

COURSE “PLANT STRESS PHYSIOLOGY”

21 April 2022

PROGRAMME

Hour (EEST time)	Topic	Speaker
10:00-10:45	Introduction to abiotic, biotic, and oxidative stresses. Extreme temperatures. Adaptations to heat, chilling, and freezing.	Tsanko Gechev, CPSBB
10:45-11:30	Salinity Tolerance: what do we learn from halophytes?	Akhtar Ali, CPSBB
11:30-12:15	How plants cope with metal stress: from exclusion to hyperaccumulation?	Giovanni dal Corso, University of Verona
12:15-13:00	<i>break</i>	
13:00-13:45	Plant biotic interactions.	Gancho Pasev, MVCRI, Plovdiv
13:45-14:30	Oxidative stress in plants. The plant antioxidant system.	Frank van Breusegem, University of Ghent
14:30-15:15	Drought stress and desiccation tolerant plants.	Dorothea Bartels, University of Bonn
15:15-16:00	Common methods to assess plant stress.	Nikola Staykov, CPSBB

What is stress in plants?

Any deviation from the optimal growth conditions that negatively influences plant growth and development:

- Retarded growth
- Impaired development
- Reduced fertility / crop productivity
- Accumulation of toxic substances (secondary metabolites)
- Early ageing / senescence
- (Programmed) cell death of plant cells, tissues, organs, or the whole plant (in the most extreme cases)

Particular conditions may be optimal for some plants but stressful for others!

Plant specificities:

Plants are sessile, they cannot escape/evade unfavorable conditions (e.g. too much sunlight, extreme temperatures etc.).

Hence, plants develop various morphological, physiological, biochemical, and molecular mechanisms for stress protection. **These stress protective mechanisms are much more diverse and comprehensive, compared with animals.**

Plants contain much more stress-related genes than animals.

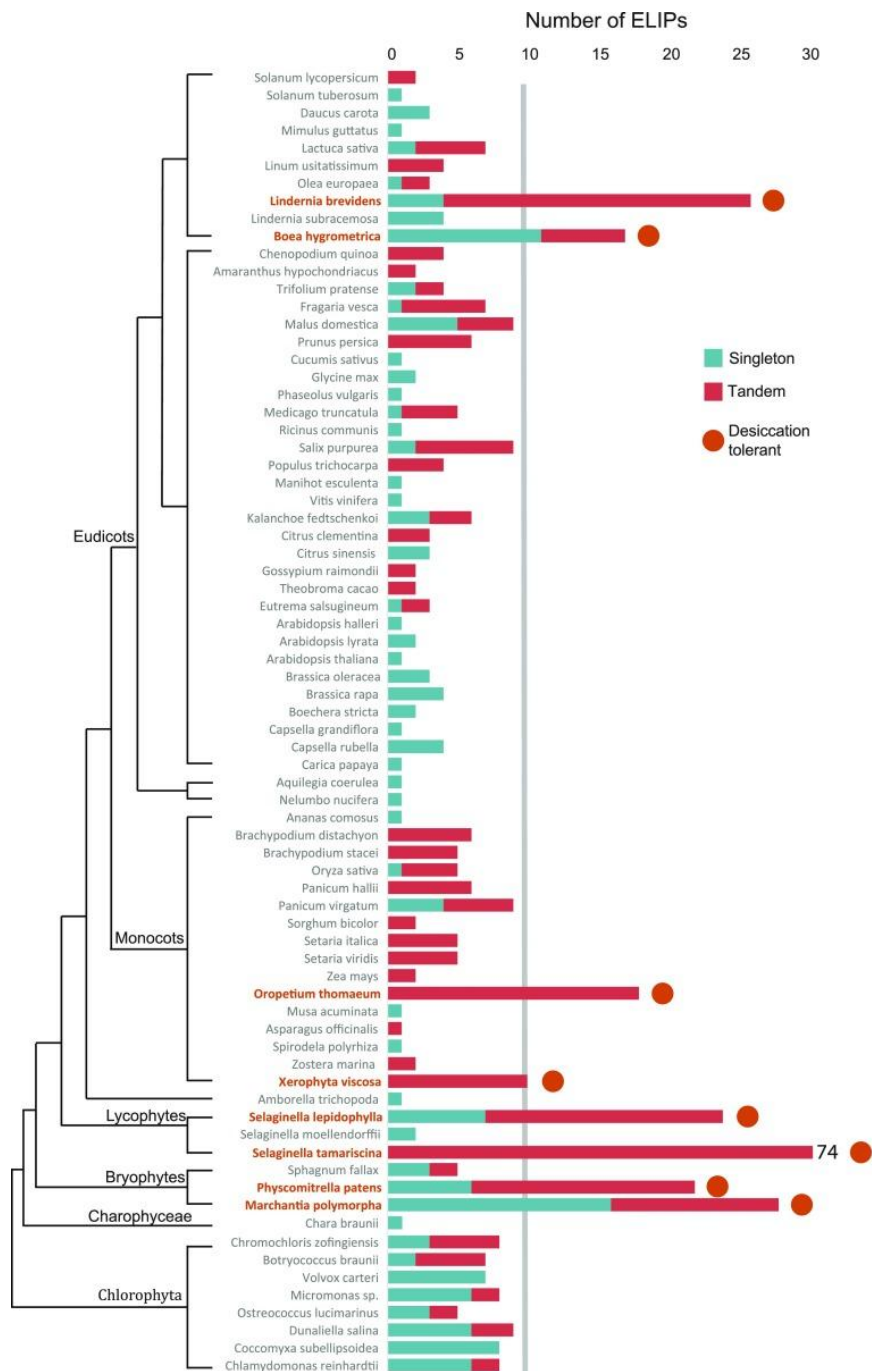
Genes for plant abiotic stress

Plants have unique stress genes encoding signalling compounds, TFs, and protective proteins:

- Pathogen-recognizing receptors
- Several kinase and phosphatase families
- ABF, ERF, WRKY plant specific TFs
- Dehydrins, ELIPs, etc.
- Asc-GSH cycle enzymes (DHAR, MDHAR)

Expansion of stress-related gene families in plants

- Single catalase gene in most animals; 3 and more genes in plants
- Huge expansion of the MAPK family in plants
- Expansion of stress genes in extremophile plants (ELIPs in resurrection plants)



Expansion of ELIPs in desiccation tolerant plants

Types of plant stress

Abiotic stress

- Drought / dehydration
- Salinity
- Extreme temperatures (heat, chilling, freezing)
- High light intensity (combined or not with low temperatures)
- Pesticides (herbicides, heavy metals, pesticides etc.)
- Hypoxia (flooding)
- UV radiation
- Nutrient deficiency (Zn, K, P etc.)

Biotic stress

- Viruses, viroids
- Bacterial pathogens
- Fungal pathogens
- Parasitic plants (e.g. *Orobancha* spp., broomrape)
- Алелопатични взаимодействия

Oxidative stress (consequence of many types of abiotic and biotic stresses)

How plants cope with stress?

Stress avoidance (ephemeral plants in deserts)

Tolerance (plants can survive stress, although with retarded growth/development)

Resistance – They grow well in stressful environments, as if there is no stress

Extremophile plants – survive extreme abiotic stress factors, like:

- Desiccation (resurrection plants, lecture of Prof. Bartels)
- High salinity (e.g. above 300 mM NaCl – halophytes in deserts and by the sea, lecture of Dr. Ali)
- Extreme temperatures (e.g. freezing)
- High UV radiation (*Pachycladon cheesemanii*)

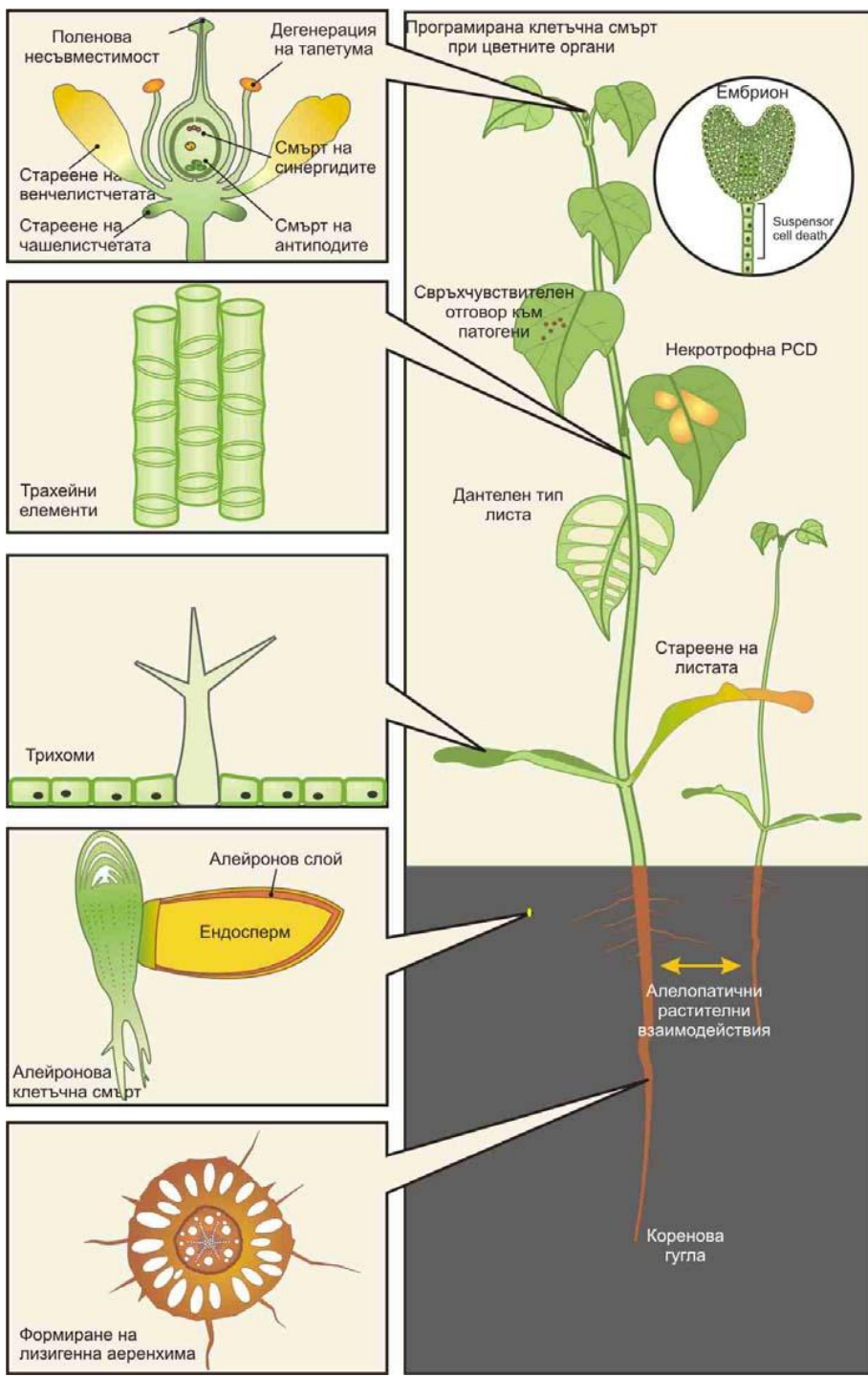
Importance of stress for plant

Stress can retard growth and development of stress sensitive species and can shrink the species area.

Stress resistant species can colonize specific habitats

Stress can accelerate ageing and trigger programmed cell death (PCD)

In crop science: Breeding for stress tolerant crops can mitigate crop losses due to unfavourable environmental conditions and has a positive effect on agro-economy.



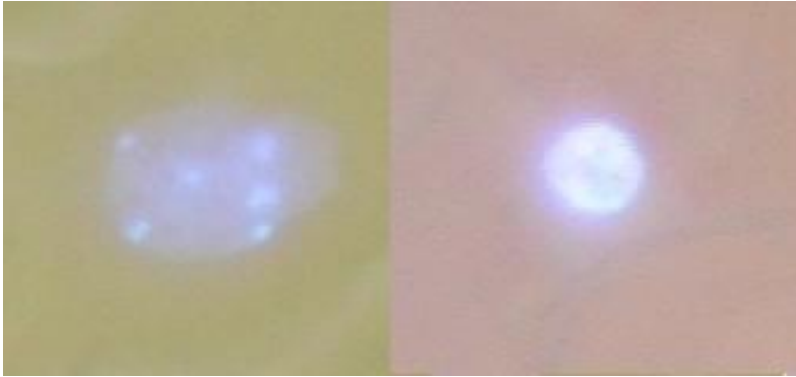
PCD is important for plant development::

- Embryo development
- Root cap
- Tracheal elements (xylem)
- Aerenchyma
- Lace leaf shape
- Trichomes
- Leaf senescence
- Aleurone layer in cereals
- Plant allelopathic interactions
- Hypersensitive response to pathogens

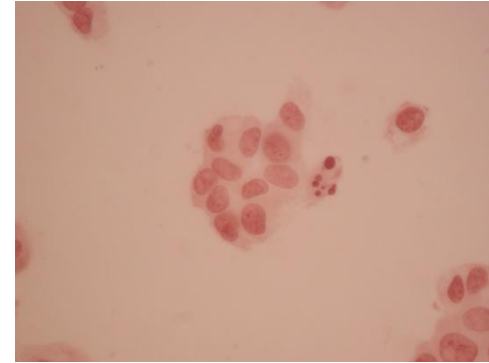
PCD unwanted consequence of abiotic and biotic stress:

- Necrotrophic pathogens (*A. alternata*, *C. victorae*)
- Abiotic stress (extreme temperatures, drought, salt stress, ozone, heavy metals etc.).

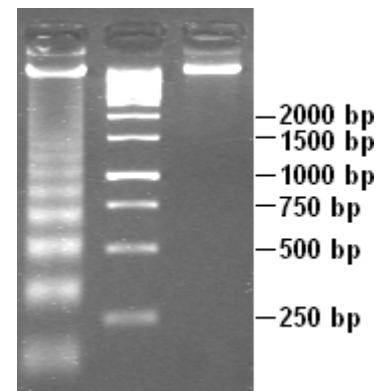
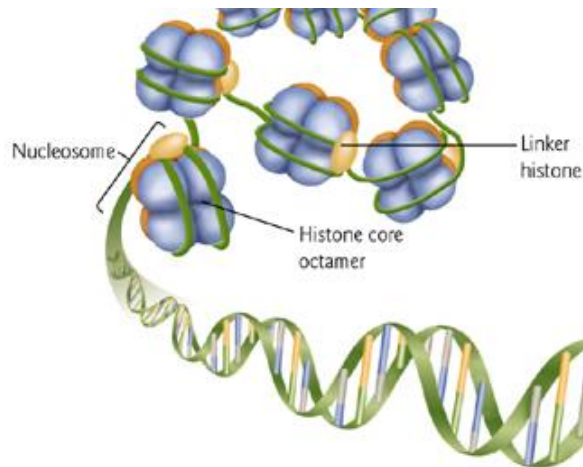
Detection of PCD



nuclear condensation in Arabidopsis
PCD triggered by AAL-toxin

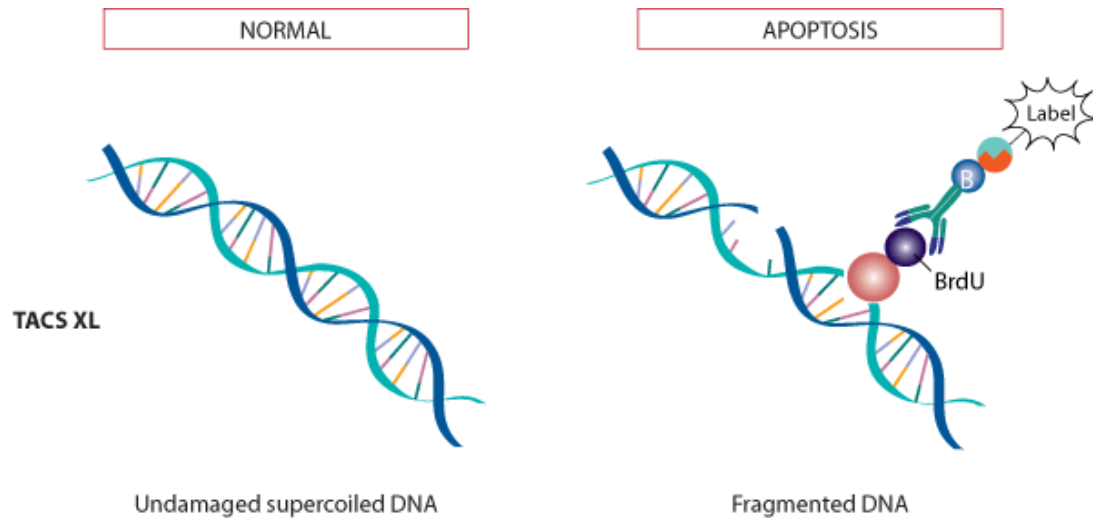


apoptotic bodies in hepatocytes
PCD triggered by $\text{TNF}\alpha$

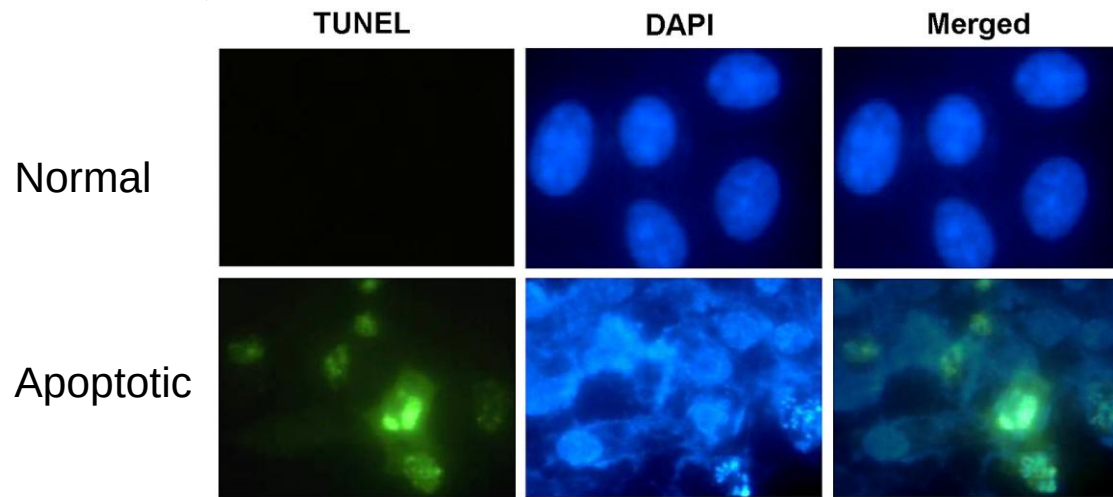


DNA laddering

TUNEL assay (Terminal deoxynucleotidyl transferase dUTP nick end labeling) with biotin or FITC



LEGEND



Drought stress

Most predominant abiotic stress in nature

Occurs with other stresses (heat, high light, salinity)

Different strategies to cope with drought stress

- delay of dehydration (maintaining high water potential, cacti)
- tolerance to dehydration (plants function at low RWC – desiccation tolerant plants)
- avoidance of the drought period (ephemeral plants with short life cycle)

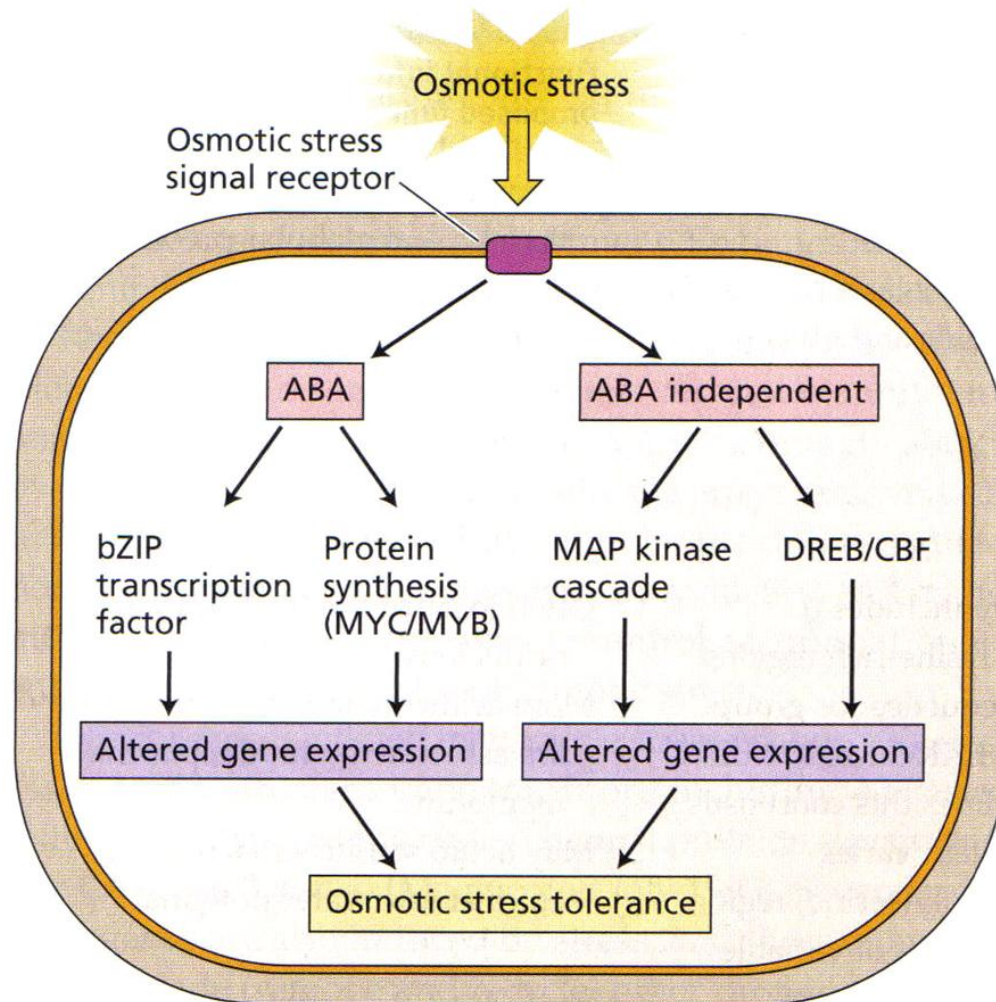
Molecular overlaps with other abiotic stresses leading to osmotic stress (salinity, low temperatures). Hence, common molecular responses/pathways:

- MAP kinases
- CaDPKs
- DREB/ABA response elements

Adaptation to drought stress

- Fast development, short life cycle
- Root architecture
- High water potential of the leaves
- Stomatal conductance
- Anatomical and morphological adaptations: wax layers, more trichomes, stomata
- Increase of membrane stability, biochemical composition
- Activation of the antioxidant system
- Osmotic regulation. Compatible solutes**

Osmotic stress signalling, common for drought and other abiotic stresses



Salt stress

Soil salinity occurs in many places of the world and is caused by diverse factors:

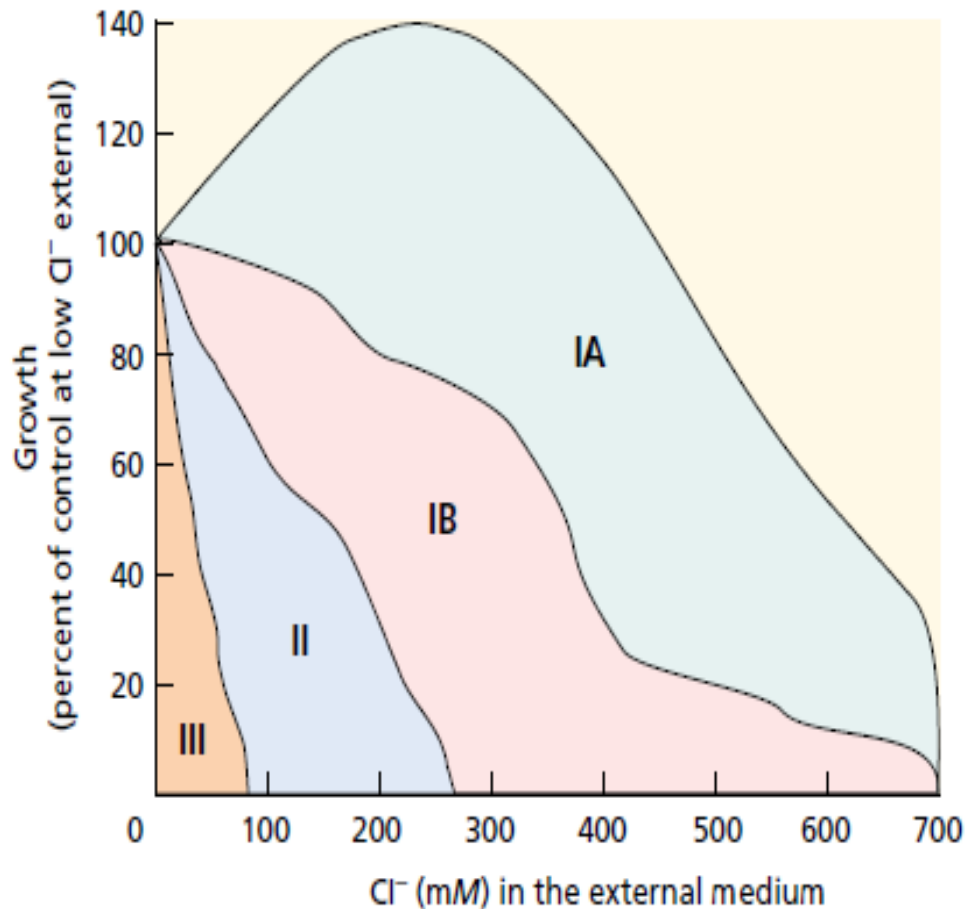
Sea water (around the sea, primary salinity; e.g. Caspian Sea).

Deserts and areas where evaporation exceeds rainfall

Salinity caused by human activities (improper irrigation etc.)

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

Plant groups according to their salt tolerance



Group IA (halophytes) includes sea blite (*Suaeda maritima*) and salt bush (*Atriplex nummularia*). These species show growth stimulation with Cl⁻ levels below 400 mM.

Group IB (halophytes) includes Townsend's cordgrass (*Spartina x townsendii*) and sugar beet (*Beta vulgaris*). These plants tolerate salt, but their growth is retarded.

Group II (halophytes and nonhalophytes) includes salt-tolerant halophytic grasses that lack salt glands, such as *Festuca rubra* subsp. red fescue (*littoralis*) and *Puccinellia peisonis*, and nonhalophytes, such as cotton (*Gossypium* spp.) and barley (*Hordeum vulgare*). All are inhibited by high salt concentrations. Within this group, tomato (*Lycopersicon esculentum*) is intermediate, and common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) are sensitive.

The species in Group III (very salt-sensitive nonhalophytes) are severely inhibited or killed by low salt concentrations. Included are many fruit trees, such as citrus, avocado, and stone fruit.

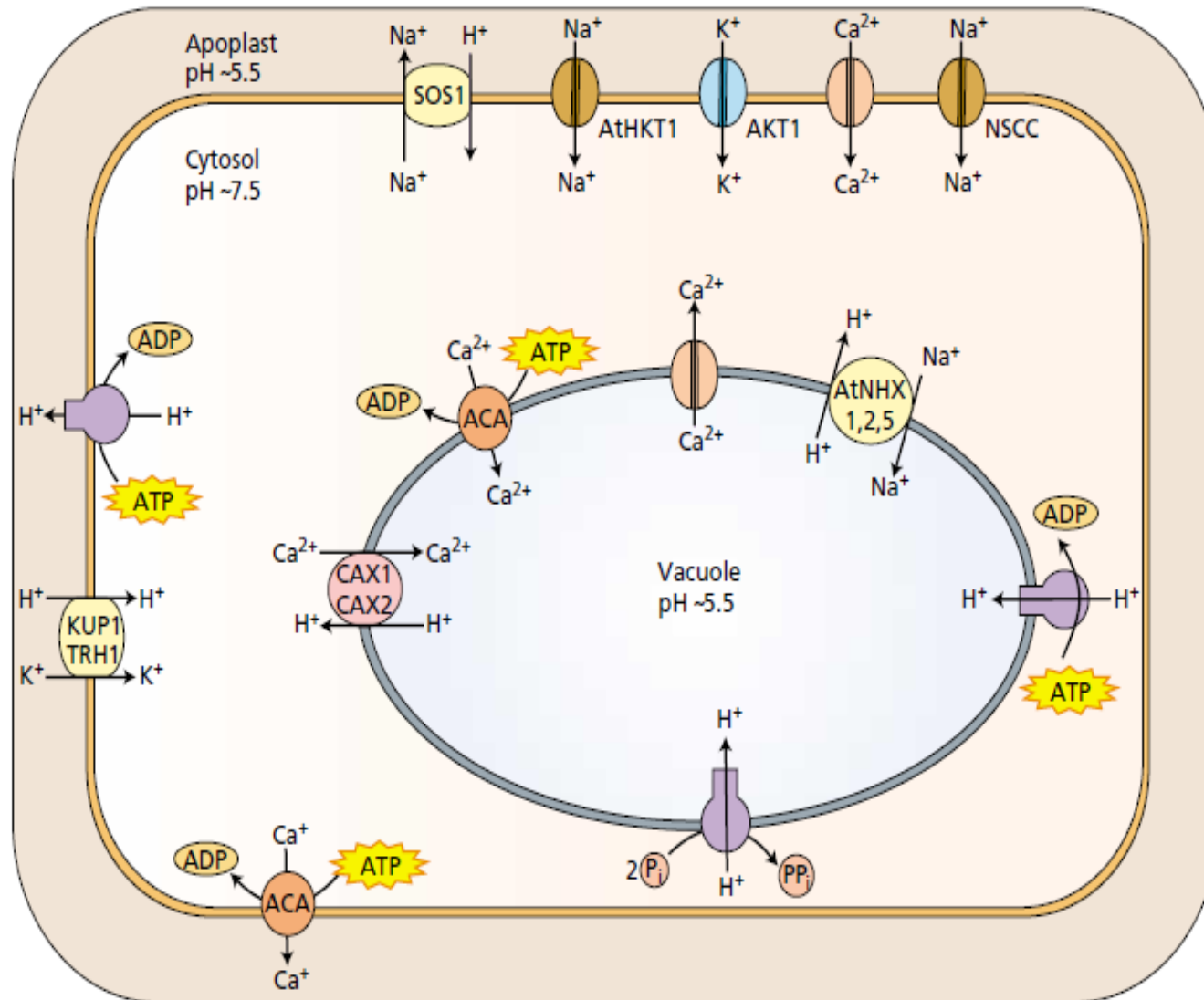
Physiological damage caused by salt stress:

- The very hard soil obstructs root development
- Inhibition of photosynthesis
- Osmotic stress
- Ion disbalance. Replacement of K^+ with Na^+ influences negatively:
 - Intake of Ca^{2+} and other ions, due to malfunction of antiporters
 - Membrane depolarisation
 - Depletion of ATP, due to osmoprotectants synthesis, which makes high affinity ion transport even more problematic
 - следствие на нарушена функция на антипортерите
- Oxidative stress
- Metabolic perturbations

Salt stress responses:

- Osmotic regulation through compatible solutes
 - Sugars, polyvalent alcohols, amino acids (proline), etc.
 - Inorganic ions (Na^+ , Cl^- , K^+)
- Extrusion of Na^+ from the roots through plasmalemma – active, energy spending process
- Sequestration inside the vacuoles
- Retention of K^+ in the cytosol, to counteract the Na^+ accumulation
- Tissue-specific accumulation (e.g. in stems). Young tissues have less salts than the old ones.
- Control of xylem transport.
- Removal of Na^+ from the aerial parts and transport to the roots
- Tolerance to oxidative stress
- Anatomic adaptations (leaf succulence, salt glands).

Membrane transporters for Na⁺, K⁺, and Ca²⁺ during salt stress



SOS1: Na⁺/H⁺ antiporter

ACA: Ca²⁺ ATPase

KUP1/TRH1: high affinity
K⁺-H⁺ cotransporter

atHKT1: Na⁺ transporter

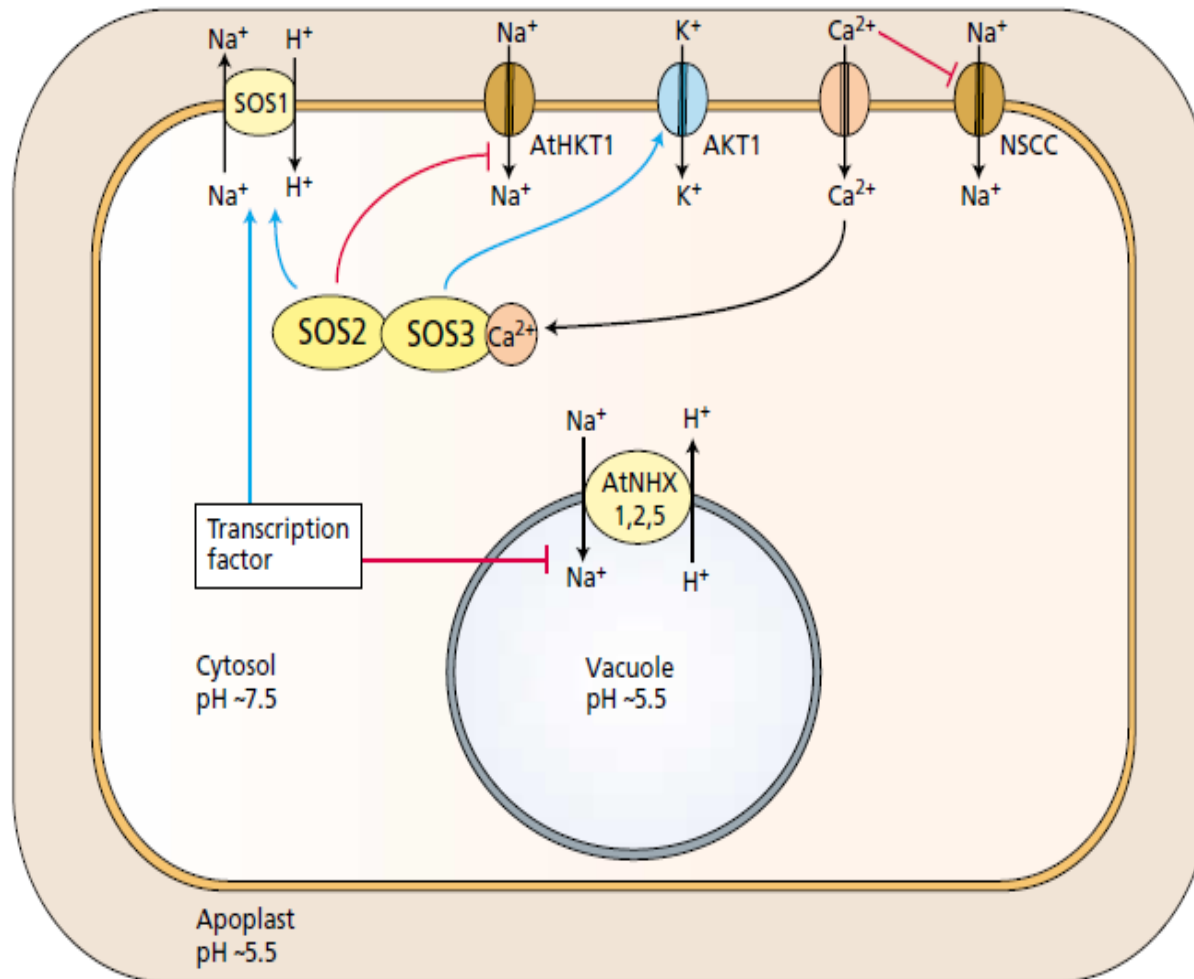
AKT1: K⁺ channel

NSCC: non-selective
cationic channel

CAX1/2:
Ca²⁺/H⁺ antiporter

atNHX1,2/5:
Endomembrane Na⁺/H⁺
antiporter

SOS response is the main mechanism of Na⁺ extrusion



SOS1: Na⁺/H⁺ antiporter

SOS2: Ser/Thre kinase

SOS3: Ca²⁺ binding protein

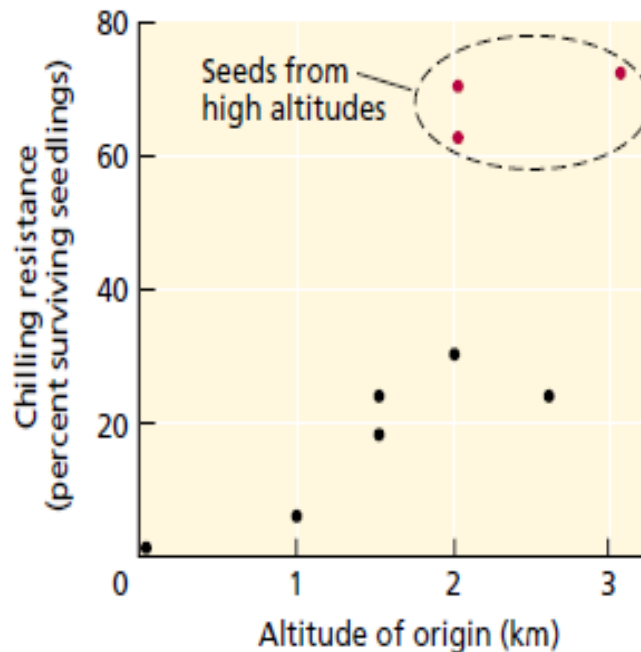
atNHX1,2,5: endomembrane
Na⁺/H⁺ antiporter

Low temperature stress

Low temperatures:

- Chilling (>0)
- Freezing (<0)

Plant tolerance to low temperatures is species specific but also with great variability inside the species:



Acclimatization: Short exposure to chilling temperatures (4-8 $^{\circ}\text{C}$) makes plants tolerant to long-term chilling/freezing

Impact of low temperature stress on plants:

- Growth retardation
- Wilting (dehydration)
- Bleaching of leaves (photooxidative damage), necrosis
- Decreased membrane fluidity, leading to malfunction of membrane enzymes, ion channels' transporters etc.
- Inhibition of photosynthesis
- Decreased protein synthesis and increased protein degradation
- Most enzymes do not function properly (low enzyme activity)

Low temperatures, combined with high light, are particularly damaging and lead to oxidative stress

Freezing temperatures lead to ice formation (both inside and outside of the cells) and can cause membrane or/and organelle rupture

Plant responses to low temperature stress:

- Increased membrane fluidity by replacing saturated fatty acids with unsaturated fatty acids
- Osmotic regulation by compatible solutes, (sucrose, proline). Resembles responses during dehydration
- Synthesis of antifreeze proteins that bind to the forming ice crystals and prevent their enlargement. In rye (*Secale cereale*), such proteins accumulate both in apoplast of the epidermal cells and in the cells surrounding intercellular space, to prevent formation of extracellular ice.

Plants tolerating low temperatures have higher percentage of unsaturated fatty acids

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

Major fatty acids ^a	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

Gene regulation during low temperatures

Low temperatures lead to transcriptional reprogramming

ICE (Inducer of CBF expression) activates the expression of CBF genes, that encode the CBF proteins (C-repeat Binding Factor), known also like DREB1 – (Drought Responsive Element Binding Factor 1): TFs of the AP2/EREBP family (APETALA 2/Ethylene-Responsive Element-Binding Protein).

CBF TFs bind to the CRT promoter elements to regulate the COR genes, encoding low temperature protection genes (dehydrins/LEA proteins, desaturases, antioxidant genes etc.)

ABA-dependent signalling pathway, activating the ABA transcription factors (ABF). **ABF bind to ABRE promoter elements in the COR genes.**

Antioxidant system at chilling temperatures

Controls			Chilling stress			
			1 day	2 days	3 days	4 days
SOD	25 °C	62.02±4.31	61.5±0.14	63.6±0.85	60.15±2.05	64.2±0.85
	5 °C		61.85±6.01	62.8±5.23	61.15±7.89	61.3±5.94
APx	25 °C	3.22±0.38	3.02±0.58	3.34±0.25	3.01±0.36	3.02±0.44
	5 °C		1.77±0.25	1.63±0.21	1.71±0.17	1.65±0.22
MDHAR	25 °C	0.204±0.005	0.208±0.0085	0.209±0.003	0.208±0.007	0.207±0.006
	5 °C		0.126±0.0015	0.125±0.001	0.120±0.005	0.117±0.0001
DHAR	25 °C	0.593±0.01	0.558±0.07	0.536±0.047	0.535±0.029	0.408±0.106
	5 °C		0.179±0.001	0.189±0.013	0.188±0.058	0.155±0.023
GR	25 °C	0.167±0.02	0.163±0.03	0.160±0.03	0.170±0.008	0.157±0.023
	5 °C		0.078±0.01	0.082±0.01	0.077±0.009	0.084±0.001
Catalase	25 °C	196.3±23.4	104.1±31.5	69±11.3	67±2.9	39.7±5.5
	5 °C		56.9±10.9	33±7.2	34±13.3	21±5.4

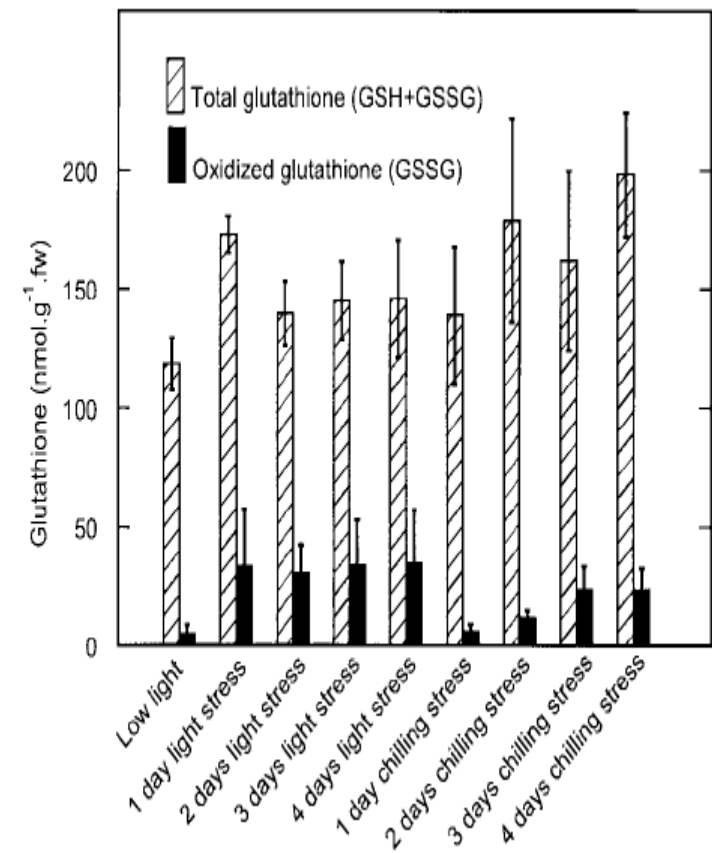
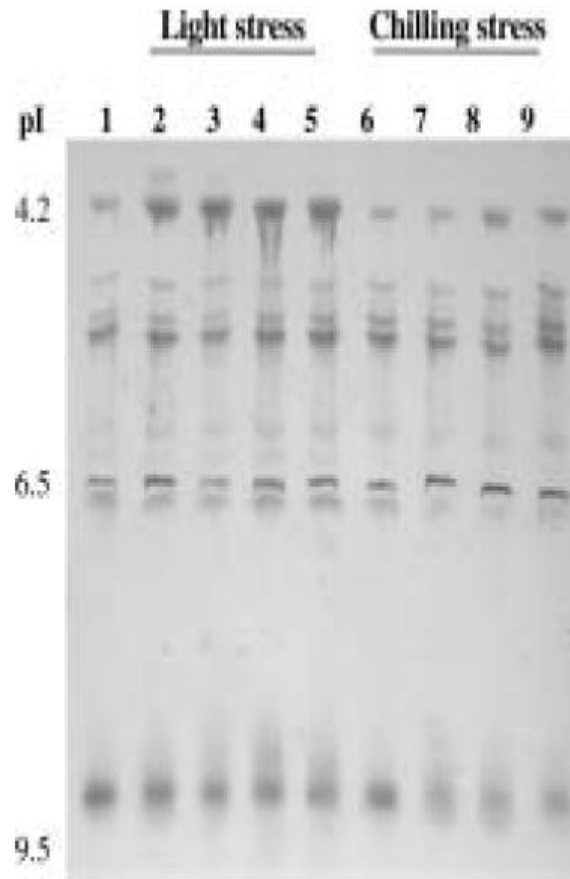
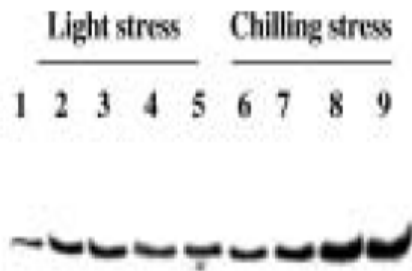
Controls		Light stress			
		1 day	2 days	3 days	4 days
SOD	62.02±4.31	63.44±0.21	64.7±1.27	63.3±1.7	64.8±1.7
Apx	3.22±0.38	3.8±0.24	3.44±0.56	5.37±0.27	6.55±0.3
MDHAR	0.204±0.005	0.202±0.014	0.206±0.007	0.206±0.01	0.204±0.003
DHAR	0.593±0.01	0.669±0.064	0.647±0.003	0.662±0.038	0.597±0.032
GR	167.17±18.1	165±1.4	155±20	160.9±25.6	161.4±11.8
Catalase	196.3±23.42	173.16±24.9	154±7.37	169.4±7.8	222±28

Antioxidant system at chilling temperatures

POX

Glutathione

GPx



HSF signalling

