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CROP PRODUCTION

# OsYUCCA2 deficiency in rice growth and development

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**ABSTRACT**: *YUCCA (YUC) proteins have critical roles in plant growth and development through their involvement in auxin production. Rice (Oryza sativa L. cv Nipponbare) has 14 OsYUC proteins, but little is known regarding their functional redundancy. In this study, transgenic rice seedlings expressing GUS-tagged antisense-OsYUC2 under the control of the ubiquitin promoter were used to investigate the effects of OsYUC2 deficiency on seedling growth and development. GUS staining showed that antisense-OsYUC2 was expressed primarily in rapidly dividing cells in callus, vegetative, and reproductive tissues. Phenotypic analysis showed that callus differentiation was compromised in OsYUC2 antisense rice. Adverse vegetative effects were observed in shoot height and embryonic, adventitious, and lateral root numbers. Reproductive tissues were also impacted, with reductions in panicle length, flower number, seed setting number, seed setting rate, and grain weight. Auxin analysis showed that indole-3-acetic acid (IAA) levels were reduced in OsYUC2 antisense rice, and exogenous addition of 3-indolebutyric acid (IBA, an analogue of IAA) partially rescued defective growth phenotypes. Taken together, these results indicated that deficiency of OsYUC2 impairs auxin biosynthesis, thereby affecting rice growth and development.*

**Key words**: *OsYUC2, auxin, rice, growth.*

# Influência da deficiência de OsYUCCA2 no crescimento e desenvolvimento do arroz

**RESUMO**: *As proteínas YUCCA (YUC) possuem importantes papéis no crescimento e desenvolvimento das plantas por meio de seu envolvimento na produção de auxinas. O arroz (Oryza sativa L. cv Nipponbare) possui 14 proteínas OsYUC, no entanto, pouco se sabe a respeito da sua redundância funcional. No presente estudo, mudas de arroz transgênico expressando OsYUC2 anti-senso com tag-GUS sob o controle do promotor de ubiquitina foram utilizadas para investigar os efeitos da deficiência de OsYUC2 no crescimento e desenvolvimento de mudas. As manchas de GUS indicaram que OsYUC2 anti-senso foi expressado primordialmente em células que se dividem rapidamente através de calos, tecidos vegetativos e reprodutivos. A análise fenotípica indicou que a diferenciação do calo foi comprometida no arroz OsYUC2 anti-senso. Os efeitos vegetativos adversos foram observados na altura do rebento e no número de raízes embrionárias, adventícias e laterais. Os tecidos reprodutivos também foram impactados, com reduções no comprimento da panícula, número de flores, número de sementes, proporção de sementes e peso de grão. A análise de auxina ilustrou que os níveis de ácido indol-3-acético (IAA) foram reduzidos no arroz anti-senso OsYUC2 e a adição exógena de ácido 3-indolbutírico (IBA, um análogo de IAA) resgatou parcialmente os fenótipos de crescimento defeituosos. Em conjunto, tais resultados indicam que a deficiência de OsYUC2 prejudica a biossíntese de auxina, afetando o crescimento e o desenvolvimento do arroz.*

**Palavras-chave**: *OsYUC2, auxina, arroz, crescimento.*

## INTRODUCTION

Auxin plays critical roles in plant growth and development and is involved in responses to abiotic and biotic stresses (FERARU et al., 2019; YAO et al., 2018a; YAO et al., 2018b; ZHANG et al., 2018; ZHAO, 2018). The regulatory roles of auxin are achieved through the activity of several proteins, including YUCCA (YUC) proteins, which are involved in auxin biosynthesis; PIN proteins, which are involved in auxin transport; and ARF receptors, which participate in auxin perception (TOGNETTI et al., 2012). Disruption of these processes affects auxin function and leads to impairment in plant growth and development (INAHASHI et al., 2018; MULLER-MOULE et al., 2016; YAMAMOTO et al., 2007; YAO et al., 2018b).

Plant auxins constitute a class of analogous compounds, including indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-Cl-IAA), and phenylacetic acid (PAA), as well as the main auxin, indole-3-acetic acid (IAA) (OLATUNJI et al., 2017). IAA in plants is synthesized by both

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tryptophan-independent and tryptophan-dependent routes. Tryptophan-independent biosynthesis uses IAA conjugates as precursors for production of IAA. Tryptophan-dependent biosynthesis, which is thought to be the predominant route for plant IAA production, uses tryptophan as the main IAA precursor (OLATUNJI et al., 2017). The tryptophan-dependent route in plants includes indole-3-pyruvic acid (IPA), tryptamine (TAM), indole-3-acetaldoxime (IAOx), and indole-3-acetamide (IAM) pathways. The IPA pathway, which involves TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA) proteins and YUC proteins, also known as the TAA/YUC pathway, is considered to be the major route for auxin synthesis in plants (OLATUNJI et al., 2017; ZHENG et al., 2016). In the TAA/YUC pathway, TAA aminotransferases convert tryptophan to IPA, which is then further converted to IAA by YUC family monooxygenases (OLATUNJI et al., 2017).

Several YUC monooxygenase proteins are involved in the regulation of auxin biosynthesis, and the activities of family members are rate-limiting in the TAA/YUC pathway. Eleven YUC family proteins were identified in Arabidopsis (YUC1-11), and 14 were identified in rice (OsYUC1-14) (ZHANG et al., 2018). Phylogenetic tree analysis divided the 14 OsYUC proteins into four groups: Group 1 (OsYUC2,3,6, and 7), Group 2 (OsYUC1,4,5, and 8), Group 3 (OsYUC9 and 10), and Group 4 (OsYUC11,12,13, and 14) (ZHANG et al., 2018).

YUC-mediated auxin biosynthesis exhibits versatility across the plant kingdom, regulating a wide range of plant physiological activities such as embryogenesis, root architecture formation, shoot development, and flower formation and development (CHEN et al., 2016; CHENG et al., 2007; YAMAMOTO et al., 2007). The disruption of single *YUC* genes may result in defects in plant physiological activities. For example, *OsYUC1* antisense rice lines had dwarf shoots and displayed inhibited root formation and elongation. By contrast, *OsYUC4* antisense rice exhibited no obvious phenotypes in rice growth and development (YAMAMOTO et al., 2007). Several combinational *YUC* gene disruption lines were constructed to analyze the functions of YUC-mediated auxin biosynthesis in plant physiological activities. For example, an Arabidopsis *yuc1yuc2yuc4yuc6* quadruple mutant showed impaired rooting ability, and the phenotype was partially rescued by addition of IAA, suggesting that YUC-mediated auxin biosynthesis was essential for *de novo* root organogenesis (CHEN et al., 2016). An Arabidopsis *yuc2yuc5yuc8yuc9* quadruple mutant showed defects in shade avoidance, indicating the additive functions of YUC2,5,8, and 9 in the shade avoidance response (MULLER-MOULE et al., 2016). Alongside biosynthesis, auxin transport has a critical role in auxin distribution in plant cells. OsAUX1, an auxin influx transporter in rice, is involved in mobilizing auxin from the root apex to the root hair zone to promote hair elongation in response to low external phosphate conditions (GIRI et al., 2018). OsPIN2, an auxin efflux transporter, regulates auxin distribution to promote root elongation growth and lateral root formation in rice (INAHASHI et al., 2018).

Our previous research showed that overexpression of OsYUC2 promoted rice growth and development. Here, an Os*YUC2* antisense rice line was constructed to further investigate OsYUC2 functions in rice. OsYUC2 expression and its essential roles in rice callus transformation, and vegetative and reproductive growth and development, are discussed.

## MATERIALS AND METHODS

### *Vector construction and rice transformation*

The coding region of *OsYUC2* gene (Os05g0528600) was amplified as described previously (HAN et al., 2020). Briefly, rice (*Oryza sativa* L. cv Nipponbare) cDNA was amplified with the forward primer 5'-taggatccctatgtgttgttcccaccag-3' and the reverse primer 5'- taggatccagctaaggagaaggtttggc-3'. The coding region was then cloned into the *pU1301* vector by the inverse ligation at *BamH*I restriction sites to generate the construct of *pUbi-antisense-OsYUC2-GUS*. The construct was further introduced into *Agrobacterium tumefaciens* strain EHA105 and used to infect rice callus. The plasmid expression in plants was screened by the selection medium containing hygromycin and GUS staining.

### *Plant materials and growth conditions*

Wild-type and transgenic plants used in the present study were all based on the background of the *Oryza sativa* L. cv Nipponbare rice seedlings. Seeds of wild-type and transgenic rice were sterilized in a solution containing 6% sodium hypochlorite and 0.1% Triton X-100 for 10 min and washed five times with deionized distilled water. The sterilized seeds were then soaked in deionized water in the dark at 37 °C for 2 d, and further transferred into Hoagland nutrient solution at 25 °C for indicated days.

### *Characterization of shoot and root growth*

The shoot height, the embryonic root length, and the adventitious root length were measured with a ruler. The number of lateral roots developed form the embryonic roots and adventitious roots were scored under a microscope with a digital camera. The length of lateral roots developed form the embryonic roots and adventitious roots were measured under a microscope with a digital camera. In each replicate, 20 plants were used and the results were expressed per plant.

*Histochemical analysis of GUS activity*

Histochemical staining for GUS activity in transgenic plants was performed as described previously (Petersson et al., 2009). Briefly, seedlings were immersed in a GUS staining reaction solution (1 mM 5-bromo-4-chloro-3-indolyl-β-D- glucuronidase, 100 mM sodium phosphate, 0.5 mM potassium ferricyanide, 0.5 mM potassium hexacyanoferrate (II), 10 mM EDTA, 0.1 % (v/v) Triton X100, 2 % (v/v) dimethyl sulfoxide, and 0.5 mM methanol, pH 7.0) and incubated at 35 °C for 10 h. After staining, the seedlings were then cleared with 70 % (v/v) ethanol and 3% (v/v) sodium hypochlorite. At least 20 plants were used for each replicate. The images were taken using a dissecting microscope (Nikon, SMZ1500) with a digital camera (Nikon D5000, Japan).

*Determination of Indole-3-acetic acid (IAA) content*

IAA content analysis was performed as described previously (Han et al., 2020). Briefly, the roots of 8-day-old rice seedlings were excised, grounded under liquid nitrogen, and 2.5 mL ice-cold 2-propanol : $H_2O$ : 37% HCl (2 : 1 : 0.002, v/v/v) was added and vortexed for extraction. The content of IAA in the exaction buffer was further analyzed by high-performance liquid chromatography coupled with mass spectrometry (HP1100 HPLC, Agilent Inc.; esquire 2000 MS Bruker Daltonics Inc., USA). Internal standards of IAA (Sigma, USA) was used.

*Analysis using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR).*

Total RNA was extracted from the roots of 9-d-old rice seedlings with Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was then treated with RNase-free DNase I (Takara, Kusatsu, Japan) to remove genomic DNA, and reverse transcribed with M-MLV reverse transcriptase (Promega, Madison, WI, USA) to get cDNA according to the manufacturer’s instructions. The cDNA was used for RT-PCR analysis with the forward primer 5’-GTCCAAAGGGAGGAGTCGTCCAG-3’ and the reverse primer 5’-GCATGATGTTTACACCCGGCCTT-3’ for *OsYUC2*, and the forward primer 5’-GGAGCGTGGTTACTCATTC -3’ and the reverse primer 5’- ACCTCAGGGCAGCGGAAA -3’ for *OsActin*.

*Statistical analysis of data.*

Data is expressed as mean ± SE. Statistical significance among at least three independent experiments was determined by one-way ANOVA, followed by multiple comparisons test (SPSS, 16.0, SPSS Inc., USA). Differences were considered significant at $P \leq 0.05$.

## RESULTS

*OsYUC2 is essential for callus cultivation in transgenic rice generation*

To investigate the effects of OsYUC2 deficiency on growth and development in rice seedlings, an antisense vector, *pUbi-OsYUC2-GUS*, was created and introduced into rice callus (*Oryza sativa* L. cv Nipponbare).

Expression of antisense-OsYUC2 was examined with β-glucuronidase (GUS) staining. Expression was uneven and was observed mainly in meristematic nodules and adventitious buds (Figure 1A, B, C, and D). The expression of antisense-OsYUC2 in rapidly dividing callus cells showed that OsYUC2 had a role in callus cultivation and generation of transgenic rice.

To investigate the role of OsYUC2 in callus cultivation, the differentiation rate, positive rate of differentiated seedlings, and survival rate of tissue culture seedlings were analyzed. Callus differentiation was lower with the antisense *pUbi-OsYUC2-GUS* construct than with empty vector controls (Figure 2A and B), and the positive rate of *OsYUC2* antisense tissue culture seedlings was 38.4% of the control (Figure 2C). Positive tissue culture seedlings were transferred from rooting medium to soil, and the survival rate of *OsYUC2* antisense rice seedlings was 28% of the control (Figure 2D). These results indicated that OsYUC2 has functions in callus transformation and transgenic seedling generation in rice.

*OsYUC2 is essential for root and shoot growth in rice*

Homozygous *OsYUC2* antisense rice seedlings were obtained by repeated screening using hygromycin and GUS staining. *OsYUC2* expression in the *OsYUC2* antisense rice seedlings was significantly lower than in control seedlings, as determined by RT-PCR detection (Figure 2E).

Expression of antisense-OsYUC2 in rice tissue organs was examined by GUS staining. As shown

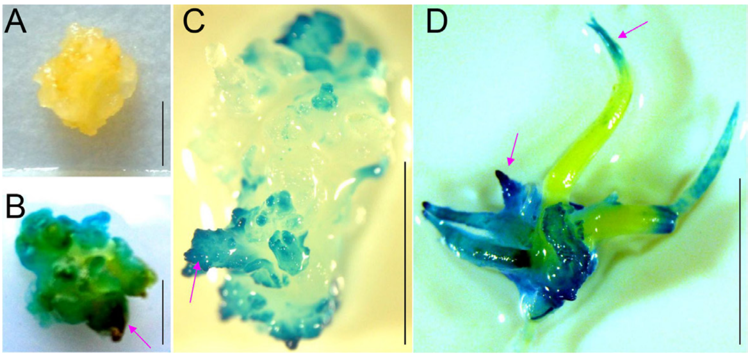

Figure 1 - The expression pattern of antisense-OsYUC2 in rice callus and tissue culture seedling by GUS staining. (A) Wild-type callus. (B) antisense-OsYUC2 in rice callus. (C) antisense-OsYUC2 in rice callus differentiated at 3 d. (D) antisense-OsYUC2 in rice seedling differentiated at 6 d. Arrows indicate antisense-OsYUC2 expression. Scale bars in (A-D) represent 15 mm.

in figures 3A-I, antisense-OsYUC2 was expressed in coleoptiles, embryonic roots, adventitious roots and lateral roots, and its expression increased in root apices and lateral root primordium. The uneven expression of antisense OsYUC2 in vegetative tissues indicated a role for OsYUC2 in rice vegetative growth and development.

Root and shoot growth traits were further investigated in *OsYUC2* antisense rice seedlings to investigate the influence of OsYUC2 deficiency on rice growth and development. As shown in figures 4A-H, 8-day-old *OsYUC2* antisense rice seedlings exhibited reductions in shoot height, adventitious

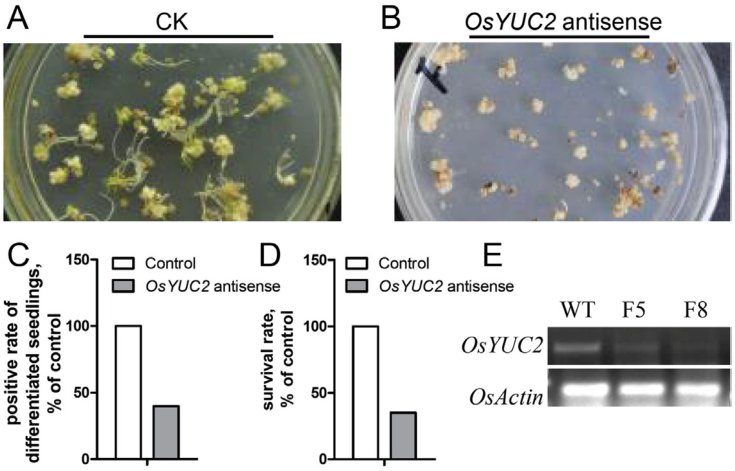

Figure 2 - The influence of OsYUC2 deficiency on rice callus transformation and transgenic seedling generation. (A) Wild-type callus tissue culture seedlings. (B) OsYUC2 antisense tissue culture seedlings. (C) Positive rate of OsYUC2 antisense tissue culture seedlings in comparison with the tissue culture control seedlings. (D) Survival rate of OsYUC2 antisense tissue culture seedlings in comparison with the tissue culture control seedlings. (E) RT-PCR analysis of gene expression in OsYUC2 antisense rice seedlings (F5 and F8). Upper panel: OsYUC2; lower panel: OsActin.

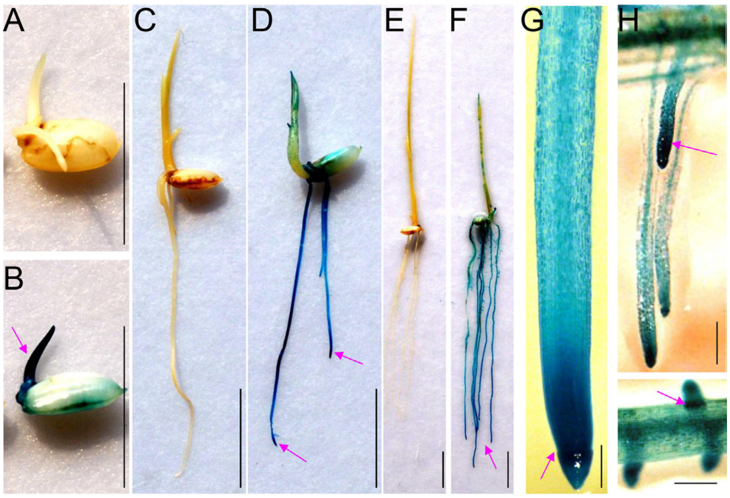

Figure 3 - The expression pattern of antisense-OsYUC2 in rice vegetative tissues. (A) Three-day-old wild-type rice seedling. (B) Three-day-old OsYUC2 antisense rice seedling. (C) Six-day-old wild-type rice seedling. (D) Six-day-old OsYUC2 antisense rice seedling. (E) Nine-day-old wild-type rice seedling. (F) Nine-day-old OsYUC2 antisense rice seedling. (G) Adventitious root apices of 9-day-old OsYUC2 antisense rice seedling. (H) Lateral root on adventitious root of OsYUC2 antisense rice seedling. (I) Early stage of lateral root on adventitious root of OsYUC2 antisense rice seedling. Arrows indicate antisense-OsYUC2 expression. Scale bars in (A-F) represent 1 cm. Scale bars in (G-I) represent 100 μm.

root numbers, and numbers of lateral roots on embryonic and adventitious roots. There were no apparent growth defects in embryonic root length, adventitious root length, or length of lateral roots on embryonic and adventitious roots. These results indicated that OsYUC2 is essential for rice vegetative growth and suggested that its deficiency could hinder rice vegetative growth.

### *OsYUC2 is essential for reproductive tissue development in rice*

Antisense-OsYUC2 expression was investigated in rice reproductive tissues using GUS staining. OsYUC2 was mainly distributed in pistil, anther, pollen, and seed tissues (Figures 5A-F).

Agronomic traits (plant height, panicle length, numbers of primary and secondary branches, flower number per panicle, grain number per panicle, seed setting rate, and thousand grain weight) were investigated in *OsYUC2* antisense rice plants to investigate the influence of OsYUC2 deficiency on rice reproductive growth. Plant height, panicle length per plant, and numbers of primary and secondary branches per panicle were significantly lower in *OsYUC2* antisense plants compared with control plants (Figures 6A-C, Table 1). Compared with wild-type plants, *OsYUC2* antisense rice plants also had lower flower numbers per panicle, seed setting numbers per panicle, seed setting rate, and thousand grain weight values (Table 2), though no apparent defects were observed in filled seeds (Figures. 6D). These results suggested that *OsYUC2* is essential for rice reproductive growth and development.

### *Auxin synthesis was impaired in OsYUC2 antisense rice seedlings*

YUC proteins catalyze a rate-limiting step in auxin biosynthesis (MASHIGUCHI et al., 2011). IAA levels were assessed in *OsYUC2* antisense rice seedlings to investigate the influence of OsYUC2 deficiency on auxin biosynthesis. As shown in Figure 7A, IAA content was significantly lower in *OsYUC2* antisense rice seedlings compared with control seedlings. This result indicated that OsYUC2 is critical for auxin biosynthesis in rice seedlings.

*OsYUC2* antisense rice seedlings were treated with IBA, an analogue of IAA, to determine whether growth defects in *OsYUC2* antisense

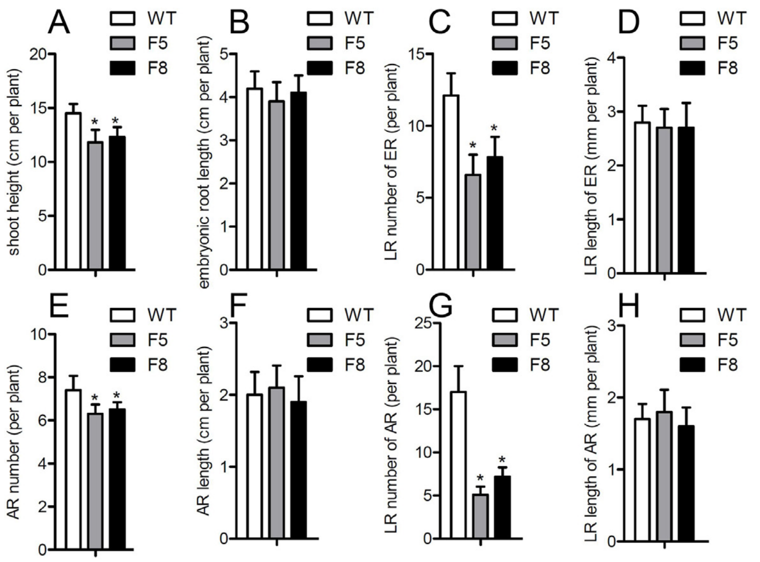

Figure 4 - The influence of OsYUC2 deficiency on rice vegetative growth. Growth traits were analyzed between wild-type and OsYUC2 antisense rice seedling (F5, F8). (A) Shoot height. (B) Embryonic root length. (C) Lateral root (LR) number of embryonic roots (ER). (D) LR length of ER. (E) Adventitious root (AR) number. (F) AR length. (G) LR number of AR. (H) LR length of AR. The experiments were repeated at least three times and displayed similar results. Twenty rice seedlings were used per experiment, and results were reported per plant. Data represent means±SD. Asterisks indicate significant differences of the means ($P < 0.05$).

rice seedlings were caused by impaired auxin biosynthesis. Growth index values, including shoot height, adventitious root numbers, and numbers of lateral roots on embryonic and adventitious roots, were partially recovered in IBA-treated *OsYUC2* antisense rice seedlings (Figures 7B-I). These results indicated that the growth defect in *OsYUC2* antisense rice seedlings is partially caused by auxin deficiency.

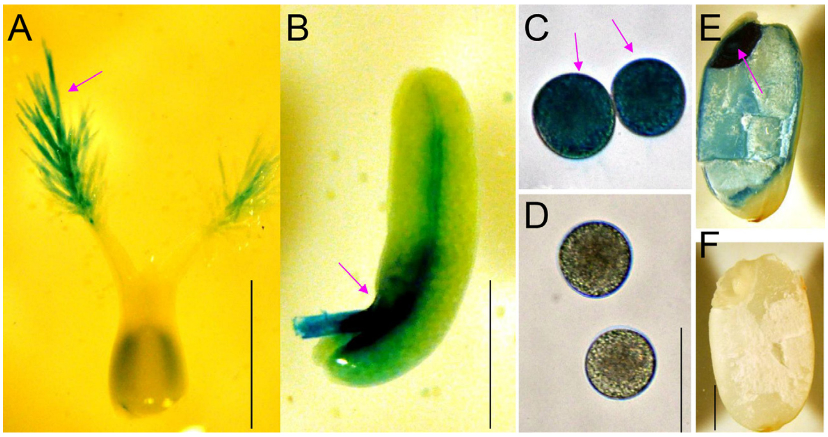

Figure 5 - The expression pattern of antisense-OsYUC2 in rice vegetative tissues. (A) Pistil in OsYUC2 antisense rice. (B) Anther in OsYUC2 antisense rice seedling. (C) Pollen in OsYUC2 antisense rice seedling. (D) Pollen in wild-type rice seedling. (E) Seed in OsYUC2 antisense rice seedling. (F) Seed in wild-type rice seedling. Arrows indicate antisense-OsYUC2 expression. Scale bars in (A-B) and (E-F) represent 1 mm. Scale bars in (C-D) represent 50 μm.

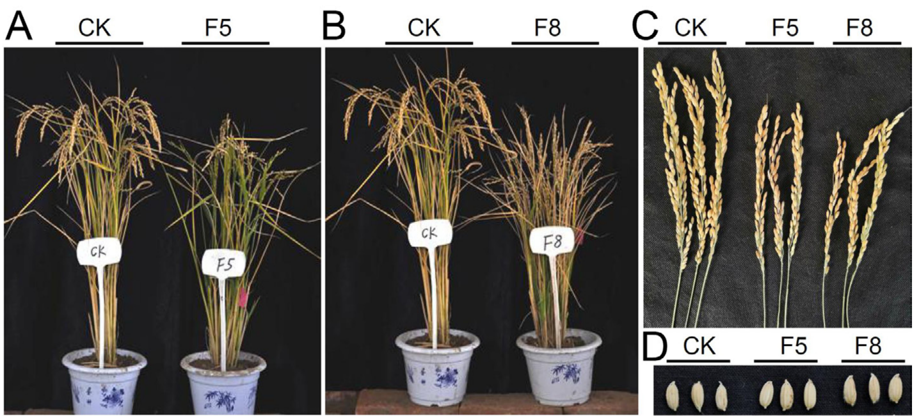

Figure 6 - The influence of OsYUC2 deficiency on rice reproductive growth. (A) Reproductive growth between wild-type and OsYUC2 antisense rice (F5). (B) Reproductive growth between wild-type and OsYUC2 antisense rice (F8). (C) Panicles in wild-type and OsYUC2 antisense rice (F5, F8). (D) Rice seed with peel and glume in wild-type and OsYUC2 antisense rice (F5, F8).

## DISCUSSION

The core phytohormone auxin is involved in nearly all aspects of plant growth and development (SAUER et al., 2013). Auxin signals are; therefore, tightly regulated, and optimal physiological cellular activities are achieved via the regulation of auxin distribution and signaling. In this study, investigation of OsYUC2-deficient rice seedlings revealed that OsYUC2 was essential for auxin biosynthesis, and for vegetative and reproductive growth and development in rice.

Auxin biosynthesis, transport, metabolism, and signaling are all involved in the regulatory functions of auxin. The TAA/YUC pathway is involved in auxin biosynthesis in plants. OsYUC proteins in rice, of which 14 have been identified, are temporally and spatially expressed and distributed in plant tissues (Zhang et al., 2018). Further research is needed to determine whether the 14 OsYUC proteins are redundant or have distinct functions. The 14 rice OsYUCs were divided into four sub-groups based on phylogenic tree analysis and, compared with Arabidopsis, more embryo YUCs and fewer root YUCs were seen in rice (Zhang et al., 2018). Expression analysis of rice YUCs showed that OsYUC1 was expressed at high levels in most rice organs, and that OsYUC3 and 4 were also expressed in most organs, albeit at low levels. OsYUC2 and OsYUC5 were expressed at low levels in specific organs including root, shoot apex, and leaf sheath tissues (Yamamoto et al., 2007). Functional analysis of rice YUCs revealed that overexpression of most OsYUCs, except OsYUC2,9,12, and 13, stimulated

Table 1 - Analysis of panicle length and branch number in *OsYUC2* antisense rice in paddy fields.

| | plant height | panicle length per panicle (cm) | number of primary branch per panicle | number of secondary branch per panicle |
|---|---|---|---|---|
| WT | 67.3±3.85$^{a}$ | 16.23±0.38$^{a}$ | 9.8±0.45$^{a}$ | 10.63±0.9$^{a}$ |
| F5 | 55.8±3.15$^{b}$ | 14.54±0.56$^{b}$ | 8.5±0.41$^{b}$ | 8.7±0.42$^{b}$ |
| F8 | 55.1±2.26$^{b}$ | 13.34±0.68$^{b}$ | 8.11±0.83$^{b}$ | 8.2±0.65$^{b}$ |

At least 20 panicles from 6 seedlings were counted and analyzed. WT: wildtype rice; F5, F8 : *OsYUC2* antisense rice. Data are means±SD. Means with different letters are significantly different ($P < 0.05$).

Table 2 - Analysis of flower number and seed setting rate in *OsYUC2* antisense rice in paddy fields.

| | flower number per panicle | seed setting number per panicle | seed setting rate per panicle (%) | 1000-grain weight (g) |
|---|---|---|---|---|
| WT | 92.15±6.3$^{a}$ | 81.76±7.1$^{a}$ | 85.4±6 $^{a}$ | 23.14±1.8$^{a}$ |
| F5 | 68.49±5.56$^{b}$ | 67.27±6.34$^{b}$ | 63.1±8 $^{b}$ | 19.49±1.2$^{b}$ |
| F8 | 56.95±4.7$^{b}$ | 62.21±8.66$^{b}$ | 55.7±6 $^{b}$ | 17.69±1.9$^{b}$ |

At least 20 panicles from 6 seedlings were counted and analyzed. WT: wildtype rice; F5, F8 : *OsYUC2* antisense rice. Data are means±SD. Means with different letters are significantly different ($P < 0.05$).

auxin overproduction and impacted crown root development (Yamamoto et al., 2007; Zhang et al., 2018). In our previous study, overexpression of OsYUC2 promoted rice growth and development in both vegetative and reproductive phases (Han et al., 2020). In this study, *OsYUC2* antisense rice plants exhibited shoot dwarfism and defects in root and grain formation. In Arabidopsis, YUCs may work redundantly to regulate plant growth and development. For example, Arabidopsis *yuc2yuc6* double mutants failed to produce viable pollen (YAO et al., 2018b), and a *yuc2yuc5yuc8yuc9* quadruple mutant did not increase hypocotyl and petiole elongation during the shade avoidance response (MULLER-MOULE et al., 2016). Further research is needed to determine whether the expression patterns and functions of the 14 rice OsYUCs exhibit similar specialization.

Auxin is transported from plant shoots to roots via a polar localized transport process. However, transported auxin is not sufficient to support

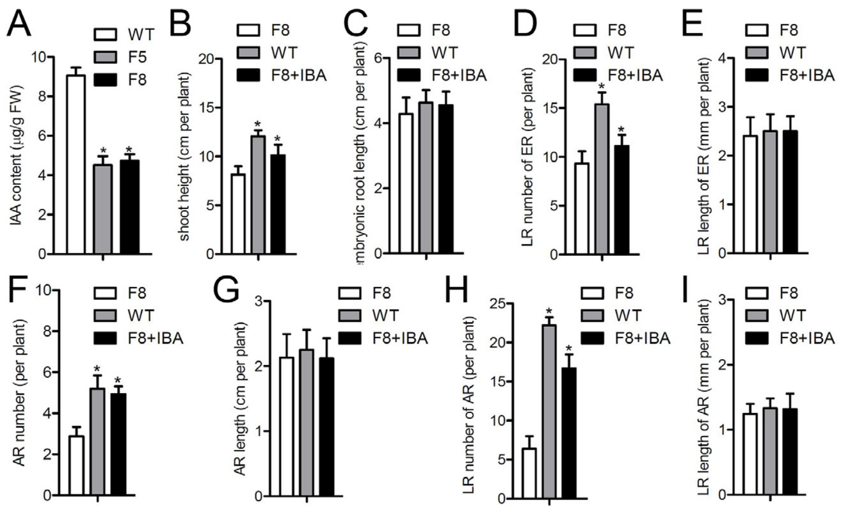

Figure 7 - Auxin analysis and the influence of exogenous addition of IBA on OsYUC2 antisense rice. (A) IAA level in wild-type and OsYUC2 antisense rice (F5, F8). (B-I) Growth traits were analyzed between wild-type and OsYUC2 antisense rice (F5, F8). (B) Shoot height. (C) Embryonic root length. (D) Lateral roots (LR) number of embryonic roots (ER). (E) LR length of ER. (F) Adventitious root (AR) number. (G) AR length. (H) LR number of AR. (I) LR length of AR. The experiments were repeated at least three times and displayed similar results. Twenty rice seedlings were used per experiment, and results were reported per plant. Data represent means±SD. Asterisks indicate significant differences of the means (P < 0.05).

root development, and local auxin biosynthesis is needed in all major plant developmental processes, including root development (ZHAO, 2018). Ectopic production of auxin in the tapetum failed to rescue pollen development defects in Arabidopsis *yuc2yuc6* double mutants, but production of auxin in either microsporocytes or microspores rescued the sterile phenotypes (YAO et al., 2018b). Arabidopsis *yuc3yuc5yuc7yuc8yuc9* mutants had short primary roots that were agravitropic. This phenotype was rescued by expression of YUC genes in roots or by exogenous addition of IAA, but YUC expression in shoots did not rescue the phenotype (CHEN et al., 2014). In this study, the root and shoot development defects in anstisense-*OsYUC2* plants were partially rescued by exogenous addition of IBA. Auxin distribution correlated with OsYUC2 expression, and we therefore propose that OsYUC2 might contribute to local auxin biosynthesis throughout all stages of rice growth and development. Expression of OsYUC2 in specific organs is needed to fully investigate the function of OsYUC2.

Our previous study showed that overexpression of OsYUC2 influenced panicle length, numbers of primary and secondary branches, and thousand grain weight (HAN et al., 2020). In this study, more agronomic traits were adversely affected, including panicle length, numbers of primary and secondary branches, flower number per panicle, grain number per panicle, seed setting rate, and thousand grain weight, in *OsYUC2* antisense plants. These results suggested that plant reproductive tissue development was more affected by auxin reduction, as in OsYUC2 antisense rice, than by auxin overproduction, as in OsYUC2-overexpression rice. Auxin in plants is actively transported by PINFORMED carrier proteins (INAHASHI et al., 2018). Overexpression of OsPIN2 in rice altered auxin distribution, resulting in increased tiller angle and numbers, and shorter plant heights (CHEN et al., 2012). Whether and how auxin transport and metabolism are involved in the regulation of plant auxin distribution in OsYUC2-overexpressing plants and antisense plants requires further study.

## CONCLUSION

OsYUC2 is essential for auxin biosynthesis in rice seedlings. OsYUC2 deficiency inhibits plant callus transformation and transgenic seedling generation, and causes defects in vegetative growth and reproductive development.

## ACKNOWLEDGEMENTS

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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