

Evaluation and Licensing Opportunities

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Patent Literature

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BLINK!

Super-synchronised stomatal control

Enhanced photosynthesis and growth while preserving water status

Stomata allow CO₂ uptake for photosynthetic carbon assimilation but often at the expense of water loss via transpiration. Stomatal opening occurs in response to changes in atmospheric CO₂ concentration, ambient light, atmospheric relative humidity and ABA. Approaches to enhance water-use efficiency in plants generally come up against this inherent trade-off between carbon assimilation and water use. Professor Mike Blatt and Professor John Christie at the University

of Glasgow have teamed up to provide a solution that accelerates the kinetics of stomatal opening and closure, thus promoting a rapid transition to carbon assimilation under high-light intensities, while maintaining plant water status when carbon demand is low.

The researchers at Glasgow have expressed a synthetic light-gated potassium channel in guard cells surrounding the stomatal pores to enhance the solute fluxes that drive effects on stomatal aperture. The K⁺ channel, BLINK1 (Cosentino et al 2015), was constructed by fusing the plant LOV2-Jα photosensory module to the miniature K⁺ channel, Kcv, of the Chlorella virus PBCV. This results in a synthetic, blue light-gated channel. When expressed in plants, and particularly in the stomatal guard cells, this results both in accelerated stomatal opening upon light irradiation and also in faster closing following a return to darker conditions. In natural crop growing conditions with fluctuating light, eg varying cloud cover or within crop canopy conditions, stomatal opening often lags behind the changing micro-environment with consequent growth penalties to the crop. With the more responsive, accelerated stomatal opening and closure afforded by expressing BLINK channels, stomatal aperture can be brought in synchrony with the environment, thus enhancing carbon-assimilation and water use.

Opening and closure of stomata is driven by ion transport across the plasma membrane of the cells of the stomatal complex – i.e. the guard cells, or in some species such as grasses like maize, the guard cells and subsidiary cells. Together with the metabolism of organic solutes, this promotes water flux and changes in cell volume and turgor, causing physical changes to the stomatal pore aperture. Blue light triggers stomatal opening, enhancing photosynthesis through the action of phototropin receptor kinases, phot1 and phot2, that lead to the activation of guard-cell proton-ATPases, in turn promoting K⁺ uptake. The expression of BLINK1 works on top of this process to enhance the kinetics of the stomatal opening and closure. The Glasgow team used a guard-cell directed promoter, pMYB60, to drive expression of BLINK1 in transiently transformed *N. benthamiana* and Arabidopsis and in stable transgenic Arabidopsis. BLINK1 not only restored BL-induced stomatal opening in phototropin-deficient mutant lines (*p1p2*) but also significantly enhanced the steady-state aperture of stomata by 17%, and 29% greater stomatal conductance compared to wild-type plants – reflecting both 40% faster and greater stomatal opening (Fig 1 below).

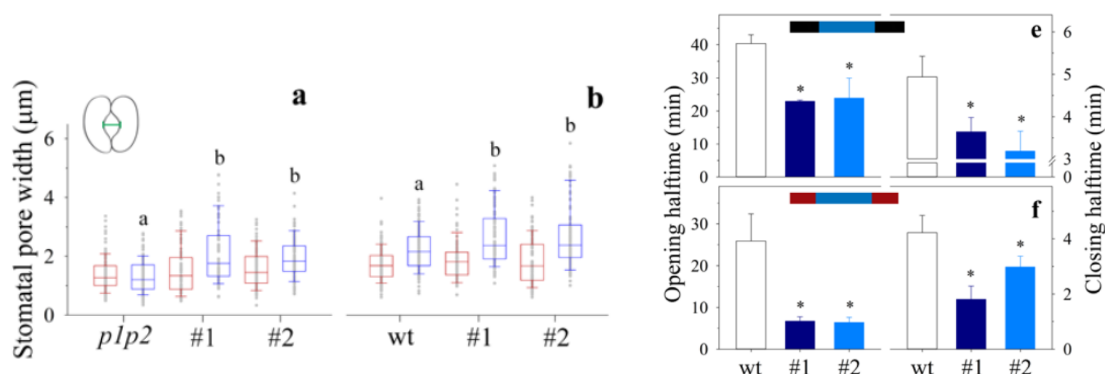


Fig 1: #1 and #2 are each independent transgenic Arabidopsis lines in either p1p2 mutant or WT backgrounds

To study Water Use Efficiency (WUE), the BLINK1 transgenic lines were grown under night/day cycles with constant low ($75 \mu\text{ mol m}^{-2} \text{ s}^{-1}$) or constant high ($190 \mu\text{ mol m}^{-2} \text{ s}^{-1}$) daytime light fluence regimes. WUE was then calculated as the ratio of accumulated dry biomass to water used over a 49-day growth period. Under these conditions there were no differences in growth (biomass accumulation, rosette area etc) or water use between BLINK plants and controls. However, in the natural environment daytime light fluctuates and while photosynthesis generally tracks light energy input, stomata are slower to respond, limiting gas exchange and leading to suboptimal assimilation when fluence rate rises and to transpiration without corresponding assimilation when the fluence rate drops quickly.

So, to better simulate natural field conditions, the researchers integrated periods of fluctuating light to give a total photon flux over the daylight period intermediate between the two continuous light regimes. They used stepped light 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. This was conducted under both water-replete and water-deficit (10% soil moisture) conditions. The BLINK plants in a WT background showed increased rosette area, increased fresh weight and, remarkably, a **2.2x increase in total biomass** grown under both watering regimes, indicating a **highly significant improvement in both growth and WUE due to BLINK expression** (Fig 2)

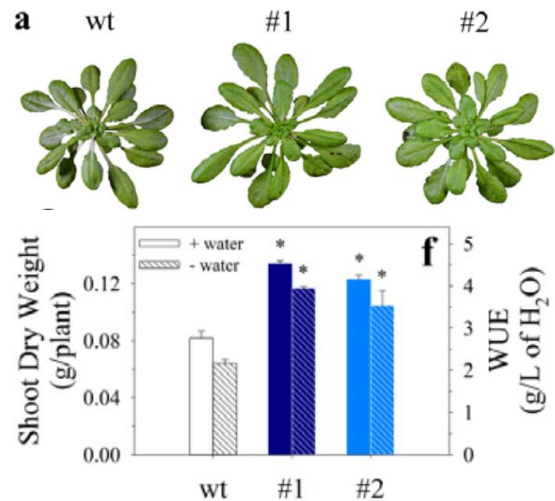


Fig 2: Plant growth and WUE under fluctuating light and different water regimes

Under water-replete conditions, BLINK plants showed a modest increase in total protein content and a small decrease in total starch, while under water deficit conditions BLINK plants had a highly significant increase in total starch. The researchers confirmed that this was not due to an alteration of photosynthesis per se, since CO_2 accumulation under saturating light was not affected in BLINK plants across the physiological range of internal CO_2 conditions.

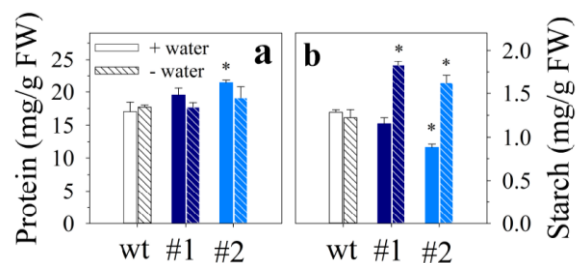



Fig 3: BLINK induces starch accumulation under water deficit

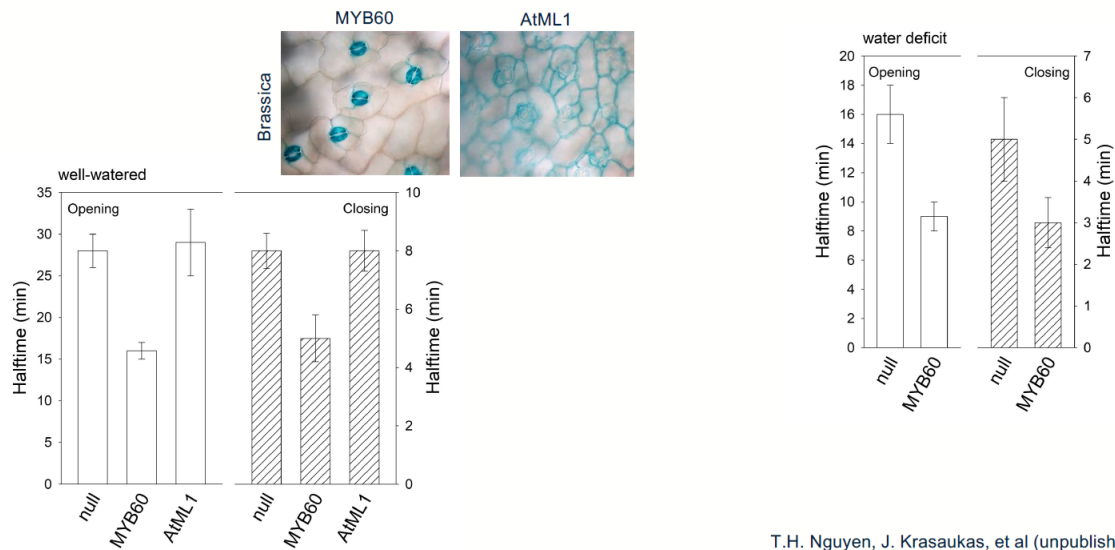
In summary, BLINK expression in plants, in a pattern directed at the cells of the stomatal complex, affords highly beneficial accelerated stomatal kinetics that are responsible for enhancing carbon assimilation without a cost in water use. This elegant innovation, using naturally derived biological components, has enormous potential for enhancement of crop performance under challenging and fluctuating environmental conditions.

The BLINK stomatal control technology is patented by PBL on behalf of the University of Glasgow. For more information or licensing interest, please contact PBL.

UPDATE March 2023

The inventors have now studied the effects of BLINK expression in transgenic **Brassica and barley**, under different promoter strategies. With expression directed to guard cells, **stomatal kinetics are markedly improved** (accelerated opening and closing) under both well-watered and water deficit conditions. Moreover under water limitation **BLINK1 expression significantly enhances growth (and WUE)** in both of these crop species.

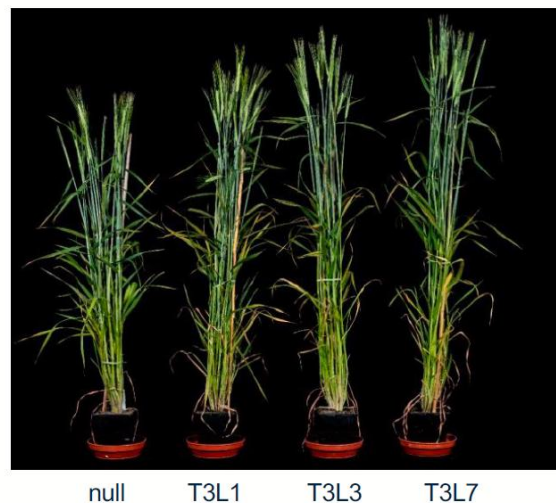
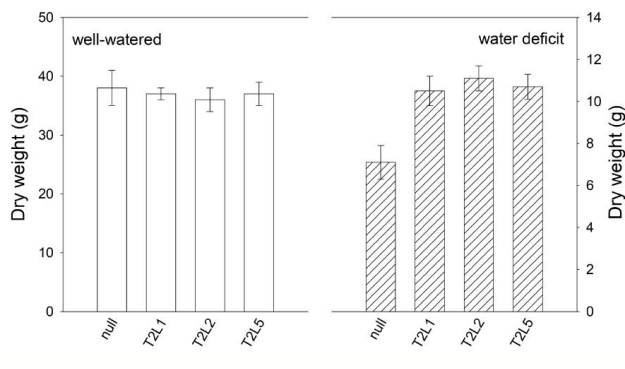
 guard cell-specific expression associates with accelerated kinetics



T.H. Nguyen, J. Krasaukas, et al (unpublished)

Above: BLINK1 promotes WUE in brassica with improved stomatal kinetics.

Below: Under water limitation, BLINK1 enhances biomass gain / growth under water limitation in Brassica (left) and barley. Barley tiller number is not affected.



This new data (March 2023) is not yet published by the inventors and therefore remains confidential under the PBL TEC Scheme. Please enquire for more information.

References:

C Cosentino et al (2015). Optogenetics. Engineering of a light-gated potassium channel. *Science*; 348: 707-710.
M Papanatsiou et al (2019). Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth. *Science*; 363 (6434): 1456-1459. DOI: 10.1126/science.aaw0046.