GENETIC MANIPULATION OF CROP PLANTS AGAINST ENVIRONMENTAL STRESSES

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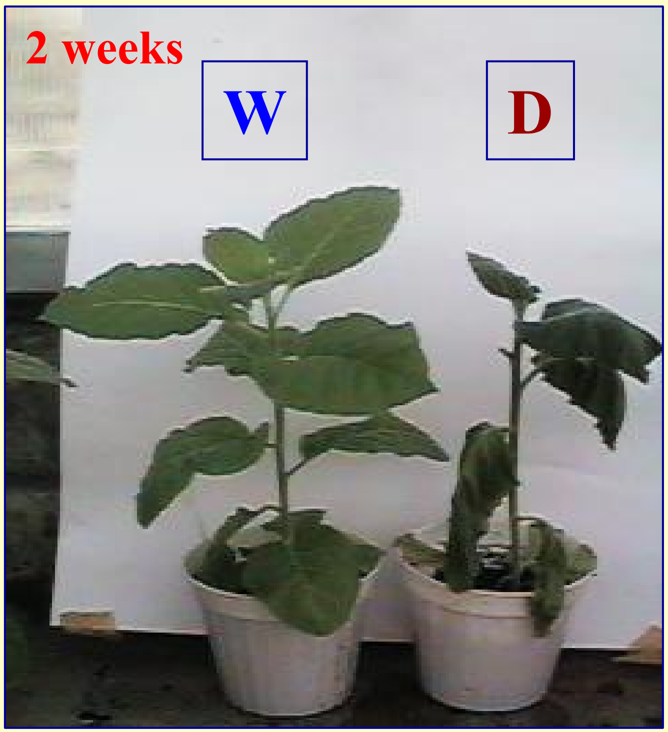
Chilling
Freezing
Heat
ABIOTIC STRESS
OSMOTIC STRESS
Flooding
Drought
Salinity

Drought Stress

Plants grown for 3 weeks under normal conditions and subjected to drought conditions (**D**) or watered (**W**) for 2 weeks.

At 4 leaf stage plants are subjected to drought stress (**D**) (6 days no water &1 day watered) or watered (**W**) regularly. Photographed after 8 weeks of treatment



Salt (NaCl) Stress

At four leaf stages plants are subjected to 0.4 M NaCl stress (NaCl) or watered regularly. Plants were photographed after 2 , 8 and 16 weeks.

Most environmental stress forms such as drought, salt, low and extreme temperature; have a common denominator.

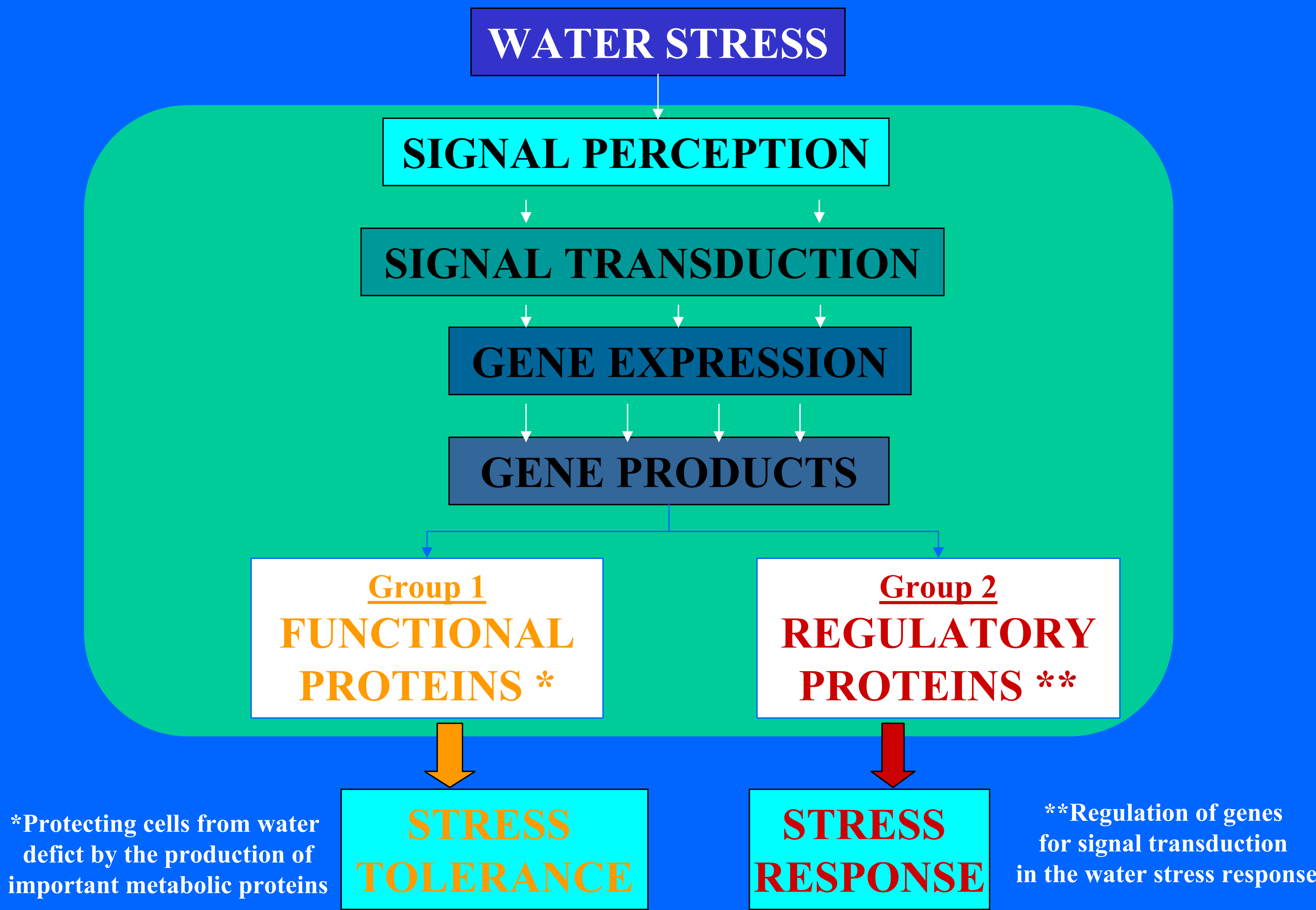
WATER DEFICIT = OSMOTIC STRESS

Since plants can not escape from these stresses they adopted a variety of mechanisms at <u>morphological</u> and <u>molecular</u> level

- ***Avoidance*: deep roots & small leaves**
- ***Escape*: fast flowering-seed set**
- ***Water saving*: stomatal closure**
- ***Osmotic adjustment*: osmolytes-osmoprotectants**

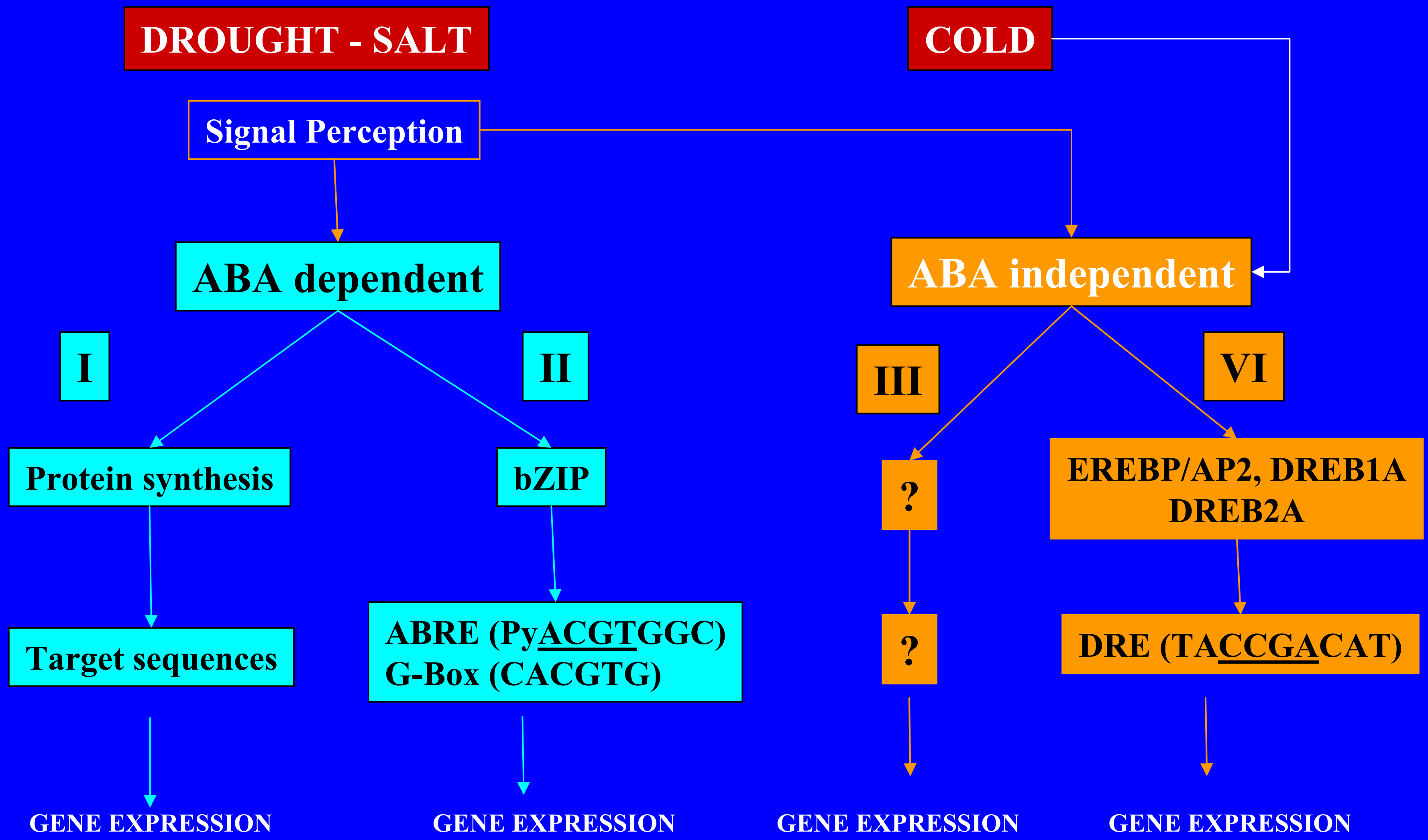
Molecular aspects of OSMOTIC STRESS

1. **Genes, their products & their functions**
2. **Strategies for genetic manipulation of plants against osmotic stress**


WATER STRESS
SIGNAL PERCEPTION
SIGNAL TRANSDUCTION
GENE EXPRESSION
GENE PRODUCTS
Group 1
FUNCTIONAL PROTEINS *
Group 2
REGULATORY PROTEINS **
STRESS TOLERANCE
STRESS RESPONSE
*Protecting cells from water deficit by the production of important metabolic proteins
**Regulation of genes for signal transduction in the water stress response

CONTROL OF GENE EXPRESSION DURRING WATER STRESS

There are 4 independent pathways for the expression of water stress induced genes.
Two of the pathways are absisic acid (ABA) dependent and the other two is ABA independent



GROUP I GENES & PRODUCTS

1) *Water channel proteins (Aguaporins) :*

Involves in movement of water through membranes

2) *Enzymes require for biosynthesis of various osmoprotectants*

sugars, proline, glycine-betaine, sorbitol

3) *Proteins that may protect macromolecules and membranes*

LEA, osmotin, dehydrins, antifreeze proteins, chaperon, mRNA binding proteins

4) *Proteases for protein turnover*

thiol protease, Clp protease, ubiquitin, (protease inhibitors-Kuintz)

5) *Detoxification enzymes*

Superoxide dismutase (SOD), glutathion-S-transferase, soluble epoxide hydroxylase, catalase, ascorbate peroxidase

6) *Transport proteins*

Na+/H+ transporter

Function in stress tolerance

GROUP II GENES & PRODUCTS

1) *PROTEIN KINASES*
MAPK, MAPKK, MAPKKK, CDPK

2) *TRANSCRIPTION FACTORS*
DREB1A, DREB1B etc.

3) *PHOSPHOLIPASE-C (PL-C)*
PIP turnover

4) *PHOSPHATASES*
calcineurin

- Function in stress response
- Regulation of signal transduction and gene expression

STRATEGIES FOR ENGINEERING OSMOTIC STRESS TOLERANCE

1) Protection against (oxygen) free radicals via overexpression of antioxidant enzymes (1990-)

2) Osmoprotectant engineering via transferring osmolyte producing enzymes (1993-)

3) Alternation in lipid membrane composition (1996-)

4) Enhanced stress related gene expression via transfer of transcriptional factors (1998-)

5) Enhanced ion compartmentalisation via Na+/H+ antiport overexpression (1999-)

6) Protection against toxic by-products via expressing detoxification enzymes (2000-)

1) Protection against (oxygen) free radicals via overexpression of antioxidant enzymes (1990-)

One of the important mechanisms by which plants are damaged during adverse environmental conditions is the excess production of *active oxygen species*.

- **Superoxide** $O_2^{\bullet -}$
- **Hydrogen peroxide** H_2O_2

- **Lignin formation in cell walls**
- **oxidative burst upon infection (hypersensitive cell death)**
- **2nd messengers (PR, phytoalexin gene expression)**

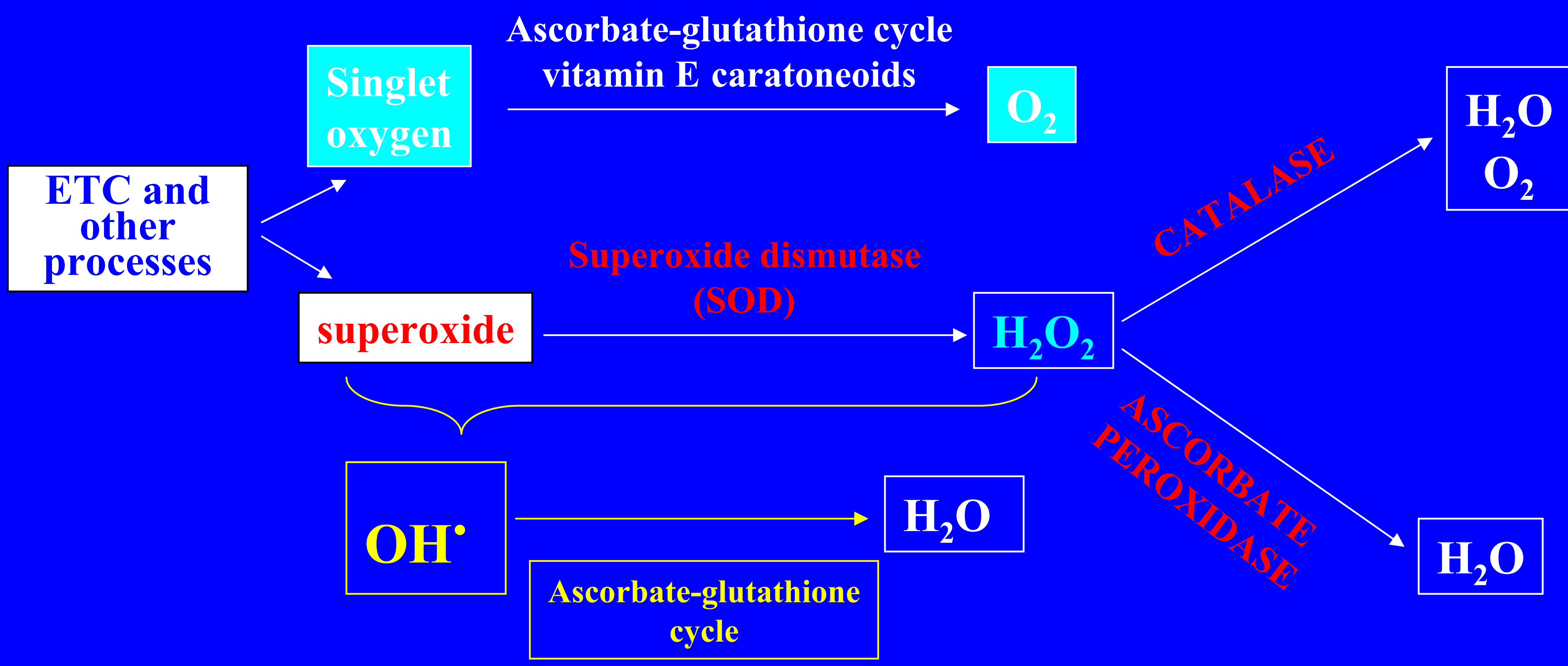
Haber-Weiss reaction (meatal (M) ion dependent)

$$O_2^{\bullet -} + H_2O_2 \xrightarrow{M^{n+},\ M^{(n+1)}} OH^{\bullet} + OH^{-} + O_2$$

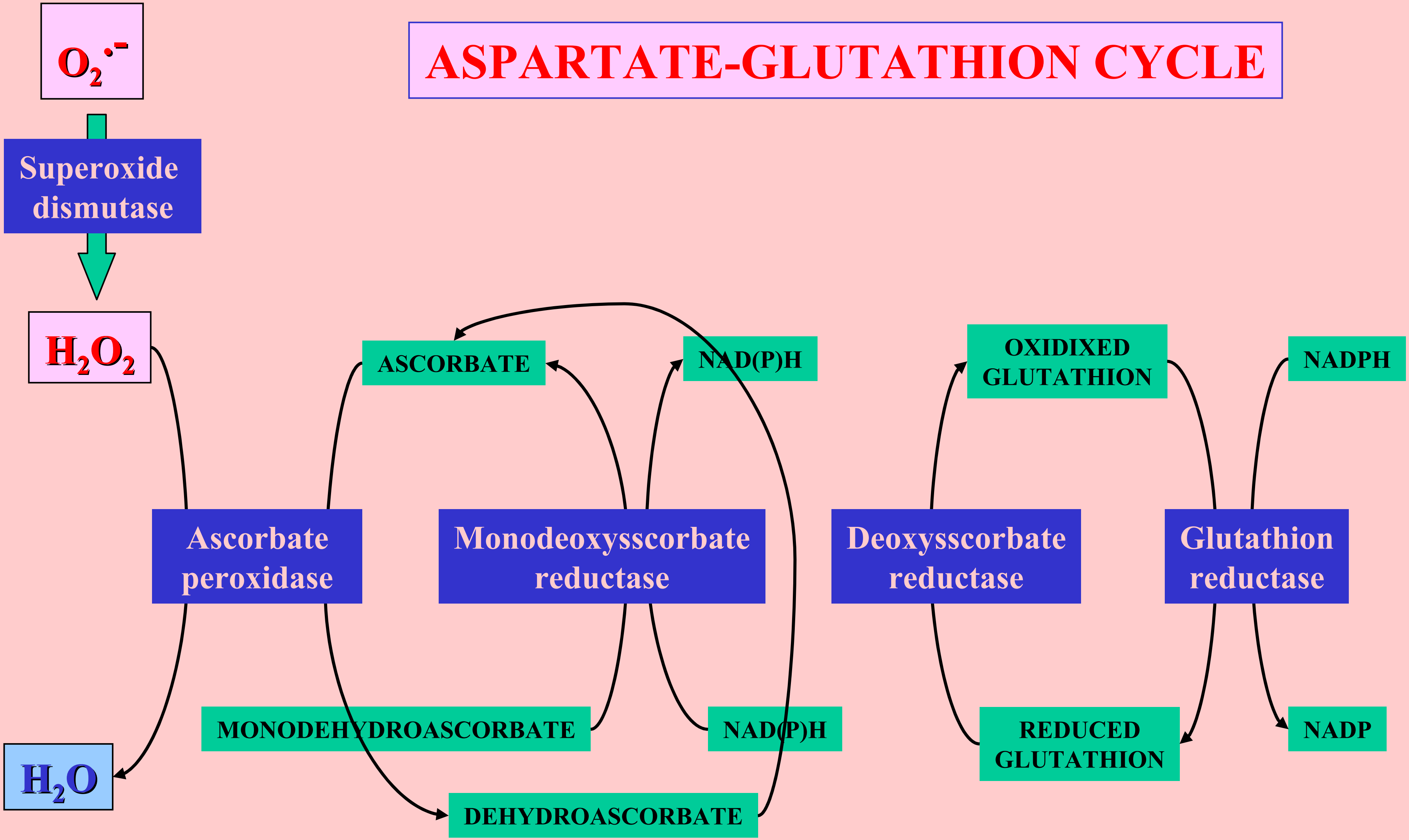
- **Hydroxyl radicals** $OH^{\bullet}$

- **One of the most reactive species known to chemistry**
- **mutate DNA, initiate chain reactions of lipid peroxidation, react with proteins**
- **cause rapid cell damage**

- Plants posses non-enzymatic and enzymatic protection mechanisms that efficiently scavenge active oxygen species.
- Antioxidants such as ascorbic acid (vitamin-C) glutathion, α-tocopherols, and carotenoids occur in high concentrations in plants.
- Hydroxyl radical are too reactive to be eleminated enzymically, but their formation is limited by scavenging of superoxide and hydrogen peroxide.



ASPARTATE-GLUTATHION CYCLE



LITERATURE DATA

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Van Camp W. et al. (1996) Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-SOD in chloroplast. Plant Physiol., 112: 1703-1714.

McKersie B.D. et al. (1996) Water deficient tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase. Plant. Physiol., 111:1171-1177.

McKersie B.D. et al. (1999) Winter survival of transgenic alfalfa overexpressing superoxide dismutase. Plant Physiol., 119: 839-847.

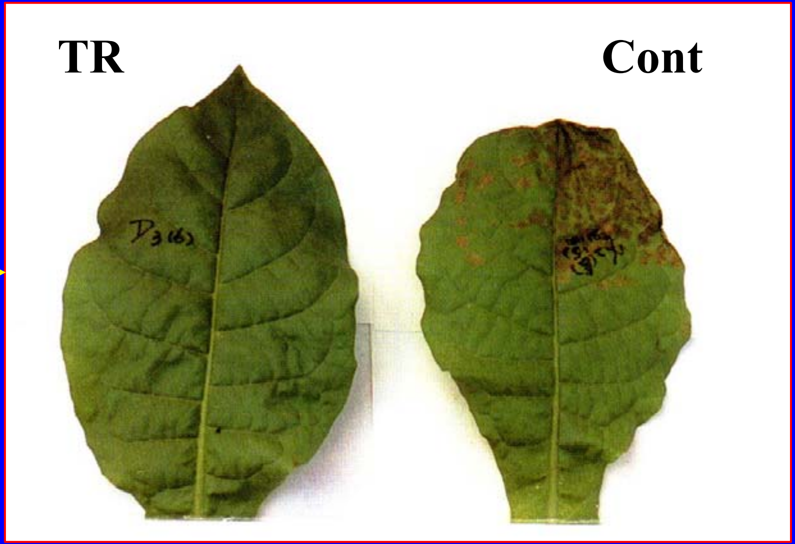
Tanaka Y. et al. (1999) Salt tolerance of transgenic rice over expressing yeast mitochondrial Mn-SOD in chloroplast. Plant Sci., 148: 131-138.

Yu Q. et al. (1999) Increased tolerance to Mn Deficiency in transgenic tobacco overproducing SOD. Annals of Botany, 84: 543-547.

McKersie B.D. et al. (2000) Iron-superoxide dismutase expression in transgenic alfalfa increases winter survival without a detectable increase in photosynthetic oxidative stress tolerance. Plant Physiol., 122: 1427-1437

Roxas V.P. et al. (1997) Overexpression of glutathion-S-transferase/ glutathion peroxidase enhances the growth of transgenic tobacco seedlings during stress. Nature Biotechnol., 15:988-991.

Wang J. et al. (1999) Overexpression of an *Arabidopsis* peroxisomal peroxidase gene in tobacco increases protection against oxidative stress. Plant Cell Physiol., 40: 725-732.



Effect of aminotriazole (catalase inhibitor) treatment on AsPrx overexpressing TR and control plants.

2. Osmoprotectant engineering via transferring osmolyte producing enzymes (1993-)

OSMOLYTES

<u>Proposed functions</u>: radical scavening, protection of enzymes, enzyme complexes or membranes, a sink for photosyntheticaly assimilated carbon under stress.

***Proline*:**

Polyamines: **spermine**

***Acyclic polyols*: Mannitol, sorbitol, galactitol.**

***Cyclic polyols*: D-Pinitol and other methylated inositols.**

Tertiatry sulfonium osmolyte: **β-Dimethylsulfoniopropionate, choline O-sulfate**

Quaternary ammonium osmolyte: **glycine betaine, proline betaine**

PROLINE

A common osmoprotectant in most organisms

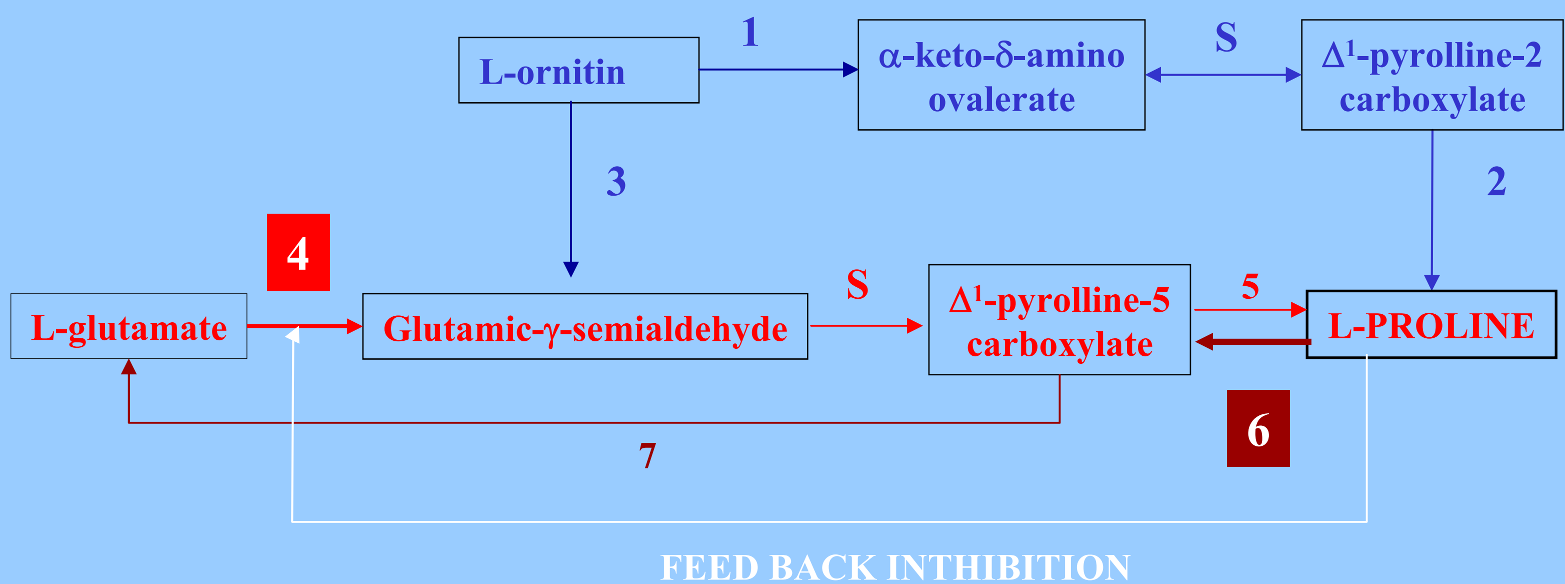
A solute that protect macromolecules against dehydration

Sink for energy to regulate redox potentials

Free radical scavenager

Reduce acidity in the cell

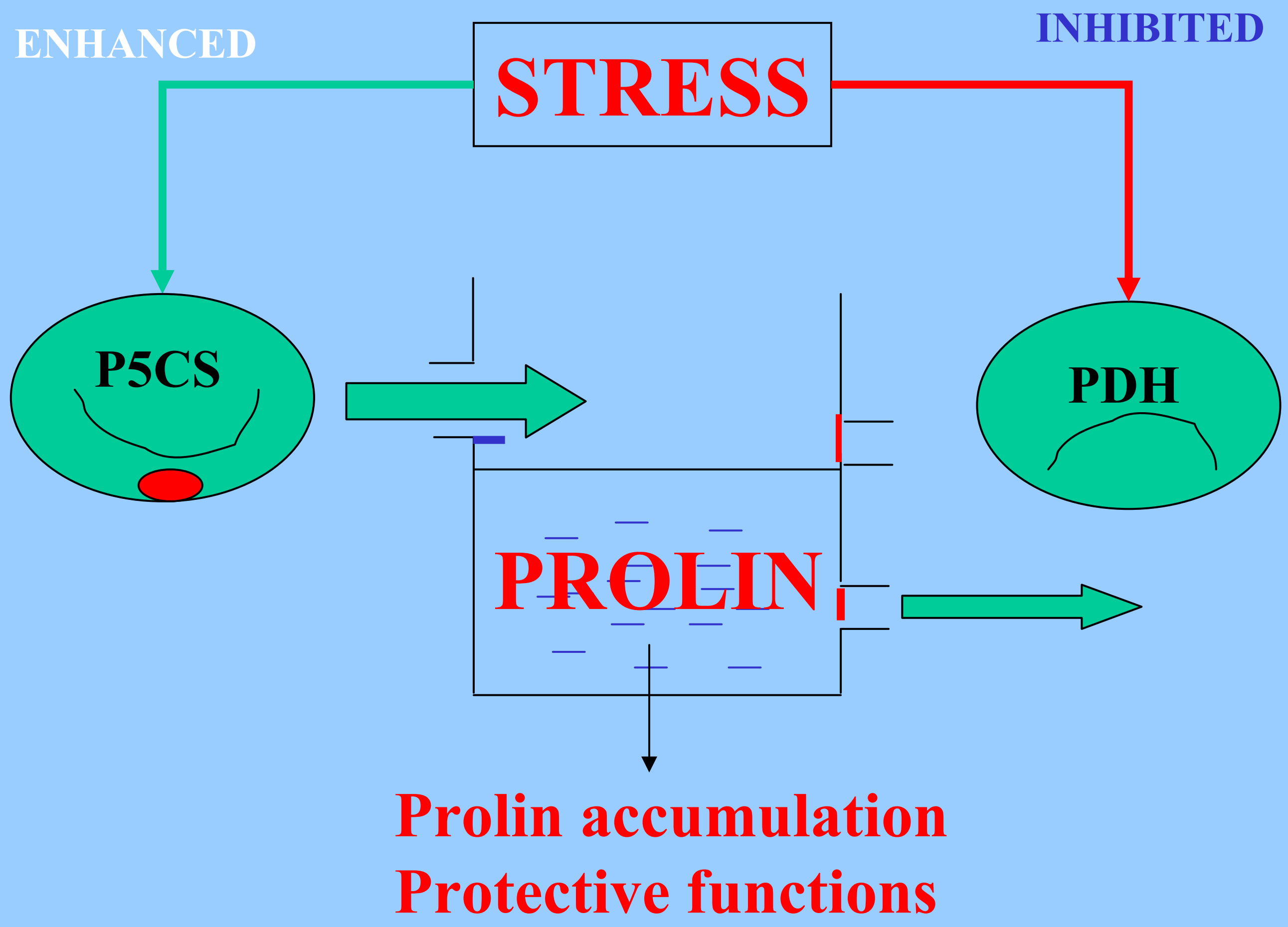
Metabolic Pathways for Proline Biosynthesis

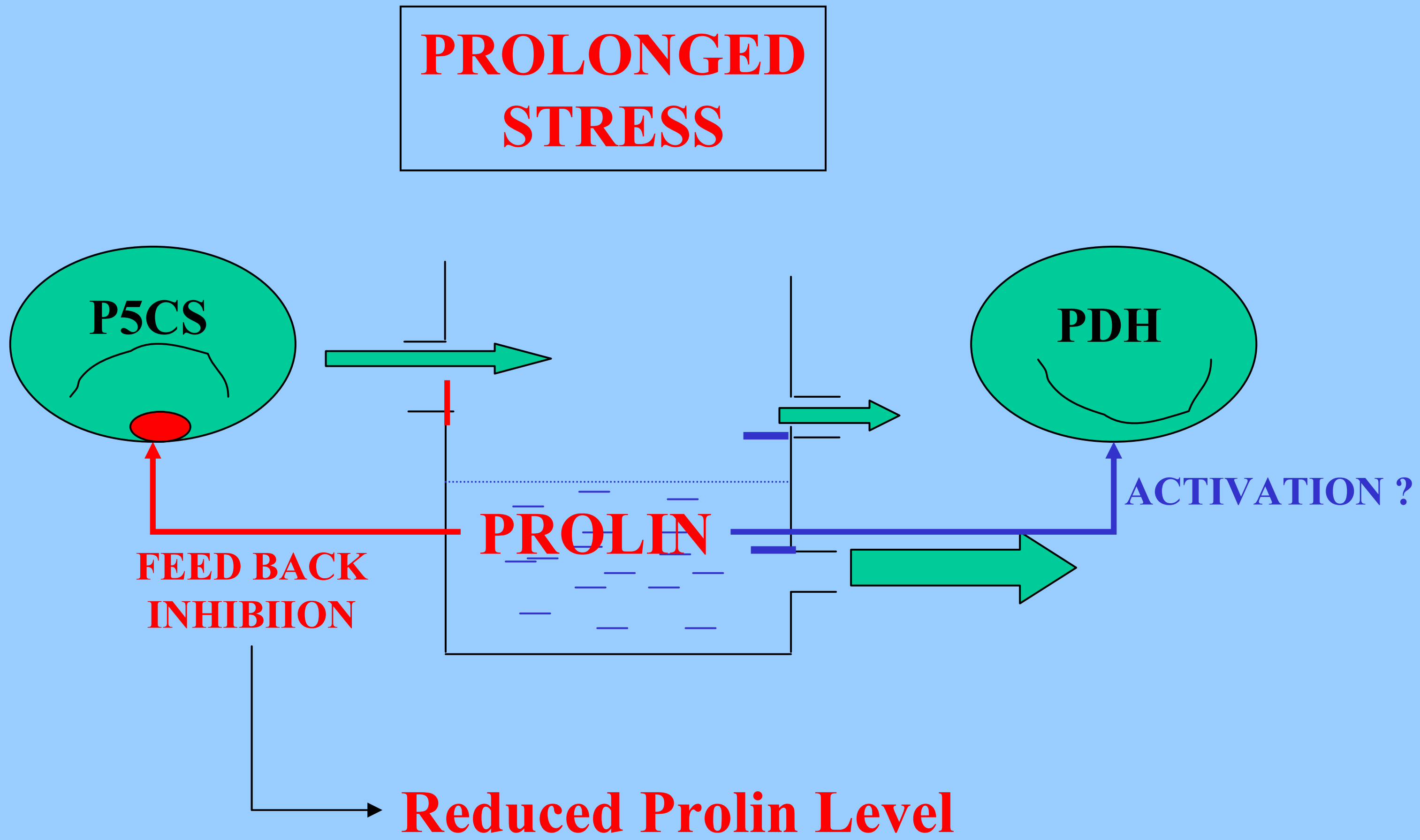


1: ornitin-αaminotransferase; 2: P2C reductase; 3: ornithine-δ-amino transferase

<u>4: P5C Synthase</u>; 5: P5C reductase; S: spontaneoues

<u>6: Proline dehydrogenase</u>; 7: P5C dehydrogenase


ENHANCED
STRESS
INHIBITED
P5CS
PDH
PROLIN
Prolin accumulation
Protective functions


PROLONGED STRESS
P5CS
PDH
PROLIN
ACTIVATION ?
FEED BACK INHIBIION
Reduced Prolin Level

STRATEGIES TO DEVELOP STRESS RESISTANT TRANSGENIC PLANTS via PROLINE METABOLIC ENGINEERING

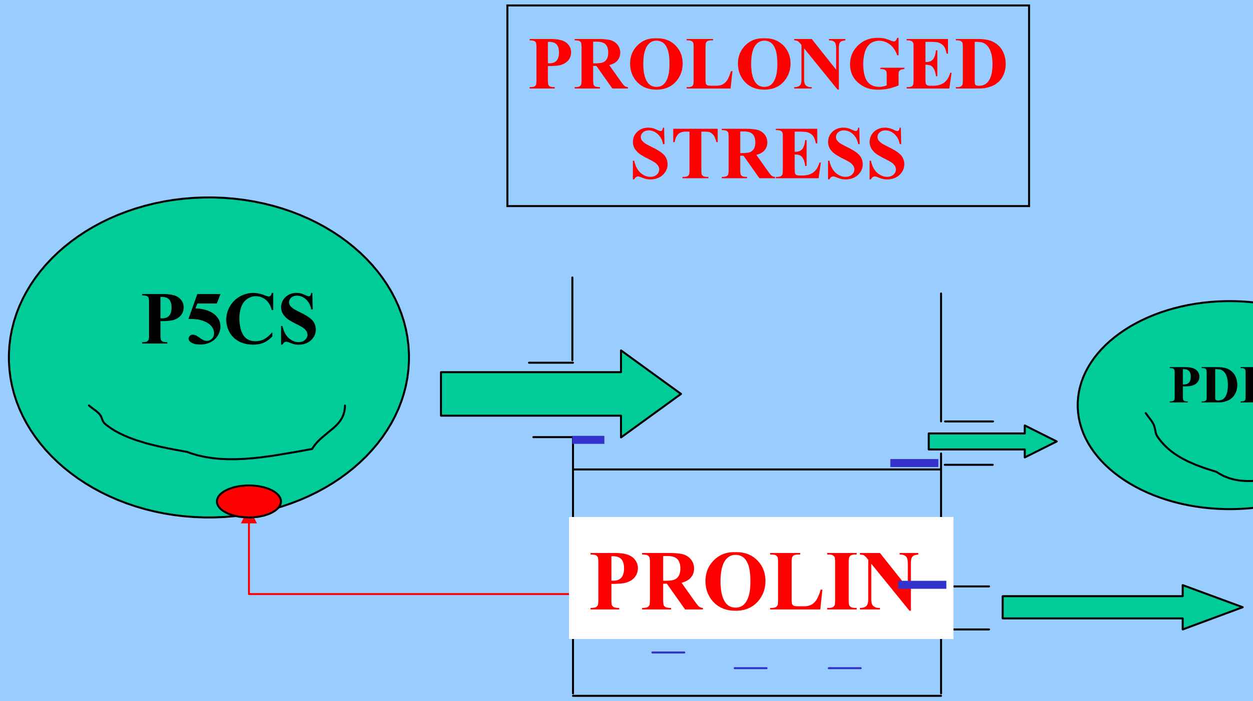
1 : Overexpress P5CS genes

2: Remove feed back inhibition on P5CS gene

3: Reduce PDH level via antisense technology

4 : Combined: Use combination of above strategies

TR STRATEGY 1 : Overexpress P5CS

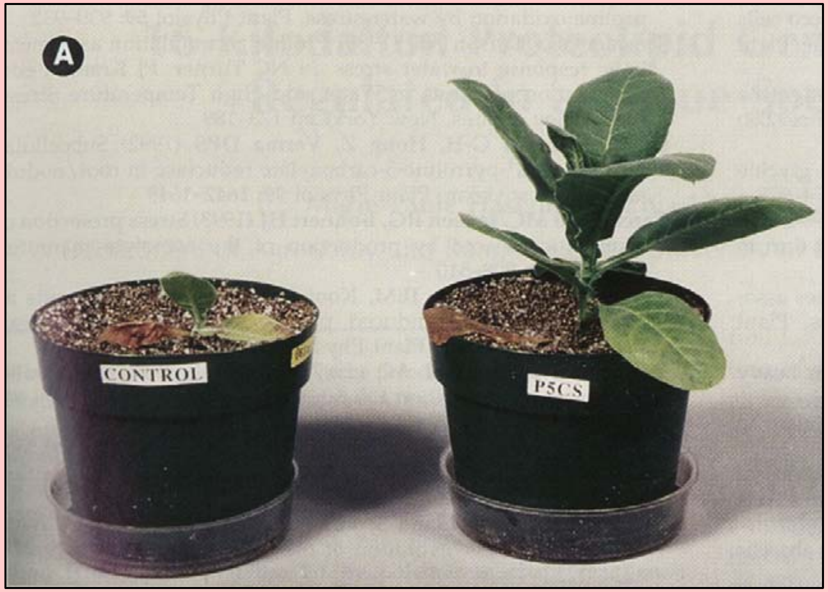


Kavi Kishor P.B. et al (1995) Overexpression of delta1-pyrroline-5-carboxylate synthase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol. 108, 1387-1394.

Zhu B. et al. (1998) Overexpression of delta1-pyrroline-5-carboxylate synthase gene and analysis of tolerance to water and salt stress in transgenic rice. Plant science 139: 41-48.

Eyidoğan F. et al. (2001) Genetic manipulation of tobacco against osmotic stress via transfer of AtP5CS gene. Manuscript in preperation.

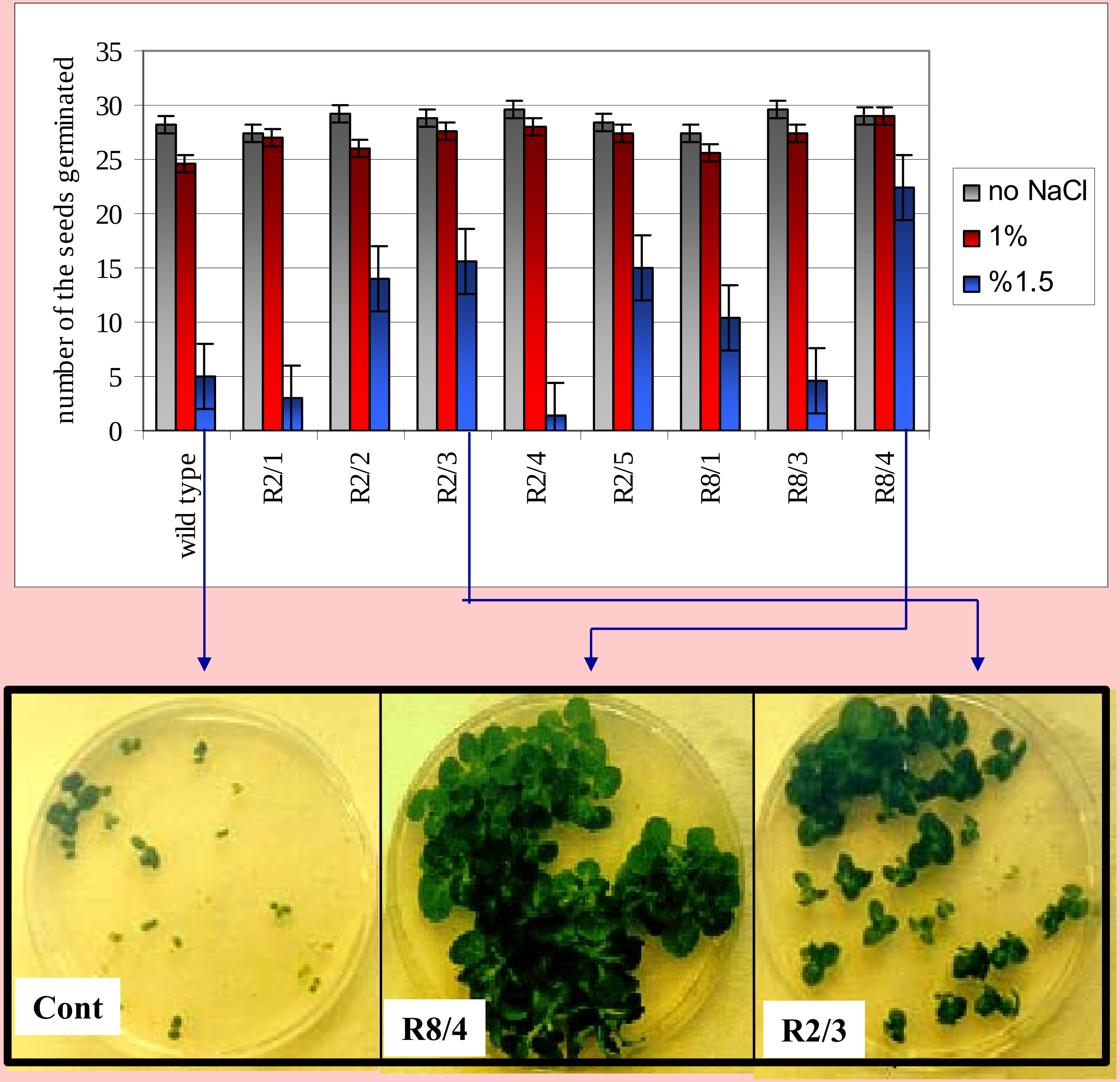
Salt-Drought Resistant TR Tobacco Plants Overexpressing *Vigna* P5CS



At four leaf stage plants were kept in trays Containing 400 mM NaCl and grown for 3 weeks.

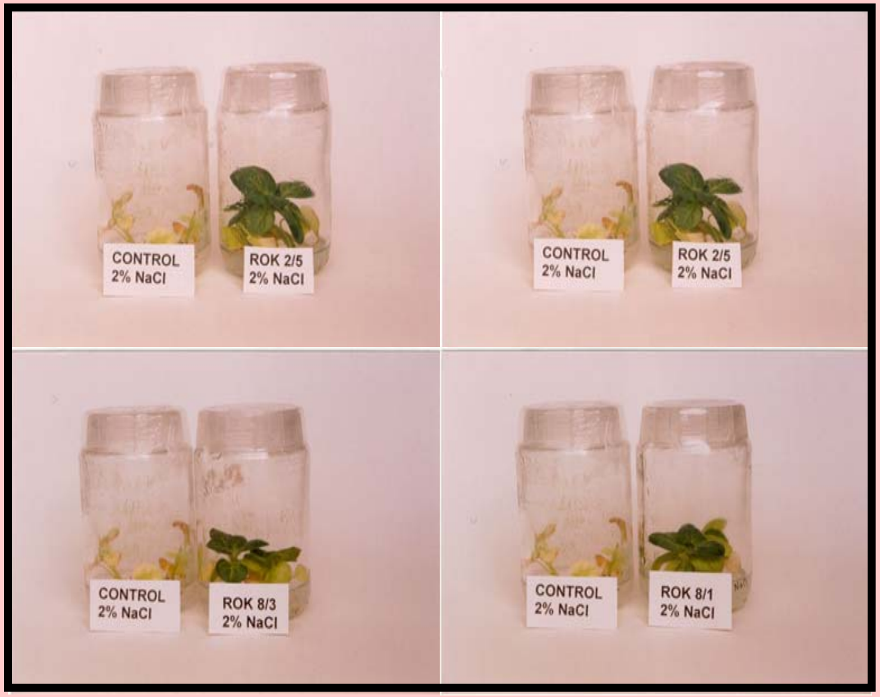
Roots of plants subjected to drought conditions. Photographed at time of flowering.

Kavi Kishor P.B. et al (1995) Plant Physiol. 108, 1387-1394.



Germination of F_1 seeds in the presence of NaCl.

Eyidoğan F. et al. (2001) Unpublished

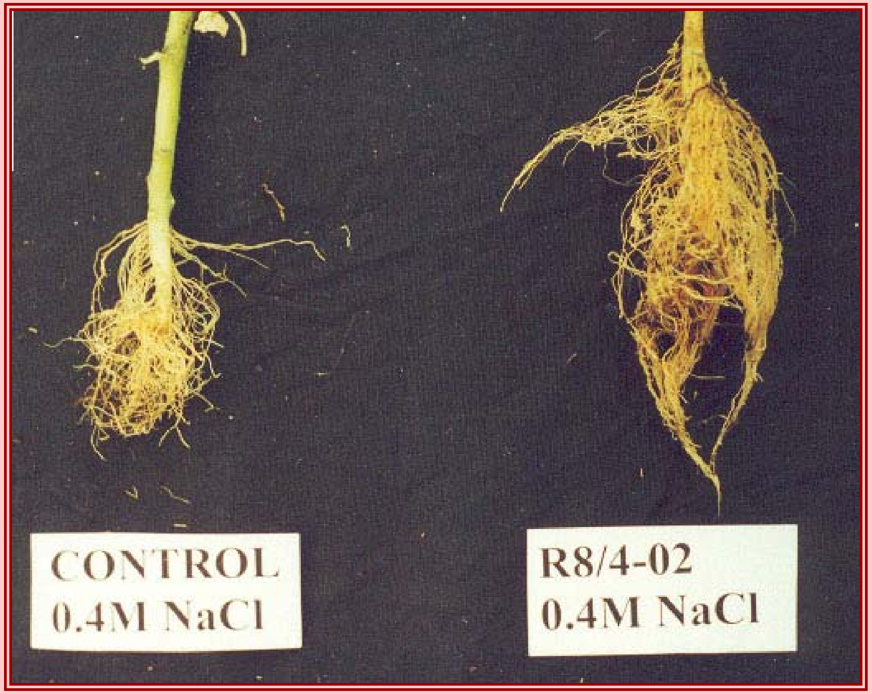


Effect of 2% NaCl treatment on growth of TR and control plants

Eyidoğan F. et al. (2001) Unpublished

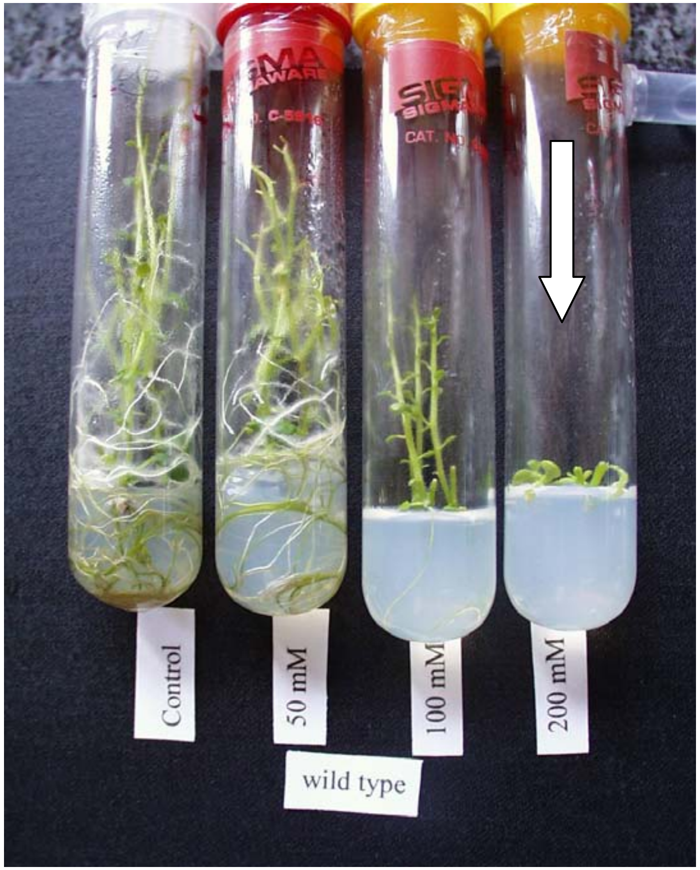
Effect of 0.4M NaCl Stress on Morphology and Growth of Transgenic and Control Plants

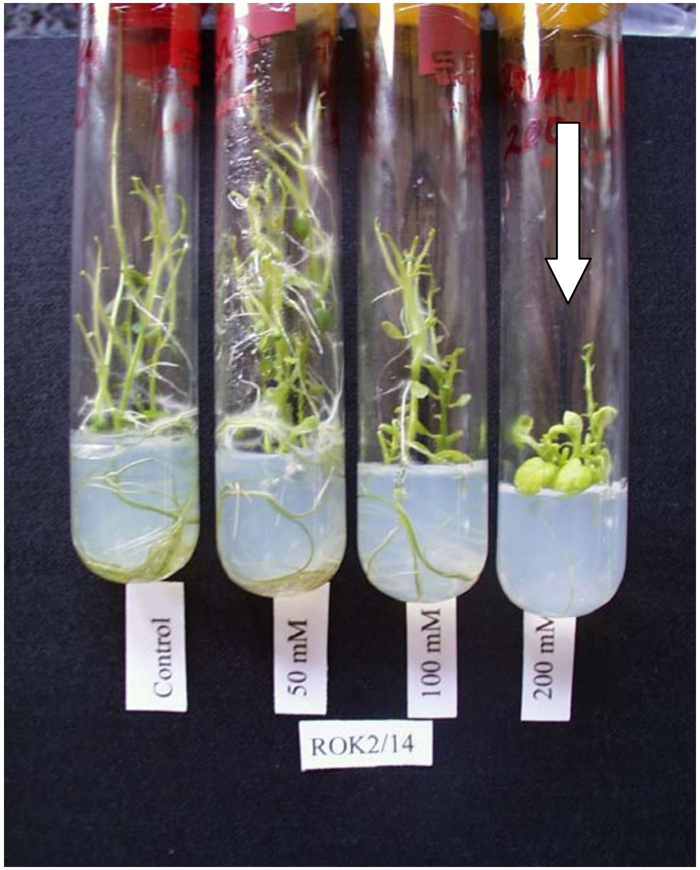
(Plants are subjected to salt stress for 3 months)



Eyidoğan F. et al. (2001)
Unpublished

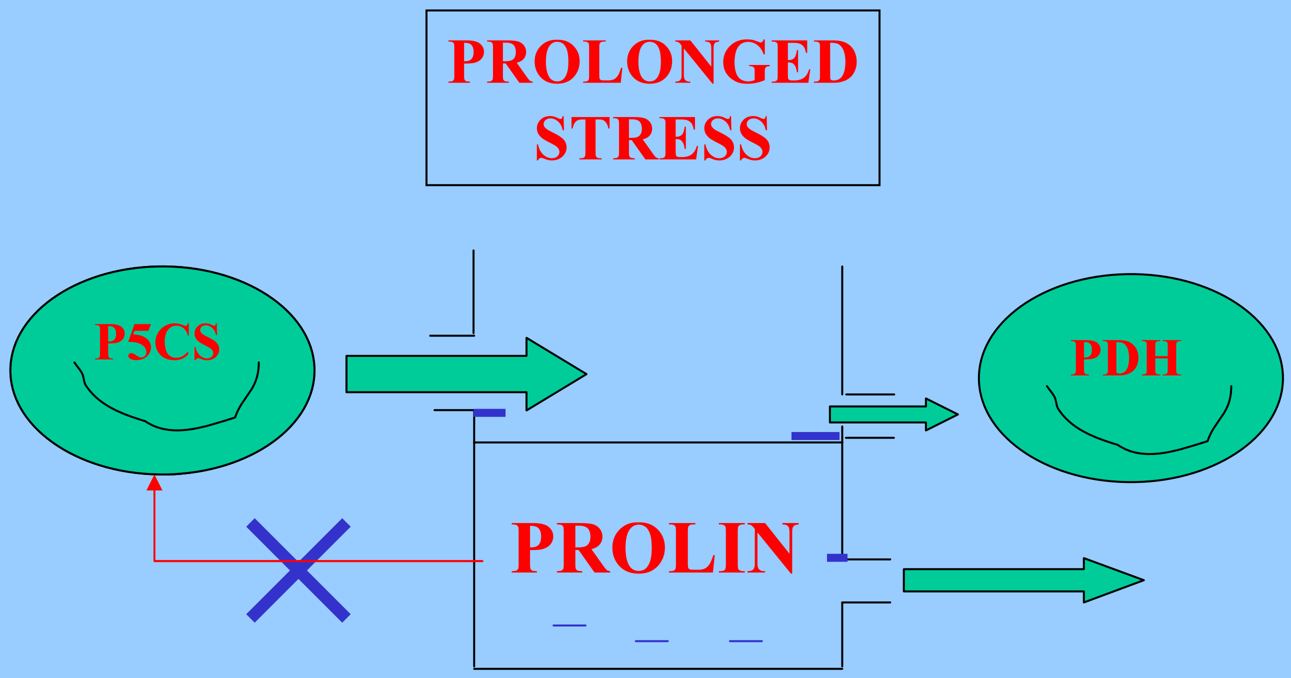
POTATO TRANSFORMATION WITH AtP5CS





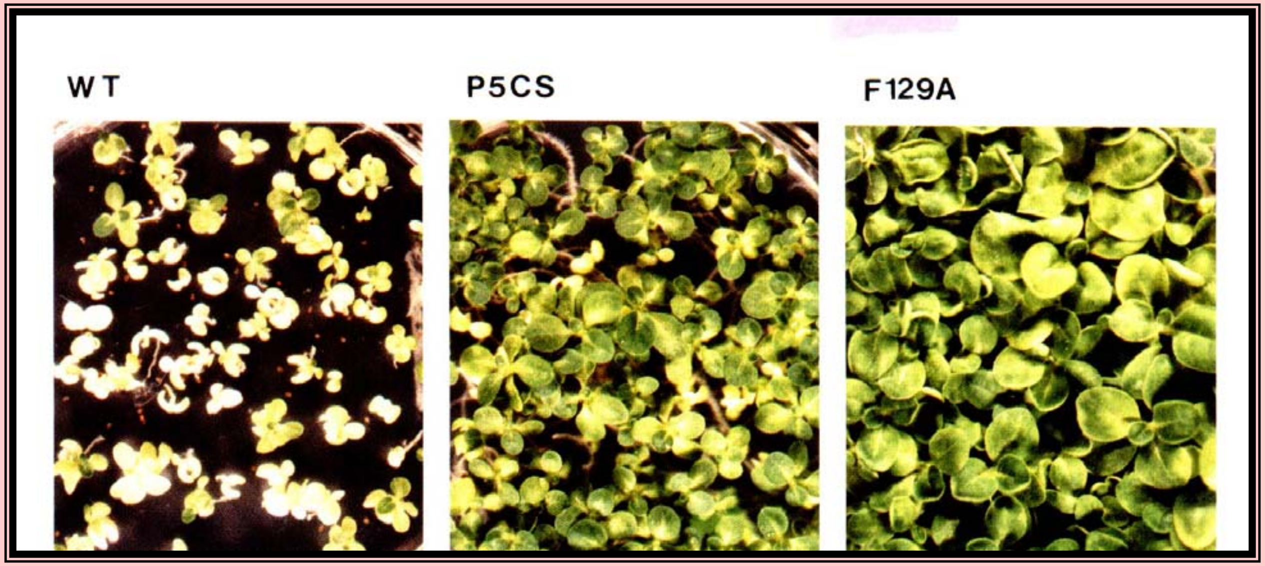
Simin Tansı, MSc. Thesis, 2002 METU, Biology

TR STRATEGY 2: Remove feed back inhibition on P5CS



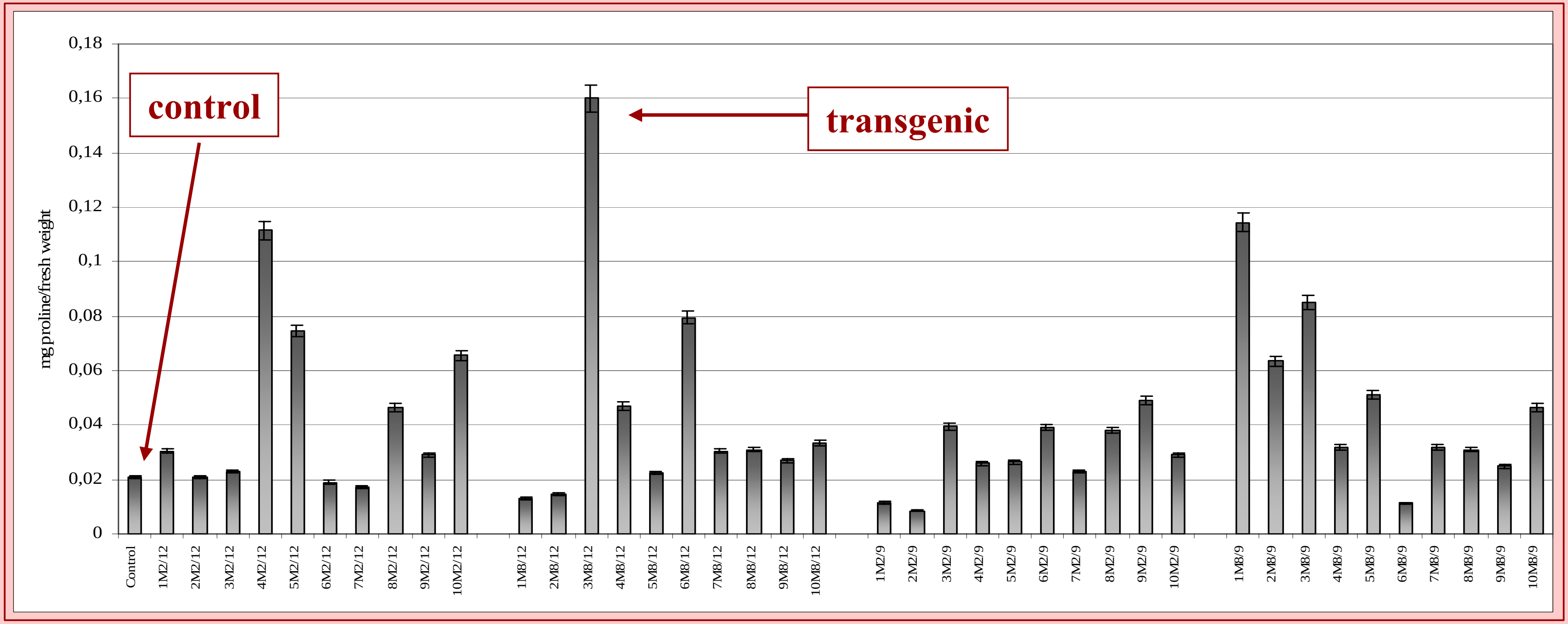
Hong, Z. et al. (2000) Removal of feedback inhibition of P5CS results in increased proline accumulation and protection of plants from osmotic stress. Plant Physiol. 122:1129-1136.

Eyidoğan F. et al. (2001) Increasing proline synthesis in tobacco by expressing the *Arabidopsis* feedback-insensitive P5CS gene. Manuscript in preperation.



Phenotype of 6 week old wild type (WT), P5CS overexpressing (P5CS) and feed back inhibition insensitive form of P5CS (F129A) transgenic seedlings as affected by salinity (200 mM NaCl). Seeds were germinated on MS medium containing 200 mM NaCl. The plates were kept in a controlled environment at 24 ^{o}C under constant light.

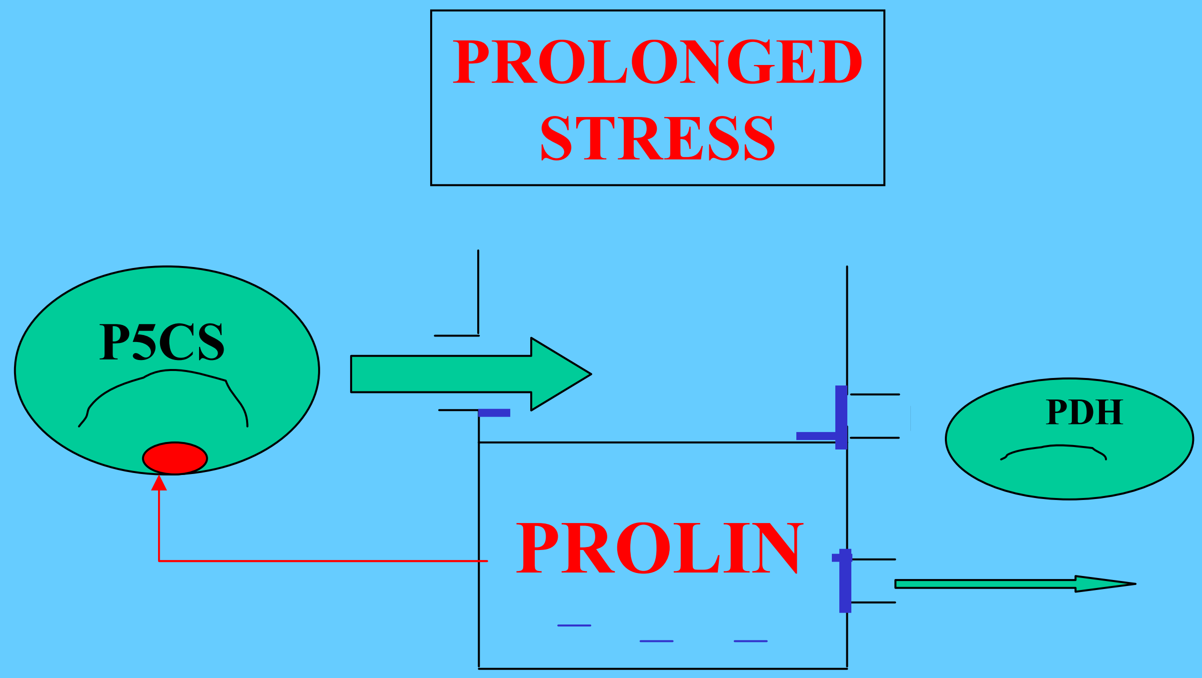
Hong, Z. et al. (2000) Plant Physiol. 122:1129-1136.



Proline contents of putative transgenic tobacco plants transformed with feedback-insentive AtP5CS gene.

Eyidoğan F. et al. (2001) Unpublished

STRATEGY 3: Reduce PDH level via antisense technology



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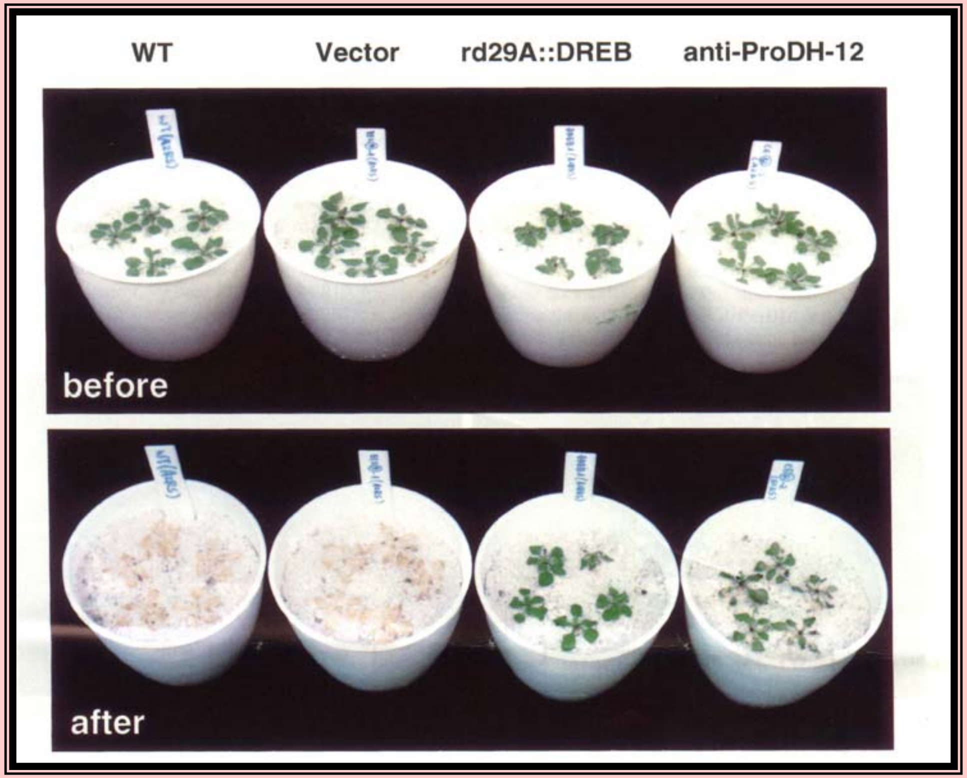
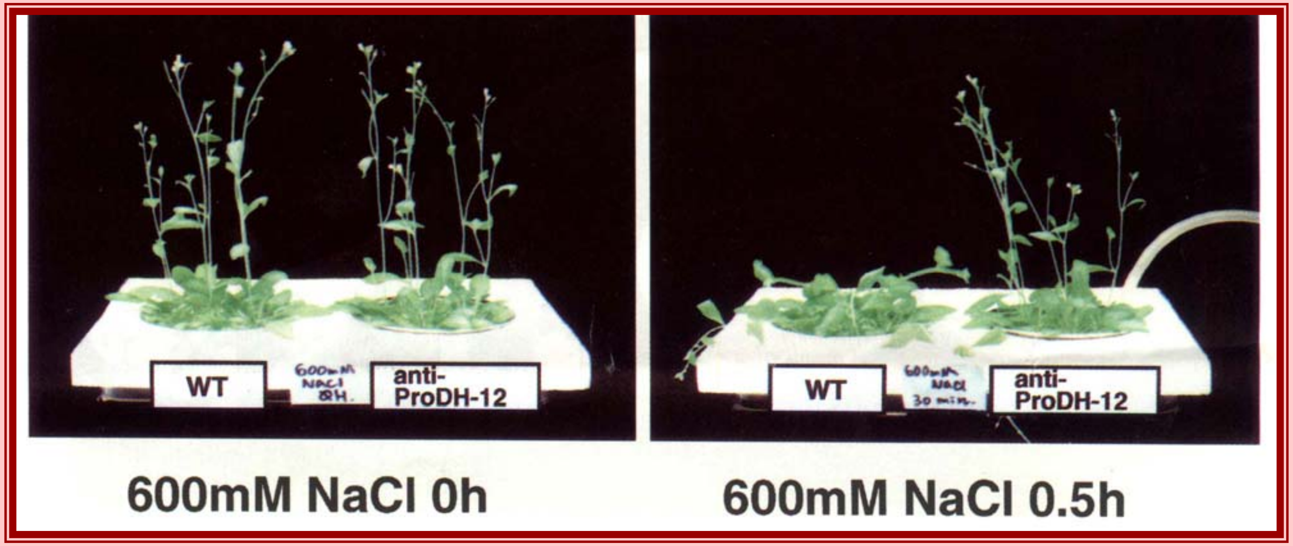
FEBS Letters 461:205-21.



Freezing tolerance of PDH antisense TR plants. Phenotypes of plants exposed to frezzing stress (-7 °C for 2 days). After stress treatment the plants were taken to 22 °C and grown for 5 days before photographed.

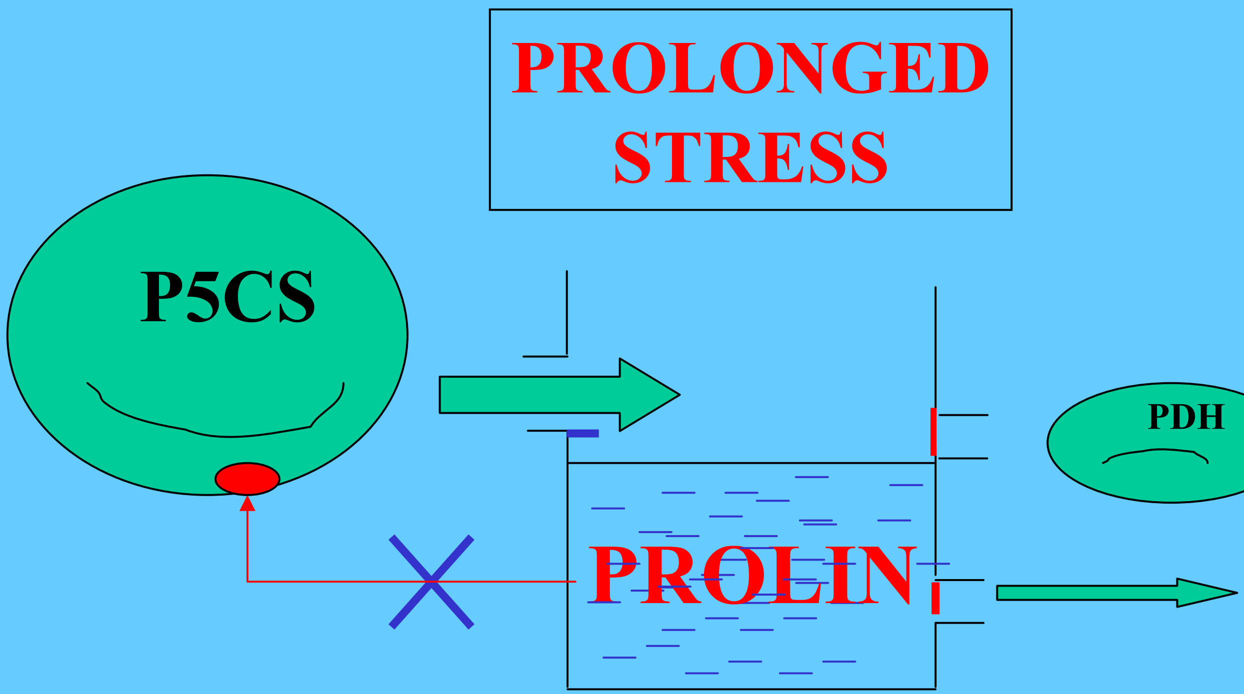
Nanjo, T. et al. (1999) FEBS Letters 461:205-21.



SALT tolerance of PDH antisense TR plants.

STRATEGY 4 : COMBINED

- Overexpress P5CS,
- Remove feed back inhibition on P5CS
- Reduce PDH levels via antisense technology



No available literature data yet

OSMOLYTE ENGINEERING FOR DEVELOPMENT OF STRESS RESISTANT TRANSGENIC PLANTS

GENE	GENE PRODUCT & FUNCTION
betA	Choline dehydrogenase - glycine betaine synthesis
codA	Choline oxidase - glycine betaine synthesis
IMT1	Myo-inositol O-methyl transferase – D-ononitol synthesis
mtlD	Mannitol-1-Phosphate dehydrogenase – mannitol synthesis
otsA	Trehalose-6-phosphate synthase – treahalose synthesis
otsB	Trehalose-6-phosphate phosphatase – treahalose synthesis
TPS1	Trehalose-6-phosphate synthase – treahalose synthesis
Odc	Ornithine decarboxylase – putrescine synthesis
SacB	Fructosyl transferase – fructan synthesis

GLYCINE-BETAINE

- Protect cells by maintaining an osmotic balance with environment
- Stabilising the quanternary structure of complex proteins

PLANTS

Choline → CHOLINE MONOOXYGENASE → Betain aldehyde → BETAIN ALDEHYDE DEHYDROGENASE → Glycine-betain

Choline: $(CH_3)_3{}^+NCH_2CH_2OH$

Betain aldehyde: $(CH_3)_3{}^+NCH_2CHO$

Arthrobacter globiformis

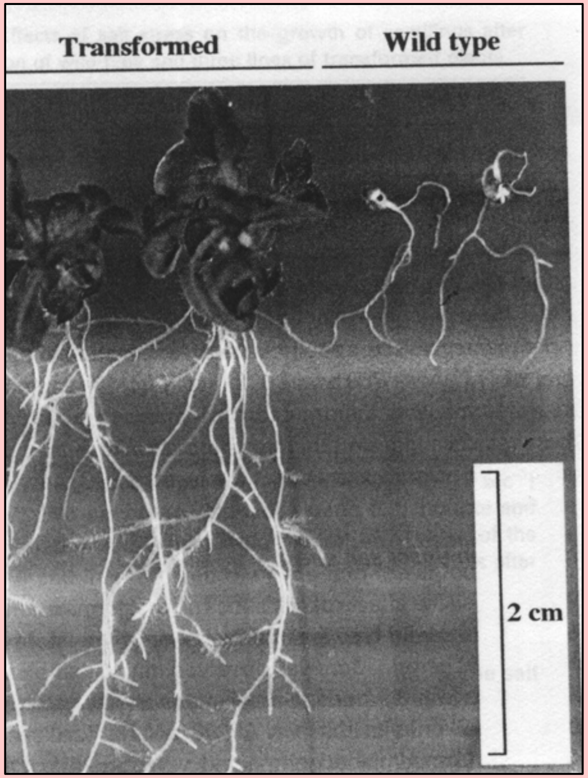
Choline → CHOLINE OXIDASE → Glycine-betain

codA gene

Glycine-betain: $(CH_3)_3{}^+NCH_2COO^-$

Arabidopsis Plants Transformed with *coda* Gene Coding for Choline Oxidase

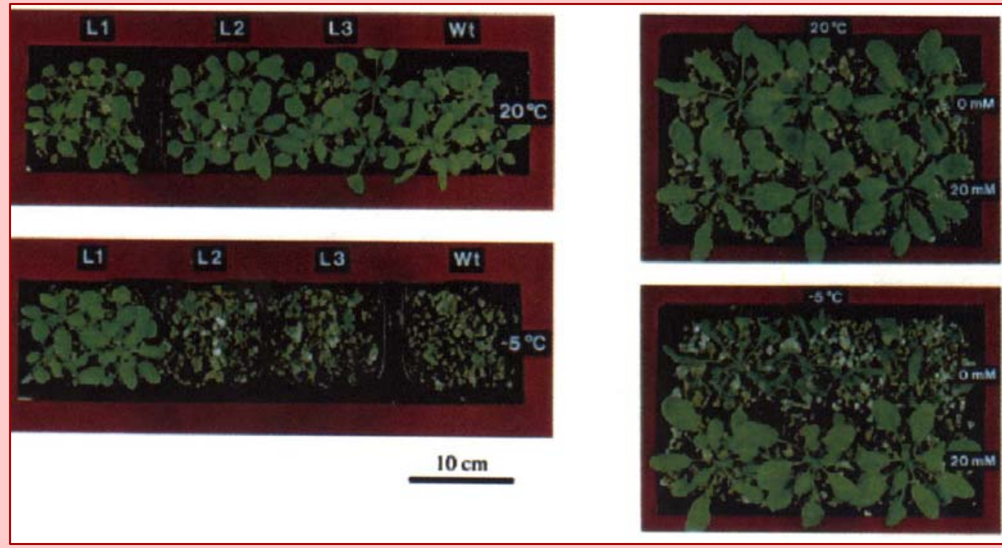
Hayashi et al. Plant J., 12:133-142, 1997

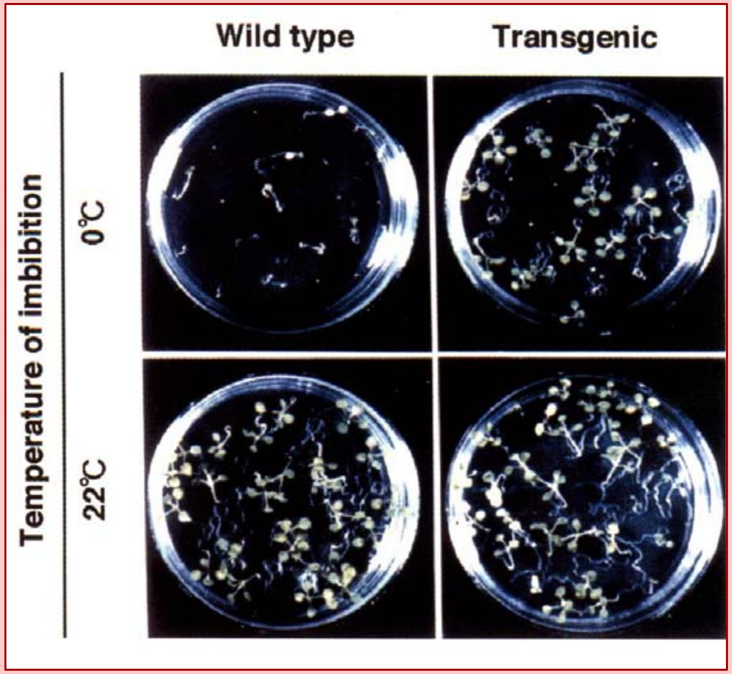


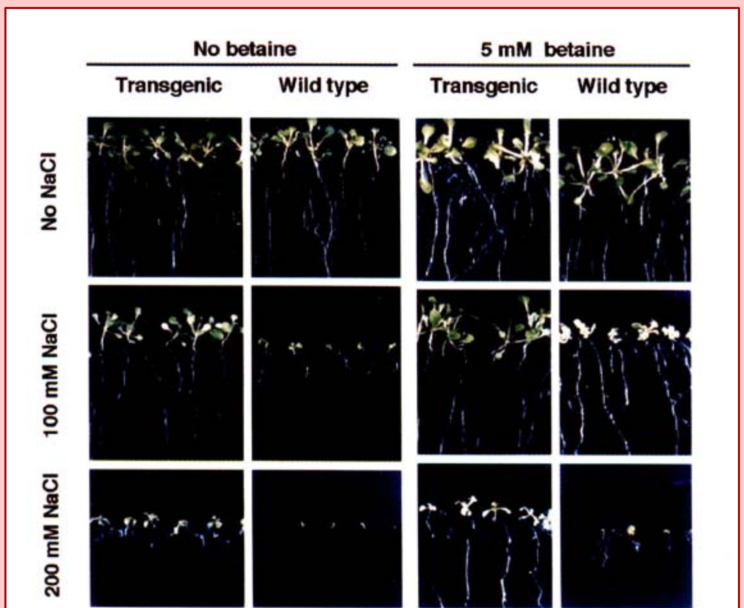
Effect of salt stress on the germination and growth of TR and WT *Arabidopsis* plants.
Seeds were germinated on MS+100mM NaCl.
After 2 days of stress, plants were grown under normal conditions for 9 days.

Low temperature induced visible dammage (arrows) to the leaves of TR and WT plants.
Treatment: 5^{o}C for 7 days.

Survival of wild-type and transgenic plants after freezing
Sakamoto A. et al. (2000) Plant J., 22: 449-453.







→ **Effect of cold and salt stress on *Arabidopsis* plants transformed with *CodA* gene.**
Sakamoto A. & Murata N. (2000)
J. Exp. Bot., 51:81-88.

TREHALOSE

- Non-reducing disacharide
- Occur in many organisms that survive complete dehydration
- Stabilizes dehydrated enzymes and lipid membranes
- In yeast trehalose synthase is responsible from synthesis
- Rare in higher plants

Glucose-6-P + UDP-glucose

↓ Trehalose-6-P-synthatase subunit (TPS1)

Trehalose-6-P

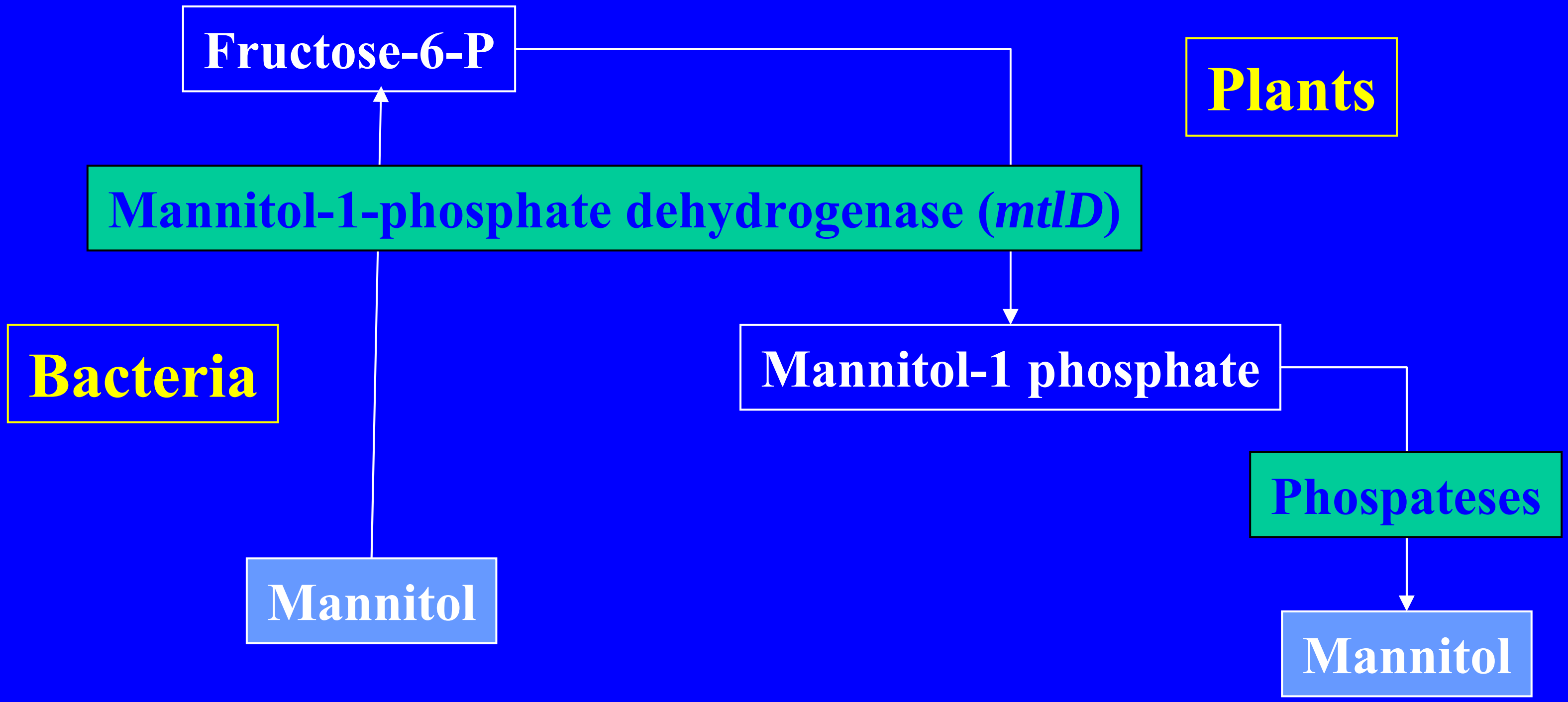
↓ Phospateses

Trehalose

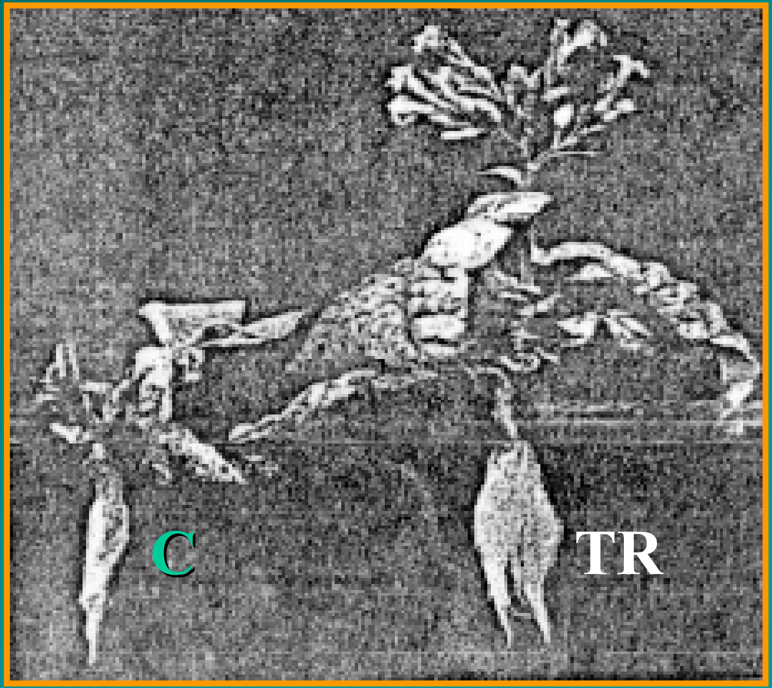
Drought response of TPS1 expressing transgenic (TR) and wild type (C) tobacco plants after 15 days of water stress. Romero C. et al. (1997) Planta, 201: 293-297.

MANNITOL

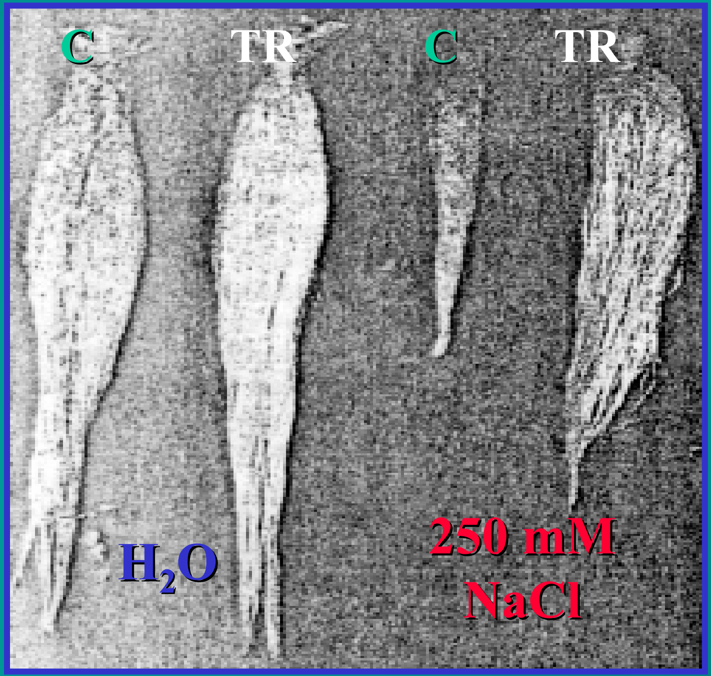
- Sugar alcohol,
- Naturally found in higher plants
- Act as an osmolyte



A



B



A: Response of Transgenic and control tobacco plants to 250 mM NaCl stress. B: Root morphology of the same plants after stress treatment.

Bohnert, H.J., Science,259, 508-510, 1993

mtlD Transformed Eggplant

- Medium with 10 % PEG (Drought)

- Medium with 10% NaCl (Salt)

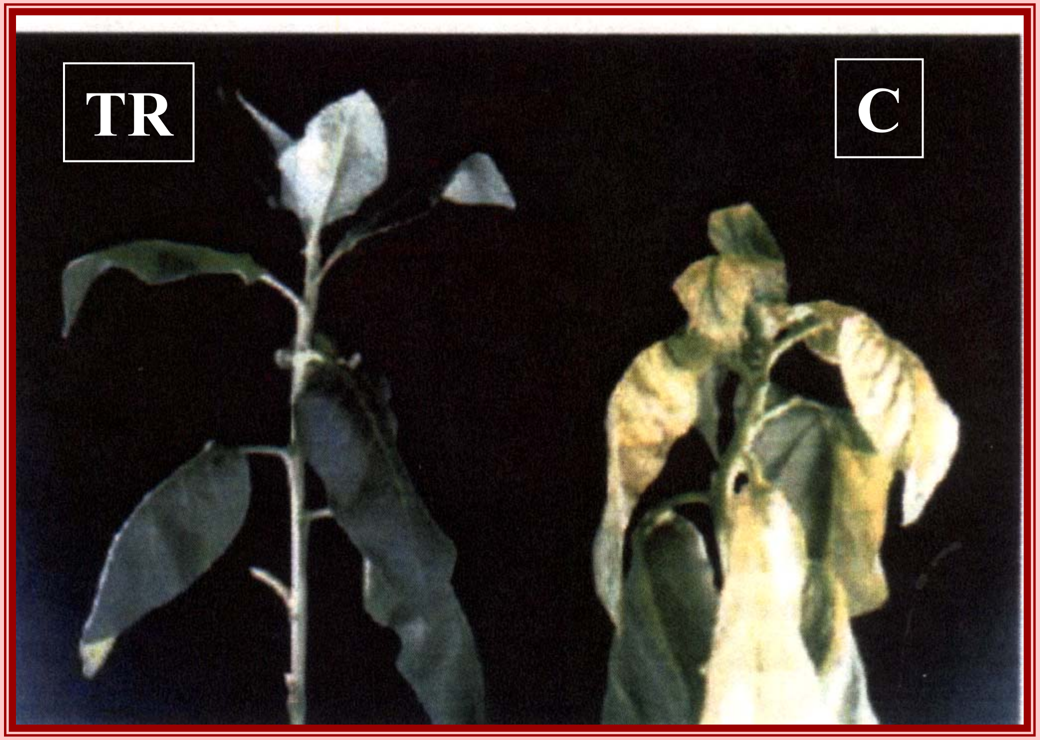
Yeast Invertase

↓

Accumulation of more sucrose and hexoses

↓

Improved salt tolerance



The plants treated with 300 mM NaCl for 122 hours

Fukishima et al. (2001) Plant Cell Physiol, 42: 245-249

3) Alternation in lipid membrane composition (1996-)

Membranes exist in two states:

- **Paracrystaline state**: At low temperature
  Lipids are tightly packed, little or no motion of acyl chains
- **Fluid state**: At a certain tempearture (characteristic for a given membrane-TRANSITION TEMPERATURE) acyl chains start rapid motion and memrane enters into a fluid state.

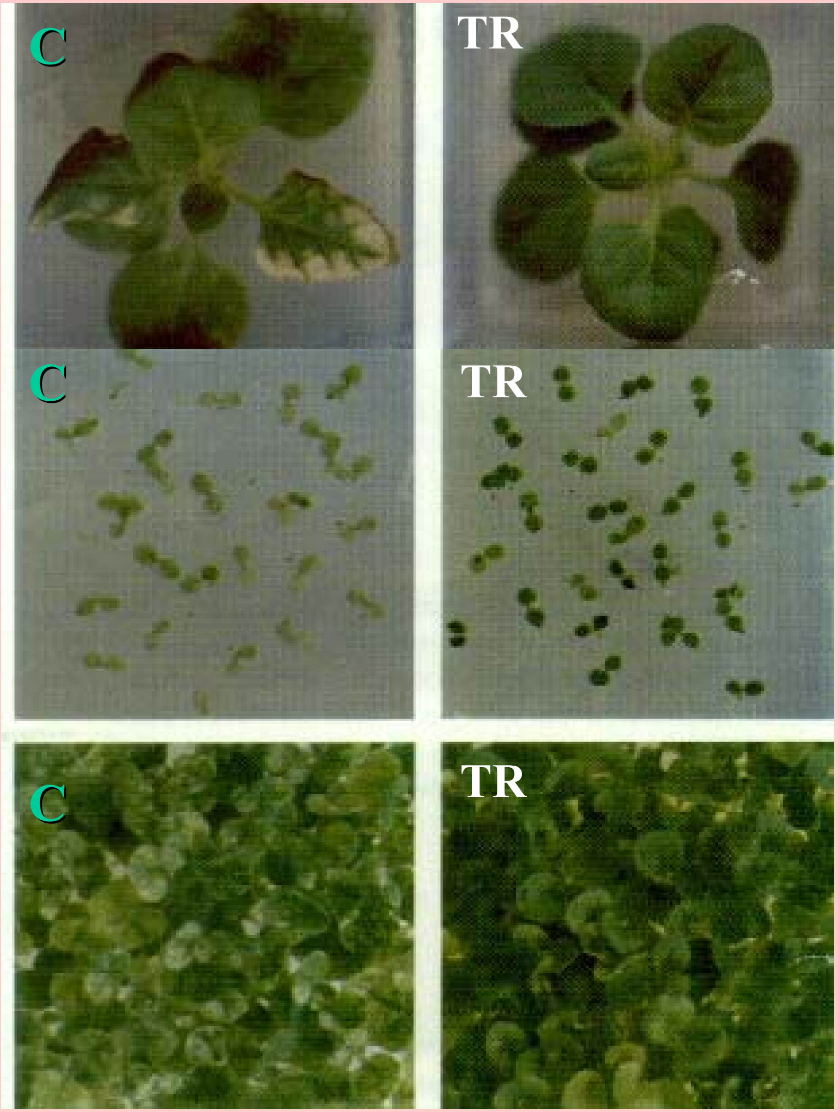
•Very low fludity at paracrystaline state. High fludity at fluid state.

•Membrane proteins are fuctional at given fludity of membs.

•So keeping membranes at certain fluid level is important for proper functioning of mebrane proteins thus the cellular activity and viability.

•Increase unsaturation level of fatty acids increase fludity of membranes at paracrystaline (low temperature) state. Therefore, maintaining high unsaturation level at low tempertures would enhance viability of plant cells.

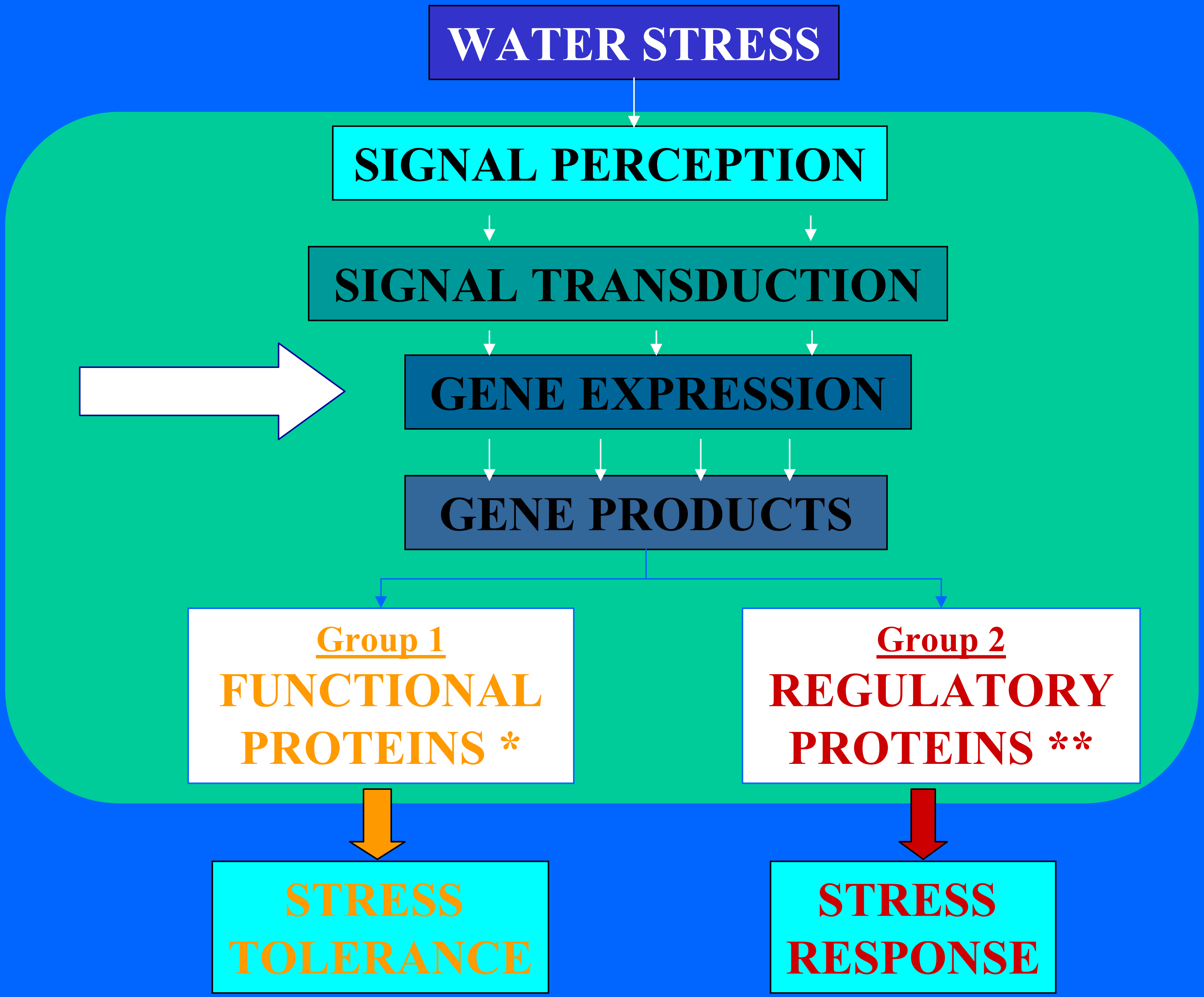
FROST RESISTANCE



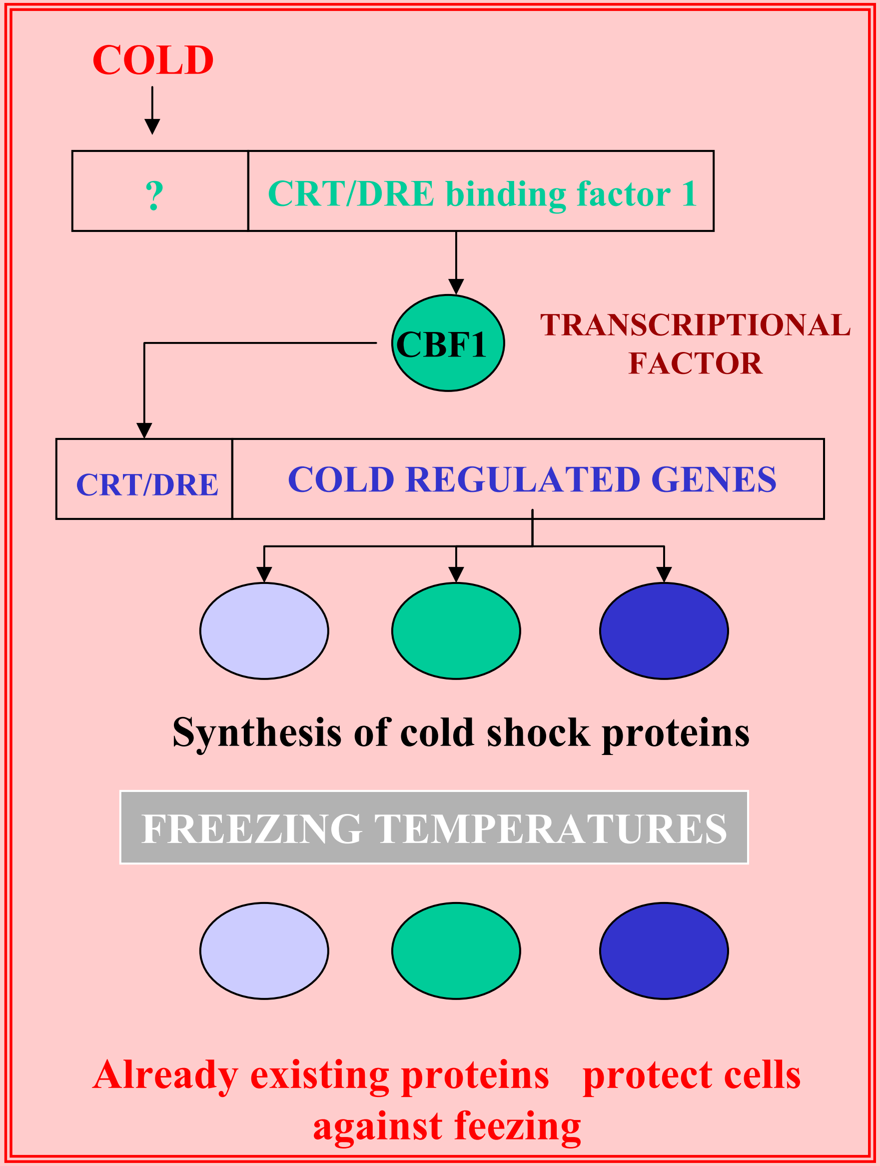
Performance of control and transgenic plants transformed with desaturase gene from *Cayanobacteria* .
After germination, seedlings were kept at 1^{o}C for 11 days.

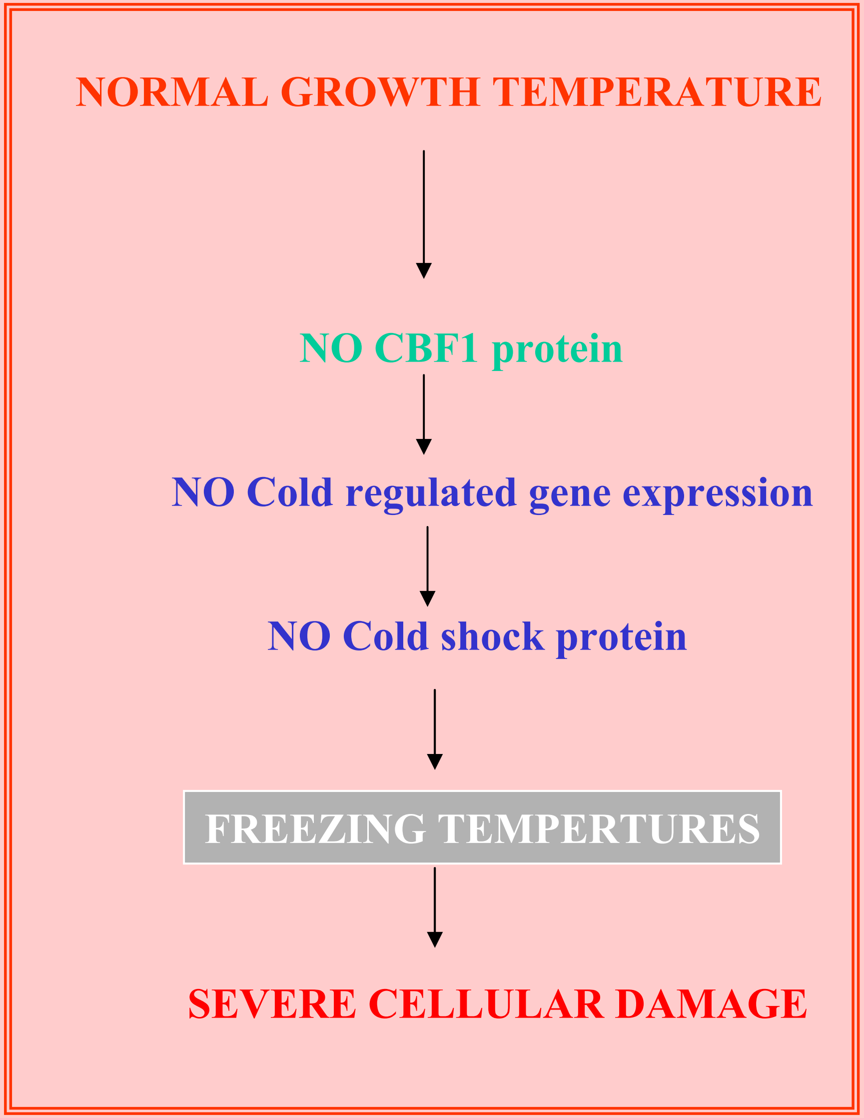
Ishizaki-N. *et al*., Nature Biotech, 14,1003-1006, 1996

4) Enhanced stress related gene expression via transfer of transcriptional factors (1998-) and regulatory proteins (2000-)


WATER STRESS
SIGNAL PERCEPTION
SIGNAL TRANSDUCTION
GENE EXPRESSION
GENE PRODUCTS
Group 1
FUNCTIONAL PROTEINS *
Group 2
REGULATORY PROTEINS **
STRESS TOLERANCE
STRESS RESPONSE

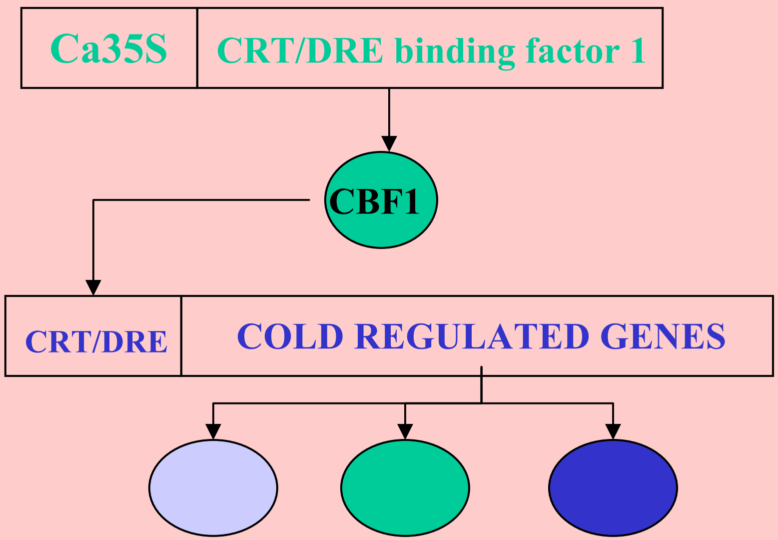
Cold Acclimation: Plants increase their tolerance to freezing in response to low non-freezing temperatures.





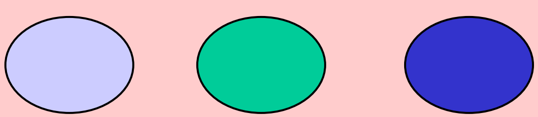
Arabidopsis CBF1 Overexpression Induces *COR* Genes & Enhances Freezing Tolerance

NORMAL GROWTH TEMPERATURES



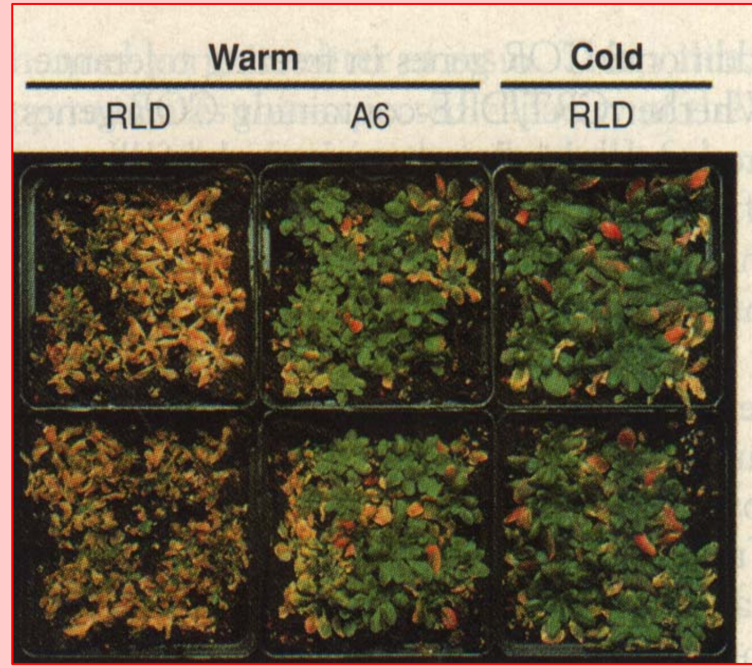
Synthesis of cold shock proteins without COLD ACCLIMATION

FREEZING TEMPERATURES



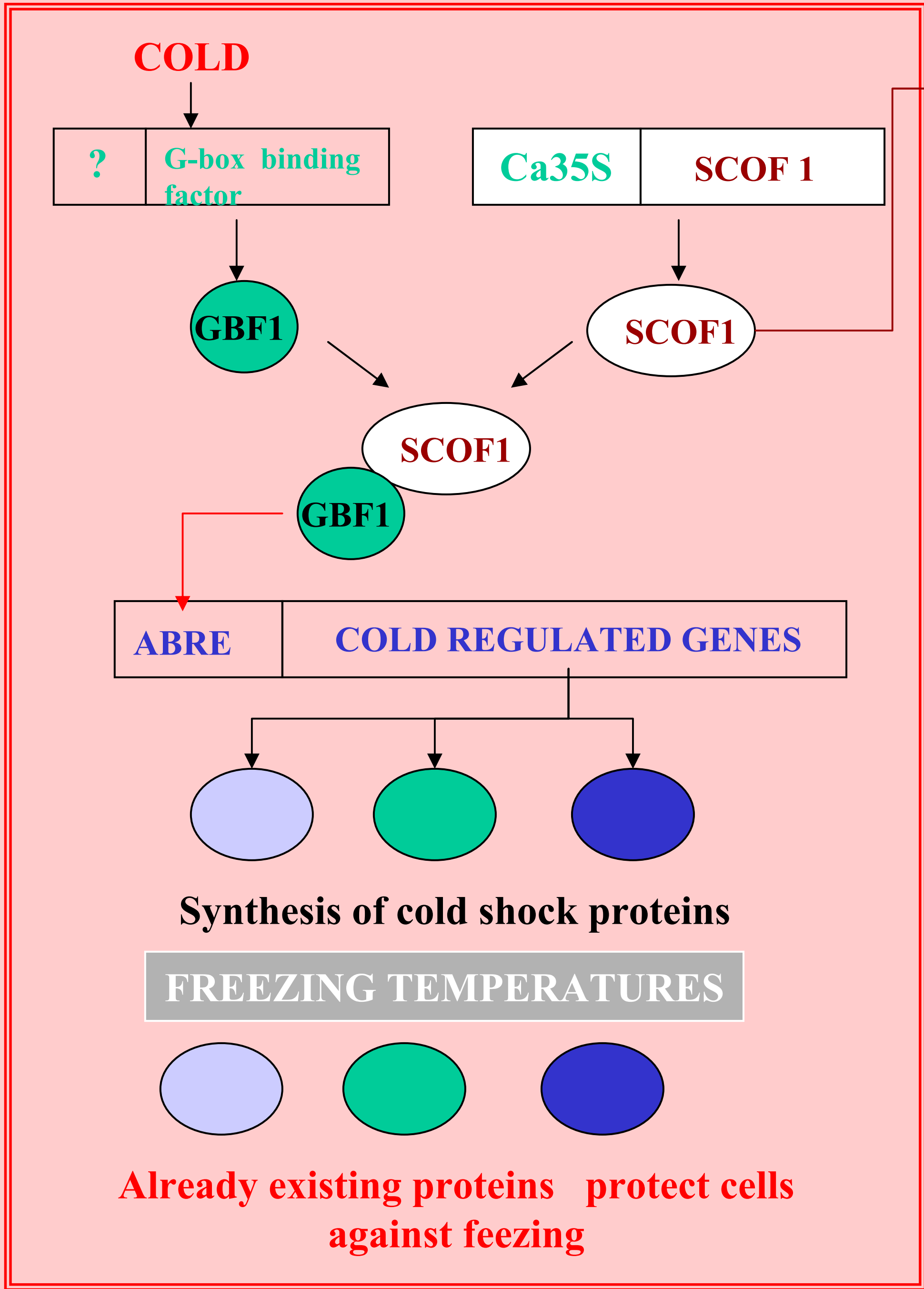
Already existing proteins protect cells against feezing

NON ACCLIMIZED ACCLIMIZED

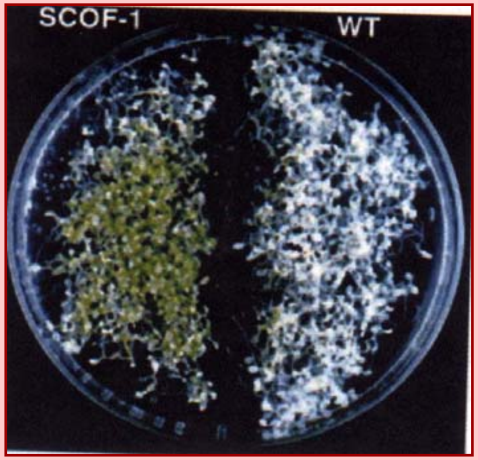


Treatment: Before treatment plants were kept at normal growth conditions (warm) or cold-acclimized for 5 days. Plants were frozen at -5°C for 2 days and then returned to a growth chamber at 22°C..
RLD: wild type plants
A6 : Transgenic plants

Jaglo-Ottosen et al. Science, 280,104-106, 1998



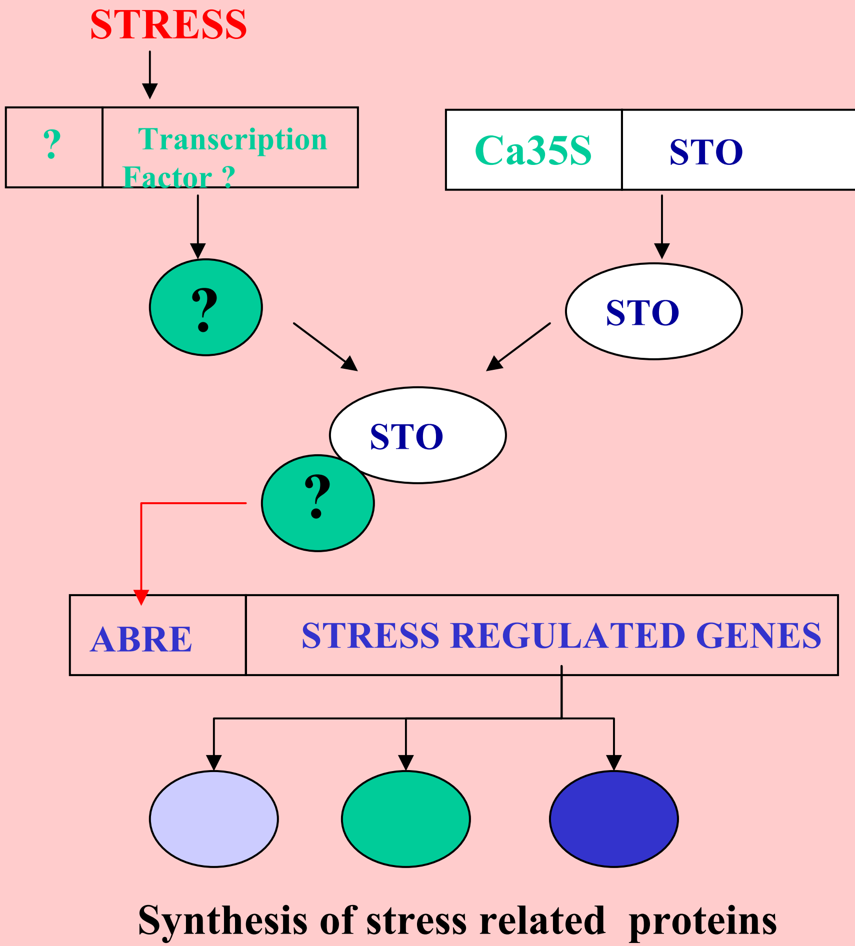
The cold and ABA inducible transcription factor SCOF1 increases COR gene expression by enhancing the DNA binding activity of GBFs (G-box binding factors).



Non-acclimated SCOF-1 TR plants and wild type plants were frozen at –7°C. Photo was taken 2 days after the return to normal growth conditions.

Control and TR plants subjected to 15 day long cold stress at 2°C and returned to normal growth temperature (25°C) and photographed after 20 days.

Kim J. et al. (2001) Plant J., 25: 247-259.



Protection of cells against stress condions

STO Transformed Tobacco

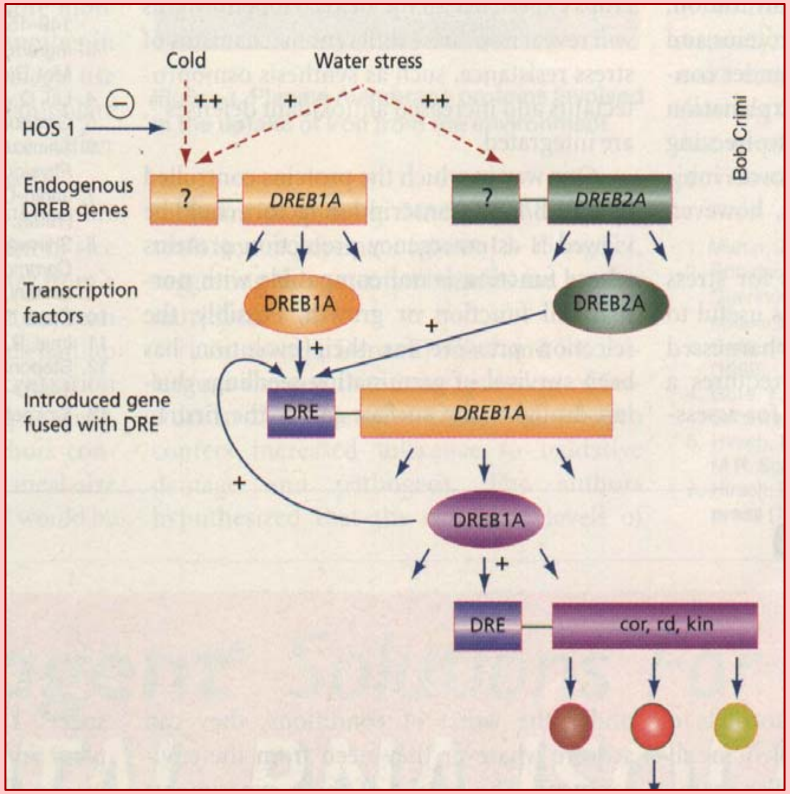
Control 200 mM NaCl Stress

Transgenic 200 mM NaCl Stress

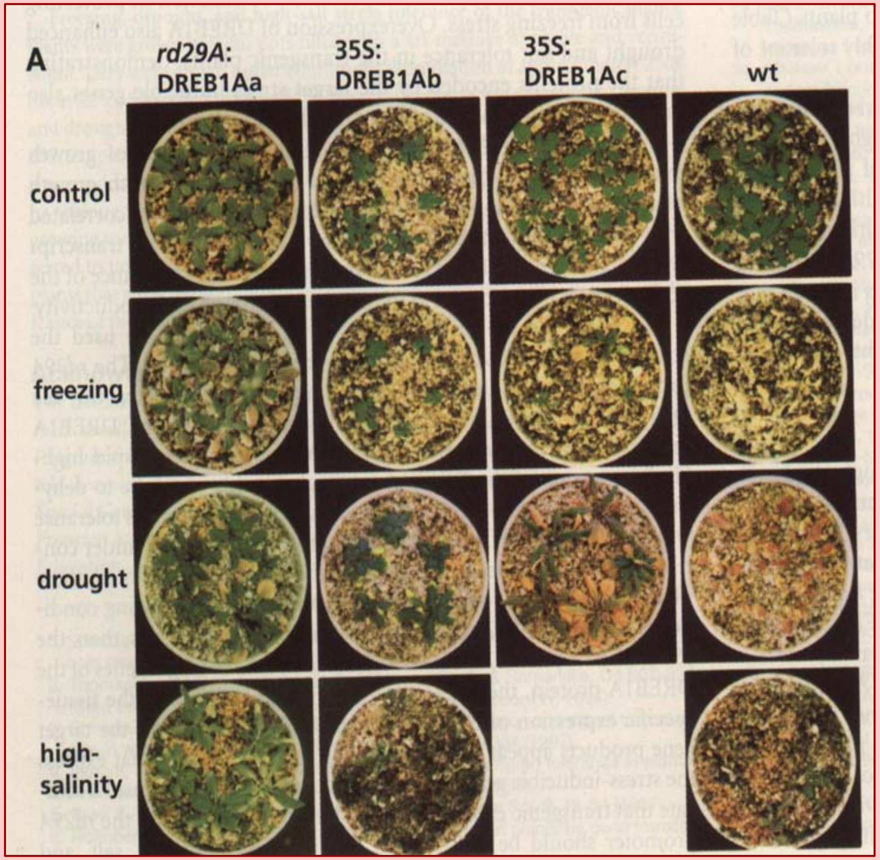
Feyza SELÇUK, PhD.Thesis, METU, Biology, 2003

Improving Plant Drought, Salt & Freezing Tolerance by Gene Transfer of a Single Stress Inducible Transcription Factor

STRATEGY



Kasuga et al., Nature Biotechnology, 17:287-291, March 1999.



FREEZING: -6°C for 2 days, 22°C for 5 days.
DROUGHT: Water withheld for 2 weeks.
SALINITY: Soaked in 600mM NaCl for 2 hours, normal growth under control conditions for 3 weeks.
CONTROL: 3 weeks old plants grown under control conditions.

ABRC1-CBF1 Transformed Tomato

Chilling Stress

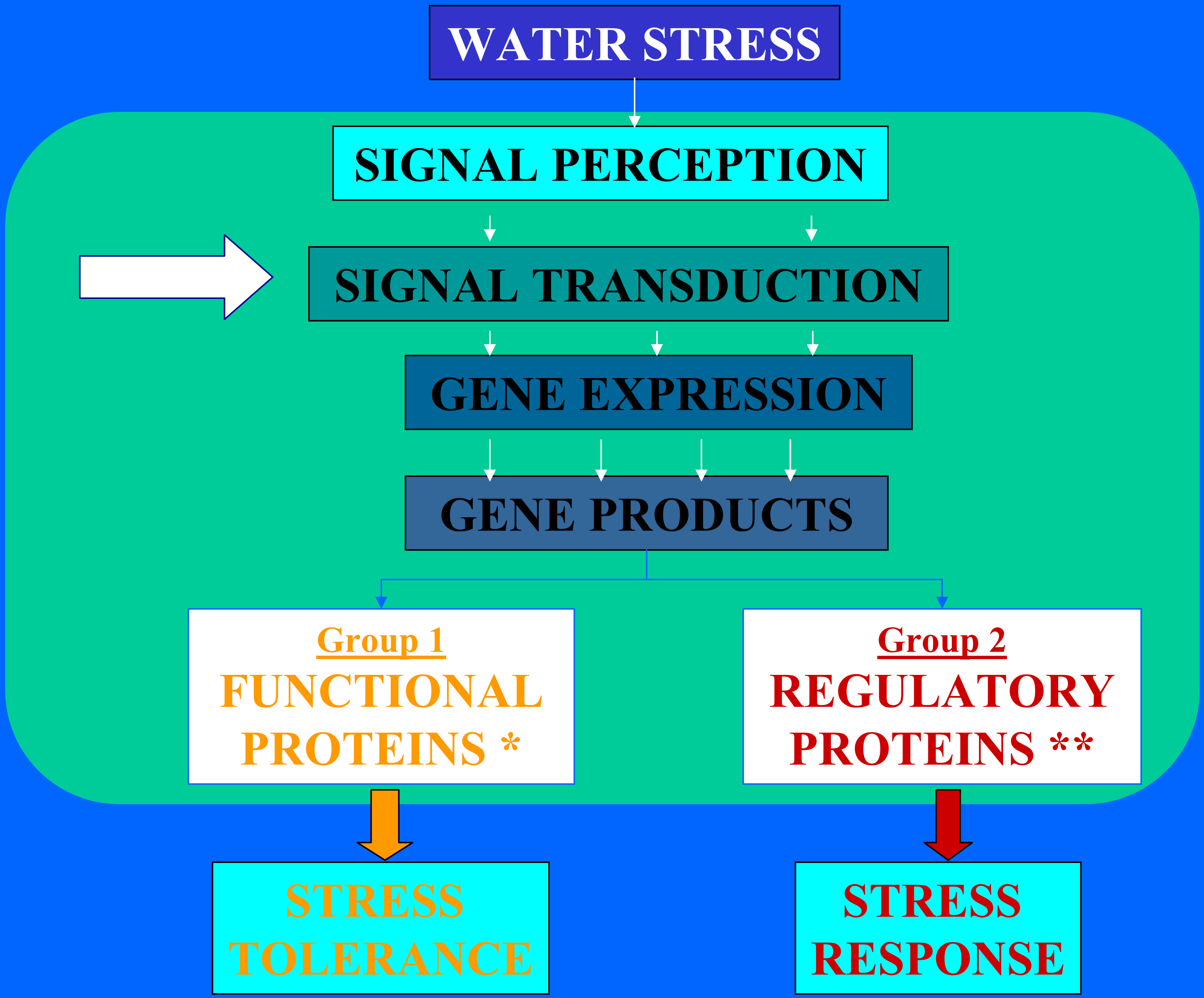
Drought Stress

Salt Stress

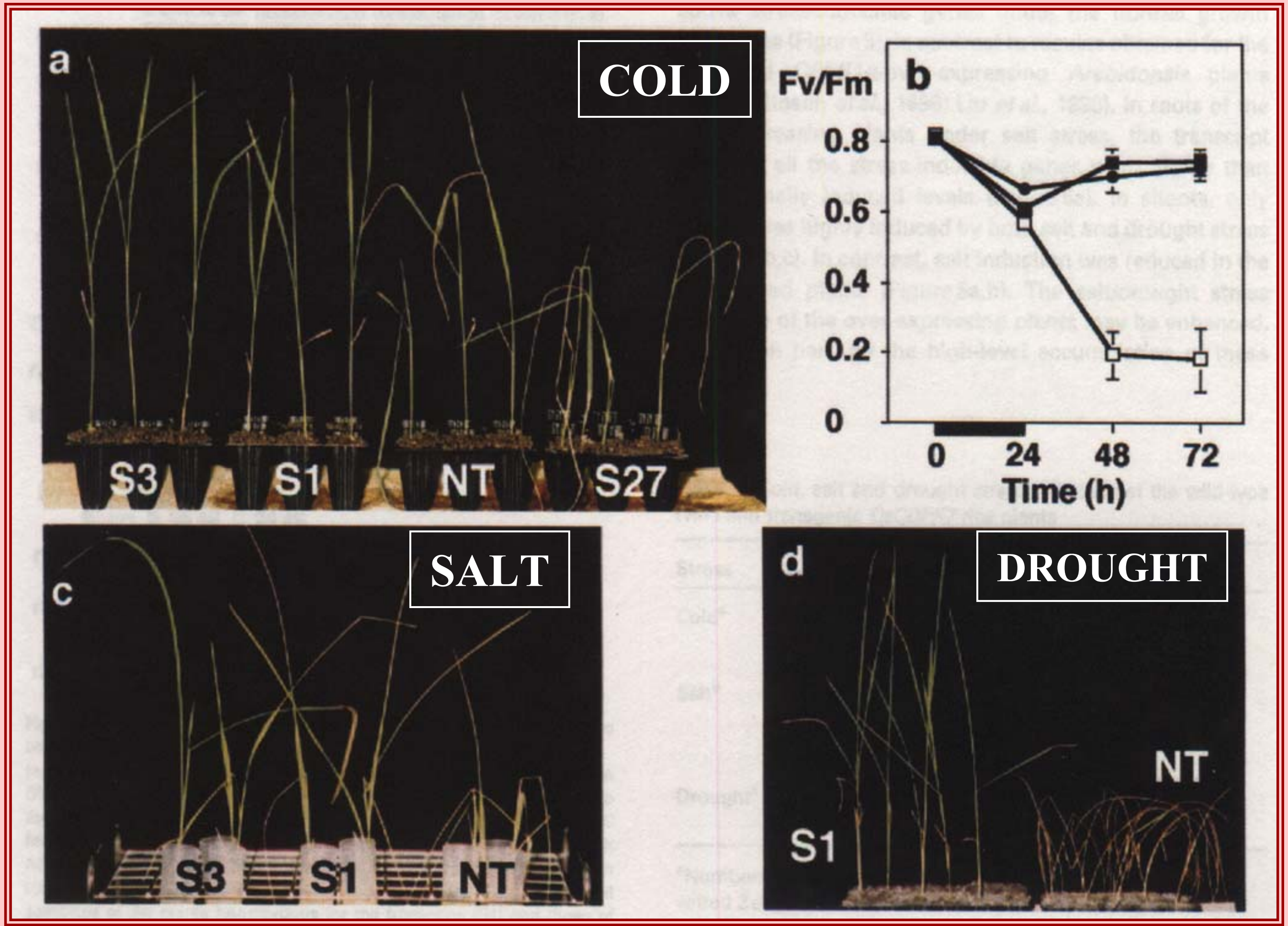
WT AC1 C5 C5+GA3

Improved agronomic performance of ABRC1-*CBF1* tomato plants. The yield of the transgenic tomato line (AC1) was equivalent to that of the untransformed plants. This condition in C5 plants (CaMV35S-*CBF1*) could be restored only after spraying the plants with GA3.

Lee J.-T. et al., Plant Cell Environment, 26:1181-1190, 2003


WATER STRESS
SIGNAL PERCEPTION
SIGNAL TRANSDUCTION
GENE EXPRESSION
GENE PRODUCTS
Group 1
FUNCTIONAL PROTEINS *
Group 2
REGULATORY PROTEINS **
STRESS TOLERANCE
STRESS RESPONSE

Cold-Salt/Drought Tolerant Transgenic Rice via Overexperssion of a Single Ca^{2+} Dependent Protein Kinase

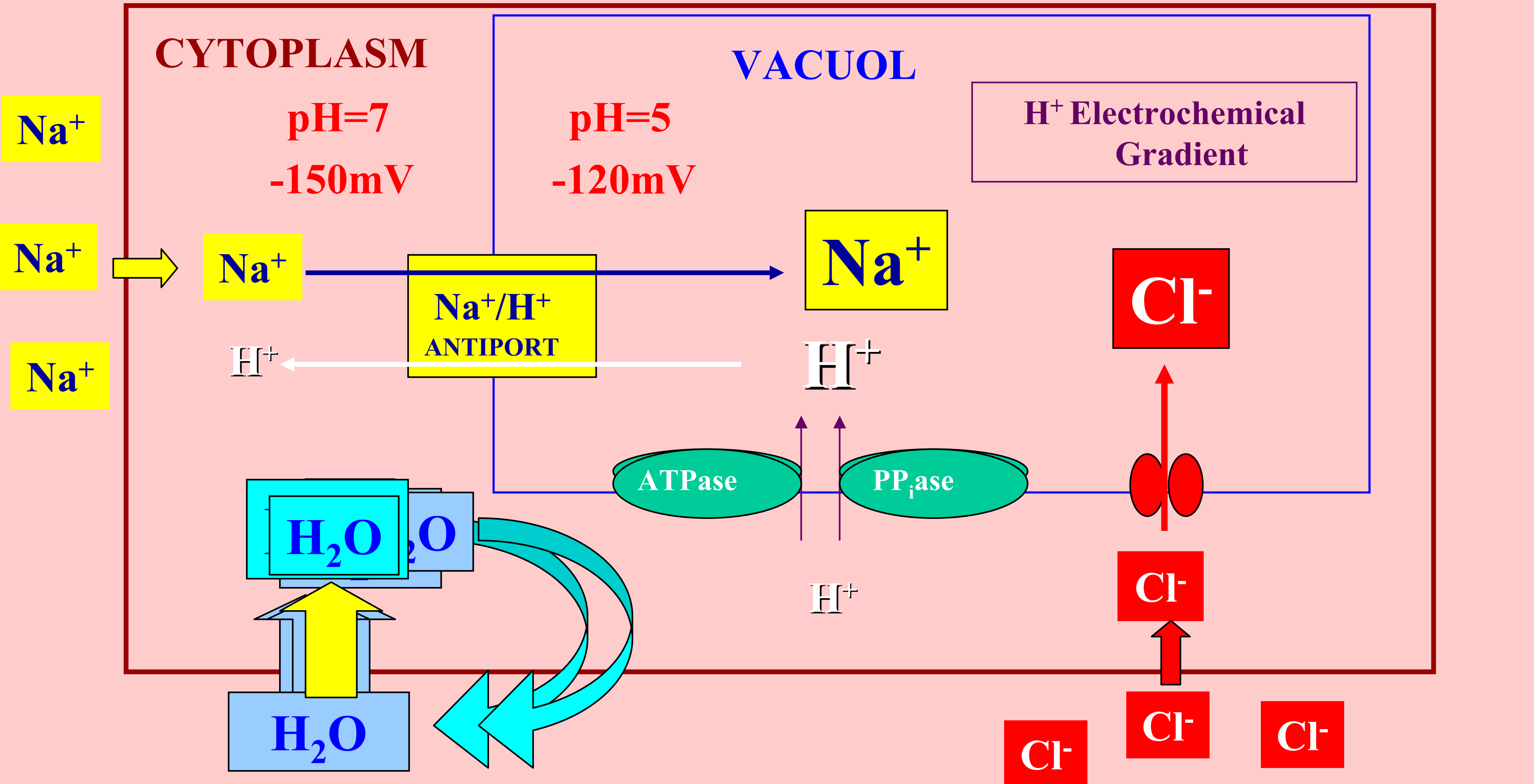


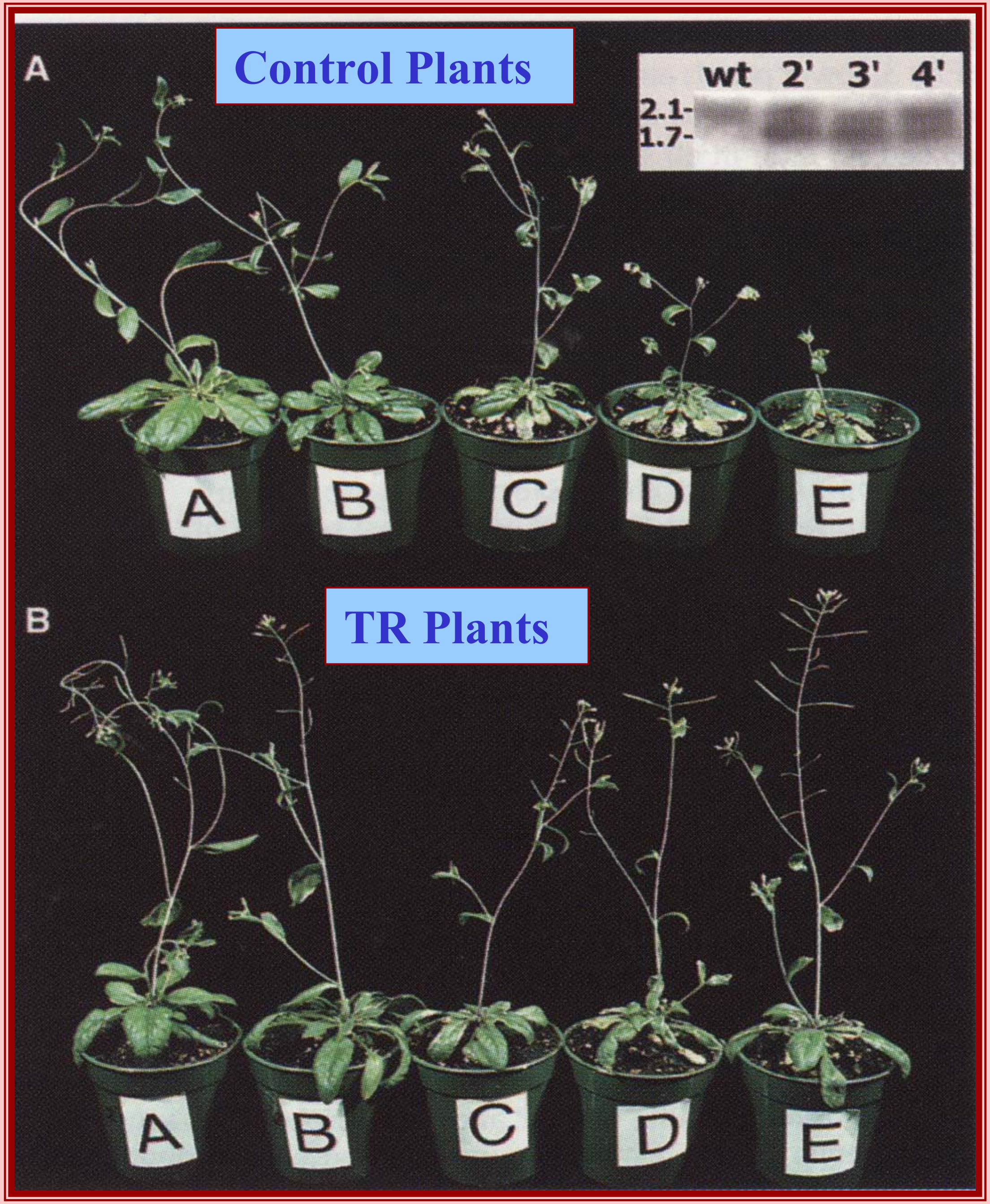
Stress tolerance of 35S:OsCDPK7 transgenic rice plants. (a) Plants 3 days after cold stress (4oC for 24 hr). (b) Chlorophyl fluorescence of young extended leaves under cold stress. Note dammage to photosynthesis in control (open rectangle) plants. (c) Plants 3 days after salt stress (200 mM NaCl for 24 hr). (d) Plants 5 days after drought stress (no water for 3 d). NT: non transgenic. Saijo Y. *et al*. The Plant Journal, 23, 319-327, 2000.

5) Enhanced ion compartmentalisation via Na+/H+ antiport overexpression (1999-)

COMPARTMENTATION OF Na^+ INTO VACUOL

The detrimental effects of salt on plants are a consequence of both a water deficit resulting in osmotic stress and the effects of excess sodium ions on critical biochemical processes.
In salt tolerant plants the compartmentation of Na^+ into vacuoles through the operation of a vacoular Na^+/H^+ antiport, provides an efficient mechanism to avert the deleterious effects of sodium in the cytosol and maintains osmotic balance by using Na^+ (and chloride) accumulated in the vacoule to drive water into the cells.





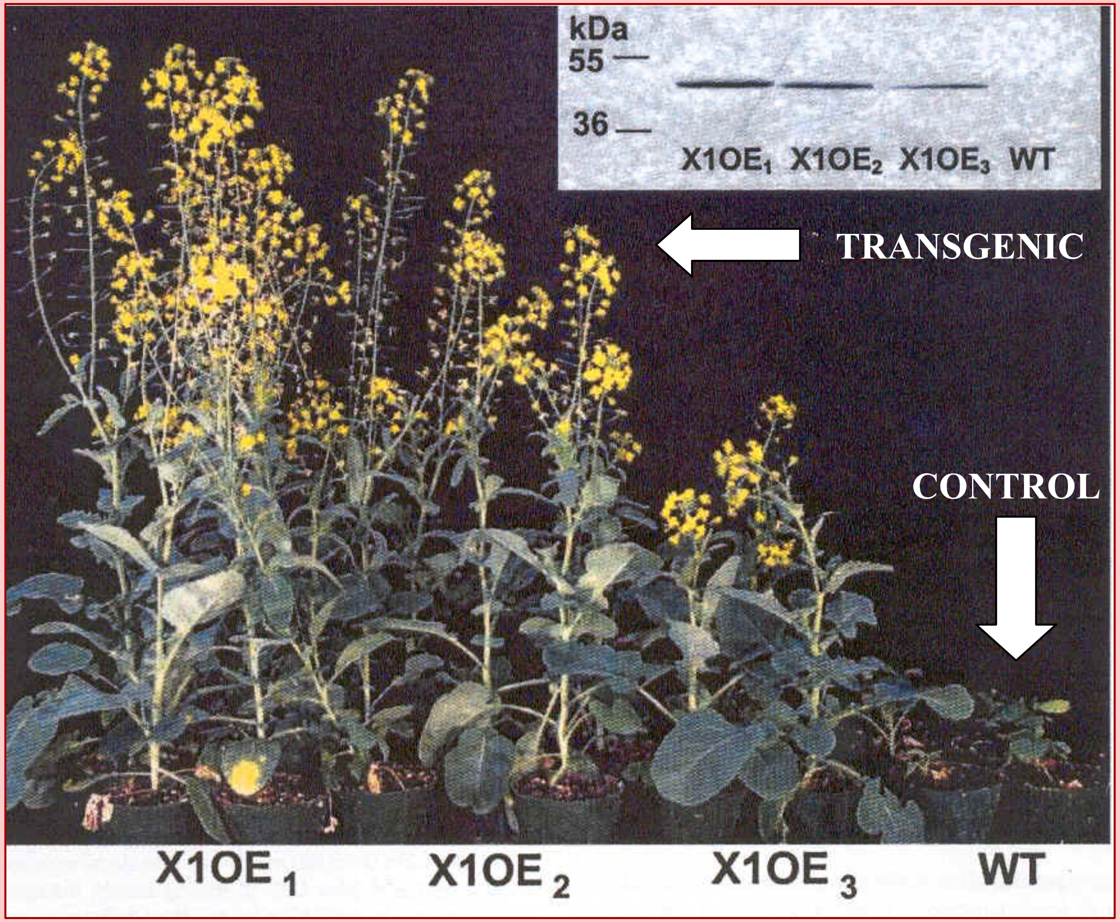
16 days old plants

Treatments

- A-Control (No NaCl)
- B-50 mM NaCl
- C-100 mM NaCl
- D- 150 mM NaCl
- E- 200 mM NaCl

Salt tolerance Conferred by Overexpression of a Vacoular Na^+/H^+ Antiport in *Arabidopsis*.

Apse et al, Science, 285:1256-1258, 1999



Salt tolerance of wild type (WT) and transgenic *Brassica* plants grown for 10 weeks under 200 mM NaCl stress.

Zhang H-X et al. (October 2001) PNAS, 98: 12832-12836

6) Protection against toxic by-products via expressing detoxification enzymes (2000-)

DETOXIFICATION OF METHYLGLYOXAL

GLYOXALASE I

MeCOCHO + GSH ⟷ MeCOC(OH)-SG → MeCH(OH)CO-SG

Methylglyoxal — Reduced Glutathion — Hemithioacetal — S-D-Lactoylglutathion

Methylglyoxal ↓

Potent cytotoxic compound.

- Arrest cell growth
- React with DNA and proteins.
- ↑ Sister chromatid exchange

Retardation of salt stress prompted senescence in TR tobacco plants by over-expressing *Gly 1* in sense.

Methylglyoxal-prompted senescence in TR tobacco plants by over-expression (NtBIS1-11) or downregulated (NtBIA-14) expression of *Gly 1*.

Venna & Sopory (1999), Plant J., 17:385-395)

STRESS

↓

ENHANCED FREE RADICAL GENERATION

↓

INTERACTION WITH MEMBRANE LIPIDS

↓

GENERATION OF PEROXIDES & HYDROXYPEROXIDES

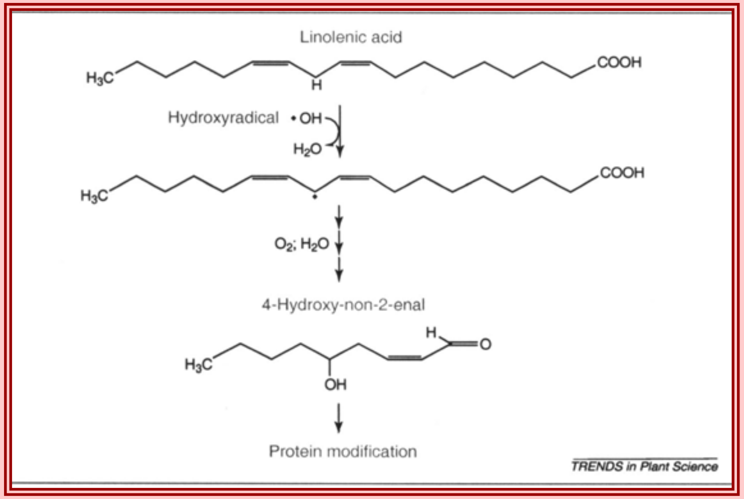
↓

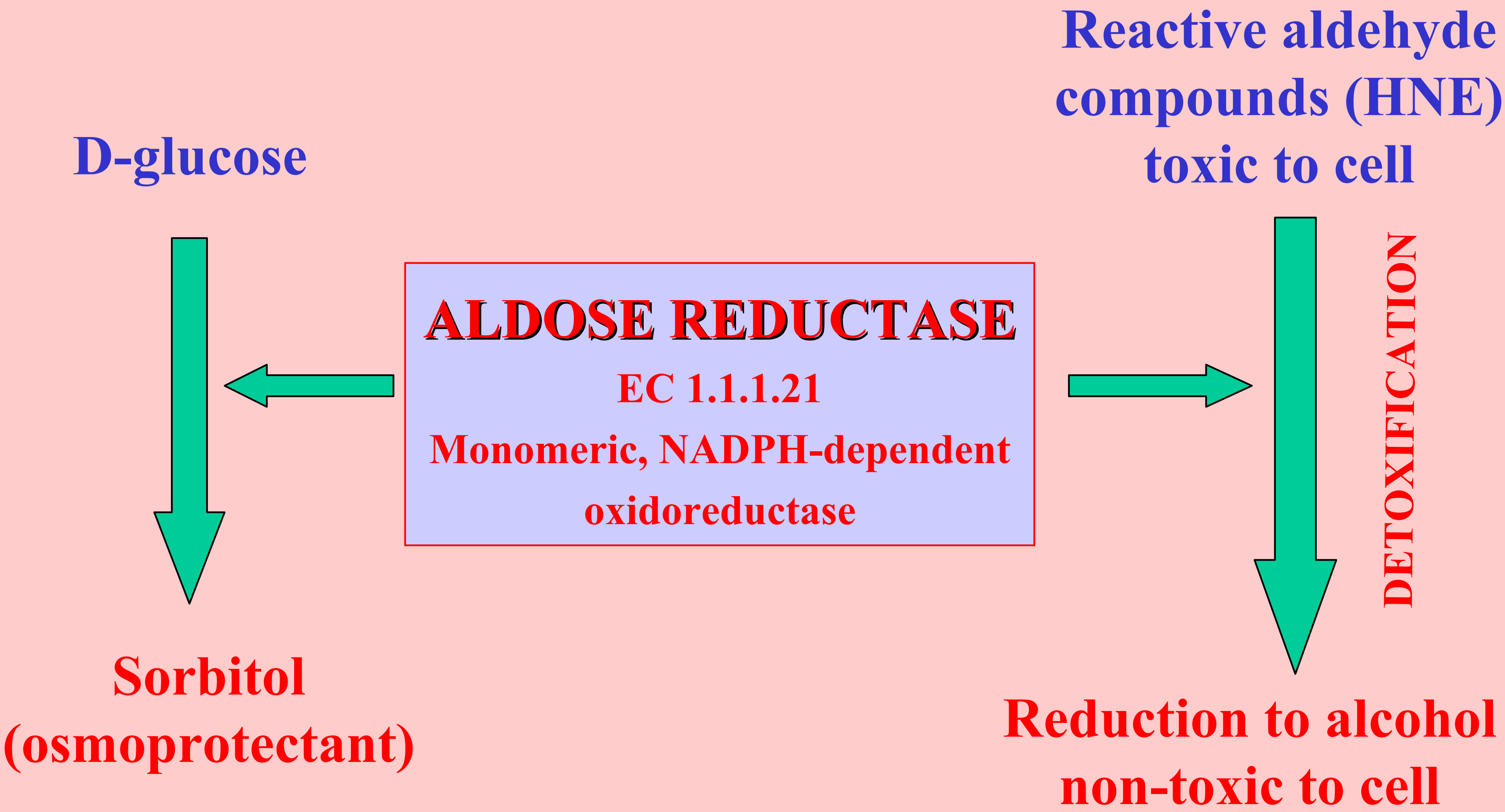
DEGRADATION AND FORMATION OF REACTIVE ALDEHYDE COMPOUNDS

↓

4-HYDROXY-NONENAL (HNE)

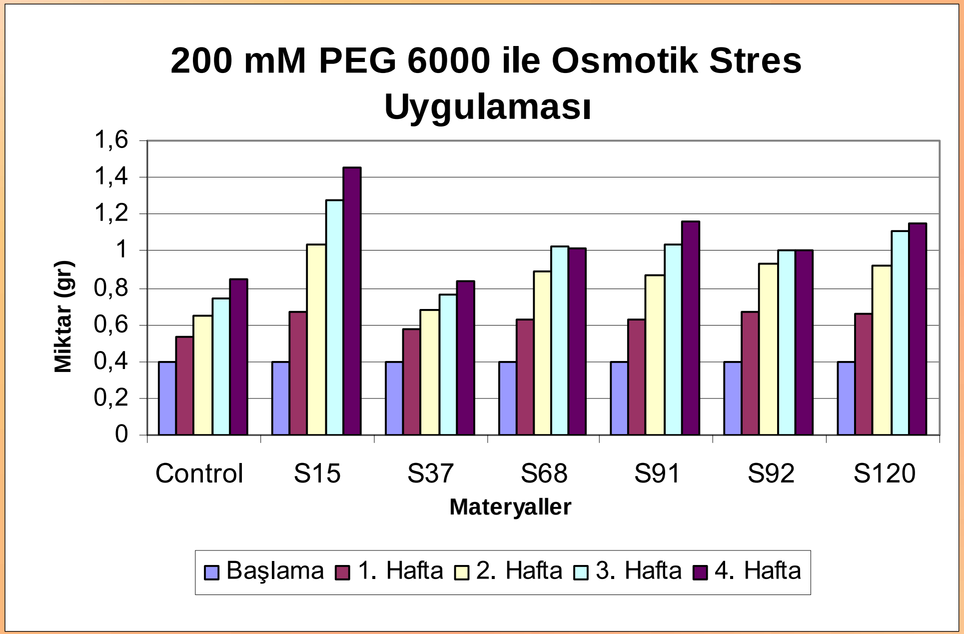
- more stable than free radicals
- migrate away from site of generation
- alter functional and structural properties of proteins
- DNA dammage at nM concentration
- Cytotoxic at mM concentration




D-glucose
Sorbitol
(osmoprotectant)
ALDOSE REDUCTASE
EC 1.1.1.21
Monomeric, NADPH-dependent
oxidoreductase
Reactive aldehyde
compounds (HNE)
toxic to cell
DETOXIFICATION
Reduction to alcohol
non-toxic to cell

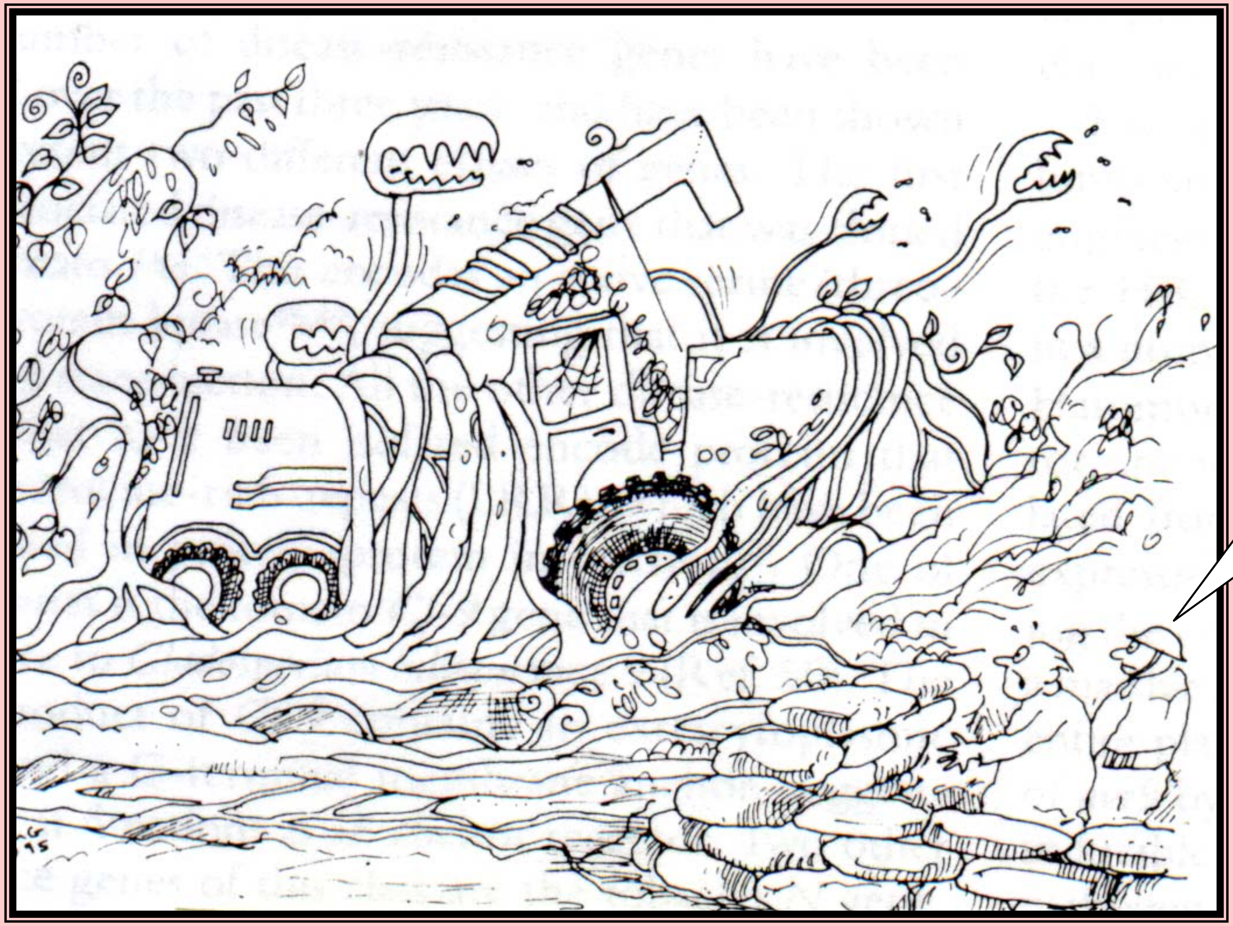
TRANSGENIC WHEAT CALLUS TRANSFORMED WITH ALDOSE REDUCTASE

Polyethylene Glycol
Treatment
(immitation of drought stress)
4 weeks post treatment



Setenci F. et al. (2001) Unpubished

REGENERATED TRANSGENIC PLANTS



Introducing the RAMBO gene for <u>total</u> resistance may have been a mistake….

Prof. Dr. Meral YÜCEL
Prof. Dr. Hüseyin Avni ÖKTEM
Dr. Füsun İNCİ EYİDOĞAN
Dr. Fahriye ERTUĞRUL
Mikail AKBULUT
Serpil APAYDIN
İrem KARAMOLLAOĞLU
Feyza SELÇUK
Çağla ALTUN
Ufuk ÇELİKKOL
İpek DURUSU
Ebru BANDEOĞLU
Simin TANSI
Ebru KARABAL
Hamdi KAMÇI
Özgür ÇAKICI
Beray GENÇSOY
Elif BOYACI GENÇ
Tarek EL-BASHITI
Betül DEÇENİ
Didem DEMİRBAŞ
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