

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

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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 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.

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Stomatal conductance influences crop photosynthesis and yield

Stomata govern gaseous diffusion between the leaf and the external atmosphere, regulating 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 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 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. K^+ , 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

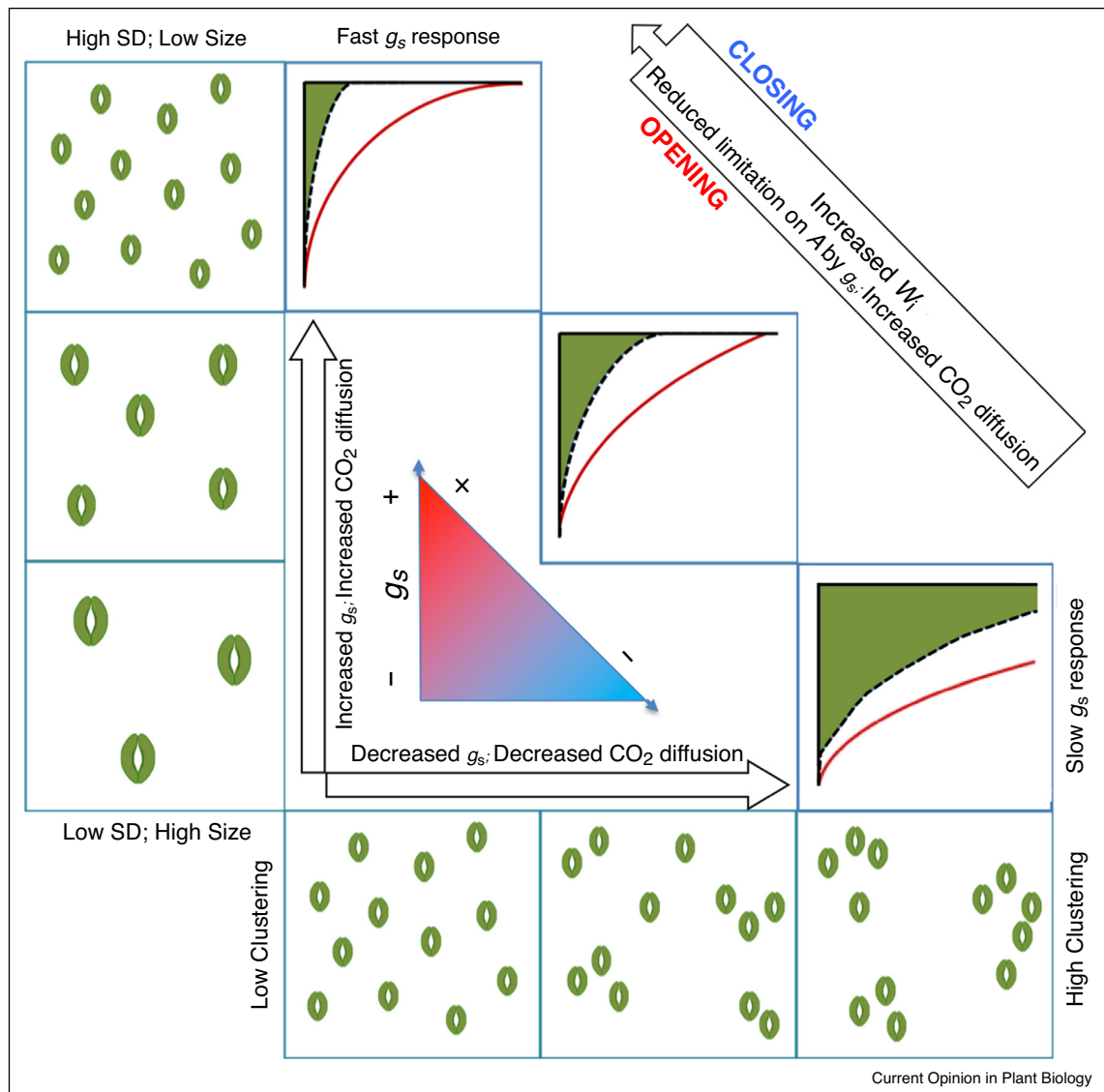


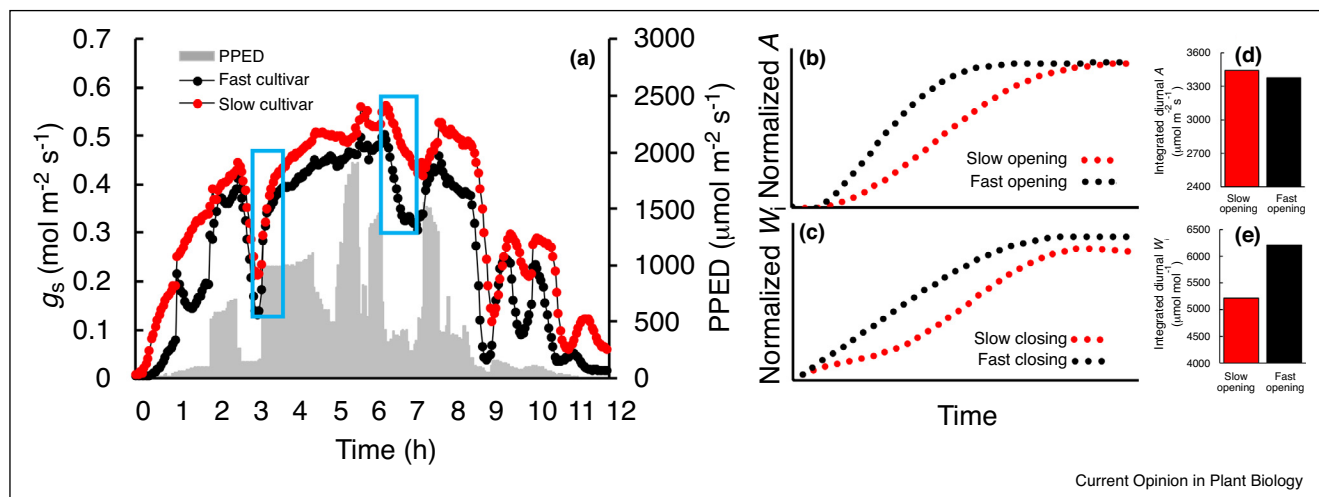
Diagram representing the influence of changes in stomatal anatomy (density and size; left panels, stomatal clustering; lower panels) on stomatal conductance (g_s , arrows) and the rate of g_s response (red lines). The impact of anatomical traits on carbon gain (A , dashed lines), the limitation of A 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 α -subunit or β -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 ⁻² s ⁻¹)	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 ₃ F ₆ 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 ₁ 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.

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