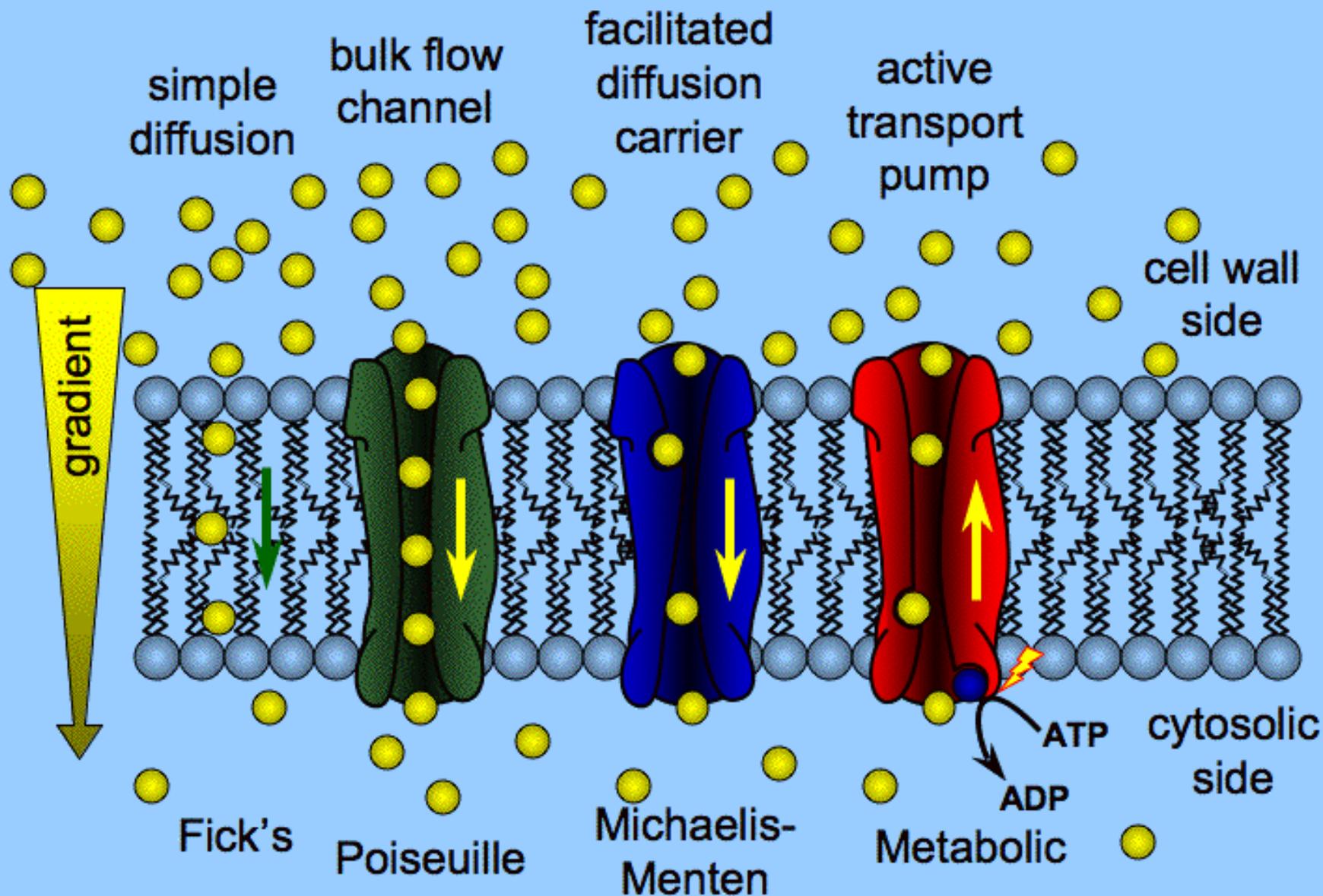
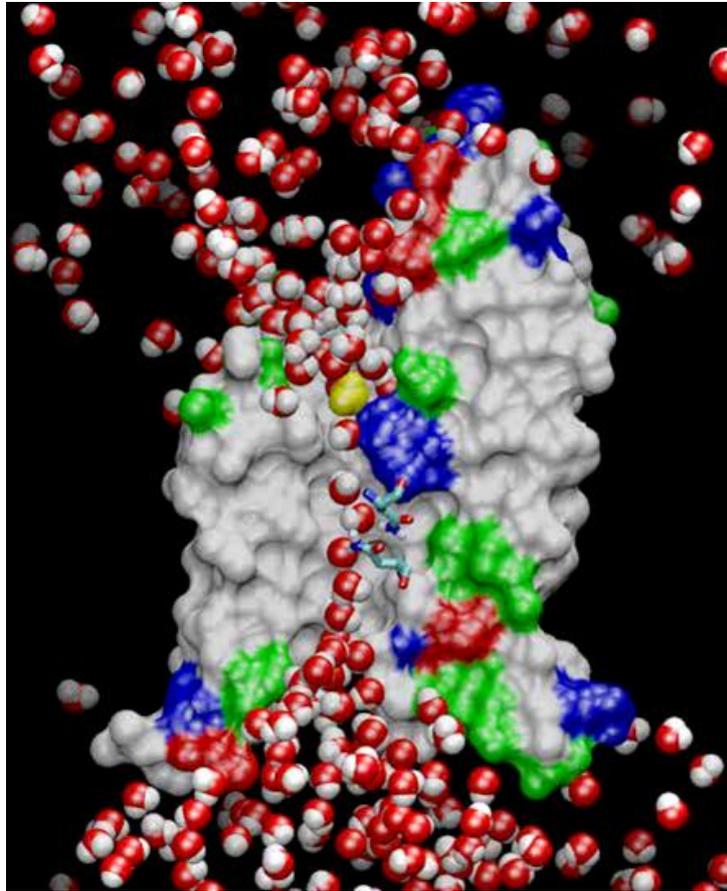


Solute movement across a membrane



Aquaporine: i canali per l'acqua



Antica famiglia multi-genica di proteine MIP (Major Intrinsic Proteins)

Presenti in animali, piante e micro-organismi

Scoperte da Peter Agre et al. nel 1992 (Nobel per la Chimica nel 2003)



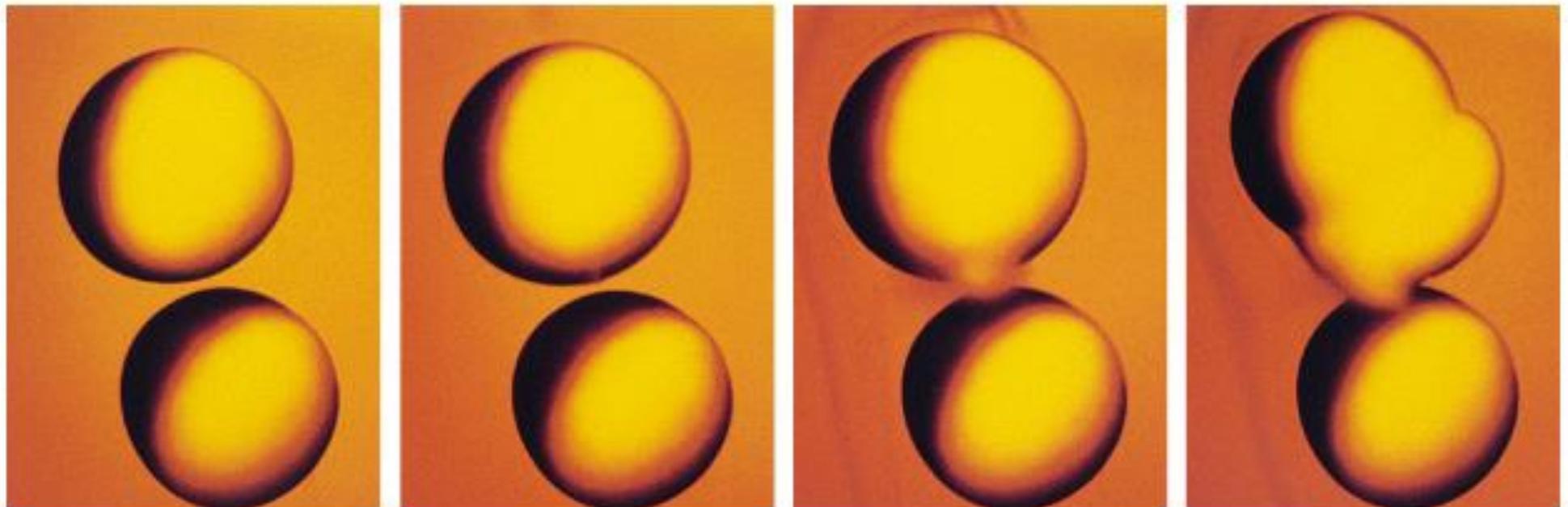
Espressione di un'aquaporina (allora chiamata Chip28) in oociti di *Xenopus laevis*

0.5 min

1.5 min

2.5 min

3.5 min



Controllo

Aquaporine

- Codificate da una famiglia multigenica
- In *Arabidopsis* identificate 35 isoforme
- 4 gruppi principali nelle piante vascolari:
 - PIP (plasmalemma intrinsic proteins)
 - TIP (tonoplast intrinsic proteins)
 - NIP (nodulin intrinsic proteins)
 - SIP (small basic intrinsic proteins)
- PIP e TIP native sono presenti nelle membrane come tetrameri
- Facilitano il passaggio di acqua attraverso le membrane biologiche, secondo gradiente di potenziale dell'acqua (= **trasporto solo passivo**)
- Altissima permeabilità all'acqua. Sino a 3×10^9 molecole di H₂O al secondo

Aquaporine

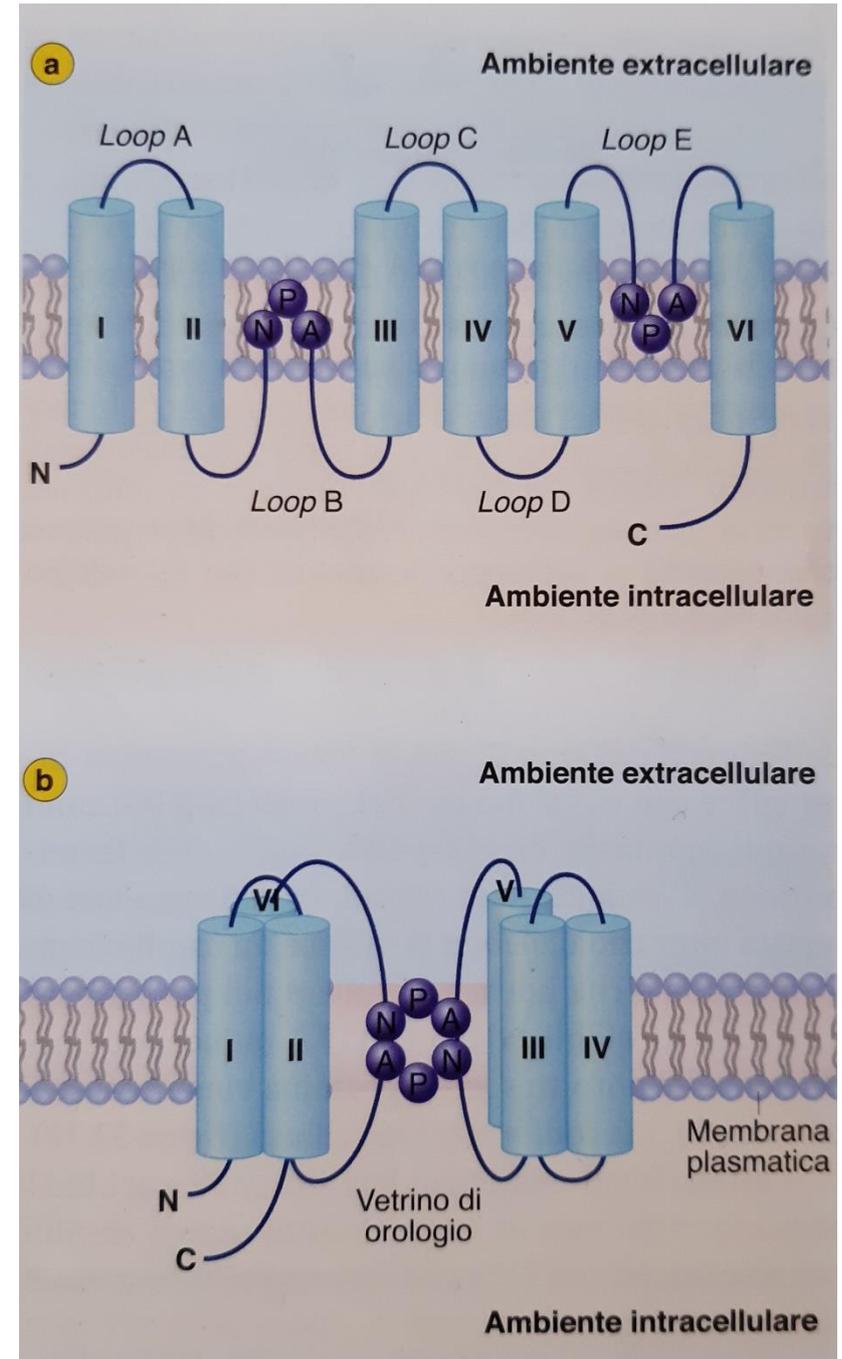
Struttura altamente conservata:
proteine di 23-31 kDa con 6 domini
transmembrana collegati da 5 loops.
Terminazioni N- e C- verso il citosol.

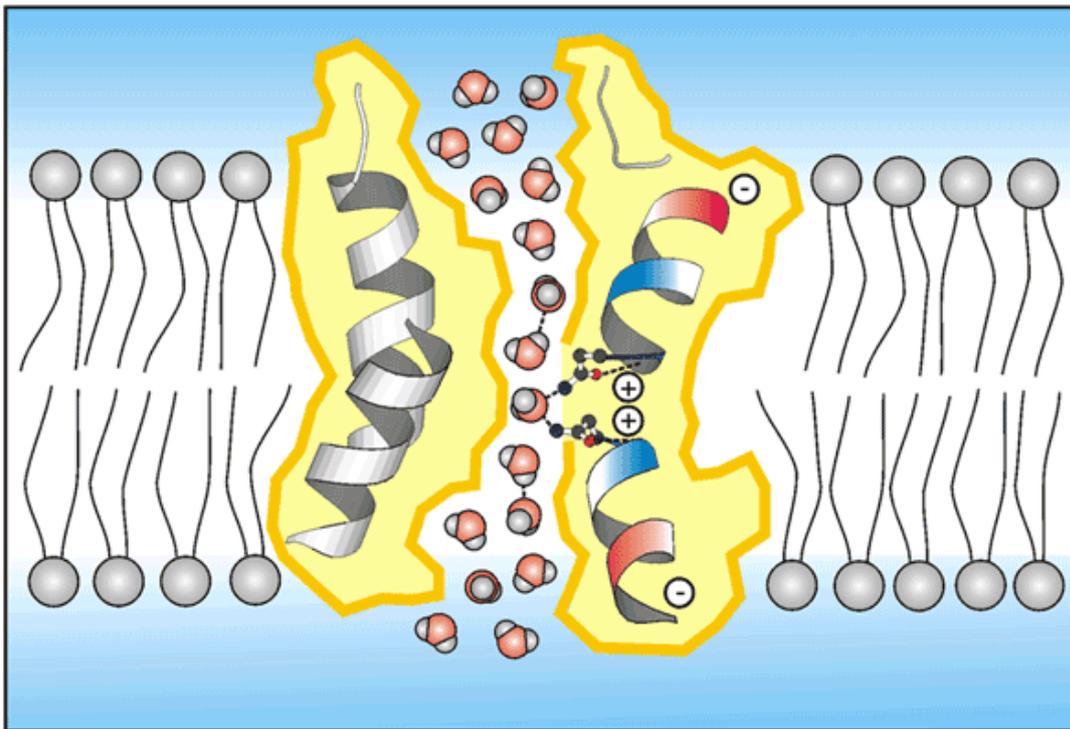
Il poro di passaggio dell'acqua è delineato
dall'interfaccia tra i domini 2 e 5, dove si
trovano residui conservati di Asn-Pro-Ala.

Selettività per l'acqua data da due siti di
costrizione:

1°: interazione delle due regioni NPA (struttura
a «vetrino di orologio»- «hourglass fold»)

2°: filtro di selettività (regione ar/R =
aromatic/arginine)



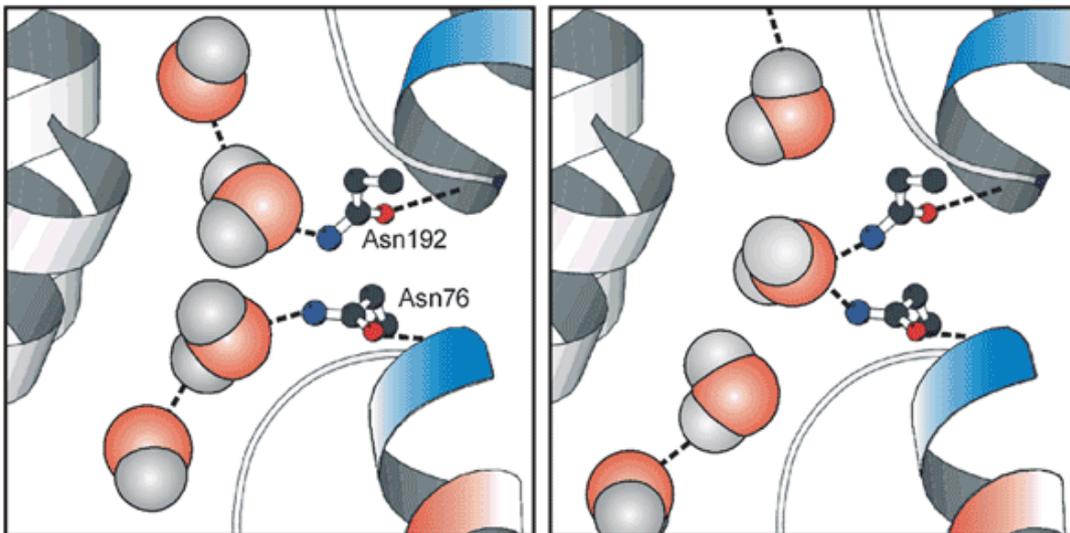


Elevata specificità delle aquaporine per il substrato:

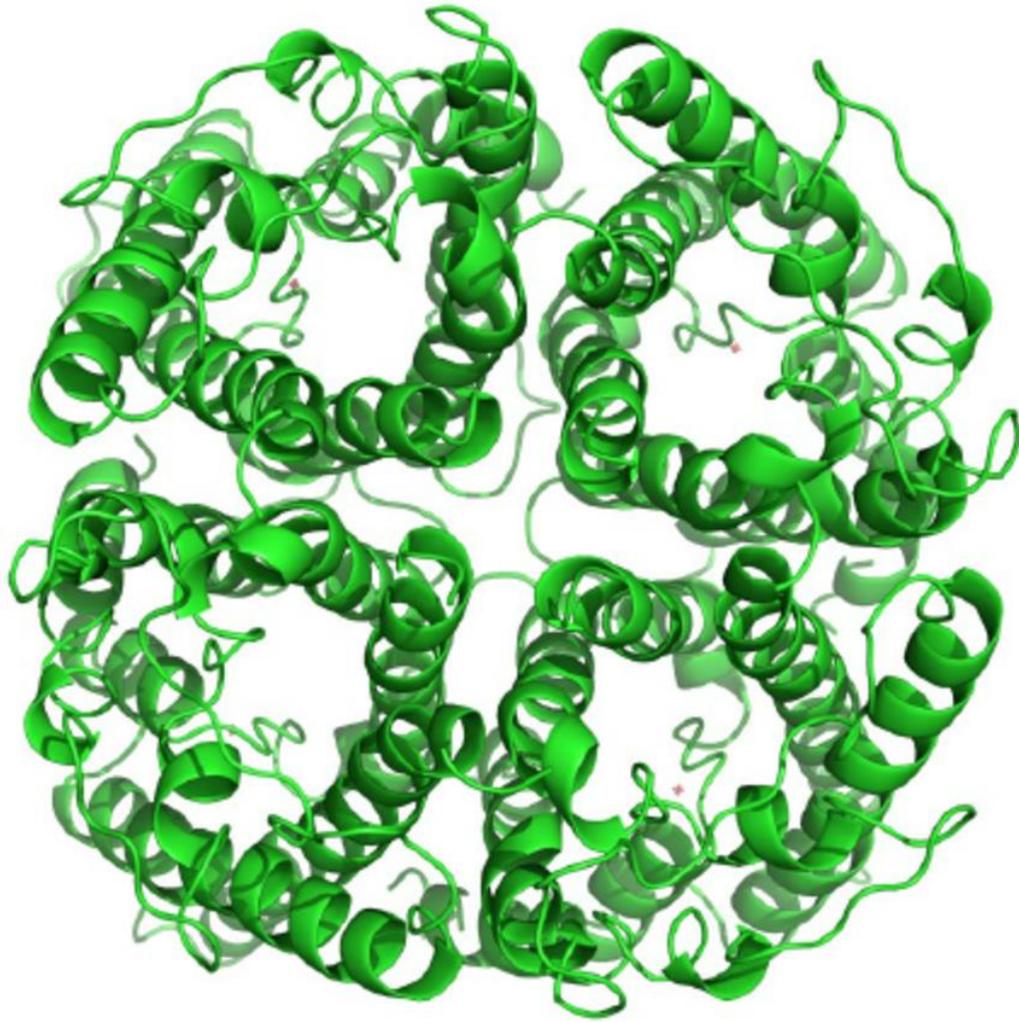
esclusione dimensionale a livello dei 2 siti di costrizione (Ar/R e NPA)

Riconoscimento stereo-specifico del substrato mediante legami idrogeno e interazioni idrofobiche

Asn76 e Asn192 formano legami idrogeno con gli atomi di ossigeno della molecola di acqua, orientando i due atomi di idrogeno e facilitando il movimento delle molecole d'acqua in una fila (single-row).



Trasporto di H^+ bloccato dalla repulsione elettrostatica nel sito Ar/R



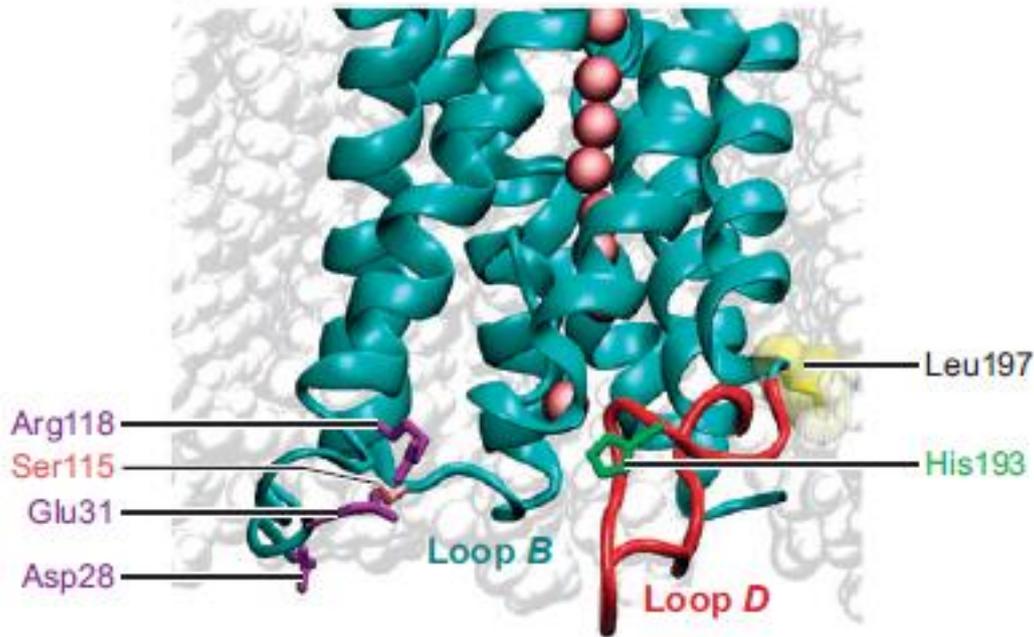
Omotetramero (PIP e TIP)

Ciascun monomero forma un poro per l'acqua + un quinto centrale meno specifico

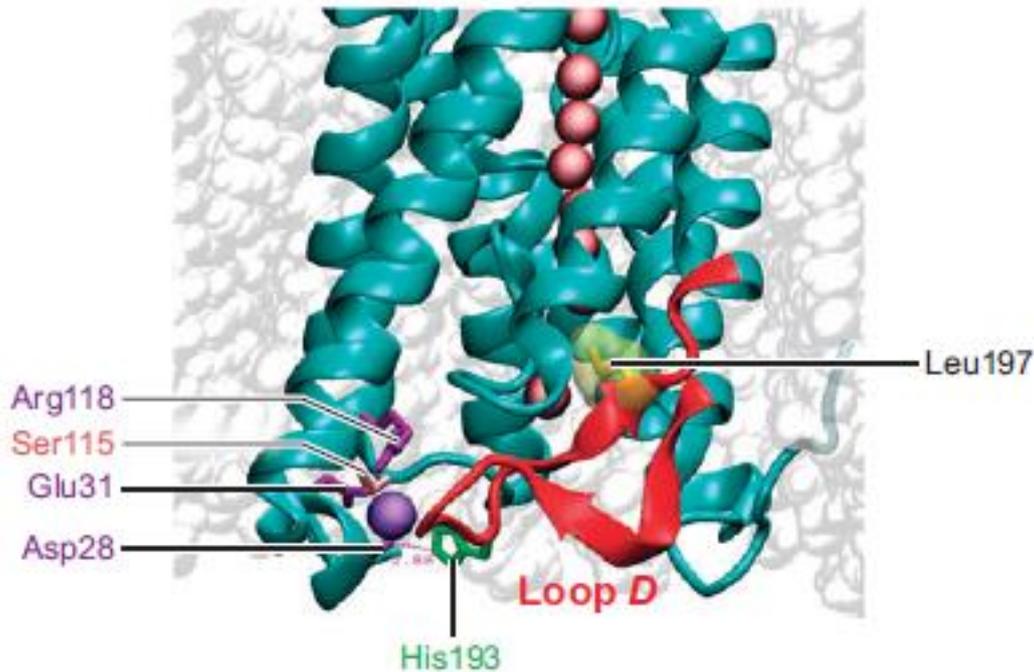
L'attività delle aquaporine dipende da:

- Abbondanza: regolata da fattori endogeni (es. fitoormoni) ed ambientali (es. stress idrico, salino..)
- Regolazione apertura-chiusura del poro (gating)

a



b



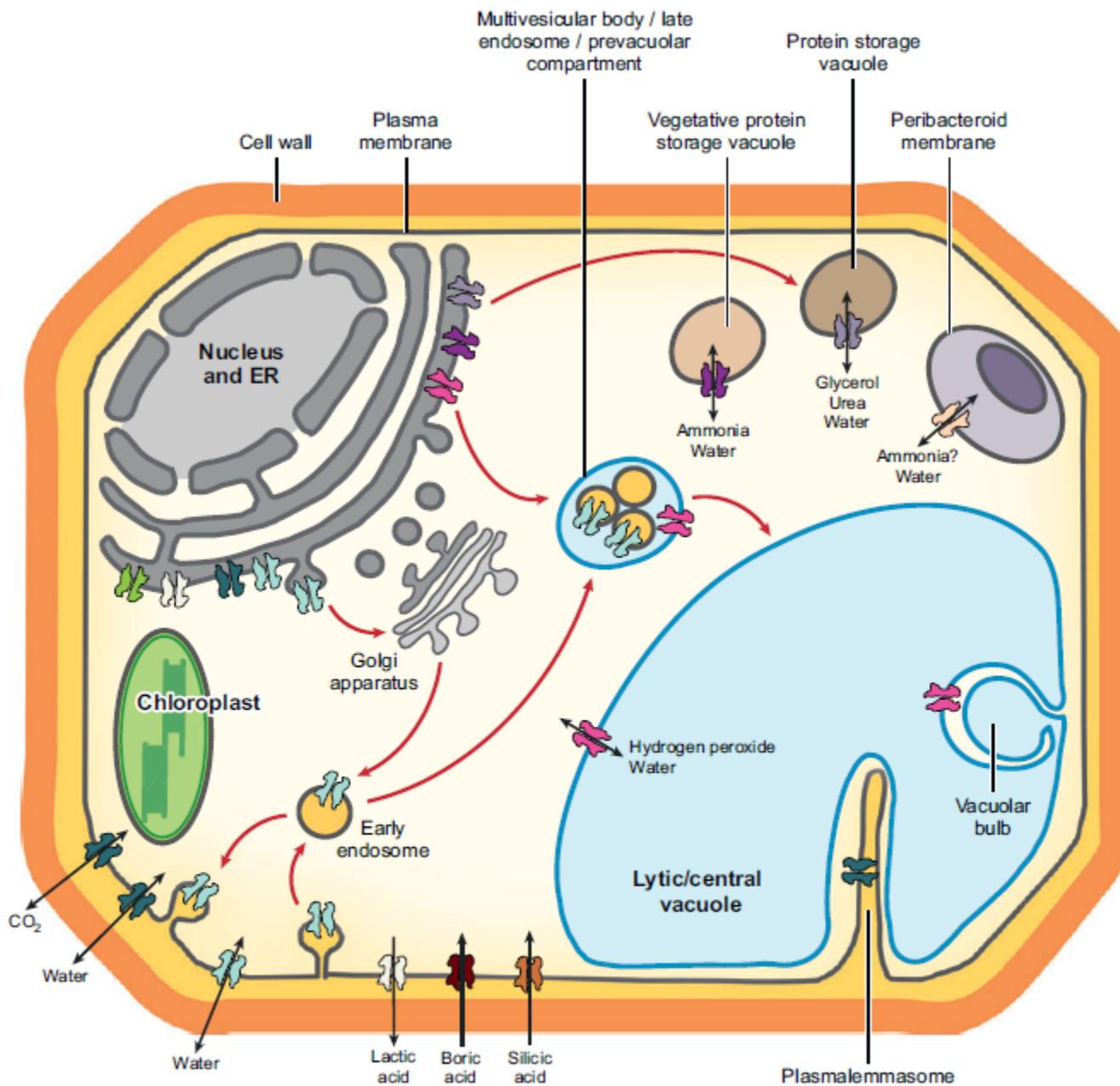
Gating (= regolazione apertura e chiusura del poro per ciascun monomero): dipende dalla posizione del loop D

Processo:

Legame H^+ con His 193 \rightarrow spostamento loop D e chiusura del poro da parte di Leu197

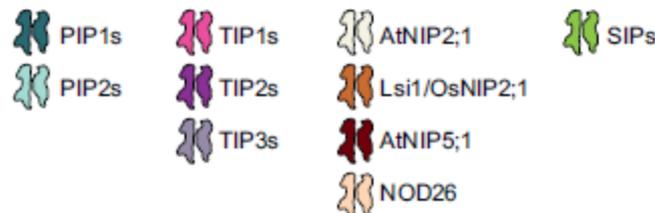
Legame cationi divalenti (es Ca^{2+}) con Asp28 e Glu31 \rightarrow stabilizzazione della conformazione chiusa (sfera color porpora)

Fosforilazione di Ser115 su Loop B (rosa) \rightarrow rilascio del loop D e apertura del poro



Aquaglyceroporine:

alcune aquaporine vegetali possono trasportare oltre all'acqua altri piccoli soluti non carichi:
 CO_2 ,
 NH_4 , H_2O_2 ,
 urea, H_4SiO_4 ..



PIP (plasmamembrane intrinsic proteins)
 TIP (tonoplast intrinsic proteins)
 NIP (nodulin intrinsic proteins)
 SIP (small basic intrinsic proteins)

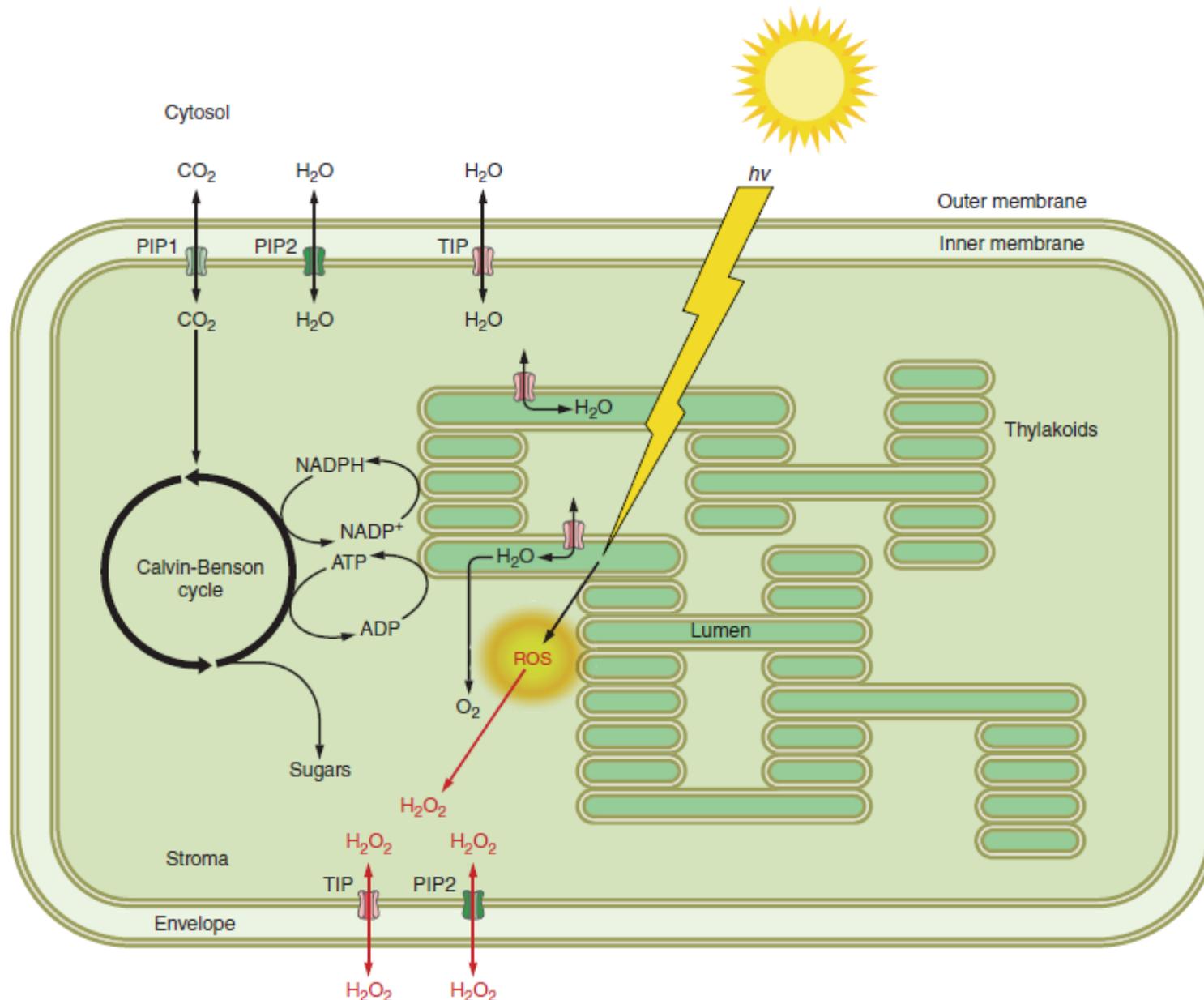


FIGURE 8. Putative functions of aquaporins in the chloroplast. Several PIPs and TIPs have been tentatively localized in the inner envelope and thylakoid membranes. They may contribute to water transport into the stroma and thylakoid lumen. In the latter compartment, photosynthesis leads to oxidation of water to molecular oxygen. Changes in incident light can result in adjustments of thylakoid and overall chloroplast volumes. A role of PIP1s in CO₂ transport across the inner envelope membrane is also indicated. Carbon fixation occurs in the stroma through carboxylation reactions within the Calvin-Benson cycle. Finally, ROS can be formed as by-products of photosynthetic activities. A putative role of PIP and TIP in facilitating H₂O₂ export from the chloroplast is shown.

Table 1. Diversity of aquaporin gene family in plants

Species	Common Name	PIPs	TIPs	NIPs	SIPs	XIPs	HIPs	GIPs	Total	Reference Nos.
<i>Selaginella moellendorffii</i>	Spike moss	3	2	8	1	3	2		19	8
<i>Physcomitrella patens</i>	Moss	8	4	5	2	2	1	1	23	59
<i>Oryza sativa</i>	Rice	11	10	10	2				33	252
<i>Arabidopsis thaliana</i>	Mouse ear-cress	13	10	9	3				35	121, 235
<i>Solanum lycopersicum</i>	Garden tomato	14	11	12	4	6			47	239
<i>Populus trichocarpa</i>	Black cottonwood	15	17	11	6	6			55	93
<i>Glycine max</i>	Soybean	22	23	13	6	2			66	334
<i>Gossypium hirsutum</i>	Upland cotton	28	23	12	7	1			71	224

Shown for all plant species, the genome of which was fully sequenced, are the number of homologs present in each of the indicated aquaporin subclasses.

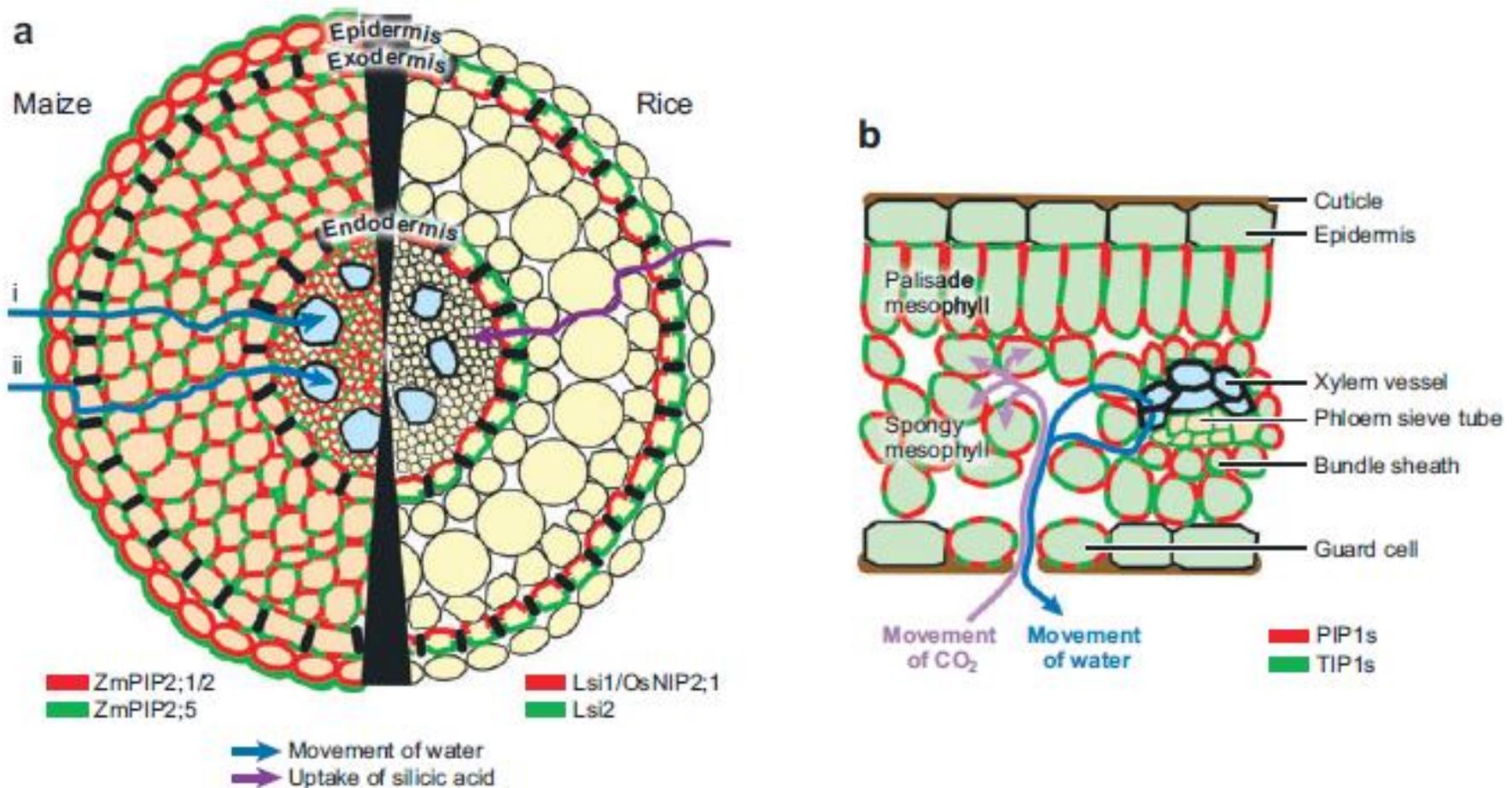


Figure 4

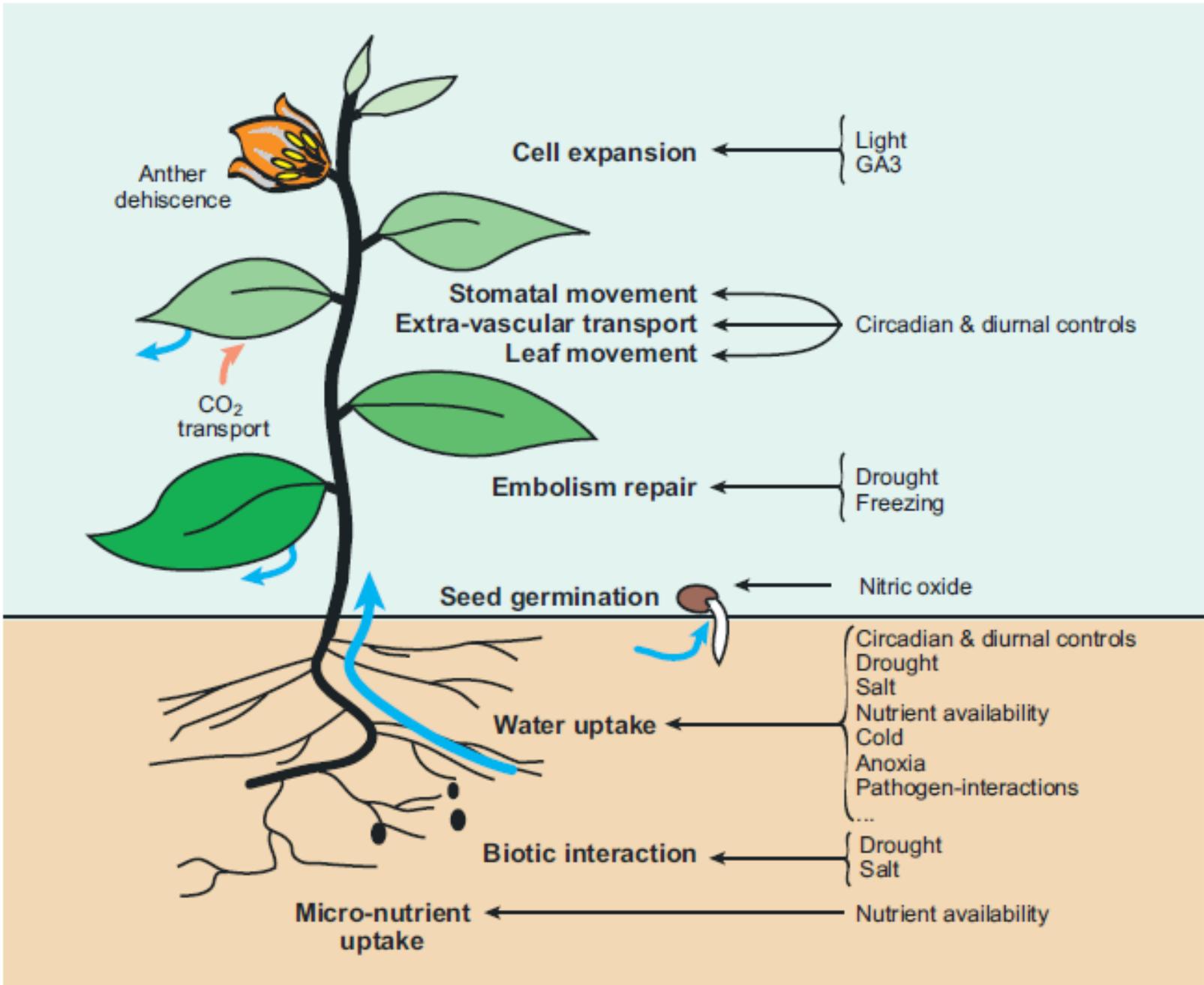
Aquaporin-mediated transport of water and solutes in roots (a) and leaves (b). Schematic cross sections with representations of the tissue-specific expression patterns of aquaporins and paths of transport are shown. Aquaporin expression and water transport in maize roots is summarized according to Reference 46, whereas uptake of silicic acid in rice roots by *Oryza sativa* Lsi1 (*OsNIP2;1*) in combination with the efflux transporter Low silicon rice 2 (*Lsi2*) is drawn according to References 82 and 83. Expression of plasma membrane intrinsic protein 1s (PIP1s) and tonoplast intrinsic protein 1s (TIP1s) in *Brassica napus* leaves was summarized according to Reference 35. The movement of water can follow the cell-to-cell (symplastic and transcellular) (i) or apoplastic (ii) path.

La permeabilità delle radici all'acqua dipende fortemente dalla presenza di aquaporine

Connessione tra respirazione radicale e assorbimento di acqua dalle radici, oppure l'appassimento di piante sottoposte a sommersione, sono spiegati dai fattori che determinano il gating delle aquaporine:

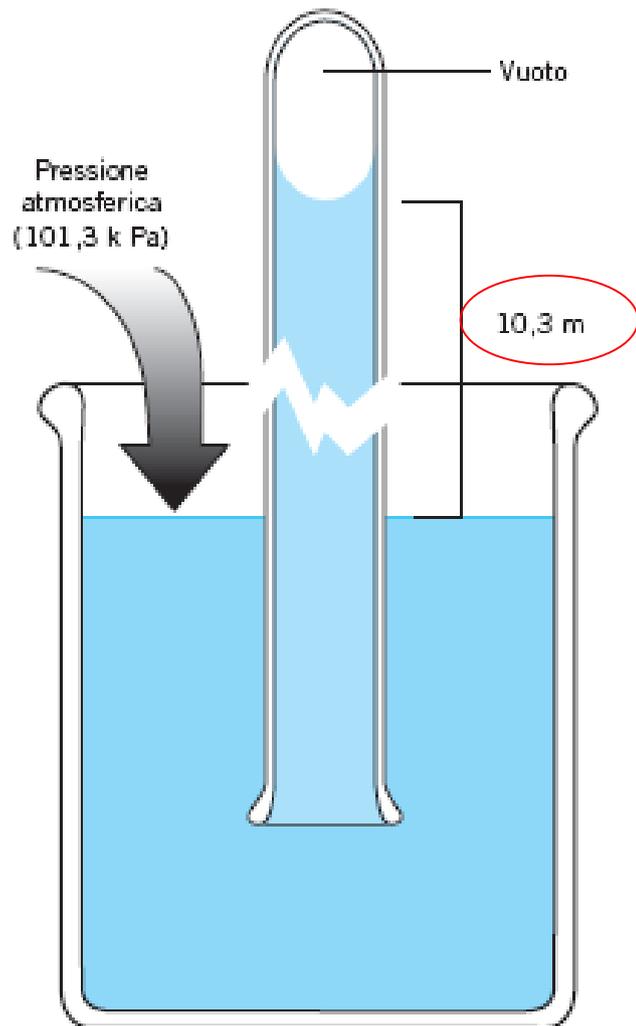
- Decremento del tasso di respirazione (dovuto alle basse temperature/condizioni anaerobiche(sommersione) può portare all'**acidificazione del citosol -> chiusura aquaporine**

Funzioni delle aquaporine nei processi fisiologici della pianta



Trasporto verticale di acqua: la teoria della Tensione-Coesione

Dixon HH, Joly J. 1894. On the ascent of sap. *Philosophical Transactions of the Royal Society London, Series B* **186**, 563-576.



Sequoia sempervirens, 112 m



Eucalyptus regnans, 150 m ?

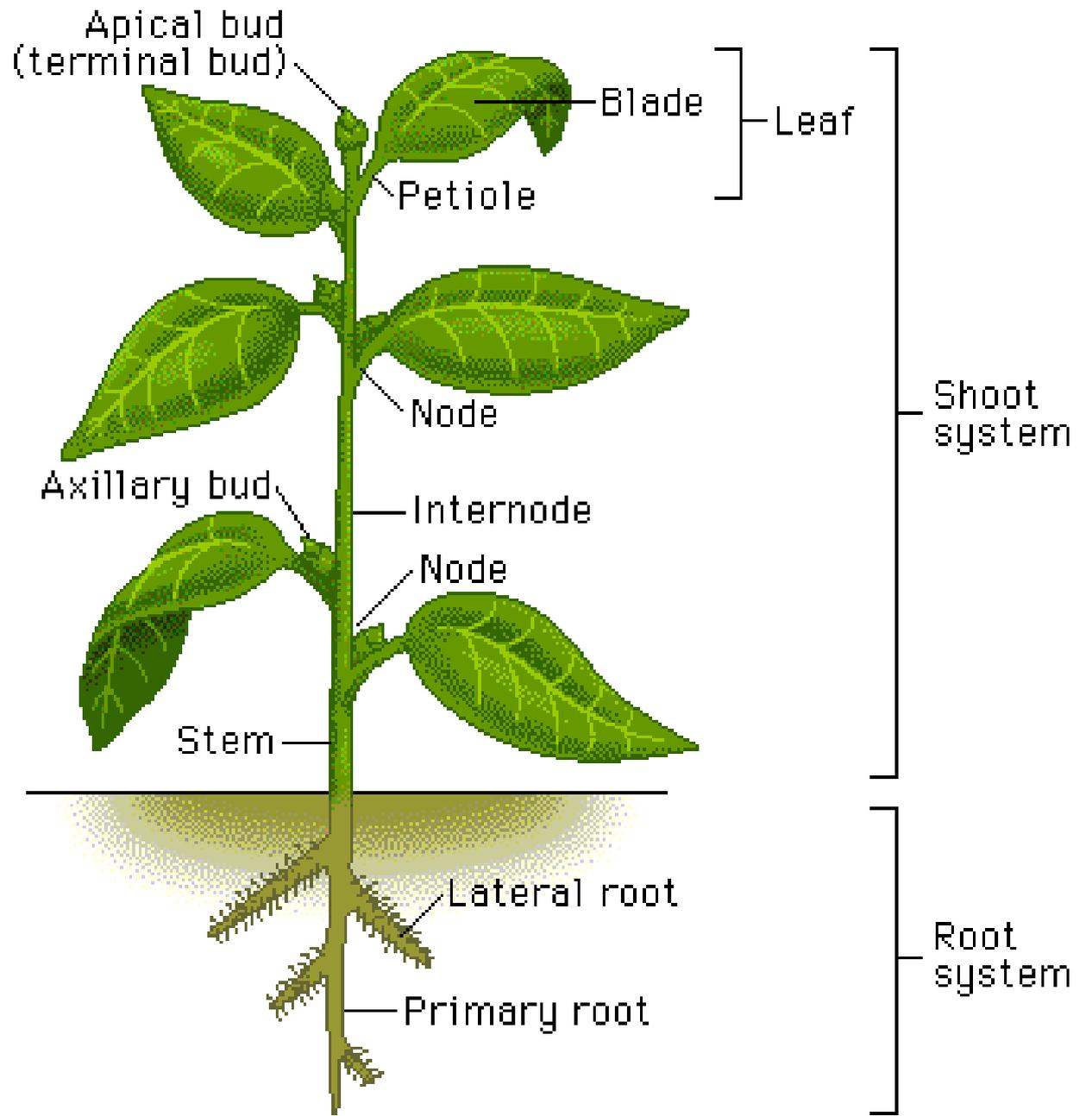
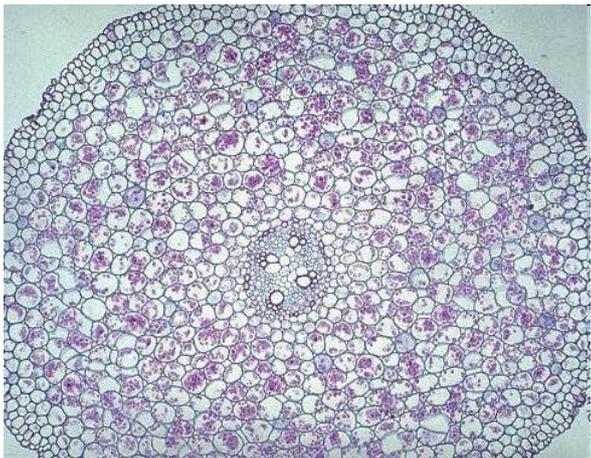
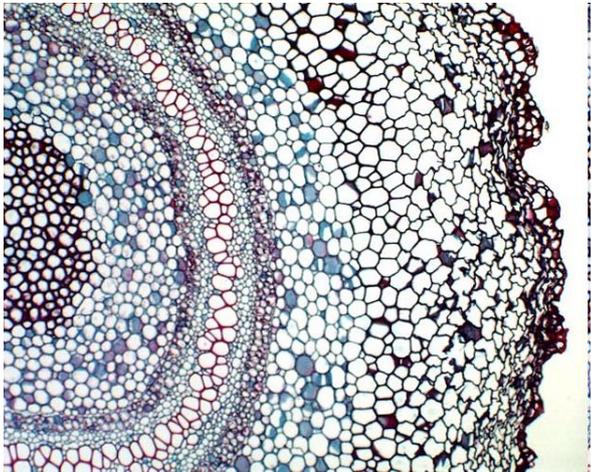
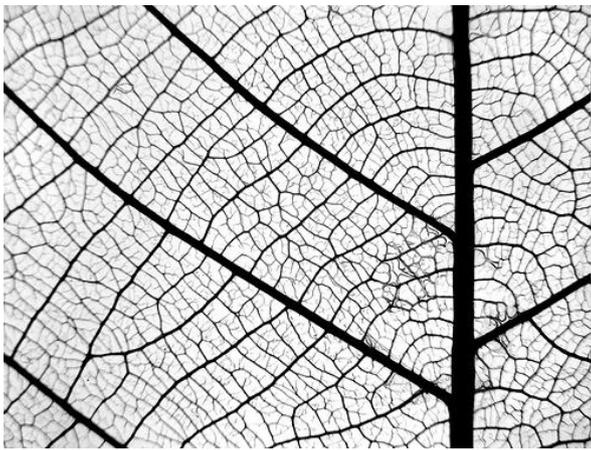
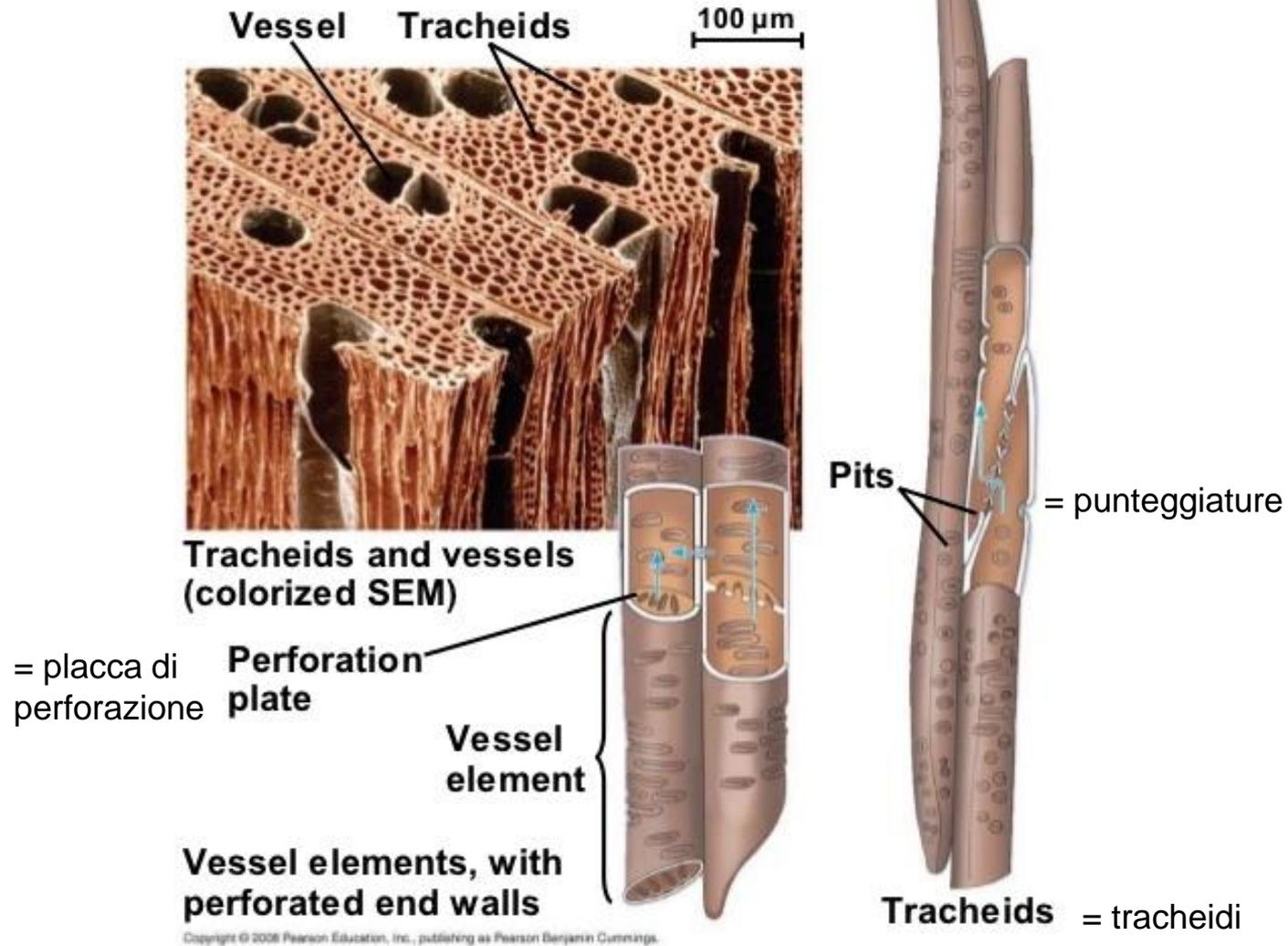


Fig. 35-10d



Gimnosperme:

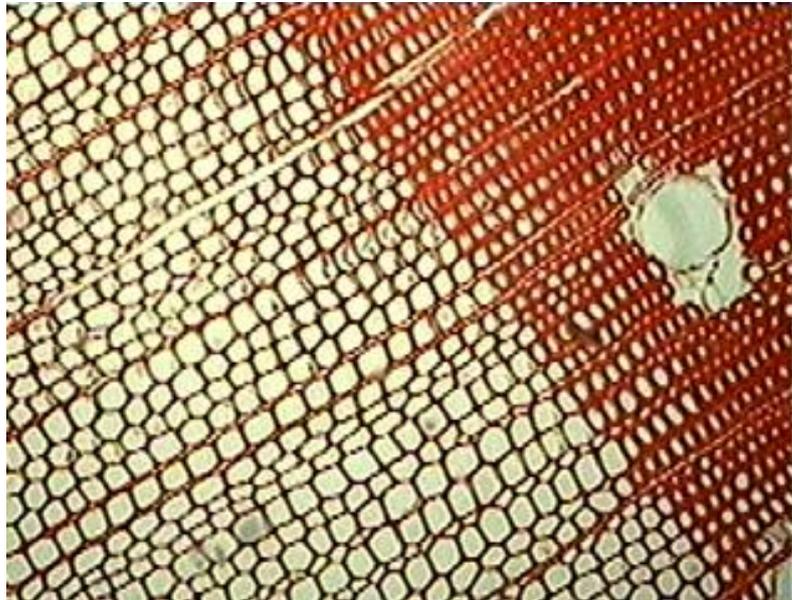
Fibrotracheidi

Singole cellule

Diametro da 5 a 80 μm

Lunghezza < 3 cm

Legno omoxilo



Angiosperme:

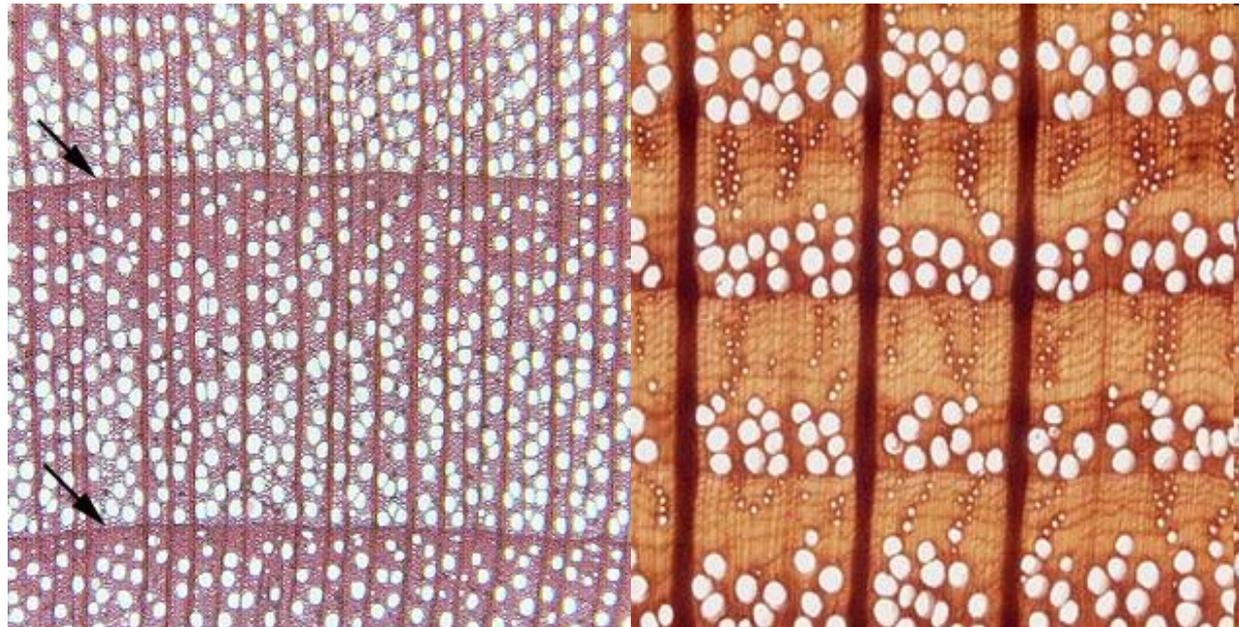
Trachee dette anche 'vasi' (+ Tracheidi)

Costituite da più cellule sovrapposte
(elementi del vaso)

Diametro da 10 a 500 μm

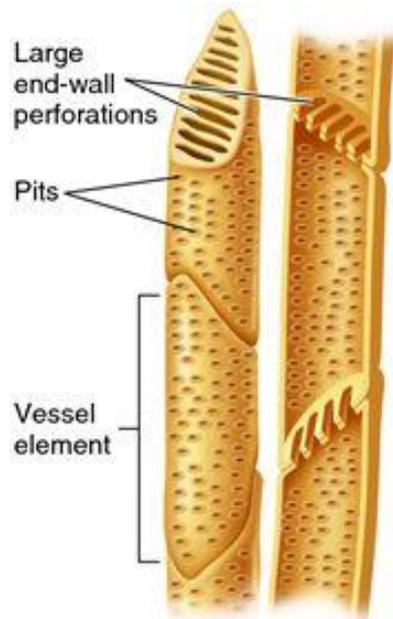
Lunghezza sino a 10-20 m
(ma più comunemente 2-20 cm)

Legno eteroxilo

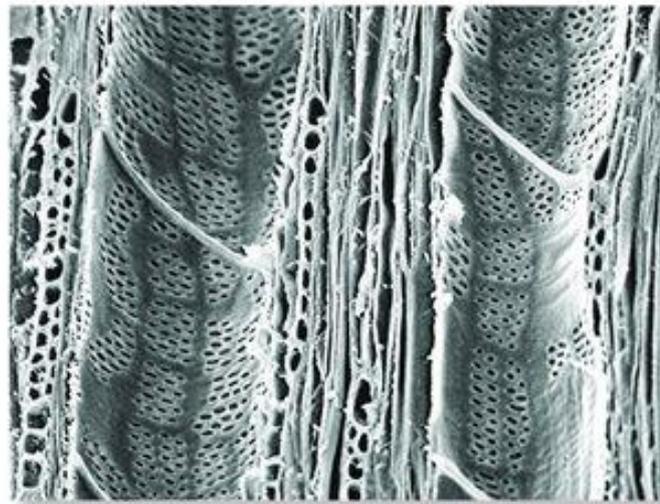


Porosità diffusa

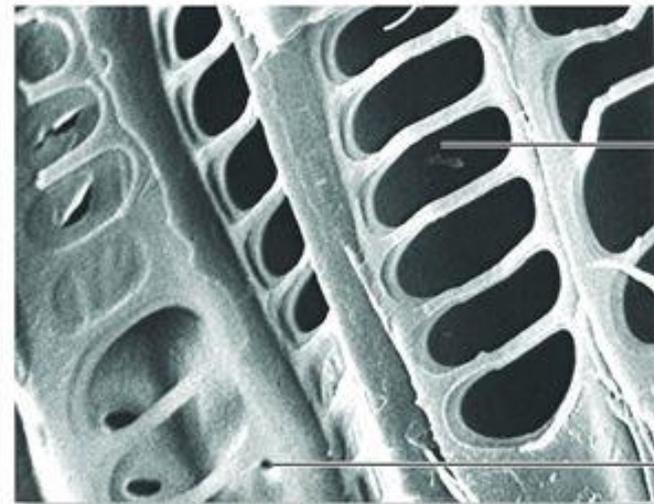
Porosità anulare



(a) Vessels made up of vessel elements



(b) Vessel elements in a walnut tree



(c) Perforations in a vessel element end wall

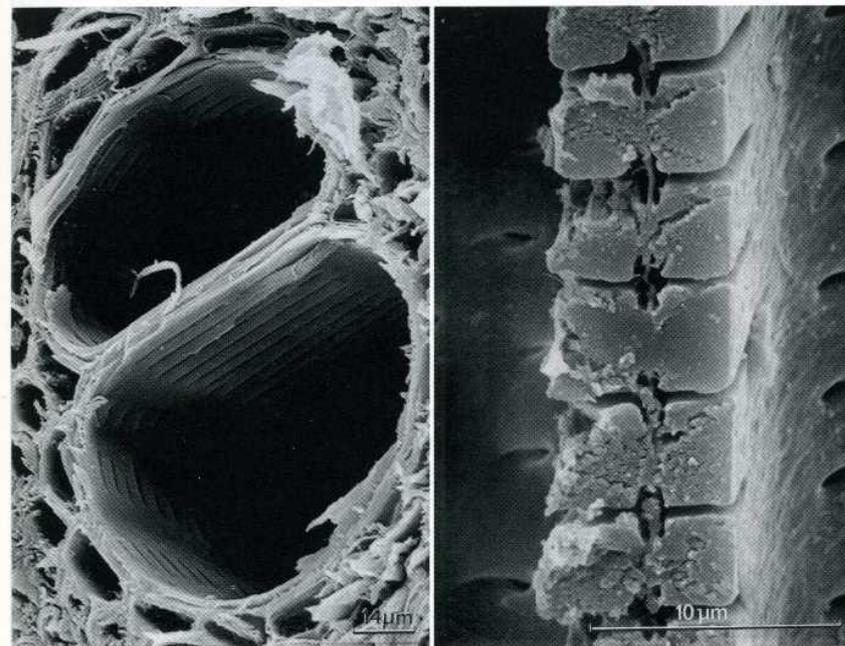
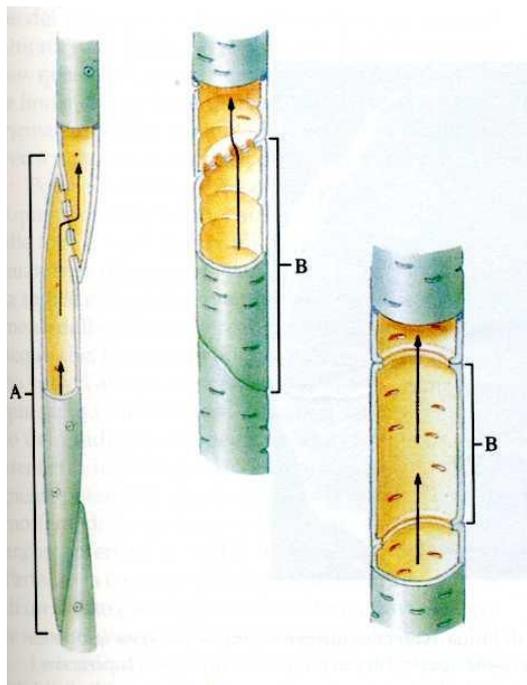
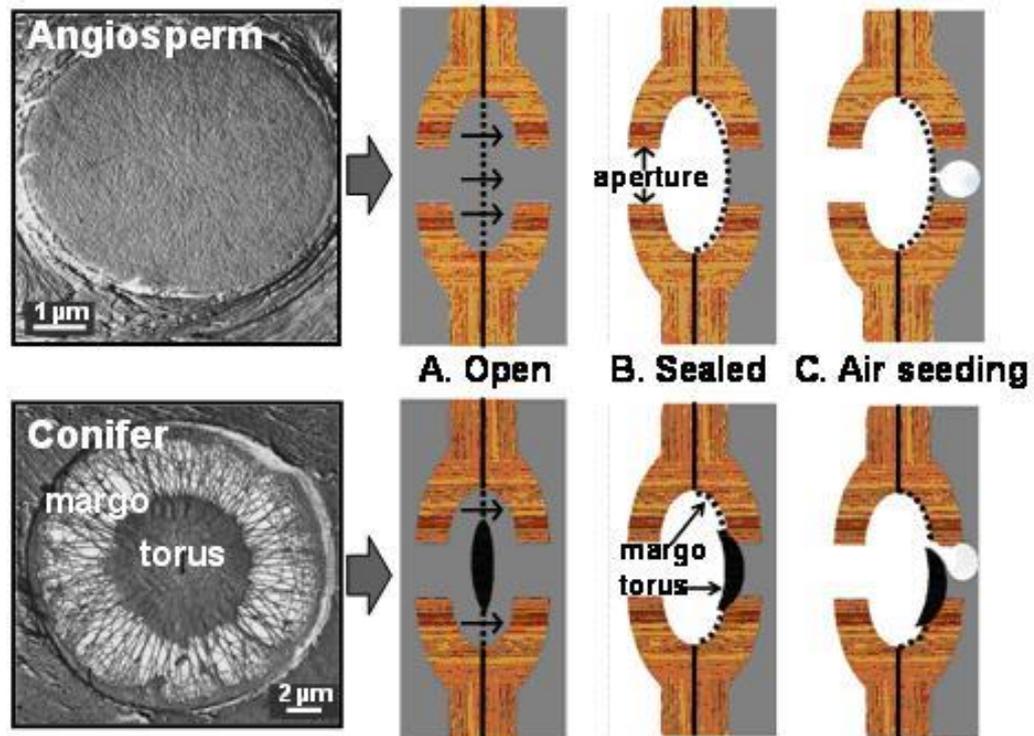


Fig. 2.9. Scanning electron micrographs of metaxylem vessels in the stem of *Rhapis excelsa*. *Left* Transversely cut stem showing interessel pit area. *Right* Interessel pits in longitudinal section (cutting across the wall). (Zimmermann et al. 1982)



Equazioni che descrivono la capacità di trasporto di acqua attraverso la pianta

$$F = V/t$$

dove F = flusso, V = volume, t = tempo

$$F = K \times \Delta\Psi$$

dove K = conduttanza idraulica, $\Delta\Psi = \Psi_{\text{suolo}} - \Psi_{\text{foglia}}$

$$K = 1/R$$

dove R = resistenza idraulica

$$F = \Delta\Psi/R$$

$$k = K \times l$$

dove k = conduttività idraulica, l = lunghezza del conduttore

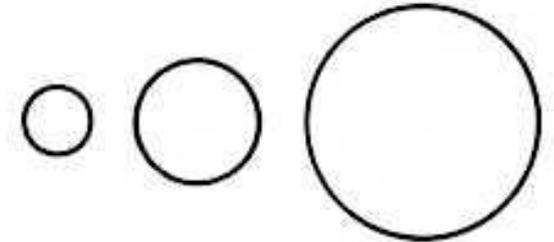
K, mmol s⁻¹ MPa⁻¹

R, MPa s mmol⁻¹

k, mmol s⁻¹ m MPa⁻¹

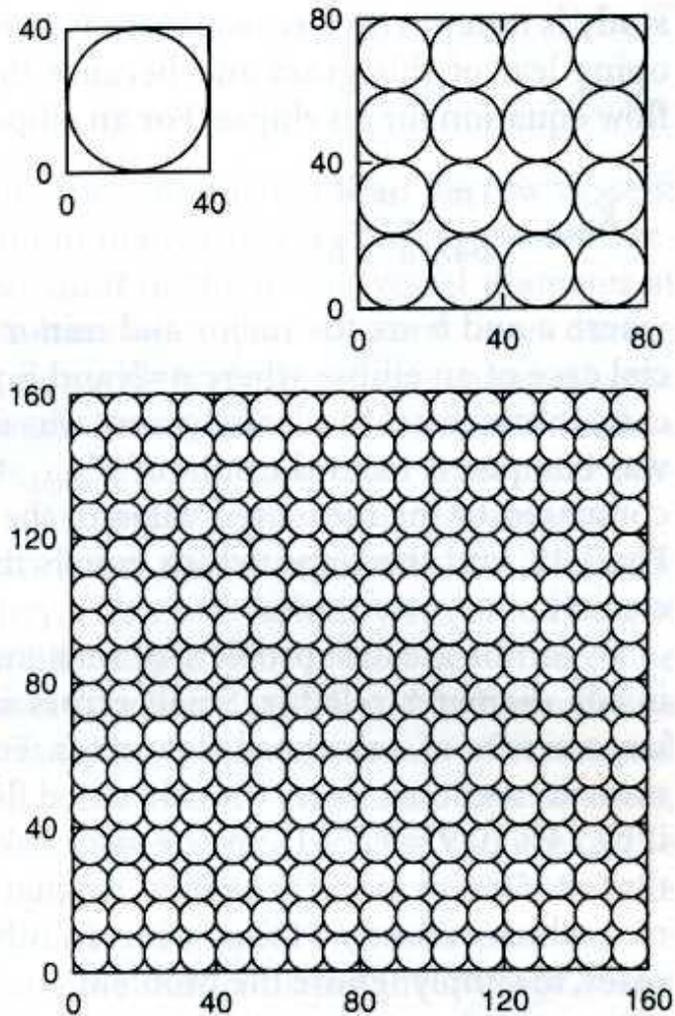
Legge di Hagen-Poiseuille

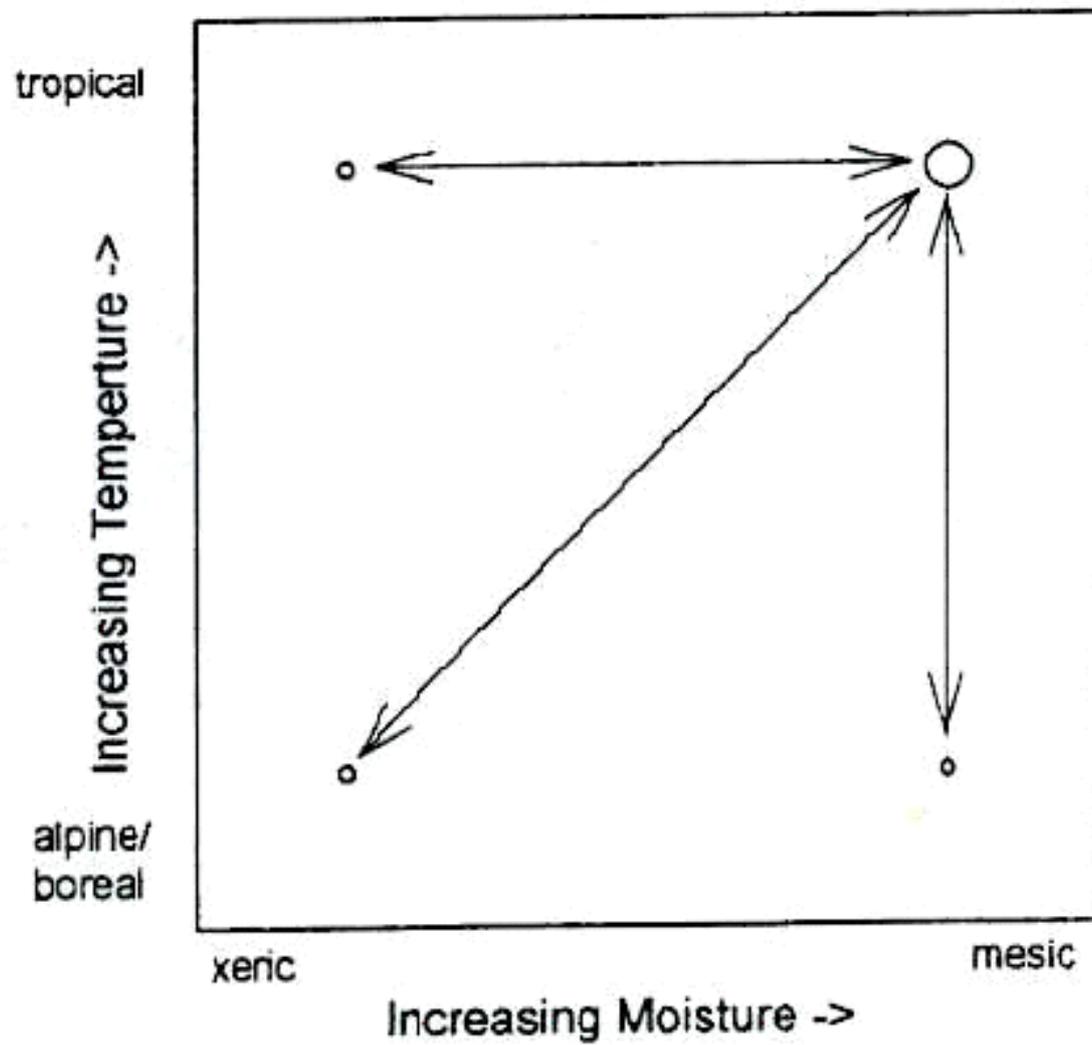
$$\text{Flusso} = \frac{\pi r^4}{8\eta L} \Delta\Psi$$



REL. DIAMETER	1	2	4
REL. T. S. AREA	1	4	16
REL. FLOW RATE	1	16	256
% FLOW RATE	0.4	5.9	93.7

Fig. 1.11. Hagen-Poiseuille law prediction of relationship between conduit diameter and conductance. The vessel blocks in **A**, **B**, and **C** have equal conductance, i.e., one vessel 40 μm diameter (**A**) is as conductive as 16 vessels 20 μm diameter (**B**), or as conductive as 256 vessels 10 μm diameter (**C**). Note that more wood cross section is needed to contain many small vessels having a combined conductance of a few large vessels





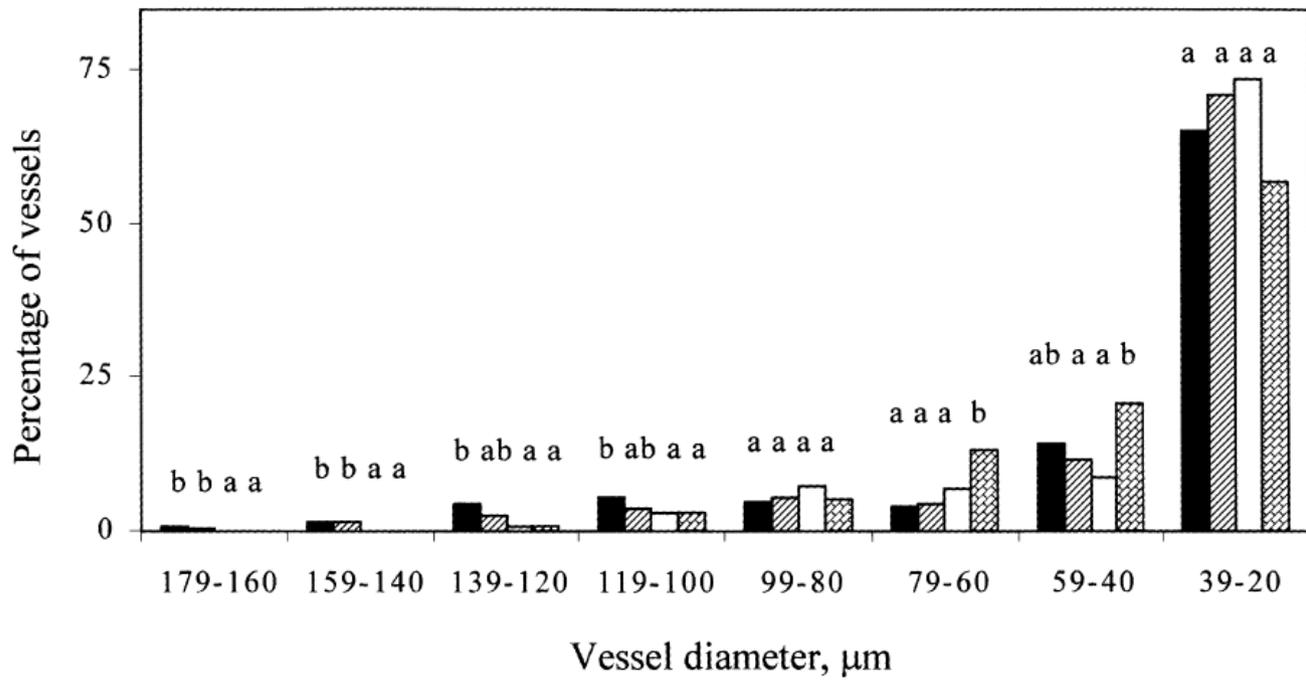
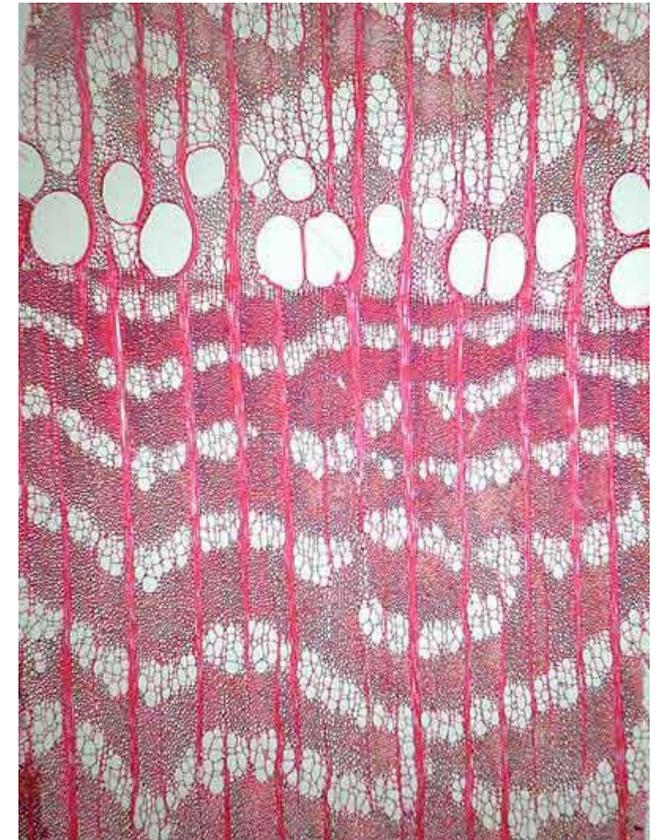
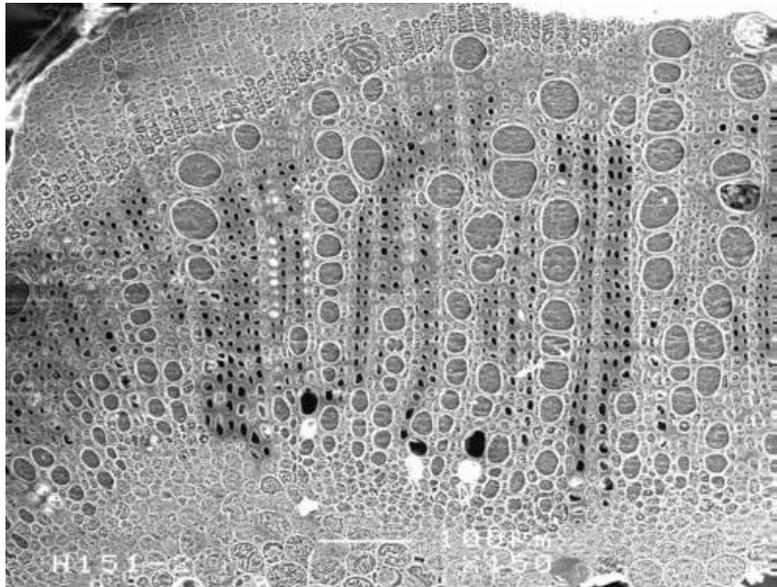
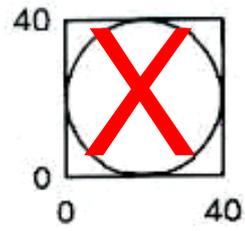


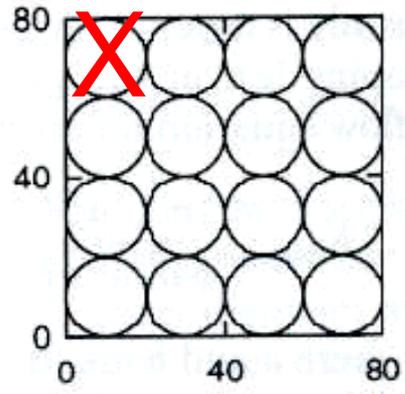
Fig. 1. Distribution of third year ring vessels (in diametric classes of 20 μm) from *U. minor* trees with high (■; n = 3), medium (▨; n = 2) and low (□; n = 2) susceptibility to DED, and from low susceptibility *U. minor* × *U. pumila* trees (▩; n = 2). Groups with a different letter differed significantly ($p \leq 0.05$, LSD)



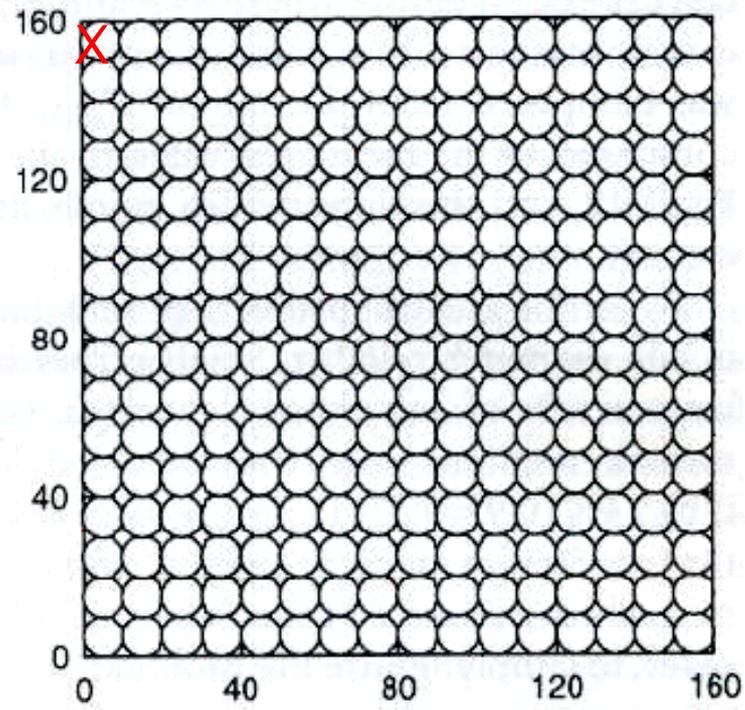
100%



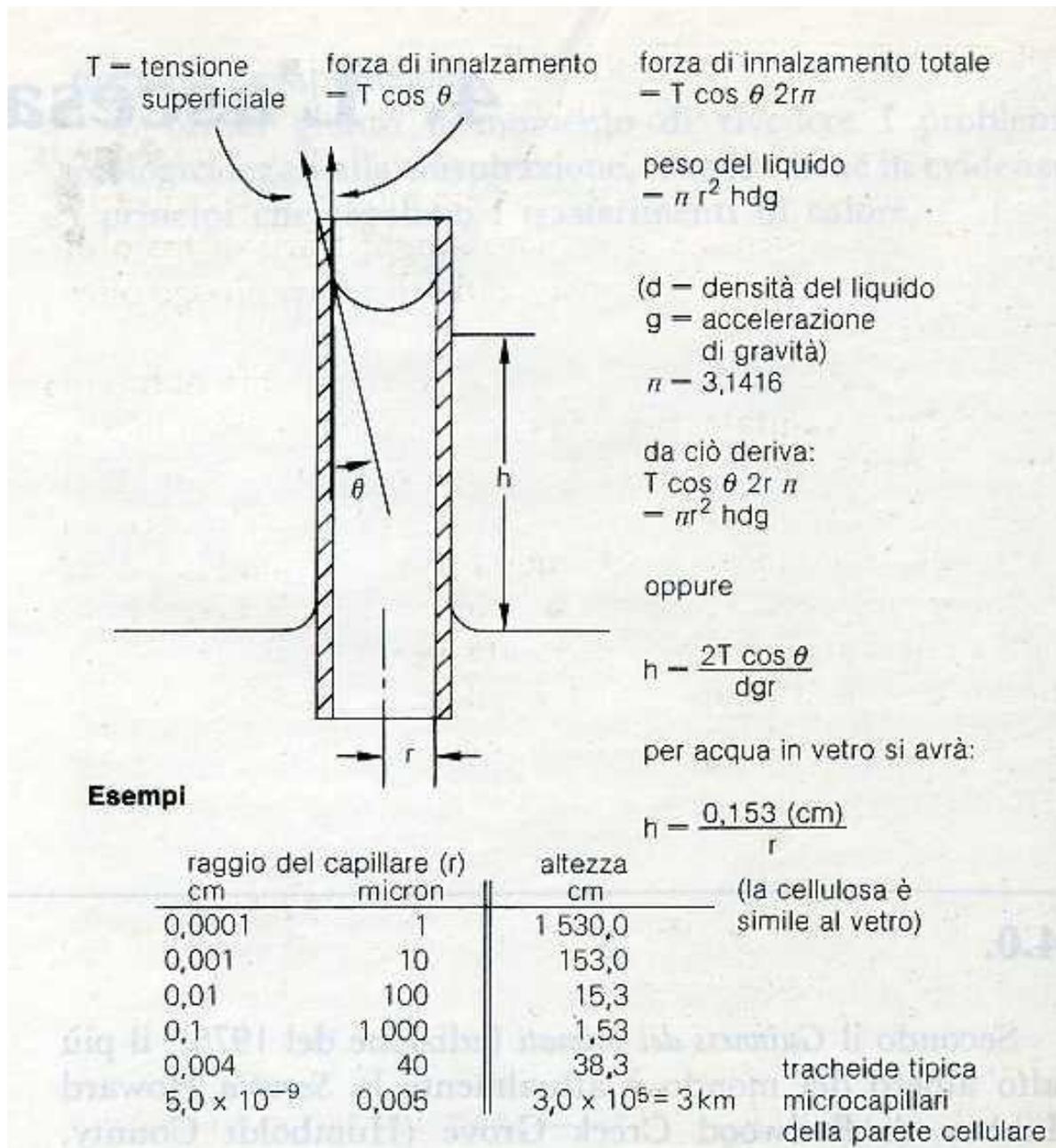
6.25%



0.39%



Come si genera la forza traente necessaria per il trasporto a lunga distanza dell'acqua nelle piante?



Il fenomeno della capillarità non può spiegare l'ascesa dell'acqua xilematica ad altezze superiori a 50 cm !!!

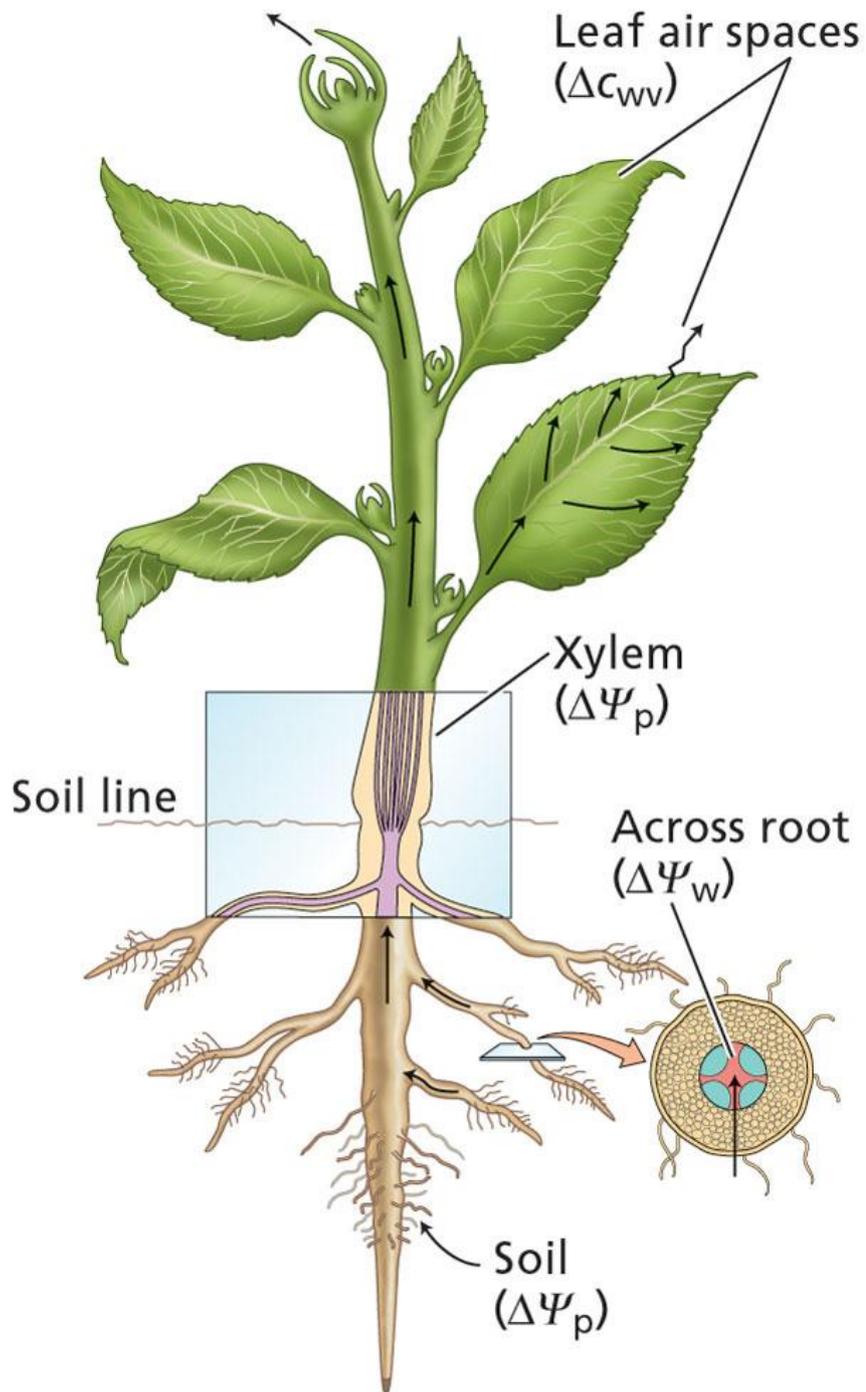
Meccanismi e forze motrici per il trasporto dell'acqua

gradiente di concentrazione del vapor d'acqua nella traspirazione

gradiente di pressione idrostatica nel trasporto a lunga distanza nello xilema

gradiente di potenziale dell'acqua nella radice

gradiente di potenziale dell'acqua nel suolo



$\Psi_{\text{suolo}} > \Psi_{\text{radice}} > \Psi_{\text{fusto}} > \Psi_{\text{foglia}} > \Psi_{\text{aria}}$



Direzione del flusso di H₂O

Air (50% relative humidity): -68.5 MPa

Leaves:
-0.5 to -2.5 MPa

Xylem sap:
-0.5 to -1.5 MPa

Roots: -0.2 to -0.4 MPa

Moist soil: -0.1 MPa

$$\Psi_{w_aria} = (RT/V_w) \times \ln (RH/100)$$

Tables 4.1. Relative air humidity (RH) and osmotic potential of a solution (MPa) as the two phases equilibrate in a closed system at 20 °C. (Recomputed from Walter 1931)

% RH	-MPa	% RH	-MPa
100	0	93.0	9.8
99.5	0.67	92.0	11.2
99.0	1.35	91.0	12.6
98.5	2.03	90.0	14.1
98.0	2.72	80.0	30.1
97.5	3.41	70.0	48.1
97.0	4.10	60.0	68.7
96.0	5.50	50.0	93.3
95.0	6.91	0	∞
94.0	8.32		