

***Engineering ion Flux of the Stomatal Complex
for Enhanced Photosynthesis and Water Use
Efficiency***

-Or-

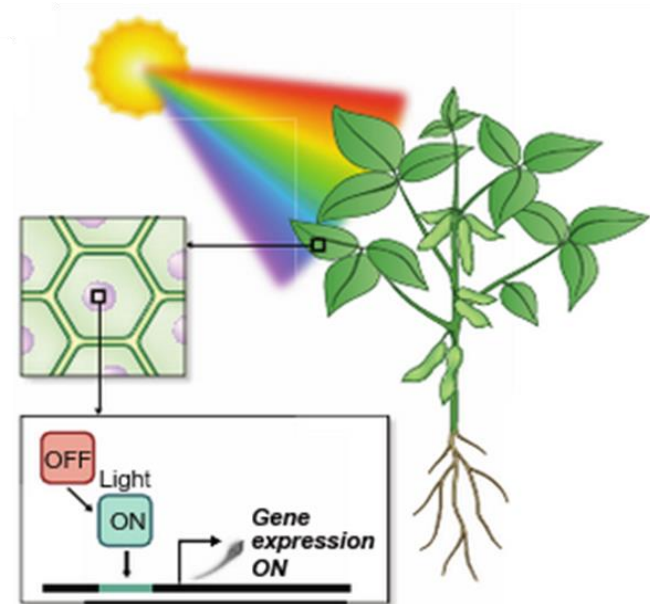
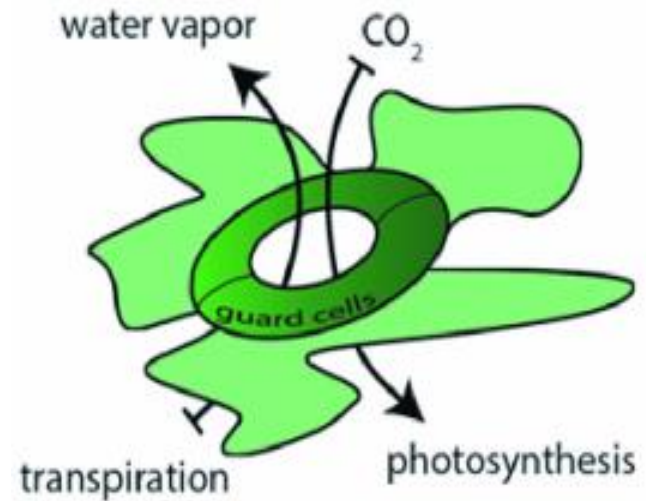
***Manipulation of Stomatal Responses for
Increased Biomass***

Background: Stomata and water use efficiency (WUE)

Stomata facilitate CO₂ influx and restrict transpiration, by changing their aperture depending on light

Optogenetics refers to the use of light-responsive proteins to control and dissect biological function

Optogenetics to enhance stomatal kinetics, improve WUE without penalty in carbon fixation

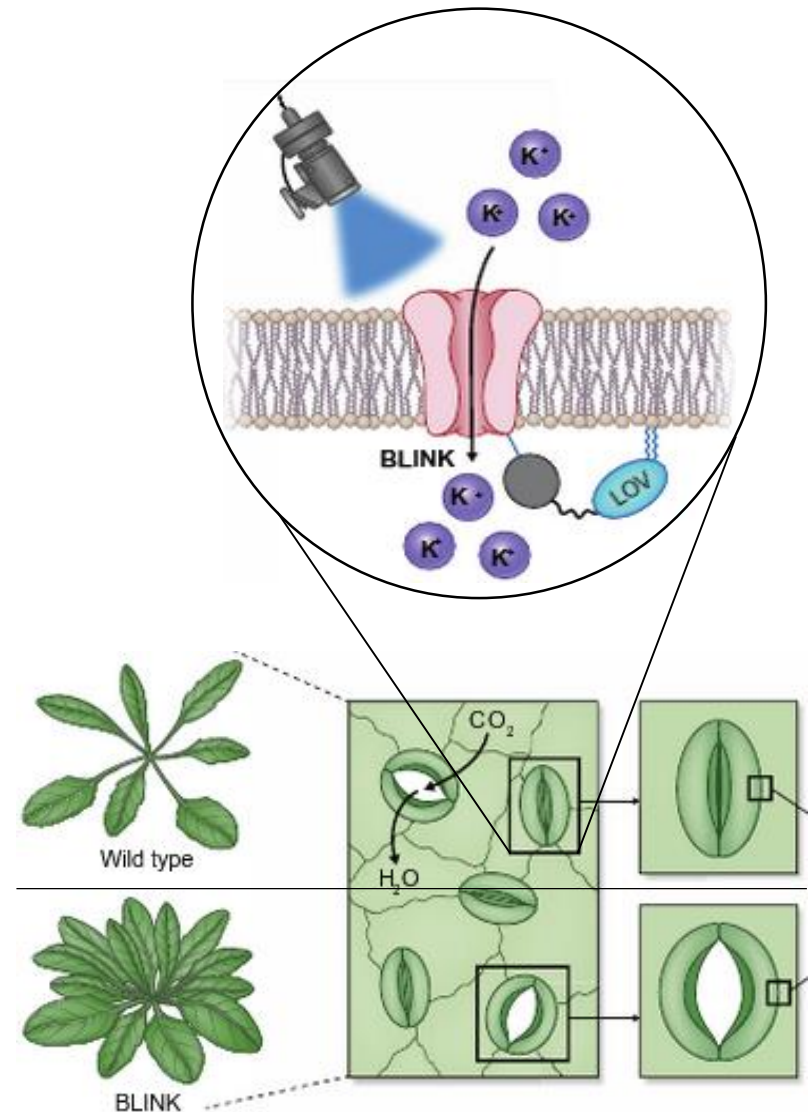


Background: BLINK1 affects stomata aperture

Fusion of LOV2-J α photoswitch from *O. sativa* PHOT1 with a viral K⁺ channel Kcv, activated by blue light

BLINK1 expressed in guard cells accelerated stomata kinetics (opening and closing) by ~40% especially in fluctuating light

BLINK1 drove a 2.2-fold increase in biomass without cost in WUE in *Arabidopsis*



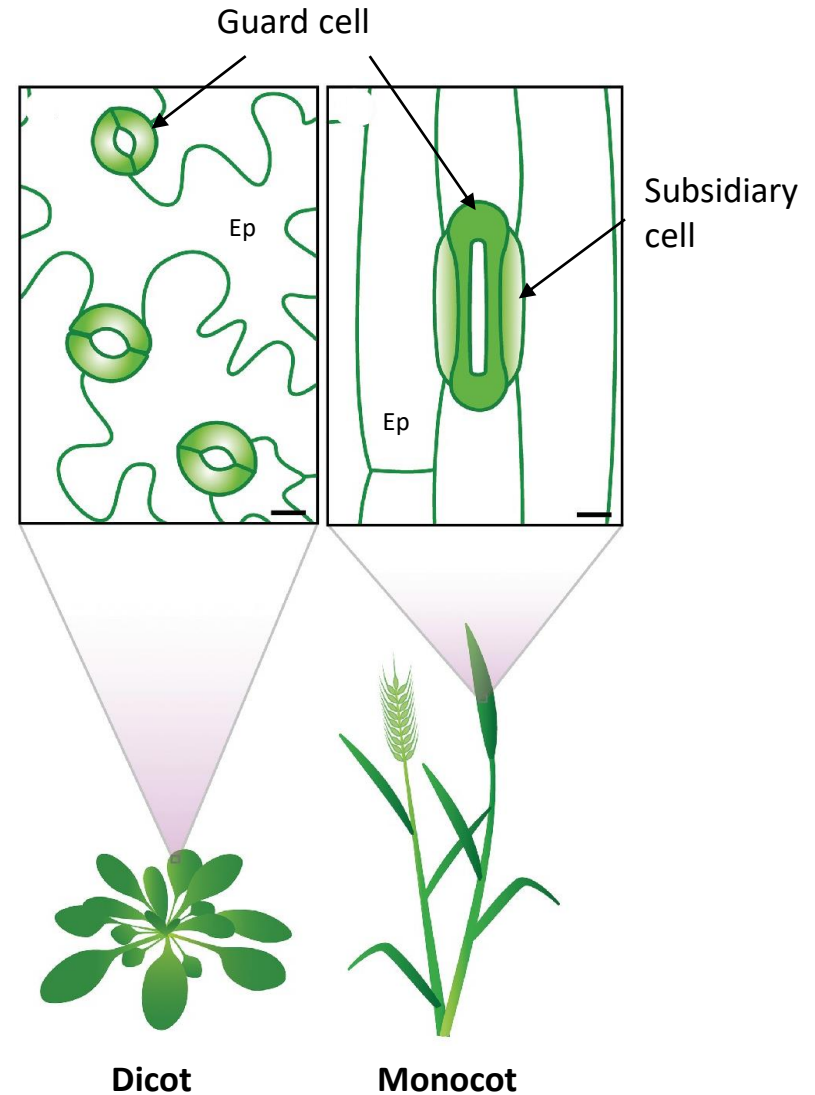
AIM: BLINK1 proof of principle

Identify epidermal cell and guard cell specific promoters in Brassica and barley

Test promoter:GUS constructs

Select a range of promoters to overexpress BLINK1

Measure stomata conductance and Assimilation in transgenics under varying conditions



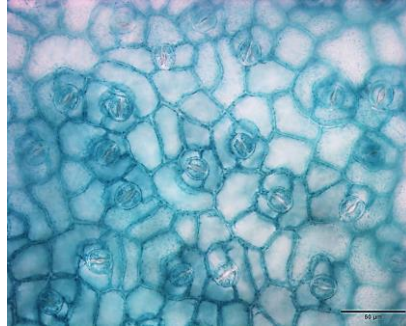
Results: GUS Expression

Epidermal cell specific

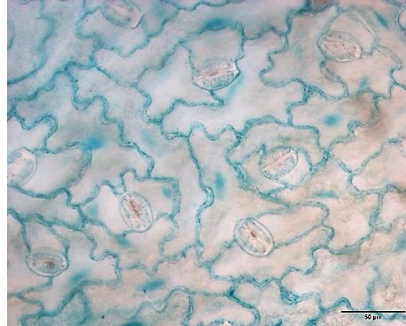
Control



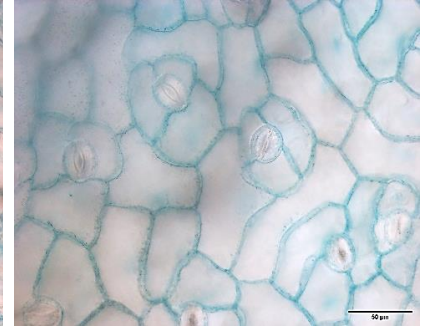
AtML1



CER6

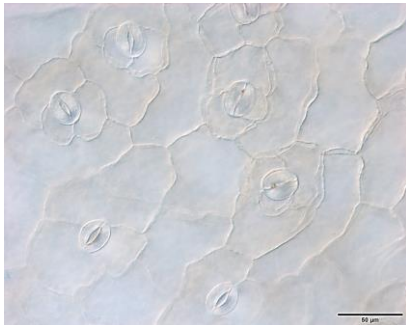


GstA+WIR1

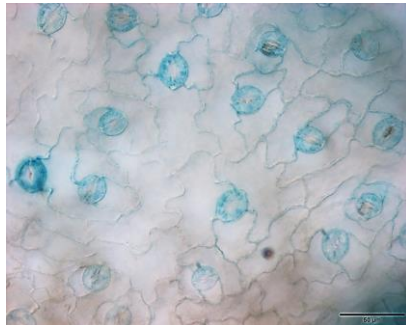


Guard cell specific

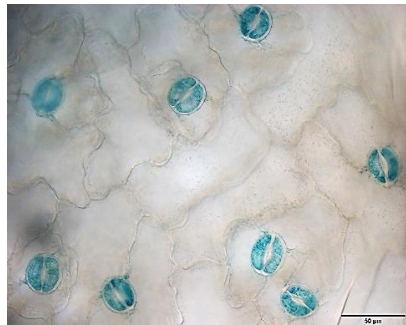
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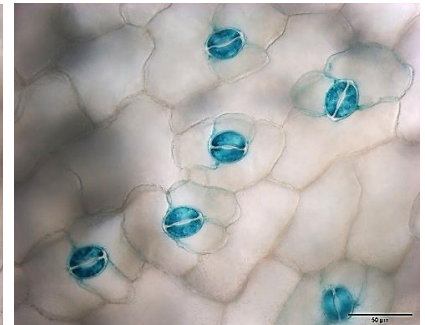
EXPA1



GC1



MYB60



T0 transgenic samples treated with 2 mM Ferri/Ferrocyanide

Results: Overview of transgenics

Promoter	Specificity	GUS Transgenics		BLINK1 Transgenics	
		Brassica	Barley	Brassica	Barley
ATML1 ^(m)	Epidermis	+++	-	✓	
CER6 ^(d)	Epidermis	+++	-	✓	
CST1 ^(m)	Guard cell	-	+++		
CYP86A2 ^(d)	Epidermis/ Guard cell	++	-		
EXPA1 ^(d)	Guard cell	++	-	✓	
GC1 ^(d)	Guard cell	++	-		
GstA1+WIR1 ^(m)	Epidermis	++	+		
KST1 ^(d)	Guard cell	++	-		
MYB60 ^(d)	Guard cell	+++	-		
SCRM2 ^(m)	Guard cell	-	-		
SNAC ^(m)	Guard cell	-	+		

d= dicot, m= monocot, b= both

Strong promoters selected to drive BLINK1 expression in guard cells and epidermal cells

T0 transgenics generated

Still to do

Identify T1 homozygous lines for promoter:GUS and promoter:BLINK1 transgenics

Set up gas exchange and stomata conductance experiments to determine stomatal kinetics for BLINK1 transgenics

- with guard cell promoter

- with epidermal cell promoters

Anticipated outcomes

Stomatal transpiration is at the centre of a crisis in water availability and crop production that is expected to unfold over the next 20-30 years.

Stomatal kinetics is now a demonstrable target for enhancing carbon gain and WUE (in *Arabidopsis*) that potentially promises wide applicability in crops.

Optogenetics may address the role of the surrounding cells in stomatal physiology.

Anticipate BLINK1 to enhance K^+ flux, promote stomatal kinetics, and enhance biomass yield and WUE, especially under fluctuating light and drought conditions in Brassica and barley.

Thank you

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Mike Blatt group and John Christie

