

Carboidrati - Parete Cellulare - Softening

The conventional approach to elucidate **fruit softening** has typically been based on two strategies:

- (1) the identification of **wall components** whose solubility increases and/or polymer size decreases in parallel with decreasing fruit **firmness** (Brummel and Harpster, 2001)
- (2) the **characterisation of proteins** that are expressed during ripening and whose biochemical activities can be mechanistically related to the observed wall changes (Brummel, 2006).

Data developed from these studies have guided the selection of genes whose expression has been enhanced or suppressed in transgenic fruits, in order to test whether they have direct roles in controlling softening, primarily using **tomato** as a model system.

In describing a cell wall, and particularly in relating cell-wall structure to food quality, it is highly appropriate to consider the cell wall as part of a hierarchy of structures (Waldron and others 1997a)

The hierarchy comprises 5 main levels of structure: the **cell wall polymers** which make up the underlying building blocks; **the cell wall**, consisting of these polymers; **the plant cell**, the morphology of which is reflected in the cell-wall shape and form; **the tissue** which comprises the constituent cells; and **the plant organ**, made up of the constituent tissues. The cell-wall-dependent characteristics of the plant organ, whether they relate to “food quality” or phenotype, will depend on the interacting properties of the different levels of structure.

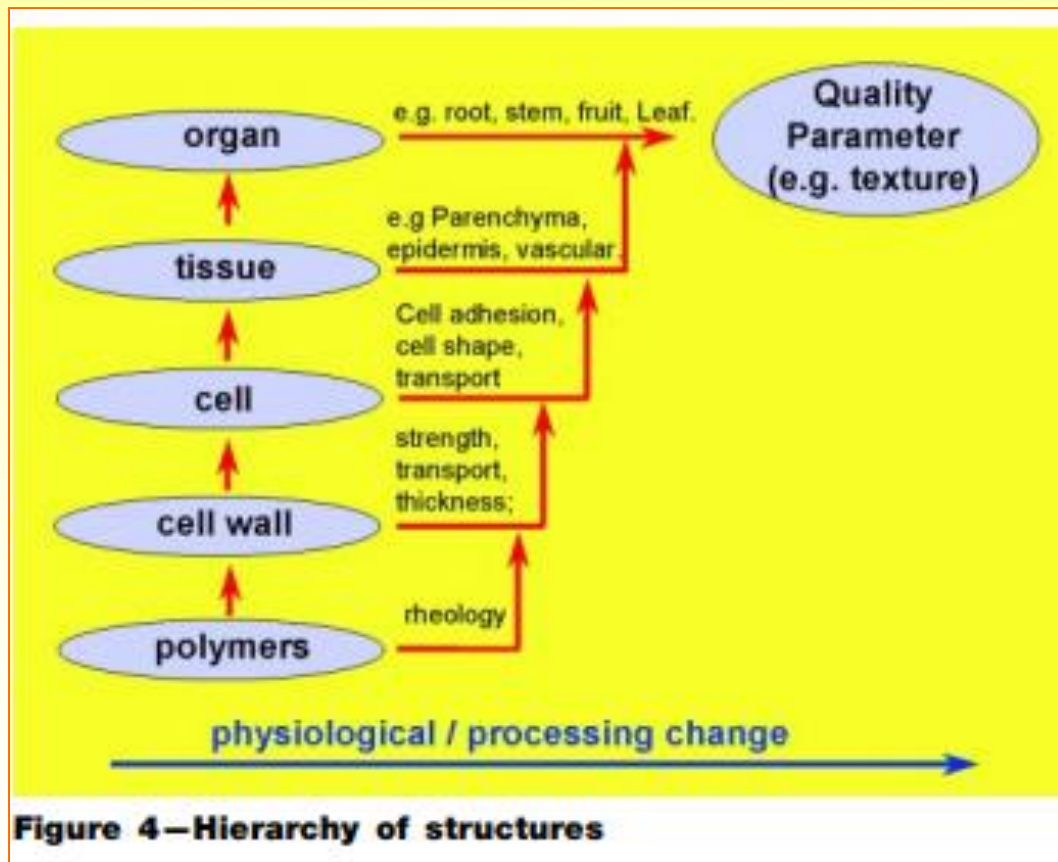
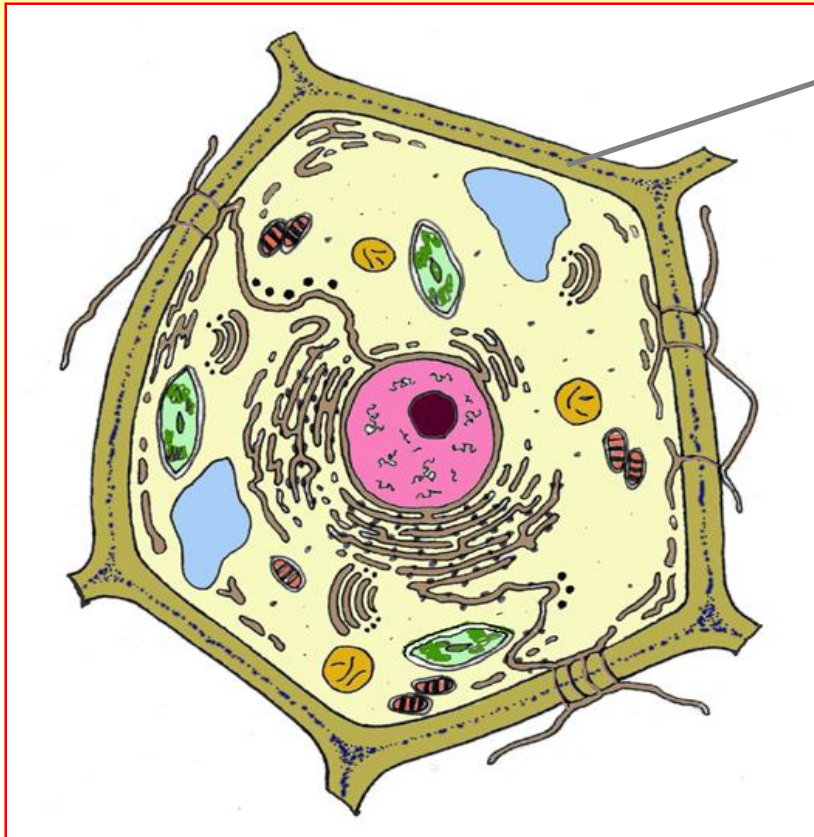
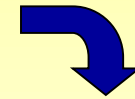


Figure 4—Hierarchy of structures

CELL WALL MODELS: CAN THEY HELP US BETTER UNDERSTAND FRUIT SOFTENING?

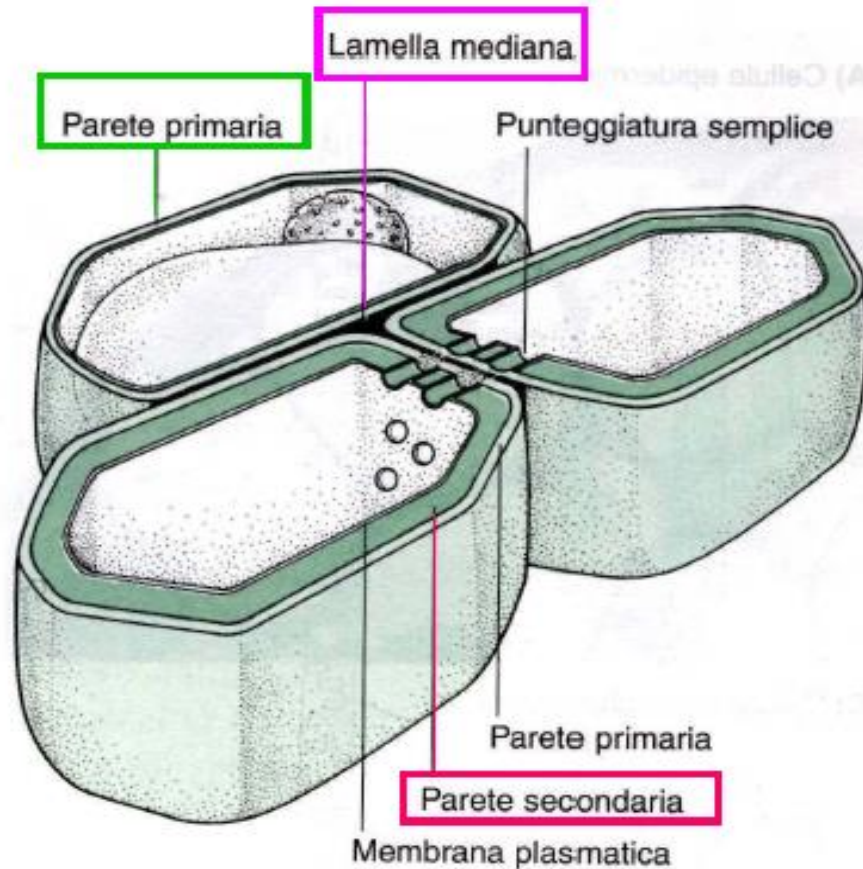


*parete
cellulare*



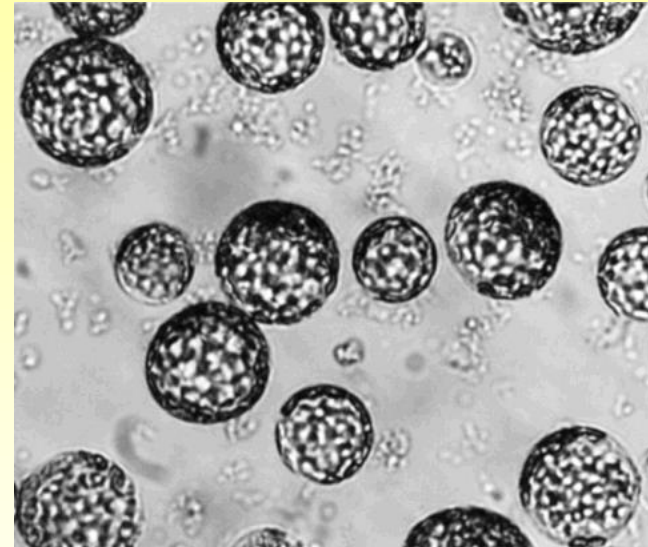
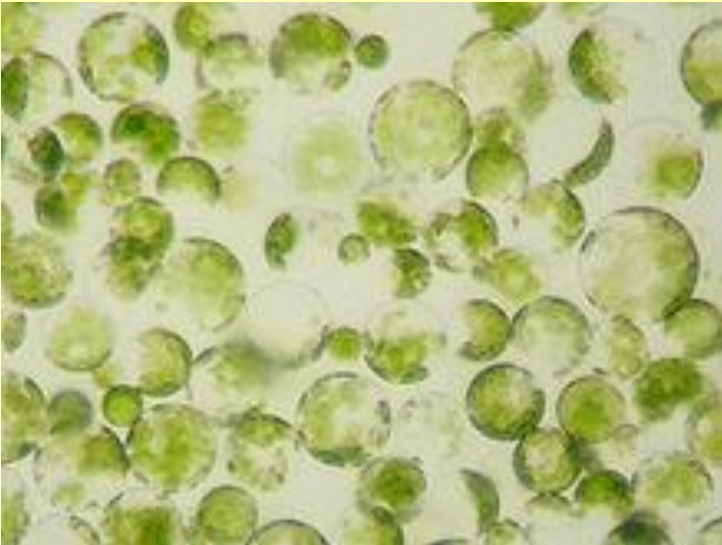
E' una struttura dinamica, che varia la composizione e le proprietà fisico-chimiche continuamente in risposta al suo sviluppo ed all'ambiente

LA PIANTA: UNA PANORAMICA SULLA STRUTTURA 11



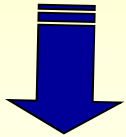
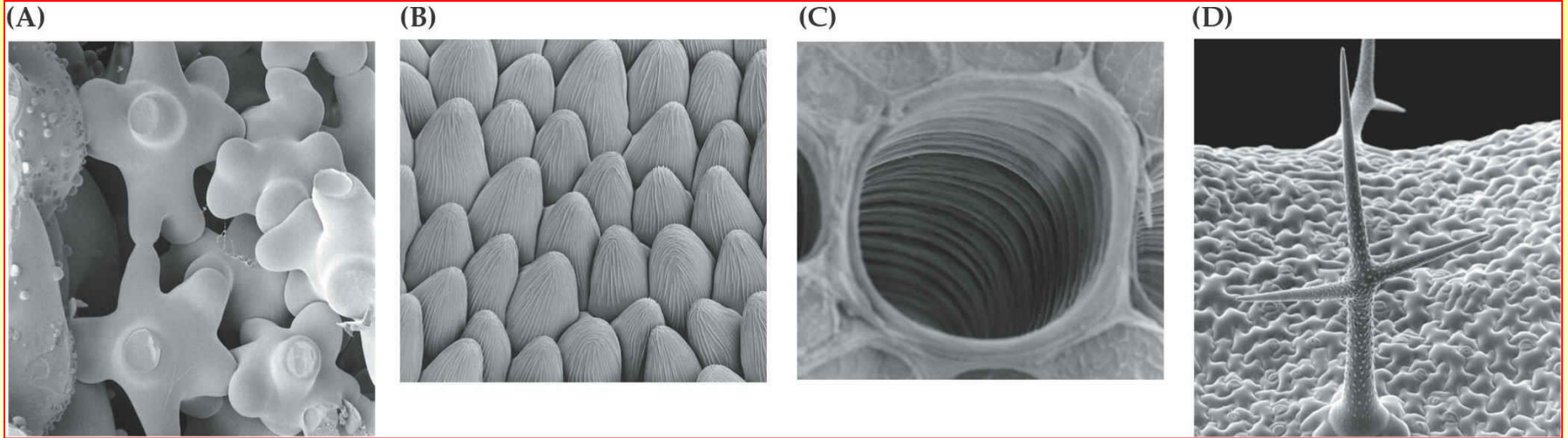
La parete ha il ruolo di controllare la velocità e la direzione dell'accrescimento cellulare, influenzando notevolmente lo sviluppo e la morfologia delle piante

Dopo digestione della parete cellulare
PROTOPLASTI

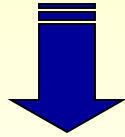


PROTOPLASTI di *Petunia Hybrida*

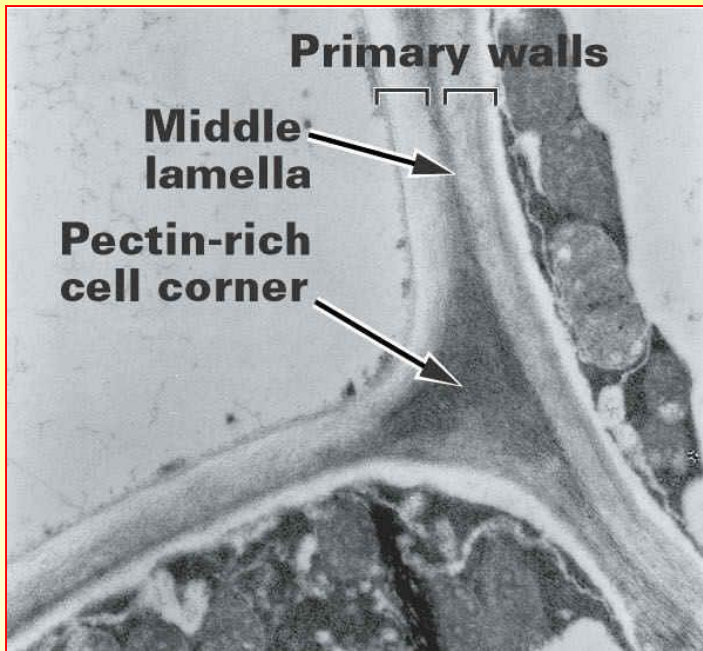
Struttura ..anche specializzata



Foglia di
Zinnia:
minimo
contatto e
max
scambio
gassoso



La forma specializzata delle cellule
epidermiche delle papille di un petalo di
bocca di leone necessarie a riflettere la
luce per arricchire i colori ed attirare
l'attenzione degli impollinatori



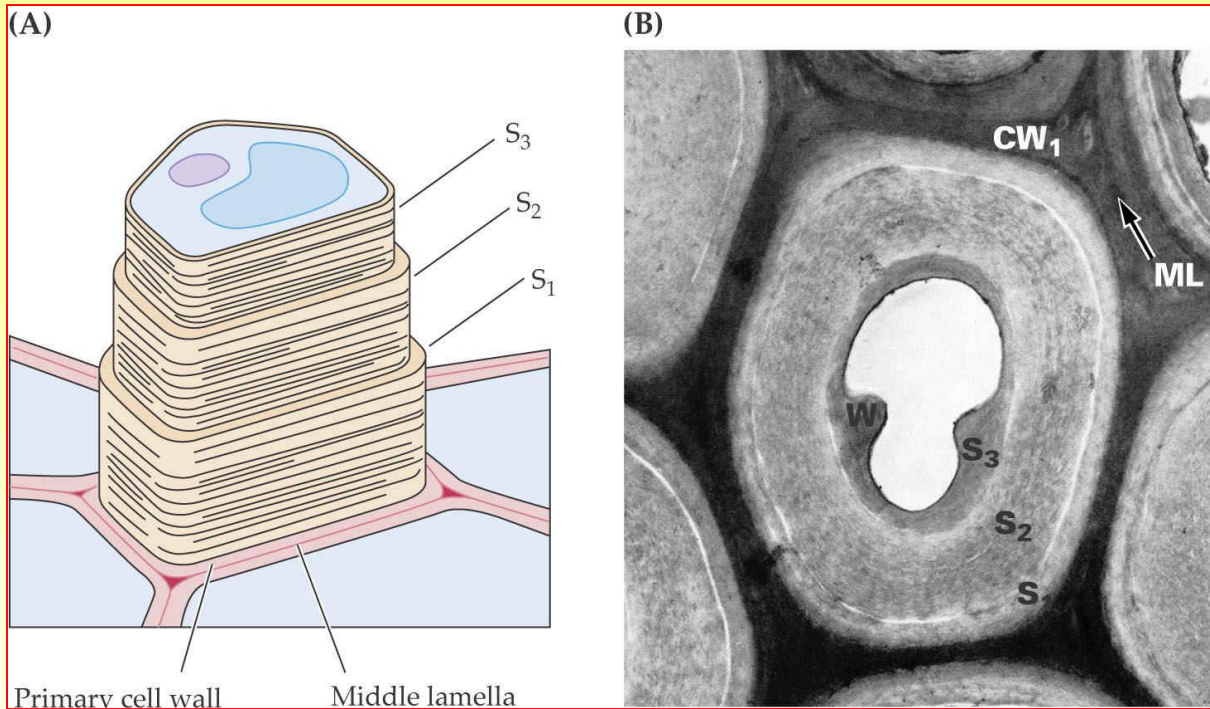
La composizione e gli arrangiamenti dei polimeri della parete cambiano in funzione della specie, del tipo di cellula e della regione della parete considerata

Non solo funzione strutturale

- Influenza processi di sviluppo
- Marca la posizione all'interno della pianta
 - Determina la comunicazione cellula-cellula e parete-nucleo
- Ha un ruolo di difesa
 - Ha un ruolo nel riconoscimento dei batteri azoto-fissatori



Parete Cellulare Primaria - Caratteristiche



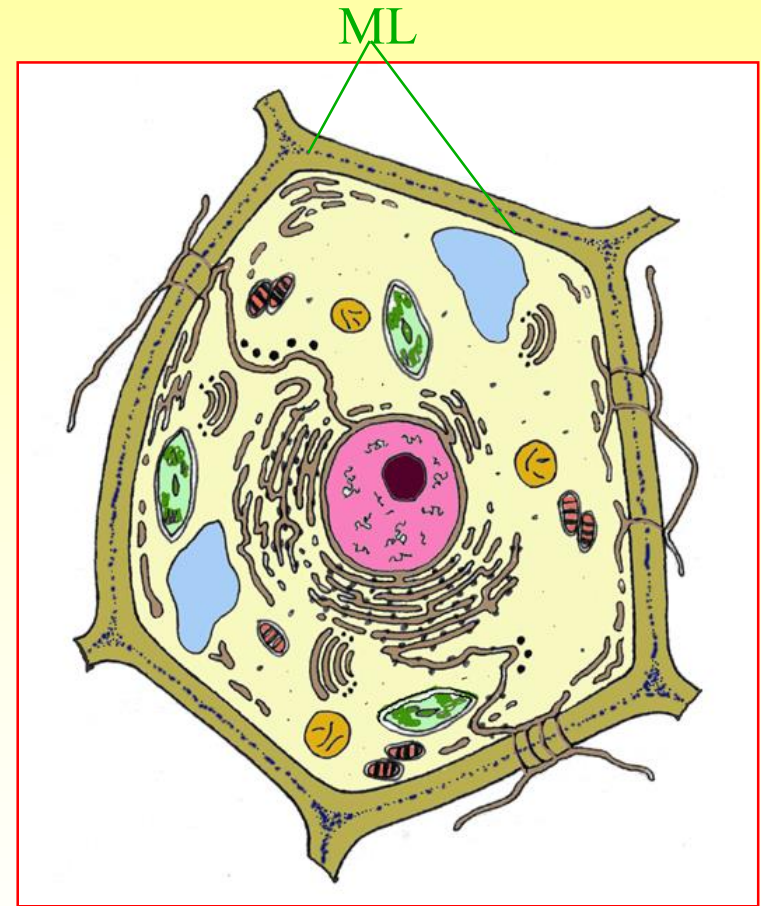
Funzioni

- supporto
- protezione
- enzimatica

- primo strato depositato in ogni cellula in accrescimento
- unico strato nelle giovani cellule in crescita, in alcune cellule di riserva,
- molto sottile (0.1 < 1 micron)
- circonda il protoplasma
- porosa all'acqua e ai minerali

Lamella mediana

- **Strato più esterno: forma**
 - Forma l'interfaccia tra le pareti primarie di cellule confinanti
- **Funzione**
 - Regola l'adesione tra le cellule
 - gel cementante
- **Spessore: 30 nm (anche meno)**
- **Composizione**
 - pectati di Ca^{2+} e Mg^{2+}
 - formano gel: **presenti nei frutti immaturi; vengono degradati durante il 'ripening'**



Parete secondaria

- si forma solo dopo che la formazione della primaria è completata
- caratteristica di cellule mature, non in crescita, elementi dello xilema
- localizzata tra PM e parete primaria
- più spessa della parete primaria
 - diversi micron
- conferisce rigidità, supporto e resistenza alla cellula
- Non è presente, se non con qualche eccezione, nella parete primaria
- Alcune pareti secondarie funzionano come materiale di deposito (riserve di carboidrati) come nei cotiledoni e nell'endosperma di semi in via di sviluppo da utilizzare successivamente durante la germinazione. Oltre a conferire robustezza per proteggere l'embrione e imporre meccanicamente la dormienza

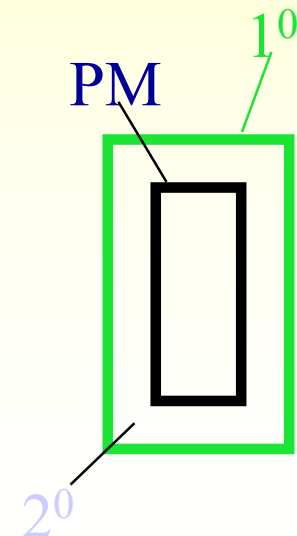
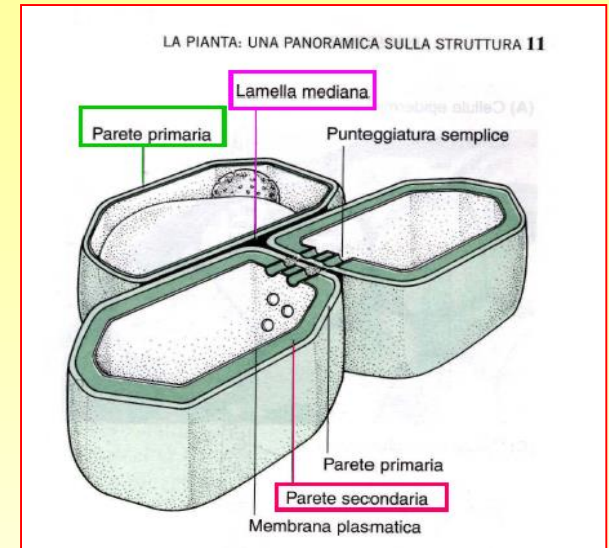


Table 4-2. Gross Composition of the Cell Wall for Apple and Strawberry: Monomers Yielded Upon Hydrolysis of Wall Polymers

<i>Component Monomers</i>	<i>% Total Accounted for</i>	
	<i>Apple</i>	<i>Strawberry</i>
Rhamnose	0.4	1.1
Fucose	0.7	ND
Arabinose	19.5	6.5
Xylose	5.9	1.9
Mannose	1.9	0.7
Galactose	5.8	7.6
Glucose	47.5	31.1
Galacturonic acid	16.6	40.3
α -Amino acid	1.7	10.7
Hydroxyproline	0.04	0.1

.....un po' di nomenclatura!!!!

Queste unità semplici di zucchero si combinano per dare i polisaccaridi che sono denominati in **funzione dello zucchero principale che essi contengono. Si parla di:**

- 1) **glucano** come **polimero del glucosio**, 2) **xilano** per il **polimero dello xilosio**, 3) **galattano** per il **polimero del galattosio**.

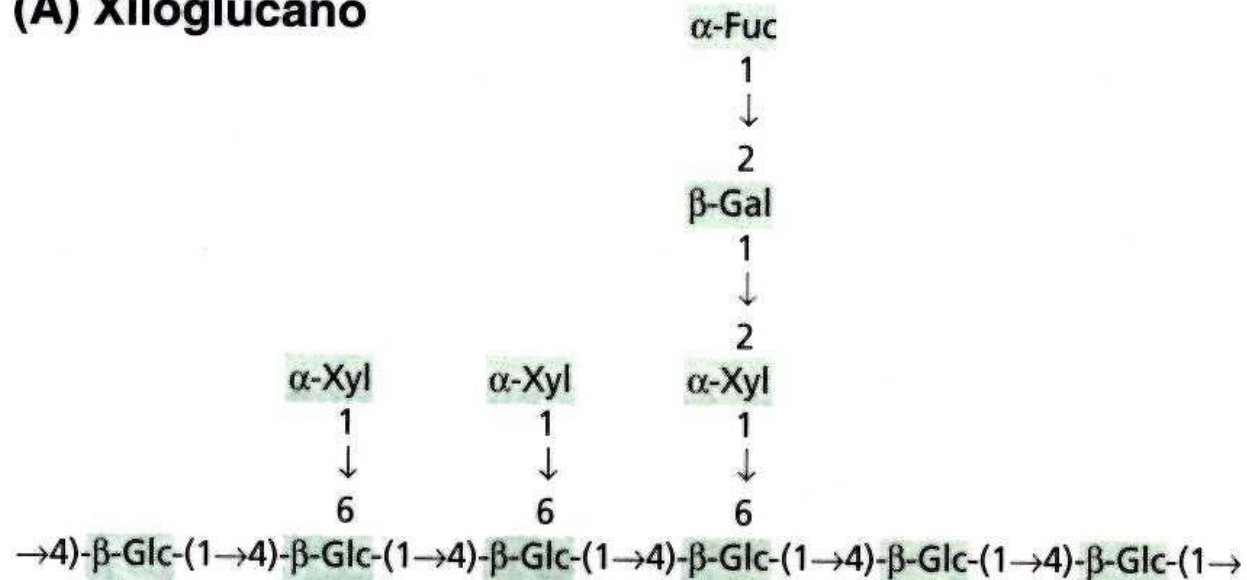
2)

Per i polisaccaridi ramificati: **la catena lineare del polisaccaride dà l'ultima parte del nome.**

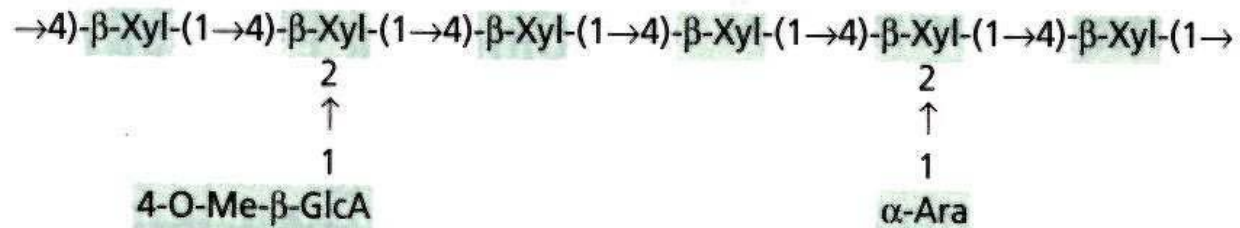
- 1) **Xiloglucani** vuol dire che hanno una **catena lineare di glucano (residui di glucosio) con xiloso attaccato nella catena laterale.**
- 2) **Arabinoxilani** vuol dire che hanno **xilano nella catena lineare e arabinosio nella laterale.**

E' chiaro che c'è qualche eccezione. Infatti i 3) **glucomannani** sono polimeri contenenti **glucosio e mannosio nella catena laterale.**

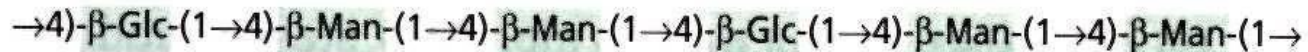
(A) Xiloglucano



(B) Xilani



(C) Glucomannani



1) Quali sono i polisaccaridi più importanti della parete; 2) come sono organizzati nelle diverse strutture (primaria, mediana, secondaria) ? 3) Quali altre componenti strutturali sono presenti ?

5 polisaccaridi
inequivocabilmente
individuati:

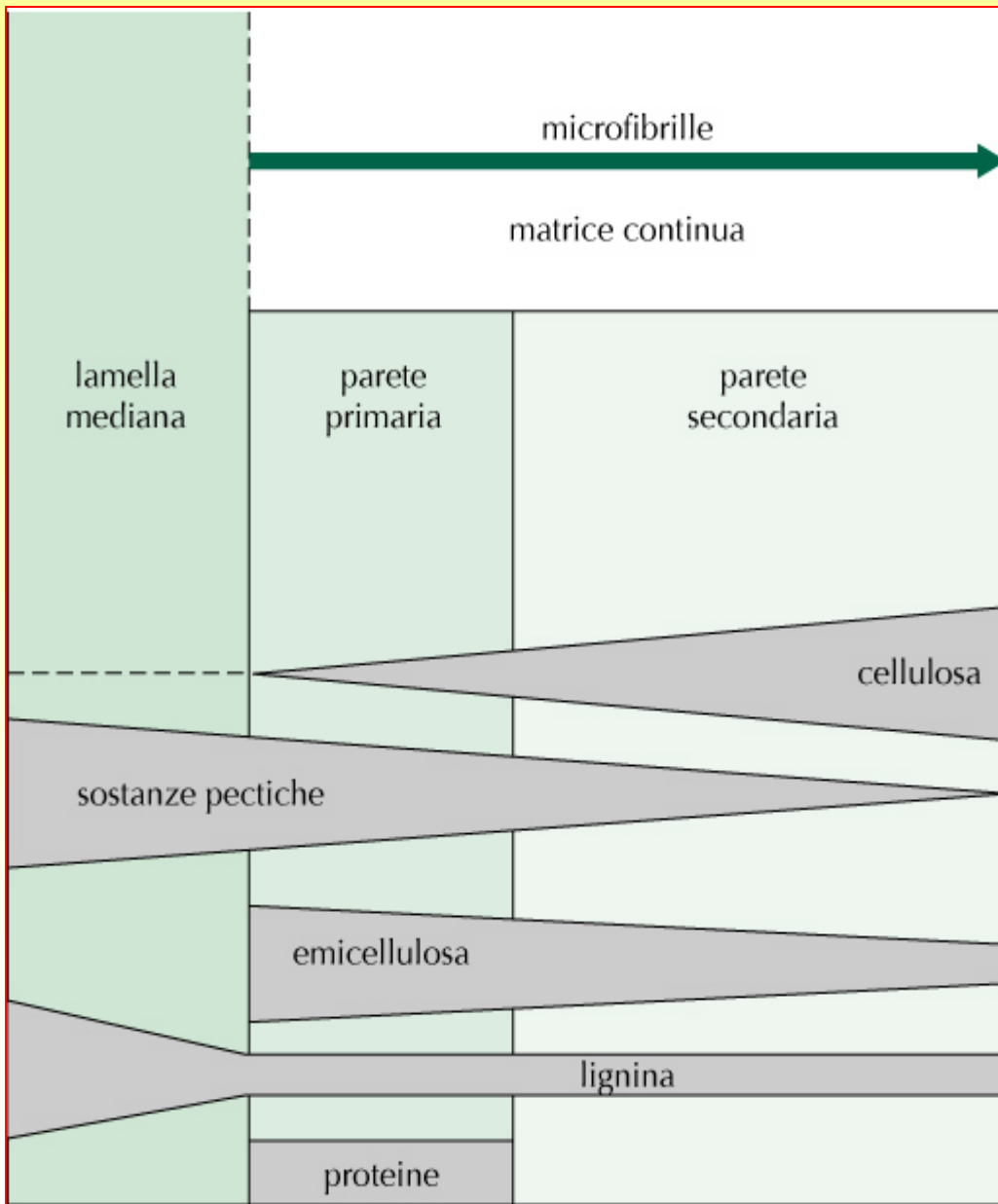
- Cellulosa
- Glucuronoarabinosilani
- Xiloglucani
- Ramnagalatturani
- Galattoglucomannani

Glicani concatenati o
cross-linking o emicellulose

Pectine

Proteine come le
Estensine

Sostanze aromatiche quali
Acidi idrossicinnamici



Componenti della parete cellulare

Fase fibrillare
Cellulosa

Fase di matrice (amorfa)

Emicellulosa
Pectina
Proteine
Fenoli
Lignina

lessuto

Estrazione con soluzioni calde di ossalato d'ammonio o EDTA

Porzione insolubile

Porzione solubile

Estrazione con KOH 2N

Precipitazione con ETOH

insolubile

solubile

solubile

insolubile

Cellulosa

emicellulose

SCARTO

Pectine

Neutralizzazione con acido acetico

insolubile

solubile

Emicellulosa A

Precipitazione con ETOH

insolubile

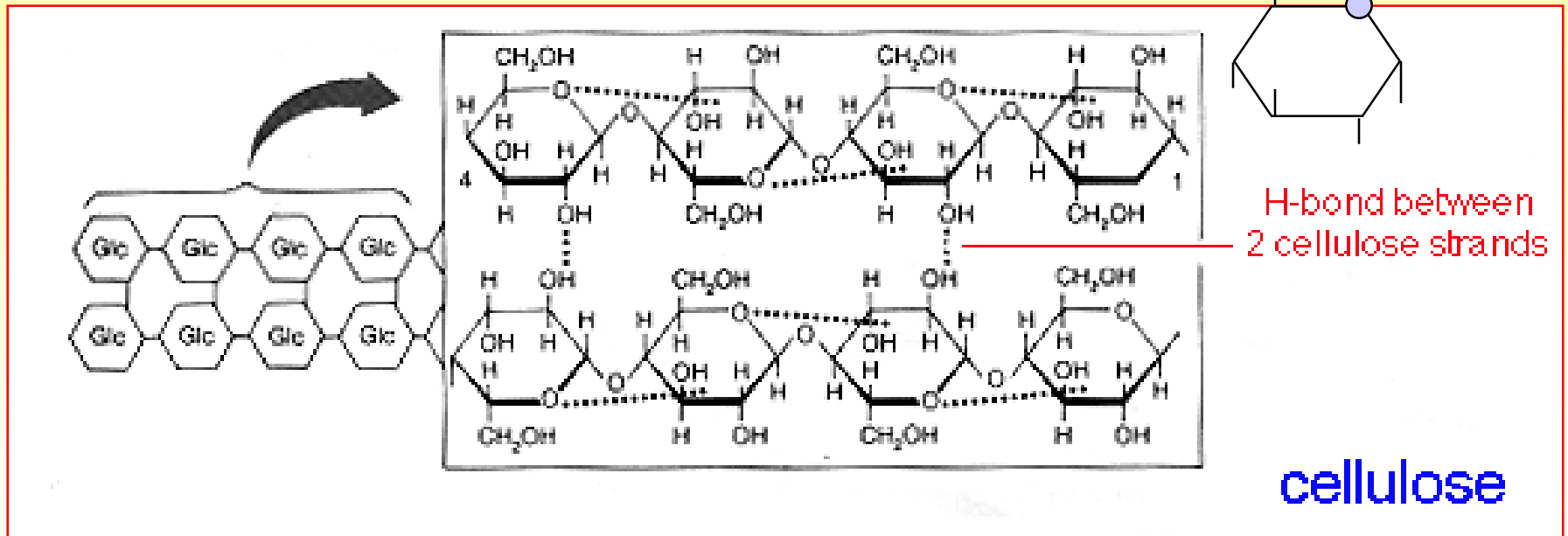
solubile

Emicellulosa B

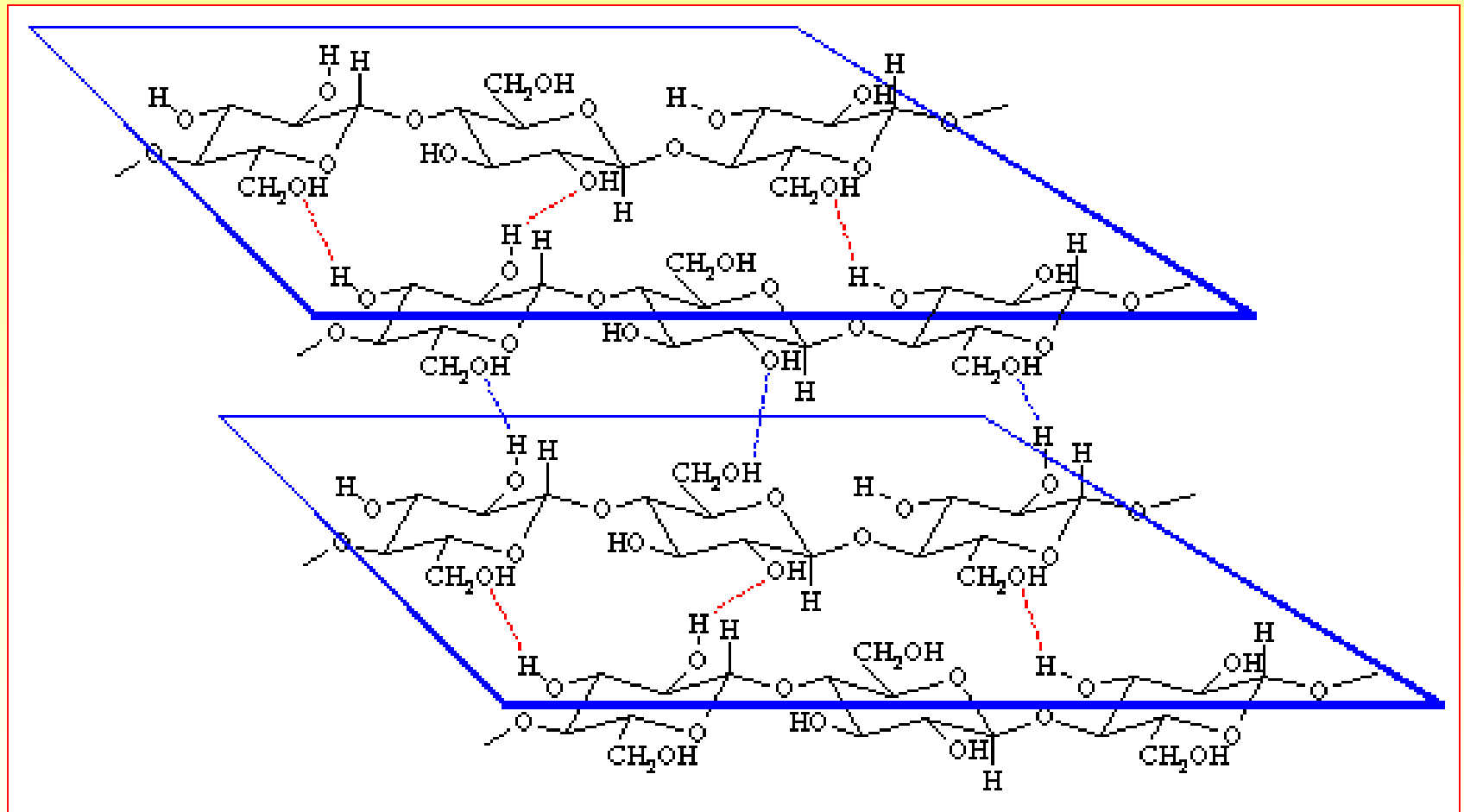
**Altri frammenti
polisaccaridici**

Cellulosa

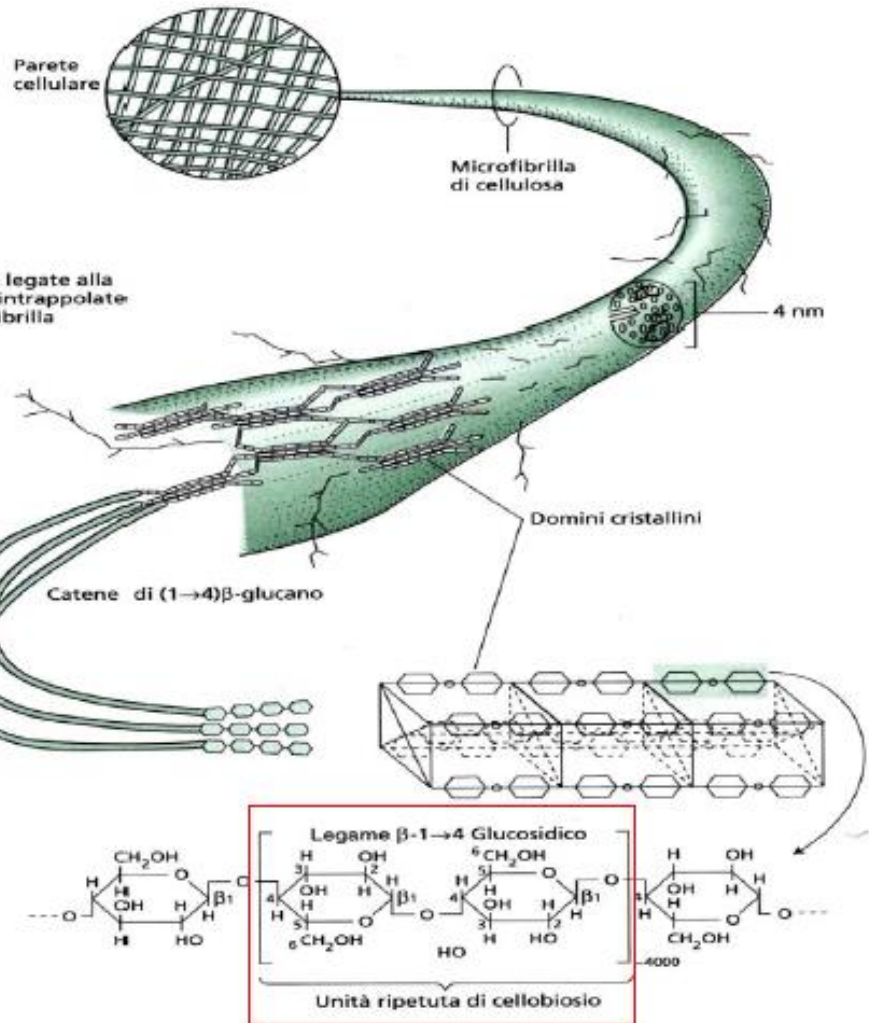
- Il più abbondante polimero biologico sulla terra
- catena lineare di monomeri di glucosio con legami β 1-4.
- Unità ripetuta è il cellobiosio
- Principale componente strutturale di tutte le pareti cellulari è circa il 15-30 % peso secco



- Forza tensile come l'acciaio (fino a 5000 bar)
 - Forze coesive (forze di van der Waals)
 - Tipicamente insolubile

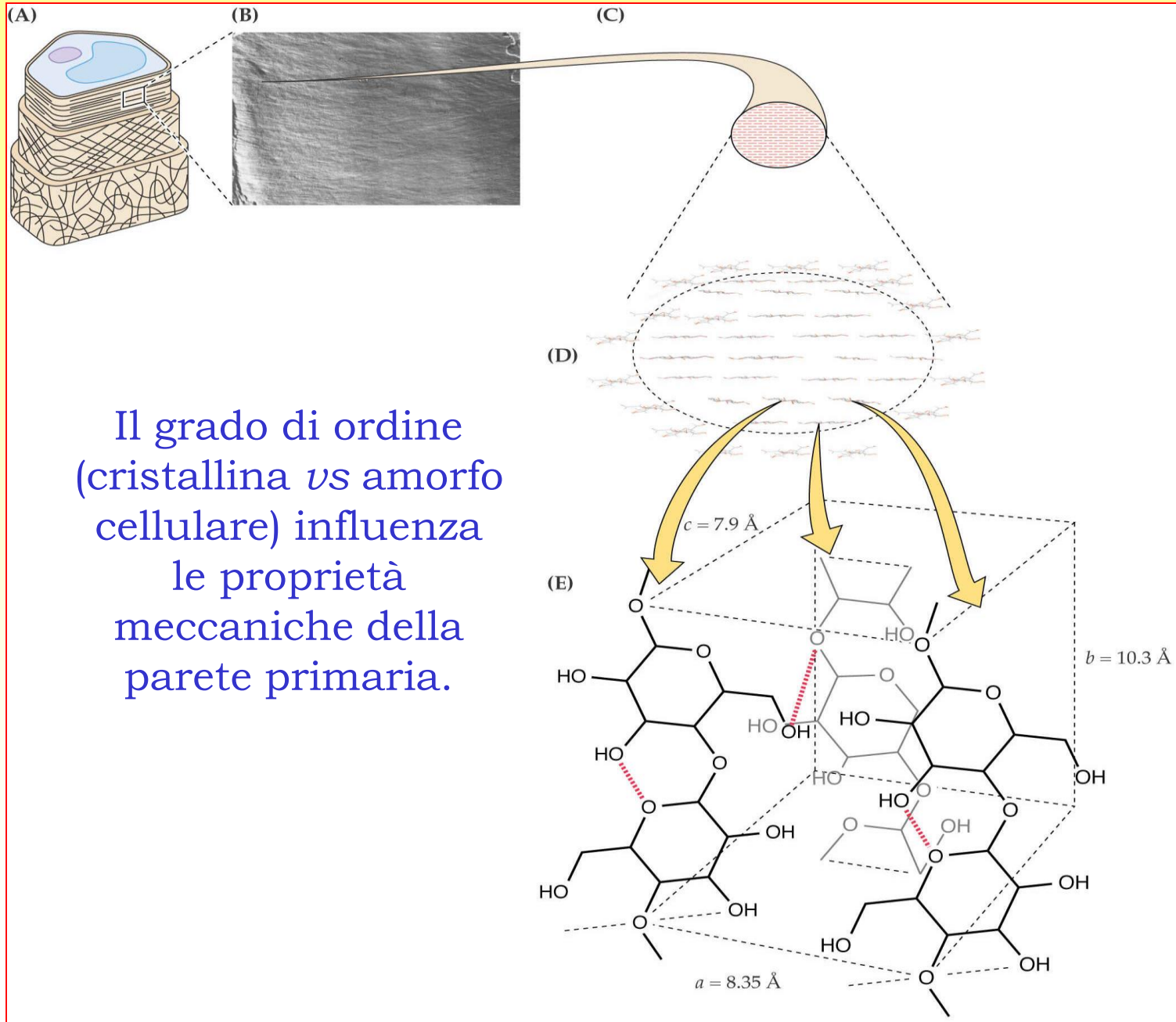


Numerosi legami H tra 20-40 catene diverse e all'interno di ciascuna catena formano una microfibrilla

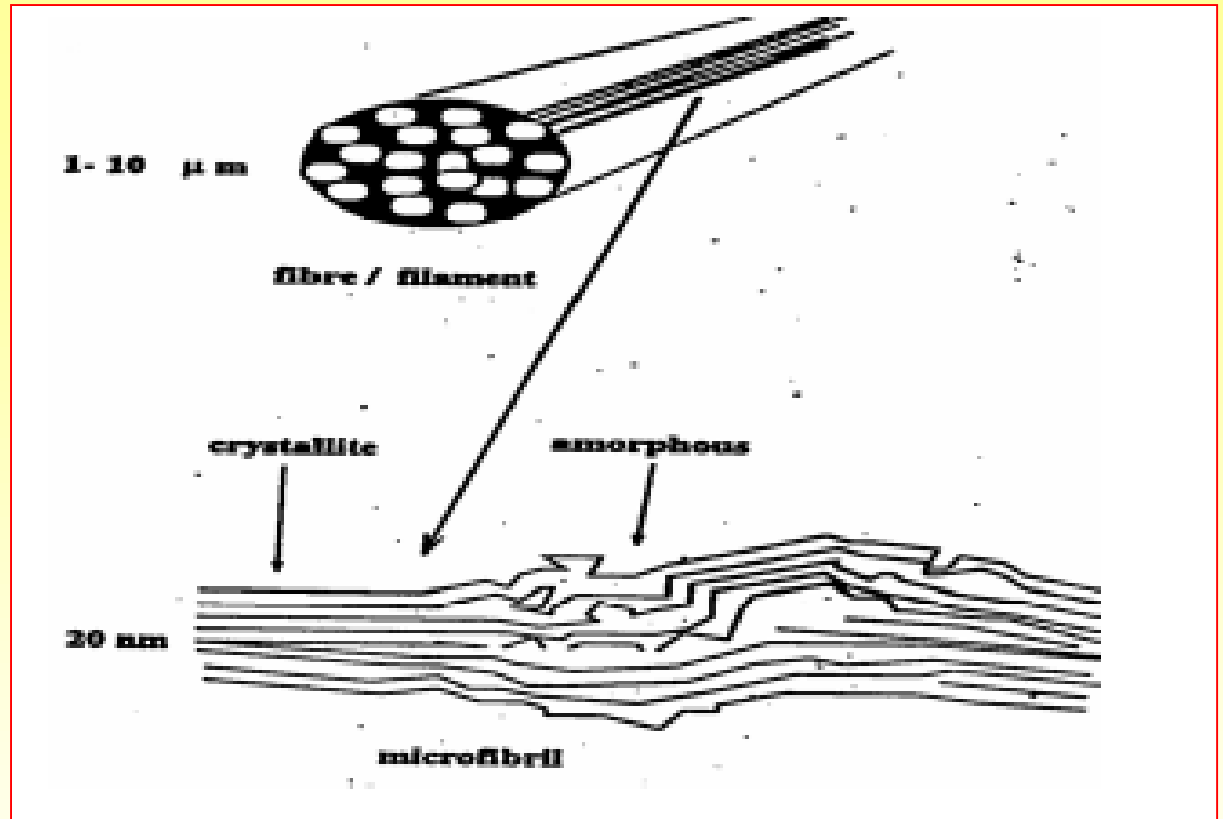


Microfibrille

- composed of many glucan chains associated in crystalline arrays to produce long microfibrils
- held together by hydrogen bonds
- 20-30 glucan chains in a microfibril
- very strong and rigid
- can measure many micrometers
- greater tensile strength than steel



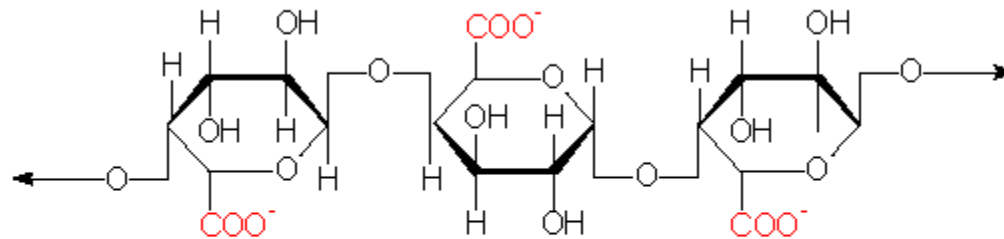
Polisaccaridi
non cellulose
molto meno
abbondanti che
saldano le
microfibrille di
alcune pareti
primarie



Le regioni amorse (emicellulose,
pectine, proteine, fenoli, lignina)
sono più sensibili all'azione di
cellulasi

Pectine

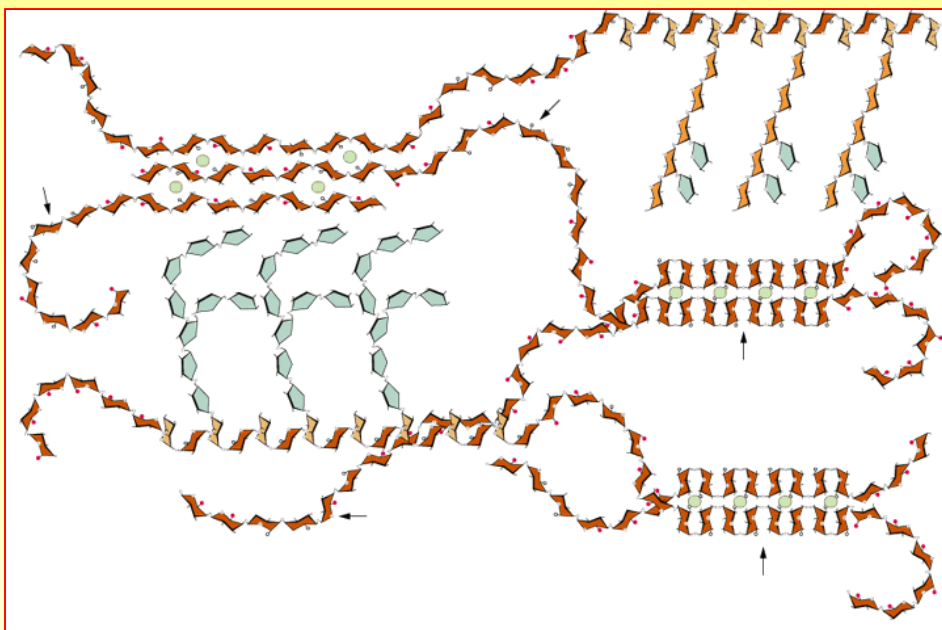
- una miscela eterogenea di polisaccaridi ramificati molto idratati e ricchi di acido galatturonico.
- è presente per il 30% della parete primaria, non presente nella parete secondaria



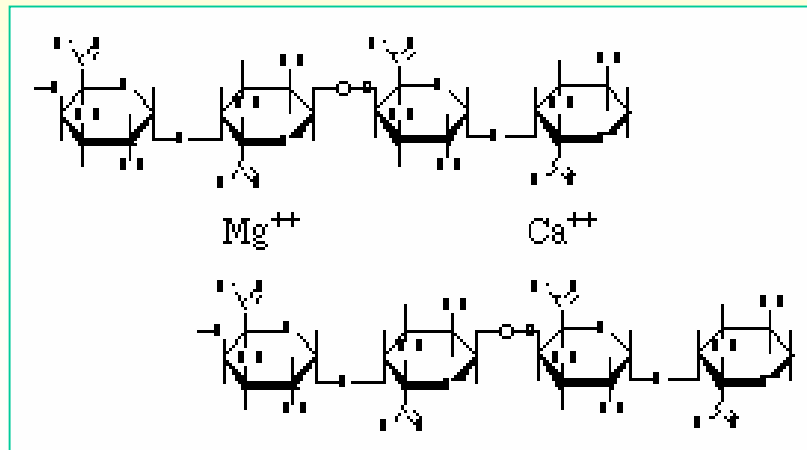
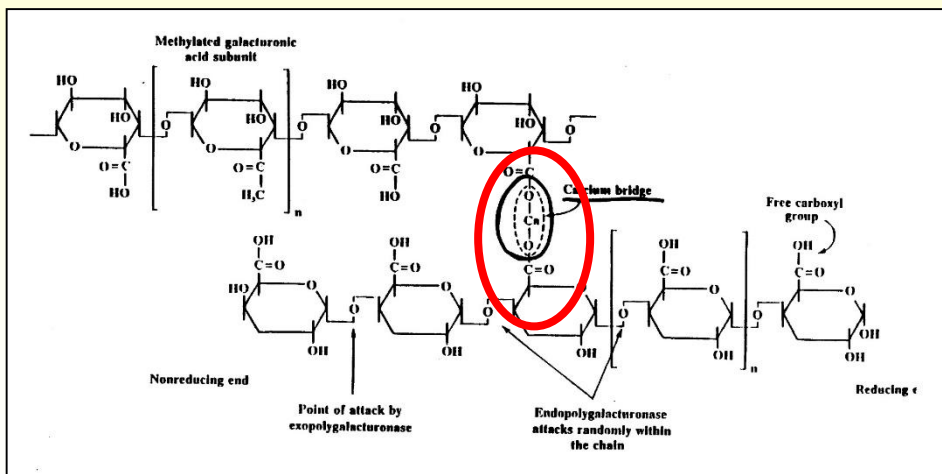
pectic acid (α -1,4-galacturonic acid)

Funzioni delle pectine

- Conferiscono porosità ed elasticità alla parete
- Forniscono superfici cariche che modulano il pH ed il bilancio ionico
- Agiscono come collanti ma anche come molecole di riconoscimento



La pectina determina la porosità della parete e quindi la texture dei prodotti



Le catene di omogalatturani presentano una forma elicoidale.
 Ioni bivalenti come il calcio possono formare legami fra catene adiacenti in modo tale da formare un gel.

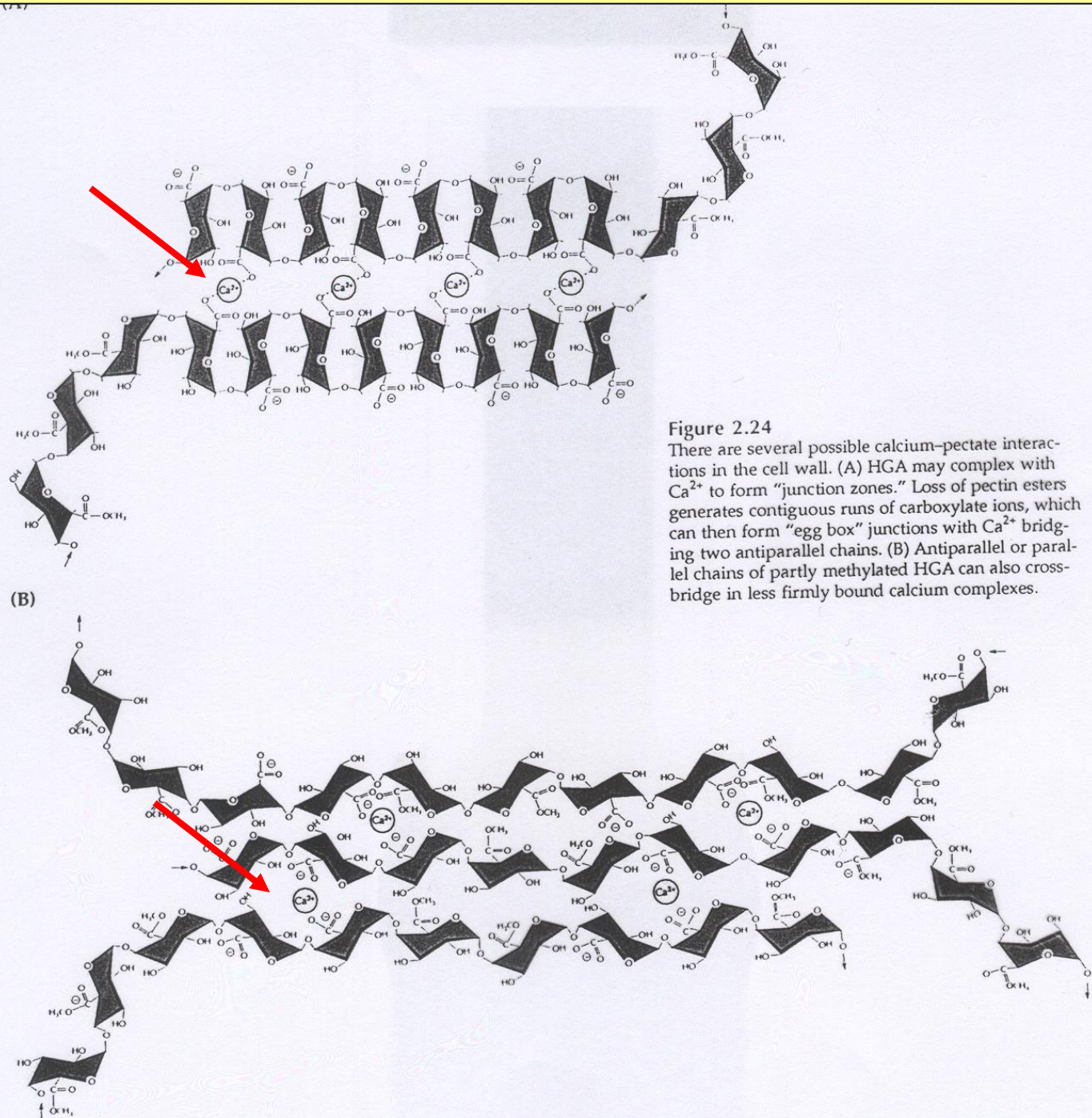


Figure 2.24
There are several possible calcium-pectate interactions in the cell wall. (A) HGA may complex with Ca²⁺ to form "junction zones." Loss of pectin esters generates contiguous runs of carboxylate ions, which can then form "egg box" junctions with Ca²⁺ bridging two antiparallel chains. (B) Antiparallel or parallel chains of partly methylated HGA can also cross-bridge in less firmly bound calcium complexes.

Ramnogalatturani

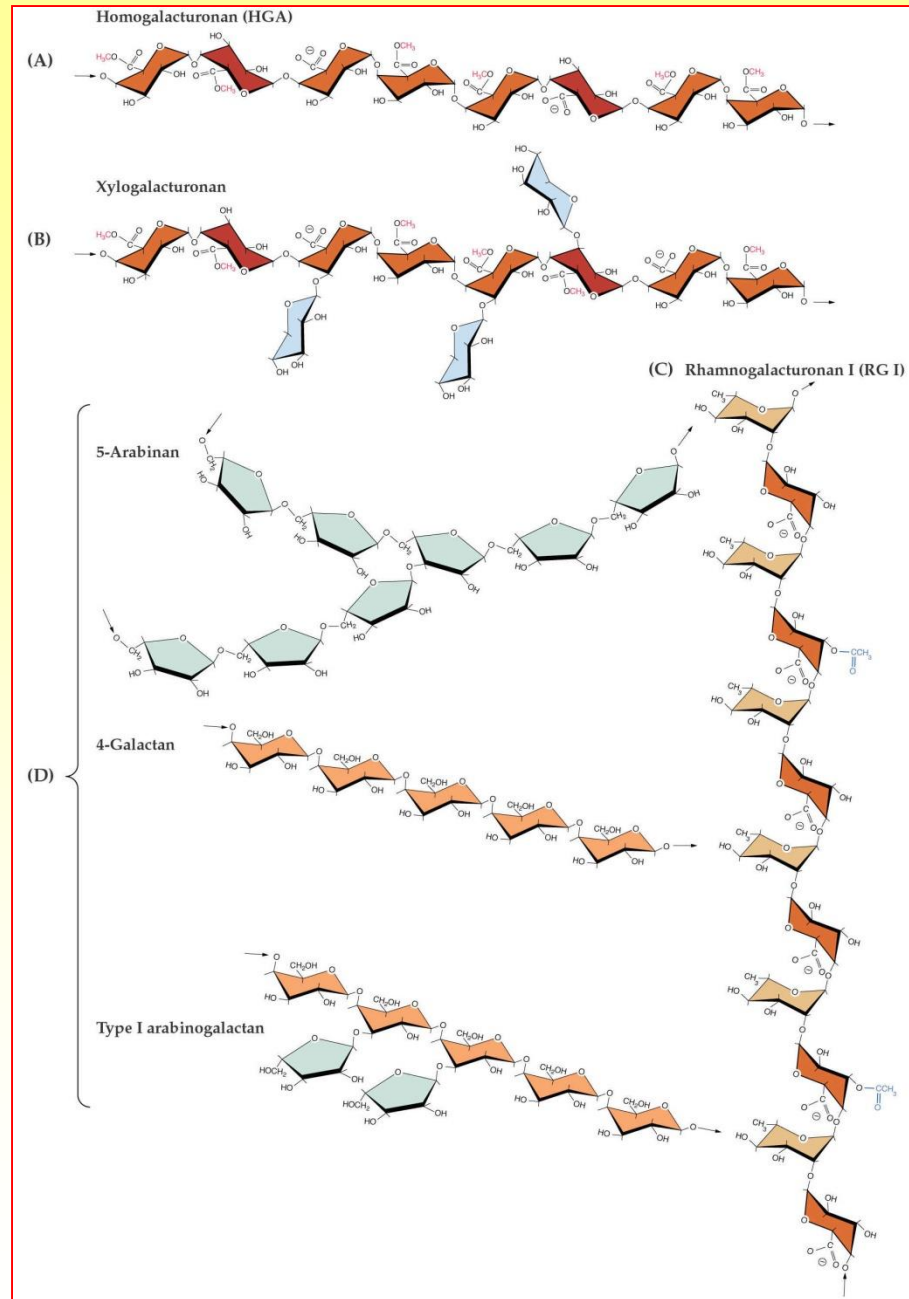
RG I

Eteropolimero di unità disaccaridi di ramnosio e galattosio

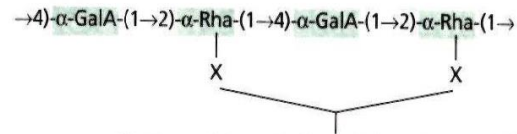
Omogalatturonani

HGA

Omopolimeri di galattosio

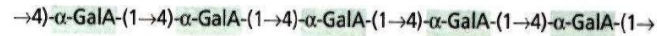


(A) Ramnogalatturonano I (RG I)

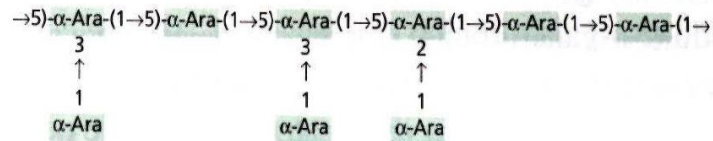


Catene laterali di arabinani e galattani

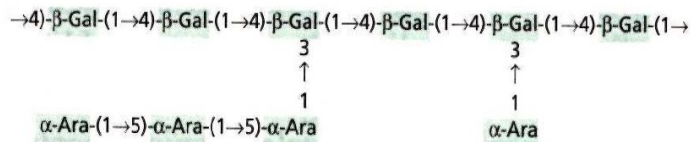
(B) Omogalatturonano (Acido poligalatturonico)



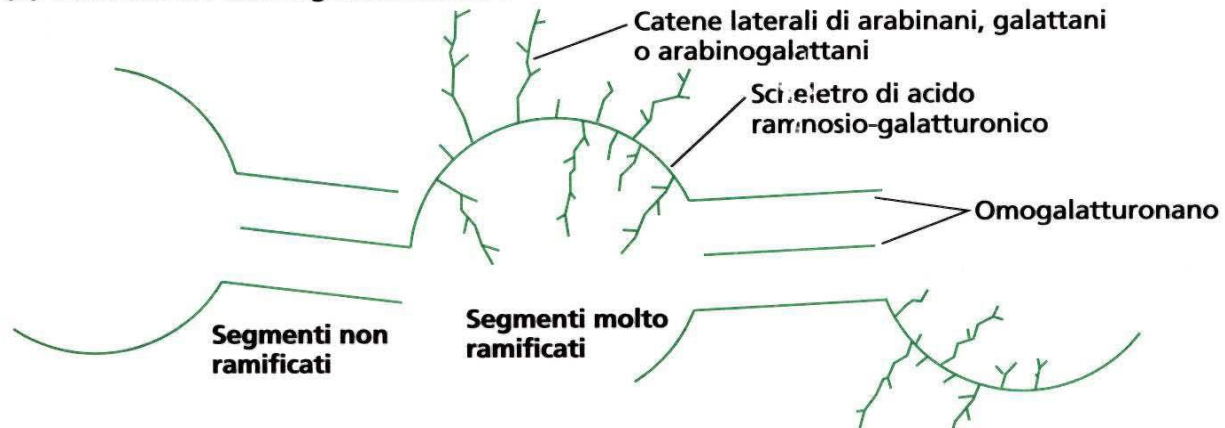
(C) Arabinani

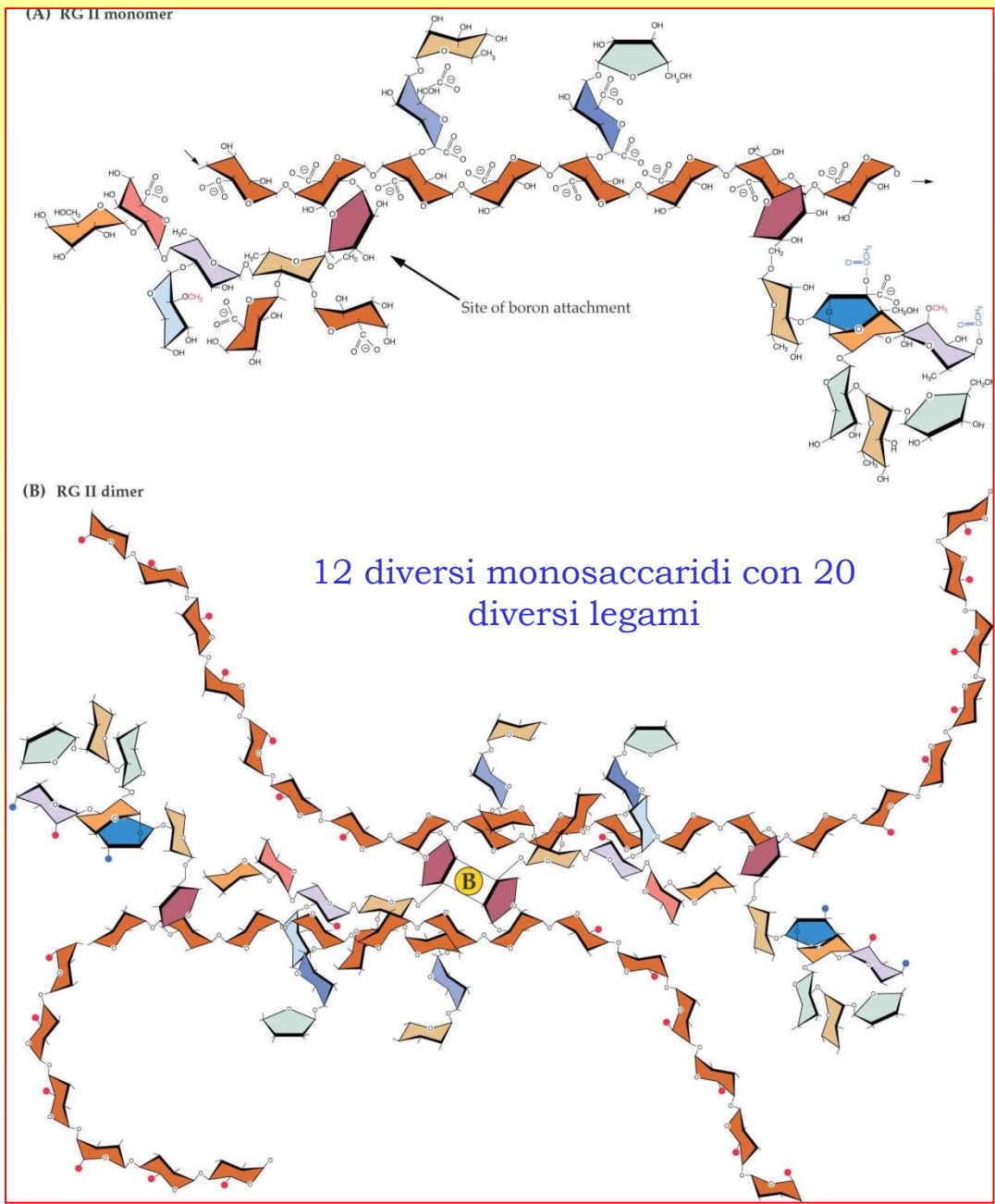


(D) Arabinogalattano I



(A) Struttura del Ramnogalatturonano I



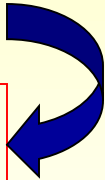


Due tipi di
OMOGALATTURANI:

Xilogalatturano

Ramnogalatturano II

RG II



Struttura
altamente
conservata,
importante
funzione, ma
di scarsa
abbondanza

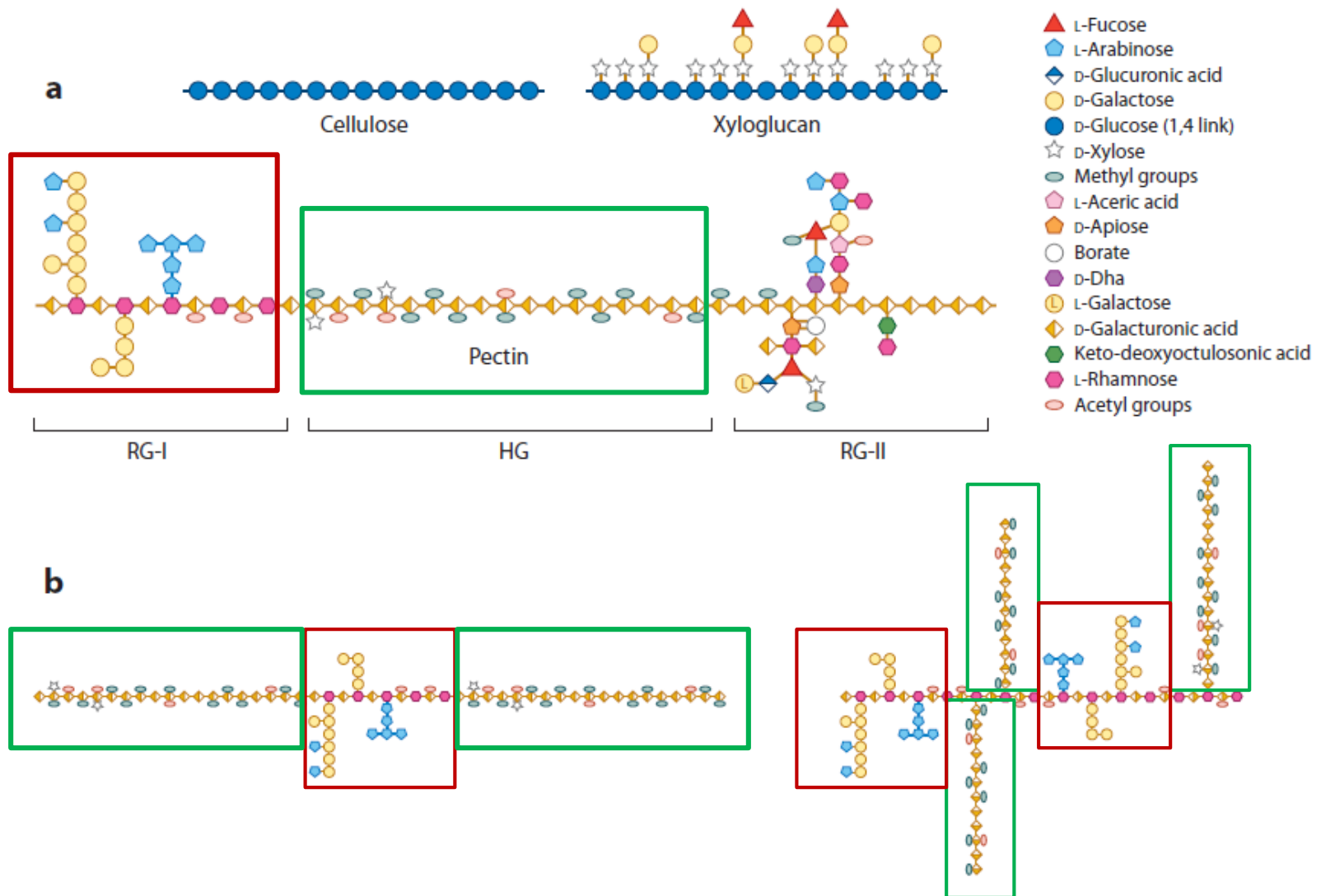
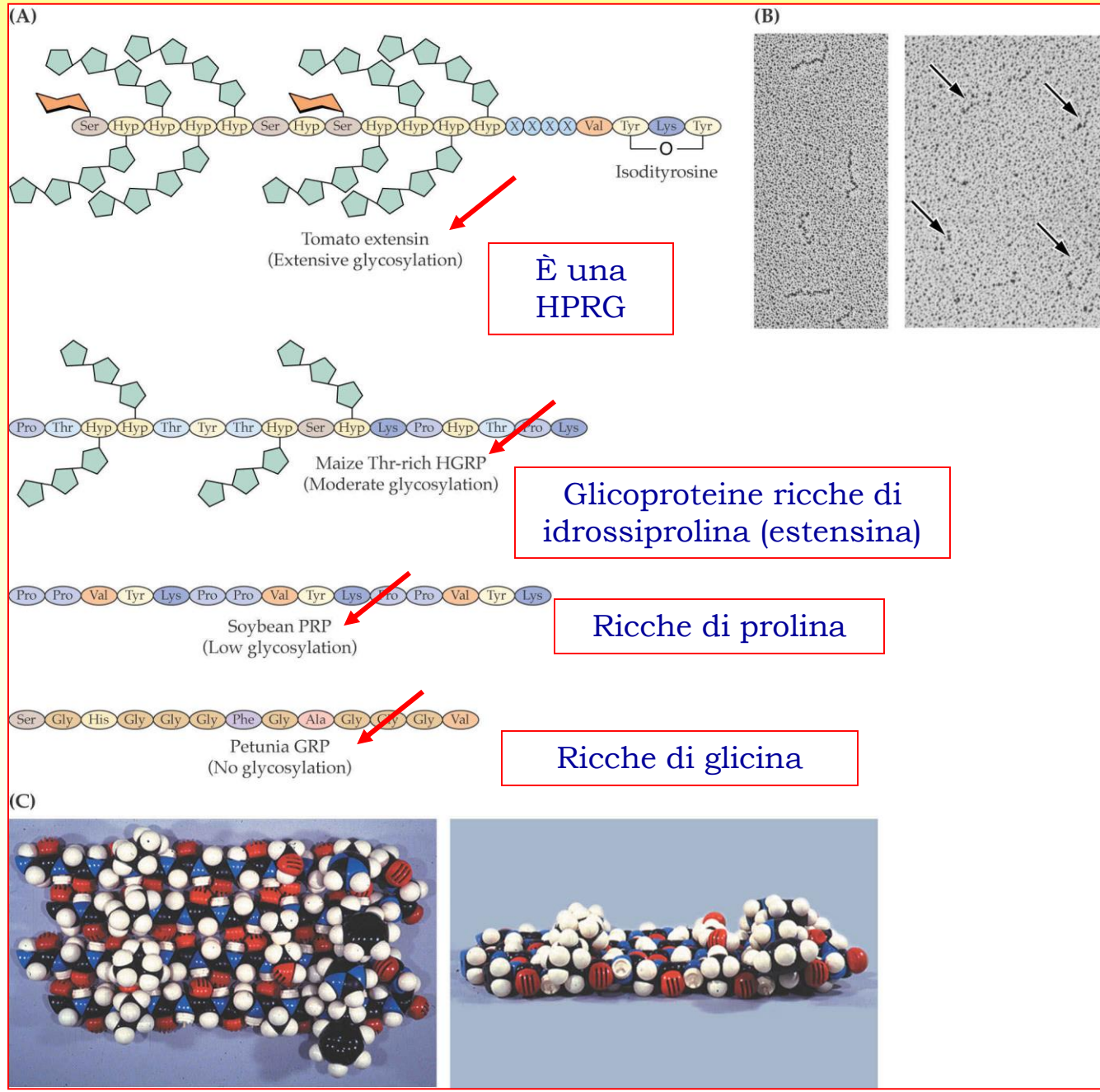


Figure 1

Structure of some cell wall components. (a) Schematic representation of cellulose, xyloglucan, and the pectic rhamnogalacturonan I (RG-I), homogalacturonan (HG), and RG-II. Adapted from Reference 22 with permission. (b) Alternative models for pectin domain organization. A linear, contiguous arrangement of HG interspersed with RG-I is shown on the left, whereas on the right, HG is drawn as side chains linked to Rha residues of the RG-I scaffold. A combination of both models may also be possible. Adapted from Reference 152 with permission.

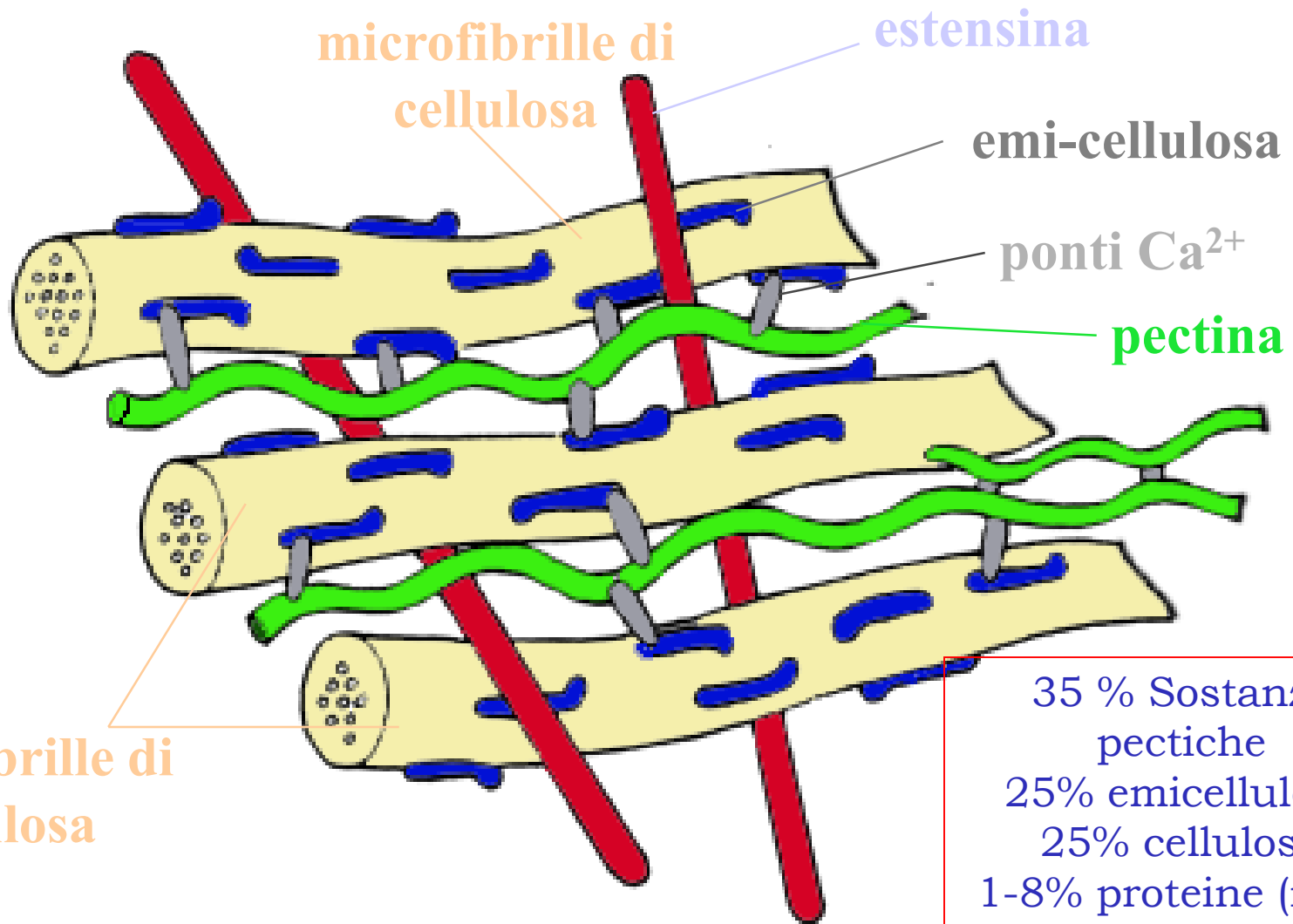
P
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HRGP (estensina), GRP e PRP

- Appena **secrete** sono **relativamente solubili**
- Diventano sempre più **insolubili** durante la maturazione cellulare o a seguito di **ferite** e attacco dei **patogeni**
- Si ritiene che il processo di **insolubilizzazione** sia dovuto a **legami intermolecolari** di difenilettere fra le **tirosine**
- Vengono **indotte** durante le ferite o attacco dei patogeni

Esempio Strutturale della parete primaria



microfibrille di
cellulosa

estensina

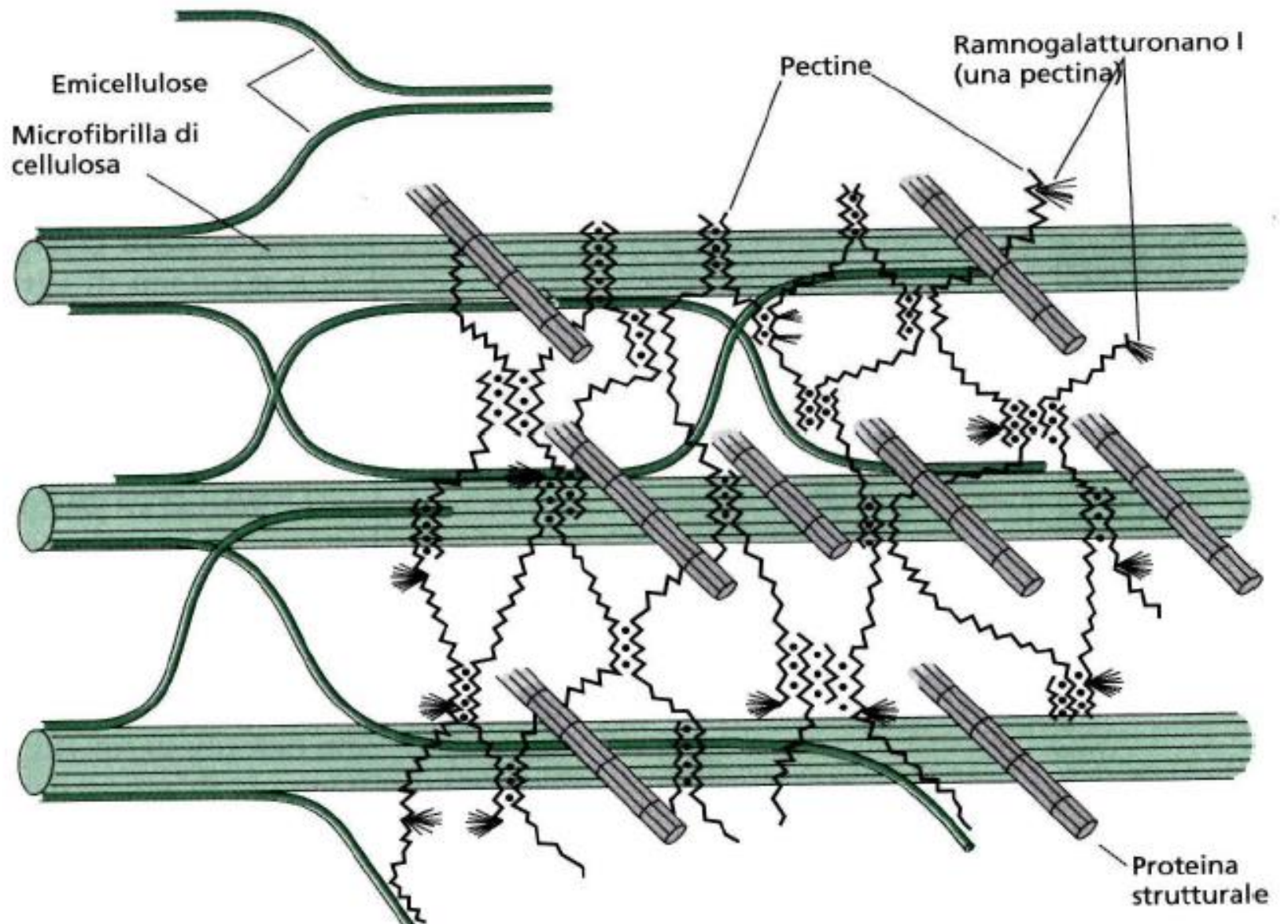
emi-cellulosa

ponti Ca^{2+}

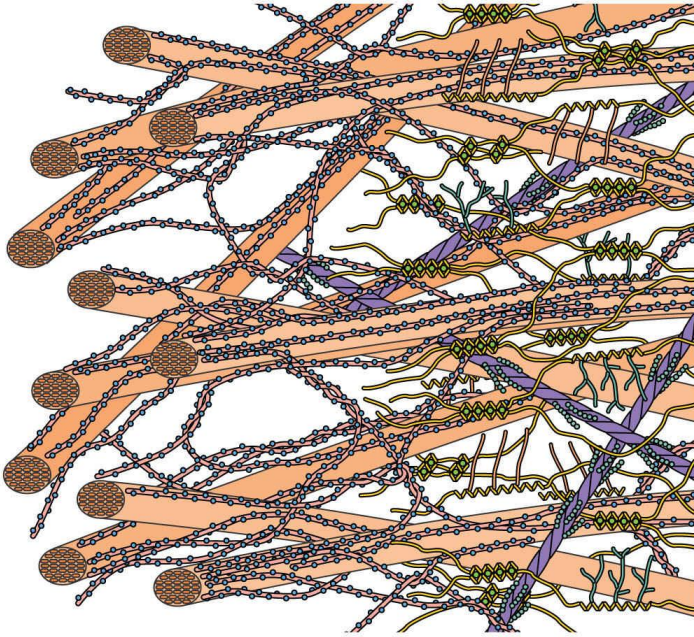
pectina

microfibrille di
cellulosa

35 % Sostanze
pectiche
25% emicellulose
25% cellulosa
1-8% proteine (nelle
dicotiledoni)

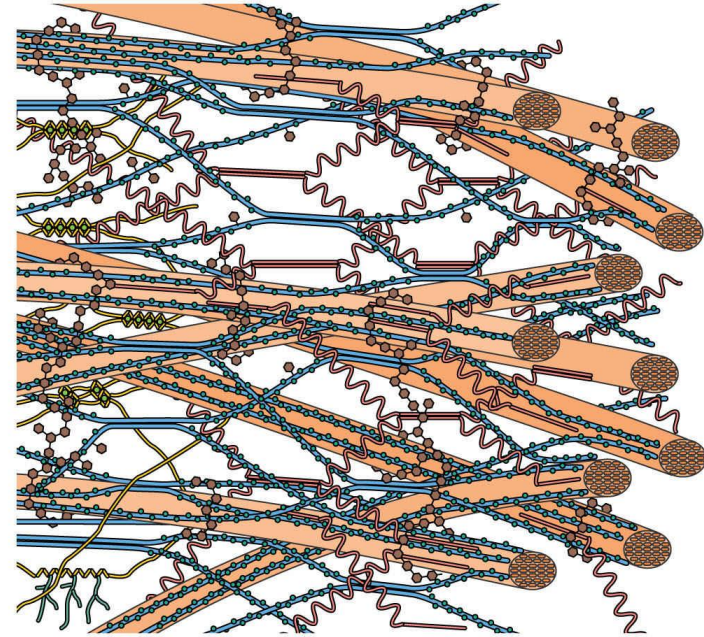


(A) Type I wall



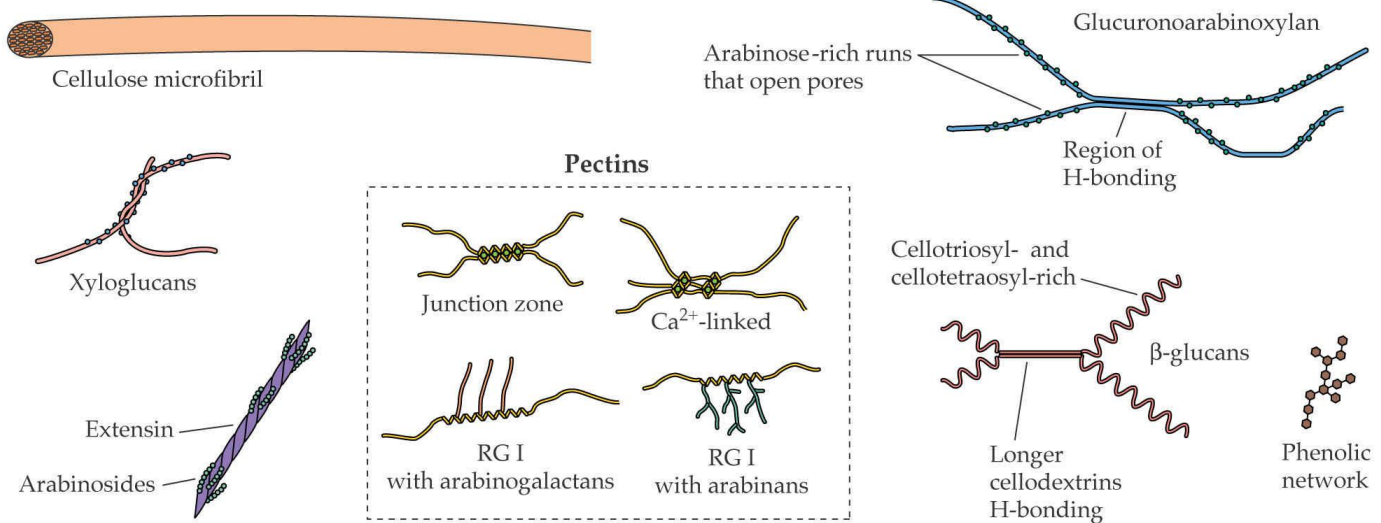
Tipo I

(B) Type II wall



Tipo II

Key:



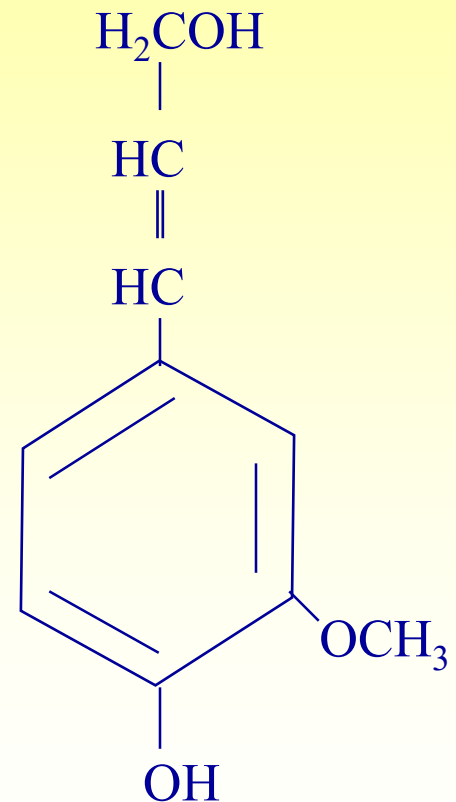
Componenti parete secondaria che conferiscono rigidità

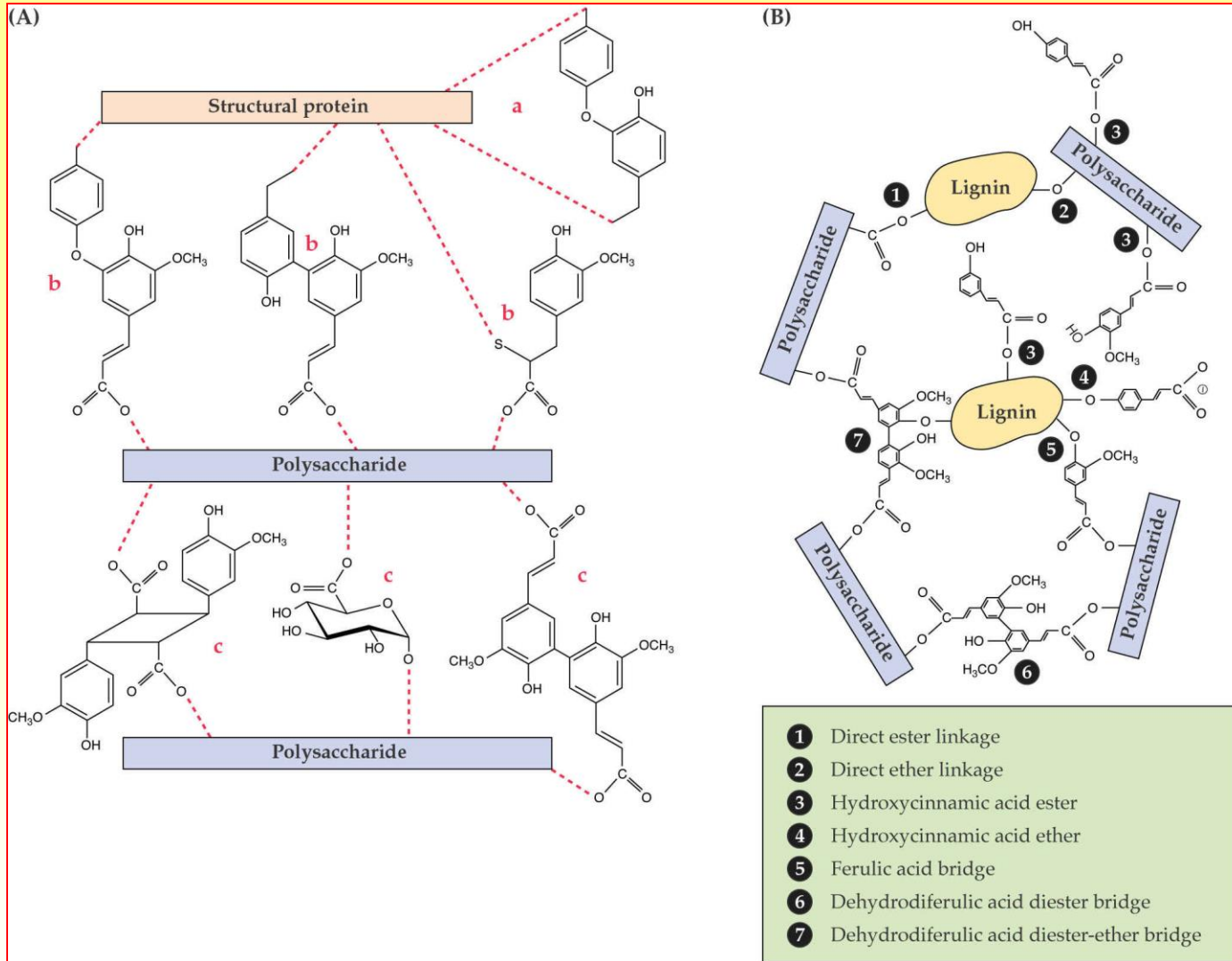
Lignina

- Macromolecole di monomeri di alcool aromatici (Fenilpropanoidi, alcoli idrossicinnamoilici ed i monolignoli –alcool *p*-cumarilico, coniferilico sinapilico)
 - alto peso molecolare
 - insolubile
 - impermeabile all'acqua
 - rigidità
- 25% del peso secco del legno

Fenilpropanoidi, alcoli idrossicinnamoilici ed i monolignoli –alcool *p*-cumarilico, coniferilico sinapilico maggiori componenti dei reticoli di lignina

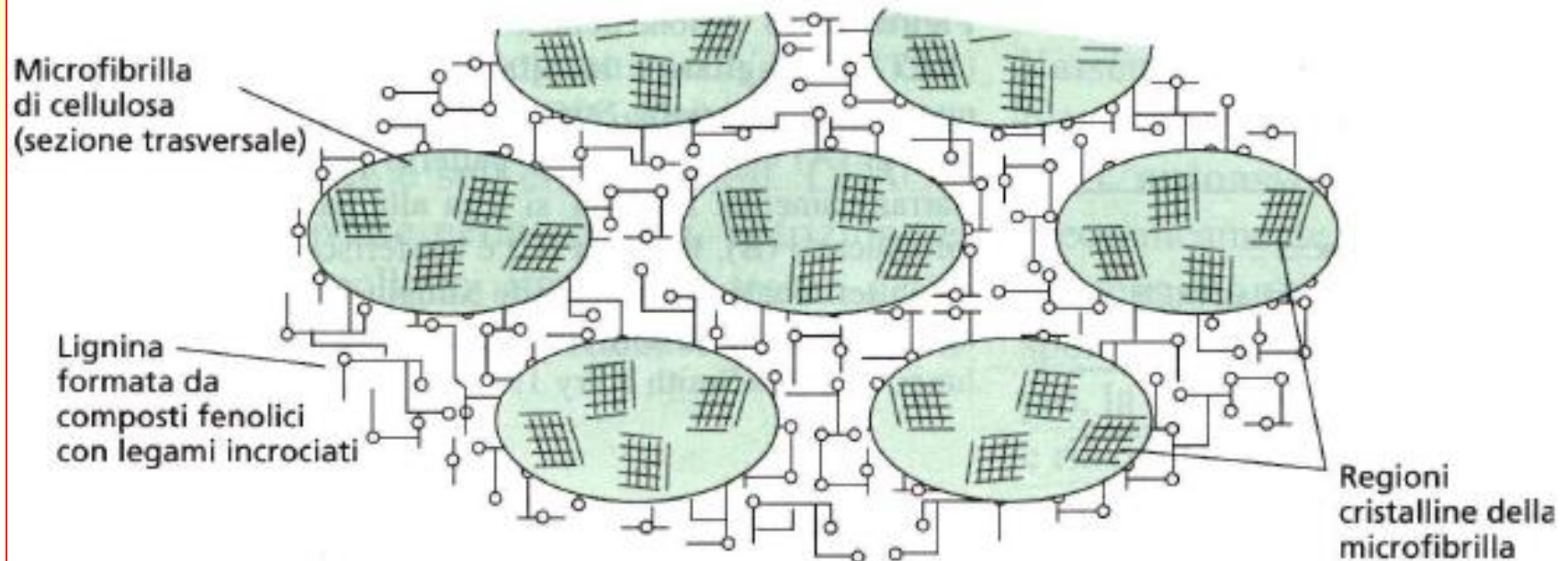
alcool coniferilico





Legami di interazione di polisaccaridi con la lignina

La sintesi di lignina **elimina H_2O** dalla parete e forma una **trama idrofobica** che lega la cellulosa e **previene l'espansione cellulare**

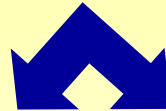


Parete cellulare e texture



Texture is one of the most prominent quality attributes to which cell walls contribute.

La presenza di una integrità strutturale della parete cellulare che gioca un ruolo nella percezione della tessitura



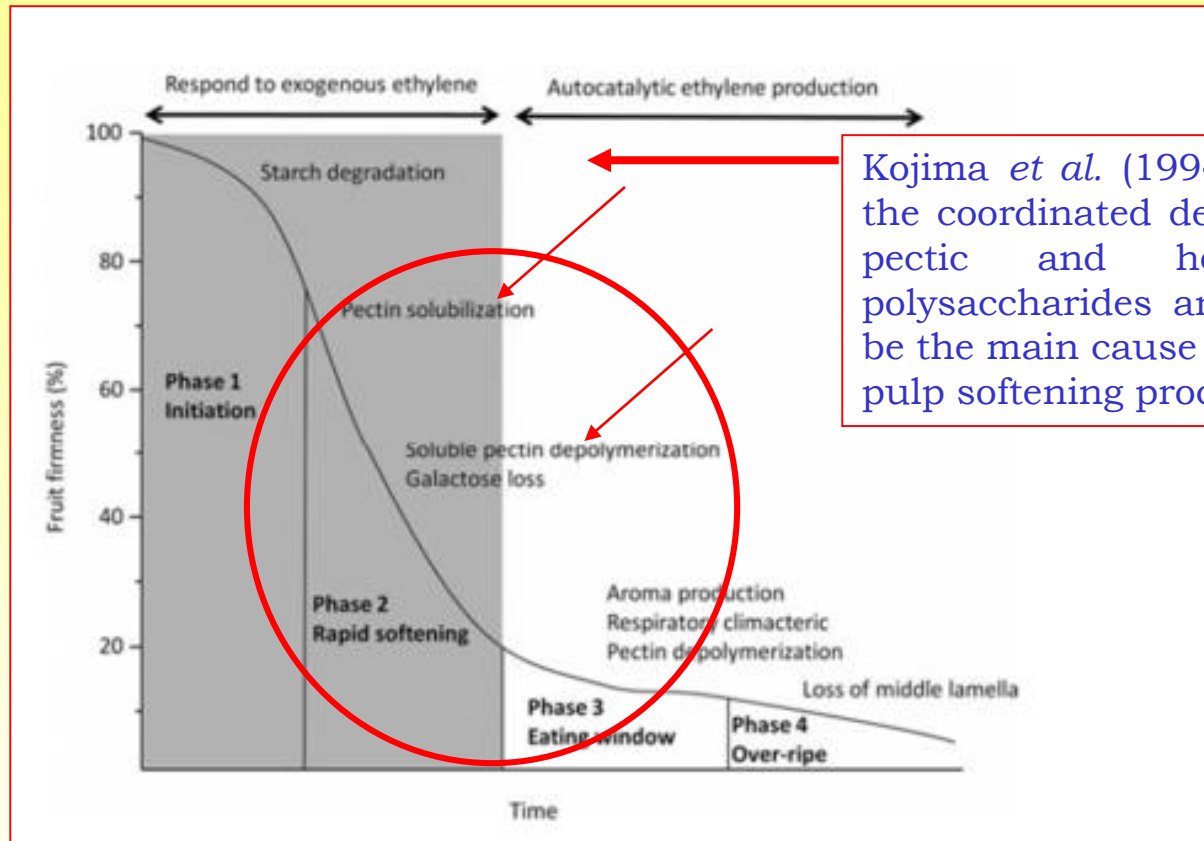
Adesione parete-parete tra cellule lamella mediana

Forza della parete primaria



“Softening” appare invariabilmente attribuibile al dissemblaggio del network di polisaccaridi della parete primaria e della lamella mediana (Brummel, 2006).

Turgore cellulare e morfologia contribuiscono all’aspetto della texture del frutto



Kojima *et al.* (1994) suggested the coordinated degradation of pectic and hemicellulosic polysaccharides and starch to be the main cause for the pulp softening process.

Figure 11.16. Schematic representation of postharvest ripening in kiwifruit, showing the timing of key physiological events. At harvest, fruit do not produce ethylene but are highly sensitive to exogenous ethylene. Softening is initiated (phase 1) and becomes rapid (phase 2). Relatively late in softening, compared with other fruit species, endogenous autocatalytic ethylene production begins, aroma volatiles are produced and fruit become soft enough to eat (phase 3). If fruit progress to the over-ripe stage (phase 4), they become unacceptably soft and exhibit 'off-flavour' notes. (Figure reproduced from Atkinson *et al.* (2011) *J. Exp. Bot.* 62, 3821-3835, with permission from the Society for Experimental Biology.)

1980
“dogma”

Correlazione del “softening” con la depolimerizzazione dei polisaccaridi mediato da idrolasi (enzimi) associate alla parete cellulare

Quelli che si ammorbidiscono notevolmente a “scioglimento” di texture (**Prugna, pomodoro e kiwi**)

Quelli che si ammorbidiscono solo moderatamente e conservano una texture friabile e fratturabile (**Mela e pera cv nashi**)

Bourne (1979) separò i frutti temperati in 2 gruppi

Nel primo gruppo l’adesione cellula-cellula è povera ed il tessuto si separa con la minima rottura;

Nel secondo gruppo è forte e dà un “sentire” di friabilità

Eventi che modulano il “softening” or “sensory texture” dei POF

- 1) dissoluzione della lamella mediana, riduzione quindi della adesione intercellulare, depolimerizzazione e solubilizzazione polisaccaridi delle emicellulose e delle pectine della parete cellulare e in alcuni casi un rigonfiamento della parete; (Brummel and Harpster,2001);
- 2) incremento della espressione di numerosi enzimi degradativi, quali idrolasi, transglicosilasi, liasi, ed altre proteine che allentano la struttura della parete come le espansine (Brummel, 2006).

Questi processi possono spiegare il “fruit ripening” (a natural and biochemically mediated process, often considered to be the initial stages of a form of senescence) e processi fisico-chimici (per es. termici) che possono influenzare la fase di postharvest storage.

In entrambe i casi, il softening è il risultato del **weakening** dell'adesione cell-cell.

Table 1. Major cell wall modifying enzymes in ripening fruits.

Cell wall enzyme	Function	Activity during ripening
Cellulase (EGase) EC 3.2.1.4	Hydrolyse β -1,4 glucan linkages in cellulose and xyloglucan	Decreases
Xyloglucan-endotransglycosylase EC 2.4.1.207	Hydrolyse and/or transglycosylate xyloglucan	Decreases
Glycosidases (i.e., β -galactosidase EC 3.2.1.23)	Terminal removal of galactosyl residues from pectin and xyloglucan	Increases
Endo-polygalacturonase EC 3.2.1.15	Hydrolytic cleavage of α -1,4-galacturonosyl linkages in unesterified pectin	Increases
Pectate lyase EC 4.2.2.2.	Cleavage of de-esterified pectin	Increases
Pectin methyl esterase EC 3.1.1.11	Removal of methyl groups from esterified pectin	Increases
Rhamnogalacturonase A	Hydrolyse α -1,2 linkages between galacturonosyl and rhamnosyl residues in pectin	Not measured

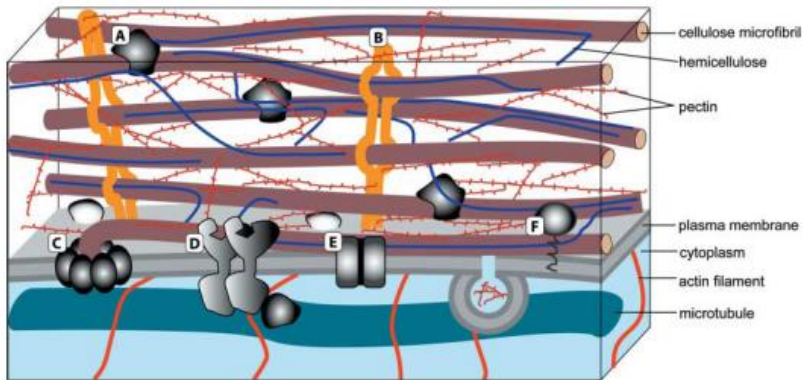
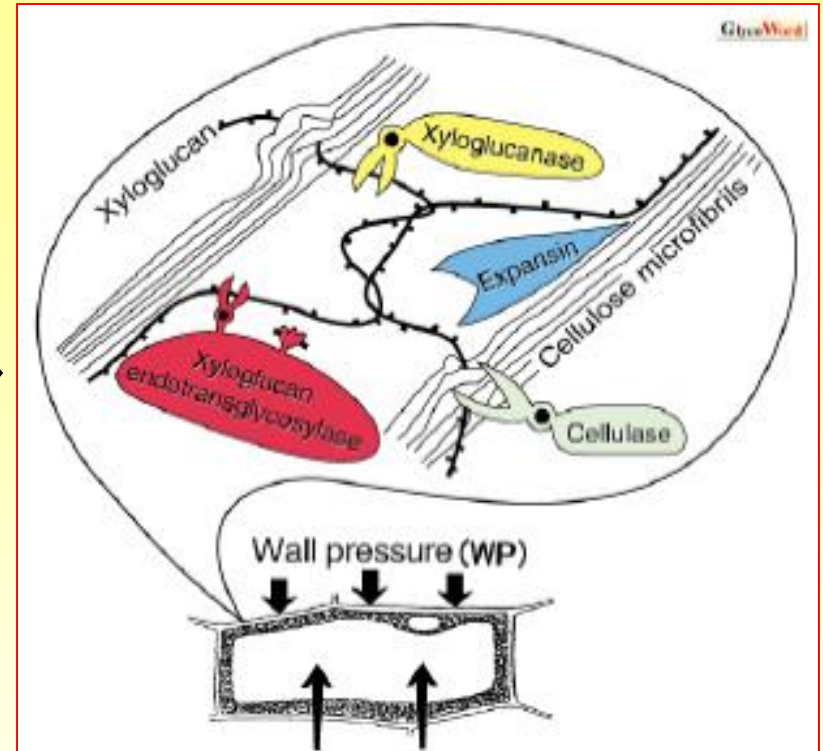
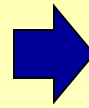
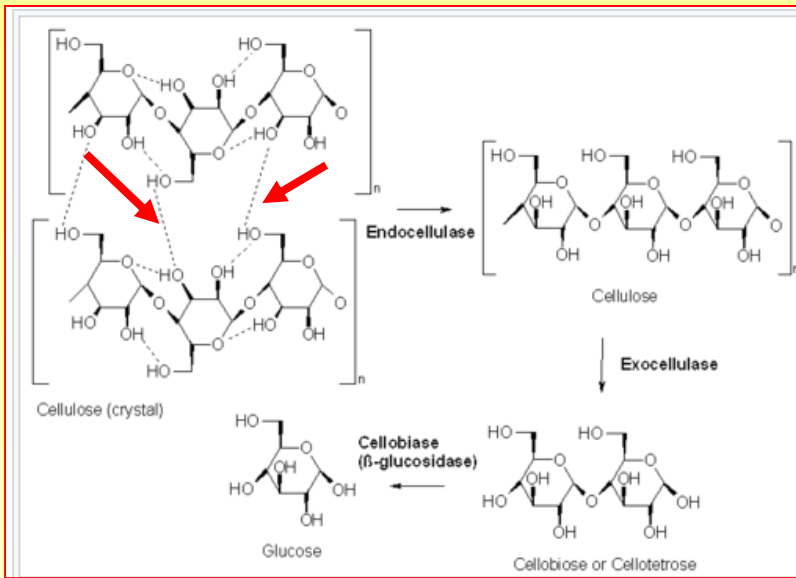


Figura 4 Composizione della parete cellulare tratta dalla review di Humphrey et al., 2007.
 A) Estensina; B) Espansina; C) Complesso della cellulosa sintasi; D) Recettore chinasi;
 E) Canali ionici F) Glicosilfosfatidilinositolo.



Una volta che il processo di softening è cominciato, la velocità dei cambiamenti in texture sono in funzione del tipo di frutto e delle condizioni con cui il prodotto viene maneggiato.

Il softening dei frutti una volta iniziato è un processo irreversibile.



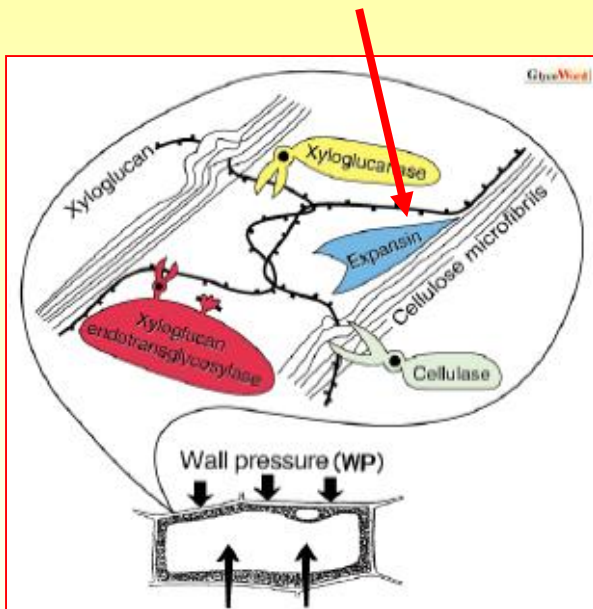
Cellulase often referred to as **EGase** (Endo (1→4) β-D glucanase) hydrolyzes internal linkages of (1→4) β-D-linked glucan chains.

EGase activity has been found in fruits of all species

Suppression or overexpression of endo- (1→4) β-D-glucanase activity had no detectable effect on fruit softening or the depolymerization of matrix glycans, and neither the substrate nor the function for this enzyme has been determined (Brummel and Harpster, 2001).

The localization of EGase in the regions of the fruit associated with abscission zones suggests the involvement of the enzyme in fruit separation as well as softening (Sexton *et al.*, 1997).

Expansins are proteins with no apparent hydrolytic enzymatic activity (Cosgrove, 2000).



Expansins appear to operate by disrupting hydrogen bonds between cellulose microfibrils and xyloglucans that bind them to one another in plant cell walls (Whitney *et al.*, 2000).

Expansins have been shown to play an important role in fruit softening (Rose *et al.*, 1997; Anjanasree and Bansal, 2003).

The ripening associated expansins might contribute to cell wall degradation by increasing the accessibility of other cell wall-modifying proteins, such as PG and cellulase to structurally important cell wall polymers (Rose and Bennett, 1999).

Expansin protein is directly correlated with fruit softening and has additional indirect effects on pectin depolymerization, showing that this protein is intimately involved in the softening process. However, the molecular mechanism by which expansins loosen cell walls has not yet been worked out on molecular terms (Neela *et al.*, 2006).

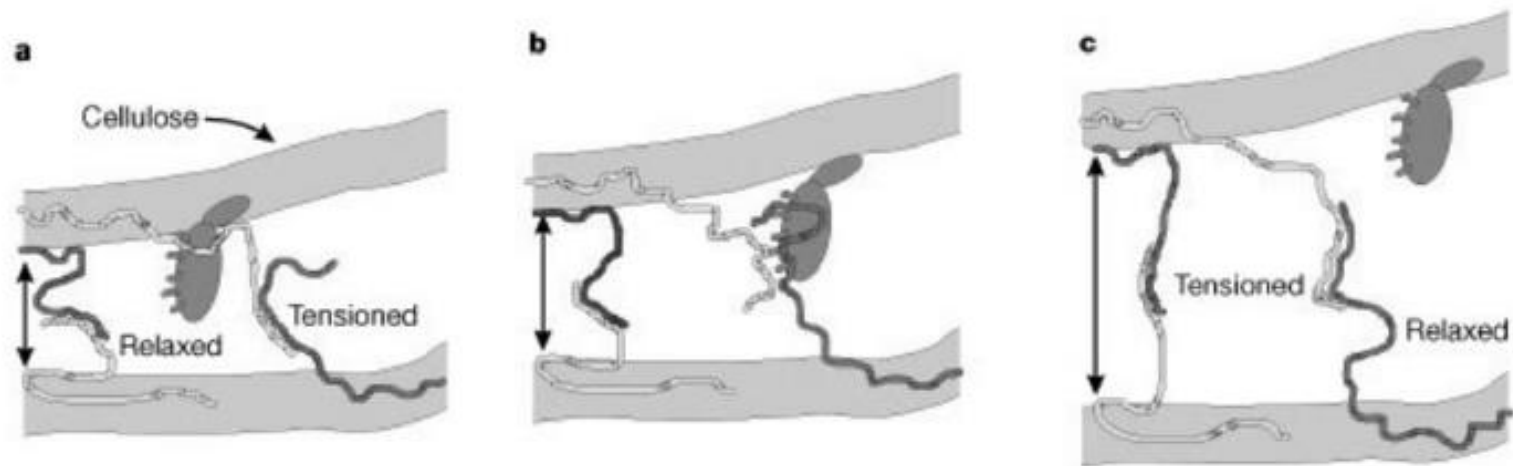
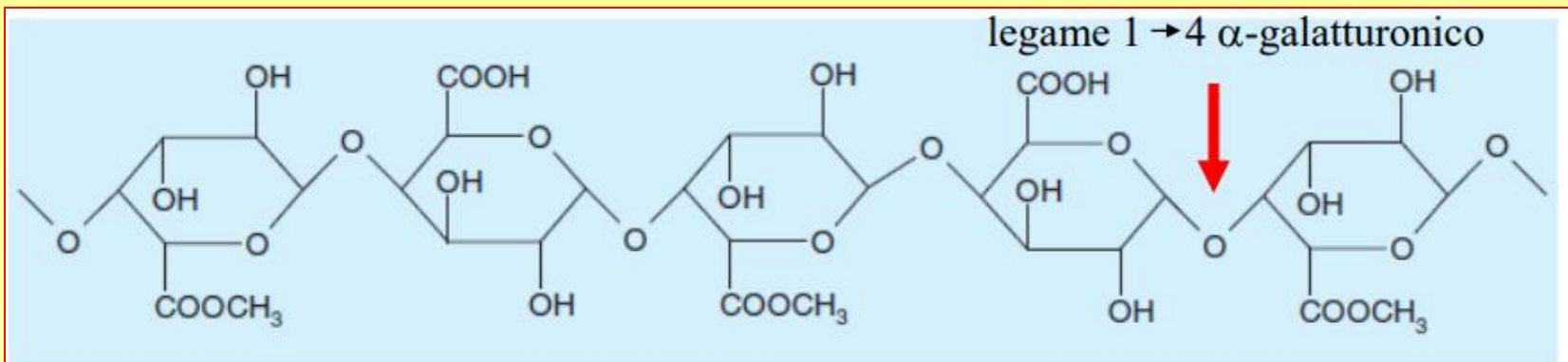


Fig. 1. Cellulose microfibrils are connected to each other by glycans (strands) that can stick to the microfibril surface and to each other. The expansin protein is hypothesized to disrupt the bonding of the glycans to the microfibril surface (a) or to each other (b). Under the mechanical stress arising from turgor, expansin action results in a displacement of the wall polymers (c) and slippage in the points of polymer adhesion (compare a and c) (Cosgrove, 2000).

These wall-degrading enzymes detected during ripening of various fruits including :

- 4) **cellulases,**
- 5) **xyloglucan endotransglycosylase (XET),**
- 6) **expansins.**

are unusual in that they exhibit no endo or exohydrolase activity. They are thought to act by disrupting hydrogen bonds between wall proteins (McQueen-Mason and Cosgrove 1994), possibly at the interface between cellulose microfibrils and matrix polysaccharides. It seems a reasonable proposition that textural changes in fruit will involve the activity of 1 or more wall-modifying enzymes. However, the exact mechanism is not completely clear.



struttura della pectina

Le **pectinasi**, o **pectina depolimerasi**, sono un complesso gruppo di enzimi coinvolti nella degradazione delle sostanze pectiche. La presenza di varie forme di questi enzimi è spiegata dal fatto che nelle cellule vegetali esistono diverse forme di sostanze pectiche.

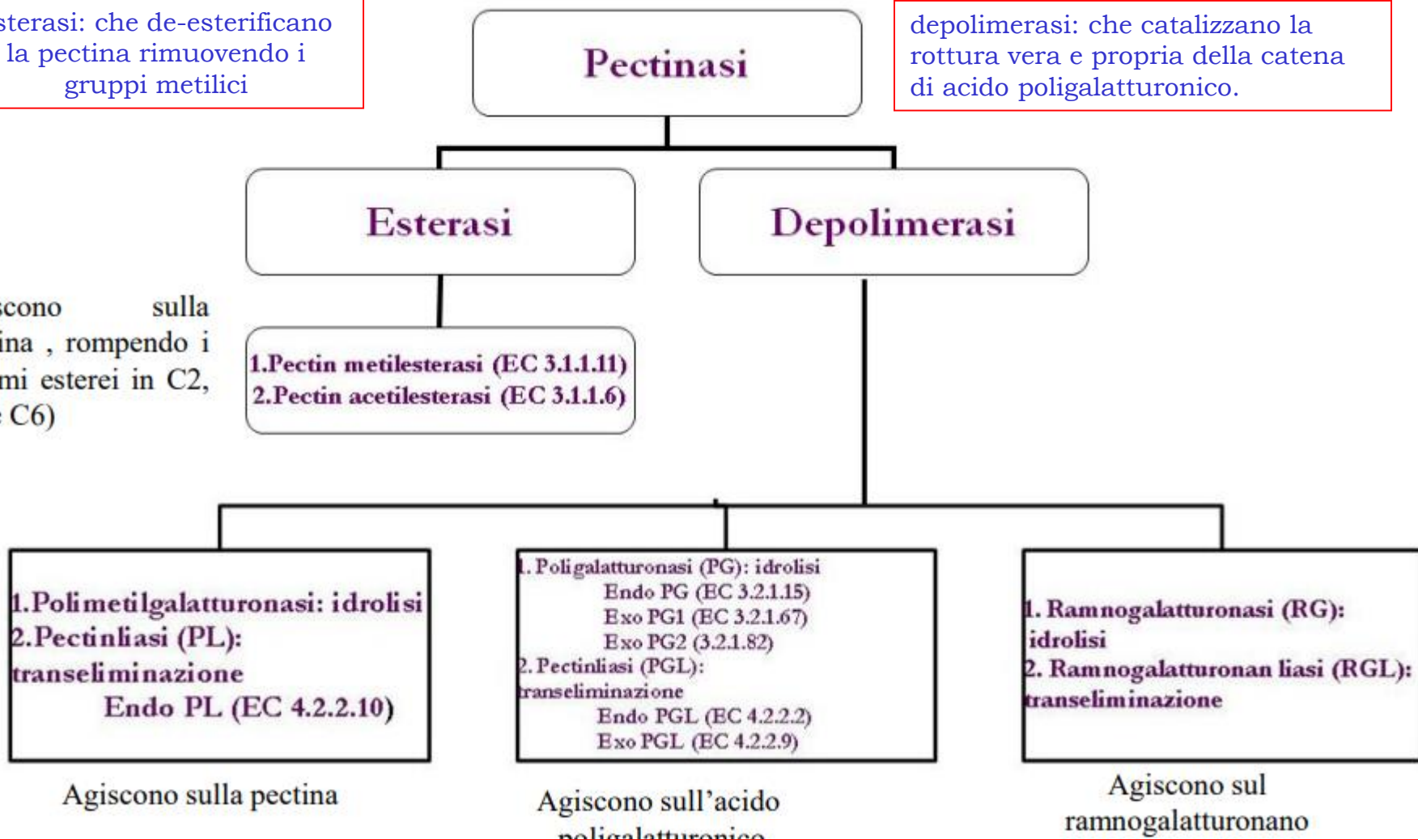
Le **pectinasi catalizzano due tipi di reazioni:**

- i) idrolisi, prendono il nome di **idrolasi**
- i) trans-eliminazione, prendono il nome di **liasi**

esterasi: che de-esterificano la pectina rimuovendo i gruppi metilici

depolimerasi: che catalizzano la rottura vera e propria della catena di acido poligalatturonico.

Agiscono sulla pectina, rompendo i legami esterei in C2, C3 e C6)



- 1) **pectin methyl esterase (PME),**
- 2) **pectin liase (PL),**
- 3) **endopolygalacturonase (endo-PG),**
- 4) **β -galactosidase.**

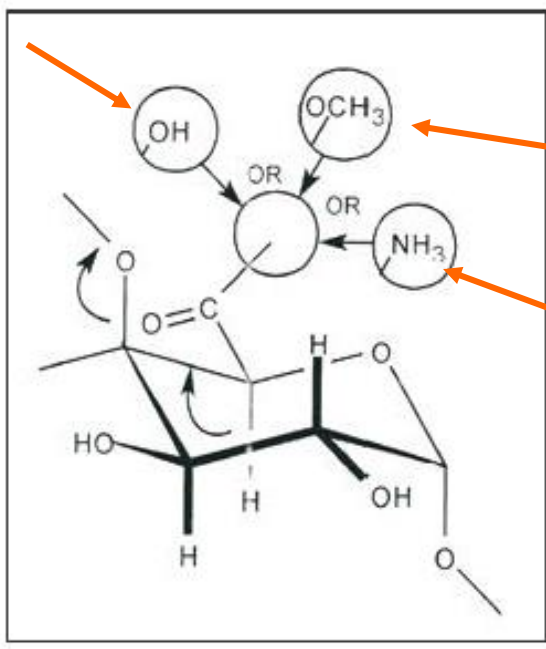
• **PG activity** is the main enzyme responsible for the depolymerisation and associated solubilization of pectic polysaccharides. Its activity requires de-esterification of pectic polymers by **PME**. Suppression of PG reduces fruit softening only slightly (but shelf life is extended).

• **PL** otherwise known as pectate transeliminases, catalyse the eliminative cleavage of de-esterified pectin, which is a major component of the primary cell walls of many higher plants (Carpita and Gibeaut, 1993)

• A **β -subunit protein of PG** limits pectin solubilization. Suppression of its accumulation increases the rate of softening by facilitating cell separation. Reducing accumulation of either PG or PME increases viscosity of tomato paste, probably through reducing depolymerisation of pectins during processing.

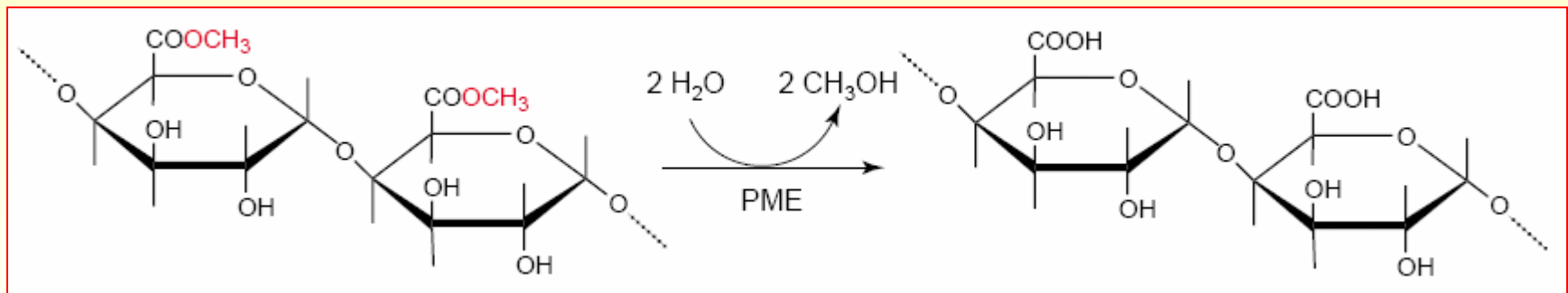
• **β -galactosidase activity**, if suppressed early in ripening, significantly reduces fruit softening, indicating that the removal of pectic galactan side chains is of significance in the ripening process.

PectinMetilEtilesterasi



L'acido galatturonico può presentare sulla funzione carbossilica un gruppo alcool o un metile.

Il gruppo ammidico è presente solo nelle pectine utilizzate a scopi alimentari.



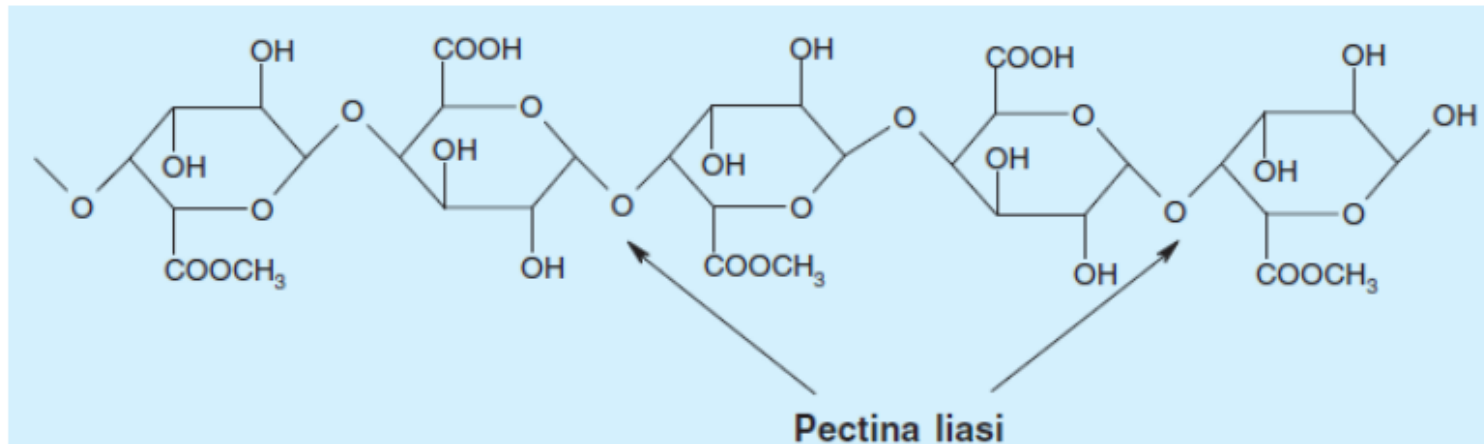
Pectina

Pectato

La pectina viene demetilata dall'enzima (PME)

Pectin liasi (PL)

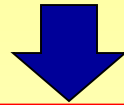
Le **PL** agiscono attraverso un meccanismo di β -eliminazione, con aggiunta di un doppio legame tra il C4 e il C5 dell'acido galatturonico.



Two *pel* genes have been reported from strawberry which are strongly and predominantly expressed in the full ripe stage (Benitez-Burraco *et al.*, 2003). *Pel* gene is an excellent candidate for biotechnological improvement of fruit softening in strawberry.

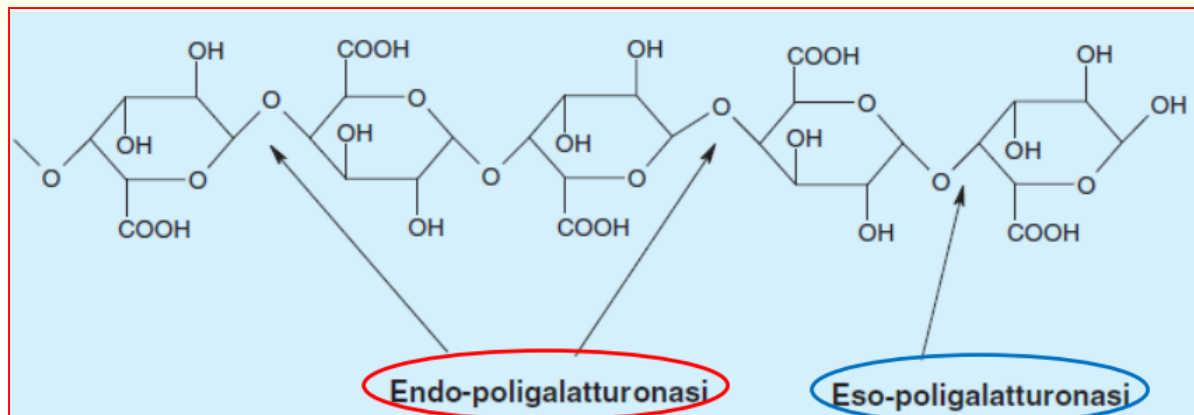
PL has been extensively studied in pathogenic bacteria, which secreted this enzyme causing depolymerization of pectins in the middle lamella and primary cell walls of higher plants, and consequently the maceration of plant tissues (Henrissat *et al.*, 1995).

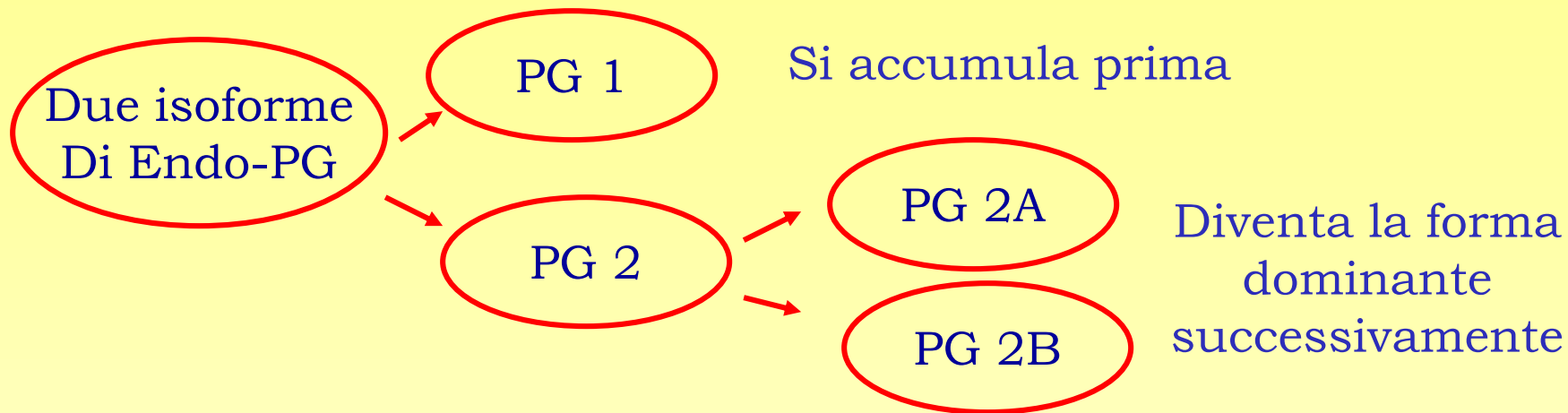
Poligalatturonasi (PG): la reazione di taglio del poligalatturonato avviene per idrolisi dei legami glicosidici all'interno del polimero e produce oligomeri. Vengono distinte in **eso- ed endo- poligalatturonasi** secondo l'azione di degradazione della pectina presente nella parete vegetale



Le *eso-poligalatturonasi* scindono la pectina di omogalatturonani liberando unità di- e mono- galatturonato a partire dall'estremità della catena;

Le *endo-poligalatturonasi* degradano la pectina scindendo anche i legami fra una unità e l'altra di acido galatturonico che si trovano all'interno della catena.



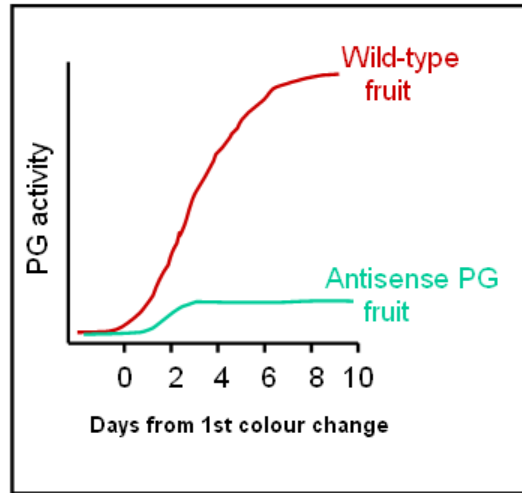


Le PG si presentano in più forme isoenzimatiche che differiscono in termini di stabilità, di attività, optimum di pH, nella preferenza del substrato e nel tipo di oligalatturonidi rilasciati (De Lorenzo et al, 1997).

PG apparently plays a minor role in strawberry softening because little or no activity was found in fruits (Abeles and Takeda, 1990). The level of PG enzyme activity and polyuronide degradation have been correlated roughly with an elevated rate of tomato fruit softening, and on this basis PG was proposed to be a major determinant of tomato fruit softening.

Altering Fruit Ripening with Antisense RNA

Polygalacturonase (PG) is an enzyme that breaks down pectin in ripening fruit walls



Plants with an antisense PG transgene produce less PG. Walls soften more slowly

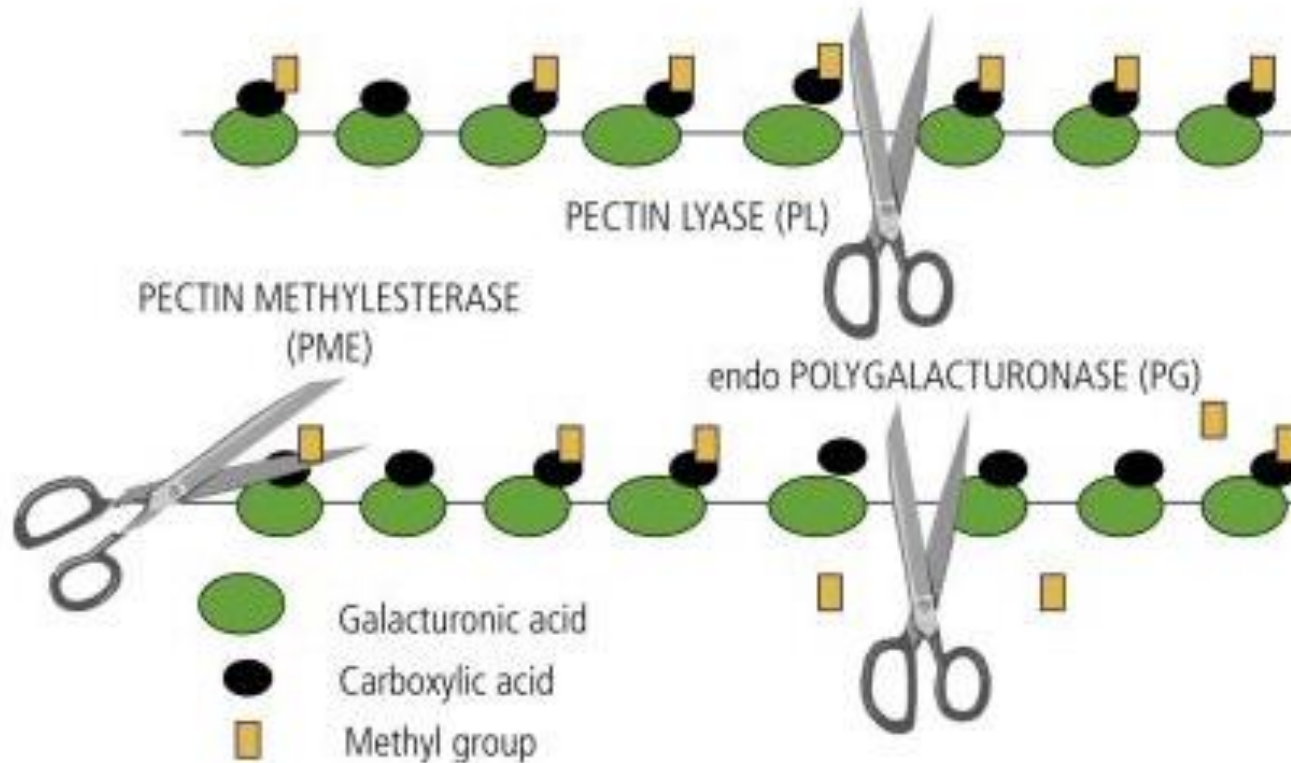
Many genes manipulated in the same way to answer basic questions:

- what is the role of hormones in ripening?
- what do particular enzymes do in fruit walls?

Giovannoni *et al.* (1989) demonstrated that high levels of PG enzyme activity and polyuronide degradation are not sufficient to induce softening in transgenic r/n (E8/PG)-2 fruit.

Analisi del softening in piante transgeniche ha riaperto il dibattito sull'importanza relativa delle PGA. In piante di pomodoro transgeniche nelle quali è stato virtualmente eliminato il livello di sintesi della PGA (99%), il softening del frutto non era significativamente differente dal normale frutto non trasformato. Allo stesso modo, quando il livello della PGA viene sovra espressa nel frutto che ne è invece carente, il softening risulta non significativamente modificato.

Mode of action of the main pectolytic enzymes



Tra le due isoforme, l'endopoligalatturonasi è molto più importante, nel senso che rompe la molecola di pectina verso il centro, producendo due molecole di circa la metà delle dimensioni originali e aumentando notevolmente la loro solubilità. La rimozione delle subunità terminali operata dalla esopoligalatturonasi, provoca tuttavia, solo una minima alterazione delle proprietà della molecola di pectina.

β -Galactosidase removes terminal non-reducing β -Dgalactosyl residue from β -D-galactoside.

Seven tomato β -galactosidase (TBG) genes are expressed during fruit development (Smith and Gross, 2000), six are known to be expressed during ripening.

β -Galactosidase proteins have also been purified and characterized from a number of fruits including kiwifruit (Ross *et al.*, 1993), coffee (Golden *et al.*, 1993), persimmon (Kang *et al.*, 1994), and apple (Ross *et al.*, 1994).

Suppression of β -galactosidase activity early in ripening significantly reduces fruit softening, suggesting that the removal of pectic galactan side-chains is an important factor in the cell wall changes leading to ripening-related firmness loss (Brummel and Harpster, 2001).

Down-regulation of tomato β -Galactosidase results in decreased fruit softening (Smith *et al.*, 2002).

Depolimerasi che agiscono sul ramnogalatturonano

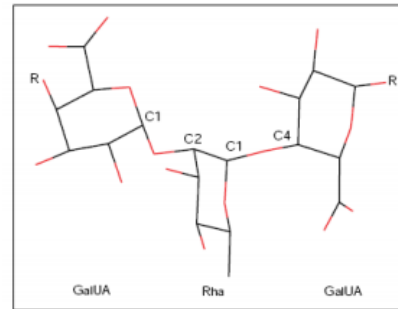
Le ramnogalatturonasi (RG)-idrolisi, sono attive sulle catene di ramnogalatturonani, si dividono in:

Endo-ramnogalatturonasi A (RGase A) idrolizza i legami α -1,2 tra ramnosio e acido galatturonico, provocando una inversione della configurazione anomerica.

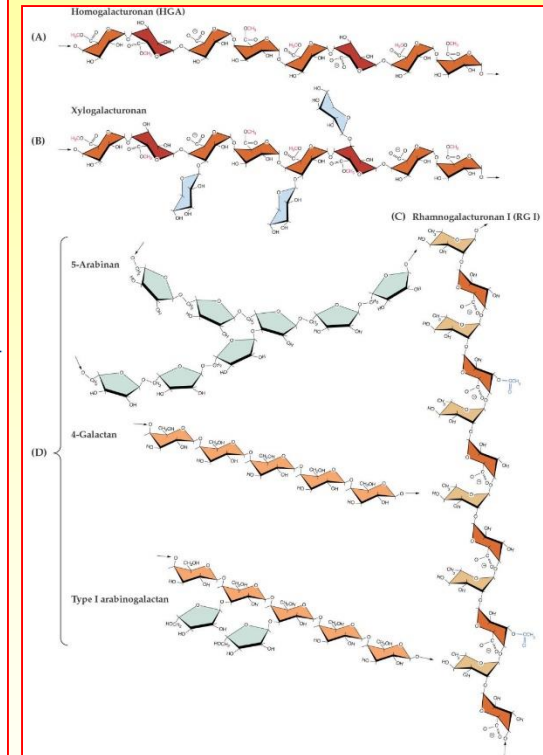
Endo-ramnogalatturonasi B (RGase B) idrolizza i legami α -1,4

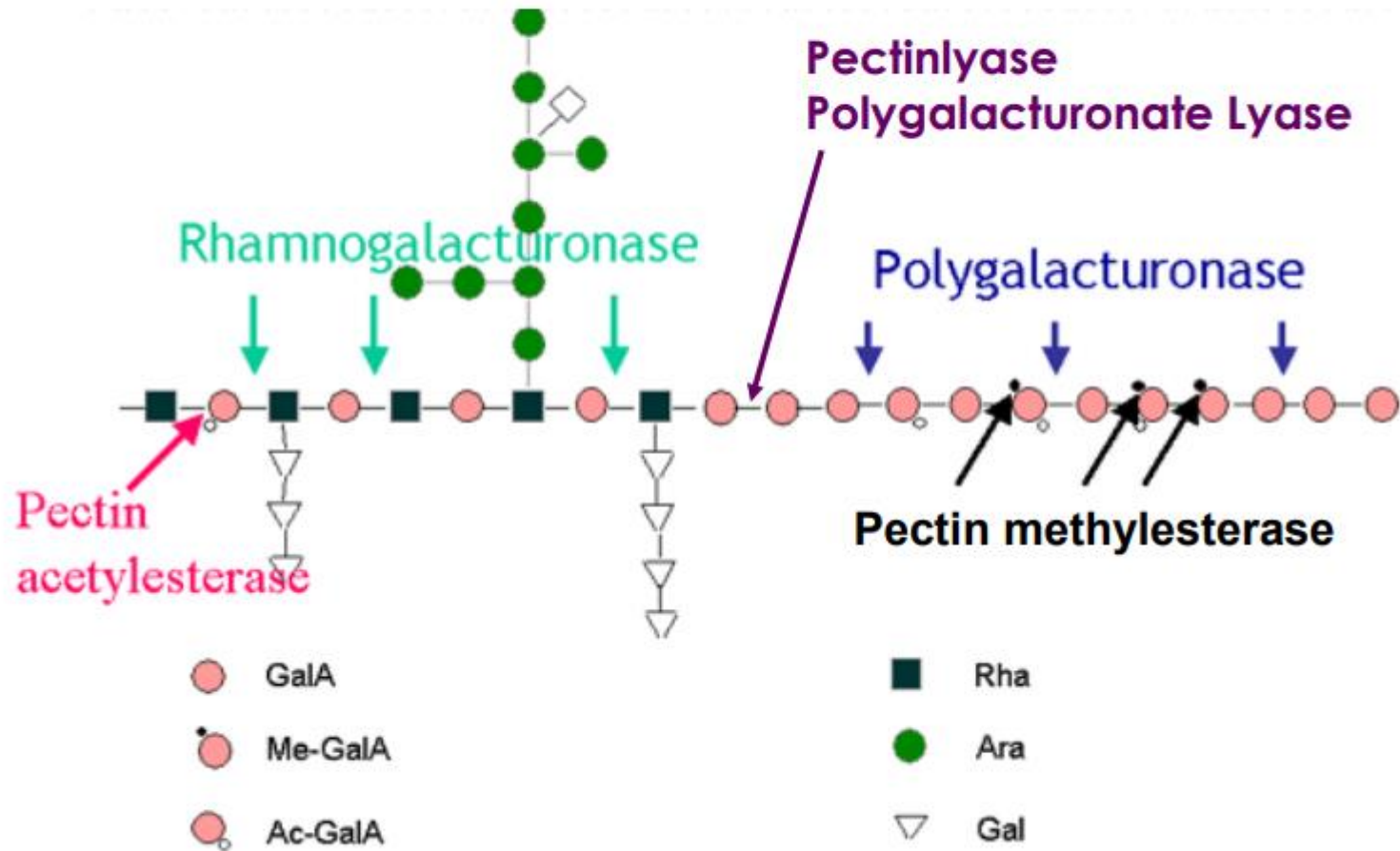
La presenza dei gruppi acetile in posizione C-2 o C-3 del galatturonato ostacola la degradazione del substrato da parte di RGase A o RGase B.

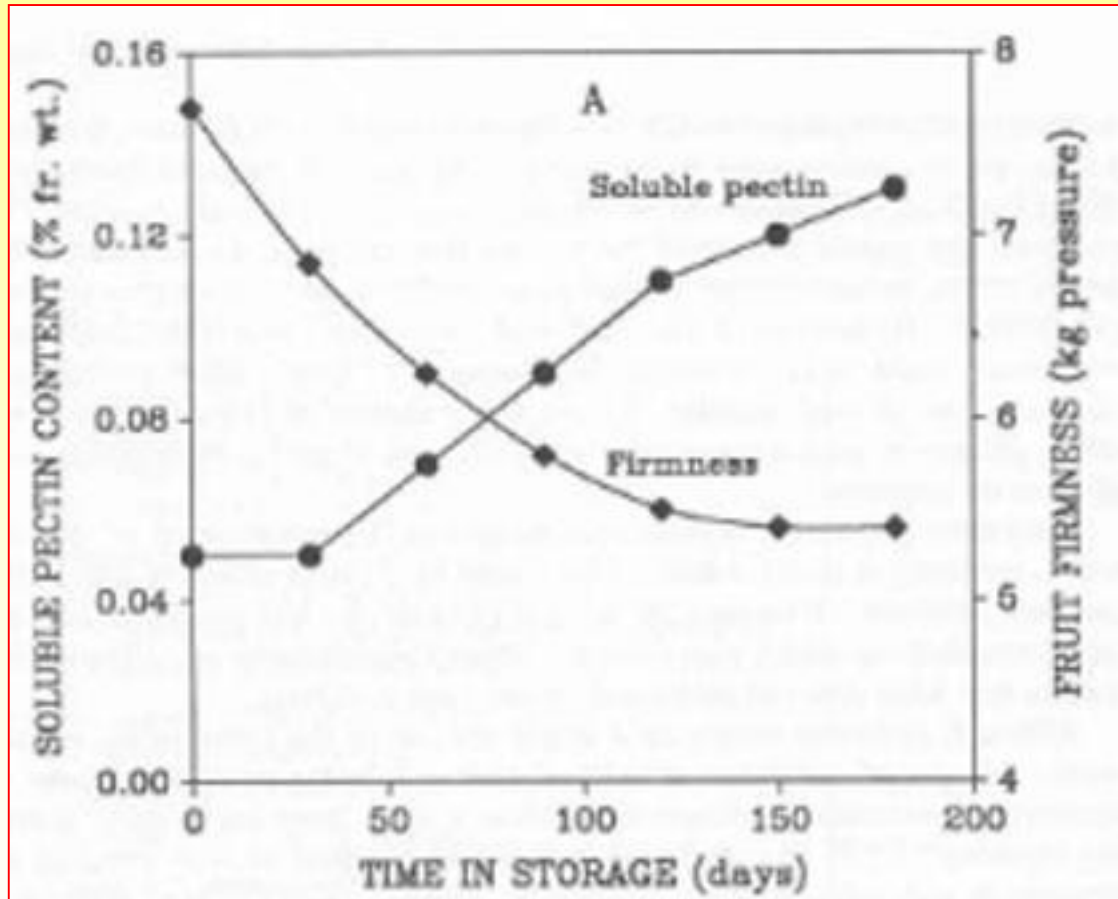
È quindi essenziale che i gruppi acetilici siano rimossi perché si verifichi l'idrolisi. La deacetilazione è catalizzata dall'enzima ramnogalatturonano acetilesterasi (RGAE)



α -1,2-L-Rha- α -1,4-D-GalUA
[Rha \rightarrow rhamnose
GalUA \rightarrow galacturonic acid]







mela

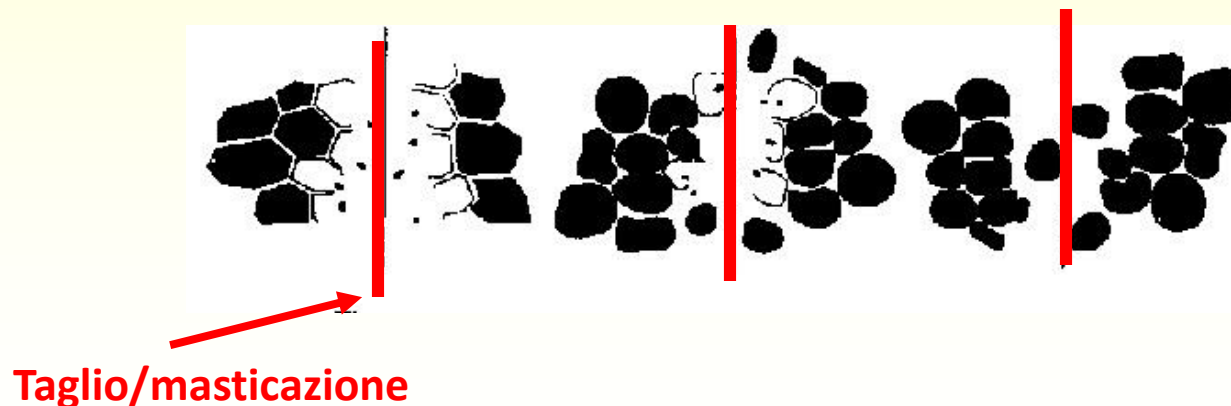
Intenerimento

- modificazioni degradative della cellulosa, emicellulosa, pectine e proteine che costituiscono la parete cellulare e la lamella mediana, ad opera di enzimi (poligalatturonasi, cellulasi, espansine etc.);
- idrolisi dei carboidrati di riserva (amido);
- aumento spazi intercellulari;
- diminuzione del turgore cellulare.

croccante-morbido

non farinoso

farinoso



The texture of ripe fruit depends on more than a simple understanding of cell adhesion. There are many structural factors that contribute to the final organoleptic texture.

For example, the component tissues within a fruit will often exhibit **different mechanical properties**, and these will be affected to varying degrees by the ripening process.

At the level of the cell, **the shape and orientation of parenchyma** (and other) cells and **intercellular spaces** can influence tissue-fracture properties. As a result, the perceived textural characteristics may be influenced by the angle at which a fruit tissue is bitten; for example, in apples (Khan and Vincent 1993).

In addition, separation of parenchyma cells may be modulated by modes of attachment throughout the wall; for example, by **plasmodesmata**. These can contribute toward the maintenance of adhesion at pit.

Similarly, **lignification** of cells such as sclereids may retard separation of adjacent parenchyma cells (Martin-Cabrejas and others 1994), preventing these areas from softening.

A Reevaluation of the Key Factors That Influence Tomato Fruit Softening and Integrity. Saladié et al., Plant Phys., 2007

The softening of fleshy fruits, such as tomato (*Solanum lycopersicum*), during ripening is generally reported to result principally from disassembly of the primary cell wall and middle lamella.

However, unsuccessful attempts to prolong fruit firmness by suppressing the expression of a range of wall-modifying proteins in transgenic tomato fruits do not support such a simple model. **'Delayed Fruit Deterioration'** (DFD) is a tomato cultivar that provides a unique opportunity to assess the contribution of wall metabolism to fruit firmness, since DFD fruits exhibit minimal softening but undergo otherwise normal ripening, unlike all known nonsoftening tomato mutants reported to date. Wall disassembly, reduced intercellular adhesion, and the expression of genes associated with wall degradation were similar in DFD fruit and those of the normally softening **'Ailsa Craig'**. However, ripening DFD fruit showed minimal transpirational water loss and substantially elevated cellular turgor. This allowed an evaluation of the relative contribution and timing of wall disassembly and water loss to fruit softening, which suggested that both processes have a critical influence. Biochemical and biomechanical analyses identified several unusual features of DFD cuticles and the data indicate that, as with wall metabolism, changes in cuticle composition and architecture are an integral and regulated part of the ripening program. A model is proposed in which the cuticle affects the softening of intact tomato fruit both directly, by providing a physical support, and indirectly, by regulating water status.



AC
(Ailsa Craig)
Normally
softening



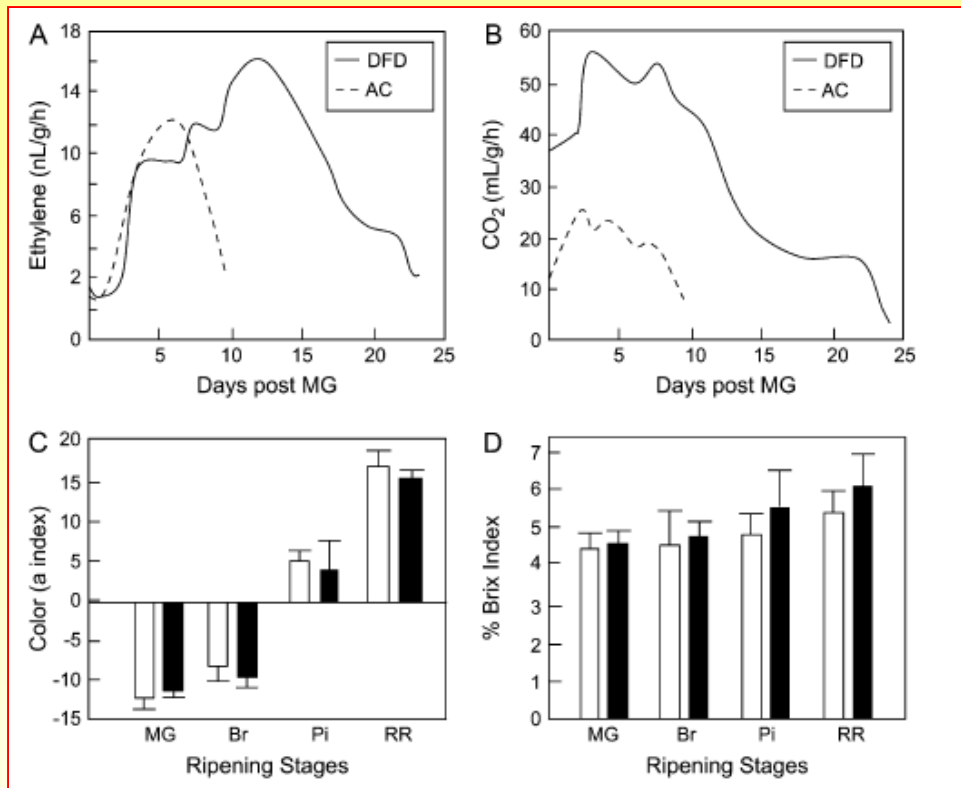
DFD
Delayed Fruit
Deterioration



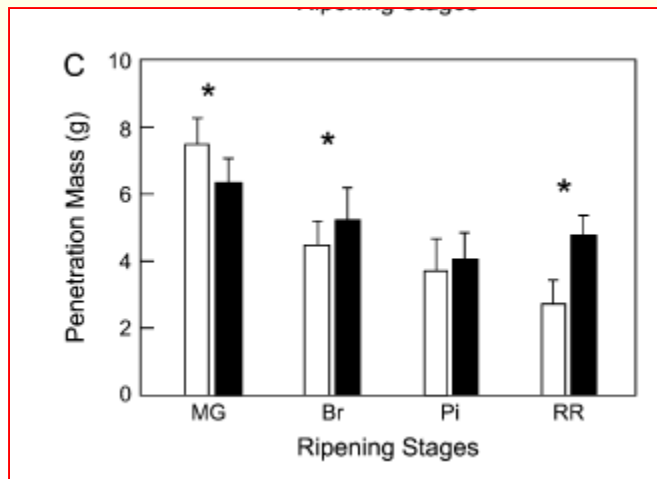
AC
(Ailsa Craig)



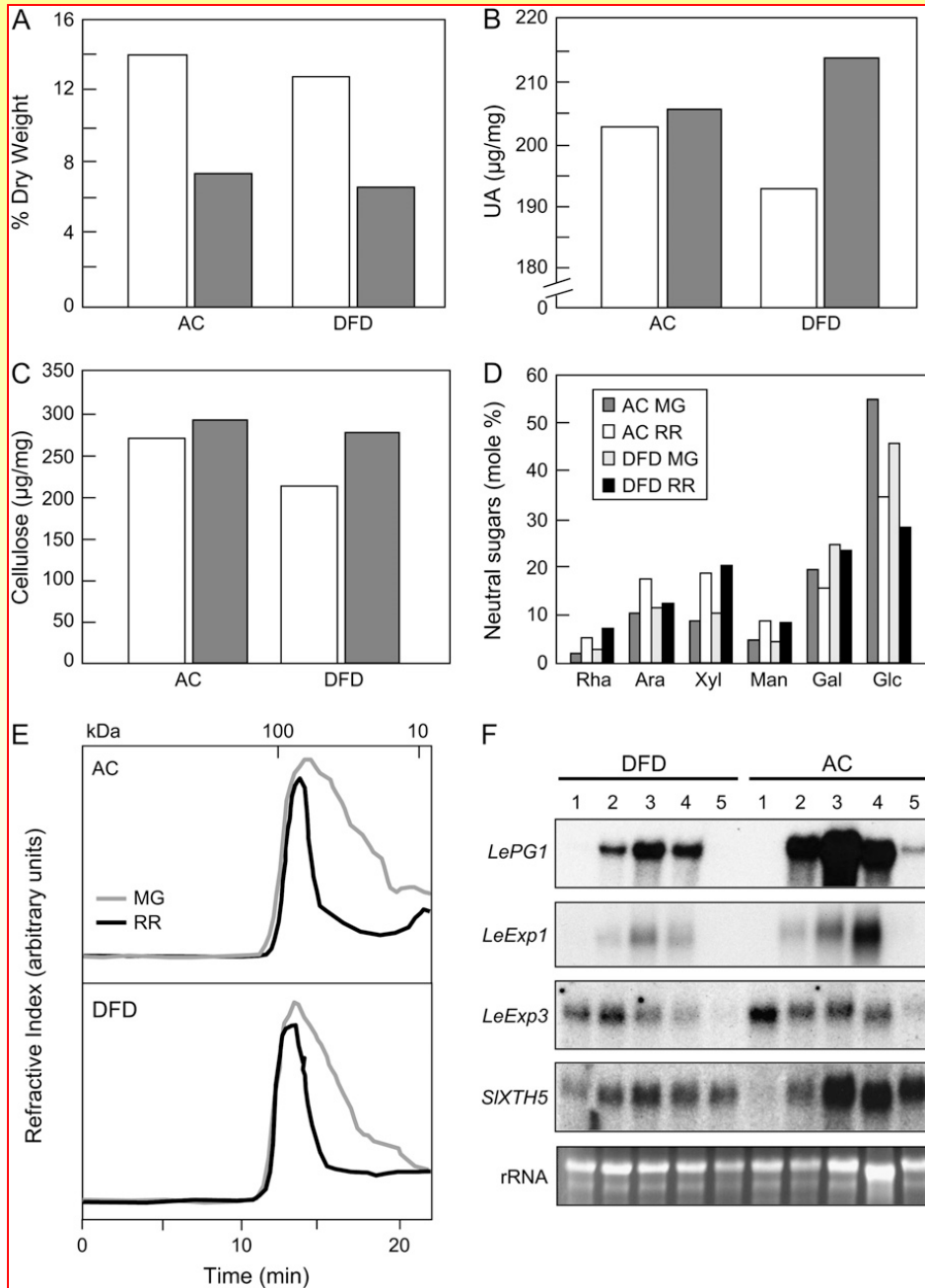
DFD



The peak in ethylene production showed a similar temporal delay, but the DFD fruits exhibited a characteristic climacteric respiratory burst and increase in ethylene synthesis at the Br stage that was more pronounced than that of AC fruit. This was not surprising, given the substantial variation that has been observed among tomato cultivars ([Guillén et al., 2006](#))

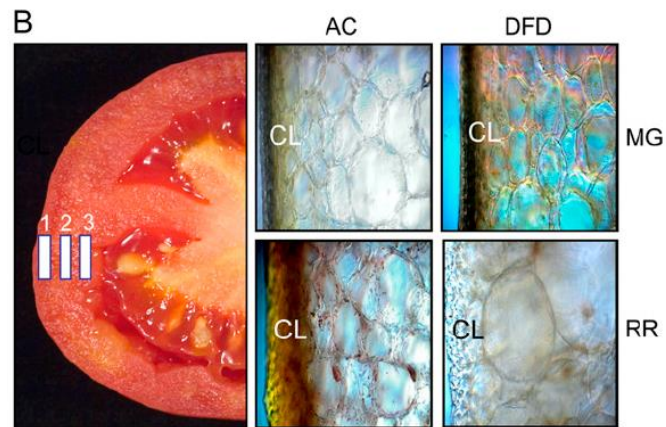
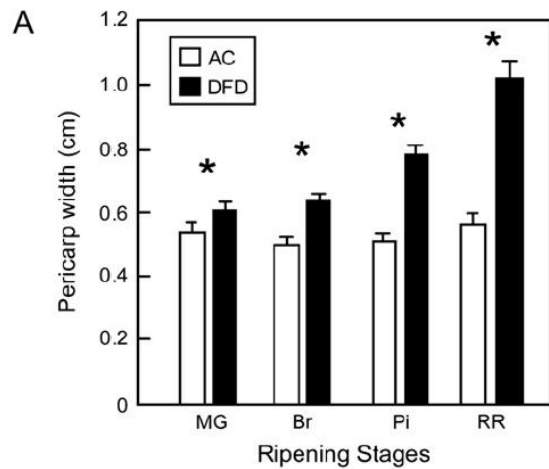


The force needed to penetrate the cuticle of intact MG fruits was greater for AC than DFD, and while the AC cuticles showed progressive weakening during ripening, those of DFD showed a minimal change, such that the penetration mass for DFD fruits at the RR stage was approximately twice that of AC.



Changes in the amounts of total wall material, based on dry weight (Fig. A), uronic acids (Fig. B), cellulose (Fig. C), and cell wall neutral sugar composition (Fig. D), showed typical ripening-related trends in both AC and DFD fruits.

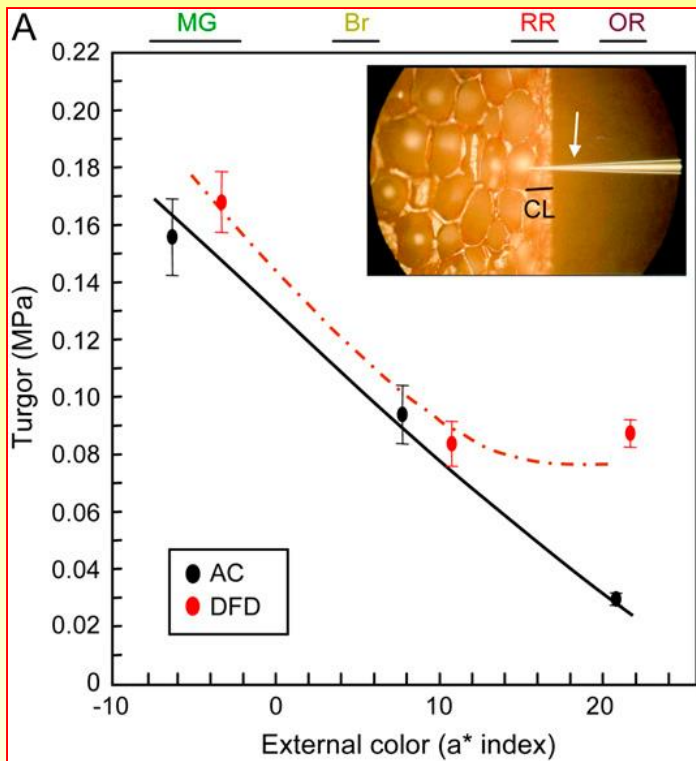
All the genes showed similar qualitative patterns of transcript accumulation and while some quantitative differences were apparent.



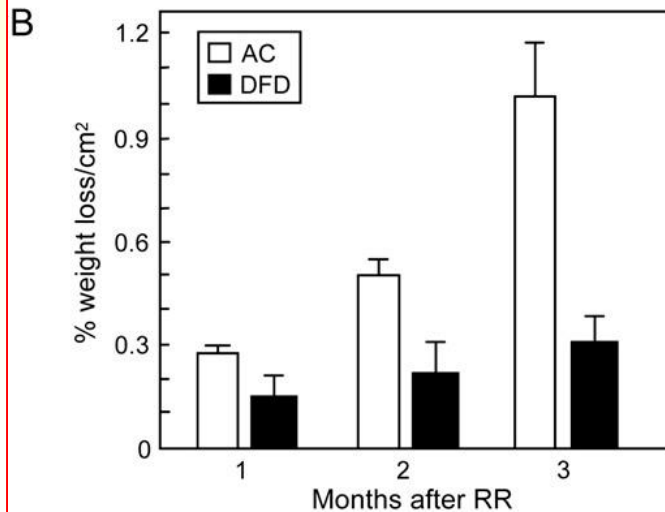
C

Even though fruit cell wall breakdown and cell separation showed similar trends in DFD and AC, the pericarp of DFD, but not AC, exhibited substantial swelling during ripening.

Microscopic analysis of three zones of the pericarp, (1–3, corresponding to outer, middle, and inner, respectively; Fig. B) showed that cell size throughout the pericarp was similar in AC and DFD fruits at the MG stage (Fig. B and C), but that by the RR stage, cells in the DFD pericarp had undergone a substantial increase in cell size.

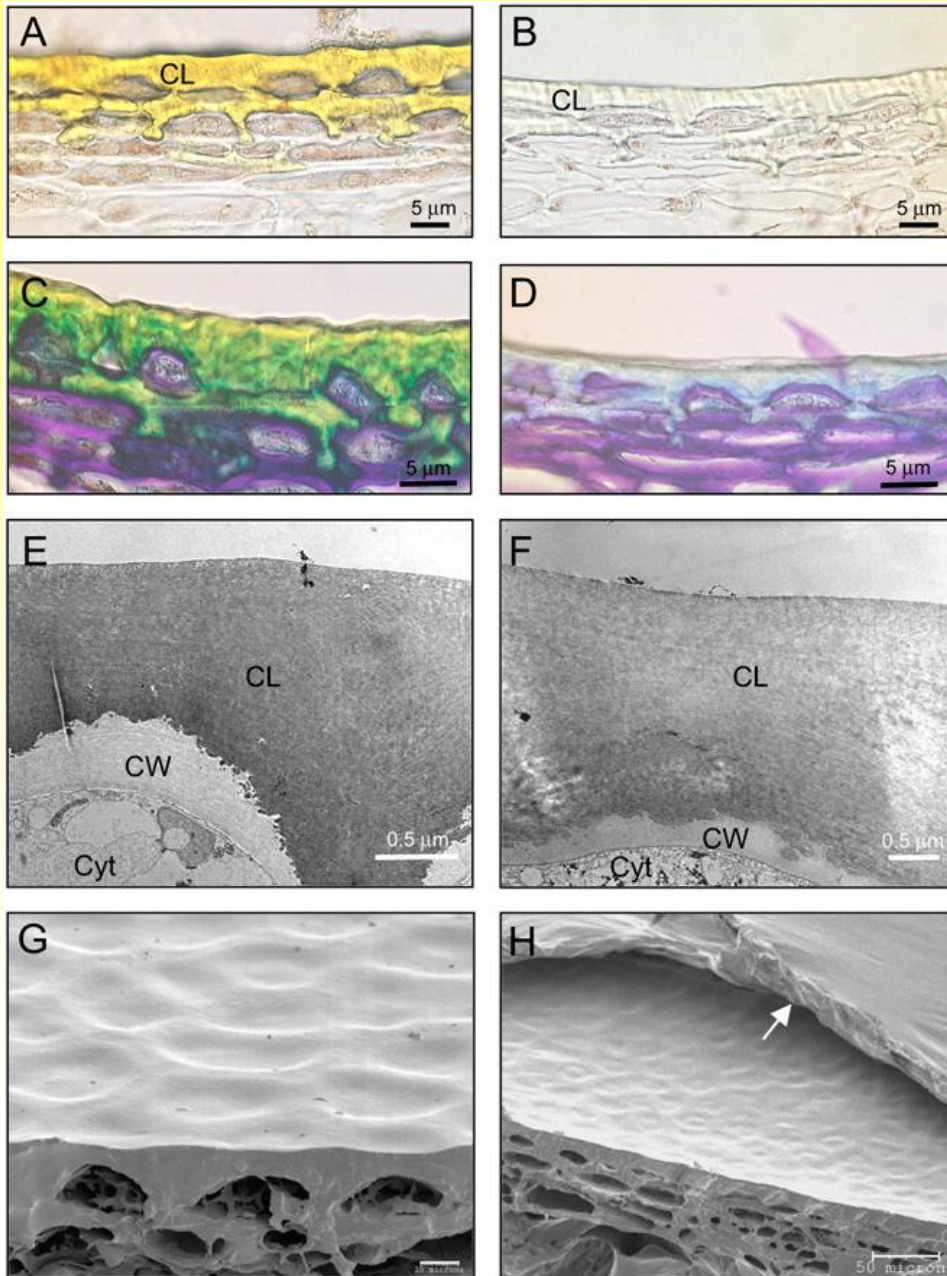


Cellular turgor in AC fruits declined prior to the onset of ripening at the MG stage and decreased linearly throughout ripening, reaching minimal values at the overripe (OR) stage.



AC fruits showed a high rate of water loss, while the loss was minimal in DFD fruits. After 3 months the AC fruits typically exhibited extensive desiccation and splitting, while the DFD fruits showed no apparent change.

cuticula



AC and DFD fruits have similar cuticle anatomies, similar patterns of cellulosic cell wall ramification within the cuticular layer

However, one notable difference was that the cuticles of AC RR fruit had a characteristic yellow-orange color, due to the presence of the flavonoid precursor naringenin chalcone ([Baker et al., 1982](#)), but no such coloration was seen in the DFD cuticles ([Fig. 7, B and D](#)).

DFD is unlike previously reported tomato mutants in that loss of fruit firmness is largely uncoupled from other aspects of ripening, thus providing a unique opportunity to examine the factors that contribute to fruit softening. Our results suggest that **multiple coordinated processes** are involved, including **disassembly of polysaccharide networks in the primary wall and middle lamella and transpirational water/turgor loss**.

In DFD fruit, the turgor and fruit firmness data indicate that softening of intact fruits results from an early decline in cellular turgor coincident with early changes in wall architecture, presumably as a result of wall relaxation. A second component is then provided by substantial **water transpiration**, which occurs in parallel with continued wall degradation and a reduction in intercellular adhesion. The **cuticle** itself is also thought to have an important influence on the biomechanical properties of ripening fruit (Petracek and Bukovac, 1995; Bargel and Neinhuis, 2004; Matas et al., 2004; Bargel and Neinhuis, 2005; Edelman et al., 2005) and studies with isolated tomato fruit cuticles (Bargel and Neinhuis, 2005) suggest that their relative contribution to tissue strength increases markedly during ripening, an idea that was previously suggested by analyses of intact tomato fruits (Jackman and Stanley, 1994).

Taken together, a growing body of evidence suggests that, as with cell wall metabolism, **dynamic changes in the structure and composition of the fruit cuticle** that lead to a reduction in fruit firmness **are likely to be an integral and regulated part of ripening**. The influence of the cuticle on fruit firmness would be both **direct**, acting as a load bearing matrix under tension, and **indirect** by regulating fruit water status. We note that our conclusions are based exclusively on studies with tomato and do not necessarily apply to all fruits.

Role of cell walls in determining additional quality parameters along the food chain.

Many of the cell-wall-dependent quality characteristics of plant based foods at different stages of the food chain relate to the role of cell walls in plant development. For example, the level of pest and pathogen damage that occurs during growth, development, and postharvest storage will be affected by the cell wall. The cell wall has **a direct role in combating pathogenic invasion by acting as a physical barrier**, important in the resistance of dormant organs such as stored seeds, roots, tubers, and bulbs, to pathogenic invasion. In addition, wall components released after degradation by enzymes from pathogens can elicit wound response mechanisms in the plant (Brett and Waldron 1996). The wall itself may then be involved in that response; for example, by enhanced cross-linking of native wall components and newly inserted molecules such as biogenic amines (Waldron and others 1997a). Hence, the cell wall can influence the marketable yield of crops and edible organs, particularly the visual appearance which may be significantly altered by evidence of previous responses to infection (for example, aberrations in organ color and visible textural characteristics). Changes in cell walls during wound responses may also affect subsequent organoleptic quality, as in the case of asparagus. The protective roles of the plant cell wall are becoming more important in organic crops where the use of pesticides and other chemicals is much reduced. Organic products are important in fulfilling purchase motives based on environmental concerns.

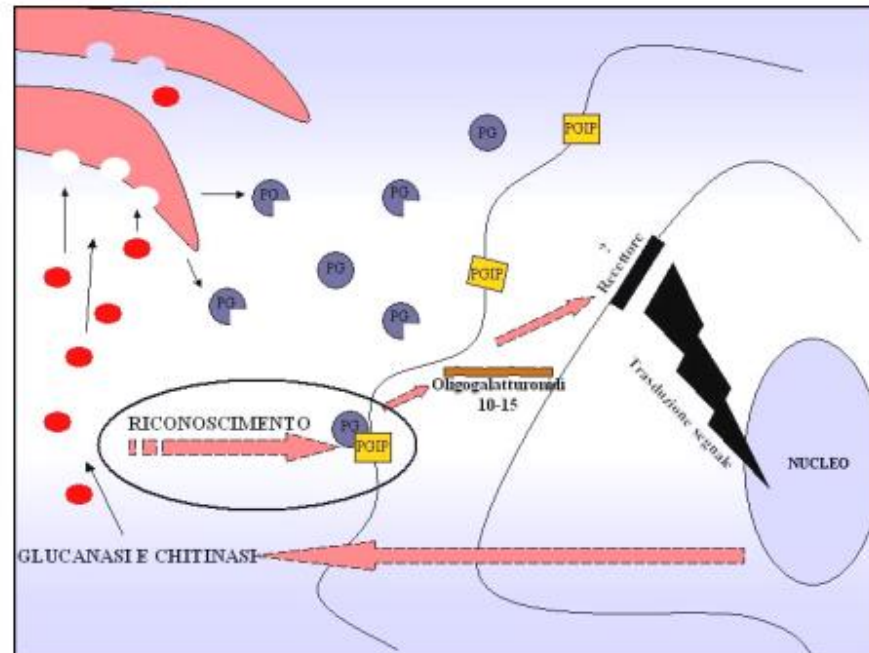


Figura 6 Rappresentazione schematica dell'interazione PGIP-PG all'inizio dell'invasione fungina e dell'attivazione dei meccanismi che portano alla trascrizione dei geni coinvolti nella risposta di difesa.

- Il fungo produce endopoligalatturonasi (PG) che digeriscono la parete della pianta durante l'infezione
- La pianta produce delle proteine (PGIP) che legano le poligalatturonasi fungine e ne rallentano l'azione
- Oligogalatturonidi (8-15) si accumulano e agiscono come elicitatori della risposta di difesa della pianta legandosi a recettori sulla membrana vegetale