- Arabidopsis -> quiescent strategy

WHAT CAN WE DO WITH NATURAL VARIATION?

HOW CAN WE FIND THE TRAITS AT THE BASE OF TOLERANCE?

Plant under flooding

Intolerant to flooding and therefore are excluded from flood-prone habitats

toleran t

escape strategy based on a suite of (inducible) morphological and anatomical traits allowing re-aeration of flooded tissues.

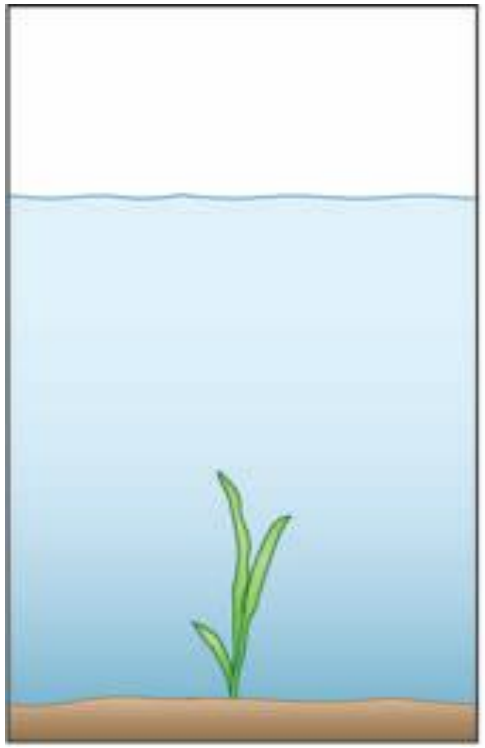
quiescence strategy composed of traits that conserve the use of energy and carbohydrates to prolong underwater survival.

(Bailey-Serres & Voesenek, 2008; Colmer & Voesenek, 2009)

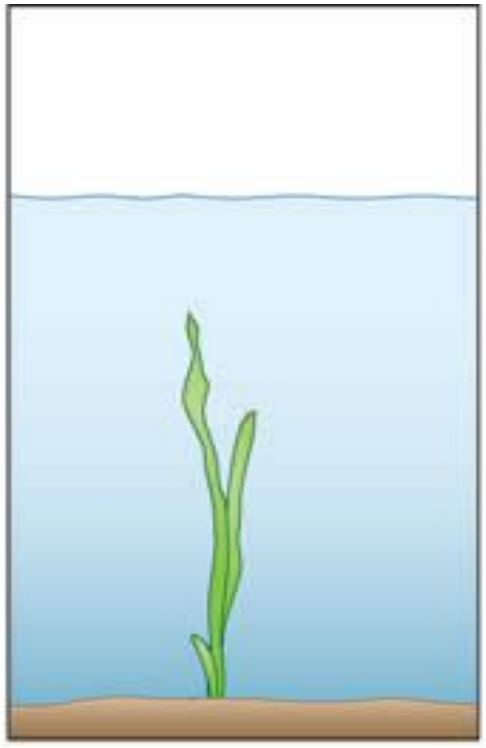
Rice responses to low O_2

Rice is remarkably well adapted to submergence and can even germinate in the complete absence of oxygen

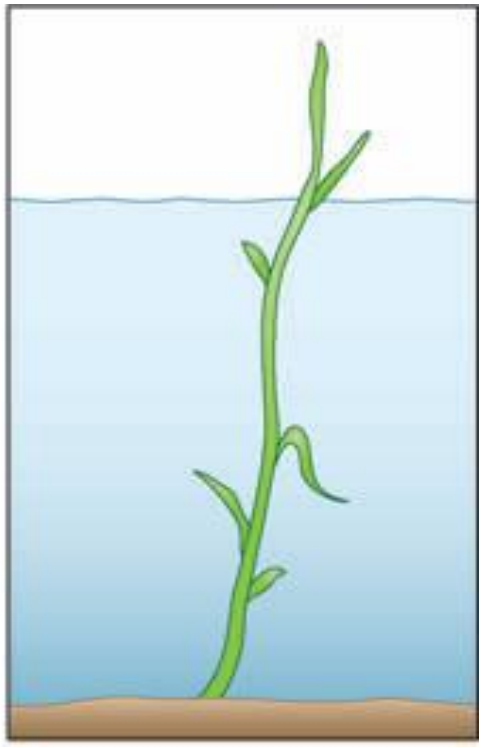
Quiescence (lowland)



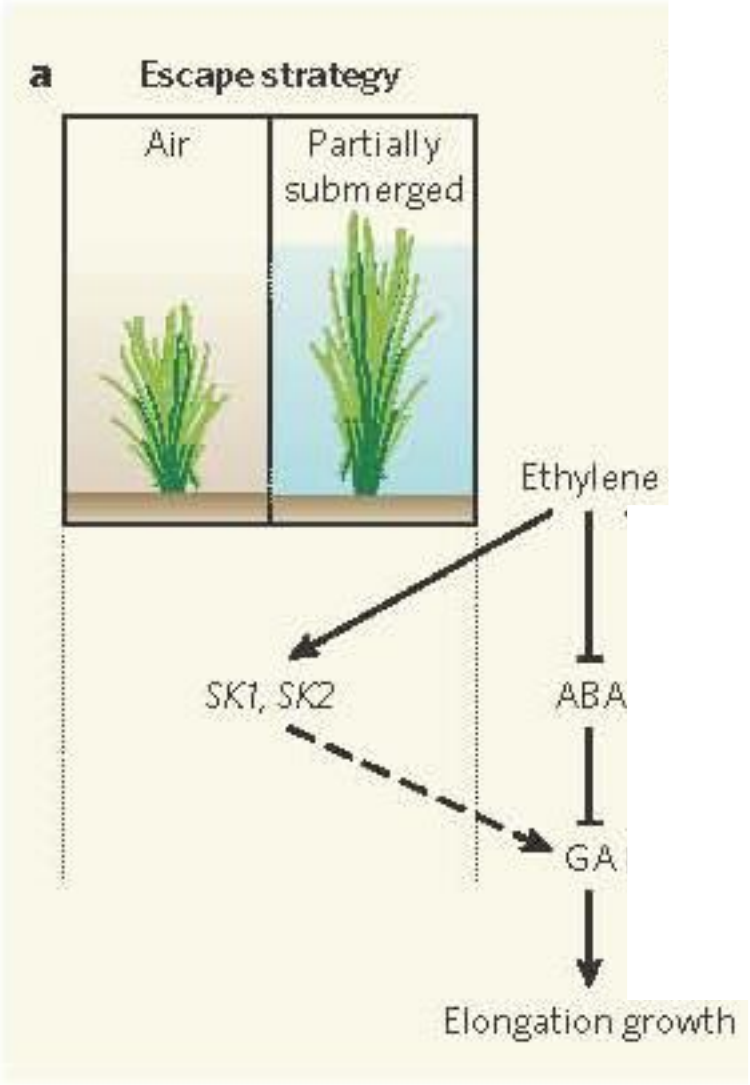
Intolerant



Escape (deep water)



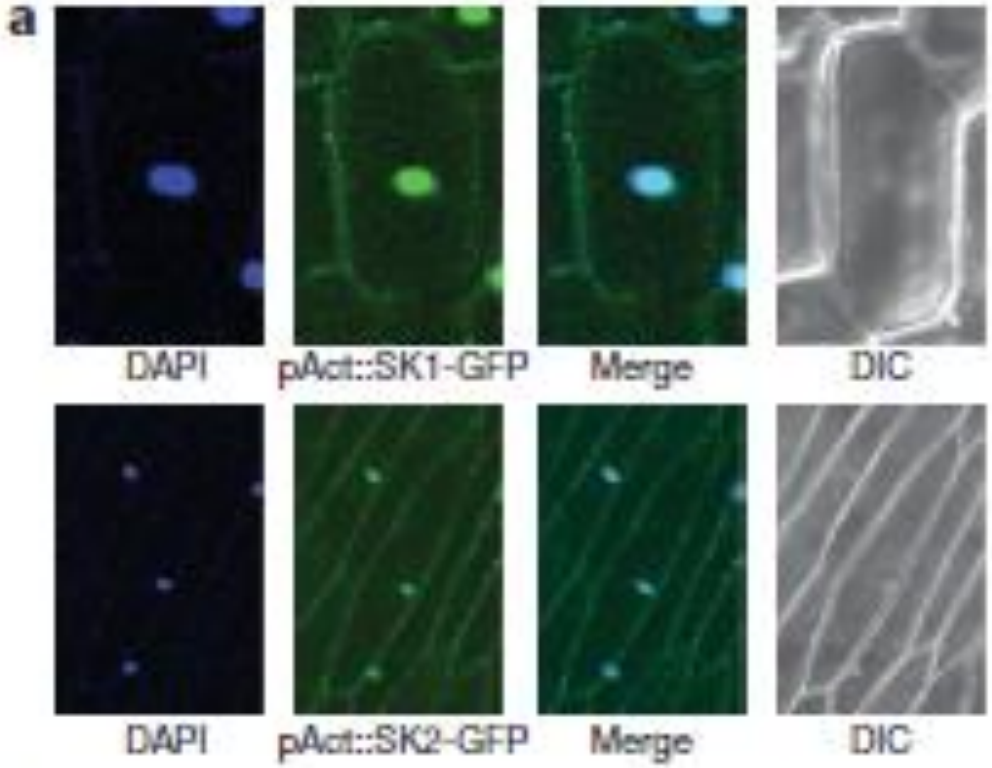
Escape and quiescence strategies for flooding tolerance



In some areas of Asia submergence occurs very rapidly and lasts for months, here rice varieties named ‘deepwater rice’ are grown. The adult plant continues to snorkel for air and keeps up with the increasing water level. This trait relies on two group VII ERF genes: SNORKEL1 and SNORKEL2 (SK1, SK2). Only present in deep water rice varieties, they activate a **gibberellin-dependent internode elongation, up to 25 cm per day**, sufficient to maintain an aerial contact with some of the leaves which allow air transfer to the submerged parts of the plant via aerenchyma

Voesenek and Bailey-Serres (2009) Nature 460:959-960

SNORKEL1 AND SNORKEL2

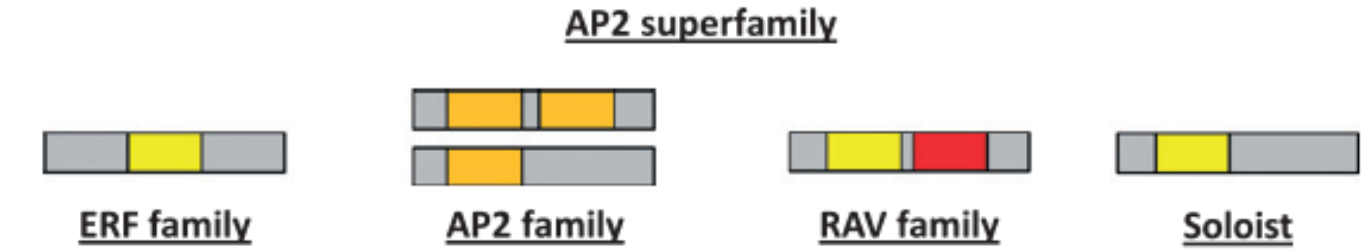


SNORKEL1 AND SNORKEL2 are transcription factors as testified by their nuclear localization.

They are also regulated by GA (Gibberellins) and CK (cytokinines)

APETALA2/Ethylene Responsive Factor (AP2/ERF) superfamily

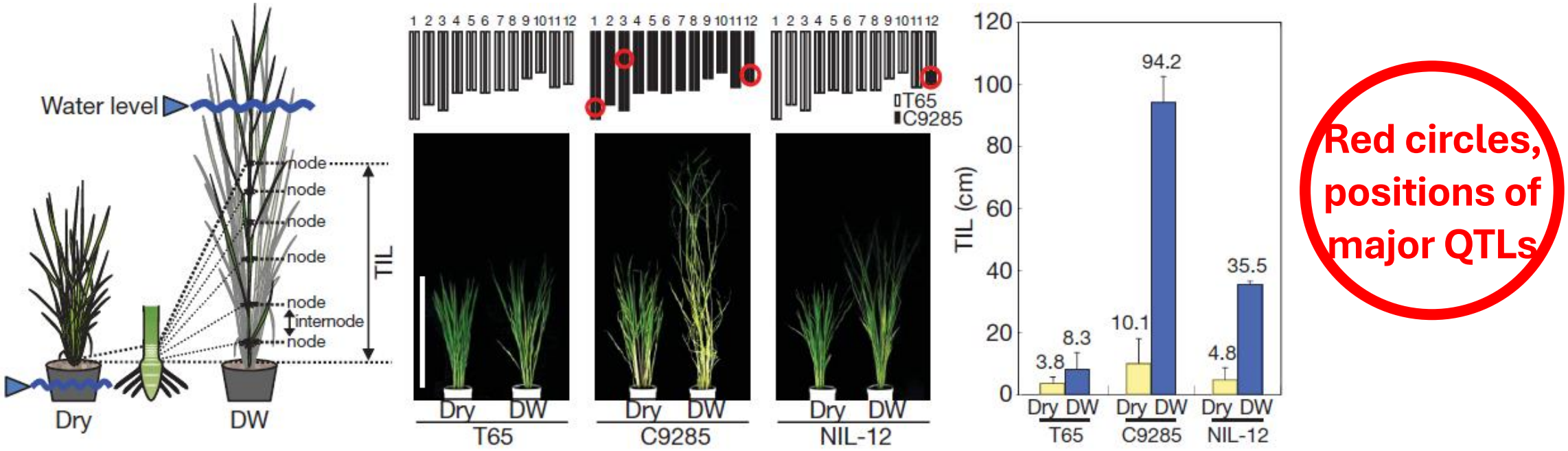
AP2/ERF proteins containing at least one DNA binding domain – named the AP2 domain – have been divided into three separate families, namely the ERF, AP2 and RAV families



Group	Subfamily	Group
I	A6	I. Double AP2 domain
II	A5	AP2
III	A1, A4, A5	ANT
IV	A2, A3	II. Single AP2 domain
V	B6	
VI	B5	
VII	B2	
VIII	B1	
IX	B3	
X	B3, B4	
VI-L	B6	
Xb-L	B6	

Fig. 1 Structure of the APETALA2/Ethylene Responsive Factor (AP2/ERF) superfamily. The AP2 superfamily is composed of single-AP2 domain proteins (ERF family), single or double ERF domain proteins (AP2 family), proteins containing one AP2 domain plus a B3 DNA binding domain (RAV family). Soloist, an ERF-related protein that appears in single copy in most of the plant genomes studied so far. ERF members have been subdivided into groups (Nakano *et al.*, 2006) or subfamilies (Sakuma *et al.*, 2002).

Submergence escape – *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*)



Red circles, positions of major QTLs

DW: Deep Water

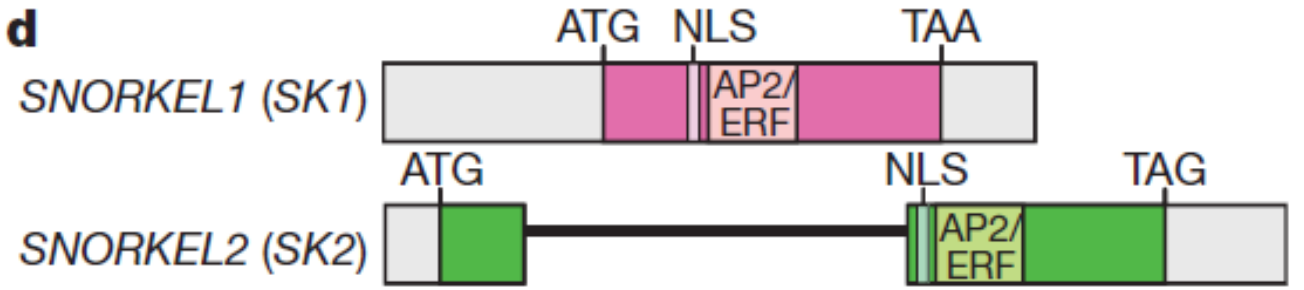
Lines

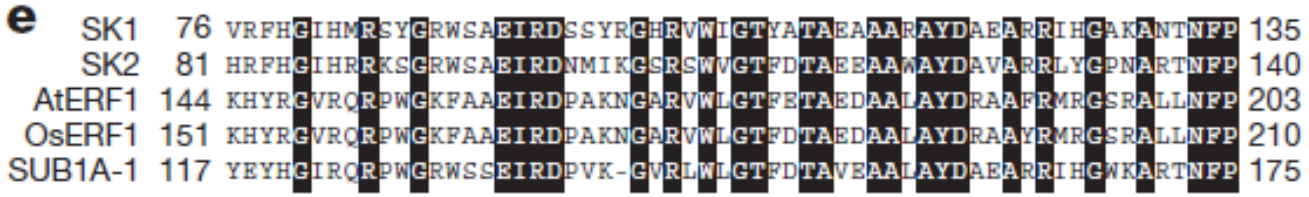
T65: non-deepwater rice cultivar
C9285: deepwater rice cultivar
NIL-12: Near Isogenic Line 12

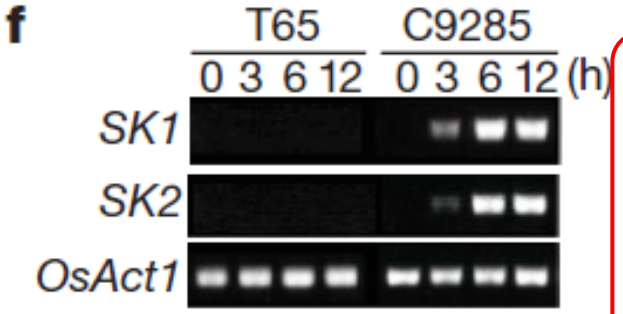
Parameters

TIL: Total Internode Elongation Length
LEI: Lowest Elongated Internode
NEI: Number Elongated Internodes

Hattori et al (2009) Nature 460:1026-1031

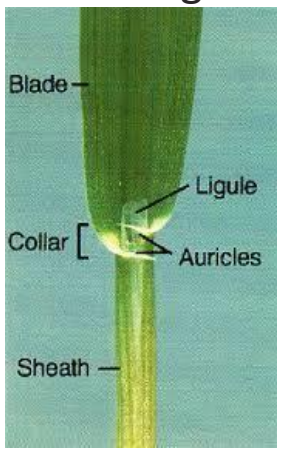




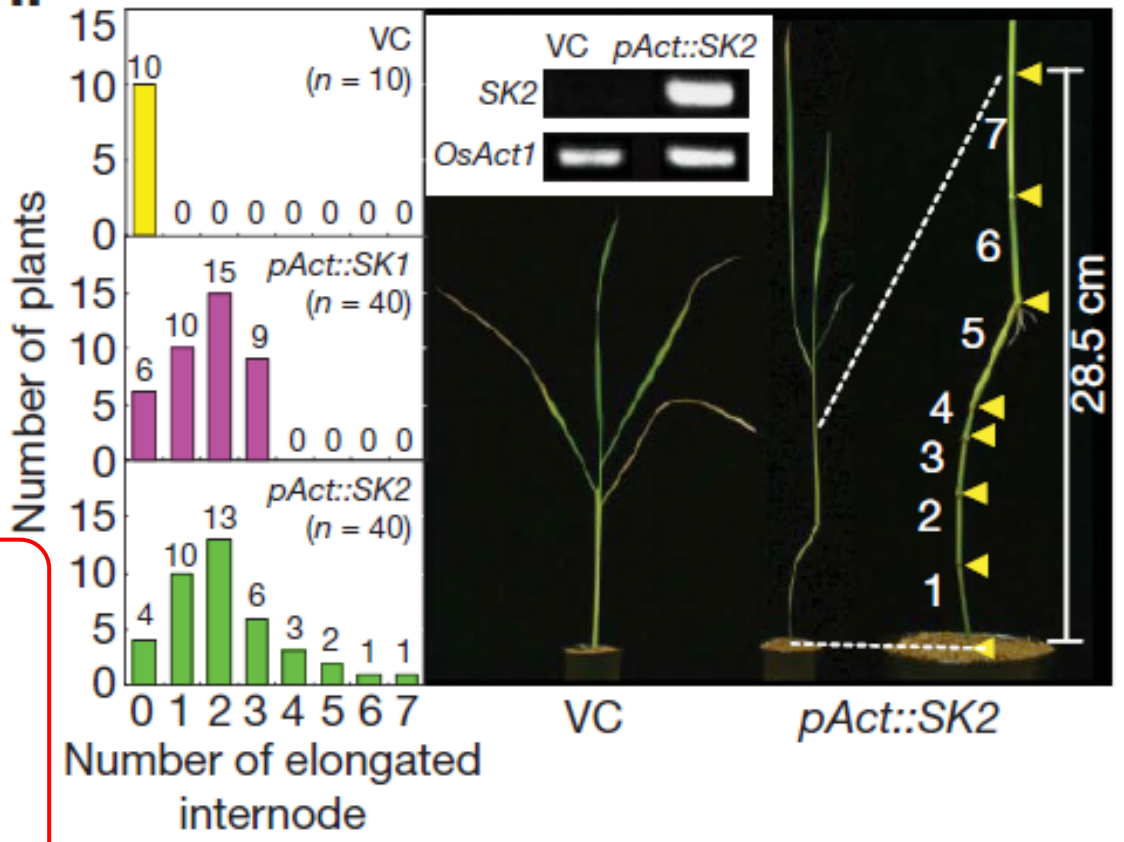


Organ-specific expression of SK genes

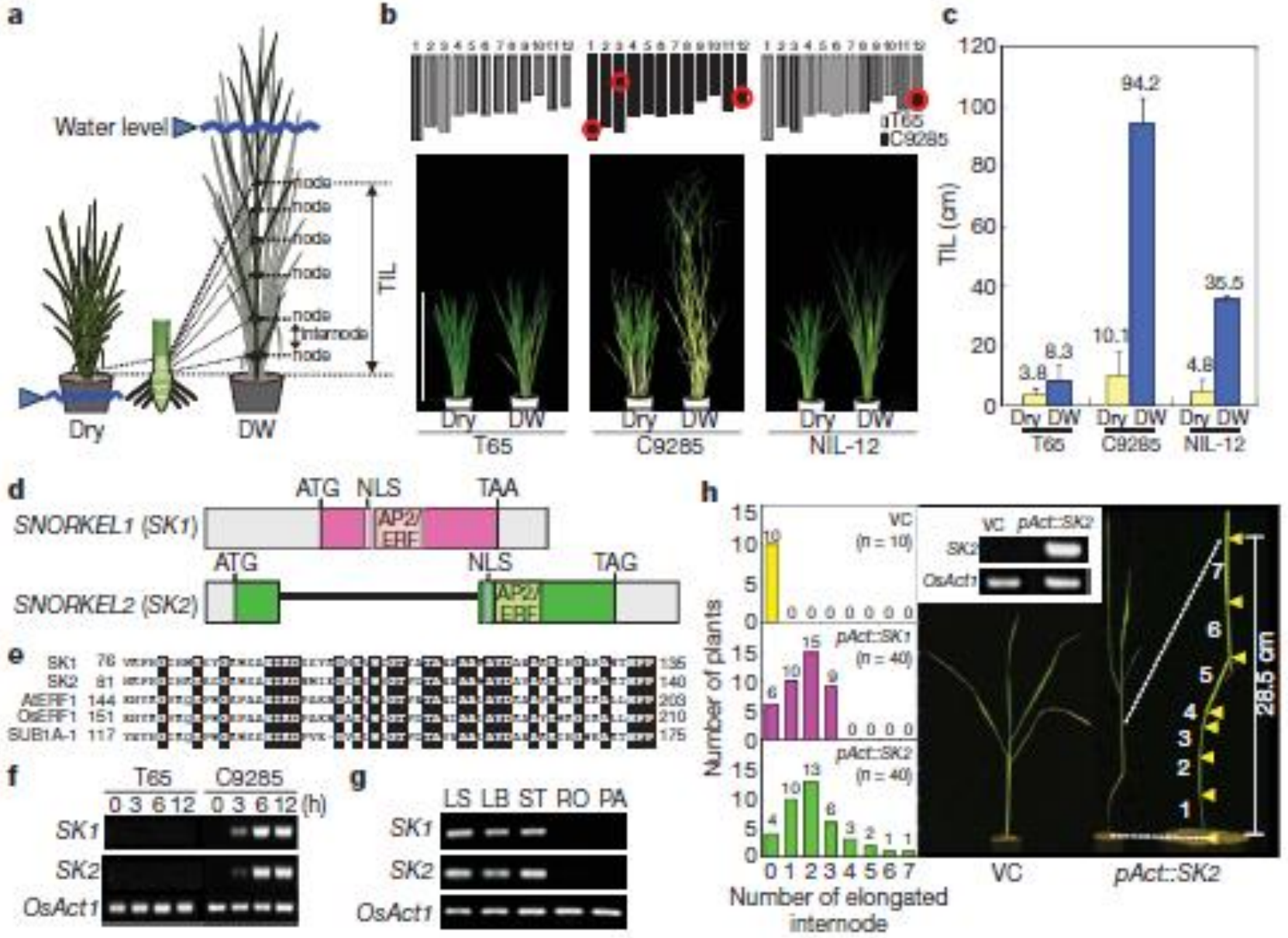
LS, leaf sheath;
LB, leaf blade;
ST, basal part of stem;
RO, root;
PA, panicle.



VC = empty vector control

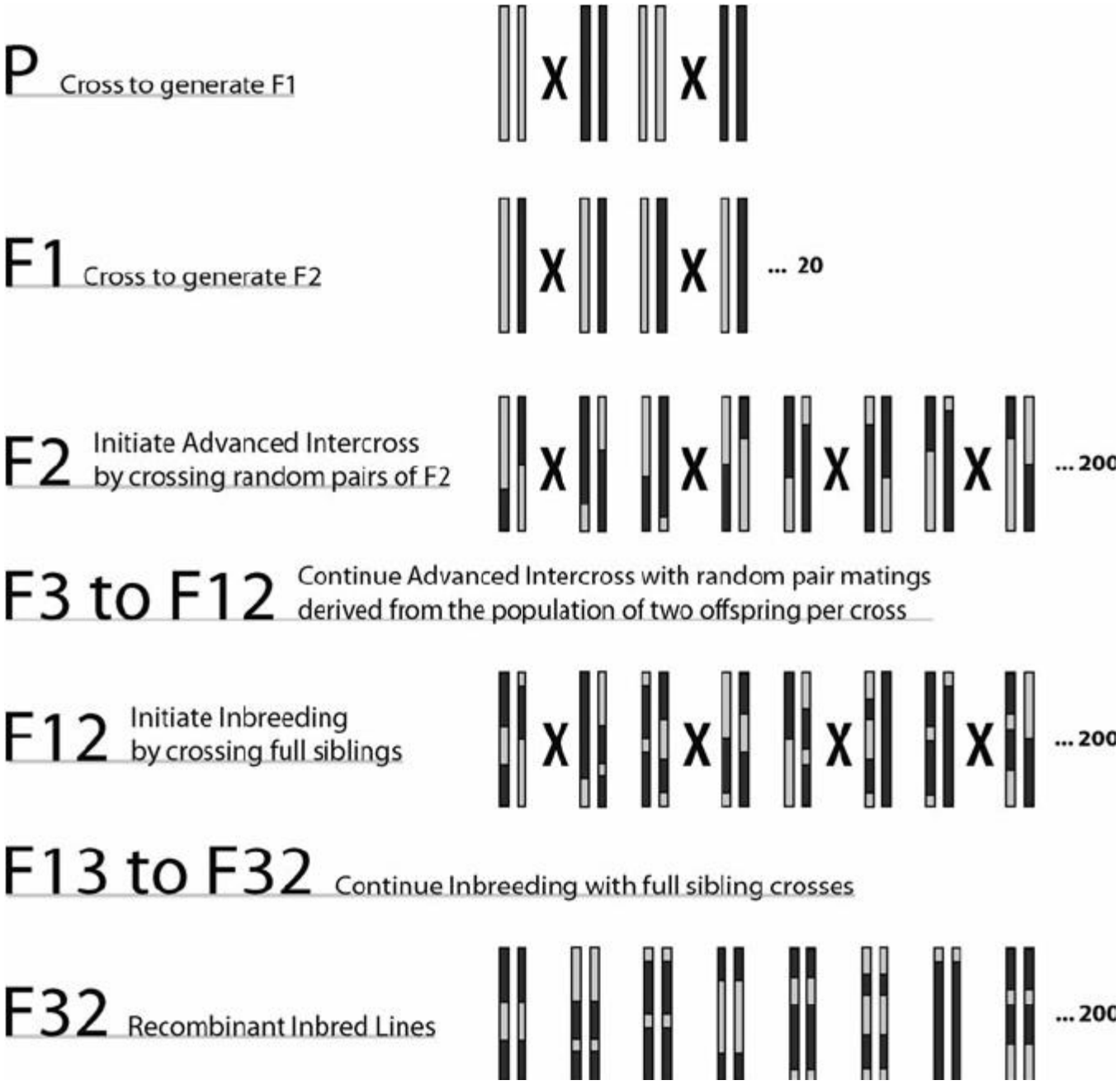


Submergence escape – *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*)



Red circles, positions of major QTLs

Hattori et al (2009) Nature 460:1026-1031



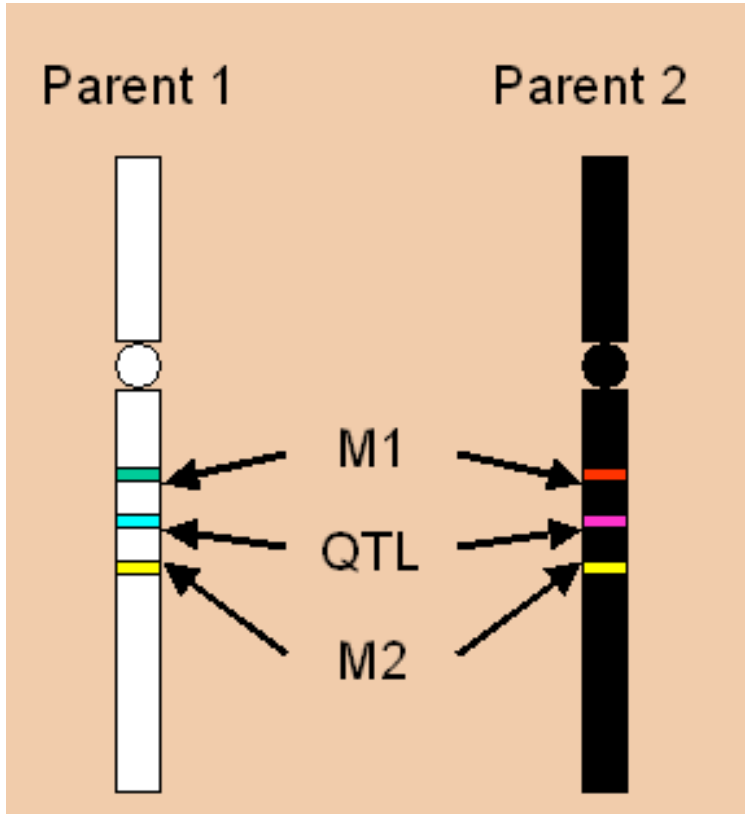
Recombinant Imbred Lines RILs

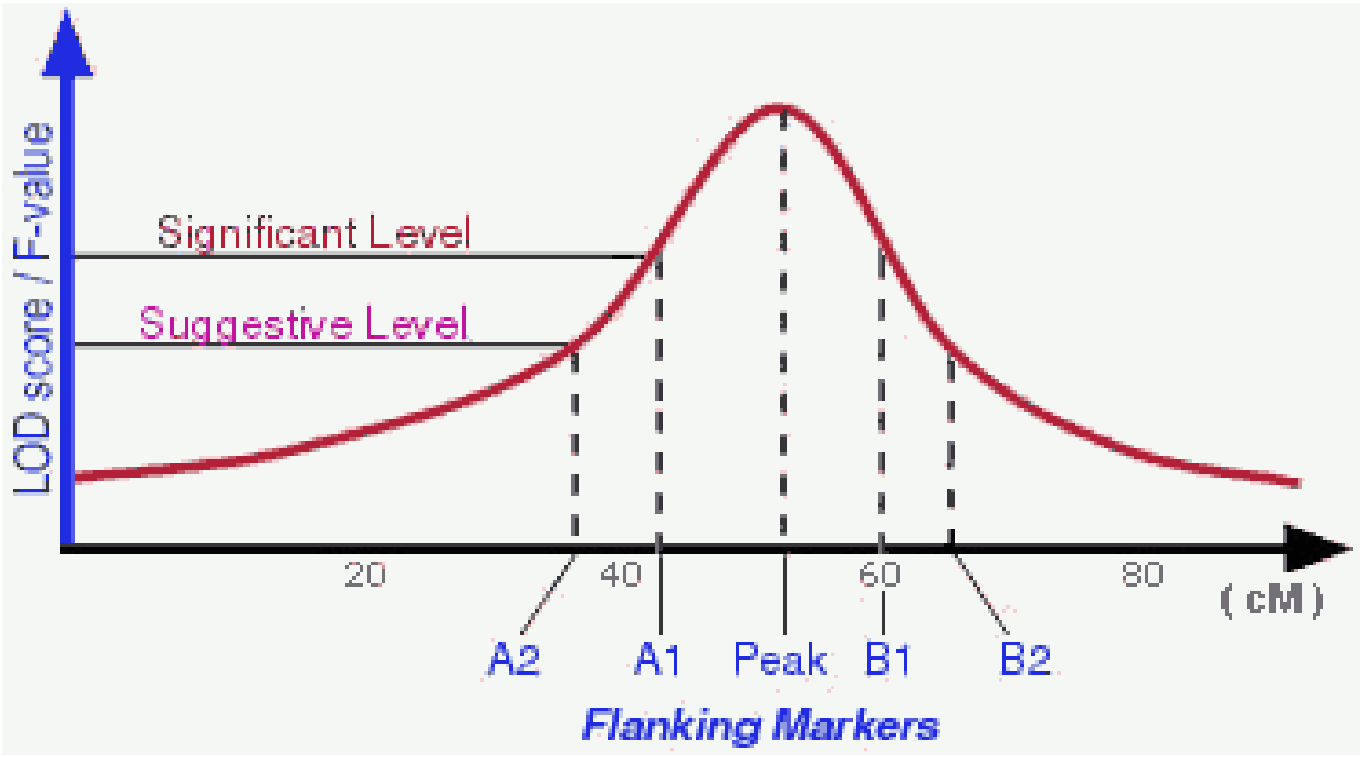
RIL, an organism with chromosomes that incorporate an **essentially permanent set of recombination events between chromosomes inherited from two or more inbred strains**. F1 and F2 generations are produced by intercrossing the inbred strains; pairs of the F2 progeny are then mated to establish inbred strains through long-term inbreeding.

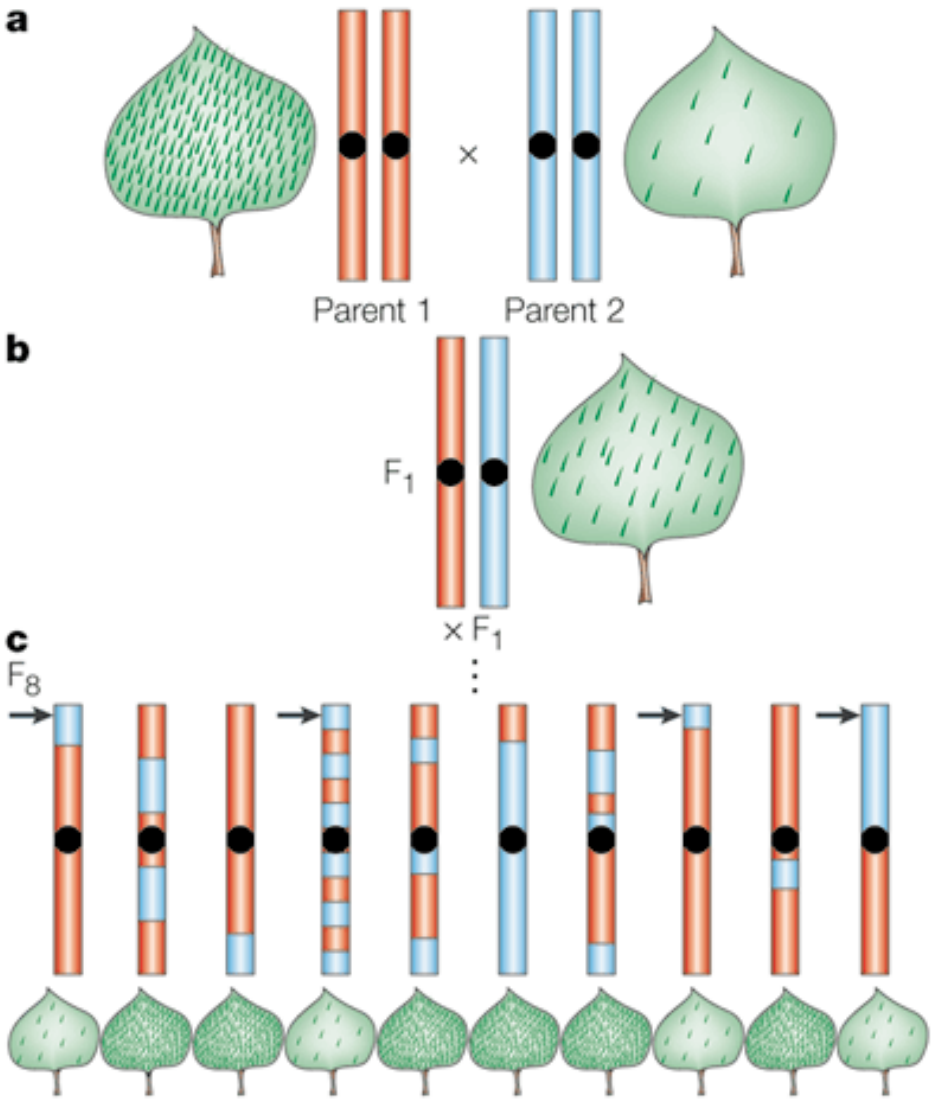
life cycle by planting >180,000 seeds representing >200 RILs at the native field sites of the parental genotypes in the period when seed dispersal occurred in the natural

What is a QTL?

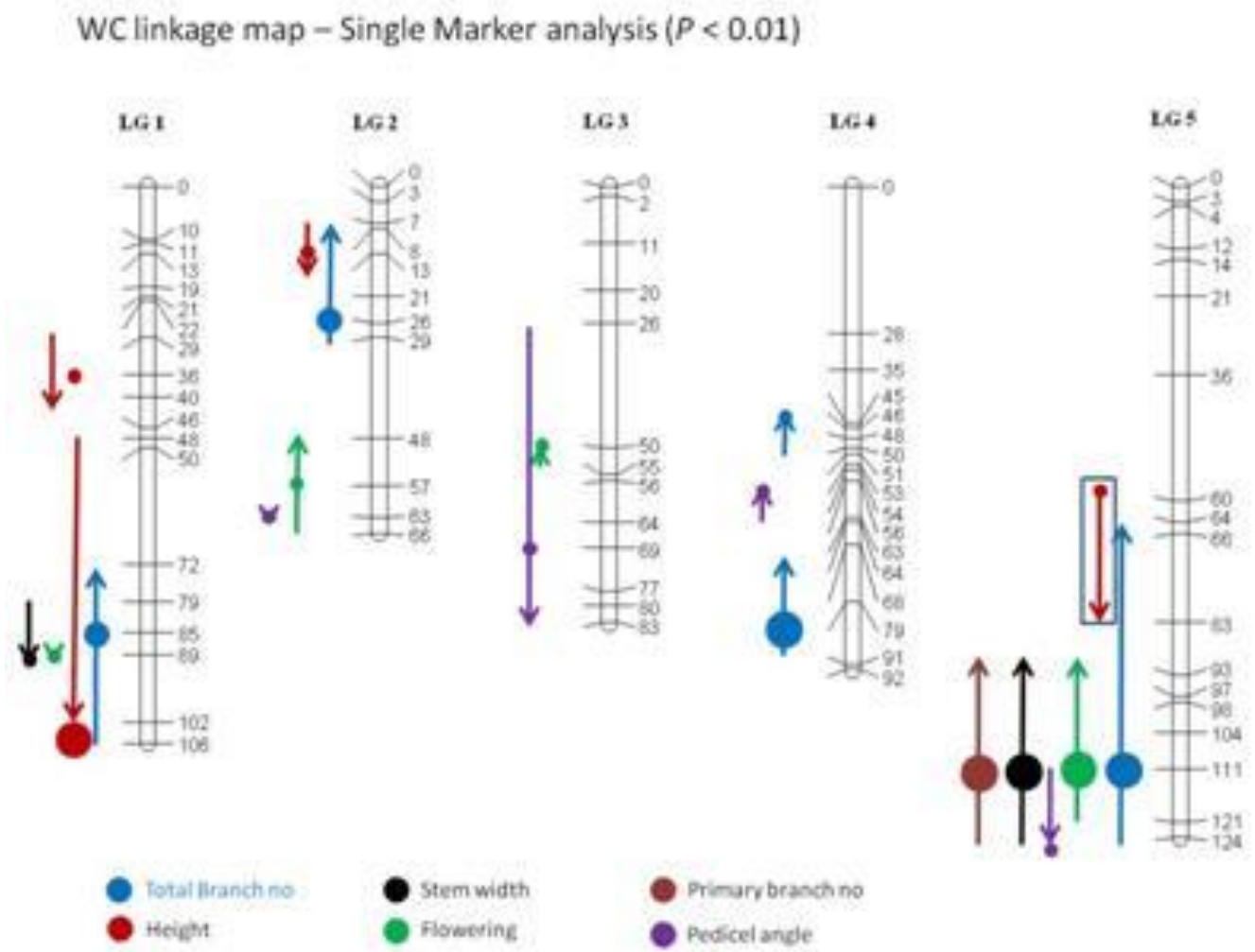
- **QTL**
  - Quantitative Trait Locus
  - A genetic locus that contributes to quantitative variation in a trait



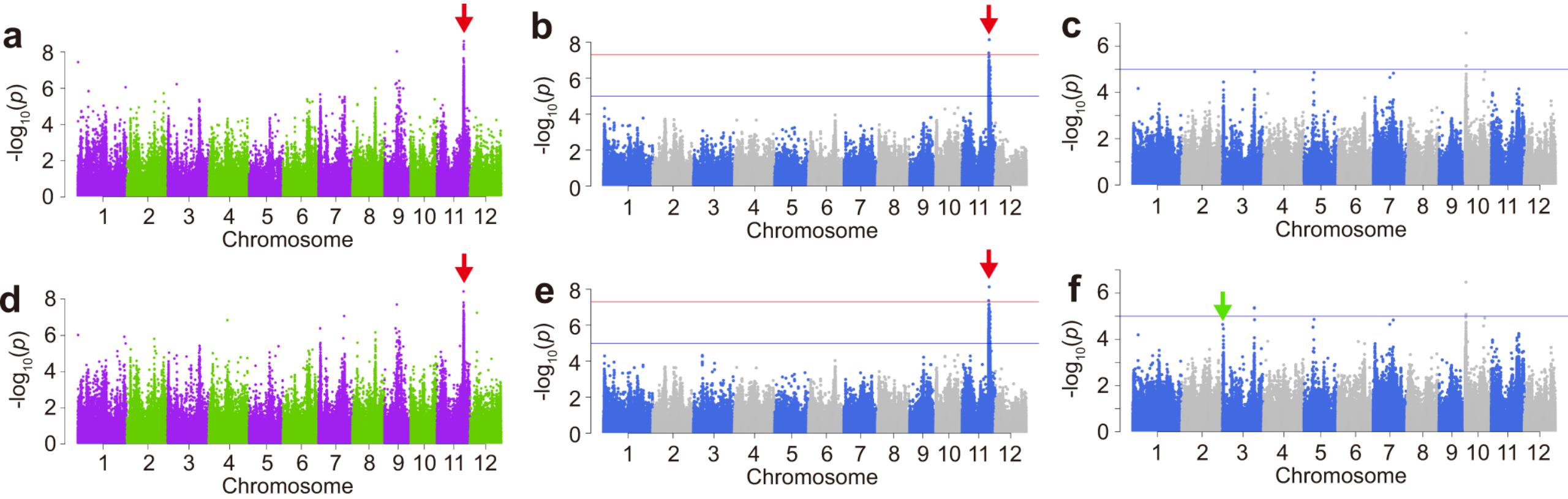




Nature Reviews | Genetics



Genome-wide association study for rice germination rate.

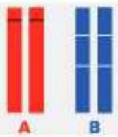


a Genotype × environment (G × E) genome-wide association study (GWAS) for germination rate at 30 °C for 24 h vs 15 °C for 96 h. GWAS for germination rate at 30 °C for 24 h (**b**) and 15 °C for 96 h (**c**). **d** G × E GWAS with the modified variant list. GWAS at 30 °C for 24 h (**e**) and 15 °C for 96 h (**f**) with the modified list. Horizontal red lines indicate 5% genome-wide significance threshold after Bonferroni-correction. Blue lines indicate -$\log_{10}$ *P* values = 5. Peak 1 and Peak 2 are shown by red and green arrows, respectively.

HOW TO DO?

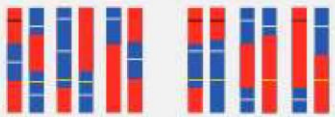
(STEPS INVOLVED)

QTL MAPPING



Select contrasting parents in trait(s) of interest

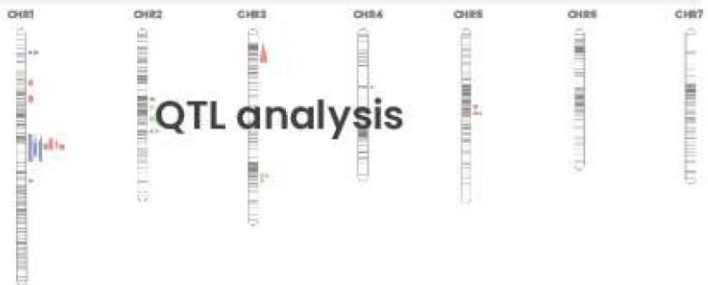
Crossing



F2, F3...Fn; DH; RILs; BCs,

Phenotyping

Genotyping



GENOME-WIDE ASSOCIATION

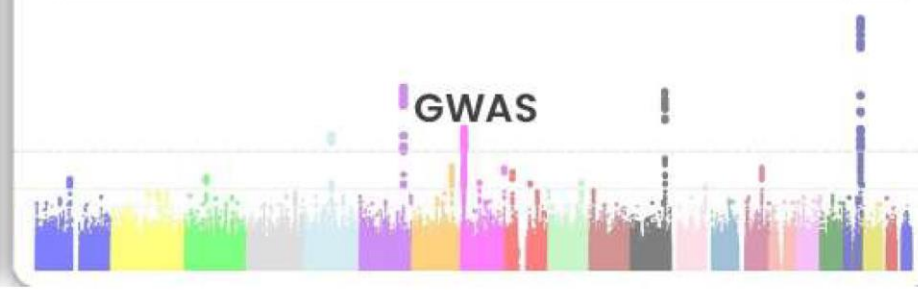
Diverse collection or multi-parental population

Crossing for multi-parental population

Genotyping

Phenotyping

LD, population structure and relatedness



How were the QTLs identified?

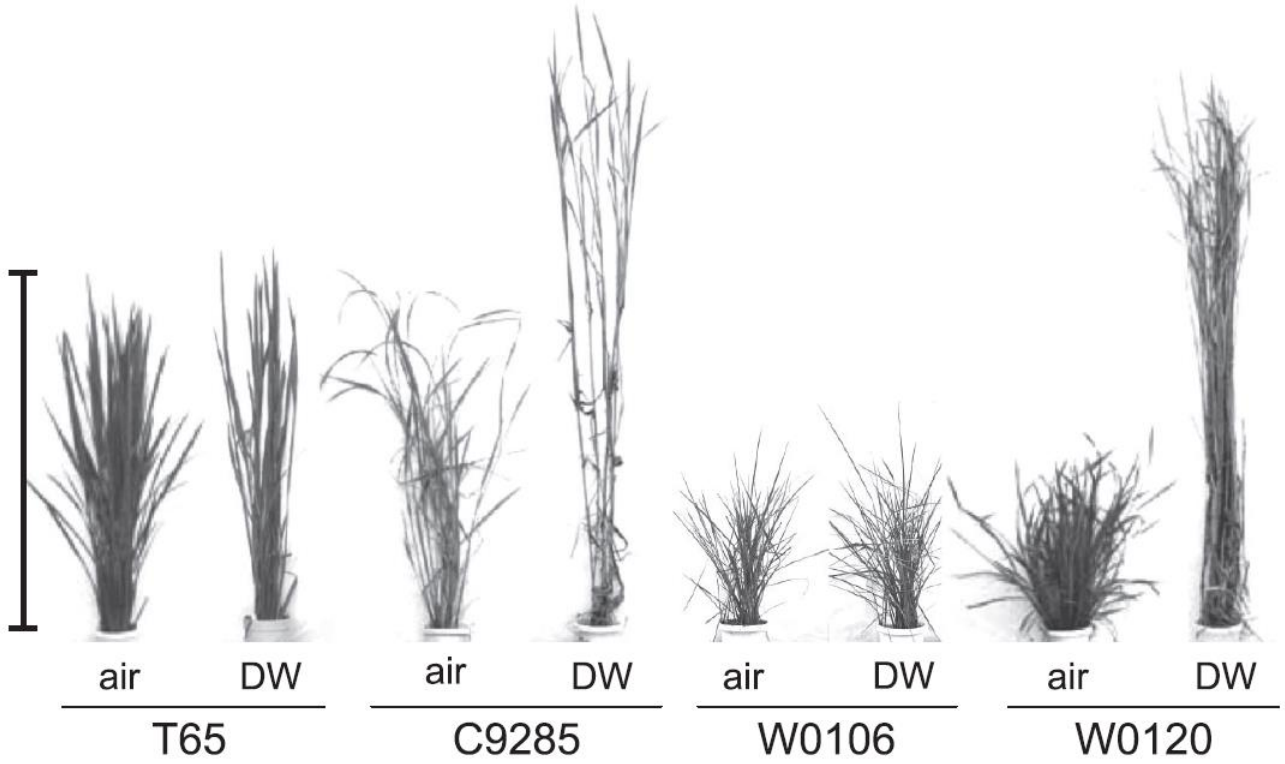


Fig. 1. Response of deepwater rice to deepwater conditions. Two deepwater strains, C9285 (*O. sativa*, ssp. *indica*) and W0120 (*O. rufipogon*), and two non-deepwater strains, T65 (*O. sativa*, ssp. *japonica*) and W0106 (*O. rufipogon*), were grown in air (air) until the ten-leaf stage and then transferred to deepwater conditions (DW) for 2 weeks. Bar, 1 m.

Breeding Science 57 : 305–314 (2007)

A Major QTL Confers Rapid Internode Elongation in Response to Water Rise in Deepwater Rice

Yoko Hattori[1,2], Kotaro Miura[1,2], Kenji Asano[1,2], Eiji Yamamoto[1], Hitoshi Mori[3], Hidemi Kitano[1], Makoto Matsuoka[1] and Motoyuki Ashikari*[1]

[1] *Bioscience and Biotechnology Center, Nagoya University*, Furo, Chikusa, Nagoya, Aichi 464-8601, Japan
[2] *Japan Society for the Promotion of Science*, 8 Ichibancho, Chiyoda, Tokyo 102-8472, Japan
[3] *Graduate School of Bioagricultural Sciences, Nagoya University*, Furo, Chikusa, Nagoya, Aichi 464-8601, Japan

Phenotypic evaluation of deepwater rice

Two types of rice plants were used in the present study: the deepwater rice cultivar C9285 (*O. sativa*, ssp. *indica*) and the wild rice species W0120 (*O. rufipogon*), which exhibit deepwater characteristics. As controls, we used a non-deepwater rice cultivar, T65 (*O. sativa*, ssp. *japonica*), and a wild rice species, W0106 (*O. rufipogon*). When grown in air, none of the lines showed significant internode elongation (Fig. 1). In contrast, growth under deepwater conditions for 2 weeks induced significant internode elongation in the C9285 and W0120 plants, while a slight internode elongation was observed in the T65 or W0106 plants.

W = wild species
T65 and C9285 = cultivar

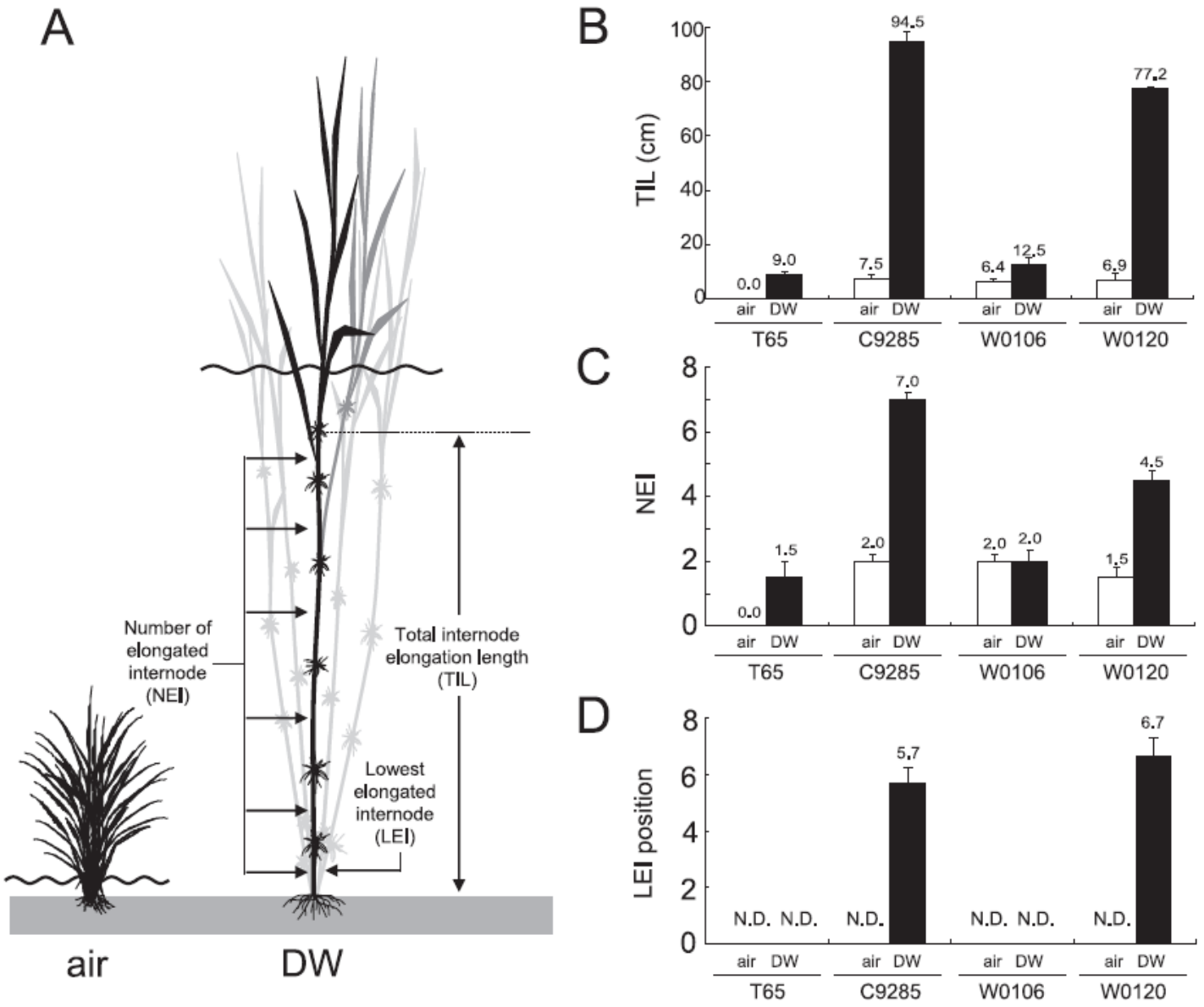


Fig. 2. Quantitative evaluation of internode elongation in deepwater rice. A, Illustration of deepwater rice behavior in air or in deepwater. Total internode elongation length (TIL) corresponds to the length from the base to the highest node. Number of elongated internodes (NEI) corresponds to the total number of elongated internodes. Lowest elongated internode (LEI) position corresponds to the internode position at which internode elongation is initiated. B, Comparison of TILs. C, Comparison of NEIs. D, Comparison of LEIs. Values in B, C and D are means with S.D. (n=5).

Preliminary characterisation

Lines

T65: non-deepwater rice cultivar
C9285: deepwater rice cultivar
W0106: wild non-deepwater rice
W0120: wild deepwater rice

Parameters

TIL: Total Internode Elongation Length
LEI: Lowest Elongated Internode
NEI: Number Elongated Internodes

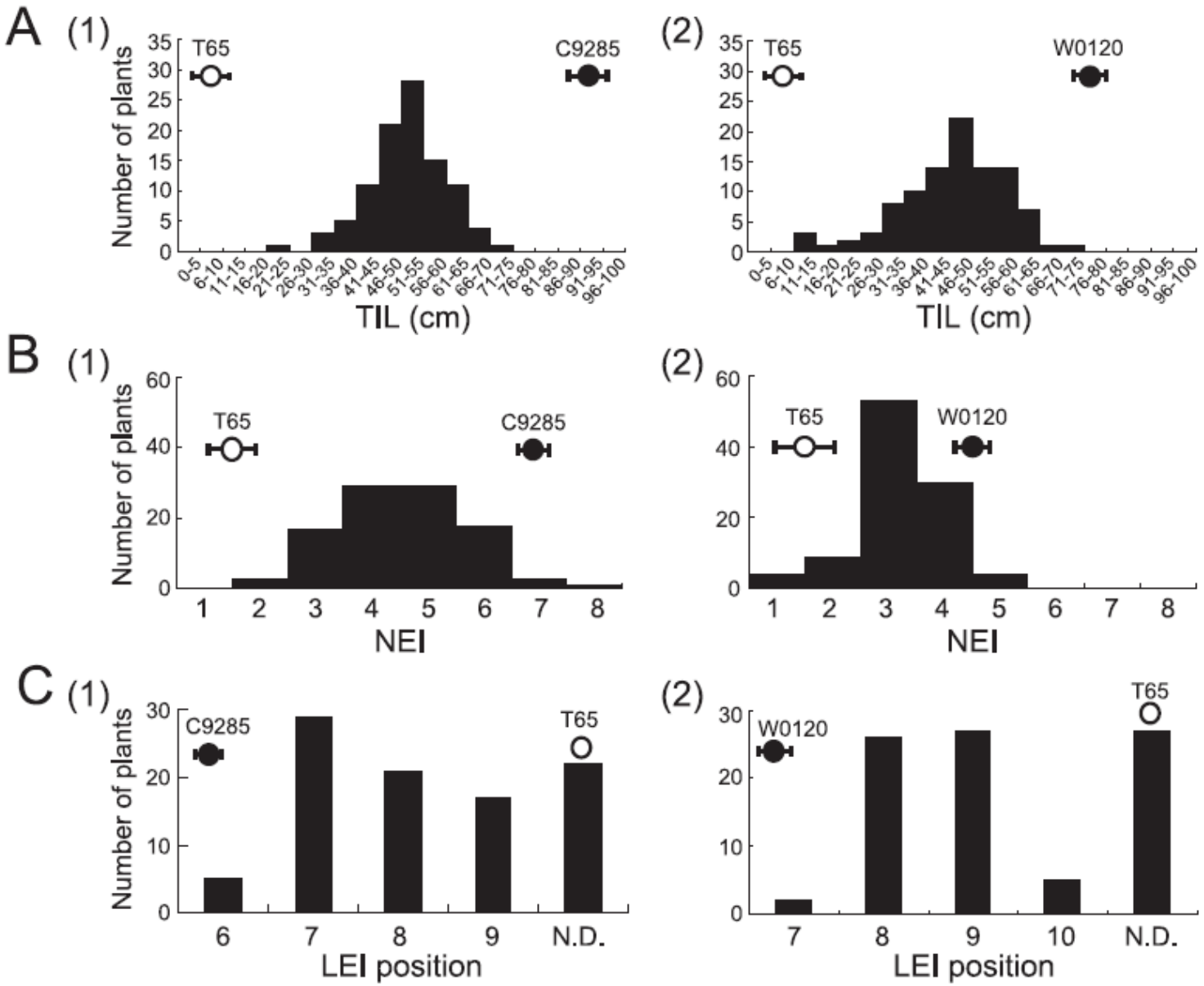


Fig. 3. Distribution frequencies of TIL, NEI and LEI for 94 F_2 individuals. A, Distribution of TIL among F_2 plants from T65/C9285 (1) and T65/W0120 (2). B, Distribution of NEI among F_2 plants from T65/C9285 (1) and T65/W0120 (2). C, Distribution of LEI among F_2 plants from T65/C9285 (1) and T65/W0120 (2). ○, T65; ●, Deepwater rice.

Two F2 populations (180 individuals) obtained after crossing T65 with a deepwater rice cultivar(C9285) and a deepwater wild rice (W0120)

The lack of 3:1 and 1:2:1 distribution means that this traits are controlled by QTLs and not by a single locus

?

Linkage maps as a tool to localize QTLs

T65/C9285: 92 molecular markers at a distance of about 19 cM

T65/W0120: 106 molecular markers at a distance of about 17 cM

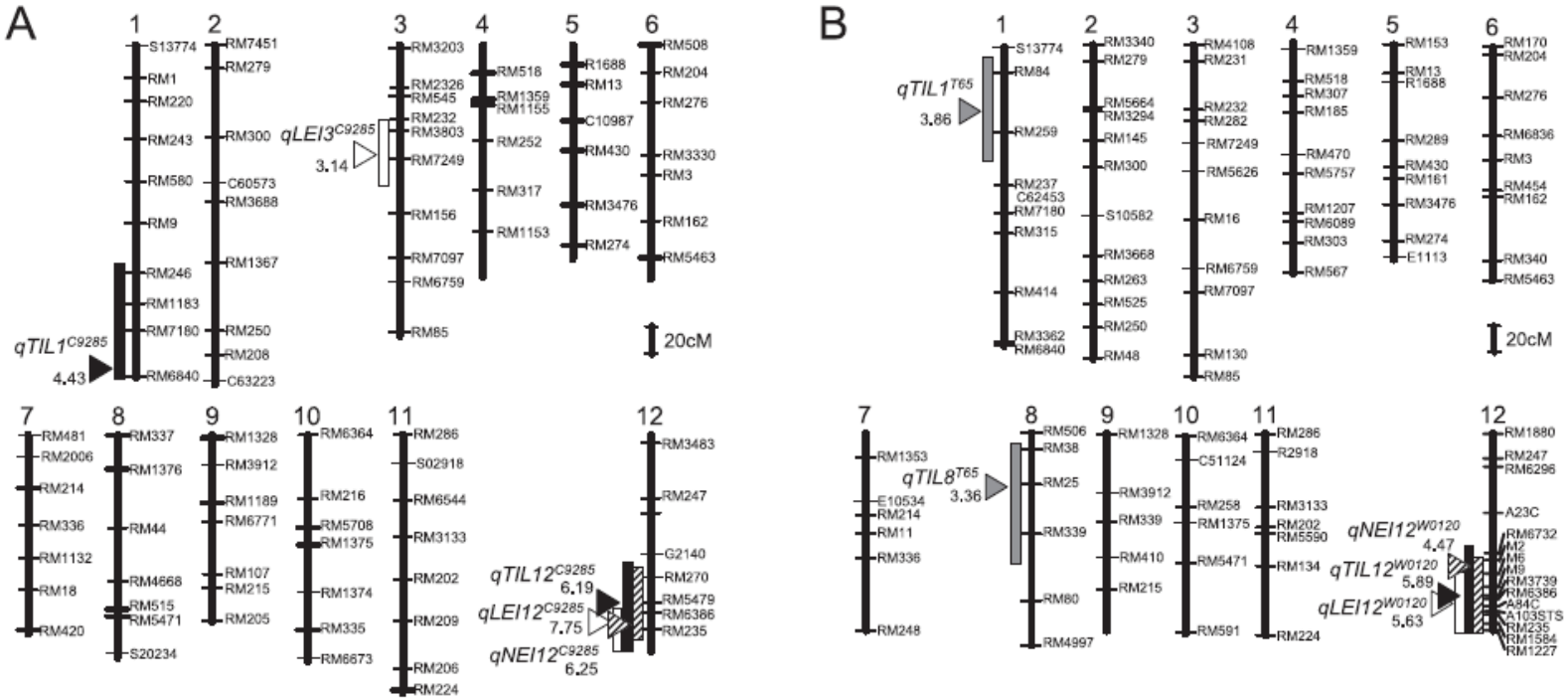


Fig. 4. QTLs for TIL, NEI and LEI. A, Location of QTLs for TIL, NEI and LEI on the linkage map from T65/C9285. B, Location of QTLs for TIL, NEI and LEI on the linkage map from T65/W0120. The region of the QTL for TIL enhanced by the deepwater allele is illustrated by a black box. The region of the QTL for TIL enhanced by the T65 allele is illustrated by a dotted box. The region of the QTL for NEI enhanced by the deepwater allele is illustrated by a hatched box. The region of the QTL for LEI enhanced by the deepwater allele is illustrated by a white box. Arrowheads indicate the QTL peaks with the LOD scores.

Major QTL, how is it confirmed?

Via Near Isogenic Lines = NIL that in this case are lines that carry this QTL in the other parent genetic background

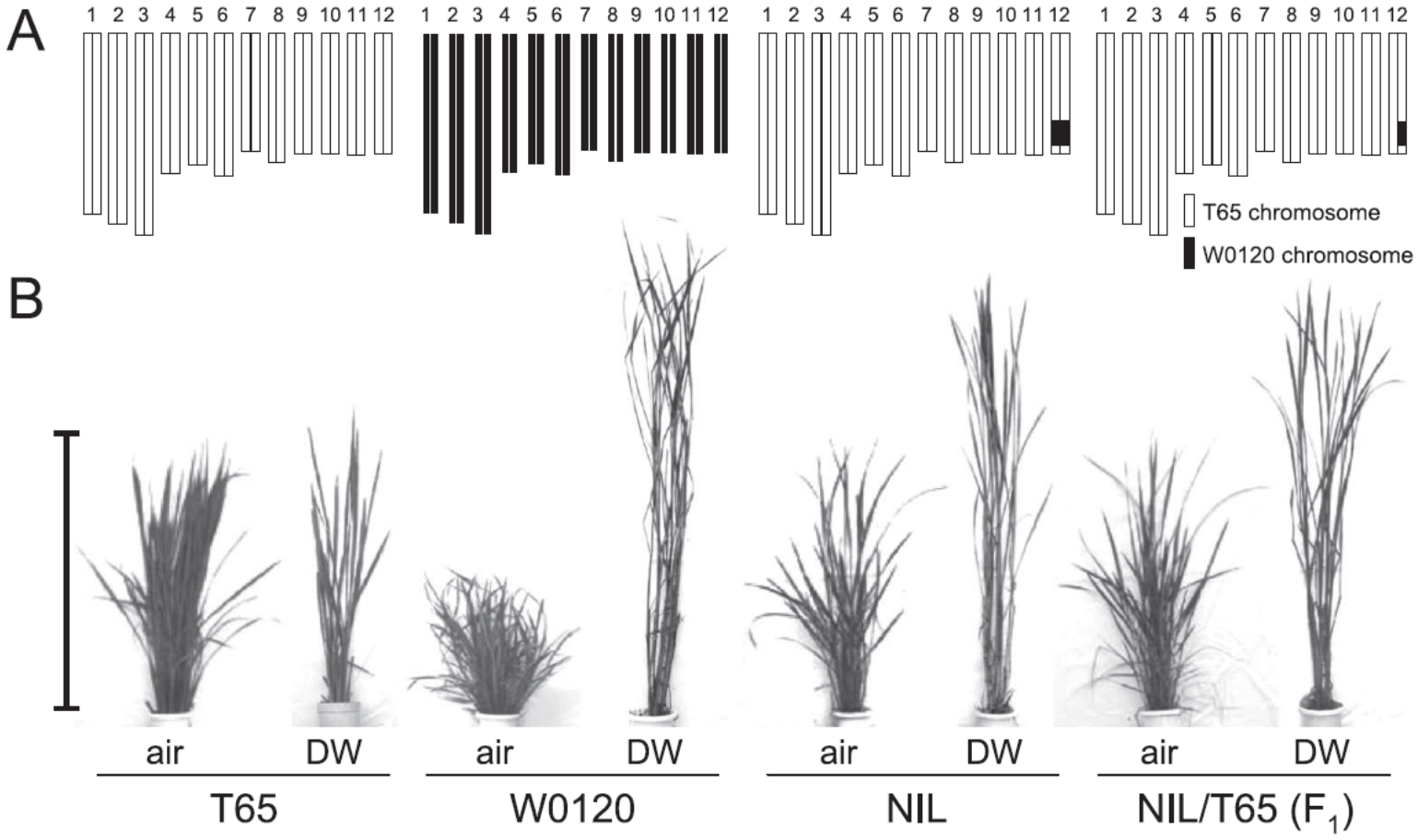


Fig. 5. Response of the NIL-12^{W0120} plants to water rise. A, Graphical genotype. From left to right: T65, W0120, NIL and F_1 (NIL/T65). NIL-12^{W0120} is abbreviated as NIL. Open bars indicate T65 chromosomes. Closed bars designate W0120 chromosomes. B, Internode elongation in water. Bar, 1 m. air, air condition; DW, deepwater condition.

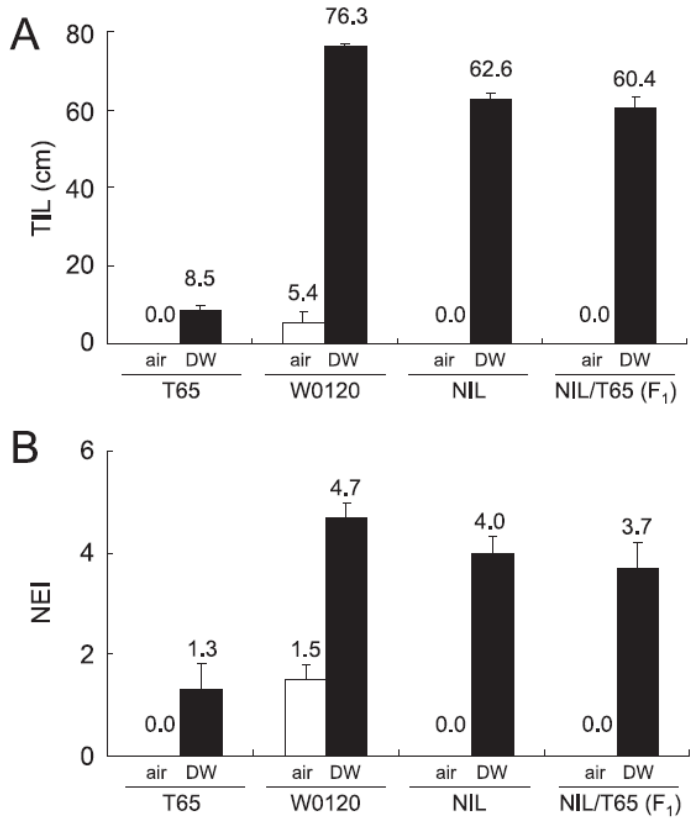


Fig. 6. Quantitative evaluation of the response of the NIL-12^{W0120} plants to water rise. A, Quantitative internode elongation, total internode elongation length (TIL). B, Quantitative internode elongation, number of elongated internodes (NEI). Values in A and B are means with S.D. (n=5). air, air condition; DW, deepwater condition. NIL, NIL-12^{W0120}.

Breeding Science 58 : 39–46 (2008)

Mapping of three QTLs that regulate internode elongation in deepwater rice

Yoko Hattori[†1,2], Keisuke Nagai[†1], Hitoshi Mori[3], Hidemi Kitano[1], Makoto Matsuoka[1] and Motoyuki Ashikari*[1]

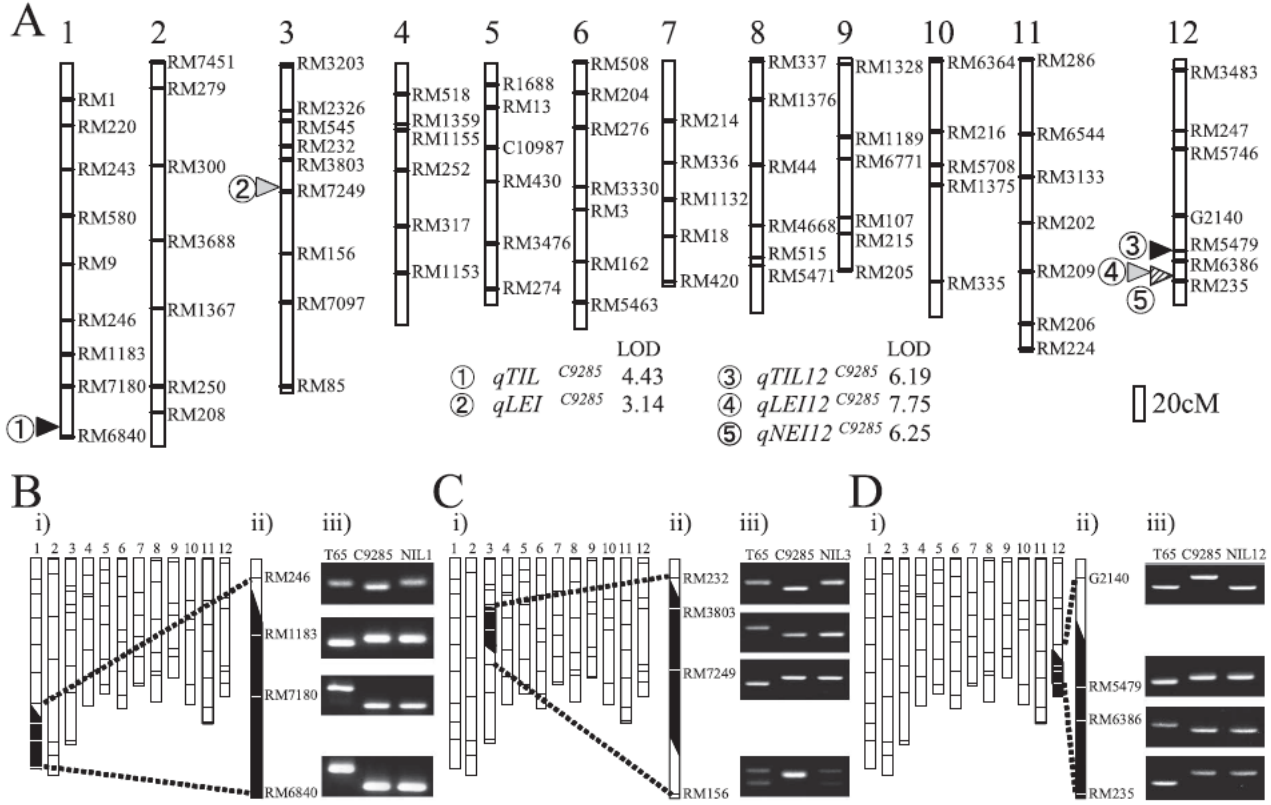
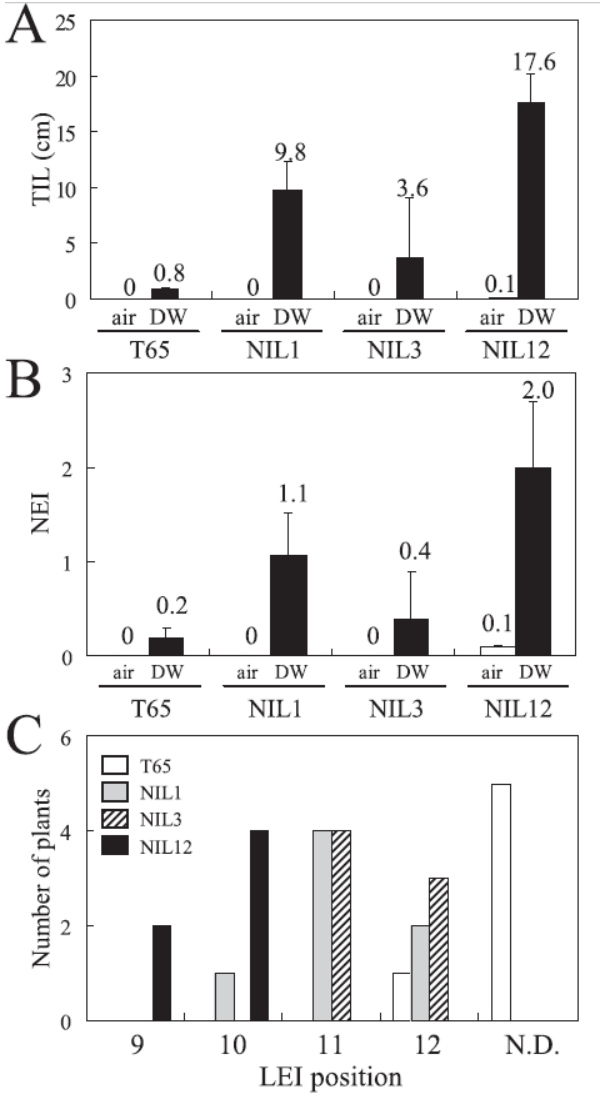
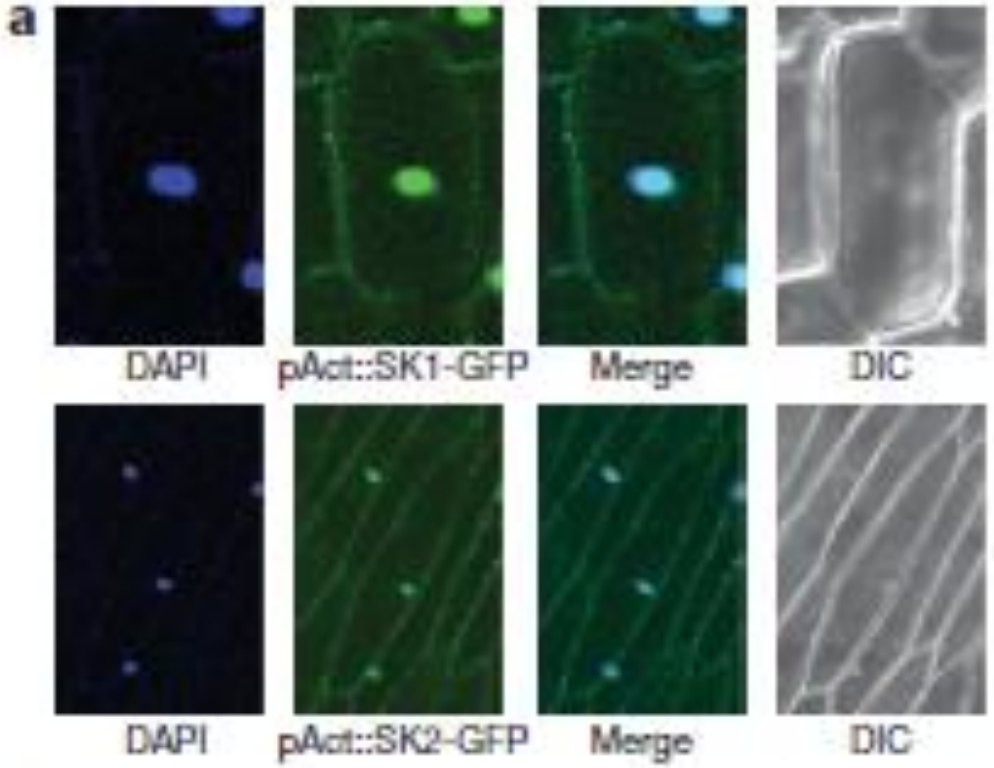


Fig. 1. Location of QTLs and graphical genotypes of nearly isogenic lines (NILs). (A) Position of the QTL for deepwater characteristics on the rice chromosome. QTL positions are illustrated based on results of QTL analysis using the deepwater rice cultivar C9285 (Hattori *et al.* 2007). The five detected QTL positions are indicated as 1–5. Arrowheads indicate QTL peaks. QTL names and LOD scores are indicated under the map. (B) i) Graphical genotypes of NIL-1^{C9285}; ii) Magnification of graphical genotype of the region for *qTIL1*^{C9285}; iii) Genotypes of markers around *qTIL1*^{C9285} in T65, C9285 and NIL-1^{C9285} (abbreviated as NIL1). (C) i) Graphical genotypes of NIL-3^{C9285}; ii) Magnification of the graphical genotype of region for *qLEI3*^{C9285}; iii) Genotypes of markers around *qLEI3*^{C9285} in T65, C9285 and NIL-3^{C9285} (abbreviated as NIL3). (D) i) Graphical genotypes of NIL-12^{C9285}; ii) Magnification of graphical genotype of region for *qTIL12*^{C9285}, *qNEI12*^{C9285} and *qLEI12*^{C9285}; iii) Genotypes of markers around *qTIL12*^{C9285}, *qNEI12*^{C9285} and *qLEI12*^{C9285} in T65, C9285 and NIL-12^{C9285} (abbreviated as NIL12). (B–D) T65 chromosome region is illustrated by a white box. C9285 chromosome region is illustrated by a black box.

Genetic markers associated to QTL and plant phenotypes

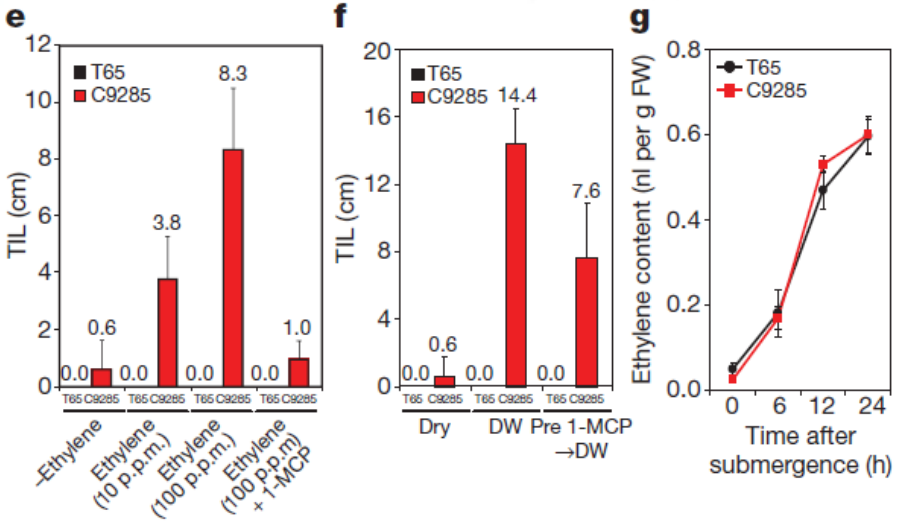


SNORKEL1 AND SNORKEL2



SNORKEL1 AND SNORKEL2 are transcription factors as testified by their nuclear localization.

They are also regulated by GA (Gibberellins) and CK (cytokinines)



- Ethylene boost TIL
- 1-MCP reduces TIL
- No difference in Ethylene production between DW and non-DW

Uniconzole is an antagonist of GA

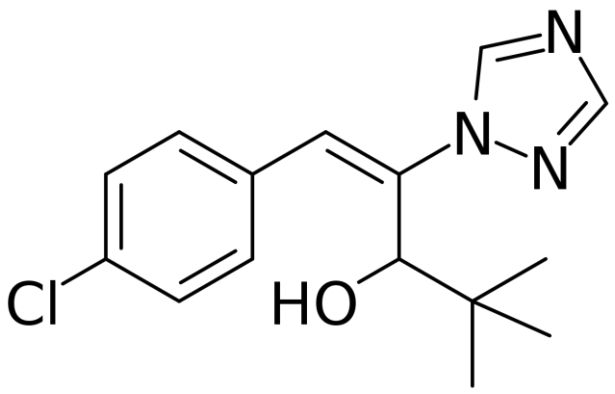
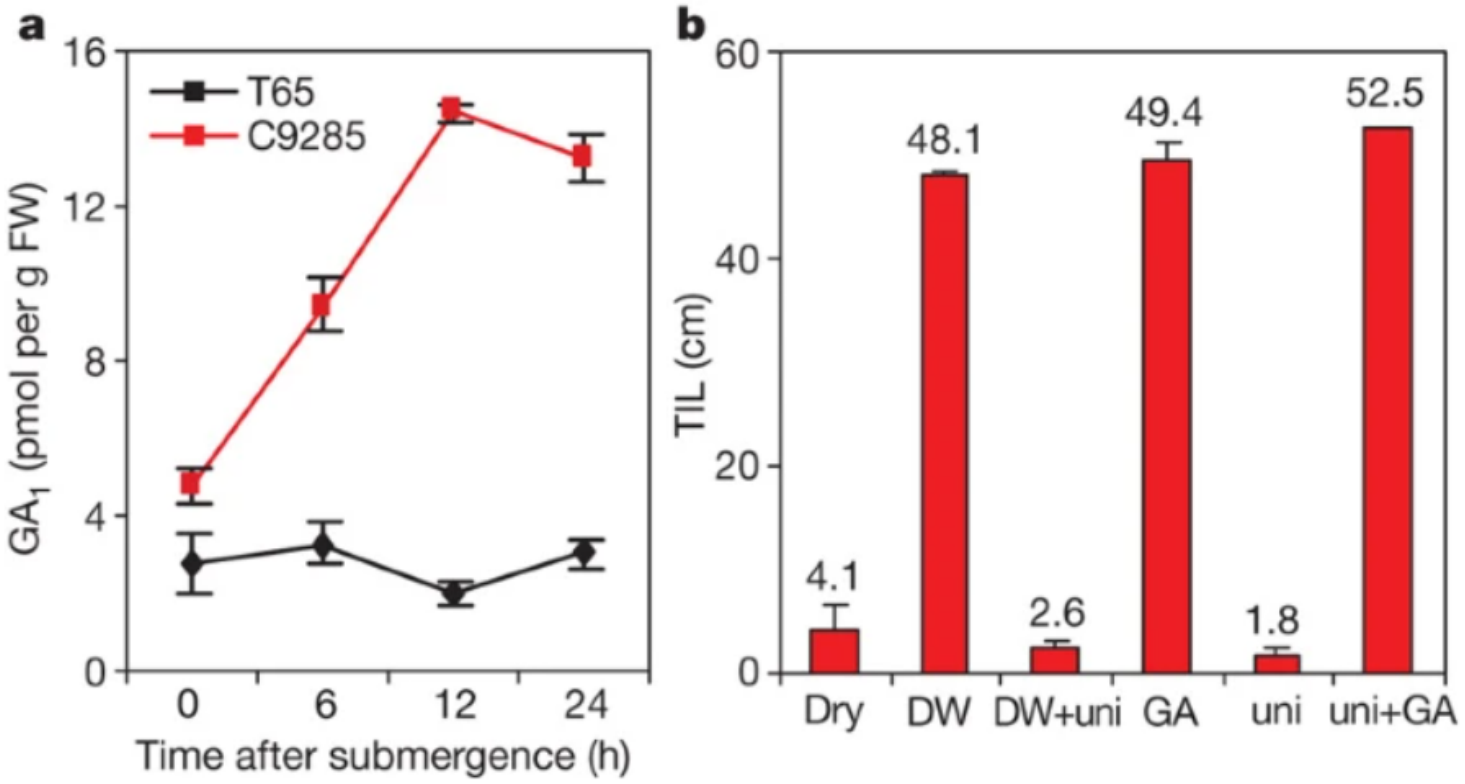
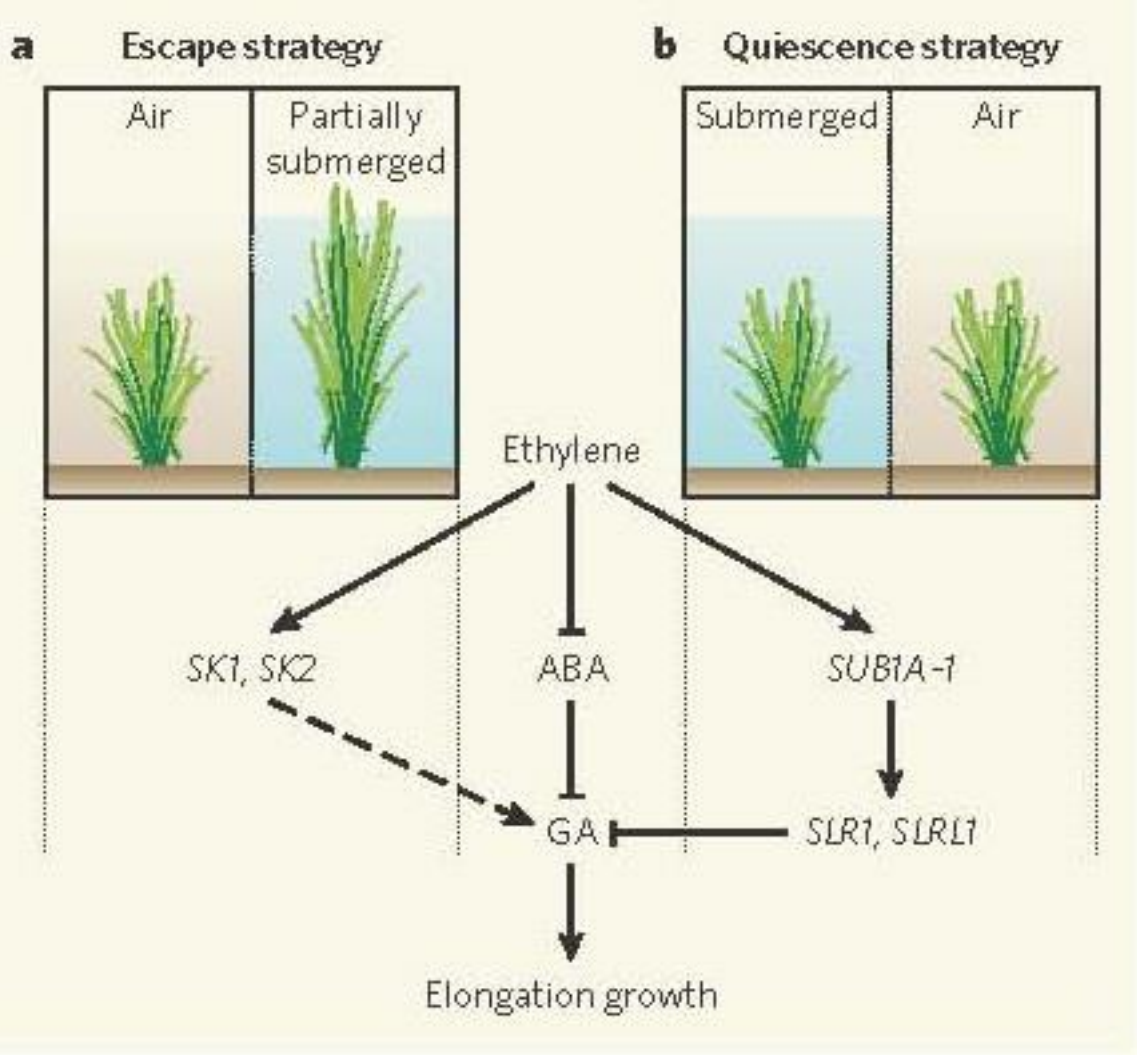


Figure 3: GA response and molecular mechanism of deepwater response.



a, GA_1 content in C9285 under deepwater conditions. Mean ± s.d., $n = 4$. **b**, GA responsiveness in C9285. Ten-leaf-stage plants were treated with 100 μM GA_3 with or without 1 μM uniconazole (uni) for one week. Mean ± s.d., $n = 8$.

Escape and quiescence strategies for flooding tolerance



Clearly the success of rice in flooded habitats is due to its ability to rapidly regain aerial contact.

Interestingly**, only a few rice varieties can survive complete submergence for an extended period of time**, a phenomena that regularly occurs in so-called flash-floods.

These varieties survive thanks to the group **VII ERF gene SUB1A**, whose product positively regulates the fermentation capacity, but represses plant growth by restricting gibberellin-signalling

SLR1, a negative regulator of GA signaling

Voesenek and Bailey-Serres (2009) Nature 460:959-960

Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice

Kenong Xu[1], Xia Xu[1], Takeshi Fukao[2], Patrick Canlas[1], Reycel Maghirang-Rodriguez[3], Sigrid Heuer[3], Abdelbagi M. Ismail[3], Julia Bailey-Serres[2], Pamela C. Ronald[1] & David J. Mackill[3]

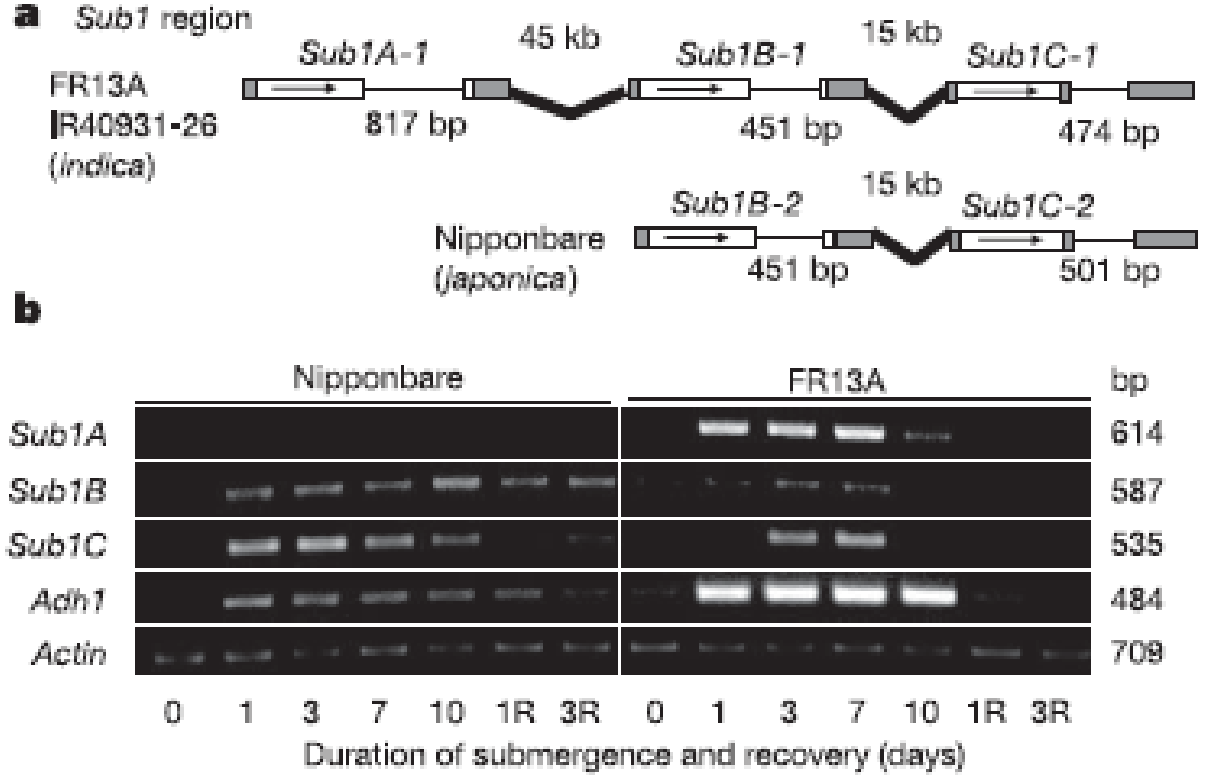


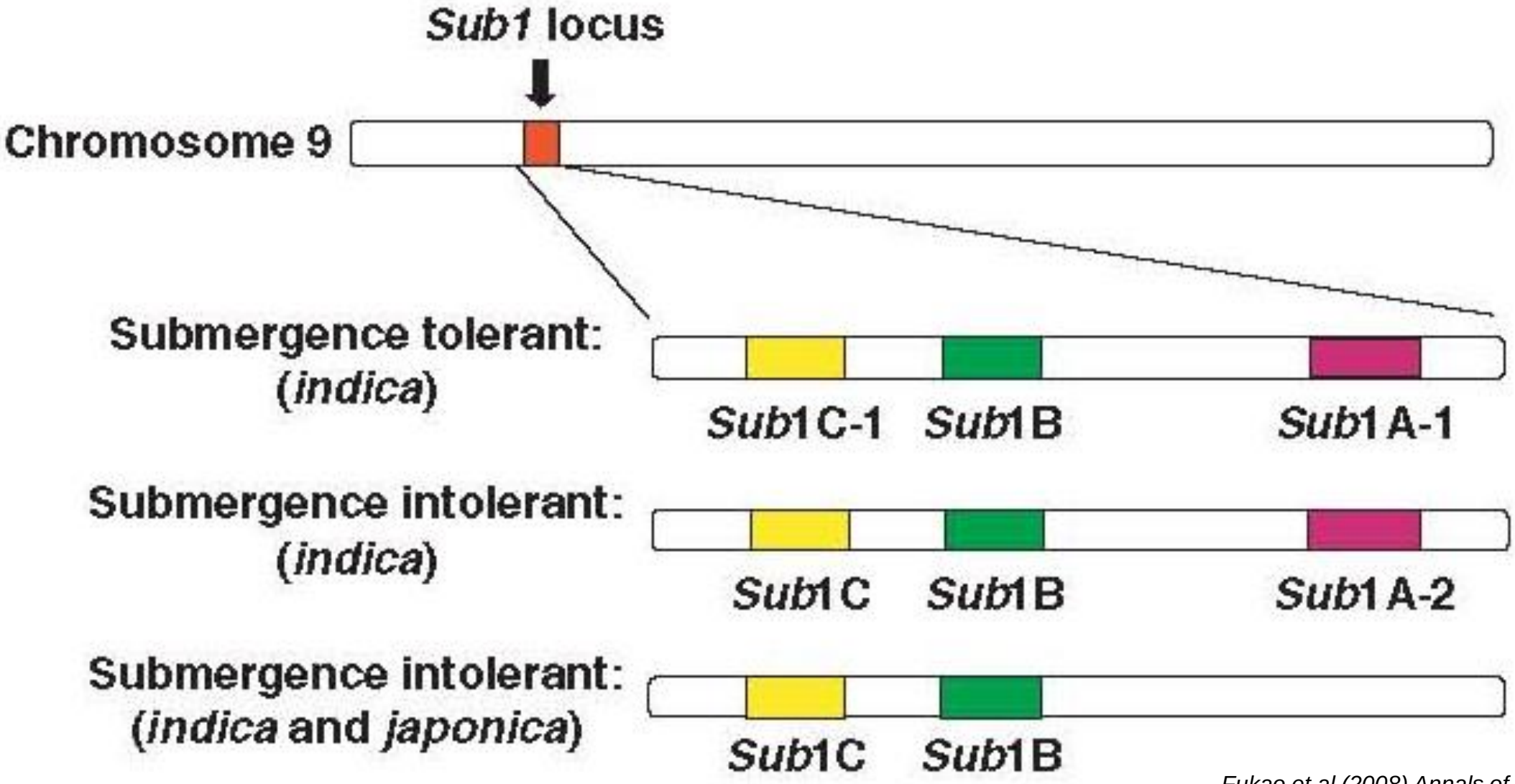
Table 1 | Haplotypes of the *Sub1* locus based on alleles of the ERF-like genes in rice varieties

Line or cultivar	Submergence phenotype	Subspecies	*Sub1A* allele	*Sub1B* allele	*Sub1C* allele
FR13A, IR40931-26, DX18-121, IR48930	Tolerant	*indica*	A-1	B-1	C-1
Goda Heenati	Tolerant	*indica*	A-1	B-6	C-1
Kurkaruppan	Tolerant	*indica*	A-1	B-3	C-1
LMNIII	ND	*indica*	A-2	B-1	C-4
Teqing, CO39, IR64, IR64-M6D6-933-1-2, 93-11	Intolerant	*indica*	A-2	B-1, B-7	C-3, C-5
IR24, IRBB21, Swarna*	Intolerant	*indica*	Absent	B-8, B-5	C-6
IR50	Intolerant	*indica*	Absent	B-9	C-7
Habiganj aman	Intolerant	*indica*	Absent	B-4	C-6
Nipponbare, Liaogeng, M-202, Taipei309	Intolerant	*japonica*	Absent	B-2	C-2

Allele designations were based on the amino-acid sequence of the putative proteins (Supplementary Figs 3, 5 and 6). The submergence-tolerant *indica*-like variety FR13A is from Orissa, in eastern India. DX18-121 is an *indica/japonica* hybrid derivative. The submergence-tolerant varieties Kurkaruppan and Goda Heenati are from Sri Lanka. IR48930, IR40931-26 and DX18-121 are derivatives of FR13A. The primary locus conferring tolerance in FR13A and Kurkaruppan was reported to be similar but different from Goda Heenati[30]. However, submergence tolerance in Goda Heenati is also largely controlled by the *Sub1* locus (K.X. and D.J.M. unpublished data). Molecular marker studies indicate considerable divergence between Goda Heenati and FR13A (D.J.M. unpublished data). GenBank accessions of 93-11 containing *Sub1A*, *Sub1B* and *Sub1C* are AAAA01009971, AAAA01020021 and AAAA01005744, respectively. ND, not determined. The varieties are grouped based primarily on common alleles of Sub1A and Sub1C.

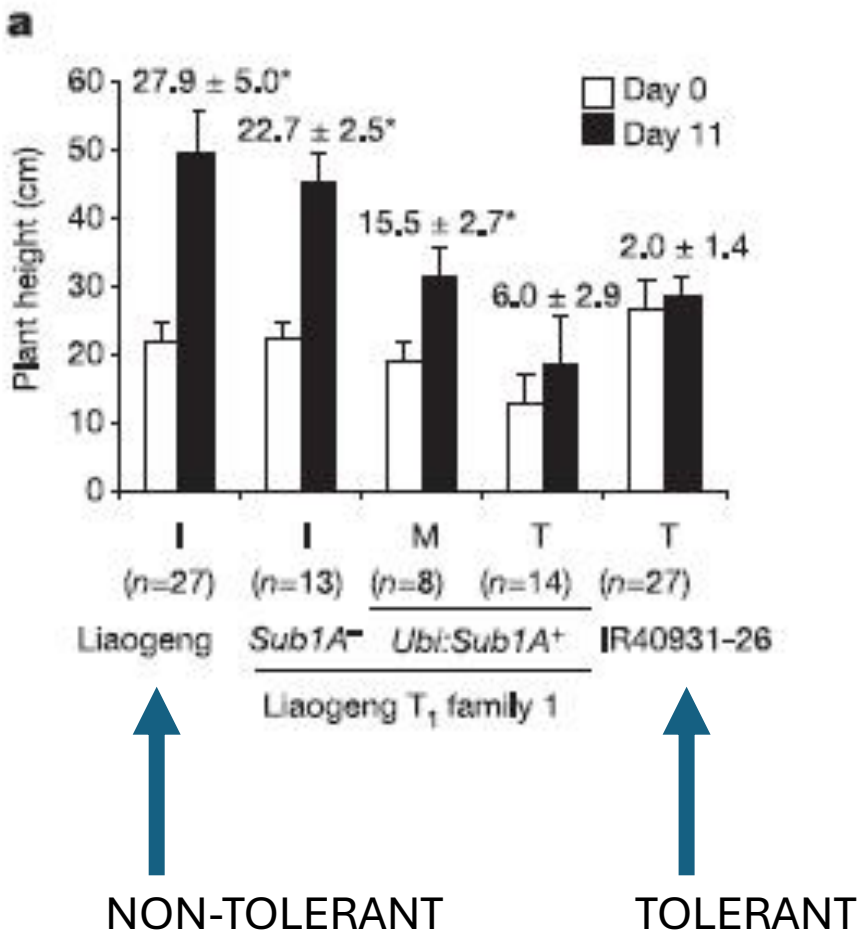
*Swarna lacks *Sub1A* and its alleles of *Sub1B* and *Sub1C* were not determined.

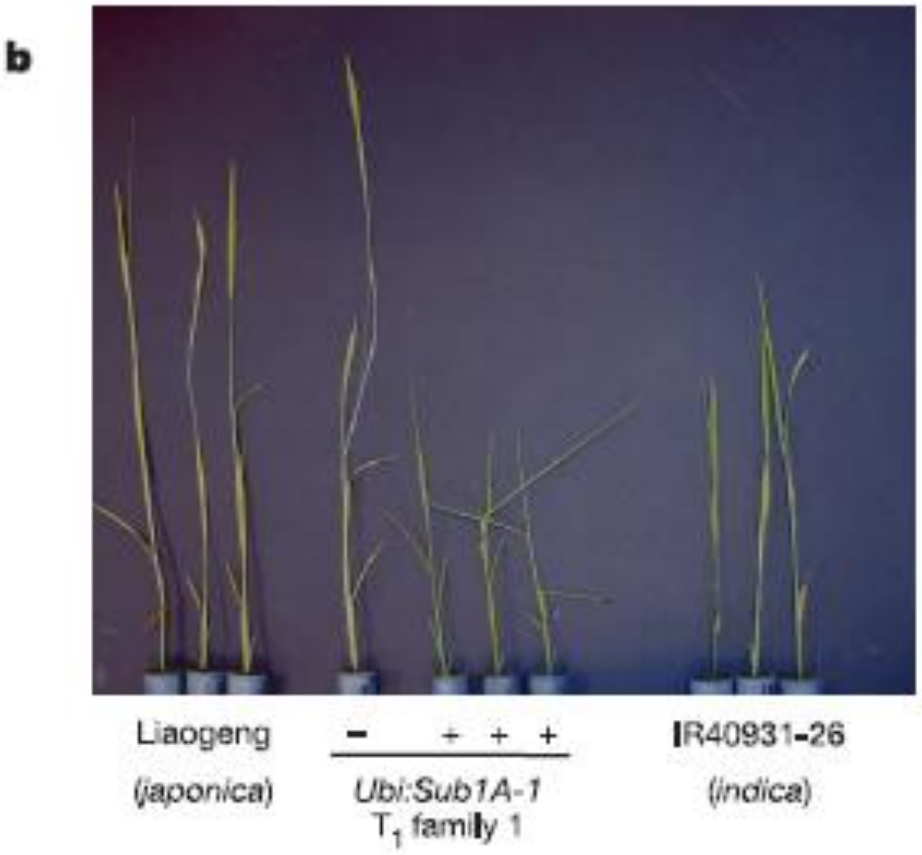
Low oxygen quiescence – *SUBMERGENCE1A-1* (*SUB1A-1*)



Fukao et al (2008) Annals of Botany 103:143

EXPRESSION OF *SUBMERGENCE1A-1* (*SUB1A-1*)

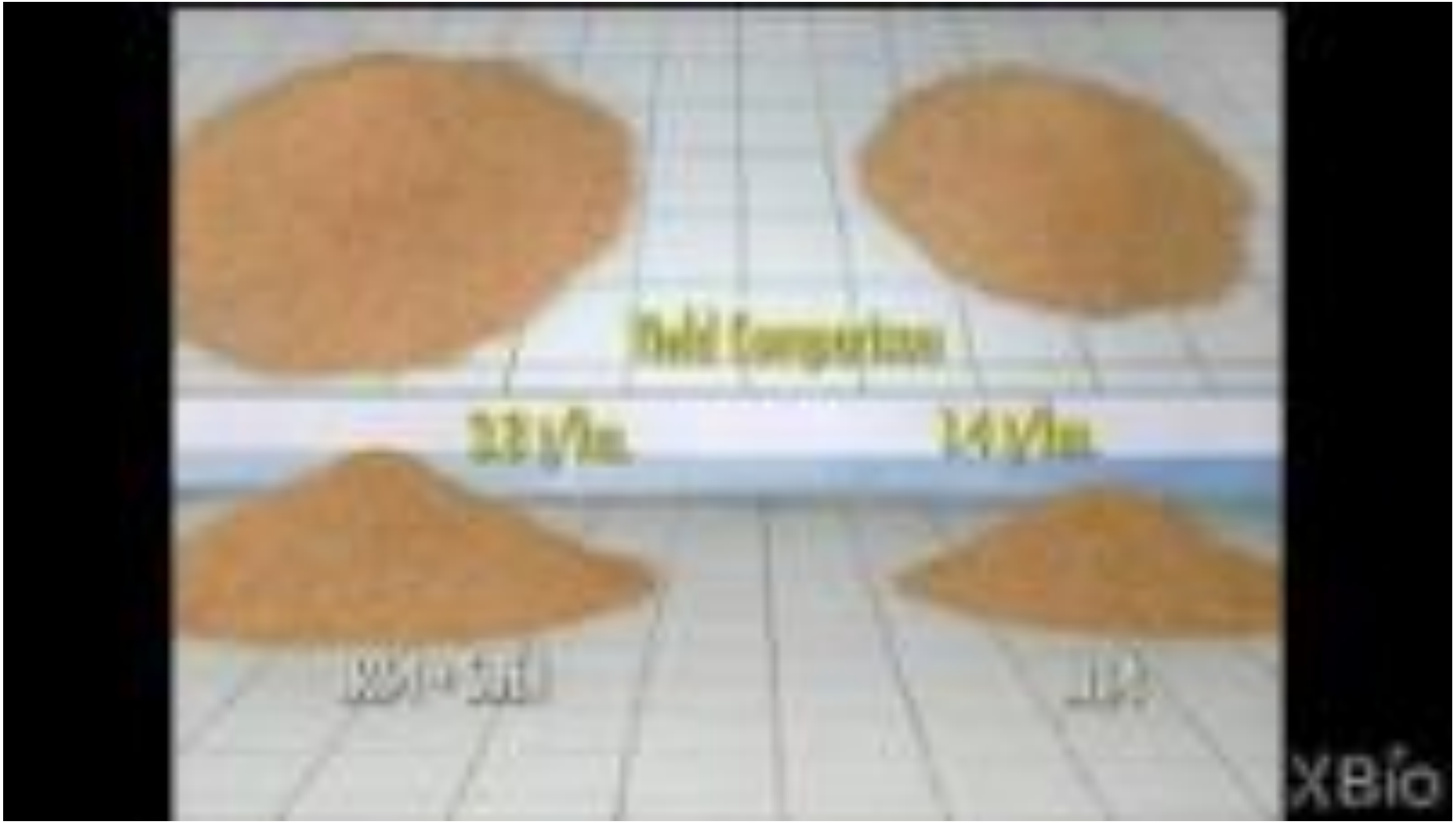




SUB1 introgression by maker assisted selection

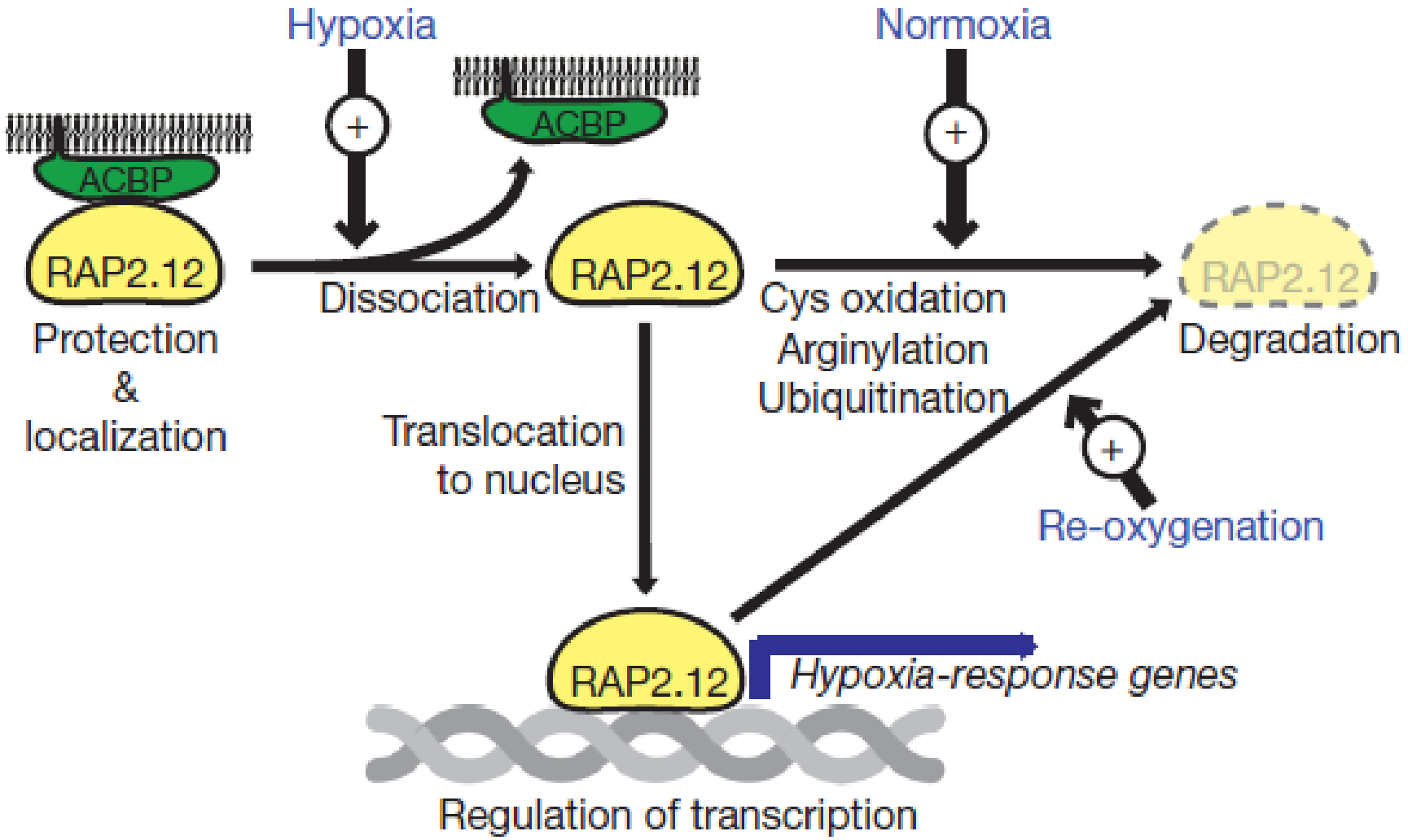
Introgression of the FR13A Sub1 haplotype into an intolerant variety by MAS confers submergence tolerance. The Sub1 region donor line IR49830 (an FR13A derivative) was introduced into the submergence intolerant indica variety Swarna by backcrossing (BC) with MAS using markers for the Sub1 region (SSR1, RM316, RM464, RM464A, RM219 and RM524) and the 12 chromosomes25–27. Individual F1 plants were selected from BC1, BC2 and BC3 that carried the FR13A Sub1 haplotype with the least IR49830 background. Fourteen-day-old seedlings were submerged for 14 days and photographed 14 d after de-submergence.

Xu et al (2006) Nature 442:705



https://www.youtube.com/watch?v=VUQwroMcoXc

In Arabidopsis



Model describing the oxygen sensor mechanism in plants. The transcription factor RAP2.12 is constitutively expressed under aerobic conditions. RAP2.12 protein is always present, bound to ACBP to prevent RAP2.12 frommoving into the nucleus under aerobic conditions and to protect it against proteasomal degradation in air. Upon hypoxia, RAP2.12 moves into the nucleus, where it activates anaerobic-gene expression. Upon reoxygenation, RAP2.12 is rapidly degraded via the N-end rule pathway and proteasome-mediated proteolysis to downregulate the hypoxic response.

In plants the “equivalent” transcription factors to HIF1a are the ERFVIIs.

LETTER

doi:10.1038/nature10536

Oxygen sensing in plants is mediated by an N–end rule pathway for protein destabilization

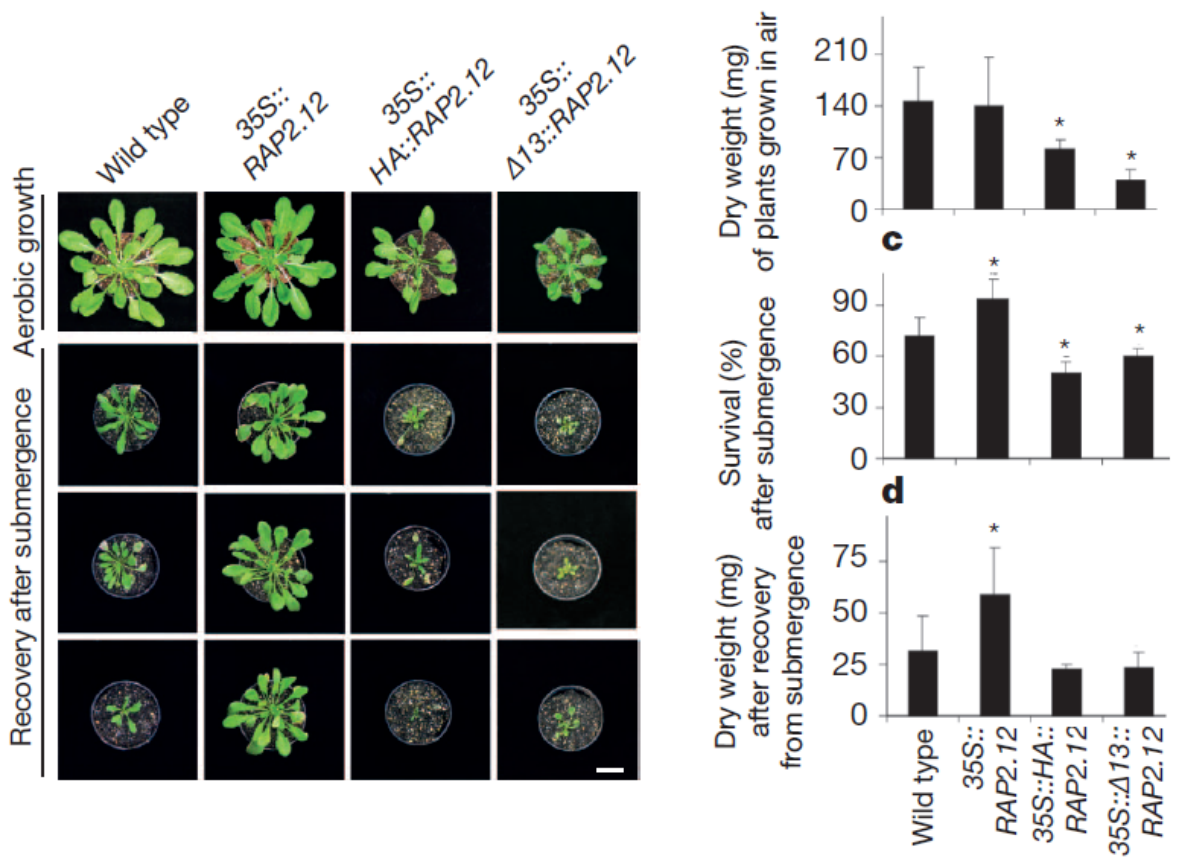
Francesco Licausi[1,2], Monika Kosmacz[1], Daan A. Weits[1], Beatrice Giuntoli[2], Federico M. Giorgi[1], Laurentius A. C. J. Voesenek[3,4], Pierdomenico Perata[2] & Joost T. van Dongen[1]

LETTER

doi:10.1038/nature10534

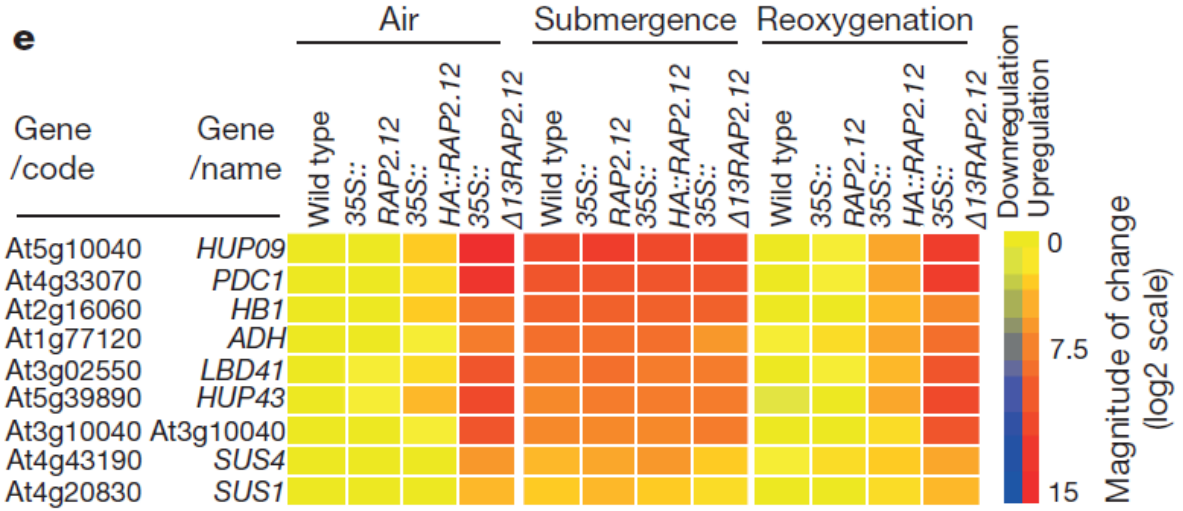
Homeostatic response to hypoxia is regulated by the N–end rule pathway in plants

Daniel J. Gibbs[1]*, Seung Cho Lee[2]*, Nurulhikma Md Isa[1], Silvia Gramuglia[1], Takeshi Fukao[2], George W. Bassel[1], Cristina Sousa Correia[1], Françoise Corbineau[3], Frederica L. Theodoulou[4], Julia Bailey-Serres[2] & Michael J. Holdsworth[1]

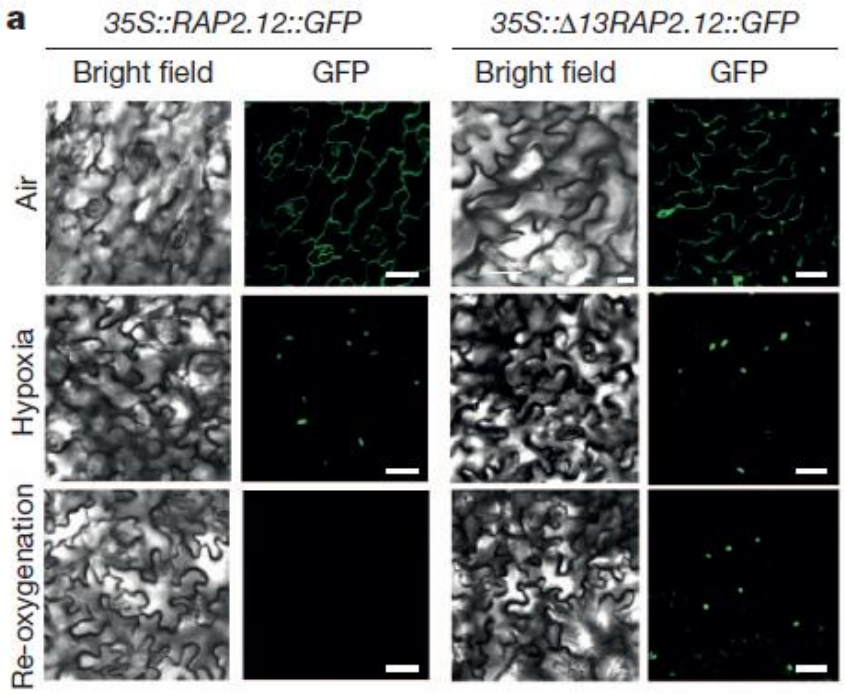


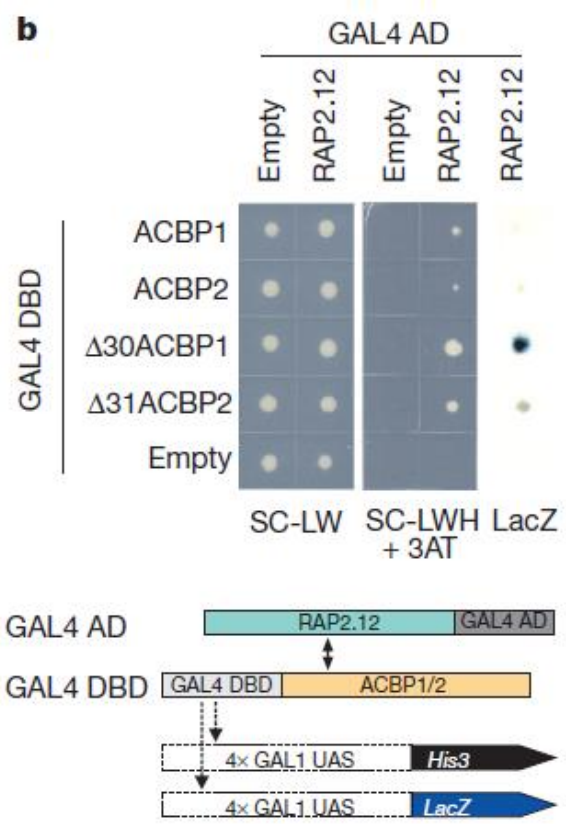
In Arabidopsis, a conserved amino-terminal amino acid sequence of the ethylene response factor (ERF)-transcription factor RAP2.12

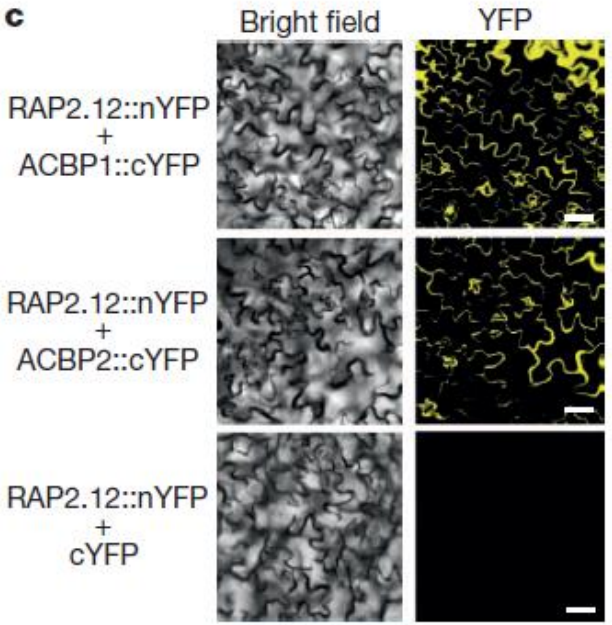
- 35S::RAP2.12 = constitutive overexpression ofRAP2.12
- 35S::HA::RAP2.12 = haemagglutinin (HA)-peptide tag at its N terminus
- RAP2.12 was expressed from which the first 13 amino acid residues were deleted (35S::Δ13RAP2.12).

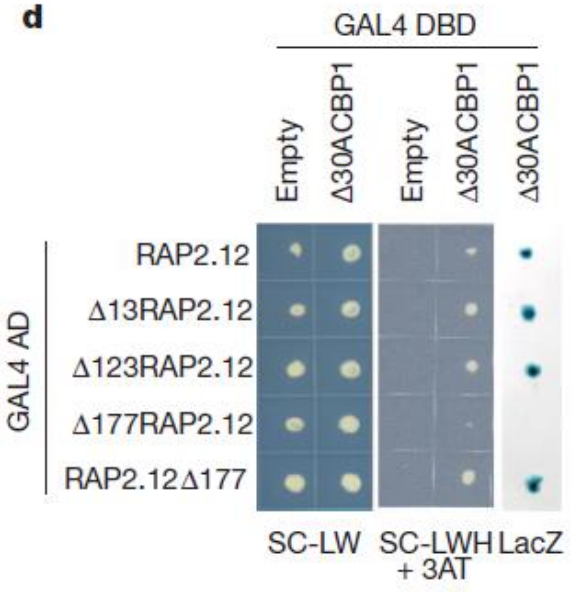


hypoxia marker genes









RAP2.12 is membrane localized and re-localizes in the nucleus upon hypoxia.

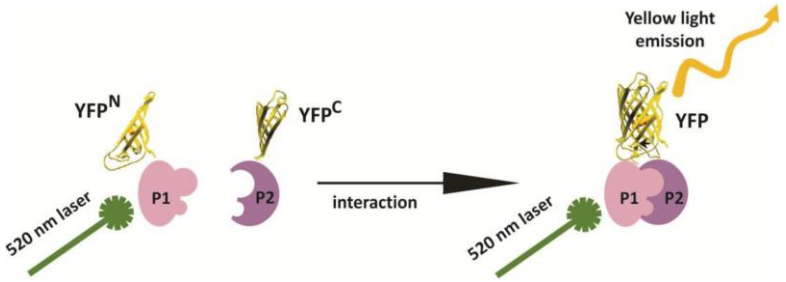
a, Subcellular localization of stably transformed GFP-fused RAP2.12 and Δ13RAP2.12.

b, Yeast two-hybrid analysis showing interaction between RAP2.12 and ACBP1 and ACBP2

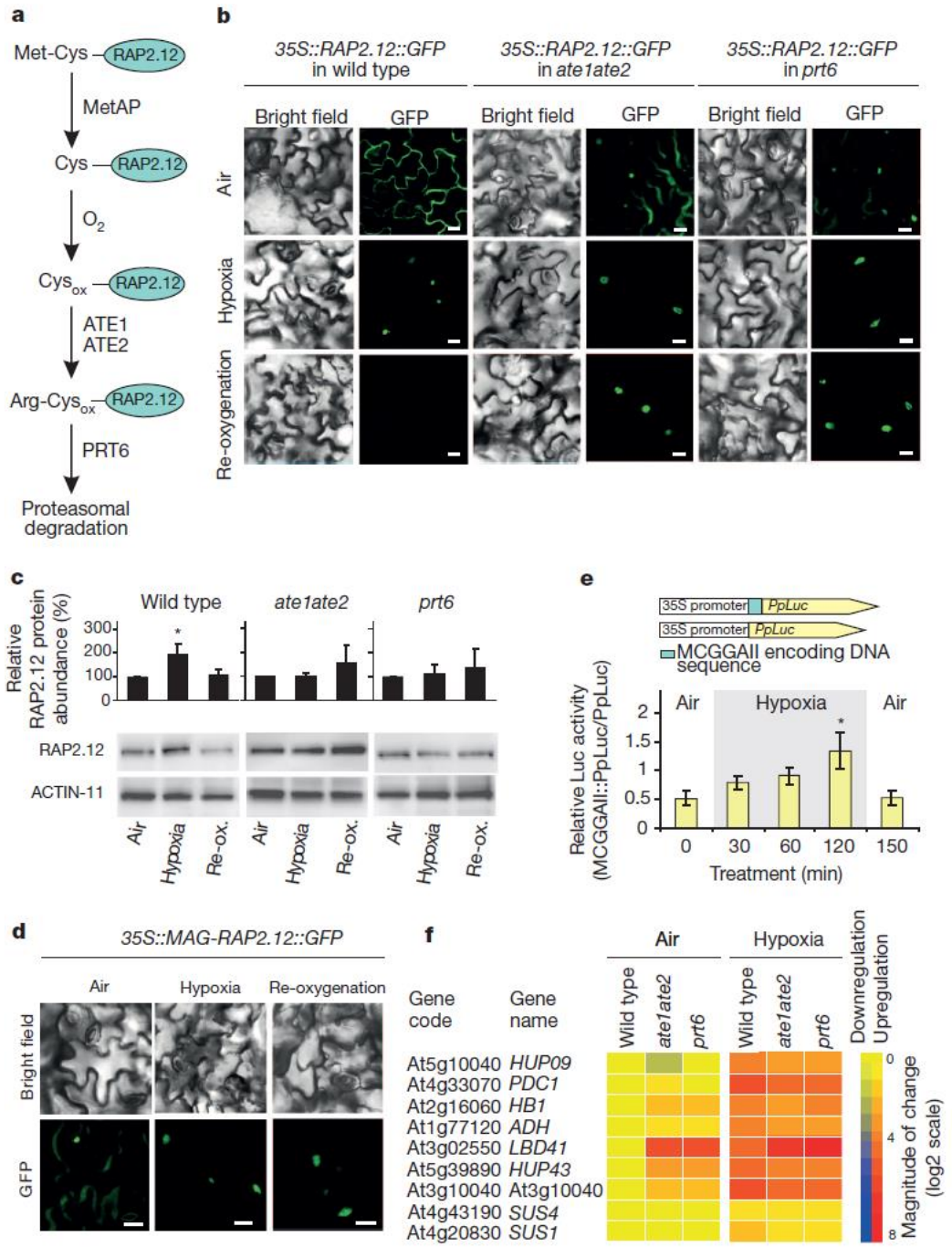
c, Bimolecular fluorescence complementation of YFP confirming interaction between RAP2.12 and ACBP1 and ACBP2.

d, Yeast two-hybrid analysis between various truncated RAP2.12 proteins and D30ACBP1.

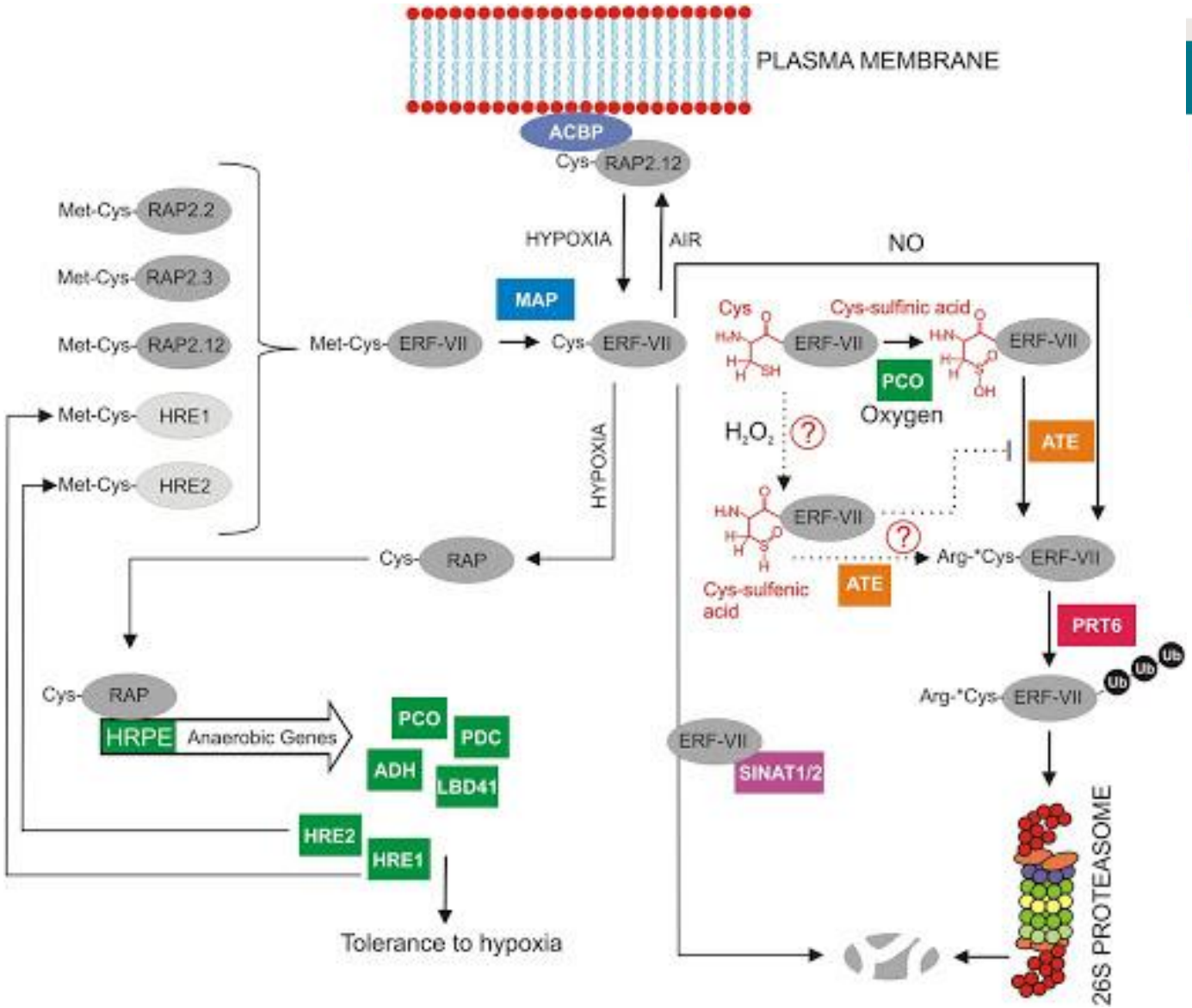
AD, activation domain; DBD, DNA-binding domain; UAS, upstream activator sequence.

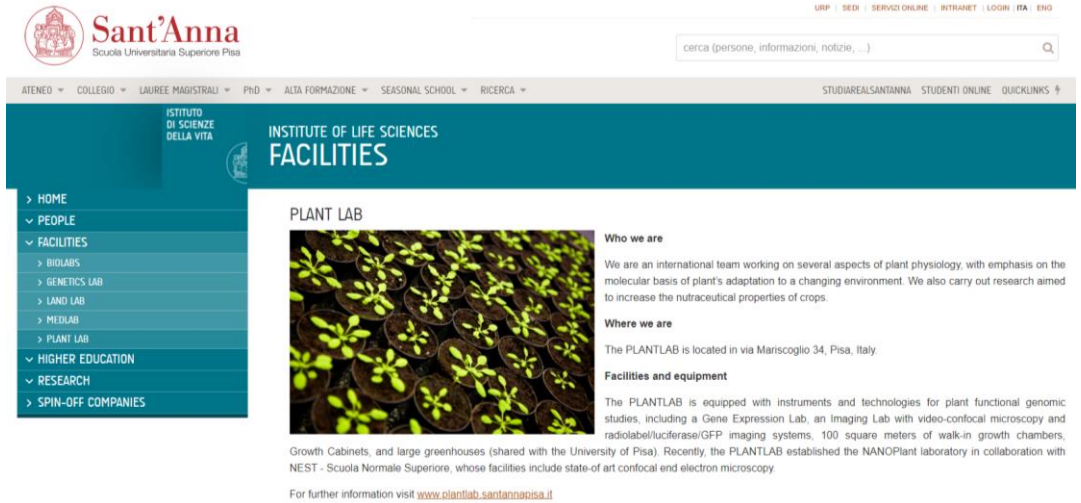


Oxygen-dependent destabilization of RAP2.12



1. According to this pathway the terminal Met is removed from the protein by methionine aminopeptidase (MetAP) when the second amino acid of the protein is Cys
2. Terminal Cys is oxidized to cysteine sulphenic acid in an oxygen-dependent manner before arginine transferase (ATE) conjugates an Arg residue to the protein
3. This triggers subsequent ubiquitination by the ligase PROTEOLYSIS 6 (PRT6) and targets the protein to the proteasome for degradation


PLASMA MEMBRANE
ACBP
Cys-RAP2.12
HYPOXIA
AIR
NO
MAP
Met-Cys-RAP2.2
Met-Cys-RAP2.3
Met-Cys-RAP2.12
Met-Cys-ERF-VII
Cys-ERF-VII
Met-Cys-HRE1
Met-Cys-HRE2
HYPOXIA
Cys-RAP
Cys-RAP
HRPE
Anaerobic Genes
PCO
PDC
ADH
LBD41
HRE2
HRE1
Tolerance to hypoxia
Cys
Cys-sulfinic acid
ERF-VII
PCO
Oxygen
H_2O_2
ATE
Cys-sulfenic acid
ATE
Arg-*Cys-ERF-VII
PRT6
Ub
Arg-*Cys-ERF-VII
ERF-VII
SINAT1/2
26S PROTEASOME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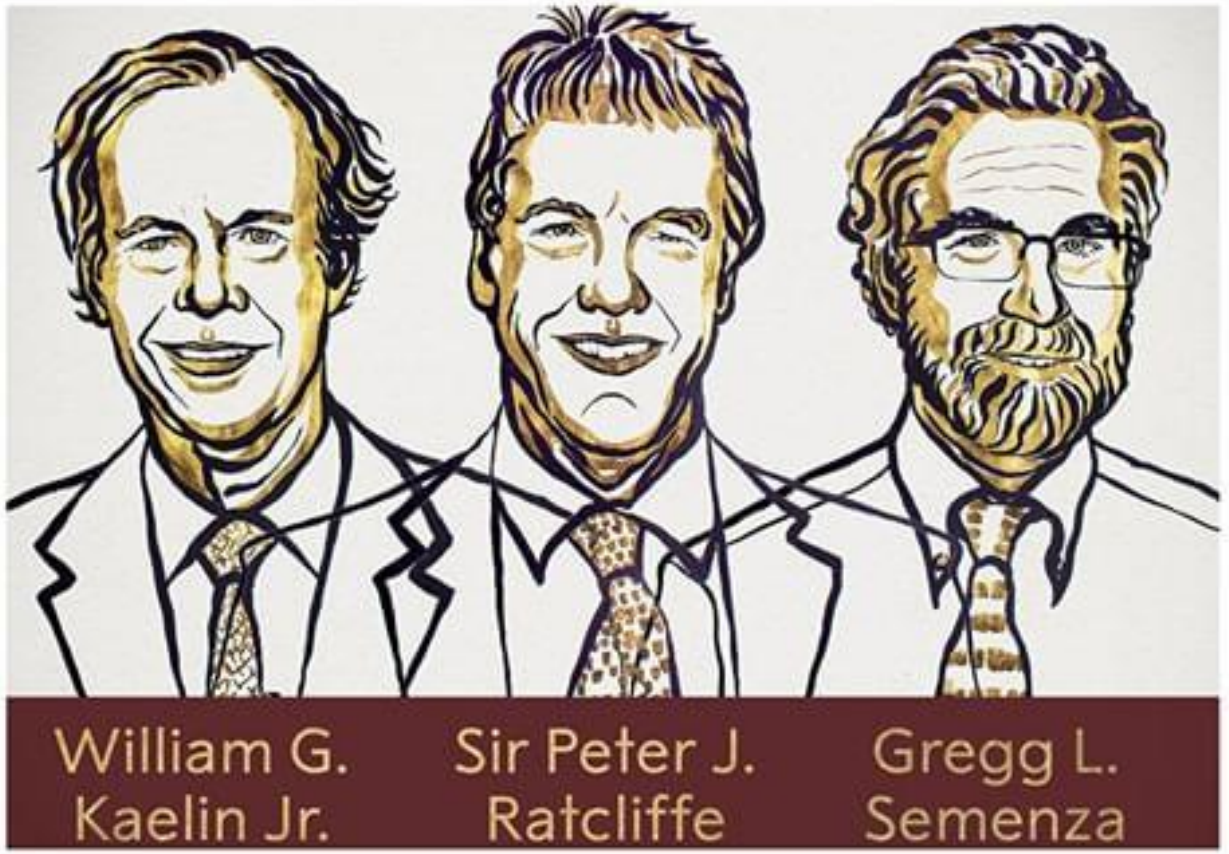
PLANT LAB
Who we are
We are an international team working on several aspects of plant physiology, with emphasis on the molecular basis of plant's adaptation to a changing environment. We also carry out research aimed to increase the nutraceutical properties of crops.
Where we are
The PLANTLAB is located in via Mariscoglio 34, Pisa, Italy.
Facilities and equipment
The PLANTLAB is equipped with instruments and technologies for plant functional genomic studies, including a Gene Expression Lab, an Imaging Lab with video-confocal microscopy and radiolabel/luciferase/GFP imaging systems, 100 square meters of walk-in growth chambers, Growth Cabinets, and large greenhouses (shared with the University of Pisa). Recently, the PLANTLAB established the NANOPlant laboratory in collaboration with NEST - Scuola Normale Superiore, whose facilities include state-of art confocal end electron microscopy.
For further information visit www.plantlab.santannapisa.it

- William G. Kaelin Jr.
- Sir Peter J. Ratcliffe
- Gregg L. Semenza

Nobel prize for medicine goes to scientists who found out how cells sense oxygen



MEDICINE PRIZE

Biologists who decoded oxygen sensing win Nobel

Laureates' discovery underpins understanding of diseases such as anaemia and cancer.

BY HEIDI LEDFORD & EWEN CALLAWAY

A trio of researchers has won the 2019 Nobel Prize in Physiology or Medicine for describing how cells sense and respond to changing oxygen levels by switching genes on and off — a discovery that has been key in understanding human diseases such as cancer and anaemia.

The three scientists are cancer researcher William Kaelin at the Dana-Farber Cancer Institute in Boston, Massachusetts; physician–scientist Peter Ratcliffe at the University of Oxford, UK, and the Francis Crick Institute in London; and geneticist Gregg Semenza at Johns Hopkins University in Baltimore, Maryland.

The team also won the Albert Lasker Basic Medical Research Award in 2016.

Their work has helped researchers to understand how the body adapts to low oxygen levels by, for example, cranking out red blood cells and growing new blood vessels.

"This is a fundamental discovery that they've contributed to," says Celeste Simon, a cancer biologist at the University of Pennsylvania in Philadelphia. "All organisms need oxygen, so it's really important."

"The field really coalesced around this discovery, which was dependent on each one of their findings," says Randall Johnson, a physiologist at the University of Cambridge, UK, and the Karolinska Institute in Stockholm, and a member of the Nobel Assembly. "This really was a three-legged stool."

L TO R: UNIV. OF OXFORD; HARVARD UNIV.; JOHNS HOPKINS MEDICINE

Nobel prizewinners Peter Ratcliffe (left), William Kaelin (centre) and Gregg Semenza (right).

OXYGEN DEPRIVATION

The body's tissues can be deprived of oxygen during exercise or when blood flow is interrupted, such as during a stroke. Cells' ability to sense oxygen is also crucial for the developing fetus and placenta, as well as for tumour growth, because the mass of rapidly growing cells can deplete oxygen in a tumour's interior.

In work conducted in the 1990s, the scientists discovered the molecular processes that cells go through to respond to oxygen levels in the body. They found that central to this is a mechanism involving proteins called hypoxia-inducible factor (HIF) and VHL.

Semenza and Ratcliffe studied the regulation of a hormone called erythropoietin (EPO), which is crucial for stimulating the production of red blood cells in response to low oxygen levels. Semenza and his team identified a pair of genes that encode the two proteins that form the protein complex HIF, which turns on certain genes and boosts EPO production when oxygen is low.

Meanwhile, Kaelin showed that a gene called *VHL* also seemed to be involved in how cells respond to oxygen. Kaelin was studying a genetic syndrome called von Hippel-Lindau's disease; families with the disease carry mutations in *VHL*, and the condition raises the risk of certain cancers.

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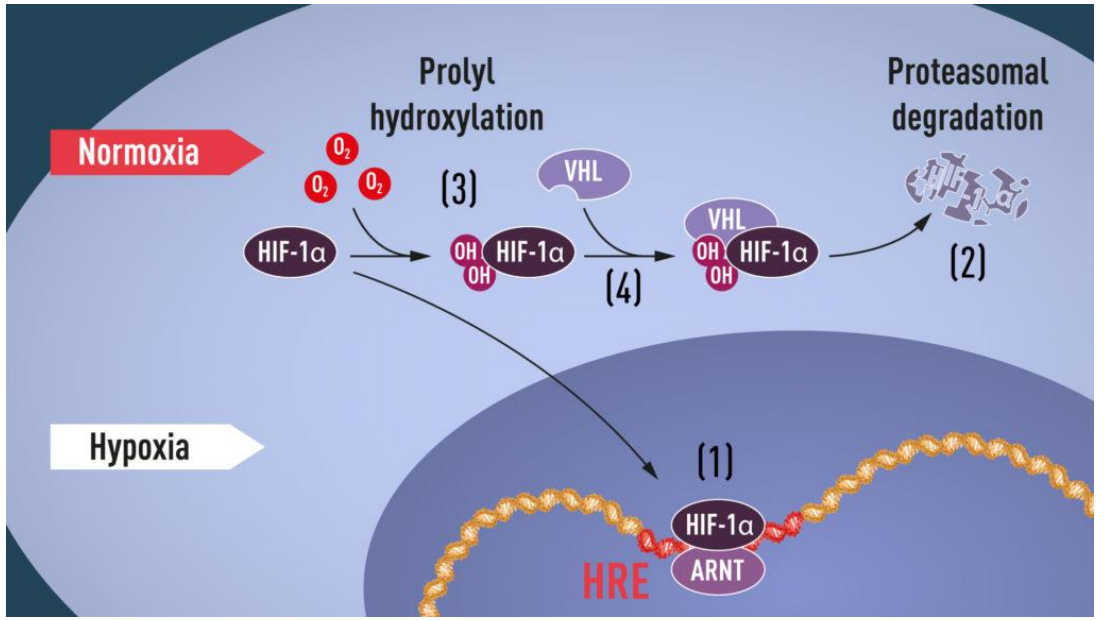


- Semenza discovered a protein complex he called "hypoxia-inducible factor" (HIF). Semenza further discovered that HIF is comprised of two transcription factors, now called HIF-1α and ARNT.
- Kaelin found that the VHL protein is needed to tag other proteins with ubiquitin. So without VHL the degradation of certain proteins is decreased, so their levels rise.
- Ratcliffe discovered that VHL interacts with HIF-1α, and is necessary for the degradation of HIF-1α at normal oxygen levels.

REVIEW SUMMARY

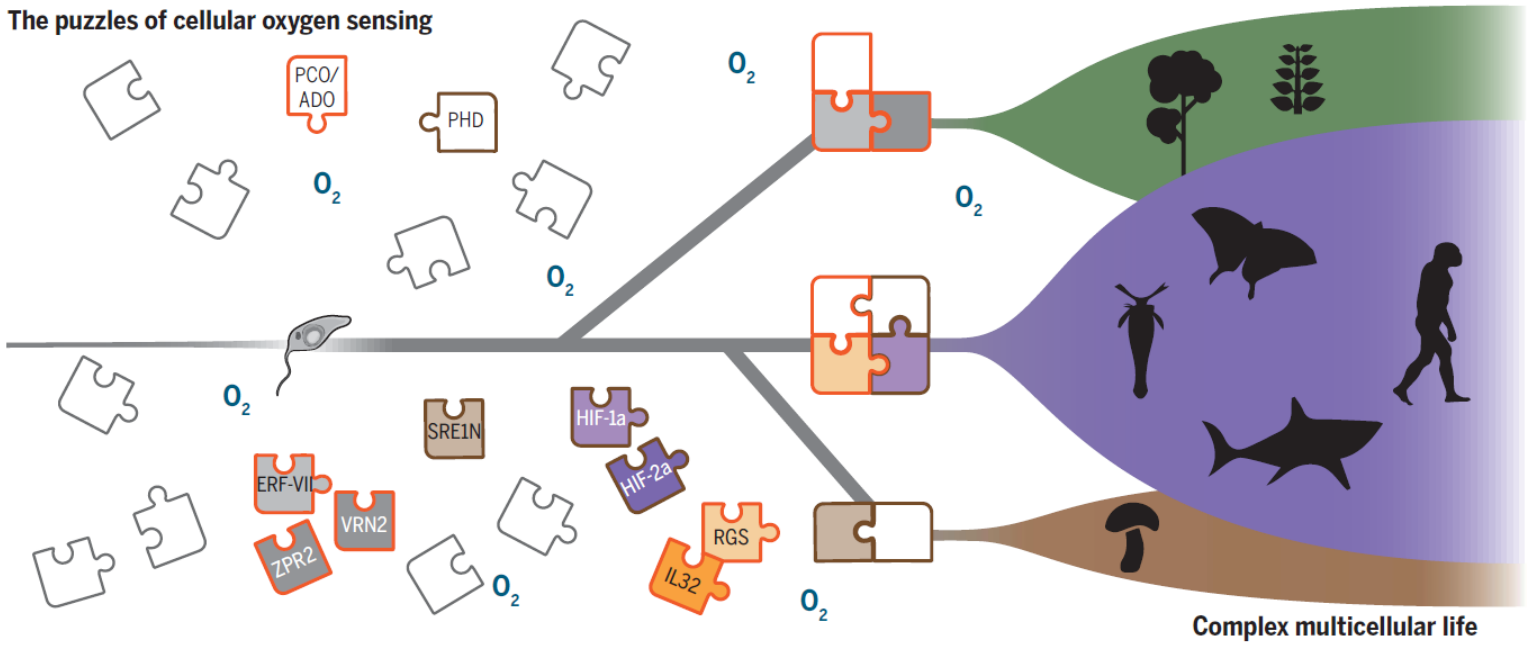
OXYGEN SENSING

Oxygen-sensing mechanisms across eukaryotic kingdoms and their roles in complex multicellularity

Emma U. Hammarlund*†, Emily Flashman, Sofie Mohlin, Francesco Licausi*†

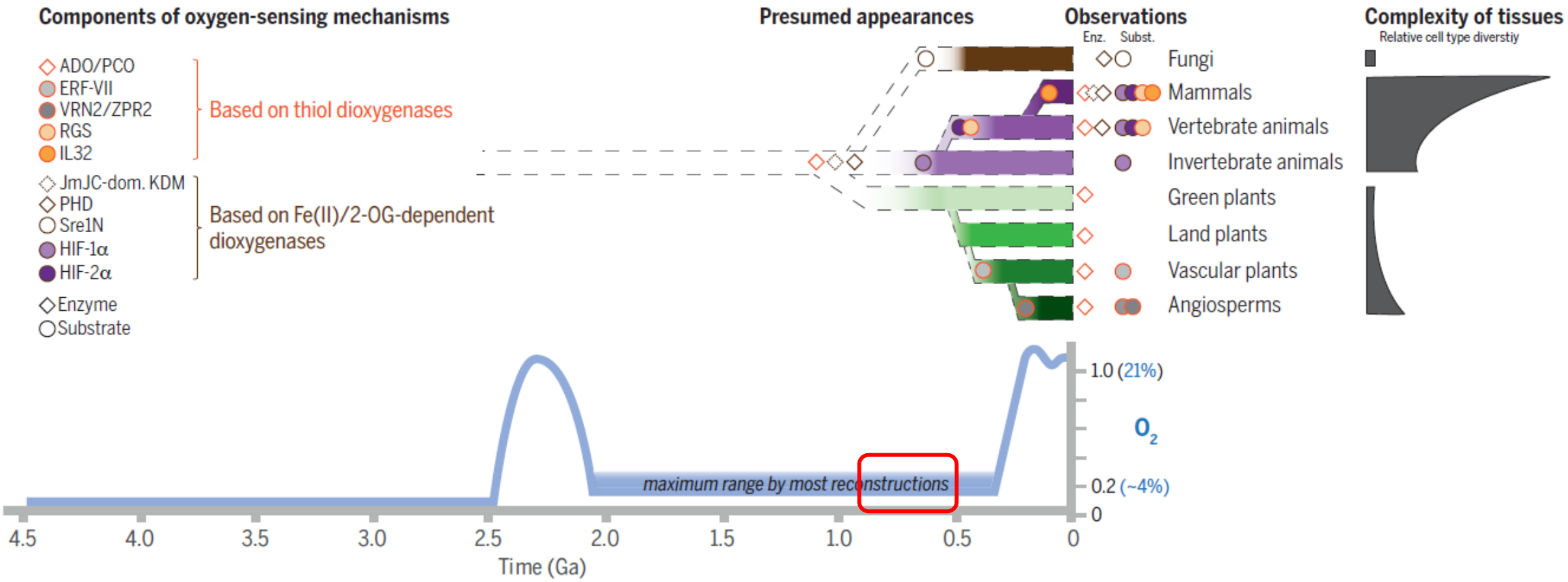
- Animals and land plants are the most **diverse** complex multicellular life-forms on Earth
- The performance of cell tasks, however, can be both dependent on and challenged by **oxygen**
- Oxygen acts as the final electron acceptor for **aerobic respiration** but also participates in reactions to **generate metabolites and structural macromolecules**
- Recently, oxygen also has come to the fore for its **signaling** role in developmental programs in animals and plants

For the rise of complex life, the capacity to link **oxygen perception to transcriptional responses** would have allowed organisms to attune cell fates to fluctuations in oxygen availability and metabolic needs in a spatiotemporal manner.



1. recruit dioxygenase enzymes to posttranslationally modify transcriptional regulators
2. proteasomal degradation at the relatively “normoxic” conditions
3. Transcriptional responses can be repressed at higher oxygen levels (which is context dependent) but are specifically elicited under hypoxia
4. the effects of prolonged hypoxia is also similar in animals and plants (transkingdom)

Increasing complexity of oxygen-sensing mechanisms and the extent of complexity within multicellular organisms over Earth's history of 4.6 Ga.



Enzymes (diamonds) and substrates (circles) form components of oxygen-sensing mechanisms, based on thiol dioxygenases (orange outlines) and Fe(II)/2-OG–dependent dioxygenases (brown outlines).

Reconstructions of atmospheric oxygen levels in the past. Eukaryotic kingdoms diversified (0.8 to 0.5 Ga ago), so the **evolution of oxygen-sensing mechanisms is rooted in hypoxic conditions**.
High atmospheric oxygen concentrations persisted at 2.5 to 2.0 Ga ago and then from 0.4 Ga ago (the Devonian Period) onward.

Oxygen sensing probes as future biotechnological application