

# Exploiting natural variation and genetic manipulation of stomatal conductance for crop improvement

Michele Faralli<sup>1</sup>, Jack Matthews<sup>1</sup> and Tracy Lawson



Rising global temperatures and more frequent episodes of drought are expected to drive reductions in crop yield, therefore new avenues for improving crop productivity must be exploited. Stomatal conductance ( $g_s$ ) balances plant  $\text{CO}_2$  uptake and water loss, therefore, greatly impacting the cumulative rate of photosynthesis and water use over the growing season, which are key determinants of crop yield and productivity. Considerable natural variation exists in stomatal anatomy, biochemistry and behavioural characteristics that impact on the kinetics and magnitude of  $g_s$  and thus gaseous exchange between the plant and atmosphere. Exploiting these differences in stomatal traits could provide novel breeding targets for new crop varieties that are potentially more water use efficient and have the ability to maintain and/or maximize yield in a range of diverse environments. Here we provide an overview of variation in stomatal traits and the impact these have on  $g_s$  behaviour, as well as the potential to exploit such variation and genetic manipulation for crop improvement.

## Address

School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, United Kingdom

Corresponding author: Lawson, Tracy ([tlawson@essex.ac.uk](mailto:tlawson@essex.ac.uk))

<sup>1</sup> Joint 1st authors.

Current Opinion in Plant Biology 2019, 49:1–7

This review comes from a themed issue on **Physiology and metabolism**

Edited by **Elizabeth A Ainsworth** and **Elizabete Carmo Silva**

<https://doi.org/10.1016/j.pbi.2019.01.003>

1369-5266/© 2018 Elsevier Ltd. All rights reserved.

## Stomatal conductance influences crop photosynthesis and yield

Stomata govern gaseous diffusion between the leaf and the external atmosphere, regulating  $\text{CO}_2$  assimilation, water loss and evaporative cooling. Stomata continually adjust aperture in response to external environmental cues (e.g. light), plant water status [1], and internal signals, that may be hormonal (e.g. ABA) [2], circadian [3], and/or a currently unidentified ‘mesophyll signal’ [4,5], to maintain an appropriate balance between  $\text{CO}_2$  uptake and water loss. Over the long-term and under

steady-state, non-limiting conditions, stomatal conductance ( $g_s$ ) has been reported to correlate strongly with the rate of photosynthesis ( $A$ ) [6], with high  $g_s$  generally associated with high  $A$  and yield [7]. However, short-term dynamic changes in the environment result in a lack of synchrony between  $g_s$  and  $A$ , as stomatal responses to changing environmental cues are often substantially slower than those observed in  $A$ , resulting in a temporal disconnect between  $A$  and  $g_s$  that can limit photosynthetic carbon assimilation and reduce plant water use efficiency ( $W_e$ , carbon assimilation as a ratio of water lost) [5,8,9\*\*]. Stomatal conductance is determined by both anatomical and behavioural characteristics, yet both vary greatly between and within species, as well as between [10] and within leaves [11], resulting in significant variation in stomatal behaviour and absolute  $g_s$  [12].

## Anatomical characteristics determine the rate of $g_s$

Anatomical features such as stomatal density (SD), size and maximum pore area, determine the calculated theoretical maximum stomatal conductance ( $g_{s,max}$ ) [13], whilst the control of stomatal opening and closure determine ‘operational’ or measured  $g_s$ , that is the fraction of  $g_{s,max}$  at which the leaf operates [14]. A positive relationship between SD and  $g_s$  has been reported within species [15], which often, but not always [16] translates into high  $A$  [17,18]. For example, [19] reported that increased SD in two near isogenic lines of barley did not result in increased  $g_s$ , due to a concurrent decrease in stomatal size. Stomatal density is also positively related to photosynthetic capacity, with several studies illustrating increases in operational and maximum  $g_s$  with increases in photosynthetic potential [20,21]. Furthermore, it is well established that significant natural variation in photosynthetic capacity exists between [22] and within species [23\*,24\*]. Stomatal size and SD also vary greatly within and between plant species [10], with differences often driven by changes in the growth environment [25], including  $[\text{CO}_2]$  [26], light intensity and spectral quality [27]. There are numerous studies that have also demonstrated significant variation in stomatal anatomical characteristics within species, cultivars, genotypes and ecotypes. For example, [28] examined 62 wild *Arabidopsis* accessions and reported significant variation in SD that was also related to other epidermal traits, including cell size, stomatal index and patterning, suggesting a common genetic basis. In [29] varietal differences in SD and aperture in rice genotypes were shown, which [16] demonstrated the importance of variation in stomatal length

that resulted in genotypic variation in  $g_s$ . Variation in SD has also been associated with differences in drought resistance, as well as photosynthetic rates in wheat cultivars [30]. Therefore, natural variation in stomatal characteristics represents an unexploited genetic resource for improving  $g_s$ ,  $A$  and plant performance. Although variation in SD is well-established there is limited information on the impact of stomatal behaviour and/or kinetics on  $A$ ,  $W_i$  and plant productivity.

### Variation in stomatal anatomy impacts on dynamic $g_s$ responses

Modifications in SD have been reported to negatively correlate with stomatal size [25], which influences not only  $g_s$  but also the speed at which stomata respond to changing environmental conditions [31,9\*\*]. Several recent studies have demonstrated that stomatal kinetics are determined by anatomical attributes including stomatal morphology and shape [31,9\*\*], size and density [32], patterning [33] and the presence or absence of subsidiary cells [9\*\*,34], and that manipulation of these features could have positive effects on the efficiency of carbon assimilation and water use [35,36\*]. Figure 1 shows the predicted impact of anatomical characters such as stomatal density and size on the magnitude and rapidity of the  $g_s$  response to a step increase in light intensity, based on the literature [9\*\*,31,32,33]. Leaves with a greater number of smaller stomata would be expected to have more rapid stomatal responses and a higher overall  $g_s$ , compared with leaves that had lower density and larger stomata. Additionally, stomatal patterning defects (i.e. stomatal clustering) have been reported to result in slower  $g_s$  responses and lower  $g_s$  values. [32] illustrated that the maximum rate of stomatal opening is driven by the surface-to-volume ratio of stomata, attributed to changes in SD and size, as species with higher stomatal densities and smaller stomata exhibited more rapid  $g_s$  kinetics [31]. [9\*\*] Quantified the impact of slow stomatal opening, in a range of species including crops, and determined on average a 10% limitation on carbon assimilation, which could equate to substantial losses in carbon gain over the course of the day, potentially negatively impacting productivity and yield [37,38]. In contrast, slow stomatal closure results in a significant decrease in intrinsic water use efficiency ( $W_i$ ) and resource use [9\*\*,39\*\*] thus potentially accelerating early soil water exhaustion [40]. Figure 2 highlights the impact on  $A$  of variation in the speed of stomatal opening and closure, between two wheat varieties (Figure 2a). Slow increases in  $g_s$  limit  $\text{CO}_2$  diffusion, reducing  $A$  (Figure 2b + d); whilst slow decreases in  $g_s$  result in lower  $W_i$  (Figure 2c + e). Synchronized behaviour and close coupling of  $A$  and  $g_s$ , therefore, have the potential to enhance carbon gain and  $W_i$ , and in turn improve performance, productivity and yield [17,39\*\*]. The wheat cultivars measured in Figure 2 showed little difference in  $A$  (Figure 2d) between the fast and slow  $g_s$  responding cultivars, (most likely due to the greater  $g_s$  in the slower

responding cultivar), whilst  $W_i$  (Figure 2e) was much greater in the cultivar with the faster  $g_s$  responses.

Although substantial progress has been made in linking stomatal anatomy to function, the size and density of stomata are not the only determinants of the speed of response [9\*\*], with stomatal patterning [33,41\*] and guard cell biochemistry [17] also playing key roles. In fact, stomatal clustering has been shown to decrease  $g_s$  and, therefore  $A$ , without any change in overall SD and size [33], and was attributed to reduced guard cell function and increased hydraulic competition with neighbouring guard cells [33,41\*] (see Figure 1). Guard cell movement is the cumulative sum of net solute fluxes (e.g.  $\text{K}^+$ ,  $\text{Cl}^-$  and Malate) integrated over time and transported across the plasma membrane and the tonoplast [17,36\*]. The density and the activity of the guard cell membrane transporters determine solute transport capacity and, inevitably, the speed and magnitude of stomatal movement [42]. Inter-specific variation in guard cells solute flux has been previously shown [17], corroborating the idea that stomatal movement is not only dependent on anatomical factors. Optimization of solute fluxes in guard cells has the potential to enhance stomatal rapidity and provides another unexploited target for crop breeding and should be given greater consideration in future research efforts.

### Genetic manipulation of $g_s$

As  $A$  is strongly correlated with  $g_s$ , a greater emphasis should be placed on recognising  $g_s$  as a major target to improve crop yields and optimize water use. There are multiple examples of the genetic manipulation of SD successfully altering  $g_s$  and influencing plant performance. Work by Gray *et al.* produced mutants with altered stomatal density by manipulating epidermal patterning factor genes [43]. Overexpression of the epidermal patterning factor EPF2 has been shown to improve long-term  $W_i$  without adversely affecting photosynthetic capacity [44] whilst also improving drought tolerance [35]. This model has been successfully applied to improve drought tolerance in barley [45\*]. In contrast, [46] manipulated another member of the EPF family, the mesophyll driven EPF9 (STOMAGEN), which increased SD and  $g_s$  resulting in a 30% increase in  $A$ , although a 40% decrease in  $W_i$  and no significant increase on growth was reported [47]. The above findings highlight that manipulation of stomatal anatomy could be a potential mechanism to increase  $g_s$  and improve crop productivity and yield. However, it is worth bearing in mind that  $g_s$  is fundamentally determined by stomatal behaviour and pore width and compensatory mechanism between density and behaviour can exist. For example work by [48] showed that reducing SD (by overexpressing the STOMATAL DENSITY AND DISTRIBUTION (SDD1) gene) in Arabidopsis, did not reduce  $g_s$  as expected, because an increase in stomatal aperture compensated

Figure 1

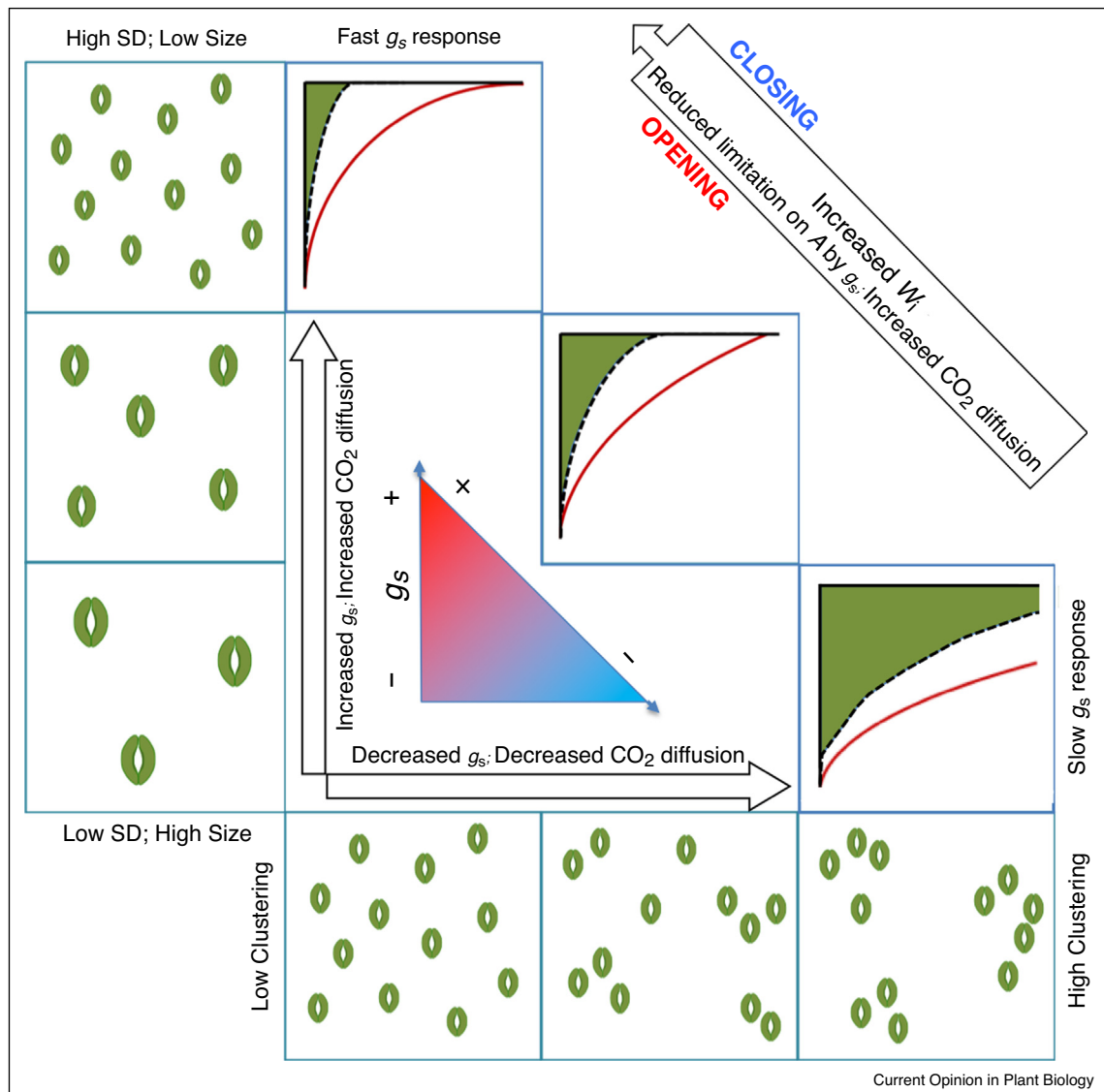


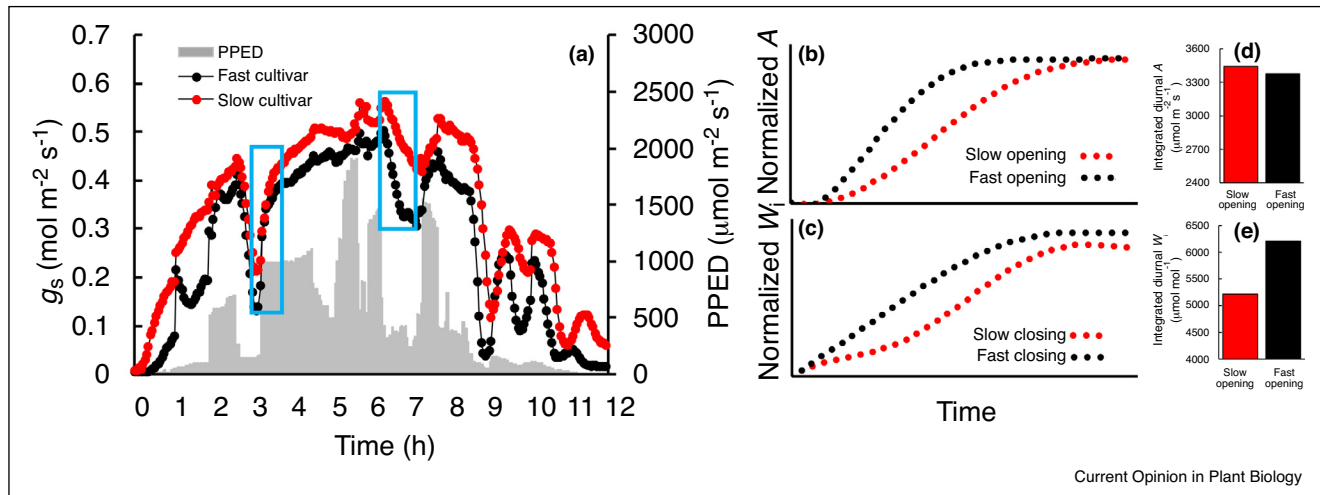
Diagram representing the influence of changes in stomatal anatomy (density and size; left panels, stomatal clustering; lower panels) on stomatal conductance ( $g_s$ , arrows) and the rate of  $g_s$  response (red lines). The impact of anatomical traits on carbon gain ( $A$ , dashed lines), the limitation of  $A$  by  $g_s$  (green area) and water use efficiency ( $W_i$ ) are illustrated. The influence of stomatal density and size (vertical arrow) and stomatal clustering (horizontal arrow) on the rate of  $g_s$  response and the maximum or operational value of  $g_s$  is highlighted.

for the lower SD and, therefore, there was no difference in  $g_s$  between the mutants and controls.

Overcoming the stomatal aperture/stomatal density trade-off was successfully shown by [49], whereby down-regulation of either the  $\alpha$ -subunit or  $\beta$ -subunit of farnesyltransferase (ERA1) increased stomatal sensitivity to ABA in canola. The increased ABA sensitivity reduced  $g_s$ , and facilitated yield maintenance in plants subjected to drought conditions through improved resource use. Increased  $g_s$  has been achieved through a number of

metabolic manipulations, for example, silencing a mitogen-activated protein kinase MPK4 in *Nicotiana attenuata* increased  $g_s$  and  $A$  threefold, as well as increased sensitivity to water stress [50]. In rice [51], tomato [52] and grapevine [53] aquaporin overexpression increased  $g_s$  and  $A$ , both under optimal and stress conditions. These studies clearly demonstrate the potential of manipulating stomatal characteristics to improve carbon assimilation and resource use. However, restrictions on growing GM crops in many countries (particular in Europe) mean that alternative methods for manipulating  $g_s$  need to be

Figure 2



Diurnal time course of  $g_s$  in two wheat cultivars with contrasting rapidity (a) under a dynamic light regime. Examples (blue sections) of the impact of slow and fast  $g_s$  responses on  $A$  after a step increase in light (b); and  $W_j$  after a step decrease in light (c). The integrated daily values of  $A$  (d) and  $W_j$  (e) for cultivars with fast and slow stomatal responses is illustrated.

Table 1

Examples of variation assessed and the range of  $g_s$  detected in cultivars or populations of different crops. The experimental design and methods for  $g_s$  estimation are shown

Authors	Crop	$g_s$ range (mol m <sup>-2</sup> s <sup>-1</sup> )	Experimental material and analysis
[54]	Wheat	0.15–0.55	Chromosome substitution lines grown under field conditions with and without supplementary irrigation. $g_s$ analysed with Li-Cor 6400 at saturating light
[55]	Wheat	0.10–0.42	Field experiment. Double haploid population grown under supplementary irrigation and no irrigation treatment. $g_s$ estimated with CI-340 portable gas-exchange system at saturating light
[7]	Spring wheat	0.34–0.57	Historical selection of wheat cultivars grown over three field seasons. $g_s$ analysed with steady state porometry on both adaxial and abaxial surface
[56]	Durum wheat	0.25–0.42	Historical selection of Italian cultivars grown over two growing seasons. $g_s$ estimated with CIRAS-1 under natural light conditions
[16]	Rice	0.25–0.85	64 accessions from a rice diversity research set of germplasm and 3 high-yielding cultivars grown under field conditions. $g_s$ estimated with Li-Cor 6400 at saturating light
[63]	Rice	0.12–0.21	Field screening under optimal and water stress condition of a BC <sub>3</sub> F <sub>6</sub> mapping population. $g_s$ analysed with Li-Cor 6400 at near-saturating light
[62]	Soybean	0.40–0.65	Greenhouse experiments including VPD manipulation and water stress application on eleven cultivars. $g_s$ analysed with Li-Cor 6400 at saturating light
[65]	Cotton	0.51–0.82	Field grown segregating population. $g_s$ analysed with steady-state porometer
[57]	Cotton	0.70–0.85	Field grown historical selection of cotton. $g_s$ estimated during sunny days with Li-Cor 1600 steady state porometry
[67]	Cotton	0.25–0.75	Field experiment on obverse and reverse F <sub>1</sub> lines. $g_s$ analysed with Li-Cor 6400 diurnally and at different light intensities and temperatures.
[58]	Tomato	0.80–1.20	Historical selection of tomatoes cultivars grown in the field and the greenhouse. $g_s$ was analysed in the field with a Li-cor 6400 at saturating light

realised. This could be achieved by exploiting the significant natural variation in stomatal characteristics and behaviour that is known to exist. However, in order to achieve this, a greater understanding of the underlying genetics that control variation as well as the compensatory mechanisms between stomatal anatomy and behaviour need to be fully understood.

### Natural variation in $g_s$ and genetic control for selection

Large natural variation in  $g_s$  under optimal, steady-state light conditions has been shown for a range of crops. In Table 1, some of the most significant and recently reported work on the variation in  $g_s$  is summarized.



Potentially useful genomic regions have been identified that could provide crucial information for future breeding programmes. For example in cereals, variation in radiation use-efficiency [59], canopy temperature and yield [7] have been attributed to differences in  $g_s$ , signifying the importance of this trait for possible further yield progress. Indeed, [7] showed that the year of release and yield genetic gain in wheat were linearly related with  $g_s$ , thus illustrating that the increase in yield was achieved by inadvertently selecting for high  $g_s$ , cooler canopy and inevitably higher  $A$ . A large normally distributed phenotypic variation for  $g_s$  was reported in two segregating populations of wheat, illustrating potential quantitative inheritance and a heritability on a family mean basis of up to 73% [60]. Subsequently, it has been shown that  $g_s$  is subject to a polygenic control which was in turn associated with QTLs for yield under stress conditions [61]. Therefore, there is strong evidence that variation in  $g_s$  is present in wheat and that marker-assisted selection could be carried out if more accurate genomic regions controlling  $g_s$  are detected.

Genotypic differences in  $g_s$  have also been detected in eleven soybean genotypes analysed under saturating light with different soil water conditions, which lead to variation in  $W_i$  in response to water stress [62]. Anatomy-driven variation in  $g_s$  was shown to be present in elite rice cultivars [16], and QTLs for steady-state  $g_s$  at saturating light in introgression lines under water stress conditions were identified on chromosomes 3 and 9 [63]. Other QTLs related to  $g_s$  were identified in rice [64] and cotton [65], thus suggesting the possibilities of selection for  $g_s$  through marker-assisted selection in several crops. Other sources of potential variation in  $g_s$  (and thus  $A$ ) include inter-specific and inter-generic crosses within the *Triticeae* [66]. The use of  $F_1$  hybrids in crops where heterosis for  $g_s$  is present (e.g. cotton; [67]) has also been shown to be successful. Hence, variation in  $g_s$  is already present in many crops with potential to be included in breeding programmes for both yield potential and enhancement in stress tolerance. Moreover, although previous research has put a great deal of emphasis on assessing the variation in stomatal anatomical characteristics or steady-state  $g_s$ , there is limited information regarding potential intra-specific variation in the rapidity of stomata responses in major food crops, with some information available in rice only [39\*\*]. Further work needs to focus on detecting the genetic basis of stomatal rapidity, thus enhancing the ability for selection of more efficient crops under naturally dynamic environmental conditions.

## Conclusions

Stomatal conductance is a major determinant of photosynthesis, and there is clear evidence that manipulating  $g_s$  can improve crop performance and yield. Natural variation in  $g_s$  exists in crops, with several genomic regions identified that could provide unexploited targets for

ongoing breeding programmes. Additionally the rapidity and kinetics of stomatal responses to changing environmental conditions have been demonstrated to greatly impact  $A$  and water use, and are the result of differences in anatomical and biochemical stomatal components [9\*\*]. As higher stomatal density is often correlated with smaller stomata, and smaller stomata have been reported to respond more rapidly to changing environmental cues, a future priority could be the selection of cultivars with these anatomical features or the identification of the genomic regions that correspond to such traits of interest. Guard cell biochemistry and the density and activity of membrane transporters play a key role in both the magnitude and rapidity of  $g_s$  responses, representing novel targets for improving crop productivity, although little is known regarding natural intra-specific variation in these functional traits. Future breeding programmes should consider the integration of *both* density and behavioural beneficial traits so that equal consideration is given to the magnitude and rapidity of  $g_s$  responses, as well as the overall steady state  $g_s$  value. In conclusion intra-specific variation in the key components governing stomatal dynamics and overall  $g_s$  represent an unexploited target for improving  $A$  and  $W_i$  for increased plant productivity.

## Acknowledgement

This work was funded by Biotechnology and Biological Sciences Research Council (BBSRC, UK) grants to TL (BB/NO16831/1; BB/S005080/1).

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
  - of outstanding interest
1. Lin YS, Medlyn BE, Duursma RA, Prentice IC, Wang H, Baig S *et al.*: **Optimal stomatal behaviour around the world.** *Nat Clim Change* 2015, **5**:459.
  2. Haworth M, Marino G, Cosentino SL, Brunetti C, De Carlo A, Avola G *et al.*: **Increased free abscisic acid during drought enhances stomatal sensitivity and modifies stomatal behaviour in fast growing giant reed (*Arundo donax* L.).** *Environ Exp Bot* 2018, **147**:116-124.
  3. Hassidim M, Dakhiya Y, Turjeman A, Hussien D, Shor E, Anidjar A *et al.*: **CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and the circadian control of stomatal aperture.** *Plant Physiol* 2017:01214.
  4. Lawson T, Terashima I, Fujita T, Wang Y: **Coordination between photosynthesis and stomatal behaviour.** *The Leaf: A Platform for Performing Photosynthesis.* Cham: Springer; 2018, 141-161.
  5. Matthews JS, Viallet-Chabrand SR, Lawson T: **Acclimation to fluctuating light impacts the rapidity and diurnal rhythm of stomatal conductance.** *Plant Physiol* 2018:01809.
  6. Wong SC, Cowan IR, Farquhar GD: **Stomatal conductance correlates with photosynthetic capacity.** *Nature* 1979, **282**:424.
  7. Fischer RA, Rees D, Sayre KD, Lu ZM, Condon AG, Saavedra AL: **Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies.** *Crop Sci* 1998, **38**:1467-1475.
  8. Lawson T, Simkin AJ, Kelly G, Granot D: **Mesophyll photosynthesis and guard cell metabolism impacts on stomatal behaviour.** *New Phytol* 2014, **203**:1064-1081.

9. McAusland L, Vialet-Chabrand S, Davey P, Baker NR, Brendel O, ●● Lawson T: **Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency.** *New Phytol* 2016, **211**:1209-1220.
- This article highlights the inter-specific variation in the rapidity of response and steady state stomatal conductance in a range of crops and other plant functional types. Particular attention is given to the impact of changes in stomatal kinetics on carbon gain and intrinsic water use efficiency.
10. Tichá I: **Photosynthetic characteristics during ontogenesis of leaves. 7. Stomata density and sizes.** *Photosynthetica* 1982, **16**:375-471.
11. Lawson T, Weyers J: **Spatial and temporal variation in gas exchange over the lower surface of *Phaseolus vulgaris* L. primary leaves.** *J Exp Bot* 1999, **50**:1381-1391.
12. Lawson T, James W, Weyers J: **A surrogate measure of stomatal aperture.** *J Exp Bot* 1998, **49**:1397-1403.
13. Franks PJ, Beerling DJ: **Maximum leaf conductance driven by CO<sub>2</sub> effects on stomatal size and density over geologic time.** *Proc Nat Acad Sci U S A* 2009, **106**:10343-10347.
14. Franks PJ, Farquhar GD: **The effect of exogenous abscisic acid on stomatal development, stomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*.** *Plant Physiol* 2001, **125**:935-942.
15. Muchow RC, Sinclair TR: **Epidermal conductance, stomatal density and stomatal size among genotypes of *Sorghum bicolor* (L.) Moench.** *Plant Cell Environ* 1989, **12**:425-431.
16. Ohsumi A, Kanemura T, Homma K, Horie T, Shiraiwa T: **Genotypic variation of stomatal conductance in relation to stomatal density and length in rice (*Oryza sativa* L.).** *Plant Prod Sci* 2007, **10**:322-328.
17. Lawson T, Blatt MR: **Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency.** *Plant Physiol* 2014, **164**:1556-1570.
18. Takahashi S, Monda K, Negi J, Konishi F, Ishikawa S, Hashimoto-Sugimoto M *et al.*: **Natural variation in stomatal responses to environmental changes among *Arabidopsis thaliana* ecotypes.** *PLoS One* 2015, **10**:e0117449.
19. Jones HG: **Transpiration in barley lines with differing stomatal frequencies.** *J Exp Bot* 1977, **28**:162-168.
20. Frank DC, Poulter B, Saurer M, Esper J, Huntingford C, Helle G: **Water-use efficiency and transpiration across European forests during the Anthropocene.** *Nat Clim Change* 2015, **5**:579.
21. McElwain JC, Yiotis C, Lawson T: **Using modern plant trait relationships between observed and theoretical maximum stomatal conductance and vein density to examine patterns of plant macroevolution.** *New Phytol* 2016, **209**:94-103.
22. Lawson T, Kramer DM, Raines CA: **Improving yield by exploiting mechanisms underlying natural variation of photosynthesis.** *Curr Opin Biotechnol* 2012, **23**:215-220.
23. Driever SM, Lawson T, Andralojc PJ, Raines CA, Parry MAJ: ●● **Natural variation in photosynthetic capacity, growth, and yield in 64 field-grown wheat genotypes.** *J Exp Bot* 2014, **65**:4959-4973.
- Driever *et al.* highlight the intra-specific variation in photosynthetic traits that exist across a panel of wheat varieties. Emphasizing the manipulation of key genes within electron transport and the Calvin cycle, the authors discuss the potential for crop improvements through breeding for this unexploited genetic resource. No evidence of a correlation between photosynthetic capacity and yield traits was reported.
24. Carmo-Silva E, Andralojc PJ, Scales JC, Driever SM, Mead A, ●● Lawson T *et al.*: **Phenotyping of field-grown wheat in the UK highlights contribution of light response of photosynthesis and flag leaf longevity to grain yield.** *J Exp Bot* 2017, **68**:3473-3486.
- This article details the natural variation of photosynthetic traits in wheat. With specific attention given to large scale phenotyping of physiological and agronomic traits, and how these may be used to inform breeding programs. A correlation between yield and operational photosynthetic carbon assimilation under natural light conditions was reported.
25. Hetherington AM, Woodward FI: **The role of stomata in sensing and driving environmental change.** *Nature* 2003, **424**:901.
26. Woodward FI, Bazzaz FA: **The responses of stomatal density to CO<sub>2</sub> partial pressure.** *J Exp Bot* 1988, **39**:1771-1781.
27. Gay AP, Hurd RG: **The influence of light on stomatal density in the tomato.** *New Phytol* 1975, **75**:37-46.
28. Delgado D, Alonso-Blanco C, Fenoll C, Mena M: **Natural variation in stomatal abundance of *Arabidopsis thaliana* includes cryptic diversity for different developmental processes.** *Ann Bot* 2011, **107**:1247-1258.
29. Maruyama S, Tajima K: **Leaf conductance in japonica and indica rice varieties: I. Size, frequency, and aperture of stomata.** *Jpn J Crop Sci* 1990, **59**:801-808.
30. Liao JX, Chang J, Wang GX: **Stomatal density and gas exchange in six wheat cultivars.** *Cereal Res Commun* 2005, **33**:719-726.
31. Franks PJ, Farquhar GD: **The mechanical diversity of stomata and its significance in gas-exchange control.** *Plant Physiol* 2007, **143**:78-87.
32. Drake PL, Froend RH, Franks PJ: **Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance.** *J Exp Bot* 2013:495-505.
33. Dow GJ, Bergmann DC: **Patterning and processes: how stomatal development defines physiological potential.** *Curr Opin Plant Biol* 2014, **21**:67-74.
34. Chen ZH, Chen G, Dai F, Wang Y, Hills A, Ruan YL *et al.*: **Molecular evolution of grass stomata.** *Trend Plant Sci* 2017, **22**:124-139.
35. Doheny-Adams T, Hunt L, Franks PJ, Beerling DJ, Gray JE: **Genetic manipulation of stomatal density influences stomatal size, plant growth and tolerance to restricted water supply across a growth carbon dioxide gradient.** *Phil Trans R Soc B* 2012, **367**:547-555.
36. Lawson T, Vialet-Chabrand S: **Speedy stomata, photosynthesis ●● and plant water use efficiency.** *New Phytol* 2018, **221**:93-99.
- This article reviews the determinants of the rapidity of stomatal conductance, including anatomical and biochemical components, and the impact of variation in these traits on photosynthesis and intrinsic water use.
37. Taylor SH, Long SP: **Slow induction of photosynthesis on shade to sun transitions in wheat may cost at least 21% of productivity.** *Phil Trans R Soc B* 2017, **372**:20160543.
38. Deans RM, Brodribb TJ, Busch FA, Farquhar GD: **Plant water-use strategy mediates stomatal effects on the light induction of photosynthesis.** *New Phytol* 2018 <http://dx.doi.org/10.1111/nph.15572>.
39. Qu M, Hamdani S, Li W, Wang S, Tang J, Chen Z *et al.*: ●● **Rapid stomatal response to fluctuating light: an under-explored mechanism to improve drought tolerance in rice.** *Funct Plant Biol* 2016, **43**:727-738.
- Qu *et al.* demonstrate, using a panel of 204 rice accessions, that the rapidity of stomatal opening and closing is strongly linked to drought resistance, highlighting the trade-off between carbon uptake and water saving. Primary focus is given to the impact of the speed of stomatal opening and closing on biomass and yield. This is the only work available on intra-specific variation for stomatal rapidity.
40. Bodner G, Nakhforoosh A, Kaul HP: **Management of crop water under drought: a review.** *Agron Sustain Dev* 2015, **35**:401-442.
41. Papanatsiou M, Amtmann A, Blatt MR: **Stomatal clustering in ●● *Begonia* associates with the kinetics of leaf gaseous exchange and influences water use efficiency.** *J Exp Bot* 2017, **68**:2309-2315.
- This article provides insights into the impact of natural stomatal clustering on stomatal kinetics and CO<sub>2</sub> assimilation under environmentally limited conditions. Particular attention is given to alterations in guard cell dynamics under light and dark treatments.
42. Blatt MR: **Cellular signaling and volume control in stomatal movements in plants.** *Ann Rev Cell Dev Biol* 2000, **16**:221-241.
43. Hunt L, Gray JE: **The signaling peptide EPF2 controls asymmetric cell divisions during stomatal development.** *Curr Biol* 2009, **19**:864-869.

44. Franks PJ, Doheny-Adams T, Britton-Harper ZJ, Gray JE: **Increasing water-use efficiency directly through genetic manipulation of stomatal density.** *New Phytol* 2015, **207**:188-195.
45. Hughes J, Hepworth C, Dutton C, Dunn JA, Hunt L, Stephens J *et al.*: **Reducing stomatal density in barley improves drought tolerance without impacting on yield.** *Plant Physiol* 2017:01844.  
Provides strong evidence that reducing stomatal density confers drought tolerance under limited water availability in barley. The transformed plants showed significant conservative water-use under water stressed conditions due to restricted leaf stomatal conductance.
46. Kondo T, Kajita R, Miyazaki A, Hokoyama M, Nakamura-Miura T, Mizuno S: **Stomatal density is controlled by a mesophyll-derived signaling molecule.** *Plant Cell Physiol* 2009, **51**:1-8.
47. Tanaka Y, Sugano SS, Shimada T, Hara-Nishimura I: **Enhancement of leaf photosynthetic capacity through increased stomatal density in Arabidopsis.** *New Phytol* 2013, **198**:757-764.
48. Büssis D, von Groll U, Fisahn J, Altmann T: **Stomatal aperture can compensate altered stomatal density in Arabidopsis thaliana at growth light conditions.** *Funct Plant Biol* 2006, **33**:1037-1043.
49. Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C: **Molecular tailoring of farnesylation for plant drought tolerance and yield protection.** *Plant J* 2005, **43**:413-424.
50. Hettenhausen C, Baldwin IT, Wu J: **Silencing MPK4 in Nicotiana attenuata enhances photosynthesis and seed production but compromises abscisic acid-induced stomatal closure and guard cell-mediated resistance to Pseudomonas syringae pv tomato DC3000.** *Plant Physiol* 2012, **158**:759-776.
51. Hanba YT, Shibusaka M, Hayashi Y, Hayakawa T, Kasamo K, Terashima I, Katsuhara M: **Overexpression of the barley aquaporin HvPIP2; 1 increases internal CO<sub>2</sub> conductance and CO<sub>2</sub> assimilation in the leaves of transgenic rice plants.** *Plant Cell Physiol* 2004, **45**:521-529.
52. Sade N, Gebretsadik M, Seligmann R, Schwartz A, Wallach R, Moshelion M: **The role of tobacco Aquaporin1 in improving water use efficiency, hydraulic conductivity, and yield production under salt stress.** *Plant Physiol* 2010, **152**:245-254.
53. Perrone I, Gambino G, Chitarra W, Vitali M, Pagliarani C, Riccomagno N *et al.*: **The grapevine root-specific aquaporin VvPIP2; 4N controls root hydraulic conductance and leaf gas exchange under well watered conditions but not under water stress.** *Plant Physiol* 2012:112.
54. Aminia R, Mohammadi S, Hoshmand S, Khodombashi M: **Chromosomal analysis of photosynthesis rate and stomatal conductance and their relationships with grain yield in wheat (Triticum aestivum L.) under water-stressed and well-watered conditions.** *Acta Physiol Plant* 2011, **33**:755-764.
55. Wang SG, Jia SS, Sun DZ, Wang HY, Dong FF, Ma HX *et al.*: **Genetic basis of traits related to stomatal conductance in wheat cultivars in response to drought stress.** *Photosynthetica* 2015, **53**:299-305.
56. De Vita P, Nicosi OLD, Nigro F, Platani C, Riefolo C, Di Fonzo N, Cattivelli L: **Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century.** *Eur J Agron* 2007, **26**:39-53.
57. De Vita P, Li Destri Nicosia O, Nigro F, Platani C, Riefolo C, Di Fonzo N, Cattivelli L: **Stomatal conductance predicts yields in irrigated Pima cotton and bread wheat grown at high temperatures.** *J Exp Bot* 1998, **49**:453-460.
58. Barrios-Masias FH, Jackson LE: **California processing tomatoes: morphological, physiological and phenological traits associated with crop improvement during the last 80 years.** *Eur J Agron* 2014, **53**:45-55.
59. Motzo R, Pruneddu G, Giunta F: **The role of stomatal conductance for water and radiation use efficiency of durum wheat and triticale in a Mediterranean environment.** *Eur J Agron* 2013, **44**:87-97.
60. Rebetzke GJ, Condon AG, Richards RA, Read JJ: **Phenotypic variation and sampling for leaf conductance in wheat (Triticum aestivum L.) breeding populations.** *Euphytica* 2001, **121**:335-341.
61. Pinto RS, Reynolds MP, Matthews KL, McIntyre L, Olivares-Villegas JJ, Chapman SC: **Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects.** *Theor Appl Genet* 2010, **121**:1001-1021.
62. Gilbert ME, Zwieniecki MA, Holbrook NM: **Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought.** *J Exp Bot* 2011, **62**:2875-2887.
63. Gu J, Yin X, Struik PC, Stomph TJ, Wang H: **Using chromosome introgression lines to map quantitative trait loci for photosynthesis parameters in rice (Oryza sativa L.) leaves under drought and well-watered field conditions.** *J Exp Bot* 2011, **63**:455-469.
64. Price AH, Young EM, Tomos AD: **Quantitative trait loci associated with stomatal conductance, leaf rolling and heading date mapped in upland rice (Oryza sativa).** *New Phytol* 1997, **137**:83-91.
65. Ulloa M, Cantrell RG, Percy RG, Zeiger E, Lu Z: **QTL analysis of stomatal conductance and relationship to lint yield in an interspecific cotton.** *J Cotton Sci* 2000, **4**:10-18.
66. Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MA, Snape JW, Angus WJ: **Raising yield potential in wheat.** *J Exp Bot* 2009, **60**:1899-1918.
67. Zeng B, Xu X, Zhou S, Zhu C, Tang C: **Effects of temperature and light on photosynthetic heterosis of an upland cotton hybrid cultivar.** *Crop Sci* 2012, **52**:282-291.