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Aspects of cell wall extensibility in *Ceratonia siliqua* L.

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Summary

The extensibility of isolated cell walls of young expanding leaves and root apices from *Ceratonia siliqua* L. (carob tree) has been investigated when: a) subjected to acidic buffers, b) treated with enzymes degrading specific cell wall components and c) applied crude protein extract from growing walls of cucumber and carob. The extension was generally restricted and smaller than that reported for rapidly growing tissues; while, specimens treated with pectolyase and pectinase rapidly extend.

Key words: cell wall, *Ceratonia siliqua*, creep assay, evergreen, leaves, roots.

Introduction

Cell enlargement in higher plants is generated by a turgor-driven extension of a loosened cell wall. Plant primary cell wall acts as a constraint to cell enlargement, while wall loosening denotes either its mechanical weakening, or a cleavage of wall structural polymers. Turgor is the physical driving force for growth, caused by the difference between intracellular and extracellular solute concentration. Turgor stretches the wall, which acts as a cellular exoskeleton that encases plant cells, giving them shape and mechanical stability, gluing them together, restraining their growth, and protecting them from assaults by pathogens and the environment (Cosgrove 1997). Cell enlargement is limited by the extensibility (Nonami 1997) of a growing cell wall that has rheological properties intermediate between those of elastic solid and viscous liquid. In plants subjected to various environmental stimuli, cell wall properties are modified (Frensch 1997; Hoson 1998). Hence, cell wall plays an active role in determining plant cell fates (Strauss 1998).

Numerous studies have documented changes in cell wall extensibility of rapidly elongating vegetative tissues, i.e. from *Arabidopsis* and various crops (Shieh & Cosgrove 1998; Yokoyama & Nishitani 2000; Kutschera 2001). In this study, experiments were conducted with cell walls from developing roots and leaves of the slowly growing *Ceratonia siliqua* L. (Caesalpiniaceae). This species is a drought tolerant, deeply rooted evergreen, and widespread as a native plant in the Mediterranean Basin (Rhizopoulou & Mitrakos 1990; Mitrakos et al. 1991; Correia & Martins-Loucao 1995). Also, it is considered a phylogenetically primitive species and an economically important plant, being a resource for afforestation in semi-arid regions (Catarino 1996), and due to the value of its beans (Ortiz et al. 1995; Kalaitzakis & Mitrakos 2000) that have been used as a primary source for alternative products (e.g. St. John's bread), fed to cattle, relished to children (Baumann 1996; Vaughan & Geissler 1999).

Cell wall extensibility of carob juvenile tissues (roots and leaves) was tested by applying: a) acidic buffers inducing wall loosening, b) enzymes capable of degrading specific cell wall components and c) expansins, a class of cell wall proteins with acidic pH optima (McQueen-Mason 1995; Cosgrove 2000; Li et al. 2003). It is likely that expansins induce extension in isolated walls *in vitro*. For example, expansins to date have

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been studied in rapidly growing cucumber hypocotyls (McQueen-Mason & Cosgrove 1995), non-growing regions of loblolly pine seedlings (Hutchison et al. 1999) and maize roots at water shortage (Wu et al. 2001). To the best of our knowledge the effect of pH, cell wall degrading enzymes and crude expansin extract on cell wall extensibility of growing tissues of *C. siliqua* has not been reported hitherto.

Materials and methods

Plant material

Seeds of *Ceratonia siliqua* L. (carob) were placed in seed trays containing vermiculite. When the two first leaves expanded, seedlings were transplanted into pots containing vermiculite, in a glasshouse (25 ± 2 °C, 50% RH, 14 h photoperiod, 500 μmol m^{-2} s^{-1}). Also, numerous carob seeds were placed in rhizotrons (i.e. boxes made by Plexiglas 5 cm × 30 cm × 50 cm) filled with vermiculite in the glasshouse, in order to investigate the elongating zone of carob roots. When carob seedlings came through and developed at the two-leaf level, stages of root elongation were detected by marking into 2 mm intervals along the main axis, on the Plexiglas foil. Each reported value is the mean of thirty measurements.

Methods

Sections 10 mm in length, from the lamina and mid-vein of expanding leaves and of root apices were excised, frozen to −20 ºC, thawed, abraded with Carborundum to disrupt the cuticle and pressed (to remove cell sap) according to a procedure that has been repeatedly used by previous investigators (Cosgrove 1989; Hyung-Taeg & Kende 1997; McQueen-Mason & Rochange 1999). The walls of growing cells exhibit a steady, long-term creep (a type of irreversible extension), that may be mimicked in isolated walls, as long as their associated proteins are not inactivated (Cosgrove 1999). Flattened specimens from expanding leaves and roots of *C. siliqua* were clamped under an applied force (10 g) and the expansion was recorded using a linear voltage displacement transducer; the specimen between the clamps was 5 mm. Actually, this is an extensometer that measures length that is subjected to a constant tension load; the magnitude of load was tested by using 2, 5, 10 and 15 g. Also, measurements were made in roots and hypocotyls of *C. siliqua* grown in darkness at 27 °C for 12 days; the results were compared with data from cucumber (*Cucumis sativum* L. cv. Burpee Pickler) hypocotyls grown in darkness at 27 °C for 3 days. The specimens were enclosed in a plastic cuvette (*c.* 1 ml volume) filled with Hepes-KOH 50 mM, pH 6.8, for 20 min. The solution surrounding the specimens was then rapidly replaced by Na-acetate 50 mM at pH 4.5 for 60 min and cell walls extended irreversibly in a time-dependent fashion under constant tension, without the complexities of living cells (i.e. wall synthesis, turgor changes). Na-acetate 50 mM at pH 4.5 was replaced, by: a) sodium acetate buffers at pH 3.5, 2.5, 1.5. b) Enzymes from Calbiochem i.e. cellulase I (from *Trichoderma viride*), cellulase II (from *Humicola insolerus*), pectinase (from *Aspergillus niger*), pectinesterase, pectolyase (from *Aspergillus japonicus*), xylanase. c) Urea (3M). d) EGTA (0.1M). e) Ca^{2+} ($CaCl_2$, 0.1M) and Sr^{2+} ($SrCl_2$, 0.1M). f) Crude expansin extract from cucumber hypocotyls (exp-*cuc*) and carob young expanding leaves (exp-*cer*). Boiling the tissues in water for 80 s inactivated the endogenous extension in such walls. The extraction of cell wall proteins was performed according to McQueen-Mason et al. (1992). Expanding leaves and root tips were harvested on ice and homogenized with a cold buffer containing 20 mM Hepes, 0.1% Triton X 200, 2 mM sodium metasulfite, pH 4.5, in a pre-chilled Waring blender. The wall fractions were collected by filtration through Miracloth (70 μm mesh), washed twice with buffer, and extracted overnight in a buffer containing 20 mM Hepes, 2 mM EDTA, 1M NaCl, 3 mM sodium metasulfite, pH 7, at 4 ºC. Cell wall fragments were removed by filtration and then it was squeezed through Miracloth. Cell wall proteins in the supernatants were precipitated with ammonium sulphate (0.35 g mL^{-1}), collected by centrifugation and stored at −80 ºC, until used in the bathing experiment.

The data were subjected to analyses of variance between tissues and among treatments, by using ANOVA.

Results and discussion

Of primary concern was to investigate the region of highest growth rate in roots of carob. The elongating zone of roots from *Ceratonia siliqua* seedlings was found to be 2−5 mm from the tip and the growth rate of root apices at *ca.* 0.8 mm h^{-1}. This growth is 4-fold lower than that of maize roots (Bret-Harte & Silk 1994).

The walls of growing cells from roots and leaves of *C. siliqua* exhibit a steady, long-term creep; mean creep rate at pH 6.8 was estimated at 1.8 μm min^{-1} in expanding leaves and at 2.9 μm min^{-1} in root apices, respectively. Isolated walls from growing leaves and roots of *C. siliqua* extended when subjected to acidic buffers (Fig. 1−2, Table 1). The maximum extensibility was detected at pH between 2.5 and 1.5, while breakage of cell walls occurred at pH < 1.5. Low pH may cause pectin dissolution. The extension of root cell walls was 0.5 to 2.5% higher than that of leaves (Fig. 2). Extension rates in expanding leaves (*c.* 3.324 μm min^{-1}) are in the same order of magnitude as earlier results with attached expanding carob leaves (*c.* 0.194 mm h^{-1}) measured continuously by using a linear variable displacement transducer (Rhizopoulou & Davies 1991). It is worth noting that sclerophyllous leaves of *C. siliqua* expand throughout 5−8 weeks during spring and exhibit about a 24-month lifespan; a secondary growth-period, though, has been observed during autumn (Rhizopoulou et al. 1991). Cell wall sectional area in carob leaves, subjected to a creep assay is 9% of the cell area,

Table 1. The effect of various buffers and bathing solutions (see Materials and Methods and the legend of Fig. 2 for concentration and period of incubation) on extensibility of isolated cell walls from leaf lamina and root apex and hypocotyls sections of *Ceratonia siliqua* L. Each value is the mean of eight replicates ± S.E.

Conditions	**Lamina of expanding leaf (μm min^{-1})**	**Mid-vein leaf section (μm min^{-1})**	**Root apex (μm min^{-1})**	**Hypocotyl (μm min^{-1})**
HEPES pH 6.8	none	none	none	none
Na-acetate pH 4.5	3.3 ± 0.02	1.5 ± 0.05	4.0 ± 0.06	1.5 ± 0.02
Na-acetate pH 3.5	4.5 ± 0.03	1.2 ± 0.05	8.0 ± 0.03	4.1 ± 0.08
Na-acetate pH 2.5	6.5 ± 0.02	5.0 ± 0.03	extension breakage	6.5 ± 0.05
Na-acetate pH 1.5	extension-breakage	8.0 ± 0.04	extension-breakage	extension-breakage
EGTA	0.9 ± 0.03	1.3 ± 0.05	0.4 ± 0.01	0.7 ± 0.03
Ca^{+2}	2.2 ± 0.05	0.4 ± 0.03	none	0.4 ± 0.02
Cellulase I	0.7 ± 0.02	0.5 ± 0.03	2.0 ± 0.05	3.2 ± 0.04
Cellulase II	0.8 ± 0.06	0.6 ± 0.01	3.8 ± 0.02	3.5 ± 0.04
Pectinase	0.9 ± 0.02	3.0 ± 0.01	1.2 ± 0.03	0.8 ± 0.02
Pectolyase	extension-breakage	extension-breakage	3.0 ± 0.06	extension-breakage
Pectinesterase	0.7 ± 0.02	1.2 ± 0.03	0.6 ± 0.03	0.3 ± 0.04
Xylanase	none	2.3 ± 0.04	none	none

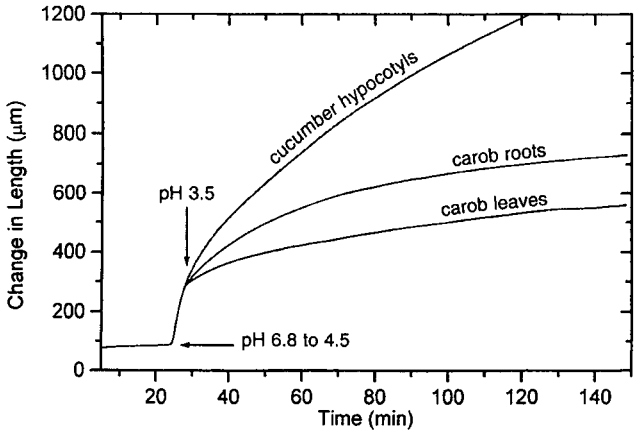

Fig. 1. Acid induced extensibility of root apices and expanding leaves of *Ceratonia siliqua*, in comparison with that of cucumber hypocotyls. The representative traces were monitored before and after switching from a neutral pH-bathing buffer at pH 6.8 to an acidic buffer, at pH 4.5 for cucumber hypocotyls, and at pH 3.5 for juvenile tissues from carob. Data are from individual samples and all experiments were repeated five times.

respectively (CHRISTODOULAKIS & ARGIROPOULOS, personal communication).

The pH of buffering solutions at 4.5 and 3.5 was measured in the cuvette by the end of the creeping experiment to verify pH, and it was found 4.7 and 3.7 respectively. This means that pH increases by 0.2 per unit pH, indicating that the apoplastic pH of *C. siliqua* is less acidic than the above-mentioned values. It has been argued that pH of xylem and apoplast of plants growing in dry soil, though, can be more alkaline than that of plants growing in wet soil, which might result in a restricted expansion (BACON et al. 1998). The lowest reported value for apoplastic pH is just above 4 and the highest just above 7 (GRIGNON & SENTENAC 1991). Changes in osmolarity in the xylem sap are transduced into pH changes, in such a way that an increase in osmolarity alkalises apoplastic fluid (FELLE 2001).

Exchange of Na-acetate with a buffer containing Ca^{2+}, after 100 min always resulted in declining cell wall extension of carob juvenile tissues (Fig. 2). Ca^{2+} antagonises acid-induced extension (Table 2); it is tightly bound to pectins of the cell wall, enhancing cell wall rigidity, affecting ion concentration and pH (FRY 1986; CLELAND et al. 1990; SAKURAI 1998). Exchange of Ca^{2+} buffering solution with Sr^{2+} buffering solution (same concentration and pH) resulted in some wall loosening (Fig. 2); Sr^{2+} displaces Ca^{2+} loosely bound to cell wall and gets in contact to carboxyl groups. The effect of the chaotropic urea on wall expansion, which disrupts hydrogen bonds, was negligible.

Cell walls from leaves and roots treated with cellulase and pectinase extended rapidly; this is probably due to a partial depolymerization of cell wall polysaccharides (Fig. 2). In segments treated with pectolyase, a sharp expansion (4-fold higher than that of pectinase) was followed by a breakage of cell wall, within 3–5 min; there may have been active several crude

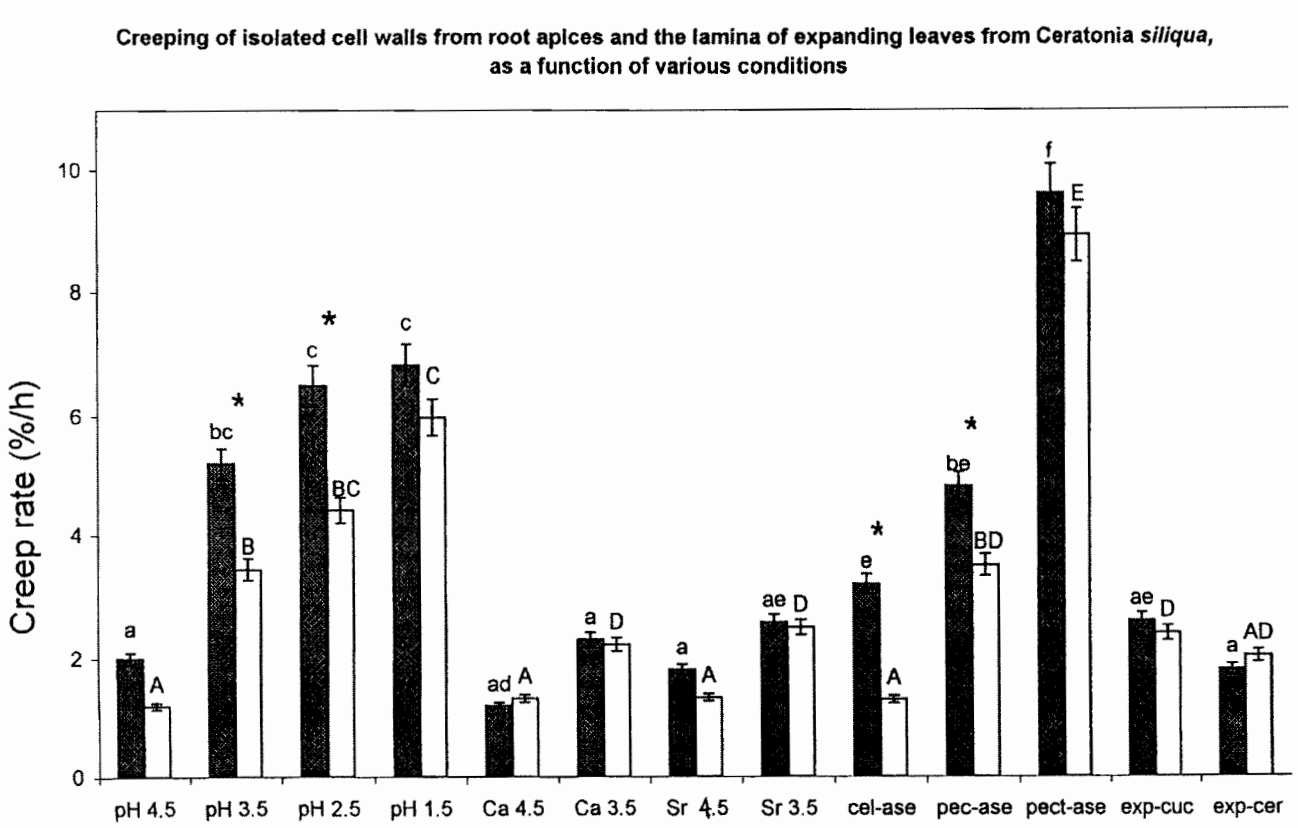

Fig. 2. Creep rate of isolated walls from root apices (closed bars) and young expanding leaves (open bars) of *Ceratonia siliqua*, under various conditions. Where: pH from 4.5 to 1.5 refers to buffers as stated in materials and methods. Ca 4.5 and Ca 3.5 refer to $CaCl_2$ 0.1 M at pH 4.5 and 3.5, as well as Sr 4.5 and 3.5 refer to $SrCl_2$ 0.1 M at pH 4.5 and 3.5, after 20 min of creeping in Na-acetate pH 4.5. Cel-ase, pec-ase and pect-ase refer to bathing solution where cellulase (cel-ase, 10 mg ml^{-1}), pectinase (pec-ase, 10 mg ml^{-1}), pectolyase (pect-ase, 3 units ml^{-1}), were added after 30 min of creeping (i.e. 10 min in Hepes pH 6.8 and 20 min in Na-acetate pH 4.5). Heat inactivated walls of juvenile tissues (roots and leaves) from carob were clamped in extensometers in 50 mM Na-acetate (pH 4.5); after 20 min the solution was replaced by crude protein extract either from cucumber hypocotyls (exp-*cuc*, 3mg ml^{-1}), or crude protein extract from carob expanding leaves (exp-*cer*, 3mg ml^{-1}). Each bar is the mean of 8 measurements ± S.E. Means followed by the same letters, within a tissue (a capital letter for leaves, a small letter for roots) are not statistically significantly different ($p > 0.05$); while * represents significant difference between roots and leaves subjected to the same treatment, at $p < 0.005$.

Table 2. Extensibility of isolated cell walls from root apices of carob and from cucumber hypocotyls as function of ranging pH and calcium concentration. Each value is the mean of eight replicates ± S.E.

	Creep rate % h^{-1}	**Creep rate % h^{-1}**	**Creep rate % h^{-1}**	**Creep rate % h^{-1}**	**Creep rate % h^{-1}**	**Creep rate % h^{-1}**
pH	**Cucumber hypocotyls**	**Carob roots**	**Cucumber hypocotyls**	**Carob roots**	**Cucumber hypocotyls**	**Carob roots**
	0.1 M $CaCl_2$	0.1 M $CaCl_2$	0.05 M $CaCl_2$	0.05 M $CaCl_2$	0.01 M $CaCl_2$	0.01 M $CaCl_2$
3.5	1.8 ± 0.02	1.4 ± 0.02	2.2 ± 0.03	2.7 ± 0.03	3.5 ± 0.03	3.5 ± 0.02
4.3	2.6 ± 0.03	1.6 ± 0.04	3.2 ± 0.01	3.6 ± 0.00	5.5 ± 0.03	4.2 ± 0.03
6.8	3.2 ± 0.04	2.2 ± 0.03	4.2 ± 0.03	4.0 ± 0.01	5.8 ± 0.02	4.5 ± 0.02
7.6	4.7 ± 0.01	3.7 ± 0.04	4.6 ± 0.04		6.0 ± 0.04	
8.2	5.8 ± 0.05					

enzymes that can cleave covalent bonds that link the sugar residues of the non-cellulosic polymers in the wall. Pectolyase splits the glycosidic linkages of pectins secreted into the apoplast, either by hydrolysis or by trans-eliminations (MICHELI 2001). Application of exp-*cuc* and exp-*cer* to juvenile tissues of carob caused a restricted extensibility, in comparison to that recorded in cucumber hypocotyls treated with exp-*cuc*. The same holds true for cucumber hypocotyls treated with exp-*cer*. It is possible that some expansins are involved in wall disassembly rather than elongation (MCQUEEN-MASON & ROCHANGE 1999).

When cell enlargement is initiated by stress-relaxation of the wall, growing cells reduce their turgor and water potential, which enables them to absorb water and to expand (COSGROVE 1997), implying that the walls are loosened during growth. In primary roots of carob longitudinal growth is maintained towards the apex at low water potential (Ψ), resulting in a shorter and thicker root than that at less negative Ψ (RHIZOPOULOU & DAVIES 1991). Turgor was approximately 0.5 MPa in the elongating zone at Ψ as high as -0.2 MPa, while it decreased around 0.3 MPa at a Ψ as low as -0.9 MPa. It would seem then, that root cells react to changes in water regime *via* interactive cellular responses to low turgor and reduced length in roots. The maintenance of apical growth in *C. siliqua* roots, despite a reduction in water supply, indicates that stress relaxation promotes cell wall loosening, allowing (greater) expansion per unit of turgor. In this species, physiological processes such as stomatal conductance and assimilation rates (NUNES et al. 1992) continue being active under drought; while, water uptake continues *via* tap roots penetrating deeply into the soil (RHIZOPOULOU & DAVIES 1991). It seems likely also that root solute potential in carob remains unchanged despite water shortage (RHIZOPOULOU & DAVIES 1991), and this may be consistent with a small dilution of cell contents by apoplastic water (PRITCHARD et al. 2000). It has been reported (PRATIKAKIS et al. 1998) that in root tips of *C. siliqua* cell wall thickenings (termed Φ) contain lignin and suberin, while in proximal sections the presence of tannins impart rigidity to the walls. However, lignins are not only deposited in the walls of specialised cells and tissues that have ceased to grow, being involved in mechanical support or protection (BOUDET 1998), but also in primary walls of young expanding cells (KUTSCHERA 2001). The Φ layer, which does not appear in all plants (MILLAY et al. 1987), does not act as a barrier to water movement from the cortex towards the xylem. On the contrary, the lignification of the Φ bands opposite the phloem could retard centrifugal water leakage from the stele to the cortex of *C. siliqua*. In fact, lignification of cell wall results in a decline in the walls' permeability to water (BLACK & CHARLWOOD 1996). Φ thickened root cells of *C. siliqua* may retard apoplastic flux and facilitate a "Huber-type" water transport between phloem and xylem.

Cell walls from expanding tissues, of the slowly growing *Ceratonia siliqua* are expected to be less extensible (tighter) than those of rapidly expanding tissues (THOMPSON & FRY 1997). Changes in cell wall components, though, that reflect *in vivo* wall-loosening and wall-stiffening in growing tissues are still a matter of speculation; cell enlargement is accompanied and controlled by numerous changes in the extracellular matrix. At the phase of extension, growth is characterized by modification of the existing wall and the insertion of new material throughout the surface (REITER 1998). In many cases, light-mediated lignification of the primary wall may be partially responsible for cell wall stiffening (SCHOPFER et al. 2001). Glucose is more likely to be constitutive for hypocotyls-expanding walls in seedlings of *C. siliqua* (SOTIRIOU et al. 1999). Galactomannans in non-expanding tissues of carob, like endosperm, are much less branched in comparison to fully branched galactomannans, and this structure changes their viscosities (BUCHANAN et al. 2000). In addition, these galactomannans have been shown to be unresponsive to expansin application (BROOKS 1999). Therefore, the peculiar cell wall elasticity of *C. siliqua* may be an adaptation to drought, as assumed by NUNES et al. (1989).

The cell wall of juvenile tissues of *C. siliqua* has to be strong enough to maintain turgor pressure at reduced rates during correlatively soil drying in sunny open fields. Under such conditions, stiffening processes of the cell wall may enhance resistance to water loss.

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References

BACON, M. A.; WILKINSON, S. & DAVIES, W. J. (1998): pH-regulated leaf cell expansion in droughted plants is abscisic acid dependent. – Plant Physiol. **118:** 1507–1515.

BAUMANN, H. (1996): Greek wild flowers and plant lore in ancient Greece. – The Herbert Press, London.

BLACK, M. & CHARLWOOD, B. (1996): Physiology and biochemistry of plant cell walls. – Chapman & Hall, London.

Boudet, A.-M. (1998): A new view of lignification. – Trends Plant Sci. **3:** 67–71.
Bret-Harte, M. S. & Silk, W. K. (1994): Fluxes and deposition rates of solutes in growing roots of *Zea mays*. – J. Exp. Bot. **45:** 1733–1742.
Brooks, S. E. C. (1999): Effect of expansins on the mechanical properties of model cell wall materials, in: Proceedings of Plant protein club workshop. Plant proteins and the mechanical properties of cell wall, Alicante, 8.
Buchanan, B. B.; Grussem, W. & Jones, R. L. (2000): Biochemistry & molecular biology of plants. – Amer. Soc. Plant Biologists, Rockville, Maryland.
Catarino, F. (1996): An old and sustainable Mediterranean crop, its potential for the future, in: Proceedings of 3rd International Carob Symposium, Tavira, 27.
Cleland, R. E.; Sarbjit, S., Taylor, D. & Björkamn, T. (1990): Calcium, cell walls and growth. In: Leonard, R. T. & Hepler, P. K. (eds.): Calcium in plant growth and development. – ASPP Symposium Series, Rockville, 9–16.
Correia, P. J. & Martins-Loucao, M. A. (1995): Seasonal variations of leaf water potential and growth in fertigated carob-trees (*Ceratonia siliqua* L.). – Plant Soil **172:** 199–206.
Cosgrove, D. J. (1989): Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls. – Planta **37:** 121–130.
Cosgrove, D. J. (1997): Relaxation in a high-stress environment: the molecular bases of extensible cell walls and cell enlargement. – Plant Cell **9:** 1031–1041.
Cosgrove, D. J. (1999): Enzymes and other agents that enhance cell wall extensibility. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **50:** 391–417.
Cosgrove, D. J. (2000): Loosening of plant cell walls by expansins. – Nature **407:** 321–326.
Felle, H. H. (2001): Signal and messenger in plant cells. – Plant Biol. **3:** 577–591.
Frensch, J. (1997): Primary responses of root and leaf elongation to water deficits in the atmosphere and soil solution. – J. Exp. Bot. **48:** 985–999.
Fry, S. C. (1986): Cross-linking of matrix polymers in the growing cell walls of angiosperms. – Annu. Rev. Plant Physiol. **37:** 165–186.
Grignon, G. & Sentenac, H. (1991): pH and ionic conditions in the apoplast. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **42:** 103–128.
Hoson, T. (1998): Apoplast as the site of response to environmental signals. – J. Plant Res. **111:** 167–177.
Hutchison, K. W.; Singer, P. B., Mc Innis, S. & Diaz-Sala, C., Greenwood, M. S. (1999): Expansins are conserved in conifers and expressed in hypocotyls in response to endogenous auxin. – Plant Physiol. **120:** 827–831.
Hyung-Taeg, C. & Kende, H. (1997): Expansins in deep water rice internodes. – Plant Physiol. **113:** 1137–1143.
Kalaitzakis, J. A. & Mitrakos, K. (2000): The sugar of the pod in the ripe carob beans. – Biol. GalloHellenica, Supplementum **26:** 9–22.
Kutschera, U. (2001): Stem elongation and cell wall proteins in flowering plants. – Plant Biol. **3:** 466–480.
Li, Y.; Jones, L. & Mc Queen-Mason, S. (2003): Expansins and cell growth. – Curr. Opin. Plant Biol. **6:** 1–8.
Mc Queen-Mason, S. (1995): Expansins and cell wall expansion. – J. Exp. Bot. **46:** 1639–1650.
Mc Queen-Mason, S. & Cosgrove, D. J. (1995): Expansin mode of action on cell walls. – Plant Physiol. **107:** 87–100.
Mc Queen-Mason, S. & Rochange, F. (1999): Expansins in plant growth and development: an update on an emerging topic. – Plant Biol. **1:** 19–25.
Mc Queen-Mason, S.; Durachko, D. & Cosgrove, D. J. (1992): Two endogenous proteins that induce cell wall extension in plants. – Plant Cell **4:** 1425–1433.
Micheli, F. (2001): Pectin methylesterases: cell wall enzymes with important roles in plant physiology. – Trends Plant Sci. **9:** 414–419.
Millay, M.; Taylor, T. N. & Taylor, E. L. (1987): Phi thickenings in fossil seed plants from Antarctica. – Iawa Bulet. **8:** 191–201.
Mitrakos, K.; Diamantoglou, S. & Christodoulakis, N. (1991): Distribution of four evergreen sclerophyllous species in Greece. – Bot. Jahrb. Syst. **113:** 1–6.
Nonami, H. (1997): Plant water relations and control of cell elongation at low water potentials. – J. Plant Res. **111:** 373–382.
Nunes, M. A.; Catarino, F. & Pinto, E. (1989): Strategies for acclimation to seasonal drought in *Ceratonia siliqua* leaves. – Physiol. Plant. **77:** 150–156.
Nunes, M. A.; Ramalho, J. D. C. & da Silva Rijo, P. (1992): Seasonal changes in some photosynthetic properties of *Ceratonia siliqua* (carob tree) leaves under natural conditions. – Physiol. Plant. **86:** 381–387.
Ortiz, P. L.; Arista, M. & Talaveras, S. (1995): Germination ecology of *Ceratonia siliqua* L. (Caesalpiniaceae), a Mediterranean tree. – Flora **190:** 89–95.
Pratikakis, E.; Rhizopoulou, S. & Psaras, G. K. (1998): A phi layer on roots of *Ceratonia siliqua* L. – Bot. Acta **111:** 93–98.
Pritchard J.; Winch S. & Gould, N. (2000): Phloem water relations and root growth. – Aust. J. Plant Physiol. **27:** 539–548.
Reiter W.-D. (1998): The molecular analysis of cell wall components. – Trends Plant Sci. **3:** 27–32.
Rhizopoulou, S. & Davies, W. J. (1991): Influence of soil drying on root development, water relations and leaf growth of *Ceratonia siliqua* L. – Oecologia **88:** 41–47.
Rhizopoulou, S. & Mitrakos, K. (1990): Water relations of evergreen sclerophylls. I. Seasonal changes in the water relations of eleven species from the same environment. – Ann. Bot. **65:** 171–178.
Rhizopoulou, S.; Meletiou-Christou, M. S. & Diamantoglou, S. (1991): Water relations for sun and shade leaves of four Mediterranean evergreen sclerophylls. – J. Exp. Bot. **42:** 627–635.
Sakurai, N. (1998): Dynamic function and regulation of apoplast in the plant body. – J. Plant Res. **111:** 133–148.
Schopfer, P.; Lapierre, C. & Nolte, T. (2001): Light-controlled growth of the maize seedlings mesocotyl: mechanical cell-wall changes in the elongation zone and related changes in lignification. – Physiol. Plant. **111:** 83–92.

SHIEH, M. K. & COSGROVE, D. J. (1998): Expansins. – J. Plant Res. **111:** 149–157.

SOTIRIOU, P.; FRY, S. C. & GANIS-SPYROPOULOS, C. (1999): D-[U-^{14}C]-glucose incorporation in carob (*Ceratonia siliqua* L.) protoplasts during cell wall regeneration. – Proc. 21st Panhellenic Meeting of Biological Sciences, Syros, 290.

STRAUSS, E. (1998): When walls can talk, plant biologists listen. – Science **282:** 28–29.

THOMPSON, J. E. & FRY, C. S. (1997): Trimming and solubilization of xyloglucan after deposition in the walls of cultured rose cells. – J. Exp. Bot. **48:** 297–305.

VAUGHAN J. G. & GEISSLER, C. A. (1999): The new Oxford book of food plants. – Oxford Univ. Pr., Oxford, 120–121.

WU, Y.; THORNE E. T., SHARP, R. E. & COSGROVE, D. J. (2001): Modifications of expansin transcript levels in the maize primary root at low water potentials. – Plant Physiol. **126:** 1471–1479.

YOKOYAMA, J. G. & NISHITANI, K. (2000): Functional diversity of xyloglucan related proteins and its implications of the cell wall dynamics in plants. – Plant Biol. **2:** 120–121.