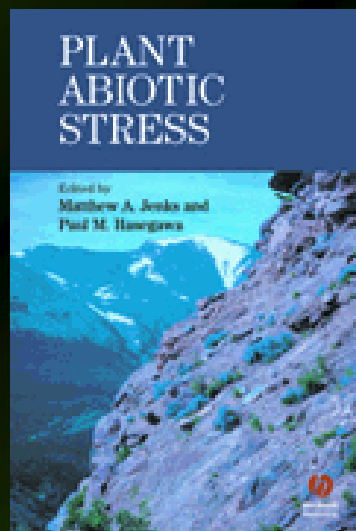


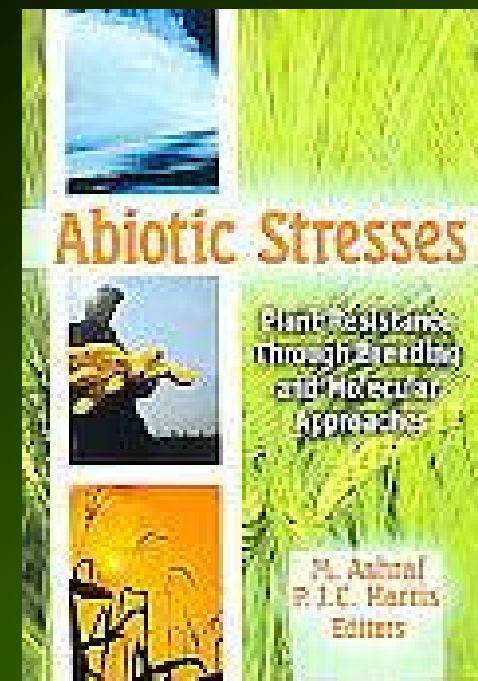
## 8) Plant responses to abiotic stress

- a) Water deficit
- b) Salt stress and osmotic adjustment and its role in tolerance to drought and salt stress
- c) Genes induced by water stress
- d) Cold stress

Jenks M *et al.* (2005)  
Plant Abiotic Stress.  
Blackwell Publishing



Ashraf M *et al.* (2005)  
Abiotic Stresses. The  
Haworth Press Inc.



## Stress factors affecting plants:

**Stress** = external factor, which makes unfavorable influence on plant

**Biotic** – triggered by other organisms

**Abiotic** – originating by influence of excess or deficit of physical or chemical factors

- water excess or drought
- high or low temperature
- much or little of light
- salinity

**Fast effects (minutes): thermal stress**

**Slow effects (day or months) : water deficiency in soil, deficiency of minerals**

**Understanding of mechanisms of adaptation, acclimatization and physiological processes triggering at the stresses is important in agriculture and ecology.**

**Tolerance to stress** - ability of plant to overcome unfavorable conditions

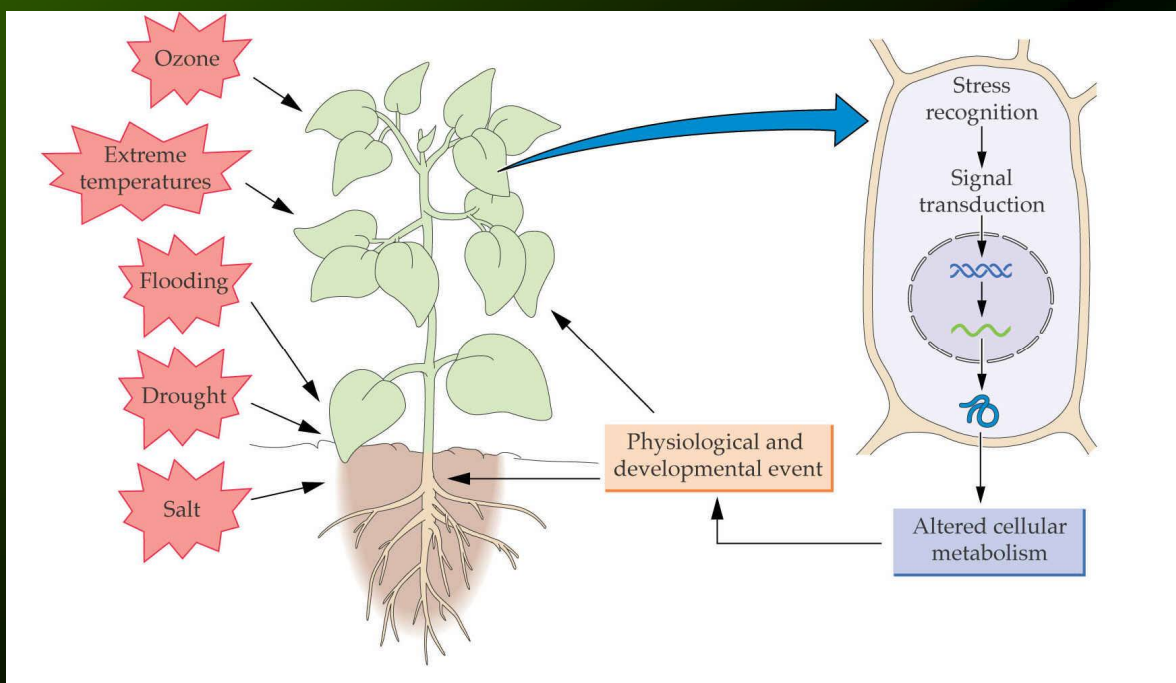
Terms of tolerance and resistance are used often as equivalent – tolerance is more correct

**Acclimatization** – increase of plant tolerance to stress as a result of gradual exposure to stress conditions

**Adaptation** – genetically determined level of tolerance obtained during selections through many generations

Stresses → changes in gene expression and cell metabolism

Changes in cell cycle and cell division, in endomembrane system and cell vacuolization, changes in cell wall architecture



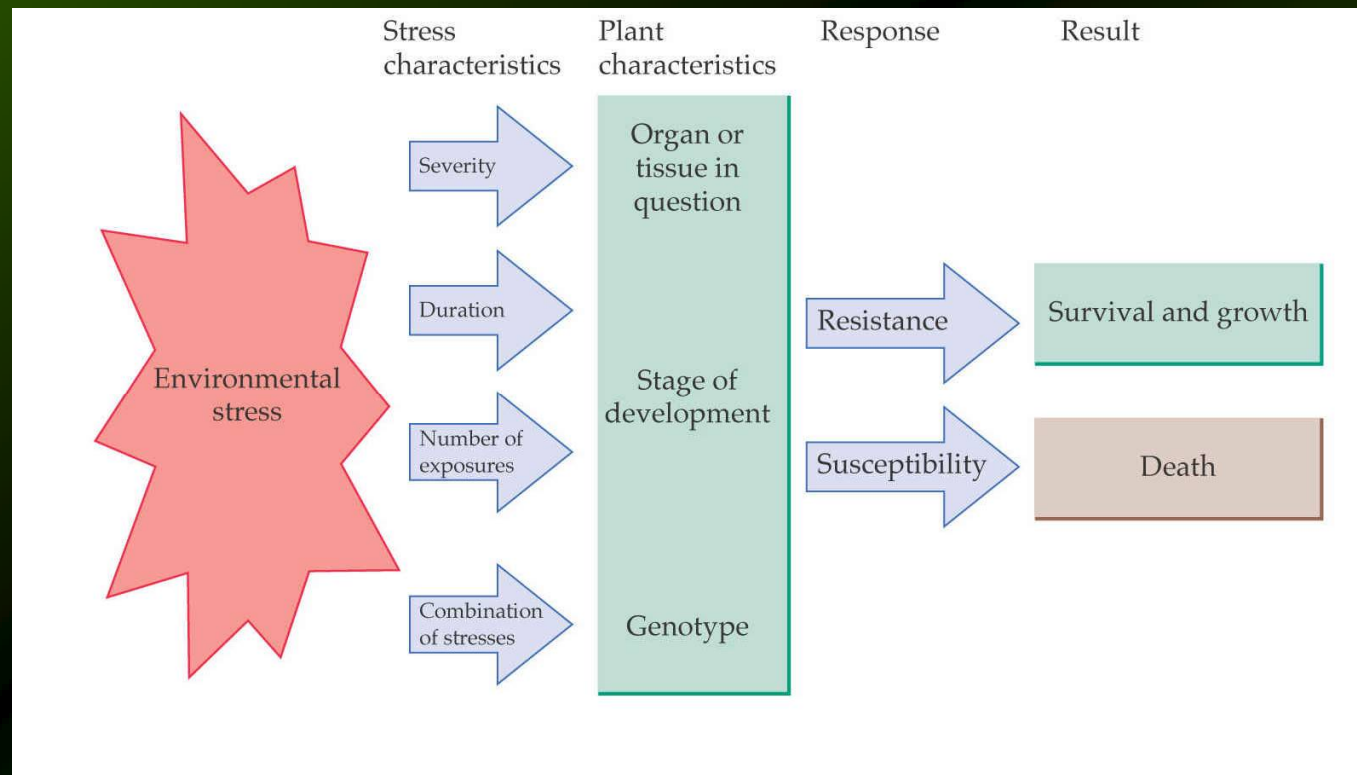
Changes in gene expression involving induction of signaling pathways.

Involvement of:

- hormones (ABA, JA, ethylene)
- secondary messengers ( $\text{Ca}^{2+}$ )

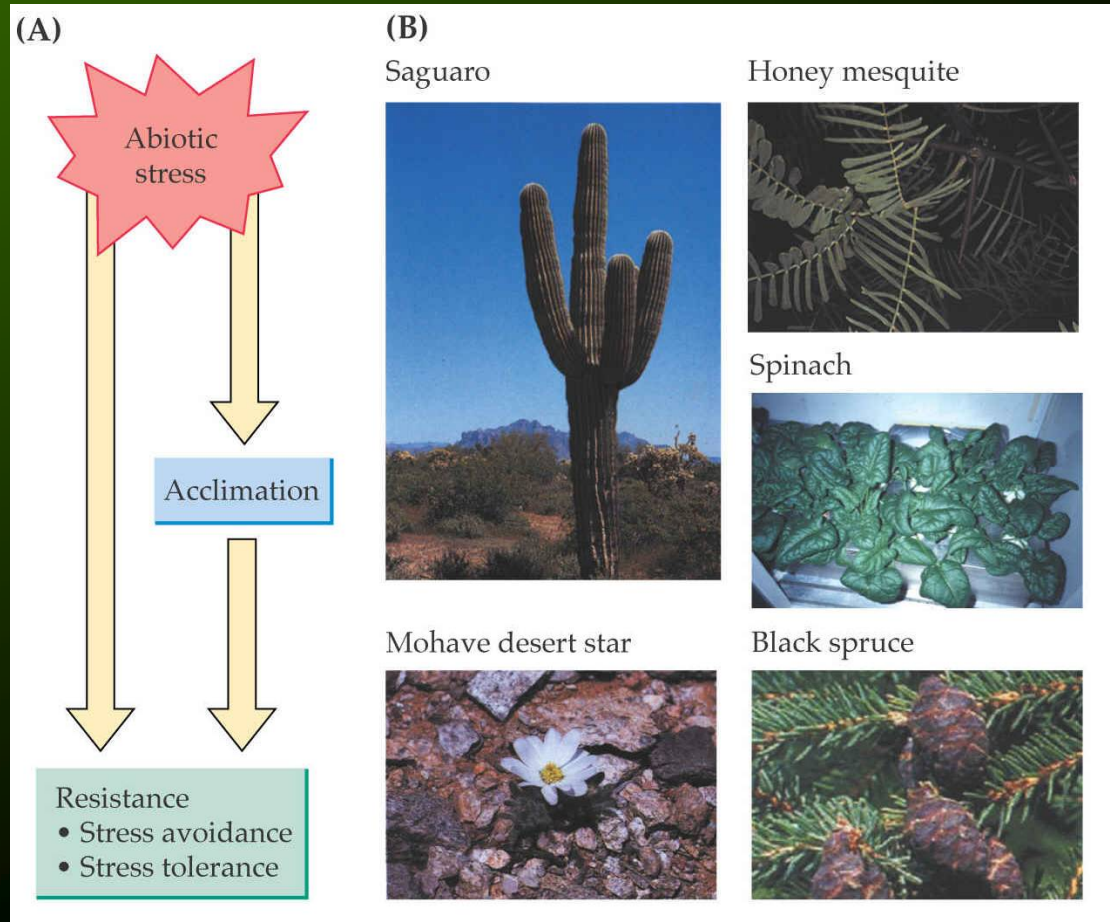
**Ability of plants to tolerate stress depends on:**

- stress intensity
- stress duration
- speed of stress coming
- plant organ



Losses in agriculture production caused by abiotic stress: 65 – 80%

### Mechanisms of resistance



Knowledge about mechanisms of resistance

Application of biotechnological methods

- timing of life cycle
- deep roots
- development of stomata and thorns

## a) Water deficit

- shortage of water in environment
- amount of water is not limited, but water is unavailable for the plant (salinity, low temperatures, high water transpiration)

### Parameters describing plant water status:

- water potential  $\Phi_w$
- relative water content (RWC)

**Water potential – expresses a level of how cell, organ and whole plant is hydrated (ability to give water)**

$$\Phi_w = \Phi_s + \Phi_p + \Phi_g + \Phi_m$$

$$\Phi = [ff]$$

**$\Phi_s$  = solute potential (osmotic potential)**

It is determined by numbers of particles dissolved in water.

$$\Phi_s \uparrow \Rightarrow \Phi_w \downarrow$$

**$\Phi_p$  = press potential**

Reflects physical powers acting on water in its surroundings.

Positive pressure (turgor) – PM press against cell wall

Negative pressure (tension) – PM press against water in cell, PM separates from cell wall

$$\Phi_p \uparrow \Rightarrow \Phi_w \uparrow$$

**$\Phi_g$  = gravitional potential**

Showed only at the transport water in plants in distance 5-10 m

**$\Phi_m$  = matric potential**

Explains how hard surfaces interact with water and suppresses  $\Phi_w$ . It is very small.

## Water potential – allows to predict water movement **into** and **out** of plant cell

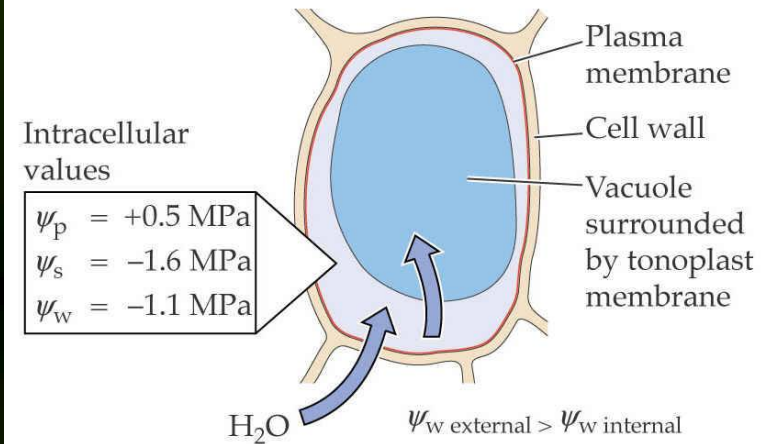
### Spontaneous water movement:

high  $\Phi_w$   $\xrightarrow{\text{H}_2\text{O}}$  low  $\Phi_w$

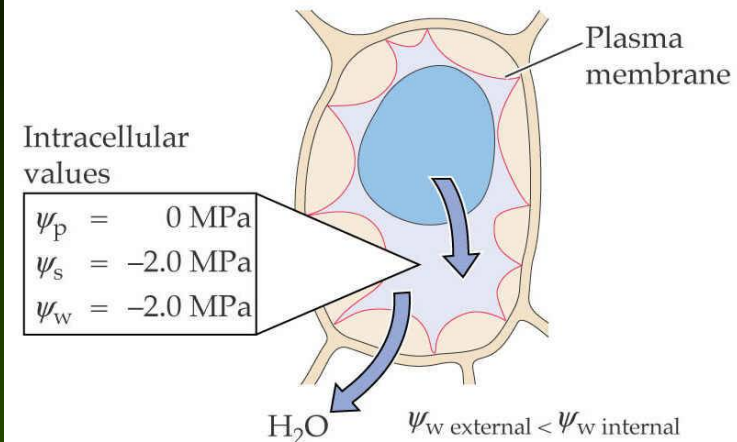
1. Cell is placed to clean H<sub>2</sub>O

2. Cell is placed to concentrated salt solution

Turgid cell:  $\psi_w \text{ external} = 0 \text{ MPa}$



Plasmolyzed cell:  $\psi_w \text{ external} = -2.5 \text{ MPa}$



Physiological and metabolic changes detected in plants exposed to water stress not always correlate with the changes in  $\Phi_w$ .



RWC – relative water content

Reception of water by roots covers loss of water by leaves => RWC = 85-95%

Critical RWC level < 50% => tissues die

Generally is in effect:  $\Phi_w$  ↓ => RWC ↓

Exceptions – plants with ability to keep high RWC at decreasing  $\Phi_w$

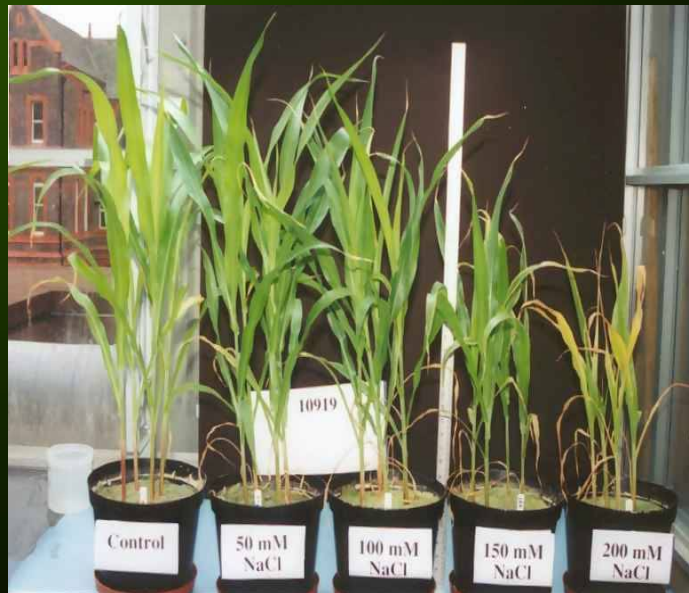
Plants able to adjust osmotically



## b) Salt stress and osmotic adjustment and its role in tolerance to drought and salt stress

### Salt stress (salinity)

Serious problem in agriculture – soil salinity essentially reduced yield:  
Coastal areas, floodplains, lower position plains



Salinity is usually determined by measurement of soil water conductivity – clean water has very low conductivity; conductivity increases with increasing salt concentration

**TABLE 25.6**  
Properties of seawater and of good quality irrigation water

Property	Seawater	Irrigation water
Concentration of ions (mM)		
Na <sup>+</sup>	457	<2.0
K <sup>+</sup>	9.7	<1.0
Ca <sup>2+</sup>	10	0.5–2.5
Mg <sup>2+</sup>	56	0.25–1.0
Cl <sup>-</sup>	536	<2.0
SO <sub>4</sub> <sup>2-</sup>	28	0.25–2.5
HCO <sub>3</sub> <sup>-</sup>	2.3	<1.5
Osmotic potential (MPa)	-2.4	-0.039
Total dissolved salts (mg L <sup>-1</sup> or ppm)	32,000	500

Quality (salt content) of flooding water differ.

Upper river: 50 mg/L

Central river: 900 mg/L



Hoover dam



LEGEND	
	STATE BOUNDRIES
	COAST LINES
	COLORADO RIVER
	OTHER RIVERS

THE COLORADO RIVER  
BASE IMAGE OBTAINED VIA THE XEROX PARC MAP SERVER AT [HTTP://PUBWEB.PARC.XEROX.COM/](http://pubweb.parc.xerox.com/)



## Division of plants based on their ability to tolerate higher level of salts:

- halophytes – very tolerant to salinity (~ 1% of world flora)
- glycophytes; nonhalophytes – sensitive to salinity

### Crops sensitive to salinity:

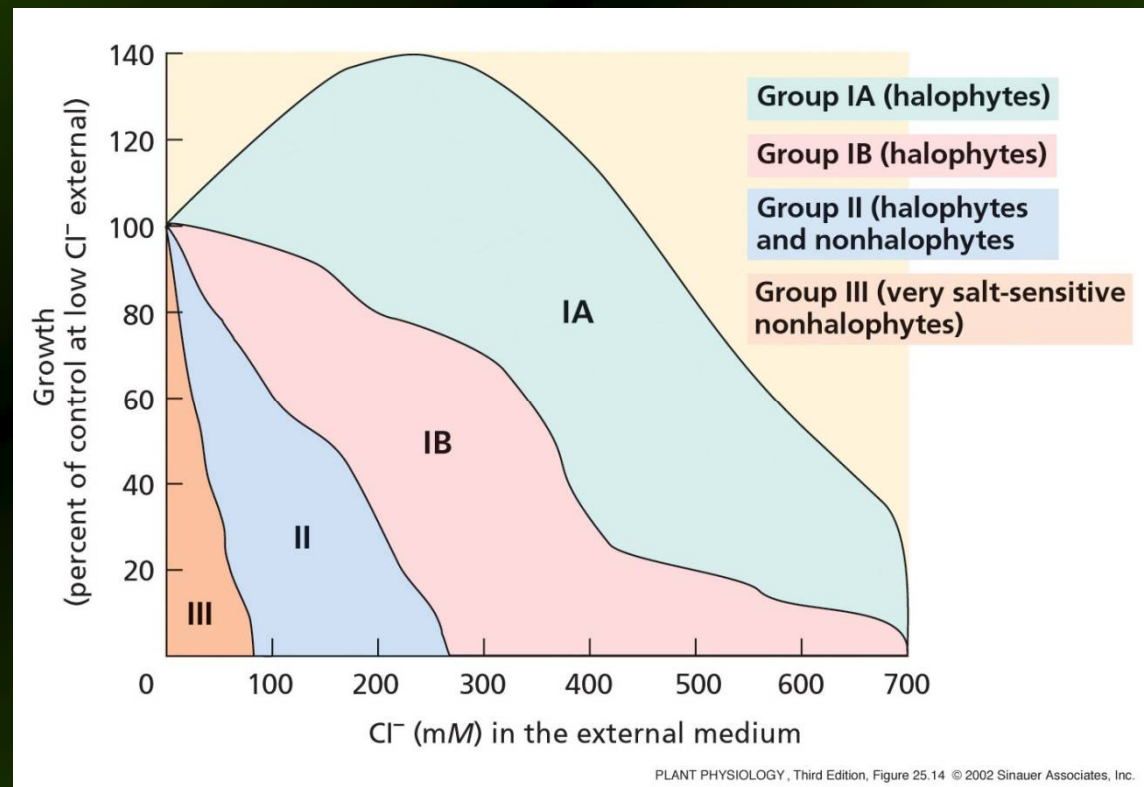
- maize
- onion
- citrus
- lettuce
- bean

### Crops less sensitive to salinity:

- cotton
- barley

### Crops tolerant to salinity:

- sugar beat
- date

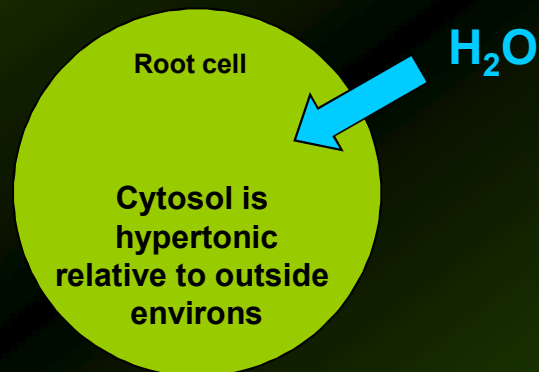


## Negative effect of salinity on plants:

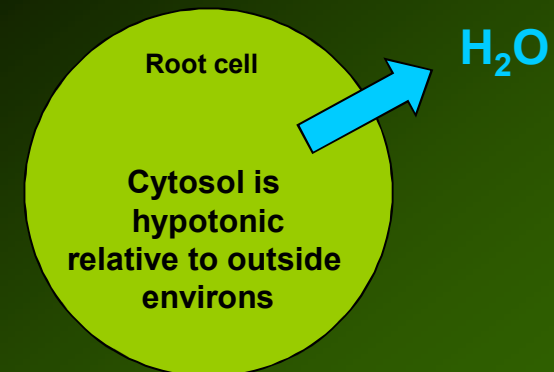
### Indirect – osmotic stress:

Increasing of osmotic pressure around the roots => decreasing the capacity of plants to take water since root cell environs is hypotonic relative to the environs around root.

#### Normal environment

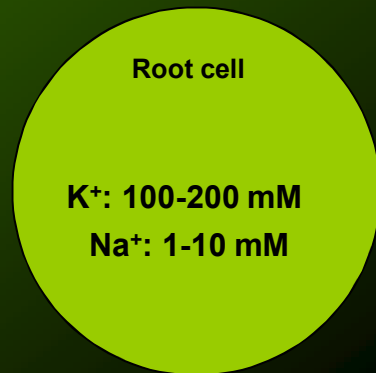


#### Salinity environment



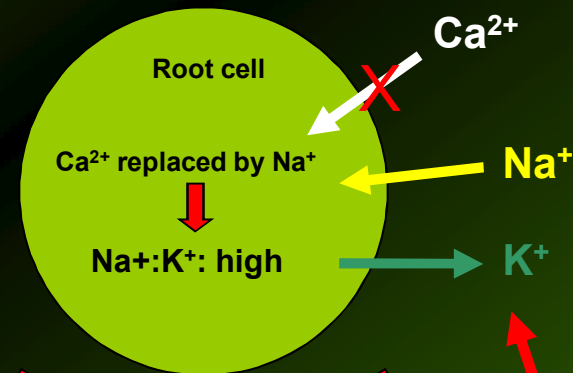
Direct - toxic effect of ions accumulating in cells:  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$

### Normal environment



Optimum function of enzymes

### Salinity environment



Inhibition of protein synthesis

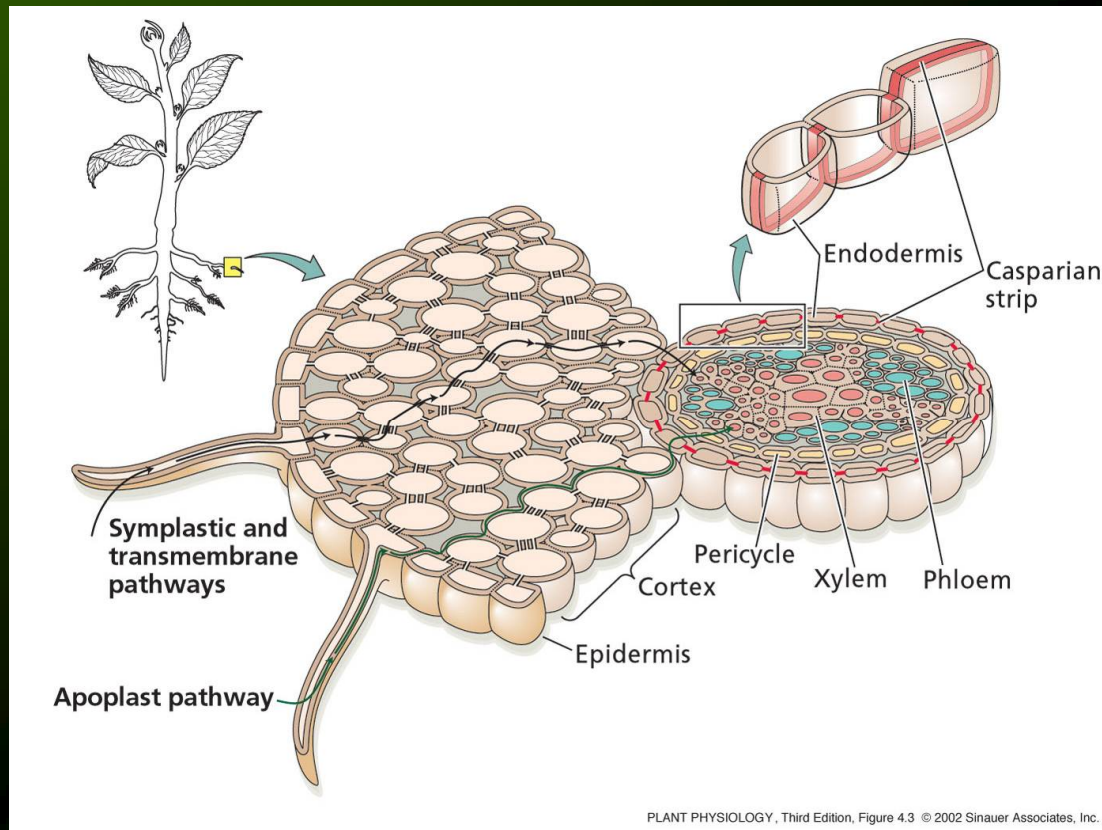
Function of enzymes inhibited

Changes in membrane permeability

Inhibition of photosynthesis:  $\text{Na}^+$  and  $\text{Cl}^-$  accumulate in chloroplasts  $\Rightarrow$  metabolism or phosphorylation is inhibited ( $e^-$  transport is not sensitive to salts).

Plants sensitive to salinity resist to salt excess in soil by limitation of ion transport to stem and leaves.

Ions use symplastic pathway to reach the xylem.  $\text{Na}^+$  reach roots in passive way (thanks to electrochem. potential) => transport  $\text{Na}^+$  from cells must happen by an active way.



PLANT PHYSIOLOGY, Third Edition, Figure 4.3 © 2002 Sinauer Associates, Inc.

Cells are able to regulate themselves amount of  $\text{Na}^+$  in cell

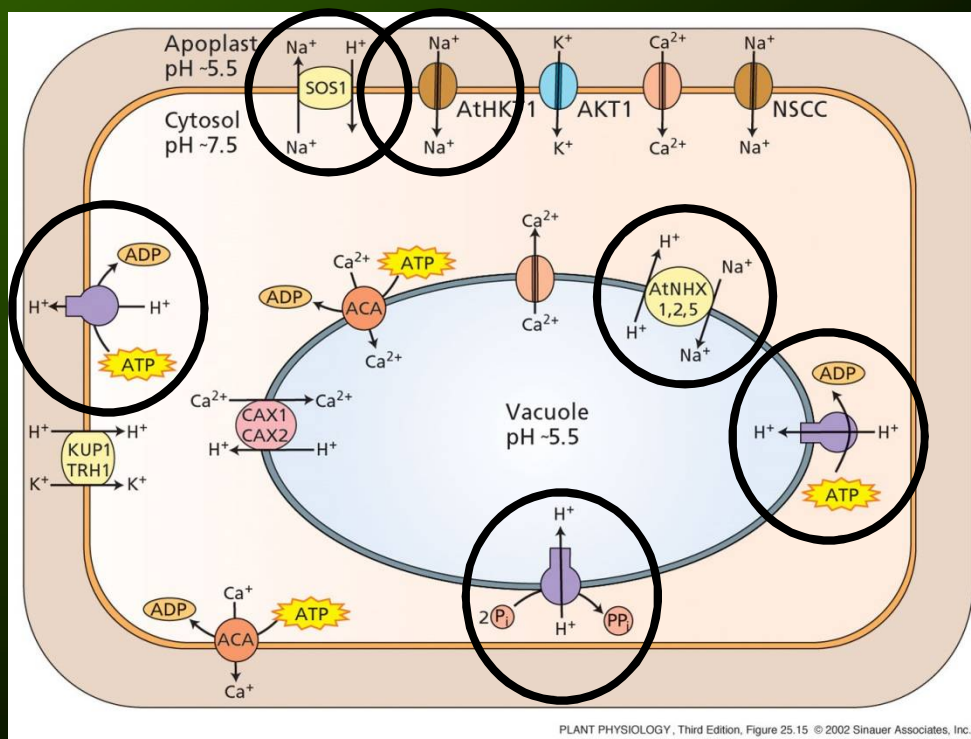
Part of  $\text{Na}^+$  is also removed by transpiration flow

Other reduction of  $\text{Na}^+$  amount from leaf

*Atriplex* – saline glands

High concentration of NaCl → Passive transport Na<sup>+</sup> into cells

Cell defense against Na<sup>+</sup> accumulation ← Accumulation of Na<sup>+</sup> in cell



1) Preferential transport of K<sup>+</sup> into the cell.

BUT at high external Na<sup>+</sup> conc., the K<sup>+</sup> channel is blocked => preferential transport of Na<sup>+</sup> into the cell.

2) Transport of Na<sup>+</sup> from cytosol out of the cell (Na<sup>+</sup>/H<sup>+</sup> antiport)

3) Transport of Na<sup>+</sup> from cytosol into the vacuole (tonoplast antiport Na<sup>+</sup>/H<sup>+</sup>)

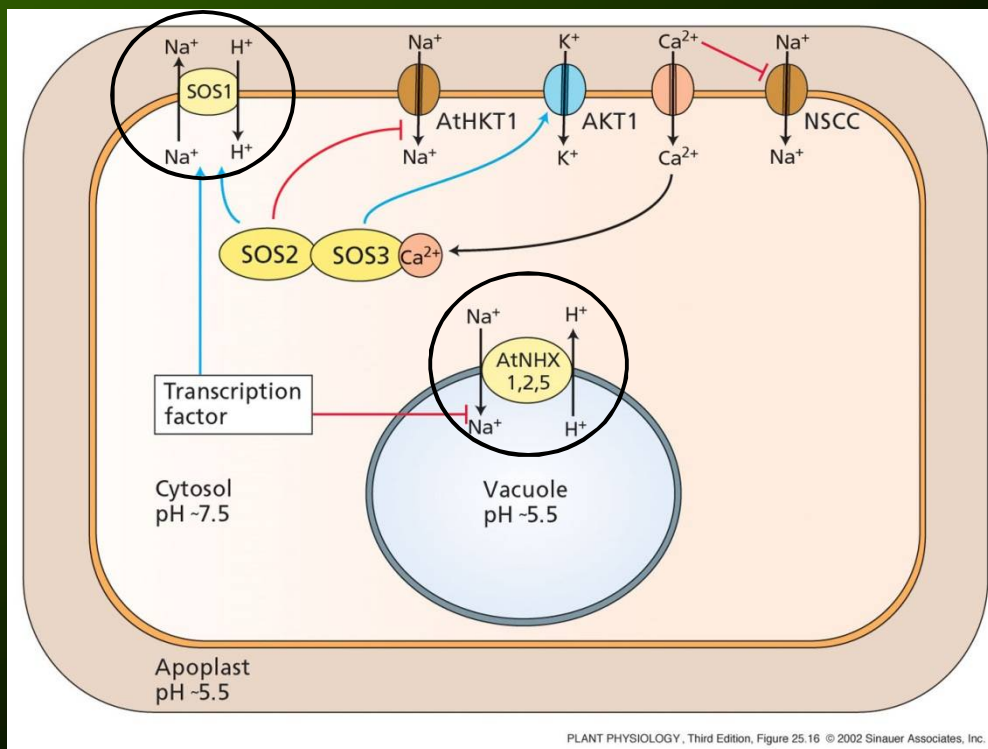
Necessity of electrochem. potential



H<sup>+</sup> pumps: H<sup>+</sup>-ATPase, H<sup>+</sup>-pyrophosphatase

Overexpression of antiport of Na<sup>+</sup>/H<sup>+</sup> in *Arabidopsis* allows the growth at 200 mM of NaCl (1/2 concentration of sea water)

**H<sup>+</sup>-ATPase initiates a differences in pH and membrane potential through plasma membrane and tonoplast. It provides power (H<sup>+</sup> electrochemical potential) for secondary ion transport. Activity of H<sup>+</sup> pump increases by salinity and by induced gene expression.**



**Cell has power to resist salt excess by the way that it transports unnecessary Na<sup>+</sup> out of the cell and into the vacuole.**

**SOS1 Na<sup>+</sup>-H<sup>+</sup> antiporter**

**SOS1 – regulated by SOS2, SOS3**

**SOS2 - serine/threonine kinase activated by Ca<sup>2+</sup> via SOS3**

**SOS3 - protein phosphatase regulated by Ca<sup>2+</sup>**

**Vacuolar compartmentalization of Na<sup>+</sup> is mediated by Na<sup>+</sup>- H<sup>+</sup> antiporter AtNHX1.**

## Osmotic adjustment and its role in tolerance to drought and salt stress

Osmotic adjustment – biochemical mechanism allowing acclimatization to drought and salinity

$\Phi_w$  in roots  $<$   $\Phi_w$  in soil

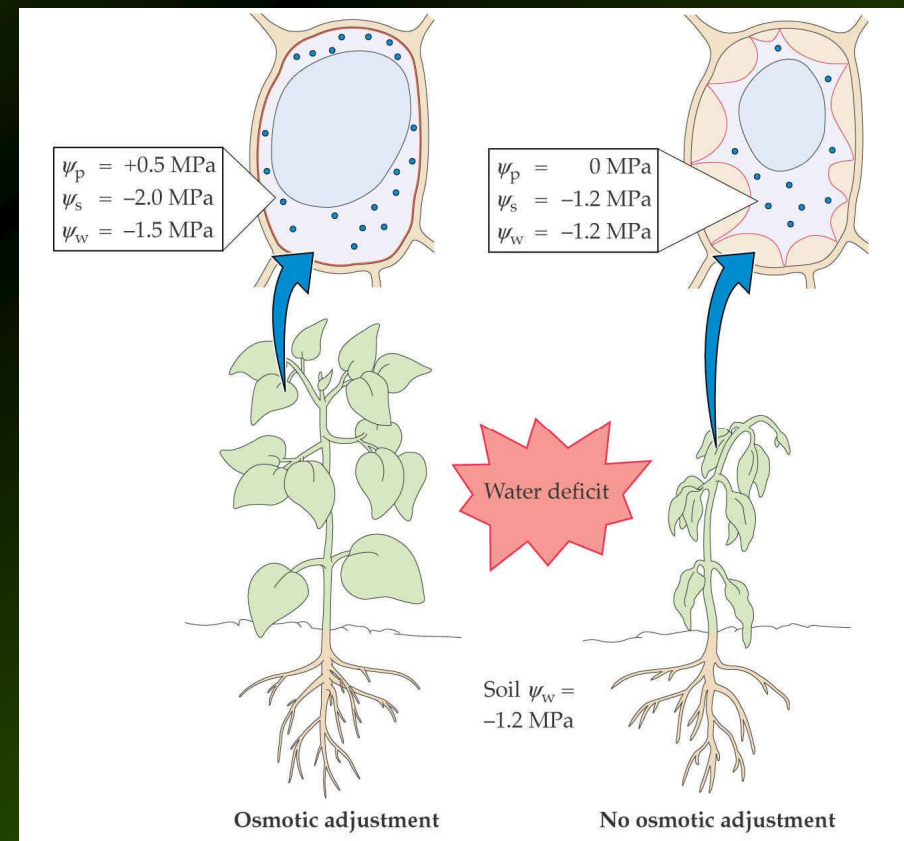


Plant is able to take water from soil

Plant is able to adjust osmotically in the case that it is able to regulate its solute potential ( $\Phi_s$ )

i.e. to increase solute amount in the cell

i.e. to decrease water potential in the cell



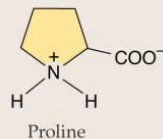
Osmotic adjustment involves:

- metabolic changes
- decrease in assimilation of low-molecular organic compounds
- increasing synthesis of low-molecular organic compounds

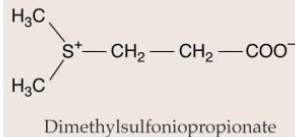
Compatible solutes = osmolytes

Compatible osmolytes

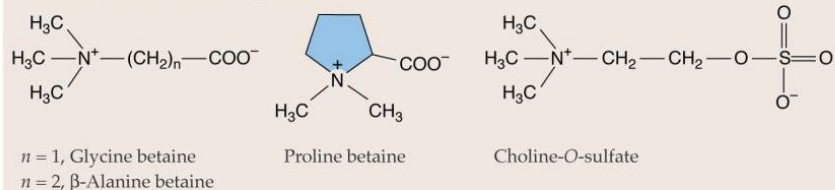
Amino acid:



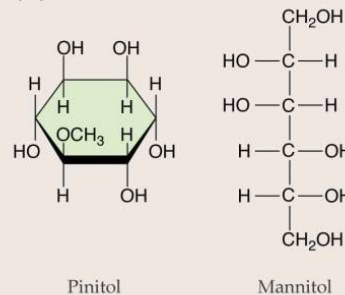
Tertiary sulfonium compound:



Quaternary ammonium compounds:



Polyhydric alcohols:



- proline

- alanine betaine, glycine betaine, proline betaine

- dimethylsulfoniopropionate

- mannitol, pinitol

## Osmotically active inorganic compounds – ions:

Charged, they bind to co-factors,  
to substrates, membranes, enzymes

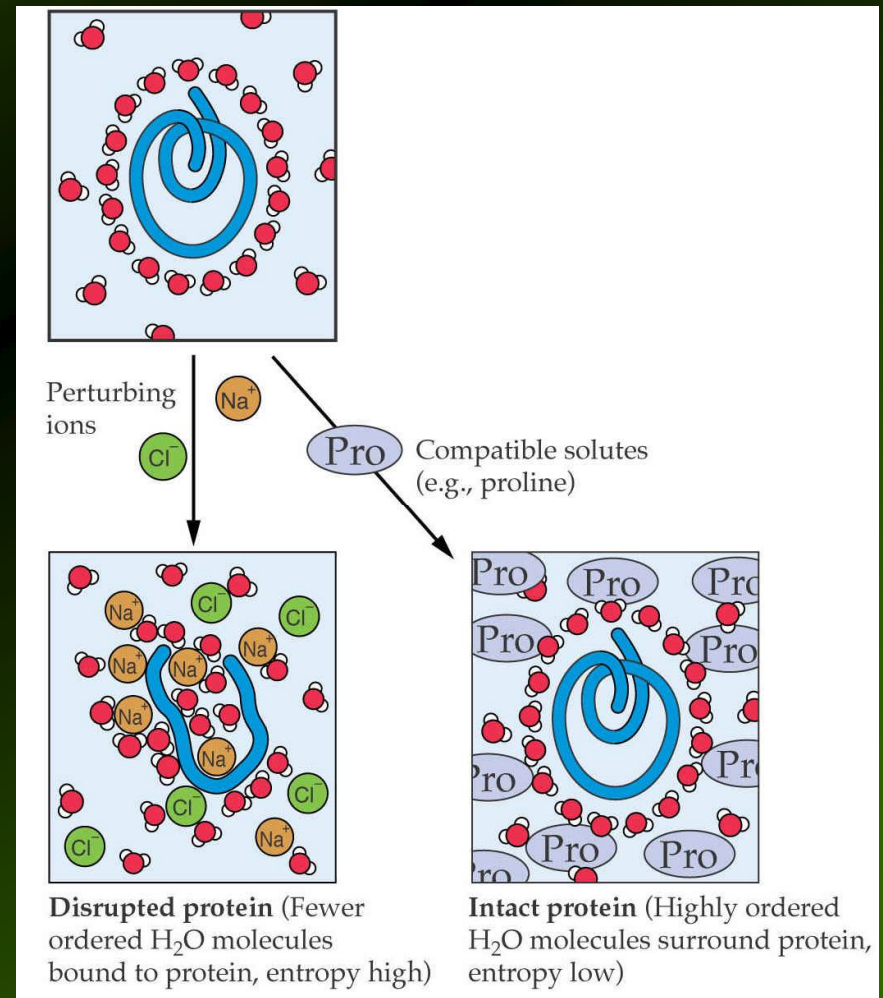


Toxic: change protein features,  
enter to hydration shells of proteins  
=> protein denaturation

Organic osmolytes – tendency to  
be neutral at physiological pH



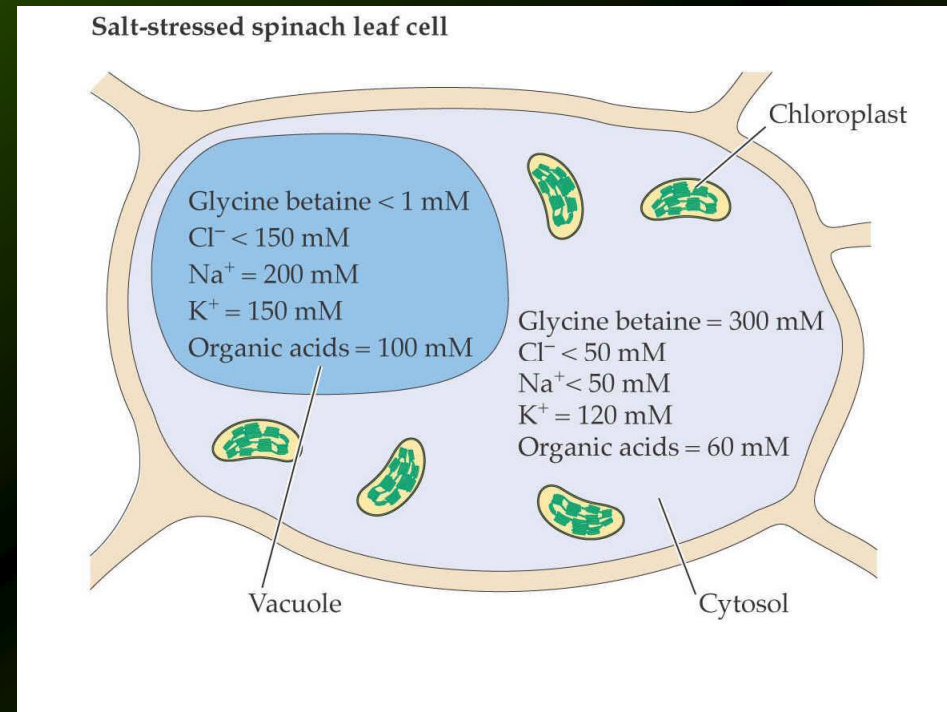
They do not enter protein  
shells



## Compatible osmolytes

↓  
**Stay in cytosol**  
↓

**Ability of cytoplasm to reach osmotic balance with vacuole**



**Transgenic plants with high level of glycine betaine have increased tolerance to abiotic stress.**

## c) Genes induced by water stress

Water shortage induces expression of genes of which products minimize negative effects of stress

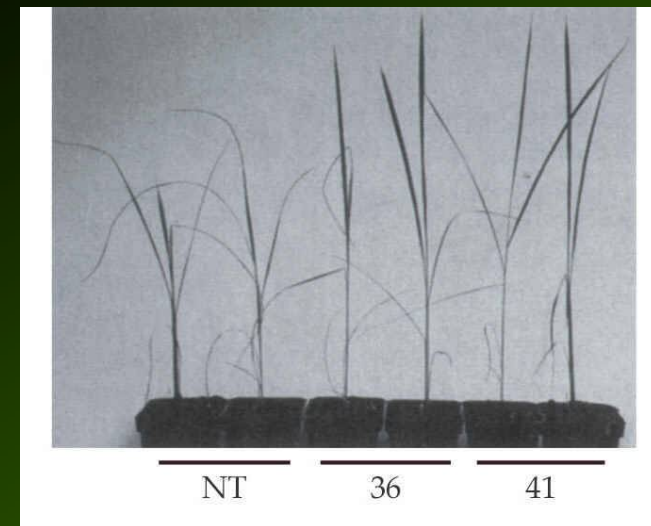
**LEA** proteins ( **L**ate **E**mbyogenesis **A**bundant) – first identified in seeds during drying

LEA are expressed in vegetative tissues exposed to stress; overexpression correlates with resistance to water stress

- LEA proteins:
- hydrophilic character => localization in cytoplasm
  - rich on alanine and glycine, lack of cysteine and tryptophan
  - importance consists in amount and way of expression

### 5 basic groups of LEA proteins

**OSMOTIN** – alkaline protein discovered in cell culture of tobacco exposed to stress of 428 mM NaCl



Transgenic lines of rice overexpression HVA1 protein (LEA protein of group 3) during salinity

Genes induced by water stress can be regulated also by ABA (level of ABA increases at water stress)

Products of genes *FLACCA*:

- 2x LEA proteins – group 2 and 5
- 1x lipid transfer protein
- 1x histone isotype H1

*ABI1, ABI3* – code proteins phosphatases, involved in transfer of ABA signal

Genes coding *tyrosine kinase*, similar to MAPK (mitogen-activated protein kinase) (function?)

No all the genes induced by water stress are regulated by ABA

Other signaling pathways involved in responses to water stress



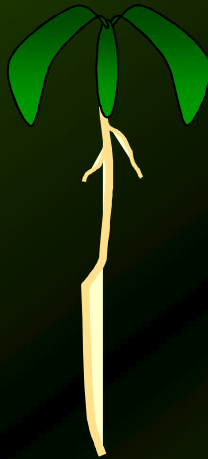
## c) Cold stress

**Cold = temperature too low for normal growth and too high for ice formation**

**Tropical and subtropical plants – sensitive to cold**

**Crops sensitive to cold: maize, bean, tomato, rice**

**25-35 °C :**  **10-15 °C :**



**Cold damage:**

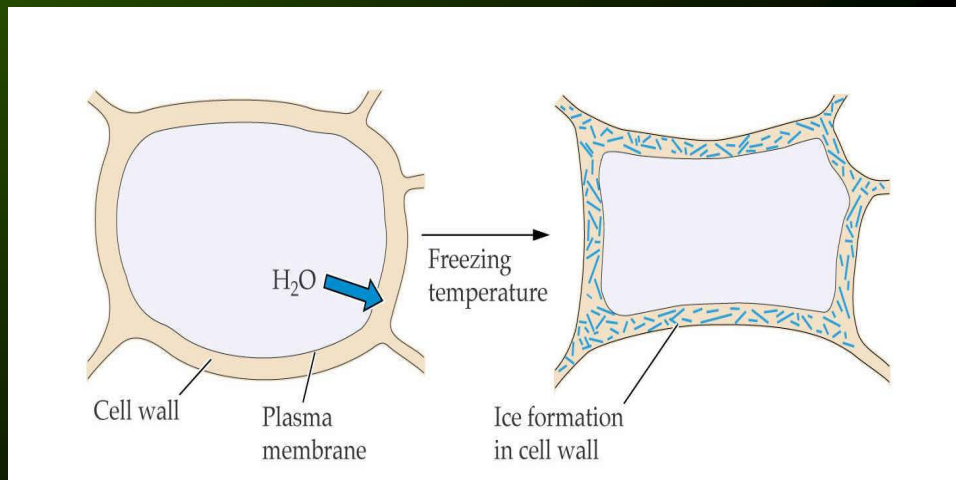
- growth deceleration
- leaf discoloration
- lesions occurrence

**Cold shock – sudden exposure of plants to temperatures around 0°C; increases risk of damage**

**Freezing damage – rises at temperatures lower than 0°C**

**Low temperature → Ice formation in intercellular areas**

**Water deficit ← H<sub>2</sub>O moves out of the cell towards ice**



### **Mechanisms of tolerance:**

- formation of extracellular ice => no ice crystals in cytosol
- +
- Accumulation of antifreezing proteins in apoplast => formation of ice slows down

**Processes during tolerance to freeze: - membrane stabilization**

- sugar accumulation, other osmolytes and antifreezing proteins
- changes in gene expression

## Freezing damages:

- leaf damages
- inhibition of photosynthesis
- decreased translocation of carbohydrates
- decreased respiration
- inhibition of protein synthesis
- Increased degradation of existing proteins

Loss of function of plasma membrane, tonoplast, membranes of chloroplasts and mitochondria



Leakages of solutes out of cell

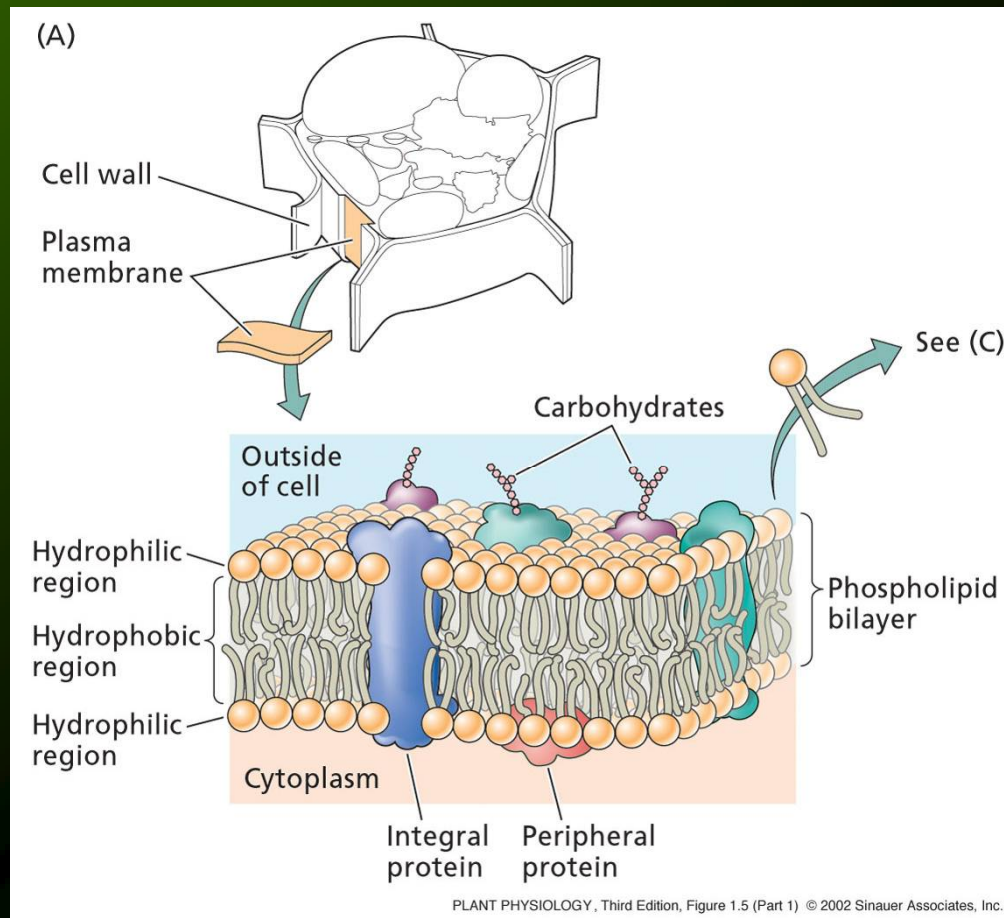


Dehydration

Why the function of membrane is damaged by cold?

## Plasma membrane – lipid bilayer containing proteins and sterols

Physical properties of lipids influence activity of integral proteins, including H<sup>+</sup>-ATPase, carriers, channels



Cold stress



Fluidity of membrane decreases



Wrong function of proteins

**Membrane lipids of plants tolerant to cold – higher portion of unsaturated fatty acids (double bond => membrane is more liquid => solidifies at lower temperatures in comparison to membranes with higher portion saturated fatty acids)**

**TABLE 25.5**

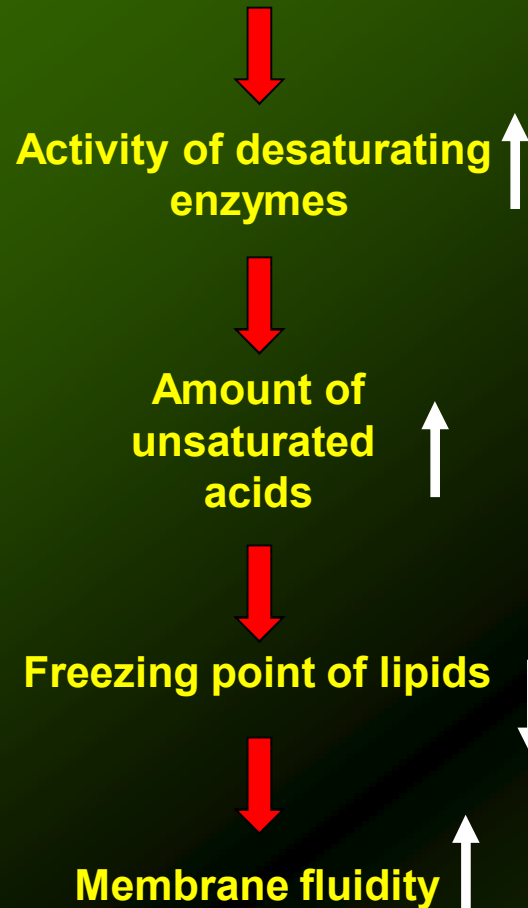
Fatty acid composition of mitochondria isolated from chilling-resistant and chilling-sensitive species

Major fatty acids <sup>a</sup>	Percent weight of total fatty acid content					
	Chilling-resistant species			Chilling-sensitive species		
	Cauliflower bud	Turnip root	Pea shoot	Bean shoot	Sweet potato	Maize shoot
Palmitic (16:0)	21.3	19.0	12.8	24.0	24.9	28.3
Stearic (18:0)	1.9	1.1	2.9	2.2	2.6	1.6
Oleic (18:0)	7.0	12.2	3.1	3.8	0.6	4.6
Linoleic (18:2)	16.4	20.6	61.9	43.6	50.8	54.6
Linolenic (18:3)	49.4	44.9	13.2	24.3	10.6	6.8
Ratio of unsaturated to saturated fatty acids	3.2	3.9	3.8	2.8	1.7	2.1

<sup>a</sup> Shown in parentheses are the number of carbon atoms in the fatty acid chain and the number of double bonds.

Source: After Lyons et al. 1964.

### Acclimatization



### Importance of membrane lipids:

Transgenic plants in *Arabidopsis* containing gene from *E. coli*, which increases portion of saturated acids

Transgenic plants are sensitive to cold

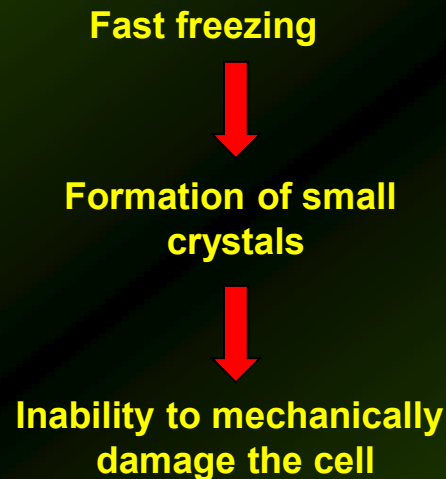
*Arabidopsis* mutant *fab1* has increased level of saturated acids

Destruction at lower temperature

**Ability to tolerate freeze differ in various tissues.**

**Dehydrated tissues (seeds, spores of fungi) – very tolerant (  $-273^{\circ}\text{C}$ )**

**Hydrated vegetative cells – sensitive; can however survive in the case of fast freezing**



**Sugar – cryoprotective effect – stabilizes proteins and membranes during dehydration induced by low temperature (cabbage – cryoprotective glykoproteins).**

**More sugar => higher tolerance to freezing – soluble sugars accumulate in cell wall, Where they limit the growth of ice crystals**

**Deep supercooling – mechanism of acclimatization to freeze – prevention (suppression) of formation of ice crystals by freezing deeply under freezing point (under  $-35^{\circ}\text{C}$ ).**

**Trees in Rocky Mountains, Colorado – pine, fir**



**Trees in South-East Canada  
East of USA – oak, elm,  
maple, birch**



**Acclimatization strongly  
decrease in the spring,  
when spring growth starts.  
The same trees may be  
damaged by temperatures  
at  $-10^{\circ}\text{C}$ .**

**Plant hormone ABA induces plant tolerance to freeze.**

**ABA induces changes in the spectrum of proteins. Induces protein synthesis often similar to proteins induced by cold.**

**Analysis of genes induced by ABA or cold**

**Proteins homologous to LEA proteins induced also by osmotic stress**



Level of ABA in plant tissues and sensitivity to ABA is associated with plant tolerance to freeze (winter wheat, rye, spinach, *Arabidopsis*)

Mutant *abi1* (*ABA insensitive*) – insensitive to ABA

Mutant *aba1* (*ABA deficient*) – low ABA level

Lack ability to acclimatize to freeze



No all genes induced by cold are induced by ABA



Expression of genes induced by ABA is not essential for freezing tolerance.

From half of 80th  Many genes induced by low temperature induced

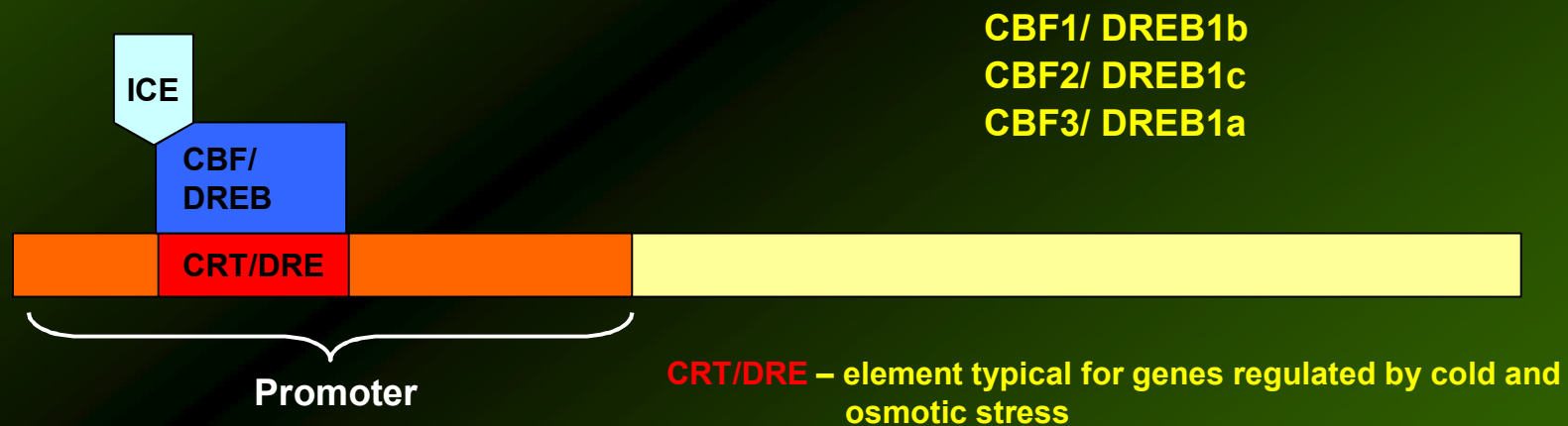
Genes induced by cold:

- Genes coding proteins with properties of chaperones; induced by heat stress as well
- Genes coding THP – thermal hysteresis proteins – water solution containing THP have properties of thermal hysteresis = transition from liquid to solid phase is induced by lower temperature than is temperature needed for transition from solid to liquid phase; belongs to antifreezing proteins – prevent ice crystal formation

## Transcription factors regulating expression of genes induced by cold

~ 100 genes induced by cold stress; their expression is activated by transcription factors CBF (C-repeat Binding Factors) (DREB – Dehydration Responsive Element Binding factors)

CBF1 contains 60-aminoacid DNA-binding domain; constitutive expression of CBF1 => increased expression of COR transcripts => tolerance to freeze



CBF1/DREB1b – controlled by transcription factors ICE (Inducer of CBF Expression)

## Newly discovered mechanism of plant tolerance to cold (2008)

