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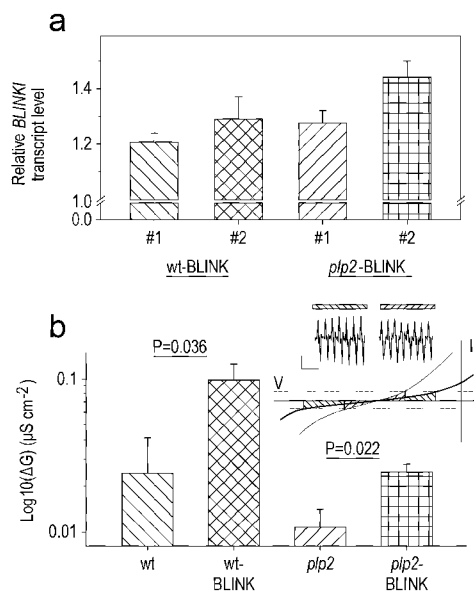


Fig. 1

(57) **Abstract:** The invention relates to methods of increasing stomatal function in plants, which as a result leads to an increase in carbon assimilation and/or water use efficiency and ultimately an increase in yield. In particular the methods of the invention relate to the expression of light-gated potassium channels in the stomatal complex. Also described are genetically altered plants characterised by the above phenotype as well as methods of producing such plants.



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Methods for optogenetic manipulation of stomatal function

FIELD OF THE INVENTION

5 The invention relates to methods of increasing stomatal function in plants, which leads to an increase in carbon assimilation and/or water use efficiency and ultimately an increase in yield. In particular the methods of the invention relate to the expression of light-gated potassium channels in the stomatal complex. Also described are genetically altered plants characterised by the above phenotype as well as methods of
10 producing such plants.

BACKGROUND OF THE INVENTION

15 Globally, water usage has increased 6-fold in the past 100 years, twice as fast as the human population, and is expected to double again before 2030, driven mainly by irrigation and agriculture. In the UK alone, irrigation has risen 10-fold in the past 30 years and this trend is expected to continue.

20 Stomata are pores in the leaf epidermis that form between pairs of guard cells. They allow CO₂ uptake for photosynthetic carbon assimilation at the expense of water loss via transpiration. As such, they provide the major route for gaseous exchange between the interior of the leaf and the atmosphere and can limit photosynthetic rates by 50% or more when demand exceeds water supply. Stomata exert a major control on the water and carbon cycles of the world, and their activity in crops has been a key factor in
25 global atmospheric modelling and weather prediction for over a quarter of a century.. Stomatal aperture is controlled by guard cell turgidity which responds to changes in atmospheric CO₂ concentration, light, atmospheric relative humidity, and abscisic acid (5-8), thereby regulating plant water use. Efforts to improve plant water use efficiency have focused on reducing stomatal density, despite its implicit penalty in carbon
30 assimilation (3, 4). Approaches that circumvent the carbon:water trade-off pose greater challenges but also much promise. 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 (3, 4).

There therefore exists a need to reduce transpiration rates from the stomata without affecting photosynthesis, particularly in crops with slow stomatal kinetics. This will ultimately lead to an increase in growth and/or yield in these crops. The present invention addresses this need.

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SUMMARY OF THE INVENTION

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Stomata serve dual and often conflicting roles, facilitating CO₂ influx into the plant leaf for photosynthesis and restricting water efflux via transpiration. Thus, strategies for reducing transpiration without a cost for photosynthesis must circumvent this inherent coupling of CO₂ and water vapour diffusion. In one example, we have expressed a synthetic, light-gated potassium channel, specifically a K⁺ channel, BLINK1, in guard cells surrounding stomatal pores to enhance the solute fluxes that drive stomatal aperture. BLINK1 introduced a K⁺ conductance and accelerated both stomatal opening in the light and closing following irradiation. Thus, we demonstrate the potential of enhancing stomatal kinetics to improve water use efficiency without penalty in carbon fixation.

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Accordingly, in one aspect of the invention there is provided a method of increasing at least one of growth, yield, drought tolerance, water use efficiency and/or carbon assimilation in a plant, the method comprising expressing a light-gated potassium channel in cells of the stomatal complex.

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In a preferred embodiment, the method increases both water use efficiency and carbon assimilation.

In one embodiment, the method comprises expressing a blue-light gated potassium channel.

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In a preferred embodiment, the method comprises introducing and expressing in a plant a nucleic acid construct comprising a nucleic acid sequence encoding a light-gated potassium channel, preferably a blue-light gated potassium channel operably linked to a regulatory sequence. More preferably, the nucleic acid sequence encodes a blue-light gated potassium channel (BLINK1) as defined in SEQ ID NO: 3 or a functional variant or fragment thereof. In a further preferred embodiment, the nucleic

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acid sequence encoding a blue-light induced potassium channel comprises a sequence as defined in SEQ ID NO: 4 or a functional variant or fragment thereof.

5 In one embodiment, the regulatory sequence is a stomatal complex promoter or a constitutive promoter. In a preferred embodiment, the stomatal complex promoter is a guard-cell specific promoter.

10 In another aspect of the invention, there is provided a nucleic acid construct comprising a nucleic acid sequence encoding a light-gated potassium channel operably linked to a regulatory sequence, wherein the regulatory sequence is a stomatal complex promoter. In one embodiment, the potassium channel is a blue-light gated potassium channel or a red-light gated potassium channel, more preferably a blue-light gated potassium channel.

15 In one embodiment, the nucleic acid sequence encodes a blue-light gated potassium channel comprising a potassium channel as defined in SEQ ID NO: 1 or a functional variant or fragment thereof. More preferably, the nucleic acid sequence encoding the potassium channel comprises a sequence as defined in SEQ ID NO: 2 or a functional variant or fragment thereof.

20 In a preferred embodiment, the nucleic acid sequence encodes a blue-light gated potassium channel (BLINK1) as defined in SEQ ID NO: 3 or a functional variant or fragment thereof. More preferably, the nucleic acid sequence encoding a blue-light induced potassium channel comprises a sequence as defined in SEQ ID NO: 4 or a functional variant or fragment thereof.

25 In a preferred embodiment, the stomatal complex promoter is a guard cell-specific promoter. More preferably, the guard-cell specific promoter is selected from Myb60, GC1 and SNAC1.

30 In another aspect of the invention, there is provided a vector comprising the nucleic acid construct described above. In a further aspect there is provided a host cell comprising the nucleic acid construct or the vector described above.

In another aspect of the invention there is provided a transgenic plant expressing the nucleic acid construct or the vector described above.

5 In another aspect of the invention, there is provided a method of producing a transgenic plant as described above, the method comprising:

- a. selecting a part of the plant;
- b. transfecting at least one cell of the part of the plant of part (a) with the nucleic acid construct or the vector described above; and
- c. regenerating at least one plant derived from the transfected cell or cells.

10 In a final aspect of the invention, there is provided a plant obtained or obtainable by the method described above.

In one embodiment the plant is a monocot or dicot.

15 DESCRIPTION OF THE FIGURES

The invention is further described in the following non-limiting figures:

20 **Figure 1** shows that BLINK1 expression in planta facilitates K⁺ fluxes across the guard cell plasma membrane. (a) qRT-PCR analysis of relative BLINK1 transcript levels normalised to reference gene ISU(29) (n=4). (b) Change in membrane conductance $\pm$ BL as means $\pm$ SE (n=4). Significance determined by student's t-test: wt/wt-BLINK, p=0.036; *p1p2/p1p2*-BLINK, P=0.022. Inset (above): Voltage deflections on current clamp with $\pm$ 100 pA in 0.5-s steps. Scale bar: 10 mV, vertical, 5 s, horizontal. Inset (below): Schematic to show the consequence for 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. Dotted lines indicate current clamp amplitude.

30 **Figure 2** shows BLINK1 photoactivation promotes stomatal opening and accelerates stomatal kinetics. (a,b) 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 $\pm$ SE (n>100). Lettering indicates statistically significant differences from the wild-type and *p1p2* backgrounds, as determined by Kruskal-Wallis ANOVA on Ranks (P<0.05). Inset: Schematic of stomatal pore width for measurement. (c,d)

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Stomatal conductances measured from p1p2 and p1p2-BLINK plants (c) and from wild-type and wt-BLINK plants (d) before, during and after 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BL treatments. (e,f) Halftimes for stomatal opening and closing of wt and wt-BLINK plants with steps from dark (e) and against a background of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ RL (f) were estimated by non-linear least-squares fitting of data following light transitions to a simple exponential function. Data are means $\pm$ 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 ($P < 0.05$).

Figure 3 shows BLINK1 expression enhances photosynthetic carbon assimilation and water use efficiency. Plants were grown under diel cycles with white light fluctuating at 1-h intervals between 10 to 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, at 390 $\mu\text{L/L}$ 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 water use efficiency (WUEi), (c) relationship of CO_2 assimilation to intracellular CO_2 concentration (C_i) at saturating (1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) white light and (d) C_i/C_a ratio at 70, 200 and 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of white light. Data are means $\pm$ SE (n=4 for each line). (e,f) Long-term plant growth (e) measured as rosette area 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, open and solid bars) and water-deficient (-water, hatched bars) conditions. Data are means $\pm$ SE (n=15 water-replete; n=6 water-deficit). Asterisks indicate statistically significant differences, determined by student's t-test ($P < 0.05$).

Figure 4 shows gas exchange characteristics of BLINK1 transgenic and wild type plants adapted to either darkness or red light.

Figure 5 shows growth characteristics of BLINK1-transgenic, wild type and p1p2 plants grown under two continuous light regimes; and under fluctuating light and two water regimes.

Figure 6 shows functional tetramer formation of BLINK1 in planta. Immunoblot analysis of total protein extracts from *N. benthamiana* leaves BLINK1 (+) or not expressing BLINK1 (-). The protein extract volumes indicated were probed with anti-Kcv monoclonal antibody recognizing specifically the tetrameric form of the channel.

Expected Mw of BLINK1 is 106.5 kDa. Lower Mw bands likely represent proteolytic cleavage products. Black arrow indicates the tetramer formation of BLINK1.

Figure 7 shows BLINK1 photoactivation drives the membrane towards the K⁺ equilibrium voltage (E_K). Representative recordings of membrane voltage in 5 mM Ca²⁺-MES, pH 6.1, with 30 mM KCl from wt (grey) and transiently BLINK1-transformed (black) Arabidopsis roots(12). Treatments with 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BL indicated by shading. Scale bar: 10 mV, vertical; 2 min, horizontal. Estimated E_K, -30 mV. Mean halftimes and voltage displacements: 89 $\pm$ 8 s (+BL); 351 $\pm$ 24 s (-BL); 15 $\pm$ 2 mV (n=10).

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Figure 8 shows BLINK1 photoactivation stimulates stomatal opening. Stomatal conductance (a, c) and CO₂ assimilation rates (b, d) measured from RL-adapted wt, wt-BLINK, p1p2 and p1p2-BLINK plants on adding 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BL. Transitions from RL to RL+BL were carried out once stomatal conductances reached a steady state. Data are means $\pm$ SE (n=5 for each line).

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Figure 9 shows BLINK1 has a marginal effect on carbon assimilation and WUE under diel cycles of continuous low light intensity. Wild type (white) and two wt-BLINK transgenic plants (dark and light blue bars and symbols) were grown under diel cycles of with steady illumination of 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Scale bar, 5 cm (above). (a) WUE_i calculated from plants exposed to 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of homogeneous white light, at 390 ppm of CO₂, 22°C and 55% relative humidity. Data are means $\pm$ SE (n=4 for each line). (b,c) Growth of plants followed for 49 days to measure rosette expansion (b) and accumulation of dry biomass and long term WUE (c). Data are means $\pm$ SE (n=10 independent experiments).

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Figure 10 shows BLINK1 has a marginal effect on carbon assimilation and WUE under diel cycles of continuous high light intensity. Wild type (white) and two wt-BLINK transgenic plants (dark and light blue bars and symbols) were grown under diel cycles of with steady illumination of 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Scale bar, 10 cm (above). (a) WUE_i calculated from plants exposed to 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of homogeneous white light, at 390 ppm of CO₂, 22°C and 55% relative humidity. Data are means $\pm$ SE (n=4 for each line). (b,c) Growth of plants followed for 49 days to measure rosette expansion (b) and accumulation of dry biomass and long term WUE (c). Data are means $\pm$ SE (n=10 independent experiments).

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Figure 11 shows BLINK1 induces starch accumulation under water limited conditions. Plants were grown under diel cycles with white light fluctuating at 1-h intervals between 10 to 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, at 390 $\mu\text{L/L}$ CO_2 , 22°C and 55% relative humidity. Plants exposed to two water regimes, water-replete (+water; open and solid bars) and water-deficit (-water; hatched bars) with 10% soil moisture. a) Total protein amount and (b) total starch amount, quantified as mg per g of fresh shoot weight. Data are means $\pm$ SE (n=4). Asterisks indicate statistically significant differences, determined by student's t-test ($P < 0.05$).

Figure 12 shows that selected LOV2-J α photoswitch components confer blue light dependence on GORK VSD interactions when separated between polypeptides. a) mbSUS assay using a GORK bait with LOV2 replacing the N-terminal binding domain and J α helix replacing the S2-S3 loop. Various isolated VSDs with and without complementary photoswitch domains used as prey. Yeast were grown in the dark or under 50 $\mu\text{mol m}^{-2}\text{s}^{-1}$ blue light (right). Growth on 50 μM Met indicates strong interaction. b) Strategies to engineer LOV2 and J α domains within a single GORK subunit using α -helical spacers (above) and myristoylated anchors (inverted black lollipops, below) to prevent LOV2-J α interactions internal to each GORK tetramer.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will now be further described. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of plant biology, microbiology, tissue culture, molecular biology, chemistry, biochemistry and recombinant DNA technology, bioinformatics which are within the skill of the art. Such techniques are explained fully in the literature.

As used herein, the words "nucleic acid", "nucleic acid sequence", "nucleotide", "nucleic acid molecule" or "polynucleotide" are intended to include DNA molecules

(e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), natural occurring, mutated, synthetic DNA or RNA molecules, and analogs of the DNA or RNA generated using nucleotide analogs. It can be single-stranded or double-stranded. Such nucleic acids or polynucleotides include, but are not limited to, coding sequences of structural genes, anti-sense sequences, and non-coding regulatory sequences that do not encode mRNAs or protein products. These terms also encompass a gene. The term "gene" or "gene sequence" is used broadly to refer to a DNA nucleic acid associated with a biological function. Thus, genes may include introns and exons as in the genomic sequence, or may comprise only a coding sequence as in cDNAs, and/or may include cDNAs in combination with regulatory sequences.

The terms "polypeptide" and "protein" are used interchangeably herein and refer to amino acids in a polymeric form of any length, linked together by peptide bonds.

The aspects of the invention involve recombination DNA technology and exclude embodiments that are solely based on generating plants by traditional breeding methods.

For the purposes of the invention, a "genetically altered plant" or "mutant plant" is a plant that has been genetically altered compared to the naturally occurring wild type (WT) plant. In one embodiment, the genetically altered plant is a transgenic plant. For the purposes of the invention, "transgenic", "transgene" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette, gene construct or a vector comprising the nucleic acid sequence or an organism transformed with the nucleic acid sequences, expression cassettes or vectors according to the invention, all those constructions brought about by recombinant methods in which either

(a) the nucleic acid sequences encoding proteins useful in the methods of the invention, or

(b) genetic control sequence(s) which is operably linked with the nucleic acid sequence according to the invention, for example a promoter, or

(c) a) and b) are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to take the form of, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues.

The natural genetic environment is understood as meaning the natural genomic or chromosomal locus in the original plant or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, most preferably at least 5000 bp. A naturally occurring expression cassette – for example the naturally occurring combination of the natural promoter of the nucleic acid sequences with the corresponding nucleic acid sequence encoding a polypeptide useful in the methods of the present invention, as defined above – becomes a transgenic expression cassette when this expression cassette is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815 both incorporated by reference.

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In one aspect of the invention there is provided a method of increasing at least one of growth, yield, drought tolerance, water use efficiency (WUE) and/or carbon assimilation in a plant, the method comprising expressing a light-gated potassium channel in cells of the stomatal complex. In a preferred embodiment, there is provided a method of increasing growth and/or yield and/or water use efficiency and carbon assimilation. In other words, the method increases both water use efficiency and carbon assimilation.

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The term "yield" in general means a measurable produce of economic value, typically related to a specified crop, to an area, and to a period of time. Individual plant parts directly contribute to yield based on their number, size and/or weight. The actual yield is the yield per square meter for a crop per year, which is determined by dividing total production per year (includes both harvested and appraised production) by planted square metres.

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The term "increased yield" as defined herein can be taken to comprise any or at least one of the following and can be measured by assessing one or more of (a) increased biomass (weight) of one or more parts of a plant, aboveground (harvestable parts), or increased root biomass, increased root volume, increased root length, increased root diameter or increased root length or increased biomass of any other harvestable part. Increased biomass may be expressed as g/plant or kg/hectare, (b) increased seed yield per plant, which may comprise one or more of an increase in seed biomass

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(weight) per plant or on an individual basis, (c) increased seed filling rate, (d) increased number of filled seeds, (e) increased harvest index, which may be expressed as a ratio of the yield of harvestable parts such as seeds over the total biomass, (f) increased viability/germination efficiency, (g) increased number or size or weight of seeds or pods or beans or grain (h) increased seed volume (which may be a result of a change in the composition (i.e. lipid (also referred to herein as oil)), protein, and carbohydrate total content and composition, (i) increased (individual or average) seed area, (j) increased (individual or average) seed length, (k) increased (individual or average) seed perimeter, (l) increased growth or increased branching, for example inflorescences on more branches, (m) increased fresh weight or grain fill (n) increased ear weight (o) increased thousand kernel weight (TKW), which may be taken from the number of filled seeds counted and their total weight and may be as a result of an increase in seed size and/or seed weight (p) decreased number of barren tillers per plant and (q) sturdier or stronger culms or stems. All parameters are relative to a wild-type or control plant. The terms "seed" and "grain" as used herein can be used interchangeably. The terms "increase", "improve" or "enhance" as used herein are also interchangeable.

In one embodiment, an increase in yield comprises at least an increase in biomass, and in one embodiment, said increase in biomass is at least one-fold, two-fold, three-fold or four-fold or more compared to a wild-type or control plant. In one embodiment, the method increases biomass by at least two-fold compared to a wild-type or control plant.

In one embodiment, the method increases at least protein and/or starch composition in the plant.

By "water use efficiency" or "WUE" is meant the ratio of water used in plant metabolism to water lost through transpiration. In one example, WUE may be measured by measuring the amount of carbon fixed per water transpired. Alternatively, WUE may be measured by measuring the biomass per water transpired over a growing period or the amount of water fed to a plant. Other methods for measuring WUE would be well known to the skilled person.

By "carbon assimilation" or carbon fixation (such terms may be used interchangeably) is meant the conversion of inorganic carbon to organic compound, for example,

through photosynthesis. In one example, carbon assimilation may be measured by measuring total biomass over time.

5 An “increase” as used herein, may refer to an increase of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90 or 95% or more compared to a control plant.

10 In some plants, stomatal opening (and closing) is controlled solely by guard cells. In other plants, such as maize and other grasses, stomatal opening (and closing) is controlled by a complex of pore-forming guard cells and adjacent subsidiary cells.

15 Accordingly, in one embodiment, the “cells of the stomatal complex” refers only to the guard cells. In other embodiments, the “cells of the stomatal complex” refer to the guard cells and adjacent subsidiary cells. In this embodiment, the light-gated potassium channel may be expressed in the guard cells and/or the subsidiary cells.

20 Preferably expression of a light-gated potassium channel in the stomatal complex affects stomatal opening, and more preferably increases the rate of stomatal opening and/or closing compared to a wild-type or control plant. In a particularly preferred embodiment, the light-gated potassium channel is a non-native or natural light-gated potassium channel. That is, the potassium channel is not an endogenous light-gated potassium channel – the channel is “exogenous” to an individual plant, which is introduced into the plant by any means other than by a sexual cross. As such, the method comprises the expression of a non-native channel. Examples of means by which this can be accomplished are described below. However, in another preferred embodiment, the potassium channel is a native channel.

30 The closing and opening of stomata is regulated by various parameters, which include endogenous light-activated signalling pathways. One such pathway acts through the photoreceptor proteins, phototropins. Phototropins are activated in response to blue light and lead, through a multi-component signalling cascade, to the opening of stomata.

35 Accordingly, by “light-gated potassium channel” is meant a transmembrane protein (i.e. the potassium channel) coupled with a photoswitch or photosensory molecule. Binding

of photons to the photosensory molecule alters the confirmation of the channel, either to an open or closed state which in turn alters the conductance of the channel. Accordingly, in one embodiment, the light-gated potassium channel comprises a transmembrane potassium channel and a photosensory molecule. In one embodiment, binding of light to the channel causes the channel to open. In an alternative embodiment, binding of light to the channel causes the channel to close. Preferably, the channel is reversible. That is, in the presence/absence of light the channel can move from an open or closed position or vice versa. In a further embodiment, the photosensory molecule alters the confirmation of the transmembrane channel in response to a wavelength of light ranging from 280-800 nm or more preferably in response to a wavelength of light in the visible spectrum. The selectivity of the channel for a particular light is determined by the choice of photosensory molecule. However, in one preferred embodiment, the photosensory molecule alters the confirmation of the transmembrane channel in response to blue light.

Thus, in one embodiment the channel is a blue-light gated channel. Blue light may be defined as light between 390-495nm. In one embodiment, the channel may open in response to blue light. In an alternative embodiment, the channel may close in response to blue light. In this example, a blue light-activated channel has the advantage that the channel will be activated at the same time as phototropins thereby leading to augmented stomatal opening.

In an alternative embodiment, the channel is a red-light gated channel. Red light may be defined as light between 600 to 750nm. In one embodiment, the channel may open in response to red light. In an alternative embodiment, the channel may close in response to red light.

In one embodiment, the photosensory molecule comprises a phototropin, more preferably a phototropin fragment. In one embodiment, the phototropin fragment is a LOV2 domain.

In one embodiment, the LOV2 domain comprises or consists of the following sequence or a functional variant or fragment thereof:

LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATV
 RKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVR
 DAAEREGVMLIKKTAE (SEQ ID NO: 14)

- 5 In a further preferred embodiment, the photosensory molecule comprises at least one Jalpha helix domain. Preferably, the Jalpha helix domain comprises or consists of the following sequence or a functional variant or fragment thereof:

AAEREGVMLIKKTAE (SEQ ID NO: 15)

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In an alternative embodiment, the photosensory molecule is selected from one of the following: a UV responsive protein (such as UVR8), a cryptochrome (such as CRY1 or CRY2), a phytochrome, a phototropin, a Zeittupe (ZTL) family protein, a neochrome, an aureochrome, a BLUF-domain containing protein, a LOV-domain containing protein or
 15 a functionally active fragment thereof (that is, the fragment can respond to light and cause the opening and/or closing of a transmembrane potassium channel that is coupled to the photosensory molecule). In one embodiment, the fragment is under 20kDa.

- 20 In one embodiment, the potassium channel itself is a native or non-native channel. That is the potassium channel may be the endogenous potassium channel that is coupled to a photosensory molecule, such as those described above. For example, where the plant is Arabidopsis, the potassium channel may be a KAT1 (NC_003076.8) or KAT2 (NC_003075.7) channel. In another example, where the plant is maize, the
 25 potassium channel may be a KZM2 (AY919830.1) or KZM3 (NM_001111691.1) channel. In another example, the native potassium channel is GORK. In one embodiment, the amino acid sequence of GORK is defined in SEQ ID NO: 10 and the nucleotide sequence defined in SEQ ID NO: 11. Also covered are functional variants and fragments thereof. Other endogenous potassium channels would be well known to
 30 the skilled person.

In an alternative embodiment, the potassium channel may be an exogenous channel. In one example, the channel may be a viral potassium channel, for example, Kcv. In one embodiment, the amino acid sequence of Kcv is defined in SEQ ID NO: 1 and the

nucleotide sequence of Kcv is defined in SEQ ID NO: 2. Also covered are functional variants and fragments of these sequences, as defined below.

5 In one particularly preferred embodiment, the potassium channel is a blue-light gated potassium channel. In one example, the blue-light gated potassium channel is the BLINK1 channel or a variant thereof described below and in Cosentino et al., 2015, which is incorporated herein by reference. The BLINK1 channel is a fusion of the plant LOV2-Japha photosensory molecule to the small viral potassium channel, Kcv. The amino acid sequence of BLINK1 is defined in SEQ ID NO: 3 and the nucleotide
10 sequence of BLINK1 is defined in SEQ ID NO: 4. BLINK1 acts as both a potassium efflux and influx channel, depending on the potassium concentration across the membrane, and is activated in response to blue-light leading to channel opening.

15 In one embodiment, a variant of BLINK1 comprises an amino acid sequence as defined in SEQ ID NO: 8 and a nucleic acid sequence as defined in SEQ ID NO: 9 or variants thereof. In this example, the BLINK1 variant is inhibited rather than activated by light.

In a further embodiment, a variant of BLINK1 comprises one or more amino acid substitutions, deletions or additions compared to the sequence presented in SEQ ID
20 NO: 3. Preferably, the one or more amino acid substitutions, deletions or additions increases or decreases photosensitivity. Preferably, the variant comprises one or more substitutions. Even more preferably, the variant comprises a I427V substitution, and decreases LOV2 photosensitivity. In an alternative example, the variant comprises a V416I substitution and increases LOV2 photosensitivity.

25 As discussed above, in one embodiment, the method comprises expressing an exogenous light-gated potassium channel. In one example this may be achieved by introducing and expressing in the plant a nucleic acid construct comprising a nucleic acid sequence that encodes a light-gated potassium channel that is operably linked to
30 a regulatory sequence. Examples of suitable light-gated and in particular blue-light gated potassium channels are described above.

In one embodiment, the method comprises introducing and expressing in the plant a nucleic acid construct comprising a nucleic acid sequence that encodes a light-gated
35 potassium channel that is operably linked to a regulatory sequence, wherein the

nucleic acid sequence encodes a blue-light gated potassium channel. In one embodiment, the potassium channel is defined in SEQ ID NO: 1, more preferably a blue-light gated potassium channel as defined in SEQ ID NO: 3 or a functional variant or fragment thereof.

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In a more preferred embodiment, the nucleic acid construct comprises a nucleic acid sequence as defined in SEQ ID NO: 4 or a functional variant or fragment thereof.

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The term “variant” or “functional variant” as used throughout with reference to any of the sequences described herein refers to a variant gene sequence or part of the gene sequence (such as a fragment) which retains the biological function of the full non-variant sequence. A functional variant also comprises a variant of the gene of interest, which has sequence alterations that do not affect function, for example in non-conserved residues. Also encompassed is a variant that is substantially identical, i.e.

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has only some sequence variations, for example in non-conserved residues, compared to the wild type sequences as shown herein and is biologically active. Alterations in a nucleic acid sequence that results in the production of a different amino acid at a given site that does not affect the functional properties of the encoded polypeptide are well known in the art. For example, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

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As used in any aspect of the invention described throughout a “variant” or a “functional variant” has at least 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%,

81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% overall sequence identity to the non-variant nucleic acid or amino acid sequence.

5 The term "operably linked" as used throughout refers to a functional linkage between the promoter sequence and the gene or nucleotide sequence of interest, such that the promoter sequence is able to initiate transcription of the gene or nucleotide sequence of interest.

10 In a preferred embodiment of the invention, the regulatory sequence may be a promoter. According to all aspects of the invention, including the method above and including the plants, methods and uses as described below, the term "regulatory sequence" is used interchangeably herein with "promoter" and all terms are to be taken in a broad context to refer to regulatory nucleic acid sequences capable of effecting
15 expression of the sequences to which they are ligated. The term "regulatory sequence" also encompasses a synthetic fusion molecule or derivative that confers, activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ.

The term "promoter" typically refers to a nucleic acid control sequence located
20 upstream from the transcriptional start of a gene and which is involved in the binding of RNA polymerase and other proteins, thereby directing transcription of an operably linked nucleic acid. Encompassed by the aforementioned terms are transcriptional regulatory sequences derived from a classical eukaryotic genomic gene (including the
25 CCAAT box sequence) and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue- specific manner. Also included within the term is a transcriptional regulatory sequence of a classical prokaryotic gene, in which case it may include a -35 box sequence and/or -10 box transcriptional
30 regulatory sequences.

More preferably the promoter may be a constitutive promoter. In an alternative
embodiment, the promoter may be a tissue-specific promoter, preferably a stomatal
complex-specific promoter (as defined above) and more preferably a guard-cell
35 promoter.

A "constitutive promoter" refers to a promoter that leads to increased or overexpression of the target gene. Examples of strong promoters include, but are not limited to, CaMV-35S, CaMV-35Somega, ubiquitin, such as UB10 and actin.

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In one example, a guard cell specific promoter may be selected from Myb60, GC1, and the SNAC1 promoter. In one embodiment, where the plant is a Brassica the promoter may be GC1 or Myb60. In another embodiment, where the plant is barley the promoter may be SNAC1 or GC1. In one embodiment, Myb60 comprises a sequence as defined in SEQ ID NO: 5 or 6 a functional variant, fragment or homologue or orthologue thereof. In another embodiment, GC1 comprises a sequence as defined in SEQ ID NO: 7 or a functional variant, fragment or homolog or orthologue thereof.

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In another embodiment, the promoter may be an epidermal promoter, which allows the light-gated potassium channel to be expressed in the subsidiary cells of the stomatal complex. Examples of epidermal promoters include CER6, ATML1, TaGstA1 and the maize CST1 promoter. In one embodiment, where the plant is a Brassica the epidermal promoter may be CER6 or ATML1. In another embodiment, where the plant is barley, the epidermal promoter is TaGstA1 or the maize CST1 promoter. In one example, the CER6 promoter sequence comprises or consists of the nucleic acid sequence defined in SEQ ID NO: 12 or a functional variant or fragment thereof. In another example, the TaGstA1 promoter sequence comprises or consists of the nucleic acid sequence defined in SEQ ID NO: 13 or a functional variant or fragment thereof

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Suitable homologs or orthologs can be identified by sequence comparisons and identification of conserved domains. The function of the homolog or ortholog can be identified as described herein and a skilled person would thus be able to confirm the function when expressed in a plant.

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For the identification of functionally equivalent promoters, the promoter strength and/or expression pattern of a candidate promoter may be analysed for example by operably linking the promoter to a reporter gene and assaying the expression level and pattern of the reporter gene in various tissues of the plant. Suitable well-known reporter genes are known to the skilled person and include for example beta-glucuronidase or beta-galactosidase.

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5 In a preferred embodiment of the methods described herein the organism is grown or cultured in fluctuating or continuous light conditions. In other words, the organism may be cultured in normal day and/or night conditions (i.e. this may mean that the plant is exposed to a suitable day/night cycle) or exposed to a fluctuating or continuous light regime. If the plant is to be grown indoors with LED lighting, the length, duration and wavelength of light may be controlled.

10 In an alternative embodiment, genome editing techniques may be used to express a light-gated potassium channel in the cells of the stomatal complex. In one embodiment, CRISPR may be used to introduce one or more copies of an light-gated potassium channel as described above into the genome at an appropriate place, that is under the control of a suitable promoter, such as the promoters described in this application.

15 In another aspect of the invention, there is provided a method of increasing stomatal opening and/or closing and/or accelerating stomatal kinetics, the method comprising expressing a light-gated potassium channel in cells of the stomatal complex, as described above.

20 In another aspect of the invention there is provided a nucleic acid construct comprising a nucleic acid sequence encoding a light gated potassium channel as described above operably linked to a regulatory sequence, wherein the regulatory sequence is a stomatal complex promoter, as described above.

25 In another aspect of the invention, there is provided a vector or expression vector comprising the nucleic acid construct described herein.

In another aspect of the invention there is provided a host cell comprising the nucleic acid construct or the vector. Preferably the host cell is a plant cell.

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In another aspect of the invention there is provided a transgenic plant where the transgenic plant expresses the nucleic acid construct or vector.

35 In one embodiment, the progeny plant is transiently transformed with the nucleic acid construct or vector. In another embodiment, the progeny plant is stably transformed

with the nucleic acid construct described herein and comprises the exogenous polynucleotide encoding the light gated potassium channel which is heritably maintained in at least one cell of the organism. The method may include steps to verify that the construct is stably integrated. The method may also comprise the additional
5 step of collecting seeds from the selected progeny plant.

In a further aspect of the invention there is provided a method of producing a transgenic plant as described herein, the method comprising at least the following steps:

- 10 a. selecting a part of the plant;
- b. transfecting at least one cell of the part of the plant of part (a) with the nucleic acid construct or the vector; and
- c. regenerating at least one plant derived from the transfected cell or cells.

15 Transformation or transfection methods for generating a transgenic plant of the invention are known in the art. Thus, according to the various aspects of the invention, a nucleic acid construct as defined herein is introduced into an organism and expressed as a transgene. The nucleic acid construct is introduced into said organism through a process called transformation. The term "transfection", "introduction" or
20 "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Such terms can also be used interchangeably in the present context. Where the organism is a plant, tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention
25 and a whole plant regenerated there from. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and
30 induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively, it may be integrated into the host genome. The resulting transformed plant cell may then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

Transformation of plants is now a routine technique in many species. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. The methods described for the transformation of an organism's cells may be utilized for transient or for stable transformation.

5 Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts, electroporation of protoplasts, microinjection into plant material, DNA or RNA-coated
10 particle bombardment, infection with (non-integrative) viruses and the like. Transgenic plants, including transgenic crop plants, are preferably produced via *Agrobacterium tumefaciens* mediated transformation.

To select transformed plants, the plant material obtained in the transformation is
15 subjected to selective conditions so that transformed plants can be distinguished from untransformed plants. For example, the seeds obtained in the above-described manner can be planted and, after an initial growing period, subjected to a suitable selection by spraying. A further possibility is growing the seeds, if appropriate after sterilization, on agar plates using a suitable selection agent so that only the transformed seeds can
20 grow into plants. Alternatively, the transformed plants are screened for the presence of a selectable marker or expression of a constitutively expressed reporter gene, as described above. Following DNA transfer and regeneration, putatively transformed plants may also be evaluated, for instance using Southern blot analysis, for the presence of the gene of interest, copy number and/or genomic organisation.
25 Alternatively or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western blot analysis, both techniques being well known to persons having ordinary skill in the art.

The generated transformed plants may be propagated by a variety of means, such as
30 by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed and homozygous second-generation (or T2) transformants selected, and the T2 plants may then further be propagated through classical breeding techniques. The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-
35 transformed cells; clonal transformants (e.g., all cells transformed to contain the

expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

5 In a further aspect of the invention, there is provided a plant obtained or obtainable by the methods described herein.

In a final aspect of the invention, there is provided the use of the nucleic acid construct or vector as described herein to increase at least one of growth, yield, drought tolerance, WUE and carbon assimilation.

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A plant according to all aspects of the invention described herein may be a monocot or a dicot plant. Preferably, the plant is a crop plant. By crop plant is meant any plant which is grown on a commercial scale for human or animal consumption or use. In a preferred embodiment, the plant is a cereal. In another embodiment the plant is
15 Arabidopsis.

15

The plant according to the various aspects of the invention may be a monocot or a dicot plant. A dicot plant may be selected from the families including, but not limited to *Asteraceae*, *Brassicaceae* (eg *Brassica napus*), *Chenopodiaceae*, *Cucurbitaceae*,
20 *Leguminosae* (*Caesalpiniaceae*, *Aesalpiniaceae*, *Mimosaceae*, *Papilionaceae* or *Fabaceae*), *Malvaceae*, *Rosaceae* or *Solanaceae*. For example, the plant may be selected from lettuce, sunflower, *Arabidopsis*, broccoli, spinach, water melon, squash, cabbage, tomato, potato, yam, capsicum, tobacco, cotton, okra, apple, rose, strawberry, alfalfa, bean, soybean, field (fava) bean, pea, lentil, peanut, chickpea,
25 apricots, pears, peach, grape vine or citrus species. In one embodiment, the plant is oilseed rape.

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Also included are biofuel and bioenergy crops such as rape/canola, sugar cane, sweet sorghum, *Panicum virgatum* (switchgrass), linseed, lupin and willow, poplar, poplar
30 hybrids, *Miscanthus* or gymnosperms, such as loblolly pine. Also included are crops for silage (maize), grazing or fodder (grasses, clover, sanfoin, alfalfa), fibres (e.g. cotton, flax), building materials (e.g. pine, oak), pulping (e.g. poplar), feeder stocks for the chemical industry (e.g. high erucic acid oil seed rape, linseed) and for amenity purposes (e.g. turf grasses for golf courses), ornamentals for public and private
35 gardens (e.g. snapdragon, petunia, roses, geranium, *Nicotiana sp.*) and plants and cut

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flowers for the home (African violets, Begonias, chrysanthemums, geraniums, Coleus spider plants, Dracaena, rubber plant).

5 A monocot plant may, for example, be selected from the families *Arecaceae*, *Amaryllidaceae* or *Poaceae*. For example, the plant may be a cereal crop, such as wheat, rice, barley, maize, oat, sorghum, rye, millet, buckwheat, turf grass, Italian rye grass, sugarcane or *Festuca* species, or a crop such as onion, leek, yam or banana.

10 Preferably, the plant is a crop plant. By crop plant is meant any plant which is grown on a commercial scale for human or animal consumption or use. Preferred plants are maize, wheat, rice, oilseed rape, sorghum, soybean, potato, tomato, grape, barley, pea, bean, field bean, lettuce, cotton, sugar cane, sugar beet, broccoli or other vegetable brassicas or poplar.

15 The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, fruit, shoots, stems, leaves, roots (including tubers), flowers, tissues and organs, wherein each of the aforementioned comprise the nucleic acid construct as described herein or carry the herein described mutations. The term "plant" also encompasses plant cells, suspension cultures, callus tissue, embryos,
20 meristematic regions, gametophytes, sporophytes, pollen and microspores, again wherein each of the aforementioned comprises the nucleic acid construct or mutations as described herein.

25 The invention also extends to harvestable parts of a plant of the invention as described herein, but not limited to seeds, leaves, fruits, flowers, stems, roots, rhizomes, tubers and bulbs. The aspects of the invention also extend to products derived, preferably directly derived, from a harvestable part of such a plant, such as dry pellets or powders, oil, fat and fatty acids, starch or proteins. Another product that may be derived from the harvestable parts of the plant of the invention is biodiesel. The
30 invention also relates to food products and food supplements comprising the plant of the invention or parts thereof. In one embodiment, the food products may be animal feed. In another aspect of the invention, there is provided a product derived from a plant as described herein or from a part thereof. In a most preferred embodiment, the plant part or harvestable part is a seed or the fruit. Therefore, in a further aspect of the

invention, there is provided a seed or fruit produced from a genetically altered plant as described herein.

5 A control plant as used herein according to all of the aspects of the invention is a plant which has not been modified according to the methods of the invention. Accordingly, in one embodiment, the control plant does not express the nucleic acid construct as described above. In an alternative embodiment, the plant has not been genetically modified, as described above. In one embodiment, the control plant is a wild type plant. The control plant is typically of the same plant species, preferably having the same
10 genetic background as the modified plant.

While the foregoing disclosure provides a general description of the subject matter encompassed within the scope of the present invention, including methods, as well as the best mode thereof, of making and using this invention, the following examples are
15 provided to further enable those skilled in the art to practice this invention and to provide a complete written description thereof. However, those skilled in the art will appreciate that the specifics of these examples should not be read as limiting on the invention, the scope of which should be apprehended from the claims and equivalents thereof appended to this disclosure. Various further aspects and embodiments of the
20 present invention will be apparent to those skilled in the art in view of the present disclosure.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is
25 to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and
30 apply equally to all aspects and embodiments which are described.

The foregoing application, and all documents and sequence accession numbers cited therein or during their prosecution ("appln cited documents") and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein
35 ("herein cited documents"), and all documents cited or referenced in herein cited

documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all
5 referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

The invention is now described in the following non-limiting examples.

10

EXAMPLE 1

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Here we have 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 water use efficiency (WUE). Thus, BLINK1, and related optogenetic tools offer ways to explore plant growth and its relationship to WUE without a cost in CO₂ availability for photosynthesis.

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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 that lead to activation of guard-cell H⁺-ATPases, in turn promoting K⁺ uptake (5, 9, 10). We therefore explored whether
25 stomatal opening could be augmented by tissue-specific expression of the optogenetic tool BLINK1.

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BLINK1 is a synthetic, blue light-gated K⁺ channel, constructed by fusing the LOV2-Jα photo-switch 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, initially we expressed BLINK1 transiently in tobacco and in *Arabidopsis* root epidermal cells (12).

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Immunoblots showed BLINK1 formed tetramers expected of the functional K⁺ channel

(Fig. 6). On treatments with $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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. 5). From the voltage kinetics, we concluded that the conductance was fully activated within 2 min +BL and decayed over 8-10 min on transfer to 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). We measured the plasma membrane conductance using two-electrode recording methods (15) on intact guard cells of p1p2-BLINK and wt-BLINK transgenic lines, comparing conductances with each to the corresponding p1p2 and wt backgrounds. Close to the free-running voltage, the membrane conductance of Arabidopsis guard 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 (Material & Methods). We therefore used a current clamp to drive 0.5-s steps of $\pm 100 \text{ pA}$ 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 $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ BL (Fig. 1b, inset) and calculated the change in membrane conductance $\pm \text{BL}$ (ΔG) 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 to the p1p2 mutant and wt controls, respectively, with a 1.6-fold increase in ΔG 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 $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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 to 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 analysed the stomatal conductances of intact plants $\pm$ BL after dark and RL adaptation (Fig. 2c-f and Fig. 8). Compared to the wt, stomatal conductance was elevated in the p1p2 background in the dark, 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BL, whereas p1p2 double-mutant plants were unresponsive to BL (Fig. 2c). BLINK1 expression in the wt background led to 22-29% enhancements in stomatal conductance in BL (Fig. 2d) despite a small reduction in stomatal size in one line. Mean stomatal opening and closing halftimes were accelerated by approximately 40% compared to the wt controls (Fig. 2e).

Pre-adapting plants to 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ BL elevated stomatal conductance in all plants (Fig. 4). However, wt-BLINK plants showed accelerated changes in stomatal conductance, with 60-70% reductions in stomatal opening and closing halftimes compared to wt plants (Figs. 2f and Fig. 8). BLINK1 activity is independent of voltage and declined over 8-10 min (Figs. 1, Fig. 7 and (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 (WUE_i). Both measures are affected by light through the combined influence on carbon demand and associated transpiration (17). We therefore 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or at a high fluence rate (HWL) of 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$. We calculated WUE_i over these periods and determined WUE as the ratio of accumulated dry biomass to water used over the 49-day growth period. Under the LWL and HWL treatment, growth of wt-BLINK and p1p2-BLINK transgenic plants showed no significant differences in biomass accumulation, rosette area expansion, or water use

when compared with that of the corresponding wt and p1p2 backgrounds (Fig. 9, Fig. 10 and Fig. 4).

In the natural environment light fluctuates, for example as clouds pass over. 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 (3, 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 therefore examined the BLINK1 transgenic lines grown with daylight periods of fluctuating WL (FWL) to give a total photon flux over the daylight period intermediate to the two continuous light regimes. We stepped fluence rates ranging between 10 and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 60-min intervals, which is close to the time normally required for stomatal opening (Fig. 2) and therefore would maximize any advantages afforded by BLINK. No change was evident in WUEi (Fig. 3b). However, rosette area and fresh weight increased in wt-BLINK transgenic plants when compared to the wt control (Fig. 3b,e and Fig. 4) and 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 decrease in starch in water-replete-grown plants, and a highly significant increase in total starch in water-deficit-grown plants (Fig. 11). The wt-BLINK plants showed significant decreases in fresh/dry weight ratios under both conditions (Fig. 5). Much of this biomass is likely therefore to be accounted for by changed in cell wall material. We confirmed that this increased biomass was not the consequence of alterations in photosynthesis *per se* (18): CO₂ assimilation under saturating light (1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was unaffected in wt-BLINK plants across the physiological range of internal CO₂ concentrations (Fig. 3c), and the C_i/C_a ratios determined at 70, 200 and 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 revolutionised 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 now 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. As many plant 'movements', growth, and morphogenic phenomena rely on solute flux to drive turgor and cell expansion, optogenetics offers new 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 CO₂ availability for photosynthesis and can slow plant growth (3, 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 of 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 now demonstrate the efficacy of introducing new 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 highlight the gains that might be achieved by enhancing stomatal kinetics under changing light environments. Furthermore, we demonstrate that stomatal speed (3) 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 gaining in photosynthetic assimilation during vegetative growth.

Material and Methods

Plant Material and growth

Wild-type (wt) *Arabidopsis thaliana* L. Heynh. (gl-1, ecotype Columbia) and the double mutant *phot1-5 phot2-1* (p1p2) were described previously (9). Plants were grown in short day conditions (8-h-light/16-h-dark cycle with, 22°C/18°C, and 60% and 70% relative humidity). Three daylight regimes were used for growth experiments, either

with steady 75 or 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$ or with light stepped at 1-h intervals at light intensities ranging between 10 and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For growth under water-deficit, after 21 d soil moisture was monitored daily with a Delta-T HH2-ML3 moisture sensor (Delta-T Devices, Cambridge UK) and maintained at 10% with water as required.

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Stable Transformation

For stable transformations, the plasmid pEZR(K)-LN (30) was digested with *SacI* and *BamHI*. The primer pairs CTCACTATAGGGAGCTCACAAGGACACAAGGACATATG (SEQ ID NO: 16) and CATCCCATTAAGCCTGCTTTTTTGTACAAAC (SEQ ID NO: 17) were used to amplify the guard-cell promoter (pMYB60) (13, 31), and the primer pairs CAGGCTTAATGGGATGTACAGTCTCTGC (SEQ ID NO: 18) and CAGCGGCAGCAGCCGTCATAAAGTTAGAACGATGAAG (SEQ ID NO: 19) were used to amplify the BLINK1 coding sequence (11). The final pEZ pMYB60::BLINK1 construct was generated by Gibson Assembly. Transformation of *Arabidopsis thaliana* gl1 and phot1-5 phot2-1 (14) with *Agrobacterium* was performed as described previously (32). T3 lines with a single transgene locus were selected by segregation of the kanamycin resistance (33).

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Transient transformation of *Nicotiana benthamiana* and *Arabidopsis*

For transient expression of BLINK1 under the control of the CaMV 35S promoter in *N. benthamiana*, the entry clone was generated by PCR using specific primers containing attB3 5'-GGGGACAACCTTTGTATAATAAAGTTGTCAACATGGGATGTACAGTCTCTGCAGAG-3' (SEQ ID NO: 20) and attB2 sites 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTATCATAAAGTTAGAACGATGAAGAACACTG-3' (SEQ ID NO: 21). The gel purified PCR product was used together with the pDONR 221 P3-P2 vector to create the final DONR vector using BP-clonase II (Life technologies) according to the manufacturer's instructions. After verifying the product of the BP-reaction by sequencing, LR-clonase II (Life Technologies) was used together with the pFRET cg-2in1-cc vector (34) to generate the final destination vector pFRET cg-2in1-cc BLINK1/mCherry. Infiltration of *N. benthamiana* leaves was performed with transformed *Agrobacterium* as described previously (35). Transient transformation of *Arabidopsis* roots was performed as described previously (12).

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Immunoblot Analysis

Total protein extracts were prepared from *N. benthamiana* leaf discs 3 days post infiltration. Plant tissue was ground with a pestle in SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 1mM DTT, 0.004% bromophenol blue) and centrifuged at 10,000 g, at 4 °C for 5 min. Half of the supernatant was directly used for immunoblot analysis of the BLINK1 tetramer (unboiled sample). Protein samples were subjected to 7.5% SDS-PAGE detection for the BLINK1 tetramer. Proteins were transferred onto nitrocellulose membrane (BioRad) by electroblotting and detected with anti-Kcv antibody (36). Blots were developed with horseradish peroxidase (HRP)-linked secondary antibodies (Promega) and Pierce ECL Plus Western Blotting Substrate (Thermo Fisher Scientific).

Gas exchange and stomatal aperture analysis

Gas exchange measurements were carried out using the LI-COR 6400 XT Infrared Gas Analyzer (LICOR Biosciences) and whole-plant Arabidopsis chamber (LI-COR 6400-17) (37). Light was adjusted using an integrated RGB light source (LI-COR 6400-18). Stomatal conductance was calculated from transpiration rates at a temperature of 22°C. Measurements were carried out over the same period of the diurnal cycle and were normalised to rosette area calculated from images using ImageJ 1.43u (<http://rsb.info.nih.gov/ij>).

Apertures were recorded from stomata in epidermal peels continuously superfused with 5 mM Ca²⁺-MES, pH 6.1, with 10 mM KCl(33, 37), in the dark and under RL and/or BL of 100 µmol m⁻² s⁻¹ for 2 h. Following measurements, the stomata were incubated for 5 min in buffer supplemented with 20 µM fluorescein diacetate to confirm viability. Only stomata with guard cells retaining a fluorescein signal under confocal fluorescence microscopy were included for analysis.

RNA extraction and real-time PCR

Total RNA was extracted from leaf tissue from 4-week old plants using Plant RNeasy Mini kit, and cDNA synthesis was carried out using Quanti-script (Qiagen) kit. Real-time PCR was carried out using Brilliant III Ultra-Fast SYBR QPCR kit (Applied Biosystems) with primer pairs ATGGAAGTGGAGCATGTCCGA (SEQ ID NO: 22) and TTTTGTCCGGGTTTGCAACA (SEQ ID NO: 23) to amplify BLINK, and GCCATCGCTTCTTCATCTGTTGC (SEQ ID NO: 24) and GTGGGGAGAGAAAGATGCTTTGCG (SEQ ID NO: 25) to amplify the reference ISU

gene (29). For each transcript, amplification was assayed over a range of cycle numbers to select optimal conditions for visualization of the PCR product and quantification.

5 **Guard Cell Electrophysiology**

Voltages and current clamp data were recorded from Arabidopsis root epidermal cells and intact guard cells in epidermal peels using Henry's EP Software Suite (<http://www.psrg.org.uk>). Double-barrelled microelectrodes (tip resistances >100 MΩ) were filled with 200 mM K⁺-acetate (pH 7.5) as described previously (37-40) after
10 equilibration against resin-bound BAPTA [for 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; Invitrogen] to prevent Ca²⁺ loading of the cytosol from the microelectrode. Voltages in root epidermal cells were recorded under continuous superfusion with 5 mM Ca²⁺-MES, pH 6.1, with 30 mM KCl. Current clamp recordings in guard cells used ±100 pA steps of 0.5 s with a 5-s cycle. Measurements were carried
15 out in 5 mM Ca²⁺-MES, pH 6.1, with 1 mM KCl to minimize background currents. Surface areas of impaled guard cells were calculated assuming a spheroid geometry (39) and voltages were analysed using Henry's EP Software Suite.

Current clamp offers advantages in quantifying small changes in conductance.
20 Between the fully closed and fully open state of the stoma, the osmotic content of an Arabidopsis guard cell rises by approximately 200 to 300 mM on a cell volume basis (5). Roughly half of this osmotic content is made up of K⁺ transported across the plasma membrane and translates to approximately 70 fmol of K⁺ per guard cell. Assuming that 60 min are required for stomatal opening, the mean K⁺ flux needed to
25 drive opening is 10 amol s⁻¹, equivalent to a current of 0.9 pA. This value is below the limit of resolution for whole-cell voltage clamp measurements from plant cells. It represents less than 0.5% of the K⁺ currents typically recorded when Arabidopsis guard cells are clamped near the voltage extremes more than ±100 mV from the free-running voltage (5).

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Total Protein and Total Starch Quantification

Leaves were harvested into liquid nitrogen and finely ground. 20 mg of material was weighed to extract total starch using Starch GO/B Assay kit (Sigma, Poole UK) according to the manufacturer's instructions. 10 mg of material was weighed to extract
35 protein using homogenization buffer [0.0625M Tris·HCl pH 6.8, 1% (wt/vol) SDS, 10%

(vol/vol) glycerol, and 0.01% (vol/vol) 2-mercaptoethanol]. Samples were incubated at 65 °C for 10 min before centrifuging at 13000 rpm for 10 min. Supernatant was collected and protein was quantified using Pierce BCA kit (Thermo Scientific, Loughborough, UK) according to the manufacturer's instructions.

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Statistical Analysis

Statistical significance was determined by Student's t-test or ANOVA at $P < 0.05$ using SigmaPlot12 (Systat) software. Data are reported as means $\pm$ SE of n observations with the exception of stomatal assays which are reported as medians $\pm$ SE along with 0.25 – 0.75 ranges.

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EXAMPLE 2

We will construct a plasmid wherein a sequence encoding BLINK1 is inserted into a pSBII vector under the control of the constitutive promoter ZmUbi or a guard-cell specific promoter, such as the maize homologue of Myb60. The resulting plasmid will be inserted into the LBA4404 *A. tumefaciens* strain. The LBA4404 strain, with the integrated pSBIII plasmid will then be used to deliver e.g. the ZmUbi:BLINK1 expression cassette into maize. Transgenic T0, T1, and T2 plants will be grown in a greenhouse under a 16h-light/8h-dark condition. Transgenic positive and the sibling transgenic negative (i.e. wild type) plants can be determined in each generation by PCR analysis for the transgene. We will also measure growth, yield, water use efficiency and carbon assimilation in these plants.

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For example, we will monitor the growth of plants by measuring rosette expansion and accumulation of dry biomass. We will also examine any increase in yield by measuring seed yield per plant.

EXAMPLE 3

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GORK engineering for optogenetic K^+ flux manipulation, The LOV2-J α photoswitch reversibly photoactivates in blue light, displacing the J α helix to uncage the LOV2 core. Several LOV-based optogenetic tools have been created using the J α helix as a steric inhibitor that is released following photoactivation. All of these tools incorporate both components within a single polypeptide, linked by residues that serve as a 'hinge.' Our

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experiments, using an mbSUS assay and using GORK as a framework for insertions, show that the LOV2 and J α domains have the potential to bind and release when incorporated in physically separate polypeptides (Figure 12).

REFERENCES

1. A. M. Hetherington, F. I. Woodward, The role of stomata in sensing and driving environmental change. *Nature*. 424, 901–908 (2003).
- 5 2. S. Jasechko et al., Terrestrial water fluxes dominated by transpiration. *Nature*. 496, 347–350 (2013).
3. T. Lawson, M. R. Blatt, Stomatal Size, Speed, and Responsiveness Impact on Photosynthesis and Water Use Efficiency. *Plant Physiology*. 164, 1556–1570 (2014).
4. L. McAusland et al., Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. *New Phytologist*. 211, 1209–1220 (2016).
- 10 5. M. Jezek, M. R. Blatt, The Membrane Transport System of the Guard Cell and Its Integration for Stomatal Dynamics. *Plant Physiology*. 174, 487–519 (2017).
6. S. M. Assmann, T. Jegla, Guard cell sensory systems: recent insights on stomatal responses to light, abscisic acid, and CO₂. *Current Opinion in Plant Biology*. 33, 157–167 (2016).
- 15 7. C. B. Engineer et al., CO₂ Sensing and CO₂ Regulation of Stomatal Conductance: Advances and Open Questions. *Trends in Plant Science*. 21, 16–30 (2016).
8. Y. Wang et al., Unexpected Connections between Humidity and Ion Transport Discovered Using a Model to Bridge Guard Cell-to-Leaf Scales. *Plant Cell*. 29, 2921–2939 (2017).
- 20 9. J. M. Christie, Phototropin Blue-Light Receptors. *Annu. Rev. Plant Biol.* 58, 21–45 (2007).
10. A. Takemiya, S. Inoue, M. Doi, T. Kinoshita, K. Shimazaki, Phototropins promote plant growth in response to blue light in low light environments. *The Plant Cell*. 17, 1120–1127 (2005).
- 25 11. C. Cosentino et al., Optogenetics. Engineering of a light-gated potassium channel. *Science*. 348, 707–710 (2015).
12. Z. Chen, C. Grefen, N. Donald, A. Hills, M. R. Blatt, A bicistronic, Ubiquitin-10 promoter-based vector cassette for transient transformation and functional analysis of membrane transport demonstrates the utility of quantitative voltage clamp studies on intact *Arabidopsis* root epidermis. *Plant Cell Environ.* 34, 554–564 (2011).
- 30 13. E. Cominelli et al., A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. *Current Biology*. 15, 1196–1200 (2005).
- 35 14. J. M. Christie, J. Gawthorne, G. Young, N. J. Fraser, A. J. Roe, LOV to BLUF: Flavoprotein Contributions to the Optogenetic Toolkit. *Mol Plant*. 5, 533–544 (2012).
15. Y. Wang et al., Systems dynamic modeling of a guard cell Cl⁻ channel mutant uncovers an emergent homeostatic network regulating stomatal transpiration. *Plant Physiology*. 160, 1956–1967 (2012).
- 40 16. T. Kinoshita et al., Phot1 and phot2 mediate blue light regulation of stomatal opening. *Nature*. 414, 656–660 (2001).
17. R. W. Pearcy, Sunflecks and photosynthesis in plant canopies. *Annu. Rev. Plant Biol.* (1990).
- 45 18. T. D. Sharkey, C. J. Bernacchi, G. D. Farquhar, E. L. Singsaas, Fitting photosynthetic carbon dioxide response curves for C(3) leaves. *Plant Cell Environ.* 30, 1035–1040 (2007).
19. J. Mattis et al., Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins. *Nat Meth.* 9, 159–172 (2012).
- 50 20. E. G. Govorunova, O. A. Sineshchekov, R. Janz, X. Liu, J. L. Spudich, Natural light-gated anion channels: A family of microbial rhodopsins for advanced optogenetics. *Science*. 349, 647–650 (2015).

21. V. Gradinaru et al., Molecular and cellular approaches for diversifying and extending optogenetics. *Cell*. 141, 154–165 (2010).
22. T. Doheny-Adams, L. Hunt, P. J. Franks, D. J. Beerling, J. E. Gray, Genetic manipulation of stomatal density influences stomatal size, plant growth and tolerance to restricted water supply across a growth carbon dioxide gradient. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 367, 547–555 (2012).
23. J. Masle, S. R. Gilmore, G. D. Farquhar, The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. *Nature*. 436, 866–870 (2005).
24. J. Hughes et al., Reducing stomatal density in barley improves drought tolerance without impacting on yield. *Plant Physiology*, pp.01844.2016 (2017).
25. Y. Tanaka, T. Nose, Y. Jikumaru, Y. Kamiya, ABA inhibits entry into stomatal-lineage development in *Arabidopsis* leaves. *The Plant Journal*. 74, 448–457 (2013).
26. J. Negi et al., CO₂ regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. *Nature*. 452, 483–486 (2008).
27. K. Kusumi, S. Hirotsuka, T. Kumamaru, K. Iba, Increased leaf photosynthesis caused by elevated stomatal conductance in a rice mutant deficient in SLAC1, a guard cell anion channel protein. *Journal of Experimental Botany*. 63, 5635–5644 (2012).
28. Y. Wang, A. Hills, M. R. Blatt, Systems analysis of guard cell membrane transport for enhanced stomatal dynamics and water use efficiency. *Plant Physiology*. 164, 1593–1599 (2014).
29. S. Bordage, S. Sullivan, J. Laird, A. J. Millar, & H. G. Nimmo, Organ specificity in the plant circadian system is explained by different light inputs to the shoot and root clocks. *New Phytologist*. 212, 136–149 (2016).
30. J. M. Christie, T. E. Swartz, R. A. Bogomolni, & W. R. Briggs, Phototropin LOV domains exhibit distinct roles in regulating photoreceptor function. *Plant Journal*. 32, 205–219 (2002).
31. E. Cominelli, et al. DOF-binding sites additively contribute to guard cell-specificity of AtMYB60 promoter. *BMC Plant Biology*. 11, 162 (2011).
32. A. M. Davis, A. Hall, A. J. Millar, C. Darrah, & S. J. Davis, Protocol: Streamlined sub-protocols for floral-dip transformation and selection of transformants in *Arabidopsis thaliana*. *Plant Methods*. 5, 3–7 (2009).
33. S. Sullivan, J. Petersen, L. Blackwood, M. Papanatsiou, & J. M. Christie, Functional characterization of *Ostreococcus tauri* phototropin. *New Phytologist*. 209, 612–623 (2015).
34. C. Grefen, & M. R. Blatt, A 2in1 cloning system enables ratiometric bimolecular fluorescence complementation (rBiFC). *BioTechniques*. 53, 311–314 (2012).
35. E. Kaiserli, S. Sullivan, M. A. Jones, K. A. Feeney, & J. M. Christie, Domain swapping to assess the mechanistic basis of *Arabidopsis* phototropin 1 receptor kinase activation and endocytosis by blue light. *The Plant Cell*. 21, 3226–3244 (2009).
36. G. Romani, et al. A virus-encoded potassium channel is a structural protein in the chlorovirus *Paramecium bursaria* chlorella virus 1 virion. *Journal of General Virology*. 94, 2549–2556 (2013).
37. M. Papanatsiou, A. Amtmann, & M. R. Blatt, Stomatal Spacing Safeguards Stomatal Dynamics by Facilitating Guard Cell Ion Transport Independent of the Epidermal Solute Reservoir. *Plant Physiology*. 172, 254–263 (2016).
38. M. R. Blatt & C. L. Slayman, KCl leakage from microelectrodes and its impact on the membrane parameters of a nonexcitable cell. *Journal of Membrane Biology*. 72, 223–234 (1983).
39. M. R. Blatt, Electrical characteristics of stomatal guard cells: The ionic basis of the membrane potential and the consequence of potassium chlorides leakage from microelectrodes. *Planta*. 170, 272–287 (1987).

40. Y. Wang & M. R. Blatt, Anion channel sensitivity to cytosolic organic acids implicates a central role for oxaloacetate in integrating potassium flux with metabolism in stomatal guard cells. *Biochemical Journal*. 439, 161–170 (2011).

SEQUENCE LISTING

SEQ ID NO: 1 Kcv amino acid sequence

5 VFSKFLTRTEPFMIHLFILAMFVMIYKFFPGGFENNFSVANPDKKASWIDCIYFGVTTHS
TVGFGDILPKTTGAKLCTIAHIVTVFFIVLTL

SEQ ID NO: 2 Kcv nucleic acid sequence

10 GTGTTTAGTAAATTTCTAACGCGAACTGAACCATTCATGATACATCTCTTTATTCTC
GCAATGTTTCGTGATGATCTATAAATTCTTCCCGGGAGGGTTCGAAAATAACTTCTC
TGTTGCAAACCCGGACAAAAAGGCATCATGGATAGATTGTATATACTTCGGAGTAA
CGACACACTCTACTGTCGGATTTCGGAGATATACTGCCAAAGACGACCGGCGCAAA
GCTTTGTACGATAGCACATATAGTAACAGTGTTCTTCATCGTTCTAACTTTATGA

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SEQ ID NO: 3 BLINK1 amino acid sequence

MGCTVSAELATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQ
GPETDRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIG
20 VQLDGEHVRDAAEREGVMLIKKTAE VFSKFLTRTEPFMIHLFILAMFVMIYKFFPGGF
ENNFSVANPDKKASWIDCIYFGVTTHSTVGFGDILPKTTGAKLCTIAHIVTVFFIVLTL

myristoylation sequence (my)

25 **LOV2 domain and Jα(underlined) (L)**

SEQ ID NO: 4 BLINK1 nucleic acid sequence

ATGGGATGTACAGTCTCTGCAGAGTTGGCTACTACACTTGAACGTATTGAGAAG
30 AACTTTGTCATTACTGACCCAAGGTTGCCAGATAATCCCATTATATTCGCGTCCG
ATAGTTTCTTGCAGTTGACAGAATATAGCCGTGAAGAAATTTTGGGAAGAACT
GCAGGTTTCTACAAGGTCCTGAAACTGATCGCGCGACAGTGAGAAAAATTAGAG
ATGCCATAGATAACCAAACAGAGGTCACTGTTTCAGCTGATTAATTATACAAAGA
GTGGTAAAAAGTTCTGGAACCTCTTTCACTTGCAGCCTATGCGAGATCAGAAGG
35 GAGATGTCCAGTACTTTATTGGGGTTCAGTTGGATGGAAGTGAAGCATGTCCGAG

ATGCTGCCGAGAGAGAGAGGGAGTCATGCTGATTAAGAAAAGTGCAGAAGTGTTTA
 GTAAATTTCTAACGCGAACTGAACCATTCATGATACATCTCTTTATTCTCGCAATGT
 TCGTGATGATCTATAAATTCTTCCCGGGAGGGTTCGAAAATAACTTCTCTGTTGCA
 AACCCGGACAAAAAGGCATCATGGATAGATTGTATATACTTCGGAGTAACGACAC
 5 ACTCTACTGTCGGATTTCGGAGATATACTGCCAAAGACGACCGGGCGCAAAGCTTTG
 TACGATAGCACATATAGTAACAGTGTTCATCGTTCTAACTTTATGA

myristoylation sequence (my)

10 **LOV2 domain and J α (underlined) (L)**

SEQ ID NO: 5 Nucleic acid sequence of Myb60_TAIR10

CACAAGGACACAAGGACATATGGTATGATGATATGCTTTGTTTCTCTGCTTCTCTT
 15 ACTAATTTGAAGCTGTTGGATTGATTTGTCTCTTCTTACGTTCCCTTCTTTTTTTTTT
 CGTTTTCTTTTGTCTATAGACCAGGCAGGGGCTAGGGCCTAGTGATGGGTATTG
 GCCCAATACTATTGGGTATTGCTGCTGTTTATTATTTGATTTTAGGTTAATTCAA
 TTTAAGAATACGTAGATTTGTTTGGTTTAGTTTGGTTTGGTTGCACTAAGTTCCGT
 TTTACATAAATAGAATCTAACACTACTAATTGTTATACGTAAAATACAACAATAA
 20 CAGATTTTTCGTTTCAATTTTCGTTTAAGAGGGTAGACATTTTGGTTTGGTTTGG
 TTCATTTTTTTTTTCCCTTTCAAATTCACATCCTTCACGTAGATGACAAAATAAAGAA
 AAACATGAATGAAAGTTGTAAGTGTGTAAGCATCAACATGGAAATCATATCACAAAG
 AACACAAATCTAACTAATGGGTCTTTTCACATATTGGTATAATTATAAGTTGTAAGA
 ATATTAGTTAAACAGAGGCAACGAGAGATGCGTGATATATGAAAAGTTGAAAACAA
 25 AAGACATGGATCTAAAGAGTCAAGCAAAATGTAATATCTTTTTTCTTCTAACTTG
 AGGATGTCCAAGTTGCAGTGAATGATTCCCTTTAATCATGGAGAAATTCAATGAAA
 TAATTGTGTTTCTTCCACACTTTATCTTTATTTATTTTCTTACCACAATTACAATA
 TTATCACAAAAATGTAAGTAACATAGCTTGTGACTCTTCTTCCATTTATGAGTTGAT
 TATCACTATATTTATAAGTAATTACCAACGAATGTTCCAAATTAAGCAAAATATTGTA
 30 ATCGATACACTATGTATTCATCTACAATATGTTAACGAGCTCCTTTTATGGAAATAT
 TTCGATTGAAAAACATTTGATGGATCGTTCACTAAATAAATAATCCAGTAACGTTT
 TCTTAAGGGAGATATACATATTCGTGTGGAGATCAACATATCTTCGTTAATTGACTA
 CGCAAAATAGTTAATGGAAAAGGCAGAGTGACTCGTGAGCTTGGCAGATCCAAAA
 GAGGTTGTCAAGAAAAAGCAGATTTAAAAGTTCTTCCCTCTTCTTTAAGTCACCCA

TTAATTTACATATATGTACATACATGTTGCATTTAACTCATATACATACatattctcacat
ctataaagagagcataagactcagagagatctagaggaagagagagagagaaagATG

SEQ ID NO: 6 Nucleic acid sequence of Myb60 used for BLINK1

5

CACAAGGACACAAGGACATATGGTATGATGATATGCTTTGTTTCTCTGCTTCTCTT
ACTAATTTGAAGCTGTTGGATTGATTTGTCTCTTCTTACGTCCCCTTCTTTTTTTTTT
CGTTTTCTTTTGTGCGCATAGACCAGGCAGGGGCTAGGGCCTAGTGATGGGTATTG
GCCCAATACTATTGGGTATTTGCCTGGTTTATTATTTGATTTTAGGTTAATTCAA
10 TTTAAGAATACGTAGATTTGTTTGGTTTAGTTTGGTTTGGTTGCACTAAGTTCGGT
TTTACATAAATAGAATCTAACTACTAATTGTTATACGTAAAATACAACAACAATAA
CAGATTTTTCTGTTTCAATTTTCTGTTAAGAGGGTAGACATTTTGGTTTGGTTTGGTT
CATTTTTTTTTTCCCTTTCAAATTCACATCCTTCACGTAGATGACAAAATAAAGAAAA
ACATGAATGAAAGTTGTAAGTTGTAAGCATCAACATGGAAATCATATCACAAAGAA
15 CACAAATCTAACTAATGGGCCTTTTCACATATTGGTATAATTATAAGCTGTAAGAAT
ATTAGTTAAACAGAGGCAACGAGAGATGCGTGATATATGAAAAGTTGAAAACAAAA
GACATGGATCTAAAGAGTCAAGCAAAATGTAATATCTTTTTTTCTTCTAACTTGAG
GATGTCCAAGTTGCAGTGAATGATTCCCTTTAATCATGGAGAAATTCAATGAAATA
ATTGTGTTTCTTCCACACTTTATCTTTATTTATTTTCTTACCACAATTACAACATT
20 ATCACA AAAAATGAAAGTAACATAGCTTGTGACTCTTCTTCCATTTATGAGTTGATTA
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CGATTGAAAAAACATTTGATGGATCGTTCACTAAATAAATAATCCAGTAACGTTTTCT
TTAAGGGAGATATACATATTCGTGTGGAGATCAACATATCTTCGTTAATTGACTAC
25 GCAAAATAGTTAATGAAAAGGCAGAGTGAAGTCTGAGCTTGGCAGATCCAAAAG
AGGTTGTCAAGAAAAAGCAGATTTAAAAGTTCTTCCCTCTTCTTTAAGTCACCCATT
AATTTACATATATGTACATACATGTTGCATTTAACTCATATACATACATATTCTCAC
ATCTATAAAGAGAGCATAAGACTCAGAGAGATCTAGAAGTAGTGATATCACAAGTT
TGTACAAAAAAGCAGGCTTA

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SEQ ID NO: 7 Nucleic acid sequence of GC1

CTTCTACAAGAAGAGTAAAGATTCAGTAACCCGATGCTCCTGCTCTTCCTCAAGAC
CTTCCTTGATTCGCCGCCGGTATGTTCTCCGTCTGTGGTAGCGCCTTTGGAACAC
35 TCTACCAACGCCGCCATGAAAGGATCTCTCATGGCCGCAGGGGACGTGTTCTTCT

TACATCTGGTGTAGGGCTATGGTTACTCCAGTGAGGAGGGAGAGGCAAGAGGTT
GCTTAATGATTCGTTTTTCCGGTGATACGAGAACTCTTTAGGTTTACCGGGAAGCT
TTTCCCATGAAAATGGGATGCCAAGTGGATGGAGAGGAGTTGCCGGAGAGTTGC
CGGAGAATAGGAGGGAATTGGAGGAGGAGGAAGAGAGTGATCGCCGGGTTGAAA
5 TGTTAACCGTCGAGGAGAATTTGACCGAGTTGGATCGTCTAGTAGGTACAATTCG
GGTCCTTGGCGAAGTATCCATTCAAATAGTGTGTTAGTTTTGGACTTGAGAAGTTG
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ATAAGAAACAAAATGGTTGCAACAGAGAGGATGAATTTATAAGTTTTCAACACCGC
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10 GGTTAGTGAATTCAAATATCTAAATTTTGTGACTCACTAACATTAACAAATATGCAT
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TCAATCTTTAGGAATTGACGATGTAGAATTGTAGATGATAAATTTTCTCAAATATAG
ATGGGCCTAATGAAGGGTGCCGCTTATTGGATCTGACCCATTTTGAGGACATTAAT
ATTTTCATTGGTTATAAGCCTTTTAATCAAATTTGTCATTAAATTGATGTCTCCCTCT
15 CGGGTCATTTTCCTTTCTCCCTCACAATTAATGTAGACTTTAGCAATTTGCACGCT
GTGCTTTGTCTTTATATTTAGTAACACAAACATTTTGACTTGTCTTGTAGAGTTTTTC
TCTTTTATTTTTCTATCCAATATGAAAACATAAAGTGTTCTCGTATACATATATTA
ATTAAAGAAACCTATGAAAACACCAATACAAATGCGATATTGTTTTAGTTTCGACGT
TTCATGTTTGTTAGAAAATTTCTAATGACGTTTGTATAAAATAGACAATTAAACGCC
20 AAACACTACATCTGTGTTTTCGAACAATATTGCGTCTGCGTTTTCTTCATCTATCTC
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AACTACCAAAGTATCAAGCTAATGTAAAAATTACTCTCATTTCCACGTAACAAATTG
AGTTAGCTTAAGATATTAGTGAACTAGGTTTGAATTTCTTCTTCTTCCATGC
ATCCTCCGAAAAAAGGGAACCAATCAAACCTGTTTGCATATCAAACCTCAACACTT
25 TACAGCAAATGCAATCTATAATCTGTGATTTATCCAATAAAAACCTGTGATTTATGT
TTGGCTCCAGCGATGAAAGTCTATGCATGTGATCTCTATCCAACATGAGTAATTGT
TCAGAAAAATAAAAAGTAGCTGAAATGTATCTATATAAAGAATCATCCACAAGTACTA
TTTTCACACACTACTTCAAATCACTACTCAAGAAAT

30 SEQ ID NO: 8: BLINK 1 variant (myLK) amino acid sequence

**MGCTVSAELATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQ
GPETDRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIG
VQLDGEHVRDAAEREGVMLIKKTAENIDEAAKELPDANLRPEDLWANHSVFSKFLT**

RTEPFMIHLFILAMFVMIYKFFPGGFENNFSVANPDKKASWIDCIYFGVTTHSTVGFGLI
LPKTTGAKLCTIAHIVTVFFIVLTL

myristoylation sequence (my)

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LOV2 domain and J α (underlined) (L)

SEQ ID NO: 9 BLINK 1 variant (myLK) nucleic acid sequence

10 ATGGGATGTACAGTCTCTGCAGAGTTGGCTACTACACTTGAACGTATTGAGAAG
AACTTTGTCATTACTGACCCAAGGTTGCCAGATAATCCCATTATATTCGCGTCCG
ATAGTTTCTTGCAGTTGACAGAATATAGCCGTGAAGAAATTTTGGGAAGAACT
GCAGGTTTCTACAAGGTCCTGAAACTGATCGCGCGACAGTGAGAAAAATTAGAG
ATGCCATAGATAACCAAACAGAGGTCAGTGTTCAGCTGATTAATTATACAAAGA
15 GTGGTAAAAAGTTCTGGAACCTCTTTCAGTTGCAGCCTATGCGAGATCAGAAGG
GAGATGTCCAGTACTTTATTGGGGTTCAGTTGGATGGAAGTGAAGCATGTCCGAG
ATGCTGCCGAGAGAGAGGGAGTCATGCTGATTAAGAAAAGTGCAGAAAATATT
GATGAGGCGGCAAAAGAACTTCCAGATGCTAATTTGAGACCAGAGGATTTGTGG
GCTAACCAGTCACTGTTTAGTAAATTTCTAACGCGAACTGAACCATTCATGATACA
20 TCTCTTTATTCTCGCAATGTTCTGTGATGATCTATAAATTTCTTCCCGGGAGGGTTCG
AAAATAACTTCTCTGTTGCAAACCCGGACAAAAAGGCATCATGGATAGATTGTATA
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25

myristoylation sequence (my)

LOV2 domain and J α (underlined) (L)

30 SEQ ID NO: 10 GORK amino acid sequence

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15 SEQ ID NO: 11 GORK genomic sequence

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SEQ ID NO: 12: CER6 promoter sequence

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SEQ ID NO: 13: TaGSTA1 promoter sequence

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15

CLAIMS:

1. A method of increasing at least one of growth, yield, drought tolerance, water use efficiency and/or carbon assimilation in a plant, the method comprising
5 expressing a light-gated potassium channel in cells of the stomatal complex.
2. The method of claim 1, wherein the method increases both water use efficiency and carbon assimilation.
3. The method of claim 1 or 2, wherein the method comprises expressing a blue-light gated potassium channel.
- 10 4. The method of any of claims 1 to 3, wherein the method comprises introducing and expressing in a plant a nucleic acid construct comprising a nucleic acid sequence encoding a light-gated potassium channel, preferably a blue-light gated potassium channel operably linked to a regulatory sequence.
5. The method of claim 4, wherein the nucleic acid sequence encodes a blue-light
15 gated potassium channel as defined in SEQ ID NO: 3 or a functional variant or fragment thereof.
6. The method of claim 5, wherein the nucleic acid sequence encoding a blue-light gated potassium channel comprises a sequence as defined in SEQ ID NO: 4 or a functional variant or fragment thereof.
- 20 7. The method of any of claims 1 to 6, wherein the regulatory sequence is a stomatal complex promoter or a constitutive promoter.
8. The method of claim 7, wherein the stomatal complex promoter is a guard-cell specific promoter.
9. The method of any of claims 1 to 8, wherein the plant is a monocot or dicot.
- 25 10. A nucleic acid construct comprising a nucleic acid sequence encoding a light-gated potassium channel operably linked to a regulatory sequence, wherein the regulatory sequence is a stomatal complex promoter.
11. The nucleic acid sequence of claim 10, wherein the potassium channel is a blue-light gated potassium channel or a red-light gated potassium channel.
- 30 12. The nucleic acid construct of claim 10 or 11, wherein the potassium channel is a blue-light gated potassium channel.
13. The nucleic acid construct of any of claims 10 to 12, wherein the nucleic acid sequence encodes a blue-light induced potassium channel as defined in SEQ ID NO: 3 or a functional variant or fragment thereof.

14. The nucleic acid construct of claim 13, wherein the nucleic acid sequence encoding a blue-light induced potassium channel comprises a sequence as defined in SEQ ID NO: 4 or a functional variant or fragment thereof.
- 5 15. The nucleic acid construct of any of claims 10 to 14, wherein the stomatal complex promoter is a guard cell-specific promoter.
16. The nucleic acid construct of claim 15, wherein the guard-cell specific promoter is selected from Myb60, GC1 and SNAC1.
17. A vector comprising the nucleic acid construct of any of claims 10 to 16.
- 10 18. A host cell comprising the nucleic acid construct of claims 10 to 16 or the vector of claim 17.
19. A transgenic plant expressing the nucleic acid construct of any of claims 10 to 16 or the vector of claim 17.
20. The transgenic plant of claim 19, wherein the plant is a monocot or dicot.
- 15 21. A method of producing a transgenic plant as defined in claim 19 or 20, the method comprising:
- a. selecting a part of the plant;
 - b. transfecting at least one cell of the part of the plant of part (a) with the nucleic acid construct of any of claims 10 to 16 or the vector of claim 17; and
 - 20 c. regenerating at least one plant derived from the transfected cell or cells.
22. A plant obtained or obtainable by the method of claim 21.
23. The method of claim 21 or the plant of claim 22, wherein the plant is a monocot or dicot.

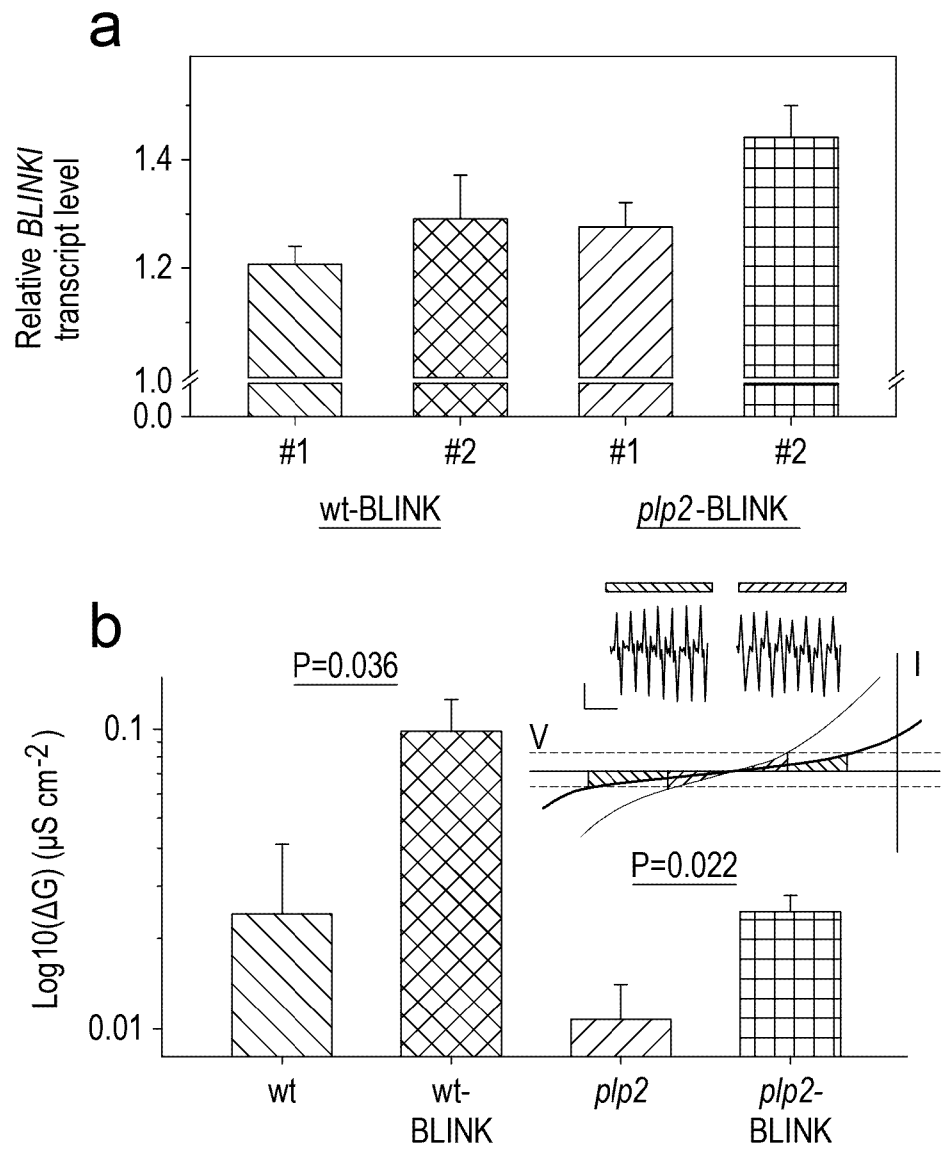


Fig. 1

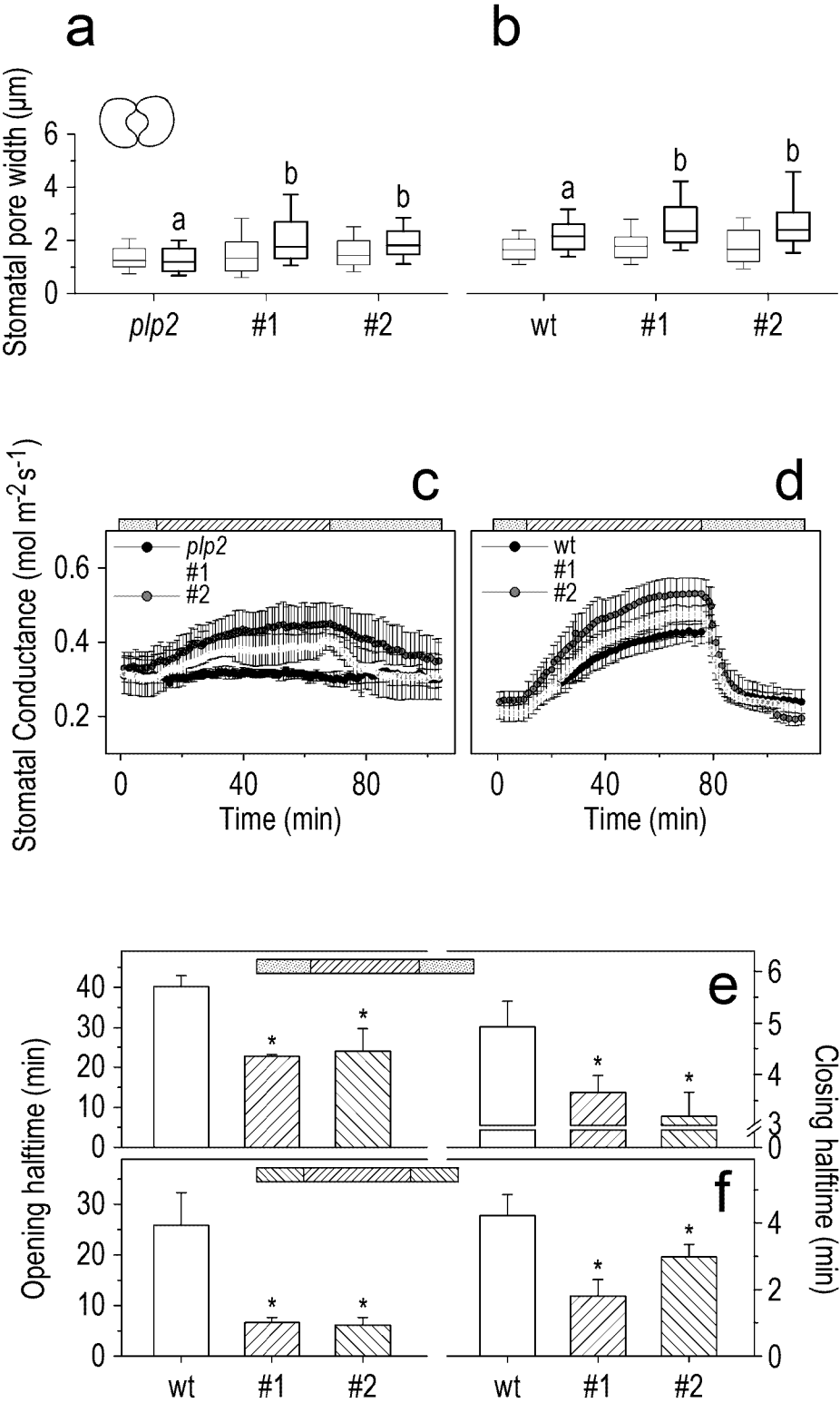


Fig. 2

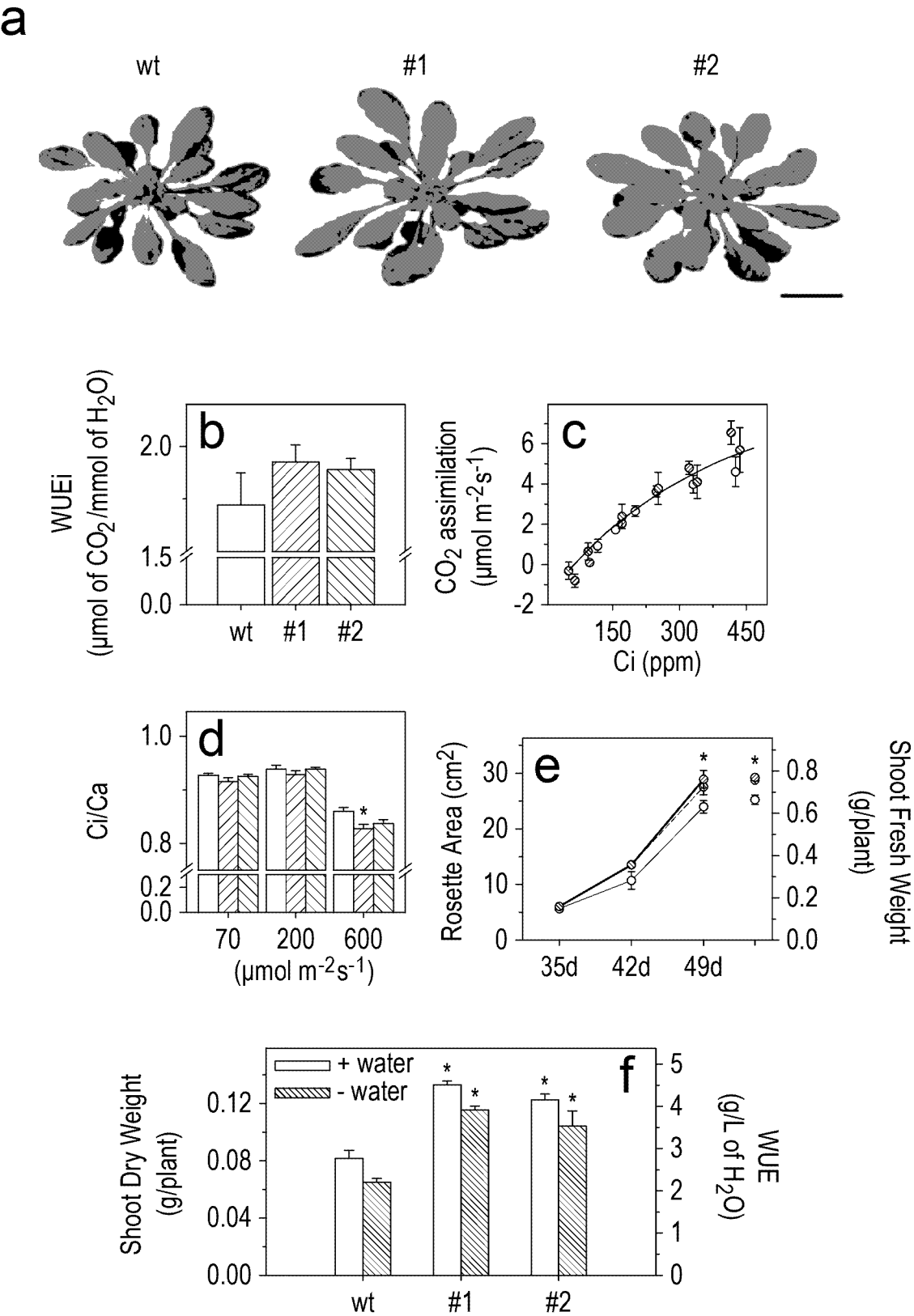


Fig. 3

		Blue light ON		Blue light OFF	
		Max. Stomatal Conductance (mol m ⁻² s ⁻¹)	Opening Halftime (min)	Min. Stomatal Conductance (mol m ⁻² s ⁻¹)	Closing Halftime (min)
Dark - Blue Light Response	wt	0.49 ±0.03	40.32 ±2.65	0.28 ±0.05	4.94 ±0.48
	#1	0.50 ±0.03	22.94 ±0.34 (<i>P</i> <0.001)	0.22 ±0.03	3.64 ±0.34 (<i>P</i> =0.03)
	#2	0.59 ±0.04	23.90 ±6.09 (<i>P</i> =0.045)	0.22 ±0.03	3.18 ±0.47 (<i>P</i> =0.019)
Red - Blue Light Response	wt	0.46 ±0.05	24.46 ±7.39	0.22 ±0.03	4.41 ±0.77
	#1	0.50 ±0.09	6.69 ±1.06 (<i>P</i> =0.012)	0.20 ±0.04	1.81 ±0.48 (<i>P</i> =0.013)
	#2	0.38 ±0.04	6.37 ±1.23 (<i>P</i> =0.007)	0.20 ±0.03	2.98 ±0.39 (<i>P</i> =0.045)

Fig. 4

		wt-BLINK			<i>p1p2</i> -BLINK		
		wt	#1	#2	<i>p1p2</i>	#1	#2
LWL (75 $\mu\text{mol m}^{-2} \text{s}^{-1}$)	Fresh Weight (g)	0.94 $\pm$ 0.09	1.21 $\pm$ 0.17	1.12 $\pm$ 0.09	0.41 $\pm$ 0.05	0.39 $\pm$ 0.08	0.44 $\pm$ 0.05
	Dry Weight (g)	0.11 $\pm$ 0.02	0.14 $\pm$ 0.01	0.10 $\pm$ 0.02	0.04 $\pm$ 0.005	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01
	Rosette Area (cm ²)	36.26 $\pm$ 3.40	36.00 $\pm$ 1.87	43.85 $\pm$ 1.95	13.67 $\pm$ 2.22	9.72 $\pm$ 0.90	13.83 $\pm$ 1.75
	WUEi (μmol of CO ₂ /mmol of H ₂ O)	2.36 $\pm$ 0.36	2.73 $\pm$ 0.36	2.47 $\pm$ 0.27	-	-	-
	WUE (g/L of H ₂ O)	4.52 $\pm$ 0.60	5.43 $\pm$ 0.48	4.00 $\pm$ 0.32	1.46 $\pm$ 0.22	1.81 $\pm$ 0.43	1.72 $\pm$ 0.33
	FW:DW	8.56 $\pm$ 0.18	8.64 $\pm$ 0.16	11.2 $\pm$ 0.13 (<i>P</i> < 0.001)	10.25 $\pm$ 0.02	7.80 $\pm$ 0.04	11.00 $\pm$ 0.06
HWL (200 $\mu\text{mol m}^{-2} \text{s}^{-1}$)	Fresh Weight (g)	2.00 $\pm$ 0.25	2.07 $\pm$ 0.21	2.16 $\pm$ 0.14	1.00 $\pm$ 0.07	0.72 $\pm$ 0.14	0.76 $\pm$ 0.13
	Dry Weight (g)	0.16 $\pm$ 0.02	0.17 $\pm$ 0.01	0.17 $\pm$ 0.007	0.08 $\pm$ 0.02	0.06 $\pm$ 0.01	0.05 $\pm$ 0.02
	Rosette Area (cm ²)	68.31 $\pm$ 5.09	58.11 $\pm$ 9.08	62.62 $\pm$ 2.62	24.26 $\pm$ 1.15	21.27 $\pm$ 3.60	19.60 $\pm$ 2.18
	WUEi (μmol of CO ₂ /mmol of H ₂ O)	2.58 $\pm$ 0.30	2.62 $\pm$ 0.45	2.36 $\pm$ 0.10	-	-	-
	WUE (g/L of H ₂ O)	6.23 $\pm$ 0.84	6.66 $\pm$ 0.46	6.69 $\pm$ 0.29	3.29 $\pm$ 0.80	2.07 $\pm$ 0.53	1.85 $\pm$ 0.30
	FW:DW	12.5 $\pm$ 0.016	12.18 $\pm$ 0.01	12.71 $\pm$ 0.004	12.5 $\pm$ 0.06	12.00 $\pm$ 0.03	15.2 $\pm$ 0.16

Fig. 5

Figure 5 continued

		wt-BLINK			<i>p1p2</i> -BLINK		
		Wt	#1	#2	<i>p1p2</i>	#1	#2
Fluctuating white light (water-sufficient)	Fresh Weight (g)	0.66 ±0.02	0.77 ±0.07 (<i>P</i> <0.001)	0.72 ±0.06 (<i>P</i> =0.001)	0.34 ±0.01	0.45 ±0.05	0.39 ±0.05
	Dry Weight (g)	0.082 ±0.005	0.13 ±0.002 (<i>P</i> <0.001)	0.12 ±0.003 (<i>P</i> <0.001)	0.05 ±0.01	0.09 ±0.03	0.07 ±0.02
	Rosette Area (cm ²)	23.87 ±1.13	28.72 ±1.67 (<i>P</i> =0.027)	27.28 ±1.45 (<i>P</i> =0.048)	11.06 ±0.72	13.77 ±0.73	12.83 ±0.96
	WUEi (μmol of CO ₂ /mmol of H ₂ O)	1.72 ±0.15	1.92 ±0.08	1.89 ±0.05	-	-	-
	WUE (g/L of H ₂ O)	2.67 ±0.16	4.19 ±0.08 (<i>P</i> <0.001)	4.15 ±0.10 (<i>P</i> <0.001)	2.18 ±0.57	2.78 ±0.79	2.84 ±0.78
	FW:DW	8.054 ±0.001	5.926 ±0.008 (<i>P</i> <0.001)	6.006 ±0.007 (<i>P</i> <0.001)	6.80 ±0.04	5.00 ±0.11	5.57 ±0.08
Fluctuating white light (water-limited)	Fresh Weight (g)	0.60 ±0.005	0.75 ±0.09	0.71 ±0.003	-	-	-
	Dry Weight (g)	0.064 ±0.003	0.12 ±0.001 (<i>P</i> <0.001)	0.11 ±0.01 (<i>P</i> =0.003)	-	-	-
	Rosette Area (cm ²)	26.78 ±1.18	26.18 ±0.69	28.07 ±1.01	-	-	-
	WUE (g/L of H ₂ O)	2.08 ±0.11	3.66 ±0.03 (<i>P</i> <0.001)	3.52 ±0.32 (<i>P</i> =0.008)	-	-	-
	FW:DW	9.46 ±0.08	6.47 ±0.09 (<i>P</i> <0.001)	6.81 ±0.03 (<i>P</i> <0.001)	-	-	-

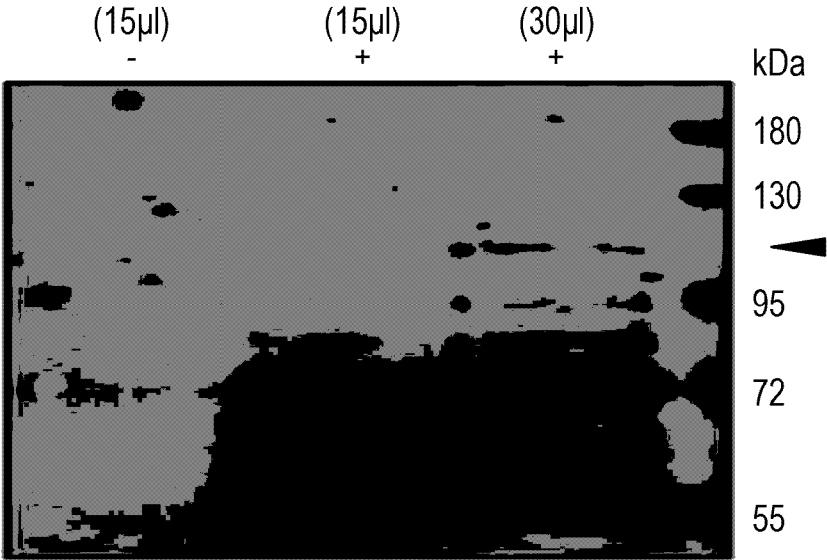


Fig. 6

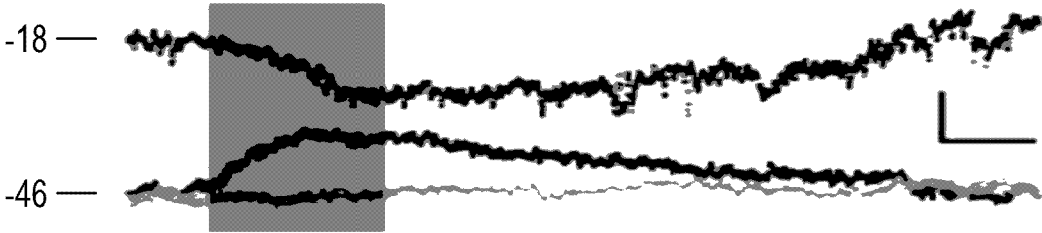


Fig. 7

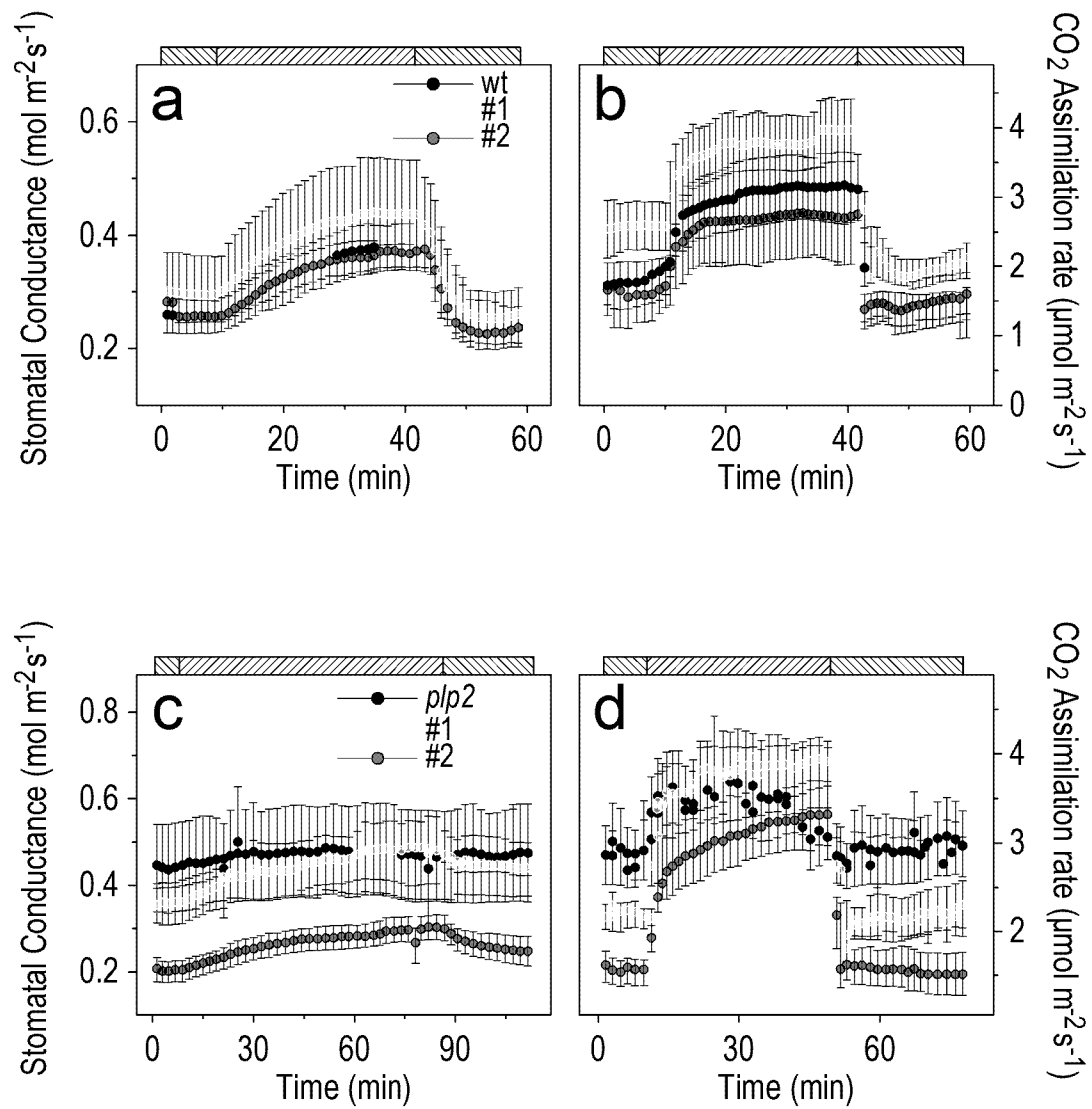


Fig. 8

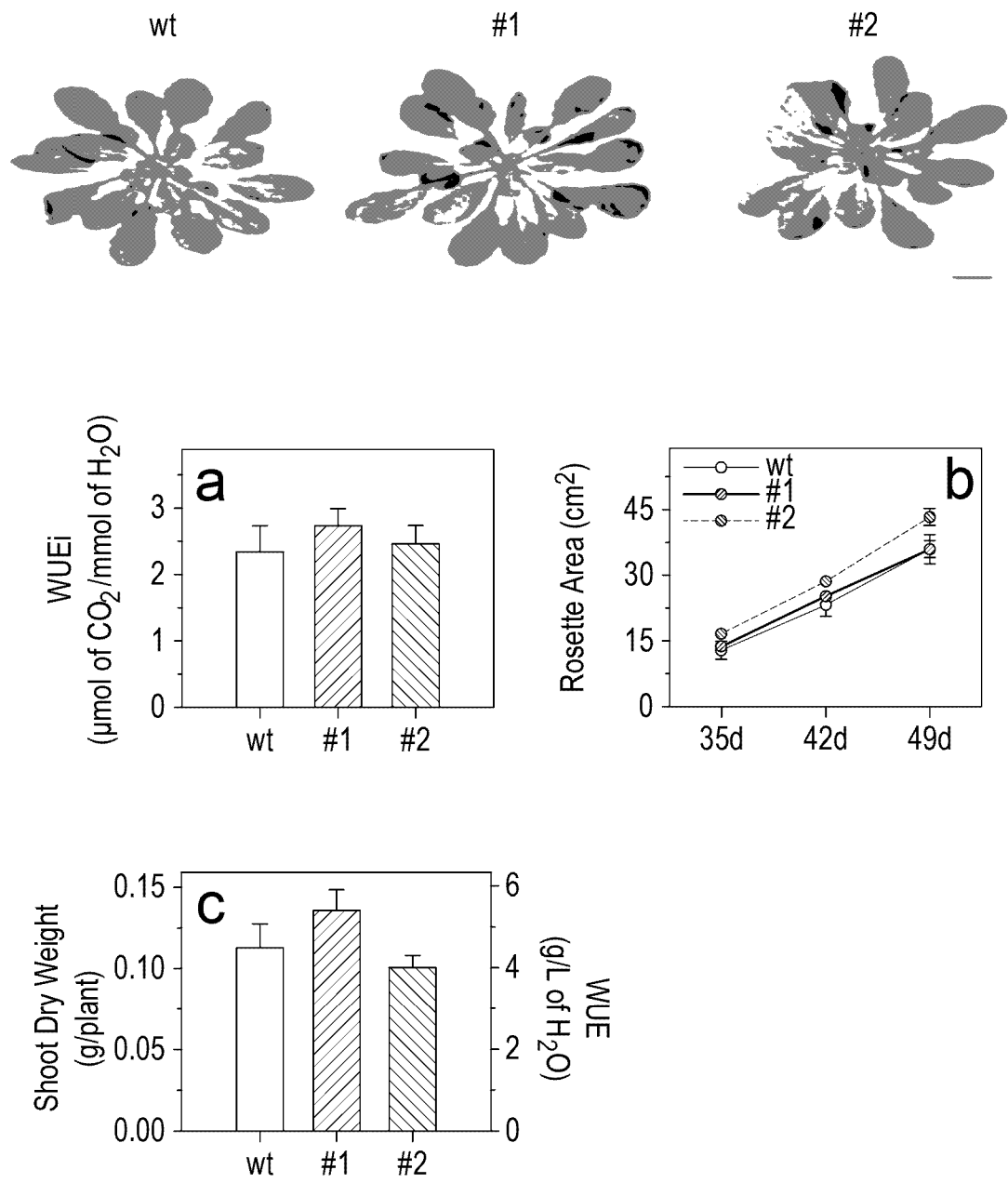


Fig. 9

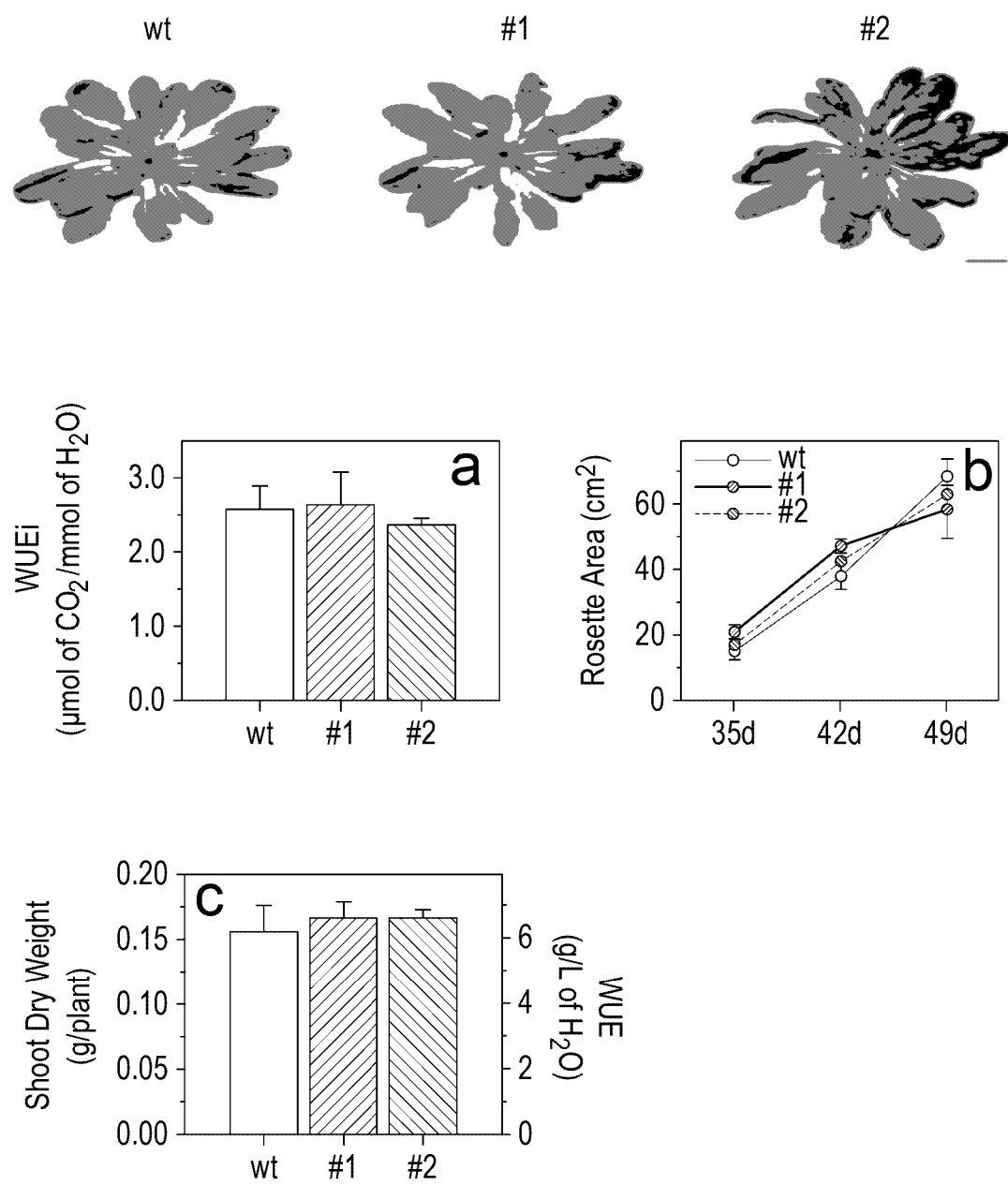


Fig. 10

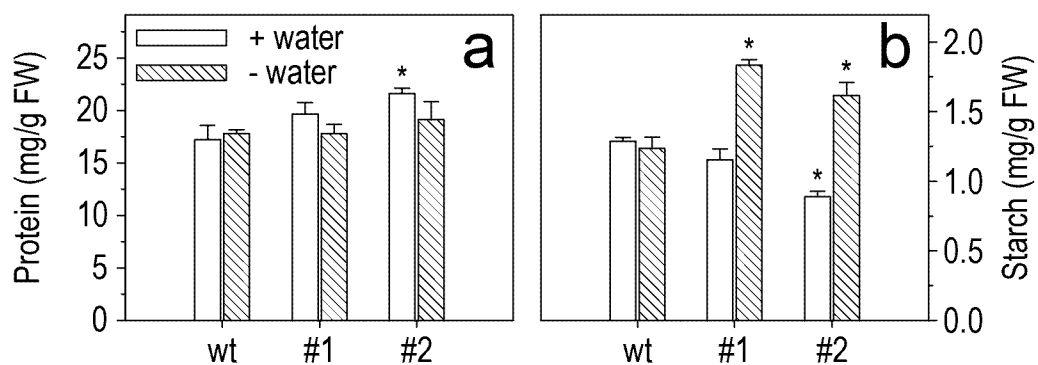


Fig. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2020/050400

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TRACY LAWSON ET AL: "Stomatal Size, Speed, and Responsiveness Impact on Photosynthesis and Water Use Efficiency", PLANT PHYSIOLOGY, vol. 164, no. 4, 27 February 2014 (2014-02-27), pages 1556-1570, XP055686211, Rockville, Md, USA ISSN: 0032-0889, DOI: 10.1104/pp.114.237107 cited in the application page 1565, left-hand column, last paragraph - page 1567, left-hand column, last paragraph ----- -/--	1

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 April 2020

Date of mailing of the international search report

07/05/2020

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Bilang, Jürg

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2020/050400

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Y. WANG ET AL: "Overexpression of plasma membrane H ⁺ -ATPase in guard cells promotes light-induced stomatal opening and enhances plant growth", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 111, no. 1, 23 December 2013 (2013-12-23), pages 533-538, XP055292816, US ISSN: 0027-8424, DOI: 10.1073/pnas.1305438111 the whole document -----	1
A	LORNA MCAUSLAND ET AL: "Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency", NEW PHYTOLOGIST, vol. 211, no. 4, 27 September 2016 (2016-09-27), pages 1209-1220, XP055686209, GB ISSN: 0028-646X, DOI: 10.1111/nph.14000 cited in the application page 1209, right-hand column, paragraph 2 - page 1210, left-hand column, last paragraph -----	1
A	C CONSENTINO ET AL: "Engineering of a light-gated potassium channel", SCIENCE, vol. 348, no. 6235, 8 May 2015 (2015-05-08), pages 707-710, XP055686650, US ISSN: 0036-8075, DOI: 10.1126/science.aaa2787 cited in the application abstract -----	1-23
X,P	M Papanatsiou ET AL: "Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth", Science (New York, N.Y.), 29 March 2019 (2019-03-29), pages 1456-1459, XP055686480, United States DOI: 10.1126/science.aaw0046 Retrieved from the Internet: URL:https://science.sciencemag.org/content/363/6434/1456.full.pdf the whole document -----	1-23