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Deepwater response in the African cultivated rice *Oryza glaberrima*

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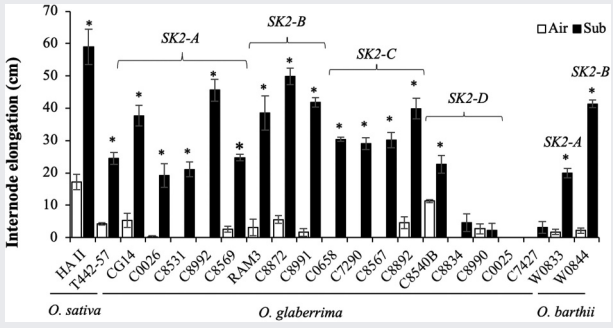
ABSTRACT

Partial submergence of *Oryza sativa* deepwater rice elicits enhancement of internodal elongation, referred to as deepwater response, conferred by three types of genes, *SNORKEL1/2* (*SK1/2*), *SEMIDWARF1* (*SD1*), and *ACCELERATOR OF INTERNODE ELONGATION 1* (*ACE1*). We investigated the presence and expression of these genes in the African cultivated rice *Oryza glaberrima* and the relationship between these genes and the deepwater response of *O. glaberrima*. In 49 of the 50 accessions tested, one or two *SK* genes were identified, which could be divided into three types of *SK1* and four types of *SK2*. The accessions with the *SK2* type whose expression was induced by submergence demonstrated rapid internodal elongation under submergence. In most of these accessions, submergence also increased the expression of *SD1* and *ACE1* genes. However, the accessions did not possess the haplotype of *SD1* that is associated with high deepwater response in *O. sativa*. In contrast, they possessed the type of *ACE1* gene similar to that in *O. sativa* deepwater rice. These results indicate that the molecular mechanisms underlying induction of deepwater response in *O. glaberrima* are similar to that found in deepwater rice of *O. sativa* and suggest that most *O. glaberrima* cultivars, including upland cultivars, can exhibit rapid internodal elongation under submergence.



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Introduction

Two cultigens, *Oryza sativa* L., Asian rice, and *Oryza glaberrima* Steud., African rice, exist in the genus *Oryza*. *O. sativa* is cultivated worldwide, including in Africa, whereas *O. glaberrima* is cultivated mainly in West Africa. Some *O. glaberrima* strains are adaptable to various abiotic and biotic stresses, such as drought, high soil iron, nematodes, and African rice gall midge (Diop et al., 2020; Jones, Dingkuhn, et al., 1997; Ndjiondjop et al., 2018). Therefore, *O. glaberrima* is a vital genetic resource for conferring stress tolerance to *O. sativa* grown in Africa (Jones, Mande, et al., 1997).

Flooding is an environmental stress that constrains the growth of most crops, thereby severely reducing crop yield. However, in the basins and deltas of large rivers in South and Southeast Asia and the inland delta of the Niger River in West Africa, deepwater rice is cultivated by taking advantage of seasonal flooding (Catling, 1992).

The adaptation of plants to flooding by promoting shoot elongation and thereby maintaining part of their foliage above the rising water surface is called deepwater response. This deepwater response, referred to as the 'escape strategy' for deepwater flooding, enables plants to continue aerobic respiration and photosynthesis even

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in flood environments, allowing them to survive. In the genus *Oryza*, cultivars and strains with deepwater response reportedly exist in the cultivated and wild species (Okishio et al., 2014; Sasayama et al., 2018, 2022), and the mechanism of the reaction has been investigated using the deepwater rice cultivars of *O. sativa* (Kende et al., 1998; Kuroha & Ashikari, 2020). In deepwater rice, shoot elongation is largely stimulated in submerged internodes, whose enhanced growth results from increased cell division in the intercalary meristem and increased elongation of the newly formed cells (Kende et al., 1998). This submergence-induced internodal elongation is triggered by ethylene accumulation in submerged tissues, which promotes gibberellin (GA) biosynthesis and/or signaling.

Three major quantitative trait loci (QTL) for submergence-induced internodal growth were detected on chromosomes 1, 3, and 12 using the progeny of the non-deepwater rice Taichung 65 (T65) and the deepwater rice C9285 (Hattori et al., 2007, 2008). The QTL pyramiding line possessing the three QTLs in the T65 genetic background exhibited almost the same internodal growth as C9285, and the QTL on chromosome 12 was the most effective (Hattori et al., 2009). Subsequently, the genes responsible for each QTL were identified. The first identified genes were *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*), which are located on chromosome 12 in deepwater cultivars of *O. sativa* and not in non-deepwater cultivars (Hattori et al., 2009). *SK1/2* genes, which encode ethylene response factor (ERF)-type transcription factors, are upregulated by ethylene and thereby may act directly or indirectly to promote GA biosynthesis and/or signaling, leading to the induction of internodal elongation through GA.

The second identified gene, located on chromosome 1, was *SEMIDWARF1* (*SD1*)/*GA20ox2* (Kuroha et al., 2018). *SD1* is a gibberellin biosynthetic gene encoding GA20-oxidase, and its null mutants, selected for the rice Green Revolution, display a semi-dwarf phenotype (Sasaki et al., 2002; Spielmeyer et al., 2002). The nucleotide sequences of *SD1* in *O. sativa* cultivars can be classified into six haplogroups (Hap-1 to −6) according to single nucleotide polymorphisms (SNPs) present in the promoter, exon, and intron regions (Kuroha et al., 2018). Rice cultivars without *SK* genes demonstrated no significant internodal elongation under submergence, regardless of the type of haplogroup of *SD1*, whereas cultivars harboring Hap-2 or Hap-6 with *SK* genes displayed significant promotion of elongation under submergence. In particular, the deepwater rice cultivars harboring Hap-6 exhibited higher *SD1* transcript accumulation under submergence, thereby exhibiting a high deepwater response.

The last identified gene, located on chromosome 3, is *ACCELERATOR OF INTERNODE ELONGATION 1* (*ACE1*), which encodes a protein of unknown function (Nagai et al., 2020). There are two genotypes of *ACE1*, deepwater and non-deepwater rice types. The genomic sequence of the deepwater rice *ACE1* has a 1-bp-deletion at position 73 compared to that in the non-deepwater rice, causing a frameshift that disrupts the nuclear localization signal in the predicted amino acid sequence. The expression of deepwater rice *ACE1* confers cells of the intercalary meristem with competence for cell division, leading to internodal growth in the presence of GA.

Most deepwater rice cultivars of *O. glaberrima* reportedly have strong elongation capacity, with some cultivars reaching a height of up to 6 m during gradual submergence (Catling, 1992). Although few reports exist on the elongation response of *O. glaberrima* deepwater rice compared to that of *O. sativa* deepwater rice (Inouye et al., 1989; Mochizuki et al., 1998; Watarai & Inouye, 1997), the mechanism of submergence-induced elongation in *O. glaberrima* deepwater rice has not been elucidated. Thus, we aimed to investigate the involvement of *SKs*, *SD1*, and *ACE1* in the growth response of African rice *O. glaberrima* to submergence. To clarify the involvement of these genes in multiple *O. glaberrima* cultivars, we determined the DNA sequence of each of these genes and examined the relationship between the sequences and expression of the genes, as well as the submergence-induced elongation response of the internodes.

Materials and methods

Plant materials

The accessions of *O. glaberrima* and *O. barthii*, *O. glaberrima*'s wild ancestral species, used in this study are listed in Supplementary Table S1. Two *O. sativa* indica cultivars, Habiganj Aman II (HA II, a deepwater rice cultivar with strong internode elongation capacity under submergence) and T442–57 (a deepwater rice cultivar with moderate internode elongation capacity under submergence) from Bangladesh and Thailand, respectively, were used as controls.

For *O. glaberrima* and *O. barthii* accessions, seeds were pretreated at 42°C in the dark to break dormancy, after which the outer and inner glumes of the seeds were removed. Seeds were surface-sterilized in 1% sodium hypochlorite solution for 30 min and then rinsed several times with tap water, after which they were germinated by soaking in water at 30°C in the dark for 2–3 days. The germinated seeds were then sown in pairs in 1 L plastic pots filled with paddy soil containing 0.2 g N, 0.2 g P_2O_5, and 0.2 g K_2O per liter of soil. At 40 days after

germination, additional fertilizer was applied at 0.07 g N, 0.07 g P_2O_5, and 0.07 g K_2O per pot. The plants were grown outdoors under natural conditions in an experimental field at Kobe University, Hyogo, Japan. The ambient temperature during the experiment was within a range of 27 to 33°C. The plants remained in the vegetative growth stage throughout the experimental period.

DNA analysis

Genomic DNA was extracted from the leaves of three-month-old plants using 200 mM Tris-HCl (pH 7.5) containing 250 mM NaCl, 25 mM EDTA, and 0.5% SDS. PCR analyses for *SK, SD1*, and *ACE1* were performed using TaKaRa Ex Taq (Takara Bio, Shiga, Japan). Primer sets and reaction conditions are listed in Supplementary Table S2. PCR amplification was performed for sequence analysis using Tks Gflex DNA Polymerase (Takara Bio). To obtain the full-length sequences of some *O. glaberrima SK2* homologs, gene-specific primers were designed based on the sequences obtained by 5′ and 3′ rapid amplification of cDNA ends (RACE) PCR. The homology of *SK1* and *SK2* genomic DNAs of *O. sativa*, *O. glaberrima*, and *O. barthii* was determined using the GENETYX software (GENETYX, Tokyo, Japan). A phylogenetic tree representing the relationship between the predicted SK1 and SK2 amino acids in *O. sativa, O. rufipogon, O. nivara, O. glumaepatula, O. glaberrima*, and *O. barthii* was constructed using UPGMA. Bootstrap analysis with 1,000 replicates was performed to estimate the statistical support for the nodes.

Gradual submergence treatment of plants

Fifty days after germination, two pots with two plants were submerged at a depth of 10 cm in 200 L plastic tanks. After two days, the water level was raised at a rate of 5 cm per day for 15 days to a final depth of 85 cm. The control plants were grown under non-submerged conditions during the same period. Plant length and total internode length on the main stem were measured before and after submergence. The position of the nodes before submergence was determined by the difference in texture of the leaf sheath with fingers. After submergence, the plants were dissected to measure total internode length.

RNA isolation and expression analysis

For the expression analysis, 50-day-old plants were partially submerged to a level where 70% of the height of each plant was below water. Total RNA was extracted from the basal 1 cm portion of the uppermost internodes of the plants grown in the air or submerged for 12 h using ISOSPIN Plant RNA (NIPPON GENE, Tokyo, Japan) according to the manufacturer's protocol. Total RNA was extracted from the basal 1 cm portion of the main culms for plants that did not form internodes. First-strand cDNA was synthesized from 1 µg total RNA using a PrimeScript II 1st Strand cDNA Synthesis Kit (Takara Bio). Expression analysis was performed as follows with three biological replicates.

Expression analysis using quantitative RT-PCR for *SK1*, *SK2*, and *SD1* was performed using TB Green *Premix Ex Taq* GC (Takara Bio) with MyGo Pro (Funakoshi, Tokyo, Japan) according to the manufacturer's instructions. The relative expression levels of the target genes were calculated based on their initial expression levels. Expression analysis using semi-quantitative RT-PCR for the *ACE1* gene was performed using Tks Gflex DNA Polymerase (Takara Bio). PCR products were separated on 1.8% agarose gels and visualized under ultraviolet light with ethidium bromide. Gene-specific primers and PCR conditions are listed in Supplementary Table S2. The *17s rRNA* gene was used as an internal control.

Results

Genomic DNA analysis of *SK* genes

We screened 50 *O. glaberrima* accessions (Supplementary Table S1) for *SK1* and *SK2* by amplifying genomic DNA using gene-specific primers (Supplementary Table S2). PCR products amplified with *SK1*- and *SK2*-specific primers were obtained from 34 and 49 accessions, respectively. Sequence analysis of these amplified products established the presence of three types of sequences in the *SK1* products, which share 82.5–95.5% identity with *OsSK1*, and the presence of four types in the *SK2* products, which share 66.6–98.0% identity with *OsSK2* (Supplementary Table S3). Therefore, the three amplified products for *SK1* were named *OglaSK1-A*, *OglaSK1-B*, and *OglaSK1-C* in descending order of their identity to *OsSK1* (Supplementary Tables S1, S3). Similarly, the four amplified products for *SK2* were named *OglaSK2-A*, *OglaSK2-B*, *OglaSK2-C*, and *OglaSK2-D* (Supplementary Tables S1, S3). The *OsSK2* gene, which contains an intron, possesses a single ERF domain in the 5′ half of the second exon. The *OglaSK2-A*, *OglaSK2-B*, and *OglaSK2-D* genes possess a similar gene structure to *OsSK2*, but the *OglaSK2-C* gene lacks the sequence of the entire first exon and the 5′ half of the second exon, thereby not possessing the ERF domain (Supplementary Figures S2, S3). Although the coding region length of *OglaSK2-C* was approximately 40% of that of *OsSK2*, the sequence of *OglaSK2-C* was 79.5% identical to that of *OsSK2*. (Supplementary Figure S2).

Table 1. Percentage amino acid identity of SK2 in *Oryza sativa* (Os), *Oryza glaberrima* (Ogla), *Oryza barthii* (Obar), and *Oryza glumaepatula* (Oglu).

	OsSK2	OglaSK2-A	OglaSK2-B	OglaSK2-C	OglaSK2-D	ObarSK2-A	ObarSK2-B	OgluSK2-2
OglaSK2-A	97.9%	—	—	—	—	—	—	—
OglaSK2-B	81.2%	81.7%	—	—	—	—	—	—
OglaSK2-C	67.7%	77.6%	67.2%	—	—	—	—	—
OglaSK2-D	59.7%	56.1%	60.7%	61.5%	—	—	—	—
ObarSK2-A	97.9%	100%	81.7%	77.6%	56.1%	—	—	—
ObarSK2-B	81.2%	81.7%	100%	67.2%	60.7%	81.7%	—	—
OgluSK2-2	59.8%	62.1%	55.9%	94.0%	82.5%	62.1%	55.9%	—
OsSKL1	59.7%	71.0%	60.7%	61.5%	100%	56.1%	60.7%	82.5%

OglaSK1-A was identified in 17 *O. glaberrima* accessions, 16 of which contained *OglaSK2-A* (Supplementary Table S1). There were 16 *O. glaberrima* accessions with *OglaSK1-B*, all of which contained *OglaSK2-B*. *OglaSK1-C* was only observed in C8569, which possessed *OglaSK2-A*. Conversely, *OglaSK2-C* or *OglaSK2-D* was identified in 15 accessions, 14 of which could not confirm the presence of *OglaSK1*. Notably, C7290, harboring *OglaSK2-C*, possessed *OglaSK1-A*. Among the 50 accessions tested, C0025 was the only accession with neither *OglaSK1* nor *OglaSK2*.

In addition to *O. glaberrima*, we investigated whether the two accessions of *O. barthii* (W0833 and W0844) possessed *SK* genes. In both accessions, two PCR products were obtained with the *SK1*-specific primers and *SK2*-specific primers. The amino acid sequences encoded by *SK1* and *SK2* amplification products of W0833 were identical to those encoded by *OglaSK1-A* and *OglaSK2-A*, respectively (Table 1; Supplementary Table S1). Furthermore, the amino acid sequences encoded by the *SK1* and *SK2* amplification products of W0844 were the same as those of *OglaSK1-B* and *OglaSK2-B*, respectively (Table 1; Supplementary Table S1).

Growth response to submergence

We investigated the growth response to gradual submergence in 17 accessions of *O. glaberrima* and two accessions of *O. barthii* with various alleles of *SK2* and compared their responses to those of the high-ability deepwater cultivar HA II and the moderate-ability deepwater cultivar T442–57 of *O. sativa*. When 50-day-old plants were submerged in water at a rate of 5 cm daily to a final depth of 85 cm, significant shoot elongation was observed in 13 *O. glaberrima* accessions and the *O. barthii* accessions, as well as in the *O. sativa* cultivars (Figure 1(a)). At the end of the experiment, all the submerged plants maintained a portion of their foliage above the water surface.

Submergence significantly promoted internodal elongation in 13 *O. glaberrima* accessions and the *O. barthii* accessions, as well as in the *O. sativa* cultivars (Figure 1(b)). In the accessions of *O. glaberrima* and *O. barthii* that demonstrated significant elongation of internodes during submergence, the increase in internodal elongation was approximately equal to or greater than that of T442–57 but less than that of HA II. All *O. glaberrima* accessions with *OglaSK2-A*, *OglaSK2-B*, or *OglaSK2-C* displayed significant internodal elongation under submergence, whereas the accessions with *OglaSK2-D*, except for C8540B, did not. Furthermore, accession C0025 without any allele of *OglaSK2* genes and accession C7427 with a loss-of-function allele of *SK2* failed to demonstrate submergence-induced internodal elongation: the *SK2* sequence of C7427 had a 13-bp deletion in the predicted coding region (Table 2; Supplementary Figure S3).

DNA analysis of *SD1* and *ACE1* genes

In the 17 *O. glaberrima* and two *O. barthii* accessions whose growth response to gradual submergence was examined as described above, we determined the genotypes of *SD1* and *ACE1* by PCR with type-specific primers. Analysis of *SD1* indicated that three *O. glaberrima* accessions possessed *SD1* Hap-4, and all other accessions possessed *SD1* Hap-2 (Table 2; Supplementary Figure S4(a)), but none of the accessions possessed *SD1* Hap-6, which exists in *O. sativa* cultivars exhibiting high deepwater response. Conversely, analysis of *ACE1* revealed that the *ACE1* gene sequence of all *O. glaberrima* and *O. barthii* accessions had a 1-bp-deletion at the same position as the *ACE1* gene sequence of *O. sativa* deepwater rice cultivars, indicating that these *O. glaberrima* and *O. barthii* accessions possess the deepwater rice type of *ACE1* (Table 2; Supplementary Figure S4(b)).

Additionally, the genomic sequences of *ACE1* of several *O. glaberrima* accessions possessing different *SK2* genes were determined. The sequences of the *ACE1* genes in the accessions examined shared more than 99.9% identity with the sequences of deepwater rice type or non-deepwater rice type of the *OsACE1* gene (Supplementary Figure S5). In *O. glaberrima* accessions possessing *SK2-A*, *SK2-B*, or *SK2-C*, the sequences of *ACE1* genes were identical (Supplementary Figure S5).

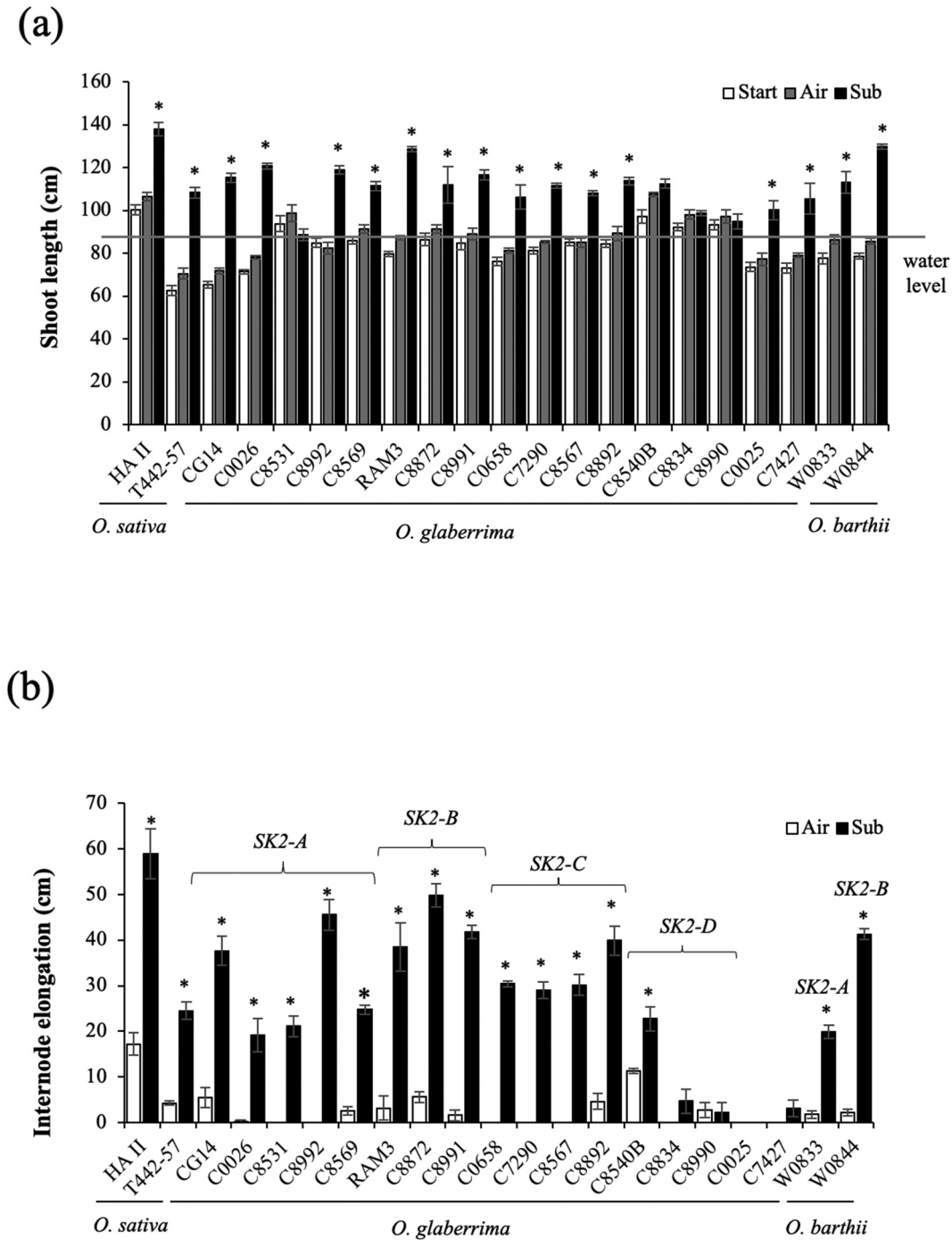

Figure 1. Effect of gradual submergence on elongation responses of *O. glaberrima* and *O. barthii* accessions and *O. sativa* deepwater cultivars. Fifty-day-old plants were submerged to a depth of 10 cm for two days, and the water level was then increased at the rate of 5 cm day $^{-1}$ for 15 days. Air-grown control plants were grown in non-submerged conditions during the same period. (a) Shoot length. The horizontal grey line denotes the final water level (85 cm). (b) Total internode elongation. The *SK2* allelic form of each accession of *O. glaberrima* and *O. barthii* is shown in the figure. Data represent the mean ± SE of four plants. Asterisks indicate a significant difference (P <0.05, Student's t-test) between the submerged and control plants.

Expression analysis of *SK1/2*, *SD1*, and *ACE1* genes

The expression of *SK1/2, SD1, and ACE1* was examined in the internodes of air-grown and submerged plants of four *O. glaberrima* accessions with different alleles of *SK2*: accessions CG14, RAM3, C7290, and C8834 possessed *SK2-A*, *SK2-B*, *SK2-C*, and *SK2-D*, respectively.

In the accessions with *SK2-A*, *SK2-B*, and *SK2-C*, *SK2* expression was induced by submergence for 12 h, but

Table 2. Allelic forms of *SK,SD1* and *ACE1* genes of *O. glaberrima* and *O. barthii* accessions and *O. sativa* deepwater cultivars used in submergence treatment.

	No.	Accession no.	*SK1*	*SK2*	*SD1*	*ACE1*
O. glaberrima	1	CG14	*SK1-A*	*SK2-A*	Hap-2	deepwater type
	2	C0026	*SK1-A*	*SK2-A*	Hap-2	deepwater type
	3	C8531	*SK1-A*	*SK2-A*	Hap-2	deepwater type
	4	C8992	*SK1-A*	*SK2-A*	Hap-2	deepwater type
	5	C8569	*SK1-C*	*SK2-A*	Hap-2	deepwater type
	6	RAM3	*SK1-B*	*SK2-B*	Hap-2	deepwater type
	7	C8872	*SK1-B*	*SK2-B*	Hap-2	deepwater type
	8	C8991	*SK1-B*	*SK2-B*	Hap-2	deepwater type
	9	C0658	-	*SK2-C*	Hap-2	deepwater type
	10	C7290	*SK1-A*	*SK2-C*	Hap-2	deepwater type
	11	C8567	-	*SK2-C*	Hap-2	deepwater type
	12	C8892	-	*SK2-C*	Hap-2	deepwater type
	13	C8540B	-	*SK2-D*	Hap-4	deepwater type
	14	C8834	-	*SK2-D*	Hap-4	deepwater type
	15	C8990	-	*SK2-D*	Hap-4	deepwater type
	16	C0025	-	-	Hap-2	deepwater type
	17	C7427	-	*SK2-A**	Hap-2	deepwater type
O. barthii	1	W0833	*SK1-A*	*SK2-A*	Hap-2	deepwater type
	2	W0844	*SK1-B*	*SK2-B*	Hap-2	deepwater type
O. sativa	1	T442-57	*OsSK1*	*OsSK2*	Hap-4	deepwater type
	2	HA II	*OsSK1*	*OsSK2*	Hap-6	deepwater type

*SK2-A**: With a 13-bp deletion at the coding region compared to the *OglaSK2-A*.

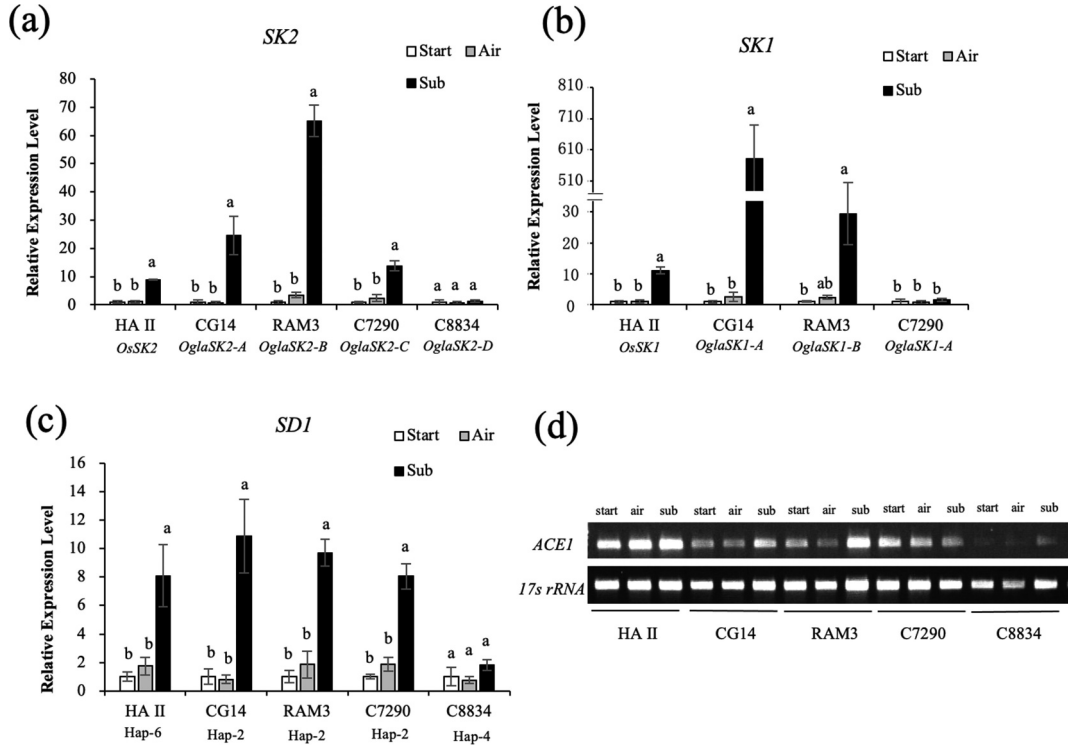

Figure 2. Expression analyses of the genes involved in the deepwater response in *O. glaberrima*. Expressions analyses of *SK2* (a), *SK1* (b), and *SD1* (c) were performed by real-time RT-PCR using gene-specific primers on the internodes of the plants submerged or grown in the air for 12 h. *17s rRNA* was used as an internal control. Data represent the means ± SE of three measurements. Different letters above bars indicate significant differences ($P < 0.05$) based on the Bonferroni test. (d) *ACE1* expression analysis was performed by RT-PCR using gene-specific primers on the internodes of the plants submerged or grown in the air for 12 h.

not in the accession with *SK2-D* (Figure 2(a)). *SK1* expression in CG14 and RAM3, which possess *SK1-A*/*SK2-A* and *SK1-B*/*SK2-B*, respectively, was induced by submergence. However, submergence-induced expression of *SK1* was not observed in accession C7290, possessing *SK1-A*/*SK2-C* (Figure 2(b)). The expression levels of *SK1* in CG14 and RAM3 were much higher than those of *OsSK1* in HA II. The expression level of *SD1* was significantly increased by submergence in CG14, RAM3, and C7290, all of which possess *SD1* Hap-2 (Figure 2(c)). Their expression levels were comparable to those of *SD1* Hap-6 in HA II. In contrast, submergence did not promote *SD1* expression in C8834, which possesses *SD1* Hap-4. Submergence-induced expression of *ACE1* was observed in CG14, RAM3, C8834, and HA II but not in C7290 (Figure 2(d)); however, these *O. glaberrima* accessions all possessed the deepwater rice type *ACE1* (Supplementary Figure S5).

Discussion

In *O. glaberrima*, the *SK1* and *SK2* genes were observed in three and four forms, respectively, whereas in *O. barthii*, both *SK1* and *SK2* were present in two allelic forms (Supplementary Figures S1, S2); the amino acid sequence encoded by *ObarSK1-A*, *ObarSK1-B*, *ObarSK2-A*, and *ObarSK2-B* was identical to that encoded by *OglaSK1-A*, *OglaSK1-B*, *OglaSK2-A*, and *OglaSK2-B*, respectively. Figure 3 shows the relationship between the presence of the respective alleles of *SK1* or *SK2* and the submergence-induced internode elongation. When comparing the different types of *SK1* alleles, the accessions that have the *SK1* genes, regardless of the allele type, displayed submergence-induced internodal elongation. The elongation was greatest in the accessions with *SK1-B* (Figure 3(a)). However, accessions without *SK1* also exhibited submergence-induced internode elongation, comparable to that observed in accessions with *SK1-A* or *SK1-C*. When comparing the different types of *SK2* alleles, the accessions with the *SK2-A*, *SK2-B*, or *SK2-C* alleles displayed submergence-induced internodal elongation, and the elongation was greatest in the accessions with *SK2-B*. In contrast, the accessions with *SK2-D* or without a functional *SK2* gene did not exhibit elongation (Figure 3(b)). The accessions harboring no *SK1* that demonstrated deepwater response possessed *SK2*, whereas accessions harboring no *SK2* did not show deepwater response (Table 1; Figure 3). These results indicate that *SK2* is critical for the deepwater response of *O. glaberrima* and *O. barthii*, as in the case of *O. sativa* and other wild species (Hattori et al., 2009). Therefore, although the accessions with *SK1-B* demonstrated the highest elongation ability compared to the different types of *SK1* alleles (Figure 3(a)), the response is probably attributed to the presence of *SK2-B* because all of the accessions harboring *SK1-B* possessed *SK2-B*, conferring the highest ability (Table 1; Supplementary Table S1; Figure 3(b)). The high elongation ability of accessions with *SK2-B* may be explained by the fact that *SK2-B* was more highly expressed by submergence than other types of *SK2* alleles (Figure 2(a)).

On the other hand, Kawano et al. (2008) reported that the seedings of *O. glaberrima* cultivar Saligbeli exhibited enhanced leaf elongation with increased dry matter accumulation during submergence. In the present study, two *O. glaberrima* accessions without *SK1/SK2* genes, C0025 and C7427, showed enhanced shoot elongation (Figure 1(a)) without internode elongation

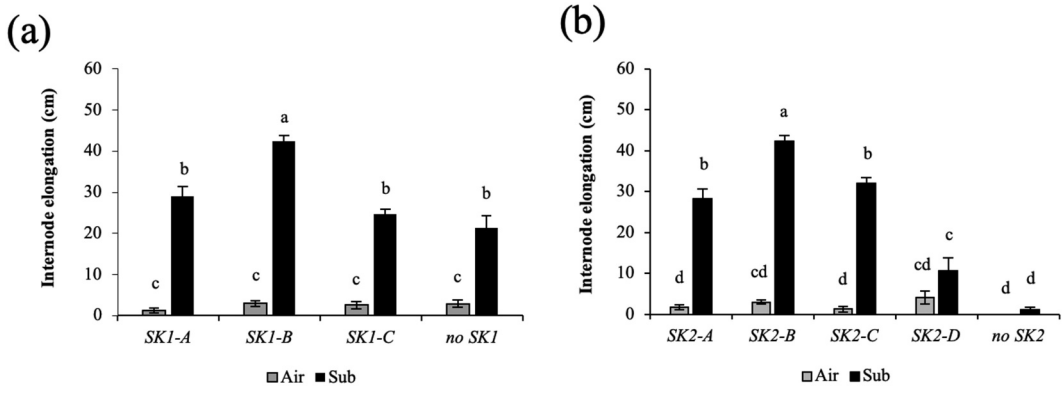

Figure 3. Comparison of internode elongation under submergence in *O. glaberrima* and *O. barthii* accessions with different *SK1* (a) and *SK2* (b) allelic forms. Fifty-day-old plants were submerged to a depth of 10 cm for two days, and then the water level was increased at the rate of 5 cm day $^{-1}$ for 15 days. Air-grown control plants were grown in non-submerged conditions during the same period. Data represent the means ± SE of 10–23 plants. Different letters above bars indicate significant differences ($P < 0.05$) based on the Bonferroni test.

(Figure 1(b)) under deepwater condition. Therefore, the shoot elongation exhibited by these two accessions is thought to be due to the promotion of leaf elongation. Such a growth response might also function as adaptation to deepwater in *O. glaberrima*.

In *O. glaberrima* accessions possessing *SK2-C*, submergence induced internodal elongation with increased expression of *SK2-C* (Figures 1(b), 2(a)). This observation suggests that this gene, as well as *SK2-A* and *SK2-B*, may function in the induction of internode elongation under submergence; however, unlike *SK1* and other *SK2* genes, *SK2-C* lacks the ERF domain (Supplementary Figures S1, S2). Transactivation activity assays of different regions of the OsSK2 protein demonstrated that the C-terminal region, which does not contain the ERF domain, has transactivation activity but not the ERF domain or N-terminal region (Hattori et al., 2009). Because OglaSK2-C covers 87% of the C-terminal region of OsSK2 (Supplementary Figure S2), the OglaSK2-C protein was expected to exhibit transactivation activity. In addition, *OglaSK2-C* shared 94% amino acid identity with the corresponding region of *OgluSK2-2*, which was identified in accessions with the high deepwater response of the wild rice species *O. glumaepatula*. However, *OglaSK2-C* shared 67.7% amino acid identity with that of *OsSK2* (Table 1). Notably, the transcription of *OgluSK2-2* was induced by submergence but not by ethylene; however, the gene has an ERF domain (Sasayama et al., 2018).

The genomic sequence of *OglaSK2-D* was 100% identical to that of *SNORKEL-LIKE1*(*SKL1*) on chromosome 12 of *O. sativa* subsp. *japonica* cv. Nipponbare (Table 1) (Nagai et al., 2022). Therefore, *OglaSK2-D* is unlikely to confer deepwater response to *O. glaberrima*. However, among the three accessions with *OglaSK2-D* investigated in the present study, one accession (C8540B) demonstrated significant internodal elongation induced by submergence, whereas the other accession did not (Figure 1). The reason for this finding remains unclear.

Of the 17 *O. glaberrima* accessions examined, all accessions possessed *SD1* Hap-2 or Hap-4 (Table 2; Supplementary Figure S4(a)): no accessions possessed *SD1* Hap-6, which was observed in *O. sativa* cultivars with high deepwater response, such as HA II. In *O. sativa* deepwater rice, the expression of *SD1* Hap-6 was much more enhanced by submergence than that of *SD1* Hap-2 and Hap-4, which may help explain the high deepwater response of the cultivar harboring *SD1* Hap-6 (Kuroha et al., 2018). In *O. glaberrima*, the accessions with *SD1* Hap-2 exhibited internodal elongation induced by submergence (Table 2; Figure 1) with increased expression levels of *SD1*, which was comparable to that of *SD1* Hap-6 in *O. sativa* HA II (Figure 2(c)), whereas the accession with *SD1* Hap-4 did not show internodal elongation or induction of expression of *SD1*. Therefore, these results suggest that submergence-enhanced expression of *SD1* may be involved in rapid internodal elongation in *O. glaberrima*, as observed in *O. sativa* deepwater rice (Kuroha et al., 2018). Furthermore, the accession with the Hap-4 did not display deepwater response, likely because *OglaSK2-D* possessed by the accession is not a functional analog of *SK2*. In *O. sativa* accessions with *OsSD1* Hap-2 or Hap-4, the presence of *SK2* is required for submergence-induced expression of *OsSD1*, thereby promoting internodal elongation (Kuroha et al., 2018). These observations also support the view that *OglaSK2-A*, *OglaSK2-B*, and *OglaSK2-C* function in the induction of internode elongation under submergence because accessions possessing the *SK2* genes exhibited submergence-induced expression of *OglaSD1* (Figure 2).

All *O. glaberrima* accessions examined possessed *ACE1* harboring a 1-bp-deletion at the same position found in the *ACE1* of *O. sativa* deepwater rice cultivars (Table 2; Supplementary Figure S4(b)); however, not all accessions with this deepwater rice type of *ACE1* exhibited deepwater response (Figure 1). This suggests that the presence of deepwater rice type *ACE1* alone may not be sufficient for inducing internode elongation during submergence in *O. glaberrima*. *ACE1* expression is induced in response to GA, leading to the formation of elongated internodes (Nagai et al., 2020). In three *O. glaberrima* accessions (CG14, RAM3, and C7290), the expression of *ACE1* in internodes was already induced before submergence treatment (Figure 2(d)), and the elongation of the internodes was promoted by submergence (Figure 1(b)). Conversely, under accession C8834, expression of *ACE1* was not observed in air-grown plants but increased in submerged plants (Figure 2(d)), which had elongated internodes (Figure 1(b)). These results suggest that deepwater rice type *ACE1* may be related to submergence-induced internode elongation in *O. glaberrima* as well as *O. sativa*.

The sequence of *OglaSK2-D* identified in this study was present in the genomes of many *O. sativa* cultivars, including *indica* and *japonica* (data not shown). In the *O. glaberrima* accessions examined here, all accessions harboring *SK2-D* possessed *SD1* Hap-4, whereas the other *O. glaberrima* accessions, and two accessions of the ancestral species *O. barthii*, possessed *SD1* Hap-2 (Table 2). Furthermore, the *ACE1* genes of three accessions harboring *SK2-D* contain thymine at position 62, as found in *ACE 1* of *O. sativa*. In contrast, the *ACE1* of the other *O. glaberrima* accessions and *O. barthii* accessions contained adenine at the corresponding position (Supplementary Figure S5). Therefore, these findings suggest that *SK2-D* of *O. glaberrima* was derived from *O. sativa*. To test this possibility, we compared the

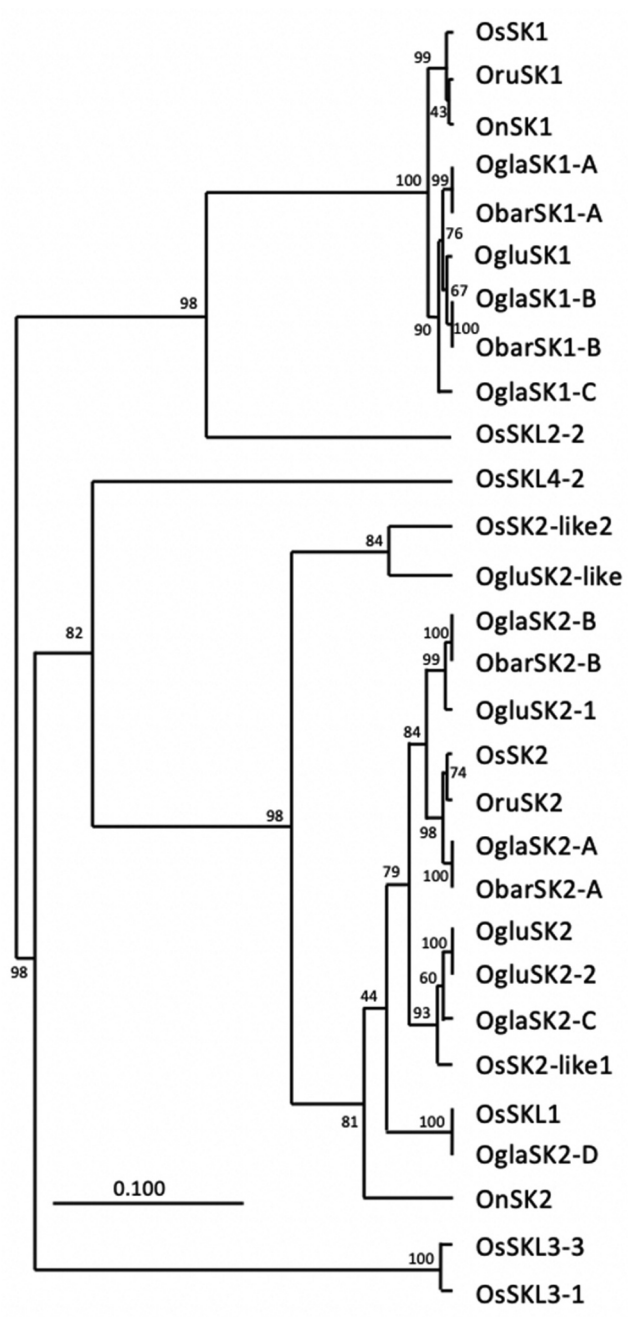

Figure 4. Phylogenetic tree of SK amino acid sequences in *Oryza* species. Phylogenetic relationships of SK amino acid sequence of *Oryza sativa* (Os), *Oryza rufipogon* (Oru), *Oryza nivara* (On), *Oryza glaberrima* (Ogla), *Oryza barthii* (Obar) and *Oryza glumaepatula* (Oglu) were constructed using the UPGMA method. The bootstrap support values from 1,000 replicants are shown at the node.

accessions with *OglaSK2-D* and other accessions in terms of the morphologies of glume hair, ligule shape, and secondary rachis-branch structure, which are generally known to be different between *O. sativa* and *O. glaberrima*. The results indicated that all the morphologies of the accessions with *OglaSK2-D*, unlike those of the other accessions, were characteristic of *O. sativa* rather than *O. glaberrima* (Supplementary Figure S6). Furthermore, as a result of examining *Osprog1*, specific to *O. sativa*, and *Oglaprog7*, specific to *O. glaberrima*, the accessions with *OglaSK2-D* possessed *Osprog1* but not *Oglaprog7* (Supplementary Figure S7) (Hu et al., 2018). These observations support our hypothesis that *SK2-D* of *O. glaberrima* was derived from *O. sativa*. Linares (2002) reported that since the late 20th century, rice cultivation in Africa had been based on a farming system in which several cultivars of *O. sativa* and *O. glaberrima* are grown in a mixed manner. In addition, Nuijten et al. (2009) reported that descendants of interspecific hybridization between these species were observed in African farms. These reports would also support the possibility that *SK2-D* of *O. glaberrima* was derived from *O. sativa*.

Catling (1992) reported that the African rice *O. glaberrima* was cultivated as upland or deepwater rice. Among the 50 *O. glaberrima* accessions tested in the present study (Supplementary Table S1), six accessions were registered as deepwater rice cultivars, and four accessions were upland rice cultivars; however, whether the remaining 40 accessions were upland or deepwater rice cultivars remains unknown. Three of the four upland accessions (CG14, C7265, and C7493) contained the *OglaSK2-A* gene (Supplementary Table S1). All accessions with *SK2-A*, including CG14, displayed enhanced internodal elongation under submergence (Figure 1(b)). Of the 40 accessions without registration of cultivation type, 22 possessed *SK2-A*, *SK2-B*, or *SK2-C*, which can confer the deepwater response to *O. glaberrima* plants (Supplementary Table S1; Figure 1). Because some accessions had been collected from Ivory Coast and Tanzania (Supplementary Table S1), where deepwater rice cultivation systems have not been practiced (Catling, 1992), they must have been cultivated in upland areas. Thus, many *O. glaberrima* cultivars grown as upland rice could possess functional *SK* genes, thereby showing rapid internodal elongation under submergence. In contrast, the existence of non-deepwater rice cultivars of *O. sativa* carrying *OsSK1/2* genes remains unknown. The domestication process of *O. glaberrima* began around 3,000 years ago, whereas that of *O. sativa* began around 9,000 years ago (Fornasiero et al., 2022). The difference in time from domestication between *O. sativa* and *O. glaberrima* might explain why *O. glaberrima* cultivars that have been cultivated in non-deepwater areas still preserve the *SK* genes.

All the known deepwater rice cultivation areas in Africa are in West Africa, and three-quarters of West Africa's deepwater rice is grown along the Niger River, mainly in Mali, Guinea, and Nigeria (Catling, 1992).

Notably, most accessions collected in Mali, which has the most extensive deepwater rice cultivation in Africa (Catling, 1992), possess the *OglaSK2-B* gene (Supplementary Table S1), which can confer the strongest deepwater response to *O. glaberrima* (Figure 3(b)). The main deepwater rice-growing area in Mali is the inland delta of the Niger River, where floodwaters may reach a height of 2.5–3 m during the rainy season (Catling, 1992). Such a severely flooded environment may have resulted in the selection of the most effective *SK2-B* allele.

Only the accessions collected in Guinea, Gambia, and Ivory Coast, located on the southwest coast of West Africa, possessed *OglaSK2-C* (Supplementary Table S1), which exhibited 94.0% amino acid identity to *OgluSK2-2* of the wild rice species *O. glumaepatula* in South America (Table 1; Figure 4). Although *O. glaberrima* was introduced to the Americas during the slave trade years and grown by enslaved Africans for decades (Carney, 2005), van Andel (2010) recently observed that *O. glaberrima* is still cultivated in the rice fields of Suriname, South America. Analysis of genetic distance calculated across the whole genome indicates that the *O. glaberrima* of Suriname is most closely related to the *O. glaberrima* accessions collected in the coastal Guinean lowlands and central highlands in *O. glaberrima* accessions collected from countries in West Africa (van Andel et al., 2016). Therefore, the presence of *OgluSK2-2* in *O. glumaepatula*, which is highly homologous to *OglaSK2-C*, might have been due to gene flow from *O. glaberrima* of Guinea origin. This may also be supported by the fact that *O. glumaepatula* strains with *OgluSK2-2* have another *SK2* gene, *OgluSK2-1*gene, which shows higher sequence similarity to *OsSK2* (Sasayama et al., 2018) and that, except for certain strains of *O. glumaepatula*, no *Oryza* species with two *SK2* genes have been identified.

SK genes are reportedly present in the Asian cultivated species *O. sativa*, its ancestral wild species *O. rufipogon*, and the South American wild species *O. glumaepatula* (Hattori et al., 2009; Sasayama et al., 2018). In the present study, *SK* genes were also identified in the African cultivated species *O. glaberrima* and its ancestral wild species *O. barthii*. Therefore, the common ancestors of these wild rice species distributed on different continents of Africa, Asia, and South America probably already possessed *SK* genes.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Carney, J. (2005). Rice and memory in the age of enslavement: Atlantic passages to Suriname. *Slavery & Abolition*, *26*(3), 325–348. https://doi.org/10.1080/01440390500319562

Catling, D. (1992). The Niger Basin and West Africa. In *Rice in Deep Water* (pp. 353–376). Macmillan Press.

Diop, B., Wang, D. R., Drame, K. N., Gracen, V., Tongoona, P., Dzidzienyo, D., Nartey, E., Greenberg, A. J., Djiba, S., Danquah, E. Y., & McCouch, S. R. (2020). Bridging old and new: Diversity and evaluation of high iron-associated stress response of rice cultivated in West Africa. *Journal of Experimental Botany*, *71*(14), 4188–4200. https://doi.org/10.1093/jxb/eraa182

Fornasiero, A., Wing, R. A., & Ronald, P. (2022). Rice domestication. *Current Biology*, *32*(1), R20–24. https://doi.org/10.1016/j.cub.2021.11.025

Hattori, Y., Miura, K., Asano, K., Yamamoto, E., Mori, H., Kitano, H., Matsuoka, M., & Ashikari, M. (2007). A major QTL confers rapid internode elongation in response to water rise in deepwater rice. *Breeding Science*, *57*(4), 305–314. https://doi.org/10.1270/jsbbs.57.305

Hattori, Y., Nagai, K., Furukawa, S., Song, X. J., Kawano, R., Sakakibara, H., Wu, J., Matsumoto, T., Yoshimura, A., Kitano, H., Matsuoka, M., Mori, H., & Ashikari, M. (2009). The ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water. *Nature*, *460*(7258), 1026–1030. https://doi.org/10.1038/nature08258

Hattori, Y., Nagai, K., Mori, H., Kitano, H., Matsuoka, M., & Ashikari, M. (2008). Mapping of three QTLs that regulate internode elongation in deepwater rice. *Breeding Science*, *58*(1), 39–46. https://doi.org/10.1270/jsbbs.58.39

Hu, M., Lv, S., Wu, W., Fu, Y., Liu, F., Wang, B., Li, W., Gu, P., Cai, H., Sun, C., & Zhu, Z. (2018). The domestication of plant architecture in African rice. *The Plant Journal*, *94*(4), 661–669. https://doi.org/10.1111/tpj.13887

Inouye, J., Hakoda, H., & NG, N. Q. (1989). Preliminary studies on some ecological characteristics of African deep water rice (*Oryza glaberrima* Steud.). *Japanese Journal of Tropical Agriculture*, *33*(3), 158–163. https://doi.org/10.11248/jsta1957.33.158

Jones, M. P., Dingkuhn, M., Aluko, G. K., & Semon, M. (1997). Interspecific *Oryza sativa* L. × *O. glaberrima* Steud. progenies in upland rice improvement. *Euphytica*, *94*, 237–246. https://doi.org/10.1023/A:1002969932224

Jones, M. P., Mande, S., & Aluko, K. (1997). Diversity and potential of *Oryza glaberrima* Steud in upland rice breeding. *Breed*

Science, *47*(4), 395–398. https://doi.org/10.1270/jsbbs1951.47.395

Kawano, N., Ito, O., & Sakagami, J. -I. (2008). Flash flooding resistance of rice genotypes of *Oryza sativa* L., *O. glaberrima* Steud., and interspecific hybridization progeny. *Environmental and Experimental Botany*, *63*(1–3), 9–18. https://doi.org/10.1016/j.envexpbot.2007.12.001

Kende, H., van der Knaap, E., & Cho, H. -T. (1998). Deepwater rice: A model to study stem elongation. *Plant Physiology*, *118*(4), 1105–1110. https://doi.org/10.1104/pp.118.4.1105

Kuroha, T., & Ashikari, M. (2020). Molecular mechanisms and future improvement of submergence tolerance in rice. *Molecular Breeding*, *40*, 41. https://doi.org/10.1007/s11032-020-01122-y

Kuroha, T., Nagai, K., Gamuyao, R., Wang, D. R., Furuta, T., Nakamori, M., Kitaoka, T., Adachi, K., Minami, A., Mori, Y., Mashiguchi, K., Seto, Y., Yamaguchi, S., Kojima, M., Sakakibara, H., Wu, J., Ebana, K., Mitsuda, N., Ohme-Takagi M., and Ashikari, M. (2018). Ethylene-gibberellin signaling underlies adaptation of rice to periodic flooding. *Science*, *361*(6398), 181–186. https://doi.org/10.1126/science.aat1577

Linares, O. F. (2002). African rice (*Oryza glaberrima*): History and future potential. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(25), 16360–16365. https://doi.org/10.1073/pnas.252604599

Mochizuki, T., Ryu, K., & Inouye, J. (1998). Elongation ability of African floating rice (*Oryza glaberrima* Steud.). *Plant production science*, *1*(2), 134–135. https://doi.org/10.1626/pps.1.134

Nagai, K., Kurokawa, Y., Mori, Y., Minami, A., Reuscher, S., Wu, J., Matsumoto, T., & Ashikari, M. (2022). *SNORKEL* genes relating to flood tolerance were pseudogenized in normal cultivated rice. *Plants*, *11*(3), 376. https://doi.org/10.3390/plants11030376

Nagai, K., Mori, Y., Ishikawa, S., Furuta, T., Gamuyao, R., Niimi, Y., Hobo, T., Fukuda, M., Kojima, M., Takebayashi, Y., Fukushima, A., Himuro, Y., Kobayashi, M., Ackley, W., Hisano, H., Sato, K., Yoshida, A., Wu, J., Sakakibara, H., Ashikari, M. … Ashikari, M. (2020). Antagonistic regulation of the gibberellic acid response during stem growth in rice. *Nature*, *584*(7819), 109–114. https://doi.org/10.1038/s41586-020-2501-8

Ndjiondjop, M. N., Semagn, K., Sow, M., Manneh, B., Gouda, A. C., Kpeki, S. B., Pegalepo, E., Wambugu, P., Sié, M., & Warburton, M. L. (2018). Assessment of genetic variation and population structure of diverse rice genotypes adapted to lowland and upland ecologies in Africa using SNPs. *Frontiers in plant science*, *9*, 446. https://doi.org/10.3389/fpls.2018.00446

Nuijten, E., van Treuren, R., Struik, P. C., Mokuwa, A., Okry, F., Teeken, B., & Richards, P. (2009). Evidence for the emergence of new rice types of interspecific hybrid origin in West African Farmers' Fields. *PLoS One*, *4*(10), e7335. https://doi.org/10.1371/journal.pone.0007335

Okishio, T., Sasayama, D., Hirano, T., Akimoto, M., Itoh, K., & Azuma, T. (2014). Growth promotion and inhibition of the Amazonian wild rice species *Oryza grandiglumis* to survive flooding. *Planta*, *240*(3), 459–469. https://doi.org/10.1007/s00425-014-2100-8

Sasaki, A., Ashikari, M., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A., Swapan, D., Ishiyama, K., Saito, T., Kobayashi, M., Khush, G. S., Kitano, H., & Matsuoka, M. (2002). Green revolution: A mutant gibberellin-synthesis gene in rice. *Nature*, *416*, 701–702. https://doi.org/10.1038/416701a

Sasayama, D., Niikawa, M., Hatanaka, T., Fukayama, H., & Azuma, T. (2022). Adaptive responses to flooding in wild rice species with various genomes other than AA. *Plant production science*, *25*(3), 350–358. https://doi.org/10.1080/1343943X.2022.2073896

Sasayama, D., Okishio, T., Hirano, T., Fukayama, H., Hatanaka, T., Akimoto, M., & Azuma, T. (2018). Internodal elongation under submergence in the Amazonian wild rice species *Oryza glumaepatula*: The growth response is induced by hypoxia but not by ethylene. *Plant Growth Regulation*, *85*(1), 123–132. https://doi.org/10.1007/s10725-018-0378-4

Spielmeyer, W., Ellis, M. H., & Chandler, P. M. (2002). Semidwarf (*sd-1*), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(13), 9043–9048. https://doi.org/10.1073/pnas.132266399

van Andel, T. R. (2010). African rice (*Oryza glaberrima* Steud.): Lost crop of the enslaved Africans discovered in Suriname. *Economic Botany*, *64*(1), 1–10. https://doi.org/10.1007/s12231-010-9111-6

van Andel, T. R., Meyer, R. S., Aflitos, S. A., Carney, J. A., Veltman, M. A., Copetti, D., Flowers, J. M., Havinga, R. M., Maat, H., Purugganan, M. D., Wing, R. A., & Schranz, M. E. (2016). Tracing ancestor rice of Suriname Maroons back to its African origin. *Nature Plants*, *2*, e16149. https://doi.org/10.1038/nplants.2016.149

Watarai, M., & Inouye, J. (1997). Effect of water conditions on LEI position in African floating rice (*Oryza glaberrima* Steud.). *Japanese Journal of Crop Science*, *66*(2), 300–306. https://doi.org/10.1626/jcs.66.300