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ORIGINAL ARTICLE

Gibberellin homeostasis and plant height control by EUI and a role for gibberellin in root gravity responses in rice

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The rice *Eui* (*ELONGATED UPPERMOST INTERNODE*) gene encodes a cytochrome P450 monooxygenase that deactivates bioactive gibberellins (GAs). In this study, we investigated controlled expression of the *Eui* gene and its role in plant development. We found that *Eui* was differentially induced by exogenous GAs and that the *Eui* promoter had the highest activity in the vascular bundles. The *eui* mutant was defective in starch granule development in root caps and *Eui* overexpression enhanced starch granule generation and gravity responses, revealing a role for GA in root starch granule development and gravity responses. Experiments using embryoless half-seeds revealed that *RAmy1A* and *GAmyb* were highly upregulated in *eui* aleurone cells in the absence of exogenous GA. In addition, the GA biosynthesis genes *GA3ox1* and *GA20ox2* were downregulated and *GA2ox1* was upregulated in *eui* seedlings. These results indicate that EUI is involved in GA homeostasis, not only in the internodes at the heading stage, but also in the seedling stage, roots and seeds. Disturbing GA homeostasis affected the expression of the GA signaling genes *GID1* (GIBBERELLIN INSENSITIVE DWARF 1), *GID2* and *SLR1*. Transgenic RNA interference of the *Eui* gene effectively increased plant height and improved heading performance. By contrast, the ectopic expression of *Eui* under the promoters of the rice GA biosynthesis genes *GA3ox2* and *GA20ox2* significantly reduced plant height. These results demonstrate that a slight increase in *Eui* expression could dramatically change rice morphology, indicating the practical application of the *Eui* gene in rice molecular breeding for a high yield potential.

***Keywords*:** ectopic expression, *Eui*, gibberellin homeostasis, gravity responses, plant height, rice
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Introduction

The plant hormone gibberellin (GA) controls diverse biological processes such as stem elongation, seed germination and flowering. Many GA-related mutants have been isolated from the model plant species *Arabidopsis thaliana* and rice (*Oryza sativa* L.), and the majority of the underlying genes have been found to encode enzymes that are related to GA metabolism or components of GA signaling [1-7]. Many GA mutants show dramatically reduced plant height and hence increased yield potential and lodging resistance of modern cereal varieties, thus greatly contributing to the success of the 'green revolution' [8-10]. Recently, GID1 (GIBBERELLIN INSENSITIVE DWARF 1) was identified as a soluble GA receptor in rice and *Arabidopsis* [11-13].

In addition to components in GA signaling, endogenous GA levels regulated by the GA metabolism also have an important role in the control of plant development. For example, a mutation in the *GA20ox* gene (*sd-1*) resulted in decreased GA levels in rice, which has been attributed to the rice 'green revolution' gene [9, 10]. Moreover, ectopic expression of *OsGA2ox1* under the control of the promoter of the GA biosynthesis gene *GA3ox2* resulted in a semi-dwarf phenotype, suggesting the potential for high yield production in rice [14]. Many GA-related genes are feedback or

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Abbreviations: double-stranded RNA (dsRNA); ELONGATED UPPERMOST INTERNODE (Eui); gibberellin (GA); GIBBERELLIN INSENSITIVE DWARF (GID); overexpression (OX); RNA interference (RNAi); slender rice (SLR); wild type (WT)
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feedforward regulated by bioactive GAs, where *GA20ox* and *GA3ox* function in GA biosynthesis with feedback regulation, and *GA2ox* functions in GA catabolism with feedforward regulation by bioactive GAs; GA metabolism is also closely related to GA signaling [4, 15].

Bioactive GA_1, GA_4, their immediate precursors GA_{20} and GA_9, and C20 GAs are deactivated by GA2-oxidases [4, 15-18]. However, 2-oxidation is not the only mechanism by which bioactive GAs are deactivated. Our previous study has demonstrated that the rice *Eui* gene (*ELONGATED UPPERMOST INTERNODE*), also known as *Eui1* [19], encodes a novel P450 monooxygenase that catalyzes 16α,17-epoxidation of non-13-hydroxylated GAs to generate bio-inactive 16α,17-$[OH]_2$-GAs, representing a novel GA deactivation mechanism in rice [20]. The 16α,17-$[OH]_2$-GAs were also found in extracts of other plant species, suggesting that 16α,17-epoxidation might participate in GA deactivation by EUI-related enzymes in a variety of plants [20]. The finding that *Eui* is a GA catabolism gene provides additional evidence that the GA metabolism pathway is a useful target for increasing the agronomic value of crops. Indeed, the *eui* mutants have been used to genetically improve the heading performance of rice male sterile cultivars in hybrid rice production [21-25].

The elevated expression of the *Eui* gene dramatically reduces the GA levels, leading to severe dwarf phenotypes in most transgenic plants that constitutively express *Eui* [20]. Therefore, fine-tuning EUI levels by either ectopic expression or RNA interference (RNAi) could effectively decrease or increase internode elongation, resulting in dwarfed or elongated transgenic plants for different breeding purposes. Interestingly, *eui* plants are still sensitive to exogenous GA_3 application, despite the highly accumulated levels of endogenous bioactive GAs in the mutants [20, 22]. Further experiments are needed to define the details of the mechanisms by which *Eui* regulates GA homeostasis and thereby GA signaling and other GA-related biological processes.

In this study, we investigated the regulation of *Eui* gene expression and its role in rice development. We generated transgenic plants that ectopically expressed *Eui* under the *GA20ox2* (*GA20ox-Eui*) and *GA3ox2* (*GA3ox-Eui*) promoters, and decreased *Eui* expression through RNAi. We show that the *Eui* gene was induced by bioactive GAs, and that it was preferentially expressed in the vascular bundles of the elongating internodes. The GA signaling genes *GID2*, *GID1* and *SLR1* were repressed in the *eui* mutant and were upregulated in the *Eui*-OX transgenic plants. The expression of *RAmy1A* and *GAmyb* was highly elevated in the *eui* mutants compared with the wild type (WT), leading to higher α-amylase activity in the aleurone layer. Accordingly, starch granule development was defective in the root-tip cells of the *eui* mutants, whereas it was enhanced in *Eui*-OX plants, resulting in altered gravity responses. Ectopic expression of the *Eui* gene driven by the GA gene promoters dramatically decreased plant height in a dose-dependent manner. We also discuss how the *Eui* gene is involved in GA homeostasis and its potential utilization in crop improvement.

Results

Differential response of eui mutants and WT plants to GAs

eui mutants accumulate extremely high levels of GA_1 and GA_4 [20]. However, our previous studies have shown that *eui* mutants remain highly sensitive to exogenous GA_3 application at the heading stage [22]. To study the responsiveness of *eui* mutants to exogenous GAs at the seedling stage, we incubated *eui-1* and WT plants with bioactive GA_3, GA_1, GA_4 and the precursor GA_9. As shown in Figure 1A and 1B, GA_3 was most effective in increasing plant height in both WT and *eui* plants. The *eui* mutants were significantly more responsive than the WT plants to GA_4 and GA_9. Because GA_4 and GA_9 are the substrates of EUI [20], they would not be deactivated by a functional EUI and therefore exhibited greater activity in *eui* plants. Although both *eui* and WT plants responded to GA_1, no significant difference was observed in their responses to this GA molecule since the control *eui* plants were found to be slightly taller than WT plants (Figure 1A and 1B). Similar results were also observed with spray treatment of GAs (data not shown). We propose that rice may have a high saturated GA response threshold. In support of this idea, we found a saturated response to about 9 mM GA_3 in WT plants at the seedling stage (data not shown).

Since EUI uses GA_4 but not GA_1 as a substrate, and GA_3 is not an endogenous GA, we next tested whether these GAs differentially induce the *Eui* gene in seedling leaves. We found that *Eui* expression was induced by GA_4 within 1-4 h (Figure 1C). *Eui* was also induced by GA_1 and GA_3 in a biphasic pattern that is different from that of GA_4 (Figure 1C). This kind of transient GA induction of *Eui* is probably in agreement with its expression pattern of strong tissue and developmental specificity [20]. Our current study indicates that the *Eui* gene is also involved in GA homeostasis at the seedling stage (also see below), even though *Eui* is not expressed at high levels in young seedlings and there is no strong phenotype of the *eui* mutants at this stage [20].

Eui is highly expressed in vascular cells

Eui is primarily expressed in rapidly elongating or dividing tissues, including the divisional zone and the

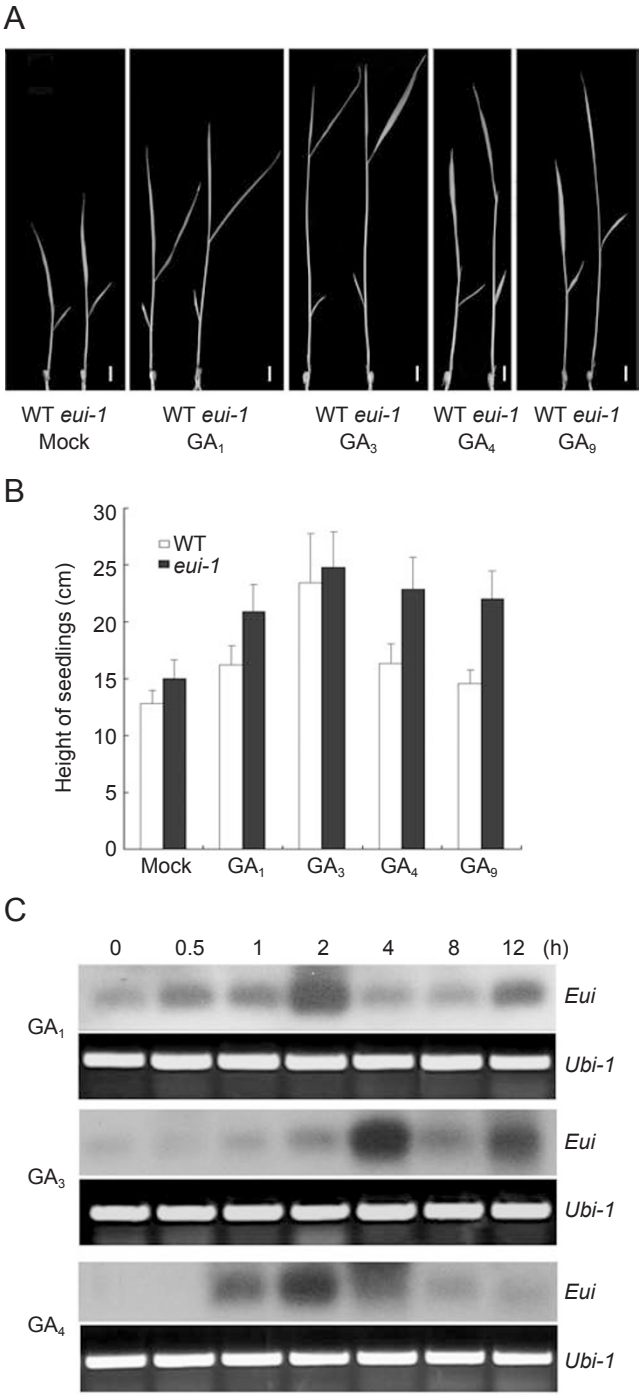

Figure 1 GA sensitivity of *eui* and wild-type plants and GA induction of *Eui*. **(A)** Sensitivity of *eui* mutant and wild-type (WT) seedlings to different GA molecules. Two-week-old seedlings were incubated with GAs (1 μM) or without GAs (mock) for 1 week. **(B)** Plant elongation of *eui* and wild-type seedlings treated with GAs. Plant height was measured at day 7 after GA incubation. Error bars show standard errors (SE). **(C)** GA induction of *Eui*. Wild-type seedlings were incubated with GA_1, GA_3 and GA_4 (1 μM) for 0-12 h. The RT-PCR products were subjected to DNA gel blot analysis to detect the *Eui* expression levels. *Ubi-1* was used as a control. Results are representative of two independent experiments with similar results.

node of the uppermost internode [20]. Bioactive GAs are thought to be produced in these regions because *GA20ox2* and *GA3ox2* are also predominantly expressed here [26]. To test whether *Eui* is expressed in specific cells in this region, we analyzed β-glucuronidase (GUS) staining of cross-sections of the divisional zone of an elongating uppermost internode of the *Eui-GUS* transgenic plant [20]. Strong GUS staining was observed in the vascular bundles of the elongating internodes (Figure 2A). Interestingly, the highest level of GUS was found in the parenchyma cells (Figure 2B), suggesting that GAs might be produced in or transported through the vascular system.

Eui regulates root starch granule development and gravity responses

An early study showed that GA plus kinetin treatment could remove starch granules in amyloplasts and therefore change gravisensitivity of cress roots [27]. We were interested in determining whether those *Eui*-mediated GA phenotypes affect starch accumulation and graviresponsiveness in rice roots. As shown in Figure 3A and 3B, starch granules were almost completely absent in *eui* root-tip cells, while their generation was enhanced in the roots of *Eui*-OX plants compared with WT plants. As a consequence of the altered starch granule development, we observed that *Eui*-OX roots were more hypersensitive than the WT to gravity. After 2 h of rotation away from vertical, most *Eui*-OX root tips bended near vertical (Figure 3C). All WT and *Eui*-OX root tips bended vertically when roots were rotated over 12 h. These results indicate that *Eui* is also involved in GA homeostasis in rice roots and reveal a novel role for GA in gravity responses.

GA signaling is regulated by Eui expression

EUI P450 deactivates bioactive GAs and knockout mutants accumulate high levels of bioactive GAs [20]. Therefore, we next investigated whether the GA signaling

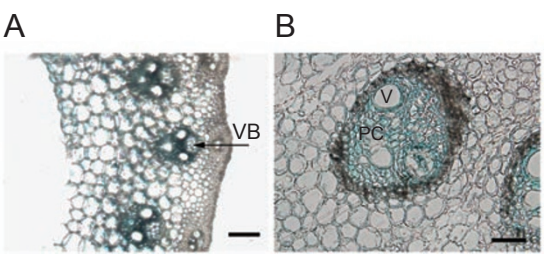

Figure 2 Cell-specific activity of the *Eui* promoter. **(A)** GUS staining was detected in the cross-section of the divisional zone of the elongating uppermost internode of the *Eui-GUS* transgenic plant. Bar=50 μm. **(B)** The highest activity was found in the parenchyma cells (PC) of the vascular bundles (VB). Bar=200 μm. V, vessel.

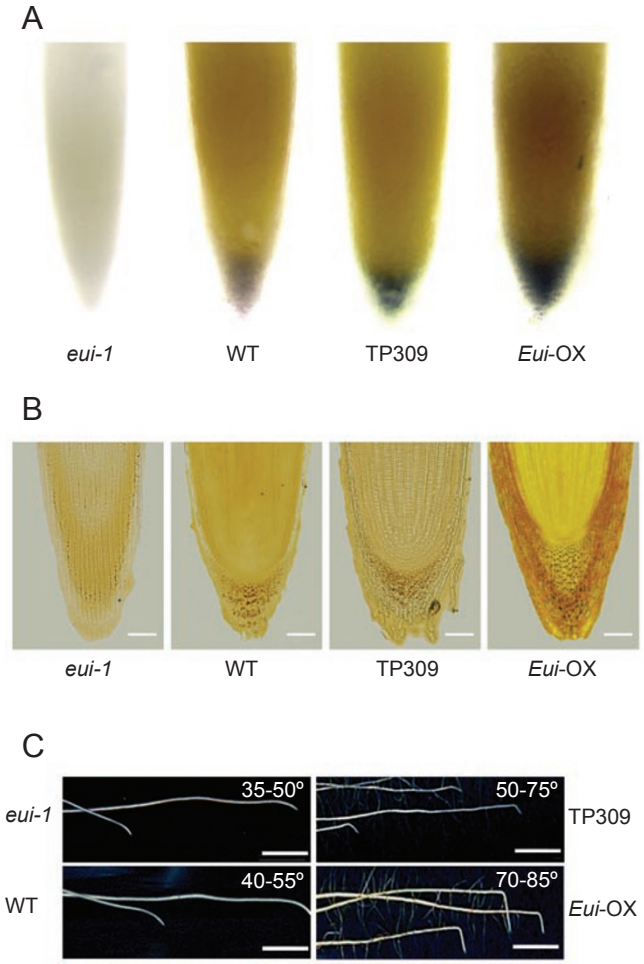

Figure 3 Effects of *Eui* on root starch granule development and gravity responses. **(A)** Starch granules in roots of *eui* mutant, wild-type and *Eui*-OX and transgenic parent (TP309) plants. Starch granules are stained as black spots in the root tips. Results are representative of three independent experiments with similar results. **(B)** Longitudinal section of stained seedling root tips. Starch granules are displayed as brown spots. Bar=100 μm. **(C)** Gravisensitivity of seedling roots. Results are representative of two independent experiments with similar results. Bending angles of the majority of roots are indicated for each line. Bar=5 mm.

genes are affected in *eui* and *Eui*-OX plants. As shown in Figure 4A, the expression of the positive GA signaling genes, the receptor gene *GID1* [11] and the F-box gene *GID2* [5] was repressed in the *eui* mutant and increased in *Eui*-OX plants. Intriguingly, the negative GA regulator *SLR1*, which encodes a DELLA protein [3], was slightly repressed in the young panicle of *eui* plants compared with WT plants. Likewise, overexpression (OX) of *Eui* slightly increased *SLR1* transcription compared with the WT. In support of our observation, SLR1 protein accumulation or stability was greater in transgenic plants constitutively expressing *Eui* [19]. By contrast, *D1*, a positive regulator of GA signaling that encodes the α-subunit of the heterotrimeric G protein [1, 2], was not affected (Figure 4A). In addition, we observed that the GA biosynthesis genes *GA3ox1* and *GA20ox2* were downregulated and *GA2ox1* was upregulated in the *eui* seedlings (Figure 4B), similar to their expression in the internode during the heading stage [20]. This result supports the above proposal that *Eui* also regulates GA homeostasis in the seedling stage.

Aleurone cells recognize GA signals and trigger expression of α-amylase [28]. We therefore assayed amylase activity to further characterize the altered GA signaling in *eui* plants. WT and *eui* mutant embryoless half-seed plants were placed on starch plates with or without 1 μM GA_3 for 3 days, and the starch was then stained with iodine. Production of α-amylase from WT half-seeds was observed only on the plate containing GA_3. By contrast, *eui* half-seeds produced strong amylase activity even in the absence of exogenous GA_3 (Figure 4C). Similar production of α-amylase was observed in the rice *slender* (*slr*) mutant [3]. Consistently, we detected higher expression levels of the α-amylase gene *RAmy1A* and *GAmyb*, a positive regulator of *RAmy1A* expression [29], compared with their expression in the WT (Figure 4D). These results demonstrate that amylase activity was high in the *eui* seeds, which indicates that GA signaling is enhanced in the *eui* aleurone cells. However, we did not observe decreased *SLR1* expression either in the *eui* aleurone cells or in the *eui* seedlings (Figure 4B and 4D), which suggests that *SLR1* might detect the GA signal differentially in different rice tissues. Together, these results indicate that the GA signaling pathway is affected in *eui* and *Eui*-OX plants owing to altered GA homeostasis.

RNAi of Eui effectively increases internode elongation

The *eui* phenotype that has increased panicle exertion (heading performance) has been used in breeding for male sterile varieties of hybrid rice [21-25]. From this we developed rice that has an *eui* phenotype using RNAi. Using a double-stranded RNA (dsRNA) transgenic approach [30], we efficiently generated *Eui* knockout/knockdown plants, of which more than 80% of independent transgenic plants showed an elongated internode phenotype with decreased or undetectable expression of *Eui* that is similar to the *eui* mutant (in Figure 5A-5C, the results from four representative T1 transgenic plants are shown). These RNAi lines were stably inherited within generations (data not shown). Consequently, this study provides a feasible approach to rapidly develop elite *eui* rice lines, which requires a long breeding term when using conventional breeding practices.

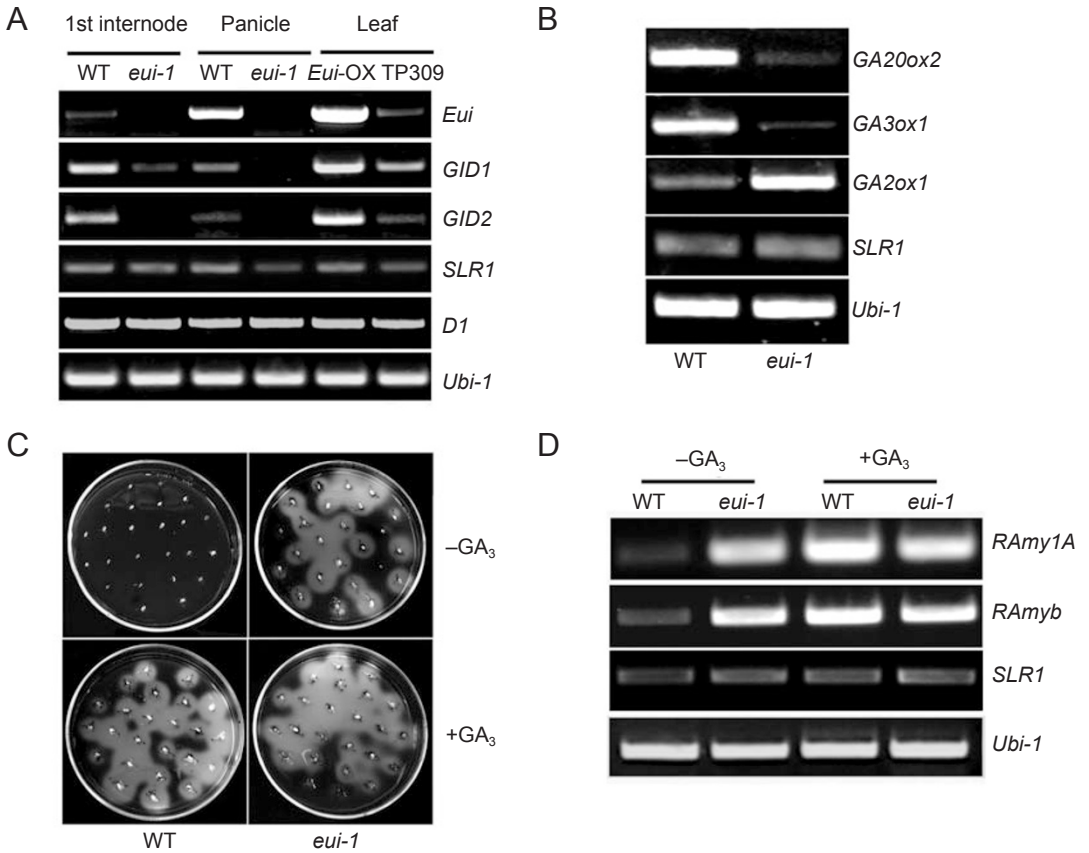

Figure 4 Effect of *Eui* expression on rice GA signaling. **(A)** Differential expression of *GID1*, *GID2*, *SLR1* and *D1* in *eui* mutant, wild-type and *Eui*-OX plants. *Ubi-1* was used as a control during RT-PCR. Results are representative of two independent experiments with similar results. TP309 is the wild type for the *Eui*-OX transgene. **(B)** Transcript levels of the rice *GA3ox1*, *GA20ox2*, *GA2ox1* and *SLR1* in seedlings, as estimated by RT-PCR. *Ubi-1* was used as a control. Results are representative of two independent experiments with similar results. **(C)** Amylase activity in the aleurone cells of the embryoless half-seeds of the wild type and *eui* mutant. Production of α-amylase was detected by staining starch with iodine in plates with or without 1 μM GA_3. Results are representative of two independent experiments with similar results. **(D)** Transcript levels of the rice *RAmy1A*, *GAmyb* and *SLR1* in the *eui* mutant and wild-type aleurone cells with or without treatment with 1 μM GA_3, as detected by RT-PCR. *Ubi-1* was used as a control. Results are representative of two independent experiments with similar results.

Ectopic expression of Eui strongly reduces plant height

The *Eui* gene dramatically reduces plant height when overexpressed in transgenic rice, leading to severe dwarfing and infertile transgenic plants [20]. We also observed rare *Eui*-OX transgenic plants that displayed different reductions in height and produced seeds but were unstable within generations (Supplementary information, Figure S1). We confirmed that the height reduction of these lines correlates well with expression levels of *Eui* (Supplementary information, Figure S1).

In order to exploit the *Eui* application in rice molecular breeding, we further transformed an elite but tall variety (R2212) with *Eui* under the control of the GA biosynthesis genes *GA20ox2* and *GA3ox2*, which function in sequential synthesis of GA9/GA20 and GA_1/GA_4, respectively [4]. As shown in Figure 6A (where results from four representative transgenic lines are shown), the independent transgenic lines expressing *GA20ox-Eui* exhibited a range of dwarf phenotypes with normal (lines 1 and 2) or less seed setting (lines 3 and 4), and later flowering. Their phenotypes were also dose-dependent on *Eui* expression (Figure 6B). Similarly, the transgenic plants with the *GA3ox-Eui* chimeric gene also exhibited plant height reduction in a dose-dependent manner. (In Figure 7A and 7C, the results from four representative T1 transgenic plants are shown.) In contrast to *GA20ox-Eui* plants, *GA3ox-Eui* plants showed less reduction in plant height, and some lines, such as line 1, exhibited a semi-dwarf phenotype with normal seed setting (Figure 7A and 7B). The different outcomes of the *GA20ox-Eui* and *GA3ox-Eui* transgenes are prob-

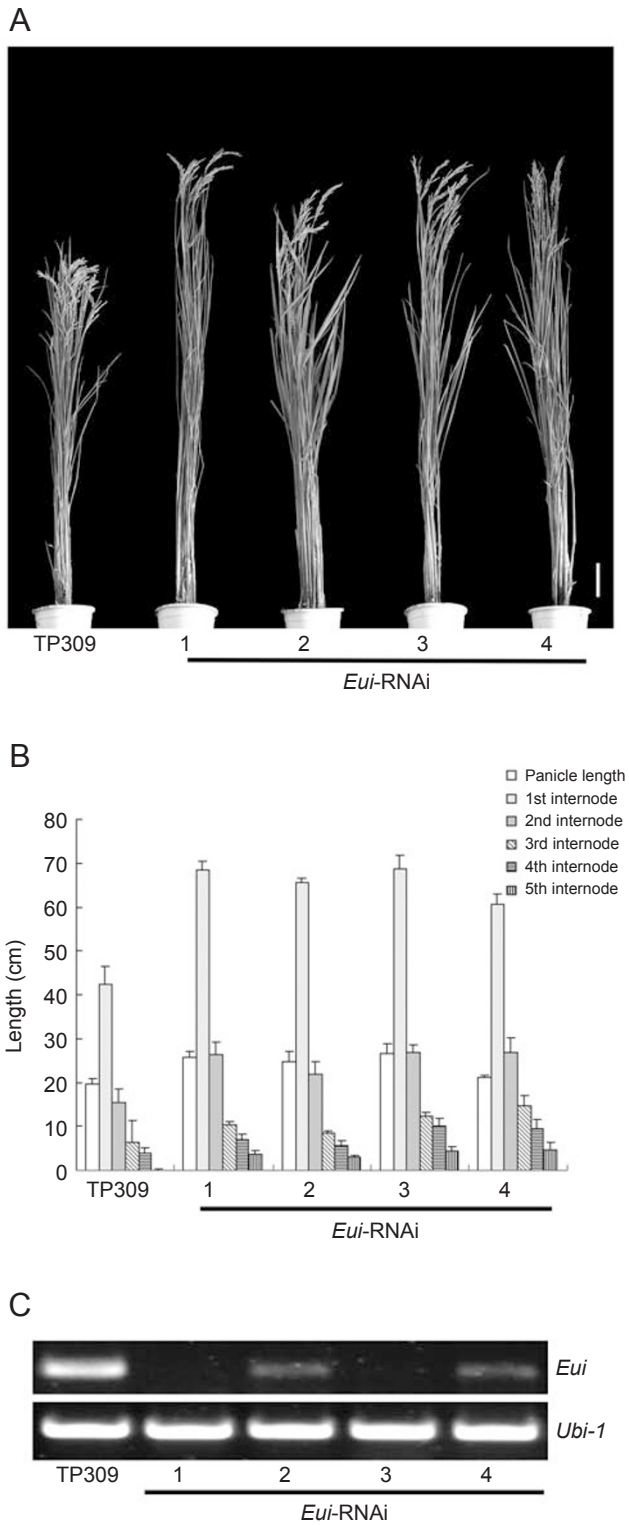

Figure 5 Efficient knockout/knockdown of the *Eui* gene by RNAi. **(A)** Morphological phenotypes of RNAi (RNA interference) and wild-type seedlings (inset) and adult plants. Four representative independent RNAi plants (T1) are shown. Bar=10 cm. **(B)** Lengths of individual internodes of wild-type and RNAi plants. **(C)** Expression levels of *Eui* detected by RT-PCR. *Ubi-1* was used as a control.

ably attributed to the tissue-specific expression patterns of *GA3ox2* and *GA20ox2* [26]. Similar results were also observed in transgenic plants expressing a *GA3ox2-GA2ox1* chimera [14]. The stable inheritance of these transgenic lines indicates the feasibility of genetic improvement of rice varieties by modulating GA catabolism with the *Eui* gene. These results also confirm that *Eui* is a strong regulator of GA homeostasis, which fine-tunes rice plant height in WT plants.

Discussion

Fine-tuning of GA homeostasis is essential for the establishment of GA-related phenotypes [4, 7, 15]. The amount of bioactive GAs is tightly maintained by both GA synthesis and catabolism. At least in rice, GA catabolism is performed by two types of enzymes: GA2-oxidases that convert bioactive GA_1, GA_4, their immediate precursors and

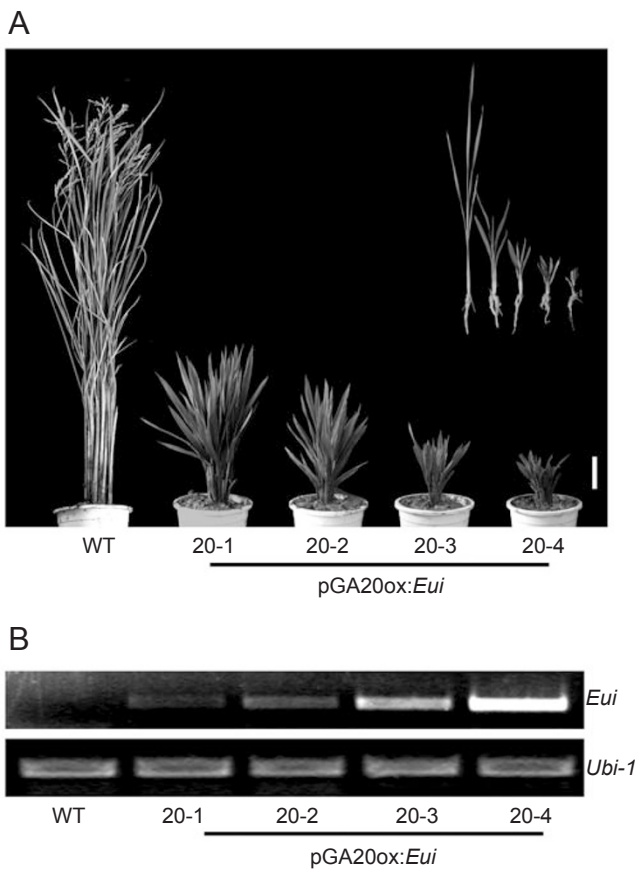

Figure 6 Ectopic expression of *Eui* under the *GA20ox2* promoter in a tall variety, R2212. **(A)** Morphological phenotypes of wild-type R2212 and *GA20ox2-Eui* seedlings (inset) and adult plants. Four representative independent transgenic plants are shown. Bar=10 cm. **(B)** Expression levels of *Eui* detected by RT-PCR in *GA20ox2-Eui* plants. *Ubi-1* was used as a control.

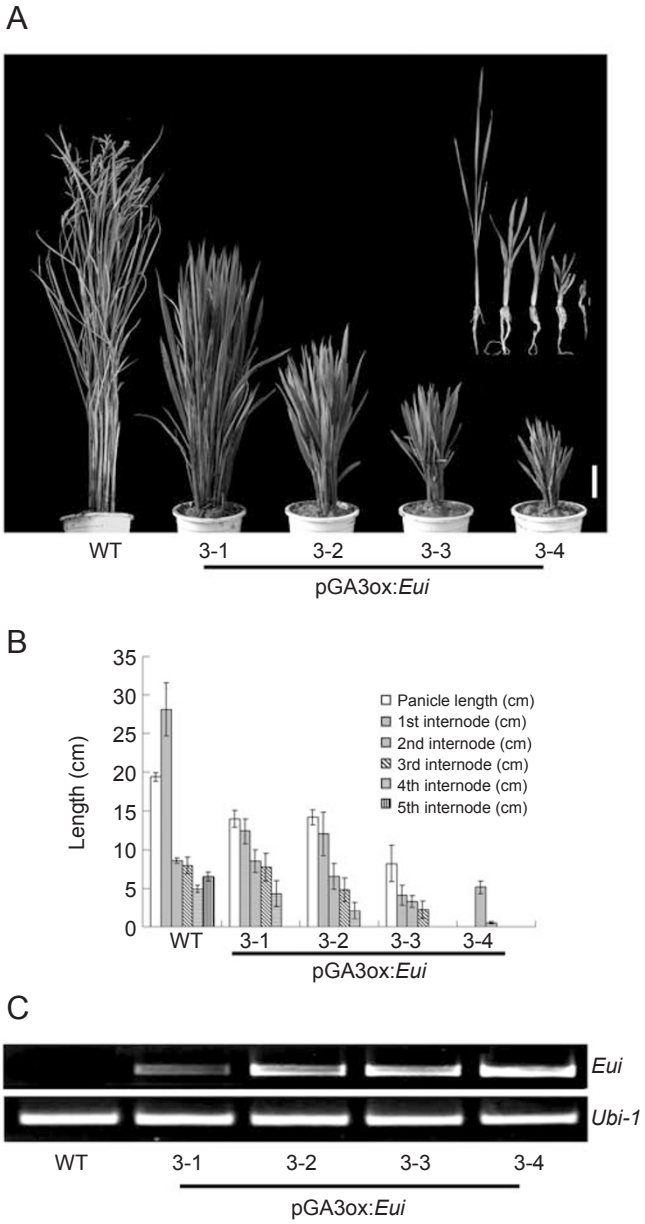

Figure 7 Ectopic expression of *Eui* under the *GA3ox2* promoter in a tall variety, R2212. **(A)** Morphological phenotypes of wild-type R2212 and *GA3ox2-Eui* seedlings (insert) and adult plants. Four representative independent transgenic plants (T1) are shown. Bar=10 cm. **(B)** Lengths of panicles and internodes of wild-type and transgenic plants. **(C)** Expression levels of *Eui* detected by RT-PCR in *GA3ox2-Eui* plants. *Ubi-1* was used as a control.

C20 GAs into inactive GAs by 2β-hydroxylation; and EUI P450 that converts bioactive GA_4 and its precursor GA_9 into inactive 16α,17 epoxy-GAs by 16α,17-epoxidation. The *Eui* expression pattern and the *eui* phenotypes suggest that EUI is a major GA catabolism enzyme in internodes of rice at the heading stage [20]. We have shown that the *eui* mutant is more sensitive than the WT to GA_4 and GA_9 at the seedling stage, and both are EUI substrates (Figure 1A and 1B). We have further indicated that the expression of the *Eui* gene responds to GA_1, GA_3 and GA_4, although GA_1 is not an EUI substrate, and GA_3 is not an endogenous GA. We propose that because GA_1 and GA_3 are structurally similar to GA_4, they would exhibit a certain capacity to trigger the *Eui* promoter with a different pattern (Figure 1C), or, alternatively, that GA_1 and GA_3 regulate GA signaling and hence *Eui* expression (see below). In addition, starch granules were almost devoid in the root tip of the *eui* mutant and were increased in those of *Eui*-OX plants, consequently affecting root gravity tropism (Figure 3). Although treatment with GA plus kinetin removed starch granules and thereby altered the gravisensitivity of cress roots [27], a reduction in the amount of GAs in maize seedlings does not significantly alter root graviresponsiveness [31]. Our current study indicates that GA has a role in starch granule generation and gravity tropism in rice roots and suggests that plants might be different in their requirements for GA in graviresponsiveness. It will be interesting to investigate whether mutants related to GA signaling such as *gid1*, *gid2*, *slr1* and *d1* also alter gravity tropism. Although there is no significant *eui* phenotype and detectable GA_1 and GA_4 at the seedling stage [20], we have found that the GA metabolism genes are indeed either feedback or feedforward regulated at this stage (Figure 4B). Collectively, our current study demonstrates that *Eui* is involved in GA homeostasis, not only at the heading stage but also at the seedling stage and in roots.

The *Eui* gene is also inducible by GA_3, a non-endogenous GA, although with a different pattern to induction by GA_4. Similar GA_3 induction was also observed for the *Arabidopsis GA2ox* genes [16]. However, the rice *OsGA2ox1* gene does not respond to exogenous GA_3 [32], suggesting that rice GA catabolism genes might differentially respond to this GA molecule. Detailed analysis of the structures and *cis*-elements of their promoters would provide further information about their regulation in GA responses. Interestingly, strong *Eui-GUS* expression was observed in vascular bundles (Figure 2). A similar cell-specific pattern was also observed for *OsGA2ox1* [32]. It is well established that GAs are produced in certain tissues, particularly in young panicles in rice [33]. However, there is no information about how bioactive GAs are delivered to other tissues/sites. Our current study raises the possibility that GAs might be transported through the vascular system.

Co-expression of GA biosynthesis and signaling genes in rice tissues indicates that the biosynthesis of active GAs occurs at the same site as GA signaling [26]. The *Eui* expression pattern also suggests that the sites of GA deactivation by EUI and the synthesis of bioactive GAs may partially

overlap at the heading stage [20]. We have shown in this study that *Eui* is involved in GA homeostasis, and therefore modulates the expression of the genes that are involved in GA signaling and α-amylase production (Figures 3 and 4). Embryoless half-seeds are used to eliminate the effect of the embryo (the site of GA production), so they are usually GA-deficient. Interestingly, the embryoless half-seeds of the *eui* mutant continued to produce and secrete large amounts of α-amylase (Figure 4C). Because no GA biosynthesis is observed in the rice aleurone layer [26], it is possible that the elevated GA levels in maturing *eui* seeds induce amylase expression and accumulation before the seed fully desiccates. An alternative explanation is that a trace amount of bioactive GAs might be present in the aleurone layer during seed development in the *eui* mutant so that once the seeds are imbibed they produce α-amylase without GA treatment. In support of the first possibility, the *GA2ox*-deficient mutant M326 of barley accumulates high levels of GA_1, resulting in high levels of premature α-amylase expression [34].

Surprisingly, we found that the expression of both positive GA signaling genes *GID1* and *GID2* and the negative signaling gene *SLR1* was repressed in the *eui* mutant internodes and increased in plants overexpressing *Eui* (Figure 4A). We propose that EUI influences the GA signaling network due to altered GA levels according to a feedback mechanism that is similar to that operating in GA biosynthesis. It is possible that the *eui* mutant plants accumulate high levels of bioactive GAs, and that the overproduction of bioactive GAs could feedback regulate GA signaling by decreasing the production of GID1 and GID2, since GA treatment reduced transcript abundance for all three *GID1* genes of *Arabidopsis* [13]. Such high GA levels could also repress *SLR1* expression as SLR1 protein accumulation was decreased or eliminated by GA treatment [5]. However, the proposed GA-mediated *SLR1* transcription and relationship between these regulation events need more study [13], as *SLR1* expression was not altered in the *eui* seedlings and aleurone cells compared with the WT in our current study.

Breeding cereal crops for a desirable plant height or morphology has been a goal of the agriculture industry. In rice, male sterile cultivars are commonly defective in elongation of the uppermost internode owing to inadequate GA production in the empty anthers [35]. The *eui* mutation provides a tool for genetically improving the heading performance of male sterile cultivars, and rice varieties carrying the *eui* mutation have been officially released in China [21-25]. However, traditional breeding of *eui* varieties takes a long time because of its recessive nature. We efficiently developed *eui* rice using an RNAi transgene (Figure 5), and we are currently using this approach to develop elite *eui* male sterile lines. On the other hand, dwarf architecture, or the so-called 'green revolution', has been valuable in rice breeding, which confers lodging resistance and high yield potential. Genetic manipulation of the levels of bioactive GA is a practical strategy to generating rice with suitable plant height for high yield. Two approaches can be adopted to lower endogenous GA levels and therefore develop elite varieties; these include decreasing GA biosynthesis and increasing GA catabolism. Indeed, by using the mutant GA biosynthesis gene *sd-1*, a mutated *GA20ox* gene [9, 10], or by using the GA catabolism gene *OsGA2ox1* to manipulate GA levels [14], breeding for semi-dwarf rice is now feasible through molecular design.

In our previous study, we developed *Eui*-OX transgenic plants that showed a severe dwarf phenotype with defects in flower development [20], and the semi-dwarf plants generated were genetically unstable (Supplementary information, Figure S1), limiting the use of the constitutive expression approach in rice molecular breeding. We therefore generated transgenic plants of a tall variety with *GA20ox-Eui* and *GA3ox-Eui* fusions (Figures 6 and 7). In contrast to the *GA20ox-Eui* transgenic approach, the *GA3ox-Eui* fusion produced more desirable semi-dwarf lines with normal seed setting. The same *GA3ox2* (*D18*) promoter was also used to drive *GA2ox1* expression and to generate semi-dwarf plants [14]. This approach can also be used in other cereals such as maize and barley. Regardless, this study suggests that manipulation of the *Eui* gene could improve rice varieties through the modulation of GA catabolism.

Materials and Methods

GA response assay

Two-week-old WT (ZS97) and *eui-1* mutant plants were incubated in 1/2 MS medium containing 1 μM GA_1, GA_3, GA_4 and GA_9; seedling height (from the base to the leaf tip) was measured at day 7 after GA treatment. For GA induction of *Eui*, seedlings were treated with 1 μM GA_1, GA_3 and GA_4, and samples were collected at different time points for RNA isolation.

Amylase activity

Matured seeds of the WT and the *eui-1* mutant were dried and stored for 3 months. Embryoless half-seeds were sterilized in 3% NaClO for 15 min, washed with sterile water three times, and then incubated on agar plates containing 0.2% starch with or without 1 μM GA_3 for 3 days at 28 °C in darkness. The plates were exposed to iodine vapor to determine α-amylase activity as described previously [36]. The same treated half-seeds were collected at day 2 for RNA preparation to detect *RAmy1A*, *GAmyb* and *SLR1* expression.

GUS staining

Histochemical assays for GUS activity in transgenic plants were performed as described previously [37]. Transverse sections (4 μm) of the divisional zone of the elongating uppermost internode of the *Eui-GUS* transgenic plant [20] were observed and photographed

under a microscope (Olympus, Tokyo, Japan).

Detection of starch granules and root gravitropism

Seedlings were grown in 1/2 MS medium. Root starch granule staining was performed as described previously [38]. Longitudinal sections (2 μm) of stained root tips were examined and photographed under a microscope (Olympus, Tokyo, Japan). For gravitropism analysis, 2-week-old seedlings grown in 1/2 MS agar were rotated by 90°. The root tip positions were recorded at 2 h after rotation.

Transgenic constructs and rice transformation

OX of *Eui* has been described previously [20]. The entire RNAi cassette of sense and antisense fragments of *Eui* was cloned into the vector pCAMBIA1300 s (provided by Dr Yinong Yang) [30], and was introduced into the WT variety TP309 to generate more than 25 independent plants by *Agrobacterium tumefaciens*-mediated transformation. Progeny plants (T1 up to T3) were assayed. For the ectopic expression of *Eui*, the promoter regions that were 2.0 kb upstream of the coding regions of *GA20ox2* (*Sd1*, accession number AF465255) and 1.38 kb upstream of the coding regions of *GA3ox2* (*D18*, accession number P0013F10.29) were fused to the full-length *Eui* cDNA (accession number AY987040) to generate *GA20ox-Eui* and *GA3ox-Eui* fusions. These were inserted into the vector pCAMBIA1300 (accession number AF234269). The plasmids were transformed into a tall *japonica* variety R2212 by *Agrobacterium tumefaciens*-mediated transformation to generate more than 30 independent transgenic lines for each construct. T0 (severe dwarf, no seed setting) or T1 (seed setting) progeny plants were analyzed.

RNA preparation and transcript analysis

Total RNA was isolated from treated tissues or transgenic plant stems using TRIzol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). The *Eui* transcripts were detected by RT-PCR and Southern blot as described previously [20]. The transcripts of *RAmy1*, *GAmyb*, *GID1, GID2, SLR1* and *D1* were detected by reverse transcriptase PCR (RT-PCR) with the following primers: Ramy-F 5′-CGCGTCGCACCGAAGCAGAGTA-3′; Ramy-R 5′-AGCAGAGCATCCAGCCCACA-3′; GAMB-F 5′-CATGTAATACTACGGTTCTTAGCC-3′; GAMB-R 5′-GAATCTGCTTTAGCGTCTGG-3′; GID1-F 5′-GAGGTCAACCGCAACGAGTGC-3′; GID1-R 5′-GCTGCCGCCGTGGAAGAATA-3′; GID2-F 5′-CGGGGAGGACCTGGTGTTCG-3′; GID2-R 5′-CCCCTCCATTCTTATCACTGTCATTT-3′; SLR1-F 5′-GGTGCGGCCAAGGATCGTCA-3′; SLR1-R 5′-AGGAGCGTGCTCGCCTGTTT-3′; D1-F 5′-AAGGAGGATGTGCTTTATG-3′; and D1-R 5′-TGGTCTAGGGCCGTAGTT-3′, using the following PCR conditions: 94 °C for 4 min, followed by 28 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and an elongation step at 72 °C for 10 min. The transcripts of *GA20ox*, *GA3ox* and *GA2ox* were detected as described previously [20].

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