Improving Stress Tolerance

Mitesh Shrestha

Stress

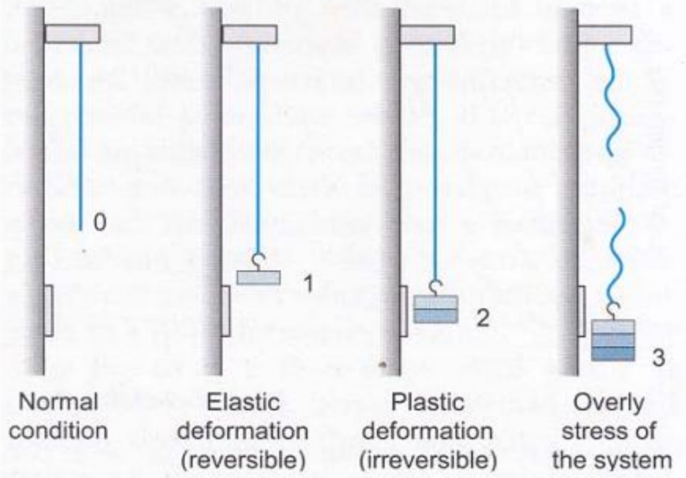
- Product of any physical, chemical or biological changes which disrupts the homeostasis.

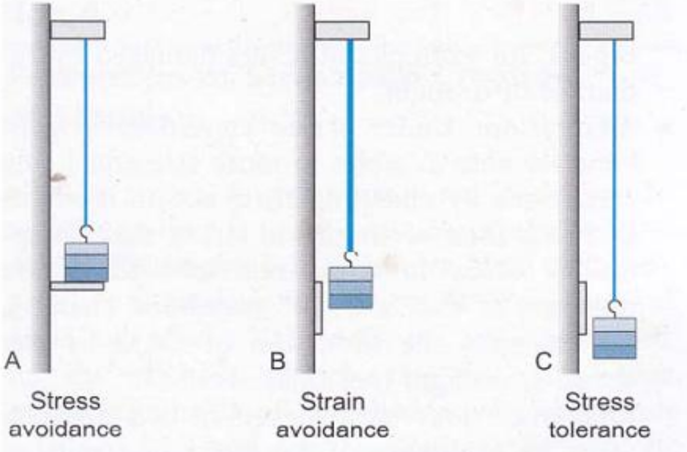
- Results in the initiation of a chain of cellular and systemic events which helps the cells to restore their homeostatic balance.

Stress

- **Stress**: Optimum range of factors- where best growth and development is observed. Deviation from the range affect physiology. Plants growing are then in stress or tension.
- All stress produce injury- the stress syndrome or **strain**
- Plant can not escape unfavourable condition by moving away like animal does. They must tolerate
- All biotic and abiotic factors may cause stress- are **stress factors**
- **Tolerance to stress**- by whole plant or by organs of plant- become normal when stress is over
- Acclimation and hardening

- **Zero stress**: exposure to most favourable condition
- All plants face environmental stress
- **Elastic stress** (return to normal after stress when condition become normal) and **plastic stress** (do not return to normal after stress)
- **Resistance mechanism**: Tolerance or avoidance/constitutive (sunken stomata) or adaptive (ABA synthesis during stress) mechanism
- **Ephimerals-** avoid unfavorable conditions


Stress, strain and damage


Three types of strain resistance (stress resistance)

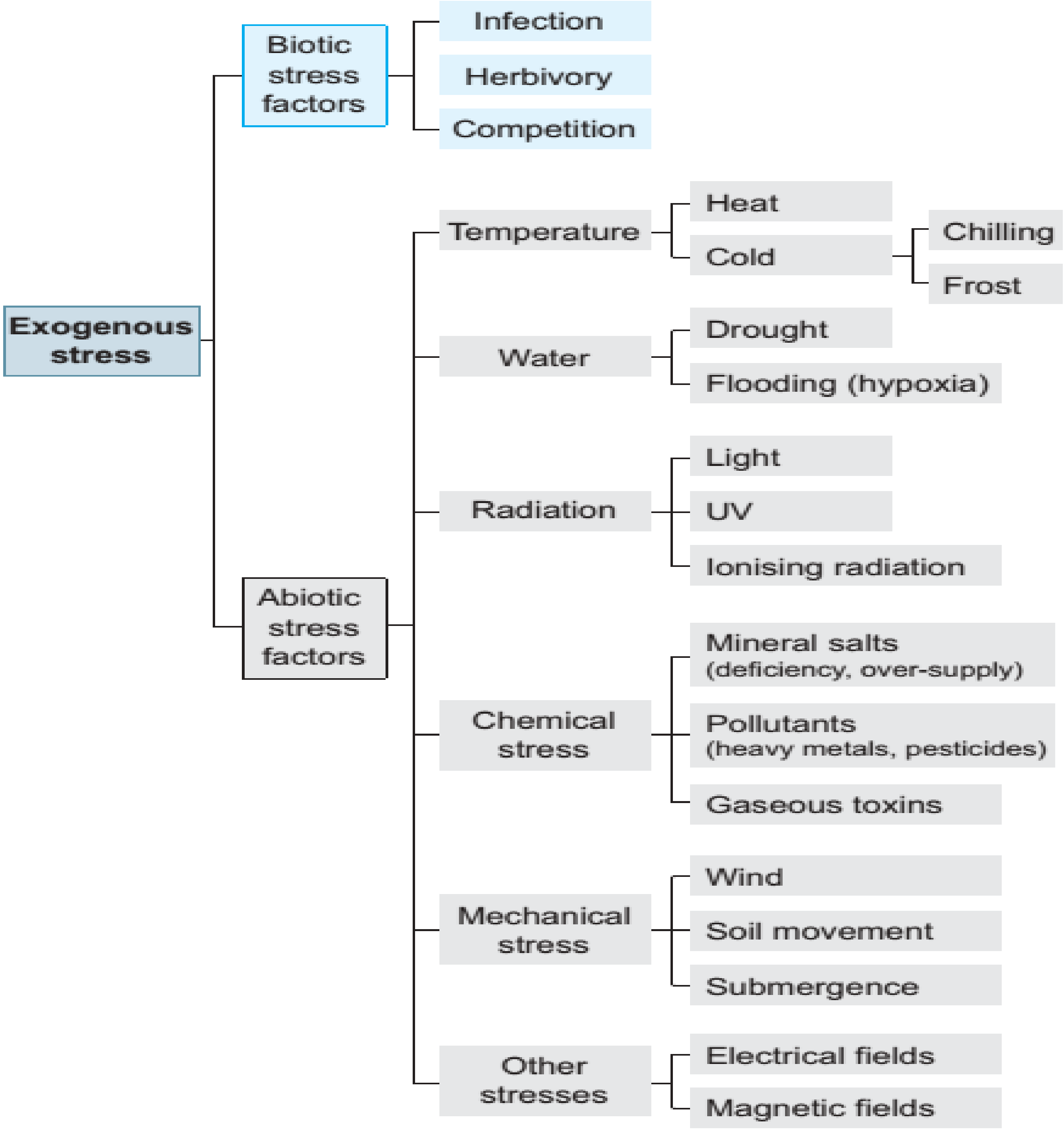
The physical stress concept of Levitt 1980

Plant stress

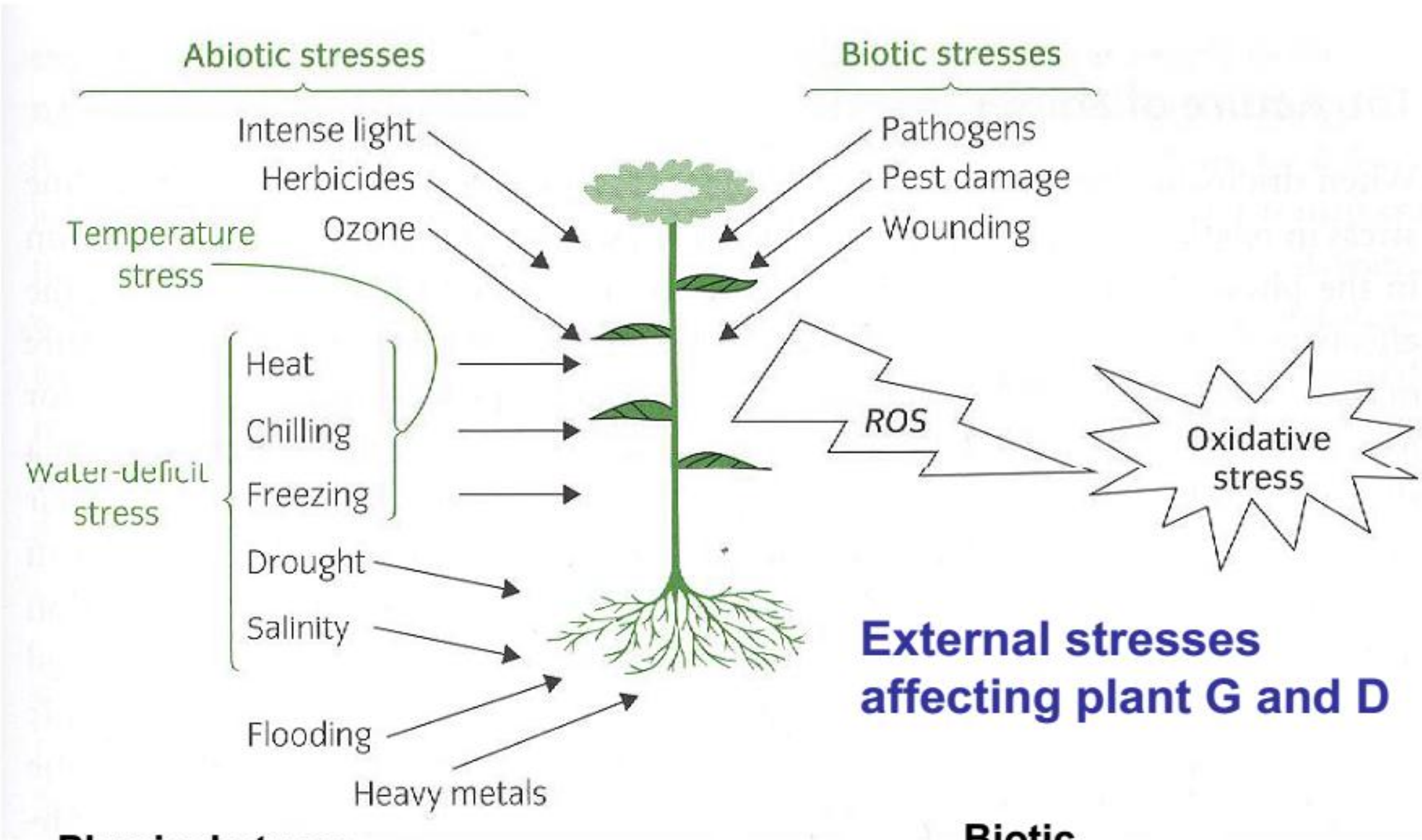
- Plants are bound to places.
- They, therefore, have to be considerably more adaptable to stressful environments and must acquire greater tolerance to multiple stresses than animals and humans.

Plant stress

- Two primary categories.
  - Abiotic stress is a physical (e.g., light, temperature) or chemical insult that the environment may impose on a plant.
  - Biotic stress is a biological insult, (e.g., insects, disease) to which a plant may be exposed during its lifetime



- **Exogenous stress**
  - Biotic stress factors
    - Infection
    - Herbivory
    - Competition
  - Abiotic stress factors
    - Temperature
      - Heat
      - Cold
        - Chilling
        - Frost
    - Water
      - Drought
      - Flooding (hypoxia)
    - Radiation
      - Light
      - UV
      - Ionising radiation
    - Chemical stress
      - Mineral salts (deficiency, over-supply)
      - Pollutants (heavy metals, pesticides)
      - Gaseous toxins
    - Mechanical stress
      - Wind
      - Soil movement
      - Submergence
    - Other stresses
      - Electrical fields
      - Magnetic fields



External stresses affecting plant G and D

Physical stress

Drought Temperature

Radiation Flooding etc

Chemicals

Pollutants nutrients

Pesticides salt pH

Biotic

Competition allelopathy

Disease Insects

Symbiosis

Plant stress

- Plant productivity is greatly influenced by environmental stresses, such as freezing, drought, salinity and flooding.
- One of the ways in which tolerance to these factors can be achieved is by the transfer of genes encoding protective proteins or enzymes from other organisms.
- Key approaches currently being examined are engineered alterations in the amounts of osmolytes and osmoprotectants, saturation levels of membrane fatty acids, and rate of scavenging of reactive oxygen intermediates.

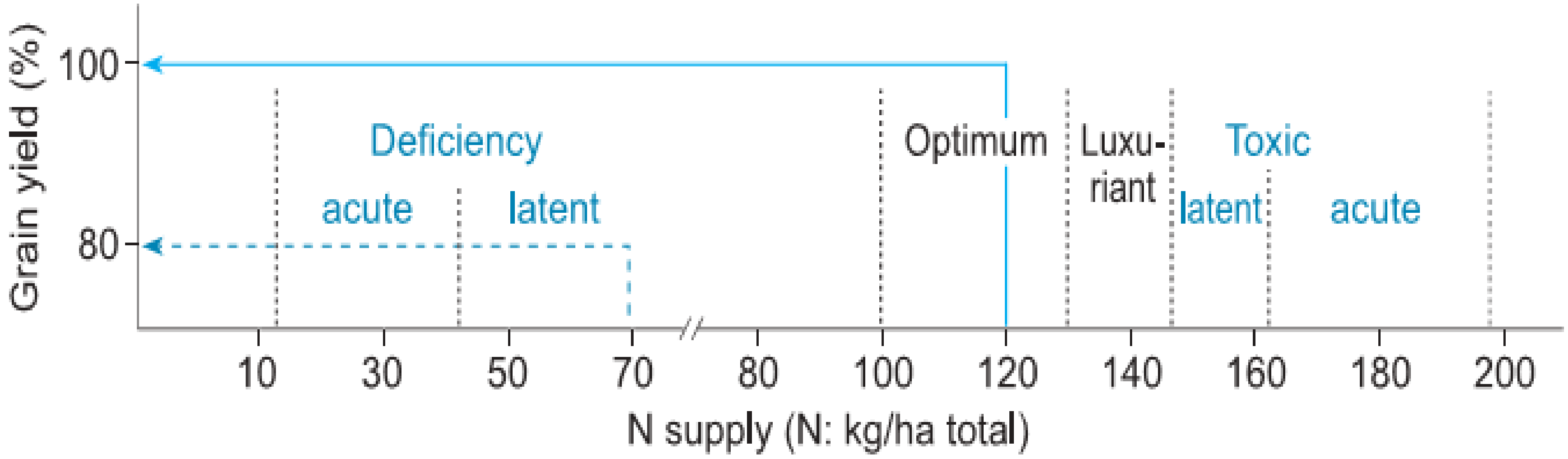
Symptoms of deficiency

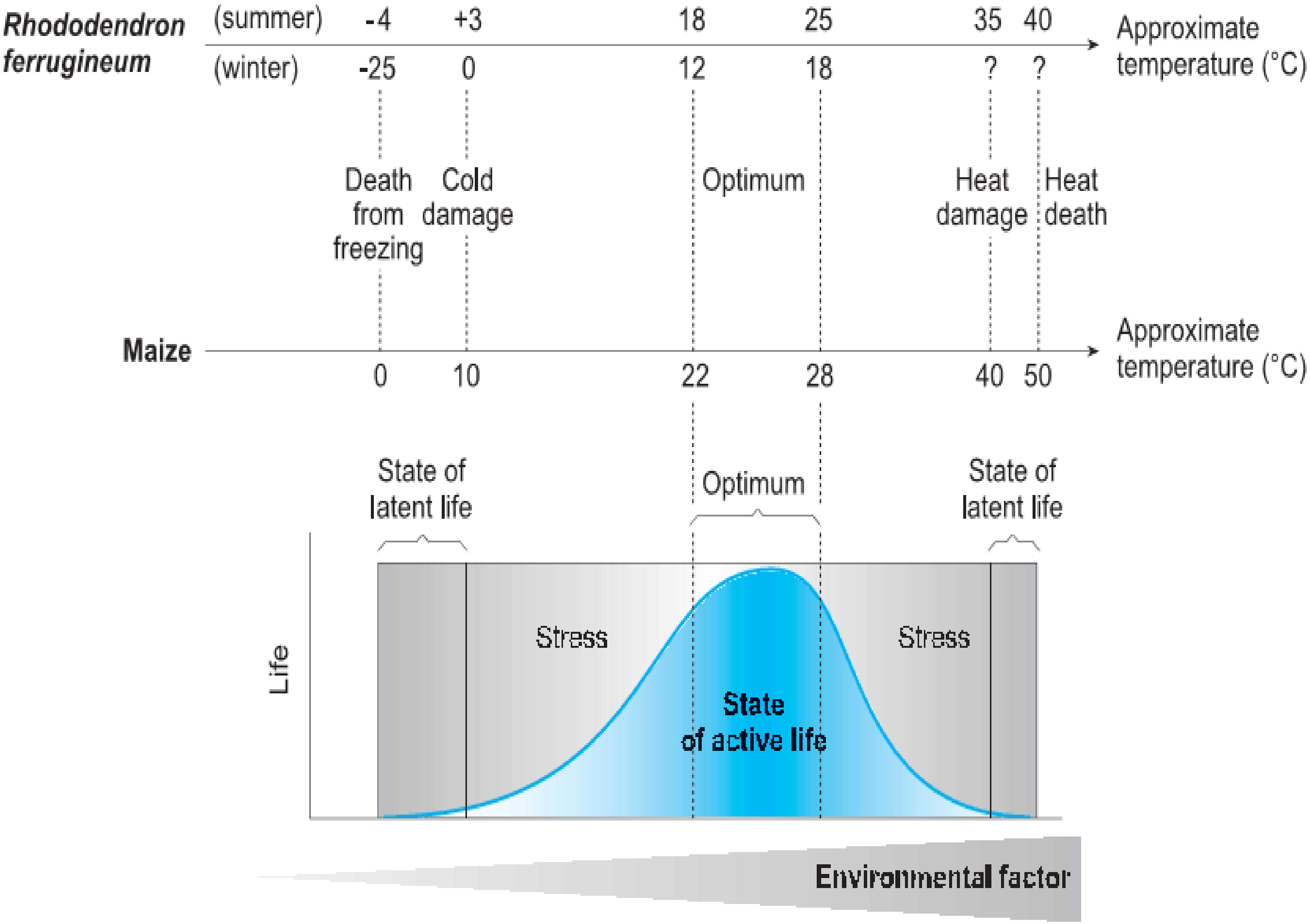
Stunted growth, small pale leaves, stiff habitus, root/shoot ratio large, lodging resistance high, premature ripening.
Limited reproductive production.
Reduced resistance to drought, increased susceptibility to fungal infections.

Symptoms of excess fertilisation

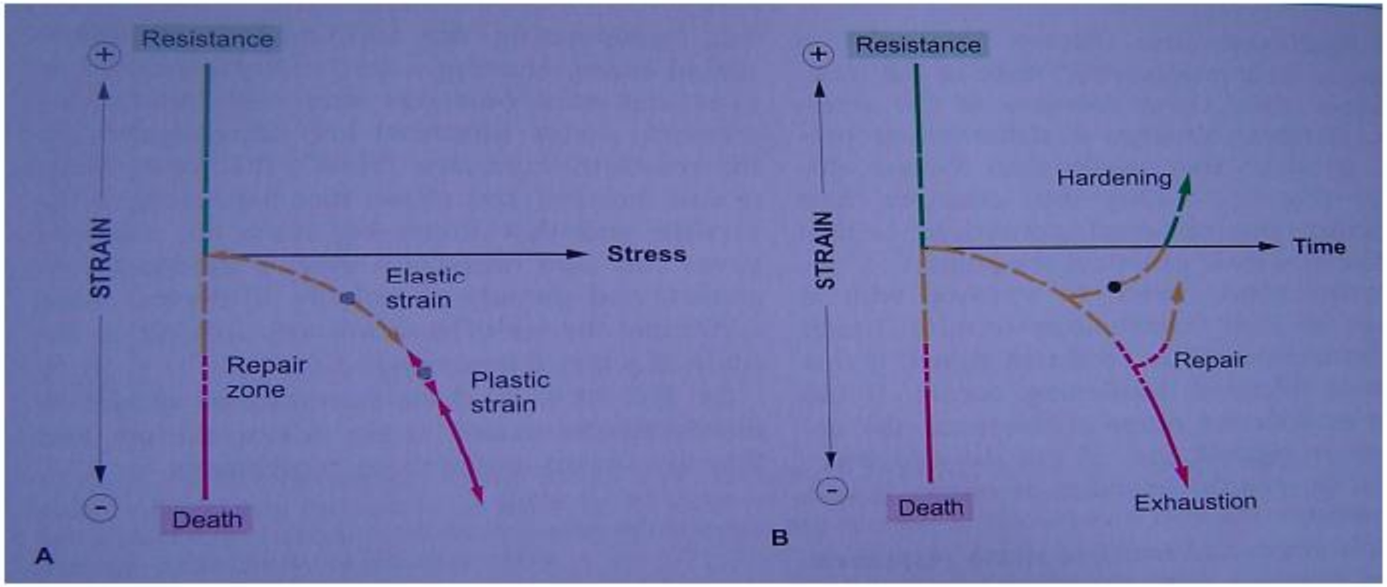
Luxuriant, large deep green leaves, soft growth, root/shoot ratio small, lodging resistance low (often lodges), maturation delayed.
Limited reproductive production.
Reduced resistance to drought, increased susceptibility to fungal infections.

Winter wheat: N requirements in spring




Rhododendron ferrugineum
(summer) -4 +3 18 25 35 40
(winter) -25 0 12 18 ? ?
Approximate temperature (°C)
Death from freezing
Cold damage
Optimum
Heat damage
Heat death
Maize
0 10 22 28 40 50
Approximate temperature (°C)
State of latent life
Optimum
State of latent life
Life
Stress
Stress
State of active life
Environmental factor

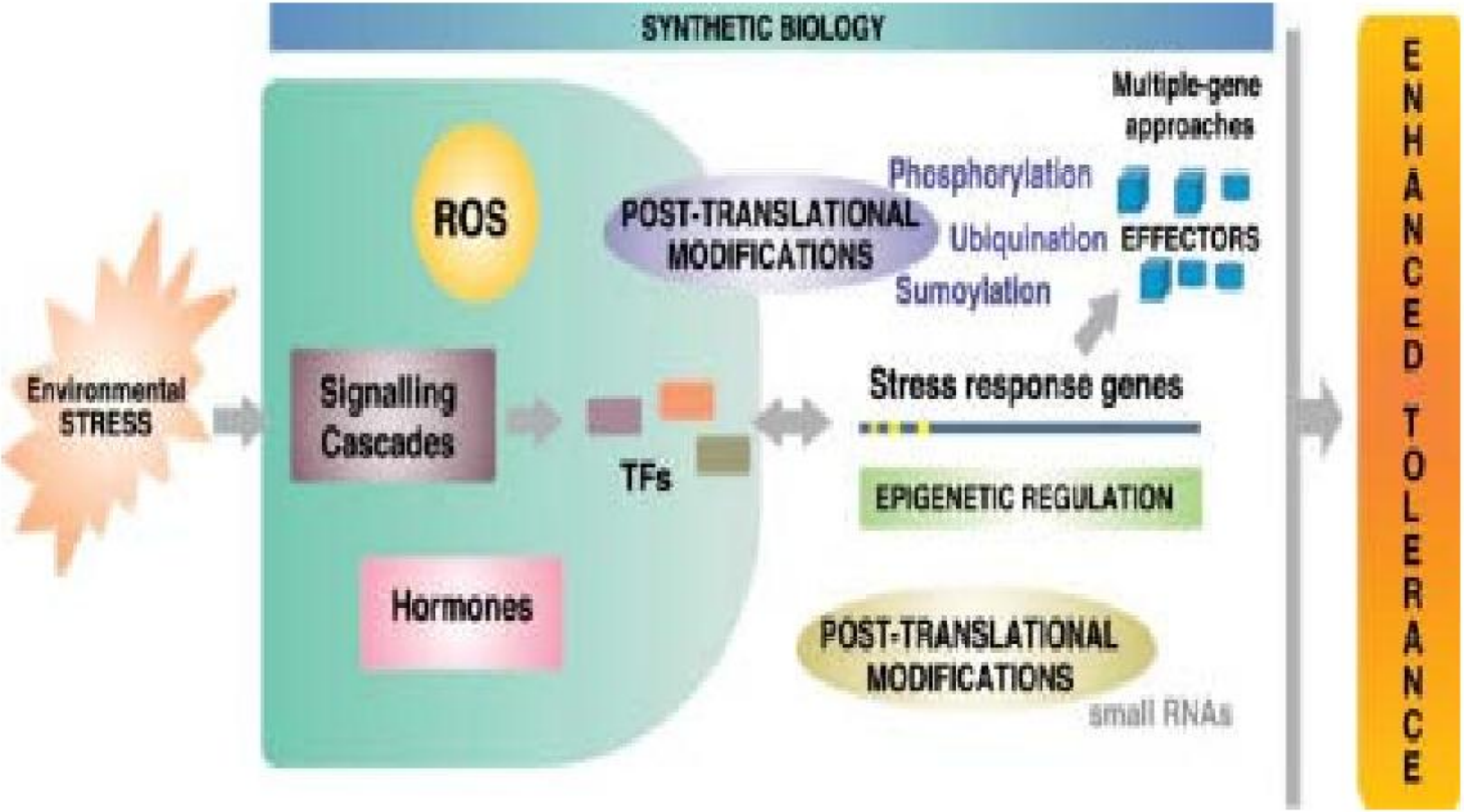
Elastic strain, repair and plastic strain



- Elastic strain changes to plastic strain depending on intensity and duration of stress
- Plastic strain is not completely reversible
- If the strain is tolerated, hardening occurs

Perception of stress and signals

- How is stress detected? How is the signal triggering stress reaction produced?
- Degradation products of pathogen wall or host cell wall termed **elicitors**-trigger response.
- How abiotic stress become a signal? Little known.
- Osmotic stress(Adapt 0.1 to 5,5 M NaCl) in *Dunaliella salina* algae without form cell wall is perceived by transitional shrinkage and adopted by accumulation of Glycerol up to 60% of cell weight and avoid change in cell volume within 30 -120 min.-**Censor located in plasmamembrane** High sterol (40%) content of PM seems important for signal creation for salt stress
- Hardening is seen without perception of stress-short day length for winter hardening in pine or low temperature at Long day also leads to frost hardening- both factors act synergetically in nature.



SYNTHETIC BIOLOGY

Environmental STRESS

ROS

Signalling Cascades

TFs

Hormones

POST-TRANSLATIONAL MODIFICATIONS

Phosphorylation

Ubiquination

Sumoylation

Multiple-gene approaches

EFFECTORS

Stress response genes

EPIGENETIC REGULATION

POST-TRANSLATIONAL MODIFICATIONS

small RNAs

ENHANCED TOLERANCE

Causes of loss	**Payment (%)**
Drought	40.8
Excess water	16.1
Cold	13.8
Hail	11.3
Wind	7.0
Insect	4.5
Disease	2.7
Flood	2.1
Other	1.5

Causes of Stress

- Inappropriate dosage (Light, Temperature, Water, Nutrients, Carbon dioxide and Oxygen)
- Environmental Noxae (UV-B, ozone, ionising radiation, xenobiotics, heavy metals and aluminium.)
- Endogenous stress

- Usually, an organism is subjected to several stress factors, e.g. lack of water and heat, or a “secondary” stress factor follows a “primary” one:
- When the plant lacks water and closes its stomata, internal CO_2 deficiency occurs when the plant is illuminated, and as a further consequence oxidative stress ensues. Combination of several stress factors is the normal case and is referred to as **multiple stress.**
- Upon elevated temperature, the modification of the basic metabolism could be interpreted as an **unspecific** reaction, whilst the production of heat shock proteins would be considered a **specific stress reaction** of the organism.
- There is yet another facet to the question of specificity of stress reactions which is described by the term **cross-protection**. Previous drought stress or salt stress (osmotic stress) is known to harden plants against temperature stress, and particularly cold stress.
- Potato plants treated with NaCl are able to tolerate lower temperatures than untreated controls. A transient increase in ABA concentration mediates this **hardening reaction**.

Biotic stress

Herbivory/infection

- Selective and some plants are not touched or used by special herbivors-**poisonous** plants
- Poisonous plants are protected by constitutively formed special chemicals- secondary metabolites accumulated in vacuoles or cell wall. Allakaloides (>6000), terpenoides (>5000), steroides (bittering agent), phenolic compounds (tanins), glycosides.
- Do not provide complete protection as some able to detoxify these compounds
  - Liver enzyme rhodonase of some animal detoxify cynogenic glycoside changing to thiocyanate
  - Butterfly *Zygaena* spp accumulate Cyanogenic glycoside and protect themselves by releasing HCN.
  - Chemical defence of plant and adaptation to defence- evolution
  - Mustard oil produced by cabbage is defence chemical but works signal for female butterfly *Pieris* to food and oviposition
- **Induced defence response**: mechanical injuries induce electric/ biochemical reaction. Long distance signal transduction path way that lead to induction of gene expression for defence. Callus in wound, inhibitor proteins -induced locally and systemically

Induced defence protein

- JA as stress hormone exhibits pleiotrophic effects, Induce senescence synersistically with ABA and Et.
- Jaxmonate induced proteins (JIP): Pin, PR proteins, phytoalexins producing enzymes, dehydrins, and induce genes, also induced by ABA and Et.
- Jasmonate increase transiently but defence is permanent due to synthesis of biologically active compounds also in non-wounded c**ells-systemic reaction/immunization**
- Promoters of *Pin 2* and *LOX1*are analysed. Element TGACG found in *LOX 1* promoter necessary for JA induction-cis element TF with bZIP element
- Plant distinguish mechanical and phytopathogenous wounds-Pin mRNA accumulate slow in mechanical.
- Specific proteins patterns induced by different insects-may be different elicitors

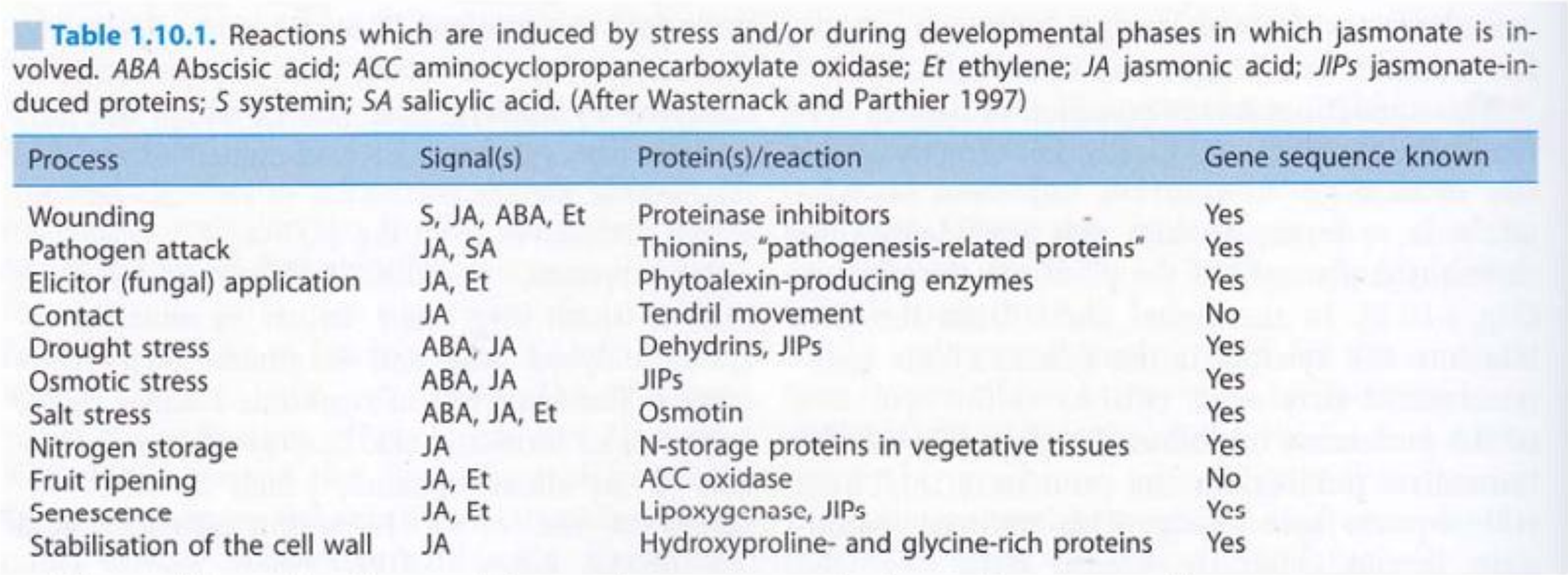


Table 1.10.1. Reactions which are induced by stress and/or during developmental phases in which jasmonate is involved. *ABA* Abscisic acid; *ACC* aminocyclopropanecarboxylate oxidase; *Et* ethylene; *JA* jasmonic acid; *JIPs* jasmonate-induced proteins; *S* systemin; *SA* salicylic acid. (After Wasternack and Parthier 1997)

Process	Signal(s)	Protein(s)/reaction	Gene sequence known
Wounding	S, JA, ABA, Et	Proteinase inhibitors	Yes
Pathogen attack	JA, SA	Thionins, "pathogenesis-related proteins"	Yes
Elicitor (fungal) application	JA, Et	Phytoalexin-producing enzymes	Yes
Contact	JA	Tendril movement	No
Drought stress	ABA, JA	Dehydrins, JIPs	Yes
Osmotic stress	ABA, JA	JIPs	Yes
Salt stress	ABA, JA, Et	Osmotin	Yes
Nitrogen storage	JA	N-storage proteins in vegetative tissues	Yes
Fruit ripening	JA, Et	ACC oxidase	No
Senescence	JA, Et	Lipoxygenase, JIPs	Yes
Stabilisation of the cell wall	JA	Hydroxyproline- and glycine-rich proteins	Yes

Reaction induced by stress and development

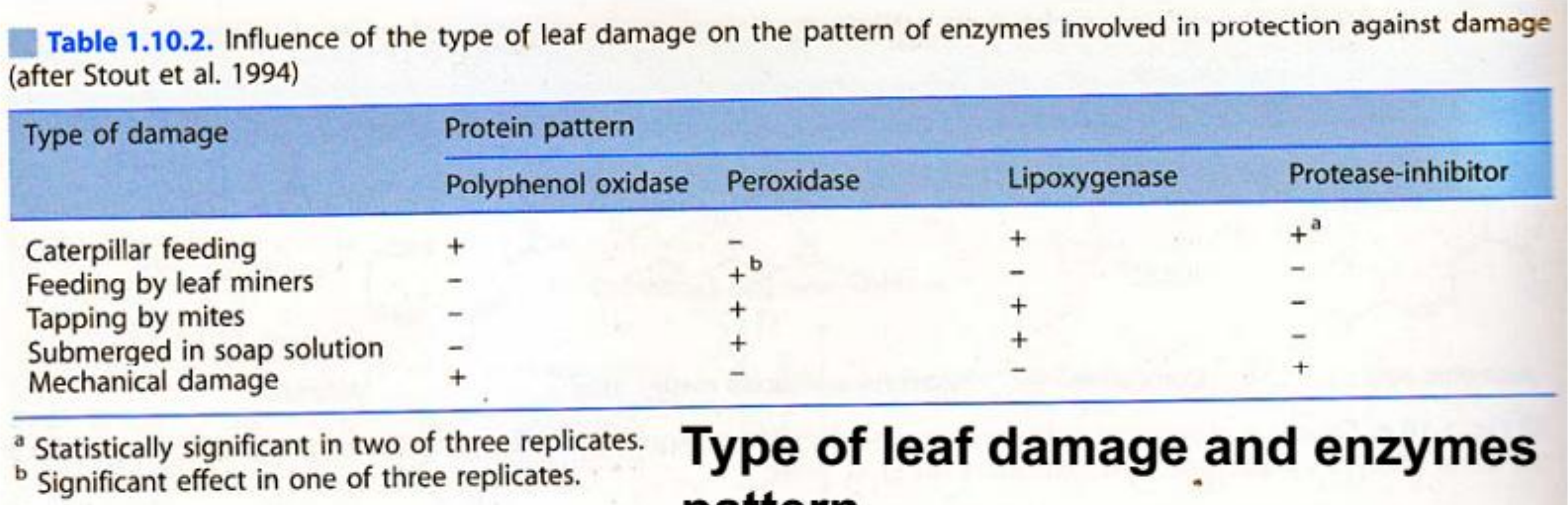


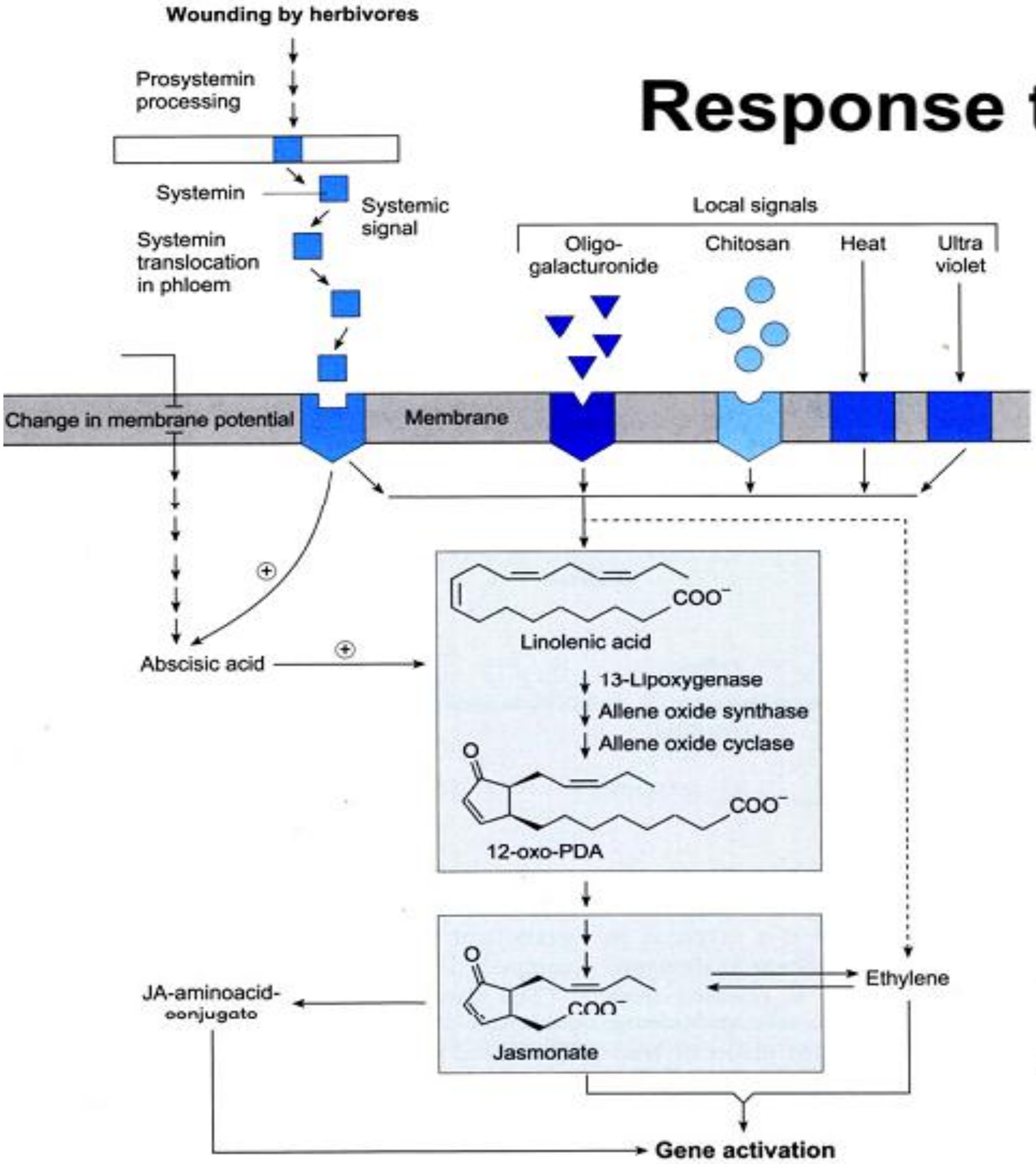
Table 1.10.2. Influence of the type of leaf damage on the pattern of enzymes involved in protection against damage (after Stout et al. 1994)

Type of damage	Protein pattern			
	Polyphenol oxidase	Peroxidase	Lipoxygenase	Protease-inhibitor
Caterpillar feeding	+	–	+	+[a]
Feeding by leaf miners	–	+[b]	–	–
Tapping by mites	–	+	+	–
Submerged in soap solution	–	+	+	–
Mechanical damage	+	–	–	+

[a] Statistically significant in two of three replicates.
[b] Significant effect in one of three replicates.

Type of leaf damage and enzymes pattern

Response to wound

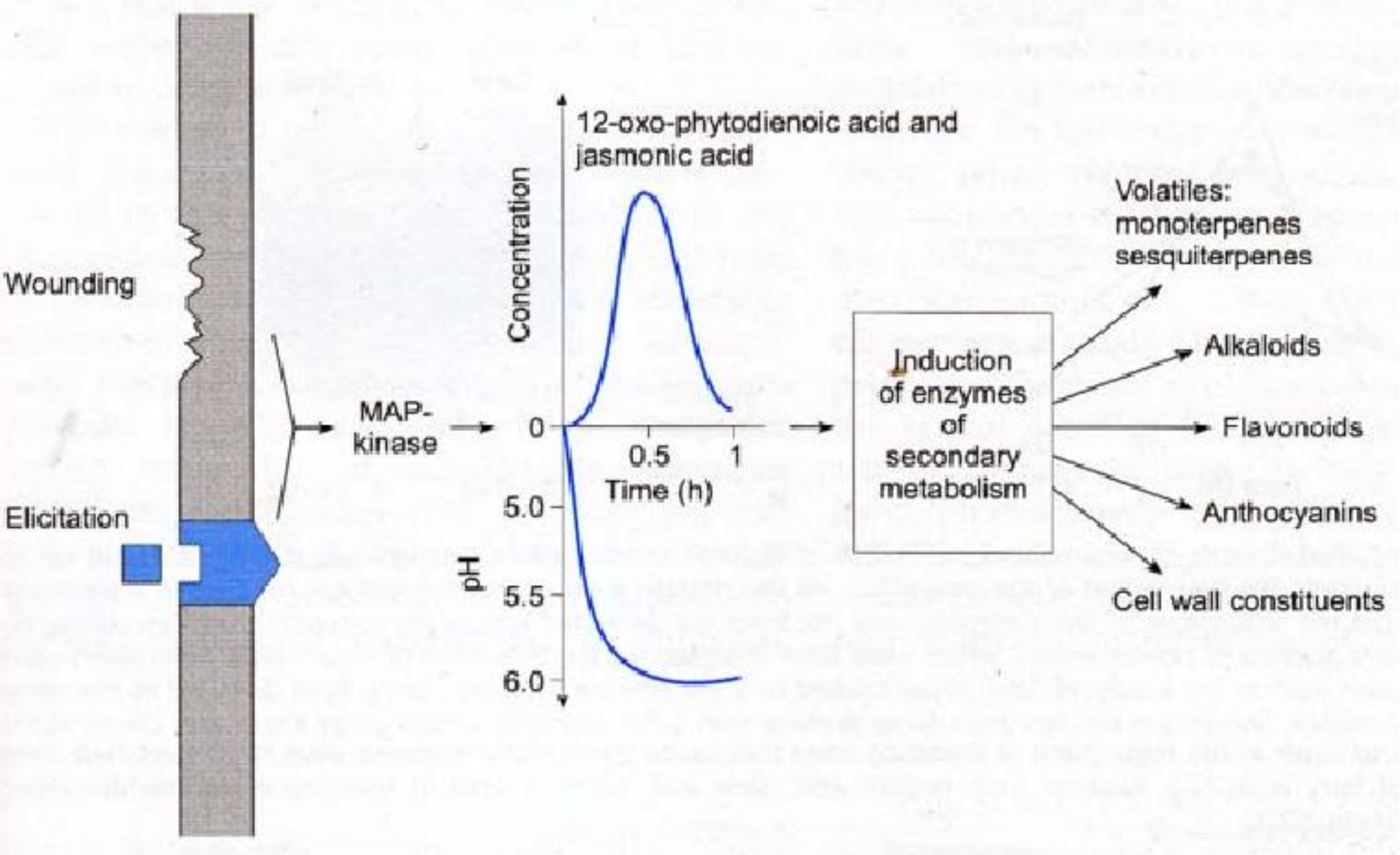


Both herbivory and infection cause wound

Responses are induced

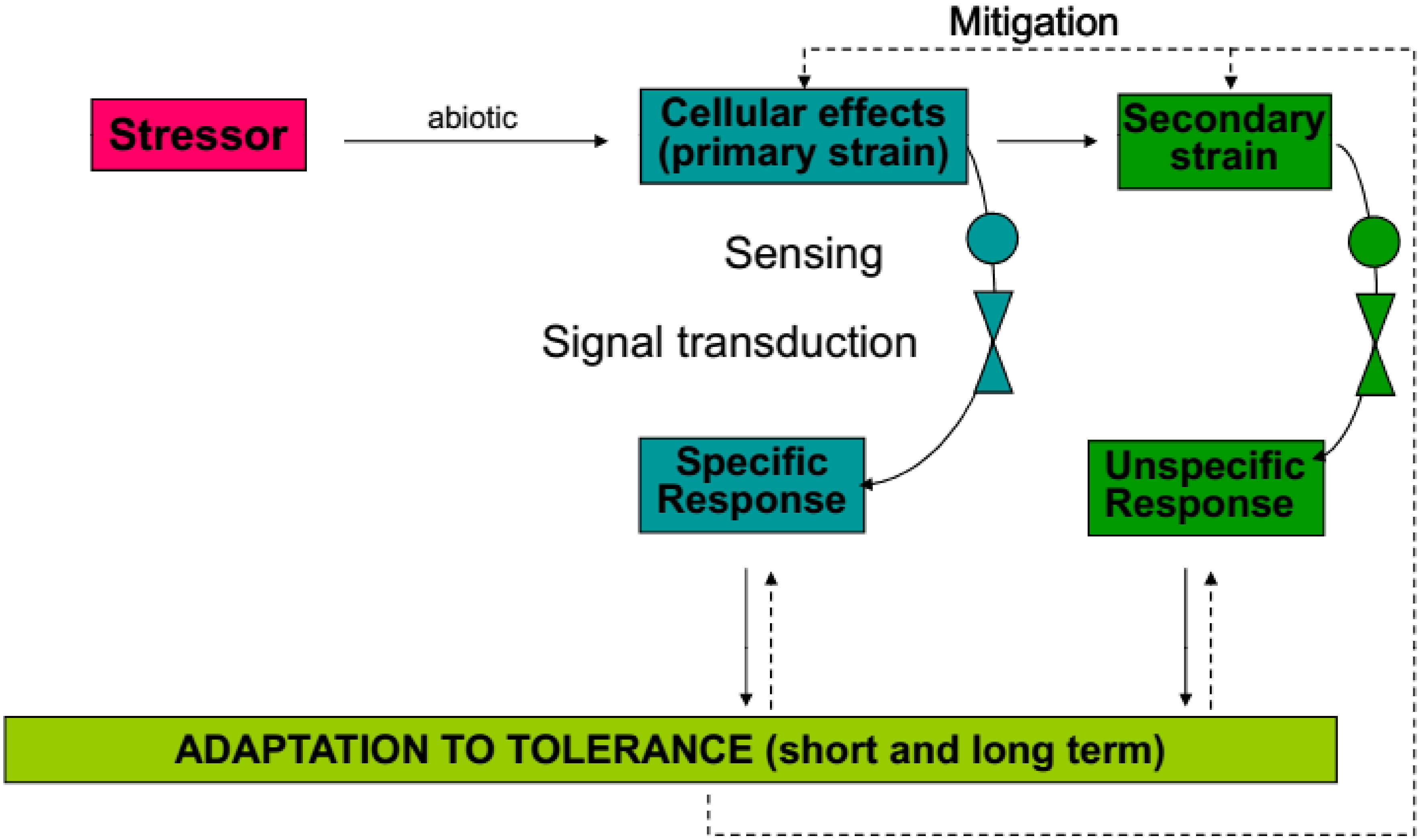
- Local
- Systemic

Induction of systemic defence



Secondary metabolites induced by JA. Wounding and elicitation leads to increase in external pH and transient rise in 12OPDA and JA, that induce gene expression encoding enzymes for sec. metabolite synthesis

General stress concept



Mitigation

Stressor

abiotic

Cellular effects (primary strain)

Secondary strain

Sensing

Signal transduction

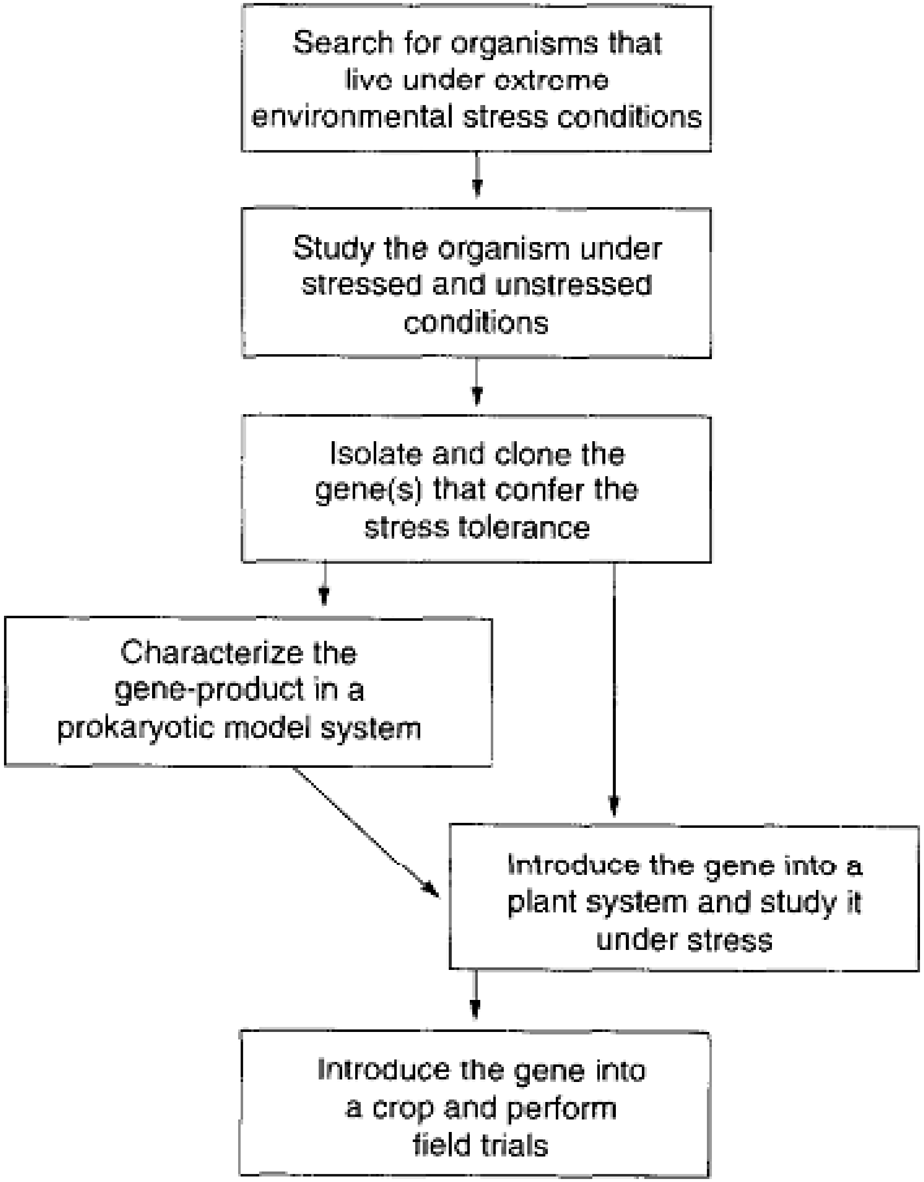
Specific Response

Unspecific Response

ADAPTATION TO TOLERANCE (short and long term)

Short: Biochemical and long: morphological

Box 1. Strategy for creating a more stress-tolerant plant using genetic engineering[a]



Abiotic stress tolerance in plants

Several abiotic stresses (heat, chilling, freezing , drought, salinity) lead to water deficit conditions

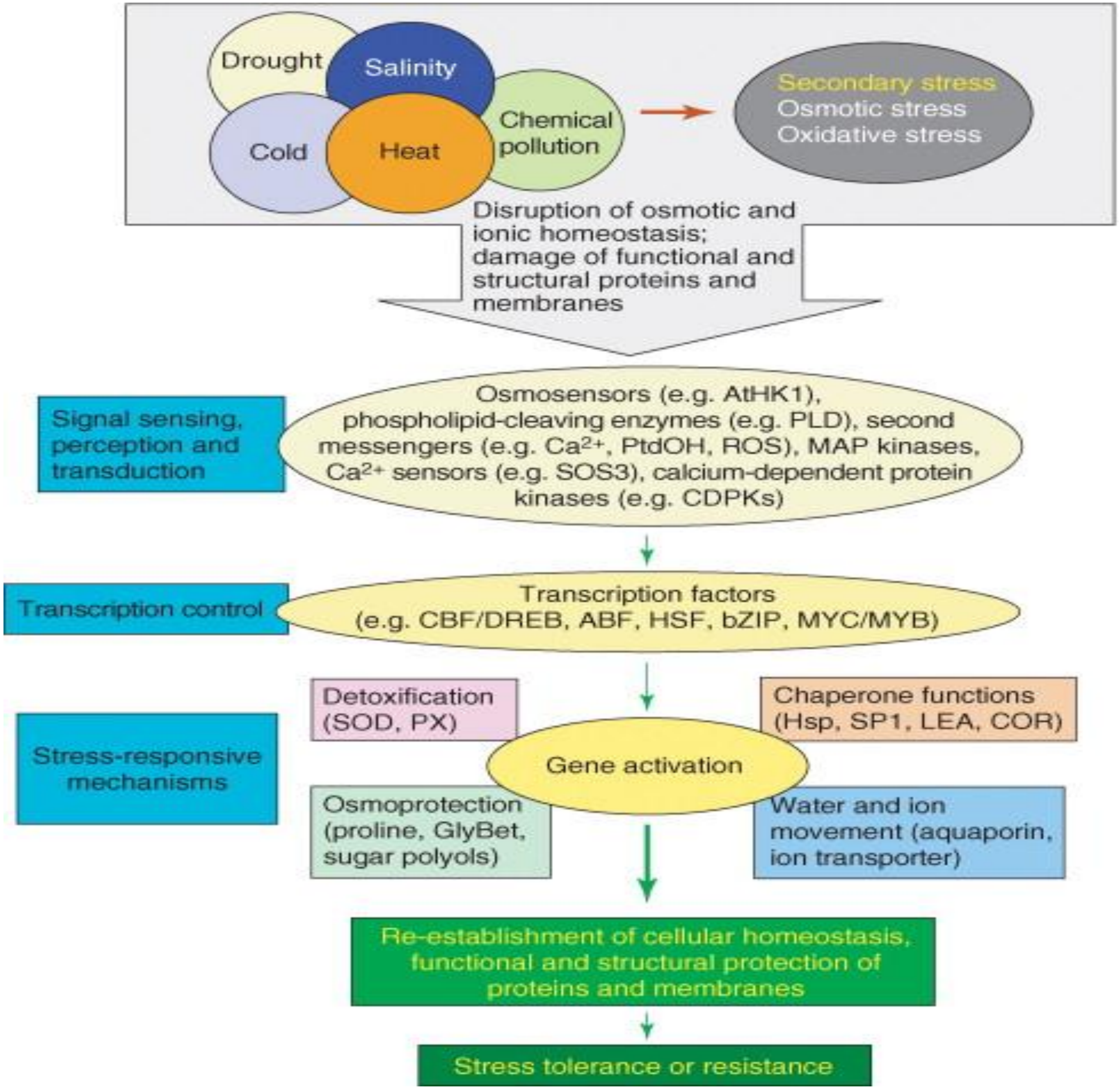
Yield of Field grown crops due to sub optimal abiotic factors in USA is only 22% of the genetic potential (Boyer, 1982)

Plants are devised with means to cope up with various stresses (avoidance/tolerance), but ability of plants to respond to stresses varies with species, environment (eg. optimal temp for growth of pea and soybean is 20 and 30 ^{0}C, resp: as temp increases pea shows signs of heat stress much sooner than soybean)

Stress avoidance mechanisms like restriction of growth to periods of high water availability (as in ephemerals), storage of water (CAM plants) or prevention of excessive water loss as in grasses, CAM plants.

Intrinsic tolerance developed by specific stress reactions like water storage, synthesis of osmolytes, biochemical and mechanical means.

Conventional breeding to accomplish improving abiotic stress tolerance due to quantitative nature of stress tolerance genes and breeding with more distant (more tolerant) relatives runs the risk of introducing undesired traits


Drought

Salinity

Cold

Heat

Chemical pollution

Secondary stress
Osmotic stress
Oxidative stress

Disruption of osmotic and ionic homeostasis; damage of functional and structural proteins and membranes

Signal sensing, perception and transduction

Osmosensors (e.g. AtHK1), phospholipid-cleaving enzymes (e.g. PLD), second messengers (e.g. Ca^{2+}, PtdOH, ROS), MAP kinases, Ca^{2+} sensors (e.g. SOS3), calcium-dependent protein kinases (e.g. CDPKs)

Transcription control

Transcription factors (e.g. CBF/DREB, ABF, HSF, bZIP, MYC/MYB)

Stress-responsive mechanisms

Detoxification (SOD, PX)

Chaperone functions (Hsp, SP1, LEA, COR)

Gene activation

Osmoprotection (proline, GlyBet, sugar polyols)

Water and ion movement (aquaporin, ion transporter)

Re-establishment of cellular homeostasis, functional and structural protection of proteins and membranes

Stress tolerance or resistance

Complexity of plant response to Abiotic stress

Drought tolerance

- All plant show certain level of tolerance to drought
- Water deficit also by salt stress and frost. In Seed development upto 90%water loss necessary so drought is regular component of plant development
- Constitutive tolerance in plant adapted to continuous drying (xerophytes)
- Mesophytes are generally non-drought tolerance
- Drought stress when their RWC is decreased to 50%.
- 50% water loss for long period is detrimental in mesophytes
- Resurrection plant (*like Craterostigma plantaginea*) survive also when lose 90%. Show constitutive tolerance to drought stress. A gene regulated character

Changes in water loss

- Plasmoysis: Shrinkage of protoplast
- Cellular solution concentrate
- Decrease or loss of turgid condition
- Changes in water potential gradient across membrane
- Disintegration of biomembrane and denaturation of protein in the worst condition
- Prolonged drought stress may cause death of cell.

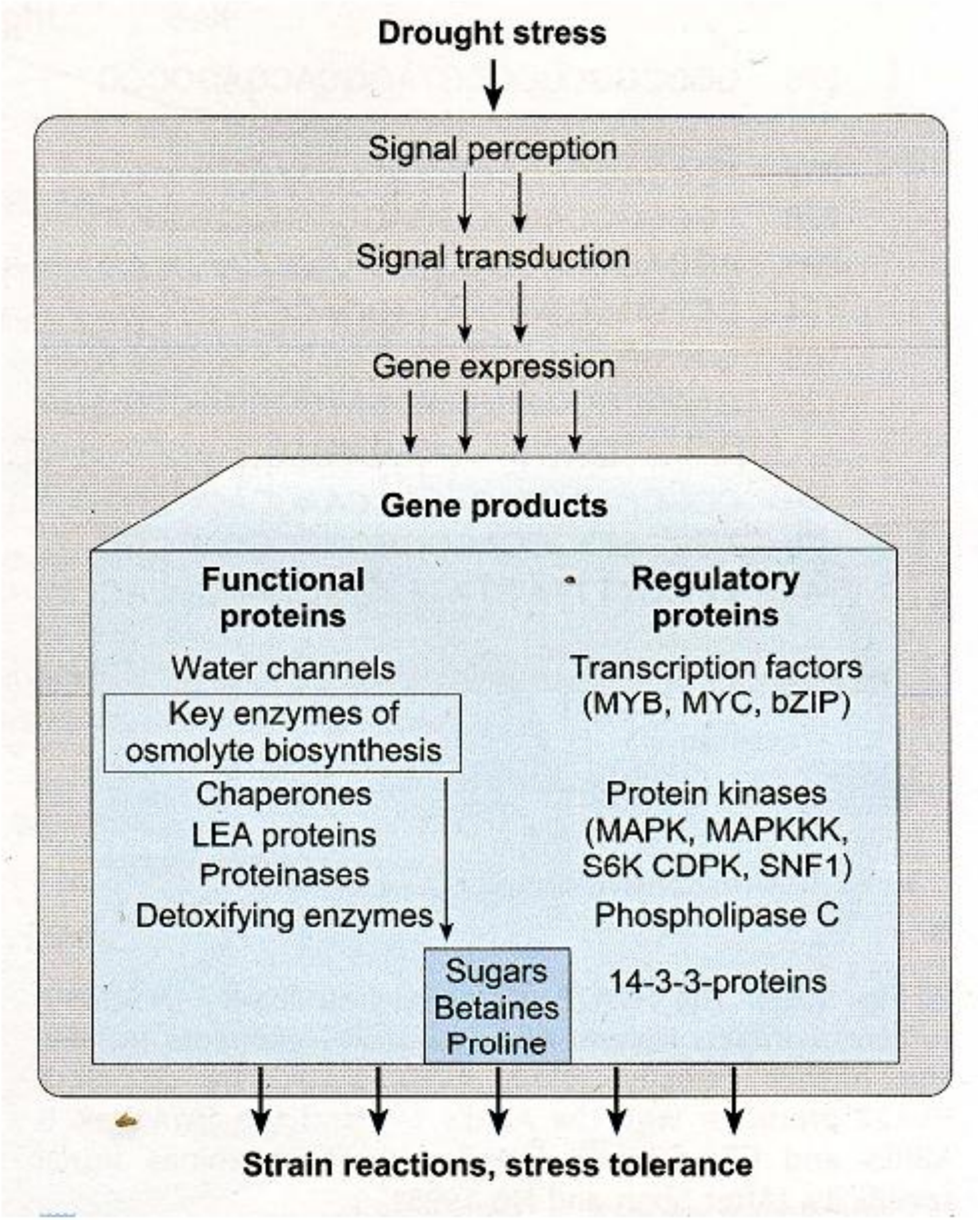
Perception of dehydration stress

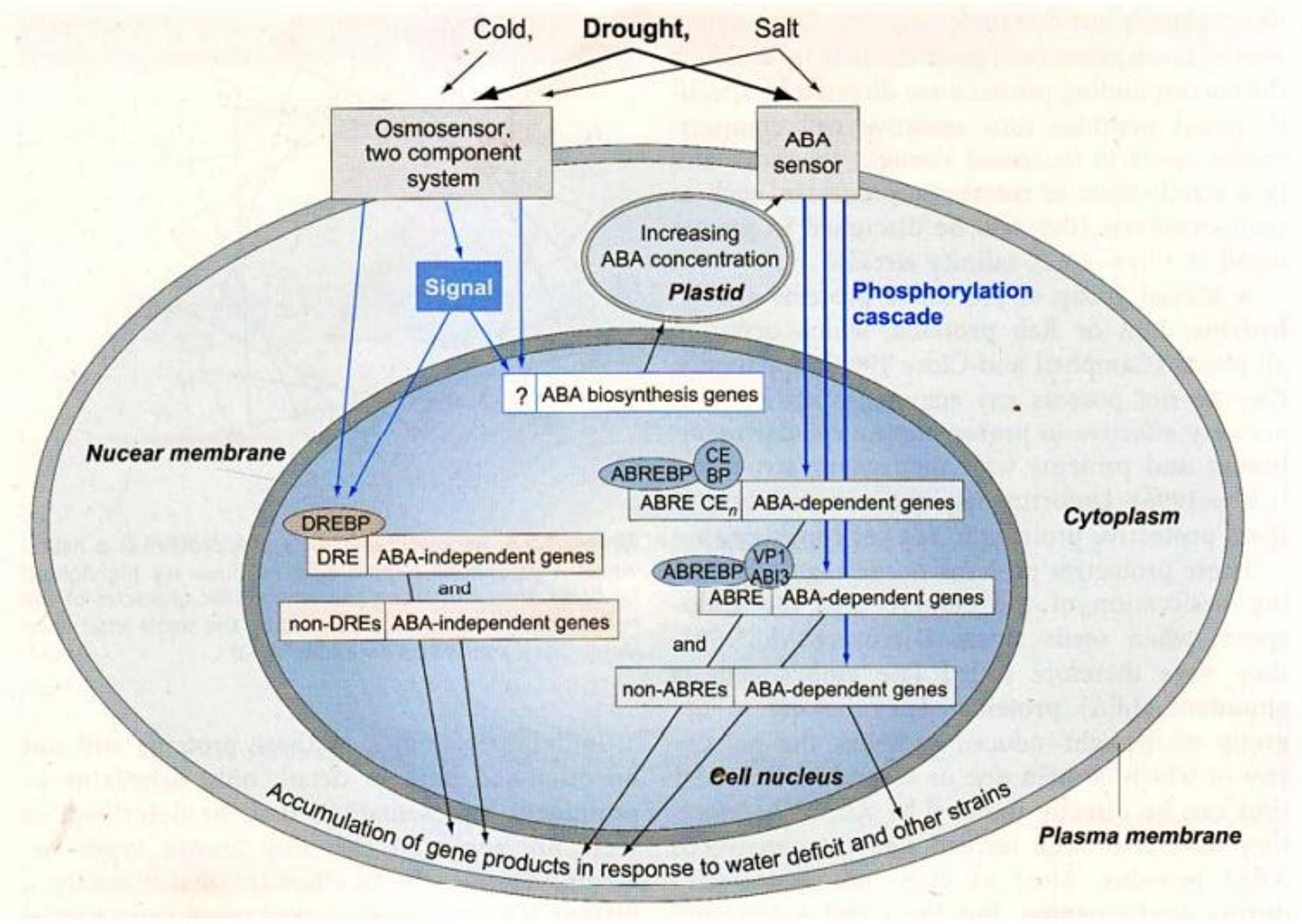
- Which of the above changes work as signal- probably change in water potential
- Osmosensers: known in *E.coli* and yeast. Yeast in dehydration the membrane peptide Sho1P activates Mitogen activated protein (MAP) kinase cascade leading to accumulation of osmoprotectant glycerol. Similar systems are reported in Arabidopsis.
- ABA mediated response: increase in drought, mutant lacking ABA are not viable as they wilt even in slightest drought stress.

Mechanism of drought tolerance

- All plants show certain tolerance to drought
- Drought tolerant plants show accumulation of ABA, sugar and osmolytes, and dehydration related proteins
- Protein synthesis is inhibited in drought but dehydration related proteins like dehydrin synthesis is promoted
- These compounds are important for stress tolerance
- Drought tolerant plant show rapid reaction and accumulate these compounds rapidly
- Drought is also a natural process- seed dry naturally in such seeds dehydrins are accumulated
- Same dehydrins are synthesised in other parts during drying

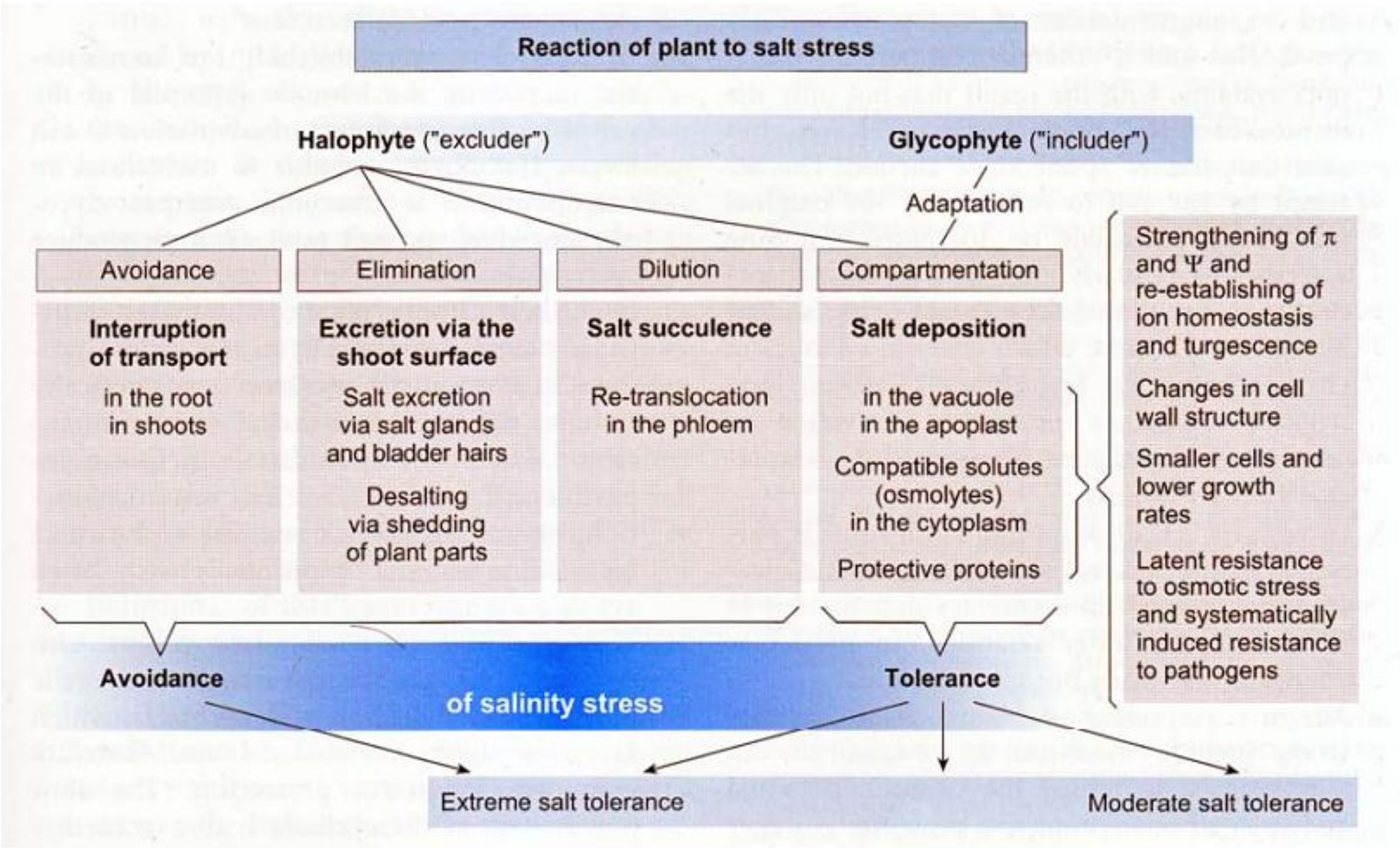
Response of plant cell to desiccation





Multiple pathways leading to induction of gene expression upon desiccation stress

mn-sod Mn-Superoxide dismutase	Dismutation of reactiveoxygen inter mediates in mitochondria	*M. sativa*	Transformants showed reduced injury from water deficit stress and increased winter survival
DREB1A (CBF3) DRE-binding protein	Transcription factor	*Arabidopsis*	Increased salt, drought and cold tolerance in nonacclimated plants
DREB1A (rd29A) DRE-binding protein	Stress-inducible promoter	*N. tabacum*	Improved drought and low-temperature stress tolerance
OsMYB3R-2 DNA-binding domain	Transcription factor	*Arabidopsis*	Overexpression of *OsMYB3R-2* leads to increased tolerance to freezing, drought, and salt stress



Reaction of plant to salt stress

Halophyte ("excluder")

Glycophyte ("includer")

Adaptation

Avoidance

Interruption of transport

in the root
in shoots

Elimination

Excretion via the shoot surface

Salt excretion via salt glands and bladder hairs

Desalting via shedding of plant parts

Dilution

Salt succulence

Re-translocation in the phloem

Compartmentation

Salt deposition

in the vacuole
in the apoplast

Compatible solutes (osmolytes) in the cytoplasm

Protective proteins

Strengthening of π and Ψ and re-establishing of ion homeostasis and turgescence

Changes in cell wall structure

Smaller cells and lower growth rates

Latent resistance to osmotic stress and systematically induced resistance to pathogens

Avoidance

Tolerance

of salinity stress

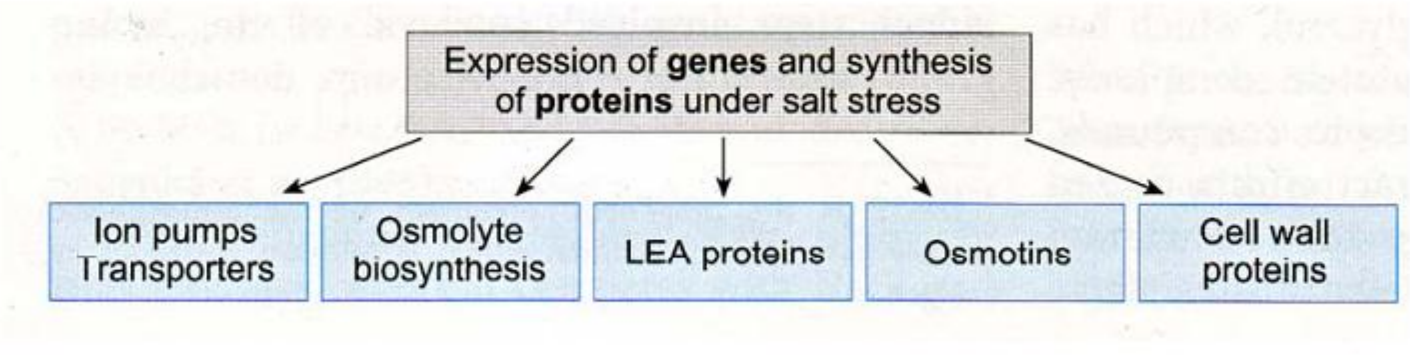
Extreme salt tolerance

Moderate salt tolerance

Plant reactions to salt stress

Adaptation

- Ion homisotasis: by salt elimination/avoidance
- Osmotic adjustment: producing osmolytes
- Induction of protective proteins: LEA proteins, osmotins



Expression of **genes** and synthesis of **proteins** under salt stress

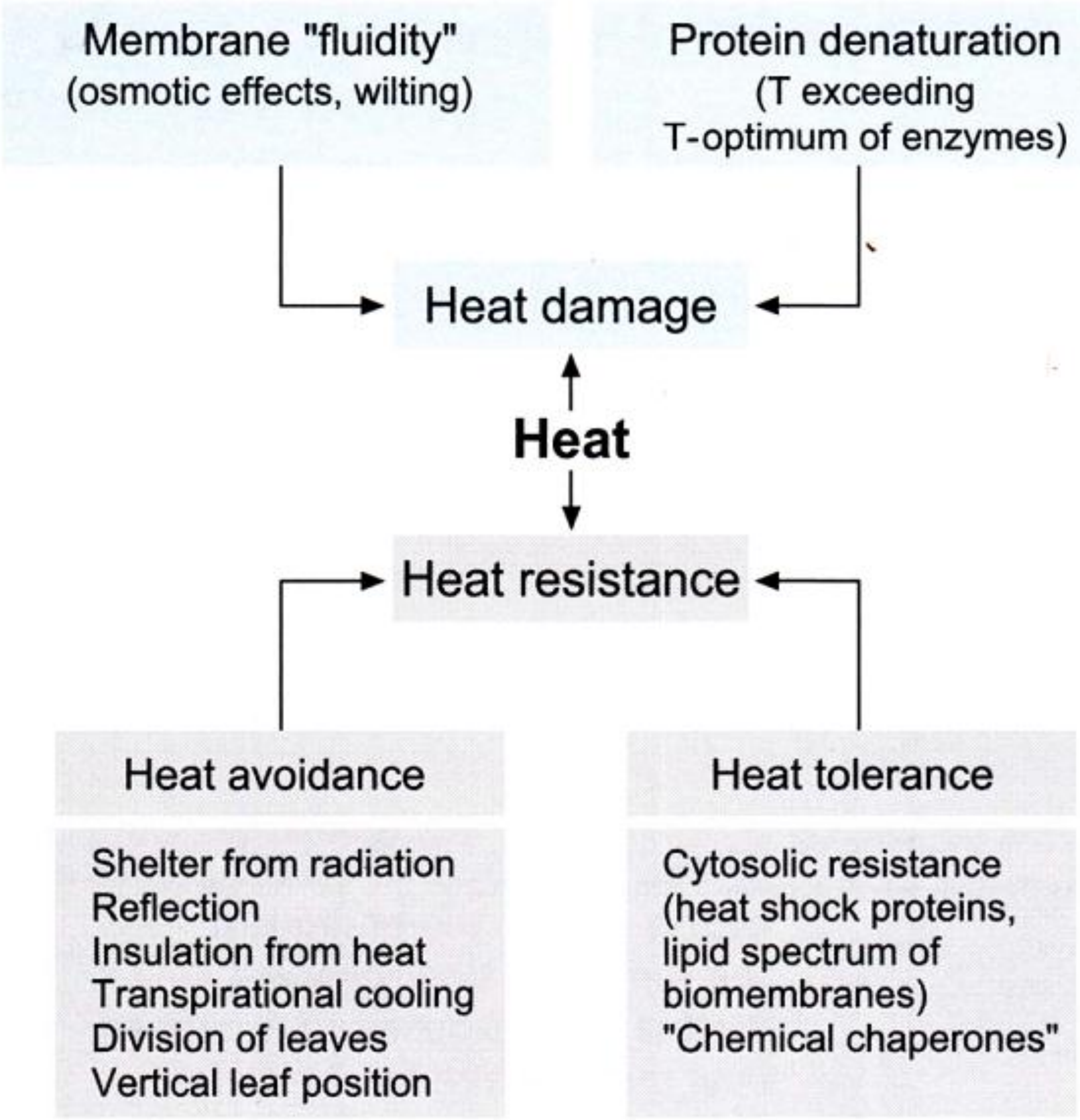
Ion pumps
Transporters

Osmolyte biosynthesis

LEA proteins

Osmotins

Cell wall proteins



Membrane "fluidity"
(osmotic effects, wilting)

Protein denaturation
(T exceeding
T-optimum of enzymes)

Heat damage

Heat

Heat resistance

Heat avoidance

- Shelter from radiation
- Reflection
- Insulation from heat
- Transpirational cooling
- Division of leaves
- Vertical leaf position

Heat tolerance

- Cytosolic resistance (heat shock proteins, lipid spectrum of biomembranes)
- "Chemical chaperones"

Effects of heat on plants and their reactions

Plant Adaptation to Heat stress

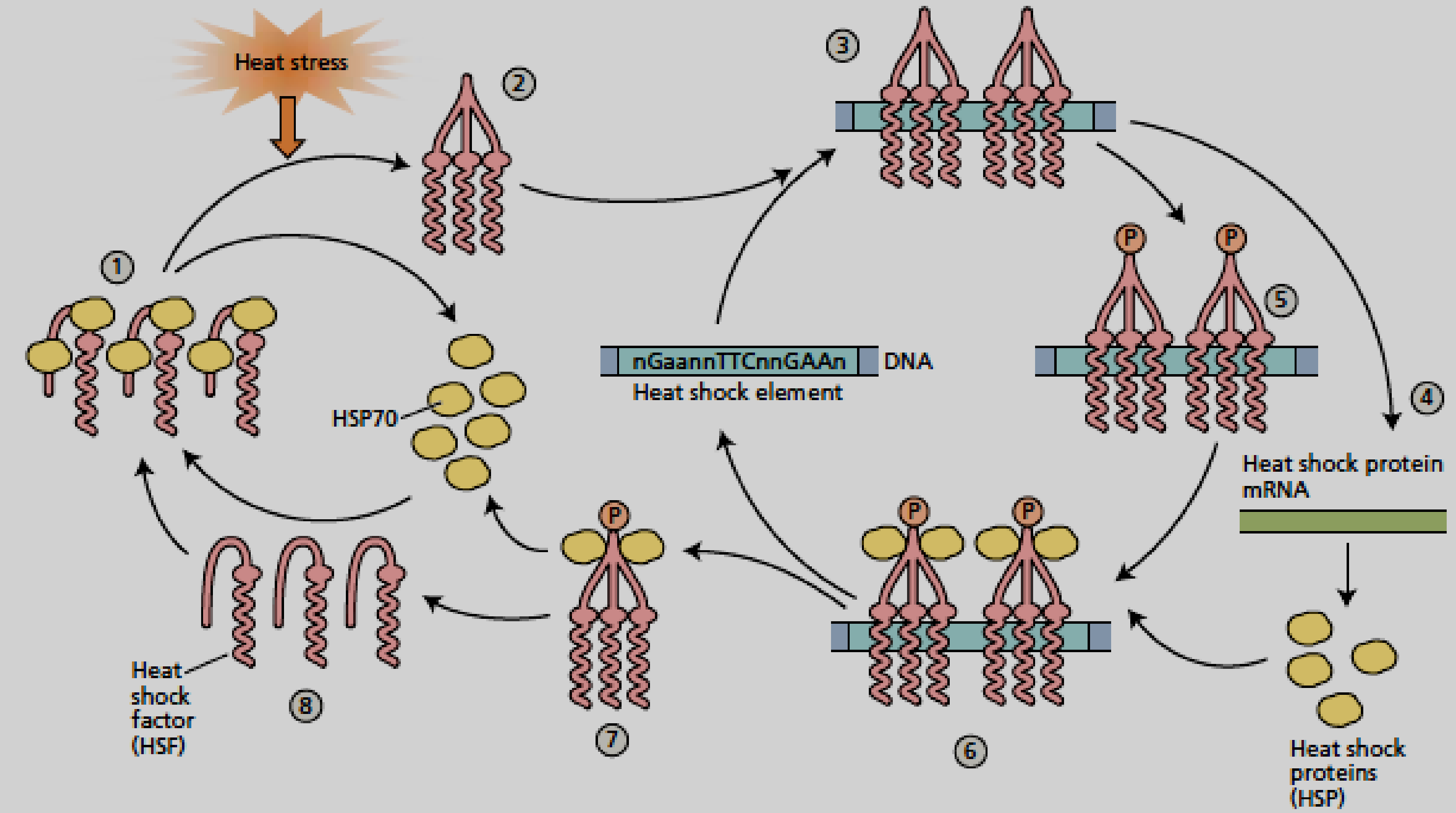


FIGURE 25.11 The heat shock factor (HSF) cycle activates the synthesis of heat shock protein mRNAs. In nonstressed cells, HSF normally exists in a monomeric state (1) associated with HSP70 proteins. Upon the onset of an episode of heat stress, HSP70 dissociates from HSF which subsequently trimerizes (2). Active trimers bind to heat shock elements (HSE) in the promoter of heat shock protein (HSP) genes (3), and activate the transcription of HSP mRNAs leading to the translation of HSPs among which are HSP70 (4). The HSF trimers associated with the HSE are phosphorylated (5) facilitating the binding of HSP70 to the phosphorylated trimers (6). The HSP70 trimer complex (7) dissociates from the HSE and disassembles and dephosphorylates into HSF monomers (8), which subsequently bind HSP reforming the resting HSP70/HSF complex. (After Bray et al. 2000.)

Plant Adaptation to water/osmotic stress

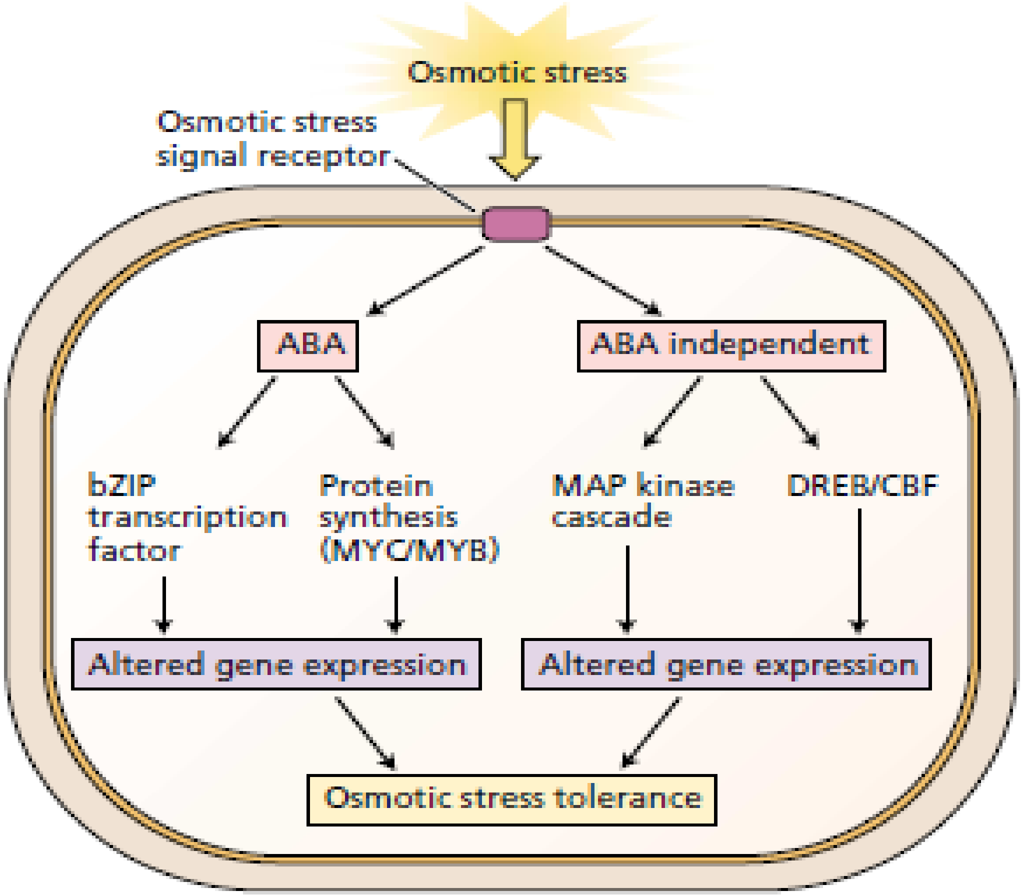


FIGURE 25.9 Signal transduction pathways for osmotic stress in plant cells. Osmotic stress is perceived by an as yet unknown receptor in the plasma membrane activating ABA-independent and an ABA-dependent signal transduction pathways. Protein synthesis participates in one of the ABA-dependent pathways involving MYC/MYB. The

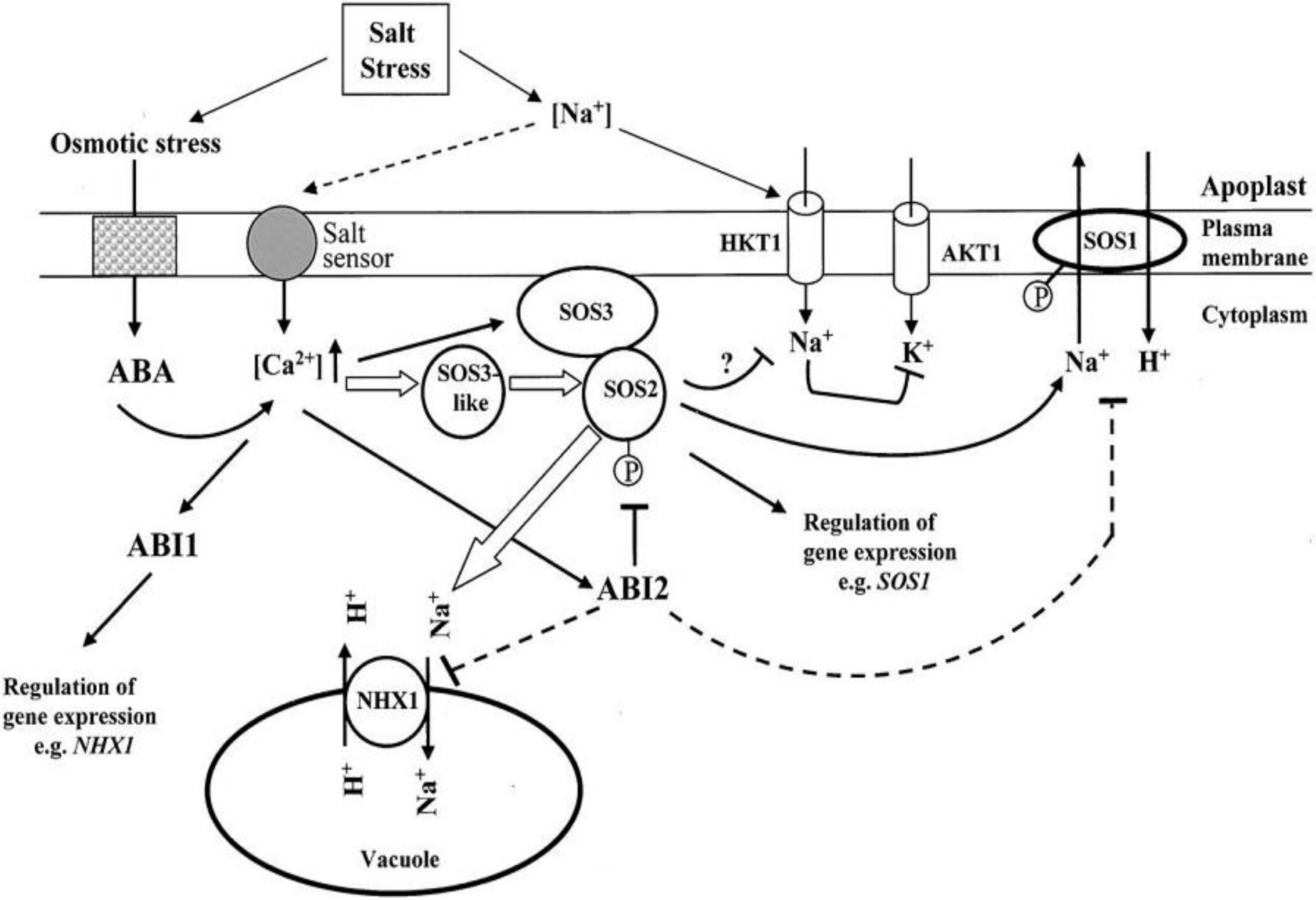

Salt Stress
Osmotic stress
[Na⁺]
Apoplast
Salt sensor
HKT1
AKT1
SOS1
Plasma membrane
P
SOS3
Cytoplasm
ABA
[Ca²⁺]↑
SOS3-like
SOS2
?
Na⁺
K⁺
Na⁺
H⁺
P
ABI1
ABI2
Regulation of gene expression e.g. *SOS1*
Regulation of gene expression e.g. *NHX1*
H⁺
Na⁺
NHX1
H⁺
Na⁺
Vacuole

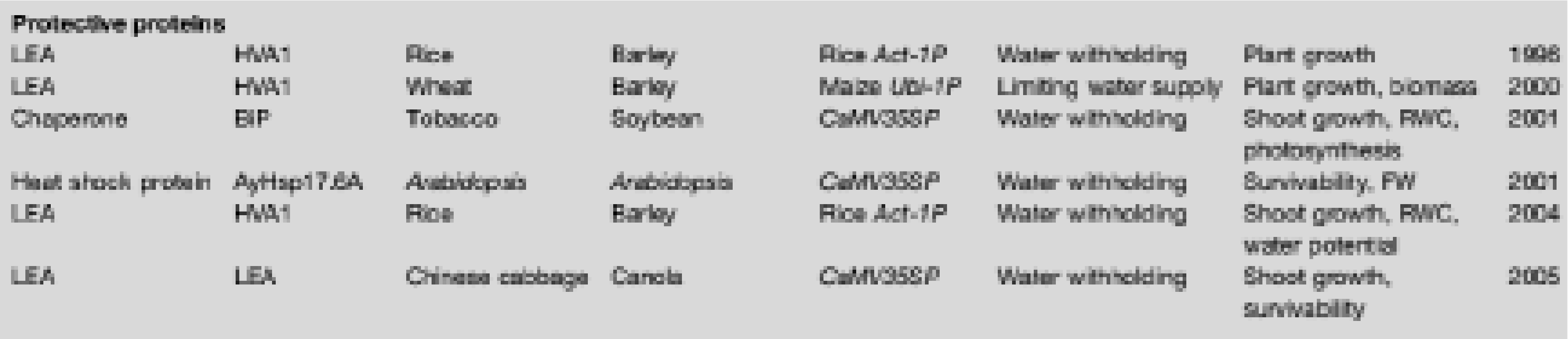
Table 2. Salt-stress tolerance of transgenic plants over-producing compatible osmolytes.

Gene and source	Transgenic plants	Stress tolerant traits
Mannitol		
E. coli mt1D (mannitol-1-phosphate dehydrogenase)	tobacco	fresh weight, plant height and flowering under salinity stress
E. coli mt1D	*Arabidopsis*	germination at 400 m*M* NaCl
E. coli mt1D	tobacco	salt-stress tolerance; mannitol contributed only to 30-40% of the osmotic adjustment
E. coli mt1D	wheat (*Triticum aestivum* L.)	only 8% biomass reduction when compared to 56% reduction in control plants in 150 m*M* NaCl stress
D-Ononitol		
IMT1 (myo-inositol *O*-methyl transferase) of common ice plant	tobacco	drought and salinity stress
Sorbitol		
Stpd1 (sorbitol-6-phosphate dehydrogenase) of apple, driven by CaMV 35S promoter	Japanese persimmon	tolerance in Fv/Fm ratio under NaCl stress
Glycine betaine		
Arthrobacter globiformis CodA (choline oxidase)	*Arabidopsis*	germination at 300 m*M* NaCl; seedling growth at 200 m*M* NaCl; retention of PSII activity at 400 m*M* NaCl
A. globiformis CodA targeted to the chloroplasts or cytosol	rice	faster recovery after 150 m*M* NaCl stress
A. globiformis CodA	*Brassica juncea* (L.) Czernj.	germination in 100–150 m*M* NaCl; seedling growth in 200 m*M* NaCl
E. coli choline dehydrogenase (*betA*) and betaine aldehyde dehydrogenase (*betB*) genes	tobacco	biomass production of greenhouse grown plants under salt stress; faster recovery from photo inhibition under high light, salt stress and cold stresses
Atriplex hortensis betaine aldehyde dehydrogenase (*BADH*) gene under maize ubiquitin promoter	wheat (*Triticum aestivum* L.)	seedling growth in 0.7% (=120 m*M*) NaCl
Barley peroxisomal *BADH* gene	rice	stability in chlorophyll fluorescence under 100 m*M* NaCl stress; accumulates less Na^+ and Cl^- ions but maintained K^+ uptake

Proline		
Vigna aconitifolia L. *P5CS* (Δ^1]-pyrroline-5-carboxylate synthetase) gene	tobacco	root growth; flower development
Vigna aconitifolia L. *P5CS* gene under barley HVA22 promoter	rice	faster recovery after a short period of salt stress
Mutated gene of *Vigna aconitifolia* L. *P5CS* which encode P5CS enzyme that lacks end product (proline) inhibition	tobacco	improved seedlings tolerance and low free radical levels at 200 m*M* NaCl
Antisense proline dehydrogenase gene	*Arabidopsis*	tolerant to high salinity (600 m*M* NaCl); constitutive freezing tolerance (−7°C)
Trehalose		
E. coli otsA (Trehalose-6-phosphate synthase) and *otsB* (Trehalose-6-phosphate phosphatase) bi-functional fusion gene (*TPSP*) under the control of ABA responsive promoter or Rubisco small subunit (*rbcS*) promoter	rice	root and shoot growth at 4 wk of 100 m*M* NaCl stress; survival under prolonged salt stress; maintenance of high K^+/Na^+ ratio; Low Na^+ accumulation in the shoot; maintained high PSII activity and soluble sugar levels
E. coli TPSP under maize ubiquitin promoter	rice	better seedling growth and PSII yield under salt, drought and cold stresses

Strategies for engineering stress tolerance in plants

Synthesis of protective proteins

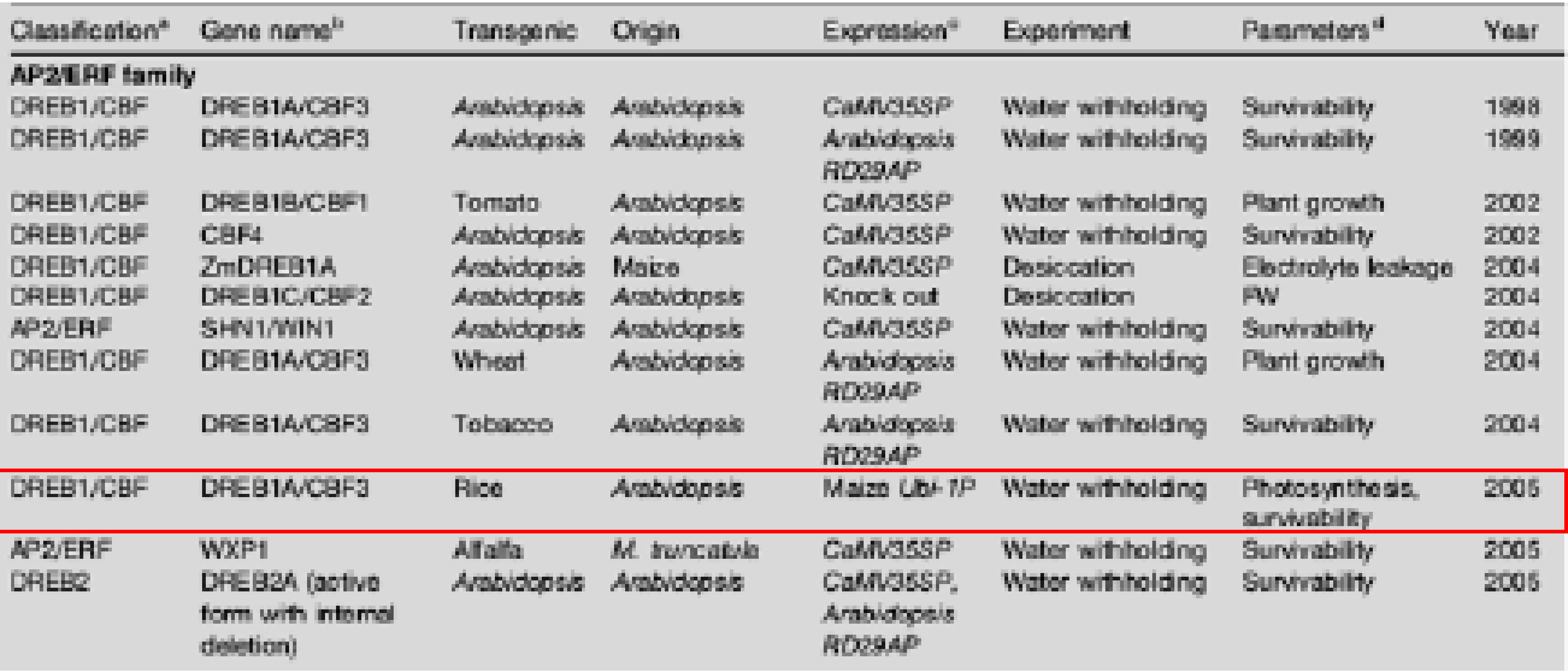


Protective proteins							
LEA	HVA1	Rice	Barley	Rice *Act-1P*	Water withholding	Plant growth	1996
LEA	HVA1	Wheat	Barley	Maize *Ubi-1P*	Limiting water supply	Plant growth, biomass	2000
Chaperone	BiP	Tobacco	Soybean	CaMV35S*P*	Water withholding	Shoot growth, RWC, photosynthesis	2001
Heat shock protein	AtHsp17.6A	*Arabidopsis*	*Arabidopsis*	CaMV35S*P*	Water withholding	Survivability, FW	2001
LEA	HVA1	Rice	Barley	Rice *Act-1P*	Water withholding	Shoot growth, RWC, water potential	2004
LEA	LEA	Chinese cabbage	Canola	CaMV35S*P*	Water withholding	Shoot growth, survivability	2005

ROS scavenging

ROS-scavenging proteins							
Detoxification	MnSOD	Alfalfa	*N. plumbaginifolia*	CaMV35S*P*	Water withholding, field trial	Photosynthesis, electrolyte leakage, yield	1996
Lipid peroxide	MsALR	Tobacco	Alfalfa	CaMV35S*P*	Water withholding	Photosynthesis	2000
NAD^+ breakdown	PARP	Canola	–	CaMV35S*P* (RNAi)	Water withholding	FW, shoot growth	2005

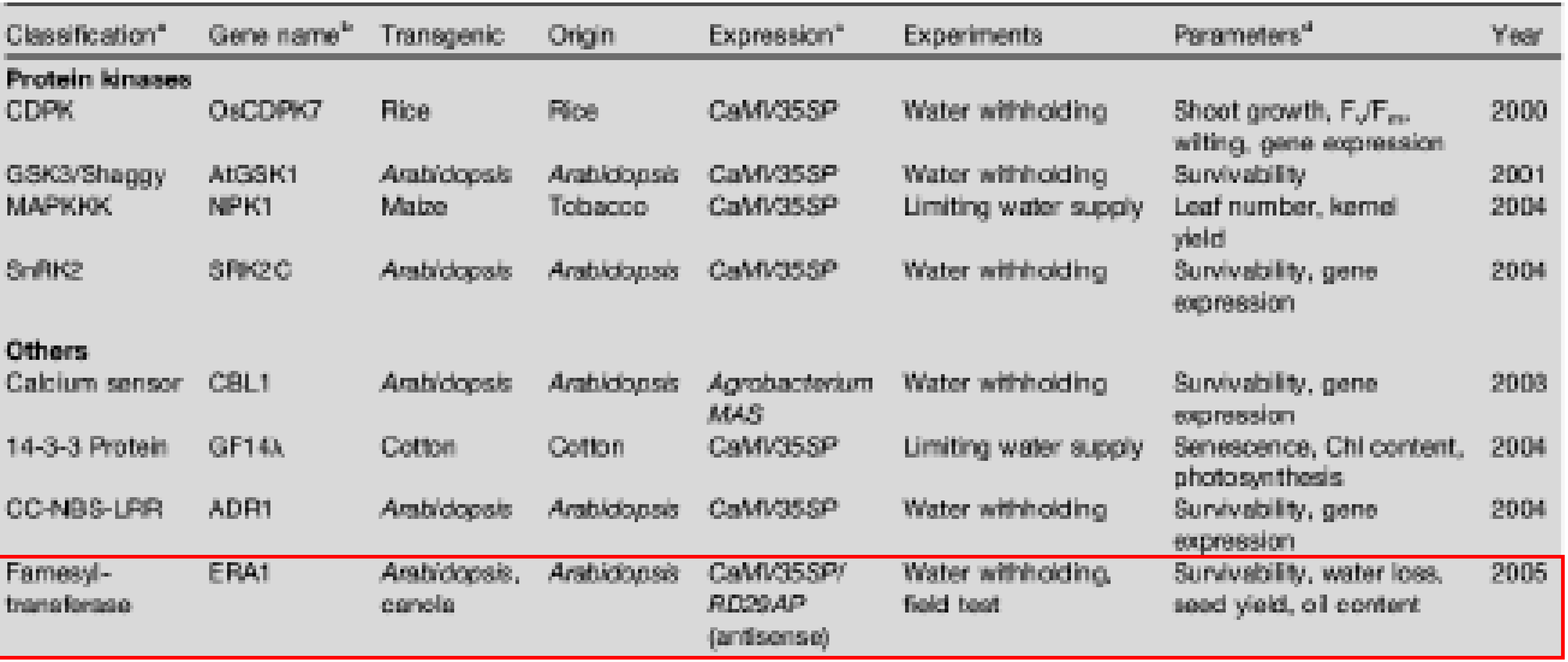
Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor



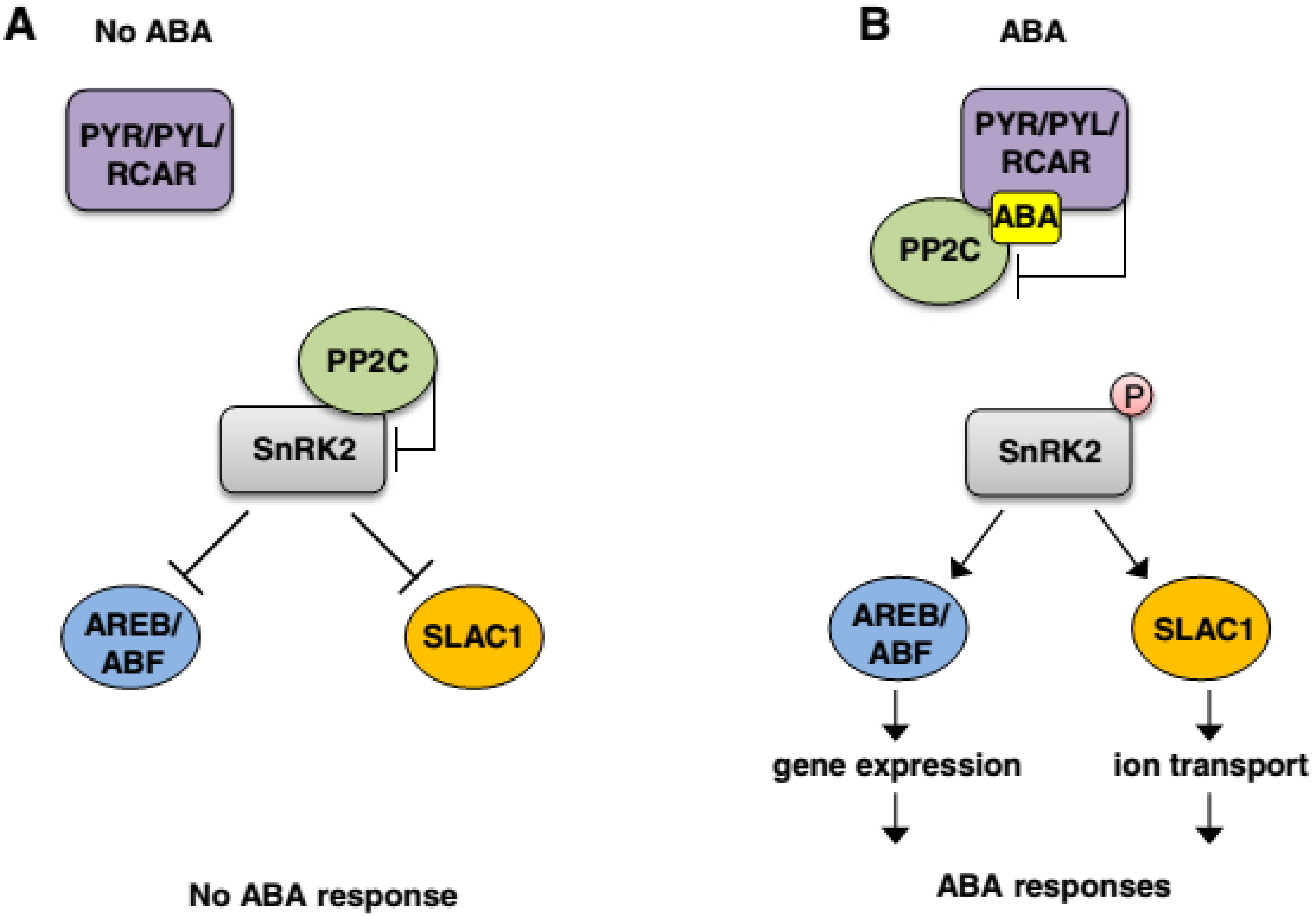
Classification[a]	Gene name[b]	Transgenic	Origin	Expression[c]	Experiment	Parameters[d]	Year
AP2/ERF family							
DREB1/CBF	DREB1A/CBF3	*Arabidopsis*	*Arabidopsis*	*CaMV35SP*	Water withholding	Survivability	1998
DREB1/CBF	DREB1A/CBF3	*Arabidopsis*	*Arabidopsis*	*Arabidopsis RD29AP*	Water withholding	Survivability	1999
DREB1/CBF	DREB1B/CBF1	Tomato	*Arabidopsis*	*CaMV35SP*	Water withholding	Plant growth	2002
DREB1/CBF	CBF4	*Arabidopsis*	*Arabidopsis*	*CaMV35SP*	Water withholding	Survivability	2002
DREB1/CBF	ZmDREB1A	*Arabidopsis*	Maize	*CaMV35SP*	Desiccation	Electrolyte leakage	2004
DREB1/CBF	DREB1C/CBF2	*Arabidopsis*	*Arabidopsis*	Knock out	Desiccation	FW	2004
AP2/ERF	SHN1/WIN1	*Arabidopsis*	*Arabidopsis*	*CaMV35SP*	Water withholding	Survivability	2004
DREB1/CBF	DREB1A/CBF3	Wheat	*Arabidopsis*	*Arabidopsis RD29AP*	Water withholding	Plant growth	2004
DREB1/CBF	DREB1A/CBF3	Tobacco	*Arabidopsis*	*Arabidopsis RD29AP*	Water withholding	Survivability	2004
DREB1/CBF	DREB1A/CBF3	Rice	*Arabidopsis*	Maize *Ubi-1P*	Water withholding	Photosynthesis, survivability	2005
AP2/ERF	WXP1	Alfalfa	*M. truncatula*	*CaMV35SP*	Water withholding	Survivability	2005
DREB2	DREB2A (active form with internal deletion)	*Arabidopsis*	*Arabidopsis*	*CaMV35SP*, *Arabidopsis RD29AP*	Water withholding	Survivability	2005

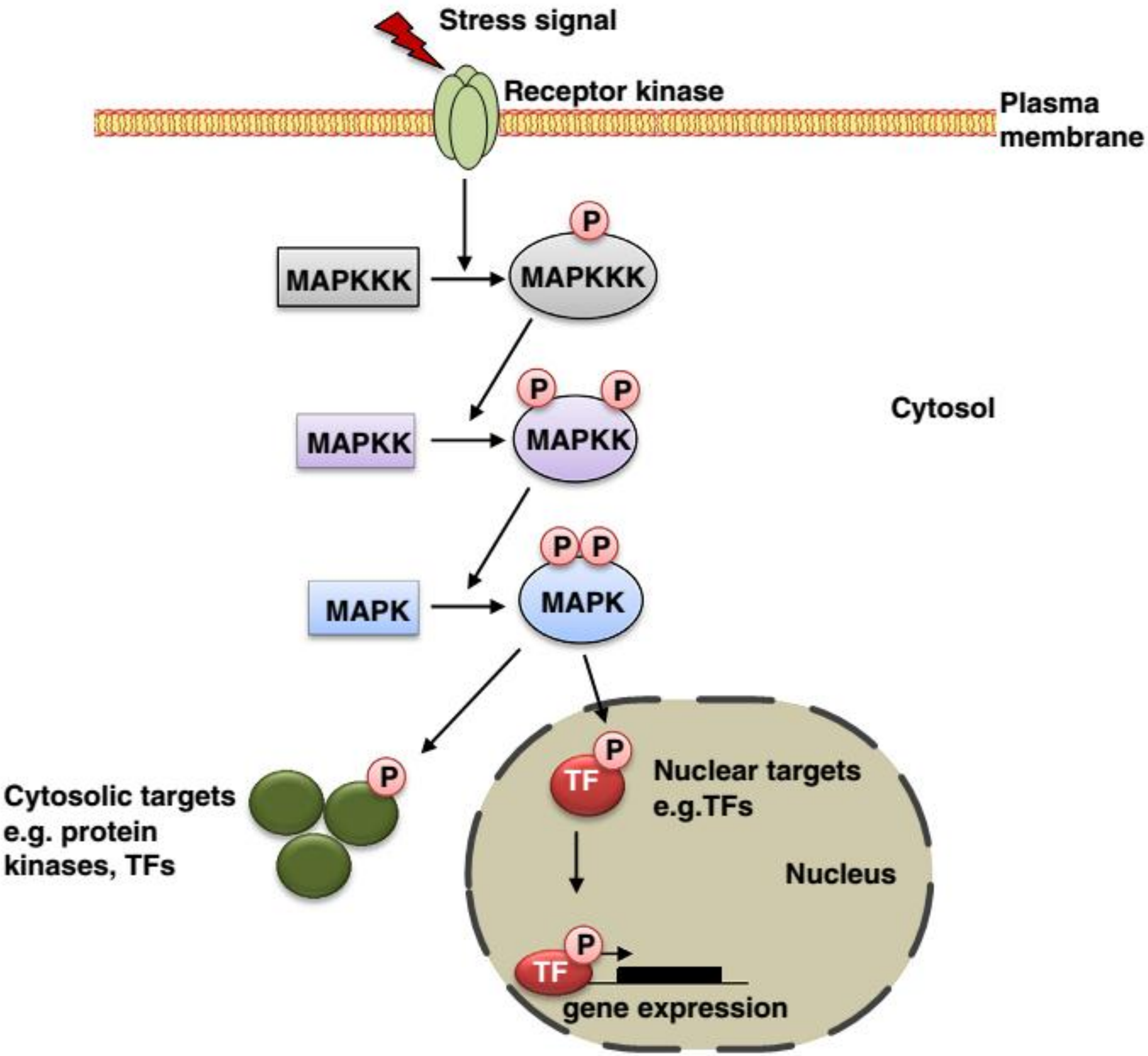
Strategies for engineering stress tolerance in plants

Use of Signalling factors



Classification[a]	Gene name[b]	Transgenic	Origin	Expression[c]	Experiments	Parameters[d]	Year
Protein kinases							
CDPK	OsCDPK7	Rice	Rice	CaMV35SP	Water withholding	Shoot growth, F_v/F_m, wilting, gene expression	2000
GSK3/Shaggy	AtGSK1	Arabidopsis	Arabidopsis	CaMV35SP	Water withholding	Survivability	2001
MAPKKK	NPK1	Maize	Tobacco	CaMV35SP	Limiting water supply	Leaf number, kernel yield	2004
SnRK2	SRK2C	Arabidopsis	Arabidopsis	CaMV35SP	Water withholding	Survivability, gene expression	2004
Others							
Calcium sensor	CBL1	Arabidopsis	Arabidopsis	*Agrobacterium MAS*	Water withholding	Survivability, gene expression	2003
14-3-3 Protein	GF14λ	Cotton	Cotton	CaMV35SP	Limiting water supply	Senescence, Chl content, photosynthesis	2004
CC-NBS-LRR	ADR1	Arabidopsis	Arabidopsis	CaMV35SP	Water withholding	Survivability, gene expression	2004
Farnesyl-transferase	ERA1	Arabidopsis, canola	Arabidopsis	CaMV35SP/*RD29AP* (antisense)	Water withholding, field test	Survivability, water loss, seed yield, oil content	2005


A
No ABA
PYR/PYL/RCAR
PP2C
SnRK2
AREB/ABF
SLAC1
No ABA response
B
ABA
PYR/PYL/RCAR
ABA
PP2C
P
SnRK2
AREB/ABF
SLAC1
gene expression
ion transport
ABA responses



Stress signal

Receptor kinase

Plasma membrane

MAPKKK → P MAPKKK

MAPKK → P P MAPKK

Cytosol

MAPK → P P MAPK

Cytosolic targets e.g. protein kinases, TFs

P

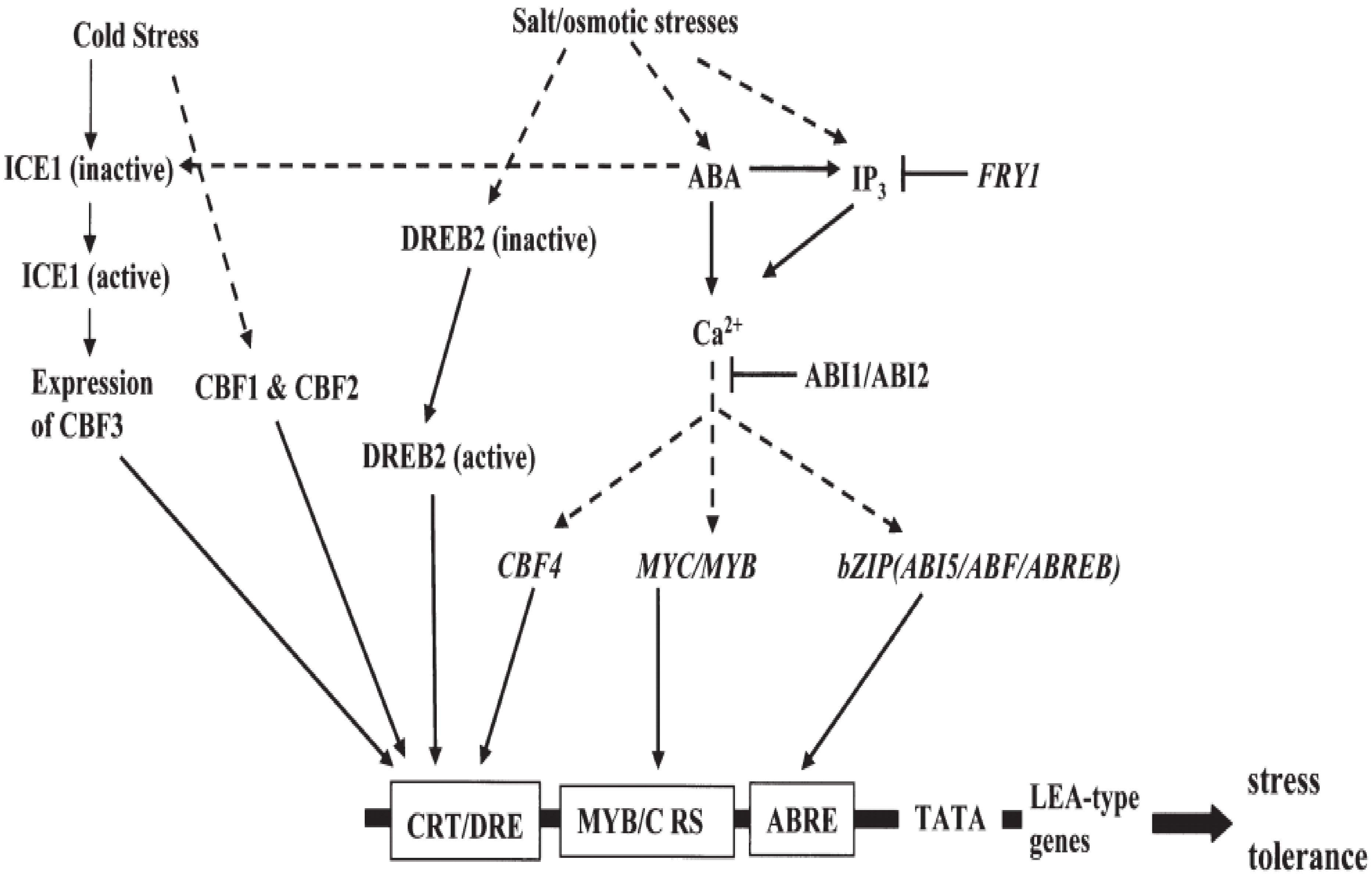
TF P

Nuclear targets e.g.TFs

Nucleus

TF P

gene expression


Cold Stress
Salt/osmotic stresses
ICE1 (inactive)
ABA
IP$_3$
FRY1
DREB2 (inactive)
ICE1 (active)
Ca^{2+}
ABI1/ABI2
Expression of CBF3
CBF1 & CBF2
DREB2 (active)
CBF4
MYC/MYB
bZIP(ABI5/ABF/ABREB)
CRT/DRE
MYB/C RS
ABRE
TATA
LEA-type genes
stress tolerance

Strategy for abiotic stress resistance in crop plant

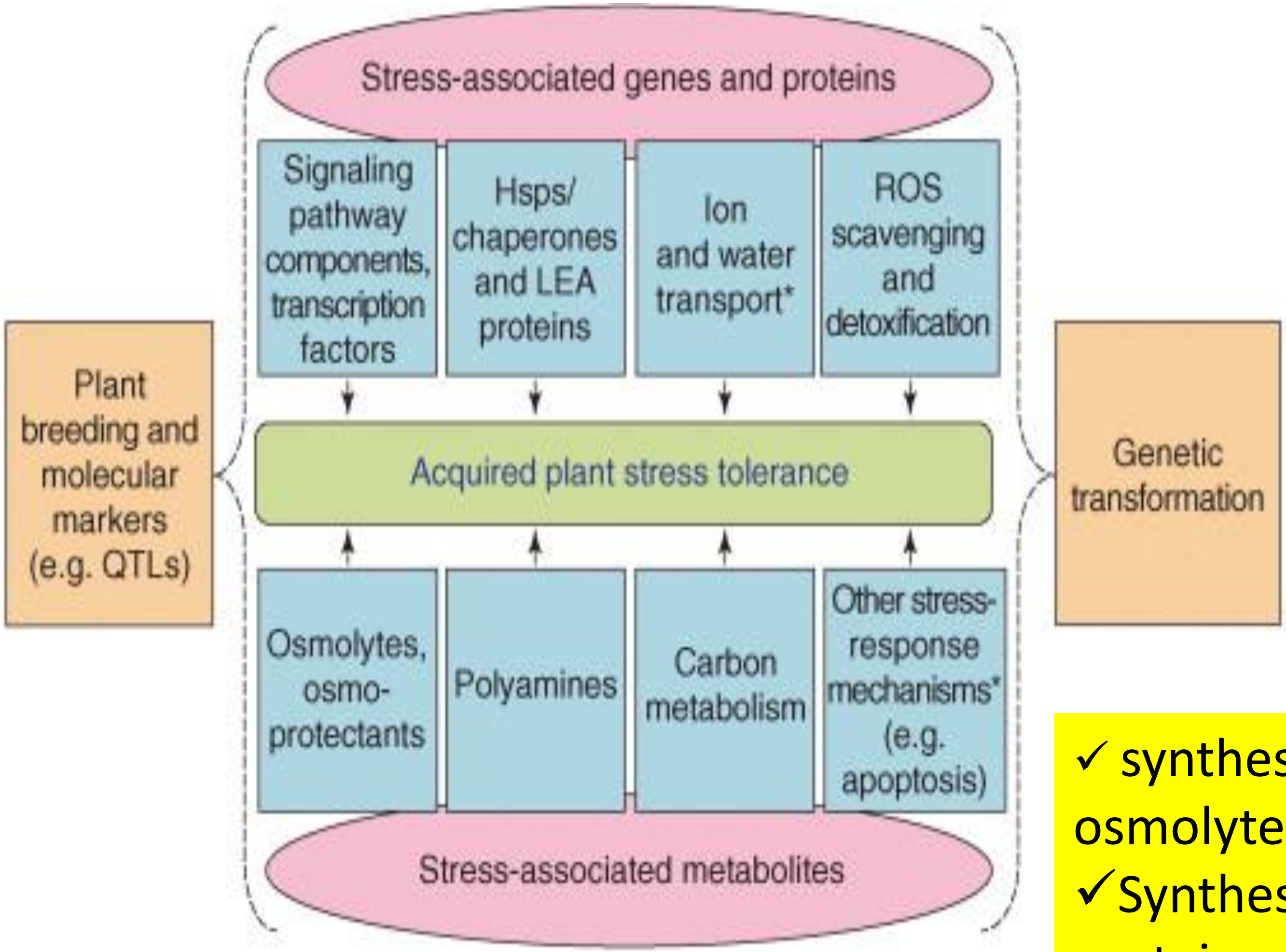


Fig: Acquired plant stress tolerance.

Hsp: heat shock protein;

LEA: late embryogenesis abundant

ROS: reactive oxygen species

- ✓ synthesis of compatible osmolytes
- ✓ Synthesis of protective proteins
- ✓ ROS scavenging
- ✓ Use of transcription factors
- ✓ Use of signaling factors

The complexity of stress adaptation: major targets for engineered stress tolerance.

Class of target	Examples	Possible mode(s) of action
Osmoprotectants	Amino acids (proline, ectoine) Dimethyl sulfonium compounds (glycine betaine, DMSP) Polyols (mannitol, D-ononitol, sorbitol) Sugars (sucrose, trehalose, fructan)	Osmotic adjustment; protein/membrane protection; reactive (OH·) scavenging
Reactive oxygen scavengers	Enzymatic (catalase, Fe/Mn superoxide dismutase, ascorbate peroxidase; glutathione cycle enzymes: glutathione S-transferase, glutathione peroxidase; gamma-glutamylcysteine synthetase, alternative oxidase) Non-enzymatic (ascorbate, flavones, carotenoids, anthocyanins)	Detoxification of reactive oxygen species
Stress proteins	Late embyogenesis abundant proteins	Unknown, protein stabilization, water binding/ slow desiccation rates; chaperones; protein/ membrane stabilization; ion sequestration
Heat shock proteins	Various heat-, cold-, salt-shock proteins in several subcellular compartments	Reversal/prevention of protein unfolding; translational modulation
Ion/proton transporters	High-affinity K^+ transporter; low-affinity K^+ channels; plasma membrane, pre-vacuolar, vacuolar and organellar proton ATPases and ion transporters (H^+/ATPase; Na^+/H^+ antiporters)	K^+/Na^+ uptake and transport; establishment of proton gradients; removal and sequestration of (toxic) ions from the cytoplasm and organelles

Membrane fluidity	Fatty acid desaturases	Increased amounts of dienoic and fluidity; chilling tolerance
Water status	Aquaporins or water channels (solute facilitators: urea, glycerol, CO_2, possibly others and including ions); CO_2 concentration	Regulation of AQP amount differentially in tonoplast and plasma membrane; regulation of membrane location; stomatal behavior
Signaling components	Homologs of histidine kinases (AtRR1/2); MAP kinases (PsMAPK, HOG); Ca^{2+}-dependent protein kinases; SNF1/kinases; protein phosphatases (ABI1/2); CNA/B signaling systems; Ca^{2+} sensors (SOS3); inositol kinases	Ca^{2+}-sensors/phosphorylation mediated signal transduction
Control of transcription	Transcription factors: EREBP/AP2 (DREB, CBF); zinc finger TF (Alfin 1); Myb (AtMyb2, CpMyb10)	Upregulation/activation of transcription
Growth regulators	Altered biosynthetic pathways or conjugate levels for abscisic acid, cytokinins and/or brassinosteroids	Changes in hormone homeostasis

Table 1. Foreign genes expressed in transgenic plants

Gene	Origin	Host	Stress	Refs
BetA	*E. coli*	Tobacco	Salinity	16
BetA	*E. coli*	Potato	Freezing	G. Lilius *et al.*, unpublished
codA	*Arthrobacter globiformis*	*Arabidopsis*	Salinity and drought	17
p5cs	*Vigna aconitifolia*	Tobacco	Drought	18
Mltd	*E. coli*	*Arabidopsis*	Salinity	19
Mltd	*E. coli*	Tobacco	Salinity	20
TPS1	*Saccharomyces cerevisiae*	Tobacco	Drought	21
SacB	*Bacillus subtilis*	Tobacco	Drought	22
fad7	*Arabidopsis*	Tobacco	Chilling	23
Des9	*Anacystis nidulans*	Tobacco	Chilling	24
HVA 1	Barley	Rice	Salinity and drought	6
Afp	Winter flounder	Tobacco	Freezing	26
afa3	Winter flounder	Tomato	Freezing	18
Mn-Sod	*Nicotiana plumbaginifolia*	Alfalfa	Drought and freezing	15
Mn-Sod	*N. plumbaginifolia*	Tobacco	Oxidative	30
Fe-Sod	*Arabidopsis*	Tobacco	Oxidative	31
Gr/Cu,Zn-Sod	*E.coli/Rice*	Tobacco	Oxidative	33
vhb	*Vitreoscilla stercoraria*	Tobacco	Hypoxia and anoxia	35