LETTERS

The ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water

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Living organisms must acquire new biological functions to adapt to changing and hostile environments. Deepwater rice has evolved and adapted to flooding by acquiring the ability to significantly elongate its internodes, which have hollow structures and function as snorkels to allow gas exchange with the atmosphere, and thus prevent drowning[1–3]. Many physiological studies have shown that the phytohormones ethylene, gibberellin and abscisic acid are involved in this response[4–8], but the gene(s) responsible for this trait has not been identified. Here we show the molecular mechanism of deepwater response through the identification of the genes *SNORKEL1* and *SNORKEL2*, which trigger deepwater response by encoding ethylene response factors involved in ethylene signalling. Under deepwater conditions, ethylene accumulates in the plant and induces expression of these two genes. The products of *SNORKEL1* and *SNORKEL2* then trigger remarkable internode elongation via gibberellin. We also demonstrate that the introduction of three quantitative trait loci from deepwater rice into non-deepwater rice enabled the latter to become deepwater rice. This discovery will contribute to rice breeding in lowland areas that are frequently flooded during the rainy season.

Deepwater rice has a unique ability to elongate its internodes with increasing water depth (Fig. 1a, Supplementary Movies 1, 2, and Supplementary Fig. 1). We attempted to identify genes that regulate deepwater responses using quantitative trait loci (QTL) analysis combined with positional cloning. The non-deepwater rice variety Taichung65 (T65) shows little elongation under deepwater conditions (Fig. 1b left panel, 1c). In contrast, the deepwater rice C9285 shows significant elongation of leaves and internodes under deep water (Fig. 1b middle, 1c). Using the progeny of T65/C9285, we previously detected three major QTLs for deepwater response on chromosomes 1, 3 and 12 (refs 9, 10; Fig. 1b middle), and also detected these QTLs in the same chromosome regions in different populations[11,12]. Among these, the QTL on chromosome 12 was the most effective for deepwater response[9–12]. To evaluate the effects of the QTL on chromosome 12, we developed the nearly isogenic line 12 (NIL-12), which possesses the C9285 genomic fragment on chromosome 12 in a T65 genetic background (Supplementary Fig. 2a). NIL-12 responded to deep water by internode elongation (Fig. 1b right, 1c).

Positional cloning and gain-of-function analysis using a transgenic approach allowed us to identify two genes, *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*), that regulated the deepwater response (Supplementary Information, Supplementary Figs 3–5, Supplementary Table 1). The deepwater rice C9285 possesses *SK1* and *SK2*, although both genes are absent from the non-deepwater rice T65 (Supplementary Fig. 3c). *SK1* and *SK2* possess a putative nuclear localization signal and a single APETALA2/ethylene response factor (AP2/ERF) domain (Fig. 1d, Supplementary Fig. 6). On the basis of phylogenetic analysis using the AP2/ERF domain, the *SK* genes were categorized as belonging to the ERF subfamily (Supplementary Fig. 7). The ERF domains in the *SK* genes showed a high similarity to those of *Arabidopsis thaliana (At)ERF1*, *Oryza sativa (Os)ERF1* and *SUB1A-1* (Fig. 1e, Supplementary Table 2)[13–15]. However, other than the AP2/ERF domain, neither *SK1* nor *SK2* showed similarity to any other known genes. The *SK* genes were significantly expressed under deepwater conditions, whereas these expressions were low under dry conditions in C9285 (Fig. 1f). Furthermore, the *SK* genes were expressed in leaf blade, leaf sheath, and basal parts of the stem, including nodes and internodes, in which the deepwater response occurs (Fig. 1g). We also generated transgenic plants that overproduced *SK* genes driven by the *OsAct1* promoter in T65 background (Supplementary Table 1). Compared to vector control plants, *SK1*-overproducers elongated one to three internodes and *SK2*-overproducers elongated one to seven internodes, even under dry conditions (Fig. 1h). These results suggest that the *SK* genes regulate internode elongation.

Subcellular localization analysis using green fluorescent protein (GFP) showed that the SK proteins localized to nuclei (Fig. 2a). In addition, analysis using the yeast one-hybrid system showed that SK proteins have transcription-activating abilities (Fig. 2b, Supplementary Information). These results suggest that *SK* genes have the potential to act as transcription factors. As *SK* genes encode ERF, we investigated their involvement in ethylene signalling. Ethylene and five other basic phytohormones—that is, gibberellin (GA), brassinosteroid (BR), auxin (IAA), cytokinin (CK) and abscisic acid (ABA)—were applied to C9285. Of these, only ethylene significantly upregulated the expression of the *SK* genes (Fig. 2c). Thus, *SK1* and *SK2* are responsive to ethylene.

Previous studies in *Arabidopsis* have indicated that the *EIN3* (*ETHYLENE-INSENSITIVE 3*) gene product activates *AtERF1* expression by binding to the *AtERF1* promoter in the ethylene signalling pathway[16]. We analysed the binding of the rice *EIN3*-like gene product (OsEIL1b, Supplementary Fig. 8) to the promoter regions of *SK1* and *SK2* (Supplementary Information). Electrophoretic mobility shift assays (EMSAs) showed that recombinant OsEIL1b protein bound to the *SK* promoter regions (Fig. 2d, Supplementary Table 3a, Supplementary Fig. 9, Supplementary Information). These two regions contained the target core sequences of the EIN3-binding site (Supplementary Table 3b)[16]. These results indicate that *SK1* and *SK2* are novel *ERFs* involved in the deepwater response of deepwater rice.

Kende *et al.* reported that application of ethylene induces internode elongation in deepwater rice[3]. We also investigated the relationship between ethylene and deepwater response. Application of

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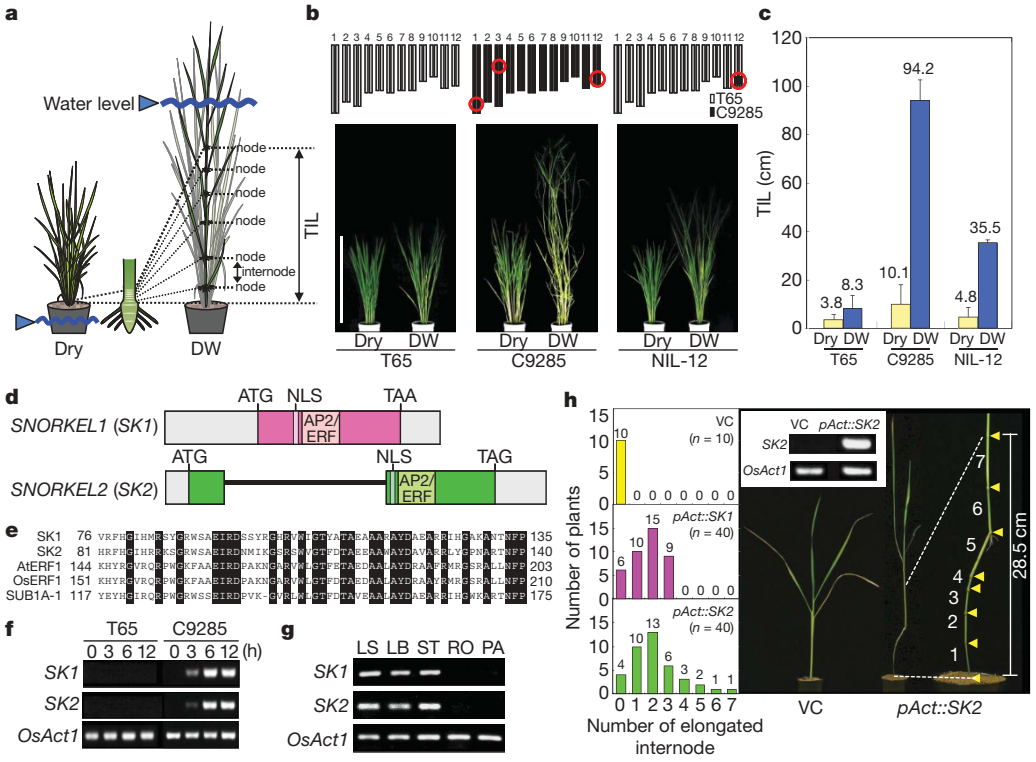

Figure 1 | Identification of genes responsible for deepwater response in rice. **a**, Image of deepwater response. Dry, water level is under the soil surface; DW, deepwater condition; TIL, total internode elongation length. **b**, Deepwater response in T65, C9285 and NIL-12. Red circles, positions of major QTLs[9,10]. Scale bar, 1 m. **c**, Quantitative evaluation of deepwater response in T65, C9285 and NIL-12. Data are mean + s.d., $n = 5$. **d**, Genome structures of *SK1* and *SK2*. Pink and green boxes, exons. White boxes, 3′ or 5′ non-coding regions. NLS, nuclear localization signal. **e**, AP2/ERF domain in SKs. Black shading, identical amino acid residues. **f**, Expression analysis of *SK* genes in T65 and C9285. **g**, Organ-specific expression of *SK* genes. LS, leaf sheath; LB, leaf blade; ST, basal part of stem; RO, root; PA, panicle. **h**, Internode elongation in transgenic plants overproducing *SK* genes in T65 background under dry conditions. VC, vector control; *pAct::SK1*, overproducing *SK1*; *pAct::SK2*, overproducing *SK2*. Ten-leaf-stage plants were submerged for two weeks (**b**, **c**), for indicated times (**f**), or for 12 h (**g**). The rice actin gene (*OsAct1*) was used as a control (**f**, **g**).

ethylene enhanced internode elongation of C9285, whereas no such effect was observed in T65 (Fig. 2e). In addition, application of ethylene along with its inhibitor 1-methylcyclopropene (1-MCP) suppressed internode elongation. We also compared the internode elongation of C9285 in deepwater conditions with or without 1-MCP pretreatment (Fig. 2f). The internode elongation of plants pretreated with 1-MCP under deepwater conditions was less than in those without pretreatment. These results indicate that ethylene is involved in deepwater response. Ethylene is known to accumulate in deepwater rice under deepwater conditions[4]. We examined whether ethylene accumulation is a common phenomenon for both deepwater and non-deepwater rice (Supplementary Fig. 10). Under deepwater conditions, ethylene content increased about 25-fold within 24 h in both T65 and C9285 (Fig. 2g). This observation indicates that ethylene accumulation under deepwater conditions is a common response of both deepwater and non-deepwater rice. The ethylene diffusion rate in water is 1/10,000 of that in air[17]. Both ethylene production and its low diffusion rate in deep water would contribute to ethylene accumulation in rice.

The above results suggest a molecular mechanism for the deepwater rice response regulated by *SK1* and *SK2*. Under deepwater conditions, ethylene accumulates in both deepwater and non-deepwater rice. The accumulated ethylene triggers the expression of the *SK* genes, leading to the induction of internode elongation in deepwater rice. In contrast, as non-deepwater rice T65 lacks *SK* genes, it shows no response to deepwater conditions.

Next, we examined the phytohormone contents of T65 and C9285 before and after submergence using liquid chromatography/mass spectrometry (LC/MS). Among the plant hormones investigated, GA_1 is the active GA, and its level in nodes was elevated after submergence of C9285, whereas the GA_1 content in T65 did not show any marked increase before or after submergence (Fig. 3a). The concentrations of other phytohormones were not significantly different between T65 and C9285 (Supplementary Table 4, Supplementary Information). GA is known as the plant growth hormone[18]. Our results suggest that GA may be involved in the deepwater response. To examine this, we investigated the GA response in deepwater rice (Fig. 3b). In the presence of the GA biosynthetic inhibitor uniconazole, internode elongation under deepwater conditions was repressed in C9285, while internode elongation was induced without uniconazole. Application of GA induced internode elongation in C9285 under dry conditions. In addition, the repression of internode elongation by uniconazole was rescued by application of GA. These results supported the findings of previous physiological experiments that GA is involved in the deepwater response[5]. Signalling by *SK* genes may be directly or indirectly connected to GA, which then induces internode elongation.

We also investigated the evolution of *SK* genes. The wild rice species *Oryza rufipogon* (W0120) and *Oryza nivara* (W0106) are progenitors of *O. sativa*[19]. We examined the deepwater responses of W0120 and W0106, and found that W0120 responded dramatically to deepwater conditions with elongated internodes, whereas W0106 showed little response (Fig. 4a). We compared the sequences of the *SK* genes in these lines, using clones selected from bacterial artificial chromosome (BAC) libraries. W0120 possessed both *SK1* and *SK2*, whereas W0106 possessed *SK1* and had a new stop-codon in exon 2 of *SK2* by insertion of a transposon (Fig. 4b). We detected the same stop-codon in other accessions of *O. nivara* (IRGC105319, IRGC105703, Supplementary Information). Gain-of-function analysis of the *SK* genes suggested that *SK2* has a more pronounced effect than *SK1* (Supplementary Information, Supplementary Fig. 5). Furthermore, recent research suggests that some *O. rufipogon* are adapted to wet areas and some *O. nivara* are adapted to dry areas[20]. This mutation in *SK2* would not

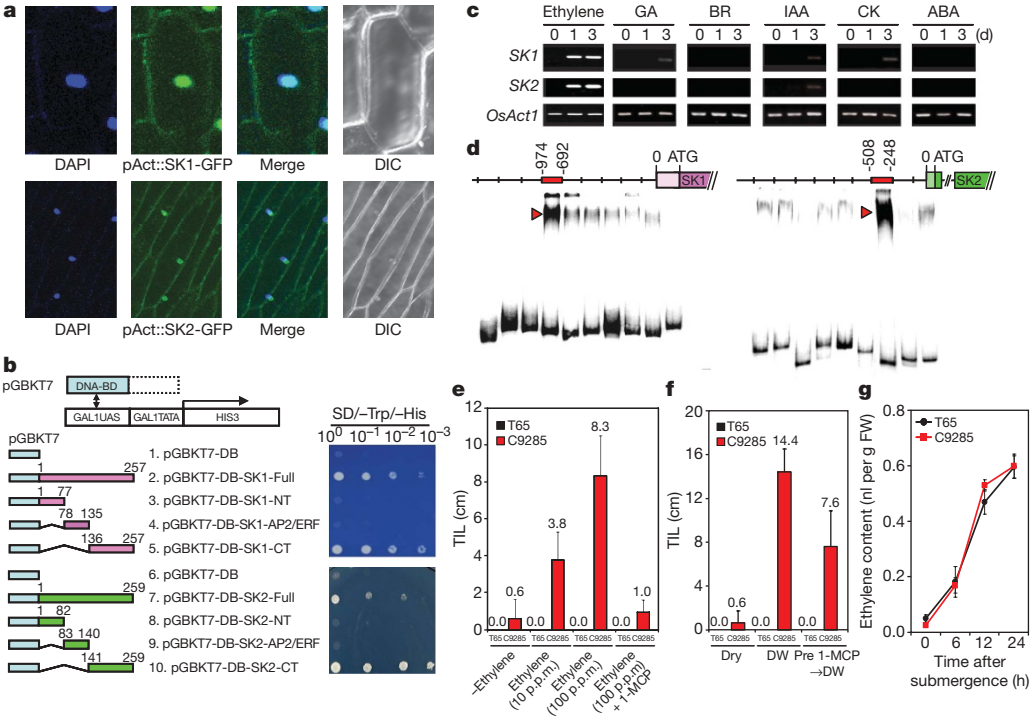

Figure 2 | Molecular characterization of *SK1* and *SK2*. **a**, Subcellular localization of SK1 and SK2 in onion epidermal cells. pAct::SK1-GFP and pAct::SK2-GFP activity was visible in nuclei. **b**, Transactivation activity assay in yeast. The corresponding full-length cDNA (full), N-terminal (NT), C-terminal (CT), or AP2/ERF domains alone (AP2/ERF) of *SK1* and *SK2* were fused to the GAL4-DNA binding domain (BD). The amino acid numbers are indicated above the fragment bars. **c**, Relationships between plant hormones and induction of *SK* genes. 10 p.p.m. ethylene, 10 μM GA, 10 nM BR, 20 μM IAA, 1 μM CK, 100 μM ABA were used. **d**, Sequence-specific binding of the recombinant OsEIL1b protein to *SK* promoters. Arrowheads indicate shifted bands. **e**, Ethylene promotes internode elongation without deepwater conditions in deepwater rice. Data are mean + s.d., $n = 8$. **f**, Internode elongation under deepwater conditions with ethylene inhibitor, 1-MCP (10 p.p.m.). Pre 1-MCP→DW, plants were pretreated by 1-MCP for 3 days before deepwater treatment; mean + s.d., $n = 8$. **g**, Measurement of ethylene content under deepwater conditions. g FW, grams fresh weight. Mean ± s.d., $n = 8$. Six-leaf-stage plants were subjected to each treatment for one week (**e**, **f**) or for the indicated time (**g**).

distinguish between *O. rufipogon* and *O. nivara*. However, in dry areas, *SK2* may be dispensable, and adaptation to such environment may have resulted in the loss of *SK2* in W0106. The wild rice species *Oryza glumaepatula* (IRGC105668), which grows in areas along the Amazon River in South America that are flooded during the rainy season, responds to deepwater conditions (Fig. 4a), and sequence analysis of its BAC clones showed that it possesses *SK2* and *SK2*-like genes, but not *SK1* (Fig. 4b). These results suggest that *SK2* is important for the deepwater response. As some wild rice species possess the *SK* genes, these genes may have been acquired before or during wild rice species divergence.

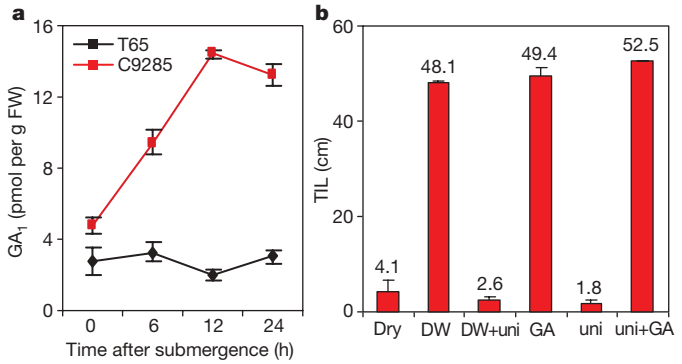

Figure 3 | GA response and molecular mechanism of deepwater response. **a**, GA_1 content in C9285 under deepwater conditions. Mean ± s.d., $n = 4$. **b**, GA responsiveness in C9285. Ten-leaf-stage plants were treated with 100 μM GA_3 with or without 1 μM uniconazole (uni) for one week. Mean ± s.d., $n = 8$.

Deepwater rice is mainly cultivated in lowland areas, and it is the only crop that can be grown in flooded areas in the rainy season. One problem with the cultivation of deepwater rice is its low yield[2]. Deepwater rice bred for these areas must possess not only internode elongation ability under deepwater conditions, but must also produce higher yields. To date, we have identified three QTLs on chromosomes 1, 3 and 12 that regulate the deepwater response[9,10]. To evaluate the deepwater responses of these QTLs, NILs and QTL pyramiding lines[21,22] were produced. Four QTL pyramiding lines were produced, possessing two QTLs on chromosomes 1 and 3 (NIL-1+3), 3 and 12 (NIL-3+12), 1 and 12 (NIL-1+12), and three QTLs on chromosomes 1, 3 and 12 (NIL-1+3+12) in a T65 genetic background (Fig. 4c, d). In particular, the QTL pyramiding line NIL-1+3+12 showed almost the same deepwater response as C9285. These results indicate that introducing these three QTLs into non-deepwater rice enables it to behave like deepwater rice. Therefore, the QTL pyramiding strategy is a powerful and efficient tool for breeding rice varieties for flood-prone areas. Recently, genes that regulate yield have been identified[21,23–26]. The combination of deepwater and yield QTLs has the potential to help breeding in these areas.

In this study, we showed that *SK* genes encoding ERFs trigger internode elongation in deepwater rice. Another water tolerance gene, *SUB1A*, which encodes an ERF (located on chromosome 9), was previously identified in rice[14,15]. *SUB1A* restricts plant elongation during flash floods at the seedling stage. In general, flash floods only continue for a few weeks. Plants carrying *SUB1A* show stunting and can survive in water for a few weeks. They avoid the energy consumption associated with plant elongation, and plant growth restarts after the flood recedes. We tested for the *SUB1A* allele and found that it is

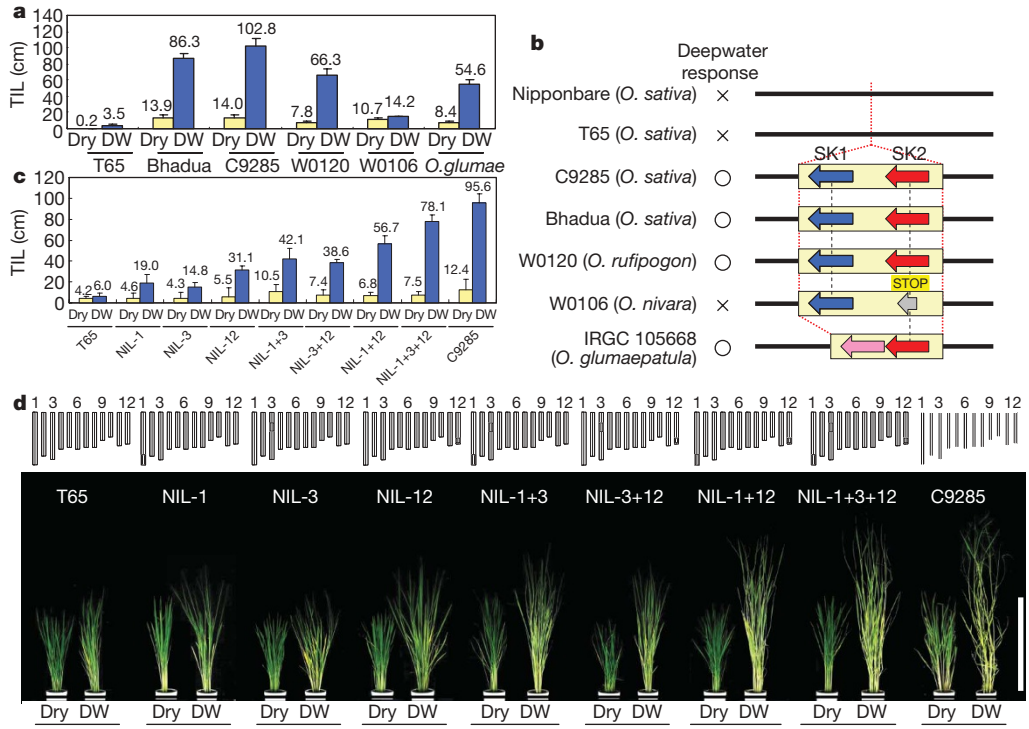

Figure 4 | *SK* genes in wild rice species and QTL pyramiding. **a**, Quantitative evaluation of deepwater response in six rice lines. *O. glumae*, *O. glumaepatula*; mean ± s.d., $n = 5$. **b**, Genome structures of the *SK* regions in cultivars and wild rice species. The region corresponding to *SK* genes in each plant are shown in yellow boxes. Blue, red and pink arrows indicate *SK1*, *SK2* and *SK2*-like gene, respectively. **c**, Quantitative evaluation of deepwater response in NILs and pyramiding lines. Mean + s.d., $n = 5$. **d**, QTL pyramiding for rice breeding. Scale bar, 1 m. Ten-leaf-stage plants were submerged for two weeks (**a**, **c**).

absent in both C9285 and T65 (Supplementary Fig. 11, Supplementary Information). *SUB1A* negatively regulates GA responses via restriction of SLR1 and SLRL1 degradation[27]. In contrast, *SK1* and *SK2* may stimulate GA. Both *SK* genes and *SUB1A* encode ERFs and are connected to GA, but they have opposing functions in regulating plant growth in response to flooding. Further investigation of the functional diversity of *SK1*, *SK2* and *SUB1A* is required.

We have identified the genes that allow rice to use natural variation to adapt to deepwater conditions. Loss-of-function mutants are commonly used to identify gene function, with cultivars as the wild type. However, as shown in this study, agriculturally important genes or unique characteristics, such as environmental adaptability, may have been lost in cultivars. In such cases, the wild rice species can be considered wild types and the cultivars as mutants. The study of wild species provides an opportunity to find agriculturally important genes that can be used to develop new varieties and improve existing cultivars.

METHODS SUMMARY

Three deepwater rice lines, W0120, C9285 and Bhadua, two non-deepwater rice lines, W0106 and T65, and one *O. glumaepatula* strain were used in this study. Plants were grown for an appropriate period and subjected to deepwater treatment in water up to 70% of the plant height, and then the total internode elongation length from base to the topmost node was measured. Molecular cloning of *SK1* and *SK2* was performed by a positional cloning and gain-of-function method. Gene expression analyses were performed by RT–PCR using gene specific primers. Microscopic observations were conducted using a GFP to detect the subcellular localization of SK proteins. Details of the above experiments and all other experiments performed in this paper are described in Methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 23 April; accepted 30 June 2009.

1. Vergara, B. S., Jackson, B. & De Datta, S. K. in *Climate and Rice* (eds IRRI) 301–319 (IRRI, Los Baños, 1976).
2. Catling, D. *Rice in Deepwater* (Macmillan, London, 1992).
3. Kende, H., Van der Knaap, E. & Cho, H.-T. Deepwater rice: a model plant to study stem elongation. *Plant Physiol.* **118,** 1105–1110 (1998).
4. Métraux, J.-P. & Kende, H. The role of ethylene in the growth response of submerged deep water rice. *Plant Physiol.* **72,** 441–446 (1983).
5. Raskin, I. & Kende, H. Role of gibberellin in the growth response of submerged deep water rice. *Plant Physiol.* **76,** 947–950 (1984).
6. Hoffmann-Benning, S. & Kende, H. On the role of abscisic acid and gibberellin in the regulation of growth in rice. *Plant Physiol.* **99,** 1156–1161 (1992).
7. Azuma, T. *et al.* Involvement of the decrease in levels of abscisic acid in the internodal elongation of submerged floating rice. *J. Plant Physiol.* **14,** 323–328 (1995).
8. Bailey-Serres, J. & Voesenek, L. A. C. J. Flooding stress: acclimations and genetic diversity. *Annu. Rev. Plant Biol.* **59,** 313–339 (2008).
9. Hattori, Y. *et al.* A major QTL confers rapid internode elongation in response to water rise in deepwater rice. *Breed. Sci.* **57,** 305–314 (2007).
10. Hattori, Y. *et al.* Mapping of three QTLs that regulate internode elongation in deepwater rice. *Breed. Sci.* **58,** 39–46 (2008).
11. Nemoto, K. *et al.* Inheritance of early elongation ability in floating rice revealed by diallel and QTL analyses. *Theor. Appl. Genet.* **109,** 42–47 (2004).
12. Kawano, R. *et al.* Mapping of QTLs for floating ability in rice. *Breed. Sci.* **58,** 47–53 (2008).
13. Nakano, T., Suzuki, K., Fujimura, T. & Shinshi, H. Genome-wide analysis of the ERF gene family in *Arabidopsis* and Rice. *Plant Physiol.* **140,** 411–432 (2006).
14. Xu, K. *et al. Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* **442,** 705–708 (2006).
15. Fukao, T., Xu, K., Ronald, P. C. & Bailey-Serres, J. A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. *Plant Cell* **18,** 2021–2034 (2006).
16. Solano, R., Stepanova, A., Chao, Q. & Ecker, J. R. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes Dev.* **12,** 3703–3714 (1998).
17. Jackson, M. B. Ethylene and responses of plants to soil waterlogging and submergence. *Annu. Rev. Plant Physiol.* **36,** 145–174 (1985).
18. Hooley, R. Gibberellins: perception, transduction and responses. *Plant Mol. Biol.* **26,** 1529–1555 (1994).
19. Cheng, C., Tsuchimoto, S., Ohtsubo, H. & Ohtsubo, E. Evolutionary relationships among rice species with AA genome based on SINE insertion analysis. *Genes Genet. Syst.* **77,** 323–334 (2002).
20. Vaughan, D. A., Lu, B.-R. & Tomooka, N. The evolving story of rice evolution. *Plant Sci.* **174,** 394–408 (2008).
21. Ashikari, M. *et al.* Cytokinin oxidase regulates rice grain production. *Science* **309,** 741–745 (2005).

22. Ashikari, M. & Matsuoka, M. Identification, isolation and pyramiding of quantitative trait loci for rice breeding. *Trends Plant Sci.* **11,** 344–350 (2006).
23. Fan, C. *et al. GS3,* a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor. Appl. Genet.* **112,** 1164–1171 (2006).
24. Song, X.-J. *et al.* A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nature Genet.* **39,** 623–630 (2007).
25. Shomura, A. *et al.* Deletion in a gene associated with grain size increased yields during rice domestication. *Nature Genet.* **40,** 1023–1028 (2008).
26. Huang, X. *et al.* Natural variation at the *DEP1* locus enhances grain yield in rice. *Nature Genet.* **41,** 494–497 (2009).
27. Fukao, T. & Bailey-Serres, J. Submergence tolerance conferred by *Sub1A* is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. *Proc. Natl Acad. Sci. USA* **105,** 16814–16819 (2008).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank I. Aichi for helping to produce the transgenic lines and M. Ito for helping to map the genes. We also thank P. Chaiyawat for the opportunity to photograph the deepwater rice specimen and M. Kojima for technical assistance with the hormone analysis. This work was supported by a grant from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Integrated Research Project for Plants, Insects, and Animals using Genome Technology, QT-2003 and QT-4002) and a research fellowship from the Japan Society for the Promotion of Science (Y.H.). The wild rice lines used in this study were obtained from the National Institute of Genetics supported by the National Bioresource Project, MEXT, Japan and the International Rice Research Institute, Philippines.

Author Contributions M.A. conceived the project and designed the experiments. Y.H. identified the genes and Y.H., K.N., S.F., X.-J.S. and R.K. performed molecular characterization of the genes. H.S. and H.M. surveyed the hormone contents. J.W. and T.M. performed BAC clone analysis. A.Y., H.K. and M.M. provided advice regarding the experiments. M.A. and Y.H. wrote the manuscript.

Author Information The DDBJ accession numbers for *SNORKEL1* and *SNORKEL2* are as follows (rice variety, accession numbers): C9285, AB510478 and AB510479; Bhadua, AB510480 and AB510481; *O. rufipogon* (W0120), AB510482 and AB510483; and in *O. nivara* (W0106), AB510484 and AB510485. *SNORKEL2* and *SNORKEL2*-like genes in *O. glumaepatula* (IRGC105668) are AB510486 and AB510487. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.A. (ashi@agr.nagoya-u.ac.jp).

METHODS

Plant materials. The Indian wild rice species W0120 (*O. rufipogon*; perennial), which tolerates deepwater conditions[28], and the deepwater rice cultivars C9285 and Bhadua (*O. sativa*, ssp. *indica*) from Bangladesh were used as deepwater rice plants. An Indian wild rice species, W0106 (*O. nivara*; also called annual type *O. rufipogon*), and the cultivar Taichung65 (T65; *O. sativa* ssp. *japonica*) were used as rice plants lacking deepwater characteristics (non-deepwater rice plants) and as crossing material. The South American wild rice species *O. glumaepatula* (IRGC105668) and other *O. nivara* strains (IRGC105319, IRGC105703, IRGC105715 and IRGC106052) were also used in this study. The lines C9285, W0120, W0106 and Bhadua were kindly provided by the National Institute of Genetics of Japan, and IRGC105668, IRGC105319, IRGC105703, IRGC105715 and IRGC106052 was obtained from the International Rice Research Institute. T65 was maintained at Nagoya University.

Growth conditions and phenotypic evaluation. Plants were germinated and transplanted into small pots. During growth, the water level was beneath the soil surface (dry conditions, described as 'Dry' in figure). Plants were submerged in water up to 70% of the plant height. To evaluate plant growth as shown in Figs 1b, c, 3b, 4a, c and Supplementary Fig. 5, plants at the ten-leaf-stage were submerged for two weeks (Figs 1b, c, 4a, c) or one week with each treatment (Fig. 3b, Supplementary Fig. 5) before determining the TIL (total internode elongation length)[9]. For expression analysis of *SK*s and other genes in Figs 1f, g, 2c and Supplementary Fig. 4, ten-leaf-stage plants were submerged for indicated times (Figs 1f, 2c, Supplementary Fig. 4) or 12 h (Fig. 1g), and in Fig. 2e–g, six-leaf-stage plants were subjected to each treatment for one week (Fig. 2e, f) or for indicated times (Fig. 2g). For measurement of phytohormones contents in Fig. 3a and Supplementary Table 4, six-leaf-stage plants were submerged for the indicated time and the nodes were sampled.

Preparation of BAC libraries. BAC libraries were constructed from young leaves of C9285 (*O. sativa*), Bhadua (*O. sativa*), W0120 (*O. rufipogon*) and IRGC105668 (*O. glumaepatula*) by conventional methods through partial DNA digestion with HindIII, size fractionation of high molecular weight DNA by pulsed-field gel electrophoresis (CHEF, Bio-Rad Laboratories), vector ligation (pIndigo BAC-5; Epicentre Biotechnologies), and transformation into *Escherichia coli* (DH10B). Positive BAC clones were screened by PCR from the pooled DNA or by Southern hybridization from high-density BAC filters of each library that contained clones with insert DNA of sufficiently large size for at least sixfold genome coverage. A BAC clone of T65 (GN_42I09) was selected from BAC libraries and a BAC clone of W0106 was purchased from the Arizona Genomic Institute of the University of Arizona.

Production of NILs and pyramiding lines. The deepwater rice cultivar C9285 was crossed with the non-deepwater cultivar T65. NILs (NIL-1, NIL-3 and NIL-12: BC_4F_2) were developed by repeated backcrossing with T65. The pyramiding lines NIL-1+3, NIL-3+12, NIL-1+12 and NIL-1+3+12 were obtained by crossing with NILs.

Molecular cloning of *SK1* and *SK2*. A total of 16,000 progeny of heterozygote NIL-12 from T65/C9285 (BC_4F_2) and 12,000 progeny of heterozygote NIL-12B from T65/Bhadua (BC_4F_2) were used for positional cloning of *SK*s. Phenotypic evaluation of *SK*s under deepwater conditions was confirmed using BC_4F_3 (see Supplementary Information for details).

Full-length genomic DNA of *SK*s including the promoter region was inserted into the binary vector pYLTAC7. These DNA fragments were introduced into T65 by *Agrobacterium tumefaciens*-mediated transformation[29]. The empty vector was also introduced into T65 as a control.

To obtain the full-length sequences of *SK1* and *SK2*, 5′ and 3′ RACE was performed using 5′-Full RACE Core Set (TaKaRa) and 3′-Full RACE Core Set (TaKaRa) in accordance with the manufacturer's instructions.

RNA isolation and expression analysis. Total RNA was prepared as described previously[30]. First-strand cDNA was synthesized from 2 μg of total RNA using Omniscript Reverse Transcription kit (Qiagen). RT–PCR was performed as described previously[31] using gene-specific primers (Supplementary Table 1).

Plasmid construction and plant transformation. To construct plants overexpressing *SK*s, their cDNA was amplified and inserted into the pBI101 binary vector containing an *OsAct1* promoter. Plasmids were transformed into T65 as described previously[32]. Transgenic plants were selected by hygromycin resistance.

To construct GFP fusion proteins, *SK*s cDNA was amplified by PCR using gene-specific primers and fragments were inserted into the destination vector pUC119 to generate fusion constructs with GFP at the C-terminal of *SK*s under the control of the *OsAct1* promoter.

Microscopic observations. Gold particles were coated with the *pAct::SK1-GFP* and *pAct::SK2-GFP* fusion constructs and used to bombard onion epidermis with a PDS-1000/He biolistic system (Bio-Rad). The onion epidermis was incubated in the dark at 22 °C. After 24 h, the cell layers were soaked in 2 μg μl^{-1} 4′,6-diamidino-2-phenylindole dihydrochloride *n*-hydrate (DAPI; Dojindo) solution for visualization of the nucleus and analysis of the nuclear localization of pAct::SK1-GFP and pAct::SK2-GFP derivatives. The stained samples were observed with a confocal microscanning laser microscope (FV500; Olympus).

Transactivation activity assay. The GAL4-based Matchmaker Two-Hybrid System 3 (Clontech) was used for the transactivation activity assay. To construct pGBKT7-BD-SKs-Full, pGBKT7-BD-SKs-NT, pGBKT7-BD-SKs-AP2/ERF and pGBKT7-BD-SKs-CT, the full-length coding sequence, N-terminal, C-terminal, or AP2/ERF domain regions alone of *SK*s were amplified using PCR and confirmed by sequencing. The validated PCR products were inserted into pGBKT7 and fused with the GAL4-binding domain after digestion with EcoRI and PstI. All constructs were transformed into the yeast strain AH109. Each yeast liquid culture was serially diluted to $A_{600} = 0.6$, and 2 μl of each dilution was inoculated onto tryptophan- and histidine-negative synthetic dropout medium.

Quantification of phytohormones. Phytohormones (auxins, cytokinins, gibberellins and abscisic acid) were extracted from samples of about 100 mg (fresh weight) of the rice nodes. The phytohormones were quantified as described[33] using an LC/MS system (UPLC/Quattro Premier XE; Waters) with an ODS column (Acquity-UPLC BEH-C_{18}, 1.7 μm, 2.1 × 100 mm; Waters). A special device was used to measure ethylene (Supplementary Fig. 10). The plants were submerged in water for indicated times and then immediately transferred into a vacuum container filled with saturated NaCl solution. To collect the internal gas, the plant body was subjected to vacuum for 90 s, and emitted gas was trapped in the graduated test tube. After release from vacuum, the tube was sealed with rubber stopper in saturated NaCl solution. One millilitre of headspace gas in the tube was injected into a gas chromatograph (model GC353, GL Sciences Inc.) fitted with a flame-ionization detector and PoraBond Q capillary column (Varian Inc.).

Phytohormones and inhibitor treatment. For analysis of phytohormone responses, ten-leaf-stage plants were transferred to the closed chamber with 10 p.p.m. ethylene or water containing 10 μM GA, 10 nM BR, 20 μM IAA, 1 μM CK, or 100 μM ABA. Uniconazole (1 μM) and 1-MCP (10 p.p.m.) were used as a GA biosynthesis inhibitor and ethylene perception inhibitor. Plants were pretreated with both inhibitors for 3 days before the experiments.

Electrophoresis mobility shift assays (EMSAs). Full-length *OsEIL1b*[34] was amplified by PCR and inserted into pET-32a(+) vector (Novagen) with the His-tag coding sequence and overexpressed in Rosetta (DE3) (Novagen) to generate the OsEIL1b. The recombinant proteins were purified with a His-tag affinity column (Bio-Rad) and Superdex-200 gel filtration chromatography (GE Healthcare). The promoter fragment probes of *SK*s (Supplementary Table 3) were labelled with [^{32}P]dATP. The DNA binding reaction was allowed to proceed for 30 min at 4 °C in 20 μl of binding buffer (12.5 mM Tris-HCl, 60 mM NaCl, 0.25 mM DTT, 12.5% glycerin, 1 mM EDTA, 0.05% NP-40, and 2 μg of poly(dI-dC)·poly(dI-dC)) containing 0.5 ng of ^{32}P-labelled oligonucleotide probe and recombinant protein, and then electrophoresed through a 13% polyacrylamide gel in 0.25 × Tris-borate-EDTA buffer. Competition experiments were performed by adding unlabelled competitor oligonucleotides to the binding reaction with the subsequent addition of labelled oligonucleotides. Mutated forms of OsEIL1b binding site were obtained by the overlapping primer method (see Supplementary Table 3) and used in competition experiments.

28. Morishima, H., Hinata, K. & Oka, H. I. Floating ability and drought resistance in wild and cultivated species of rice. *Ind. J. Genet. Plant Breed.* **22,** 1–11 (1962).
29. Hood, E. E., Helmer, G. L., Fraley, R. T. & Chilton, M.-D. The hypervirulence of *Agrobacterium tumefaciens* A281 is encoded in a region of pTiBo542 outside of T-DNA. *J. Bacteriol.* **168,** 1291–1301 (1986).
30. Sambrook, J., Fritsch, E. F. & Maniatis, T. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1989).
31. Kaneko, M. *et al.* Where do gibberellin biosynthesis and gibberellin signaling occur in rice plants? *Plant J.* **35,** 104–115 (2003).
32. Hiei, Y., Ohta, S., Komari, T. & Kumashiro, T. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J.* **6,** 271–282 (1994).
33. Hirano, K. *et al.* Comprehensive transcriptome analysis of phytohormone biosynthesis and signaling genes in microspore/pollen and tapetum of rice. *Plant Cell Physiol.* **49,** 1429–1450 (2008).
34. Mao, C., Wang, S., Jia, Q. & Wu, P. *OsEIL1*, a rice homolog of the *Arabidopsis EIN3* regulates the ethylene response as a positive component. *Plant Mol. Biol.* **61,** 141–152 (2006).