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Rapid Paper

EUI1, Encoding a Putative Cytochrome P450 Monooxygenase, Regulates Internode Elongation by Modulating Gibberellin Responses in Rice

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Elongation of rice internodes is one of the most important agronomic traits, which determines the plant height and underlies the grain yield. It has been shown that the elongation of internodes is under genetic control, and various factors are implicated in the process. Here, we report a detailed characterization of an *elongated uppermost internode1* (*eui1*) mutant, which has been used in hybrid rice breeding. In the *eui1-2* mutant, the cell lengths in the uppermost internodes are significantly longer than that of wild type and thus give rise to the elongated uppermost internode. It was found that the level of active gibberellin was elevated in the mutant, whereas its growth in response to gibberellin is similar to that of the wild type, suggesting that the higher level accumulation of gibberellin in the *eui1* mutant causes the abnormal elongation of the uppermost internode. Consistently, the expression levels of several genes which encode gibberellin biosynthesis enzymes were altered. We cloned the *EUI1* gene, which encodes a putative cytochrome P450 monooxygenase, by map-based cloning and found that *EUI1* was weakly expressed in most tissues, but preferentially in young panicles. To confirm its function, transgenic experiments with different constructs of *EUI1* were conducted. Overexpression of *EUI1* gave rise to the gibberellin-deficient-like phenotypes, which could be partially reversed by supplementation with gibberellin. Furthermore, apart from the alteration of expression levels of the gibberellin biosynthesis genes, accumulation of SLR1 protein was found in the overexpressing transgenic plants, indicating that the expression level of *EUI1* is implicated in both gibberellin-mediated SLR1 destruction and a feedback regulation in gibberellin biosynthesis. Therefore, we proposed that *EUI1* plays a negative role in gibberellin-mediated regulation of cell elongation in the uppermost internode of rice.

Keywords: Cytochrome P450 monooxygenase — Elongated uppermost internode 1 — Gibberellin — Internode elongation — Rice.

Abbreviations: BAC, bacterial artificial chromosome; CaMV, cauliflower mosaic virus; CAPS, cleaved amplified polymorphic sequence; ELISA, enzyme-linked immunosorbent assay; EMS, ethylmethane sulfonate; EUI, elongated uppermost internode; ORF, open reading frame; RNAi, RNA interference; RT–PCR, reverse transcription–PCR.

Introduction

The rice culm is a major organ that affects plant height, lodging, panicle exsertion and the grain yield. A culm consists of a series of internodes interconnected by nodes. The sum of the lengths of elongated internodes accounts for a large fraction of plant height. These internodes usually begin to elongate in a sequential manner around the initiation of panicle primordial, and this continues during heading. Elongation of internodes is influenced by various factors such as growth duration, photoperiod and stress environments (Yoshida 1981), suggesting that different signaling pathways are involved. Previous studies on rice dwarf mutants have revealed that gibberellin is the most important phytohormone that determines the plant height (Hong et al. 2003). Both gibberellin-deficient and gibberellin-insensitive rice mutants showed alterations in plant height. Mutations in the rice gene *Dwarf 1*, which encodes an α-subunit of GTP-binding protein, block the gibberellin-mediated induction of α-amylase activity and cause a gibberellin-insensitive phenotype, suggesting that the GTP-binding protein may be associated with gibberellin signal transduction (Ashikari et

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Table 1 The culm lengths, panicle exsertion and internode lengths of *eui1* alleles and their original cultivars

Genotype	Culm length (cm)	Panicle exsertion (cm)	Length of internode (cm)				
			First	Second	Third	Fourth	Fifth
Wild-type (Teqing2)	92.1 ± 1.4	0.7 ± 0.3	33.7 ± 1.3	22.6 ± 0.8	17.4 ± 0.8	9.0 ± 0.5	6.3 ± 0.6
eui1-2 (Teqing2)	136.5 ± 1.7	21.1 ± 1.0	57.5 ± 1.5	39.8 ± 1.3	22.3 ± 0.5	9.2 ± 1.2	5.5 ± 0.2
Wild-type (Zhonghua11)	87.8 ± 3.0	6.2 ± 0.7	36.2 ± 1.5	20.7 ± 0.9	14.0 ± 0.6	8.0 ± 0.8	3.3 ± 0.7
eui1-3 (Zhonghua11)	110.7 ± 1.1	14.2 ± 1.4	50.0 ± 1.5	31.5 ± 0.6	14.4 ± 0.8	9.3 ± 0.6	5.1 ± 0.9
eui1-4 (Zhonghua11)	108.9 ± 1.6	15.8 ± 0.6	50.4 ± 0.7	25.1 ± 0.5	17.3 ± 0.6	9.6 ± 0.5	5.3 ± 0.7
Wild-type (Xieqingzao)	57.8 ± 1.1	1.1 ± 1.0	30.0 ± 1.5	14.4 ± 0.7	7.9 ± 0.4	4.4 ± 0.4	0.7 ± 0.1
eui1-5 (Xieqingzao)	102.9 ± 1.3	23.2 ± 1.2	52.6 ± 1.9	29.2 ± 1.3	13.8 ± 0.6	5.4 ± 1.9	1.4 ± 0.5

al. 1999). *GID2* isolated from a gibberellin-insensitive dwarf mutant, *gid2*, encodes a positive regulator of gibberellin signaling, and regulated degradation of SLR1 is initiated through gibberellin-dependent phosphorylation and finalized by an SCF(GID2)–proteasome pathway (Sasaki et al. 2003). *GIBBERELLIN INSENSITIVE DWARF1* isolated from the gibberellin-insensitive dwarf mutant *gid1* encodes a soluble receptor for gibberellin (Ueguchi-Tanaka et al. 2005). The rice 'Green Revolution' semi-dwarf mutant gene *semi-dwarf1* (*sd1*), which was widely used in breeding, encodes gibberellin-20-oxidase. In the *sd1* mutant, the last step of gibberellin metabolism is affected, leading to a low gibberellin level and a semi-dwarf phenotype (Sasaki et al. 2002, Spielmeyer et al. 2002). Consistently, the wheat 'Green Revolution' gene, *RHT*, encoding a DELLA protein in gibberellin signaling, affects gibberellin responses and alters plant height (Peng et al. 1999). Therefore, elongation of rice internodes has been used as a unique system for the study on biosynthesis and signaling of gibberellin.

Panicle enclosure is a typical phenotype of almost all male-sterile lines (Shen et al. 1987, Yang et al. 2002), which greatly reduces seed production of hybrid rice due to its blocking of normal pollination. Therefore, a large amount of exogenous gibberellin has to be used for inducing panicle exsertion. However, gibberellin application not only increases the cost of seed production, but also greatly increases the rate of seed germination on the panicle, resulting in decreased quality and shortened storage life of hybrid seeds. A recessive rice *eui* (elongated uppermost internode) mutant was first reported by Rutger and Carnahan (1981), which is characterized by the near doubling length of the uppermost internode, excess panicle exsertion and an increased panicle length in comparison with the wild type. The *eui* mutant shows notably rapid elongation of the uppermost internode at the heading stage. Because of its prospective application to amend panicle enclosure in the male-sterile lines, this recessive trait, along with male sterility, maintainer and restorer, was referred to as the fourth genetic element of hybrid rice production (Rutger and Carnahan 1981). Indeed, the *eui* locus has been successfully incorporated into many male-sterile plants through conventional breeding methods. In these *eui* male-sterile plants, the panicle enclosure can be eliminated, and the cost and the side effects resulting from gibberellin application can be reduced or avoided (He and Shen 1991, He and Shen 1994).

So far, many rice *eui* mutants have been reported (Maekawa et al. 1989, Yang et al. 2001, Zhang and Yang 2003, Zhu et al. 2003). Genetic studies have shown that most of these mutants are allelic to *eui* initially identified by Rutger and Carnahan (1981), with only one exception. So, the former is renamed *eui1*and the latter is renamed *eui2* (Yang et al. 2001).

The *EUI1* locus has been mapped to chromosome 5 using primary trisomics and subsequently to a 98 kb region (Librojo and Khush 1986, Xu et al. 2004). In this report, we describe the molecular cloning and the functional analysis of the *EUI1* gene. Our data indicate that *EUI1* plays a negative role in gibberellin-mediated regulation of cell elongation in the rice uppermost internode.

Results

Characteristics of eui1 mutants

Two *eui* mutants were previously identified by their notable elongation of the uppermost internode at the heading stage (Rutger and Carnahan 1981, Yang et al. 2002); we have identified three new mutants, derived from spontaneous mutation or ethylmethane sulfonate (EMS)-mutagenized populations in different genetic backgrounds (see Materials and Methods), that were phenotypically similar to *eui1*. Genetic analysis revealed that these new mutants were allelic to *eui1*. Accordingly, we renamed these five mutants *eui1-1* through *eui1-5*, respectively (see Table 1).

During vegetative growth, no detectable difference between *eui1* and wild-type plants was observed under different growth and light conditions (data not shown). In all *eui1* mutants, whereas all internodes were longer than that of the wild type to different extents, the dramatic elongation occurred in the first (uppermost) and the second internodes, thereby leading to the increase in height in the mutants (Table 1; Fig. 1A, B). The panicle exsertion, however, showed substantial variations among different mutant alleles. For example, the length of panicle exsertion in *eui1-2* and *eui1-3* was 29.3- and 2.3-fold that of the wild type, respectively, while other alleles showed an intermediate phenotype of panicle exsertion (Table 1).

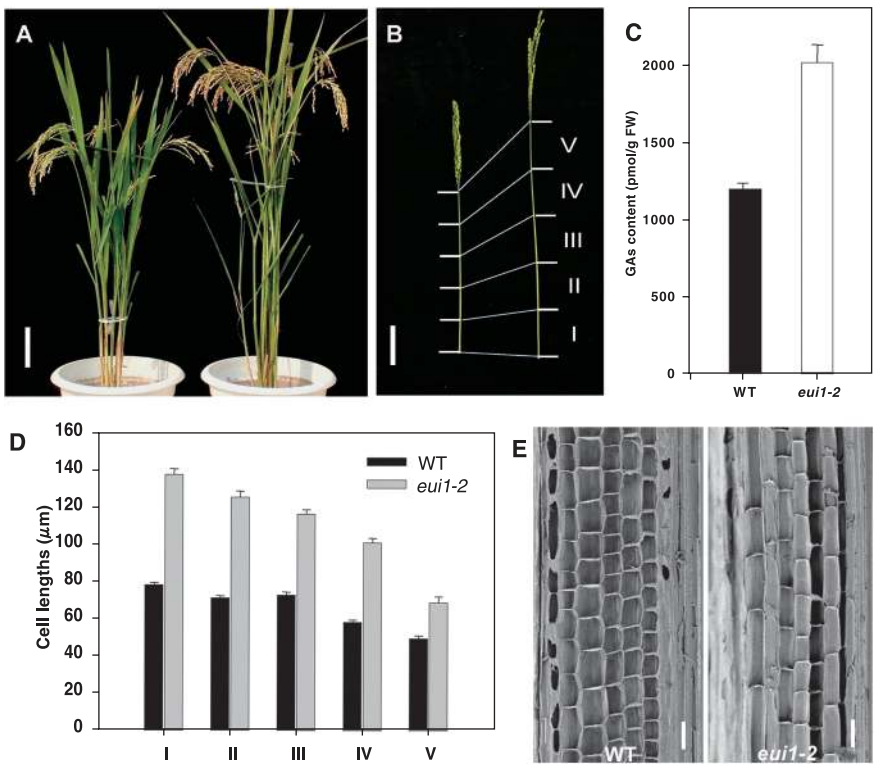

Fig. 1 The characteristics of *eui1-2* mutants. (A) Phenotypes of the *eui1-2* mutant and the wild type at 4 months after sowing (scale bar = 10 cm). (B) Comparison of the uppermost internode between the wild type and *eui1-2* mutants. The uppermost internodes were equally divided into five sections (indicated as I, II, III, IV and V) and each section was then sampled for scanning electron microscopic examination (scale bar = 10 cm). (C) The contents of GA_1 and GA_4 in the elongating uppermost internode. (D) The average cell lengths of the respective region of the uppermost internode. (E) Representation of a microscopic longitudinal section in region III of the uppermost internode (scale bar = 50 μm).

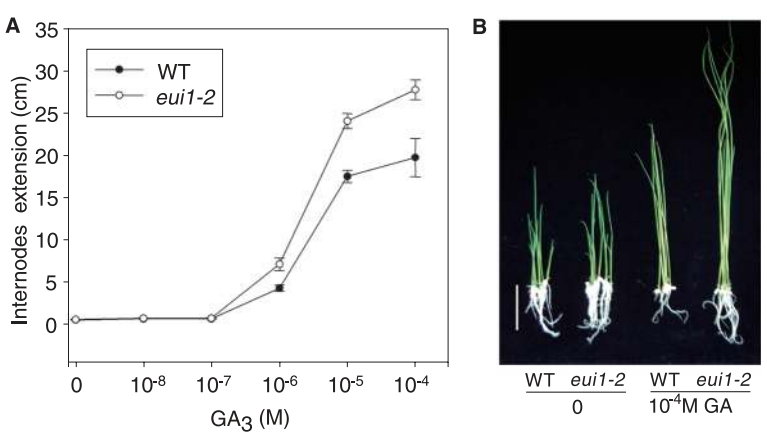

Fig. 2 Gibberellin responses in the wild type and *eui1* mutant. (A) Effect of GA_3 on growth and internode extension in the wild type and *eui1-2* mutant under greenhouse conditions. (B) Effect of GA_3 treatment on growth of rice seedlings. Scale bar = 10 cm.

In rice, internode elongation is attributed to cell divisions in the intercalary meristem, followed by cell elongation in the elongation zone (Hoshikawa 1989). To investigate whether the excess elongation of the uppermost internode in *eui1* mutants was caused by abnormal cell elongation and/or cell division, we compared the longitudinal sections of uppermost internodes in the late stage of heading. Five equally divided sections were subjected to microscopic observation (Fig. 1B). In both wild-type and *eui1-2* plants, the cell length decreased gradually from bottom to top. However, in each section, the cell length of *eui1-2* was longer than that of the wild type, and the cell length increased ratios were 1.76-, 1.76-, 1.60-, 1.74- and 1.39-fold from I to V, respectively. Except for section V, the longitudinal increased ratio of cell length was in nearly the same proportion to the ratio of the uppermost internode elongation (Fig. 1D, E). Similar results were also observed in *eui1-3* and *eui1-4* plants (data not shown). Therefore, the increased length of the uppermost internode in *eui1* should be mainly attributed to the increased cell elongation.

Altered gibberellin contents and response in the eui1-2 mutant

Gibberellin has been found as a major phytohormone promoting cell elongation. Among various gibberellin derivatives identified in plants so far, only active forms, such as GA_1 and GA_4, are able to promote cell elongation. The observation that *eui1* caused increased cell elongation prompted us to analyze

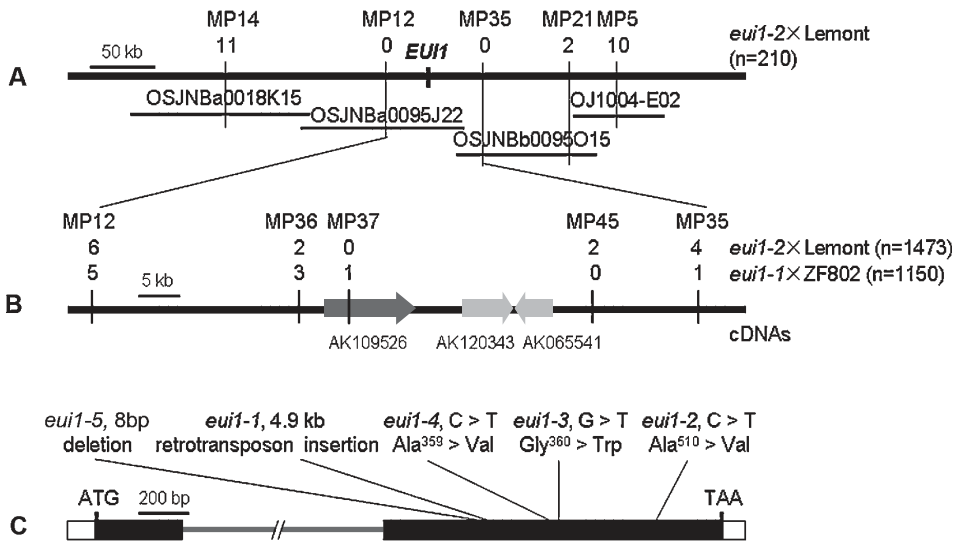

Fig. 3 Map-based cloning of the *EUI1* gene. (A) The *EUI1* gene was primarily mapped on chromosome 5. (B) The fine physical map of the *EUI1* gene based on two F_2 populations; the numbers below the vertical line indicate the numbers of the recombinants between the respective markers and the *EUI1* locus. (C) Structure of the *EUI1* gene. Mutations of five respective alleles are shown as indicated, two exons shown as black boxes, and an intron shown as a broken line.

the active gibberellin contents in the mutant. Indeed, the contents of GA_1 and GA_4 extracted from the uppermost internode of *eui1-2* were nearly doubled in comparison with that from the wild type (Fig. 1C).

To test internode extension in response to GA, 2-week-old rice seedlings were transferred into soil with different concentrations of GA_3, and extension lengths were measured after 4 weeks. In the absence of GA_3, no apparent internode elongation was observed. However, upon being treated with 10^{-6} M GA_3, substantial internode extension was observed in both the wild type and *eui1-2* (Fig. 2A). Notably, the extension length of *eui1-2* was significantly longer than that of the wild type (Fig. 2A). When germinated and grown on agar medium in the presence and absence of 10^{-4} M GA_3, whereas leaf sheath elongation was similar between *eui1-2* and the wild type (data not shown), the *eui1-2* shoot was longer than that of the wild type upon GA_3 treatment (Fig. 2B). Taken together, these results indicated that *eui1* has a higher active gibberellin content in planta, and also shows an altered gibberellin response to GA_3.

Map-based cloning of the EUI1 gene

The *EUI1* gene was previously mapped on the central part of the long arm of chromosome 5, and located within two overlapping bacterial artificial chromosome (BAC) clones, OSJNBa0018K15 and OSJNBa0095J22, in an interval of 98 kb between markers M0387 and M01 (Xu et al. 2004). By analyzing 210 F_2 mutant progeny derived from a cross between *eui1-2* and Lemont, we narrowed down the *EUI1* locus to within two cleaved amplified polymorphic sequence (CAPS) markers MP14 and MP5 (Fig. 3A). To fine-map *EUI1*, we developed additional CAPS markers (Table 2) which were used for mapping two F_2 populations derived from crosses between *eui1-1* and ZF802, and *eui1-2* and Lemont, respectively. Finally, the *eui1-2* mutation was mapped between MP36 and MP45, spanning a 34 kb sequence in the BAC clone OSJNBa0095J22 (Fig. 3B). Similarly, *eui1-1* was mapped between markers MP37 and MP35, in an interval of 41 kb that partially spanned two adjacent BAC clones, OSJNBa0095J22 and OSJNBb0095O15 (Fig. 3B). Therefore, *EUI1* should be located between MP37 and MP45, approximately in a 30 kb region (Fig. 3A).

Table 2 The CAPS markers used to locate the *EUI1* gene

Marker	Forward primer sequence (5′ to 3′)	Reverse primer sequence (5′ to 3′)	Size (kb)	Enzyme
MP14	GAATAGTTTGAAAGATACACCT	ATGAAACTACTCTTGTGTTGT	0.73	*Hae*III
MP12	AGAACAGCATGGTAAGATATAAGA	GGTGTTGTGCCATTCTTGA	0.81	–[a]
MP36	GAAGAACATCACAGGAGGGA	AGAGTTGATTGTGATGAGCAGA	0.90	*Ava*II
MP37	ACACGGTCTCCAAAACACAA	TAGCACATGACGGCCACAA	0.75	*Dde*I
MP45	ACCTGACCGAATCGAAGGCT	ATCGCCGAACACTTCCAACA	0.58	*Sty*I
MP35	TTAACCGATACCAGTCCCAGG	ACGCTCTACGCCTCTCACCA	0.69	*Ava*II
MP21	AACGCAAATAGAAAGGGGAA	GCTGATTTGGAGAGAATTGGA	0.90	*Bgl*I
MP5	TACTTTATGCGTGACTTATGT	GATTCTCGTCGTAATCAAGA	0.32	*Dra*I

[a] The PCR product of the marker MP12 is 0.81 kb in *eui1-1* and Lemont; no product is amplified in ZF802 and *eui1-2*.

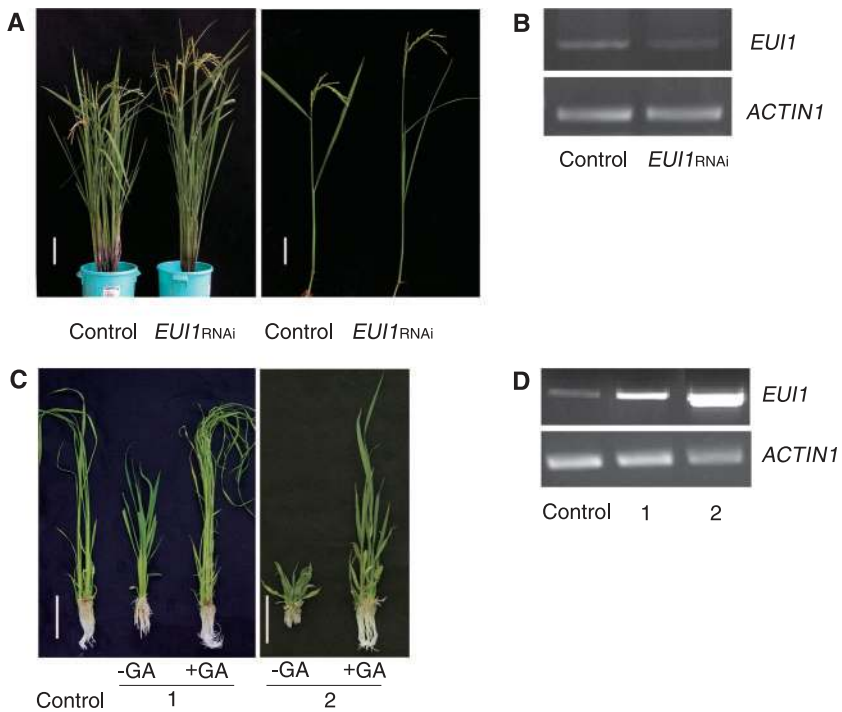

Fig. 4 Phenotypes of the high and reduced expression of the *EUI1* gene in transgenic rice. (A) Transgenic plants carrying *pCaMV 35S: EUI1-RNAi* showed *eui1*-like phenotypes. (B) The reduced *EUI1* transcripts in the transgenic plant shown in (A). (C) Overexpression of *EUI1* affects plant growth and gibberellin responses. (D) The level of *EUI1* transcript was high in the transgenic lines shown in (C). Line 2, with higher expression of *EUI1*, showed more severe phenotypes.

Database (Kikuchi et al. 2003) searches revealed that there were three open reading frames (ORFs) in this region which were confirmed by full-length cDNA clones. DNA sequence analysis of these ORFs indicated that whereas no mutation was found in two genes, mutations of various natures were identified in the third gene, *AK109526*, in all five *eui1* alleles (Fig. 3C). The *eui1-1* allele has an insertion of a 4.9 kb putative retrotransposon, resulting in the disruption of the ORF. Single base pair substitutions were found in three mutant alleles, including a C to T substitution in *eui1-2*, G to T in *eui1-3*, and C to T in *eui1-4*, which result in Ala510 changing to Val510 in *eui1-2*, Gly360 to Try360 in *eui1-3*, and Ala359 to Val359 in *eui1-4*, respectively. The *eui1-5* allele has an 8 bp deletion, resulting in a frameshift mutation in the ORF (Fig. 3C).

Moreover, knock-down of AK109526 expression by RNA interference (RNAi) using two *p35S:EUI1-RNAi* vectors (see Materials and Methods) showed a phenotype similar to that of *eui1* mutants. The RNAi transgenic plants displayed the increased panicle exsertion and uppermost internode elongation (Fig. 4A), correlated to a reduced level of AK109526 expression (Fig. 4B and data not shown). Transgenic plants carrying an empty vector did not show an apparent phenotype.

Comparison of genomic and cDNA sequences revealed that *EUI1* contained two exons and a 7,874 bp intron. BLAST searches suggested that *EUI1* encodes a putative cytochrome P450 monooxygenase with a conserved heme-binding domain that is presumably involved in the oxidative degradation of various compounds. According to the nomenclature of the P450 superfamily (Nelson et al. 1996), the EUI1 protein was designated as CYP714D1, and was phylogenetically grouped into the CYP714 subfamily, which belongs to the CYP72 clan. The protein exhibits the highest similarity to three previously reported proteins, *Arabidopsis* CYP735A1 (29% identity and 46% similarity; GenBank accession No. NM_123206), *Arabidopsis* CYP735A2 (29% identity and 45% similarity; GenBank accession no. NM_105381) and the CYP72A1 of periwinkle (28% identity and 42% similarity; GenBank accession no. Q05047) (Fig. 5).

Expression pattern of EUI1

To understand better the function of *EUI1*, we analyzed its expression pattern. By Northern analysis using total RNAs prepared from different tissues and organs, we were unable to detect *EUI1* expression (data not shown). Hence, a semi-quantitative reverse transcription–PCR (RT–PCR) analysis was performed to estimate the transcript levels. It was found that *EUI1* is expressed in most tested tissues and organs, including the young leaves, young roots, shoot apical meristem, the first and the second elongating internodes, flag leaf and young panicles (Fig. 6). *EUI1* is expressed at a relatively low level in the first elongating internodes, and the highest expression level was found in the young panicles during heading.

Overexpression of EUI1 alters gibberellin responses

To gain more insight into the function of *EUI1*, we generated transgenic rice plants overexpressing *EUI1.* To this end, we placed an entire *EUI1* ORF under the control of the cauliflower mosaic virus (CaMV) 35S promoter (*pCaMV35S: EUI1*), and then transformed this into rice cultivar Zhonghua

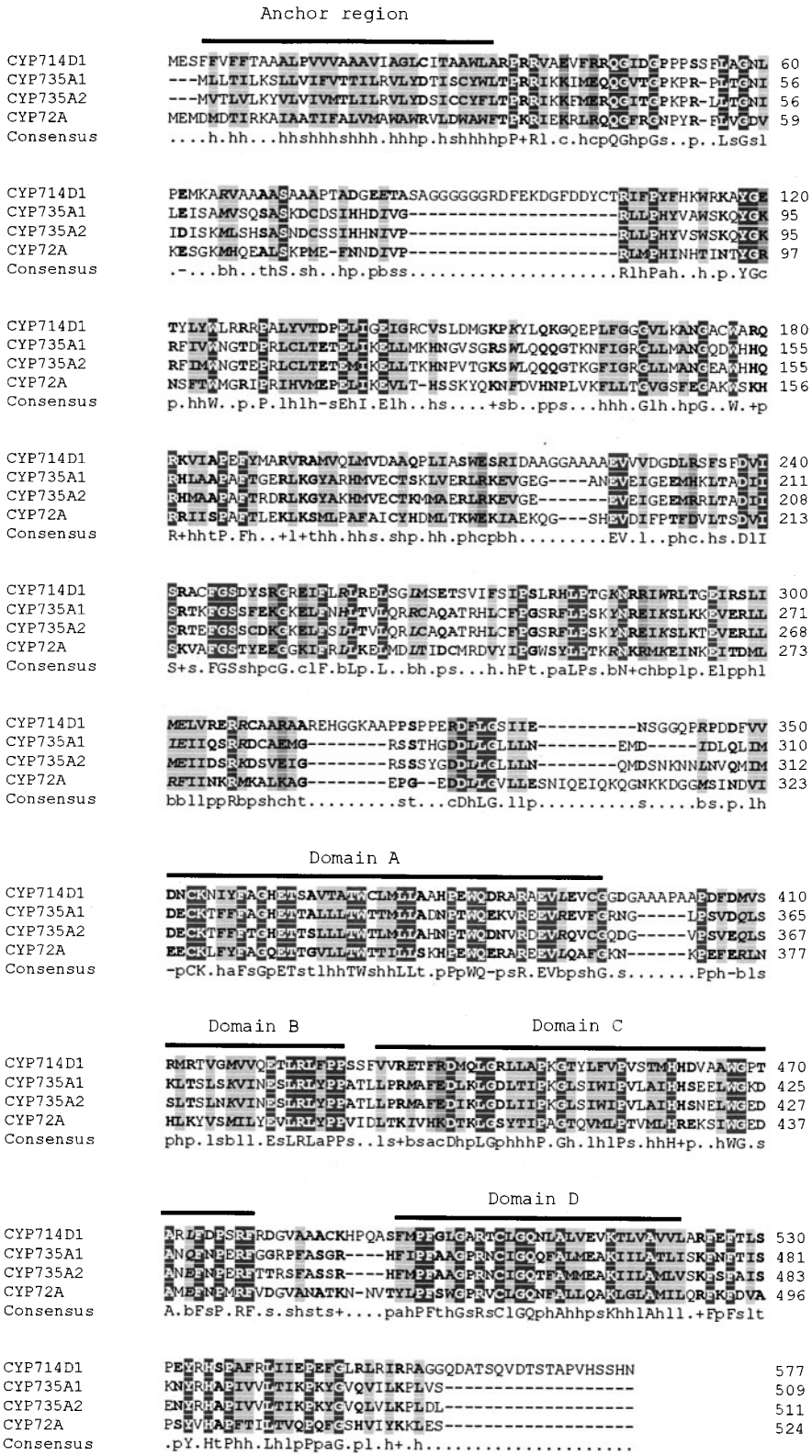

Fig. 5 Sequence alignments of CYP-714D1 and P450 homologs with defined functions using ClustalW. The conserved P450 domains, the anchor domain and domains A-D are labeled. The consensus of each residue is shown under the alignment with the following symbols: '–', negatively charged amino acid; '+', positively charged amino acid; 'c', charged amino acid; '|', aliphatic amino acid; 'a', aromatic amino acid; 't', tiny amino acid; 's', small amino acid; 'b', big amino acid; 'p', polar amino acid; 'h', hydrophobic amino acid.

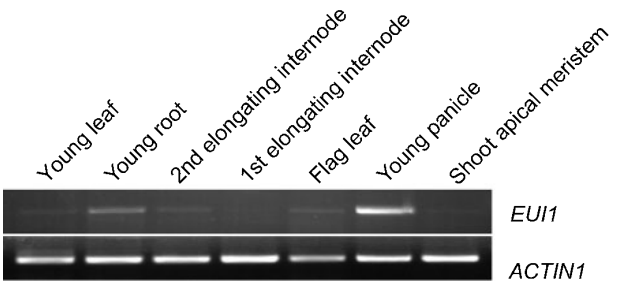

Fig. 6 Expression patterns of *EUI1* in rice. The RNA was extracted from young shoots, young roots, the first and second elongating internodes, flag leaf, young panicles and shoot apical meristem. RT–PCR was performed; *ACTIN1* was used as control.

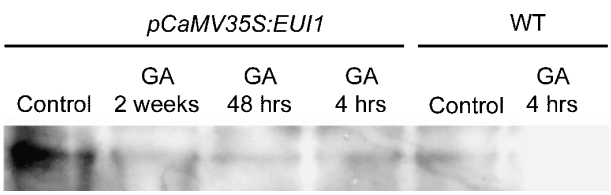

Fig. 7 Overexpression of *EUI1* caused accumulation of SLR1 protein, and delayed the gibberellin-mediated SLR1 protein destruction. The young plants were treated with 10^{-4} M GA_3 for 4 and 48 h, and with 10^{-5} M GA_3 for 2 weeks, with water as control. Each lane contains 10 μg of total protein.

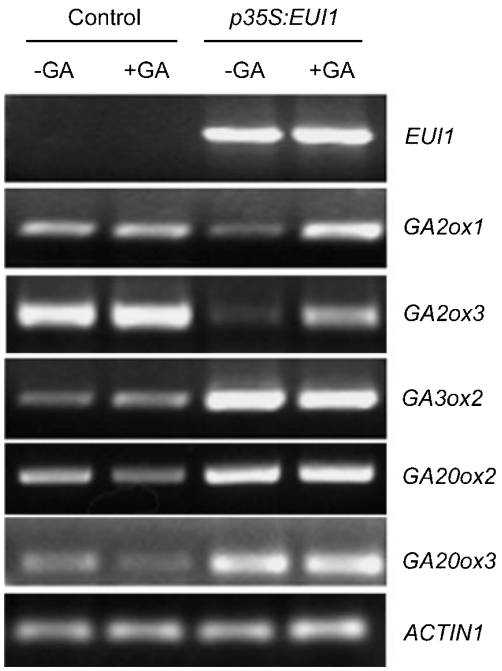

Fig. 8 The transcriptional patterns of genes involved in the gibberellin metabolic pathway in wild-type and transgenic rice lines carrying the *pCaMV35S:EUI1* construct. RT–PCR was performed as descried in Materials and Methods with *ACTIN1* as control.

11. Multiple independent transgenic lines were analyzed and similar results were obtained. In contrast to *eui1* mutants, overexpressors of *EUI1* exhibited remarkably dwarf phenotypes characterized by dark green leaves as well as short and thick roots; the phenotype strength of the transgenic plants is strictly dependent on the expression level of *EUI1* (Fig. 4C, D).

We next analyzed transgenic plants overexpressing *EUI1* in response to gibberellin. Upon treatment with 10^{-5} M GA_3 for 2 weeks, the growth and development of the transgenic plants were rescued to a varying degree. In particular, growth and development of leaves were nearly as normal as those of wild-type plants (Fig 4C). The observation that exogenous gibberellin application was able partially to rescue the dwarf phenotype of the transgenic plants suggests that EUI1 may function as a negative regulator of the gibberellin level and/or signaling.

Overexpression of EUI1 delays gibberellin-induced SLR1 destruction and modulates gibberellin biosynthesis in transgenic rice

The data presented above indicate that the loss-of-function mutations in *EUI1* cause an elevated level of active gibberellin and also hypersensitivity to gibberellin, suggesting that the putative cytochrome P450 monooxygenase is involved in gibberellin metabolism and/or signaling. As a major regulatory mechanism, gibberellin acts to destabilize the DELLA protein SLR1, a negative regulator of gibberellin signaling (Itoh et al. 2002). To test if *EUI1* is involved in regulating SLR1 stability, and thereby gibberellin signaling, we analyzed SLR1 at the protein level in *EUI1*-overexpressing transgenic plants. A higher level of SLR1 protein accumulation was found in the transgenic plants in comparison with the wild type (Fig. 7). Upon treatment with gibberellin, SLR1 was rapidly degraded, with an undetectable level in the wild type in 4 h. However, a substantial amount of SLR1 was detected in gibberellin-treated transgenic plants under identical assay conditions, even after 2 weeks of treatment. These results suggest that EUI1 is capable of stabilizing SLR1.

In both Arabidopsis and rice, expression of gibberellin-20-oxidase and gibberellin-3-oxidase, which encode enzymes that catalyze relatively late steps in the gibberellin biosynthetic pathway, are positively regulated by DELLA protein (Peng et al. 1997, Fu et al. 2001). To test if an increased SLR1 protein level also plays a role in regulating these biosynthetic pathway genes, we analyzed expression of gibberellin-20-oxidase, gibberellin-3-oxidase and gibberellin-2-oxidase in young seedlings of *EUI1* overexpressors. It was found that expression of gibberellin-20-oxidase2, gibberellin-20-oxidase3 and gibberellin-3-oxidase2, which catalyzed active gibberellin formation, was remarkably up-regulated in transgenic plants (Fig. 8). Conversely, expression of gibberellin-2-oxidase1 and gibberellin-2-oxidase3, which catalyzed active gibberellin destruction, was greatly down-regulated.

It is known that endogenous gibberellin levels are subjected to negative feedback regulation (Hedden and Phillips

2000). In *Arabidopsis*, for example, expression of gibberellin-20-oxidase and gibberellin-3-oxidase is down-regulated by active gibberellin (Chiang et al. 1995, Phillips et al. 1995, Xu et al. 1995, Cowling et al. 1998). In wild-type plants treated with 10^{-5} M gibberellin for 2 weeks, whereas expression of gibberellin-20-oxidase3 and gibberellin-20-oxidase2 was slightly down-regulated, expression of gibberellin-3-oxidase2, gibberellin-2-oxidase1 and gibberellin-2-oxidase3 remained nearly unchanged. In transgenic plants treated with gibberellin for 2 weeks, however, expression of gibberellin-2-oxidase1 and gibberellin-2-oxidase3 was greatly increased, while expression of gibberellin-20-oxidase and gibberellin-3-oxidase did not show apparent alterations compared with the control (Fig. 8). Similar observations were made in plants treated with gibberellin for 4 and 48 h (data not shown). These results suggest that whereas gibberellin-20-oxidase and gibberellin-3-oxidase transcriptional levels were positively regulated by EUI1, gibberellin-2-oxidase transcriptional levels were negatively regulated by EUI1.

Discussion

Elongation of the uppermost internodes in rice is an important agronomic trait, yet the genetic control and the molecular mechanism remain largely unknown. In this study, we have cloned and functionally characterized the *EUI1* gene, which is a key regulator in the control of elongation of the uppermost internode in rice during heading. Molecular cloning revealed that the *EUI1* gene encodes a putative cytochrome P450 protein, CYP714D1. Because all five allelic *eui1* mutants (*eui1-1* through *eui1-5*) carry loss-of-function mutations in the *EUI1* gene, and transgenic plants carrying a RNAi construct of this gene displayed a phenotype similar to that of *eui1* mutants, we conclude that the increased elongation of the uppermost internode during heading was caused by mutations in the cytochrome P450 protein.

Cytochrome P450 proteins have been known as heme-binding enzymes with mono-oxygenase activities. Some members of the plant P450 family are known to be involved in biosynthesis of plant growth regulators such as gibberellins, jasmonic acid, auxin and brassinosteroids (Nelson et al. 1996). The phylogenetic analysis of rice and *Arabidopsis* cytochrome P450 proteins shows that the EUI1/CYP714D1 protein belongs to the CYP714 subfamily of the CYP72 group. The CYP72 clan seems primarily to have conserved functions involved in plant hormone homeostasis (Nelson et al. 1996). Three known proteins, CYP735A1, CYP735A2 and CYP72A1, belonging to the CYP72 group exhibit the greatest similarity to the EUI1 protein. CYP735A1 and CYP735A2 have been identified as cytokinin biosynthetic enzymes (Takei et al. 2004), and CYP72A1 is a secologanin synthase (Irmler et al. 2000). According to the sequence similarity and the phylogenetic relationship between EUI1 and the three proteins, the *EUI1* gene possibly encodes a putative cytochrome P450 involved in gibberellin homeostasis.

The *eui1* mutants have increased cell elongation, higher levels of endogenous active gibberellin, and also a higher sensitivity to exogenously applied GA_3. Expression of genes involved in gibberellin biosynthesis, such as gibberellin-20-oxidase and gibberellin-3-oxidase, was elevated in the uppermost internodes of *eui1* mutants in comparison with that of the wild type (data not shown). In contrast, transgenic rice plants overexpressing *EUI1* exhibit typical gibberellin-deficient-like phenotypes, such as dark green leaves and dwarf architecture, which can be partially rescued by exogenous gibberellin applications. Consistent with these observations, overexpression of *EUI1* causes increased expression of several gibberellin biosynthesis genes encoding gibberellin-20-oxidase2, gibberellin-20-oxidase3 and gibberellin-3-oxidase2 (*D18* gene), that are responsible for the conversion of active gibberellin, but decreased expression of the genes encoding gibberellin-2-oxidase1 and gibberellin-2-oxidase3, that are involved in gibberellin deactivation. Similar results were observed in transgenic *Arabidopsis* plants with a low active gibberellin level (Magome et al. 2004, Alcazar et al. 2005).

In the *EUI1-RNAi* transgenic plants, the reduction of *EUI1* expression led to increased lengths of panicle exsertion and uppermost internode elongation, but not comparable with that in *EUI1*-null mutants. We also found that the increased lengths of panicle exsertion and uppermost internode elongation in our five allelic *eui1* mutants varied significantly (Table 1). Taking these data together, we conclude that EUI1 plays a negative role in controlling the elongation of rice internodes during heading, and this negative regulation should be correlated with the EUI1 level or activity.

However, the *EUI1* gene is constitutively expressed at a low level in all tested organs, except that it is preferentially expressed in the young panicles during heading. In *eui1* mutant plants, the most excessive elongation occurred in the first (uppermost) and second internodes, and therefore it was unexpected that the expression of the *EUI1* gene in the first and second elongating internodes was relatively low. It is possible that the substrate of EUI1, which controls internode elongation, could exist mainly in the young panicles and might be transported to the action site, the first and the second internodes. Further experiments are needed to elucidate the action of *EUI1*.

We demonstrated that the gibberellin-deficient-like phenotypes in the *EUI1* overexpressors can be restored more or less by exogenous gibberellin application, and that the SLR1 protein in the *EUI1* overexpressors is accumulated to a high level in comparison with the wild type. It has been shown that the SLR1 protein is triggered to undergo rapid degradation by GA_3 treatment (Itoh et al. 2002). After application of exogenous gibberellin, the SLR1 protein can still be detected at a high level, even after 48 h or 2 weeks treatment, which strongly suggested that EUI1 is a fine tuner for modulating gibberellin biosynthesis and/or modifying the stability of SLR1. Taking all the

data together, it is reasonable to postulate that EUI1, the putative P450 monooxygenase, may work as an enzyme for gibberellin inactivation in gibberellin metabolism.

The rice *eui1* mutation, as a valuable genetic resource for rice hybrid breeding, has been introduced into male-sterile lines to increase panicle exsertion (He and Shen 1991, Yang et al. 2002, Zhang and Yang 2003). *eui1* has also been brought into pollinator parents (restorers) that are 10–20 cm taller than sterile parents to increase pollen shedding onto the female panicles (Virmani et al. 1988). Thus, further elucidation of the function of the *EUI1* gene would not only give an insight into the new components in the gibberellin signaling pathway, but might also provide a new approach to improve hybrid rice breeding.

Materials and Methods

Plant materials

The *eui1-1* mutant (originally named *eui*) was identified by Rutger and Carnahan (1981). Four other alleles (*eui1-2* through *eui1-5*) were obtained from our screening and collections, of which *eui1-4* and *eui1-5* (originally named *eB1*; Yang et al. 2001) were derived from EMS- and γ-ray irradiation-mutagenized populations, respectively. The *eui1-2* and *eui1-3* mutants were recovered from breeding practices, and the nature of the mutagens was unclear. The genetic background of the mutants is listed in Table 1. Plants were grown in experimental fields under natural conditions unless indicated otherwise.

Two F_2 mapping populations were generated by crossing *eui1-1* (*japonica*) with ZF802 (*indica*), and *eui1-2* (*indica*) with Lemont (*japonica*), respectively.

DNA extraction and positional cloning

Genomic DNA was prepared from leaves according to the method of Dellaporta et al. (1983). More than 40 pairs of primers for CAPS markers were designed to cover an 8.3 cM interval (94.5–102.8 cM) on the long arm of chromosome 5, to which the *eui* locus had previously been mapped (Wu et al. 1998, Xu et al. 2004). Most of the CAPS primers were designed according to the DNA sequences of chromosome 5 of *indica* and *japonica* (http://www.ncbi.nih.gov/), except that some were adopted from the Gramene Database (http://www.gramene.org/Oryza_sativa) or the Rice Genomic Project Database (http://rgp.dna.affrc.go.jp/publicdata/). The PCR was run for 34 cycles at 94°C for 30 s, 59°C for 30 s and 72°C for 90 s, with an additional extension at 72°C for 5 min. PCR products were cleaved and then separated by electrophoresis on 1.2–3.0% (w/v) agarose gels.

Sequence analysis of candidate genomic region

Because the candidate P450 gene has two predicted exons split by a 7,874 bp long intron, we amplified the two genomic regions covering the two exons separately through 32 PCR cycles (94°C for 30 s, 58°C for 30 s and 72°C for 120 s) using the primer pairs designed from the genomic sequence. The primer pairs were 5′-TGTGTAACCGTCCAGGGTGA-3′ and 5′-TGATAGCTGATTAATTGTGGGT-3′, and 5′-AGATCACATAGGCACGCATCA-3′ and 5′-TCCTCTGTTGTCTCTGTATGTG T-3′, respectively. Amplified products from *eui1* mutant alleles and corresponding wild-type plants were purified and cloned into the pMD18-T cloning vector (TaKaRa, Dalian, China). The cloned DNA fragments were sequenced and the nucleotide sequences were compared against the GenBank database. The nucleotide mutations in some *eui1* mutants resulted in CAPS polymorphisms with its wild type. In these cases, we developed new CAPS markers to confirm the *eui1* mutations further.

RT–PCR and Western blotting analysis

Total RNA was extracted using the guanidinium isocyanate/acidic phenol method described by Chomczynski and Sacchi (1987).

The first-strand cDNA was synthesized from 2 μg of total RNA (pre-treated with DNase I) using oligo(dT) as primers (Promega). The first strand cDNA products equivalent to 50 ng of total RNA were used as templates in a 20 μl PCR. The following primer pairs were used in amplification of the candidate genes: *AK109526* (P450, *EUI1*), 5′-AAGGACGGGTTCGACGACTA-3′ and 5′-TTGCAGTTGTCCACCACGAA-3′; *AK120343*, 5′-CCTTCAACCACCTCCTCCAT-3′ and 5′-ACTAAGCCGAAAGGTATCATCA-3′; *AK065541*, 5′-TGACATGGGTAACAGTGAGGT-3′ and 5′-AGCAATCTCAAGGGCCTTCA-3′.

For expression analysis of gibberellin-20-oxidases, gibberellin-3-oxidase and gibberellin-2-oxidases, which play important roles in GA metabolism, RT–PCR was performed with total RNAs isolated from young seedlings of *EUI1* overexpressors and the primers as described by Sakamoto et al. (2004).

The rice *actin1* gene (*ACTIN1*) was used as an internal control in the RT–PCR analysis with primer pairs 5′-ACATCGCCCTGGACTATGACCA-3′ and 5′-GTCGTACTCAGCCTTGGCAAT-3′.

The extraction of protein and Western blotting analysis were performed as described by Fu et al. (2002).

Plasmid construction

Two RNAi constructs, p4501 and p4502, were constructed as previously described (Luo et al. 2005), Briefly, the *OsGRF* fragment in pCGI was replaced with the ORF fragments of *EUI1* (p4501 targeting –60 to + 355 bp; p4502 targeting +761 to +1,173 bp). DNA fragments consisting of a sense and an antisense strand separated by an intron were inserted into pXQ35S [a derivative of pCAMBIA2300 carrying the CaMV 35S promoter and the octopine synthase (OCS) terminator]. For the overexpression construct of *EUI1*, an entire *EUI1* ORF was PCR amplified, sequenced and inserted into pXQ35S, yielding the p4503 construct. These constructs were introduced into *Agrobacterium tumefaciens* EHA105.

Rice transformation

Transgenic rice plants of p4501, p4502 and p4503 were generated by using the *Agrobacterium*-mediated transformation method described previously with some modifications (Chen et al. 2001). Embryogenic calli derived from mature seeds of the rice cultivar Nipponbare and Zhonghua11 were used for transformation. Antibiotic G418 was used for the selection of transformed calli. After regeneration and rooting, resistant rice plants were transferred to soil and grown in a field. Transgenic plants of the T_0 generation were subjected to phenotypic and RT–PCR analysis. As a control, the pXQ35S vector was also introduced into Nipponbare and Zhonghua11.

Gibberellin preparation and ELISA analysis

The ground frozen tissues were extracted in 80% methanol and then centrifuged at 13,000 rpm for 15 min at 4°C; the total concentrations of GA_1 and GA_4 were then determined by enzyme-linked immunosorbent assay (ELISA; Nanjing Agricultural University, China) as described by Cui et al. (2005).

Gibberellin response in shoot elongation

Elongation of shoots was measured according to the method of Ueguchi-Tanaka et al. (2000) with minor modifications. Rice seeds were divested of glumes and surface sterilized, washed three times

with sterile distilled water, then imbibed at 30°C for 1 d. The seeds were placed on agar containing various concentrations of GA_3 and grown at 30°C under continuous light. After an 8 d incubation, the lengths of the second leaf sheaths and the plant height were measured.

To measure internode extension in response to gibberellin, rice seeds were sown in soil and grown for 2 weeks in a greenhouse. The seedlings were then transferred to pots containing various concentrations of GA_3. After 4 weeks, the total lengths of elongated internodes were measured. In the absence of GA_3, no elongation of the internodes was observed under our assay conditions.

Electron microscopy

The uppermost internodes collected from natural grown mature plants were used for scanning electron microscope analysis. Briefly, samples were fixed with 2.5% glutaraldehyde solution. The fixed samples were dehydrated with a gradual ethanol series, dried by a critical-point drying method using liquid carbon dioxide (Model HCP-2, Hitachi, Tokyo, Japan), gold-coated with an Edwards E-1010 ion sputter coater (Hitachi, Tokyo, Japan) and then observed with a S-3000N variable pressure scanning electron microscope (Hitachi, Tokyo, Japan).

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References

Alcazar, R., Garcia-Martinez, J.L., Cuevas, J.C., Tiburcio, A.F. and Altabella, T. (2005) Overexpression of ADC2 in Arabidopsis induces dwarfism and late-flowering through GA deficiency. *Plant J.* 43: 425–436.

Ashikari, M., Wu, J., Yano, M., Sasaki, T. and Yoshimura, A. (1999) Rice gibberellin-insensitive dwarf mutant gene *Dwarf 1* encodes the alpha-subunit of GTP-binding protein. *Proc. Natl Acad. Sci. USA* 96: 10284–10289.

Chen, S., Qu, N., Cao, S.Y., Bauwe, H., Chen, S.Y., Tian, W.Z. and Chu, C.C. (2001) Expression pattern of *gdcs*P promoter from C_3–C_4 intermediate plant *Flaveria anomala* in transgenic rice. *Chin. Sci. Bull.* 46: 1635–1638.

Chiang, H.H., Hwang, I. and Goodman, H.M. (1995) Isolation of the Arabidopsis GA_4 locus. *Plant Cell* 7: 195–201.

Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal. Biochem.* 162: 156–159.

Cowling, R.J., Kamiya, Y., Seto, H. and Harberd, N.P. (1998) Gibberellin dose–response regulation of GA_4 gene transcript levels in Arabidopsis. *Plant Physiol.* 117: 1195–1203.

Cui, D., Neill, S.J., Tang, Z. and Cai, W. (2005) Gibberellin-regulated XET is differentially induced by auxin in rice leaf sheath bases during gravitropic bending. *J. Exp. Bot.* 56: 1327–1334.

Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983) A rapid method for DNA extraction from plant tissue. *Plant Mol. Biol. Rep.* 1: 19–21.

Fu, X., Richards, D.E., Ait-Ali, T., Hynes, L.W., Ougham, H., Peng, J. and Harberd, N.P. (2002) Gibberellin-mediated proteasome-dependent degradation of the barley DELLA protein SLR1 repressor. *Plant Cell* 14: 3191–3200.

Fu, X., Sudhakar, D., Peng, J., Richards, D.E., Christou, P. and Harberd, N.P. (2001) Expression of Arabidopsis GAI in transgenic rice represses multiple gibberellin responses. *Plant Cell* 13: 1791–1802.

He, Z.H. and Shen, Z.T. (1991) Inheritance of panicle exsertion and improvement of male sterile line in rice. *Chin. J. Rice Sci.* 5: 1–6.

He, Z.H. and Shen, Z.T. (1994) Sensitivity of elongated internode gene to GA_3 and improvement of MS line in rice. *Acta Agronom. Sin.* 20: 161–167.

Hedden, P. and Phillips, A.L. (2000) Gibberellin metabolism: new insights revealed by the genes. *Trends Plant Sci.* 5: 523–530.

Hong, Z., Ueguchi-Tanaka, M., Umemura, K., Uozu, S., Fujioka, S., Takatsuto, S., Yoshida, S., Ashikari, M., Kitano, H. and Matsuoka, M. (2003) A rice brassinosteroid-deficient mutant, *ebisu dwarf* (*d2*), is caused by a loss of function of a new member of cytochrome P450. *Plant Cell* 15: 2900–2910.

Hoshikawa, K. (1989) The Growing Rice Plant. pp. 123–148. Nobunkyo, Tokyo.

Irmler, S., Schroder, G., St-Pierre, B., Crouch, N.P., Hotze, M., Schmidt, J., Strack, D., Matern, U. and Schroder, J. (2000) Indole alkaloid biosynthesis in *Catharanthus roseus*: new enzyme activities and identification of cytochrome P450 CYP72A1 as secologanin synthase. *Plant J.* 24: 797–804.

Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M. and Matsuoka, M. (2002) The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. *Plant Cell* 14: 57–70.

Kikuchi, S., Satoh, K., Nagata, T., Kawagashira, N., Doi, K. et al. (2003) Collection, mapping, and annotation of over 28, 000 cDNA clones from japonica rice. *Science* 301: 376–379.

Librojo, A.L. and Khush, G.S. (1986) Chromosomal location of some mutant genes through the use of primary trisomics in rice. *In* Rice Genetics. Edited by Khush, G.S. pp. 249–255. International Rice Research Institute, Manila.

Luo, A.D., Liu, L., Tang, Z.S., Bai, X.Q., Cao, S.Y. and Chu, C.C. (2005) Down-regulation of *OsGRF1* gene in rice *rhd1* mutant results in reduced heading date. *J. Integrative Plant Biol.* 47: 745–752.

Maekawa, M., Maekawa, T., Shinbashi, N. and Kinoshita, T. (1989) Allelism of genes for elongation of uppermost internode from two different sources. *Rice Genet. Newslett.* 6: 101–103.

Magome, H., Yamaguchi, S., Hanada, A., Kamiya, Y. and Oda, K. (2004) *dwarf and delayed-flowering 1*, a novel Arabidopsis mutant deficient in gibberellin biosynthesis because of overexpression of a putative AP2 transcription factor. *Plant J.* 37: 720–729.

Nelson, D.R., Koymans, L., Kamataki, T., Stegeman, J.J., Feyereisen, R. et al. (1996) P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 6: 1–42.

Peng, J., Carol, P., Richards, D.E., King, K.E., Cowling, R.J., Murphy, G.P. and Harberd, N.P. (1997) The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev.* 11: 3194–3205.

Peng, J., Richards, D.E., Hartley, N.M., Murphy, G.P., Devos, K.M. et al. (1999) 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400: 256–261.

Phillips, A.L., Ward, D.A., Uknes, S., Appleford, N.E., Lange, T., Huttly, A.K., Gaskin, P., Graebe, J.E. and Hedden, P. (1995) Isolation and expression of three gibberellin 20-oxidase cDNA clones from Arabidopsis. *Plant Physiol.* 108: 1049–1057.

Rutger, J.N. and Carnahan, H.L. (1981) A fourth genetic element to facilitate hybrid cereal production. A recessive tall in rice. *Crop Sci.* 21: 373–376.

Sakamoto, T., Miura, K., Itoh, H., Tatsumi, T., Ueguchi-Tanaka, M. et al. (2004) An overview of gibberellin metabolism enzyme genes and their related mutants in rice. *Plant Physiol.* 134:1642–1653.

Sasaki, A., Ashikari, M., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A. et al. (2002) Green revolution: a mutant gibberellin-synthesis gene in rice. *Nature* 416: 701–702.

Sasaki, A., Itoh, H., Gomi, K., Ueguchi-Tanaka, M., Ishiyama, K., et al. (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. *Science* 299: 1896–1898.

Shen, Z.T., Yang, C.D. and He, Z.H. (1987) Studies on eliminating panicle enclosure in WA type MS line of rice *Oryza sativa* subsp. *indica*. *Chin. J. Rice Sci.* 1: 95–99.

Spielmeyer, W., Ellis, M.H. and Chandler, P.M. (2002) *Semidwarf* (*sd-1*), 'green revolution' rice, contains a defective *gibberellin 20-oxidase* gene. *Proc. Natl Acad. Sci. USA* 99: 9043–9048.

Takei, K., Yamaya, T. and Sakakibara, H. (2004) Arabidopsis CYP735A1 and CYP735A2 encode cytokinin hydroxylases that catalyze the biosynthesis of trans-zeatin, *J. Biol. Chem.* 279: 41866–41872.

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., et al. (2005) *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. *Nature* 437: 693–698.

Ueguchi-Tanaka, M., Fujisawa, Y., Kobayashi, M., Ashikari, M., Iwasaki, Y., Kitano, H. and Matsuoka, M. (2000) Rice dwarf mutant d1, which is defective in the alpha subunit of the heterotrimeric G protein, affects gibberellin signal transduction. *Proc. Natl Acad. Sci. USA* 97: 11638–11643.

Virmani, S.S., Dalmacio, R.D. and Lopez, M.T. (1988) *eui* gene for elongated uppermost internode transferred to indica rice. *Int. Rice Res. Newslett.* 13: 6.

Wu, Y.L., He, Z.H., Dong, J.X., Li, D.B., Lin, H.X., Zhuang, J.Y., Lu, J. and Zheng, K.L. (1998) The RFLP tagging of *eui* gene in rice. *Chin. J. Rice Sci.* 12: 119–120.

Xu, Y.H., Zhu, Y.Y., Zhou, H.C., Li, Q., Sun, Z.X., Liu, Y.G., Lin, H.X. and He, Z.H. (2004) Identification of a 98-kb DNA segment containing the rice *EUI* gene controlling uppermost internode elongation, and construction of a TAC transgene sublibrary. *Mol. Gen. Genet.* 272: 149–155.

Xu, Y.L., Li, L., Wu, K., Peeters, A.J., Gage, D.A. and Zeevaart, J.A. (1995) The GA_5 locus of *Arabidopsis thaliana* encodes a multifunctional gibberellin 20-oxidase: molecular cloning and functional expression. *Proc. Natl Acad. Sci. USA* 92: 6640–6644.

Yang, R.C., Zhang, S.B., Huang, R.H., Yang, S.L. and Zhang, Q.Q. (2002) Breeding technology of *eui* hybrids of rice. *Sci. Agric. Sin.* 35: 233–237.

Yang, S.L., Yang, R.C., Qu, X.P., Zhang, Q.Q., Huang, R.H. and Wang, B. (2001) Genetic and microsatellite analysis of a new elongated uppermost internode gene *eui2* of rice. *Acta Bot. Sin.* 43: 67–71.

Yoshida, S. (1981) Fundamentals of Rice Crop Science. International Rice Research Institute, Los Banos, Laguna, The Philippines.

Zhang, Q.Q. and Yang, R.C. (2003) The effect of different *eui* genes on biological characters of *eui*-hybrid rice. *Sci. Agric. Sin.* 36: 735–739.

Zhu, X.D., Chen, H.Q. and Min, S.K. (2003) Allelism analysis and comparison of plant height, panicle and internode lengths in tall-culm mutants of rice (*Oryza sativa* L.). *Acta Agronom. Sin.* 29: 591–594.

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