Elastic and collapsible: current understanding of cell walls in succulent plants

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Highlight

Although cell walls of succulent plants are a key aspect of how they function, they remain largely unexplored, yet they hold potential for climate change mitigation.
Abstract/Summary

Succulent plants represent a large functional group of drought-resistant plants which store water in specialized tissues. Several co-adaptive traits accompany water-storage capacity to constitute the succulent syndrome. A widely reported anatomical adaptation of cell walls in succulent tissues allows them to fold in a regular fashion during extended drought, thus preventing irreversible damage and allowing for reversible volume changes. Although ongoing research on crop and model species continuously reports the importance of cell walls and their dynamics in drought resistance, cell walls of succulent plants have received relatively little attention to date, despite the potential of succulents as natural capital to mitigate the effects of climate change. In this review, we summarize the current knowledge of cell walls in drought-avoiding succulents and their effects on tissue biomechanics, water relations and photosynthesis. We also highlight the existing knowledge gaps and propose a hypothetical model for regulated cell wall folding in succulent tissues upon dehydration. Future perspectives of methodological development in succulent cell wall characterization, including the latest technological advances in molecular and imaging techniques, are also presented.

Keywords

Cell wall composition; cell wall folding; cell wall remodelling; collapsible cell walls; drought avoidance; plant cell walls; plant glycomics; polysaccharides; succulent plants
Abbreviations

Cell wall polymers
AGP       Arabinogalactan protein
CWSP      Cell wall storage polysaccharide
HG        Homogalacturonan
RG-I      Rhamnogalacturonan I
RG-II     Rhamnogalacturonan II
DM        Degree of methyl-esterification (of homogalacturonan or pectin in general)

Physiological parameters
C         Hydraulic capacitance
ε         Volumetric modulus of elasticity
gi        Internal conductance to CO\textsubscript{2} (in photosynthetic tissues)
K         Hydraulic conductance: \( K_{\text{tissue/organ}} = (K_{X}^{-1} + K_{OX}^{-1})^{-1} \) (see Sack and Scoffoni, 2013)
K\textsubscript{X}     Xylem hydraulic conductance
K\textsubscript{OX}    Outside-xylem hydraulic conductance
Ψ         Water potential; simplified formula: \( \Psi = \Psi_{p} + \Psi_{s} \) (see Taiz et al., 2014)
Ψ\textsubscript{p}    Pressure potential, hydrostatic potential or turgor pressure
Ψ\textsubscript{s}    Solute or osmotic potential
Introduction

With their peculiar appearance and their capacity to thrive under extreme conditions, succulent plants have long captivated botanists and plant enthusiasts (Eggli, 2017). Drought-avoiding succulent plants store water in living cells for later remobilization, which renders them temporarily independent of external water supply (see Box 1; Eggli and Nyffeler, 2009; Griffith and Males, 2017). Water-storage capacity in succulents is usually accompanied by several co-adaptive traits, such as certain xeromorphic features and different degrees of crassulacean acid metabolism (CAM), so that succulence emerges as a complex adaptive syndrome (Ogburn and Edwards, 2010; Winter et al., 2015; Males, 2017). The link between succulence and CAM is an ongoing debate: succulence has long been regarded as a pre-requisite for CAM, and succulence and strong CAM are highly correlated (Kluge and Ting, 1978; Sayed, 2001; Lüttege, 2004), but it remains unclear whether the co-occurrence of CAM and succulence is due to mutual facilitation or just a result of co-selection under similar selective pressures (Ogburn and Edwards, 2010; Heyduk et al., 2016; Edwards, 2019; Leverett et al., 2021). Succulence and its co-adaptive traits have evolved in numerous lineages across the plant tree of life (Fig. 1; Nyffeler and Eggli, 2010; Edwards, 2019). Among photosynthetic succulent organs, a widely used functional classification coined by Ihlenfeldt (1985) considers two types of succulence: all-cell succulence (e.g. Crassulaceae; Fradera-Soler et al., 2021), with all cells both performing photosynthesis and storing water, and storage succulence (e.g. Aloe, Asphodelaceae; Ni et al., 2004a), with a functional demarcation between photosynthetic tissue (i.e. chlorenchyma) and water-storing tissue (i.e. hydrenchyma). In reality, the anatomical diversity of succulent organs is even larger when considering the intermediate states between all-cell and storage succulence and the various arrangements of hydrenchyma and chlorenchyma within an organ. The term “succulent tissue” is usually applied to those tissues in succulent organs responsible for water storage, which are comprised primarily of highly vacuolated parenchyma cells with thin, elastic primary cell walls (Kluge and Ting, 1978; Gibson and Nobel, 1986; von Willert et al., 1992). Thus, “succulent tissue” may refer specifically to the hydrenchyma in a storage succulent or to all parenchyma cells in an all-cell succulent organ.

Across the plant tree of life, variation in cell wall structure and composition governs plant morphology and physiology and has undoubtedly played a crucial role in the adaptation to different evolutionary pressures (Sarkar et al., 2009; Sørensen et al., 2010). Primary cell walls are complex and dynamic systems capable of deformation due their intrinsic viscoelasticity (Niklas, 1992; Braybrook et al., 2012; Cosgrove, 2018). They are composed of three coextensive polymeric networks: (1) a tension-bearing cellulose-hemicellulose network, (2) a water-retentive, gel-forming pectin network, and (3) a structural protein network (Fig. 2; Cosgrove, 2005; Albersheim et al., 2011; Carpita et al., 2015). Hemicelluloses, pectins and structural proteins are highly diverse, and differing abundance and arrangement of these components result in contrasting cell wall characteristics (Showalter, 1993;
Willats et al., 2001; Harholt et al., 2010; Scheller and Ulvskov, 2010). These characteristics can be modified through cell wall remodelling, which affects cell wall structure and/or composition (see Box 2). Some cell wall polysaccharides, known as cell wall storage polysaccharides (CWSPs), appear to have been evolutionarily repurposed for storage and other functions across several plant lineages (see Box 3).

Cell wall properties are expected to be decisive in overcoming the alleged biomechanical and physiological challenges posed by the succulent syndrome. Besides being involved in mechanical support, cell walls in succulent tissues are capable of folding, which allows for reversible volume changes of succulent organs during dehydration/rehydration cycles while preventing catastrophic cell collapse and irreversible damage (von Willert et al., 1992; Christensen-Dean et al., 1993; Mauseth, 1995; Burgoyne et al., 2000; Bobich and North, 2009). Secondly, cell walls are the gas-liquid interface in the parallel processes of CO₂ diffusion and water movement in photosynthetic organs, thus influencing the interplay of factors linked to photosynthesis limitation (Barbour, 2016; Gago et al., 2019). Therefore, water relations and CO₂ uptake in succulents are expected to be tightly controlled by cell wall characteristics (Flexas et al., 2013; Xiong et al., 2017; Xiong and Nadal, 2020). Despite the general assumption that cell wall characteristics play a pivotal role in the succulent syndrome, cell walls of succulent plants have received little research attention to date. Studies have been hampered by the challenges of applying standard histological and biochemical techniques to water-rich tissues, which often demand methodological modifications in order to investigate succulent tissues (e.g. Ahl et al., 2018; Mozzi et al., 2021).

Increasing surface temperature and expanding aridity in many parts of the world (IPCC, 2007) exacerbate the need for deeper insights into the mechanisms of drought resistance and water management in plants. CAM-performing succulent plants have been identified as natural capital to mitigate the effects of climate change (Grace, 2019), including the possibility of engineering CAM into crops (Borland et al., 2014; Yang et al., 2015). However, while several succulence-related traits will probably allow many succulent groups to better withstand future climatic conditions (Willis, 2017), other succulent taxa are facing a high risk of extinction (Goettsch et al., 2015; Guo et al., 2016; Young et al., 2016). A better understanding of the mechanisms underlying the succulent function would reaffirm the role of succulent plants as natural capital and would help to promote conservation efforts. This review focuses on the current knowledge of cell walls in drought-avoiding succulent plants and their influence on the function of the succulent syndrome, and highlights the knowledge gaps in these topics. Future perspectives of the characterization of cell walls in succulents and its challenges are also presented.
Functional relations between cell wall components and responses to drought

Cell wall responses to drought and other abiotic stresses, most of which involve differential gene expression leading to cell wall remodelling, have been widely studied and reviewed in crop and model plants (Le Gall et al., 2015; Tenhaken, 2015; Ezquer et al., 2020). These acclimation processes highlight the importance of cell walls in drought resistance and can also hint at cell wall adaptations in succulents which may have shaped their evolution into drought-prone habitats.

Since the highly labile pectin network strongly influences many interrelated cell wall properties (e.g. thickness, porosity, hydration, elasticity), changes in pectin are likely crucial to drought-induced cell wall remodelling (Harholt et al., 2010; Braybrook et al., 2012; Levesque-Tremblay et al., 2015; Bidhendi and Geitmann, 2016). The nature of pectin gels is determined, at least partially, by the degree of methyl-esterification (DM) of homogalacturonans (HGs), which is regulated by pectin methylesterases (PMEs), resulting in the formation of either “strong” gels that stiffen the cell wall or “weak” gels that soften it (Hocq et al., 2017). Other pectin-modifying enzymes, such as pectin acetyltransferases (PAEs), polygalacturonases (PGs) and pectate lyases (PLs), also influence the properties of the pectin matrix. Xyloglucan, the most abundant hemicellulose in primary cell walls of spermatophytes, is targeted by xyloglucan endo-transglycosylases/hydrolases (XTHs), which can perform two different catalytic activities and either strengthen or soften the cell wall (Eklöf and Brumer, 2010; Scheller and Ulvskov, 2010; Nishikubo et al., 2011). Contrasting regulation patterns in response to drought have been reported among pectin- and xyloglucan-modifying enzymes (Pelloux et al., 2007; He et al., 2009; Clauw et al., 2015; Nguyen et al., 2016; Xuan et al., 2016), which highlights the complex relationship between these enzymatic activities and cell wall properties. On the other hand, drought stress has been strongly linked to upregulation of a large portion of expansin isoforms (Harb et al., 2010; Chen et al., 2019, 2020; Jin et al., 2020), which suggests that adjustments of cell wall loosening and extensibility are general responses against drought.

Pectin gel properties are also determined by rhamnogalacturonan I (RG-I), whose side chains influence cell wall hydration and elasticity (Willats et al., 2001; Harholt et al., 2010). Drought stress has been associated with an increase in the amount of arabinan, galactan and arabinogalactan RG-I side chains (Leucci et al., 2008; Gribaa et al., 2013). Due to the high mobility of RG-I arabinans and galactans in the cell wall, they have been postulated as cell wall plasticizers which maintain the fluidity of the pectin network and stabilize the cell wall during dehydration and rehydration (Harholt et al., 2010). This is a particularly relevant feature for cells that undergo drastic changes in shape as water is lost during drought. Structurally highly complex rhamnogalacturonan II (RG-II) side chains, which are thought to provide mechanical strength to the cell wall by forming borate cross-links.
(O’Neill et al., 2004), also seem to increase in number in response to drought stress (Leucci et al., 2008), although the interpretation of this response is not as clear since the exact physiological role of RG-II is still relatively unknown.

Drought stress has also been associated with upregulation of arabinogalactan proteins (AGPs; Cui et al., 2012; Mareri et al., 2019). Periplasmic AGPs, many of which are anchored to the plasma membrane, seem to occur in a reticulate pattern along the external face of the cell membrane, where they help to maintain the membrane-cell wall continuum by interacting with cell wall components (Gens et al., 2000; Liu et al., 2015). Given that this continuum can be compromised during abiotic stress, the upregulated AGPs are believed to form a “buffer zone” which stabilizes the membrane by preventing its direct interaction with the cell wall (Lamport et al., 2006). Indeed, a decrease in AGP epitopes and their rearrangement have been linked to the disruption of the membrane-wall continuum in senescing fruits (Leszczuk et al., 2020). AGPs have also been postulated as cell wall plasticizers (Lamport et al., 2006) and may be performing a similar role to that of the aforementioned RG-I side chains during dehydration. Another cell wall structural protein, extensins, are generally thought to form self-assembling scaffolds which strengthen the wall (Cannon et al., 2008). However, gene expression studies have given contrasting results regarding the regulation of different extensin genes upon drought (Molina et al., 2008; Cevher-Keskin, 2019), which suggests that different extensin isoforms may be performing different functions in the cell wall. Several functions of cell wall structural proteins and their involvement in drought response remain largely hypothetical, which presents many research opportunities.

**Structure and function of cell walls in succulents**

**Biomechanics**

Succulent organs tend to have a low surface-area-to-volume ratio to minimize water loss and enhance water storage (Males, 2017), but the considerable weight of stored water poses a biomechanical problem. Cell walls in succulent organs are thus expected to have inherent mechanical properties allowing for efficient mechanical support. Small, globose or prostrate succulent plants possess succulent organs mostly lacking support tissues, which is the case for the leaves of Aizoaceae, Crassulaceae and succulent Asteraceae, and the stems of small members of Cactaceae and some succulent Asteraceae and Asclepiadoideae (Apocynaceae; Gibson, 1996; Ogburn and Edwards, 2010). High cell turgor pressure in these succulent organs generates high hydrostatic pressure and provides most of the mechanical support (Niklas, 1992; Gibson, 1996; Bobich and North, 2009), which also makes them capable of drastic shrinking upon drought (Mauseth, 2006). As a remarkable exception, despite their relatively large size, succulent leaves of *Aloe* and closely related genera lack support tissues and are also primarily supported by hydrostatic pressure on a reinforced epidermis (Gibson, 1996).
However, most large succulent organs usually exhibit support tissues, such as hypodermis, fibres, and wood and bark from secondary growth (Blunden, 1973; Koller and Rost, 1988a; Mauseth, 2004a, b, 2006). There has been a growing interest in the support tissues and their cell walls in certain succulent lineages due to their adaptive and evolutionary relevance or their useful applications, such as the different types of wood of Cactaceae (Vázquez-Sánchez et al., 2017; Reyes-Rivera et al., 2018; Maceda et al., 2019) and the sclerenchyma fibres of Agave (Asparagaceae; Ferreira et al., 2014; Hidalgo-Reyes et al., 2015). Despite having support tissues, most large succulent plants are still capable of a high degree of volume change, which may be facilitated by morphological adaptations such as ribs in many Cactaceae and succulent Apocynaceae and Euphorbiaceae (Gibson and Nobel, 1986; Nobel, 1988; Felger and Henrickson, 1997; Eggli and Giorgetta, 2020). Most succulents undergo successive cycles of dehydration and rehydration following external water availability, which is reflected in shrinking and swelling of their succulent organs as the water stores are emptied and refilled (Gibson and Nobel, 1986; von Willert et al., 1992). Even in large succulents with support tissues, turgor pressure still plays an important role in mechanical support compared with non-succulent plants (Schulte et al., 1989; Bobich and North, 2009).

Since drastic volume changes of succulent organs can compromise tissue function, succulent taxa capable of extreme shrinking often exhibit secondary cell wall thickenings which provide structural support during dehydration and restrict the shrinkage direction of cells. In the notoriously drought-resistant genus Sansevieria (syn. Dracaena, Asparagaceae), many species exhibit secondary cell wall bands in the hydrenchyma (Koller and Rost, 1988a, 1988b). Similarly, wide-band tracheids occur in the vascular tissues of succulent organs in many genera of succulent families of the Caryophyllales, namely Cactaceae, Aizoaceae, Anacampserotaceae and Didiereaceae; these tracheids have annular or helical secondary wall thickenings which extend deeply into the lumen (Landrum, 2001, 2006; Mauseth, 2004c). Wide-band tracheids are believed to increase hydraulic adaptability, as they preserve the function of vascular tissues by preventing both cavitation and occlusion during drought-induced shrinking of succulent organs (Landrum, 2006; Mauseth, 2006).

**Water relations**

Unlike non-succulent “true” xerophytes, succulent plants are able to maintain a relatively high water potential ($\Psi$) even during extended drought (Nobel and Jordan, 1983; von Willert et al., 1992; Griffiths and Males, 2017). This is due to high values of hydraulic capacitance ($C$) and low values of volumetric modulus of elasticity ($\varepsilon$) in succulent organs, which is related to highly elastic cell walls (Ogburn and Edwards, 2010). $C$ can be defined as:

$$C = \frac{\Delta V}{\Delta \Psi}$$
where $\Delta V$ is the change in volume, and $\Delta \Psi$ is the change in water potential (Nobel, 2009). On the other hand, $\varepsilon$ can be defined as:

$$\varepsilon = \frac{\Delta \Psi_p}{\Delta V/V}$$

where $\Delta \Psi_p$ is the change in pressure potential or turgor pressure, and $\Delta V/V$ is the relative volume change; lower values of $\varepsilon$ indicate higher elasticity (Nobel, 2009). Cell wall thickness has long been assumed to affect $\varepsilon$ (i.e. thicker walls are generally more rigid; Tyree and Jarvis, 1982), and a strong positive correlation has recently been reported (Peguero-Pina et al., 2017). These two formulas suggest that cell wall properties influence the trade-offs between maintaining tissue volume and tissue $\Psi$. High $C$ and low $\varepsilon$ means that succulents maintain higher turgor pressure for longer with decreasing $\Psi$ and lose relatively large amounts of water before turgor loss occurs (Bobich and North, 2009; Ogburn and Edwards, 2010). The turgor loss point (TLP, i.e. the $\Psi$ at which turgor loss occurs) has generally been interpreted as an indicator of drought tolerance (i.e. tolerating low $\Psi$) among non-succulent plants (Lenz et al., 2006; Blackman et al., 2010). Many arid-adapted non-succulents respond to drought by lowering their already low TLP through physiological adjustments, primarily osmotic adjustments (Bartlett et al., 2012; Turner, 2018; Signori-Müller et al., 2021). On the other hand, measurements of TLP and the closely related osmotic potential ($\Psi_S$; see formula in Bartlett et al., 2012) in drought-avoiding succulents have shown that they exhibit relatively high TLP values (Walter and Stadelmann, 1974; Smith and Lütge, 1985; von Willert et al., 1992; Donatz and Eller, 1993; Gotsch et al., 2021; Leverett et al., 2021); their ability to maintain high $\Psi$ seems to relax the need for a low TLP. Indeed, drought-avoiding succulents are assumed to have a relatively limited capacity for osmotic adjustment (Walter and Stadelmann, 1974; Griffiths and Males, 2017). Given this limitation, if turgor loss is to be prevented during severe, extended drought, elastic adjustment by further decreasing $\varepsilon$ may be an important process among drought-avoiding succulents (Schulte, 1992; A. Leverett, pers. comm.). Such elastic adjustment likely involves rapid changes of the cell wall driven by wall remodelling, particularly of the pectin fraction (Peaucelle et al., 2011; Bethke et al., 2016; Roig-Oliver et al., 2020b, 2021). Indeed, changes in DM of cell wall HGs have been reported as a response to dehydration in the hydrenchyma of Aloe species (Fig. 3E; Ahl et al., 2019b). In succulent organs of storage succulents, cell wall heterogeneity between tissues in terms of wall thickness and elasticity allow for preferential water loss and tissue-to-tissue remobilization. As $\Psi$ decreases during early stages of drought, water is preferentially lost from the large-celled hydrenchyma, given that hydrenchyma cell walls are thinner and more elastic (i.e. lower $\varepsilon$) than those of the chlorenchyma, and this water can then be remobilized to the chlorenchyma to maintain photosynthesis (Schmidt and Kaiser, 1987; Goldstein et al., 1991; Nobel, 2006). This remobilization process seems to be driven by minor osmotic adjustments primarily involving polymerization or depletion of organic solutes, which create an osmotic gradient
(ΔΨₛ) between hydrenchyma and chlorenchyma (Barcikowski and Nobel, 1984; Schulte and Nobel, 1989; Schulte et al., 1989; Nerd and Nobel, 1991; Herrera et al., 2000).

Despite adaptations of the vascular system to optimize hydraulic connectivity (e.g. Mauseth, 2006; Ogburn and Edwards, 2013; Melo-de-Pinna et al., 2016), succulent organs are generally assumed to have reduced hydraulic conductance (K), with outside-xylem hydraulic conductance (Kₒₓ) expected to be particularly limiting due to long outside-xylem hydraulic pathways (Brodribb et al., 2007; de Boer et al., 2012; Ferrio et al., 2012; Sack and Scoffoni, 2013). Water movement in succulents is tightly controlled: emptying of succulent tissues during drought is remarkably slow, whereas refilling upon rain events can happen strikingly quickly (Gibson and Nobel, 1986; Smith and Nobel, 1986; Flach et al., 1995). In transpiring non-succulent leaves, recent evidence suggests that water flow predominantly follows the apoplastic pathway (Buckley, 2015; Buckley et al., 2015). Assuming that the dominance of the apoplastic pathway can be extrapolated to other photosynthetic organs, such as succulent leaves and stems, cell wall features such as thickness, effective porosity and cell-to-cell connectivity are expected to be among the strongest determinants of Kₒₓ (Buckley, 2015; Buckley et al., 2015; Bidhendi and Geitmann, 2016; Xiong et al., 2017). Since such features can be modulated through cell wall remodelling, water movement in succulents is likely controlled, at least partially, by cell wall modifications. Among these modifications, pectin remodelling has been postulated as the strongest contributor: conformational changes of pectin due to different enzymatic activities can affect cell wall porosity (McKenna et al., 2010; Levesque-Tremblay et al., 2015; Bidhendi and Geitmann, 2016), and increased cell wall pectin content has been linked to lower cell wall thickness and higher elasticity and hydration (Roig-Oliver et al., 2020a, b, 2021; Carriquí et al., 2020). Other factors such as pH and ion concentration also influence cell wall thickness and extensibility (Demarty et al., 1984; Cosgrove, 2005).

Although the largest reservoir of water in succulent tissues is symplastic, apoplastic water contributes to stored water in some succulent groups, most notably in suborder Portulacineae (Nyffeler, 2007), and is facilitated by a matrix of highly hydrophilic apoplastic polysaccharides known as mucilage (Nobel et al., 1992; von Willert et al., 1992; Ogburn and Edwards, 2010). The term mucilage has also been used interchangeably (and arguably mistakenly) to refer to all water-extractable polysaccharides from succulent tissues (e.g. Sáenz et al., 2004; Ni et al., 2004a). Mucilage has been extensively reported in seeds and/or fruits of numerous land plant lineages, which in many cases has also been linked to water retention (Phan and Burton, 2018). Mucilage in succulents occurs in the apoplastic space, either partially filling the space between cells or within the wall of specialized mucilage cells (Nobel et al., 1992; Mauseth, 2006). Mucilage in Cactaceae has been considerably studied and its composition resembles that of pectins, particularly RG-I, with a highly branched structure rich in arabinose and galactose (Cárdenas et al., 1997; Goycoolea and Cárdenas, 2003). Mucilage has also been reported in succulent species of
Aizoaceae, Anacampserotaceae, Crassulaceae, Didiereaceae, Portulacaceae and Vitaceae (Landrum, 2002; Mauseth, 2004a), although its role and composition remain unclear.

**Photosynthesis**

Besides imposing a limitation on \( K_{\text{OX}} \), a recent review by Flexas *et al.* (2021) has highlighted the often-neglected effect of cell wall properties on limiting internal conductance to \( \text{CO}_2 \) \( (g_i) \) and, thus, on photosynthesis. Several interrelated cell wall properties, such as thickness, \( \varepsilon \) and effective porosity, have been postulated as some of the strongest determinants of \( g_i \) (Evans *et al.*, 2009; Tosens *et al.*, 2012; Ellsworth *et al.*, 2018; Nadal *et al.*, 2018). However, the influence of cell wall composition on \( g_i \) is still scarcely understood, as indicated by contrasting findings regarding the relationship between pectin content and \( g_i \) (Clemente-Moreno *et al.*, 2019; Carriquí *et al.*, 2020; Roig-Oliver *et al.*, 2020a, c, 2021). Correlations between \( g_i \) and \( K \) and their relationship with cell wall parameters indicate coordination between these two parameters and demonstrate the shared cell wall pathway for \( \text{CO}_2 \) and water (Flexas *et al.*, 2013; Xiong *et al.*, 2017; Xiong and Nadal, 2020; Roig-Oliver *et al.*, 2021).

Throughout land plant evolution, both \( g_i \) and \( K \) have generally increased with enhanced photosynthetic capacity (de Boer *et al.*, 2012; Flexas and Carriquí, 2020), and such increases have likely been facilitated by changes in cell wall characteristics such as thickness and \( \varepsilon \) (Nadal *et al.*, 2018; Carriquí *et al.*, 2020; Gago *et al.*, 2019). Thin cell walls and a peripheral distribution of chloroplasts against the cell membrane in succulent tissues (Gibson and Nobel, 1986; von Willert *et al.*, 1992) would suggest that in succulents the cell wall poses a relatively low limitation on \( g_i \) (Evans *et al.*, 2009; Gago *et al.*, 2019; Flexas *et al.*, 2021). However, contrary to the aforementioned evolutionary trend, CAM-performing succulent plants have regressed to states of relatively low \( g_i \), with values being as low as in gymnosperms, which is thought to increase CAM capacity by limiting internal \( \text{CO}_2 \) efflux (Maxwell *et al.*, 1997; Flexas *et al.*, 2008; Ripley *et al.*, 2013). Even though such low \( g_i \) has been previously attributed primarily to anatomical features related to intercellular air spaces (Nelson *et al.*, 2005; Nelson and Sage, 2008), the role of cell wall characteristics in limiting \( g_i \) in succulents remains unexplored.
Cell walls of succulent tissues under drought

Succulent tissues are characterized by having thin and highly flexible primary cell walls, yet little is known of the mechanism that translates into drought avoidance. Early academic works on succulent tissues noted that distinctive cell wall folding patterns could be observed as cells shrink during drought (Westermaier, 1884; Haberlandt, 1904; Engmann, 1934). Since those early studies, these collapsible cell walls have been reported for a few succulent taxa and are often assumed as a general anatomical feature of succulents, allowing for controlled regular wall folding and reversible volume changes in succulent organs (Fig. 3). Studies on the cortex hydrenchyma in stems of Cactaceae (Mauseth, 1995) and the hydrenchyma in leaves of Aloe (Ahl et al., 2019b) have given the most detailed descriptions to date of collapsible cell walls in succulents. This type of cell wall has also been reported in succulent stems of Euphorbia (Euphorbiaceae) and Asclepiadoideae (Apocynaceae; Mauseth, 2004b), and in succulent leaves of Sansevieria (Koller and Rost, 1988a, b) and Pyrrosia (Polypodiaceae; Ong et al., 1992). Although the presence of collapsible cell walls has not been systematically surveyed, histological images from an even broader body of research suggests that collapsible cell walls occur in many more succulent lineages: folding patterns can be observed in succulent tissues of Aizoaceae (e.g. Melo-de-Pinna et al., 2014; Ogura et al., 2018), Crassulaceae (e.g. Jiménez et al., 1983; Sandoval-Zapotitla et al., 2019), Bromeliaceae (e.g. Gomes-da-Silva et al., 2012; Reinert et al., 2013), Gesneriaceae (e.g. Pereira-Dias and Santos, 2015) and Piperaceae (e.g. Horner et al., 2017). When cells in non-succulent plants reach the TLP under severe drought, negative turgor pressures can develop and result in dehydration injury due to plasmolysis and/or collapse of the cell walls around the plasmolyzed protoplasts (Ristic and Cass, 1991; Palomäki et al., 1994; Ding et al., 2014; Vollenweider et al., 2016). On the other hand, succulents maintain relatively high cell turgor pressures and rarely reach the TLP, even during extended drought. As cells in succulent tissues shrink, the convoluted regular folding of collapsible cell walls, coupled with the maintenance of high turgor, points towards a coordinated response that preserves the cell membrane-cell wall continuum and prevents irreversible damage due to mechanical stress. In fact, it is likely that even cells that have apparently collapsed in succulent tissues retain some turgor, as the protoplasm seems to remain mostly in contact with the cell wall (A. Leverett, pers. comm.; J. D. Mauseth, pers. comm.). Similarly, cell wall folding in resurrection plants (see Box 1) is thought to prevent the development of negative turgor and subsequent irreversible damage (Vander Willigen et al., 2001).

Besides the cell wall and its polysaccharidic components, plant cells also contain carbohydrates within the symplastic domain; all carbohydrates in a tissue, organ or a whole plant can be referred to as the glycome. The glycome of some economically important succulent groups has received particular attention due to its multiple applications in pharmaceutics, food, cosmetics, bioremediation, bioenergy and material sciences (Borland et al., 2009; Grace, 2019). Studies have therefore focused on taxa such as Aloe (e.g. Reynolds and Dweck, 1999; Ni et
The interest in Aloe vera (L.) Burm.f. and its relatives in Asphodelaceae due to their widespread medicinal uses has fostered one of the most detailed cell wall characterizations in succulent tissues. In the leaf hydrenchyma of A. vera, besides structural cell wall polysaccharides, cell contents are rich in storage polysaccharides and free sugars, including the prized acetylated glucomannans with putative medicinal properties (Reynolds and Dweck, 1999; Ni et al., 2004a, b). Subsequent studies have shown that monosaccharide profiles of the hydrenchyma across Aloe species and relatives are phylogenetically constrained, and that well-developed hydrenchyma is the main predictor for medicinal use (Grace et al., 2013, 2015). More recent studies have highlighted the usefulness of high-throughput polysaccharide screening methods such as comprehensive microarray polymer profiling (CoMPP) to characterize the glycomic profiles of succulent tissues (Ahl et al., 2018). Among four Aloe species, such profiles exhibited abundant mannans and were shown to vary seasonally (Ahl et al., 2019a), which suggests that acclimation processes affecting storage polysaccharides and/or cell walls occur in response to seasonal changes.

Another study on two species of Aloe (A. helenae and A. vera) has confirmed the existence of a tightly regulated cell wall folding process during dehydration (Ahl et al., 2019b). Drought-induced pectin remodelling of hydrenchyma cell walls in these Aloe species is thought to cause the loss of low-DM HG (Fig. 3E), which is believed to enhance cell wall elasticity and initiate the cell wall folding process. Remarkably, the same study also reported changes in cell wall mannans (incl. (galacto)(gluco)mannans and acetylated glucomannans), which accumulated inside the cells upon drought in a granular form that resembles that of starch (Fig. 3F). Granular forms of mannans have also been observed in storage organs of Dendrobium (Orchidaceae; He et al., 2017) and Amorphophallus (Araceae; Ohtsuki, 1968; Chua et al., 2013). The presence of cell wall mannans in the hydrenchyma of Aloe was shown to decrease sharply during drought, whereas intracellular mannans increased in the chlorenchyma (Ahl et al., 2019b). It has been postulated that, despite not being directly involved in the folding process, mannans in Aloe could be acting as CWSPs (see Box 3) by providing energy storage, particularly during drought periods with stalled photosynthesis, and by helping to maintain an osmotic gradient between hydrenchyma and chlorenchyma (Ahl et al., 2019b). Mannan mobilization from storage organs has also been reported in orchids and geophytes, and it has been linked to certain growth stages and to drought stress response by establishing osmotic gradients and promoting water transfer between tissues (Stancato et al., 2001; Tan et al., 2007; Wang et al., 2008; Chua et al., 2013). The reason why Aloe and perhaps other succulents seem to rely on mannans as storage during drought rather than the more widespread starch probably stems from their different physicochemical properties: starch granules are highly packed and insoluble, thus exhibiting extremely low osmotic activity, whereas soluble mannans possess high osmotic activity and water-holding capacity, and are also mobilized more readily and rapidly than starch (Meier and Reid, 1982; Buckeridge et al., 2000).
of some orchids and geophytes the mobilization of mannans occurs before that of coexisting starch (Matsuo and Mizuno, 1974; Franz, 1979), whereas during flowering of Oncidium (Orchidaceae) mannans are mobilized from the pseudobulb and subsequently degraded and converted to starch, which is temporarily accumulated before further catabolic reactions (Wang et al., 2008). Either way, these observations indicate that mannans can be more easily mobilized than starch, which may be the basis of the use of mannans as CWSPs in Aloe.

From different studies, it seems clear that collapsible cell walls in succulents maintain their high elasticity or even increase it further during drought through elastic adjustment, a process which is likely driven by cell wall remodelling (Mauseth, 1995; Ahl et al., 2019b). However, the exact mechanism behind this highly regulated process is still largely unknown. Anatomical peculiarities of collapsible cell walls hint at the mechanism behind the folding process: in Sansevieria the collapsible walls in the hydrenchyma exhibit bands of secondary thickening (Koller and Rost, 1988a, 1988b), and it is possible this ridged spatial patterning of stiffer and softer regions determines how the wall folds. However, most succulent tissues lack secondary wall thickening. Instead, cell wall remodelling can create patterns of local softening and/or loosening and induce phase separation phenomena in the wall, as seen in many developmental and acclimation processes which require cell growth or change in cell shape (Peaucelle et al., 2011; Miedes et al., 2013; Amsbury et al., 2016; Bidhendi and Geitmann, 2016; Chebli and Geitmann, 2017; Bidhendi et al., 2019; Haas et al., 2020, 2021). Thus, similar processes leading to localized cell wall softening and/or loosening could be involved in the initiation of the regular cell wall folding process in succulent tissues.

A hypothetical model, based on the observations of Moore et al. (2013) on leaves of resurrection plants, those of Bidhendi et al. (2019) on pavement cells of Arabidopsis and those of Ahl et al. (2019b) on leaves of Aloe, is presented in Fig. 4. Cell wall folding can also be observed in plant tissues and organs frequently subjected to desiccation, such as seeds of some plant lineages (Webb and Arnott, 1982) and leaves of resurrection plants (Cooper and Farrant, 2002; Moore et al., 2006). In resurrection plants, cell wall folding upon dehydration has been linked to expansin-mediated cell wall loosening, which enhances wall extensibility, and to wall remodelling affecting primarily pectin (Jung et al., 2019), with arabinose-rich polymers (e.g. RG-I arabinans/arabinogalactans and AGPs) postulated as cell wall plasticizers which allow for elastic adjustment (Moore et al., 2013). These cell wall components could act as plasticizers in collapsible cell walls of succulent plants as well. Observations in resurrection plants also suggest that upregulation of certain proteins during dehydration-driven cell wall folding, such as glycine-rich proteins (GRPs; Wang et al., 2009; Giarola et al., 2016) and wall-localized dehydrins (Layton et al., 2010), may help to maintain cell wall integrity and enable repair. Since these proteins are ubiquitous among land plants (Sachetto-Martins et al., 2000; Hanin et al., 2011), it is possible that they also play a role in dehydration response in succulent plants and in regulating the cell wall folding process. However, the
high values of cell wall thickness found in resurrection plants makes drawing parallels with drought-avoiding succulents challenging (Flexas et al., 2021; Nadal et al., 2021).

**Future perspectives**

The cell wall is a central aspect of drought resistance in plants, yet much remains to be determined about the molecular and physiological mechanisms of cell wall folding processes in drought-avoiding succulents. Cell wall folding in resurrection plants, which has received special attention over the last decades, relies on different mechanisms in different lineages, most of which involve arabinose-rich polymers acting as cell wall plasticizers (Moore et al., 2013). More research is thus needed to elucidate how cell wall folding is regulated in the numerous succulent lineages and whether a shared mechanism exists. In Aloe, for instance, it has been recently postulated that HGs and mannans are involved in the folding process (Ahl et al., 2019b). Changes in DM of HGs reinforce the idea that cell wall elasticity is optimized during wall folding, whereas the involvement of mannans suggests that CWSPs and soluble sugars likely play a crucial role during dehydration. Whether similar processes occur in other succulent lineages and whether other cell wall components are involved in the folding process remain to be explored.

Since studies of separate cell wall components tend to overlook the complexity of the cell wall and the interactions between different components, holistic approaches should be favoured for cell wall characterization in succulents. Advancing cell wall analytical methods provide promising prospects, with a growing demand for high-throughput methods for rapid screening and profiling of cell wall components (Persson et al., 2011). Spectroscopic methods have been widely used for cell wall characterization (Bauer, 2012; Mansfield et al., 2012; Pettolino et al., 2012; Gierlinger, 2018; Zhao et al., 2020) in combination with imaging techniques (Zhao et al., 2019; Bidhendi et al., 2020; DeVree et al., 2021; Xu et al., 2021). Recent advances in non-destructive real-time imaging, such as light-sheet fluorescence microscopy (LSFM), could allow us to observe changes in cell walls of succulent tissues under drought in near-physiological conditions (Grossmann et al., 2018; Ovečka et al., 2018). Comprehensive microarray polymer profiling (CoMPP), a method based on the specificity of molecular probes, allows for high-throughput screening of numerous cell wall components across a wide range of samples (Møller et al., 2007; Rydahl et al., 2018). CoMPP has recently been used alongside immunolocalization to characterize the cell wall and glycomic composition of several Aloe species and relatives and to provide a deeper insight into cell wall dynamics under drought (Ahl et al., 2018, 2019b). However, the semiquantitative nature of CoMPP poses some limitations, and it should usually be employed as a complementary method to quantitative biochemical techniques (Møller et al., 2007; Persson et al., 2011). Another disadvantage of CoMPP is the difficulty to isolate succulent tissues within a succulent organ, which is not feasible in most cases and requires whole organs. The latest technological developments include imaging techniques which allow for 3D
visualization of cell wall structure, composition and connectivity, including serial-sectioning scanning electron microscopy (ssSEM; Oi et al., 2017; Harwood et al., 2020, 2021; Antreich et al., 2021) among other high-resolution microscopy techniques (Zeng et al., 2017; Haas et al., 2020), X-ray microcomputed tomography (X-ray microCT; Théroux-Rancourt et al., 2017; Earles et al., 2018) and magnetic resonance imaging (MRI; Malik et al., 2016; Hesse et al., 2020; Mylo et al., 2021). These methods have the potential to elucidate how succulent tissues are built and their anatomical complexity from a 3D perspective.

While omics studies have shed light on cell wall-related genes and their respective products (Carpita et al., 2001; Minic et al., 2009; Albenne et al., 2013; Houston et al., 2016), genetic tools and resources to specifically study succulents are still largely missing. Genome sequencing of a few succulent taxa over the last decade (Cai et al., 2015; Ming et al., 2015; Copetti et al., 2017; Yang et al., 2017; Jaiswal et al., 2021) offers the possibility of establishing them as models to study drought resistance and/or CAM performance (Yang et al., 2019). Given that succulence has often been regarded as a pre-requisite for CAM, engineering CAM into crops and other economically important plants to enhance their water-use efficiency would probably first require the engineering of succulence (Borland et al., 2014; Yang et al., 2015). Since cell walls are expected to play a central role in succulence, next-generation sequencing can be used for future omics studies to mine candidate genes involved in cell wall remodelling in succulent plants (Egan et al., 2012; Strickler et al., 2012; Gross et al., 2013), which would provide opportunities for ongoing (e.g. Lim et al., 2020) and future efforts of engineering tissue succulence into crops.
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Boxes

Box 1. Ecology of succulents

Drought can lead to different degrees of water stress in plants, defined as “situations in which plant water potential and turgor are reduced enough to interfere with normal functioning”, although the “exact cell water potential at which this occurs depends on the kind of plant” (Kramer, 1983). Many drought-resistant plants (sensu Levitt, 1980) are drought tolerant and are able to track soil water potential to exceptionally low values (Walter and Stadelmann, 1974; Pockman and Sperry, 2000; Griffiths and Males, 2017); this category includes “true” xerophytes and the extreme case of resurrection plants, which are additionally desiccation tolerant. However, most succulent plants do not tolerate low water potentials (Ψ) and are therefore regarded as drought avoiders, with stored water delaying or completely preventing the effects of water stress at the cellular/tissue level (Eggli and Nyffeler, 2009; Ogburn and Edwards, 2010); this review focuses on drought-avoiding succulents. Succulence may be linked to other ecological strategies, most notably halophytism (Kadereit et al., 2003; Flowers and Colmer, 2008), although halophytic succulents are functionally distinct from drought-avoiding succulents. Despite being traditionally associated with arid and hyper-arid deserts (“true” deserts sensu Laity, 2008), drought-avoiding succulents need to refill their water stores periodically and are therefore dependent on seasonally predictable rainfall typical of semi-arid habitats (von Willert et al., 1992). Thus, the hotspots of succulent diversity tend to occur in semi-arid habitats and desert fringes (Burgess and Shmida, 1988; Ogburn and Edwards, 2010). Drought-avoiding succulents are also well represented in xeric microhabitats within relatively hydric habitats (Fig. 1H-J), as is the case with many epiphytes (Zotz, 2016) and plants in some alpine niches (Körner, 2003).
Box 2. Cell wall remodelling

The primary cell wall is a dynamic system whose properties can be tightly controlled via cell wall remodelling, which involves controlled modification, rearrangement, degradation and/or reconstruction of the cell wall in both growing and mature cells in response to various stimuli (Barnes and Anderson, 2018; Anderson and Kieber, 2020). Cell wall extension and contraction are generally regarded as a consequence of cell wall remodelling through the processes of cell wall loosening (i.e. cell wall stress relaxation and increased extensibility) and/or softening (i.e. reduced stiffness and increased deformability; Cosgrove, 2018; Zhang et al., 2019). Cell wall loosening is thought to be mediated by expansins, a class of non-enzymatic proteins which weaken non-covalent bonds in the cellulose-hemicellulose network and allow for slippage among cell wall components, whereas the activity of several hemicellulose- and pectin-modifying enzymes can lead to cell wall softening and secondary loosening (Cosgrove, 2016, 2018). These enzymes comprise xyloglucan endo-transglycosylases/hydrolases (XTHs), pectin methylesterases (PMEs), pectin acetylenes (PAEs), polygalacturonases (PGs) and pectate lyases (PLs), among others (Eklöf and Brumer, 2010; Sénéchal et al., 2014). There has been a growing interest in cell wall remodelling in response to abiotic stress due to its potential applications in near-future climate change scenarios (e.g. Le Gall et al., 2015; Tenhaken, 2015; Ezquer et al., 2020). A large proportion of plant genes are involved in cell wall synthesis, assembly and remodelling (~15% of the genome in Arabidopsis; AGI, 2000; Carpita et al., 2001), and shifts in expression patterns of these genes in response to different stresses have been widely reported (Houston et al., 2016), which highlights the relevance of cell walls in stress response.
Box 3. Cell wall storage polysaccharides

Cell wall storage polysaccharides (CWSPs) are apoplastic polysaccharides associated with the cell wall which can be repurposed for energy storage and other functions (Meier and Reid, 1982). They comprise mannans, xyloglucans and (arabino)galactans and are mobilized from the cell wall via various enzymatic activities (Buckeridge et al., 2000; Buckeridge, 2010). In many cases, CWSPs occur as a special deposition inside the ordinary primary cell wall. Among mannan CWSPs, insoluble “pure” mannans have been linked to increased hardness and are abundant in seeds, whereas soluble mannans, formed by substitution with galactosyl residues (i.e. galacto(gluco)mannans) and/or acetylation, have been reported in succulent-like storage organs, such as orchid pseudobulbs and underground organs of geophytes, where they are believed to play a role in cellular water relations and water storage (Stancato et al., 2001; Wang et al., 2006; Ranwala and Miller, 2008; Chua et al., 2013).
Figure captions

Figure 1. Succulence can occur in any plant organ, with leaf succulents and stem succulents being the most familiar. (A-G) Examples of drought-avoiding succulent plants from arid and semi-arid regions of the world: (A) Lithops ruschiiorum (Aizoaceae); (B) Crassula deceptor (Crassulaceae); (C) Anacampseros filamentos (Anacampserotaceae); (D) Aloe striata (Asphodelaceae); (E) Carnegiea gigantea (Cactaceae); (F) Hoodia gordonii (Asclepiadoideae, Apocynaceae); (G) Agave shawii (Asparagaceae). (H-J) Examples of drought-avoiding succulent plants from xeric microhabitats: (H) Sempervivum montanum (Crassulaceae); (I) Peperomia galapagensis (Piperaceae); (J) Dendrobium kratense (Orchidaceae). All photos from iNaturalist (see photo credits below).

Figure 2. Three-dimensional molecular model of the type I primary cell wall typical of most angiosperms (except the commelinids), showing the molecular interactions between the cell wall polysaccharides. Boxes show some representatives of the two groups of non-cellulosic cell wall polysaccharides, and of cell wall structural proteins (not included in the 3D model). Modified from Carpita et al. (2015) [The Cell Wall. In: Buchanan BB, Gruissem W, Jones RL, eds. Biochemistry and Molecular Biology of Plants. 2nd edition. 45-110. © 2015 John Wiley and Sons, Ltd]. Created with BioRender.com.

Figure 3. (A-F) Drought-response in succulent tissues of Aloe helenae (Asphodelaceae). (A) Morphology of a succulent leaf. (B,C) Section stained with toluidine blue of a leaf under (B) well-watered and (C) severe drought conditions; note the extreme degree of shrinking of the hydrenchyma upon dehydration. (D) Close-up of the shaded area, showing highly convoluted collapsible cell walls in the hydrenchyma, in contrast with the mostly smooth cell walls in the chlorenchyma. (E) In situ detection of highly de-methyl-esterified HGs using the monoclonal antibody COS (green signal); note the loss of signal in hydrenchyma cell walls compared with chlorenchyma (arrowhead). (F) In situ detection of acetylated mannans using the monoclonal antibody CCRCM-170 (red signal), with calcofluor white used to stain cellulose in cell walls (blue signal); note the intracellular accumulation of granular mannans (arrowhead). Hyd: hydrenchyma; Chl: chlorenchyma. (G-I) Drought-response in succulent tissues of Facheiroa sp. (Cactaceae). (G) Morphology of a succulent stem of F. cephaliomelana [© Pierre Braun, CC-BY-SA-4.0]. (H,I) Stem sections of F. ulei stained with Safranin O/Fast Green FCF of cortex hydrenchyma under (H) well-watered and (I) severe drought conditions. (A-C) Modified from Ahl et al. (2019b); (H, I) modified from Mauseth (2020).
**Figure 4.** Diagram of the hypothetical cell wall folding process in succulent tissues during drought conditions. (A) Detail of contact region between two cells in a succulent tissue. From a highly hydrated state, initial decreases in relative water content may result in different responses among different succulent lineages: cell wall remodelling may occur in some taxa to increase overall cell wall elasticity and/or to mobilize CWSPs, as seen in *Aloe* (Ahl et al., 2019b), whereas other taxa may exhibit constitutively highly elastic cell walls and may not need any modifications at this stage. (B) As relative water content decreases further during extended drought and the cells lose volume, the cell walls experience buckling due to local mechanical stress (in red), which triggers a subcellular response that initiates localized cell wall remodelling (orange arrows). (C) Cell wall remodelling results in patterning of softened and/or loosened regions along the cell wall (in blue), which may act as hinges and facilitate the regular cell wall folding process. Created with BioRender.com.
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Figure 1
