PLANT SCIENCE

Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth

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Stomata serve dual and often conflicting roles, facilitating carbon dioxide influx into the plant leaf for photosynthesis and restricting water efflux via transpiration. Strategies for reducing transpiration without incurring a cost for photosynthesis must circumvent this inherent coupling of carbon dioxide and water vapor diffusion. We expressed the synthetic, light-gated K^+ channel BLINK1 in guard cells surrounding stomatal pores in *Arabidopsis* to enhance the solute fluxes that drive stomatal aperture. BLINK1 introduced a K^+ conductance and accelerated both stomatal opening under light exposure and closing after irradiation. Integrated over the growth period, BLINK1 drove a 2.2-fold increase in biomass in fluctuating light without cost in water use by the plant. Thus, we demonstrate the potential of enhancing stomatal kinetics to improve water use efficiency without penalty in carbon fixation.

Stomata are pores in the leaf epidermis that form between pairs of guard cells. They allow CO_2 uptake for photosynthetic carbon assimilation at the expense of water loss via transpiration, thereby influencing global carbon and hydrological cycles (*1*, *2*). Stomatal aperture is controlled by guard cell turgidity, which responds to changes in atmospheric CO_2 concentration, light, atmospheric relative humidity, and abscisic acid (*3*–*6*), thereby regulating plant water use. Efforts to improve plant water use efficiency (WUE) have focused on reducing stomatal density, despite its implicit penalty in carbon assimilation (*7*, *8*). Approaches that circumvent the carbon–water trade-off pose greater challenges but are also very promising. In particular, accelerating the kinetics of stomatal opening and closing could be used to promote carbon assimilation under high light intensities, while maintaining plant water status when carbon demand is low (*7*, *8*). In this study, we used the synthetic, blue light–induced K^+ channel 1 (BLINK1) as a tool for modulating guard cell K^+ conductance and accelerating changes in stomatal aperture with light. We demonstrate that a strategy of enhancing stomatal kinetics is sufficient to promote photosynthetic carbon assimilation and WUE. Thus, BLINK1 and related optogenetic tools offer ways to explore plant growth and its relationship to WUE without a cost in CO_2 availability for photosynthesis.

Opening and closing of stomata is driven by ion transport across the guard cell plasma membrane, which, together with the metabolism of organic solutes, promotes water flux and changes in guard cell volume and turgor. Blue light (BL) triggers stomatal opening, among other responses, enhancing photosynthesis through the action of the phototropin receptor kinases phot1 and phot2 in activating guard cell H^+–adenosine triphosphatases that, in turn, promote K^+ uptake (*3*, *9*, *10*). We therefore explored whether stomatal opening could be augmented by tissue-specific expression of the optogenetic tool BLINK1.

BLINK1 is a synthetic, blue light–gated K^+ channel constructed by fusing the LOV2-Jα photoswitch from *Avena sativa* phot1 to the small viral K^+ channel Kcv; when expressed in human embryonic kidney cell cultures, it introduces a K^+ conductance that is independent of voltage and activated by BL with half-maximal saturation near 40 μmol m^{-2} s^{-1} (*11*). To confirm that BLINK1 also functions in plants, we first expressed BLINK1 transiently in tobacco and in *Arabidopsis* root epidermal cells (*12*). Immunoblots showed that BLINK1 formed the tetramers expected of a functional K^+ channel (fig. S1). On treatment with 100 μmol m^{-2} s^{-1} BL, membrane voltages of root epidermal cells bathed in 30 mM K^+ showed mean displacements of 15-mV amplitude toward the predicted K^+ equilibrium voltage, as expected on activating a K^+ conductance (fig. S2). From the voltage kinetics, we concluded that the conductance was fully activated within 2 min of BL treatment (+BL) and decayed over 8 to 10 min on transfer to the dark.

To analyze BLINK1 function in guard cells, we used a strong guard cell–specific promoter (*13*) to express the synthetic channel in wild-type (wt) *Arabidopsis* (wt-BLINK) and, as a background control, in the *phot1phot2* (*p1p2*) (*14*) double mutant (*p1p2*-BLINK). Transcript analysis showed that *BLINK1* was expressed at comparable levels in two independent *p1p2*-BLINK and wt-BLINK transgenic lines (Fig. 1B and fig. S3). We measured the plasma membrane conductance using two-electrode recording methods (*15*) on intact guard cells of *p1p2*-BLINK and wt-BLINK transgenic lines and compared conductances in each line to the corresponding *p1p2* and wt backgrounds. Close to the free-running voltage, the membrane conductance of *Arabidopsis* guard

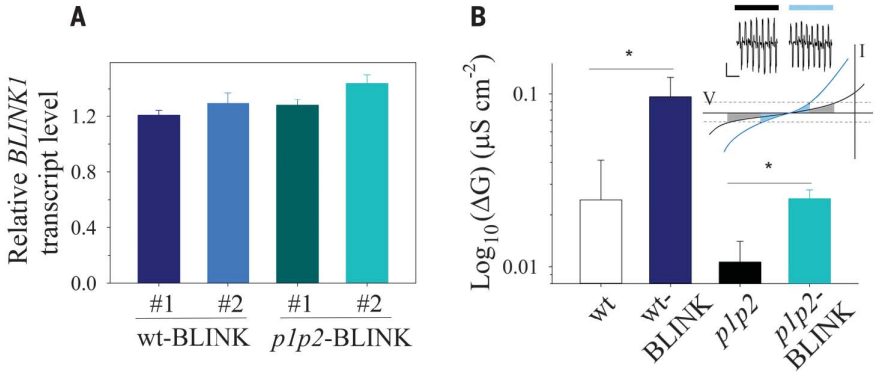

Fig. 1. BLINK1 expression in planta facilitates K^+ fluxes across guard cell plasma membrane. (**A**) Quantitative reverse transcription polymerase chain reaction analysis of relative *BLINK1* transcript levels normalized to reference gene *ISU* (*29*) (n = 4). (**B**) Change in membrane conductance ±BL as means ± SE (n = 4). Significance determined by Student's t test: wt/wt-BLINK, *P = 0.036; *p1p2*/*p1p2*-BLINK, *P = 0.022. (Inset, top) Voltage deflections on current clamp with ±100 pA in 0.5-s steps. Scale bar: 10 mV, vertical; 5 s, horizontal. (Inset, bottom) Schematic to show the consequence of fixed-amplitude current steps on membrane voltage before (black) and during (blue) BL to introduce an increase in conductance. Grey and blue shading indicates the range of voltage deflections. Dashed lines indicate current-clamp amplitude.

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cells is normally small, making it difficult to resolve, by voltage clamp, the conductance changes that would suffice to enhance K^+ flux and accelerate stomatal movements (see supplementary materials and methods). We therefore used a current clamp to drive 0.5-s steps of ±100 pA at intervals across the plasma membrane of dark-adapted guard cells isolated in epidermal peels. We monitored the resulting changes in voltage before, during, and after illuminating with 100 μmol m^{-2} s^{-1} BL (Fig. 1B, inset) and calculated the change in membrane conductance ±BL (Δ*G*) from Ohm's law (Fig. 1B). Photoactivation of BLINK1 led to increased conductance in guard cells of *p1p2*-BLINK and of wt-BLINK plants compared with the *p1p2* mutant and wt controls, respectively, with a 1.6-fold increase in Δ*G* of wt-BLINK plants (Fig. 1B). Thus, we concluded that BLINK1 introduces a BL-dependent K^+ conductance in the plasma membrane of guard cells.

To examine whether BLINK1 photoactivation can alter stomatal opening, we recorded stomatal apertures in epidermal peels exposed to either red light (RL) or BL fluence rates of 100 μmol m^{-2} s^{-1} for 2 hours. BLINK1 restored BL-induced stomatal opening in the *p1p2* double-mutant background (Fig. 2A) and enhanced the steady-state apertures of wt-BLINK plants, on average, by 17% compared with the wt background in BL (Fig. 2B). Similar apertures were observed for all plants under RL, indicating that the effects were BL-specific and demonstrating the potential for BLINK1 to augment stomatal opening in vivo. To assess stomatal kinetics with BLINK1, we used gas exchange and analyzed the stomatal conductances of intact plants ±BL after dark and RL adaptation (Fig. 2, C to F, and fig. S4). Compared with the wt stomatal conductance was elevated in the *p1p2* background in the dark, which is consistent with previous observations (*16*). Against this background, significant increases in stomatal conductance were recovered in each case in the *p1p2*-BLINK transgenics with 100 μmol m^{-2} s^{-1} BL, whereas *p1p2* double-mutant plants were unresponsive to BL (Fig. 2C). BLINK1 expression in the wt background led to enhancements of 22 to 29% in stomatal conductance in BL (Fig. 2D), despite a small reduction in stomatal size in one line (fig. S7). Mean stomatal opening and closing halftimes were accelerated by ~40% compared with the wt controls (Fig. 2E).

Preadapting plants to 200 μmol m^{-2} s^{-1} RL ensures a substantial background of photosynthetic energy input to reduce CO_2 concentration within the leaf and reflects a more natural background for analyzing stomatal movements. As expected, no significant differences in steady-state transpiration, and hence in stomatal conductances, were observed between the wt-BLINK and wt plants; in this background, adding 100 μmol m^{-2} s^{-1} BL elevated stomatal conductance in all plants (table S1). However, wt-BLINK plants showed accelerated changes in stomatal conductance, with 60 to 70% reductions in stomatal opening and closing halftimes compared with wt (Fig. 2F and fig. S4). BLINK1 activity is independent of voltage and declined over 8 to 10 min (Fig. 1 and fig. S2) (*11*), so the accelerated kinetics for stomatal closing is consistent with BLINK1-promoted K^+ efflux as well as influx subject to the electrochemical potential for K^+ across the guard cell membrane.

One measure of plant productivity is water use efficiency, defined either as the amount of dry mass produced per unit water transpired (WUE) or as the ratio of the instantaneous rates of carbon assimilation over transpiration (WUEi). Both measures are affected by light through the combined influence on carbon demand and associated transpiration (*17*). We examined the BLINK1 transgenic lines grown under diel cycles with daylight periods of constant white light, either at a low fluence rate (LWL) of 75 μmol m^{-2} s^{-1} or at a high fluence rate (HWL) of 190 μmol m^{-2} s^{-1}. We calculated WUEi over these periods and determined WUE as the ratio of accumulated dry biomass to water used over the 49-day growth period. By measuring growth under LWL and HWL treatment, we determined that wt-BLINK and *p1p2*-BLINK transgenic plants showed no significant differences in biomass accumulation, rosette area expansion, or water use when compared with wt and *p1p2* controls (figs. S5 and S6 and table S1).

Light fluctuates in the natural environment, for example, as clouds pass overhead. Photosynthesis generally tracks light energy input, but stomata are slower to respond. The slower stomatal kinetics limits gas exchange and can lead to suboptimal assimilation when fluence rate rises and to transpiration without corresponding assimilation when the fluence rate drops quickly (*7*, *17*). Because BLINK1 accelerated stomatal movements (Fig. 2), we predicted that, when integrated over periods of fluctuating light, BLINK1 could benefit carbon assimilation and water use. We examined the BLINK1 transgenic lines grown with daylight periods of fluctuating white light to give a total photon flux over the daylight period intermediate to that of the two continuous light regimes. We stepped fluence rates ranging between 10 and 150 μmol m^{-2} s^{-1} at 60-min intervals, which is close to the time normally required for stomatal opening (Fig. 2) and therefore would maximize any

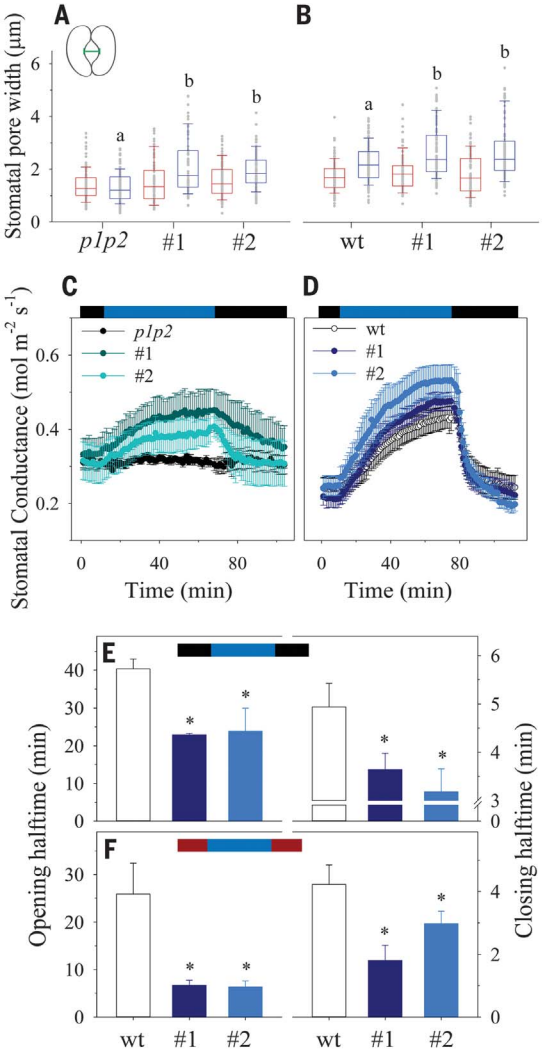

Fig. 2. BLINK1 photoactivation promotes stomatal opening and accelerates stomatal kinetics. BLINK1 restoration of BL-induced stomatal opening in the *p1p2* double mutant (**A**) and enhanced BL-induced stomatal opening in the wt background (**B**). Data are means ± SE (n > 100). Lettering indicates statistically significant differences from the wt and *p1p2* backgrounds, as determined by Kruskal-Wallis analysis of variance on ranks (P < 0.05). (A, inset) Schematic of stomatal pore width for measurement. Stomatal conductances measured in *p1p2* and *p1p2*-BLINK plants (**C**) and in wt and wt-BLINK plants (**D**) before, during, and after 100 μmol m^{-2} s^{-1} BL treatments. Halftimes for stomatal opening and closing of wt and wt-BLINK plants with steps from dark (**E**) and against a background of 100 μmol m^{-2} s^{-1} RL (**F**) were estimated by nonlinear least-squares fitting of data after light transitions to a simple exponential function. Data are means ± SE (n = 5) from wt (white) and the two wt-BLINK lines (dark and light blue) in each case. Asterisks indicate statistically significant differences, as determined by Student's t test (*P < 0.05).

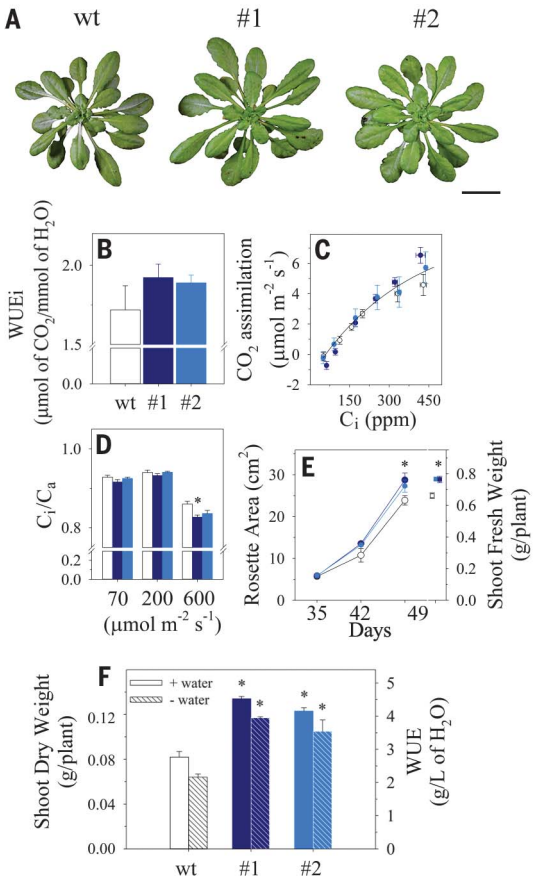

Fig. 3. BLINK1 expression enhances photosynthetic carbon assimilation and WUE. Plants were grown under diel cycles with white light fluctuating at 1-hour intervals between 10 to 150 μmol m^{-2} s^{-1}, at 390 μl/liter CO_2, 22°C, and 55% relative humidity. Scale bar, 5 cm. (**A**) Representative wt (white) and two wt-BLINK plants (cross-referenced below in dark and light blue). (**B**) Instantaneous WUE (WUEi), (**C**) relationship of CO_2 assimilation to intracellular CO_2 concentration (C_i) at saturating (1000 μmol m^{-2} s^{-1}) white light and (**D**) C_i/C_a ratio at 70, 200, and 600 μmol m^{-2} s^{-1} of white light. Data are means ± SE (n = 4 for each line). Long-term plant growth measured as rosette area and shoot fresh weight (**E**) and as shoot dry weight and WUE (**F**) determined for each experiment as dry biomass per liter of water applied. Data in (F) is for plants grown under water-replete (+water, solid white and blue bars) and water-deficit (−water, hatched bars) conditions. Data are means ± SE (n = 15 water-replete; n = 6 water-deficit). Asterisks indicate statistically significant differences compared with wt by Student's t test (*P < 0.05).

advantages afforded by BLINK. No significant difference was evident in WUEi (Fig. 3B). However, rosette area and fresh weight increased in wt-BLINK transgenic plants compared with rosette area and fresh weight in wt control plants (Fig. 3E and tables S2 and S3). We found a 2.2-fold increase in total dry biomass of plants grown under both water-replete and water-deficit conditions, which, for similar rates of steady-state transpiration, translates to an equivalent and highly significant improvement in WUE in the wt-BLINK plants (Fig. 3F). We observed a modest increase in total protein content and a decrease in starch in plants grown under water-replete conditions and a highly significant increase in total starch in plants grown under water-deficit conditions (fig. S8). The wt-BLINK plants showed significant decreases in fresh/dry weight ratios under both conditions (tables S2 and S3); much of this biomass can likely be accounted for by changes in cell wall material. We confirmed that the biomass increase was not the consequence of alterations in photosynthesis per se (*18*): CO_2 assimilation under saturating light (1000 μmol m^{-2} s^{-1}) was unaffected in wt-BLINK plants across the physiological range of internal CO_2 concentrations (C_i) (Fig. 3C), and the C_i/C_a ratios (where C_a is the ambient CO_2 concentration) determined at 70, 200, and 600 μmol m^{-2} s^{-1} of white light were similar to wt plants in each case (Fig. 3D). Thus, we conclude that guard cell expression of BLINK1 and the accelerated stomatal kinetics afforded by the synthetic channel are responsible for enhancing carbon assimilation without a cost in water use.

Optogenetics has revolutionized the study of the mammalian nervous circuitry (*11*, *19*). Because of the high output gain possible in regulating neuronal membrane voltage, the ion fluxes introduced by rhodopsin-based pumps and channels have proven sufficient to control rapid nervous signal transmission (*20*, *21*). Introducing BLINK1 into guard cells demonstrates the application potential for optogenetics to manipulate net ion flux in plant cells, which, over periods of many minutes, can directly alter cell volume and osmotically related physiology. Because many plant movements, growth, and morphogenic phenomena rely on solute flux to drive turgor and cell expansion, optogenetics offers strategies with which to study and control these processes.

Our findings also have implications for strategies to improve crop WUE and enhance net photosynthetic carbon assimilation. Much research to date has focused on enhancing WUE by reducing stomatal densities, an approach that suppresses the overall conductance of the leaf but also reduces CO_2 availability for photosynthesis and can slow plant growth (*7*, *22*–*25*). Manipulating the native populations of ion channels and pumps has been shown to affect stomatal conductance and photosynthesis, but generally at the expense of carbon assimilation or WUE (*15*, *26*–*28*). Indeed, a systems analysis of stomatal physiology shows that manipulating transporter populations alone is unlikely to improve stomatal performance and that alterations targeting the control of transport, including channel gating, are more likely to be effective (*28*). Our findings demonstrate the efficacy of introducing controls on guard cell membrane transport: incorporating BLINK1 adds a light-driven conductance that accelerates stomatal opening and closing to match the temporal demands for guard cell ion flux. Our findings also highlight the gains that might be achieved by enhancing stomatal kinetics under changing light environments. Furthermore, we demonstrate that stomatal speed (*7*) can improve WUE without a cost in carbon assimilation. Enhancing guard cell ion flux with available light is an effective strategy to match stomatal movements with the often conflicting demands of safeguarding water use, at the same time yielding gains in photosynthetic assimilation during vegetative growth.

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ACKNOWLEDGMENTS

We thank A. Moroni for providing the anti-Kcv antibody and for commenting, along with T. Lawson, on the manuscript. **Funding:** This research was supported by the Biotechnology and Biological Sciences Research Council (grants BB/L019025/1,

BB/L001276/1, and BB/M001601/1 to M.R.B.; and BB/M002128/1 and BB/R001499/1 to J.M.C.). **Author contributions:** M.P., J.P., J.M.C., and M.R.B. designed the study; J.P. generated constructs, screened and isolated the *Arabidopsis* transgenic lines, and performed transient expression in tobacco; Y.W. carried out transient transformations and measurements in roots; M.P. performed the physiological and electrophysiological characterization of transgenic lines with assistance from L.H.; M.P., J.M.C., and M.R.B. analyzed the data; M.P., J.M.C., and M.R.B. wrote the manuscript. All authors discussed and commented on the manuscript. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** Requests for materials should be addressed to M.R.B. and J.M.C. Stable BLINK1 transgenic lines of *Arabidopsis* in the wt and *p1p2* backgrounds are available under a material agreement with Plant Bioscience Ltd., Norwich, and the University of Glasgow. All of the data pertaining to the work are contained within the figures and supplementary materials.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/363/6434/1456/suppl/DC1
Material and Methods
Figs. S1 to S8
Tables S1 to S3
References (*30*–*40*)

9 November 2018; accepted 27 February 2019
10.1126/science.aaw0046

Science

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Science **363** (6434), 1456-1459.
DOI: 10.1126/science.aaw0046

Speeding up stomatal responses

A plant's cellular metabolism rapidly adjusts to changes in light conditions, but its stomata—pores that allow gas exchange in leaves—are slower to respond. Because of the lagging response, photosynthesis is less efficient, and excess water is lost through the open pores. Papanatsiou *et al.* introduced a blue light–responsive ion channel into stomata of the small mustard plant *Arabidopsis.* The channel increased the rate of stomata opening and closing in response to light. The engineered plants produced more biomass, especially in the fluctuating light conditions typical of outdoor growth.

Science, this issue p. 1456

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