2024/10/10

Analysis of light-induced stomatal opening and enhancement of photosynthesis and plant yield

Toshinori Kinoshita

Institute of Transformative Bio-Molecules (ITbM), Nagoya University

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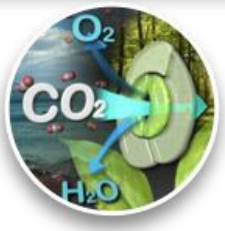


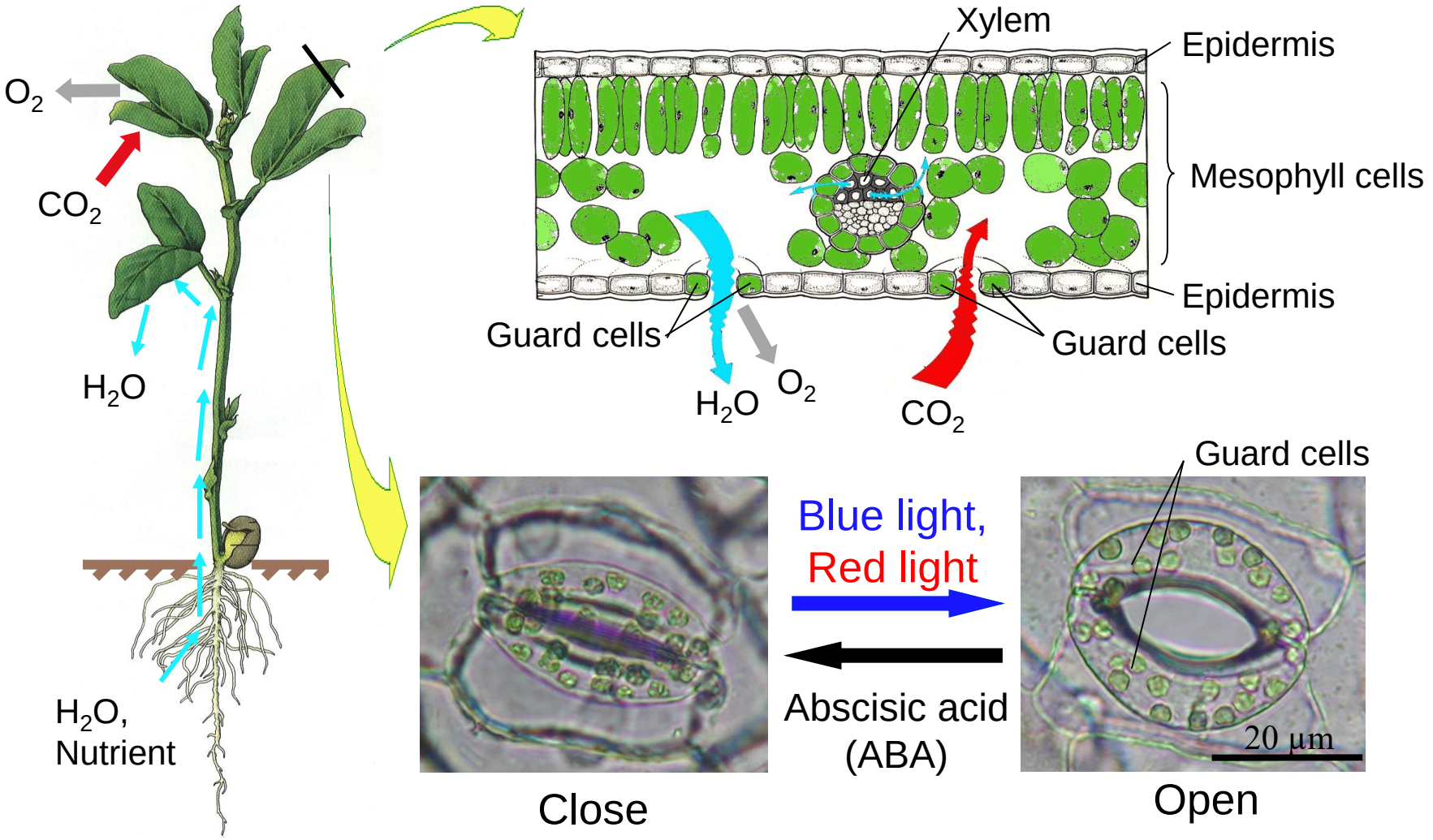
Topics

1. Molecular mechanism of light-induced stomatal opening

2. Importance of stomatal opening and PM H^+-ATPase in plant growth

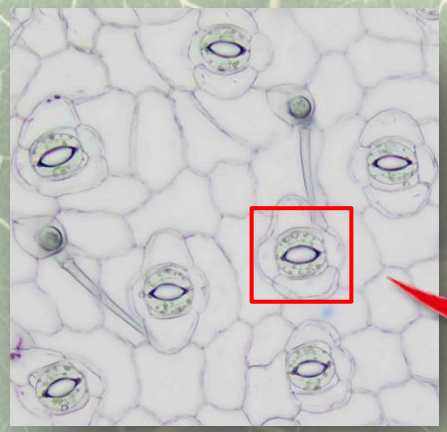
3. Chemicals confer drought tolerance to plants

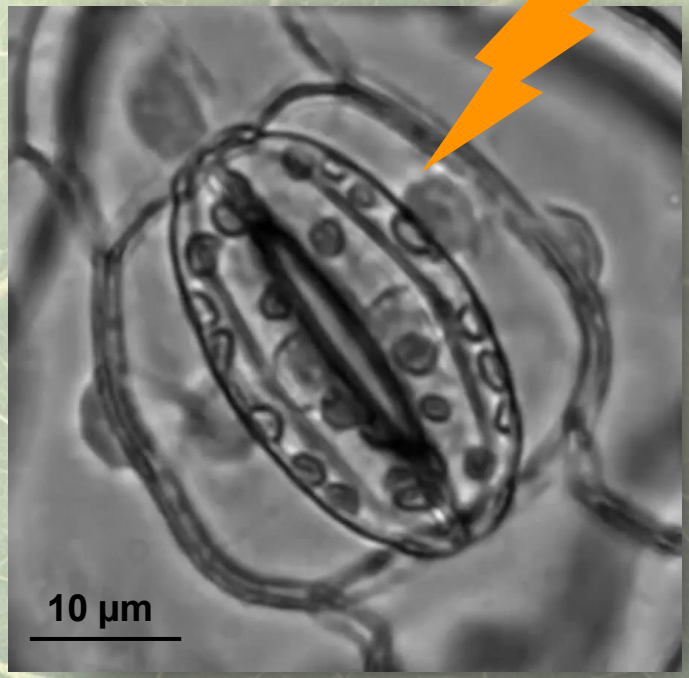
Stomata regulate gas exchange



Stomatal opening in Commelina （x 1,200）
(Tsuyukusa)

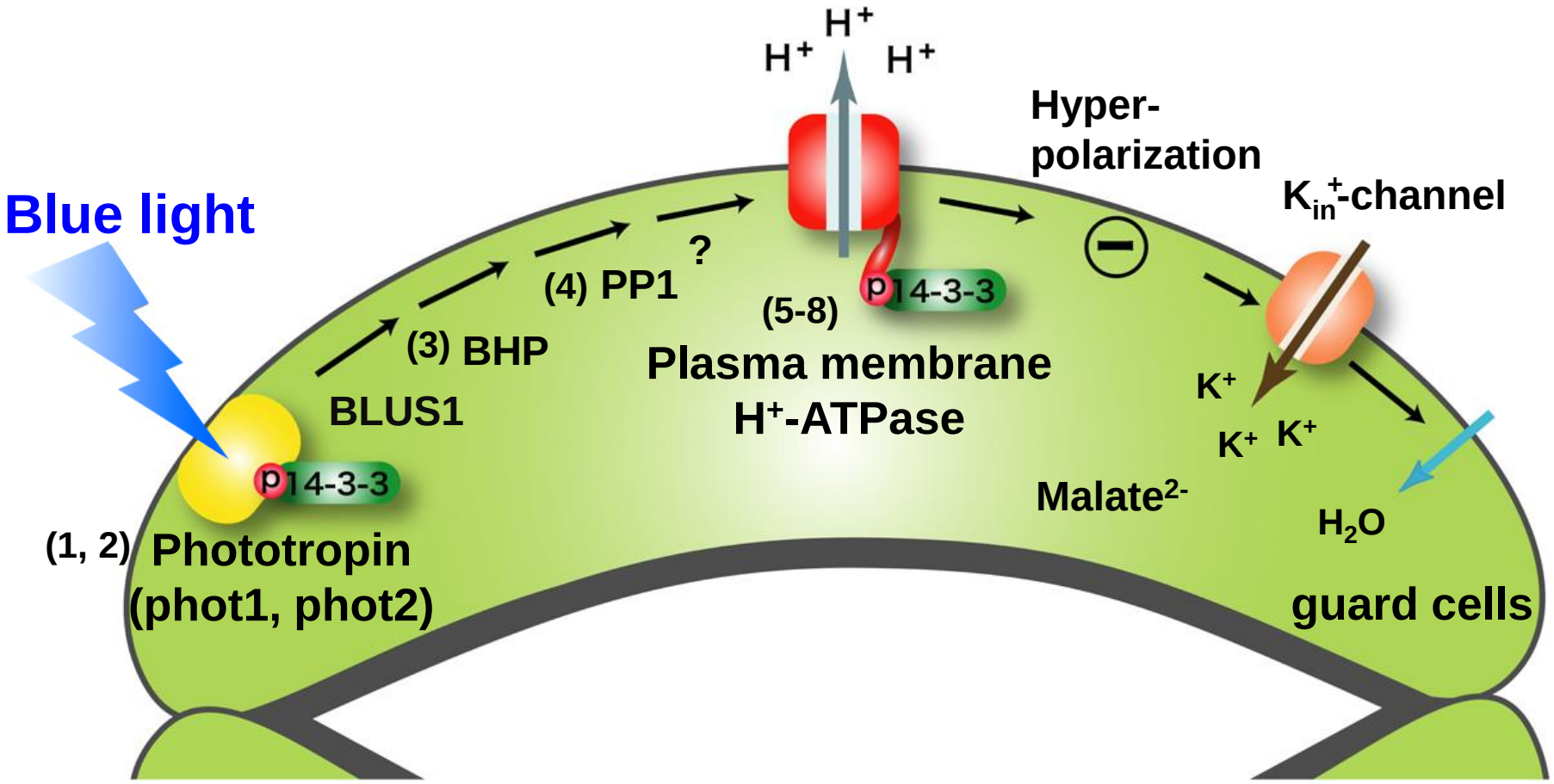
Light



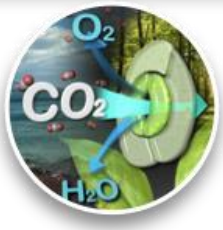


Complete stomatal opening takes more than one hour.

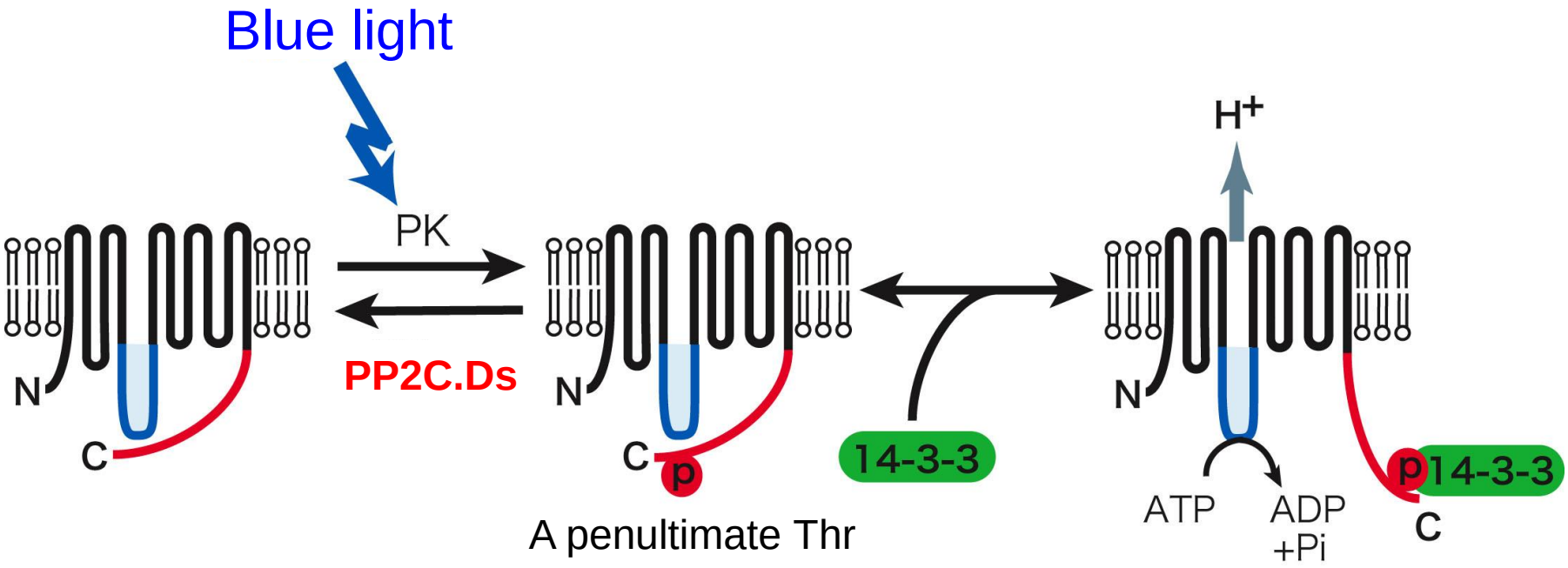
Blue light-induced stomatal opening



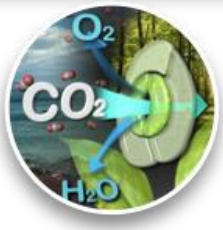
(1) Kinoshita et al. (2001) Nature
(2) Kinoshita et al. (2003) Plant Physiol
(3) Hayashi et al. (2017) Sci Rep
(4) Takemiya et al. (2006) PNAS
(5) Kinoshita & Shimazaki (1999) EMBO J
(6) Wang et al. (2014) PNAS
(7) Aihara et al. (2023) Nature Commun
(8) Hayashi et al. (2024) Nature Commun



Blue light-induced activation of H+-ATPase in guard cells



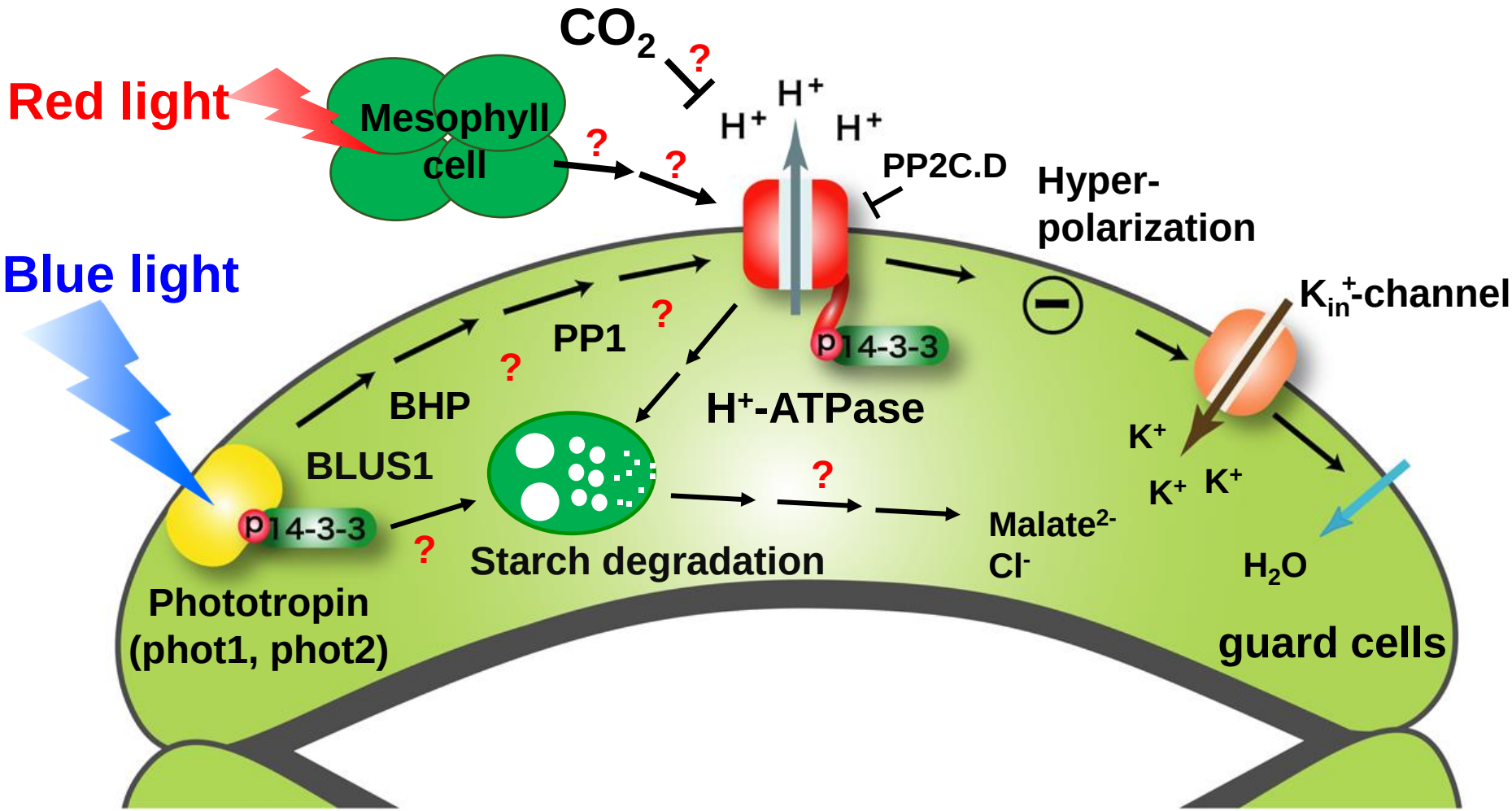
Kinoshita & Shimazaki (1999) EMBO J.
Inoue & Kinoshita (2017) Plant Physiol.
Akiyama et al. (2022) Plant Physiol.



Phosphorylation of Thr881, in addition to Thr948, needs full activation of PM H+-ATPase and blue light-induced stomatal opening

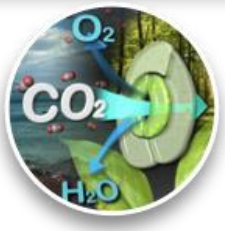
Hayashi et al. (2024) Nature Commun.

Light-induced stomatal opening



Hayashi et al. (2017) Sci Rep
Ando & Kinoshita (2018) Plant Physiol
Aihara et al. (2023) Nature Commun
Commun

Akiyama et al. (2022) Plant Physiol
Ando et al. (2022) New Phytol
Hayashi et al. (2024) Nature



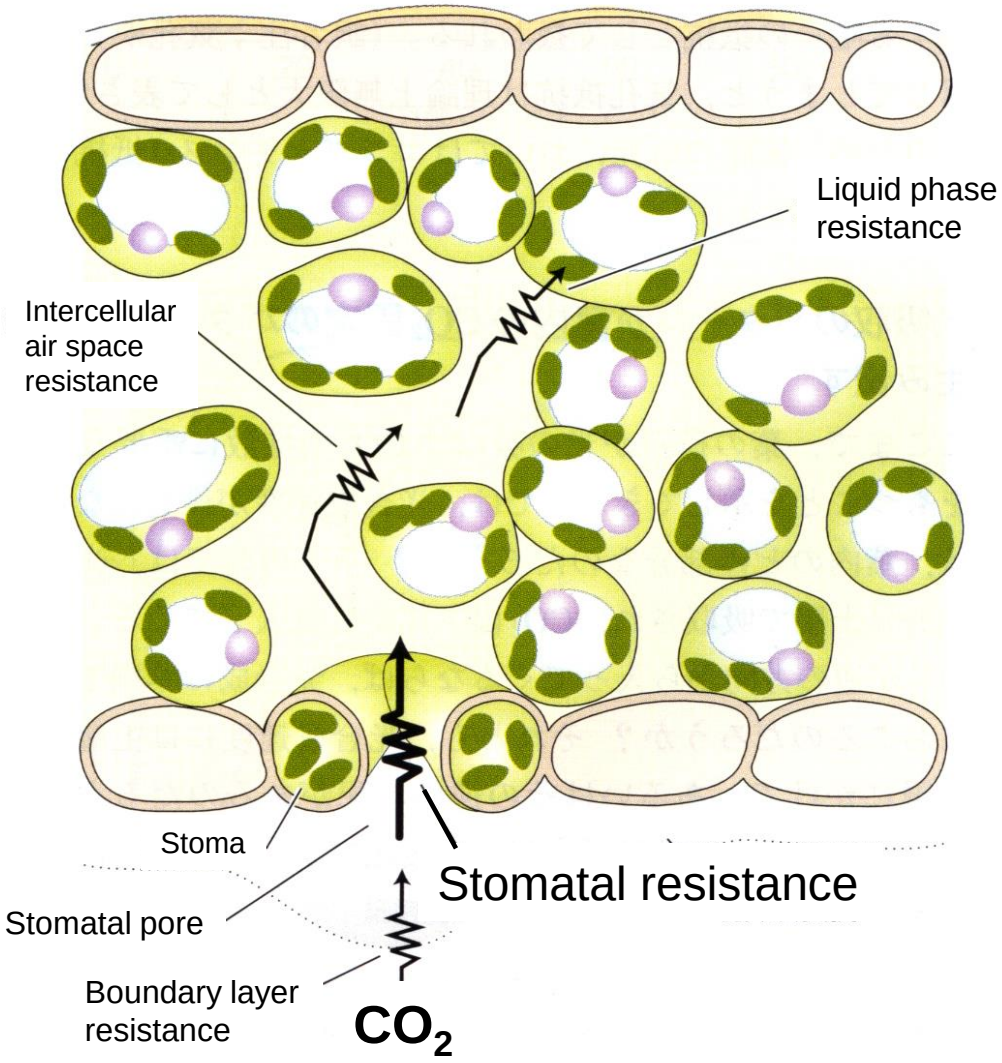
Topics

1. Molecular mechanism of light-induced stomatal opening

2. **Importance of stomatal opening and PM H^+-ATPase in plant growth**

3. Chemicals confer drought tolerance to plants

Stomatal resistance is a major limiting factor for CO_2 uptake and photosynthesis



Promotion of stomatal opening

↓

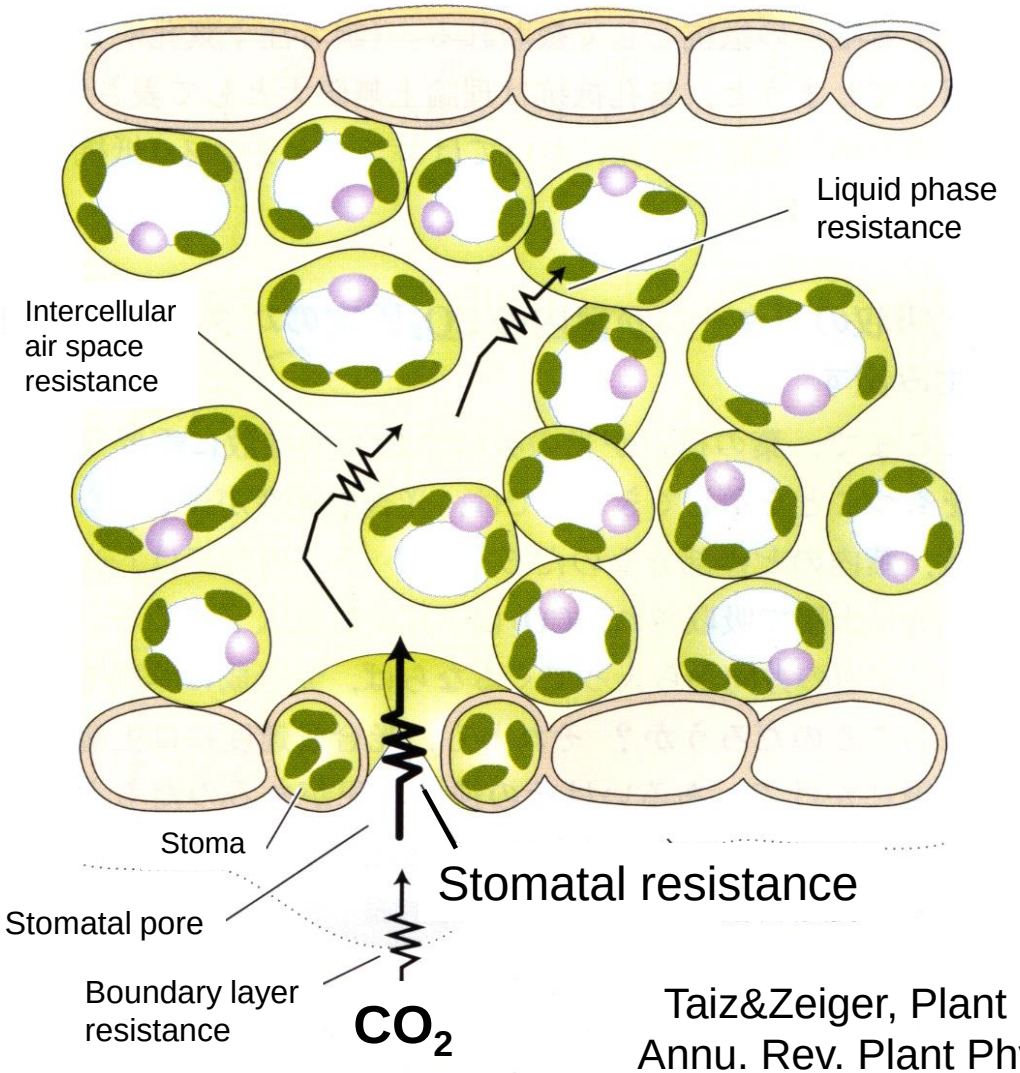
Decrease of stomatal resistance

↓

Increase of photosynthesis

Taiz&Zeiger, Plant Physiology
Annu. Rev. Plant Physiol. (1982)

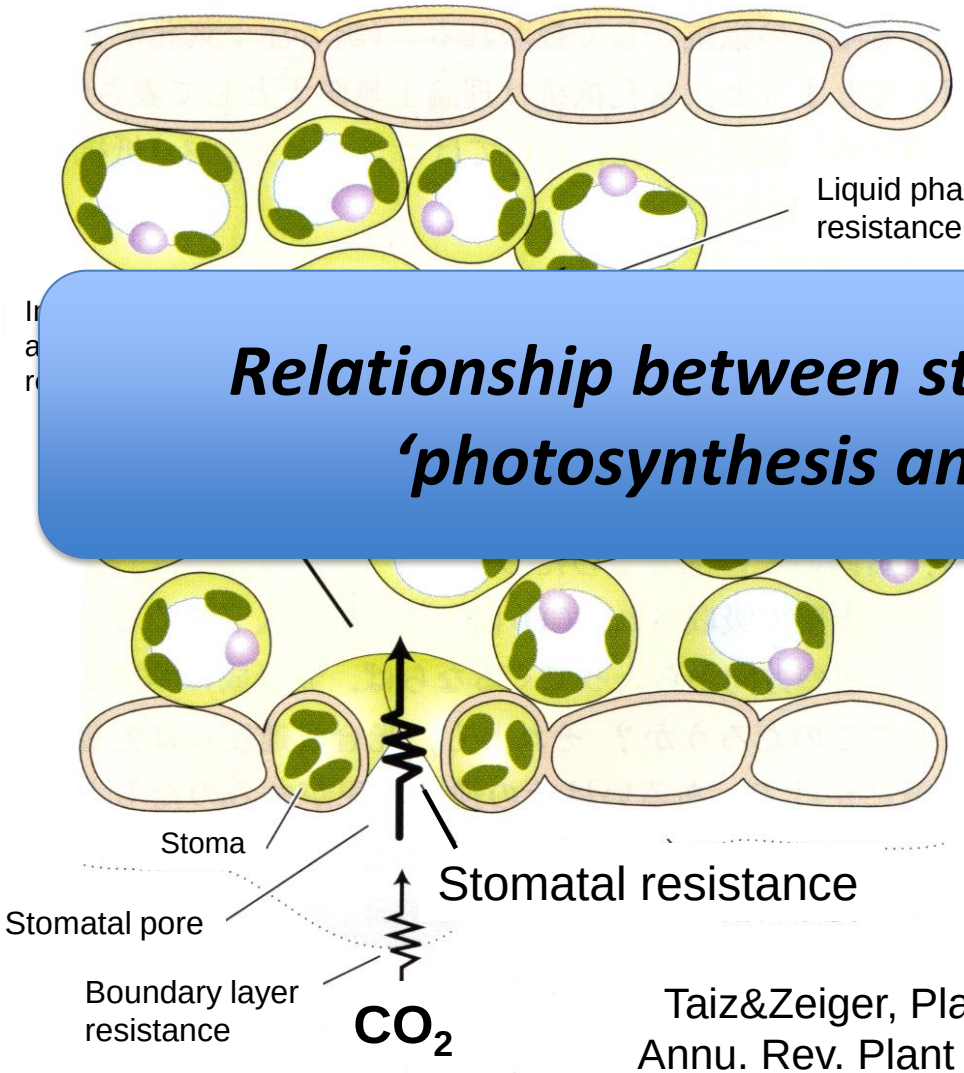
Stomatal resistance is a major limiting factor for CO_2 uptake and photosynthesis



There was no experimental evidence for this.

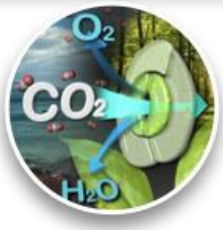
Taiz&Zeiger, Plant Physiology
Annu. Rev. Plant Physiol. (1982)

Stomatal resistance is a major limiting factor for CO_2 uptake and photosynthesis



Relationship between stomatal aperture and 'photosynthesis and plant growth'

Taiz&Zeiger, Plant Physiology
Annu. Rev. Plant Physiol. (1982)



Enhancement of stomatal opening by overexpression of H+-ATPase in guard cells using guard cell strong promoter GC1, increases photosynthesis and plant growth

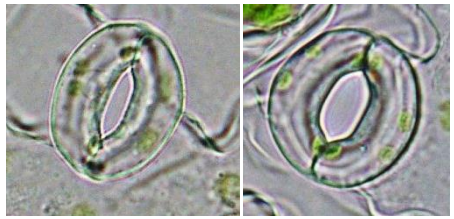
25-day-old plants

WT ***GC1::AHA2 #1***

WT ***GC1::AHA2 #1***

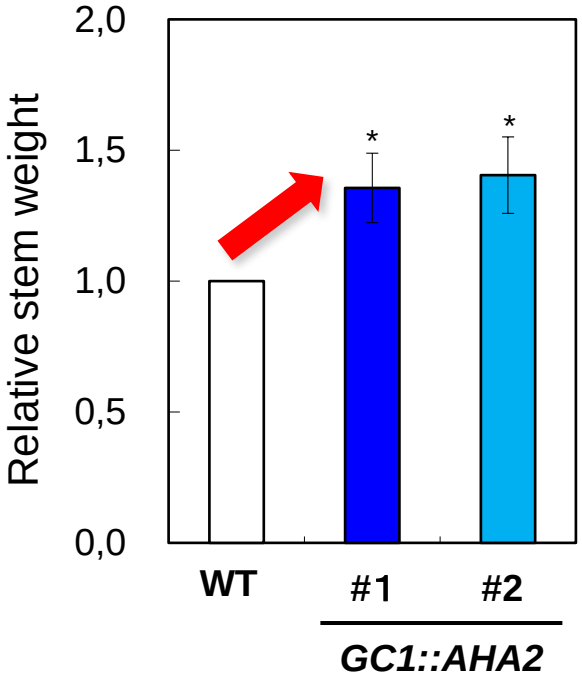
AHA2 : PM H+-ATPase isoform in Arabidopsis

WT ***GC1::AHA2 #1***



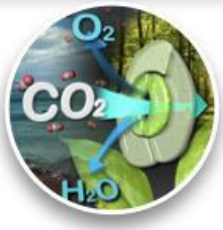
WT ***GC1::AHA2 #1***

45-day-old plants

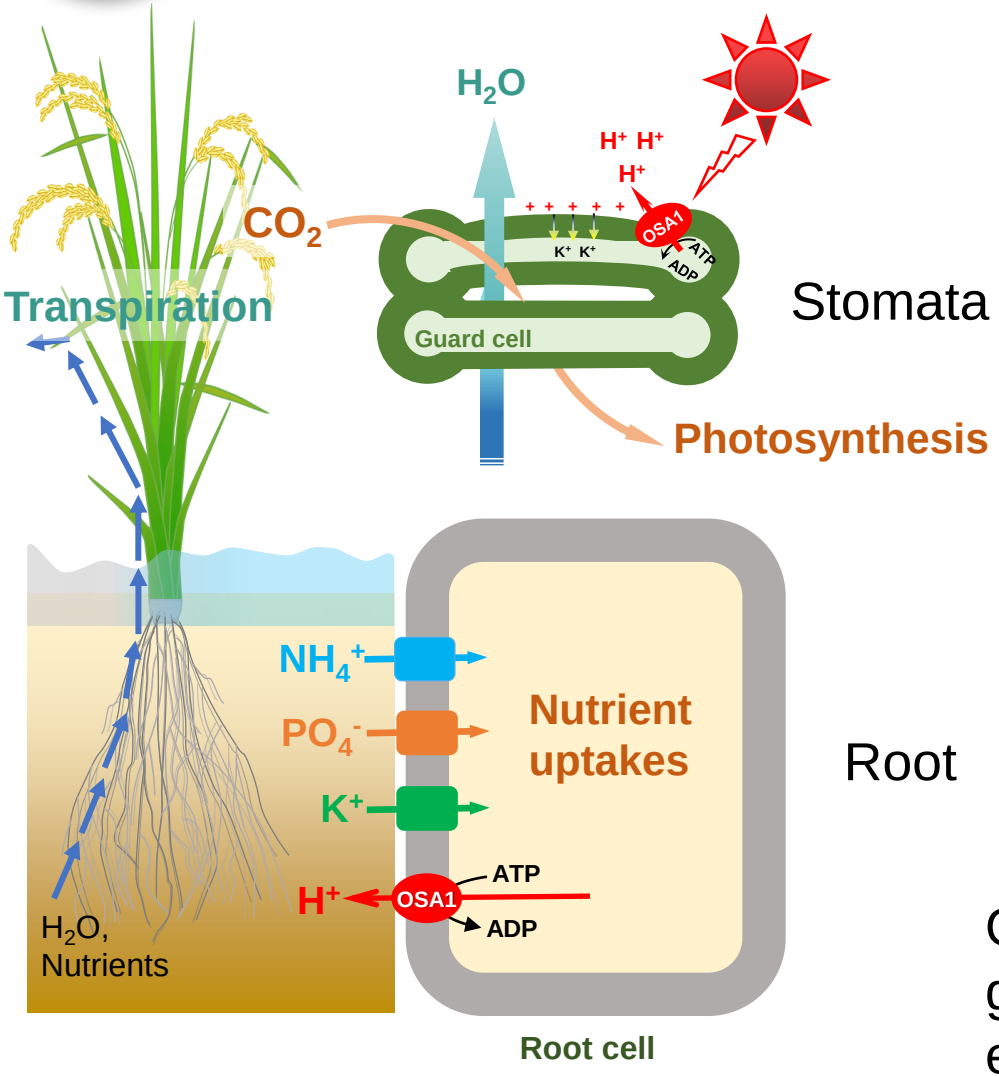


Wang et al. (2014) PNAS

Stomatal aperture is the limiting factor for photosynthesis and plant growth in Arabidopsis plants.



PM H+-ATPase mediates both stomatal opening and NH_4^+ uptake in rice roots



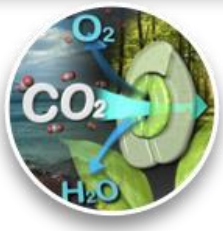
(OSA1: *Oryza sativa* plasma membrane H+-ATPase 1)

H+-ATPase has an important role for stomatal opening in rice

Toda et al. (2016) Plant Cell Physiol.

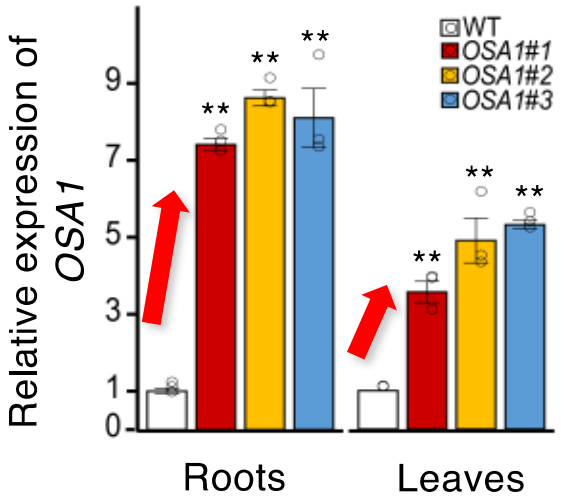
H+-ATPase mediates nutrient uptakes in roots coupled with the secondary transporters, including ammonium in rice roots

Overexpression of H+-ATPase in guard cells and roots would efficiently improve both CO_2 and NH_4^+ uptakes in rice

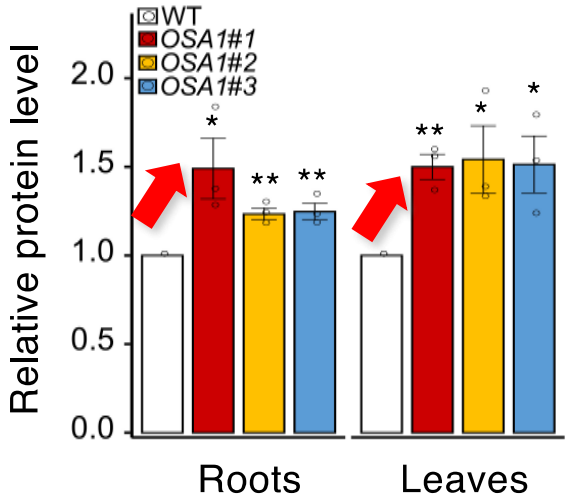


PM H+-ATPase-oxs showed higher expression, protein level, and activity

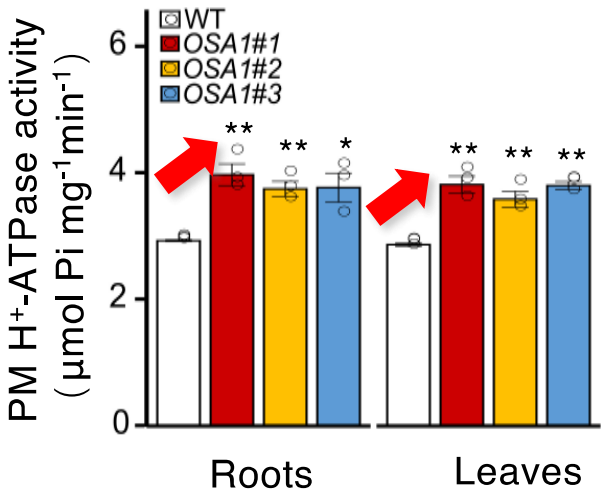
Expression level



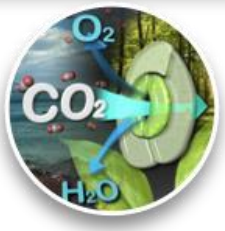
Protein level



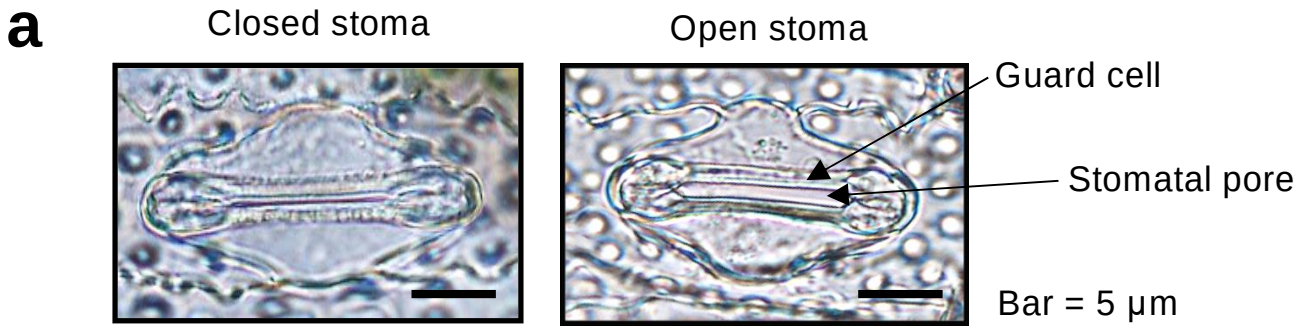
PM H+-ATPase activity



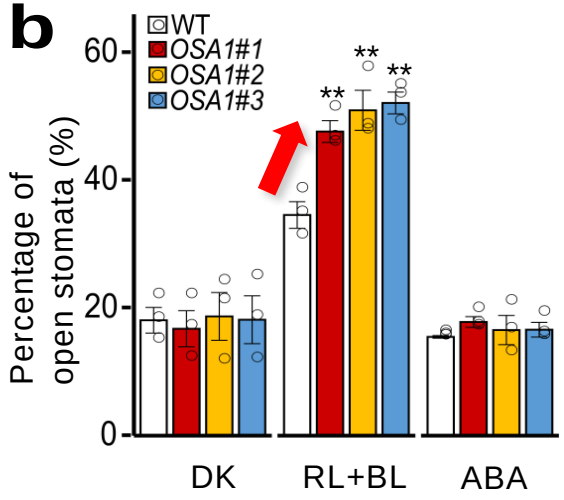
PM H+-ATPase overexpression by 35S-CaMV promoter
OSA1: a typical PM H+-ATPase isoform in rice



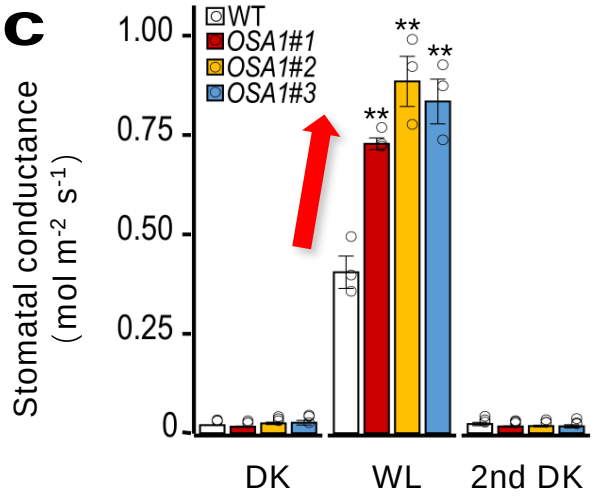
PM H+-ATPase-oxs showed higher stomatal opening, conductance, and photosynthesis



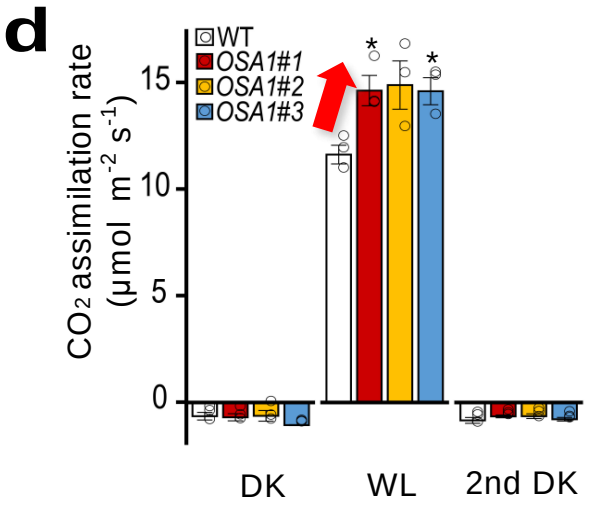
Stomatal aperture

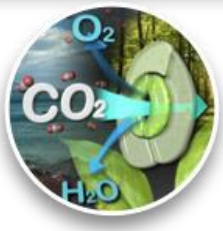


Conductance



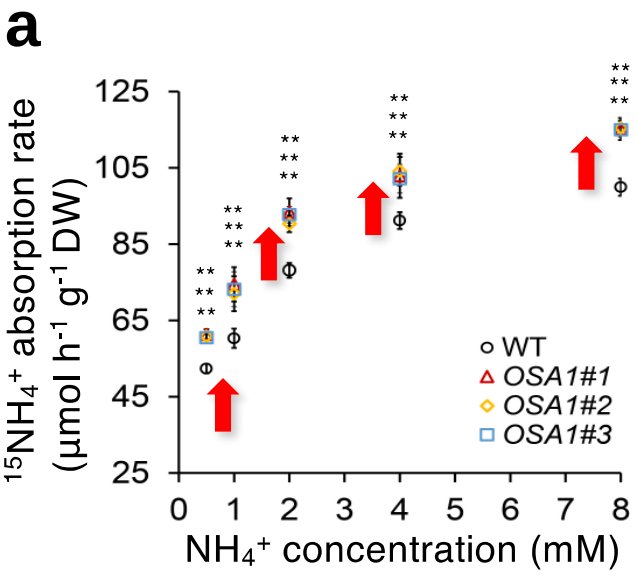
Photosynthesis



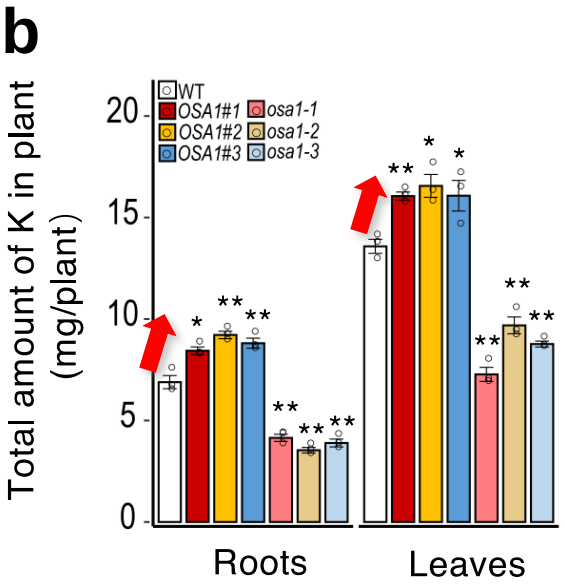


PM H+-ATPase-oxs showed higher NH_4^+ uptake rate, and K^+ and P amount

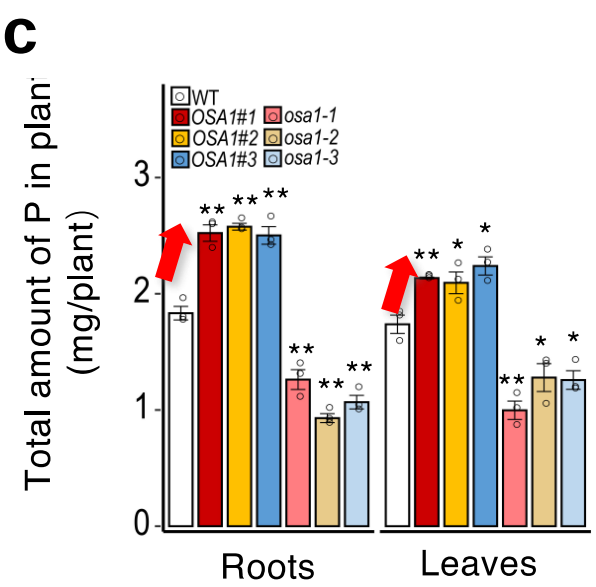
NH_4^+ uptake rate

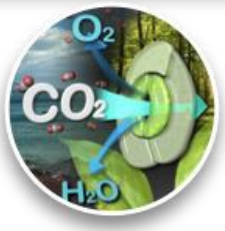


K^+ amount



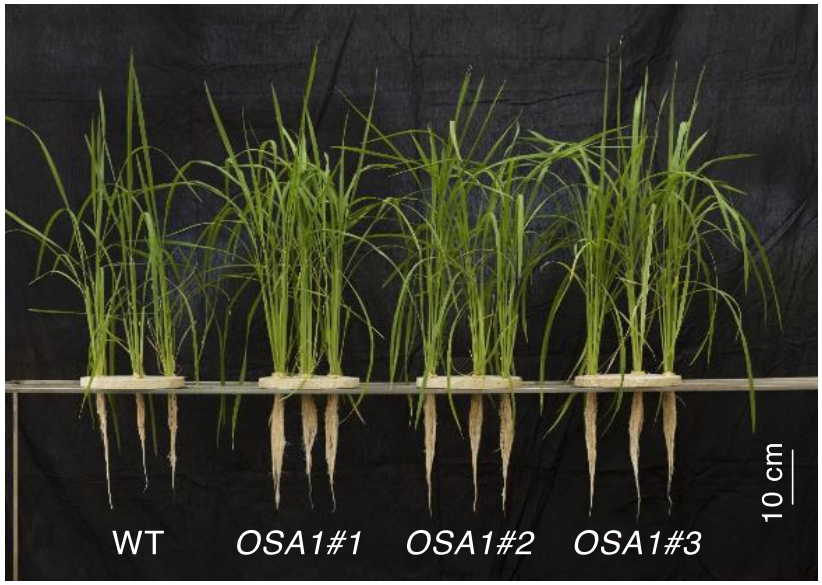
P amount





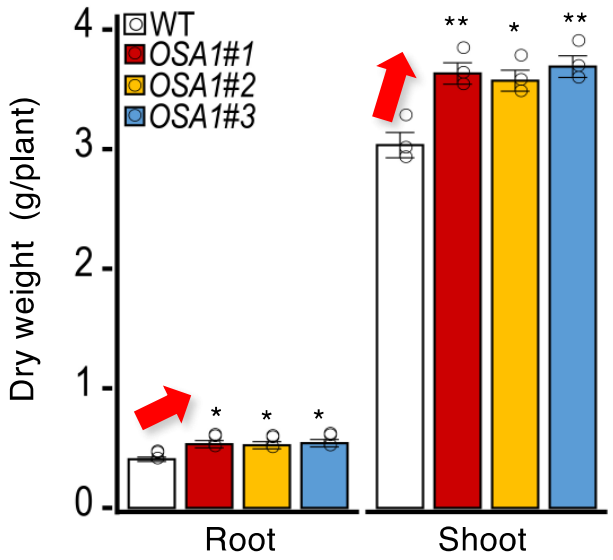
PM H+-ATPase-oxs showed over 20% grater dry weight compared to wild type

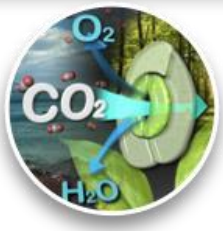
a



(Four-week-old plants)

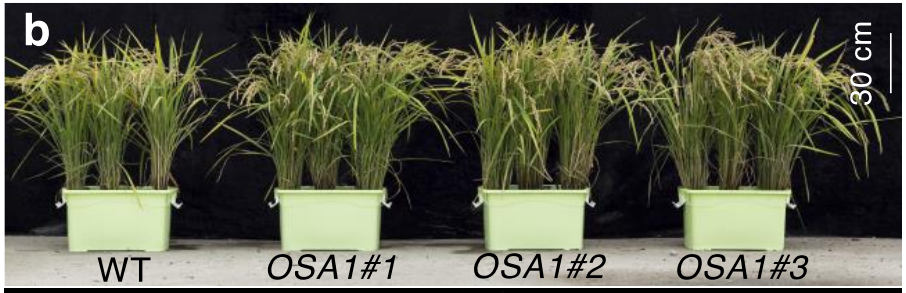
b

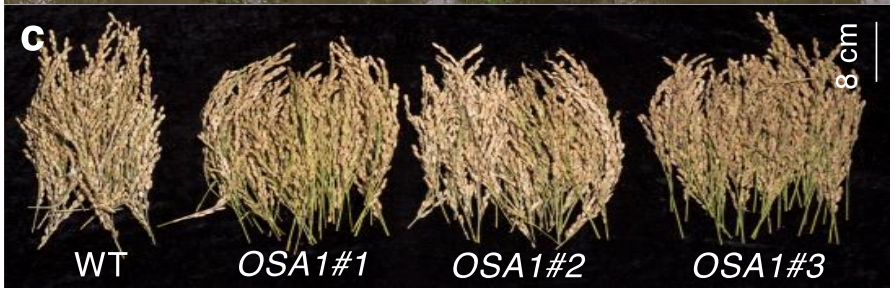




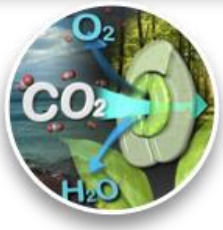
Field tests over two growing seasons at three different locations in the middle of China (Nanjing-S in 2016, and Nanjing-N and Fengyang in 2017).

Nanjing-N in 2017

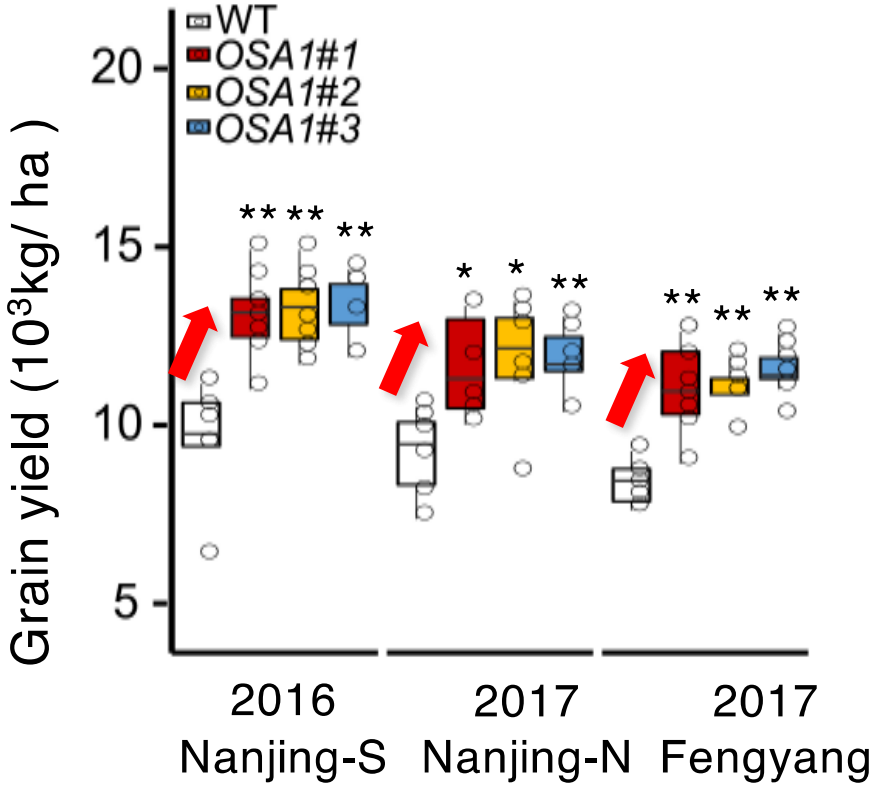


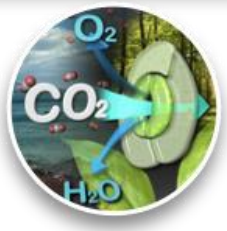


Collaboration with Professor Yiyong Zhu in Nanjing Agricultural University

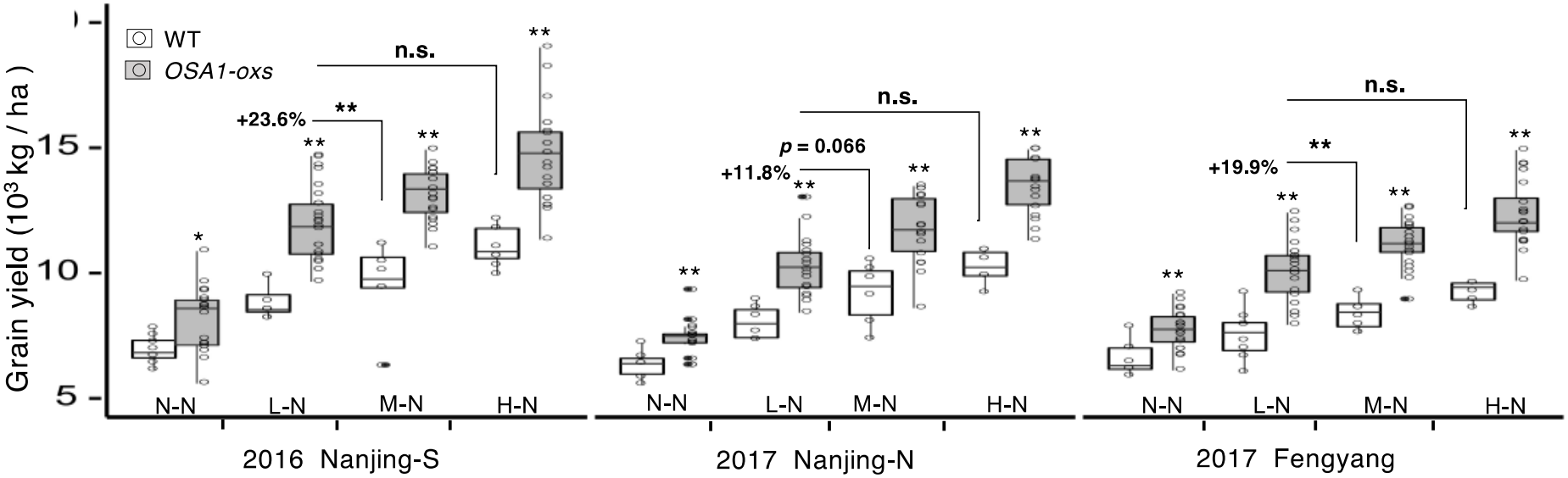


PM H+-ATPase-oxs showed over 30% higher grain yields in all locations



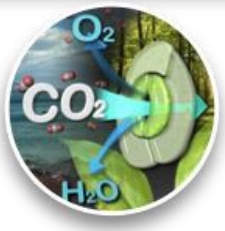


Grain yields were increased NH_4^+-dependent manner and PM H^+-ATPase-oxs showed higher grain yields in all NH_4^+ conditions

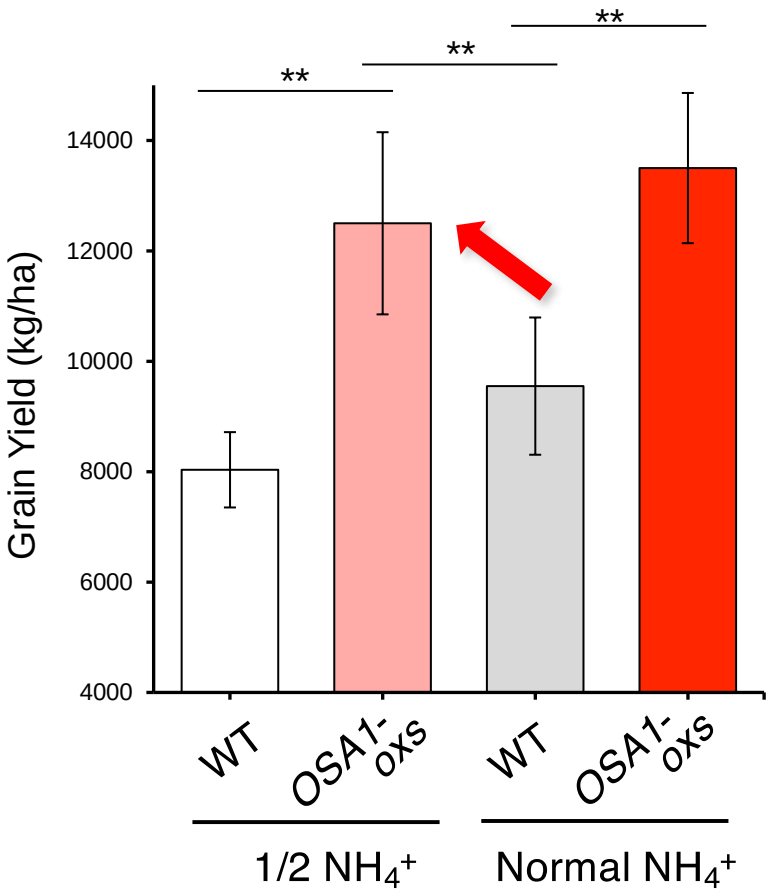


Effect of NH_4^+ concentration for grain yield.

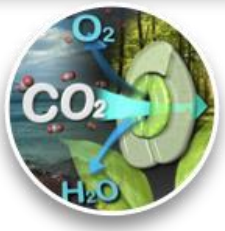
- N-N: 0 NH_4^+
- L-N: ½ NH_4^+
- M-N: normal NH_4^+
- H-N: 150% NH_4^+



PM H^+-ATPase-oxs under ½ NH_4^+ showed higher grain yield compared to WT under normal NH_4^+



➡ We can save the nitrogen fertilizer by using H^+-ATPase-oxs



Summary

PM H^+-ATPase-oxs showed higher nutrient uptakes, stomatal opening, and photosynthesis.

PM H^+-ATPase-oxs showed over 30% higher grain yields in the field conditions.

It is possible that we can reduce fertilizers by using PM H^+-ATPase-oxs.

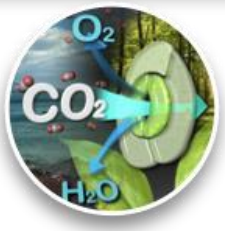
PM H^+-ATPase overexpression plants as **PUMP** plants from **P**romotion and **U**pregulation of plasma **M**embrane **P**roton-ATPase.

Dr. Zhang
(Nagoya Univ.)

Prof. Zhu
(Nanjing Agricultural Univ.)

For the future:
If H^+-ATPase-ox can be realized using non-transgenic methods such as genome editing and chemical biology, these crops could have great potential for practical use.

Zhang et al. (2021) Nature Commun.



Topics

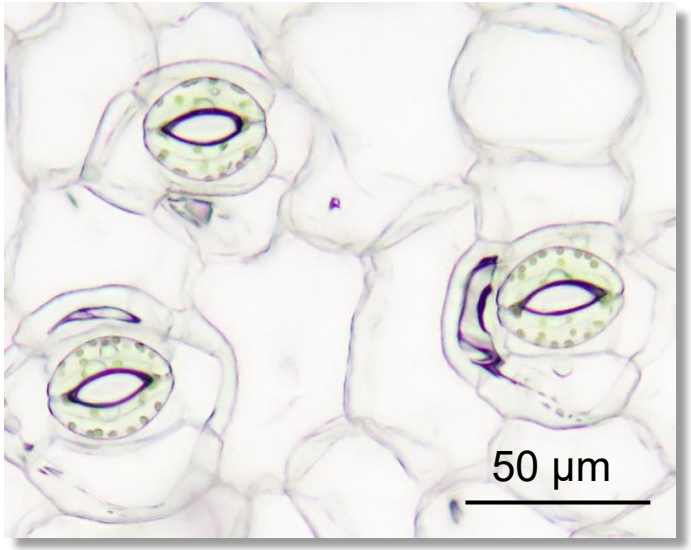
1. Molecular mechanism of light-induced stomatal opening

2. Importance of stomatal opening and PM H^+-ATPase in plant growth

3. **Chemicals confer drought tolerance to plants**

Screening of chemicals, which affect stomatal aperture

<Plant material>



Commelina benghalensis

<Chemical Library>

From ITbM

This library is an original library and the compounds are collected from low molecular weight various compounds.

Progress of 2nd screening using ITbM library

Opening activity

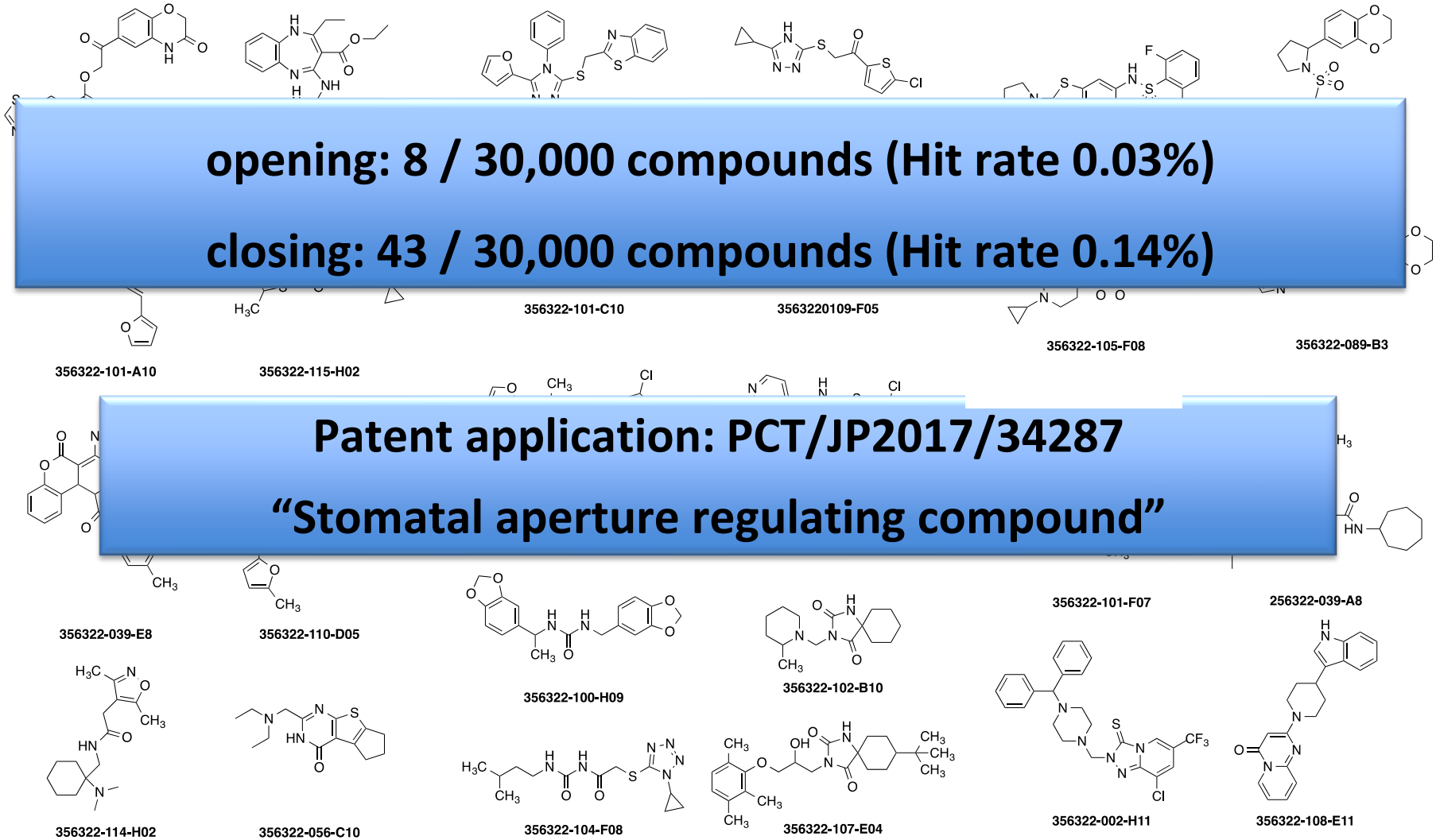
Closing activity

opening: 8 / 30,000 compounds (Hit rate 0.03%)

closing: 43 / 30,000 compounds (Hit rate 0.14%)

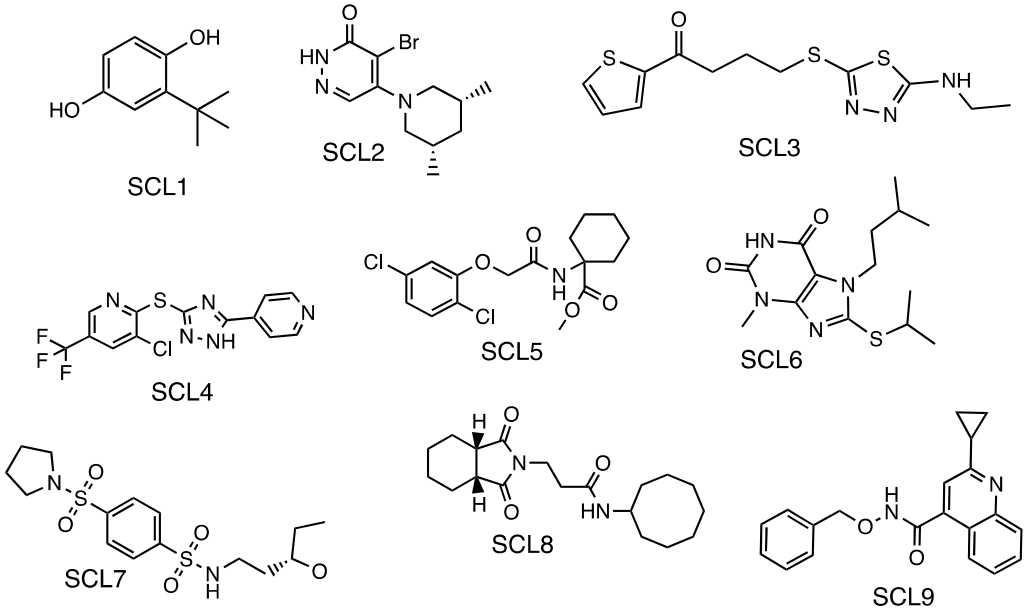
Patent application: PCT/JP2017/34287

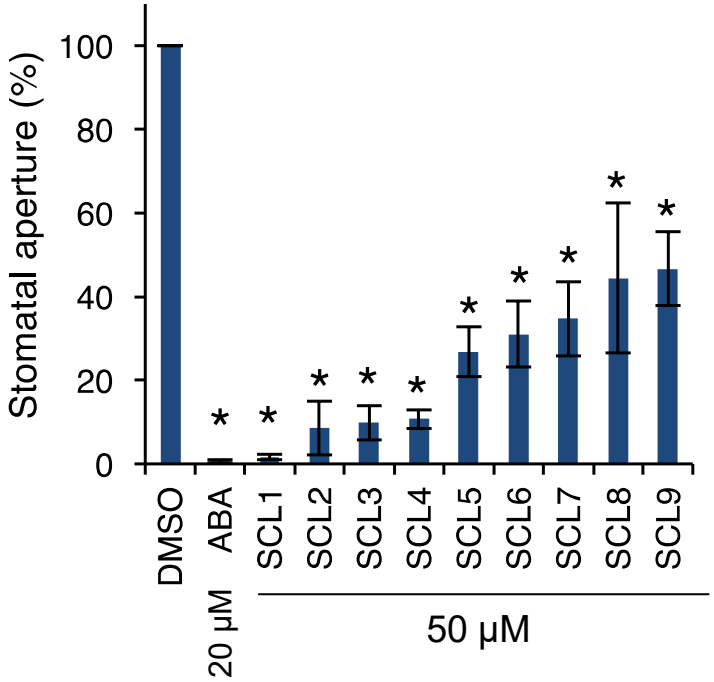
“Stomatal aperture regulating compound”



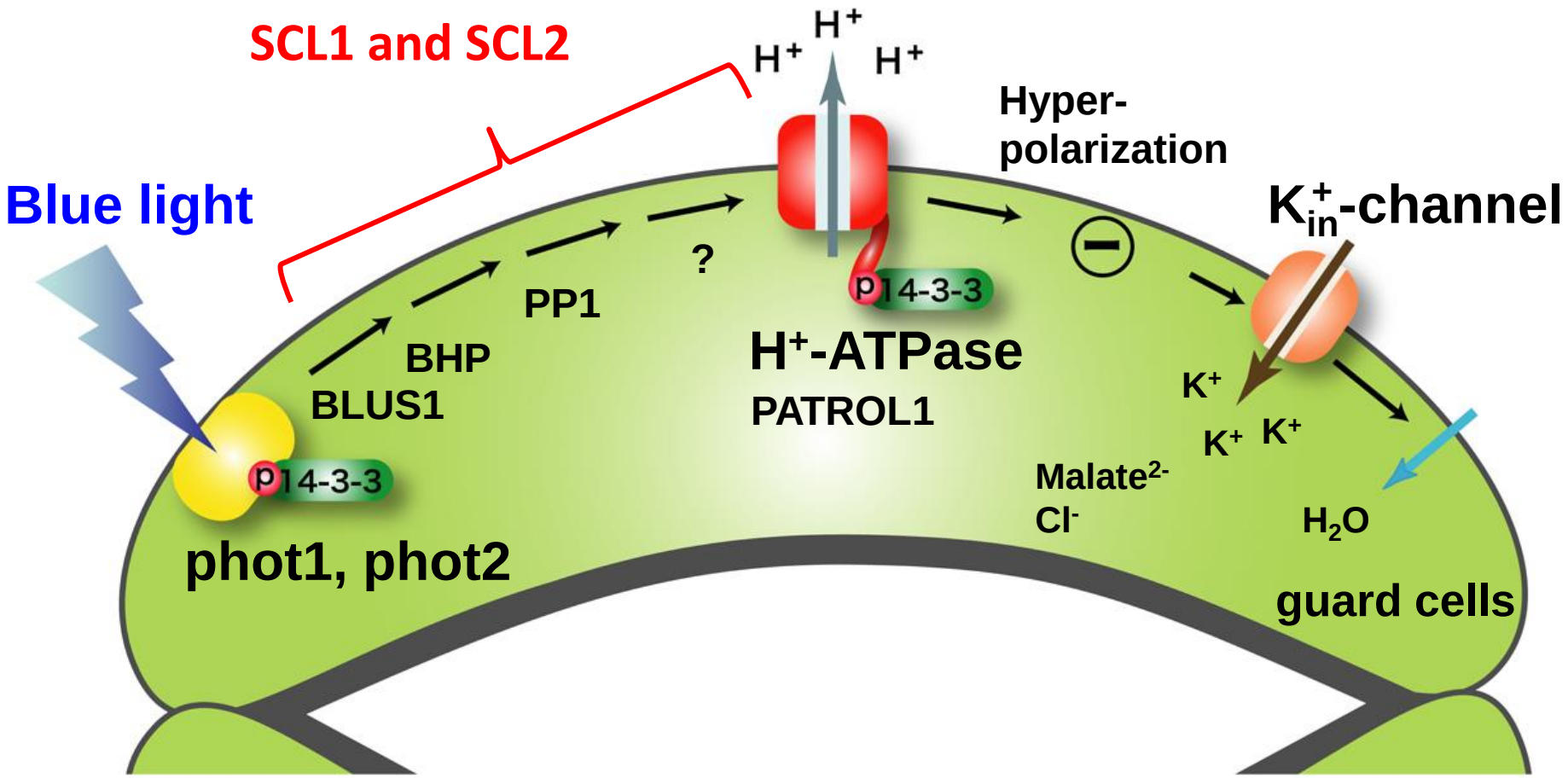
SCLs suppress light-induced stomatal opening

Stomatal Closing Compounds (SCLs)



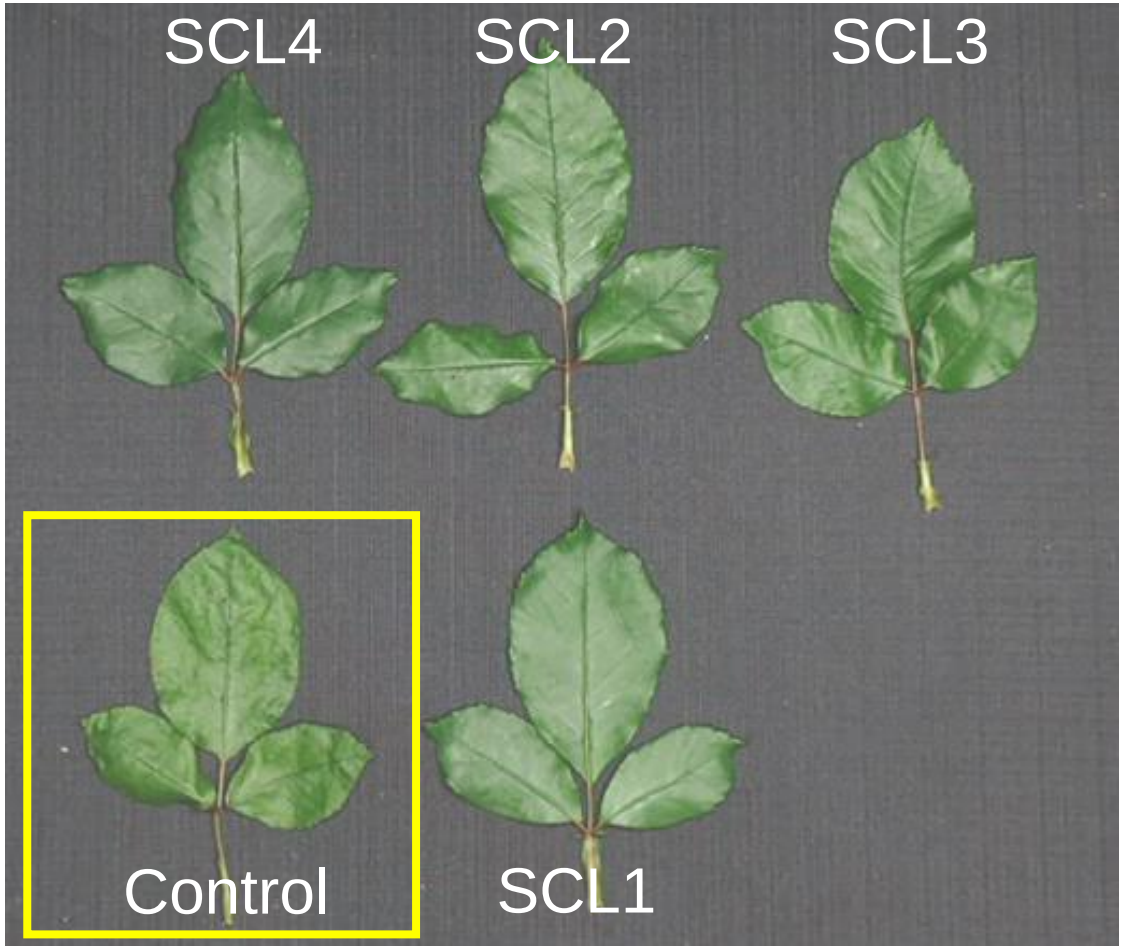


SCL1 and 2 likely affect the signaling components between downstream of phototropin and H+-ATPase activation



Toh and Inoue et al. (2018) Plant Cell Physiol. “Plant Chemical Biology”

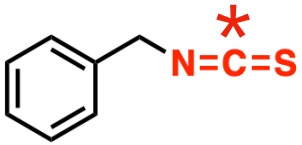
Spray of SCLs suppressed wilting of detached leaves



SCL1 strongly suppressed wilting of detached leaves 0～8 hr

Organic chemical BITC

Benzyl isothiocyanate (BITC)

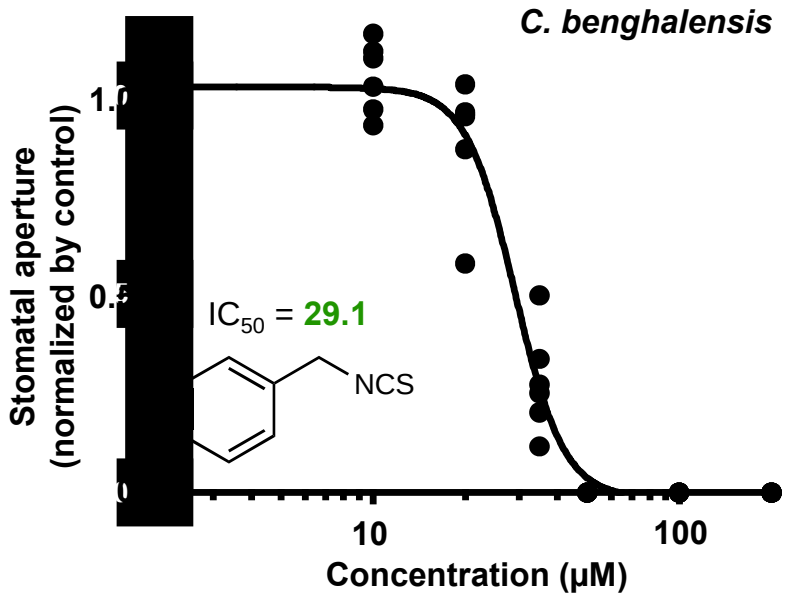


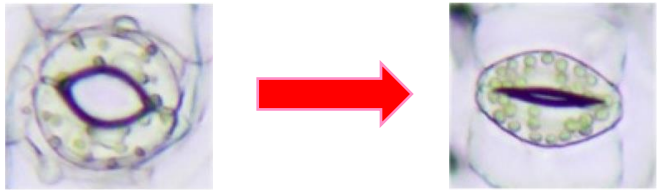
- **It is special metabolites in Bracicaceae family plants**
- **It is contained in mustard, so we eat BITC sometimes**

Target of BITC in plants is unknown

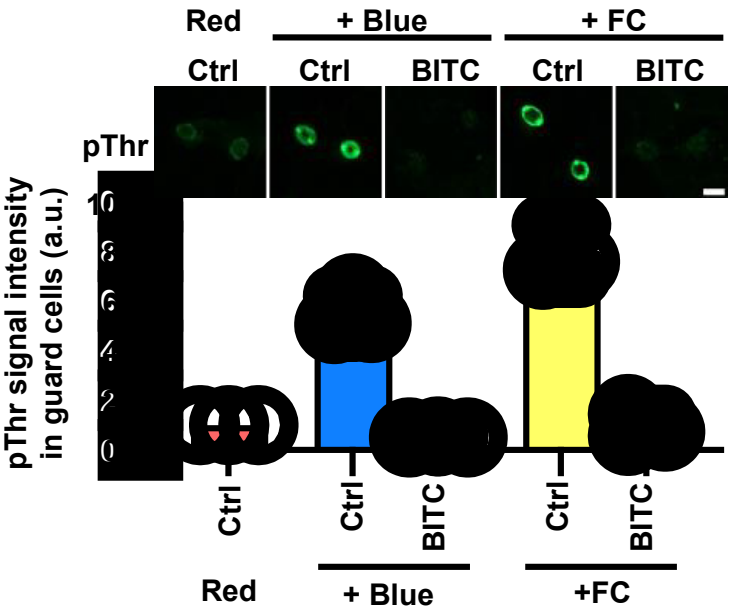
Yusuke Aihara

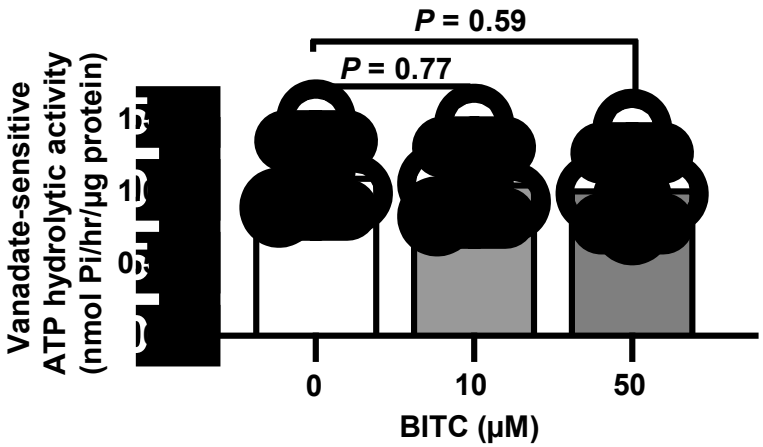
Effect of BITC on light-induced stomatal opening

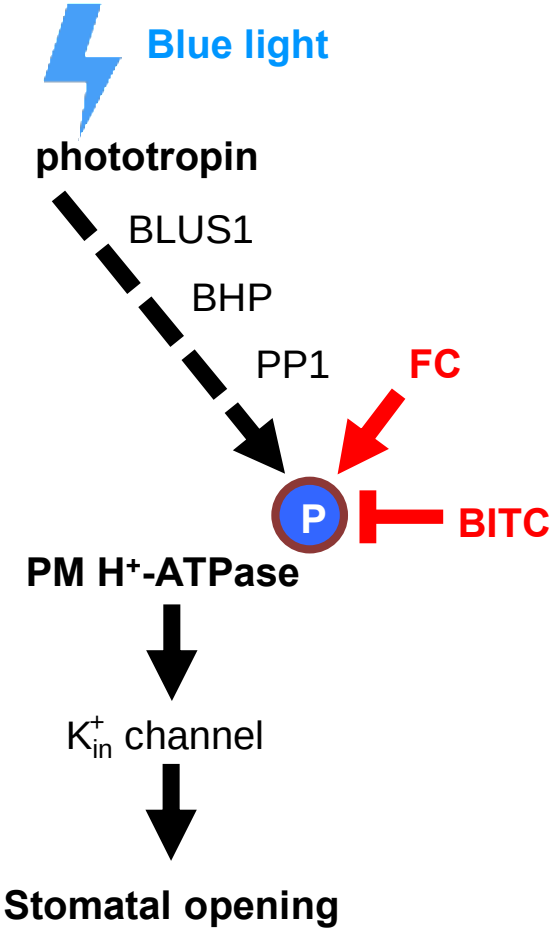




BITC suppresses PM H⁺-ATPase phosphorylation/activaton

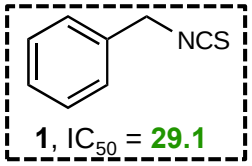




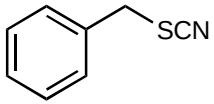


FC:カビ毒素フシコクシン
プロトンポンプ活性化剤
ポンプのリン酸化を誘導

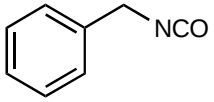
BITC derivatives



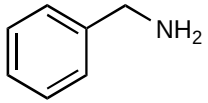
1, IC_{50} = **29.1** (μM)



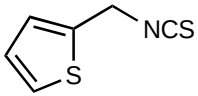
2, IC_{50} = **166.8**



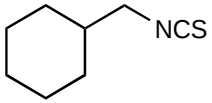
3, IC_{50} = **>200**



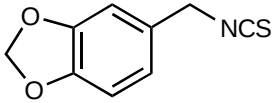
4, IC_{50} = **>200**



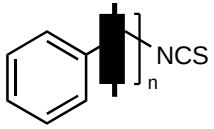
5, IC_{50} = **20.6**



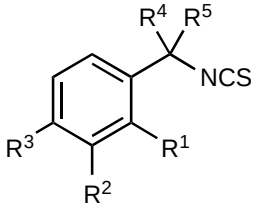
6, IC_{50} = **>200**



7, IC_{50} = **>200**



8, n=0 IC_{50} = **>200**
1, n=1 IC_{50} = **29.1**
9, n=2 IC_{50} = **26.4**
10, n=3 IC_{50} = **36.6**
11, n=4 IC_{50} = **37.1**
12, n=6 IC_{50} = **35.7**



13, R^1 = -Me IC_{50} = **25.8**
14, R^1 = -Cl IC_{50} = **27.5**
15, R^1 = -I IC_{50} = **23.5**
16, R^1 = -Ph IC_{50} = **7.0**

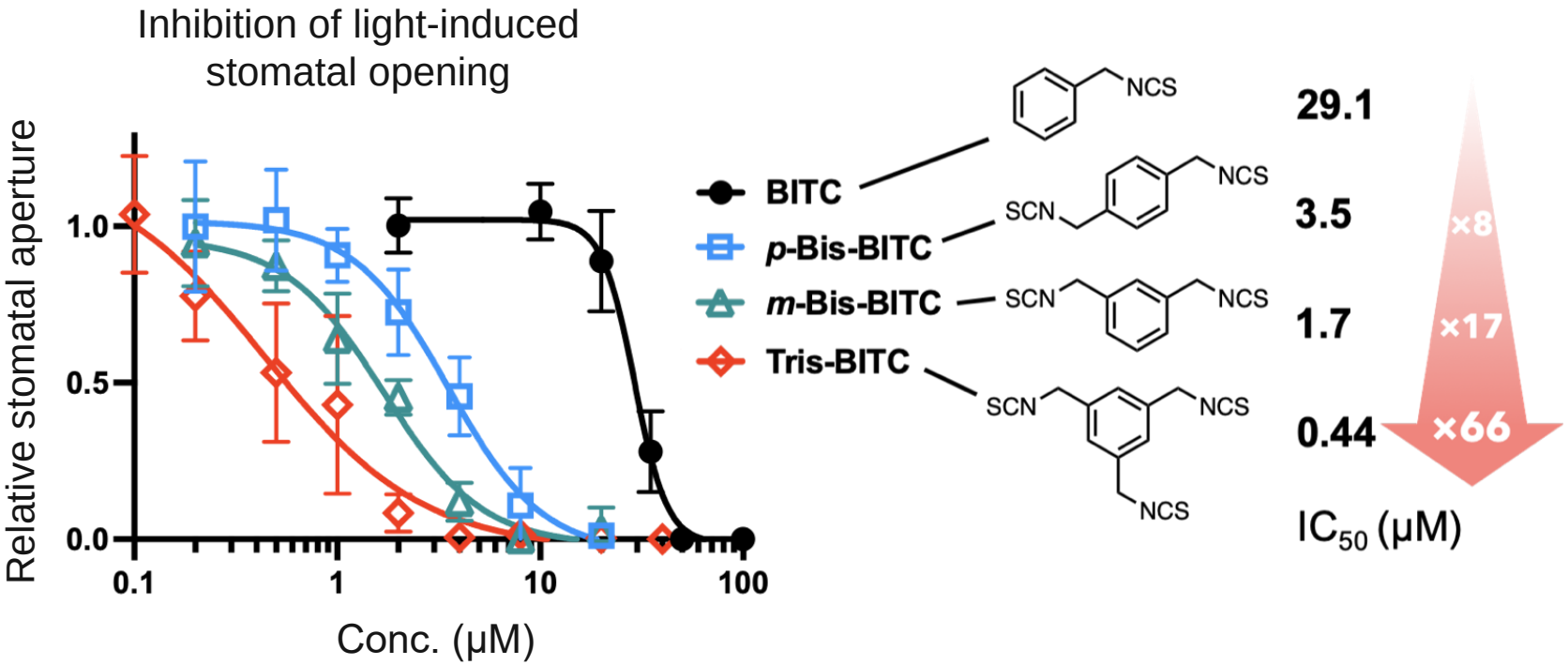
17, R^2 = -Me IC_{50} = **22.5**
18, R^2 = -Cl IC_{50} = **26.6**
19, R^2 = -I IC_{50} = **6.5**
20, R^2 = -Ph IC_{50} = **5.3**

21, R^3 = -Me IC_{50} = **51.9**
22, R^3 = -Cl IC_{50} = **23.7**
23, R^3 = -I IC_{50} = **10.7**
24, R^3 = -Ph IC_{50} = **6.2**
25, R^3 = -F IC_{50} = **30.2**
26, R^3 = $-OCF_3$ IC_{50} = **36.6**
27, R^3 = $-(CH_2)_3CH_3$ IC_{50} = **48.6**
28, R^3 = $-C(CH_3)_3$ IC_{50} = **35.3**
29, R^3 = -NHBoc IC_{50} = **>200**
30, R^3 = -CN IC_{50} = **10.5**
31, R^3 = $-COOCH_3$ IC_{50} = **13.2**

32, R^4 = -Me, R^5= -H IC_{50} = **14.2**
33, R^4 = -Ph, R^5= -H IC_{50} = **16.1**
34, R^4 = $-CH_2CH_3$ (*R*), R^5= -H IC_{50} = **79**
35, R^4 = $-CH_2CH_3$ (*S*), R^5= -H IC_{50} = **56.1**
36, R^4, R^5 = =O IC_{50} = **>200**

Collaboration with Professor Kei Murakami in Kwansei Gakuin University

Modification of BITC



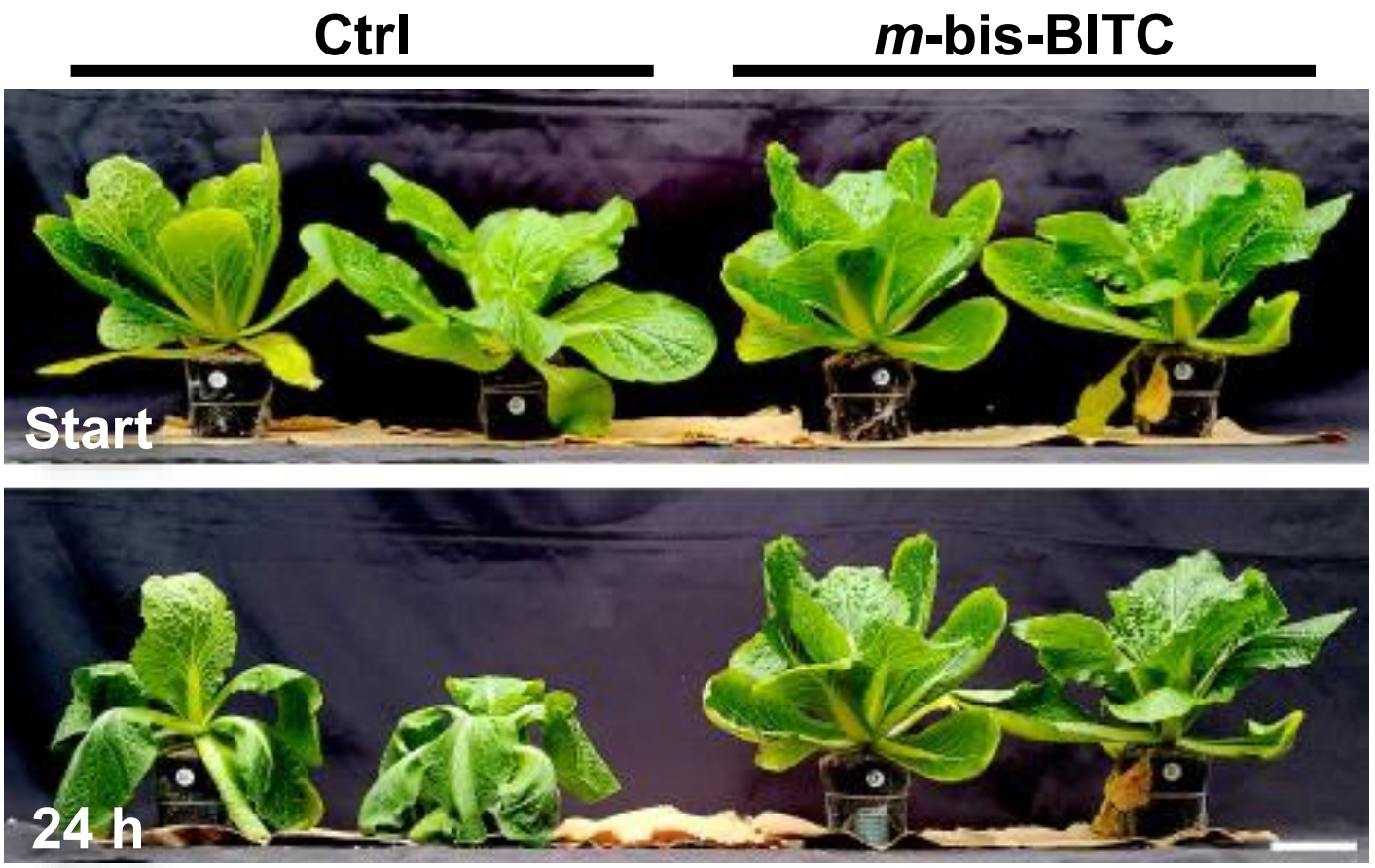
BITC derivatives have higher stomatal closing activity than plant hormone ABA (IC_{50} = 2.9 μM)

Aihara et al. (2023) Nature Commun

Spray of BITC suppresses witling of plants

We sprayed BITC to the cut flowers and checked leaf wilting without watering for 1.5 hr.

Spray of BITC suppresses witling of plants



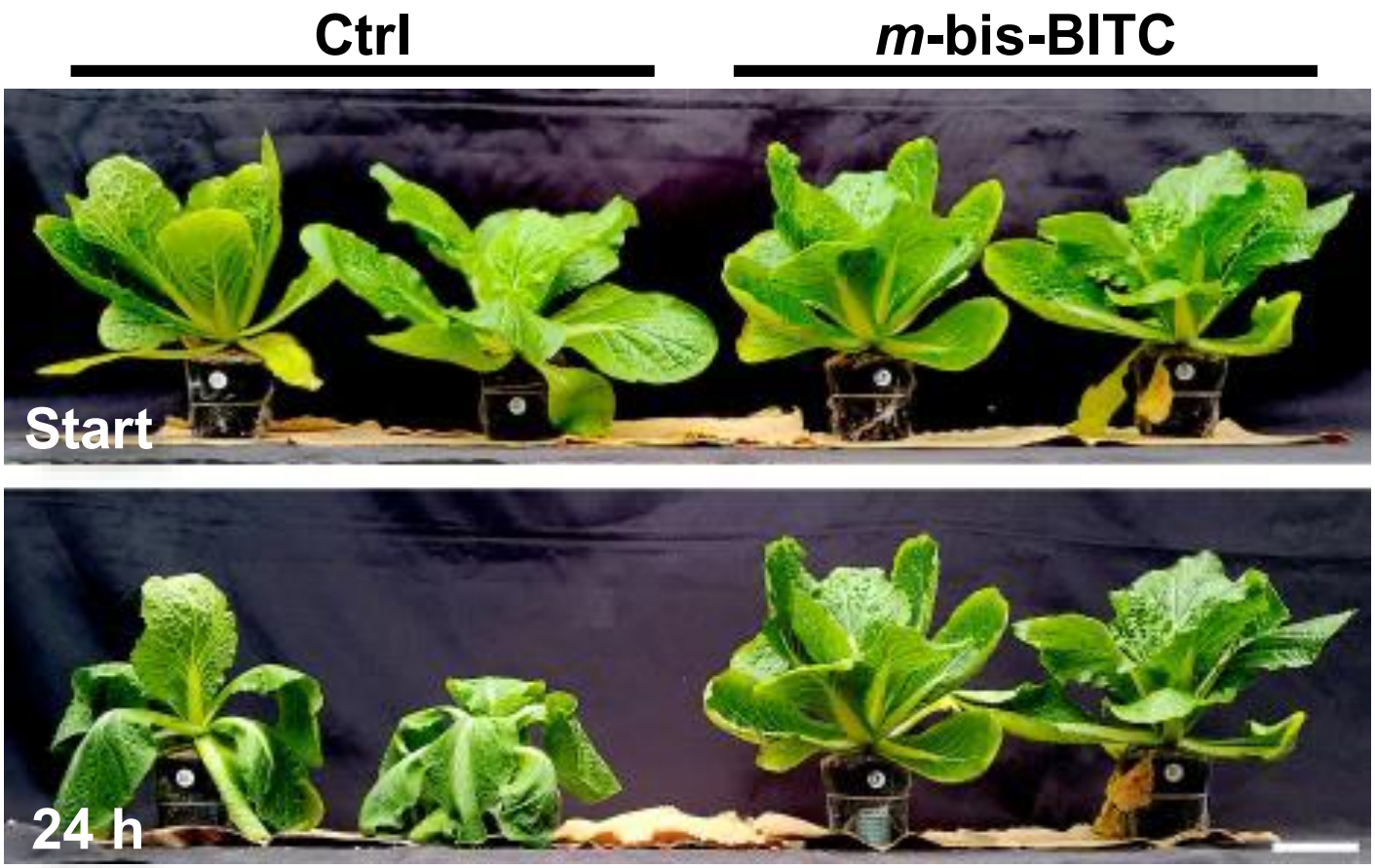
No water for 24 hr

(Soil growing Chinese cabbage)

If we stop watering, it will wilt in 24 hours.

Aihara et al. (2023) Nature Commun

BITC suppresses witling of plants



No water for 24 hr (Chinese cabbage)

BITC confers drought tolerance and could be useful as an agrochemical for enhancing drought resistance

Aihara et al. (2023) Nature Commun

Acknowledgements

Nagoya University

Yuki Hayashi
Maki Hayashi
Taku Sakakibara
Koji Takahashi

Kohei Fukatsu
Mitsumasa Akiyama
Hodaka Sugimoto
Yohei Takahashi

Zhang Maoxing

Yoshikatsu Matsubayashi

University of Minnesota
William M. Gray

Nanjing Agricultural University
Yiyong Zhu

Peking University
Yin Wang
Wenxiu Ye

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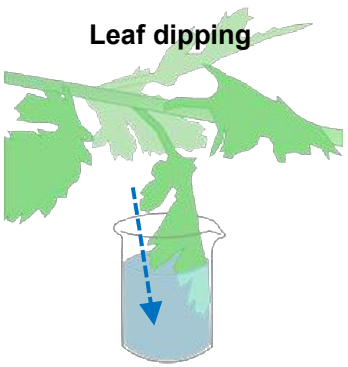
BITC suppresses witling of plants



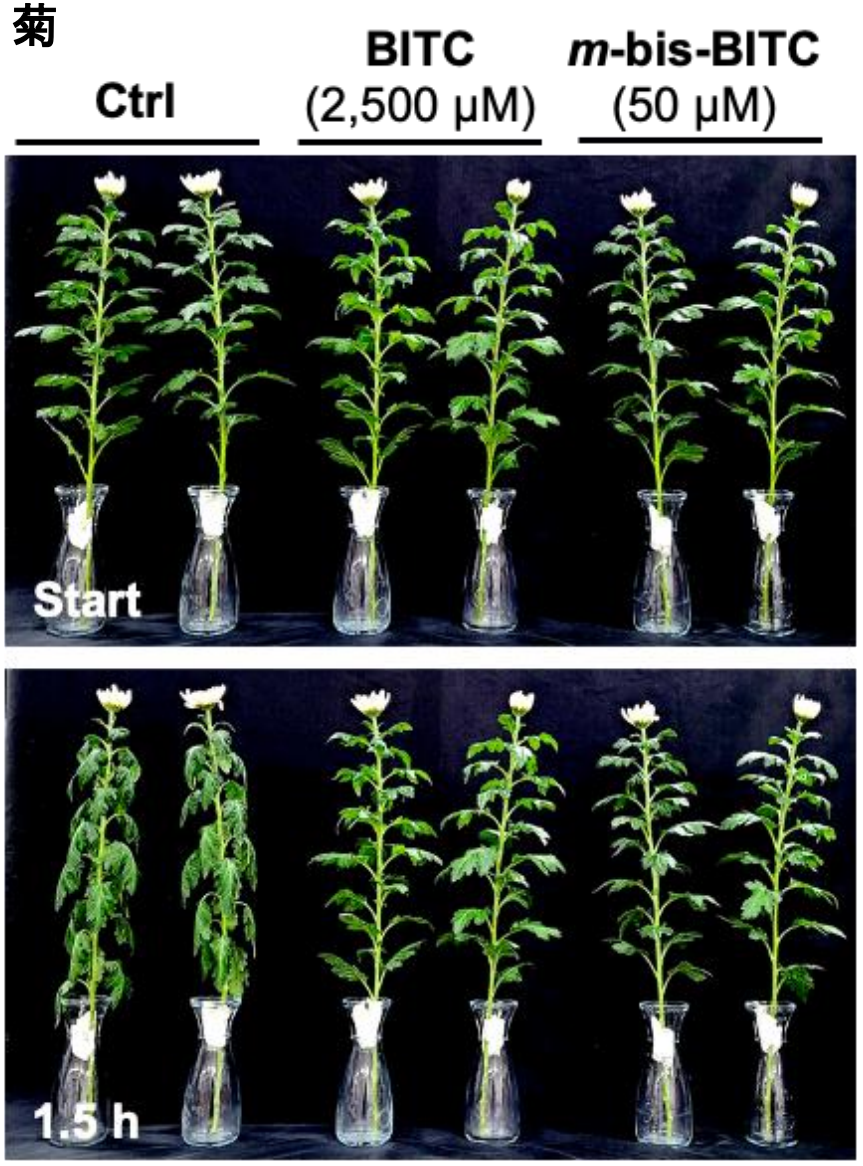
Light 3 h

Microscopic
observation

Dehydration

1.5時間水を
与えずに放置

菊



*スプレー処理では***bis***-の方が***tris***-より若干性能良い