La réponse des plantes aux stress environnementaux

Le Stress hydrique ou osmotique

I. Introduction – définition du stress hydrique
II. Les effets du stress hydrique – végétaux adaptés
III. Les modèles d'étude du stress hydrique
IV. Paramètres décrivant le statut hydrique des plantes
V. Les osmolytes
VI. Autres facteurs importants
VII. Les voies de signalisation

I. Introduction

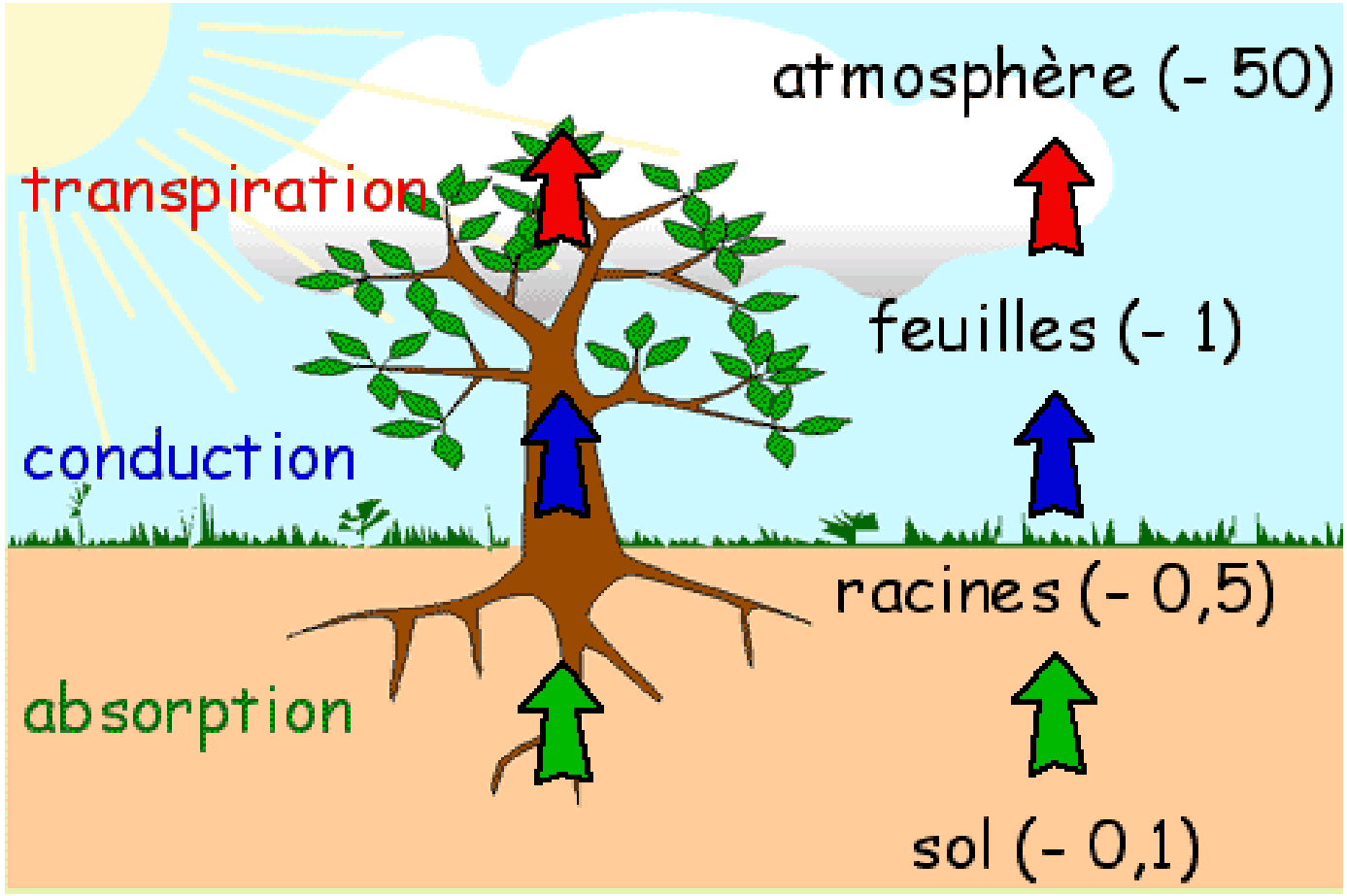


Figure 1. La plante et son environnement hydrique. L'eau absorbée dans le sol par les racines est conduite dans toutes les parties de la plante. Une partie est éliminée dans l'atmosphère par la transpiration. A droite, sont notées les valeurs du potentiel hydrique en Méga-Pascal (MPa) des différents partenaires. © Biologie et Multimedia

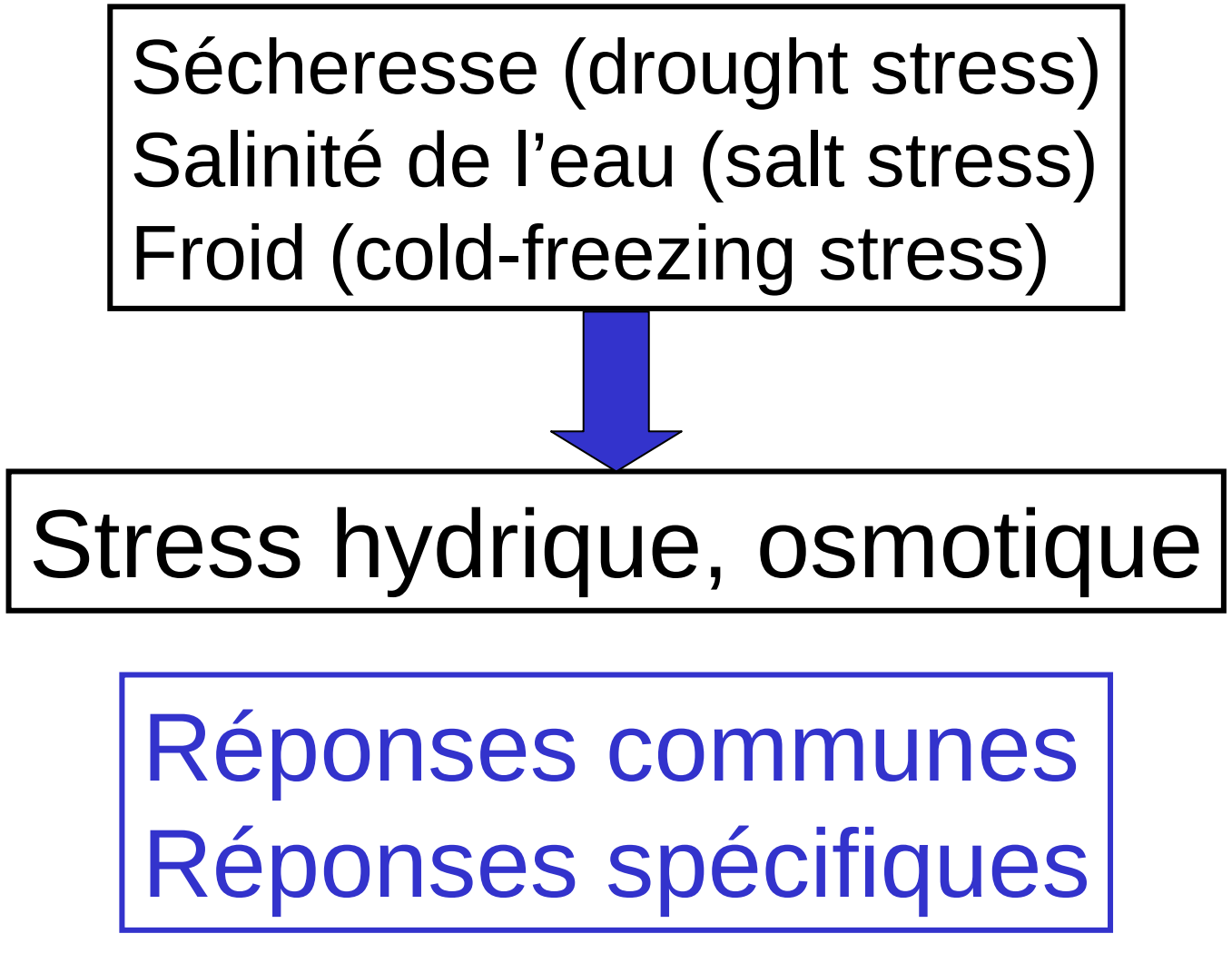
Quand la plante est-elle en déficit hydrique ?

Déficit hydrique : quantité d'eau transpirée supérieure à la quantité d'eau absorbée.

Les réactions des plantes à la sécheresse dépendent :

- de la vitesse d'évaporation de l'eau,
- de la durée du déficit hydrique,
- de l'espèce (mais aussi de la variété, donc du génotype)

Au niveau cellulaire, les réactions varient en fonction de :
- l'organe considéré,
- du type de cellule,
- du stade de développement de la plante.


Sécheresse (drought stress)
Salinité de l'eau (salt stress)
Froid (cold-freezing stress)
Stress hydrique, osmotique
Réponses communes
Réponses spécifiques

La salinité

• 6 % de la surface terrestre sont affectés par la salinité (notamment 20% des cultures irriguées)

• Salinité naturelle

Remarque : Eau de mer :
Na^+ : 10 g/kg 470 mM
Cl^- : 20 g/kg 550 mM

• Salinité induite par l'agriculture

La salinité

En général : les plantes n'utilisent pas le Na^+ ni le Cl^-

Salinité -> hyperosmolarité et toxicité ionique

Glycophytes

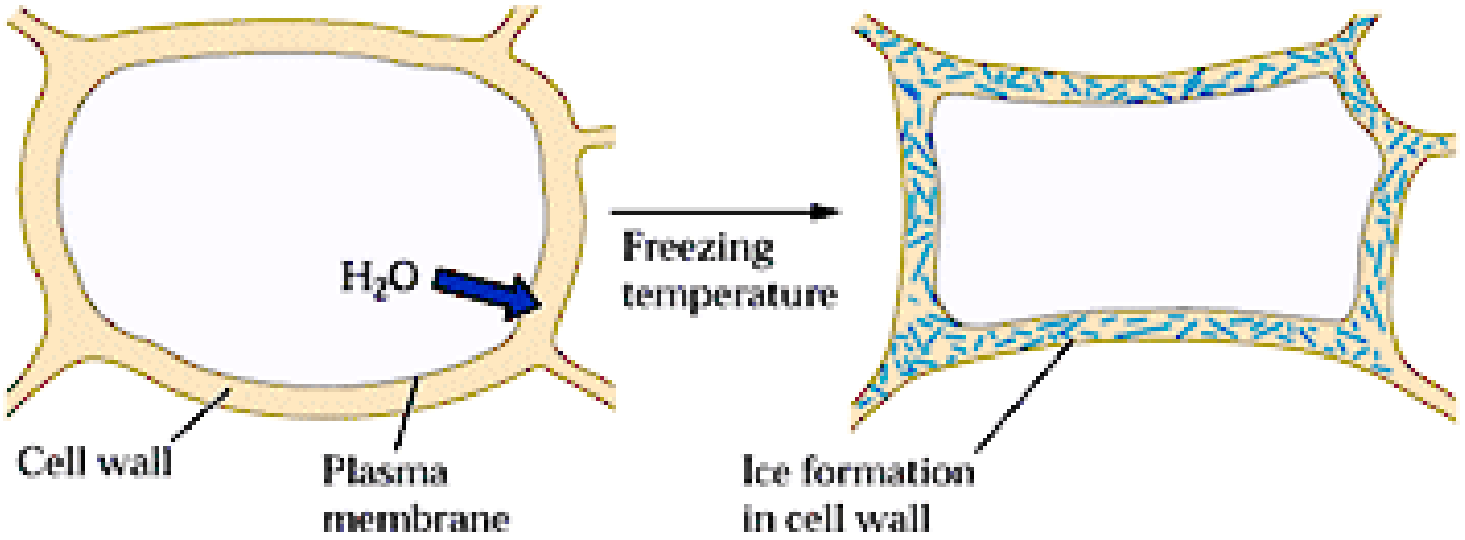
Halophytes :

- besoin de plus d'électrolytes pour une croissance optimale ([NaCl]sol: 20 à 500 mM), utilisation comme osmoticum
- extrusion du Na+ (cellules spécialisées)
- compartimentation vacuolaire
- transport vers les parties aériennes jeunes (limitation du Na+ au niveau racinaire)

Palétuvier

Salicorne

Le froid



➔ Protection membranaire indispensable

Distribution de l’eau dans la plante

- Bois : 60%
- Feuilles de blé : 12%
- Fruit de tomate : 94%

- Graines d’orge : 20 %
- Graines de blé : 12 %
- Cacahuète : 5%

Fonctions mécaniques et physiologiques chez les plantes

➔Nécessaire pour :

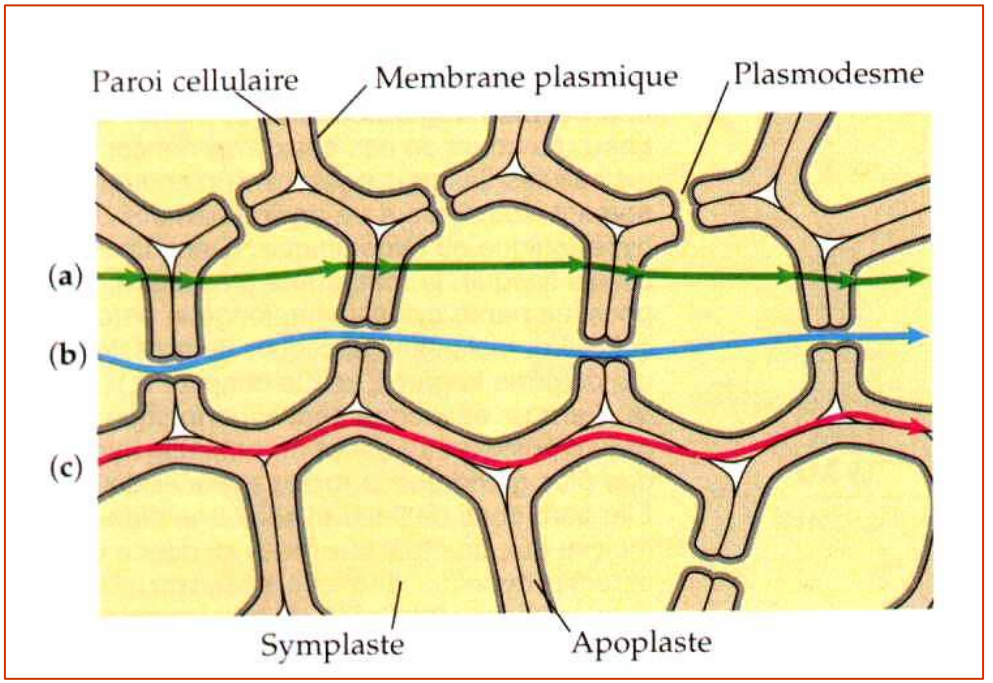
- ➔ La photosynthèse (donneur d'électrons)
- ➔ La croissance
- ➔ Les transports de solutés
- ➔ Le port érigé (turgescence)
- ➔ Les mouvements
- ➔ Le refroidissement par évapotranspiration

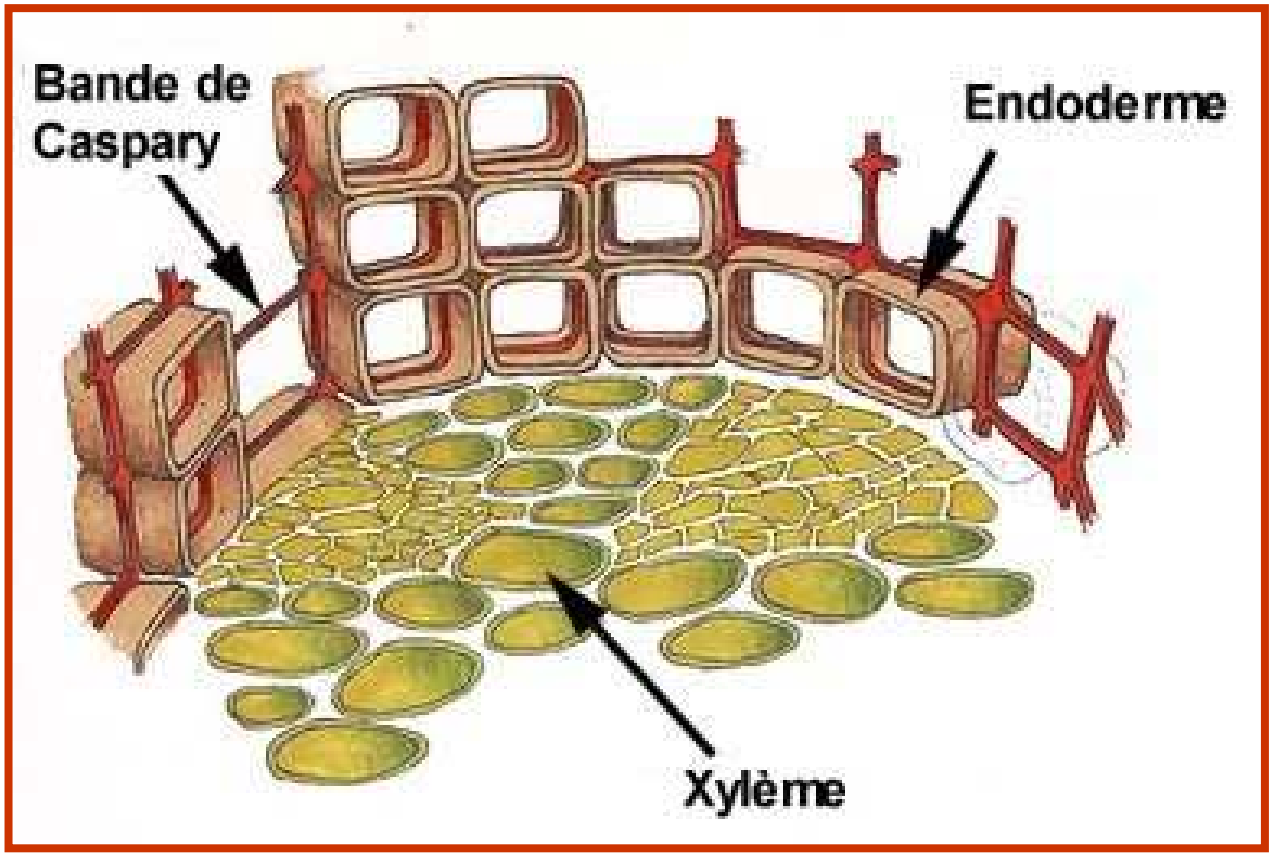
L ’eau traverse la racine en empruntant 3 voies:

a. En passant à travers la membrane des cellules = voie transcellulaire

b. En passant de cellule en cellule par les plasmodesmes = voie **symplaste.**

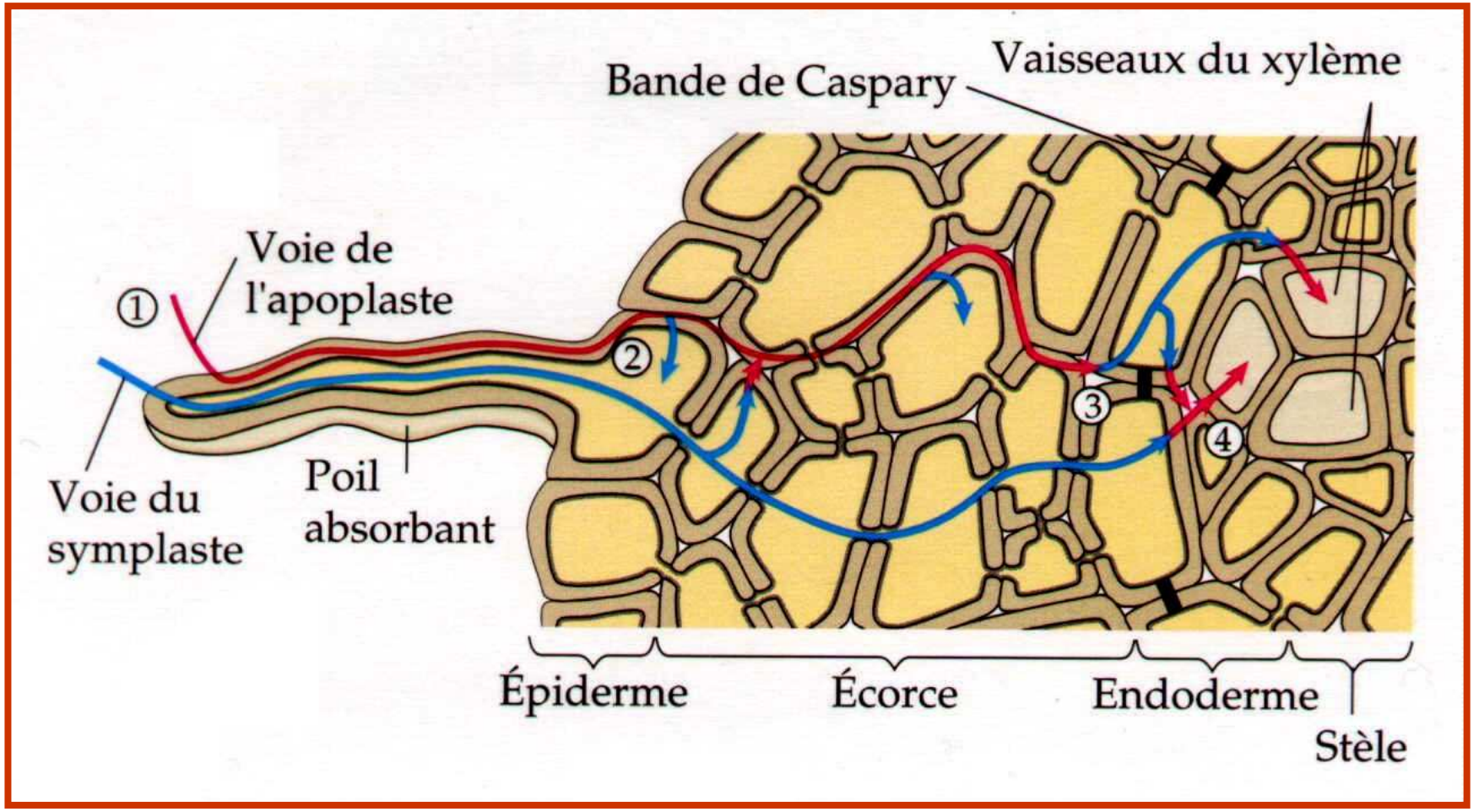
c. En passant entre les cellules ou dans les cellules mortes = voie **apoplaste.**





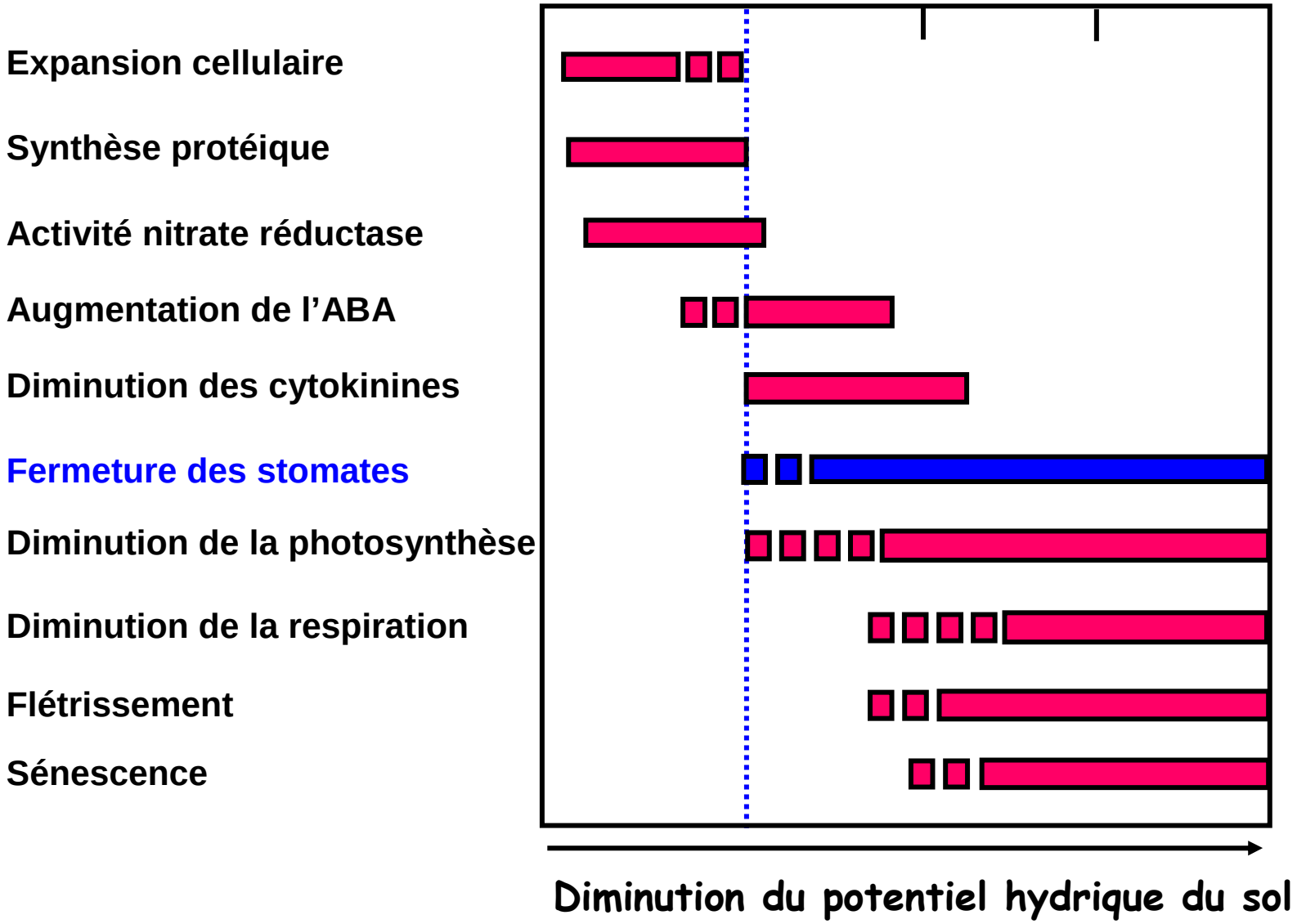
Les parois des cellules de l ’endoderme sont imprégnées de cire (subérine) = *bande de Caspary* . L ’eau ne peut pas s ’y infiltrer par apoplaste.

Avant d ’atteindre le xylème, l ’eau doit absolument traverser une membrane au moins une fois = **filtre**.


Bande de Caspary
Vaisseaux du xylème
Voie de
l'apoplaste
①
②
③
④
Voie du
symplaste
Poil
absorbant
Épiderme
Écorce
Endoderme
Stèle

II. Les effets du stress hydrique

II. 1. Réponses au stress hydrique : Végétaux non adaptés



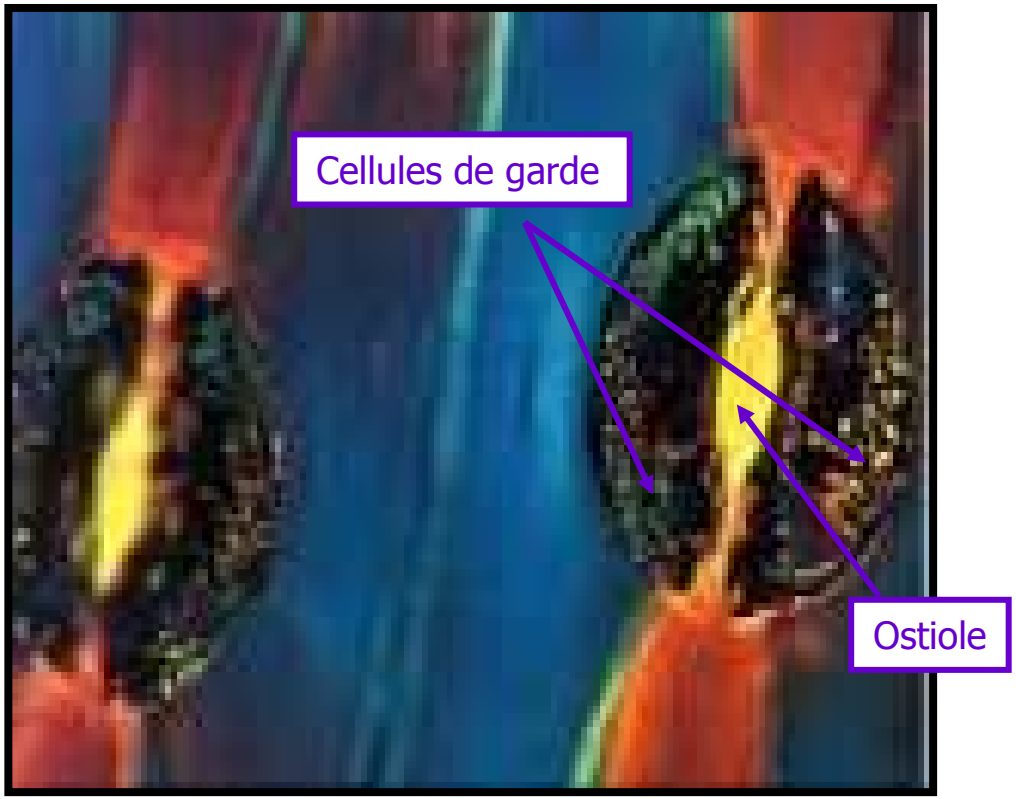
Rappel

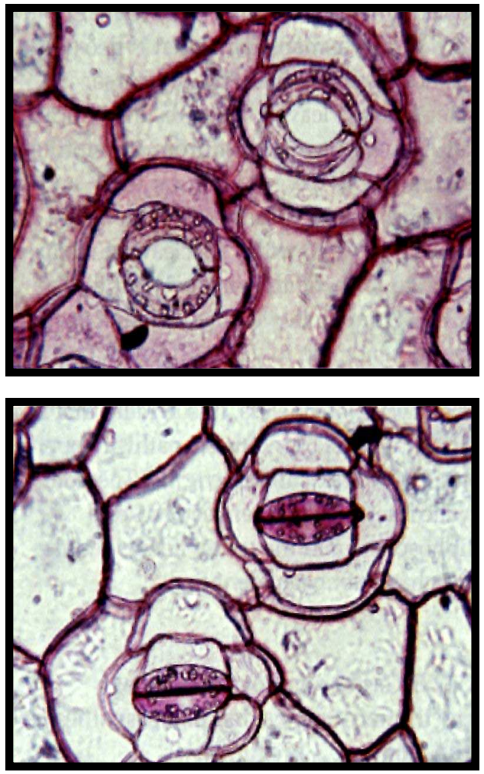
Les stomates

Siège des échanges gazeux (O_2, CO_2) et lieu de la transpiration (évaporation de l'eau sous forme de vapeur d'eau).

La transpiration : stomates ouverts, fixation du CO_2 atmosphérique (sous forme dissoute, pour la photosynthèse)

Fermeture des stomates lors d'un stress hydrique





• Au niveau cellulaire :

- Dégâts mécaniques liés à la perte de turgescence

- Modifications structurales : macromolécules, membranes, …organites…

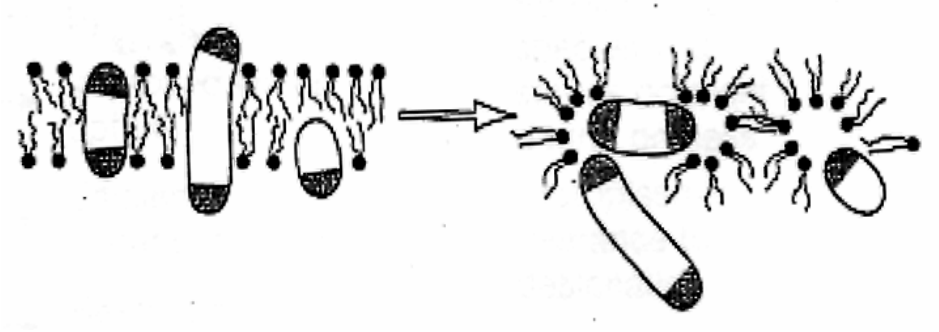


Fig. 6.62. Possible orientation of lipid and protein components in membranes in relation to their degree of hydration. Hydrated state (*left*) orientation of the hydrophilic poles (*heads*) of the phospholipids and of the membrane proteins (*stippled areas*) toward the external aqueous medium. Dehydrated state (*right*) reversed orientation of the polar ends of phospholipids and proteins toward interior water channels of the membrane. (Bewley and Krochko 1982)

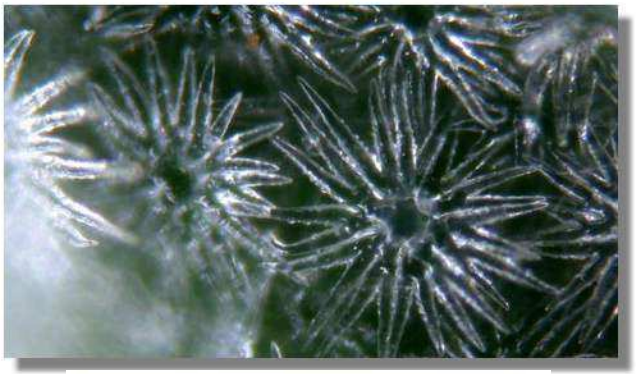
- Modifications du métabolisme, production de radicaux libres…

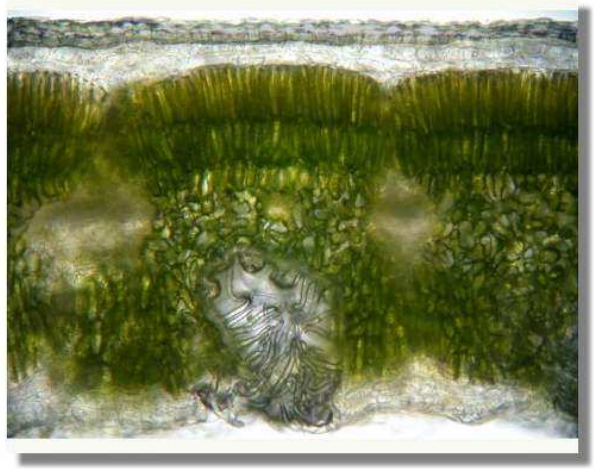
II. 2. Réponses au stress hydrique : Végétaux adaptés (Xérophytes)

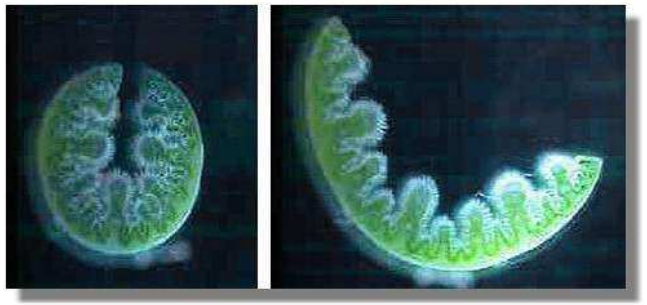
⇨Adaptations anatomiques et morphologiques

- ✓Système racinaire de surface
- ✓Système racinaire profond
- ✓Accumulation d’eau
- ✓Réduction surface foliaire

✓Protection des stomates







⇨ Adaptations métaboliques

• Photosynthèse (CAM)

• Biosynthèse de composés protecteurs (osmotiques, structuraux)

• Mise en place de systèmes de détoxification (des espèces réactives d'oxygène).

• Systèmes de réparation

Plantes au métabolisme CAM

CAM = Crassulacean Acid Metabolism

Une modification du métabolisme C4:
la capture de la CO_2 et la photosynthèse sont séparés dans le temps, plutôt que dans l'espace

Ce type de métabolisme est présent dans de nombreuses autres familles de plantes (~ 20 familles).
Ex. Cactus, Ananas, Orchidées

La nuit:

- Ouverture des stomates.
- Absorption de CO_2.
- CO_2 réagit avec un composé à 3C (*pyruvate*) pour former un composé acide à 4C (*oxaloacétate* et puis *malate*).

CO_2 + C3 ⇢ C4 (*malate*)

- Le malate s'accumule dans les vacuoles au cours de la nuit

Le jour:

- Les stomates se ferment (ce qui limite la perte d'eau).
- Le malate est converti en un composé à 3C (*pyruvate*) et en CO_2 (→ Calvin).

III. Les modèles d'étude du stress hydrique

1. Les plantes reviviscentes

➔ Tolérance à la dessiccation,
➔ Réhydratation

- Lichens : 13.000 espèces
- Mousses : 10.000 espèces dont *Tortula ruralis*
- Fougères : 70 espèces (Selaginelles…)
- Angiospermes : 60 espèces sur 250.000 connues.

Ex : *Craterostigma plantagineum*
Plante de la résurrection

Tolérance à la déshydratation : Plante de la résurrection

Craterostigma plantagineum

Hydratée Déshydratée (7 J) Réhydratée (24 h)

Les plantes reviviscentes. Biofutur, Fév. 2000, p39-41

Fig. 3. Green, fresh (a) and purple, dried (b) *Craterostigma pumilum* (Scrophulariaceae) plant. This angiosperm resurrection plant accumulates anthocyanins during dehydration, which are thought to be involved in the defense against oxidative stress[11]. The plant originates from the Mount Elgon region in Kenya. Scale bars = 1 cm.

Trends in plant science
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III. Les modèles d'étude du stress hydrique

III. 2. Les graines orthodoxes

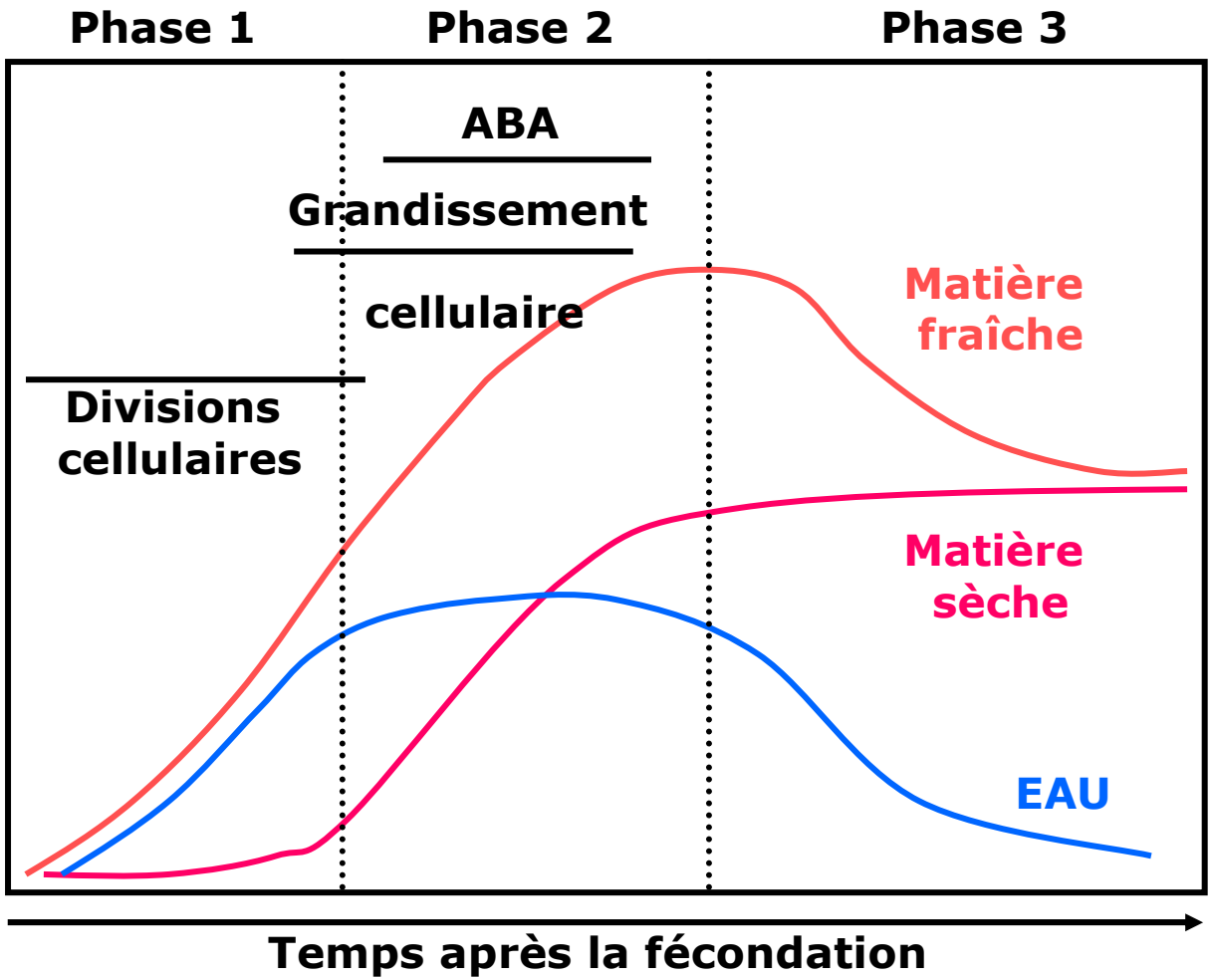
Dessiccation des graines :

Pré-requis pour la survie et l'accomplissement du cycle complet de développement

Intervient au cours de la maturation des graines

Stades de développement des graines

Développement des semences



Phase 1: embryogénénèse
Phase 2: accumulation des réserves
Phase 3: deshydratation

III. 3. Les plantes modèles

Approche de génétique classique :

Recherche de mutants hypersensibles ou hyper-résistants

Clonage positionnel de la mutation

Approche de génétique inverse : tester les hypothèses

IV. Paramètres décrivant le statut hydrique des plantes

La quantité relative d'eau (RWC : relative water content)

RWC = [(pf-ps)/(pf turg.-ps)]x100

pf : poids frais, ps : poids sec, turg. : turgescent*

RWC feuilles qui transpirent normalement : 85 %
Seuil critique : environ 50% : mort

Plantes tolérantes : perte de la quasi-totalité
de l'eau protoplasmique : anhydrobiose

le potentiel hydrique

$\psi_w = \psi_s + \psi_p$

ψ_s : potentiel du aux solutés

lié au nombre de particules solubles dissoutes dans l'eau
[solutés] $\uparrow$: ψ_s diminue, donc ψ_w diminue

ψ_p : potentiel de pression

Forces physiques exercées par l'eau sur l'environnement
Tension : $\psi_p < 0$
Turgescence $\psi_p > 0$

Le transport de l'eau s'effectue si :
ψ_w racines < ψ_w milieu extérieur

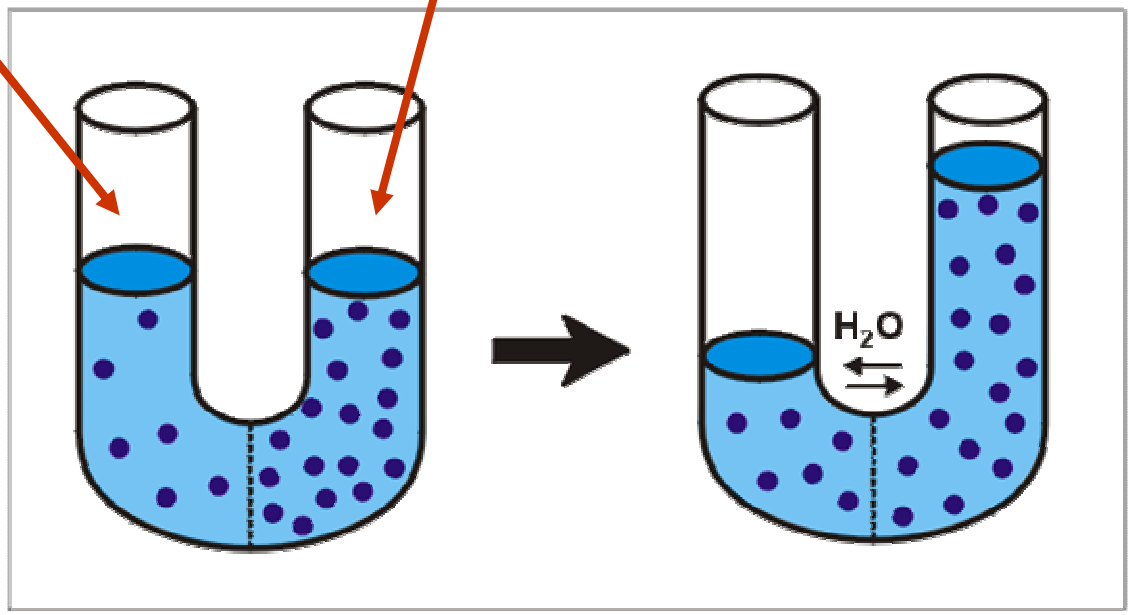
Osmose et potentiel hydrique

Osmose = diffusion de l 'eau

[eau] ⟶ [eau]

Milieu hypotonique

Milieu hypertonique

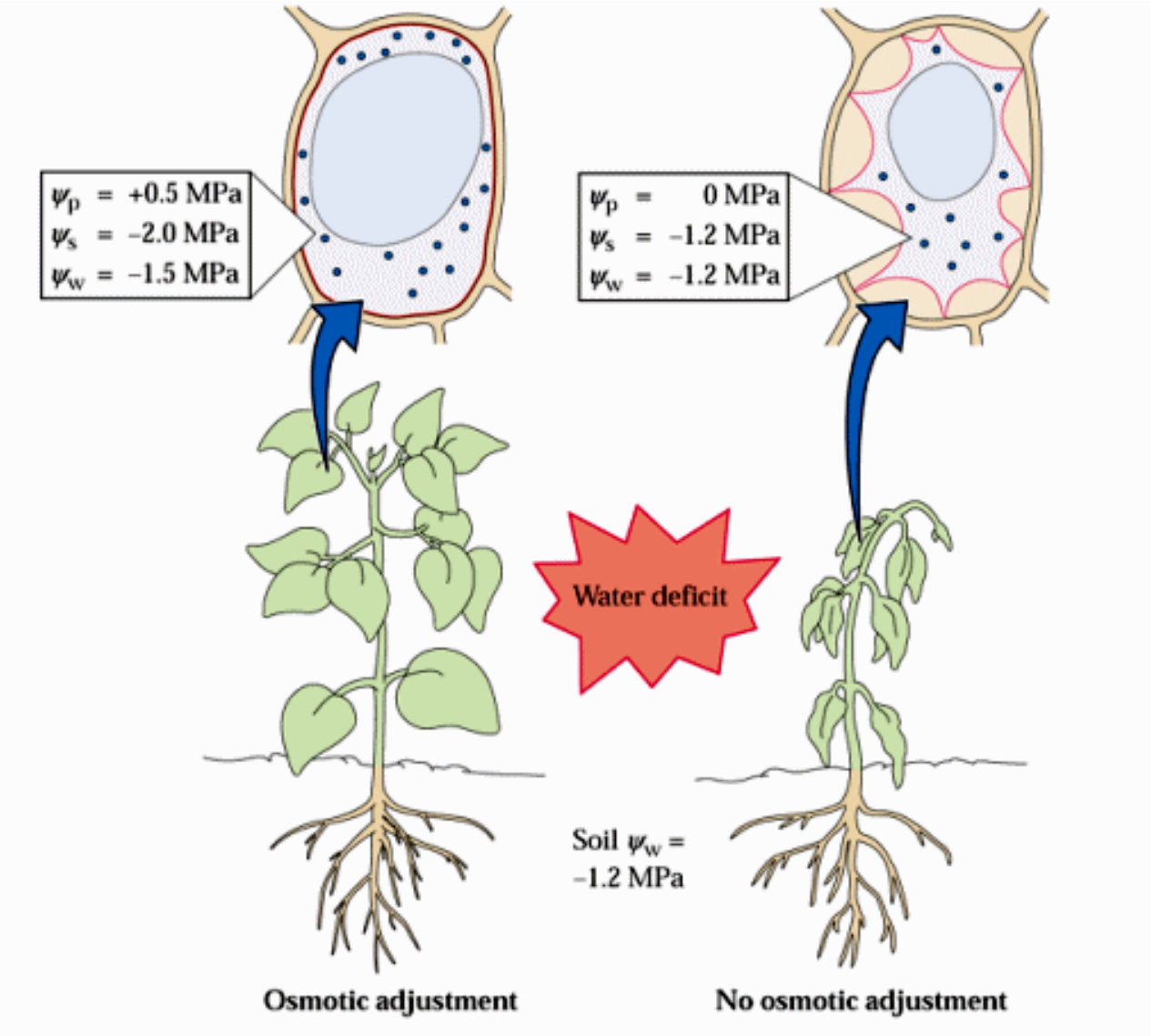


Eau se déplace du milieu hypotonique au milieu hypertonique

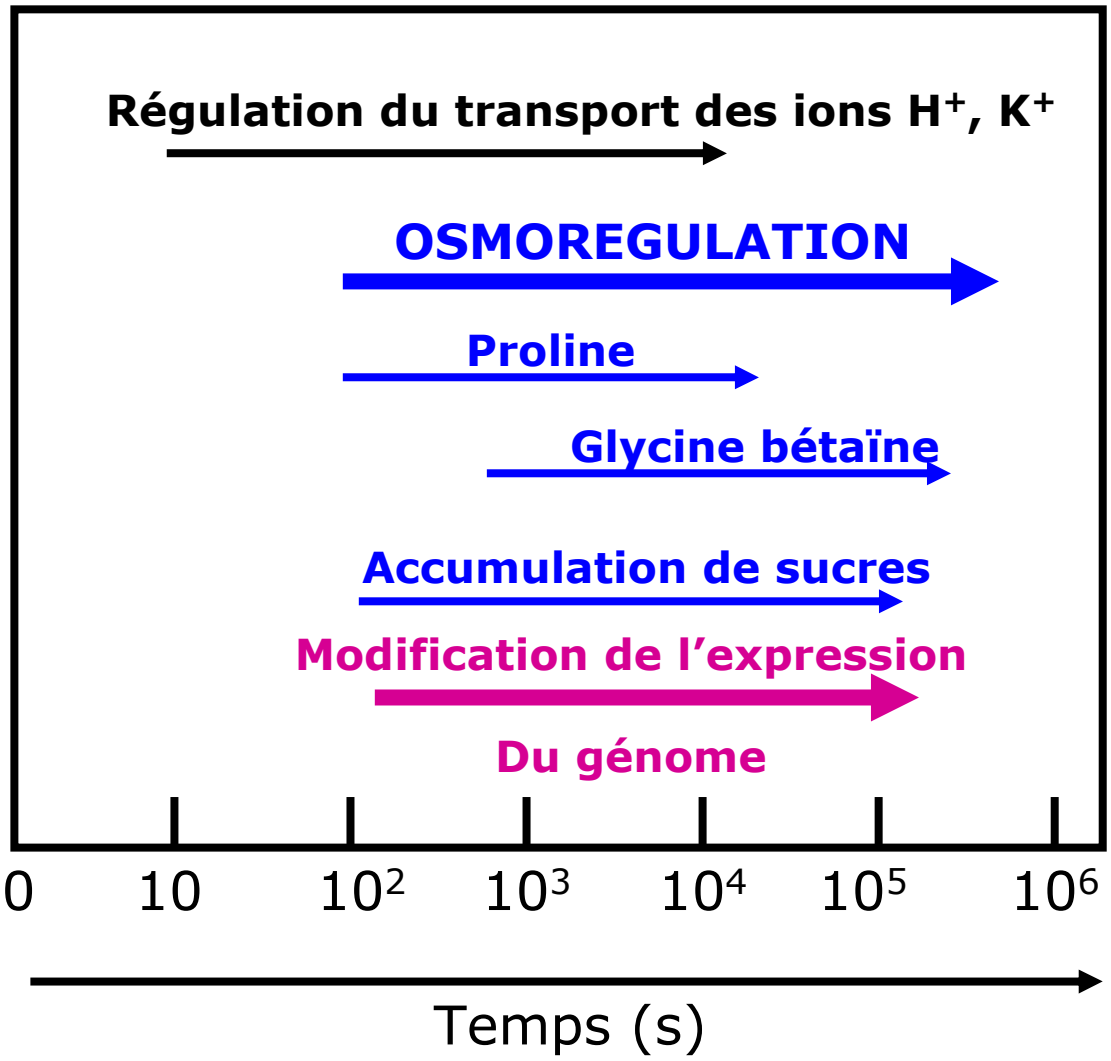
L’ajustement osmotique

➔ Maintien de la turgescence des cellules

➔ Accumulation dans le cytoplasme de composés osmoprotecteurs (osmolytes)



➔Réponses à la perte de turgescence
Plantes adaptées



V. Les osmolytes

= Osmoprotectants, = Composés osmoprotecteurs
= Composés solubles compatibles (compatible solute)

→ **Composés organiques**
→ **Propriétés physiques et biologiques <u>compatibles</u>, même à forte concentration, avec les fonctions métaboliques**

- Molécules très solubles
- Molécules neutres au pH physiologique
(non ionisées ou dipôles)

Contraire des solutés inorganiques = ions :
leur accumulation est toxique pour les cellules :
dénaturation des protéines

Mise en évidence chez les bactéries

Les osmolytes

A. Fonction : protection contre les stress abiotiques

1°- Ajustement osmotique

2°- Stabilisation des membranes

3°- Stabilisation de la conformation des protéines

4°- Propriétés : antioxydants ?

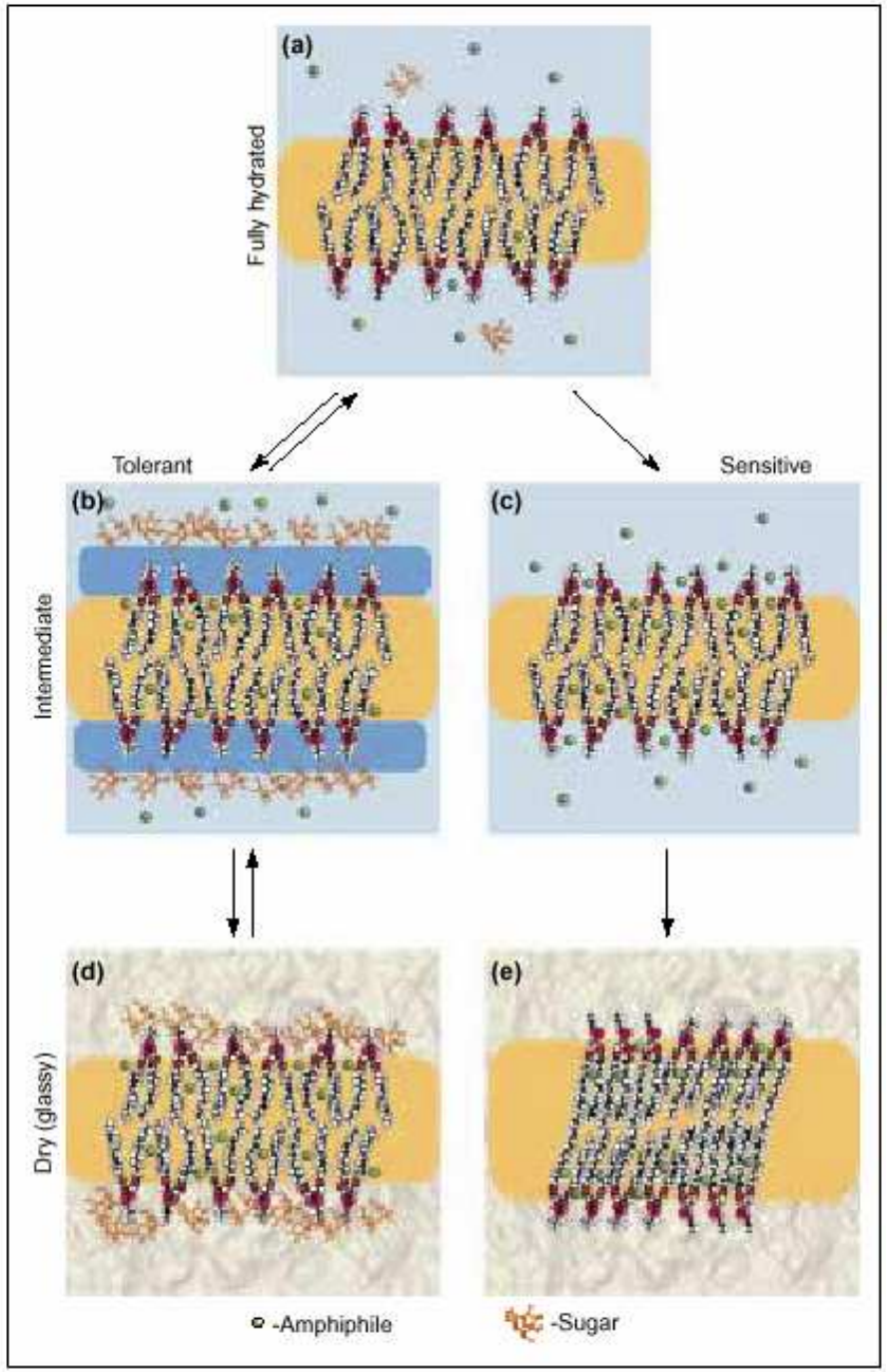


Fig. 2. Membrane behavior at different stages of water loss. In fully hydrated cells (a), membrane lipids are in an undisturbed liquid-crystalline state. Upon water loss (intermediate water contents), cytoplasmic amphiphilic compounds increase in concentration and partition into membranes, which can be considered as a preferential interaction. This causes membrane disturbance in both tolerant (b) and sensitive (c) cells. Amphiphile partitioning into membranes is complete with the dissipation of bulk water. In the intermediate water range, the presence of preferentially excluded solutes (sugars) in tolerant cells (b) keeps the membrane surface preferentially hydrated (indicated by the blue band) and prevents membrane fusion. The absence of these solutes in the sensitive cells (c) might result in membrane fusion, as in model membrane systems. On further drying below 0.3 (g H_2O) (g dry weight)$^{-1}$, the sugar molecules in tolerant cells replace water in the hydration shell of the membranes, thereby maintaining the spacing between phospholipid molecules. The bilayer remains in the liquid-crystalline phase. In sensitive cells, the removal of water from the hydration shell in the absence of sugars results in packing of the phospholipid molecules, which leads to a phase transition into the gel phase. This might lead to lateral phase separations and irreversible membrane damage. Below 0.1 (g H_2O) (g dry weight)$^{-1}$, cytoplasmic components are immobilized in a glassy matrix, which might differ in properties between tolerant (d) and sensitive (e) cells. Whether preferential exclusion has an influence on amphiphile partitioning into membranes is not known. The arrows indicate the reversibility of the processes during rehydration. The slow repartitioning of amphiphiles from membranes into the cytoplasm on rehydration might cause

1°- Rôle protecteur des sucres au niveau membranaire

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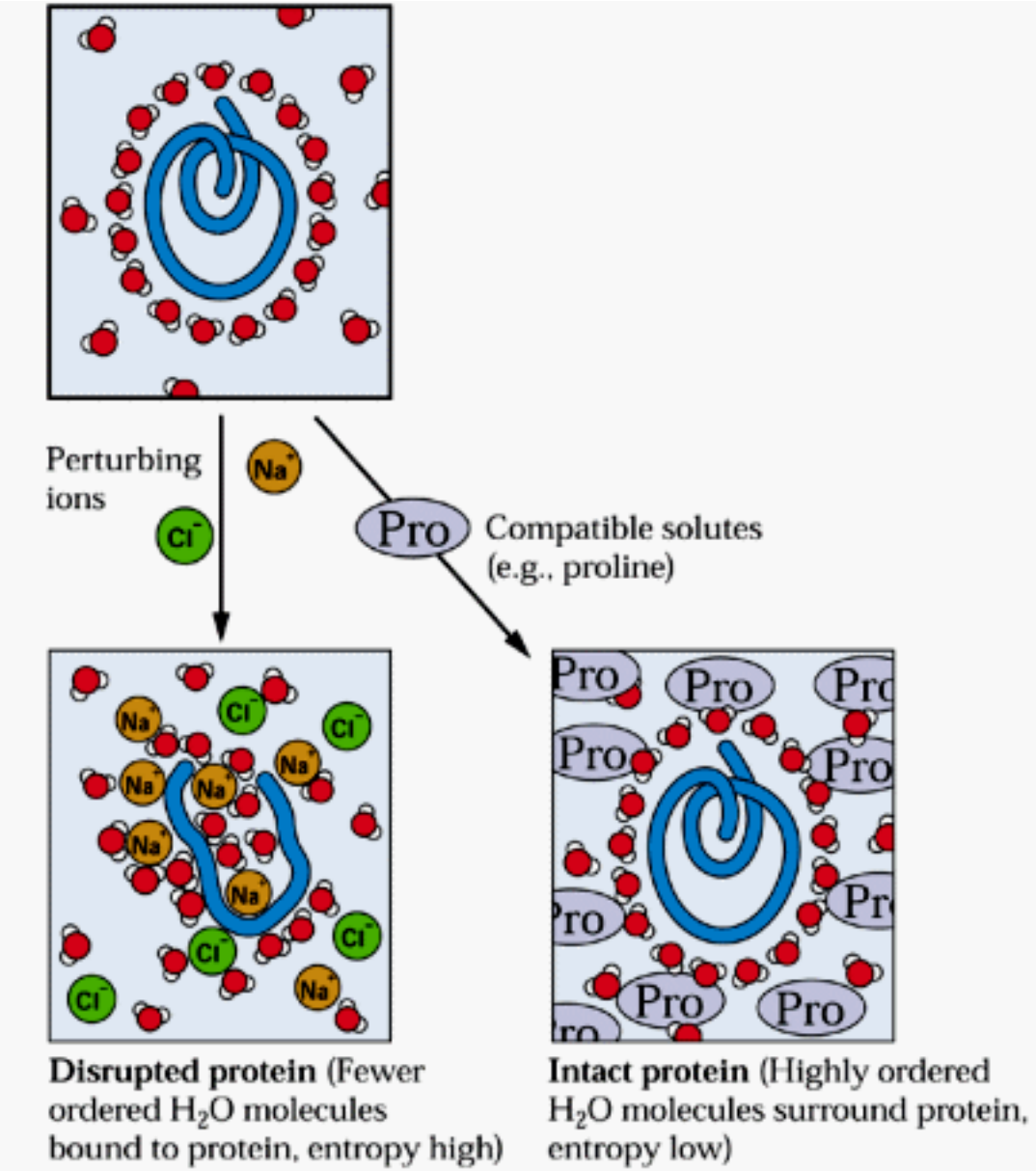
3°- Les osmolytes : rôle dans le maintien de la confo rmation des protéines



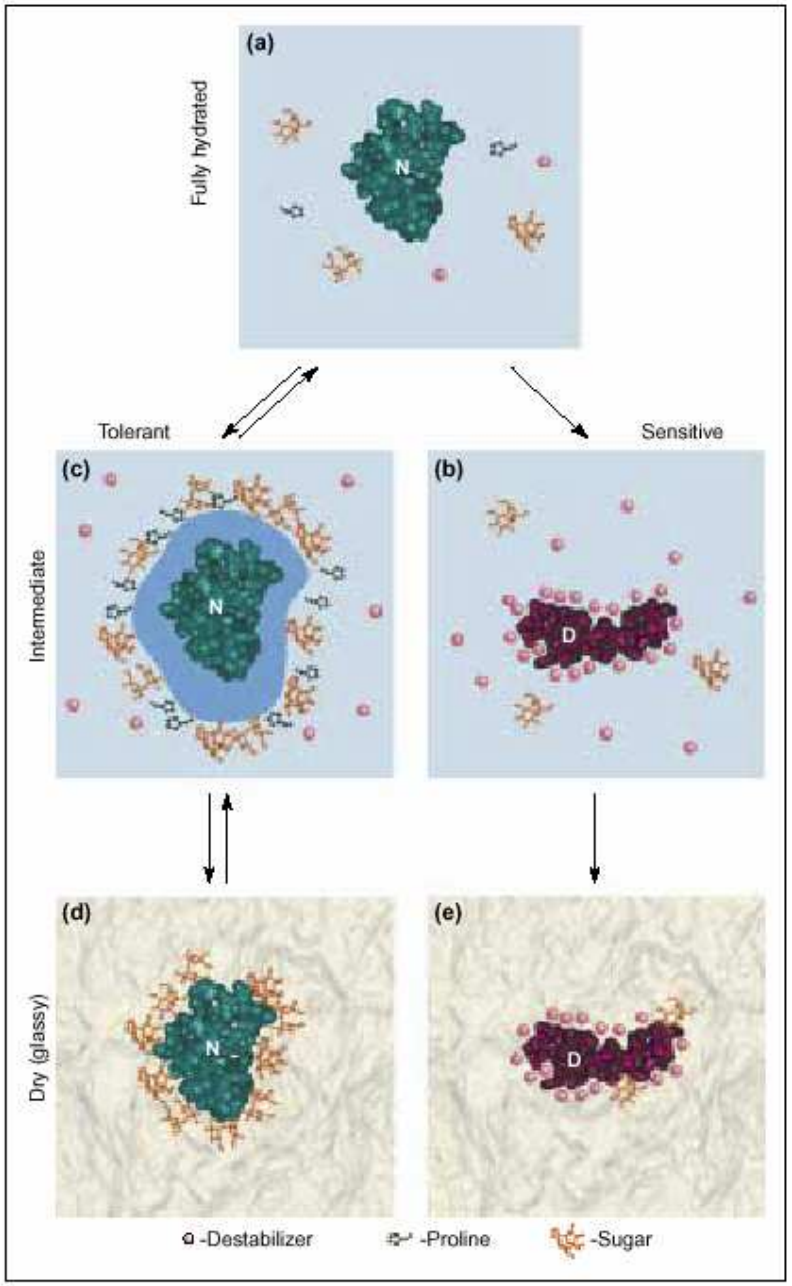


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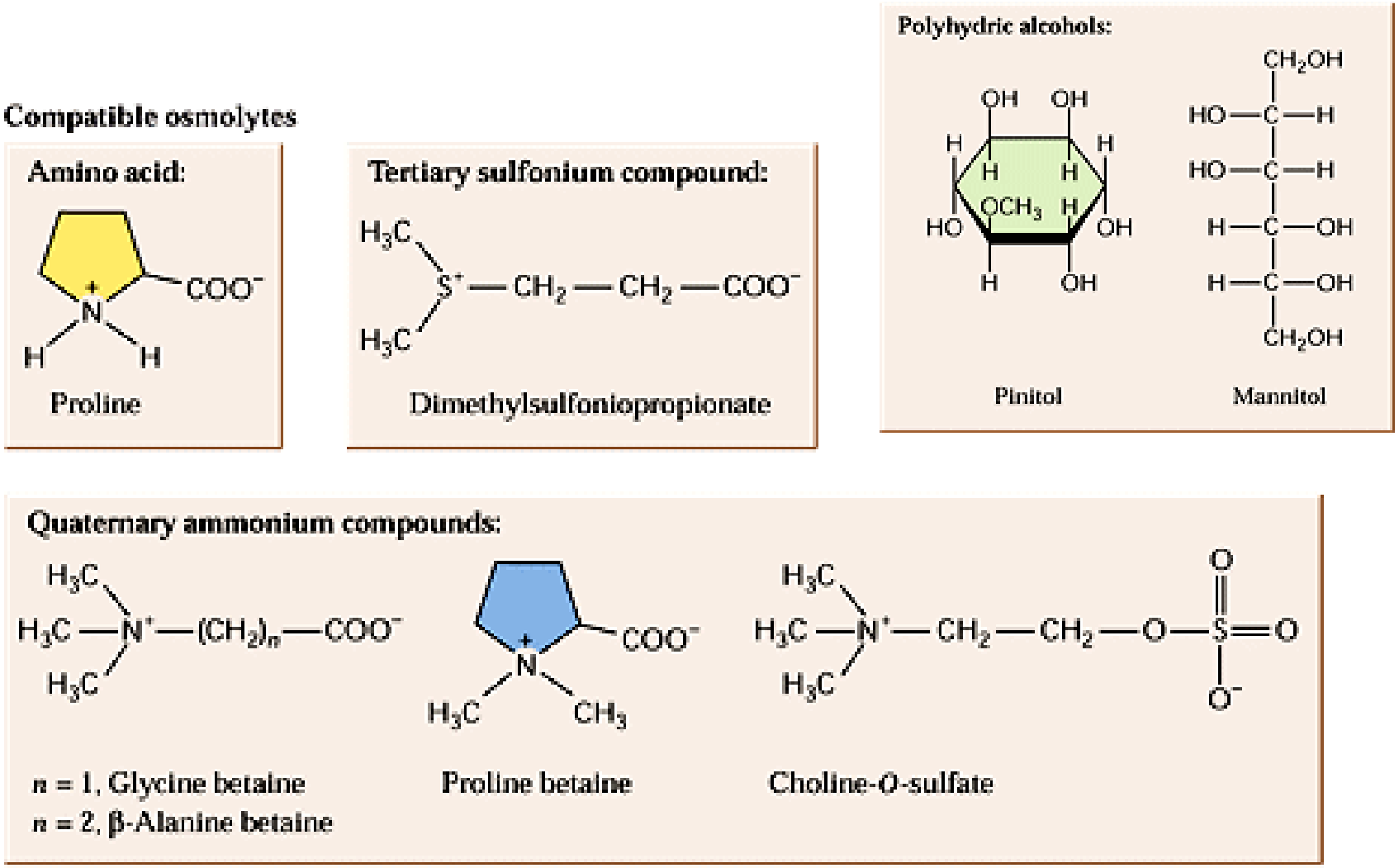
Fig. 1. Mechanisms of protein structure stabilization at different stages of water loss. In fully hydrated cells (a), the native (folded) form of a protein (N) is thermodynamically favorable. Molecular crowding during water loss increases the probability of the cytoplasmic solutes interacting with the protein surface. In sensitive cells (b), the lack of compatible solutes (for example, proline and sugar) causes preferential binding to dominate over preferential exclusion, which leads to protein unfolding and denaturation (D). Preferentially bound molecules act as destabilizers. In tolerant cells (c), preferential exclusion from the protein surface dominates over preferential binding, which maintains proteins in their native conformation at the intermediate water contents. Preferential exclusion of compatible solutes causes a preferential hydration of the protein surface (indicated as the blue ring around the protein). With the disappearance of the water shell from the proteins below 0.3 (g H_2O) (g dry weight)$^{-1}$, sugar molecules that were previously excluded from the protein surface replace water via hydrogen bonding, thus stabilizing the native protein structure in the dried (glassy) cytoplasm in tolerant cells (d). Compatible solutes other than sugars fail to stabilize proteins in the dried state. In dried, sensitive cells (e), the previously formed unfolded conformation (D) is fixed in a cytoplasmic glass. The reversibility of the processes occurring during dehydration and rehydration is indicated by arrows.

B- La structure chimique de quelques osmolytes



➔ Accumulation : biosynthèse (irréversible ou non), modifications de du catabolisme, du transport :

- des acides aminés et de leurs dérivés

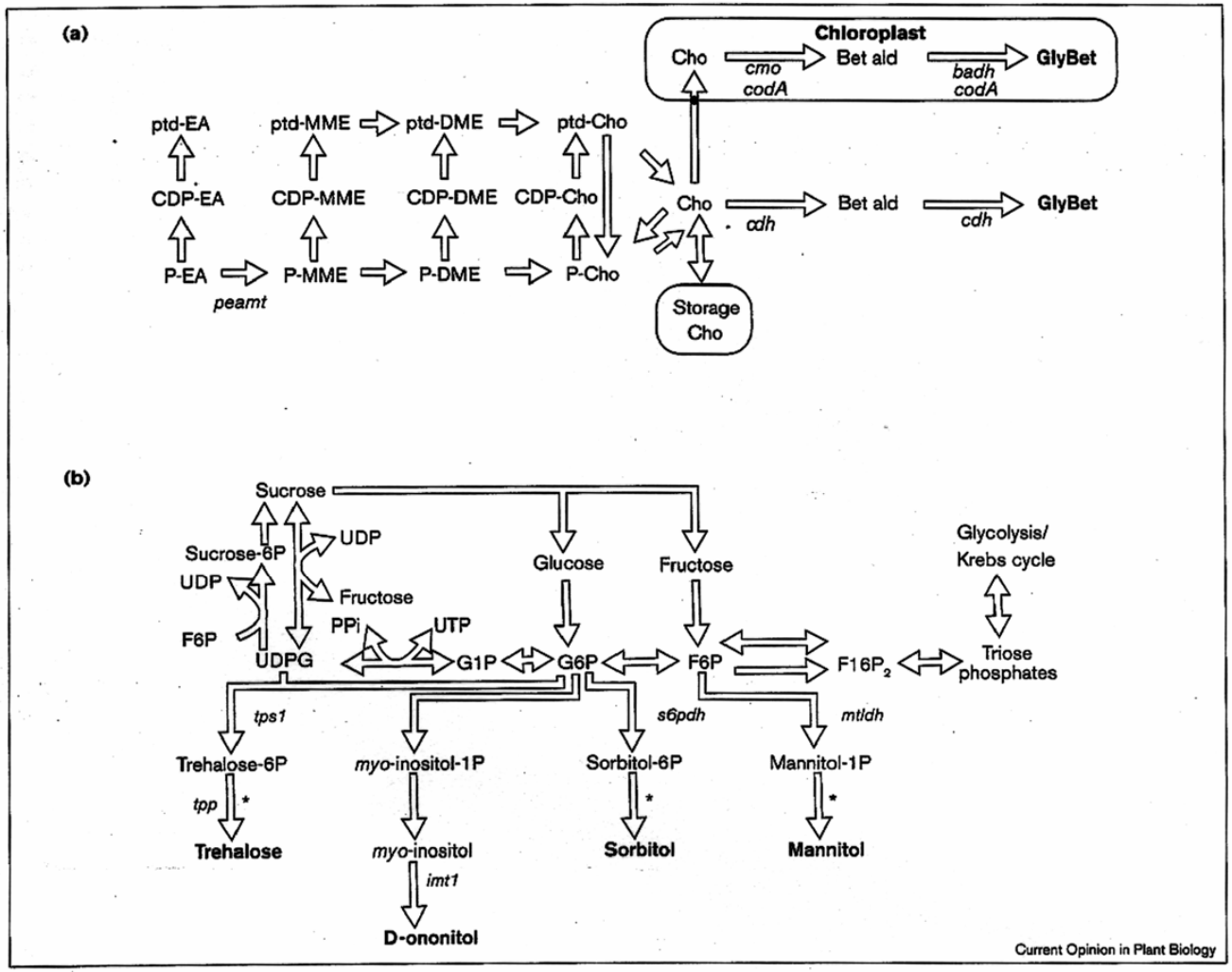
- des sucres

- de divers alcools

➔ distribution dans le règne végétal

Large : proline

Restreinte : glycine bétaïne

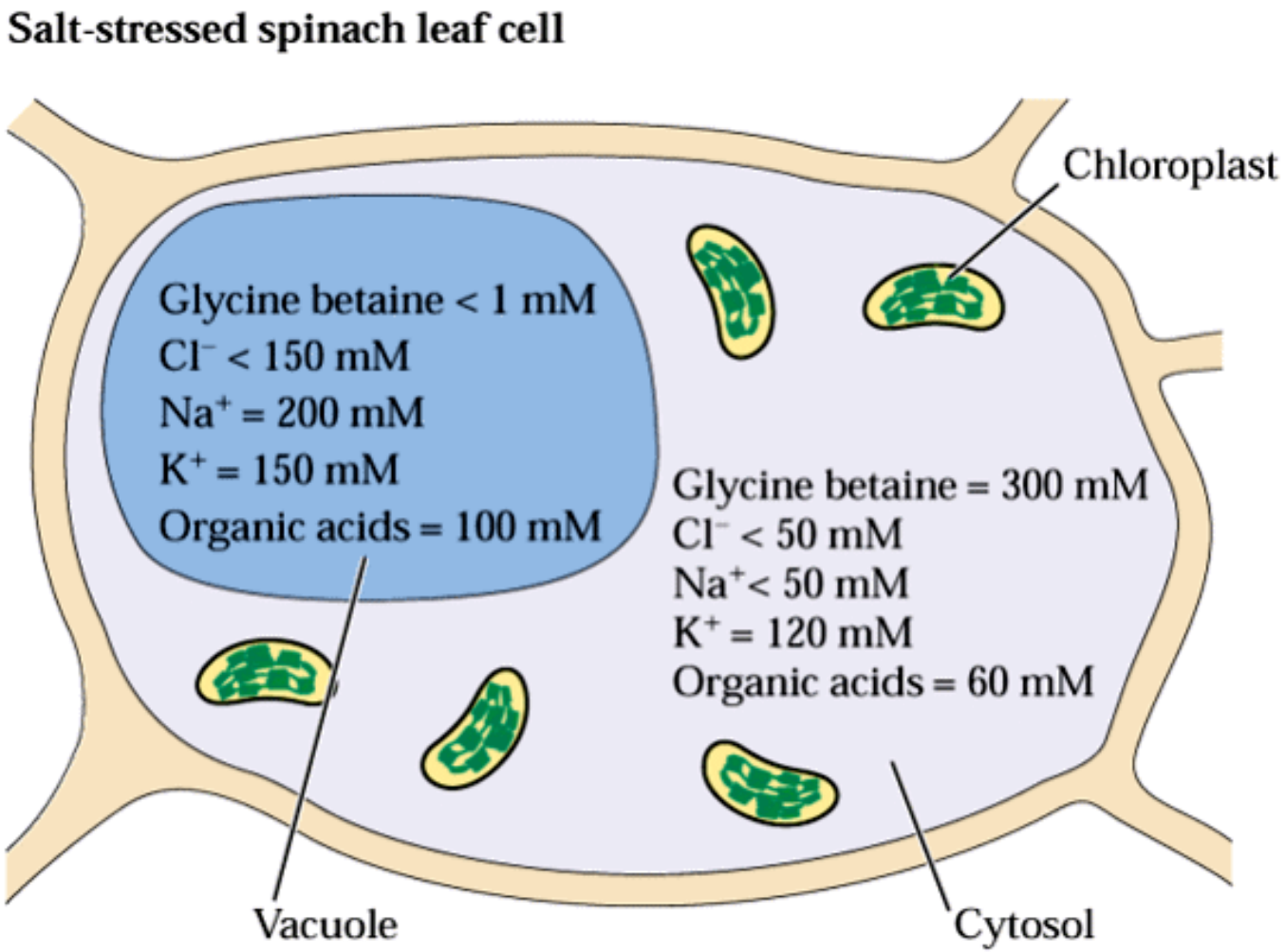


Metabolic pathways associated with GlyBet, polyol and trehalose synthesis. **(a)** GlyBet synthesis (modified from [12••]) and **(b)** polyol and trehalose synthesis. The osmoprotectants are given in bold. Abbreviations are CDP-, cytidyldiphospho-; EA, ethanolamine; MME, monomethylEA; DME, dimethylEA; Bet ald, betaine aldehyde; *cmo*, choline monooxygenase; *nsdh*, endogenous non-specific aldehyde dehydrogenase activity; *cdh*, Cho dehydrogenase; *codA*, Cho oxidase; *peamt*, P-EA-*N*-methyltransferase; *mtldh*, mannitol dehydrogenase; *s6pdh*, sorbitol dehydrogenase; *imt1*, inositol methyltransferase; *tps1*, trehalose-6-phosphate synthase; *tpp*, trehalose-6-phosphate phosphatase. Reactions with an asterisk (*) are non-specific phosphatase activity.

➔Localisation intracellulaire

compartimentation → cytoplasme majoritairement

composés toxiques : stockés dans la vacuole



1°- Les Sucres

Composés et distribution

✓ Animaux, champignons, levures, bactéries : tréhalose

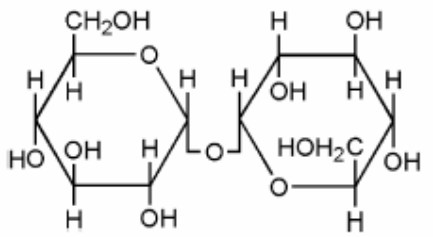


Fig. 1. Structure of trehalose.

✓ Plantes : saccharose + autres sucres

ex : plante de la résurrection :
saccharose et 2-D octulose
tréhalose

Table 1 Major sugars (mg g^{-1} of lyophilized material) found in *Craterostigma plantagineum* leaves[a]

Sugar	Fresh leaves	Dried leaves	Rehydrated leaves			
			4 h	21 h	28 h	5 days
Glucose	10(1)[b]	13(3)	96(32)	178(30)	60(12)	4(1)
Fructose	10(2)	8(2)	60(20)	128(21)	184(36)	3(1)
Sucrose	25(5)	374(90)	140(42)	151(27)	5(1)	7(2)
2-Octulose	430(89)	17(4)	3(1)	73(12)	215(42)	352(94)
Myo-inositol	5(1)	4(1)	2(1)	6(1)	14(3)	4(1)
Other sugars	tr	tr	tr	54(9)	30(6)	3(1)
Total	480	416	301	590	508	373

[a]Means of three determinations each made on samples of several different plants.
[b]Percentages (in parentheses) are rounded off to the nearest percentage unit.
tr = traces.

Rôle protecteur des sucres

- ✓ Vitrification du cytoplasme
- ✓ Protection des protéines : stabilisation de la conformation
- ✓ Protection des membranes

Ex : Le tréhalose :

The function of trehalose biosynthesis in plants
Wingler A. (2002) Phytochemitry 60:437-440

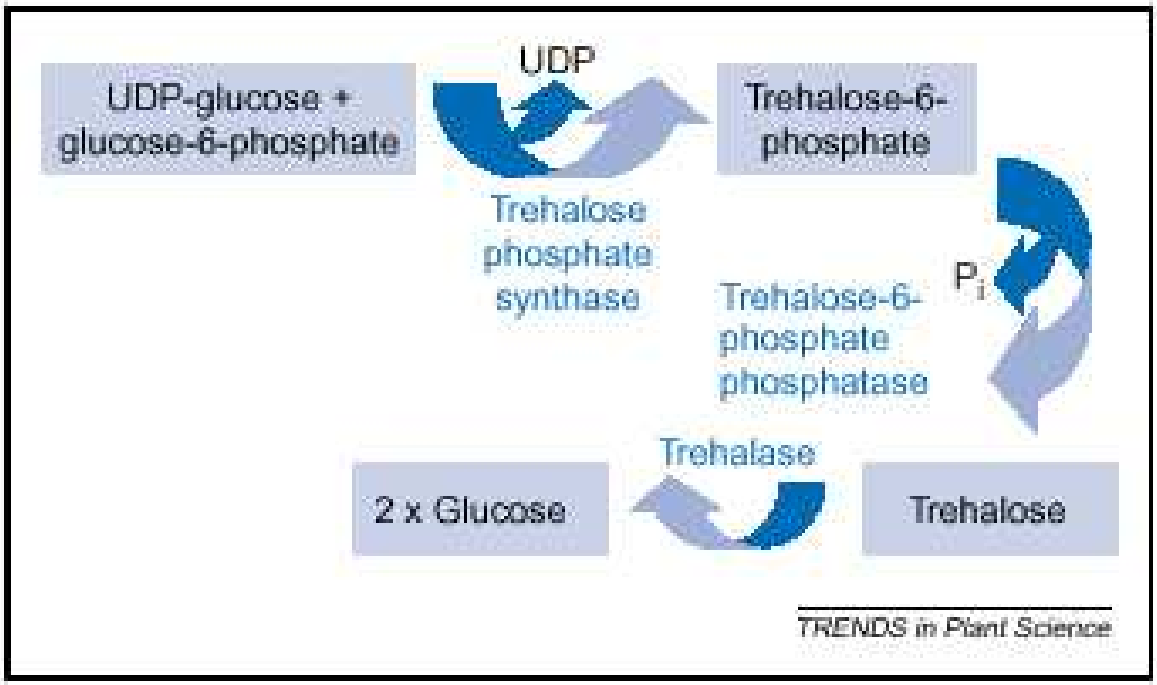
Trehalose metabolism in plants :
Goddjin et al., (1999) Trends in plant Science 4:315-319

Building stress tolerance through over-producing trehalose in plants
Penna S, (2003)Trends in plant Science 8:355-357

pas de tréhalose chez les plantes sauf certaines plantes de la résurrection :

-> introduction de la voie de biosynthèse du tréhalose chez les plantes à partir de gènes d ’origine procaryotique

TPS1 (yeast)
TPS (E. coli, OtsA)



TPS2 (yeast)
TPP (E. coli, OtsB)

NB : Yeast :
TPS3 : su régulatrice

Fig. 2. The expression of the *E. coli otsA* (trehalose-6-phosphate synthase activity) gene in tobacco leads to enhanced trehalose biosynthesis and also results in various pleiotropic effects. The *otsA* transgenic plant (right) has small, dark-green, lancet-shaped, thick leaves and a reduced senescence compared with the wild-type control plant (left).

-> Effets pléïotropiques

Table 1. Expression of trehalose biosynthetic genes in transgenic plants

Origin	Gene used	Promoter	Target plant	Prominent effects	Refs
E. coli	*otsA, otsB*	Constitutive (CaMV35S)	Tobacco	Improved growth under stress conditions, morphological alterations	[3,12]
Yeast	*TPS*	Tissue-specific rbcS	Tobacco	More trehalose levels	[8]
Yeast	*TPS1*	Constitutive (CaMV35S)	Tobacco	Stunted growth, lancet-shaped leaves, reduced sucrose content and improved drought tolerance	[6]
E. coli	*TPS*	Tuber specific (Patatin)	Potato	No trehalose levels detected	[3]
E. coli and *yeast*	*TPS* *TPP*	Constitutive (CaMV35S)	Tobacco	Enhanced rate of photosynthesis (TPS) Reduced photosynthesis (TPP)	[13]
E. coli	*otsA, otsB*	Tissue specific (rbcS) and stress dependant (abscisic acid inducible)	Rice	Sustained plant growth, less photo-oxidative damage, favorable mineral balance (under salt, drought and low temperature stress) and more trehalose. Increased stress tolerance	[9]

Chez *Arabidopsis* : pas de tréhalose mais :
- forte activité tréhalase
- mise en évidence de gènes de biosynthèse du tréhalose :

-> 1998 : complémentation d 'un mutant de levure *tps2* avec une banque d 'ADNc d '*Arabidopsis* (ARNm issus d 'un mélange de tissus).

- complémentation du phénotype thermosensible du mutant
- MEE de tréhalose par HPLC et diminution du pool de tréhalose 6-P (mais partielle par rapport au gène *TPS2*)

-> 2001 : analyse bioinformatique du génome d 'Arabidopsis : 11 gènes putatifs codant TPS/TPP

-> Rôle physiologique ?

15898–15903 | PNAS | December 10, 2002 | vol. 99 | no. 25

Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses

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Departments of *Molecular Biology and Genetics, ‡Plant Biology, and §Horticulture, Cornell University, Ithaca, NY 14853; †Department of Biological Science, Myongji University, Yongin, Kyonggi-Do 449-728, Korea; ¶School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; and ‖U.S. Department of Agriculture–Agriculture Research Service, Plant, Soil, and Nutrition Laboratory, Cornell University, Ithaca, NY 14853

Trehalose is a nonreducing disaccharide of glucose that functions as a compatible solute in the stabilization of biological structures under abiotic stress in bacteria, fungi, and invertebrates. With the notable exception of the desiccation-tolerant "resurrection plants," trehalose is not thought to accumulate to detectable levels in most plants. We report here the regulated overexpression of *Escherichia coli* trehalose biosynthetic genes (*otsA* and *otsB*) as a fusion gene for manipulating abiotic stress tolerance in rice. The fusion gene has the advantages of necessitating only a single transformation event and a higher net catalytic efficiency for trehalose formation. The expression of the transgene was under the control of either tissue-specific or stress-dependent promoters. Compared with nontransgenic rice, several independent transgenic lines exhibited sustained plant growth, less photo-oxidative damage, and more favorable mineral balance under salt, drought, and low-temperature stress conditions. Depending on growth conditions, the transgenic rice plants accumulate trehalose at levels 3–10 times that of the nontransgenic controls. The observation that peak trehalose levels remain well below 1 mg/g fresh weight indicates that the primary effect of trehalose is not as a compatible solute. Rather, increased trehalose accumulation correlates with higher soluble carbohydrate levels and an elevated capacity for photosynthesis under both stress and nonstress conditions, consistent with a suggested role in modulating sugar sensing and carbohydrate metabolism. These findings demonstrate the feasibility of engineering rice for increased tolerance of abiotic stress and enhanced productivity through tissue-specific or stress-dependent overproduction of trehalose.

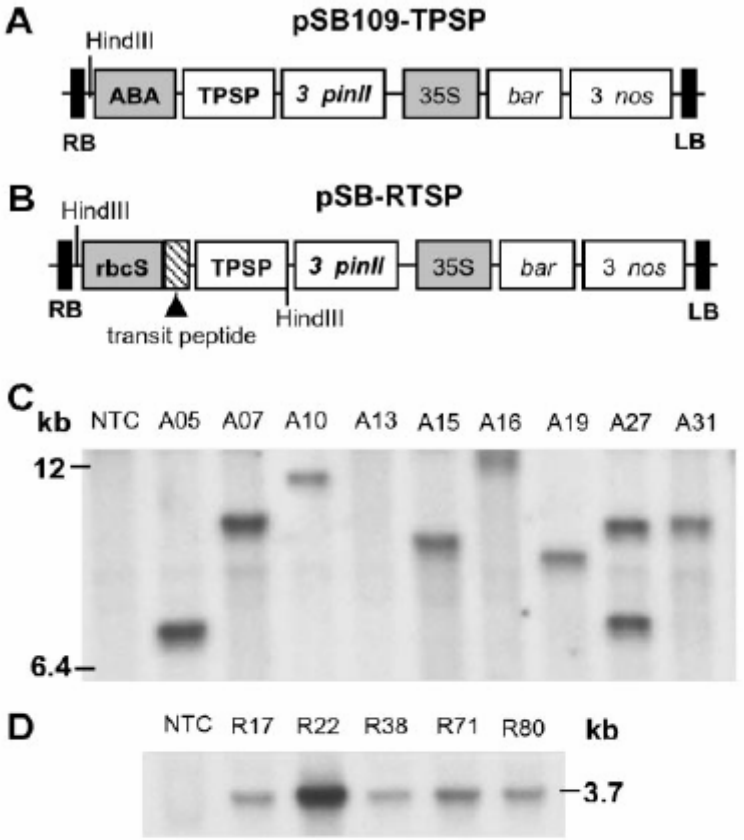


Fig. 1. Schematic representation of the expression vectors and DNA-blot hybridization analysis. Two binary plasmids, each containing the trehalose biosynthetic fusion gene (*TPSP*) that includes the coding regions of the *E. coli otsA* and *otsB* genes (encoding TPS and TPP, respectively), were constructed and transformed into *indica* rice, as described in *Materials and Methods*. (*A*) pSB109-TPSP plasmid. (*B*) pSB-RTSP plasmid. Shaded boxes represent promoter elements (ABA, ABA-inducible; rbcS, rice *rbcS*; 35S, cauliflower mosaic virus 35S); RB and LB represent T-DNA border on the right and left sides, respectively. Shown is DNA-blot hybridization analysis from nontransformed control (NTC) plant, and representative transgenic plants of nine A-lines (*C*) and five R-lines (*D*) that were transformed with the plasmid pSB109-TPSP and pSB-RTSP, respectively. The rice genomic DNA was digested with *Hin*dIII (a unique site in the plasmid pSB109-TPSP, whereas two sites are present in the plasmid pSB-RTSP) and DNA blot hybridization analysis was performed with the 2.2-kb *TPSP* fusion gene as the probe. Molecular sizes (kb) are indicated.

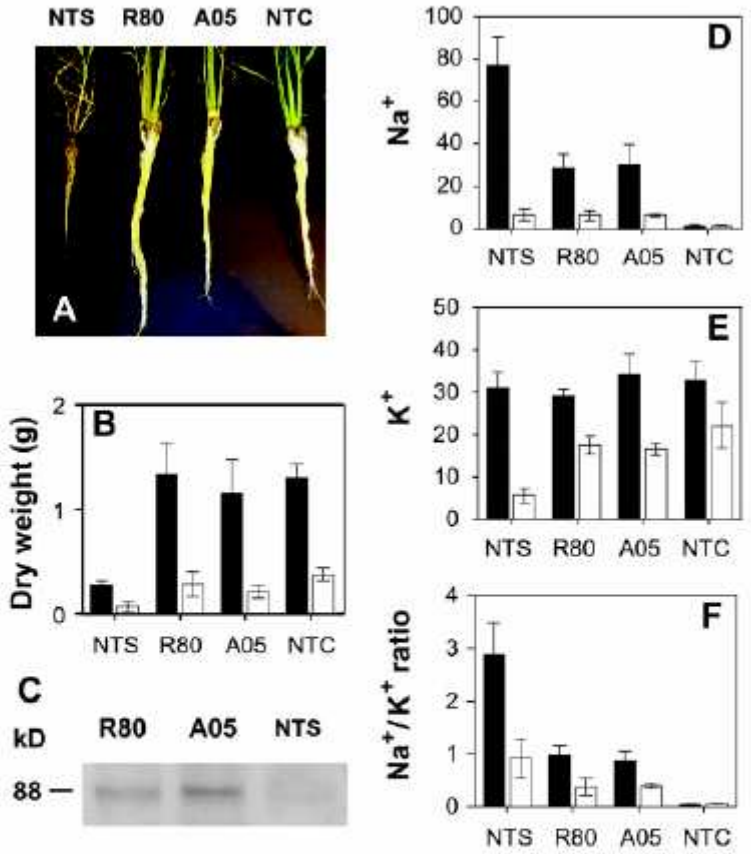


Fig. 2. Salt tolerance of rice plants and changes in mineral nutrition caused by salt stress. (*A*) Plant roots after 4 weeks of continuous 100 mM NaCl stress; the plants were not stressed in NTC. (*B*) Dry weight of shoots (black bars) and roots (white bars) of plants grown under salt stress (NTS, R80, and A05) or no stress (NTC) conditions. (*C*) Western blots of leaf extracts (20 μg of proteins) immediately after salt stress of plants. (*D–F*) Plant mineral nutrient content in shoots (black bars) and roots (white bars) under salt stress (NTS, R80, and A05) or no stress (NTC) conditions. (*D*) Na^+. (*E*) K^+. (*F*) Na^+/K^+ ratio. The ionic concentration is presented as mg/g dry weight. Values are the means ± SD ($n = 5$).

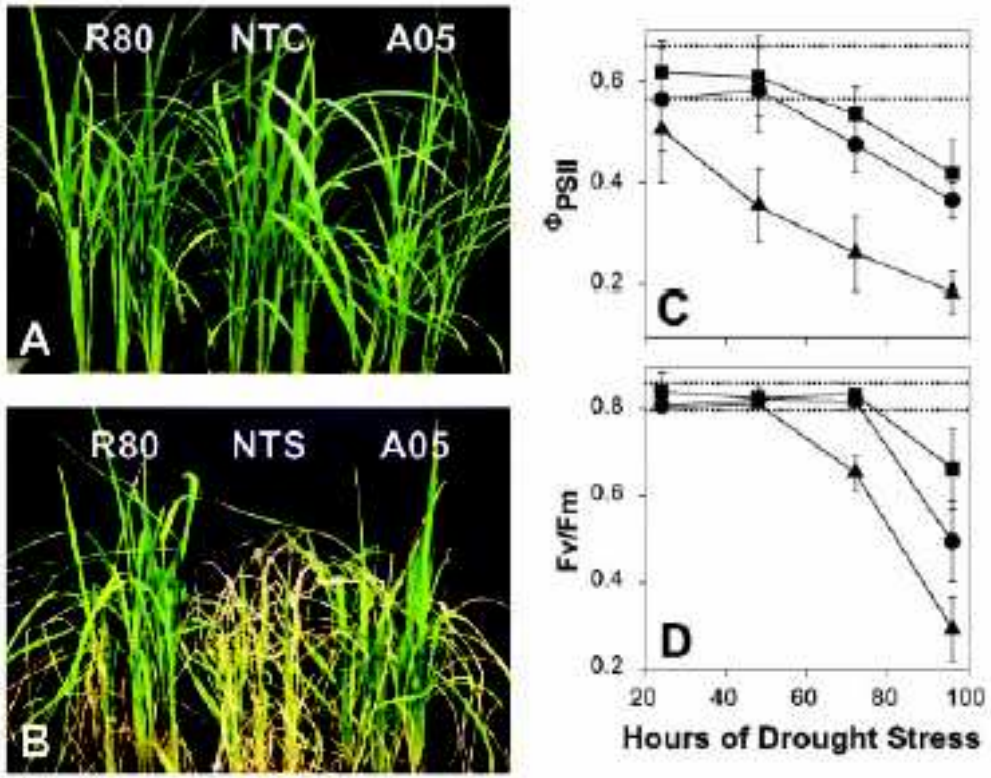


Fig. 3. Appearance of plants and chlorophyll fluorescence parameters during drought stress. Five-week-old nontransformed and T_4 generation transgenic (R80 and A05) seedlings grown in soil were subjected to two cycles of 100 h of drought stress followed by watering for 3 weeks. (*A*) Plants grown under well watered conditions (NTC, nontransgenic plants). (*B*) Plants of the same age after two cycles of drought-stress treatment (NTS, nontransgenic plants after drought stress). (*C* and *D*) Chlorophyll fluorescence measurements on young, fully expanded leaves during the first cycle of 100 h of continuous drought stress. (*C*) Φ_{PSII}, a measure of the efficiency of PS II photochemistry under ambient growth conditions. (*D*) Decreases in Fv/Fm are a measure of photooxidative damage to PS II. ▲, nontransformed plants; ■, R80; ●, A05. Dotted lines represent the range of values for nonstressed control plants of all lines. Data represent means ± SD ($n = 5$) from independent plants.

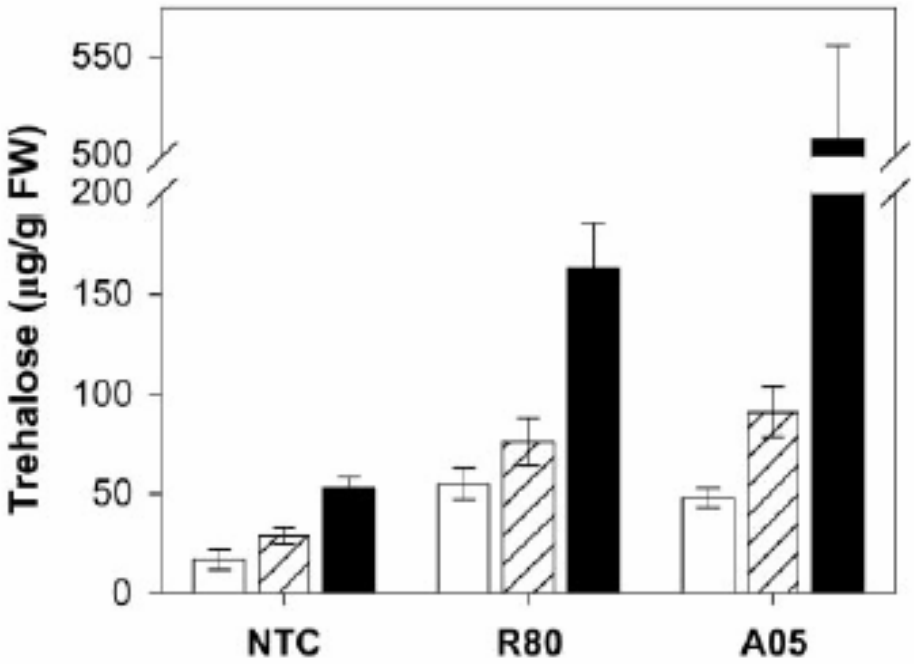


Fig. 4. Trehalose content in shoots of transgenic (R80 and A05) and non-transgenic plants with or without stress. Trehalose accumulation under non-stressed (white bars), salt-stressed (100 mM NaCl for 4 weeks, hatched bars), or drought-stressed (100 h, black bars) conditions.

2°- Les polyols

Polyalcools :

Bactéries marines, algues, levures, plantes, animaux

• Aliphatiques :

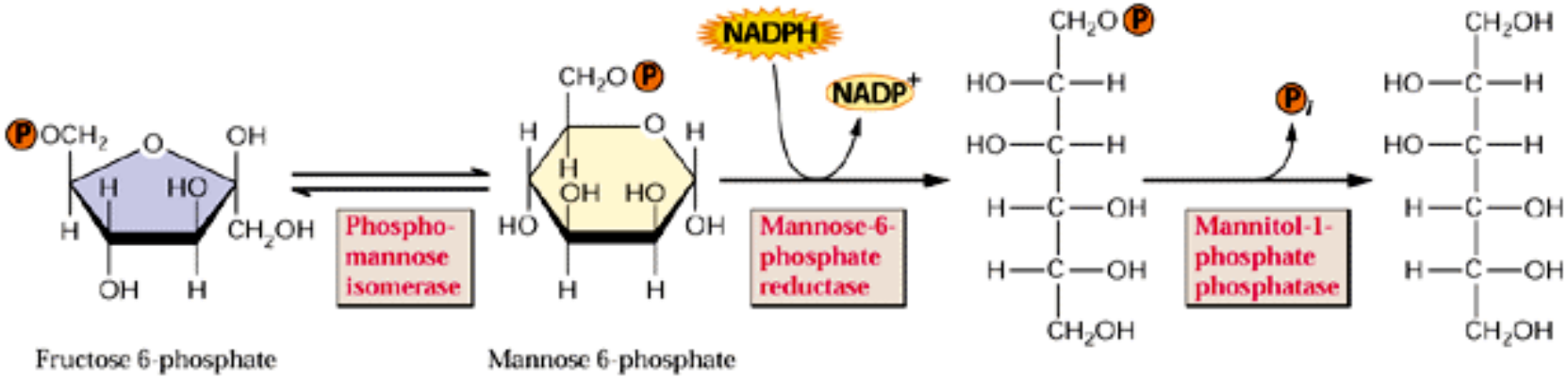
Mannitol	précurseur : mannose		nombreuses plantes
Sorbitol=glucitol		glucose	Pomme
Dulcitol =galacticol		galactose	Melon

• Cycliques :
myo-inositol, D-ononitol, D-pinitol

Ex 1 : Le mannitol

Source de carbone chez le céleri et d'autres plantes.
(50% mannitol, 50% saccharose)
-> Constitue une source importante des photoassimilats

Biosynthèse du mannitol à partir du fructose-6-P



M6PR

Métabolisme du mannitol chez le céleri

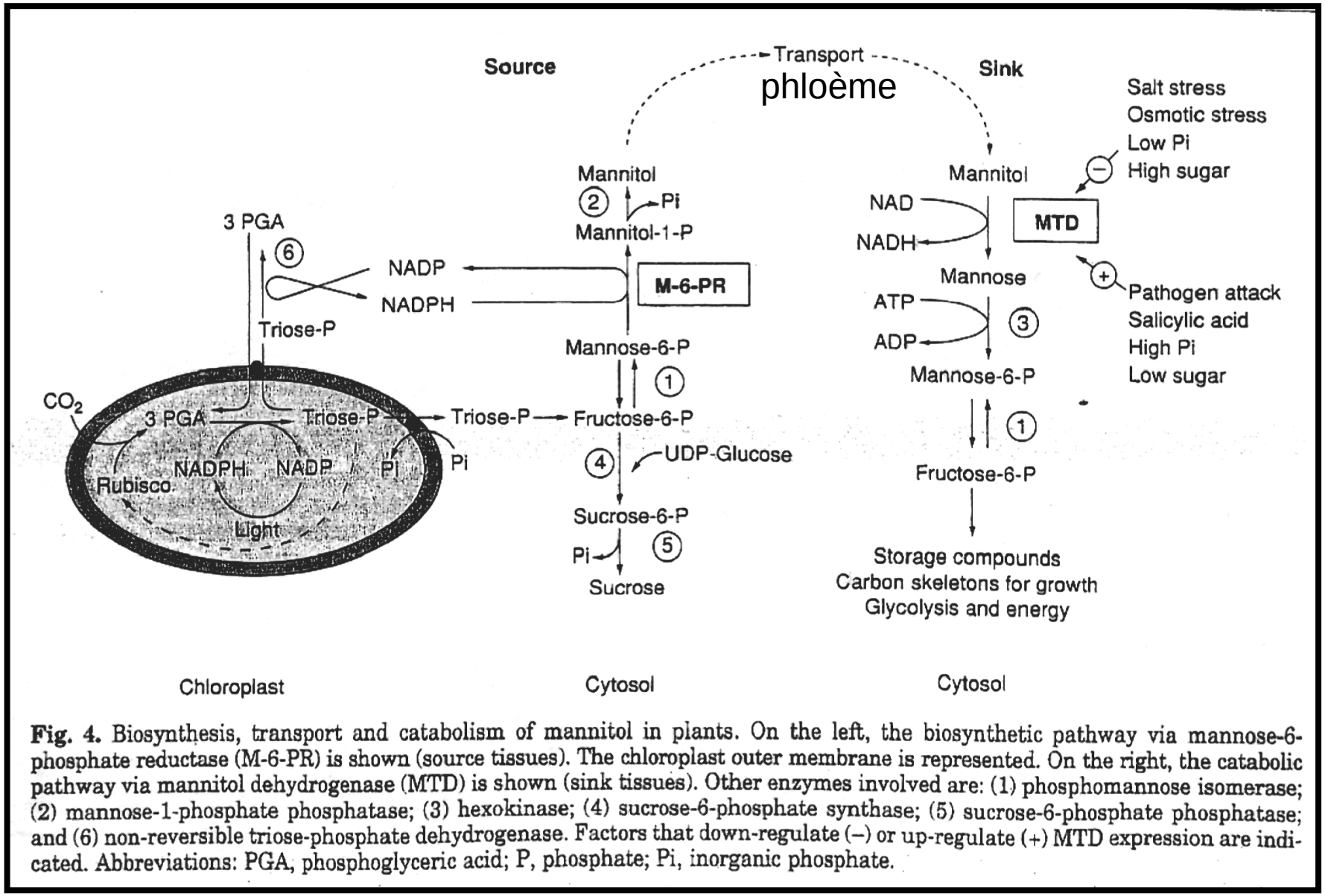


Fig. 4. Biosynthesis, transport and catabolism of mannitol in plants. On the left, the biosynthetic pathway via mannose-6-phosphate reductase (M-6-PR) is shown (source tissues). The chloroplast outer membrane is represented. On the right, the catabolic pathway via mannitol dehydrogenase (MTD) is shown (sink tissues). Other enzymes involved are: (1) phosphomannose isomerase; (2) mannose-1-phosphate phosphatase; (3) hexokinase; (4) sucrose-6-phosphate synthase; (5) sucrose-6-phosphate phosphatase; and (6) non-reversible triose-phosphate dehydrogenase. Factors that down-regulate (–) or up-regulate (+) MTD expression are indicated. Abbreviations: PGA, phosphoglyceric acid; P, phosphate; Pi, inorganic phosphate.

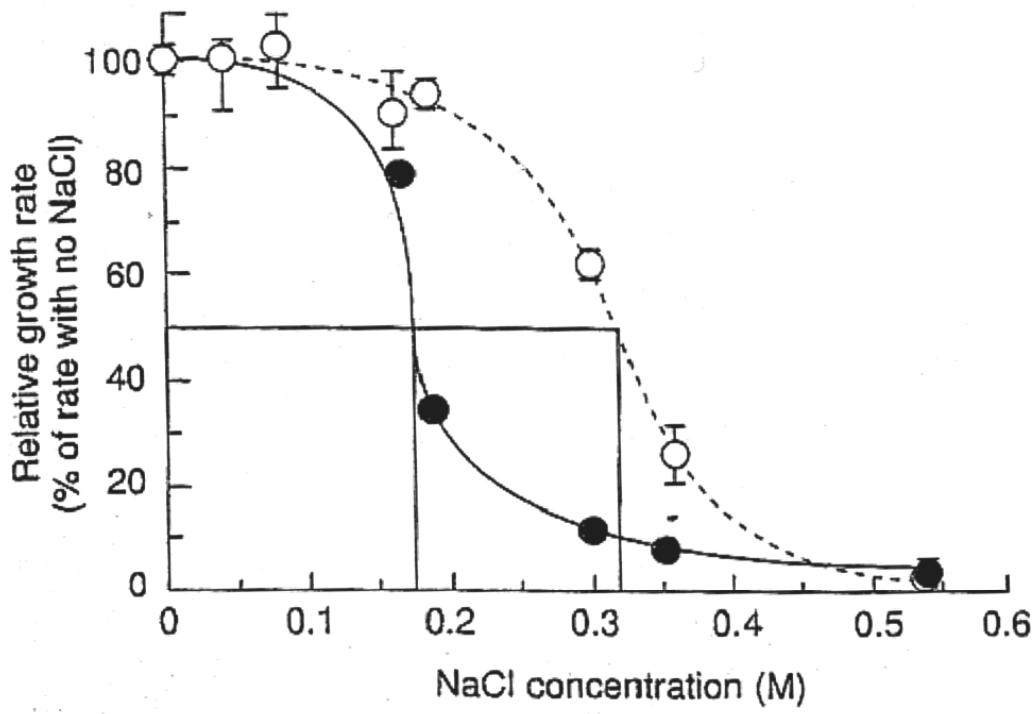
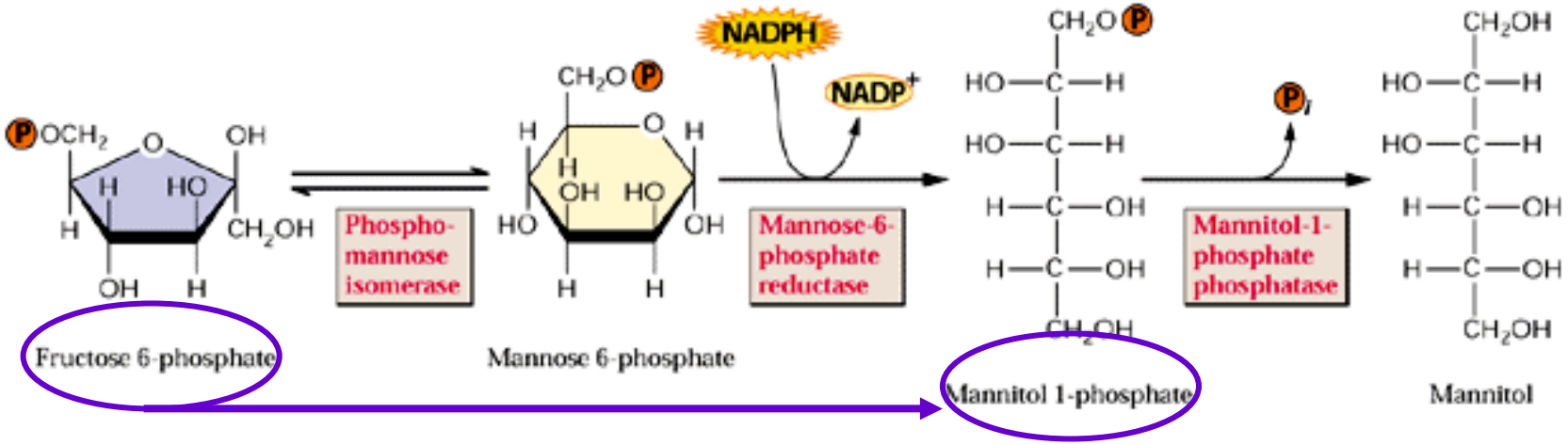


Fig. 3. Effect of mannitol on salt tolerance in celery (*Apium graveolens*) suspension cells. Celery suspension cells were grown under increasing concentrations of NaCl in medium containing either sucrose (filled circles: solid line) or mannitol (open circles: dashed line) as the sole carbon source. The I_{50}s (NaCl concentration at which growth was inhibited by 50%) were 0.17 M for sucrose-grown cells and 0.32 M for mannitol-grown cells. Bars indicate the standard error of three replicates. *Reproduced, with permission, from Ref. 18.*

Biosynthèse du mannitol à partir du fructose-6-P
Chez *E. coli : 1 gène mtlD pour 2 réactions*



mtlD
Mannitol-1-P-déshydrogénase

Introduction du gène *mtlD* chez le blé

-> céréale ne produisant pas de mannitol comme source de carbone

Tolerance of Mannitol-Accumulating Transgenic Wheat to Water Stress and Salinity[1]

Tilahun Abebe[2], Arron C. Guenzi*, Bjorn Martin, and John C. Cushman[3]

Plant Physiology (2003), vol 131, 1748-1755

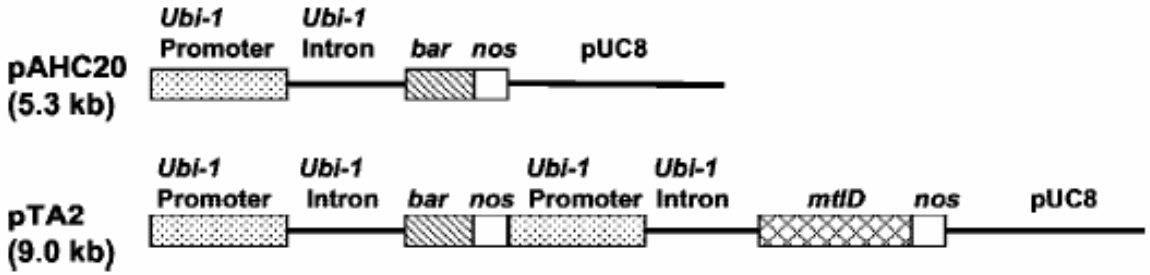


Figure 1. Plasmids used for wheat transformation. Plasmid pAHC20 contains only the selectable marker *bar*. Plasmid pTA2 contains *bar* and the *E. coli mtlD* gene for biosynthesis of mannitol-1-phosphate. Both genes were under the control of the maize (*Zea mays*) *ubi*-1 promoter. Calli and plants transformed with pTA2 were used as mannitol-accumulating lines (+mtlD), and those transformed with pAHC20 served as negative controls (−mtlD).

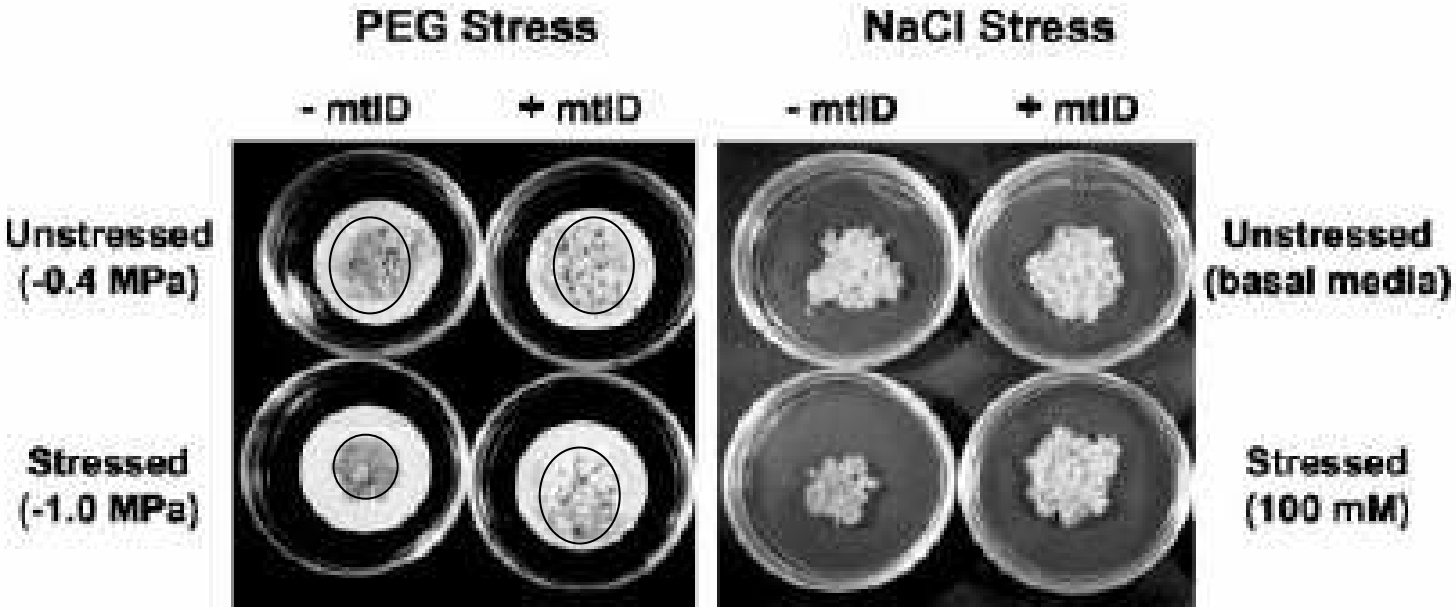


Figure 2. Effect of osmotic stress on the growth of transgenic wheat calli. The mannitol-accumulating callus line C2-20 (+mtlD) and the nonaccumulating line C1-11 (−mtlD) were grown in Murashige and Skoog medium containing PEG 8,000 (−1.0 MPa) or 100 mM NaCl for 60 d.

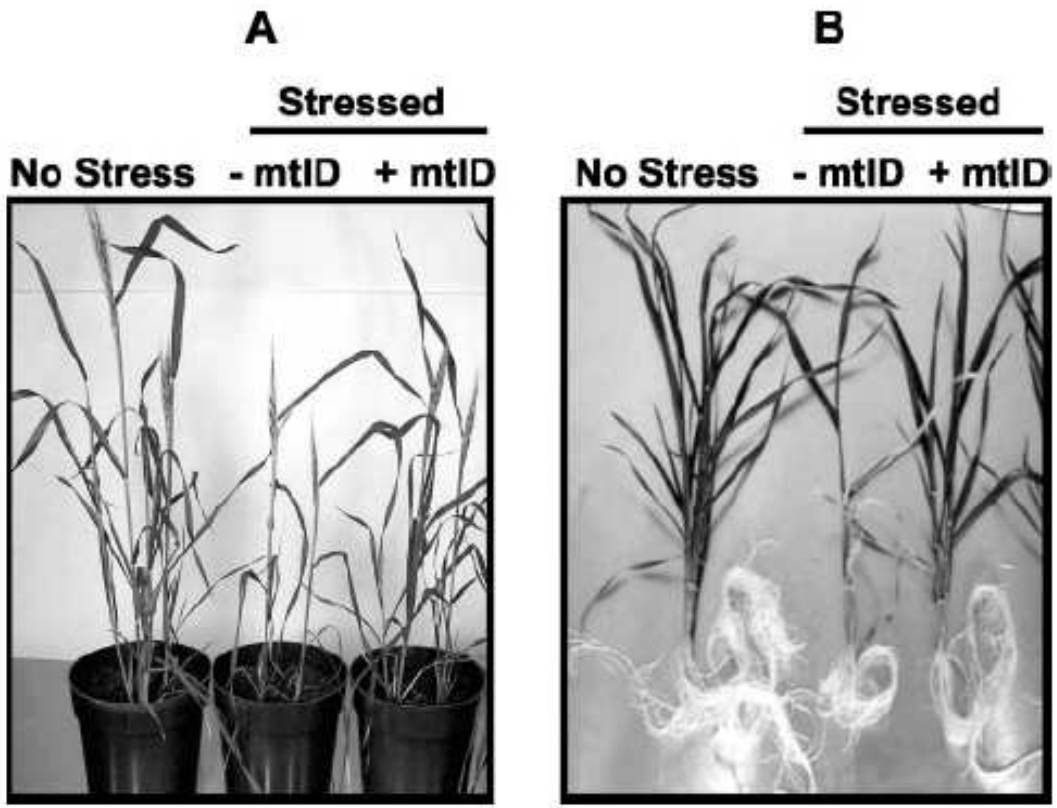


Figure 3. Effect of water stress and salinity on the growth of +mtlD and −mtlD plants. The mannitol-accumulating transgenic wheat line P2-19-1 (+mtlD) and the nonaccumulating P1-13-1 (−mtlD) were stressed by withholding water (A) or by supplementing the nutrient solution with 150 mM NaCl (B) for 30 d. Pictures were taken 30 d after the imposition of water stress and 20 d after NaCl stress. In the absence of stress, −mtlD and +mtlD plants were similar in size; thus, for unstressed controls, only the −mtlD plants are shown.

Ex 2 : Le pinitol

Mesembryanthemum cristallinum
Ice plant (Halophyte)

Désert de Namibie
Croissance en milieu sec, salin et froid
Modèle d'étude du stress hydrique

Régulation de l'absorption des ions
Compartimentation des osmolytes

Osmoprotection

Evite les effets toxiques des sels

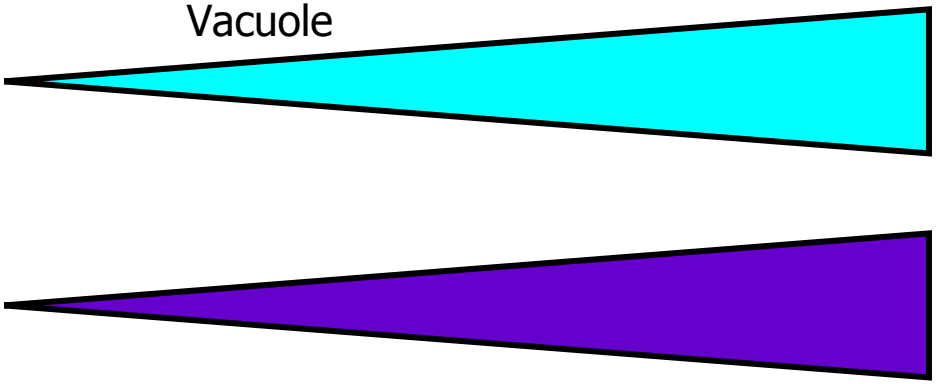
Mesembryanthemum cristallinum

Ice plant (Halophyte)

Absorption du Na^+

Gradient : maximum tissus jeunes

Vacuole



[D-pinitol] ↑

Cytoplasme et chloroplastes

Cellules épidermiques : ↑ Taille

Cellules polyploïdes

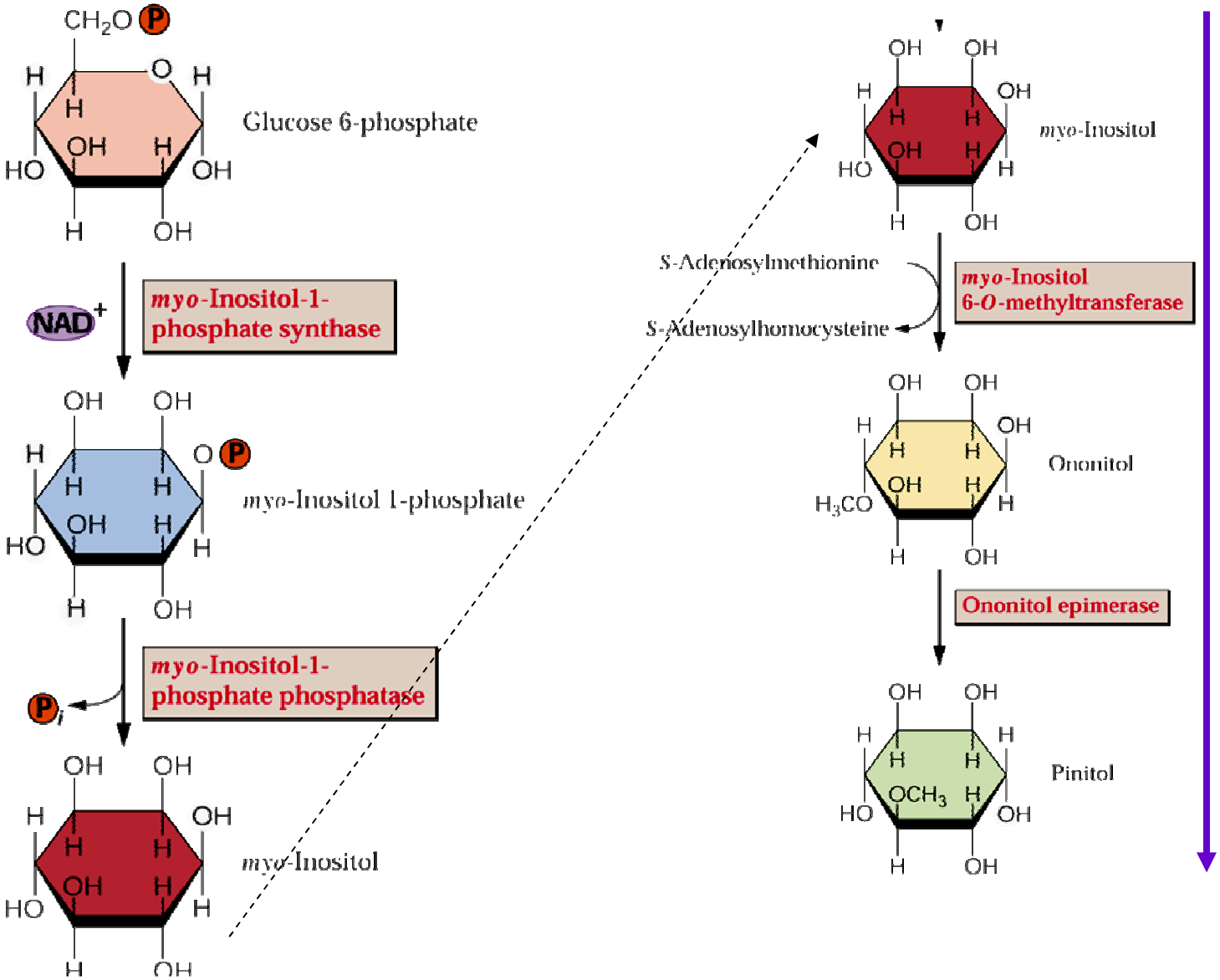
->2 µm volume cellulaire -> 3 µl

[D-pinitol] élevée -> 700 mM

[Na^+] > 1M

Ex 2 : Le pinitol

biosynthèse du pinitol : voie induite par le stress

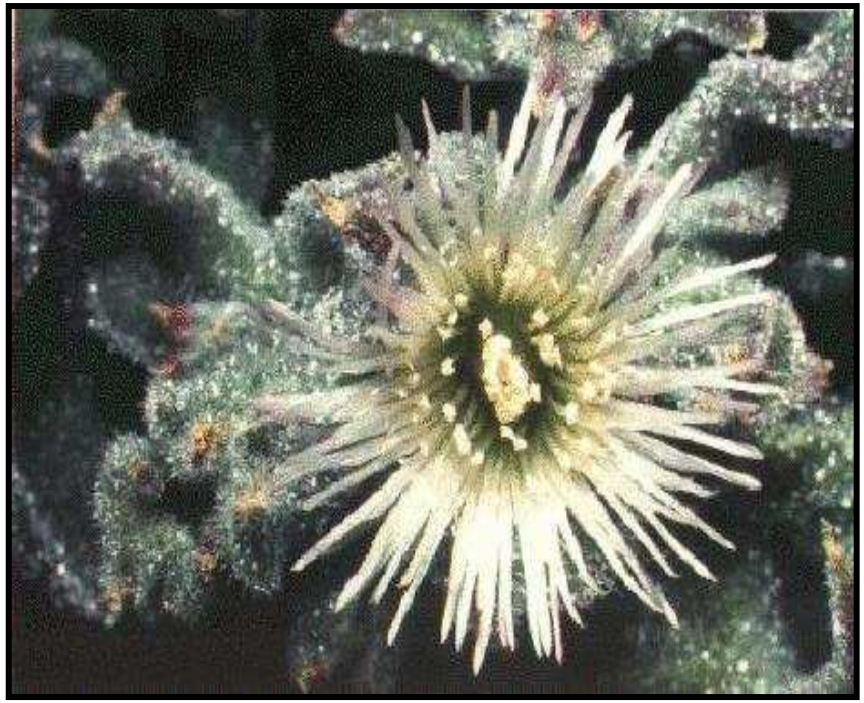


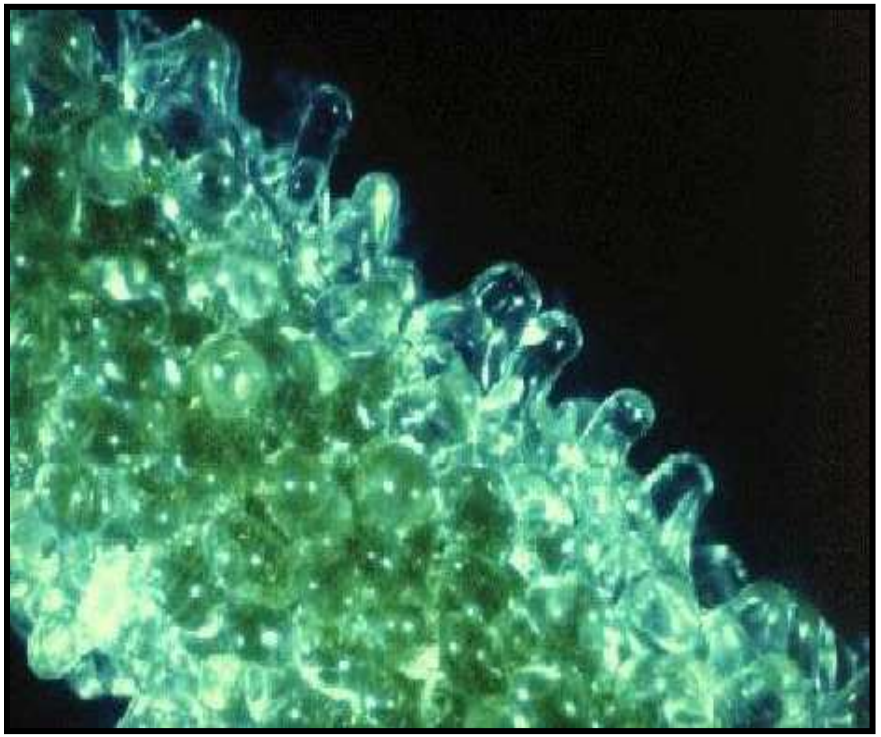
Mesembryanthemum cristallinum

10 Jours

2-3 Semaines

4 Semaines 8 Semaines

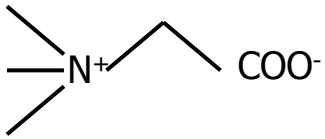




3°- Les acides aminés et leurs dérivés

Molécules zwitterions

Acides aminés : proline



Dérivés des acides aminés

- **QAC (Quaternary ammonium compounds)**

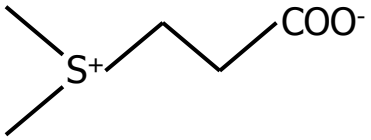
  Glycine bétaïne

  Choline-O-sulfate, β-alanine bétaïne, proline-bétaïne,

  Hydroxyproline-bétaïne

- **TSC (tertiary sulfonium compounds)**

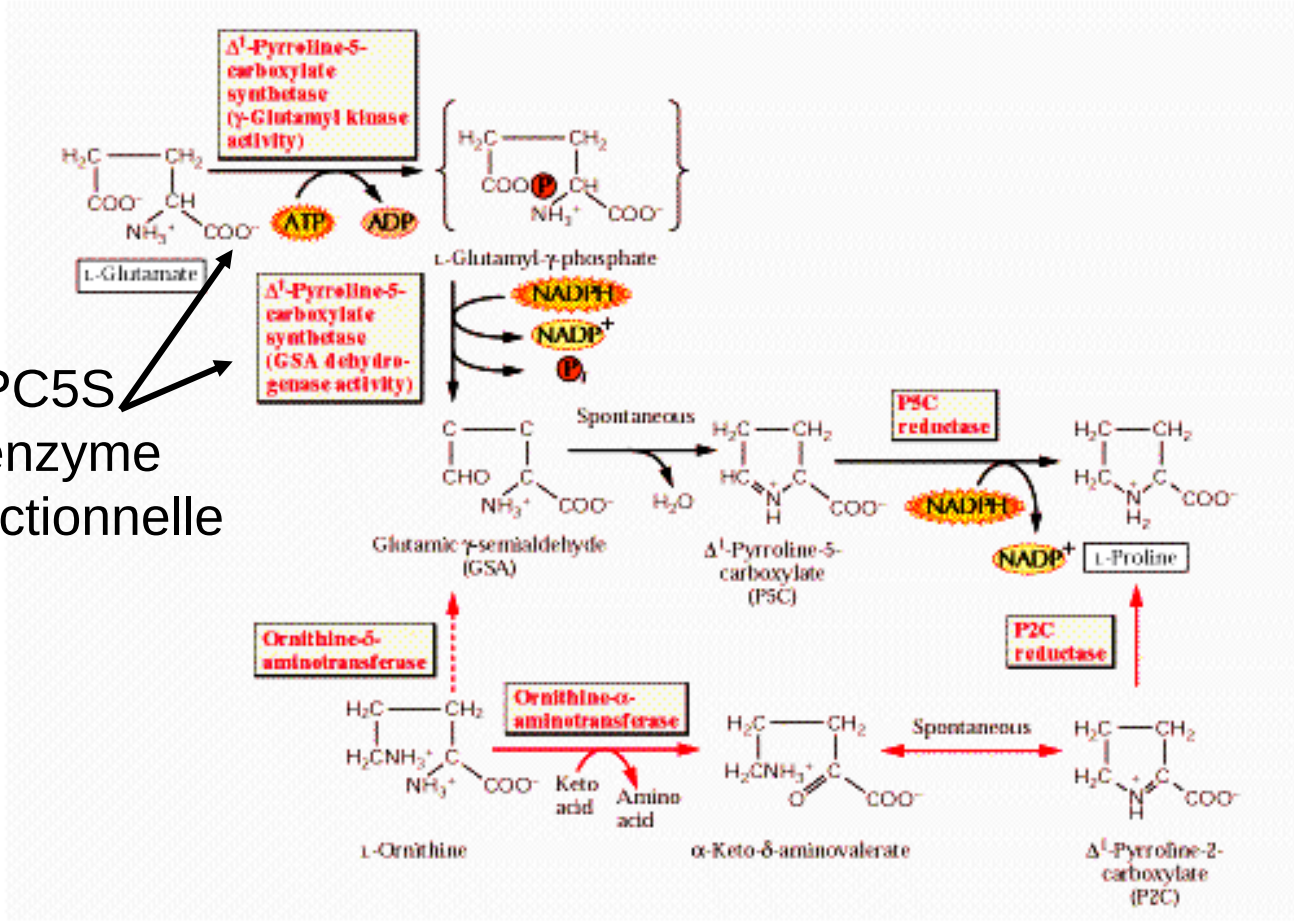
  DMSP 3-diméthylsulfoniopropionate



Ex 1. La proline

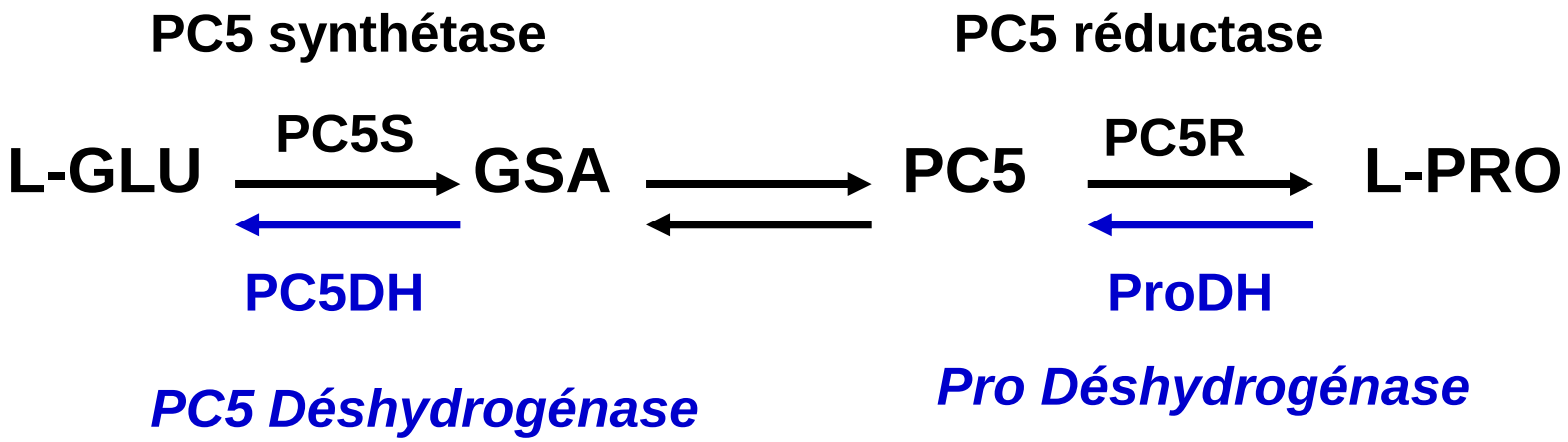
Voies de biosynthèse

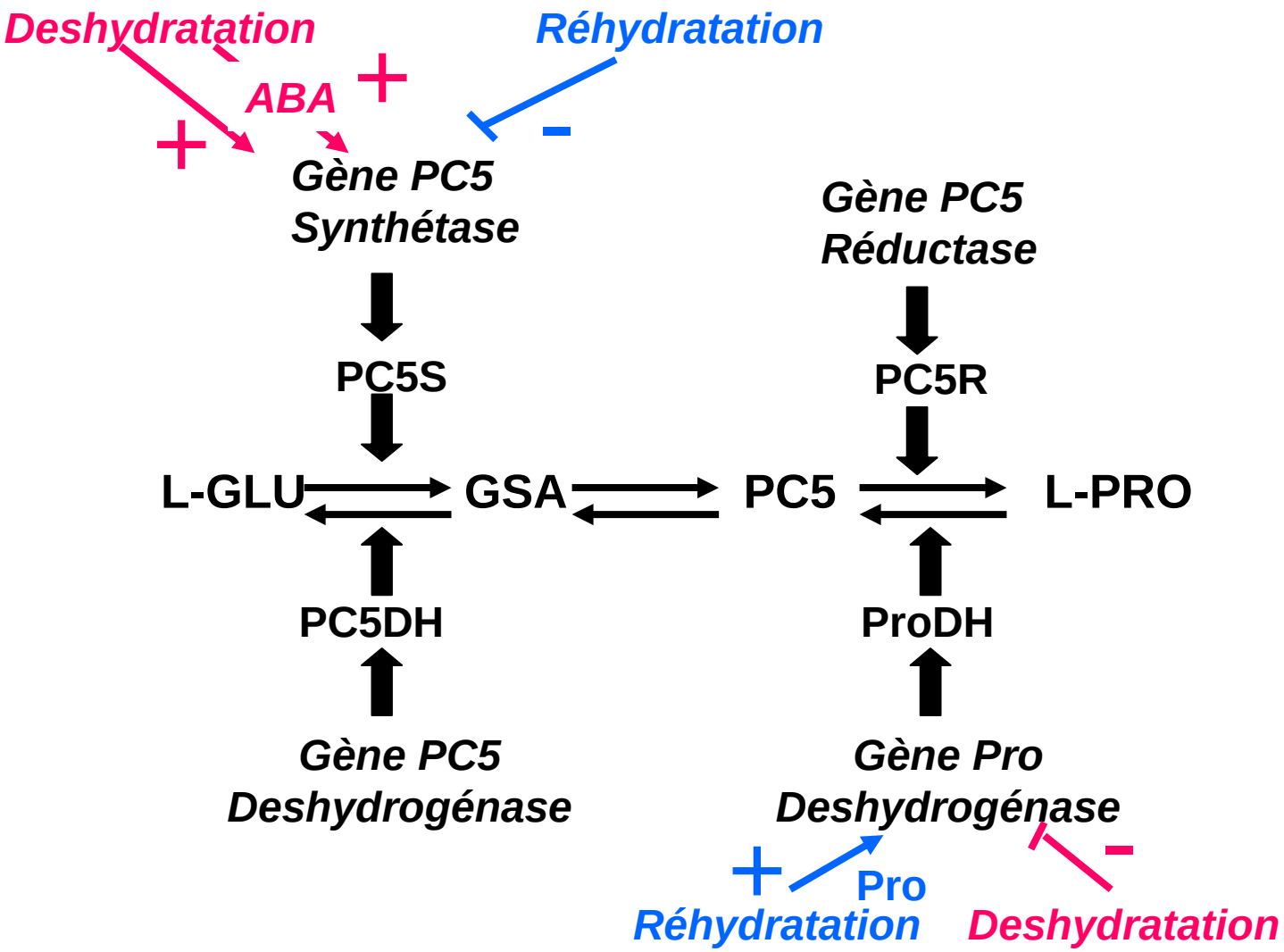
PC5S
1 enzyme
bifonctionnelle



La proline

Voie de biosynthèse principale lors d ’un stress osmotique




Deshydratation
Réhydratation
ABA
+
+
-
Gène PC5
Synthétase
Gène PC5
Réductase
PC5S
PC5R
L-GLU
GSA
PC5
L-PRO
PC5DH
ProDH
Gène PC5
Deshydrogénase
Gène Pro
Deshydrogénase
+
Pro
Réhydratation
-
Deshydratation

Kishor P.B.K. *et al.*, 1995
Plant Physiol., 108:1387-1394

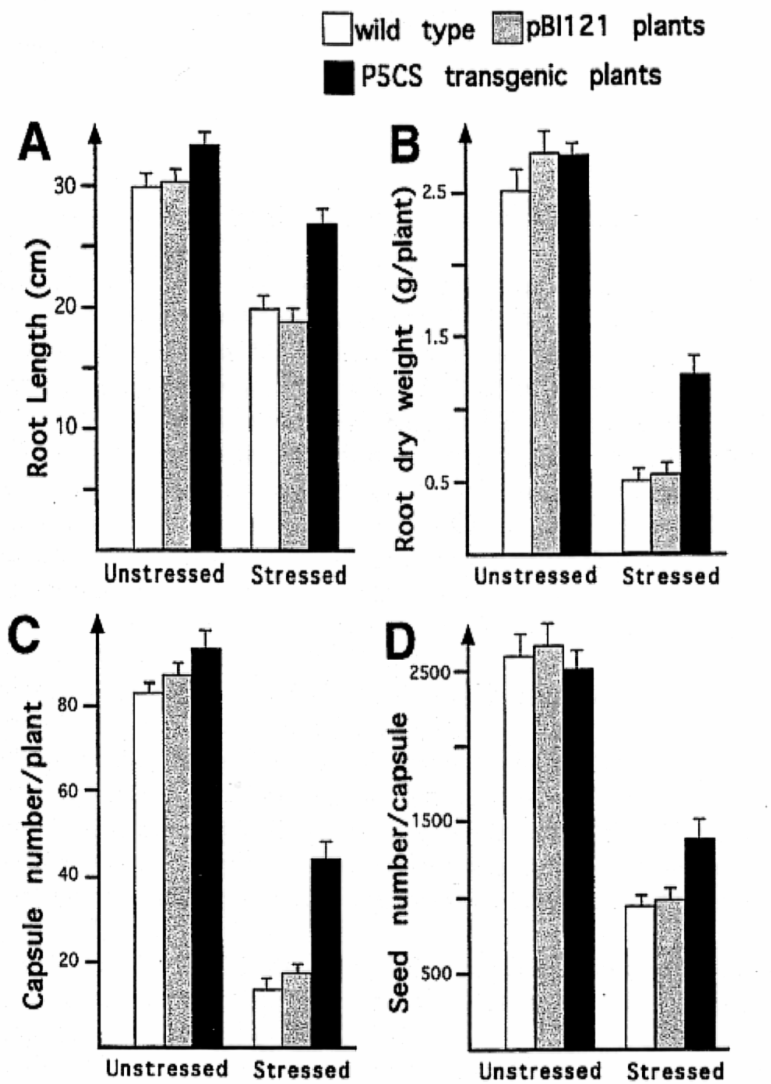


Figure 6. Comparison of the wild-type (open box), pBI121 (stippled box), and P5CS transgenic plants (solid box) in root length (A), root dry weight (B), pod number (C), and seed number (D). The plants were grown to maturity in Metromix, supplied with 0.5 M NaCl. Ten independent transgenic lines (T_1) with six plants each were used for this analysis, and only an average value is shown.

Kishor P.B.K. *et al.*, 1995
Plant Physiol., 108:1387-1394

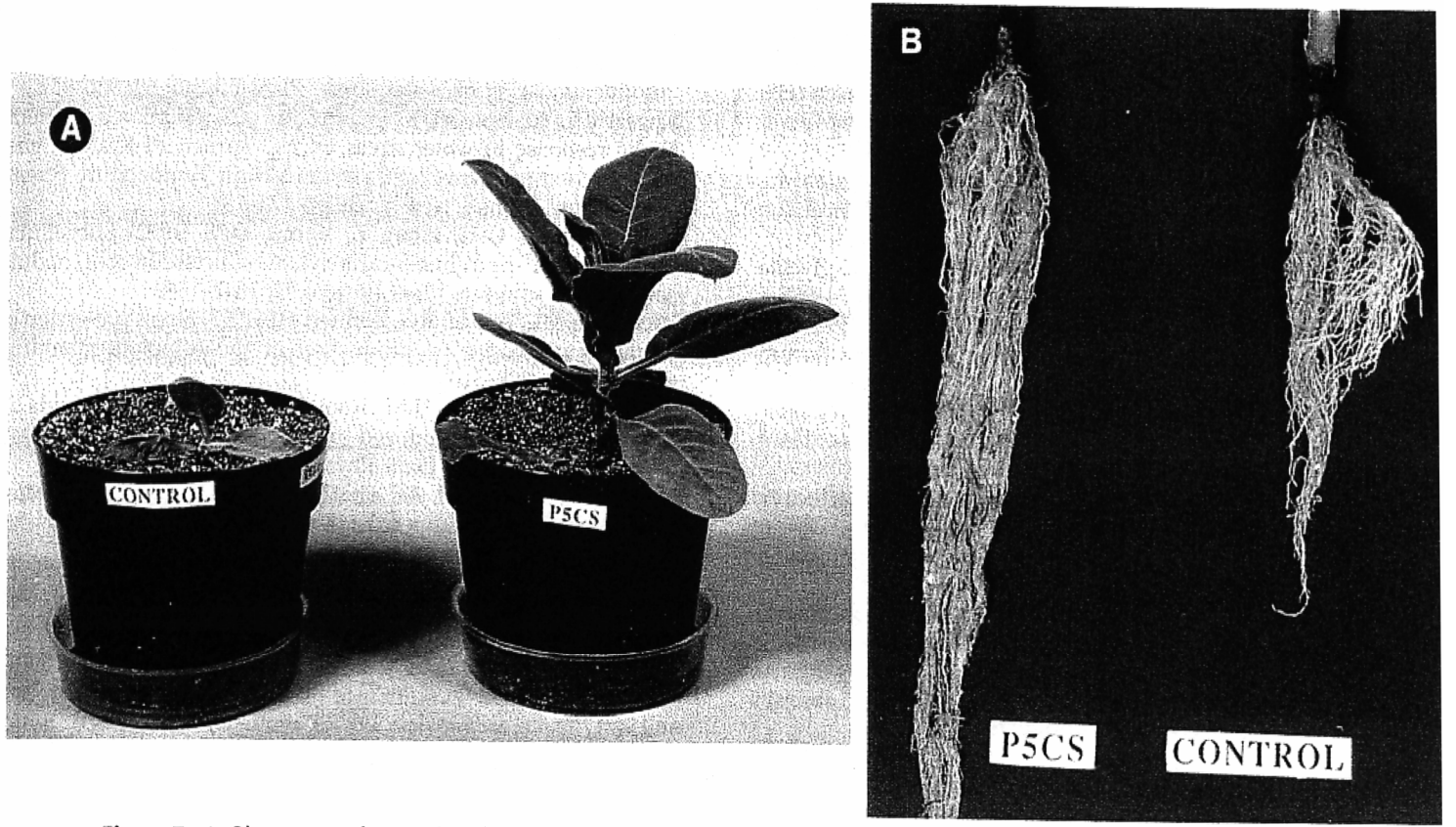


Figure 7 A, Phenotype of control and P5CS transgenic plants treated with salinity stress. Plants of wild type and transgenic line 22 (T_1) were grown in vermiculite, and at the four-leaf stage, the pots were transferred to trays containing 0.4 M NaCl and allowed to stand in the solution for 3 weeks. B, Root phenotype of wild type and transgenic line 22 (T_1) treated with drought stress. The plants were potted in Metromix, and 6-week-old plants were subjected to drought conditions until flowering. The roots at the time of flowering were washed and photographed.

L ’accumulation de la proline est aussi contrôlée au niveau post-traductionnel :

-> inhibition de la PC5S par la proline

Plant Physiology, April 2000, Vol. 122, pp. 1129–1136, www.plantphysiol.org © 2000 American Society of Plant Physiologists

Removal of Feedback Inhibition of Δ^1-Pyrroline-5-Carboxylate Synthetase Results in Increased Proline Accumulation and Protection of Plants from Osmotic Stress[1]

Zonglie Hong, Karuna Lakkineni, Zhongming Zhang, and Desh Pal S. Verma*

Department of Molecular Genetics and Plant Biotechnology Center, The Ohio State University, 1060 Carmack Road, Columbus, Ohio 43210–1002

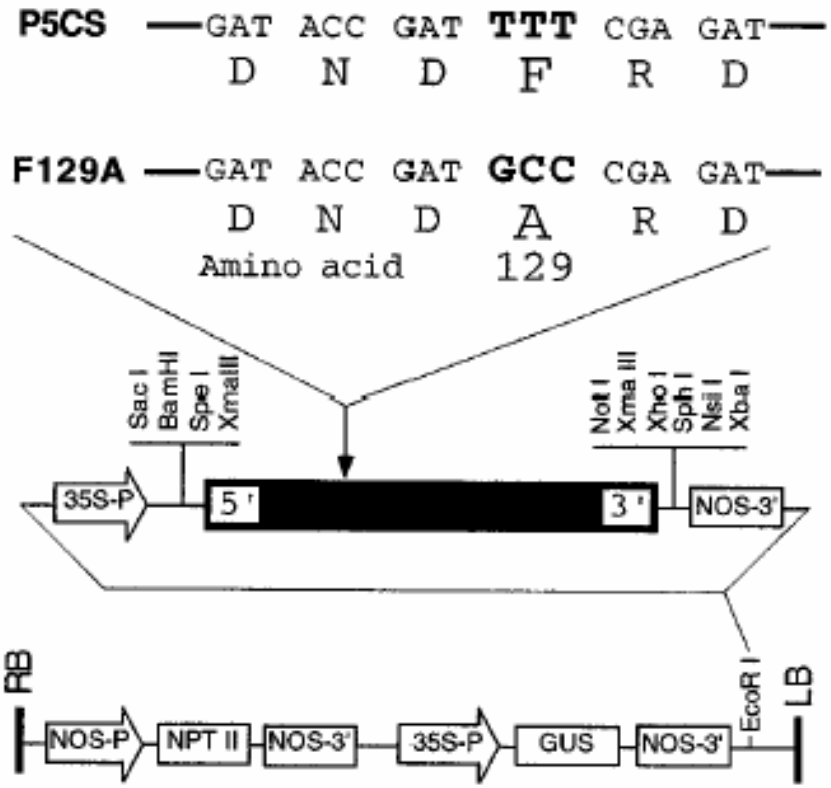


Figure 1. Mutation site of P5CS that removed feedback inhibition by Pro, and restriction map of pBI-P5CSF129A. Codon TTT at nucleotide positions 421 to 423 of the *V. aconitifolia* P5CS cDNA (Hu et al., 1992) was changed to GCC by site-directed mutagenesis so that Phe (F) at amino acid position 129 of the P5CS peptide is replaced by Ala (A), generating P5CSF129A. The mutant enzyme retains similar kinetic characteristics as the wild-type P5CS, except that its allosteric regulation by Pro is eliminated (Zhang et al., 1995).

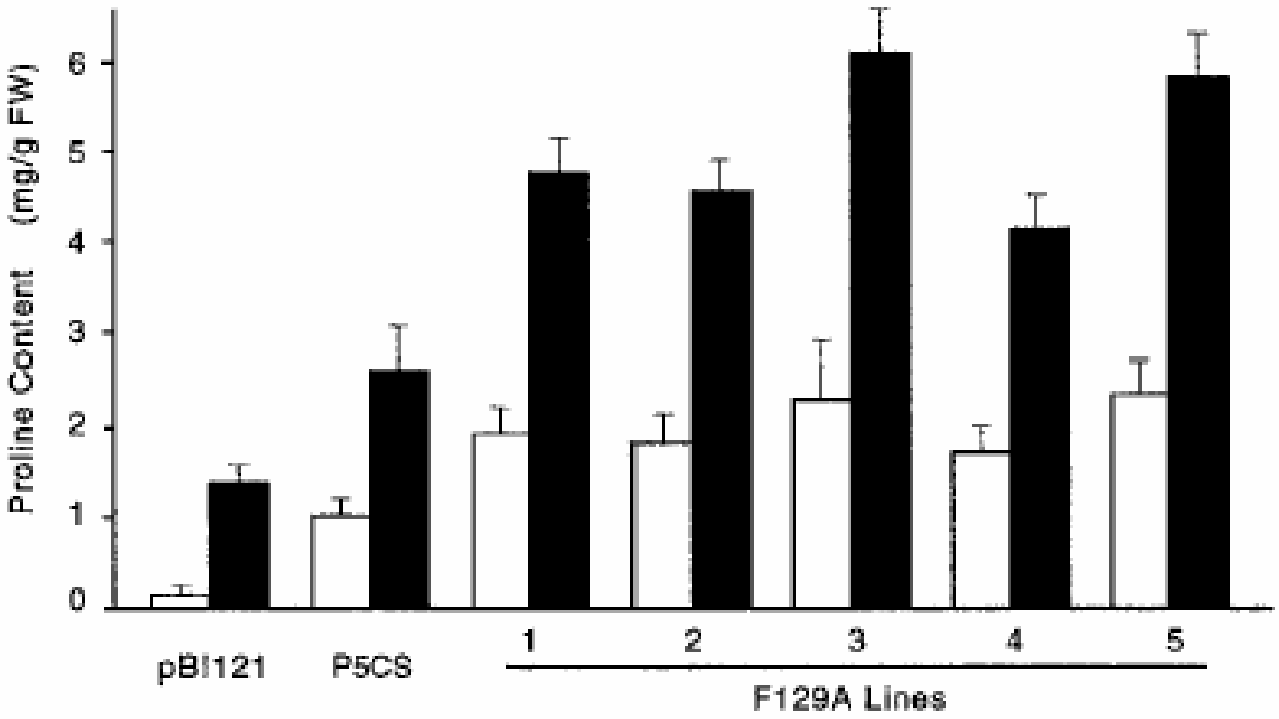


Figure 2. Pro levels in independent P5CSF129A transgenic lines. Seeds of five independent P5CSF129A lines were germinated on MS medium containing no NaCl (control; white bars) or 200 mM NaCl (black bars). The plants were grown under constant light at 24°C in a growth room. Plants transformed with vector pBI121 and P5CS served as controls. Pro content was measured in leaf extracts.

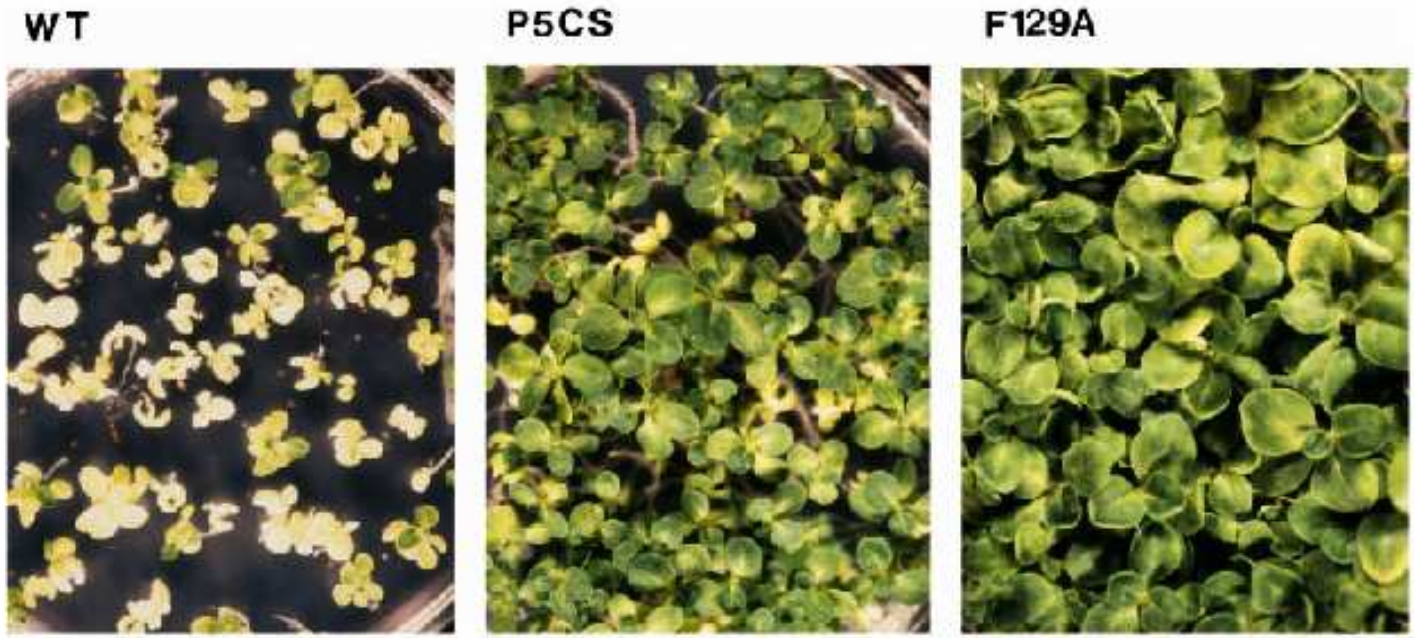
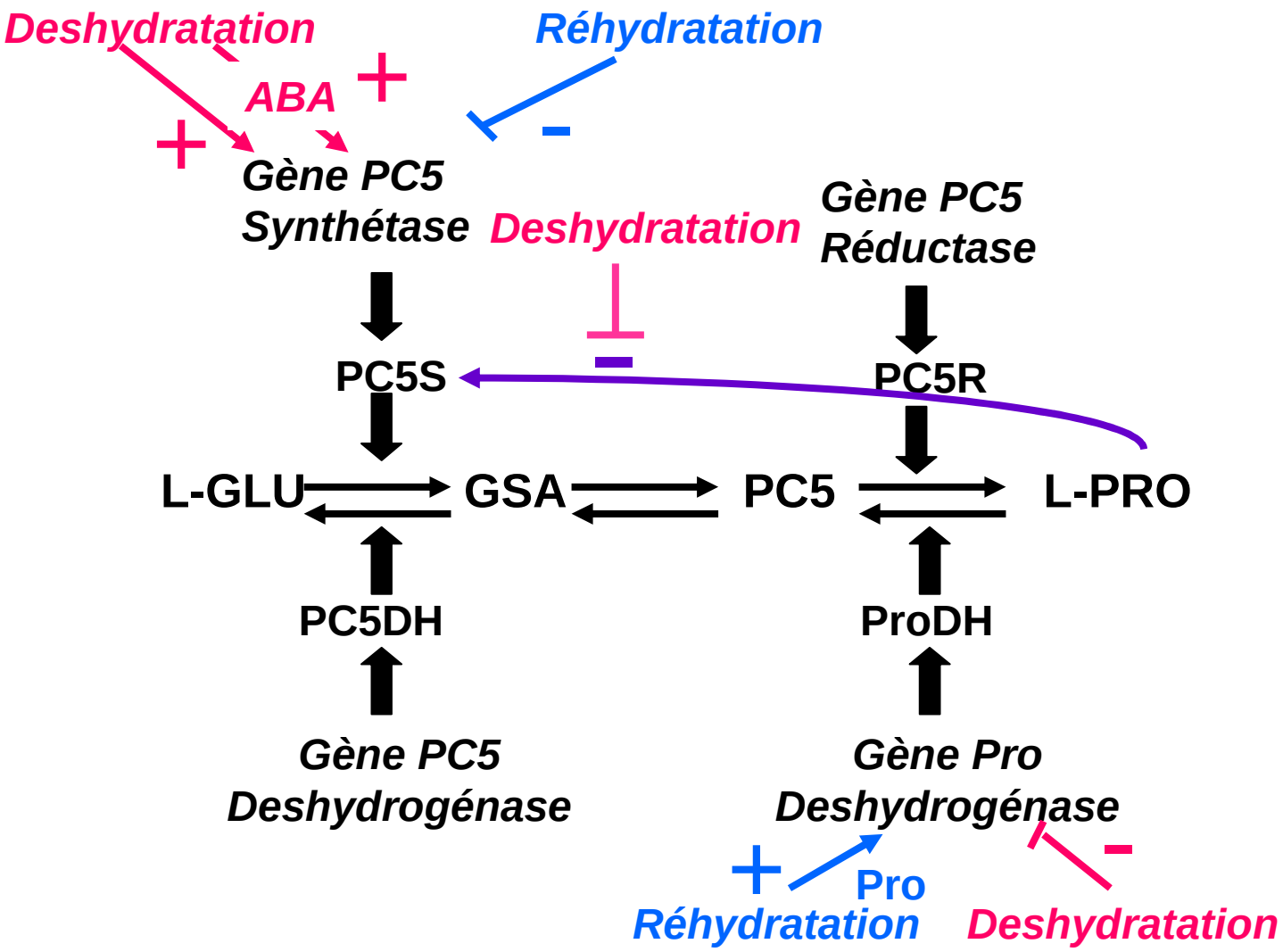


Figure 4. Phenotype of 6-week-old wild-type, P5CS, and P5CSF129A seedlings as affected by salinity (200 mM NaCl) stress. Seeds were germinated and maintained on MS medium containing 200 mM NaCl. The plates were kept in a controlled environment at 24°C under constant light.


Deshydratation
Réhydratation
ABA
+
+
-
Gène PC5
Synthétase
Deshydratation
Gène PC5
Réductase
PC5S
PC5R
L-GLU
GSA
PC5
L-PRO
PC5DH
ProDH
Gène PC5
Deshydrogénase
Gène Pro
Deshydrogénase
+
Pro
Réhydratation
-
Deshydratation

Ex 2. La glycine bétaine

V. Les autres facteurs importants pour la résistance au stress hydrique

1°- Les protéines LEA

2°- Les aquaporines

Autres molécules protectrices : des protéines hydrophiles
Les protéines LEA

LEA : late embryogenesis abundant proteins

Protéines très solubles et très hydrophiles

Mise en évidence

• Maturation des graines (phase de dessiccation)
• Plantes soumises au stress hydrique

Classification

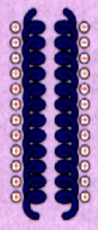
• **5 groupes** : séquence primaire en acides aminés
et conformation

Fonctions proposées

Rôle structural dans la protection cellulaire aux dommages liés à la dessication :

• liaisons avec des molécules d'eau→ maintien d'un état d'hydratation en des sites déterminés (solvatation de structures cytoplasmiques).

• molécules chaperonnes

Group	Hypothetical structures (none proven)	Representative proteins
Group 1 (D-19 family[a])		Em (early methionine-labeled protein, wheat)
Group 2 (D-11 family[a])		DHN1 (maize) D-11 (cotton)
Group 3 (D-7 family[a])		HVA1 (*Hordeum vulgare* ABA-induced, barley) D-7 (cotton)
Group 4 (D-95 family[a])		D-95 (soybean)
Group 5 (D-113 family[a])		LE25 (tomato) D-113 (cotton)

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ORIGINAL ARTICLE

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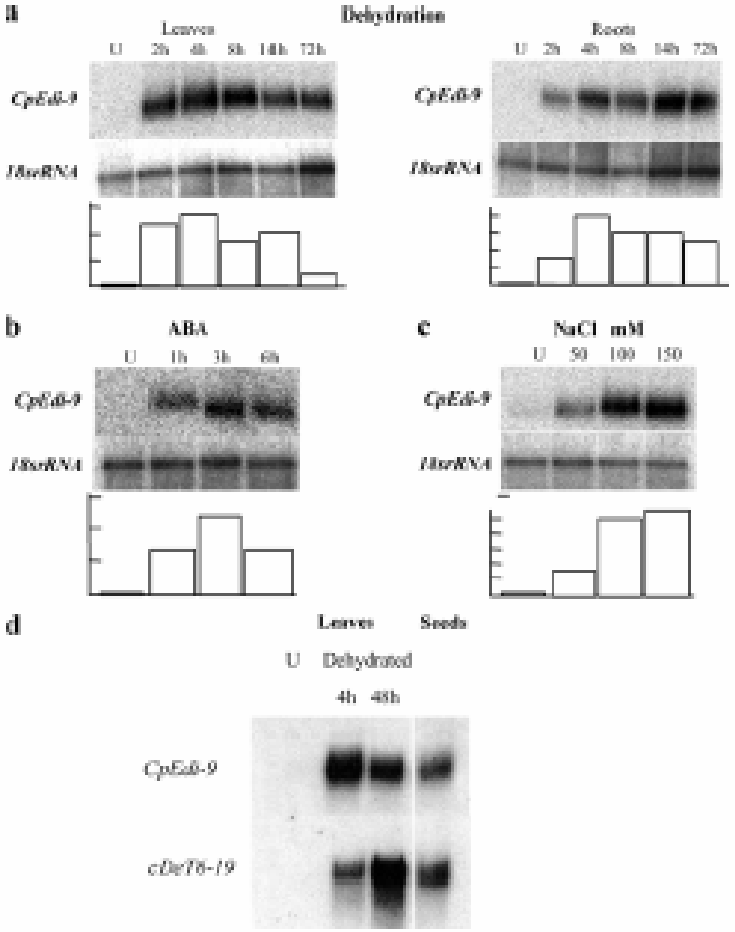
The novel gene *CpEdi-9* from the resurrection plant *C. plantagineum* encodes a hydrophilic protein and is expressed in mature seeds as well as in response to dehydration in leaf phloem tissues



Temps
Deshydratation 0h, 2h, 4h, 8h, 14h, 72h

RWC 100%, 62, 39, 25, 18, 15

Fig. 4 **a–c** RNA blot analysis showing the expression of the *C. plantagineum* CpEdi-9 transcript in response to **a** dehydration, **b** ABA (100 µM) and **c** salt treatment (NaCl for 6 h). Membranes carrying 2 µg of poly(A)$^+$RNA were probed with the CpEdi-9 cDNA insert. All filters were hybridised with a ribosomal probe to monitor loading of RNA. The relative intensity of the hybridising bands was calculated using the 18srRNA signal as a reference. The graph below each autograph gives the relative signal intensity. In the dehydration kinetics the relative water content (RWC) was set to 100% in untreated plants (*lane U*) and the corresponding RWC values for dehydrated plants were 62%, 39%, 25%, 18% and 15% for 2, 4, 8, 14 and 72 h of dehydration. **d** The CpEdi-9 transcript accumulates in mature seeds. Total RNA (30 µg in each lane) from untreated (*U*) and dehydrated (4 h and 48 h) leaves and mature seeds of *C. plantagineum* was hybridised with the CpEdi-9 cDNA insert to compare relative expression levels. The blot was also hybridised with the dehydrin CDeT6-19 cDNA insert to compare expression levels (Bartels et al. 1990)

Les aquaporines

MIP : Major Intrinsec Protein

✓ TIP : sur la membrane vacuolaire
✓ PIP : sur la membrane plasmique = plasmalemme

✓ Quand les aquaporines sont-elles synthétisées en condition normale ?

Etude par promoteur TIP (tonoplastic intrinsec protein) fusionné à un gène rapporteur
- cellules méristématiques
- cellules en extension

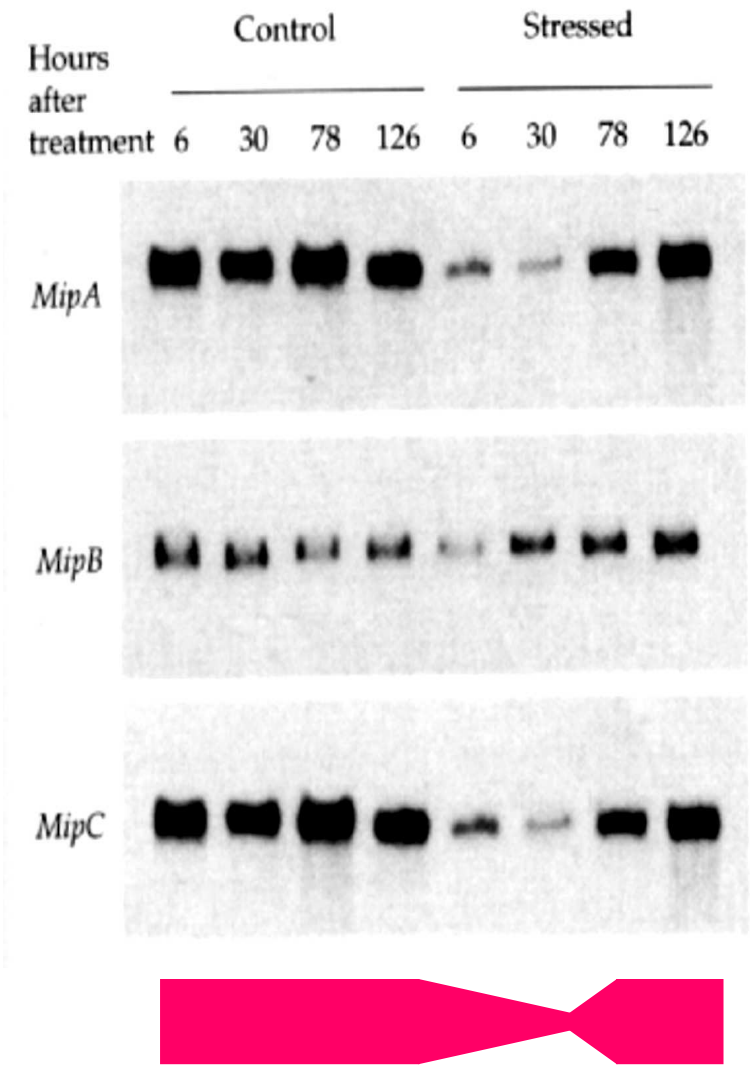
✓ Sont-elles synthétisées en condition de stress ?

Arabidopsis thaliana : RD 28
Forte accumulation des ARNm lors d'un stress hydrique

Mesembryanthemum cristallinum :
Criblage différentiel plantes normales/ + 400 mM NaCl
5 MIP identifiées

Expression des aquaporines
lors d'un stres salin (400 mM NaCl)
Ice plant

Northern-blot



Turgescence des cellules