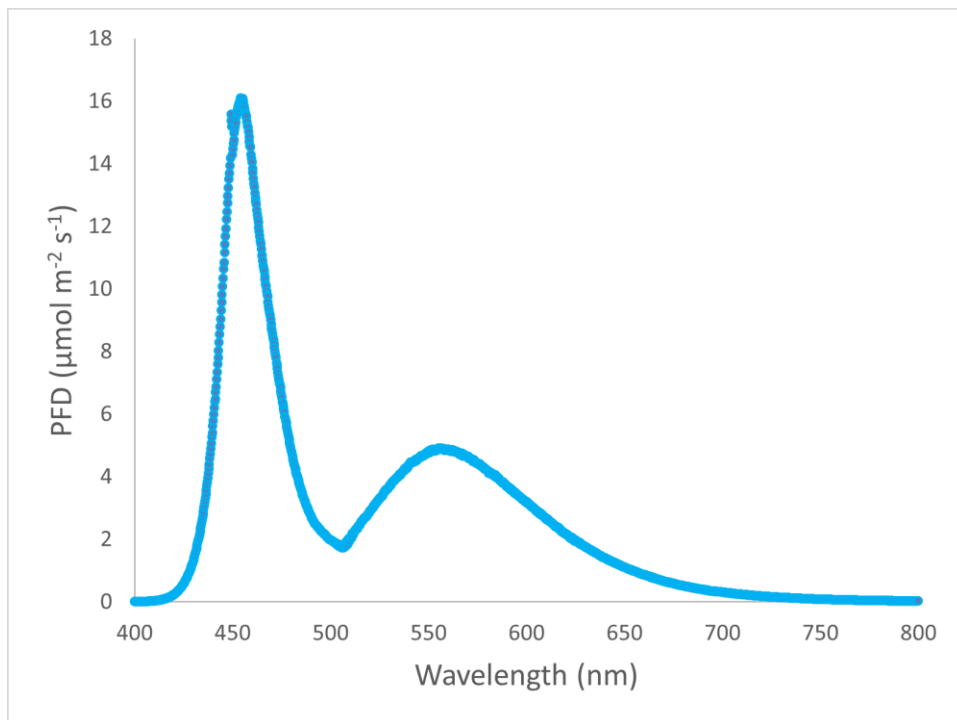


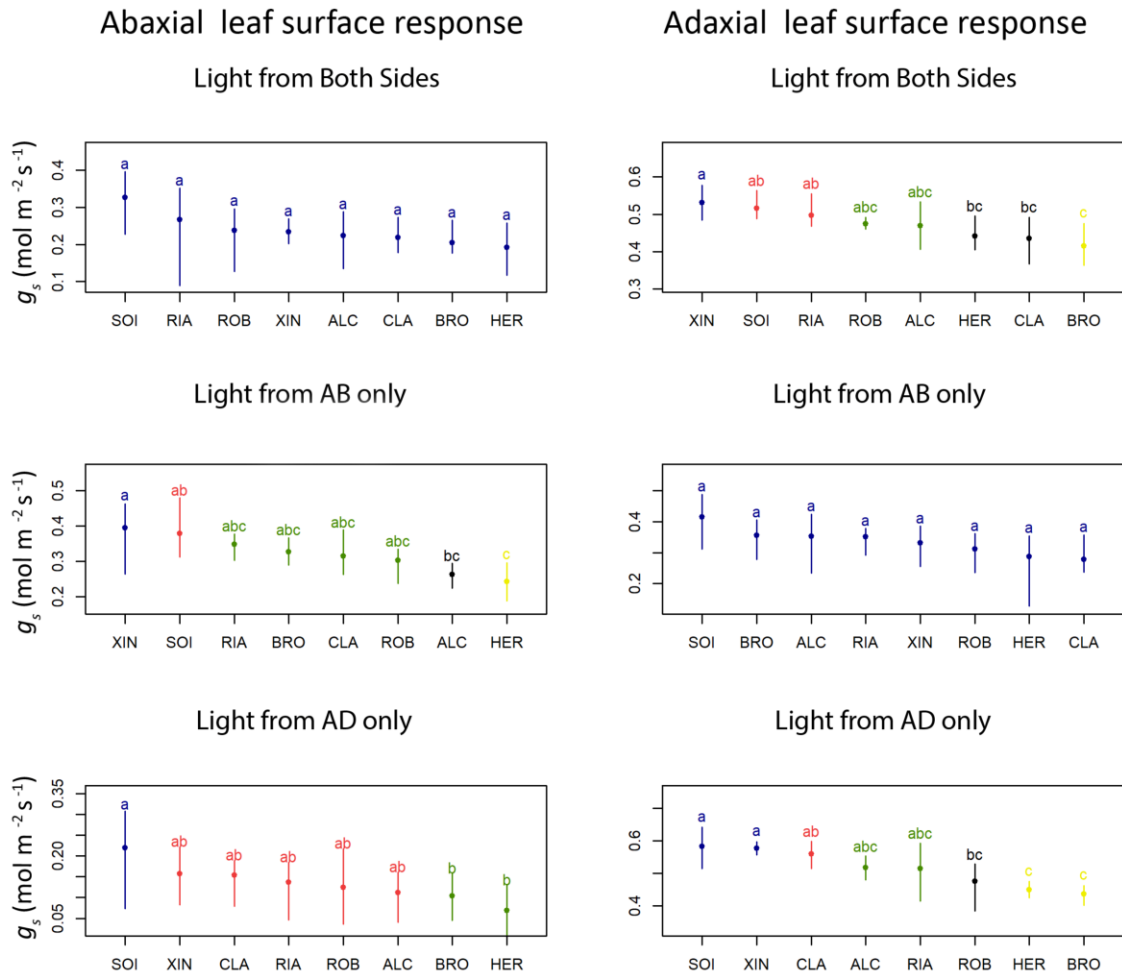
New Phytologist Supporting Information Article title: **Stomata on the abaxial and adaxial leaf surface contribute differently to leaf gas exchange and photosynthesis in wheat**

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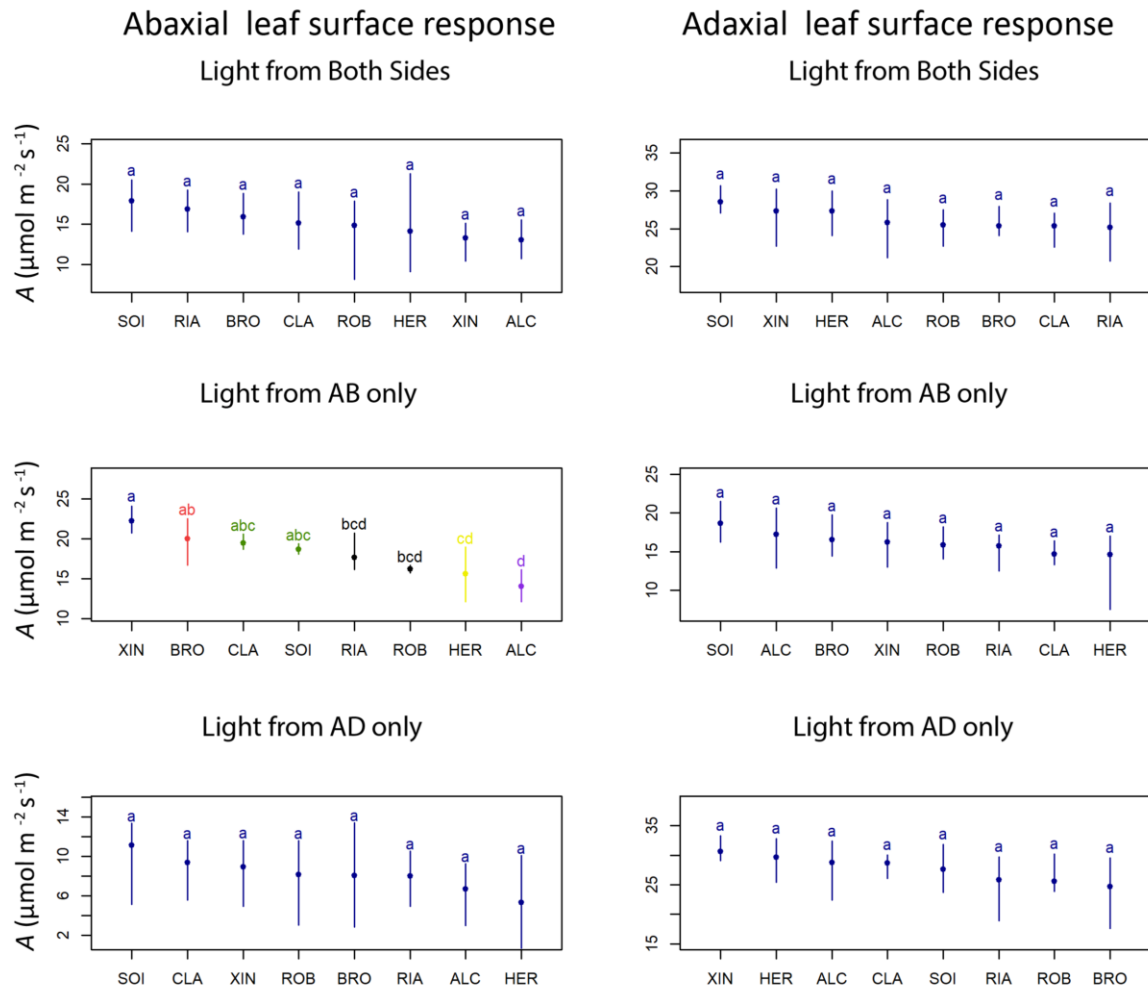
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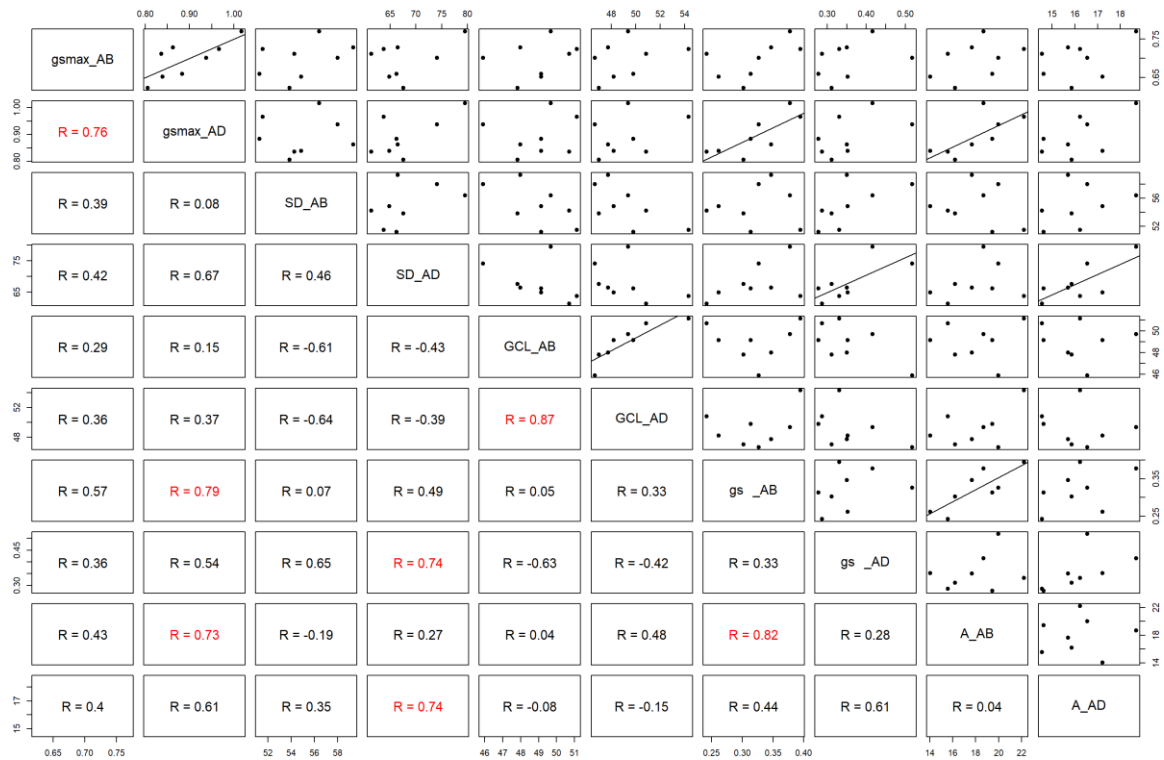
**Fig. S1:** Spectrum of white actinic LED light source (Iso light, Technologica) used with the split chamber, measured by a spectroradiometer (Spectrapen, PSI Systems, Czech republic).



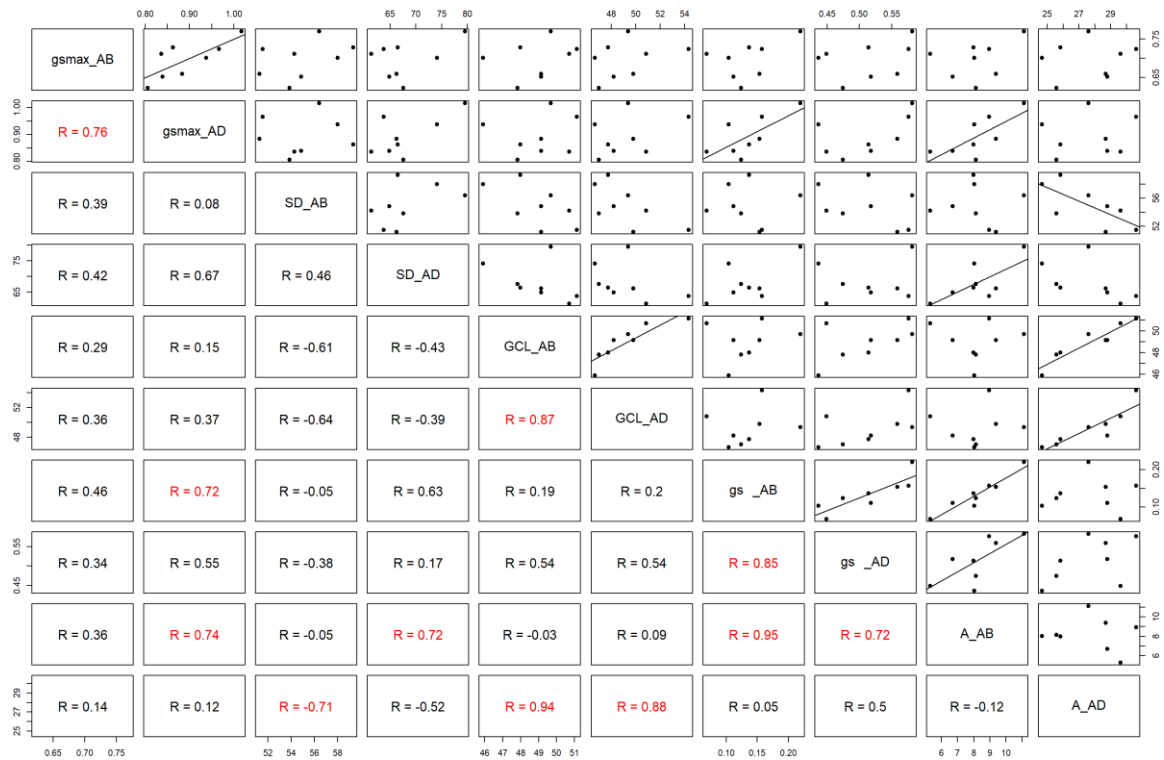
**Fig. S2:** Statistics for end responses of stomatal conductance ( $g_s$ ;  $\text{mol m}^{-2} \text{s}^{-1}$ ) to a step increase in PPFD for eight wheat cultivars, Alchemy (ALC), Brompton (BRO), Claire (CLA), Hereward (HER), Rialto (RIA), Robigus (ROB), Soissons (SOI) and Xi19 (XIN) using the split-chamber cuvette. Separate responses are shown for the adaxial and abaxial leaf surfaces, in addition to the combined response of both surfaces. Headings 'Light from AD only' and 'Light from AB only' represent the lighting regime, where plants were lit from the adaxial and abaxial leaf surface respectively ( $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD), and 'Light from Both sides' represents plants lit from both sides ( $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD). Gas exchange parameters were recorded at 60 min, leaf temperature,  $[\text{CO}_2]$  and leaf VPD were maintained at  $22 \text{ }^\circ\text{C}$ ,  $400 \mu\text{mol mol}^{-1}$  and  $1 \pm 0.2 \text{ kPa}$  respectively. Different letters represent statistically significant differences ( $p < 0.05$ ) between means of each leaf surface of the light treatment ( $n = 4-6$ ).



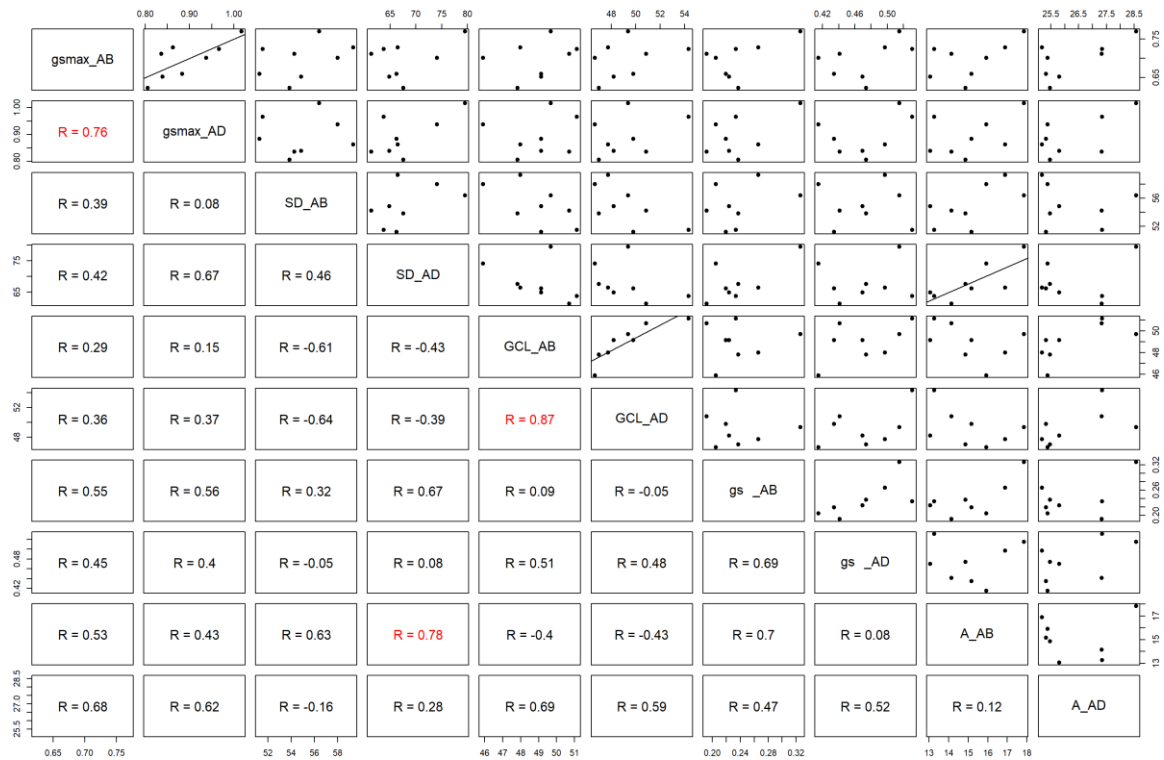
**Fig. S3:** Statistics for end responses of net CO<sub>2</sub> assimilation ( $A$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) to a step increase in PPFD for eight wheat cultivars, Alchemy (ALC), Brompton (BRO), Claire (CLA), Hereward (HER), Rialto (RIA), Robigus (ROB), Soissons (SOI) and Xi19 (XIN) using the split-chamber cuvette. Separate responses are shown for the adaxial and abaxial leaf surfaces, in addition to the combined response of both surfaces. Headings 'Light from AD only' and 'Light from AB only' represent the lighting regime, where plants were lit from the adaxial and abaxial leaf surface respectively ( $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD), and 'Light from Both sides' represents plants lit from both sides ( $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD). Gas exchange parameters were recorded at 60 min, leaf temperature, [CO<sub>2</sub>] and leaf VPD were maintained at 22 °C,  $400 \mu\text{mol mol}^{-1}$  and  $1 \pm 0.2$  kPa respectively. Different letters represent statistically significant differences ( $p < 0.05$ ) between means of each leaf surface of the light treatment ( $n = 4-6$ ).



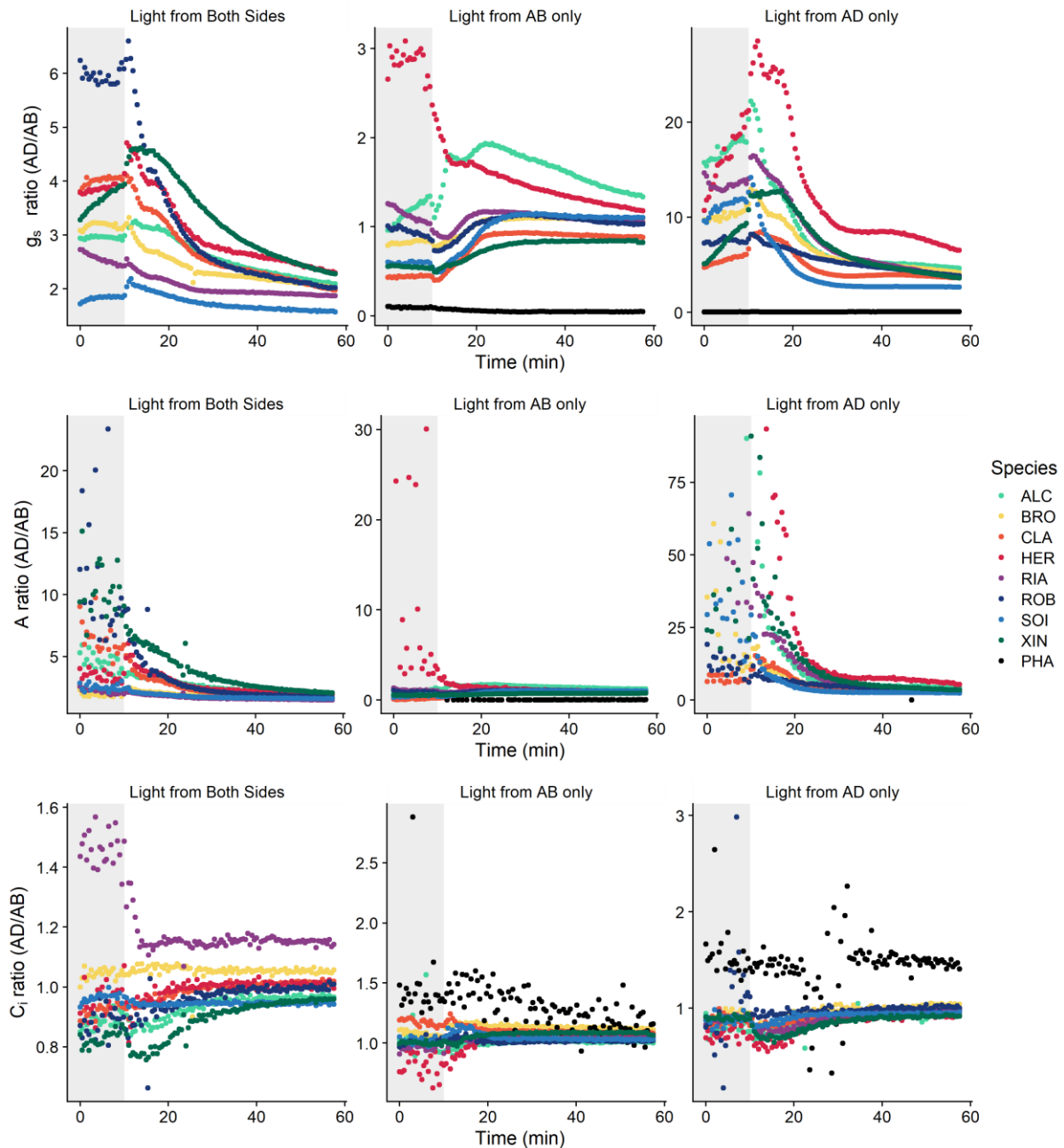
**Fig. S4:** Correlation between leaf gas exchange and leaf anatomy parameters from a leaf lit from the Abaxial leaf surface. Parameters with suffixes “AB” and “AD” represent the values for the abaxial and adaxial leaf surface respectively. gsmax: maximum stomatal conductance, SD; stomatal density, GCL; guard cell length,  $g_s$ ; steady state stomatal conductance under  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , A: steady state net  $\text{CO}_2$  assimilation under  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . R values for significant correlations are highlighted in red.



**Fig. S5:** Correlation between leaf gas exchange and leaf anatomy parameters from a leaf lit from the Adaxial side. Parameters with suffixes “AB” and “AD” represent the values for the abaxial and adaxial leaf surface respectively.  $g_{smax}$ : maximum stomatal conductance, SD; stomatal density, GCL; guard cell length,  $g_s$ ; steady state stomatal conductance under  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , A: steady state net  $\text{CO}_2$  assimilation under  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . R values for significant correlations are highlighted in red.

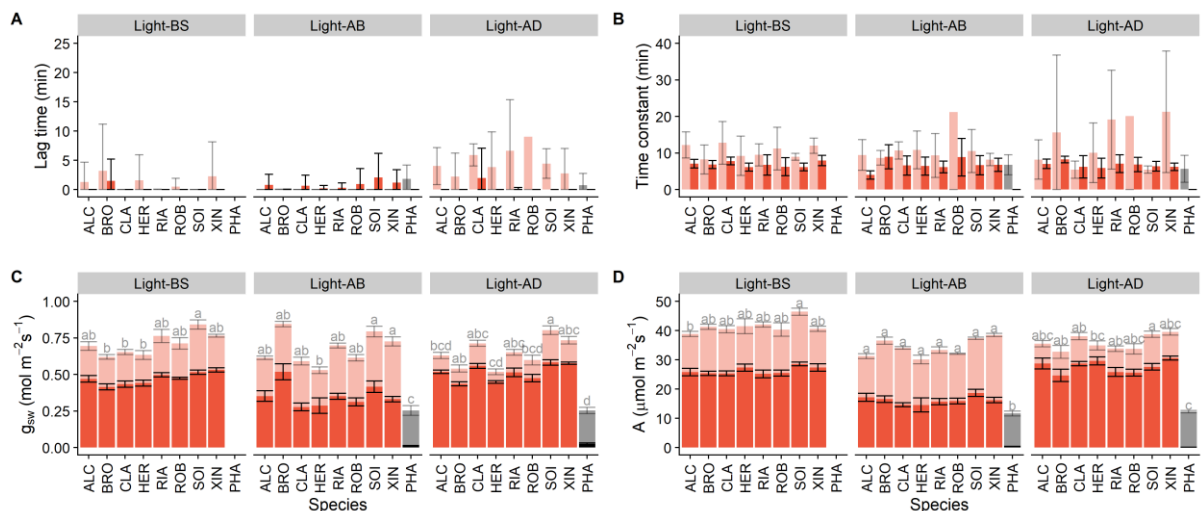


**Fig. S6:** Correlation between leaf gas exchange and leaf anatomy parameters from a leaf lit from both sides. Parameters with suffixes “AB” and “AD” represent the values for the abaxial and adaxial leaf surface respectively.  $g_{smax}$ : maximum stomatal conductance, SD; stomatal density, GCL; guard cell length,  $g_s$ ; steady state stomatal conductance under  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , A: steady state net  $\text{CO}_2$  assimilation under  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ . R values for significant correlations are highlighted in red.

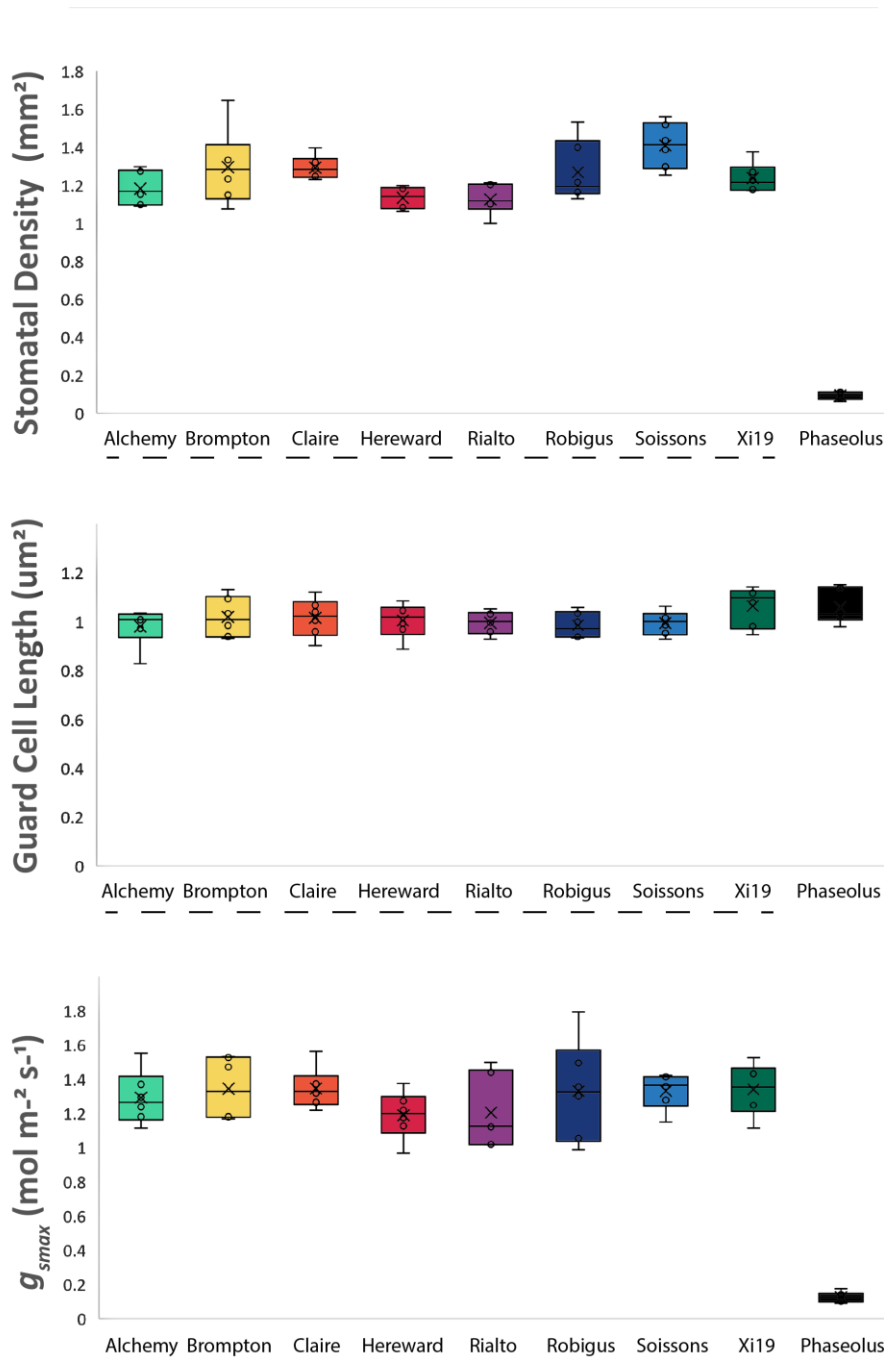


**Fig. S7:** Ratio of adaxial to abaxial values for stomatal conductance ( $g_s$ ), net  $\text{CO}_2$  assimilation ( $A$ ) and intercellular  $\text{CO}_2$  concentration ( $C_i$ ) in response to a step increase in PPF for eight wheat cultivars, Alchemy (ALC), Brompton (BRO), Claire (CLA), Hereward (HER), Rialto (RIA), Robigus (ROB), Soissons (SOI) and Xi19 (XIN) using the split chamber cuvette. Headings 'Light from AD only' and 'Light from AB only' represent the lighting regime, where plants were lit from the adaxial and abaxial leaf surface respectively ( $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPF), and 'Light from Both sides' represents plants lit from both sides ( $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPF). Gas exchange parameters were recorded at 60 min, leaf temperature,  $[\text{CO}_2]$  and leaf VPD were maintained at  $22^\circ\text{C}$ ,  $400 \mu\text{mol mol}^{-1}$  and  $1 \pm 0.2 \text{ kPa}$  respectively.





**Fig. S8:** Variation of lag time in stomatal opening (A), time constant for stomatal opening (B), steady state stomatal conductance (C), net  $\text{CO}_2$  assimilation (D) for the abaxial (AB) and adaxial (AD) leaf surfaces of *Phaseolus Vulgaris* (P.V, grey) and eight wheat cultivars (Alchemy, Brompton, Claire, Hereward, Rialto, Robigus, Soissons and Xi19) to a single step change in PPFD. Headings 'Light-AD' and 'Light-AB' (grey banner) represents the lighting regime, where plants were lit from the adaxial and abaxial leaf surface respectively ( $100 - 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD) and 'Light-BS' (grey banner) represents plants lit from both sides ( $50 - 500 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD). Error bars in (A) and (B) represent 95% confidence intervals and in (C) and (D) the standard error of mean. Different letters represent statistically significant differences ( $p < 0.05$ , Tukey HSD) between means of the added value for both leaf surfaces ( $n = 4-6$ ).



**Fig. S9:** Boxplots of stomatal anatomy ratios the abaxial and adaxial leaf surfaces in eight wheat (*Triticum aestivum*) cultivars (underlined in a broken line) and one French bean (*Phaseolus vulgaris*) cultivar. Variation (box and whisker plots displaying the distribution of biological replicates) and mean (black x) of SD ratios, GCL ratios and  $g_{smax}$  ratios between the abaxial and adaxial leaf surfaces. (n = 6).

