Tansley insight

Speedy stomata, photosynthesis and plant water use efficiency

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Contents

Summary 1
I. Introduction 1
II. Influence of the speed of $g_s$ responses on $A$ and $W_i$ 1
III. Determinants of the rapidity of $g_s$ responses 3
IV. Conclusion 3
Acknowledgements 5
References 5

New Phytologist (2018)
doi: 10.1111/nph.15330

Key words: dynamic environments, photosynthesis, speed of response, stomatal conductance, temporal kinetics, water use efficiency.

Summary

Stomatal movements control CO$_2$ uptake for photosynthesis and water loss through transpiration, and therefore play a key role in plant productivity and water use efficiency. The predicted doubling of global water usage by 2030 mean that stomatal behaviour is central to current efforts to increase photosynthesis and crop yields, particularly under conditions of reduced water availability. In the field, slow stomatal responses to dynamic environmental conditions add a temporal dimension to gaseous fluxes between the leaf and atmosphere. Here, we review recent work on the rapidity of stomatal responses and present some of the possible anatomical and biochemical mechanisms that influence the rapidity of stomatal movements.

I. Introduction

Stomata control gas exchange between the leaf interior and the external environment, and therefore adjustments in stomatal aperture in response to both environmental factors and internal signals determine CO$_2$ diffusion into the leaf and water loss via transpiration. Regulation of gaseous fluxes in and out of the leaf is essential to meet mesophyll demand for CO$_2$, maintaining appropriate leaf temperature, whilst conserving overall plant water status. Early work by Wong et al. (1979) demonstrated a close relationship between photosynthesis $A$ and stomatal conductance $g_s$; however, stomatal responses to changing conditions are generally an order of magnitude slower than photosynthetic responses (see Lawson & Blatt, 2014). Sluggish stomata can cause nonsynchronous behaviour between $A$ and $g_s$, which under dynamic conditions can result in far from optimal intrinsic water use efficiency ($W_i = A/g_s$; Matthews et al., 2017; Vialet-Chabrand et al., 2017). The quantification of stomatal kinetics has recently received a great deal of attention; however, the majority of this work focused on the consequences and has provided little mechanistic understanding of the underlying causes. Our review will centre on the physical and metabolic constraints that influence the speed at which stomatal aperture responds to changing conditions; and while we will exemplify dynamic responses using photosynthetic photon flux density (PPFD)-driven changes, we acknowledge that other environmental factors greatly influence kinetics.

II. Influence of the speed of $g_s$ responses on $A$ and $W_i$

Light is one of the most dynamic environmental signals that influence both photosynthetic rate $A$ and stomatal conductance $g_s$. Passing clouds and overlapping leaves in a canopy result in leaves experiencing ‘sun flecks’ and ‘shade flecks’ that can occur in timeframes of seconds to hours. Additionally, the variation in light...
energy received by the plant can create rapid and extreme fluctuations in leaf temperature and leaf–air vapour pressure deficit to which stomata will respond in conjunction with other environmental cues. However, plant responses do not all occur within the same timescale: PPFD-driven changing in A responding and reaching a new steady state within several tens of seconds to minutes, whereas changes in gs can take minutes to hours (Barradas & Jones, 1996; Lawson & Morison, 2004; Lawson et al., 2010; Vico et al., 2011; McAusland et al., 2016). The slow gs increase often limits A, whilst the slow gs decreases result in a lag between the drop in A and the gs response, which can result in unnecessary water loss for a limited carbon gain, reducing Wj (Hetherington & Woodward, 2003; Franks & Farquhar, 2007; Brodribb et al., 2009; Vico et al., 2011; Lawson et al., 2012; Drake et al., 2013; McAusland et al., 2016). The disconnection between A and gs and resulting gs limitation of A depends on the change in PPFD, the photosynthetic capacity of the plant, the initial and final gs and the speed of the stomatal response. This is illustrated in Fig. 1, which shows the relationship between A and gs at different light intensities and the possible trajectories of A and gs following a step change in light. In this example, the red line illustrates a typical temporal response of A, in which A rapidly increases to a new steady state (determined by the initial gs value), with any further increase in A dependent upon the rapidity of the gs response. The blue line represents the theoretical gs required to achieve 95% A, and the trajectory if A and gs were fully synchronized, and could represent a target for improved stomatal behaviour. Values to the left of the trajectory (blue line) represent gs; limitation of A (red shading), whilst those to the right (blue shading) represent unnecessary water loss relative to CO2 gain.

Considerable variation between species in both the rapidity and magnitude of gs responses to changing PPFD has been reported in both laboratory (e.g. Elliott-Kindston et al., 2016; McAusland et al., 2016) and field studies (e.g. Cardon et al., 1994, 1995; Barradas & Jones, 1996; Qu et al., 2016), depending on guard cell type (Hetherington & Woodward, 2003; Franks & Farquhar, 2007; McAusland et al., 2016), growth conditions (Elliott-Kindston et al., 2016; Qu et al., 2016; Matthews et al., 2017; Hepworth et al., 2018) and the magnitude and type of signal that initiates these responses (Elliott-Kindston et al., 2016; Hepworth et al., 2018). Fig. 2 provides an example of the diversity of A and gs responses to a step change in irradiance in Vicia faba and Avena sativa. The difference between the initial and final steady-state g, along with the rapidity of response resulted in different limitations on A and Wj. In V. faba, A took longer to reach a plateau due to the slow temporal response of g, (Fig. 2a) limiting CO2 diffusion, whilst slow stomatal closure in A. sativa resulted in unnecessary water loss (Fig. 2b). Modeled synchronous behaviour in g, and A has been shown to theoretically increase Wj by 20% in Phaseolus vulgaris subjected to dynamic light (Lawson & Blatt, 2014).

Two important components of the PPFD signal that will determine the temporal response of A, g, and Wj are the intensity of the new light level and its duration (Vialet-Chabrand et al., 2016). The intensity impacts on the magnitude of the g, response, whilst the duration determines the level that can be achieved within the timescale, as illustrated in Fig. 3. Owing to slow stomatal responses, variations in PPFD of a short duration or low magnitude do not significantly impact on g, despite a change in A (Lawson et al., 2012). However, changes in light intensity of a greater magnitude and/or longer duration result in a typical exponential response of A and g, that can be used to quantify the rapidity of g, response, the degree of limitation of A and the impact on Wj (Fig. 3). In this example Wj is improved by the slow increase in g,; however, this is at the expense of limiting A, which illustrates the challenge of optimizing Wj whilst maintaining high A. Slow reaction time of stomata can also result in a g, continuing on the same trajectory even after the light stimulus has ceased (Kirschbaum et al., 1988; Tinoco-Ojanguren & Peary, 1993), which can be defined as a stomatal ‘overshoot’. Overshoots in stomatal opening are a common feature of well-watered plants, resulting in unnecessary water loss and decreasing Wj (McAusland et al., 2016), but may be important for maintaining leaf temperature or maximizing A in a dynamic environment.

McAusland et al. (2016) showed significant variation in the opening and closing kinetics in a range of different species, reporting an average 10% stomatal limitation on A. Kaiser et al. (2015) suggested that stomatal limitation of A was minimal (1–3%) compared with the biochemical limitations imposed by activation of Rubisco. However, these findings could be due to the experimental protocol, as plants were dark adapted before being exposed to a step increase in illumination of 1000 µmol m⁻² s⁻¹ PPFD. Plants in a ‘real’ environment would never experience such extreme changes in light intensity, which could induce high levels of...
stress due to a lack of induction and activation of key photosynthetic enzymes.

III. Determinants of the rapidity of $g_s$ responses

The determinants of the rapidity of the $g_s$ response can be simply categorized into anatomical (e.g. density and size of stomata), structural (e.g. cytoskeleton and cell wall elasticity) and biochemical (e.g. number and activity of transporters or ion channels) features.

Anatomical considerations

Smaller stomata have often been proposed to have faster kinetics (Drake et al., 2013), thought to be due to a greater guard cell membrane surface area to volume ratio, which enables more rapid
changes in solutes than with larger guard cells (Hetherington & Woodward, 2003; Drake et al., 2013; Raven, 2014). Although a relationship between size and speed holds up in closely related species of the same genus (Drake et al., 2013), this is not conserved over a wide range of species (Elliott-Kingston et al., 2016; McAusland et al., 2016) and may only be valid within or between species with similar stomatal features. Whilst diverse species with different stomatal features may have mechanisms influencing the speed independently of the size (e.g. including the number and size of subsidiary cells for solutes exchange (Franks & Farquhar, 2007) and differences in biochemistry, gene expression and sensitivity). For example, Elliott-Kingston et al. (2016) examined rapidity of stomatal closure in an evolutionary diverse set of species (including fern, cycad, conifers and angiosperms) and found no relationship with size or density of stomata, but suggested that species diversification in low atmospheric [CO₂] led to faster stomatal responses (Elliott-Kingston et al., 2016). The differences in the rapidity of g observed by McAusland et al. (2016) in a range of crop plants could also not be explained by the size of stomata in species with elliptical (or kidney-shaped) guard cells. However, in species with dumbbell-shaped guard cells, size impacted on the speed and amplitude of response, and generally resulted in faster g, responses (McAusland et al., 2016), suggesting involvement of biochemical mechanisms. Interestingly, the same authors also suggested that mesophyll photosynthetic metabolites might be important in the rapidity, as both opening and closing responses were faster in C₄ dumbbell species than in C₃ (McAusland et al., 2016), although the exact mechanism that links these two processes has yet to be identified.

Franks & Farquhar (2007) illustrated that morphology of stomatal complexes, including guard cell shape and the presence or absence of subsidiary cells, also influences stomatal function. This research illustrated that fully turgid subsidiary cells convey a mechanical advantage, and maximum stomatal aperture in grass species such as Triticum aestivum cannot be achieved without reductions in subsidiary cell turgor pressure. The rapid exchange of osmotica between subsidiary cells and guard cells enables rapid switching of turgor pressure between these two cells, providing a possible mechanism for the rapid stomatal movement in grasses (Franks & Farquhar, 2007). A recent study supporting this mechanism, in which a transcription factor necessary for subsidiary cell development was manipulated in Brachypodium distachyon, has resulted in impaired stomatal kinetics and reduced g (Raisig et al., 2017). The evolution of dumbbell-shaped guard cells in grass species (which includes a number of major crops) and the relationship between the guard cells and subsidiary cells has provided these species with the functional advantage of faster stomatal responses (Drake et al., 2013; McAusland et al., 2016; Chen et al., 2017) and optimal patterning (Hepworth et al., 2018) that has been attributed to the evolutionary success of these species (Chen et al., 2017).

Structural considerations

Stomatal movements involve pronounced changes in the shape and volume of the guard cell that are partially controlled by the reorganization of actin filaments (Kim et al., 1995; Hwang et al., 1997; Higaki et al., 2010; Eisinger et al., 2012) and cell wall properties (Carter et al., 2017; Woolfenden et al., 2017). For example, Carter et al. (2017) recently proposed a new model of guard cell structural changes in response to turgor, involving a pectin-based pinning down of the guard cell ends that promotes increase in stomatal width during opening. The importance of these properties in the rapidity of guard cell movements has recently been assessed using actin-related protein 2 (arp2) and arp3 mutant, which showed impaired vacuolar fusion and slower opening than wild-type (WT) controls and complementation lines (Jiang et al., 2012; Li et al., 2013; Isner et al., 2017).

Biochemical considerations

Stomatal movements result from changes in guard cell turgor due to osmotic adjustments in response to fluxes of potassium ions (K⁺), chloride and organic anions (e.g. malate and sucrose), and their transport across the plasma membrane and tonoplast (Blatt, 2000). The extent and rapidity of stomatal movements is therefore intrinsically linked to the capacity for solute transport and the speed with which transport responds to environmental cues (reviewed by Lawson & Blatt, 2014). Transport capacity is determined by the density and activity of guard cell membrane transporters, which is connected to the surface area to volume ratio and has been suggested to relate stomatal size with speed (Franks & Farquhar, 2007; Raven, 2014). However, several studies have reported considerable variation in solute fluxes in different species independent of cell size (reviewed by Lawson & Blatt, 2014), which could explain the lack of correlation between the size of stomata and stomatal speed. In addition to the mechanical advantage provided by the four-celled stomatal complex, grass species exhibit the fastest response due to the rapid transport of ions and osmolytes between guard cells and subsidiary cells (see Cai et al., 2017; Chen et al., 2017; Jezek & Blatt, 2017). A systematic approach for exploring potential targets to manipulate solute fluxes and the speed of stomatal responses is the use of quantitative system modelling. For example, Wang et al. (2014a) used the OnGuard model and found that only primary hydrogen ion transport and transporters directly influencing calcium ion (Ca²⁺) fluxes affected stomatal movements, and that modest changes of separate ion channels is largely ineffective. This study provided promising targets in the form of manipulation of the voltage-dependent characteristics of the K⁺ channels of the plasma membrane in guard cells. The authors reported that a voltage shift in the gating of the outward-rectifying K⁺ channel accelerated stomatal closure by 30%; however, the model found that doubling the number of channels actually resulted in a slower rate of closing. In complement, Viala-Chabrand et al. (2017) analysed the synergies between transporters in different cellular compartments and identified subsets of transporters associated with [Ca²⁺], which represent potential targets to enhance plant performance. These studies illustrate the complexity of stomatal osmoregulation and signal transduction pathways, highlighting the difficulty of finding viable targets for manipulating the rapidity of stomatal responses at a biochemical level. However, it emphasizes how reverse engineering may provide...
practical solutions for improving $A$ and $W_i$ in a dynamic environment. For example, McLachlan et al. (2016) demonstrated that the breakdown of triacylglycerols is required to supply ATP for hyperpolarization of the plasma membrane and K$^+$ uptake through inward-rectifying K$^+$ channels for stomatal opening. Wang et al. (2014b) overexpressed H$^+$$\cdot$ATPase in the guard cells of Arabidopsis and reported greater $g_c$ and $A$, which enhanced plant growth but at the expense of $W_i$. This suggested that stomatal closure rates did not parallel the accelerated stomatal opening (Lawson & Blatt, 2014). Asymmetry of opening and closing responses has been reported with longer delays in opening or slower opening responses relative to closure (Vico et al., 2011), which may indicate water conservation strategies rather than optimizing carbon gain (Vico et al., 2011; McCausland et al., 2016). However, stomatal responses also depend on the growth environment, including the influence of water status (Qu et al., 2016; Haworth et al., 2018) and lighting regime (Matthews et al., 2018), illustrating further complexities.

It is worth remembering that the cost of stomatal movements, in terms of energy and solute requirements, could be too great if stomata responded continuously to environmental changes to maximize photosynthesis and water use efficiency. Therefore, the more conservative (buffered) responses, as well as the reported asymmetry in the rapidity of stomatal opening and closing, could reflect a trade-off between cost of stomatal movements, CO$_2$ uptake and water loss under a specific environment.

IV. Conclusion

Improving the rapidity of stomatal responses could greatly improve $A$ and $W_i$ and aid plant productivity. Although many studies have investigated the rapidity of stomatal responses and attributed differences to anatomical features, a full mechanistic understanding is still lacking. Guard cell membrane transport and channel activity are key to balancing ionic fluxes for stomatal movement; however, the manipulation of a single channel is unlikely to increase the rapidity of $g_c$, as coordination of multiple channels is required, as well as coordination of fluxes at both the plasma membrane and tonoplast. Further studies are therefore needed to generate extensive data sets on stomatal kinetics from existing mutants, as well as the identification of new targets for guard cell manipulation. Restricting studies to a single genus will minimize genetic effects, reducing the complexity of responses, and may be the most effective procedure for screening and selecting for faster stomata (Drake et al., 2013).

Acknowledgements

S.V-C. and T.L. were supported through BBSRC grants a BB/1001187/1 & BB/N021061/1 awarded to T.L. We thank the three reviewers for their comments that have improved the manuscript.

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