conditions

- Engineering chloroplastic **superoxide dismutase (SOD)** in tobacco led to an increase of chilling tolerance of photosynthesis

- Tobacco plants that overproduced cytosolic **ascorbate peroxidase** showed increased tolerance to oxidative stress

- Over expression of **glutathione S-transferase** or **glutathione peroxidase** provided some protection against cold and salt stress in tobacco

3. Engineering of genes encoding LEA Proteins

- A genes encoding LEA (**Leaf Embryo Abundance**) proteins is activated under osmotic stress

- LEA proteins play a role in desiccation tolerance during seed development and in response to dehydration, salinity and cold stress

  - **Maintenance of protein or membrane structure**
  - **Sequestration of ions,**
  - **Binding of water**
  - **Operating as molecular chaperones**

Example:

- *HVA1*, a group 3 LEA protein from barley
- *LE25*, a group 4 LEA protein from tomato

- Rice plants transformed with barley LEA gene *HVA1* possessed increased tolerance to water deficit and salinity

- Improved salinity and freezing resistance has been observed in yeast transformed with *LE25*

4. Engineering of genes encoding heterologous enzymes with different temperature optima

- Homologous proteins and their specific enzymatic activities from different plant species differ in terms of their temperature
  - E.g: When a gene of **NADH-hydroxypyruvate reductase** from cucumber (higher optimum growth temperature) was transformed into tobacco (lower optimum growth temperature), the optimum temperature of transgenic tobacco was increased

5. Genetic Engineering of molecular Chaperones

‘**Chaperone**’ : assist and maintain correct folding and trafficking of cell proteins, are crucial for plants to survive in abiotic stresses

- E.g: An antisense inhibition of the *HSP70* family prevented Arabidopsis plants from acquiring thermotolerance

- Heat shock proteins *HSP101* from soybean, corn, wheat and tobacco complemented a thermo tolerance defect in yeast caused by deletion of yeast *HSP104*

6. Engineering of genes encoding Transcription Factors

- Responses of plants to abiotic stresses are multigenic and a single gene is not likely to induce the whole cascade

- Recently, transcription factors for abiotic stress-induced genes have been identified, cloned and used in transgenic experiments

- Simultaneous expression of downstream stress-inducible genes have been achieved with parallel increase in stress tolerance

Example:

- Over expression of the transcriptional activator ***CBF1*** ('C-repeat binding factor') induced the expression of four **COR** ('cold-regulated') genes and increased freezing tolerance of *Arabidopsis* plant

- By over expressing a single stress-inducible transcription factor (***DREB1A***), lead to plant tolerance to freezing, salinity and dehydration

7. Genetic Engineering of Cell Membranes

- Membranes are critical sites of injury by chilling, freezing, heat and osmotic stresses

- Increased levels of unsaturated membrane lipids leads to higher membrane fluidity which improves plant-chilling tolerance and photosynthetic parameters

Example:

- Tobacco transformed with acyl-ACP:glycerol-3-phosphate acyltransferase (*GPAT*) from chilling- tolerant species of *Arabidopsis* acquired chilling tolerance

Thank you !!!