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REVIEW PAPER

Cell wall thickness and composition are involved in photosynthetic limitation

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Abstract

The key role of cell walls in setting mesophyll conductance to CO_2 (g_m) and, consequently, photosynthesis is reviewed. First, the theoretical properties of cell walls that can affect g_m are presented. Then, we focus on cell wall thickness (T_{cw}) reviewing empirical evidence showing that T_{cw} varies strongly among species and phylogenetic groups in a way that correlates with g_m and photosynthesis; that is, the thicker the mesophyll cell walls, the lower the g_m and photosynthesis. Potential interplays of g_m, T_{cw}, dehydration tolerance, and hydraulic properties of leaves are also discussed. Dynamic variations of T_{cw} in response to the environment and their implications in the regulation of photosynthesis are discussed, and recent evidence suggesting an influence of cell wall composition on g_m is presented. We then propose a hypothetical mechanism for the influence of cell walls on photosynthesis, combining the effects of thickness and composition, particularly pectins. Finally, we discuss the prospects for using biotechnology for enhancing photosynthesis by altering cell wall-related genes.

Keywords: Cell wall composition, cell wall thickness, cellulose, CO_2 diffusion, hemicellulose, land plants, leaf anatomy, mesophyll conductance, pectin.

Abbreviations: A_n, net assimilation rate; CW, cell wall; D, diffusivity; DT, desiccation tolerance; ΔL, apparent diffusion path length; ε, bulk modulus of elasticity; g_{cw}, cell wall conductance to CO_2 diffusion; g_m, mesophyll conductance to CO_2 diffusion; g_s, stomatal conductance to CO_2 diffusion; p, cell wall porosity; p/τ, effective porosity; PME, pectin methylesterase; PRE, pectin-remodelling enzyme; r_{cw}, cell wall resistance to CO_2 diffusion; S_c/S, chloroplast surface area exposed to intercellular air spaces per unit of leaf area; T_{cw}, mesophyll cell wall thickness; τ, tortuosity.

Introduction

Cell walls (CWs) have a well recognized importance in plant physiology and biotechnology. As such, they are intensively studied from different perspectives. A reflection of their importance is that different aspects of CW biology have been repeatedly reviewed and updated over the last 70 years, for example in the *Annual Review* series (Frey-Wyssling, 1950; Albersheim *et al.*, 1969; Cleland, 1971; Northcote, 1972; Cassab and Varner, 1988; Popper *et al.*, 2011; Wolf *et al.*, 2012; Anderson and Kieber, 2020) as well as in many other journals, including the *Journal of Experimental Botany* (e.g. Sarkar *et al.*, 2009; Carpita and McCann, 2015; Sorek and Turner, 2016). Interestingly, among all these reviews, the word 'photosynthesis' is only mentioned by Sarkar *et al.* (2009), to say that photosynthesis influences CWs, which contrasts with the more recent view that CWs can be crucial in regulating photosynthesis. Hence, the global aim of this review is to highlight the CW as a mechanistic limitation on photosynthesis.

Photosynthesis is an essential plant process, and improving crop photosynthesis is a major goal (Zhu *et al.*, 2010; Ray *et al.*, 2013; Ort *et al.*, 2015) that up to now has been addressed mostly by attempting to modify either stomata (Drake *et al.*, 2013; de Boer *et al.*, 2016; Lawson and Vialet-Chabrand, 2019; de Sousa *et al.*, 2020), Rubisco and Calvin cycle enzymes (Price *et al.*, 2013; Schlüter and Weber, 2016; South *et al.*, 2019), or dynamic sun–shade aspects (Kromdijk *et al.*, 2016). These attempts reflect a long-standing view of the scientific community in which photosynthesis is mainly limited by either stomatal closure or restricted biochemical and photochemical activity. However, it is now widely recognized that mesophyll conductance (g_{m})—the ease of diffusion of CO_2 from substomatal cavities to Rubisco active sites inside chloroplast stroma—plays a similarly important role to those of stomata and photo-biochemistry (Gago *et al.*, 2020*a*). Indeed, g_{m} often shows the most consistent limitation of photosynthesis across the terrestrial plant phylogeny (Gago *et al.*, 2019; Flexas and Carriquí, 2020).

Mesophyll conductance limits photosynthesis to an extent similar to that exerted by the well-known stomatal and biochemical limitations, which results in a globally significant correlation between net photosynthesis (A_{n}) and g_{m} (Fig. 1). Because of this, based on standard photosynthesis modelling (Farquhar *et al.*, 1980), it has been estimated that only small improvements (typically 10–30%) in photosynthesis can be expected from alleviating a single limitation alone. Larger improvements require two or more limitations to be alleviated simultaneously (Flexas, 2016; Gago *et al.*, 2019). The optimal combination would improve mesophyll conductance and photo-biochemistry without altering stomatal limitations, achieving simultaneous increases in photosynthesis and photosynthetic water use efficiency (Flexas, 2016; Flexas *et al.*, 2016). Consequently, g_{m} is a significant target—alone or in combination with other components of photosynthesis—for enhancing crop photosynthesis using biotechnological approaches (Flexas *et al.*, 2016; Bailey-Serres *et al.*, 2019).

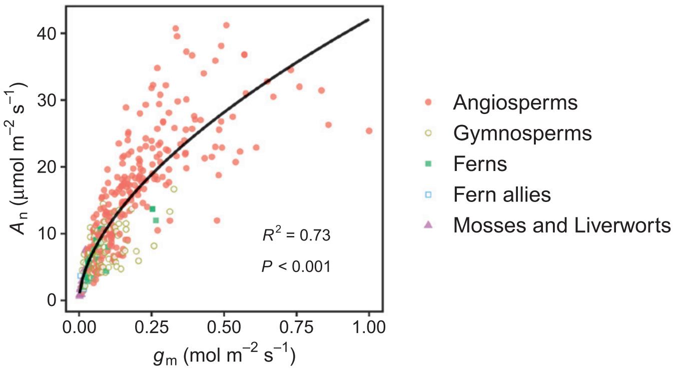

Fig. 1. Relationship between net assimilation rate (A_{n}) and mesophyll conductance to CO_2 diffusion (g_{m}) in C_3 plants. The line represents an exponential standardized major axis (SMA) fit (y=42.103 $x^{0.5796}$) of the pooled data across all phylogenetic groups (412 entries from 271 species). SMA fittings were performed using the function sma() in the 'smatr' R package. Data and references are available at Dataset S1 at Dryad. Gymnosperm species from Veromann-Jürgenson *et al.* (2020) are also included.

Although the role of g_{m} in determining A_{n} is now widely recognized within the scientific community, limitations arise from lack of knowledge of the mechanistic basis of g_{m}. Mesophyll conductance is a composite conductance of an intercellular gas phase (g_{ias}) and a liquid phase (g_{liq}):

$$g_{\mathrm{m}} = \frac{1}{\frac{1}{g_{\mathrm{ias}}} + \frac{RT_{\mathrm{k}}}{H \times g_{\mathrm{liq}}}} \tag{1}$$

where $H/(RT_{\mathrm{k}})$ is the dimensionless form of Henry's law constant, needed to convert g_{liq} to a gas phase equivalent conductance, as g_{m} is defined as a gas-phase conductance (Niinemets and Reichstein, 2003; Tosens *et al.*, 2012*a*). At the same time, g_{liq} is a composite conductance of the apoplastic and cellular components of the CO_2 pathway comprised from the mesophyll CW surface to the carboxylation site in the chloroplast. Thus, g_{liq} can be expressed on a leaf projected surface area basis by scaling it by the chloroplast surface area exposed to intercellular airspaces per unit of leaf area (S_{c}/S):

$$g_{\mathrm{liq}} = \frac{S_{\mathrm{c}}}{S} \times \frac{1}{r_{\mathrm{cw}} + r_{\mathrm{pl}} + r_{\mathrm{cyt}} + r_{\mathrm{en}} + r_{\mathrm{st}}} \tag{2}$$

where g_{cw}, g_{pl}, g_{cyt}, g_{en}, and g_{st} account for the CW, plasma membrane, cytosol membrane, chloroplast membrane, and stroma conductances, respectively. Concomitantly, each partial conductance is determined by several anatomical properties and/or biochemical properties. While biochemical components, probably explaining the observed rapid variations in response to varying environment (Flexas *et al.*, 2007; Vrábl *et al.*, 2009; Douthe *et al.*, 2012), are still a matter of debate (Gu and Sun, 2014; Théroux-Rancourt and Gilbert, 2017; Carriquí *et al.*, 2019*a*; Shrestha *et al.*, 2019; Evans, 2021), there is ample agreement that leaf anatomical properties are important drivers of g_m variability, at least at the interspecific level (Evans *et al.*, 2009; Terashima *et al.*, 2011; Tomás *et al.*, 2013; Peguero-Pina *et al.*, 2017; Veromann-Jürgenson *et al.*, 2017, 2020; Carriquí *et al.*, 2019*b*; Gago *et al.*, 2019). Previous studies have highlighted the importance of anatomical properties, such as mesophyll porosity (Earles *et al.*, 2018; Lundgren *et al.*, 2019; Baillie and Fleming, 2020) or the chloroplast distribution, in particular the S_c/S (Ren *et al.*, 2019), in determining g_m. Although not negligible, the contribution of mesophyll air space porosity to the overall g_m has been shown to be much lower than that of S_c/S (Han *et al.*, 2018; Carriquí *et al.*, 2020; Hu *et al.*, 2020), except perhaps in succulent leaves (Earles *et al.*, 2018). Still, mesophyll porosity may have indirect important effects as apparently it determines the formation of functional stomata during leaf development (Lundgren *et al.*, 2019; Baillie and Fleming, 2020). Besides CW thickness (T_{cw}) and associated traits [e.g. porosity (p) or tortuosity (τ)], S_c/S has been generally suggested to be the most limiting anatomical factor for g_m (Tosens *et al.*, 2016; Peguero-Pina *et al.*, 2017; Carriquí *et al.*, 2019*b*; Ren *et al.*, 2019; Veromann-Jurgenson *et al.*, 2020). However, a dominant role for T_{cw} in g_m has also been described, and the anatomical limitation analysis of g_m introduced by Tomás *et al.* (2013) has shown that, in fact, CW limitations are frequently equal to or greater than S_c/S and that together these constitute the primary anatomical factors for setting the maximum g_m that a species can achieve (Tomás *et al.*, 2013; Tosens *et al.*, 2016; Carriquí *et al.*, 2019*a*, *b*; Veromann-Jurgenson *et al.*, 2020). In addition to thickness, recent evidence points to an effect of CW composition on photosynthesis (Weraduwage *et al.*, 2016, 2018; Ellsworth *et al.*, 2018; Clemente-Moreno *et al.*, 2019; Carriquí *et al.*, 2020; Roig-Oliver *et al.*, 2020*b*, *c*; Evans, 2021).

The aim of this review is to focus on CWs, highlighting their importance in setting g_m and, hence, photosynthesis. For this, we first conceptualize which are the CW properties expected to affect g_m. Then, we review the available empirical evidence of T_{cw} and CW composition effects on g_m both within and between species. Afterwards, we propose a hypothetical mechanism for the influence of CWs on g_m and photosynthesis, and we speculate on potential CW-mediated trade-offs between photosynthesis, leaf water relations, and hydraulics. Based on all the evidence, we briefly discuss prospects for improving photosynthesis by CW manipulation. It is important to highlight that all concepts reviewed here account mostly for C_3 plants. Until very recently, it was technically difficult to estimate g_m in C_4 plants, limiting an understanding of the role of CWs and other anatomical components in this group (Tosens *et al.*, 2016; Cousins *et al.*, 2020; Pathare *et al.*, 2020).

Cell wall properties affecting g_m

The plant CW is a complex biological structure with multiple functions, including maintaining cell structural integrity, providing flexibility for cell division and expansion, and acting as a barrier for pathogens and protecting cells from the environment (Anderson and Kieber, 2020). Depending on the plant group, cell type, and developmental stage, the CW exhibits variable composition and structural complexity (Sarkar *et al.*, 2009; Popper *et al.*, 2011; Maron, 2019; Yokoyama, 2020). In particular, photosynthetic cells possess a primary CW mainly composed of a variable proportion and content of cellulose, hemicellulose, pectin, structural proteins, and some additional components (Cosgrove, 2005; Sarkar *et al.*, 2009; Cosgrove and Jarvis, 2012). The universal presence of the CW of photosynthetic cells of all species constitutes a barrier that hinders CO_2 diffusion (Evans *et al.*, 2009; Terashima *et al.*, 2011; Tholen and Zhu, 2011).

In the journey of atmospheric CO_2 molecules through the photosynthetic organs to the carboxylation sites inside chloroplast stroma, CWs are the first resistance in unistratose organs (e.g. most moss phyllids and filmy fern fronds), and the second in multistratose organs with intercellular air spaces, that CO_2 molecules must face (Hanson *et al.*, 2014; Flexas *et al.*, 2018; Carriquí *et al.*, 2019*b*). However, the role of CWs in limiting photosynthesis has often been neglected (e.g. Sims and Pearcy, 1989; Ellsworth and Reich, 1993; Niinemets, 1999) for several reasons. For instance, the CW represents only a tiny fraction of the apparent CO_2 pathway length of most photosynthetic organs since its thickness ranges between 0.1 μm and 4 μm in land plants (Han *et al.*, 2016; Carriquí *et al.*, 2019*b*; Coe *et al.*, 2019; Sugiura *et al.*, 2020), while the mesophyll can be up to several millimetres or even centimetres thick (Earles *et al.*, 2018; Carriquí *et al.*, 2020; Herrera, 2020). Moreover, CW pore size, ranging between 30 Å and 50 Å (3–5 nm), is sufficient to allow the free crossing of CO_2 molecules, which have a kinetic diameter of only 3.3 Å (Carpita *et al.*, 1979). Additionally, CW properties were considered to be quite static concerning their influence on photosynthesis (Evans *et al.*, 2009). However, there is now broad consensus that the physicochemical properties of the CW constitute a key determinant of A_n and g_m (Flexas *et al.*, 2018; Gago *et al.*, 2020*a*, *b*; Sugiura *et al.*, 2020; Evans, 2021).

g_{cw}, the inverse of CW resistance (r_{cw}), is determined, as in the other components of the CO_2 pathway, by four main physicochemical properties: diffusivity (D, 1.79×10^{-9} m^2 s^{-1} at 25 °C), porosity (p, expressed in m^3 m^{-3}), apparent diffusion path length (ΔL, expressed in m), and tortuosity (τ, expressed in m m^{-1}), which are inter-related as follows (Evans *et al.*, 2009; Terashima *et al.*, 2011):

$$g_{cw} = \frac{1}{r_{cw}} = \frac{D \times p}{\Delta L \times \tau} \quad (3)$$

Diffusion of CO_2 in CW occurs in the liquid phase (Rondeau-Mouro *et al.*, 2008), for which D is at least 10^4 times lower than in the intercellular gas phase (Terashima *et al.*, 2011). In turn, D is also determined by the atmospheric pressure, Henry's law constant (which accounts for the equilibrium air–water partition coefficient), and the combination of pH and [CO_2], which modifies the diffusion coefficient by influencing the fraction of CO_2 that crosses the CW in the form of bicarbonate. However, considering the slightly acidic pH in the apoplast, the influence of the conversion of CO_2 to bicarbonate on CO_2 diffusivity might be small (Niinemets and Reichstein, 2003; Terashima *et al.*, 2011; Tholen and Zhu, 2011; Xiao and Zhu, 2017). Finally, D is perhaps also affected by carbonic anhydrases, as their presence has been reported by Chen *et al.* (2009) in the CW apoplast of rice calli. Certainly, further studies about the effects of pH and the presence of carbonic anhydrases are still required.

The other three properties determining g_{cw} largely depend on the amount, structure, organization, and interaction between CW components. Very briefly, CWs are composed of cellulose microfibrils to which hemicellulosic polysaccharides link, and all of this is embedded in a pectin matrix, forming a tangled web (Cosgrove, 2005; Anderson and Kieber, 2020; Yokoyama, 2020). As the CW assembly determines several properties, such as p and τ, at the same time, it influences g_{cw} in a very complex (as further discussed in the following sections) and dynamic way, given the constant remodelling and reconstruction to which the wall is subjected (Sarkar *et al.*, 2009; Bellincampi *et al.*, 2014; Houston *et al.*, 2016; Clemente-Moreno *et al.*, 2019; Maron, 2019; Zhang *et al.*, 2019).

ΔL is equivalent to T_{cw} (Evans *et al.*, 2009; Tosens *et al.*, 2012*b*; Tomás *et al.*, 2013), and g_m has been reported to be lower at larger T_{cw}, and therefore ΔL (Evans *et al.*, 2009; Carriquí *et al.*, 2015; Peguero-Pina *et al.*, 2017; Sugiura *et al.*, 2020). This has been further confirmed by the analytical models based on anatomical traits (Tosens *et al.*, 2012*b*; Tomás *et al.*, 2013; Xiao and Zhu, 2017), that—although also based on several assumptions that are debatable, such as the actual value for wall porosity—predict g_m quite accurately (Tosens and Laanisto, 2018; Flexas and Carriquí, 2020) and emphasize the importance of T_{cw} to g_m limitation (Veromann-Jürgenson *et al.*, 2017, 2020; Han *et al.*, 2018; Carriquí *et al.*, 2019*b*). For its ease of measurement, ΔL is the most characterized CW property, with hundreds of measurements in different species and conditions. Although never proved experimentally, potentially ΔL could be assocaited with CW composition.

p is determined by the quantity of wall pores of sufficient size for the passage of CO_2. On the other hand, τ is related to path lengthening due to non-linearity in the diffusion path that CO_2 molecules must face due to the cellulose and hemicellulose microfibril entanglement (Burgert and Dunlop, 2011). These two properties are the least known and the most difficult parameters to determine due to their close intercorrelation. In fact, there are still no direct measurements for these properties and, in most approximations of CO_2 diffusion through walls, both are usually combined as a single parameter, p/τ (i.e. effective porosity) (Nobel, 2004; Terashima *et al.*, 2006; Evans *et al.*, 2009; Tosens *et al.*, 2012*b*; Tomás *et al.*, 2013). Based on simple physicochemical estimations, it was traditionally considered that effective porosity was large enough for g_{cw} not limiting g_m and photosynthesis (Nobel, 2004). Later, Terashima *et al.* (2006), considering also the relationship between g_m and the mesophyll anatomical traits, and studies on wall permeability to water on algae (Gutknecht, 1967) with extremely thick walls (Okuda *et al.*, 1997), suggested that p/τ might be actually limiting g_m and inversely proportional to T_{cw}. Evans *et al.* (2009), based on microscopy images showing pore size in onion (McCann *et al.*, 1990), also suggested the possibility that p/τ may be limiting g_m under certain conditions. Evans (2021) proposed that the important variability of $g_m/S_c/S$ for a given T_{cw} (see Fig. 2E) could be largely explained by differences in p/τ and membrane permeability. Thus, most analytical models of g_m have considered a variable p/τ closely related to T_{cw} (Tosens *et al.*, 2012*b*, 2016; Tomás *et al.*, 2013; Veromann-Jürgenson *et al.*, 2017, 2020), although Carriquí *et al.* (2020) proposed that this might not be the case in species with large T_{cw}. The underlying problem is that the assumption of one porosity or another can radically change the conclusions of the study (Evans, 2021). Nowadays, despite advances in tools to quantify p/τ (Liu *et al.*, 2019), complicated interactions among CW basic components and physical microenvironments such as polysaccharides, wall proteins, and apoplastic pH, a precise understanding of how these parameters interact and affect CO_2 diffusion (see 'Effects of cell wall composition on A_n and g_m' for further details) remains elusive.

Relationships among mesophyll conductance, cell wall thickness, and leaf water relations and hydraulics across species and phylogenetic groups

Historically, studies linking photosynthesis, g_m, and T_{cw} have been mostly dedicated to angiosperms (Flexas *et al.*, 2012). However, in recent years, measurements of a significant number of previously understudied gymnosperms (Veromann-Jürgenson *et al.*, 2017, 2020; Carriquí *et al.*, 2020) ferns and fern allies (Carriquí *et al.*, 2015; Tosens *et al.*, 2016), and mosses and liverworts (Carriquí *et al.*, 2019*b*; Coe *et al.*, 2019) have allowed the exploration of the relationships between A_n, g_m, and its anatomical drivers from a phylogenetic and evolutionary perspective. Photosynthetic capacity, A_n, and its underlying parameters [stomatal conductance (g_s), g_m, and biochemical capacity] display a progressive phylogenetic increase from bryophytes to angiosperms (Gago *et al.*, 2019). A similar increasing tendency is observed for S_c/S, while a decreasing tendency is observed for T_{cw} (Gago *et al.*, 2019). When all plant groups are pooled together, T_{cw} strongly correlates with g_m and A_n ($P<0.001$ Fig. 2A, B). On the other hand, a weaker but equally significant correlation between g_m and S_c/S is observed (Fig. 2C), despite the correlation between S_c/S and T_{cw} (Fig. 2D). In addition, the g_m/S_c is significantly decreased with increasing T_{cw}

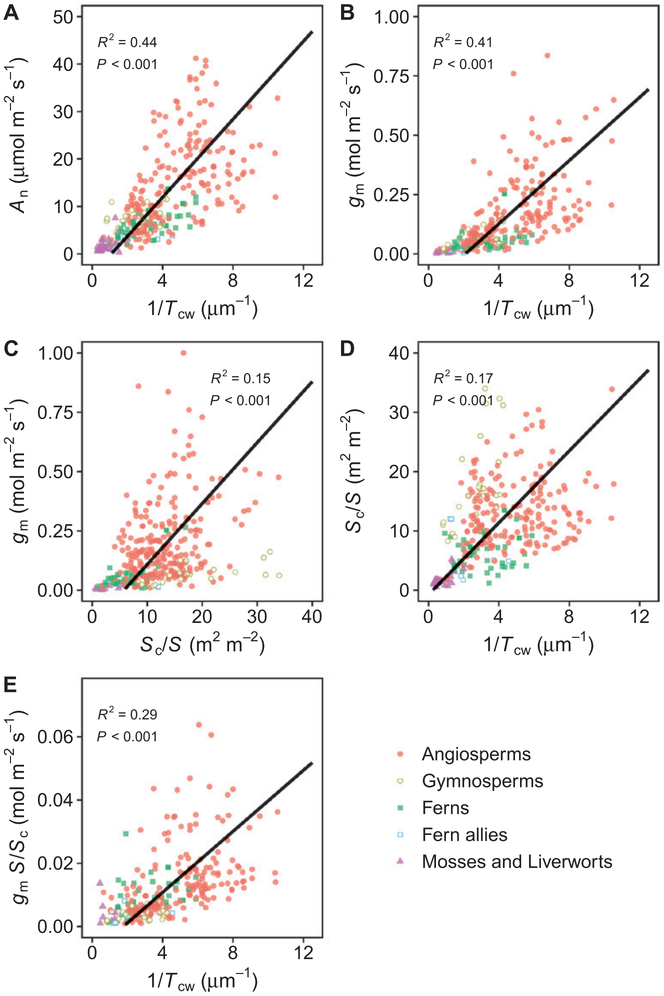

Fig. 2. Relationships among photosynthesis and anatomical traits in C_3 plants. Lines represent linear SMA fittings ($y=a\,x+b$) on data pooled across all phylogenetic groups. (A, $y=4.110\,x–4.504$) Net assimilation rate (A_n) and cell wall thickness (T_{cw}) (303 entries from 204 species). (B, $y = 0.067\,x–0.140$) Mesophyll conductance to CO_2 diffusion (g_m) and T_{cw} (280 entries from 183 species). (C, $y = 0.026\,x–0.148$) g_m and chloroplast surface area exposed to intercellular air spaces per leaf area (S_c/S) (327 entries from 201 species). (D, $y=3.032\,x–0.750$) S_c/S and T_{cw} (293 entries from 195 species). (E, $0.005\,x–0.008$) Mesophyll conductance expressed on a chloroplast surface area basis ($g_m\,S/S_c$) and T_{cw} (273 entries from 178 species). SMA fittings were performed using the function sma() in the 'smatr' R package. Data and references are available at Dataset S1 at Dryad.

(Fig. 2E) (Terashima *et al.*, 2011; Tomás *et al.*, 2013). While the correlations showed here may not necessarily implicate causality, they agree with the observations based on mechanistic g_m limitation analysis showing that S_c/S and T_{cw} are indeed the two strongest anatomical limitations to the maximum g_m achievable by a given species or genotype (Tomás *et al.*, 2013; Tosens *et al.*, 2016; Carriquí *et al.*, 2019*a*, *b*; Gago *et al.*, 2019; Veromann-Jurgenson *et al.*, 2020). T_{cw} and S_c/S may vary independently and compensate for each other to achieve substantial g_m in some cases, as described for Mediterranean *Quercus* species (Peguero-Pina *et al.*, 2017) and, to an extreme extent, in resurrection plants (Fernández-Marín *et al.*, 2020; Nadal *et al.*, 2021). These hardy species have been found to achieve similar or greater photosynthesis than their non-resurrection counterparts by breaking the often-reported trade-off between S_c/S and T_{cw}, so that they have a much larger S_c/S concomitant with larger T_{cw} than non-resurrection species. However, it has to be considered that, while T_{cw} can be easily determined from TEM images as a 2D trait, the 3D estimation of S_c/S is critical (Théroux-Rancourt *et al.*, 2017; Earles *et al.*, 2019; Harwood *et al.*, 2020). Indeed, the S_c/S values Dataset S1 available at the Dryad Digital Repository, https://doi.org/10.5061/dryad.qbzkh18gs) were derived from 2D images with broad assumptions regarding cell and chloroplast shapes. In this sense, the methodological issues in estimating S_c/S could perhaps partly contribute to the variation of g_m–S_c/S relationship between and within groups.

There is strong support for the concept that an increased photosynthetic rate was likely to be an important adaptive process contributing to the successful angiosperm radiation (Brodribb and Feild, 2010). The observed trajectory of increasing g_m due largely to decreasing T_{cw} (Gago *et al.*, 2019) fits well into this broader narrative, possibly explaining one of the primary limitations that delayed a transition from an ancestral low photosynthetic poikilohydric state (observed in most extant bryophytes), to a derived homeohydric high photosynthetic condition in the vascular plant lineage. Among the diverse morphological innovations that occurred during land plant diversification, such as stomata development, the hydrophobic cuticle, and the hydraulic system (Kenrick and Crane, 1997), evolution in T_{cw} has received little attention. This allows us to hypothesize and speculate about some evolutionary aspects that may deserve future experimental attention.

Concerning the general decline of T_{cw} along the phylogeny, from bryophytes to angiosperms, one interesting possibility is that a large T_{cw} may have been a fundamental constraint on the evolution of high A_n, and that T_{cw} could not be reduced until homeohydric features such as stomatal regulation of gas exchange and efficient vasculature had evolved. An explanation for this may be that desiccation tolerance (DT) requires thick CWs to prevent tissue damage during dehydration. DT is a prerequisite for terrestrial species with low vascular efficiency to carry out photosynthesis at humidities below 100% (Brodribb *et al.*, 2020). These plants can survive dehydration to <30% relative water content or water potentials < −100 MPa for extended periods (Alpert and Oliver, 2002; Gaff and Oliver, 2013). Bryophytes constitute the group of land plants with the highest percentage of studied desiccation-tolerant species, although desiccation-tolerant ferns and lycophytes are also common (Oliver *et al.*, 2005; Vitt *et al.*, 2014; Fernández-Marín *et al.*, 2016). There is evidence that species exposed to frequent changes in cell volume appear to require thick CWs with high elasticity preventing damaging leaf deformation upon dehydration/rehydration (Balsamo *et al.*, 2003). The occurrence of thick CWs in angiosperms that have recently evolved DT (as a homoplasy with DT in bryophytes) provides strong support for the idea that thick CWs are a prerequisite for DT (Perera-Castro *et al.*, 2020). If this is the case, it may be that a transition from DT and poikilohydry was a critical step to developing high A_n because the thin CWs required for high A_n are only effective where cell hydration is maintained within narrow limits. Assuming that this connection between T_{cw} and DT is constitutive, it is easy to envisage an evolutionary sequence in land plants whereby evolution in water relations (stomatal regulation of transpiration and efficient water transport) enabled a shift away from DT, enabling a decline in T_{cw} and an increase in g_m and A_n.

Homeohydry in most seed plants may enable cell T_{cw} to be much lower (and g_m to be much higher) than in early branching land plant clades, but it also adds an important water transport limitation on A_n. Evidence of this is clearly seen in the coordination between hydraulic conductance (K_{leaf}) and A_n among land plants (Brodribb *et al.*, 2007; Flexas *et al.*, 2013; Xiong *et al.*, 2017; Lu *et al.*, 2019; Xiong and Nadal, 2020), since water loss and CO_2 uptake share a common path at the leaf surface. The observed coordination between water supply and A_n across land plants broadly agrees well with the theoretical resource allocation in leaves based on optimality theory (Deans *et al.*, 2020), and can be observed in patterns of leaf vein evolution across the land plant phylogeny (Boyce *et al.*, 2009; Brodribb and Feild, 2010). Efficient water supply to leaves can enable high rates of water and CO_2 exchange across the epidermis enabling high A_n, but this could only happen with a parallel evolution of stomata on the leaf surface (Brodribb *et al.*, 2017). Early vascular plants are characterized by large relatively immobile guard cells, but evolution in guard cell size and shape occurred in parallel with leaf vascular systems such that modern angiosperms possess very dense arrays of small, highly mobile stomata (Franks and Beerling, 2009; de Boer *et al.*, 2012) and very dense leaf venation (Roddy, 2019), which together provide sufficient g_s to enable angiosperm leaves to profit from the high g_m that is allowed by low T_{cw}.

Dynamic changes in cell wall thickness and their effects on mesophyll conductance and photosynthesis

Photosynthetic CW thickness has been considered as a relatively static parameter with only interspecific variations (Evans *et al.*, 2009). However, recent studies demonstrate that T_{cw} changes very dynamically even in mature leaves (Table S1 at Dryad). The responses of g_m during acclimation to environmental changes can be explained by the variation of anatomical traits (e.g. T_{cw} and/or S_c/S), although physiological factors such as aquaporins and carbonic anhydrases can also be involved (Flexas *et al.*, 2006; Perez-Martin *et al.*, 2014; Groszmann *et al.*, 2017; Momayyezi *et al.*, 2020; Evans, 2021). In general, g_m variation in response to internal and external factors is not so tightly coupled with either T_{cw} or S_c/S as are the differences in the maximum g_m among species, seen in the previous section.

T_{cw} has been reported to change during leaf development (Niinemets *et al.*, 2012). After leaf emergence, T_{cw} increases, even after full expansion (Miyazawa and Terashima, 2001; Miyazawa *et al.*, 2003; Tosens *et al.*, 2012*a*; Borniego *et al.*, 2020; Carriquí *et al.*, 2021), and then it may remain stable or decrease with ageing in some species (Hanba *et al.*, 2001; Saito *et al.*, 2006). Effects of the age-dependent changes in T_{cw} on g_m and A_n vary greatly depending on growth environment, leaf life span, and plant species. In some trees, g_m increases regardless of the increase in T_{cw} possibly because S_c/S also increases during ontogeny (Hanba *et al.*, 2001; Miyazawa and Terashima, 2001; Tosens *et al.*, 2012*a*). On the other hand, some herbaceous plants show a marked decrease in g_m during ontogeny. For example, in tomato and soybean, T_{cw} increases by ~40–80% in leaves 10–14 d after full expansion, while S_c/S remains virtually unchanged, and g_m decreases (Berghuijs *et al.*, 2015; Sugiura *et al.*, 2020). An observed decrease in g_m in bean during leaf ontogeny (Miyazawa *et al.*, 2003) was due more to the decrease in S_c/S than to any change in T_{cw} (Sugiura *et al.*, 2020).

T_{cw} can also change according to light environment (Syvertsen *et al.*, 1995; Oguchi *et al.*, 2003; Gratani *et al.*, 2006; Fini *et al.*, 2016), although this might depend on species-specific light requirements. Generally, plants grown under high light conditions tend to show higher g_m without changing T_{cw}, which could be explained by an increase in S_c/S (Syvertsen *et al.*, 1995; Hanba *et al.*, 2002; Tosens *et al.*, 2012*a*; Peguero-Pina *et al.*, 2016). However, Fini *et al.* (2016) reported that three woody species of the Oleaceae family with different light requirements changed g_m and T_{cw} in a different manner when grown under 30% or 100% of full sunlight. While sun-requiring species increased g_m despite an increase in T_{cw}, shade-tolerant species decreased g_m despite unchanged T_{cw} and S_c/S. Whether light requirement is involved in the predominance of anatomical or biochemical components of g_m needs further confirmation.

The effect of CO_2 concentration during growth in leaf anatomy and g_m has been less frequently assessed. Teng *et al.* (2006) reported an increase in T_{cw}, cellulose, and pectin in Arabidopsis plants grown at elevated CO_2. Unfortunately, g_m was not determined in that study. However, Mizokami *et al.* (2019) did not observe a significative variation of g_m despite the increase of T_{cw} in response to high [CO_2] in this species. Using free-air CO_2 enrichment (FACE) technology, rice developed leaves with thicker CWs and decreased g_m, while in wheat higher [CO_2] resulted in unchanged T_{cw} and g_m (Zhu *et al.*, 2012). Another factor affecting T_{cw} is the sink–source balance, which varies interspecifically and depends greatly on environmental conditions. Sugiura *et al.* (2017) showed that the amount of CW materials accumulated in leaves was higher for radish plants grown under lower nitrogen and higher CO_2 conditions (i.e. lower sink), while it was lower in radish with larger hypocotyls (i.e. higher sink activity). Furthermore, soybean and bean showed coordinated increases in T_{cw} and decreases in g_m and A_n after a substantial decrease in sink activity by defoliation, which supports the idea that photosynthesis may be down-regulated by anatomical changes in response to changes in the sink–source ratio (Sugiura *et al.*, 2020).

In some studies, g_m decreases in response to long-term drought together with a general decrease in S_c/S and a slight increase in T_{cw} (Miyazawa *et al.*, 2008; Tosens *et al.*, 2012*a*; Galmés *et al.*, 2013; Tomás *et al.*, 2014; Han *et al.*, 2016; Ouyang *et al.*, 2017; Du *et al.*, 2019). However, intercultivar and interspecific differences in the response of S_c/S and T_{cw} to water stress have been observed. Ouyang *et al.* (2017) reported that many cultivars of rice showed drought-induced increases in T_{cw} and decreases in g_m without changes in S_c/S. Instead, wheat showed an increase in S_c/S under drought conditions (Ouyang *et al.*, 2017).

Effects of cell wall composition on g_m and A_n

Changes in CW components and their biochemical properties can modify CW thickness, conformation, and complexity, which could affect the CO_2 diffusion by physicochemical interactions through the CW (Le Gall *et al.*, 2015; Houston *et al.*, 2016; Clemente-Moreno *et al.*, 2019). Only recently, researchers have begun to study the role of CW composition on g_m using genetic approaches. Although some studies testing mutants with changes in their CW composition have reported reductions in the photosynthetic rate (Zhang *et al.*, 2020) and/or anatomical changes that alter mesophyll architecture and, potentially, leaf CO_2 diffusion (Weraduwage *et al.*, 2016), to the best of our knowledge only two studies have directly addressed the effect of CW composition on g_m. Firstly, Ellsworth *et al.* (2018) described a strong g_m reduction (83%) in mutants with disrupted synthesis and accumulation of the hemicelluloses (1,3)- and (1,4)-linked β-glucosyl polysaccharides, implying photosynthesis rate reductions of ~30–40%. This allowed them to propose that CW mixed-linkage glucans content improves p/τ in rice. However, a significant part of the g_m reduction with the mutation was due to pleiotropic effects affecting other anatomical traits (Evans, 2021), since at least 23% of the g_m constraint was caused by a reduction of S_c/S. However, other reduced parameters such as lower leaf mass area (LMA), leaf thickness, and T_{cw} could facilitate higher g_m, implying that g_m was at least partially constrained by altered CW composition. More recently, Roig-Oliver *et al.* (2020*c*) reported a g_m reduction related to diminished galacturonic acid amounts in Arabidopsis mutants with alterations in pectin acetyl- and methylesterases, although whether these mutations affected other key anatomical traits determining g_m was not assessed.

Besides genetic approaches, interspecific comparisons may also help in disentangling the role of CW components on g_m. Recently, Carriquí *et al.* (2020) performed an interspecific study in which seven conifer species were evaluated under non-stress conditions. The advantage of using conifers is that they lie on the quasi-flat region of the g_m to T_{cw} exponential decay, where variations of g_m are less dependent on variations of T_{cw} (Fig. 2). Although no significant correlation between CW components and A_n was found, probably because g_m was not the main limitation to photosynthesis in these species, hemicellulose and cellulose contents were negatively correlated with g_m. Also, while pectin content itself was not related to g_m, the pectin to cellulose and hemicellulose ratio strongly correlated with g_m. In another rercent study in rice (Ye *et al.*, 2020), genotypes with higher LMA also presented greater leaf thickness and T_{cw}, and lower g_m. In this study, the amount of celluloses, hemicelluloses, and pectins also scaled positively with LMA and T_{cw}, while the pectin to cellulose and hemicellulose ratio declined (Ye *et al.*, 2020). Hence, in rice, co-variation of T_{cw} and CW composition makes it difficult to establish whether variation in g_m is due only to T_{cw}, composition, or an interaction of both.

Additional evidence for a possible effect of CW composition on g_m and A_n arises from studies of photosynthesis response to abiotic stress. In tobacco exposed to salinity and drought, pectins showed a significant negative relationship with both A_n and g_m, whilst the hemicellulose/pectin ratio correlated positively with both g_m and A_n, in contrast to the cellulose/hemicellulose ratio, which correlated negatively with g_m. Interestingly, these changes were associated with the activity of antioxidant apoplastic enzymes linked to CW alterations as well as with specific CW primary metabolites such as galactose, glucosamine, and hydroxycinnamate, which were exclusively correlated with g_m and not with other photosynthetic traits (Clemente-Moreno *et al.*, 2019). In grapevines subjected to stresses including high and low temperature and water stress, it was observed that stress-induced variations in cellulose concentrations were negatively related to changes in g_m and A_n (Roig-Oliver *et al.*, 2020*b*). In sunflower plants acclimated to different water availability conditions, lignin accumulation was significantly associated with g_m reductions (Roig-Oliver *et al.*, 2020*a*). Interestingly, the same study showed that the proportion between cellulose and hemicelluloses to pectins was positively correlated with changes in T_{cw}. Altogether, these results indicate that abiotic stresses provoke species-dependent changes in CW composition. These affect the different main CW compounds, so that the relative proportions between them varies, probably altering the physicochemical CW environment and affecting CO_2 diffusion. In addition, minor CW compounds have also been suggested to affect photosynthesis. For instance, in *Triticale*, increased amounts of CW-bound phenolics, especially ferulic acid, resulted in increased photosynthesis (Hura *et al.*, 2009, 2012), whilst in sunflower coumarate content in CWs was negatively correlated with g_m (Roig-Oliver *et al.*, 2020*a*).

Hypothetical mechanism for the influence of cell walls on photosynthesis

As discussed in the previous sections, both T_{cw} and chemical composition (which in turn also potentially determines T_{cw}) appear to be involved in setting and perhaps regulating g_m. However, the empirical evidence concerning both factors is limited. The correlation between g_m and T_{cw} is strong regardless of the phylogenetic groups considered (Fig. 2A), as might be expected due to the central position of T_{cw} in the theoretical equation for g_m. For CW composition, instead, data are still scarce and discrepancies appear between studies. These apparent discrepancies are likely to reflect, on one hand, the different and complementary roles of thickness and composition in setting g_m, and, on the other, that the system is complicated and some CW components (e.g. pectins) play a variety of roles in the physicochemical properties defining diffusive conductance of the CW (g_{cw}, a major component of g_m). As a result, these may potentially act in opposite directions and/or have pleiotropic effects.

What is the position of CW components in the equation of g_{cw}? We suggest that CW composition—with a major role for pectins and/or the proportion between pectins and other compounds—determines p and, very probably, τ, yet in a complex manner (Fig. 3). If so, CW composition (even a single compound) may have effects on both the numerator and the denominator of the g_m equation, and in a non-proportional way, meaning that strong simple correlations between g_m and the concentration of any single CW component is unlikely.

Among principal CW compounds, pectins are often considered the master regulators of p (Ochoa-Villareal *et al.*, 2012; Bidhendi and Geitmann, 2016; Cosgrove, 2016; Novakovic *et al.*, 2018). While the general view is that pectins decrease porosity, we argue that p/τ could instead be positively related to an increased fraction of pectins, based on experiments in which pectin degradation results in larger molecules capable of traversing the wall (Baron-Epel *et al.*, 1988; Fleischer *et al.*, 1999). This is because pectins exhibit hydrocolloid properties and can bind several times their own volume of water (Panchev *et al.*, 2010; Schiraldi *et al.*, 2012). Because CO_2 is a very small molecule as compared with average CW pore sizes (Carpita *et al.*, 1979), slight reductions of p may not affect its diffusion significantly. However, as CO_2 diffuses in solution, it might be the hydrophilic fraction of the pore which affects its diffusion. In this sense, pectins may increase what could be called the 'effective porosity to water and CO_2'. Regardless of these speculative thoughts about the effect of pectin abundance on p, there is no doubt that increased pectin content modifies p due to interactions with other molecules (Fig. 3).

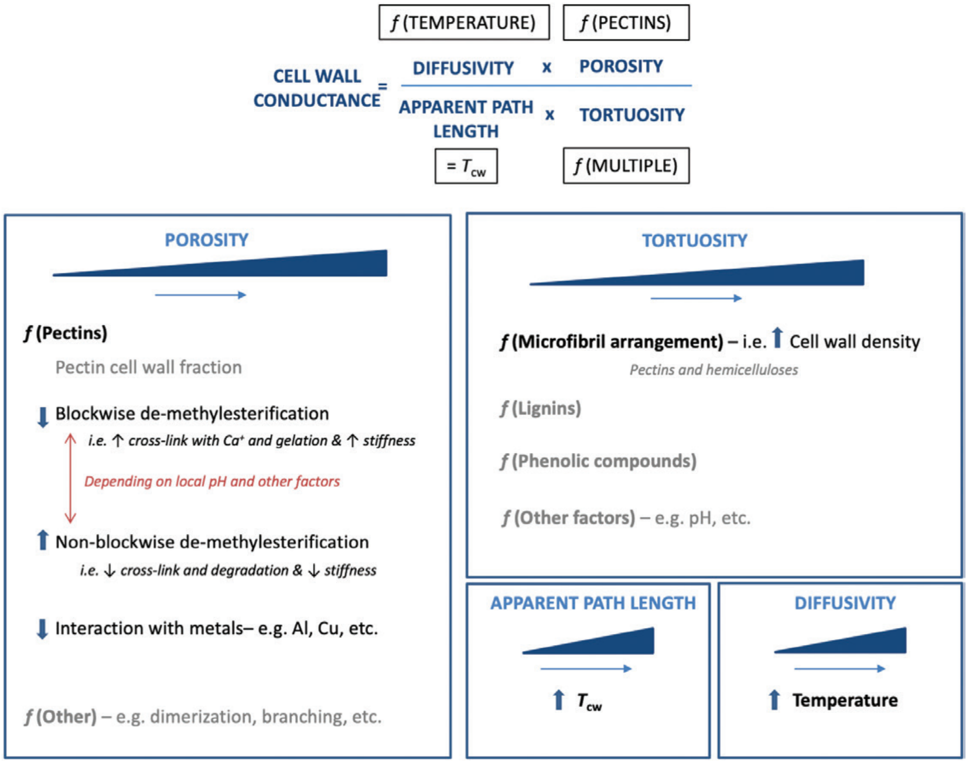

Fig. 3. Summary of the hypothetical mechanisms for the effects of cell wall properties on cell wall conductance. The relationship between the four properties determining cell wall conductance (g_{cw}) is displayed at the top of the diagram. Below the equation, the physicochemical factors that determine (black) or potentially determine (grey) each cell wall property are detailed. Arrows indicate the known or suggested direction of influence of each factor.

The action of pectin-remodelling enzymes (PREs) promotes changes in pectin physicochemical properties, which modify the pectin matrix status as well as pectin interaction with other CW components, thus determining CW characteristics (Pelloux *et al.*, 2007; de Souza and Pauly, 2015). In particular, the degree of pectin methylesterification is also an important feature that affects *p*. However, paradoxically, de-esterification has been related to both increased and decreased *p* and CW stiffness (Bidhendi and Geitmann, 2016). Apparently, the effect depends on how pectin de-esterification occurs. Basically, there are two mechanisms of de-esterification, blockwise and non-blockwise. Blockwise de-esterification results in a continuous region of de-esterified pectin, which allows better cross-linking of pectins with Ca^{2+} bonds, resulting in gelation, which increases wall stiffness and, presumably, decreases *p*. On the contrary, non-blockwise de-esterification pectin methylesterases (PMEs) act discontinuously, resulting in reduced cross-linking of pectins with Ca^{2+} and also facilitating pectin degradation by polygalacturonases, hence resulting in wall softening and increased porosity (Bidhendi and Geitmann, 2016). The occurrence of blockwise or non-blockwise de-esterification depends on which specific PMEs are involved, which in turn depends on pH, initial stage of methylesterification, and cation concentration (Osorio *et al.*, 2008). This implies that *p* may not even be constant through the T_{cw}, since all these parameters can change from one side to another in the apoplast. For instance, a pH gradient may occur from slightly acidic apoplast to weakly alkaline symplast, thus through the CW pathway HCO_3^- will tend to increase in concentration assuming that there is an equilibrium between CO_2 aqueous and HCO_3^- (Evans *et al.*, 2009). Apoplast redox status and the activity of several antioxidant apoplast enzymes such as peroxidases (POXs) varies depending on stress conditions (O'Brien *et al.*, 2012; Tenhaken, 2015). Under stress, apoplast water pH tends to alkalinize (Geilfus, 2017), reducing the pH gradient with the symplast, and at equilibrium $[HCO_3^-]/[CO_{2aq}]$, the anion HCO_3^- will increase in concentration relative to CO_2 along the apoplast and CW.

Besides methylesterification, other factors have been related to *p*. For instance, interaction of pectins with ion metals such as aluminium or copper is known to reduce *p* and affect the hydraulic conductivity of the pectic matrix, and pectin dimerization and branching have also been suggested to affect *p* somehow (Bidhendi and Geitmann, 2016). In addition, the apoplast redox status will be modified by the role of reactive oxygen species (ROS) and antioxidant apoplastic enzymes, which are also involved in modifying CW chemistry, especially, in response to stress (Tenhaken, 2015). Apoplastic ROS and their related enzymes are linked to different CW modification processes such as cell elongation, lignin and suberin formation, and cross-linking of CW components directly influencing the cell stiffening/loosening properties (Schmidt *et al.*, 2016). The large complexity of mechanisms potentially involved in pectin-dependent regulation of *p* may explain why correlations with g_m are not as clear as those observed between g_m and T_{cw}, but a hypothetical understanding of the action of these compounds will help in elucidating the precise mechanisms in future studies.

Concerning τ, this might be mostly related to the specific arrangement of cellulose and hemicellulose fibrils, which may strongly depend on hemicelluloses and pectins because these bind covalently among them (Burgert and Dunlop, 2011). It may also depend on phenolic compounds and lignins, because these also form covalent bounds with hemicelluloses (Burgert and Dunlop, 2011; Anderson and Kieber, 2020) and increase CW hydrophobicity (Niklas *et al.*, 2017), which might be relevant as CO_2 diffuses dissolved in water. Indeed, lignins have been shown to negatively correlate with g_m in one study (Roig-Oliver *et al.*, 2020*a*). Additionally, the role of structural proteins can also be an extra factor to be evaluated as, depending on species, they can range from 1% to 10% of the total CW, modifying CW properties in response to abiotic and biotic stress (Olmos *et al.*, 2017) which may also affect CO_2 diffusion through the CW.

It is worth saying that the physical equation and terms that we use in this section (conductance, porosity, and tortuosity) rely on the assumption that CO_2 moves across CWs in a simple, non-facilitated diffusion manner—following Fick's first law. However, this may not necessarily be the case, and chemical interactions between CO_2 molecules—or water molecules where CO_2 is dissolved—and wall components may eventually occur inside the CW pores (Terashima *et al.*, 2011). If so, the actual τ would be a combination of the 'physical' τ (i.e. the twisting of the path length due to the non-linear position of the pores across the fibre matrix) and the 'chemical' τ (i.e. any kind of chemical interactions between CO_2 and CW compounds that could slow CO_2 diffusion). Rondeau-Mouro *et al.* (2008) reported that the aqueous phase diffusion in Arabidopsis CW residues (i.e. CWs in which pectins, proteins, and starch have been removed mechanically) approximates that in free water. For this reason, for several years, chemical τ, also referred as r_f [i.e. a dimensionless factor that accounts for the reduction of *D* compared with free water (Weisiger, 1998)], has been considered negligible in studies in which mesophyll conductance was modelled (Tosens *et al.*, 2012*a*, 2016; Tomás *et al.*, 2013; Carriquí *et al.*, 2019*b*). However, phenolic compounds, one of the potential components of this chemical τ, have been shown to negatively correlate with g_m in Roig-Oliver *et al.* (2020*a*) and with A_n in a multispecies comparison (Sumbele *et al.*, 2012), and, in general, any other compound capable of establishing molecular interactions such as transient van der Waals bonds with diffusing water and/or CO_2 itself may significantly reduce chemical τ; that is, increase r_f.

Such potential chemical interactions between water, CO_2, and CW composition have not been investigated *in vivo* in plants, but they are known and used in industrial procedures related to phenol transformation, cellulose-derived materials, etc. (Luo *et al.*, 2016; Gunnarsson *et al.*, 2017, 2018). Local conditions across the apoplast would also determine the chemical τ because, as CO_2 diffuses in water, the temperature determines its solubility and the pH determines its chemical form as carbon dioxide or bicarbonate (Terashima *et al.*, 2011). We suggest that studying these interactions *in planta* deserves future research as they are potentially key, but unknown, regulators of CO_2 diffusion across CWs.

Prospects for improving photosynthesis by cell wall manipulation

Up until recently, most of the attempts to improve photosynthesis through anatomical changes have been focused on stomatal traits such as size, shape, and density (Gago *et al.* 2020*b*, and references therein), mesophyll cell density, distribution, and cell to cell contact (Masle *et al.*, 2005; Takai *et al.*, 2013; Lehmeier *et al.*, 2017), and S_c/S (Tholen *et al.*, 2008; Li *et al.*, 2013; He *et al.*, 2017). This topic has been reviewed by Tholen *et al.* (2012) and Ren *et al.* (2019). However, biotechnological attempts to specifically study the relationship between CW composition and g_m (and, thus, A_n) have been scarce.

Based on the evidence discussed in the sections above, the most obvious ways to increase g_{cw} would be achieving lower T_{cw} and increasing p/τ. Unfortunately, the genes regulating these properties are unknown, and changing a single gene can have important pleiotropic consequences. The genes regulating the metabolic pathways of the major CW compounds and enzymatic activities related to their synthesis and degradation would directly affect leaf expansion, growth, cell density, and cell to cell contact (Tenhaken, 2015). However, knowledge of how those alterations could also affect CO_2 diffusion through differential physicochemical interactions in the CW matrix remains unclear (Clemente-Moreno *et al.*, 2019). Molecular strategies focused on altering any of these components could have huge consequences on the highly coordinated network to sustain CW functionality. For instance, virus-induced gene silencing (VIGS) of CESA1 and CESA2 (genes encoding enzymes catalysing cellulose synthesis) in *Nicotiana benthamiana* showed strong reductions of cellulose, but this alteration promoted PME gene regulation, avoiding CW weakness by a reduction in the PME degree accompanied by an increase in Ca^{2+} cross-linkages (Burton *et al.*, 2000; Weraduwage *et al.*, 2018).

Because of the pivotal role of the cell in plant physiology, any alteration in CW genetics would have dramatic effects on the plant's anatomy and performance (Weraduwage *et al.*, 2018), making it more difficult to use CW mutants to establish specific cause–effect relationships between cell composition affecting g_{cw}. So, the question emerges: how can we disentangle the CW changes that affect general plant physiological status (and also their photosynthetical capacity) from those specifically altering CO_2 diffusion?

p, τ, and intrinsic physicochemical properties interacting with the CO_2 diffusion will mostly depend on the matrix network developed by the major polysaccharide compounds, glycoproteins, and phenolic compounds, defining both the final CW conformation and general leaf anatomy (thus, affecting g_m) (Ren *et al.*, 2019). Reductions in cellulose content will drive strong effects in the CW and weaken it. In order to strengthen the weakened CW, increased levels of pectins (esterified and unesterified, with increased Ca^{2+}-mediated cross-linkage with xyloglucan and extensins), reduced levels of arabinogalactan proteins (AGPs), and changes in extensins have been observed (Burton *et al.*, 2000; Mélida *et al.*, 2009, and references therein). Additionally, these changes in CW profile can be accompanied by general mesophyll rearrangements—T_{cw} and cell elongation—as observed in the cellulose-defective mutants AtCesA7 in Arabidopsis (Zhong *et al.*, 2003), that could also affect g_m (Evans *et al.*, 2009; Evans, 2021).

Despite the key role in CW modifications described for pectins (Tenhaken, 2015), just a few studies focused on how altering PREs, including PMEs and pectin acetylesterases, could affect photosynthesis. Weraduwage *et al.* (2016) showed that Arabidopsis mutants with reduced and overexpressed levels of PME showed minor differences in their A_n. Contrarily, Roig-Oliver *et al.* (2020c) tested other Arabidopsis mutants with alterations in pectin acetyl and methylesterases, and showed that reductions in both A_n and g_m were paralleled by a diminished pectin amount.

In summary, the CW is a highly coordinated molecular and physiological network that performs an essential role in cells, and any change in its composition dynamically activates a response to compensate and sustain their main functionality from genes to enzymatic activity (Burton *et al.*, 2000; Weraduwage *et al.*, 2018). These changes will affect not just its intrinsic composition, but also a general rearrangement of leaf anatomy that will also affect leaf gas exchange and plant growth (Weraduwage *et al.*, 2016, 2018). Indeed, most studies show that relationships with g_m are significantly stronger when considerd as ratios among all the major compounds rather than with any single compound, indicating a complex interaction that could affect p/τ (Clemente-Moreno *et al.*, 2019; Carriquí *et al.*, 2020; Roig-Oliver *et al.*, 2020*b*, *c*).

Before altering CW CO_2 diffusion properties using biotechnological approaches becomes feasible, further efforts are required to elucidate how the different interactions between CW compounds, including structural proteins, can affect g_{cw}. Unfortunately, CW mutants often display strong phenotypes due to the essential role of the CW in plant physiology. Specific CW-inducible transformants become highly desirable to avoid dramatic phenotypes that can alter the whole-plant functioning, complicating specific examination of the effect of CW composition on g_m and A_n. In parallel, additional technologies should be employed to further characterize CW properties, including immunolocation assays to determine the position and distribution of their main components, precise CW characterization from its main monomers to its principal components and their conformations, and ultrastructural *p* determination, among others, in combination with gas exchange measurements.

Concluding remarks

In addition to their multiple and recognized functions in plant cells, there is now ample evidence for a role for CWs in g_m and photosynthesis. It has been shown that a phylogenetic trend exists from thick CWs and low photosynthetic capacities in bryophytes to thin walls and high photosynthesis in angiosperms. Besides thickness, an emerging role of CW composition in g_m is highlighted. While the role of thickness is well understood, further studies are required to better understand the mechanisms by which changes in composition may affect CO_2 diffusion. We hypothesize that these effects might be multiple and complex, complicating efforts to disentangle the particular role of each compound. Yet with current perspectives, we can hypothesize a major role for pectins and/or the pectin to cellulose plus hemicellulose ratio. More genetic approaches are needed to investigate the possibility of improving crop photosynthesis by means of CW manipulation. While already presenting thin CWs, which may preclude improvement, the possibility of improving crop photosynthesis by manipulating CW composition has yet to be evaluated. In addition, such studies should also evaluate the possibility that CW-related improvement of photosynthesis could have detrimental effects on plant water relations, plant tolerance to water stress, and/or any of the other fundamental functions that CWs have in plant cells.

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Author contributions

JF and MC: conceptualization; MC, AVP-C, MJC-M: data compilation; MC and MN: data analysis. JF and MJC-M: homogenization of language and style between the sections; JF, MJC-M, JB, TJB, JG, YM, MN, AVP-C, MR-O, DS, DX, and MC: writing, revision, and approval of the final manuscript.

Data availability

Dataset included in the figures of this review are available at the Dryad Data Repository: https://doi.org/10.5061/dryad.qbzkh18gs (Flexas *et al.*, 2021). Dataset S1. Photosynthetic and anatomical data compilation from previously published papers.

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