INDITTO2 transposon conveys auxin-mediated *DRO1* transcription for rice drought avoidance

Yiting Zhao[1,2,3,8*], Lixia Wu[1,2,3*], Qijing Fu[1,2,3*], Dong Wang[6], Jing Li[7], Baolin Yao[7], Si Yu[1], Li Jiang[1], Jie Qian[1], Xuan Zhou[1,2,3], Li Han[1,2,3], Shuanglu Zhao[1], Canrong Ma[5], Yanfang Zhang[1], Chongyu Luo[1,2,3], Qian Dong[1], Saijie Li[1], Lina Zhang[1], Xi Jiang[1], Youchun Li[1], Hao Luo[1], Kuixiu Li[1,2,3], Jing Yang[1,2,3], Qiong Luo[1,2,3], Lichi Li[9], Sheng Peng[1,2,3], Huichuan Huang[1,2,3], Zhili Zuo[6], Changning Liu[7], Lei Wang[5], Chengyun Li[1,2,3], Xiahong He[1,2,3], Jiří Friml[4], Yunlong Du[1,2,3#]

[1] College of Plant Protection, Yunnan Agricultural University, Kunming 650201, China.

[2] State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming 650201, China.

[3] Key Laboratory of Agro-Biodiversity and Pest Management of Education Ministry of China, Yunnan Agricultural University, Kunming 650201, China.

[4] Institute of Science and Technology Austria (IST Austria), Klosterneuburg, Austria.

[5] Key Laboratory of Economic Plants and Biotechnology, Yunnan Key Laboratory for Research and Development of Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China.

[6] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China.

[7] CAS Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun,

Mengla 666303, Yunnan, China.

[8] Shanxi Agricultural University/Shanxi Academy of Agricultural Sciences. The Industrial Crop Institute, Fenyang 032200, China.

[9] International Agriculture Research Institute, Yunnan Academy of Agricultural Sciences. Beijing Rd.2238, Kunming 650205, China

[*] Yiting Zhao, Lixia Wu and Qijing Fu should be considered joint first authors

[#] **Corresponding authors**

College of Plant Protection, Yunnan Agricultural University, Kunming 650201, China.

Tel: +86 18206799459

Email: yunlongdu@aliyun.com

ORCID ID: yunlongdu@aliyun.com

Funding information

This work was supported by grants from the National Natural Science Foundation of China (Grant Nos. 31460453, 31660501, 31860064 and 31470382); the Major Special Program for Scientific Research, Education Department of Yunnan Province (Grant No. ZD2015005); SRF for ROCS, SEM (Grant No. [2013] 1792); the Major Science and Technique Programs in Yunnan Province (Grant No. 2016ZF001); the Key Projects of Applied Basic Research Plan of Yunnan Province (Grant No. 2017FA018); the National Key R&D Program of China (2018YFD0201100); the China Agriculture Research System (CARS-21); the National Key Research Development Program of China (2016YFD0100600), the Program for Innovative Research Team in University of Yunnan Province (IRTSTYN) and the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement No 742985).

Running head

INDITTO2 conveys auxin signal in rice root growth

Abstract

Transposable elements exist widely throughout plant genomes and play important roles in plant evolution. Auxin is an important regulator that is traditionally associated with root development and drought stress adaptation. The *DEEPER ROOTING 1* (*DRO1*) gene is a key component of rice drought avoidance. Here, we identified a transposon that acts as an autonomous auxin-responsive promoter and its presence at specific genome positions conveys physiological adaptations related to drought avoidance. Rice varieties with high and auxin-mediated transcription of *DRO1* in the root tip show deeper and longer root phenotypes and are thus better adapted to drought. The *INDITTO2* transposon contains an auxin response element and displays auxin-responsive promoter activity; it is thus able to convey auxin regulation of transcription to genes in its proximity. In the rice Acuce, which displays *DRO1*-mediated drought adaptation, the *INDITTO2* transposon was found to be inserted at the promoter region of the *DRO1* locus. Transgenesis-based insertion of the *INDITTO2* transposon into the *DRO1* promoter of the non-adapted rice variety Nipponbare was sufficient to promote its drought avoidance. Our data identify an example of how transposons can act as promoters and convey hormonal regulation to nearby loci, improving plant fitness in response to different abiotic stresses.

Keywords: Indoleacetic acid, *Oryza*, Drought, DNA transposable elements, Stress

Introduction

Transposons are important mobile DNA elements and are frequently found cross the genome. Transposons have been found to be involved in a broad range of phenotypes, including changes in gene promoter activity (Bureau et al., 1996; Sun et al., 2013), genome diversity (Oki et al., 2008), plant responses to drought stress (Yan et al., 2011), plant morphogenesis (Jiang et al., 2003; Kikuchi et al., 2003; Momose et al., 2010; Nakazaki et al., 2003) as well as encoding transposases (Zhang et al., 2001; Zhang et al., 2004). Miniature inverted-repeat transposable element (MITE) families, including *Tourist* and *Stowaway*, play important roles in rice genome diversity (Oki et al., 2008). The *Tourist* transposable elements, including a non-autonomous DNA transposon named *INDITTO*, have been found in the genomes of rice, maize, barley and sorghum (Bureau & Wessler, 1992, 1994; Jiang & Wessler, 2002). *Tourist* elements can be present within the proximal promoter of auxin-binding protein 1 in maize and teosinte and form multimers in the maize genome (Elrouby & Bureau, 2000, 2012; Jiang & Wessler, 2001). This implies that cross-talk between auxin and the *Tourist* elements may be involved in plant development. However, the possible developmental and physiological roles of *INDITTO* and its homologues remain unclear.

Drought avoidance is of critical importance in the development of many plants. Drought avoidance is the mechanism by which plants alter the angles of root growth to take up water and nutrients from the soil, or roll their leaves to reduce transpiration (Kadioglu et al., 2012; Uga et al., 2015a). The major plant hormone auxin is involved in the regulation of rice drought avoidance. Auxin may be associated with the binding of auxin response elements (AREs) and may also negatively regulate the level of rice *DEEPER ROOTING 1* (*DRO1*) gene, which functions to enhance rice drought

avoidance (Uga et al., 2013). The *DRO1* gene, located on chromosome 9, has been identified as a major quantitative trait locus (QTL) controlling rice root growth angle and promoting rice yield (Arai-Sanoh et al., 2014; Uga et al., 2011). The rice variety Kinandang Patong, which contains full *DRO1* sequences, shows stronger drought avoidance than the variety IR64, which has a nucleotide adenine (A) deletion in exon 4 that results in the pre-termination of *DRO1* translation (Uga et al., 2013). Furthermore, the function of *DRO1* may be regulated by a few major QTLs located on chromosomes 2, 4, 6 and 7 (Kitomi et al., 2015; Uga et al., 2015b). The *DRO1* gene is a member of the IGT family (Dardick et al., 2013; Guseman et al., 2017), which also includes the Os*TAC1*, Os*LAZY1* and *qSOR1* genes (Godbole et al., 1999; Kitomi et al., 2020; Li et al., 2007; Yoshihara & Iino, 2007; Yu et al., 2007). The *DRO1* homologues in rice (Kitomi et al., 2020), *Arabidopsis* and peach also regulate root and shoot architecture (Guseman et al., 2017). The rice auxin efflux carrier from the PIN-FORMED (PIN) family is known to play an important role in adventitious root emergence (Xu et al., 2005), tiller number and tiller angle (Chen et al., 2012), and root growth angle and crown root development (Wang et al., 2018; Zhang et al., 2012) by regulating polar auxin transport. Recombinant OsARF1 protein can bind to the AREs of the rice *DRO1* promoter as well as other *cis*-regulatory elements involved in the expression divergence of *DRO1-like* wheat homologues (Ashraf et al., 2019; Uga et al., 2013). However, the mechanism underlying the enhancement of rice root drought avoidance by *DRO1* remains poorly understood. We hypothesize that auxin may interact with some kinds of genes to regulate *DRO1* transcription involving in rice root drought avoidance mediated by controlling auxin transport.

The *indica* rice Acuce is a paddy rice landrace with a more than 100 years of planting history in the Yuanyang Hani's terraced fields at altitudes of 1600-2000 m in

Yunnan Province, China. Acuce is a dominant rice variety with favourable agronomic traits, including stable yields and resistance to pathogens. Revealing the genetic basis of the excellent Acuce agronomic traits, especially that of root development and particularly with regard to *DRO1* and transposons, will be of great value in improving rice responses to abiotic and biotic stresses in agricultural production.

Here, we found that the *DRO1* gene controls root architecture via a mechanism related to auxin transport. Further study revealed that an *INDITTO2* transposon located in the upstream promoter region of the Acuce *DRO1* gene has promoter activity and can convey auxin-dependent transcriptional regulation of *DRO1* to enhance rice drought avoidance. These findings provide new insights into how transposons can regulate plant fitness under different environmental stresses.

Materials and Methods

Plant materials

The rice varieties used in this study included Nipponbare, Li jiang xin tuan hei gu (LTH) and Wen lu dao 4 (Wld) with *japonica* background; IR64 with *indica* background; and Kasalath with *aus* background. The following rice germplasms Acuce (Oryza sativa cv. indica, Acuce), Ai jiao gu (Ajg), Ai zhe gu (Azg), Bai gu (Bg), Ban jiu gu (Bjg), Che bu (Cb), Che jia (Cj), Che zuo (Cz), Chuan bai gu (Cbg), Da leng shui (Dls), Da pi gu (Dpg), Duo dian (Dd), Ga niang hong gu (Gnhg), Gan di gu (Gdg), Gan tian nuo (Gtn), Hei gu (Hg), Hong jiao lao geng (Hjlg), Hong yang 1 (Hy 1), Hua ke nuo (Hkn), Jian shui gu (Jsg), Jiu yue nuo (Jyn), Kou ni he lve (Knhl), Le che che ma (Lccm), Ma xian gu (Mxg), Ma zhe nuo (Mzn), Man che hong nuo (Mchn), Mao lai gu (Mlg), Meng la gu (Mlg), Meng la nuo (Mln), Qi xian gu (Qxg), Shi yue bai gu (Sybg), Si ma che (Smc), Xi bai gu (Xbg), Xiao gu (Xg), Xiao hua gu

(Xhg), Xiao hua nuo (Xhn), Xiao pi gu (Xpg), Ye bai gu (Ybg), Yun hui 290 (Yh 290) and Yun xiang (Yx) (Supplemental Table S1) are paddy rice landraces, all obtained from the Yuanyang Hani's terraced fields, Yunnan Province, China.

Drought treatment of rice seedlings

Rice seeds were germinated in water and grown for three weeks, and then the seedlings were transplanted into experimental fields located in Kunming and Jinghong, Yunnan Province, China. The root architectures of rice seedlings grown under irrigation and drought conditions for three months were observed.

To observe the root phenotypes of rice seedlings grown under polyethylene glycol-6000 (PEG6000) treatment, rice seeds were surface-sterilized with 70% alcohol for 90 seconds, treated with 2% sodium hypochlorite for 14 minutes, and then washed five times with sterilized water. Seeds were cultured on Murashige-Skoog (MS) medium for two days under dark conditions, and seedlings were then transferred to MS medium containing with or without 15% PEG6000 to simulate drought stress as previously reported (Liu et al., 2017), and they were grown for four days under light conditions.

Antibody preparation and immune detection

In order to test subcellular localization of auxin efflux carrier, we conducted an immunocytochemical experiment. To prepare the anti-OsPIN1 and anti-OsPIN2 antibodies, the synthetic peptides QSSRNPTPRGSSFNC and QTSREPTPRASSFNC for OsPIN1b (Os02g0743400) and OsPIN2 (Os06g0660200), respectively, were separately injected into rabbit, and the rabbit serum was then purified to obtain the antibodies. All the antibodies were prepared at Huaan Company (Hangzhou Huaan

Biotechnology Co., LTD). Rice seeds were germinated and grown on MS medium for 2 days in the dark, and the rice seedlings were then transferred to MS medium containing with 15% PEG6000 and grown for 3 days under 12 h light per day. The primary root tips from the rice seedlings were then subjected to whole-mount root immune detection as previously described (Sauer et al., 2006). The primary anti-rabbit OsPIN1 antibody (1:200) and anti-rabbit OsPIN2 antibody (1:200) were used separately to incubate with the rice root tips. The secondary antibody was anti-rabbit IgG Alexa488-conjugated antibody (Jackson Immuno Research) (1:500).

Genomic DNA isolation and cloning of the *DRO1* gene, *INDITTO2* and *INDITTO4* transposons

Genomic DNA from each of the rice varieties Nipponbare, LTH, Wld, IR64, Kasalath as well as 40 rice varieties collected from the Yuanyang Hani's terraced fields (including Acuce) (Supplemental Table S1) was isolated using the CTAB method. The gene-specific primer pairs Transposon-FP and Transposon-RP, PDI-FP and PDI-RP, Cas9-FP and Cas9-RP and PUV3-R and gRNA-R5 were used to clone the *INDITTO2*/ *INDITTO4* transposons, the *INDITTO2* transposon containing a flanking sequence from the partial *DRO1* promoter, *Cas9* and *gRNA* in different rice varieties, respectively (Supplemental Table S2). The PCR mix contained 0.3 μL of DNA template, 4 μL of 5 × Transtart FastPfu Fly buffer, 0.4 μL of Transtart FastPfu Fly DNA polymerase (TransGen Biotech, Beijing), 1.5 μL of 2.5 mM dNTPs, and 1.5 μL of each 10 μM primer pair. PCR was performed under the following conditions: denaturation at 95°C for 3 minutes, followed by 30 cycles of 95°C for 50 seconds, 56°C for 50 seconds and 72°C for 90 seconds, and a final extension at 72°C for 10 minutes. The PCR products of the *INDITTO2* and *INDITTO4* transposons were

checked using electrophoresis on 2% agarose gels and sent to the Tsingke Company for sequencing.

RNA isolation, cDNA synthesis and real-time PCR analysis

Primary roots or leaves derived from rice seedlings that were grown in experimental fields or tissue culture seedlings that were grown on different plates were pooled together. The total RNA from the pooled tissues was isolated with the *EasyPure*® Plant RNA kit (TransGen Biotech). To synthesize first-strand cDNA, 100 ng of DNase I-treated RNA, oligo-dT primer and TransScript® II One-Step gDNA Removal and cDNA Synthesis Super Mix (TransGen Biotech) were used to perform the reverse transcription reactions. The relative quantitative expression levels of the *DRO1*, *OsYUCCA2a*, *OsYUCCA5b*, *GUS*, *OsPIN1b*, *OsPIN2* and *OsPIN3t* genes were determined using an ABI QuantStudio 7 Flex Real-Time PCR system (Applied Biosystems, USA). The 10 μL reaction mixture was prepared with 5 μL PowerUp ™ SYBR ™ Green Master Mix (Thermo Fisher Scientific) containing 0.8 μL the primer pairs DRO1-rFP and DRO1-rRP, OsYUCCA2a-rFP and OsYUCCA2a-rRP, OsYUCCA5b-rFP and OsYUCCA5b-rRP, GUS-rFP and GUS-rRP, OsPIN1b-rFP and OsPIN1b-rRP, OsPIN2-5FP and OsPIN2-5RP, and OsPIN3t-FP and OsPIN3t-RP (Supplemental Table S2) for *DRO1*, *OsYUCCA2a*, *OsYUCCA5b*, *GUS*, *OsPIN1b*, *OsPIN2* and *OsPIN3t*, respectively, and 0.5 μL cDNA template. The *actin* gene (GenBank no. AK060893.1) acted as the internal control, and was amplified with the primer pair OsActin-FP and OsActin-RP (Supplemental Table S2). PCR was performed under the following conditions: denaturation at 95°C for 2 minutes, followed by 40 cycles of 95°C for 45 seconds, 56-58°C for 30 seconds and 72°C for 1 minute. Three biological replicates were made. The relative expression level was

calculated using the $2^{-\Delta\Delta Ct}$ method. SPSS version 19.0 (IBM, Inc., Armonk, NY, USA) was used to analyze the differences in gene expression. $P < 0.05$ indicated a statistical difference, and $P < 0.01$ indicated a statistically significant difference.

Results

Acuce roots showed drought avoidance

To analyze root development in response to drought stress, the *indica* rice Acuce and the *japonica* rice Nipponbare were grown in fields either with irrigation (Figure 1A, Supplemental Figure S1A) or under drought conditions (Figure 1B, Supplemental Figure S1B) conditions. The above-ground parts of Acuce grew better than that of Nipponbare (Figure 1B, Supplemental Figure S1B) under drought stress. Furthermore, Acuce showed a deep-rooting phenotype with a smaller root growth angle (Figure 1G, 1I) than Nipponbare (Figure 1C), which had a shallow-root phenotype when grown in fields with irrigation. Acuce also showed a smaller root growth angle (Figure 1H, 1I) than Nipponbare (Figure 1D) when exposed to drought stress. Nipponbare showed a larger root growth angle when grown under drought stress (Figure 1D, 1I) than when under irrigation conditions (Figure 1C, 1I). In contrast, the Acuce root growth angle did not show a significant difference (Figure 1I) between growth under irrigation conditions (Figure 1G) and drought stress (Figure 1H). The root lengths of Acuce (Supplemental Figure S1D, S1H) and Nipponbare (Supplemental Figure S1C, S1G) did not show any significant difference when grown under irrigation conditions (Supplemental Figure S1K). However, under drought stress, Acuce (Supplemental Figure S1F, S1J S1K) had a longer root length than did Nipponbare (Supplemental Figure S1E, S1I, S1K). Overall, Acuce had a higher root fresh weight than Nipponbare (Supplemental Figure S1L). To further investigate the root phenotypes,

Acuce and Nipponbare were grown on medium containing 15% PEG6000. Compared with the unstressed control (Supplemental Figure S2A, S2C), root growth was normal in Acuce seedlings (Supplemental Figure S2B, S2E), but the primary root of Nipponbare was shorter than that of the control (Supplemental Figure S2D, S2E). These results demonstrated that the Acuce rice variety is better adapted to drought than Nipponbare, and its root development showed obvious drought avoidance.

Acuce roots had high *DRO1* expression

Next, we tested whether the previously identified *DRO1* gene, which is known to be involved in rice drought avoidance (Uga et al., 2013), might underpin this difference between the Acuce and Nipponbare varieties. The Acuce DRO1 amino acid sequences shared 99.6%, 99.6% and 89.6% identity with the DRO1 proteins from the rice varieties Kinandang Patong, Nipponbare and IR64, respectively (Supplemental Figure S3). We further analyzed the deduced tertiary structures of the DRO1 proteins from the rice varieties Acuce, Kinandang Patong, Nipponbare and IR64. The DRO1 amino acid sequence from Acuce is differs at position 163 from the varieties Nipponbare, Kinandang Patong and IR64 (Supplemental Figure S3); however, the different amino acid did not alter the deduced tertiary structures of the DRO1 proteins from Acuce, Kinandang Patong and Nipponbare, although that of IR64 was different (Supplemental Figure S4). Next, we tested the transcription of the *DRO1* gene in the primary root, revealing that *DRO1* levels were strongly upregulated in the meristem region but showed no obvious change in the elongation and differentiation zones in Acuce grown under drought stress (Supplemental Figure S2F). However, *DRO1* transcription was not altered in Nipponbare (Supplemental Figure S2F). These observations show that the drought avoidance in Acuce correlates with the specific

capability of this variety to upregulate *DRO1* transcription in the root meristem region in response to drought stress.

DRO1 **mutation altered the root architecture**

To test whether the *DRO1* gene was relevant to the drought-mediated changes in Acuce root development, we obtained *dro1*-cc mutant lines using the CRISPR/Cas9 method. In the Acuce *dro1*-cc mutant, one additional nucleotide, thymine (T), was inserted into the second exon, thereby altering the open reading frame and leading to the pre-terminated translation of DRO1 protein (Supplemental Figure S5A, S5B). When the Acuce homozygous *dro1*-cc mutant was grown in a greenhouse with irrigation, we found that compared with wild-type plants (Supplemental Figure S6A), it showed shallow root (Supplemental Figure S6A) and short root length phenotypes (Supplemental Figure S6B). The expression levels of *OsPIN1b* and *OsPIN3t* were upregulated and downregulated, respectively, but the level of *OsPIN2* showed no obvious change in the Acuce *dro1*-cc mutant root (Supplemental Figure S6C). These results suggested the involvement of *DRO1* in regulating auxin transport in root growth.

DRO1 is a member of the IGT family (Dardick et al., 2013; Guseman et al., 2017). To further analyze the role of *DRO1* in root growth, we searched the IGT family genes and *DRO1* homologues in Nipponbare, including the *OsLAZY1*, *OsTAC1* and *qSOR1* genes, which contain IGT and EAR-like motifs (Supplemental Figure S7). The expression analysis of IGT family genes revealed low expression levels of *OsLAZY1*, *OsTAC1* and *qSOR1*, but high expression levels of *DRO1* in rice roots (Supplemental Figure S8). Next, we generated Nipponbare *dro1*-cc mutants using the CRISPR/Cas9 method (Supplemental Figure S9). We obtained 11 *dro1*-cc mutant lines and randomly

selected 3 lines (#5, #10 and #17) to analyze the root phenotype. These T2 *dro1*-cc lines showed a shorter root length than the original Nipponbare variety (Figure 2F). When the homozygous rice *dro1*-cc line #17, in which the CRISPR cassette Cas9 was selected out (Supplemental Figure S10), was grown under irrigation conditions, it showed a shallower root phenotype (Figure 1E) than wild-type Nipponbare (Figure 1C, 1D), with a significantly larger root growth angle (Figure 1I). However, when line #17 was grown under drought stress (Figure 1F), it was small (Figure 1B) and had a shallow root phenotype (Figure 1F, 1I). When the rice seedlings were grown in MS medium supplemented either without (Supplemental Figure S11A, S11C) or with (Supplemental Figure S11B, S11D) 15% PEG6000, both Nipponbare and *dro1*-cc (#17) rice seedlings showed shorter root lengths under drought stress compared with the control (Supplemental Figure S11E). Additionally, the *dro1*-cc (#17) (Supplemental Figure S11C, S11E) seedlings also showed a shorter root length than the wild-type (Supplemental Figure S11A, S11E) when grown on MS medium.

We then determined the expression levels of the auxin biosynthesis genes *OsYUCCA2a* and *OsYUCCA5b* in the Nipponbare *dro1*-cc (#17) mutant grown under drought stress. Under drought conditions, the *OsYUCCA5b* gene was upregulated, but the levels of *OsYUCCA2a* were not obviously different in wild-type Nipponbare and the *dro1*-cc (#17) mutant (Supplemental Figure S12). When seedlings of wild-type Nipponbare and the *dro1*-cc (#17) mutant were grown on MS medium supplemented with 15% PEG6000 plus either DMSO (as a control) (Supplemental Figure S13A, S13C) or 1 μM auxin biosynthesis inhibitor L-Kynurenine (Kyn) (He et al., 2011) (Supplemental Figure S13B, S13D), both wild-type and *dro1*-cc (#17) mutant seedlings had longer root lengths (Supplemental Figure S13E). These data suggest that *DRO1* does not function to regulate auxin biosynthesis.

Root architecture is largely determined by auxin and, in particular, by polarized auxin transport, which is dependent on PIN auxin exporters (Adamowski & Friml, 2015; Benkova et al., 2003). Therefore, we analyzed the influence of DRO1 activity on PIN expression. Under drought conditions, expression levels of *OsPIN1b* were downregulated in wild-type seedlings but were unchanged in the *dro1*-cc (#17) mutant, expression levels of *OsPIN2* were unchanged in the wild-type seedlings and upregulated in the *dro1*-cc (#17) mutant, and expression levels of *OsPIN3t* were downregulated in the wild-type but upregulated in the *dro1*-cc (#17) mutant (Supplemental Figure S11F). To further investigate whether mutation of *DRO1* disturbed auxin transport, we checked the root phenotype during growth under gravity stimulation. The results showed that the root of *dro1*-cc (#17), which had a larger gravitropic curvature than the wild-type (Supplemental Figure S14E), was associated with a disturbance in auxin transport (Supplemental Figure S14F). Compared with the larger root growth angle in the wild-type treated with auxin grown under gravitropic stimulation (Supplemental Figure S14C, S14E), the primary root growth angle is smaller in the *dro1*-cc (#17) mutant treated with auxin (Supplemental Figure S14D, S14E) and did not show significant difference compared with wild-type treated with DMSO (Supplemental Figure S14E). We further investigated the subcellular localization of OsPIN1b and OsPIN2 in the root meristem region of the *dro1*-cc (#17) mutant grown in MS medium supplemented either without or with 15% PEG6000. Compared with the wild-type grown under drought stress (Figure 3B, 3I), the subcellular localization of OsPIN1b did not obviously change on the plasma membrane in *dro1*-cc (#17) (Figure 3D, 3I), and OsPIN2 showed reduced localization in the plasma in both the wild-type (Figure 3F, 3I) and *dro1*-cc (#17) (Figure 3H, 3I)

grown under drought stress. These results show that mutation of *DRO1* disturbs auxin transport.

Auxin differentially regulates *DRO1* transcription in Acuce and Nipponbare

Auxin is known to negatively regulate the expression of *DRO1* (Uga et al., 2013). Therefore, we tested the potential auxin-mediated regulation of *DRO1* expression in Nipponbare and Acuce. Rice seedlings were treated with 0.01 μM, 0.1 μM and 1 μM NAA and 2,4-D (Supplemental Figure S15). An increased auxin concentration inhibited the primary root length (Supplemental Figure S15M, S15N). The expression levels of *DRO1* increased following 0.01 μM NAA and 2,4-D treatment in Acuce, and decreased following treatment with higher concentrations of auxin (Supplemental Figure S15O, S15P). In contrast, in Nipponbare, no such transcription increase was observed following either treatment with 0.01 μM NAA and 2,4-D or with higher concentrations of auxin (Supplemental Figure S15O, S15P). These results suggest that in Acuce, auxin has the capacity to regulate *DRO1* expression.

We next conducted the complementary experiment and treated rice seedlings with the auxin biosynthesis inhibitor Kyn. The primary roots of Acuce and Nipponbare were obviously longer compared to the untreated control following treatment with increasing concentrations of Kyn (Supplemental Figure S16A-I). Notably, Acuce was more sensitive than Nipponbare to Kyn treatment, as 20 μM Kyn could induce a longer root length in Acuce but not in Nipponbare (Supplemental Figure S16I). In addition, *DRO1* gene expression was upregulated when Acuce was treated with higher concentrations of 30 μM Kyn, this is in contrast to the unchanged *DRO1* expression levels in Nipponbare following treatment with Kyn (Supplemental Figure S16J). These data show a pronounced difference in auxin sensitivity between the Acuce and

374 Nipponbare rice varieties; both at the level of root growth and the regulation of *DRO1*
375 gene expression.
376
377 **The *DRO1* promoter in Acuce and many other rice varieties contains the**
378 ***INDITTO2* transposon**
379 To investigate the possible mechanism of the *DRO1*-dependent differences in drought
380 avoidance and auxin-mediated *DRO1* transcription regulation in Acuce, we compared
381 its *DRO1* promoter to those of the varieties IR64, Nipponbare and Kinandang Patong
382 (Supplemental Figure S17). We found that the *DRO1* promoters of Acuce and IR64
383 contained an additional 266 nucleotides (Supplemental Figure S17). We analyzed this
384 sequence with online software (www.repeatmasker.org, www.girinst.org) and found
385 that it shared 93.2% identity with the *INDITTO* transposon, which is a
386 non-autonomous DNA transposon belonging to the *Tourist* superfamily (Jiang &
387 Wessler, 2002). Therefore, we named the sequence inserted in the *DRO1* promoter as
388 the *INDITTO2* transposon.
389 We conducted a BLAST search in the NCBI database (www.ncbi.nlm.nih.gov)
390 using the sequence of the *INDITTO2* transposon. Interestingly, a rice Teqing
391 homologue sequence was located between two genes that encoded a putative
392 ADP-glucose pyrophosphorylase subunit SH2 (GenBank no. AAB58473.1) and a
393 putative NADPH-dependent reductase A1 (GenBank no. AAB58474.1)
394 (Supplemental Figure S18A). Further analysis of this new sequence with online
395 software (www.repeatmasker.org, www.girinst.org) showed that it also belonged to
396 the *Tourist* family and shared 93.6% and 97.7% identity with the *INDITTO* and
397 *INDITTO2* transposons, respectively (Supplemental Figure S18B). Thus, we named
398 the sequence as *INDITTO3* transposon (Supplemental Figure S18A, S18B). Because

no homologues of *INDITTO2* were found in the *DRO1* promoter region in Nipponbare (Supplemental Figure S17), we further amplified the *INDITTO2* transposon in the Nipponbare genome using PCR. An *INDITTO2* homologue was found in the Nipponbare genome, and we named it *INDITTO4* (Supplemental Figure S18B). *INDITTO4* is inserted in yet another different locus, and its sequence shared 95.1% and 95.5% identity with *INDITTO* and *INDITTO2*, respectively (Supplemental Figure S18B). We then conducted a BLAST search using the *INDITTO4* sequence in the Nipponbare genome. A total of 94 homologues of the *INDITTO4* gene were found in the Nipponbare genome, of which 15 *INDITTO4* homologues were inserted into known gene regions, and 12.76% genes contained an ARE (Supplemental Table S3). These findings suggest that *INDITTO* transposons and its homologues can jump around the rice genome and can potentially disrupt or regulate different loci.

To further characterize the *INDITTO2* transposon in the rice genome, we cloned *INDITTO2* from the *DRO1* promoter region in 45 rice lines, including IR64, Kasalath, LTH, Nipponbare, Wld and 40 rice varieties collected from the Yuanyang Hani's terraced fields, with gene-specific primers PDI-FP and PDI-RP using PCR (Supplemental Figure S19A). We found that from 45 rice lines investigated, there were 40 lines in which the homologues of the *INDITTO2* transposon could be detected in the *DRO1* promoter region (Supplemental Figure S19B) and show high sequence conservation (Supplemental Figure S20). A BLAST search of publicly available sequences further revealed that the rice varieties Shuhui498 and RP Bio-226 contained the *INDITTO2* transposon in the *DRO1* promoter (Supplemental Figure S20). These results show that the *DRO1* promoter regions of many rice varieties, including Acuce, contain the *INDITTO2* transposon. A phylogenetic analysis showed that the *INDITTO2* transposon and its homologues formed two main subgroups,

which contain with or without AREs, and that these subgroups are distinct from those of the *INDITTO*, *INDITTO3*, *INDITTO4* and its homologues from the different rice varieties (Supplemental Figure S21).

Presence of *INDITTO2* transposon conveys auxin-responsiveness to *DRO1* expression

The presence of the *INDITTO2* transposon in the *DRO1* promoter of Acuce but not Nipponbare (Supplemental Figure S17) provides a potential mechanism for the differential *DRO1* transcription regulation between these two rice varieties. Sequence analysis showed that the Acuce *INDITTO2* transposon contained an ARE (Supplemental Figure S17) and various transcription factor binding sites, including those related to the BBR-BPC, HD-ZIP, C2H2, HD-ZIP, bZIP and the MYB-related families (Supplemental Table S4). In addition, the *INDITTO2* homologue in nine of the studied rice varieties (Shuhui498, RP Bio-226, Bg, Qxg, Cz, Cj, Xhn, Xhg and Kasalath) contained an ARE (Supplemental Figure S20). Three further AREs also occur in the *DRO1* promoter outside the *INDITTO2* sequence (Supplemental Figure S17).

The *INDITTO2* transposon contained various transcription factor binding sites (Supplemental Table S4). We constructed different expression vectors to study the role of the *INDITTO2* transposon in the regulation of *DRO1* transcription by auxin (Supplemental Figure S22A). We placed the *GUS* reporter gene under the *DRO1* promoter of Acuce and Nipponbare with or without the *INDITTO2* transposon (Supplemental Figure S22A) and infiltrated it into tobacco leaves using an *Agrobacterium tumefaciens*-mediated method. The full-length *DRO1* promoter from Acuce conveyed stronger *GUS* expression than the Acuce *DRO1* promoter without the

INDITTO2 transposon (Supplemental Figure S22B). In addition, deletion of the ARE in the *INDITTO2* transposon from the Acuce *DRO1* promoter also resulted in the downregulation of its activity (Supplemental Figure S22B). This result implies that the *INDITTO2* transposon containing the ARE is involved in the regulation of gene expression. Consistent with this result, the level of the *GUS* gene controlled by the Nipponbare *DRO1* promoter was lower than that of the *GUS* transcribed by the Acuce *DRO1* promoter (Supplemental Figure S22C). However, when the *INDITTO2* transposon was inserted into the Nipponbare *DRO1* promoter, the expression level of *GUS* was obviously upregulated (Supplemental Figure S22C).

The *INDITTO2* transposon acts as an auxin-responsive promoter

To further investigate the potential promoter function of the *INDITTO2* transposon, we placed the *INDITTO2* transposon only (without the genomic context of the *DRO1* promoter) upstream of the *GUS* gene. Compared with the expression under the control of the Acuce *DRO1* promoter, *GUS* expression controlled by the *INDITTO2* transposon was upregulated (Supplemental Figure S22D). However, when *GUS* transcription was controlled by the *INDITTO2* transposon without the ARE, its activity was obviously downregulated compared with that of the full-length *INDITTO2* (Supplemental Figure S22D). These findings suggest that the *INDITTO2* transposon exerts promoter activity that depends on the presence of the ARE.

Next, we tested whether the promoter activity of the *INDITTO2* transposon was responsive to auxin. Exogenous NAA (1 μM) was added to a solution of the *Agrobacterium tumefaciens* strain EHA105 containing a full-length *INDITTO2* transposon construct and infiltrated into tobacco leaves. *GUS* expression was obviously upregulated relative to the untreated control (Supplemental Figure S22D).

Furthermore, *GUS* transcription controlled by the *INDITTO2* transposon with the ARE deletion did not obviously respond to auxin treatment (Supplemental Figure S22D). Taken together, these results show that the *INDITTO2* transposon exerts auxin-regulated promoter activity.

To further investigate the role of the *INDITTO2* transposon regulated by auxin in the *DRO1* promoter, *Agrobacterium* strains EHA105 containing the GUS gene fused to either the whole Acuce *DRO1* promoter or with the *INDITTO2* transposon deleted were infiltrated into tobacco leaves. The *GUS* expression levels were found to be downregulated under NAA treatment (Supplemental Figure S22E). When the whole Nipponbare *DRO1* promoter or the promoter containing the *INDITTO2* transposon were fused with the *GUS* gene and transiently expressed in tobacco leaves, both constructs resulted in lower levels of *GUS* gene expression under NAA treatment (Supplemental Figure S22F). However, when the whole Acuce *DRO1* promoter with ARE deletion in the *INDITTO2* transposon fused with the *GUS* gene was expressed in tobacco leaves, the *GUS* gene expression levels were upregulated under NAA treatment (Supplemental Figure S22E).

Three elements located in the *DRO1* promoter can respond to auxin treatment (Uga et al., 2013). To further determine the effect of these three elements in responding to auxin treatment, we constructed two expression vectors containing the Acuce or Nipponbare *DRO1* promoter with the three AREs deleted and fused with the *GUS* gene (Supplemental Figure S22A). When these two constructs were expressed in tobacco leaves under NAA treatment, the expression levels of the *GUS* gene were downregulated in the Acuce construct but upregulated in the Nipponbare construct compared with the control without NAA treatment (Supplemental Figure S22G).

We further investigated the role of the *INDITTO2* transposon in regulating the expression of the *DRO1* gene in rice seedlings. The *DRO1* promoter of Nipponbare was inserted with the *INDITTO2* transposon containing with or without ARE and fused with the genomic *DRO1* gene of Nipponbare (Figure 4A). A solution of *A. tumefaciens* strain EHA105 transformed with the expression vector (Figure 4A) and containing 30 μM Kyn was used to infiltrate the rice leaf veins (Figure 4B). Compared with the control, the *DRO1* gene expression transcribed under the Nipponbare *DRO1* promoter containing the whole *INDITTO2* transposon was obviously upregulated in rice leaves (Figure 4C), and this was in contrast to the expression pattern of the *GUS* gene transcribed under Acuce *DRO1* or Nipponbare *DRO1* containing the *INDITTO2* transposon and induced with 1 μM NAA (Supplemental Figure S22E, S22F). However, the *DRO1* gene expression transcribed under the Nipponbare *DRO1* promoter containing the *INDITTO2* transposon with the ARE deletion was obviously downregulated (Figure 4C), and this was in contrast to the expression levels of the *GUS* gene transcribed under the Acuce *DRO1* promoter carrying the *INDITTO2* transposon with the ARE deletion induced with 1 μM NAA (Supplemental Figure S22E). These results show that the ARE of the *INDITTO2* transposon plays an important role in the transcriptional activity of the *DRO1* promoter regulated by auxin.

The *INDITTO2* transposon is sufficient to promote Nipponbare drought avoidance

The rice variety Nipponbare showed low drought avoidance correlating with low and auxin-insensitive expression of *DRO1* (Figure 1, Supplemental Figure S2, S15). To test whether the *INDITTO2* transposon, which can convey auxin regulation of *DRO1*,

would be sufficient to induce *DRO1* transcription and enhance rice drought avoidance, we obtained 11 transgenic Nipponbare rice lines expressing the *INDITTO2* transposon. The *INDITTO2* transposon was inserted into the *DRO1* promoter of Nipponbare (Supplemental Figure S23A), and the insertion position of the *INDITTO2* transposon was the same as that in the *DRO1* promoter of Acuce (Supplemental Figure S17). The T1 transgenic Nipponbare lines containing the *INDITTO2* transposon were verified by PCR, and lines #8, #11, and #14 were grown on MS medium containing with 15% PEG6000. These transgenic rice lines expressing the whole *INDITTO2* transposon showed drought avoidance accompanied by an unchanged root length (Supplemental Figure S23C-E, S23G-I, S23J) and upregulated *DRO1* expression levels in the root tips (Supplemental Figure S23K). Compared with the control grown under irrigation conditions (Figure 5B-D, 5J-L), the T2 transgenic Nipponbare lines (#1, #4, and #8) grown under drought stress (Figure 5F-H, 5N-P) did not show an obvious difference in root growth angle (Figure 5Q) or root length (Figure 5R); however, the wild-type Nipponbare showed a larger root growth angle (Figure 5Q) and a shorter root length (Figure 5R). When the Nipponbare *DRO1* gene containing the *INDITTO2* transposon in the Nipponbare *DRO1* promoter (Supplemental Figure S23A) was transformed into the Nipponbare *dro1*-cc (#17) mutant (Figure 2E-F), the T1 rescued rice mutant lines (#1, #5, #6, and #20) showed a longer root length compared with the wild-type Nipponbare (Figure 2F). These results show that rice variety with the presence of the *INDITTO2* transposon in the *DRO1* locus is sufficient for enhancing drought avoidance and better adaptation to drought stress.

The *INDITTO2* transposon shows functional diversity in rice adaptation to drought stress

548 The *INDITTO2* transposon is involved in the regulation of *DRO1* expression. We
549 next investigated root development of the rice varieties containing the *INDITTO2*
550 transposon under drought stress. We randomly selected rice varieties Kasalath and
551 Xhn, which contained the *INDITTO2* transposon with an ARE (Supplemental Figure
552 S20), and the rice varieties Knhl and Azg, which contained the *INDITTO2* transposon
553 but without ARE (Supplemental Figure S20). These four rice varieties were grown on
554 MS medium supplemented with 1μM NAA (Supplemental Figure S24A-H).
555 Compared with the control (Supplemental Figure S24A-D), the primary root lengths
556 of the seedlings treated with NAA were significantly shorter than those in the control
557 (Supplemental Figure S24I), and the expression levels of *DRO1* were downregulated
558 in the varieties Kasalath and Xhn, unchanged in Knhl, but upregulated in the variety
559 Azg (Supplemental Figure S24J). However, when these rice varieties were grown on
560 MS medium containing with 15% PEG6000 (Supplemental Figure S25E-H), Kasalath,
561 Xhn and Knhl showed shorter primary root length, but that of Azg was not obviously
562 changed (Supplemental Figure S25I) compared with the control grown on MS
563 medium without 15% PEG6000 (Supplemental Figure S25A-D). Furthermore, the
564 expression levels of *DRO1* were downregulated in Kasalath, unchanged in Xhn and
565 Knhl, but upregulated in Azg (Supplemental Figure S25J). When the four rice
566 varieties were grown in the field, compared with the control (grown with irrigation),
567 Kasalath and Xhn showed no change in root growth angles, but Knhl and Azg had
568 larger root growth angles when the rice seedlings were grown under drought stress
569 (Supplemental Figure S26I). However, compared with the control grown with
570 irrigation, Kasalath, Knhl and Azg had shorter root lengths, while Xhn did not have
571 obvious change in root length when the rice seedlings grown under drought stress
572 (Supplemental Figure S26J). This demonstrates that Xhn has a deep root phenotype

and that the varieties Kasalath, Knhl and Azg have a shallow root phenotype. When we further analyzed this correlation between root length and root growth angle, compared with seedlings grown in an irrigated field (Supplemental Figure S27A, S27C, S27E, S27G), we found a significant negative correlation in both rice Xhn and Knhl (Supplemental Figure S27D, S27F), no significant negative correlation in Kasalath (Supplemental Figure S27B), and a positive correlation tendency in the variety Azg (Supplemental Figure S27H). These results suggest that the *INDITTO2* transposon is able to regulate the transcription of the *DRO1* gene in a mechanism mediated by auxin to enable plants to adapt to drought stress, but this effect varies among the different rice genetic backgrounds.

Discussion

Rice is a worldwide crop with several different subspecies, including the *japonica* and *indica*. However, drought, an important abiotic stress, may alter rice seedling development and reduce crop quality and yields. Various rice cultivars exist differing genetic background and plant morphogenesis (for example in root architecture) to drive important adaptation in drought stress (Uga et al., 2013). In this study, we found that rice drought avoidance can be enhanced through *INDITTO2* transposon-mediated promoter activity and conveyed auxin-dependent transcriptional regulation of the *DRO1* gene.

Rice varieties with increased *DRO1* expression had deeper root phenotypes and increased root lengths than transgenic rice lines with *DRO1* mutation. Furthermore, *DRO1* is involved in the regulation of auxin distribution and the subcellular localization of the auxin transporter OsPIN1b. Furthermore, an *INDITTO2* transposon inserted into the Acuce *DRO1* promoter could react to auxin and show promoter

activity, and the transgenic Nipponbare line with an *INDITTO2* insertion in the *DRO1* promoter region showed enhanced drought avoidance. These observations suggest that *DRO1* interacts with auxin to control rice drought avoidance. *INDITTO2* conveys the auxin signal to mediate *DRO1* activity, thus providing a mechanism by which rice can adapt to environmental drought stress.

During drought stress, auxin plays an important role in rice root growth. *DRO1* is an early auxin-response gene and is a member of the IGT family (Uga et al., 2013), which controls plant architecture by forming an asymmetric auxin distributions (Dong et al., 2013; Godbole et al., 1999; Li et al., 2007; Taniguchi et al., 2017; Yoshihara & Iino, 2007; Yoshihara & Spalding, 2017; Yoshihara et al., 2013). In the current study, mutation of *DRO1* disturbed the localization and levels of the auxin efflux carrier (Figure 3, Supplemental Figure S11) as well as the auxin content (Supplemental Figure S14). Therefore, it was plausible that the effect of *DRO1* on drought avoidance was related to auxin transport. The *DRO1* gene showed higher expression levels than those of the other IGT family members expressed in the roots (Supplemental Figure S8), which suggests that the *DRO1* gene is the main regulating factor altering root architecture in the control of root drought avoidance. A previous research shows that auxin can negatively regulate the level of *DRO1* gene (Uga et al., 2013), whereas, we found that auxin could not negatively regulate the levels of *DRO1* gene in certain rice varieties (Supplemental Figure S24J), which implies that the role of auxin in regulating the expression levels of *DRO1* is dependent on the rice genetic background.

Transposons are involved in various aspects of plant growth, including rapeseed vernalization (Hou et al., 2012), potato tuber skin colour (Momose et al., 2010), rice glume development and maize latitudinal adaptation (Huang et al., 2018; Nakazaki et

623 al., 2003). The rice variety IR64 has low drought avoidance resulting from a single
624 adenine nucleotide deletion in exon 4 of the *DRO1* gene (Uga et al., 2013). The
625 deduced tertiary structure of Nipponbare *DRO1* was not different from that of the
626 variety Acuce (Supplemental Figure S4), but that Nipponbare was sensitive to drought
627 stress while Acuce was not (Figure 1). However, given that the *INDITTO2* transposon
628 conveyed the auxin signal to regulate the transcription of the *DRO1* gene (Figure 4C)
629 and promoted Nipponbare drought avoidance (Figure 5), it is plausible that drought
630 avoidance is regulated through a mechanism by which *INDITTO2* impacts on the
631 *DRO1* gene, and that high levels of *DRO1* are necessary for rice adaptation to drought
632 stress. However, we also noticed that the root growth phenotypes of the rice varieties
633 Xhn and Azg (Supplemental Figure S26I) were not consistent with the expression of
634 *DRO1* gene when rice were grown under drought stress (Supplemental Figure S25J).
635 This suggests that unknown factors associated with the particular genetic background
636 of the variety may be involved in the regulation of drought avoidance mediated by
637 *DRO1*. The *INDITTO2* transposon was found to be present in the *DRO1* promoter of
638 the various rice varieties (Supplemental Figure S18, S20) and could be detected in
639 different loci of the Nipponbare genome (Supplemental Table S3). Thus, the
640 *INDITTO2* transposon increased rice genetic diversity and was enable it to adapt to
641 different abiotic stresses, including drought stress, during rice development.

642 The impact of the *INDITTO2* transposon on regulation of *DRO1* is similar to that of
643 certain other transposons, which have been proposed to regulate transcription of
644 *BnFLC.A10* and *ABP1* (Elrouby & Bureau, 2000, 2012; Huang et al., 2018). However,
645 the *INDITTO2* and its homologues were found both with or without AREs and
646 putative transcription factor binding sites (Supplemental Figure S20, Supplemental
647 Table S4), which is similar to the role of *siR441* and *siR446* derived from *Stowaway1*

transposon to regulate ABA signalling (Yan et al., 2011). Therefore, the *INDITTO2* transposon functions as an auxin-inducible promoter, which as described in *PdMLE1* transposon (Sun et al., 2013), and an alternative auxin signalling pathway might regulate *INDITTO2*-mediated drought avoidance. However, the *INDITTO2* could not induce upregulation of *DRO1* genes in certain rice varieties under drought stress (Supplemental Figure S25J). Furthermore, the four AREs that are located in the *DRO1* promoter region and the *INDITTO2* transposon show different patterns when responding to the transcriptional activity of the *DRO1* promoter under auxin treatment (Figure 4, Supplemental Figure S22). These findings suggest that the functional differences of AREs may contribute to the differential roles of *DRO1* gene in the responses of rice to drought stress, and the rice genetic diversity is an important factor for the role of *INDITTO2* in regulating gene expression profile.

The *INDITTO2* transposon was identified from the variety Acuce as well as certain rice landraces collected from the Yuanyang Hani's terraced fields, and which grow on terraced fields at altitudes of 1600-2000 m. This implies that the *INDITTO2* transposon, jumping to different positions in the rice genome, can function as an auxin-regulated promoter to accelerate the evolution of developmental and physiological traits in different rice species, and its presence is sufficient to convey plant adaptation under different environmental stresses in agricultural production.

In summary, the *INDITTO2* transposon and its homologues are found widely throughout the rice genomes and are able to convey auxin-mediated *DRO1* transcription to enhance rice drought avoidance through an as yet undiscovered mechanism as shown in a putative model (Supplemental Figure S28). The *INDITTO2* transposon and its homologues increase the rice genetic diversity and may enable it to adapt to different environmental stresses.

Acknowledgments

We thank Professor Jianqiang Wu (Kunming Institute of Botany, Chinese Academy of Sciences) for providing generous support for the IAA measurements. This work was supported by grants from the National Natural Science Foundation of China (Grant Nos. 31460453, 31660501, 31860064 and 31470382), the Major Special Program for Scientific Research, Education Department of Yunnan Province (Grant No. ZD2015005), SRF for ROCS, SEM (Grant No. [2013] 1792), the Major Science and Technique Programs in Yunnan Province (Grant No. 2016ZF001), the Key Projects of Applied Basic Research Plan of Yunnan Province (Grant No. 2017FA018), the Earmarked Fund for Modern Agro-industry Technology Research System (Grant No. CARS-21), the National Key Research Development Program of China (2016YFD0100600), the Program for Innovative Research Team in University of Yunnan Province (IRTSTYN), and the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Programme (Grant Agreement No. 742985).

Conflict of Interest Statement

The authors declare that they have no competing interests.

Accession numbers

Sequence data for the Acuce *DRO1* and Nipponbare *DRO1*, *qSOR1*, *OsLAZY1* and *OsTAC1* genes described in this study can be found in the NCBI database under the following accession numbers: MH939159, Os09g0439800, Os07g0614400, Os11g0490600 and Os09g0529300, respectively.

Authorship

Y.D. conceived the original screening and research plans; Y.D. supervised the experiments; Y.Z., L-X.W., Q.F., D.W., J.L., B.Y., S.Y., L.J., J.Q., X.Z., L.H., S.Z., C.M., Y-F.Z., C-Y.L., Q.D., S.L., L.Z., X.J., Y.L., H.L., and K.L. performed the majority of the experiments; Y.D., J.Y., Q.L., L.L., S.P., H.H., Z.Z., C.L., L.W., C.L., X.H., and J.F. provided technical assistance to Y.Z., L-X.W., Q.F., D.W., J.L., B.Y., S.Y., S.Y., L.J., J.Q., X.Z., L.H., S.Z., C.M., Y-F.Z., C-Y.L., Q.D., S.L., L.Z., X.J., Y.L., H.L., and K.L.; Y.D., Y.Z., L-X.W., and Q.F. designed the experiments and analysed the data; Y.D. conceived the project and wrote the article with contributions from all the authors; Y.D. and J.F. supervised and completed the manuscript writing. Y.D. agrees to serve as the author responsible for contact and will ensure communication.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

References

Adamowski, M., & Friml, J. (2015). PIN-dependent auxin transport: action, regulation, and evolution. *Plant Cell, 27*(1), 20-32.

Arai-Sanoh, Y., Takai, T., Yoshinaga, S., Nakano, H., Kojima, M., Sakakibara, H., Kondo, M., & Uga, Y. (2014). Deep rooting conferred by DEEPER ROOTING 1 enhances rice yield in paddy fields. *Scientific Reports, 4*, 5563.

Ashraf, A., Rehman, O. U., Muzammil, S., Léon, J., Naz, A. A., Rasool, F., Ali, G.

723 M., Zafar, Y., & Khan, M. R. (2019). Evolution of Deeper Rooting 1-like
724 homoeologs in wheat entails the C-terminus mutations as well as gain and loss
725 of auxin response elements. *Plos One, 14*(4), e0214145.
726 Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G.,
727 & Friml, J. (2003). Local, efflux-dependent auxin gradients as a common
728 module for plant organ formation. *Cell, 115*(5), 591-602.
729 Bureau, T. E., Ronald, P. C., & Wessler, S. R. (1996). A computer-based systematic
730 survey reveals the predominance of small inverted-repeat elements in
731 wild-type rice genes. *Proceedings of the National Academy of Sciences of the*
732 *United States of America, 93* (16), 8524-8529.
733 Bureau, T. E., & Wessler, S. R. (1992). Tourist: a large family of small inverted
734 repeat elements frequently associated with maize genes. *Plant Cell, 4*(10),
735 1283-1294.
736 Bureau, T. E., & Wessler, S. R. (1994). Mobile inverted-repeat elements of the
737 Tourist family are associated with the genes of many cereal grasses.
738 *Proceedings of the National Academy of Sciences of the United States of*
739 *America, 91*(4), 1411-1415.
740 Chen, Y. N., Fan, X. R., Song, W. J., Zhang, Y. L., & Xu, G. H. (2012).
741 Over-expression of OsPIN2 leads to increased tiller numbers, angle and
742 shorter plant height through suppression of OsLAZY1. *Plant Biotechnology*
743 *Journal, 10*(2), 139-149.
744 Dardick, C., Callahan, A., Horn, R., Ruiz, K. B., Zhebentyayeva, T., Hollender, C.,
745 Whitaker, M., Abbott, A., & Scorza, R. (2013). PpeTAC1 promotes the
746 horizontal growth of branches in peach trees and is a member of a functionally
747 conserved gene family found in diverse plants species. *Plant Journal, 75*(4),

618-630.

Dong, Z. B., Jiang, C., Chen, X. Y., Zhang, T., Ding, L., Song, W. B., Luo, H. B., Lai, J. S., Chen, H. B., Liu, R. Y., Zhang, X. L., & Jin, W. W. (2013). Maize LAZY1 mediates shoot gravitropism and inflorescence development through regulating auxin transport, auxin signaling, and light response. *Plant physiology, 163*(3), 1306-1322.

Elrouby, N., & Bureau, T. E. (2000). Molecular characterization of the Abp1 5 '-flanking region in maize and the teosintes. *Plant physiology, 124*(1), 369-377.

Elrouby, N., & Bureau, T. E. (2012). Modulation of auxin-binding protein1 gene expression in maize and the teosintes by transposon insertions in its promoter. *Molecular Genetics and Genomics, 287*(2), 143-153.

Godbolé, R., Takahashi, H., & Hertel, R. (1999). The *lazy* mutation in rice affects a step between statoliths and gravity-induced lateral auxin transport. *Plant Biology, 1*(4), 379-381.

Guseman, J. M., Webb, K., Srinivasan, C., & Dardick, C. (2017). DRO1 influences root system architecture in *Arabidopsis* and *Prunus* species. *Plant Journal, 89*(6), 1093-1105.

He, W. R., Brumos, J., Li, H. J., Ji, Y. S., Ke, M., Gong, X. Q., Zeng, Q. L., Li, W. Y., Zhang, X. Y., An, F. Y., Wen, X., Li, P. P., Chu, J. F., Sun, X. H., Yan, C. Y., Yan, N., Xie, D. Y., Raikhel, N., Yang, Z. B., Stepanova, A. N., Alonso, J. M., & Guo, H. W. (2011). A small-molecule screen identifies L-Kynurenine as a competitive inhibitor of TAA1/TAR activity in ethylene-directed auxin biosynthesis and root growth in *Arabidopsis*. *Plant Cell, 23*(11), 3944-3960.

Hou, J. N., Long, Y., Raman, H., Zou, X. X., Wang, J., Dai, S. T., Xiao, Q. Q., Li, C.,

Fan, L. J., Liu, B., & Meng, J. L. (2012). A tourist-like MITE insertion in the upstream region of the BnFLC.A10 gene is associated with vernalization requirement in rapeseed (*Brassica napus* L.). *BMC plant biology, 12*, 238.

Huang, C., Sun, H. Y., Xu, D. Y., Chen, Q. Y., Liang, Y. M., Wang, X. F., Xu, G. H., Tian, J. G., Wang, C. L., Li, D., Wu, L. S., Yang, X. H., Jin, W. W., Doebley, J. F., & Tian, F. (2018). ZmCCT9 enhances maize adaptation to higher latitudes. *Proceedings of the National Academy of Sciences of the United States of America, 115*(2), E334-E341.

Jiang, N., Bao, Z. R., Zhang, X. Y., Hirochika, H., Eddy, S. R., McCouch, S. R., & Wessler, S. R. (2003). An active DNA transposon family in rice. *Nature, 421*(6919), 163-167.

Jiang, N., & Wessler, S. R. (2001). Insertion preference of maize and rice miniature inverted repeat transposable elements as revealed by the analysis of nested elements. *Plant Cell, 13*(11), 2553-2564.

Jiang, N., & Wessler, S. R. (2002). INDITTO: a non-autonomous DNA transposon from *Oryza sativa*. *In Repbase Reports*, 2(7), 21.

Kadioglu, A., Terzi, R., Saruhan, N., & Saglam, A. (2012). Current advances in the investigation of leaf rolling caused by biotic and abiotic stress factors. *Plant Science, 182*, 42-48.

Kikuchi, K., Terauchi, K., Wada, M., & Hirano, H. Y. (2003). The plant MITE mPing is mobilized in anther culture. *Nature, 421*(6919), 167-170.

Kitomi, Y., Hanzawa, E., Kuya, N., Inoue, H., Hara, N., Kawai, S., Kanno, N., Endo, M., Sugimoto, K., Yamazaki, T., Sakamoto, S., Sentoku, N., Wu, J., Kanno, H., Mitsuda, N., Toriyama, K., Sato, T., & Uga, Y. (2020). Root angle modifications by the DRO1 homolog improve rice yields in saline paddy

fields. *Proceedings of the National Academy of Sciences, 117*(35), 21242-21250.

Kitomi, Y., Kanno, N., Kawai, S., Mizubayashi, T., Fukuoka, S., & Uga, Y. (2015). QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of DEEPER ROOTING 1. *Rice, 8*. doi:ARTN 1610.1186/s12284-015-0049-2

Li, P. J., Wang, Y. H., Qian, Q., Fu, Z. M., Wang, M., Zeng, D. L., Li, B. H., Wang, X. J., & Li, J. Y. (2007). LAZY1 controls rice shoot gravitropism through regulating polar auxin transport. *Cell Research, 17*(5), 402-410.

Liu, X. L., Li, X., Zhang, C., Dai, C. C., Zhou, J. Y., Ren, C. G., & Zhang, J. F. (2017). Phosphoenolpyruvate carboxylase regulation in C4-PEPC-expressing transgenic rice during early responses to drought stress. *Physiologia Plantarum, 159*(2), 178-200.

Momose, M., Abe, Y., & Ozeki, Y. (2010). Miniature inverted-repeat transposable elements of *Stowaway* are active in potato. *Genetics, 186*(1), 59-66.

Nakazaki, T., Okumoto, Y., Horibata, A., Yamahira, S., Teraishi, M., Nishida, H., Inoue, H., & Tanisaka, T. (2003). Mobilization of a transposon in the rice genome. *Nature, 421*(6919), 170-172.

Oki, N., Yano, K., Okumoto, Y., Tsukiyama, T., Teraishi, M., & Tanisaka, T. (2008). A genome-wide view of miniature inverted-repeat transposable elements (MITEs) in rice, *Oryza sativa* ssp. *japonica*. *Genes & Genetic Systems, 83*(4), 321-329.

Sauer, M., Paciorek, T., Benková, E., & Friml, J. (2006). Immunocytochemical techniques for whole-mount in situ protein localization in plants. *Nature Protocols, 1*(1), 98-103.

823 Sun, X. P., Xu, Q., Ruan, R. X., Zhang, T. Y., Zhu, C. Y., & Li, H. Y. (2013).
824 PdMLE1, a specific and active transposon acts as a promoter and confers
825 *Penicillium digitatum* with DMI resistance. *Environmental Microbiology*
826 *Reports, 5*(1), 135-142.
827 Taniguchi, M., Furutani, M., Nishimura, T., Nakamura, M., Fushita, T., Iijima, K.,
828 Baba, K., Tanaka, H., Toyota, M., Tasaka, M., & Morita, M. T. (2017). The
829 Arabidopsis LAZY1 family plays a key role in gravity signaling within
830 statocytes and in branch angle control of roots and shoots. *Plant Cell, 29*(8),
831 1984-1999.
832 Uga, Y., Kitomi, Y., Ishikawa, S., & Yano, M. (2015a). Genetic improvement for root
833 growth angle to enhance crop production. *Breeding Science, 65*(2), 111-119.
834 Uga, Y., Kitomi, Y., Yamamoto, E., Kanno, N., Kawai, S., Mizubayashi, T., &
835 Fukuoka, S. (2015b). A QTL for root growth angle on rice chromosome 7 is
836 involved in the genetic pathway of DEEPER ROOTING 1. *Rice, 8*. doi:ARTN
837 810.1186/s12284-015-0044-7
838 Uga, Y., Okuno, K., & Yano, M. (2011). *Dro1*, a major QTL involved in deep rooting
839 of rice under upland field conditions. *Journal of Experimental Botany, 62*(8),
840 2485-2494.
841 Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai,
842 Y., Ono, K., Kanno, N., Inoue, H., Takehisa, H., Motoyama, R., Nagamura, Y.,
843 Wu, J. Z., Matsumoto, T., Takai, T., Okuno, K., & Yano, M. (2013). Control
844 of root system architecture by DEEPER ROOTING 1 increases rice yield
845 under drought conditions. *Nature Genetics, 45*(9), 1097-1102.
846 Wang, L. L., Guo, M. X., Li, Y., Ruan, W. Y., Mo, X. R., Wu, Z. C., Sturrock, C. J.,
847 Yu, H., Lu, C. G., Peng, J. R., & Mao, C. Z. (2018). LARGE ROOT ANGLE

1, encoding OsPIN2, is involved in root system architecture in rice. *Journal of Experimental Botany, 69*(3), 385-397.

Xu, M., Zhu, L., Shou, H. X., & Wu, P. (2005). A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. *Plant and Cell Physiology, 46*(10), 1674-1681.

Yan, Y. S., Zhang, Y. M., Yang, K., Sun, Z. X., Fu, Y. P., Chen, X. Y., & Fang, R. X. (2011). Small RNAs from MITE-derived stem-loop precursors regulate abscisic acid signaling and abiotic stress responses in rice. *Plant Journal, 65*(5), 820-828.

Yoshihara, T., & Iino, M. (2007). Identification of the gravitropism-related rice gene LAZY1 and elucidation of LAZY1-dependent and -independent gravity signaling pathways. *Plant and Cell Physiology, 48*(5), 678-688.

Yoshihara, T., & Spalding, E. P. (2017). LAZY genes mediate the effects of gravity on auxin gradients and plant architecture. *Plant physiology, 175*(2), 959-969.

Yoshihara, T., Spalding, E. P., & Iino, M. (2013). AtLAZY1 is a signaling component required for gravitropism of the *Arabidopsis thaliana* inflorescence. *Plant Journal, 74*(2), 267-279.

Yu, B., Lin, Z., Li, H., Li, X., Li, J., Wang, Y., Zhang, X., Zhu, Z., Zhai, W., Wang, X., Xie, D., & Sun, C. (2007). TAC1, a major quantitative trait locus controlling tiller angle in rice. *The Plant Journal, 52*(5), 891-898.

Zhang, Q., Li, J. J., Zhang, W. J., Yan, S. N., Wang, R., Zhao, J. F., Li, Y. J., Qi, Z. G., Sun, Z. X., & Zhu, Z. G. (2012). The putative auxin efflux carrier OsPIN3t is involved in the drought stress response and drought tolerance. *Plant Journal, 72*(5), 805-816.

Zhang, X. Y., Feschotte, C., Zhang, Q., Jiang, N., Eggleston, W. B., & Wessler, S. R.

(2001). *P* instability factor: an active maize transposon system associated with the amplification of *Tourist*-like MITEs and a new superfamily of transposases. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(22), 12572-12577.

Zhang, X. Y., Jiang, N., Feschotte, C., & Wessler, S. R. (2004). PIF- and pong-like transposable elements: distribution, evolution and relationship with tourist-like miniature inverted-repeat transposable elements. *Genetics, 166*(2), 971-986.

Figure

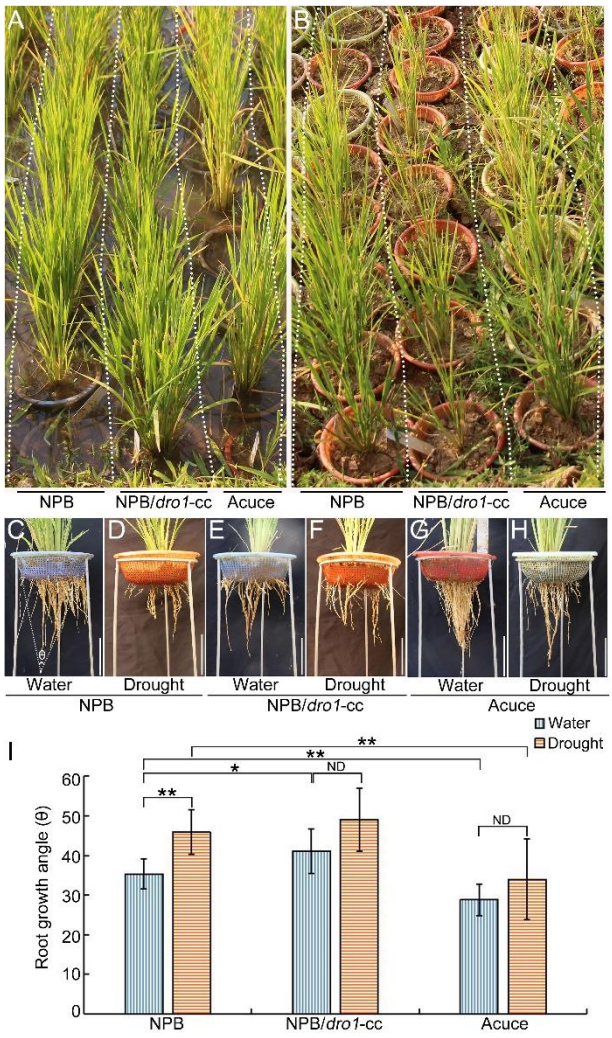
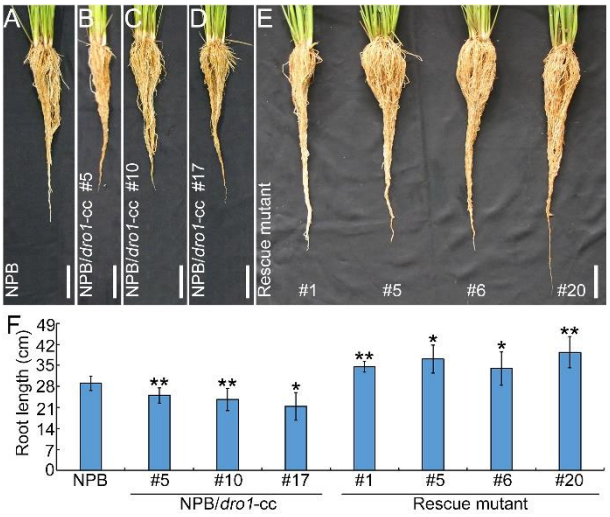


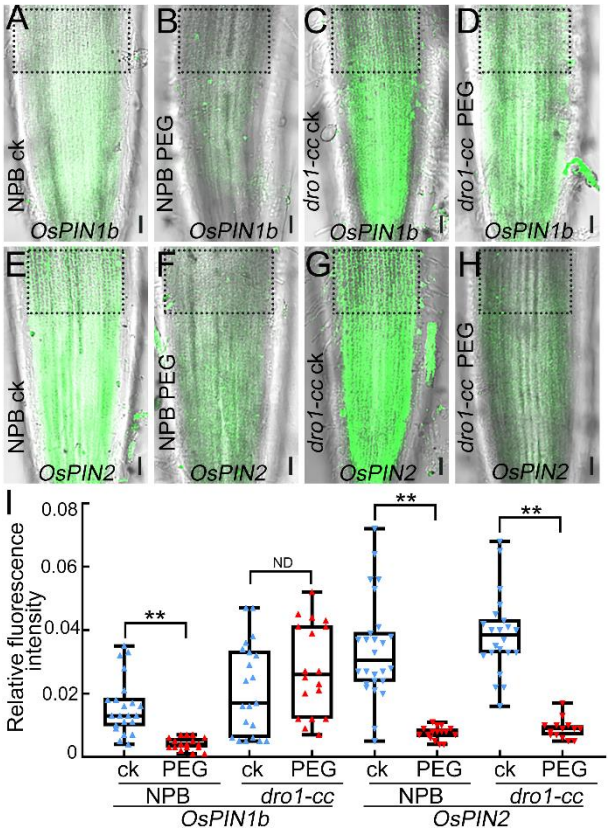
Figure 1. Phenotypes of rice varieties grown in the field. The rice varieties Acuce, Nipponbare, and the Nipponbare *dro1*-cc mutant were grown with irrigation conditions (A) or under drought conditions (B) for 3 months, and the root architecture was observed (C-H). Quantification of the root growth angle (I) (Acuce: n_{water} = 13, $n_{drought}$ = 11; NPB: n_{water} = 9, $n_{drought}$ = 6; NPB/*dro1*-cc: n_{water} = 9, $n_{drought}$ = 5). Data are means ± SD; * $P < 0.05$, ** $P < 0.01$ (Student's *t*-test). NPB = Nipponbare, NPB/*dro1*-cc = Nipponbare *dro1*-cc mutant, ND = no difference, θ = root growth angle. Bar = 10 cm.



893

894 **Figure 2. Root lengths in the Nipponbare *dro1*-cc and rescue mutant.** Rice
895 varieties Nipponbare (A), *dro1*-cc lines #5 (B), #10 (C), and #17 (D) and the T1
896 rescue mutant lines (#1, #5, #6 and #20) (E) were grown in an experimental field with
897 irrigation conditions for 3 months. Quantification of root length (F) (NPB: n=10;
898 NPB/*dro1*-cc #5: n=7, #10: n=8, #17: n=12; rescue lines #1: n=4, #5: n=4, #6: n=8,
899 #20: n=4). Data are means ± SD; * $P < 0.05$, ** $P < 0.01$ (Student's *t*-test). NPB =
900 Nipponbare. Bar = 10 cm.

901



902

903 **Figure 3. The subcellular localization of OsPIN1 and OsPIN2 in the Nipponbare**
904 ***dro1*-cc mutant.** Rice seedlings of wild-type Nipponbare (A, B, E, F) and the *dro1*-cc
905 (#17) mutant (C, D, G, H) grown on MS medium supplemented without (A, C, E, G)
906 or with (B, D, F, H) 15% PEG6000. Immunolocalization of OsPIN1b (A-D) and
907 OsPIN2 (E-H) in root epidermal cells of wild-type Nipponbare and the *dro1*-cc (#17)
908 mutant. Quantification of the fluorescence intensity of OsPIN1b and OsPIN2 at the
909 plasma membrane (I) (NPB/OsPIN1b: $n_{ck} = 22$, $n_{PEG6000} = 17$; *dro1*-cc/OsPIN1b: $n_{ck} =$
910 21, $n_{PEG6000} = 18$; NPB/OsPIN2: $n_{ck} = 26$, $n_{PEG6000} = 16$; *dro1*-cc/OsPIN2: $n_{ck} = 22$,
911 $n_{PEG6000} = 14$). The rectangle shows the region used to measure fluorescence intensity.
912 Data are means ± SD; ** $P < 0.01$ (Student's *t*-test). ck = Seedlings grown on MS
913 medium. PEG = Seedlings grown on MS medium supplemented with 15% PEG6000.
914 NPB = Nipponbare. Bar = 1 cm.

915

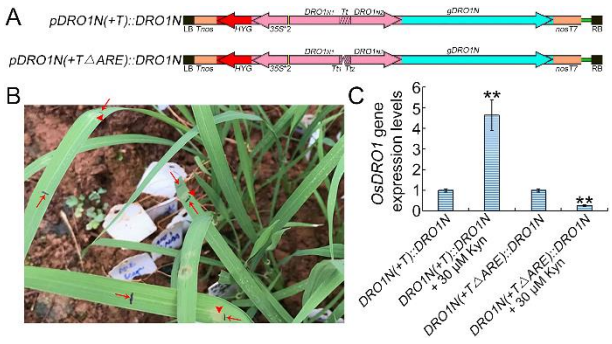
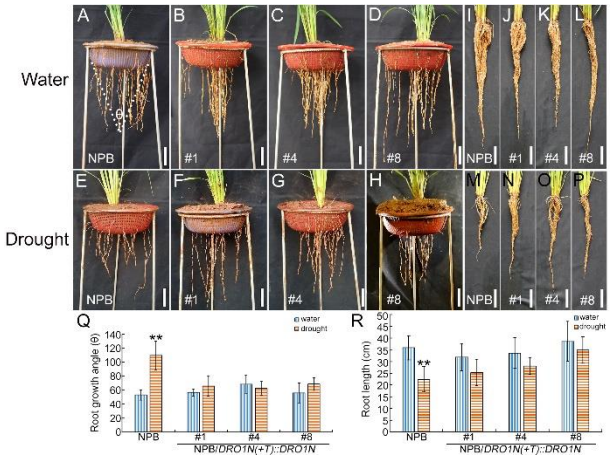


Figure 4. Expression levels of the *DRO1* in Nipponbare leaves. Schematic diagram of the rice expression constructs (A). A solution of *Agrobacterium* strain EHA105 transformed with the expression vector with the addition of 30 μM L-Kynurenine was infiltrated into rice leaf veins (B). The expression levels of the *DRO1* gene in rice leaves were determined by real-time PCR (C). The *OsActin* gene was used as an internal control. Data are means ± SD. ** $P < 0.01$ (SPSS analysis). *pDRO1N (+T)::DRO1N* = the Nipponbare *DRO1* promoter containing the *INDITTO2* transposon and fused with the Nipponbare genomic *DRO1* gene, *pDRO1N (+T△ ARE)::DRO1N* = the Nipponbare *DRO1* promoter containing the *INDITTO2* transposon with a deletion of the auxin response element and fused with the Nipponbare genomic *DRO1* gene. gDRO1N = Nipponbare genomic *DRO1* gene. Kyn = L-Kynurenine. The arrowheads point to the injection position in the leaf veins; the arrows point to the region of the *Agrobacteriun* solution in the leaf veins.



931

932 **Figure 5. Root architectures of transgenic Nipponbare rice lines with *INDITTO2***
933 **transposon insertion in the *DRO1* promoter.** The Nipponbare (A, E, I, M) and T2
934 transgenic Nipponbare rice lines #1 (B, F, J, N), #4 (C, G, K, O) and #8 (D, H, L, P)
935 were grown with irrigation conditions (A-D, I-L) or under drought stress (E-H, M-P)
936 for 3 months. Quantification of the root growth angle (Q) (NPB: $n_{water} = 8$, $n_{drought} = 7$;
937 #1: $n_{water} = 7$, $n_{drought} = 8$; #4: $n_{water} = 7$, $n_{drought} = 9$; #8: $n_{water} = 6$, $n_{drought} = 4$) and root
938 length (R) (NPB: $n_{water} = 10$, $n_{drought} = 8$; #1: $n_{water} = 7$, $n_{drought} = 7$; #4: $n_{water} = 6$, $n_{drought}$
939 $= 5$; #8: $n_{water} = 7$, $n_{drought} = 7$). Data are means ± SD; ** $P < 0.01$ (Student's *t*-test).
940 NPB = Nipponbare, θ = root growth angle. Bar = 5 cm.

941

Supporting Information

INDITTO2 transposon conveys auxin-mediated *DRO1* transcription for rice drought avoidance

Yiting Zhao[1,2,3,8*], Lixia Wu[1,2,3*], Qijing Fu[1,2,3*], Dong Wang[6], Jing Li[7], Baolin Yao[7], Si Yu[1], Li Jiang[1], Jie Qian[1], Xuan Zhou[1,2,3], Li Han[1,2,3], Shuanglu Zhao[1], Canrong Ma[5], Yanfang Zhang[1], Chongyu Luo[1,2,3], Qian Dong[1], Saijie Li[1], Lina Zhang[1], Xi Jiang[1], Youchun Li[1], Hao Luo[1], Kuixiu Li[1,2,3], Jing Yang[1,2,3], Qiong Luo[1,2,3], Lichi Li[9], Sheng Peng[1,2,3], Huichuan Huang[1,2,3], Zhili Zuo[6], Changning Liu[7], Lei Wang[5], Chengyun Li[1,2,3], Xiahong He[1,2,3], Jiří Friml[4], Yunlong Du[1,2,3#]

Supplemental Experimental Procedures

Gravity stimulation of rice roots

Rice roots were subjected to gravity stimulation as previously described (Du et al., 2013). Five-day-old rice seedlings were transferred to Murashige-Skoog (MS) medium containing with 0.01 μM NAA or the equivalent amount of DMSO as control. The plates were then turned 90° compared with the original vertical position. Seedlings were gravity stimulated for 18 hours under light conditions.

Plasmid construction

Genomic DNA of the rice varieties Acuce and Nipponbare was isolated from rice leaves. To clone the *DRO1* promoter of Acuce (*DRO1A*) and Nipponbare (*DRO1N*), the gene-specific primers PrDRO1-FP and PrDRO1-RP containing the *Eco*31I site (Supplemental Table S2) were synthesized based on the known *DRO1* sequence

967 (GenBank no. AB689742.1). The amplification product was cloned into the vector
968 *pBWA(V)HG-ccdB* digested with *Eco*31I, and was sequenced at BGI. Constructs
969 containing the *DRO1* promoter of Acuce and Nipponbare fused with the *gusA* gene
970 were named *pDRO1A* and *pDRO1N*, respectively.

971 To construct the expression vectors *pDRO1A*(*△T*) and *pDRO1A*(*T△ARE*) carrying
972 the *INDITTO2* transposon deletion or the *INDITTO2* transposon without the ARE,
973 respectively, the PCR fragment was amplified with the *pDRO1A* template, fused with
974 the *gusA* gene, and inserted into the vector *pBWA*(*V*)*HG-ccdB*.

975 To construct the expression vector *pDRO1N*(*+T*), the PCR fragment of the
976 *INDITTO2* transposon was amplified and inserted into the *DRO1* promoter of
977 Nipponbare, fused with the *gusA* gene, and inserted into the vector
978 *pBWA*(*V*)*HG-ccdB*.

979 To construct the expression vectors *pT* and *pT*(*△ARE*) carrying the *INDITTO2*
980 transposon with or without AREs and the transcribed *gusA*, respectively, the PCR
981 fragment containing the *INDITTO2* transposon was amplified with the *pDRO1A*
982 template and inserted into the vector *pBWA*(*V*)*HG-ccdB*.

983 To construct the expression vectors *pDRO1A*(*△RE1/2/3*) and *pDRO1N*(*△RE1/2/3*),
984 in which the three AREs RE1 (TGTCTC), RE2 (TGTC) and RE3 (GACA) (Uga et al.,
985 2013) were deleted, respectively, the PCR fragments of the respective Acuce and
986 Nipponbare *DRO1* promoters were amplified and fused with the *gusA* gene, and
987 inserted into the vector *pBWA*(*V*)*HG-ccdB*.

988 To construct the expression vectors *pDRO1N* (*+T*)*::DRO1N* and *pDRO1N(+T△*
989 *ARE)::DRO1N*, the PCR fragments of the *INDITTO2* transposon with or without ARE
990 were amplified with the *pDRO1A* template and inserted into the *DRO1* promoter of
991 Nipponbare, respectively. The insertion position of the *INDITTO2* transposon was the

same as that in the Acuce *DRO1* promoter. The Nipponbare *DRO1* gene was subsequently amplified, fused with the Nipponbare *DRO1* promoter, and inserted into the vector *pBWA*(*V*)*HG-ccdB*.

All the expression vectors were constructed at BIORUN Company (Wuhan BIORUN Bio-Tech Co., LTD).

Construction of the CRISPR/Cas9 plasmids

Codon-optimized hSpCas9 was linked to the maize ubiquitin promoter (UBI) in an intermediate plasmid (Cong et al., 2013), and this expression cassette was then inserted into the binary vector *pCAMBIA1300* (Cambia, Australia), containing the HPT (hygromycin B phosphotransferase) gene. The original *Bsa*I site in the *pCAMBIA1300* backbone was removed using a point mutation kit (Transgen, China). A fragment comprising an *OsU6* promoter (Feng et al., 2013), a negative selection marker gene *ccdB* flanked by two *Bsa*I sites and an sgRNA derived from *pX260* was inserted into this vector using the In-fusion Cloning kit (Takara, Japan) to produce the CRISPR/Cas9 binary vector *pBGK032* (Cong et al., 2013). *Escherichia coli* strain DB3.1 was used to maintain this binary vector.

The 23-bp targeting sequences (including the protospacer adjacent motif (PAM)) were selected within the target genes, and their targeting specificity was confirmed using a BLAST search against the rice genome (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Hsu et al., 2013). The designed targeting sequences were synthesized and annealed to form oligo adaptors. The vector *pBGK032* was digested with *Bsa*I and purified using a DNA purification kit (Tiangen, China). A ligation reaction (10 μL) containing 10 ng of the digested *pBGK032* vector and 0.05 mM oligo adaptor was performed and directly transformed into *E. coli* competent cells to produce CRISPR/Cas9 plasmids.

1017 The CRISPR/Cas9 vector was constructed at Biogle Company (Hangzhou Biogle Co.,
1018 LTD.)
1019
1020 **Construction of the *DRO1* knockout mutant and transgenic rice lines**
1021 The rice *dro1*-cc mutant lines were produced using the CRISPR/Cas9 method. The
1022 specific guide-RNA (gRNA) sequence was designed as follows:
1023 5'-GAAGGCGCAGAAGAATTTGC-3'. The target gene sequence that added the
1024 PAM of *DRO1* was 5'-TAAGGCGCAGAAGAATTTGCGGG-3', and the GGG
1025 trinucleotide acted as the PAM. The CRISPR/Cas9 plasmids were introduced into
1026 *Agrobacterium tumefaciens* strain EHA105. Genomic DNA was extracted from rice
1027 transformants using the CTAB method, and the primer pair OsDRO1-ccFP and
1028 OsDRO1-ccRP (Supplemental Table S2) flanking the designed target site was used for
1029 PCR amplification. The PCR products were sequenced directly and identified using
1030 the degenerate sequence decoding method (Ma et al., 2015). To obtain transgenic
1031 Nipponbare lines containing the *INDITTO2* transposon in the Nipponbare *DRO1*
1032 promoter, the construct *pDRO1N*(*+T*)*::DRO1N* was transformed into *Agrobacterium*
1033 *tumefaciens* strain EHA105. Rice transformation was performed as previously
1034 described by the *Agrobacterium*-mediated method at Biogle Company (Hangzhou
1035 Biogle Co., LTD) (Nishimura et al., 2006).
1036
1037 **Measurement of auxin content**
1038 Roots of the *dro1*-cc mutant were ground to a powder, and the phytohormone IAA
1039 was measured by HPLC-MS/MS as previously described (Luo et al., 2016). For IAA
1040 extraction, the root powder was dissolved with ethyl acetate spiked with 5 ng of
1041 D5-IAA (OlhemIm) as an internal standard. Multiple reaction monitoring was

performed to monitor the analytic parent ion → product ion process: mass-to-charge ratio [m/z] 176.00 → 130.00 (CE, -15 V; Q1 pre bias, -19 V; Q3 pre bias, -22 V) for IAA; m/z 181.00 → 134.05 (CE, -18 V; Q1 pre bias, -23 V; Q3 pre bias, -23 V) for D5-IAA.

Transient gene expression in tobacco and rice leaves

Agrobacterium strain EHA105 containing the expression constructs was cultured in liquid LB medium supplemented with antibiotics (50 mg/L rifampicin and 50 mg/L kanamycin) at 28°C. The precipitate of the bacterial liquid culture was washed three times with sterilized water followed by injection buffer (containing 2.132 g/L MES, 10 mM $CaCl_2$ and 0.2 mM acetosyringone) and diluted with injection buffer to an OD_{600} of 0.7. Leaves of *Nicotiana benthamiana* and Nipponbare leaf veins were infiltrated with *Agrobacterium* solution with or without 1 μM NAA or 30 μM of the auxin biosynthesis inhibitor L-Kynurenine, respectively. Infiltrated leaves of tobacco and rice were grown in chambers or greenhouse for 4 and 5 days, respectively, and then harvested to detect the expression levels of the *GUS*, *DRO1*, *NaActin* (GenBank no. JQ256516.1) and *OsActin* genes (GenBank no. AK060893.1) amplified with the gene-specific primer pairs GUS-rFP and GUS-rRP, DRO1-rFP and DRO1-rRP, NaActin-rFP and NaActin-rRP, and OsActin-FP and OsActin-RP (Supplemental Table S2), respectively, using quantitative real-time PCR performed under denaturation at 95°C for 2 minutes, followed by 40 cycles of 95°C for 45 seconds, 52°C for 30 seconds and 72°C for 1 minute. Three biological replicates were tested. The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method. The *NaActin* and *OsActin* genes served as internal controls.

Identification of IGT family genes in the rice genome

BLASTP searches were performed to identify the IGT family genes in Nipponbare. The homologues genes of *DRO1*, *TAC1*, and *LAZY1* from *Arabidopsis* were used as query sequences against the rice protein sequences.

Conserved motif analysis of IGT family proteins

The online Multiple Expectation Maximization for the Motif Elucidation (MEME) toolkit was used to identify all conserved motifs (http://meme-suite.org/) (Bailey et al., 2009). All IGT family protein sequences were used for the queries. The parameters were set as follows: minimum width = 6, maximum width = 150, motif number = 15 and minimum number of sites = 2.

Expression profile analysis of IGT family genes

All IGT gene expression data in eleven tissues (ovary, anther, embryo, pistil, inflorescence, root, lemma and palea, leaf blade, endosperm, stem, and leaf sheath) of rice were retrieved from the RiceXPro database (https://ricexpro.dna.affrc.go.jp/) (Sato et al., 2013). All normalized data were log2 transformed. The expression profile was displayed in a heat map using the R package pheatmap (https://cran. r-project. org/ web/packages/pheatmap/index. html).

Identification of *INDITT04* copies in the Nipponbare genome

INDITT04 copies were identified by local BLASTN. The reference sequence was the *Oryza sativa* Japonica RGAP7 genome and was used to construct the local BLASTN database. The specific parameters were set as follows: word size = 4, e-value = 1e-10, best hit score edge = 0.05 and best hit overhang = 0.25. The sequences of all

INDITT04 copies shared more than 95% identity with the original *INDITT04*. The positions of all *INDITT04* copies were compared with the positions of all genes in the *Oryza sativa* Japonica RGAP7 genome to obtain the inserted loci of all *INDITT04* copies.

Phylogenetic tree construction

The sequences of *INDITTO* and its homologues in different rice varieties were used to construct a phylogenetic tree using the neighbour-joining method in MEGA 5.1. Each bootstrap value was analyzed with 1000 replicates.

Analysis of the transposon and root phenotype

The online software (http://bioinf.cs.ucl.ac.uk/introduction/, www.repeatmasker.org and www.girinst.org) was used to analyse the tertiary structures of DRO1 and the transposons. The online software (http://plantregmap.cbi.pku.edu.cn) was used to analyze the transcription factor binding sites of the transposons. Vector NTI Suite 6, DNAMAN and Discovery Studio 4.0 were used to analyze the sequence alignment, protein sequence and conformation of the DRO1 protein, respectively. ImageJ 1.41 was used to measure the root growth angle, root length and fluorescence intensities. To measure the root growth angle with ImageJ 1.41, the longest root tip acted as vertex and joined both sides of the most upward apex root end that emerged from the basket edge when the rice seedlings were grown in a basket. Pearson's correlation coefficient was calculated using SPSS software and used to analyze the correlation between the root length and angle. All images were processed with Photoshop.

SI References

1117 Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., Ren, J.
1118 Y., Li, W. W., & Noble, W. S. (2009). MEME SUITE: tools for motif
1119 discovery and searching. *Nucleic Acids Research, 37*(Web Server issue),
1120 W202-W208.
1121 Cong, L., Ran, F. A., Cox, D., Lin, S. L., Barretto, R., Habib, N., Hsu, P. D., Wu, X.
1122 B., Jiang, W. Y., Marraffini, L. A., & Zhang, F. (2013). Multiplex genome
1123 engineering using CRISPR/Cas systems. *Science, 339*(6121), 819-823.
1124 Du, Y. L., Tejos, R., Beck, M., Himschoot, E., Li, H. J., Robatzek, S., Vanneste, S., &
1125 Friml, J. (2013). Salicylic acid interferes with clathrin-mediated endocytic
1126 protein trafficking. *Proceedings of the National Academy of Sciences of the*
1127 *United States of America, 110*(19), 7946-7951.
1128 Feng, Z. Y., Zhang, B. T., Ding, W. N., Liu, X. D., Yang, D. L., Wei, P. L., Cao, F.
1129 Q., Zhu, S. H., Zhang, F., Mao, Y. F., & Zhu, J. K. (2013). Efficient genome
1130 editing in plants using a CRISPR/Cas system. *Cell Research, 23*(10),
1131 1229-1232.
1132 Hsu, P. D., Scott, D. A., Weinstein, J. A., Ran, F. A., Konermann, S., Agarwala, V.,
1133 Li, Y. Q., Fine, E. J., Wu, X. B., Shalem, O., Cradick, T. J., Marraffini, L. A.,
1134 Bao, G., & Zhang, F. (2013). DNA targeting specificity of RNA-guided Cas9
1135 nucleases. *Nature Biotechnology, 31*(9), 827-832.
1136 Luo, J., Wei, K., Wang, S. H., Zhao, W. Y., Ma, C. R., Hettenhausen, C., Wu, J. S.,
1137 Cao, G. Y., Sun, G. L., Baldwin, I. T., Wu, J. Q., & Wang, L. (2016).
1138 COI1-regulated hydroxylation of jasmonoyl-L-isoleucine impairs *Nicotiana*
1139 *attenuata's* resistance to the generalist herbivore *Spodoptera litura*. *Journal of*
1140 *Agricultural and Food Chemistry, 64*(14), 2822-2831.
1141 Ma, X. L., Chen, L. T., Zhu, Q. L., Chen, Y. L., & Liu, Y. G. (2015). Rapid decoding

of sequence-specific nuclease-induced heterozygous and biallelic mutations by direct sequencing of PCR products. *Molecular Plant, 8*(8), 1285-1287.

Nishimura, A., Aichi, I., & Matsuoka, M. (2006). A protocol for *Agrobacterium*-mediated transformation in rice. *Nature Protocols, 1*(6), 2796-2802.

Sato, Y., Takehisa, H., Kamatsuki, K., Minami, H., Namiki, N., Ikawa, H., Ohyanagi, H., Sugimoto, K., Antonio, B. A., & Nagamura, Y. (2013). RiceXPro version 3.0: expanding the informatics resource for rice transcriptome. *Nucleic Acids Research, 41*(D1), D1206-D1213.

Supplemental Figures and Tables

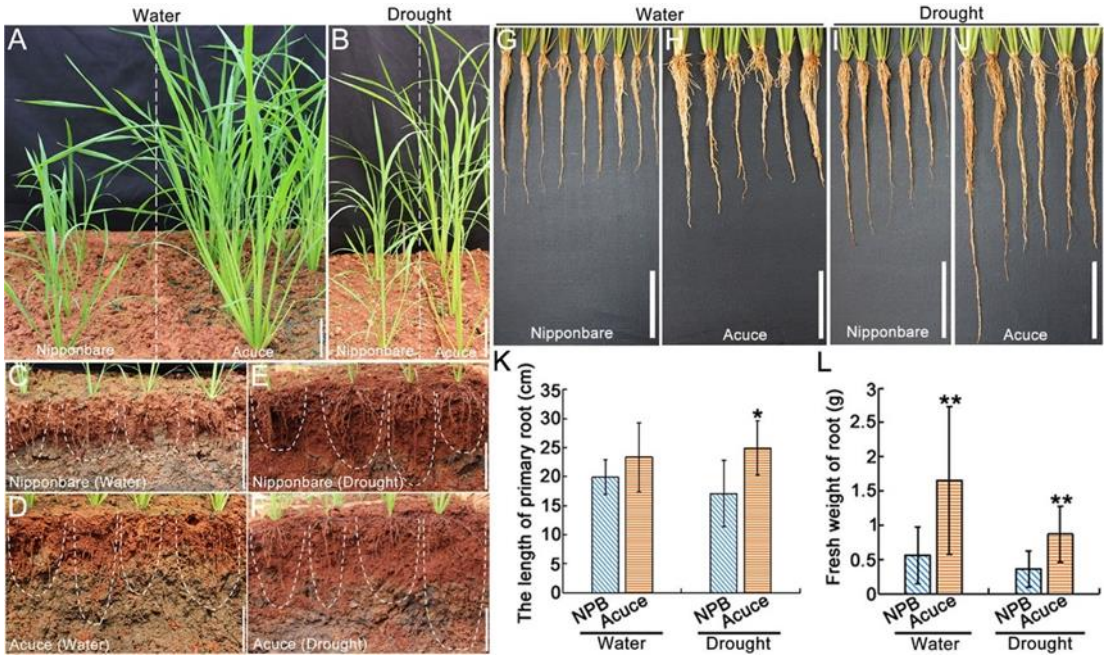


Figure S1. Root phenotypes of Acuce and Nipponbare grown in the field.

Root architectures of Acuce (A, B, D, F) and Nipponbare (A, B, C, E) grown in the field with irrigation (A, C, D) or under drought stress (B, E, F). Root phenotypes of Acuce (H, J) and Nipponbare (G, I) grown with irrigation (G, H) or under drought stress (I, J). Quantification of primary root length (K) (NPB: $n_{water} = 10$, $n_{drought} = 6$; Acuce: $n_{water} = 12$, $n_{drought} = 12$) and root fresh weight (L) (NPB: $n_{water} = 11$, $n_{drought} = 7$; Acuce: $n_{water} = 12$, $n_{drought} = 12$). Data are means ± SD; * $P < 0.05$, ** $P < 0.01$ (Student's *t*-test). Bar = 10 cm.

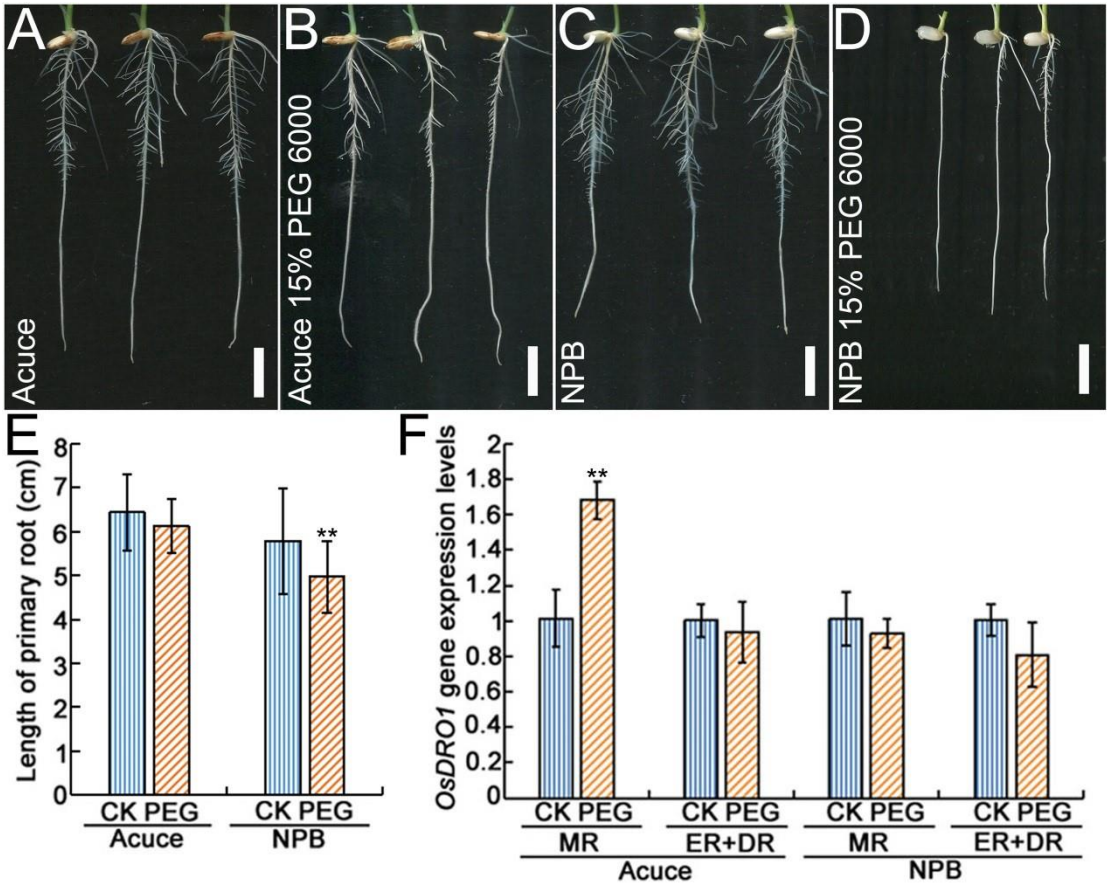


Figure S2. Root phenotypes of Acuce and Nipponbare treated with PEG6000.

Seedlings of Acuce (A and B) and Nipponbare (C and D) grown on MS medium supplemented either with or without 15% PEG6000 for 4 days. The primary root length was measured and quantified (E) (Acuce: $n_{CK} = 42$, $n_{PEG6000} = 30$, NPB: $n_{CK} = 29$, $n_{PEG6000} = 30$). The expression levels of *DRO1* (F) in the root meristem region, elongation region and differentiation region were determined using real-time PCR. The *OsActin* gene was used as an internal control. Data are means ± SD. ** $P < 0.01$ (Student's *t*-test for root length analysis, SPSS analysis for gene expression). CK = rice seedlings grown on MS medium, PEG = rice seedlings grown on MS medium supplemented with 15% PEG6000, NPB = Nipponbare, MR = meristem region, ER = elongation region, DR = differentiation region. Bar = 1 cm.

Figure S3. Amino acid sequences of DRO1 in different rice varieties.

The DRO1 amino acid sequences of the *indica* rice IR64 and Acuce and the *japonica* rice Kinandang Patong and Nipponbare were aligned. KP = Kinandang Patong.

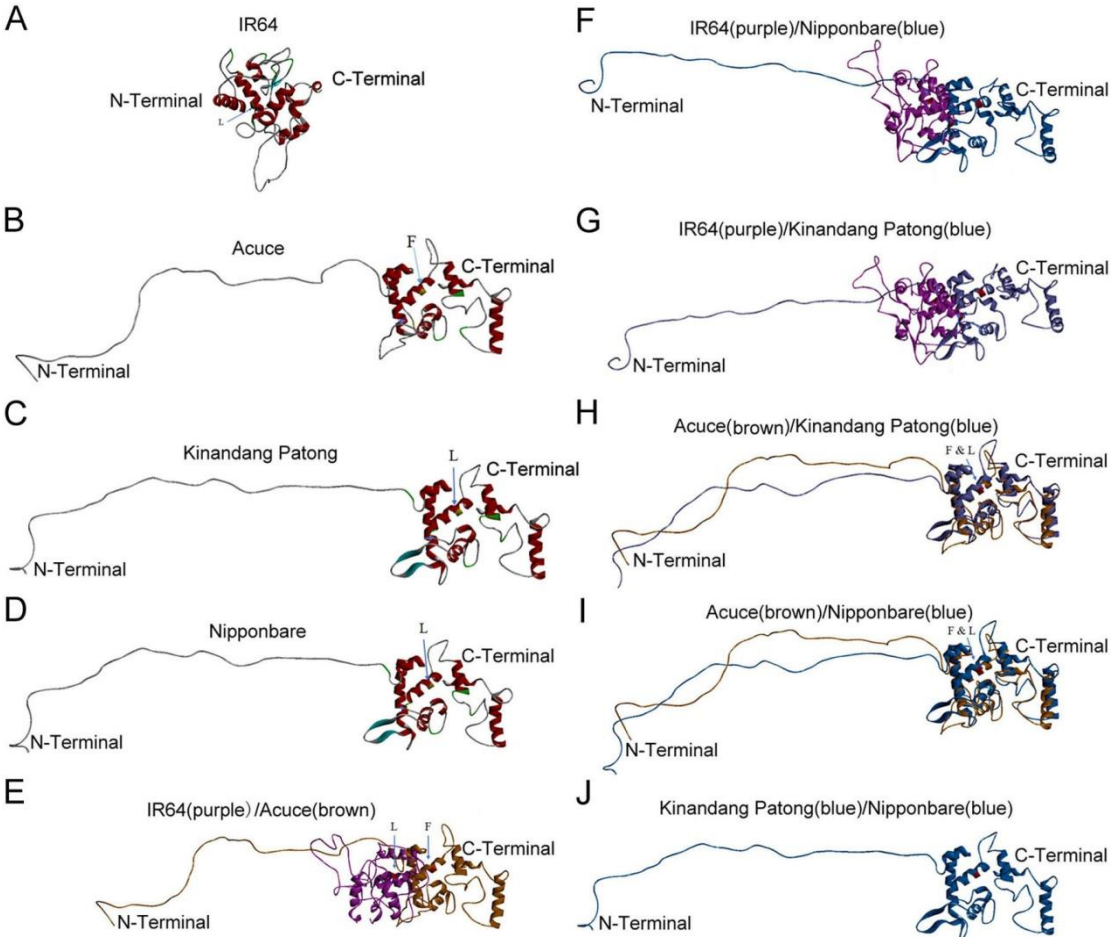


Figure S4. Deduced tertiary structures of the DRO1 protein.

The deduced tertiary structures of DRO1 amino acids from IR64 (A), Acuce (B), Kinandang Patong (C) and Nipponbare (D) were analyzed with the online software (http://bioinf.cs.ucl.ac.uk/introduction/). Merged images show the structural differences between Acuce and IR64 (E), IR64 and Nipponbare (F), IR64 and Kinandang Patong (G), Acuce and Kinandang Patong (H), Acuce and Nipponbare (I) and Kinandang Patong and Nipponbare (J). The amino acid of DRO1 at position 163 is labelled with a blue arrow. The capital F and L indicate Phe and Leu, respectively.

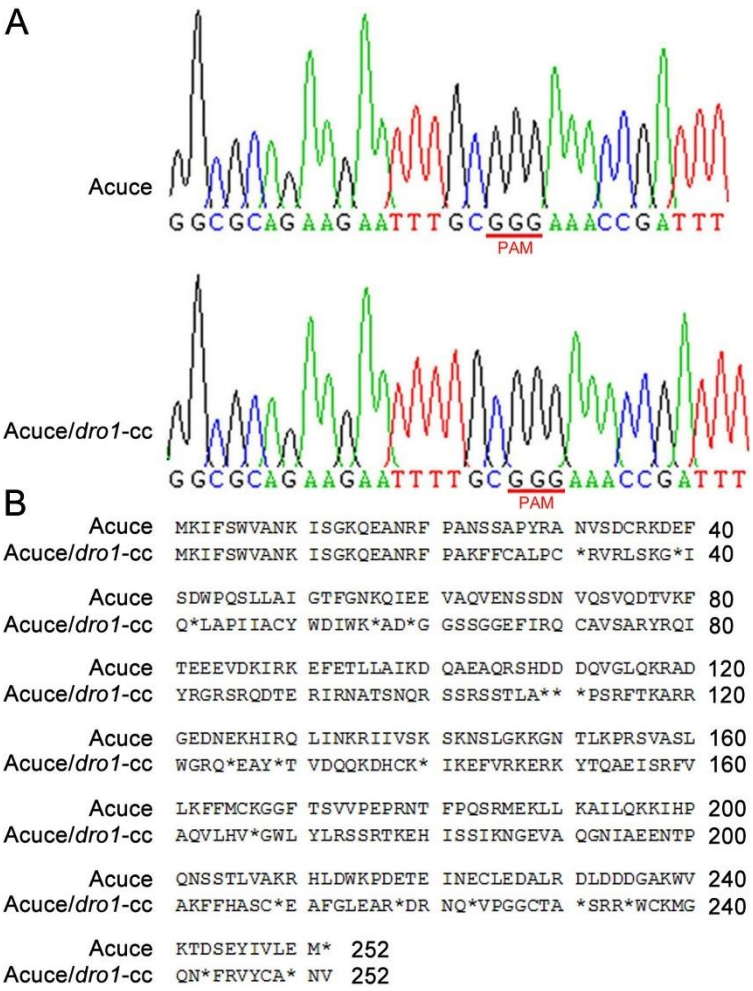


Figure S5. Sequence profiles of the *DRO1* gene in Acuce and *DRO1* knockout mutant.

The genomic *DRO1* gene of Acuce was knocked out using the CRISPR/Cas9 method. Sequence map of the PCR product of the genomic *DRO1* gene (A), and the amino acid sequences encoded by the *DRO1* gene (B) in wild-type Acuce and the *dro1*-cc mutant. PAM = protospacer adjacent motif. Asterisks indicate translation termination.

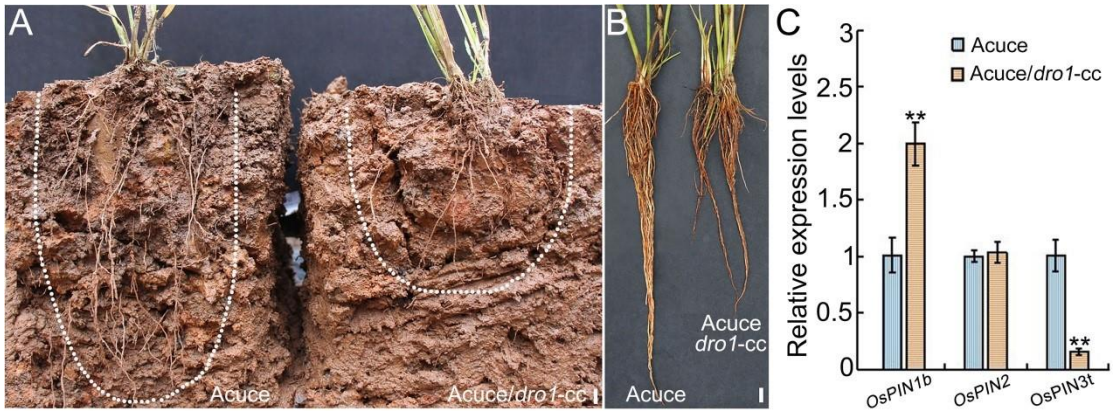


Figure S6. Root phenotypes and gene expression levels in the Acuce *dro1*-cc mutant.

Root phenotypes of wild-type Acuce (left) and the *dro1*-cc mutant (right) (A, B). Bar = 5 cm. Expression levels of *OsPIN1b*, *OsPIN2* and *OsPIN3t* in the primary roots (C). Data are means ± SD. ** $P < 0.01$ (SPSS analysis).

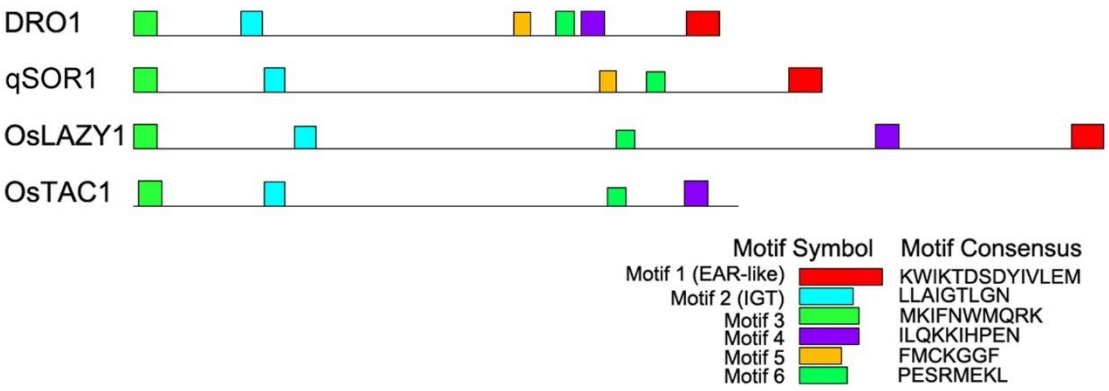


Figure S7. Motif profiles in the rice IGT family.

The motifs in IGT family proteins DRO1, qSOR1, OsLAZY1 and OsTAC1 are shown in different colors. The color codes indicate different motifs.

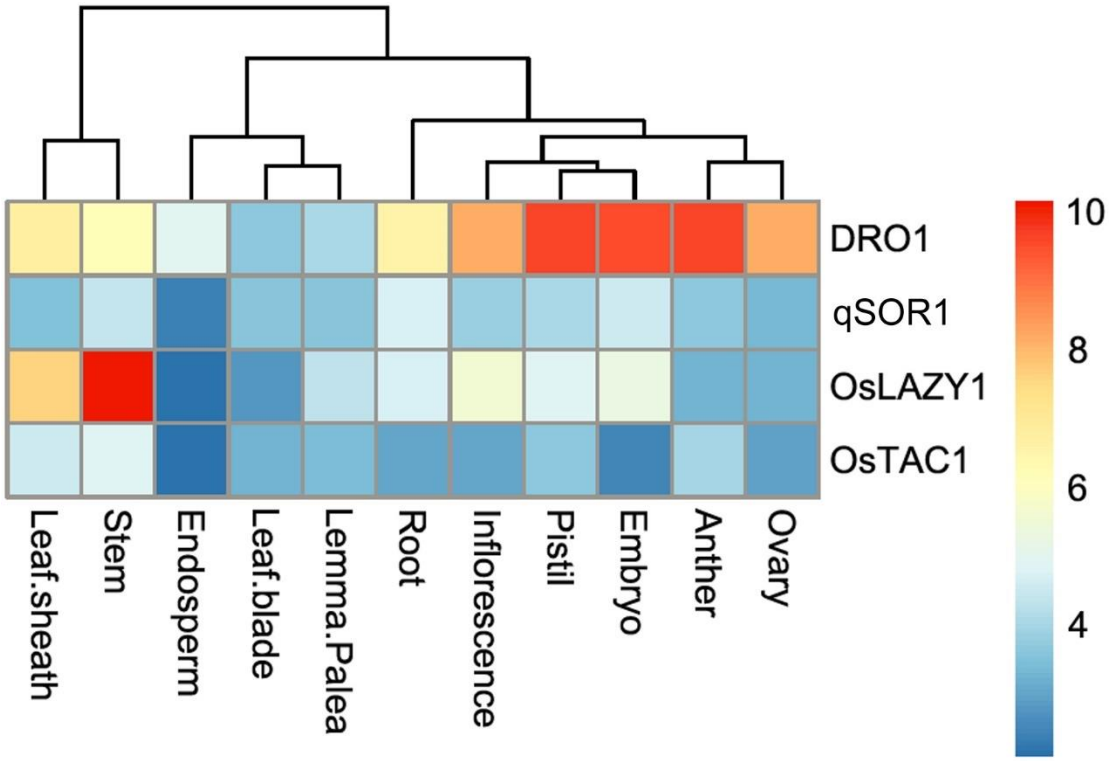


Figure S8. Heat maps showing the expression profiles of IGT family genes in Nipponbare.

The transcriptional levels of the IGT family genes *DRO1*, *qSOR1*, *OsLAZY1* and *OsTAC1* in different rice tissues are indicated by different colors. The relative expression intensity is color coded: blue = low; yellow = medium; and red = high.

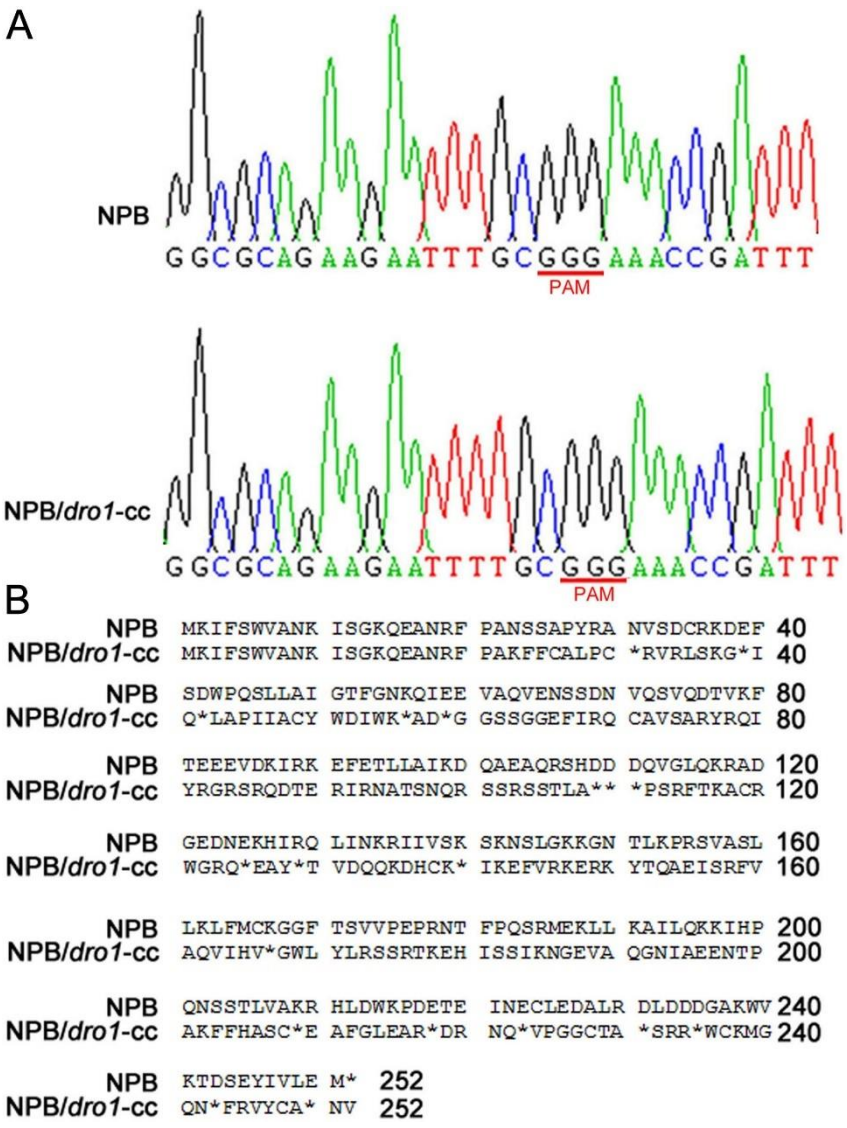


Figure S9. Sequence profiles of the *DRO1* gene in Nipponbare and *DRO1* knockout mutant.

The genomic *DRO1* gene of Nipponbare was knocked out using the CRISPR/Cas9 method. Sequence map of the PCR product of the genomic *DRO1* gene (A), amino acid sequences encoded by the *DRO1* gene (B) in wild-type Nipponbare and the *dro1*-cc mutant. PAM = protospacer adjacent motif. Asterisks indicate translation termination.

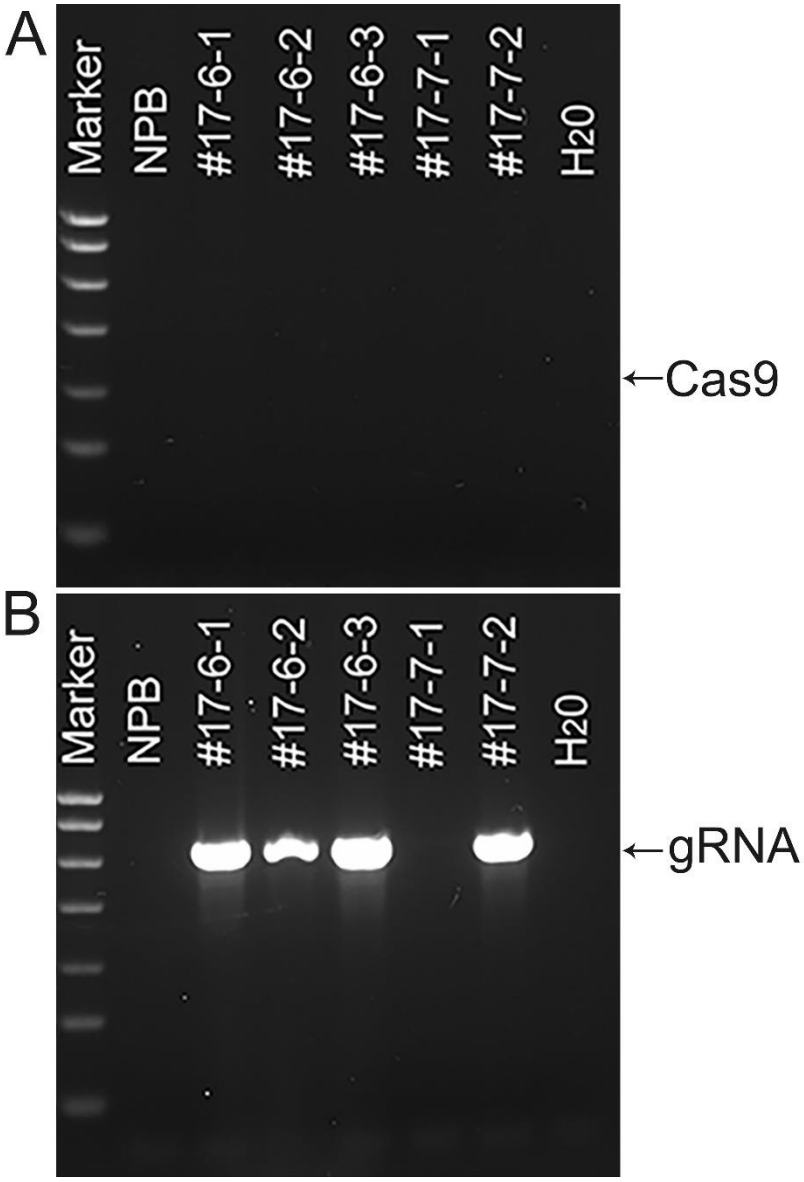


Figure S10. PCR detection of the CRISPR cassette in Nipponbare *dro1*-cc line 17. The *Cas9* (A) and *gRNA* (B) genes in the wild-type Nipponbare and *dro1*-cc mutant line 17 (#17-6-1, #17-6-2, #17-6-3, #17-7-1, #17-7-2) genomes were amplified by PCR using genomic DNA as the template. The arrow shows the PCR product of the *Cas9* and *gRNA* genes with sizes of 371 bp and 550 bp, respectively. The negative controls were used genomic DNA of wild-type Nipponbare and sterilized water as a template in PCR. NPB = Nipponbare, gRNA = guide-RNA.

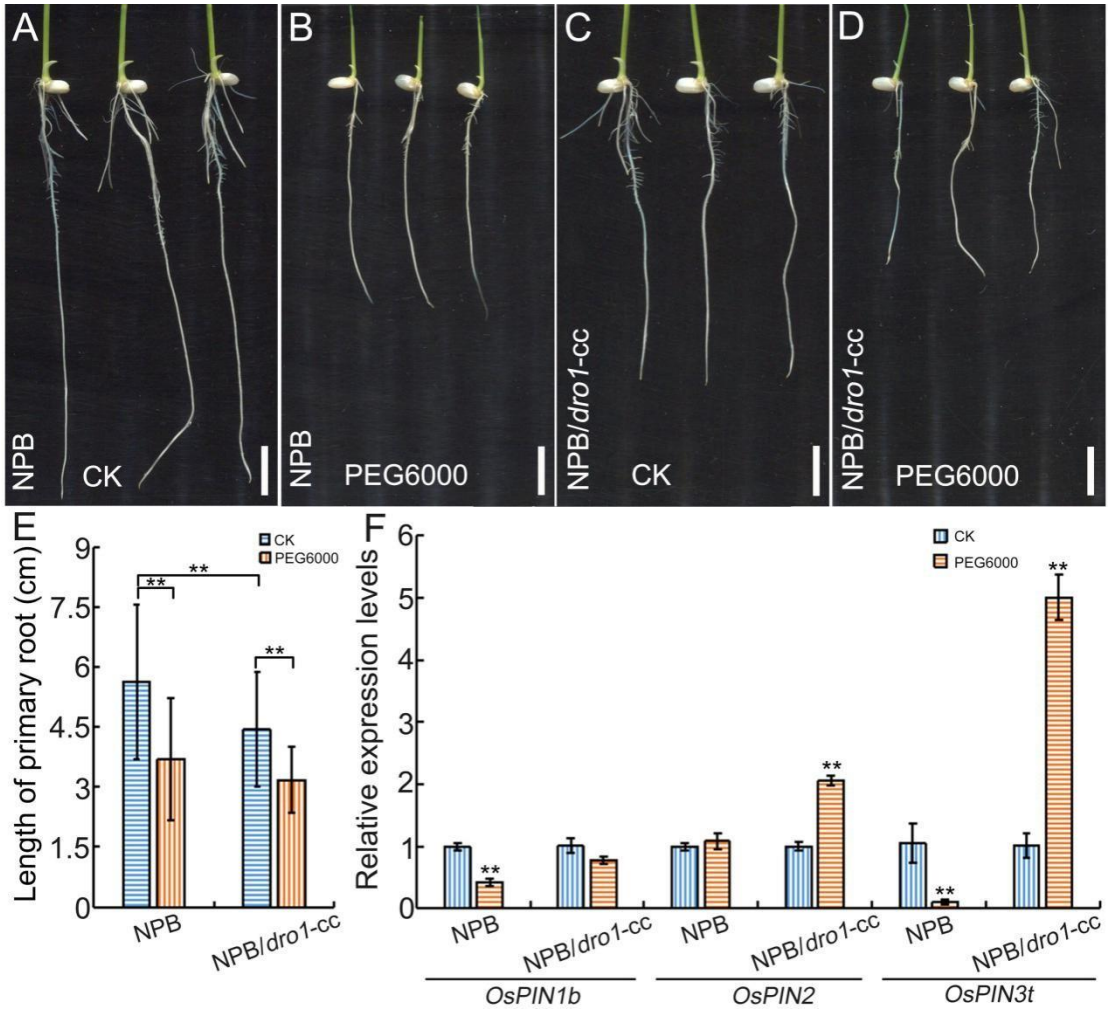


Figure S11. Root phenotypes of the Nipponbare *dro1*-cc mutant.

Seedlings of Nipponbare (A, B) and the *dro1*-cc mutant (#17) (C, D) grown on MS medium (A, C) or supplemented with 15% PEG6000 (B, D). Quantification of the primary root lengths in Nipponbare and the *dro1*-cc mutant (E) (Nipponbare: n_{CK}=31, $n_{PEG6000}$= 35; NPB/*dro1*-cc: n_{CK} = 41, $n_{PEG6000}$ = 25). The expression of *OsPIN1b*, *OsPIN2* and *OsPIN3t* (F) in the primary root meristem region. CK = rice seedlings grown on MS medium, PEG = rice seedlings grown on MS medium supplemented with 15% PEG6000, NPB = Nipponbare. Bar = 1 cm. Data are means ± SD; ** $P <$ 0.01 (Student's *t*-test for root length analysis, SPSS analysis for gene expression).

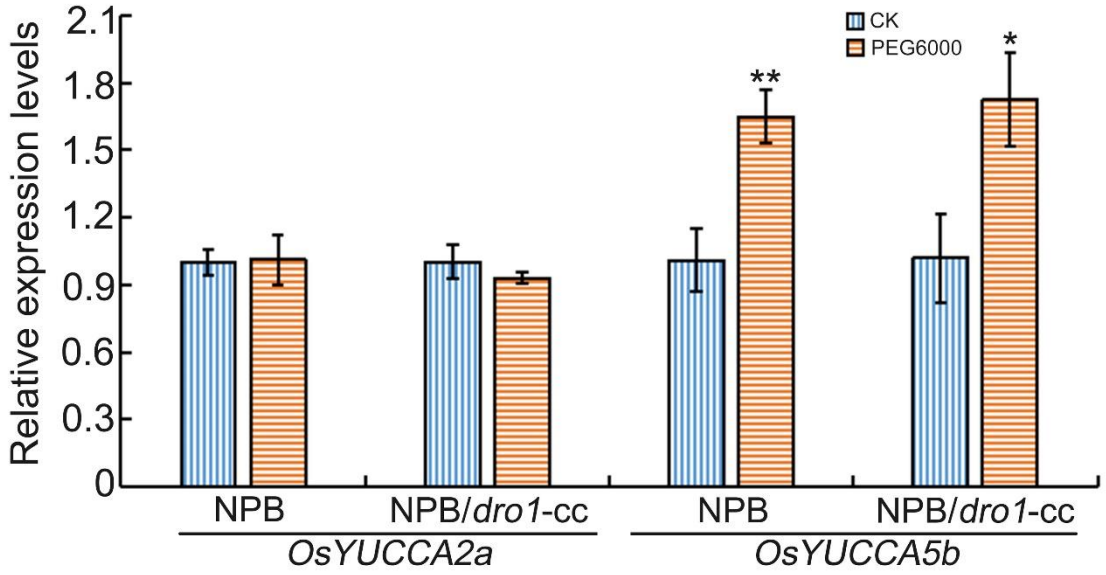


Figure S12. The expression levels of *OsYUCCA2a* and *OsYUCCA5b* in the Nipponbare *dro1-cc* mutant.

Seedlings of Nipponbare and the *dro1-cc* mutant grown on MS medium supplemented with 15% PEG6000 for 4 days. The expression levels of the *OsYUCCA2a* and *OsYUCCA5b* genes in the rice root meristem region were determined using real-time PCR. The *OsActin* gene was used as an internal control. CK = seedlings grown on MS medium, PEG = seedlings grown on MS medium supplemented with 15% PEG6000, NPB = Nipponbare. Data are means ± SD. * $P < 0.05$, ** $P < 0.01$ (SPSS analysis).

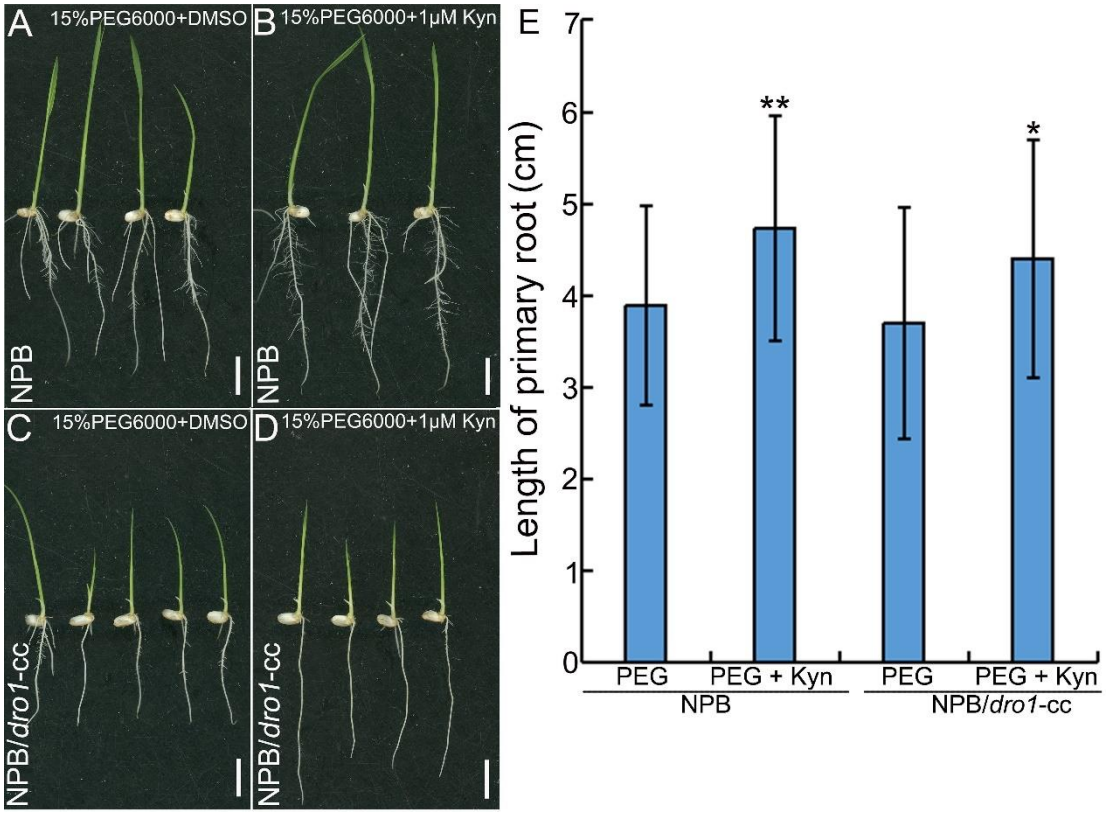


Figure S13. Root lengths of the Nipponbare *dro1*-cc mutant grown under drought stress co-treated with L-Kynurenine.

Seedlings of Nipponbare (A, B) and the *dro1*-cc mutant (C, D) grown on MS medium (A, B) supplemented with 15% PEG6000 and co-treated with 1 μM Kyn (B, D) or DMSO (A, C). Seedlings grown on the MS medium containing DMSO was acted as a control. Quantification of primary root lengths in Nipponbare and the *dro1*-cc mutant (E) (NPB: $n_{PEG6000} = 29$, $n_{PEG6000+Kyn} = 34$; NPB/*dro1*-cc: $n_{PEG6000} = 24$, $n_{PEG6000+Kyn} = 30$). Kyn = L-Kynurenine, NPB = Nipponbare, PEG = seedlings grown on MS medium supplemented with 15% PEG6000. Data are means ± SD; * $P < 0.05$, ** $P < 0.01$ (Student's *t*-test). Bar = 1 cm.

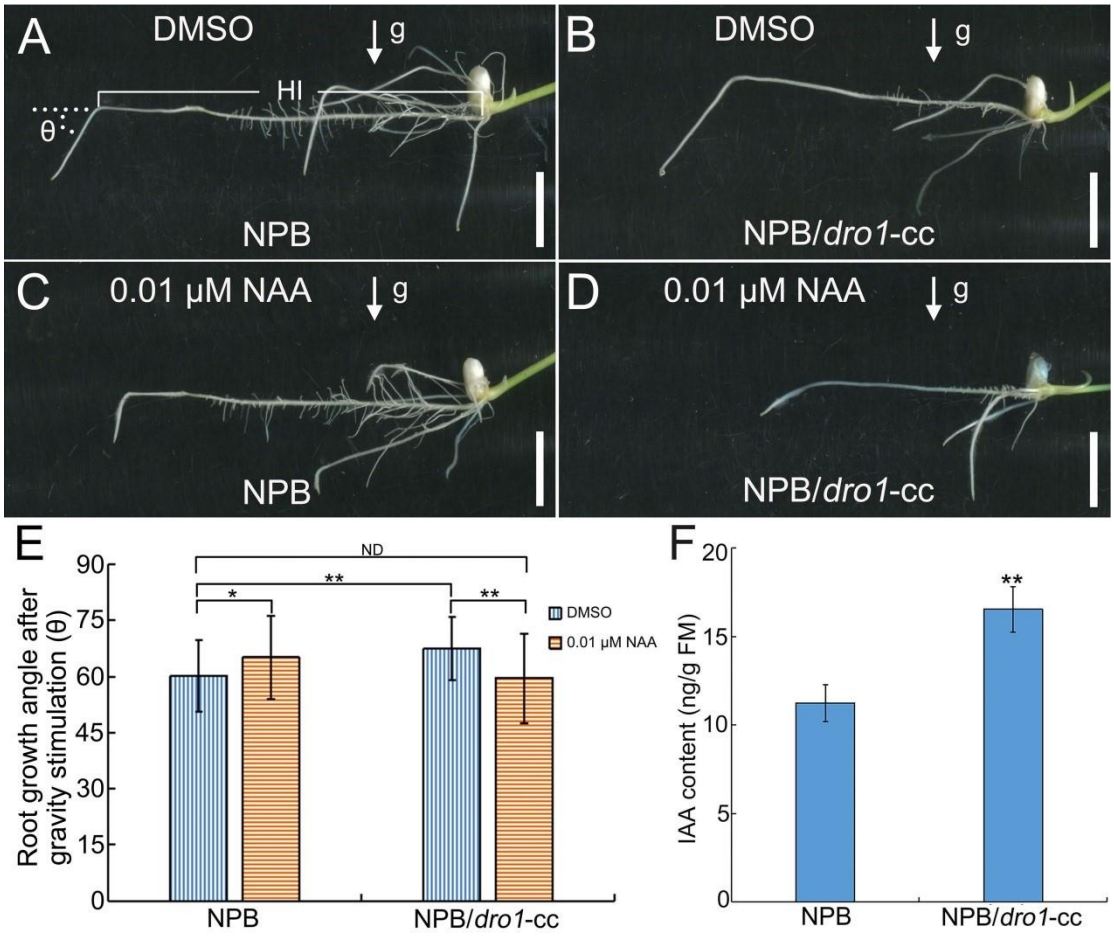


Figure S14. Auxin transport was disturbed in the Nipponbare *dro1*-cc mutant.

Root phenotypes of wild-type Nipponbare (A, C) and the *dro1*-cc (#17) mutant (B, D) grown on MS medium supplemented with DMSO (A, B) or 0.01 μM NAA (C, D) under gravity stimulation. Seedlings grown on the MS medium containing DMSO was acted as a control. Quantification of root growth angles (E) (NPB: n_{DMSO} =39, n_{NAA}= 41; NPB/*dro1*-cc: n_{DMSO}=32, n_{NAA}= 42). IAA content in rice primary roots grown in MS medium under gravity stimulation (F) (NPB: n = 3, NPB/*dro1*-cc: n = 4). Data are means ± SD; * $P < 0.05$, ** $P < 0.01$ (Student's *t*-test). NPB = Nipponbare, HI = IAA content in parallel primary roots, g = gravity. Bar = 1 cm. The arrow shows the direction of gravity.

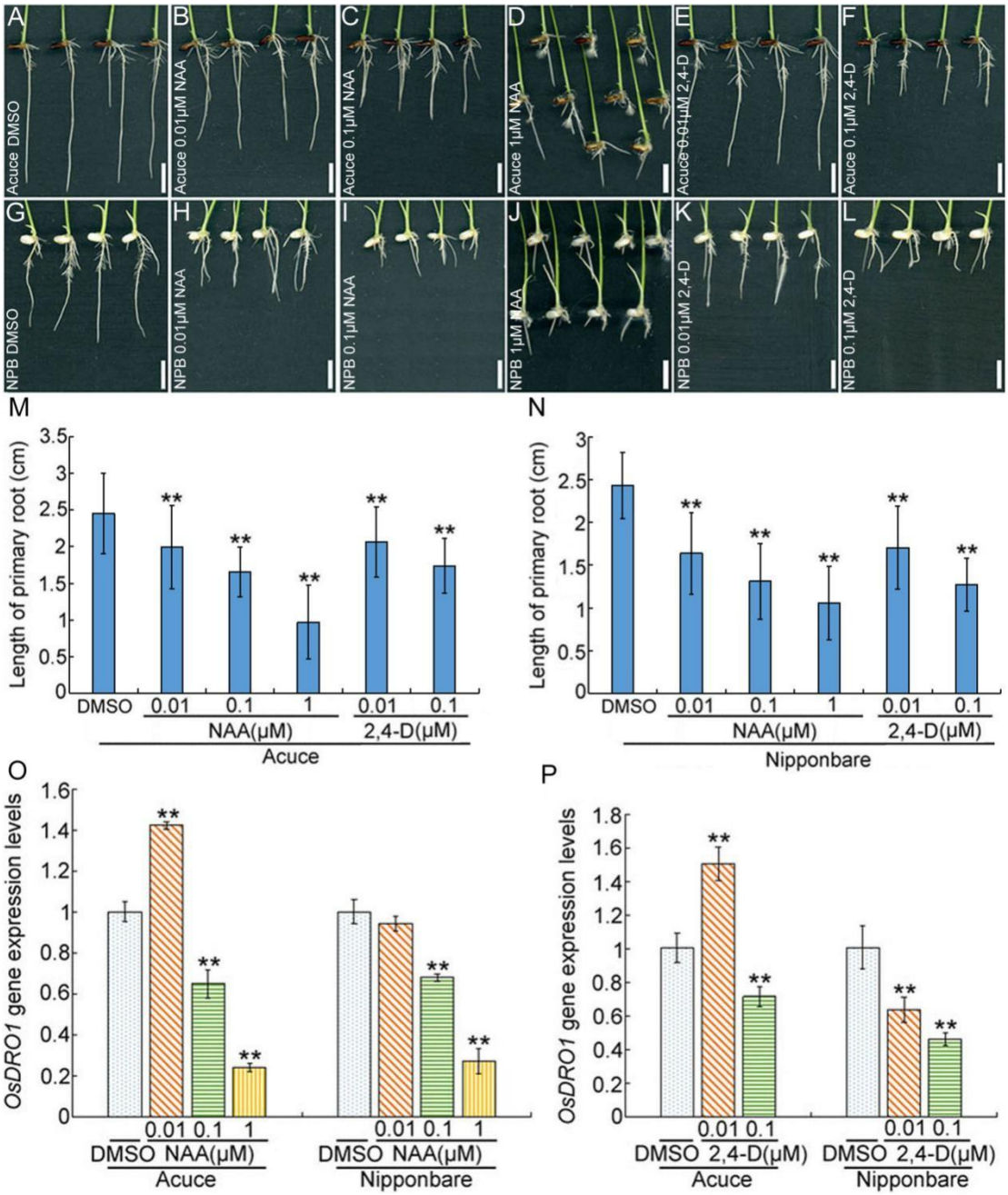


Figure S15. Root phenotypes of rice seedlings treated with auxin.

Seedlings of Acuce and Nipponbare cultured with MS medium containing DMSO (A and G), 0.01 μM NAA (B and H), 0.1 μM NAA (C and I), 1 μM NAA (D and J), 0.01 μM 2,4-D (E and K), and 0.1 μM 2,4-D (F and L) for 4 days. Seedlings grown on the MS medium containing DMSO was acted as a control. The primary root lengths were measured and quantified (M and N) (Acuce: $n_{DMSO} = 49$, $n_{0.01\ \mu M\ NAA} = 38$, $n_{0.1\ \mu M\ NAA} = 38$, $n_{1\ \mu M\ NAA} = 42$, $n_{0.01\ \mu M\ 2,4\text{-}D} = 37$, $n_{0.1\ \mu M\ 2,4\text{-}D} = 53$; NPB: $n_{DMSO} = 23$, $n_{0.01\ \mu M\ NAA} =$

20, $n_{0.1\ \mu M\ NAA} = 19$, $n_{1\ \mu M\ NAA} = 42$, $n_{0.01\ \mu M\ 2,4\text{-}D} = 20$, $n_{0.1\ \mu M\ 2,4\text{-}D} = 32$). The expression levels of *DRO1* (O and P) in the rice root meristem region were determined using real-time PCR. The *OsActin* gene was used as an internal control. Data are means ± SD. ** $P < 0.01$ (Student's *t*-test for root length, SPSS analysis for differential gene expression). NPB = Nipponbare. Bar = 1cm.

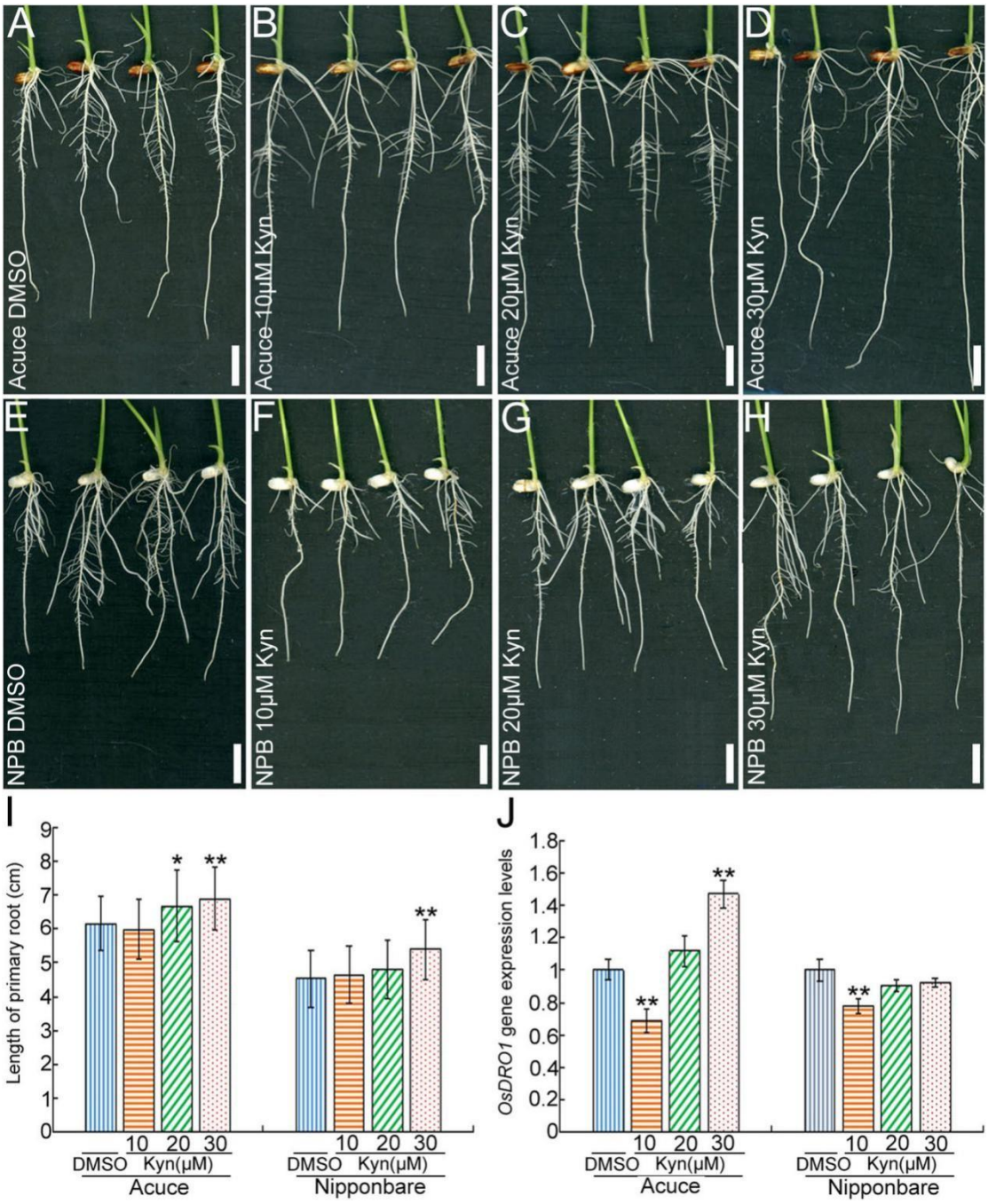


Figure S16. Root phenotypes of rice seedlings treated with L-Kynurenine.

Four-day-old seedlings of Acuce and Nipponbare cultured with MS medium containing DMSO (A and E) or 10 μM (B and F), 20 μM (C and G), and 30 μM (D and H) L-Kynurenine for 4 days. Seedlings grown on the MS medium containing DMSO was acted as a control. The primary root lengths were measured and quantified (I) (Acuce: $n_{DMSO} = 34$, $n_{10\ \mu M\ Kyn} = 46$, $n_{20\ \mu M\ Kyn} = 40$, $n_{30\ \mu M\ Kyn} = 38$; NPB:

1303 $n_{DMSO} = 23$, $n_{10\ \mu M\ Kyn} = 26$, $n_{20\ \mu M\ Kyn} = 30$, $n_{30\ \mu M\ Kyn} = 28$). The expression levels of

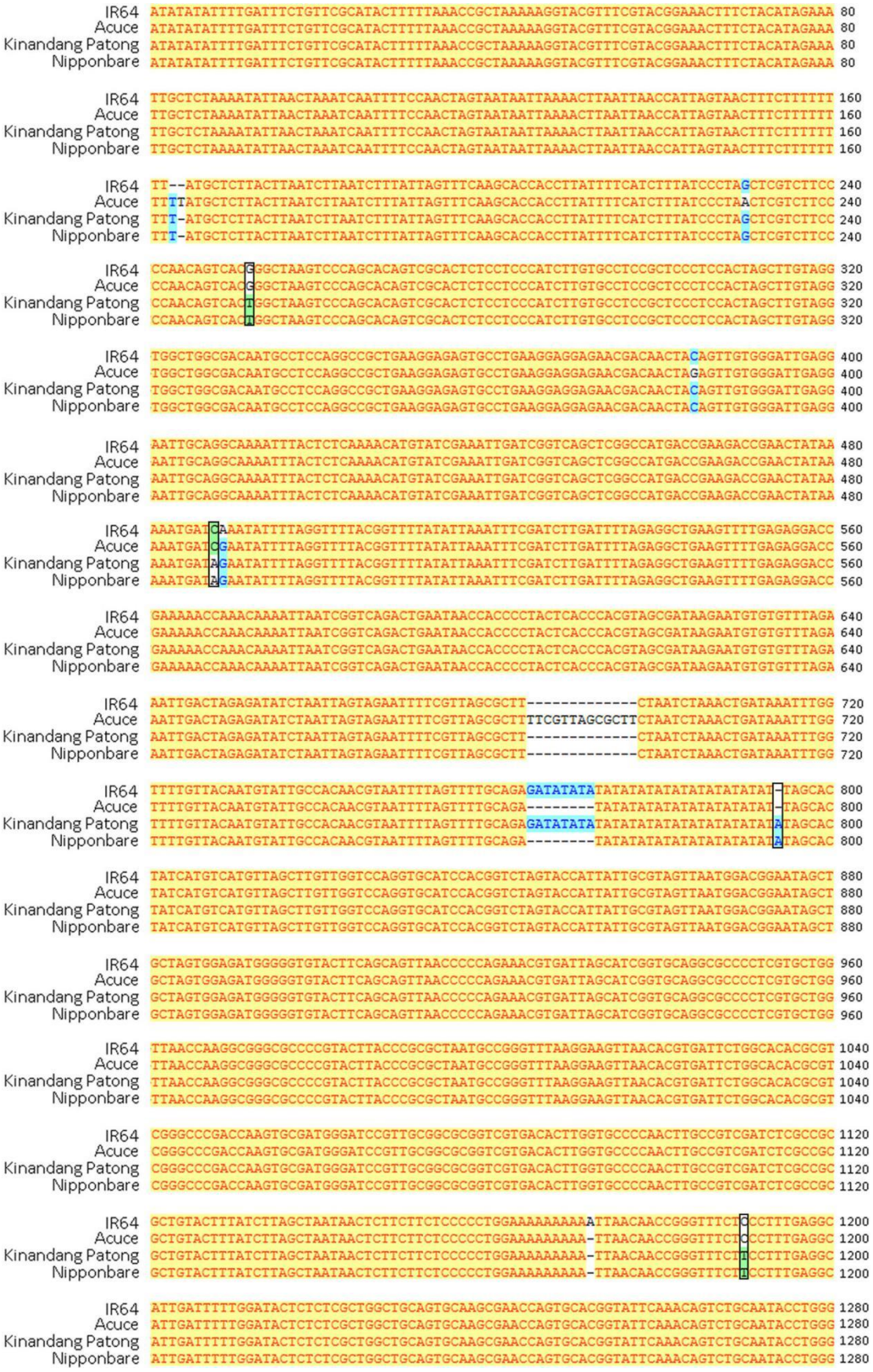
1304 *DRO1* in the rice root meristem region were determined using real-time PCR (J). The

1305 *OsActin* gene was used as an internal control. Data are means ± SD. * $P < 0.05$, ** P

1306 < 0.01 (Student's *t*-test for root length, SPSS analysis for differential gene expression).

1307 NPB = Nipponbare, Kyn = L-Kynurenine. Bar = 1 cm.

1308


IR64
Acuce
Kinandang Patong
Nipponbare

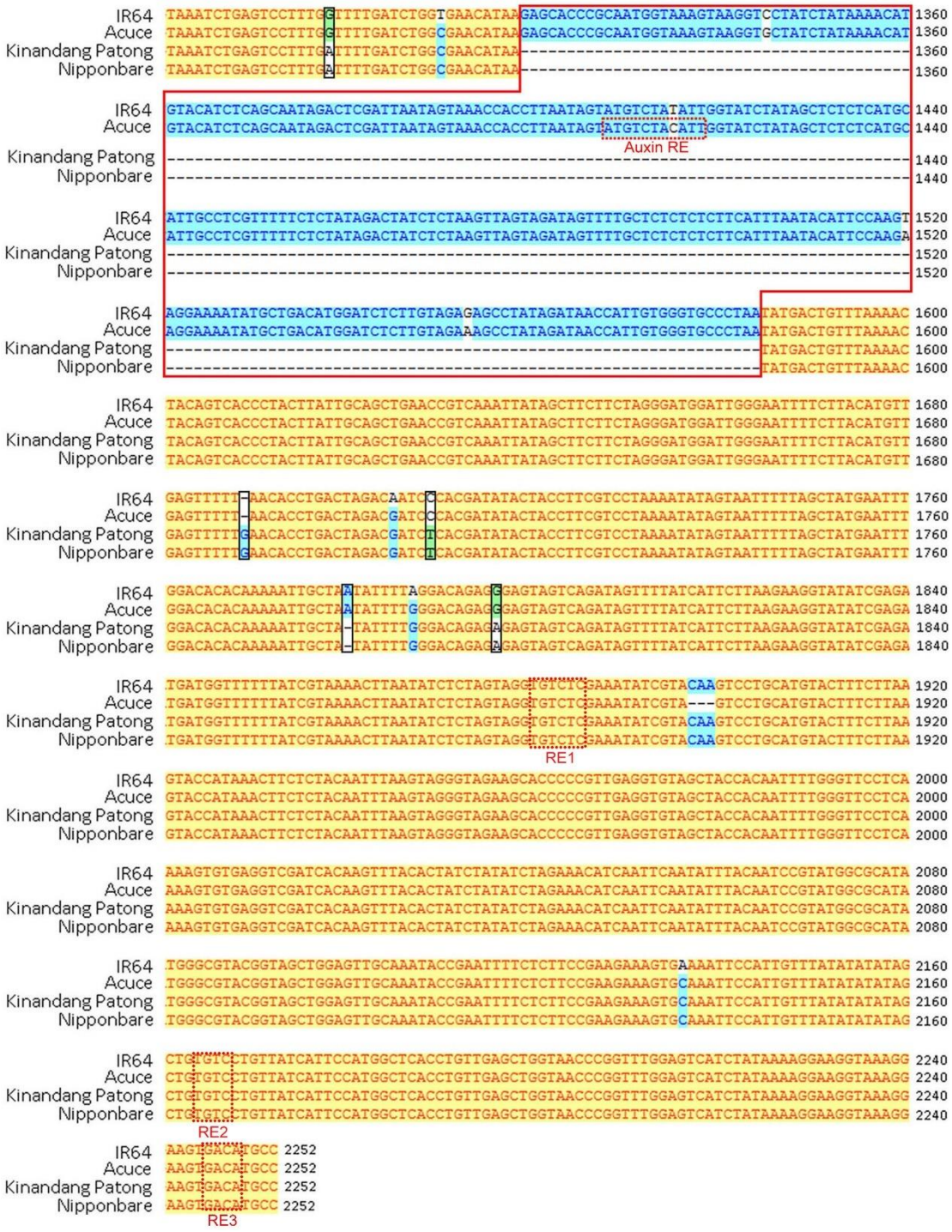


Figure S17. Sequences of the *DRO1* promoter in different rice varieties.

Sequences of *DRO1* promoters from the rice varieties Acuce, Nipponbare, IR64 and Kinandang Patong were aligned. The conserved sequences are indicated by black boxes. The sequences of *INDITTO2* transposons are labelled with red boxes. Auxin RE = auxin response element. RE1, 2, 3 = auxin response element 1, 2 and 3,

1316 respectively.

1317

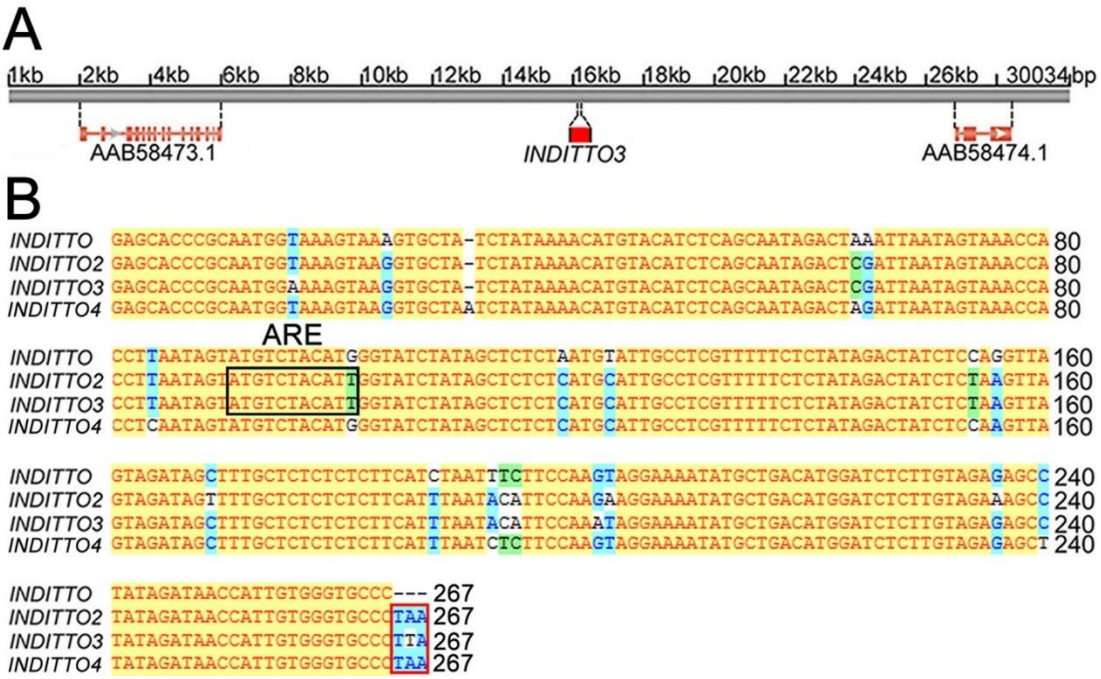


Figure S18. Positions and sequences of the *INDITTO2* transposon and its homologues genes in different rice varieties.

Position of the *INDITTO3* transposon in the Teqing genome obtained from the NCBI database (A). Sequence alignments of the *INDITTO*, *INDITTO2*, *INDITTO3* and *INDITTO4* transposons (B). The *INDITTO4* transposon was amplified from the Nipponbare genome. The red box represents the target site duplication (TSD) sequence. The black box represents the auxin response element (ARE).

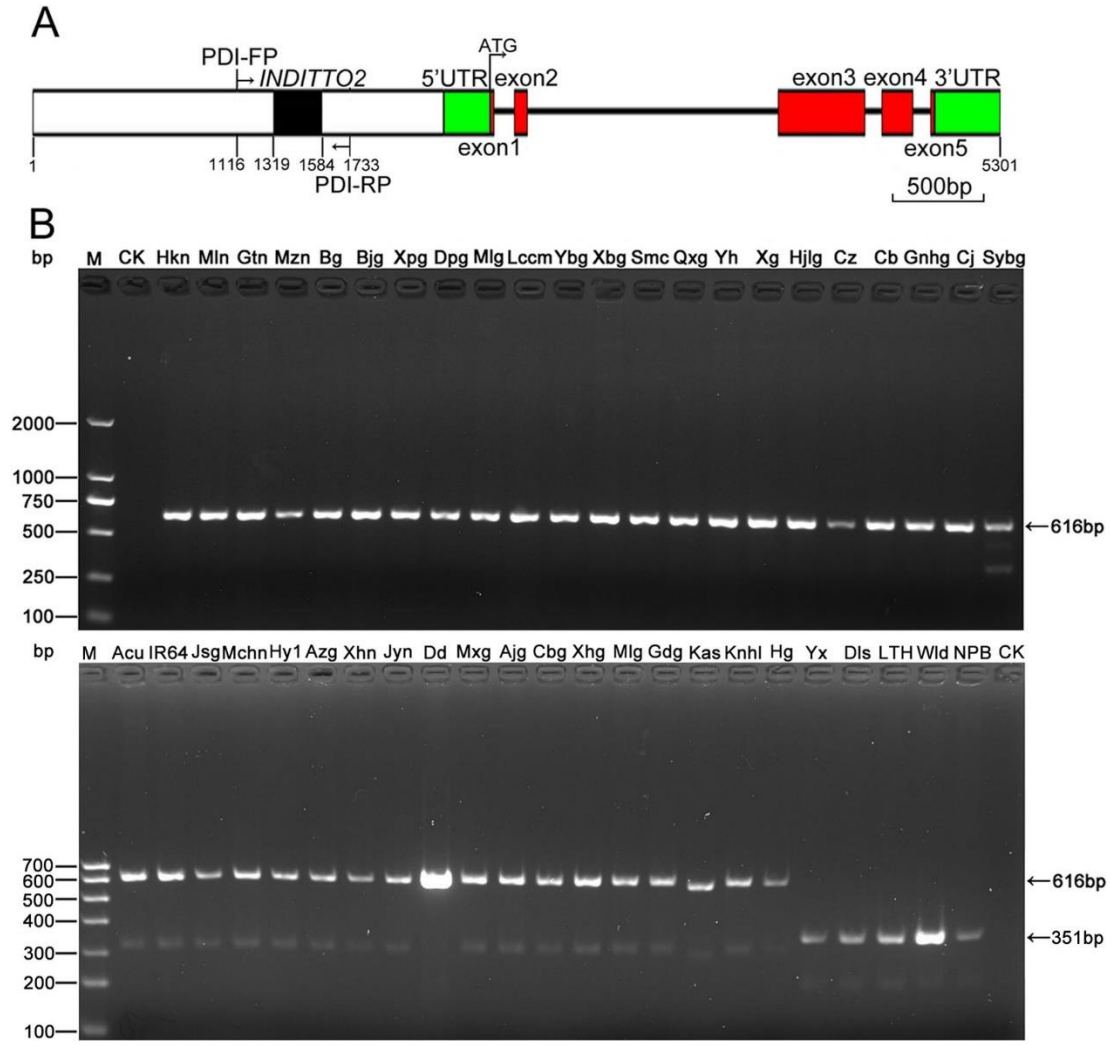
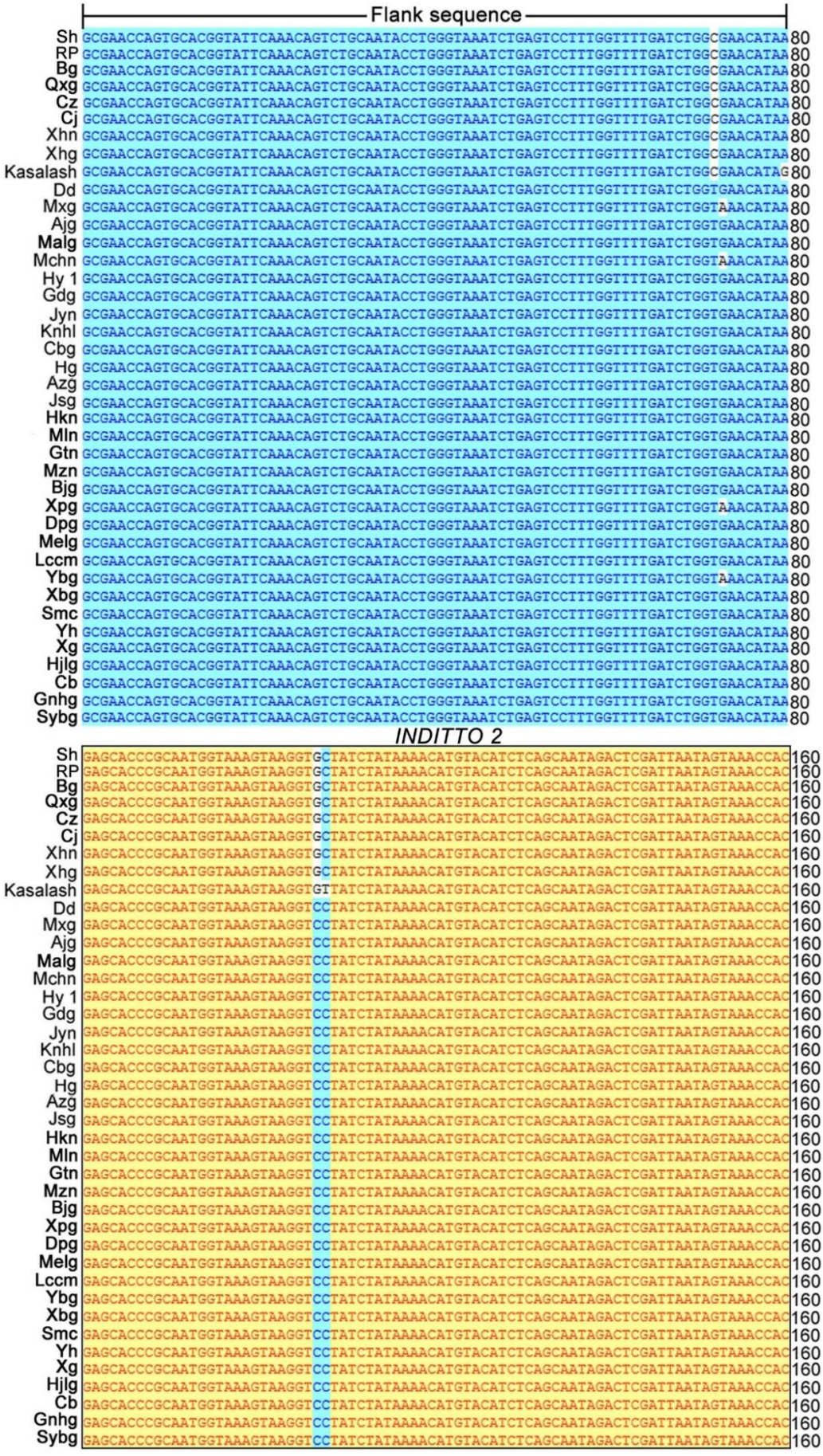


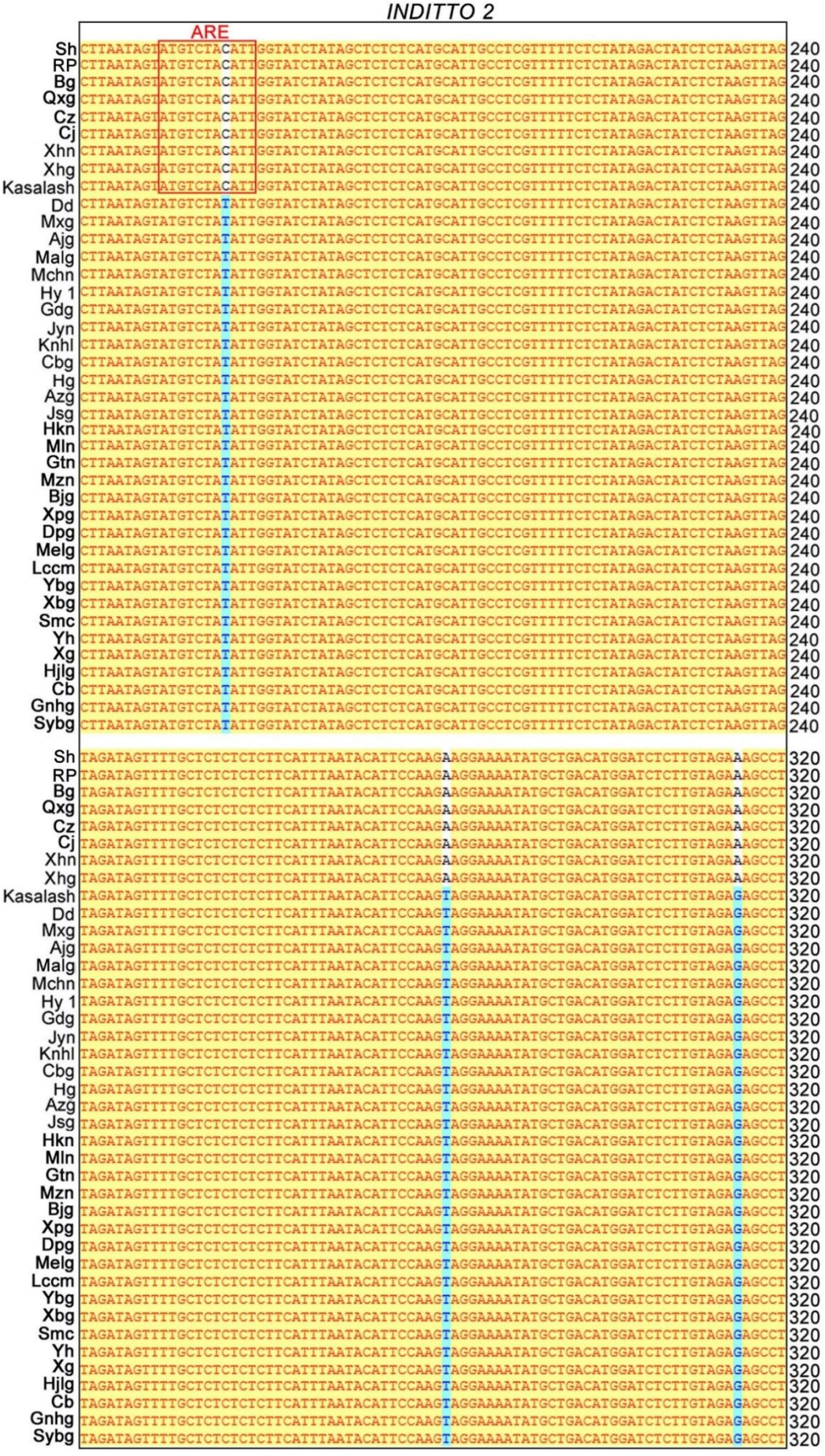
Figure S19. Amplification of the *INDITTO2* transposon and its homologues genes from different rice genomes.

Schematic diagram of the *INDITTO2* transposon located in the *DRO1* promoter of Acuce (A). Agarose gel electrophoresis shows the PCR product of the *INDITTO2* transposon amplified with the primer pair PDI-FP and PDI-RP from 45 rice varieties (B). The arrow shows the PCR product of the *INDITTO2* transposon with a size of 616 bp amplified with primers PDI-FP and PDI-RP. The 351 bp PCR product was non-specifically amplified. Acu = Acuce, Ajg = Ai jiao gu, Azg = Ai zhe gu, Bg = Bai gu, Big = Ban jiu gu, Cb = Che bu, Cbg = Chuan bai gu, Cj = Che jia, Cz = Che zuo, Dd = Duo dian, Dls = Da leng shui, Dpg = Da pi gu, Gdg = Gan di gu, Gnhg = Ga niang hong gu, Gtn = Gan tian nuo, Hg = Hei gu, Hjlg = Hong jiao lao geng, Hkn

= Hua ke nuo, Hy 1 = Hong yang 1, IR64 = IR64, Jsg = Jian shui gu, Jyn = Jiu yue nuo, Kas = Kasalath, Knhl = Kou ni he lve, Lccm = Le che che ma, LTH = Li jiang xin tuan hei gu, Mchn = Man che hong nuo, Mlg = Mao lai gu, Mlg = Meng la gu, Mln = Meng la nuo, Mxg = Ma xian gu, Mzn = Ma zha nuo, NPB = Nipponbare, Qxg = Qi xian gu, Smc = Si ma che, Sybg = Shi yue bai gu, Wld = Wen lu dao 4, Xbg = Xi bai gu, Xg = Xiao gu, Xhg = Xiao hua gu, Xhn = Xiao hua nuo, Xpg = Xiao pi gu, Ybg = Ye bai gu, Yh = Yun hui 290, Yx = Yun xiang. M = DNA marker, CK = H_2O, which served as a negative control.


Flank sequence
Sh GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGCGAACATAA 80
RP GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGCGAACATAA 80
Bg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGCGAACATAA 80
Qxg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGCGAACATAA 80
Cz GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGCGAACATAA 80
Cj GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGCGAACATAA 80
Xhn GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGCGAACATAA 80
Xhg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGCGAACATAA 80
Kasalash GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGCGAACATAG 80
Dd GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Mxg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTAAACATAA 80
Ajg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Malg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Mchn GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTAAACATAA 80
Hy 1 GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Gdg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Jyn GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Knhl GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Cbg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Hg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Azg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Jsg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Hkn GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Mln GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Gtn GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Mzn GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Bjg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Xpg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTAAACATAA 80
Dpg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Melg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Lccm GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Ybg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTAAACATAA 80
Xbg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Smc GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Yh GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Xg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Hjlg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Cb GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Gnhg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
Sybg GCGAACCAGTGCACGGTATTCAAACAGTCTGCAATACCTGGGTAAATCTGAGTCCTTTGGTTTTGATCTGGTGAACATAA 80
INDITTO 2
Sh GAGCACCCGCAATGGTAAAGTAAGGTGCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
RP GAGCACCCGCAATGGTAAAGTAAGGTGCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Bg GAGCACCCGCAATGGTAAAGTAAGGTGCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Qxg GAGCACCCGCAATGGTAAAGTAAGGTGCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Cz GAGCACCCGCAATGGTAAAGTAAGGTGCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Cj GAGCACCCGCAATGGTAAAGTAAGGTGCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Xhn GAGCACCCGCAATGGTAAAGTAAGGTGCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Xhg GAGCACCCGCAATGGTAAAGTAAGGTGCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Kasalash GAGCACCCGCAATGGTAAAGTAAGGTGTTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Dd GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Mxg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Ajg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Malg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Mchn GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Hy 1 GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Gdg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Jyn GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Knhl GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Cbg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Hg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Azg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Jsg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Hkn GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Mln GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Gtn GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Mzn GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Bjg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Xpg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Dpg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Melg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Lccm GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Ybg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Xbg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Smc GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Yh GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Xg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Hjlg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Cb GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Gnhg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160
Sybg GAGCACCCGCAATGGTAAAGTAAGGTCCTATCTATAAAACATGTACATCTCAGCAATAGACTCGATTAATAGTAAACCAC 160

1348


INDITTO 2
ARE
Sh CTTAATAGTATGTCTACATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
RP CTTAATAGTATGTCTACATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Bg CTTAATAGTATGTCTACATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Qxg CTTAATAGTATGTCTACATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Cz CTTAATAGTATGTCTACATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Cj CTTAATAGTATGTCTACATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Xhn CTTAATAGTATGTCTACATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Xhg CTTAATAGTATGTCTACATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Kasalash CTTAATAGTATGTCTACATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Dd CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Mxg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Ajg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Malg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Mchn CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Hy 1 CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Gdg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Jyn CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Knhl CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Cbg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Hg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Azg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Jsg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Hkn CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Mln CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Gtn CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Mzn CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Bjg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Xpg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Dpg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Melg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Lccm CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Ybg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Xbg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Smc CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Yh CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Xg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Hjlg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Cb CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Gnhg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Sybg CTTAATAGTATGTCTATATTGGTATCTATAGCTCTCTCATGCATTGCCTCGTTTTTCTCTATAGACTATCTCTAAGTTAG 240
Sh TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGAAGGAAAATATGCTGACATGGATCTCTTGTAGAAAGCCT 320
RP TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGAAGGAAAATATGCTGACATGGATCTCTTGTAGAAAGCCT 320
Bg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGAAGGAAAATATGCTGACATGGATCTCTTGTAGAAAGCCT 320
Qxg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGAAGGAAAATATGCTGACATGGATCTCTTGTAGAAAGCCT 320
Cz TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGAAGGAAAATATGCTGACATGGATCTCTTGTAGAAAGCCT 320
Cj TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGAAGGAAAATATGCTGACATGGATCTCTTGTAGAAAGCCT 320
Xhn TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGAAGGAAAATATGCTGACATGGATCTCTTGTAGAAAGCCT 320
Xhg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGAAGGAAAATATGCTGACATGGATCTCTTGTAGAAAGCCT 320
Kasalash TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Dd TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Mxg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Ajg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Malg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Mchn TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Hy 1 TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Gdg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Jyn TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Knhl TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Cbg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Hg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Azg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Jsg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Hkn TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Mln TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Gtn TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Mzn TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Bjg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Xpg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Dpg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Melg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Lccm TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Ybg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Xbg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Smc TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Yh TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Xg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Hjlg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Cb TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Gnhg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320
Sybg TAGATAGTTTTGCTCTCTCTCTTCATTTAATACATTCCAAGTAGGAAAATATGCTGACATGGATCTCTTGTAGAGAGCCT 320

1349

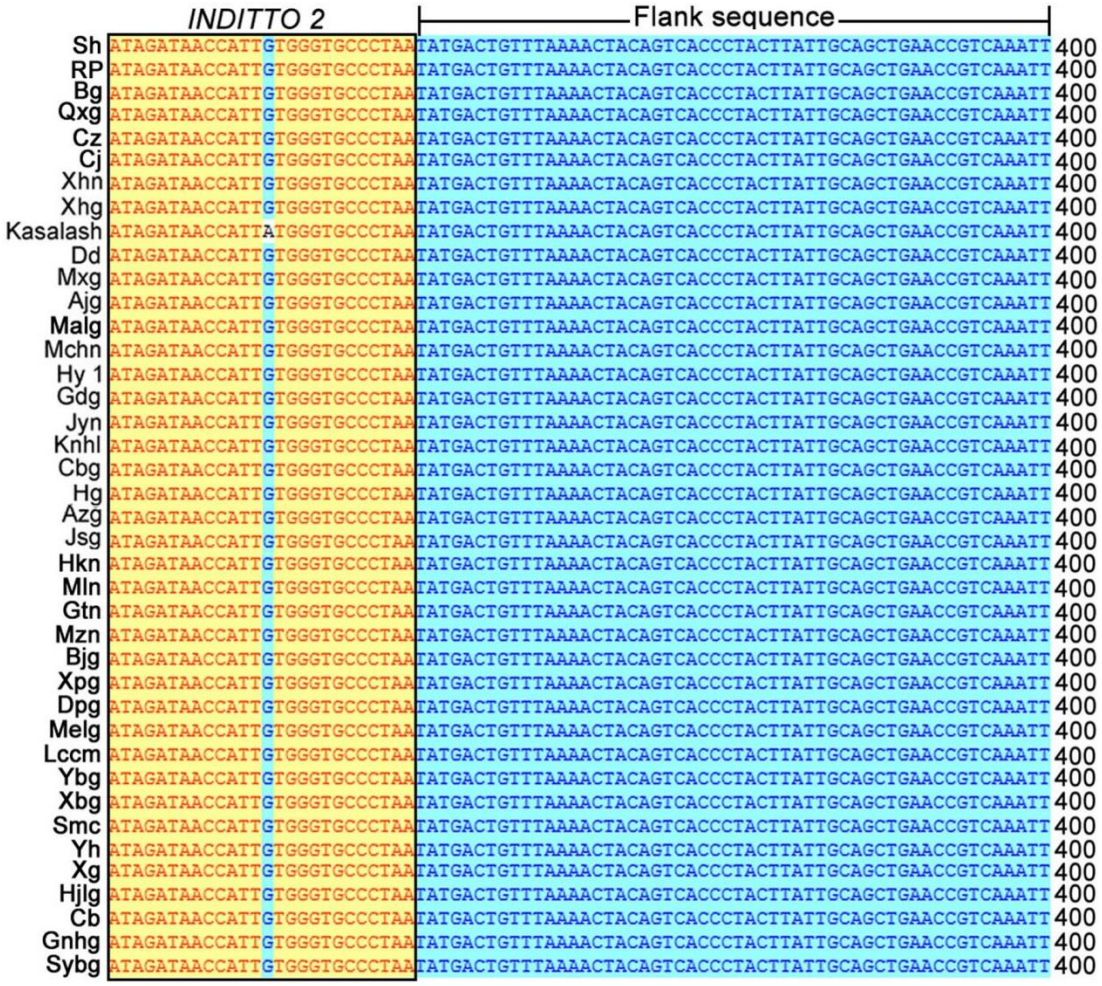


Figure S20. Sequence alignments of the *INDITTO2* transposon and its homologues genes in the *DRO1* promoters of different rice varieties.

Forty sequences of the *INDITTO2* transposon were amplified from 38 rice varieties and two sequences from Shuhui498 and RP Bio-226 obtained from the NCBI database. The red box represents an auxin response element (ARE). Ajg = Ai jiao gu, Azg = Ai zhe gu, Bg = Bai gu, Bjg = Ban jiu gu, Cb = Che bu, Cbg = Chuan bai gu, Cj = Che jia, Cz = Che zuo, Dd = Duo dian, Dpg = Da pi gu, Gdg = Gan di gu, Gnhg = Ga niang hong gu, Gtn = Gan tian nuo, Hg = Hei gu, Hjlg = Hong jiao lao geng, Hkn = Hua ke nuo, Hy 1 = Hong yang 1, Jsg = Jian shui gu, Jyn = Jiu yue nuo, Knhl = Kou ni he lve, Lccm = Le che che ma, Malg = Mao lai gu, Mchn = Man che hong nuo, Melg = Meng la gu, Mln = Meng la nuo, Mxg = Ma xian gu, Mzn = Ma zhe nuo, Qxg

= Qi xian gu, RP = RP Bio-226, Sh = Shuhui498, Smc = Si ma che, Sybg = Shi yue bai gu, Xbg = Xi bai gu, Xg = Xiao gu, Xhg = Xiao hua gu, Xhn = Xiao hua nuo, Xpg = Xiao pi gu, Ybg = Ye bai gu, Yh = Yun hui 290.

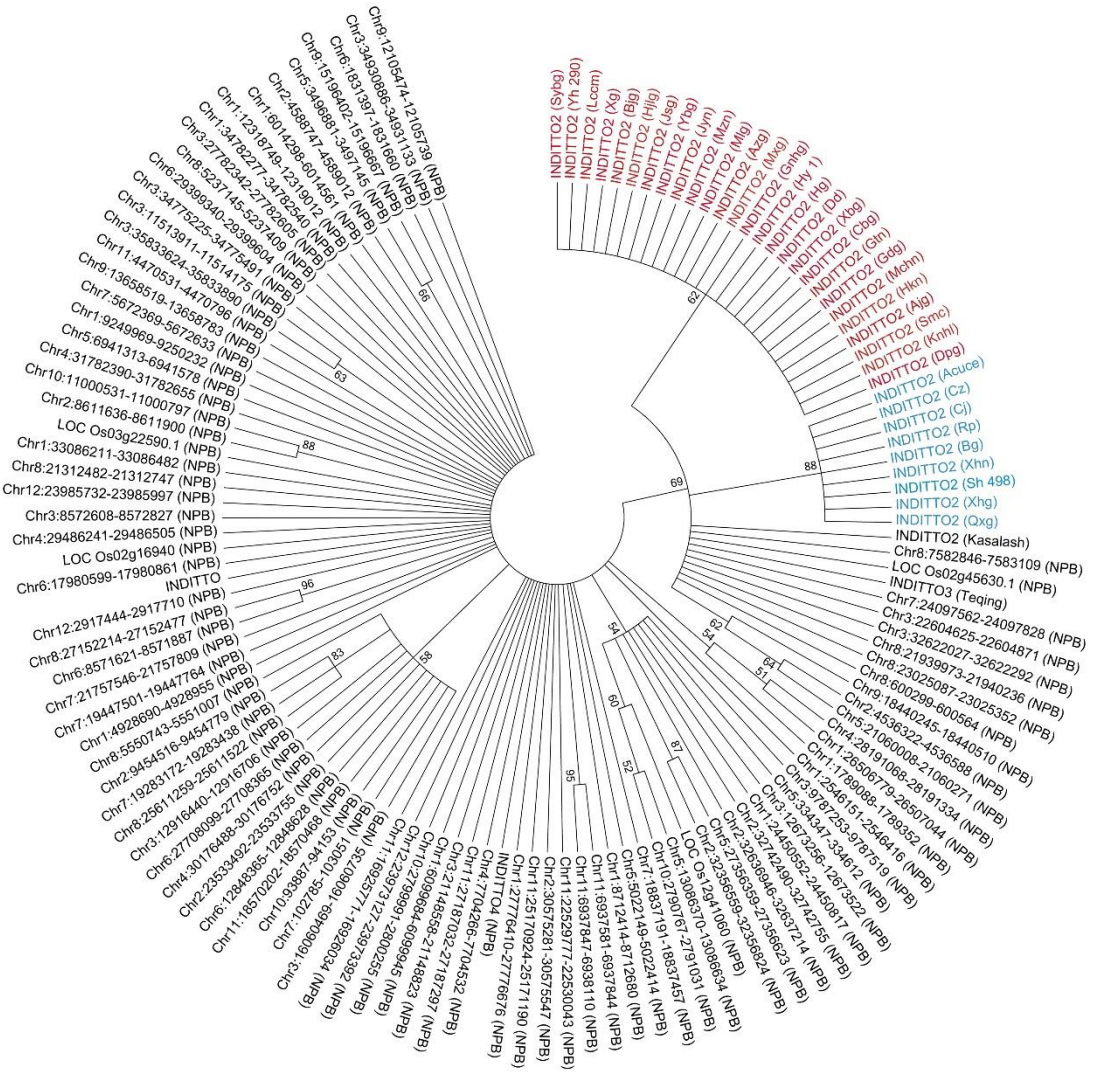


Figure S21. Phylogeny of *INDITTO* and its homologues from different rice varieties.

Phylogenetic tree constructed based on neighbour-joining (NJ) analysis. The numbers indicate bootstrap values at the branching points. The name of homologues of the *INDITTO4* transposon was represented by the gene position in the Nipponbare genome. Ajg = Ai jiao gu, Azg = Ai zhe gu, Bg = Bai gu, Bjg = Ban jiu gu, Cbg = Chuan bai gu, Cj = Che jia, Cz = Che zuo, Dd = Duo dian, Dpg = Da pi gu, Gdg = Gan di gu, Gnhg = Ga niang hong gu, Gtn = Gan tian nuo, Hg = Hei gu, Hjlg = Hong jiao lao geng, Hkn = Hua ke nuo, Hy 1 = Hong yang 1, Jsg = Jian shui gu, Jyn = Jiu yue nuo, Knhl = Kou ni he lve, Lccm = Le che che ma, Mchn = Man che hong nuo,

Mlg = Meng la gu, Mxg = Ma xian gu, Mzn = Ma zhe nuo, NPB = Nipponbare, Qxg = Qi xian gu, RP = RP Bio-226, Sh = Shuhui498, Smc = Si ma che, Sybg = Shi yue bai gu, Xbg = Xi bai gu, Xg = Xiao gu, Xhg = Xiao hua gu, Xhn = Xiao hua nuo, Ybg = Ye bai gu, Yh 290= Yun hui 290.

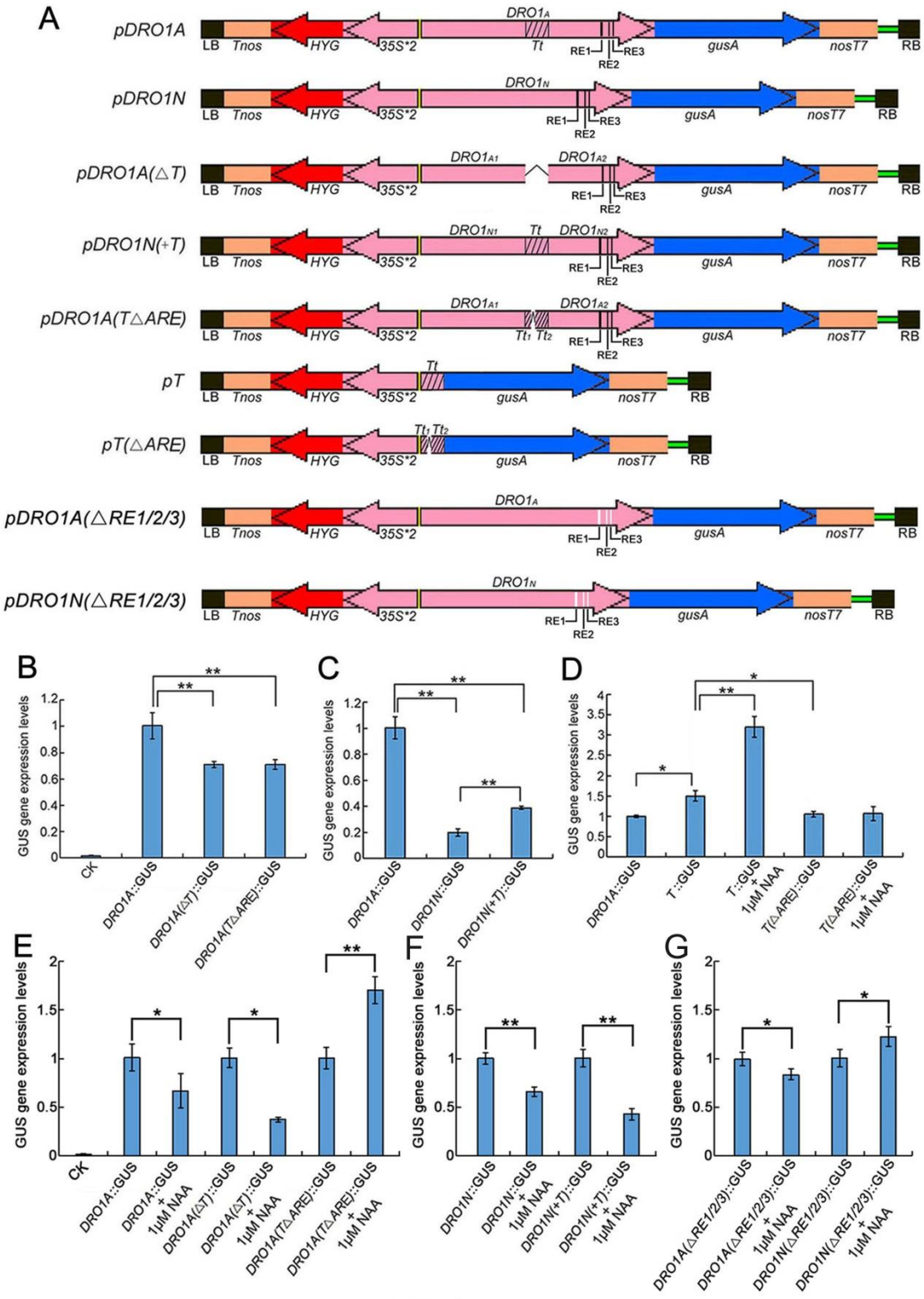


Figure S22. Expression levels of the *GUS* gene in tobacco leaves.

Schematic diagram of the expression vector containing the *DRO1* promoter and *INDITTO2* transposon (A). A solution of *Agrobacterium* strain EHA105 transformed with the expression vector was infiltrated into tobacco leaves with or without 1 μM

NAA supplementation. The expression levels of the *GUS* gene (B-G) in tobacco leaves were determined using real-time PCR. The *NaActin* gene was used as an internal control. Data are means ± SD. * $P < 0.05$, ** $P < 0.01$ (SPSS analysis). CK = tobacco leaf without treatment (negative control). *DRO1A* = the Acuce *DRO1* promoter, *DRO1N* = the Nipponbare *DRO1* promoter, *DRO1A*(△*T*) = the Acuce *DRO1* promoter with deletion of the *INDITTO2* transposon, *DRO1A*(*T*△*ARE*) = the Acuce *DRO1* promoter containing the *INDITTO2* transposon with an ARE deletion, *DRO1N*(+*T*) = the *INDITTO2* transposon inserted into the Nipponbare *DRO1* promoter, *pT* = the expression construct containing the *INDITTO2* transposon, *pT*(△*ARE*) = the expression construct containing the *INDITTO2* transposon with an ARE deletion, *DRO1A*(△*RE1/2/3*) = the Acuce *DRO1* promoter with deletion of 3 AREs, *DRO1N*(△*RE1/2/3*) = the Nipponbare *DRO1* promoter with deletion of 3 AREs, Tt = *INDITTO2* transposon, RE 1, 2, 3 = auxin response element 1, 2 and 3, *gusA* = *GUS* gene, ARE = auxin response element.

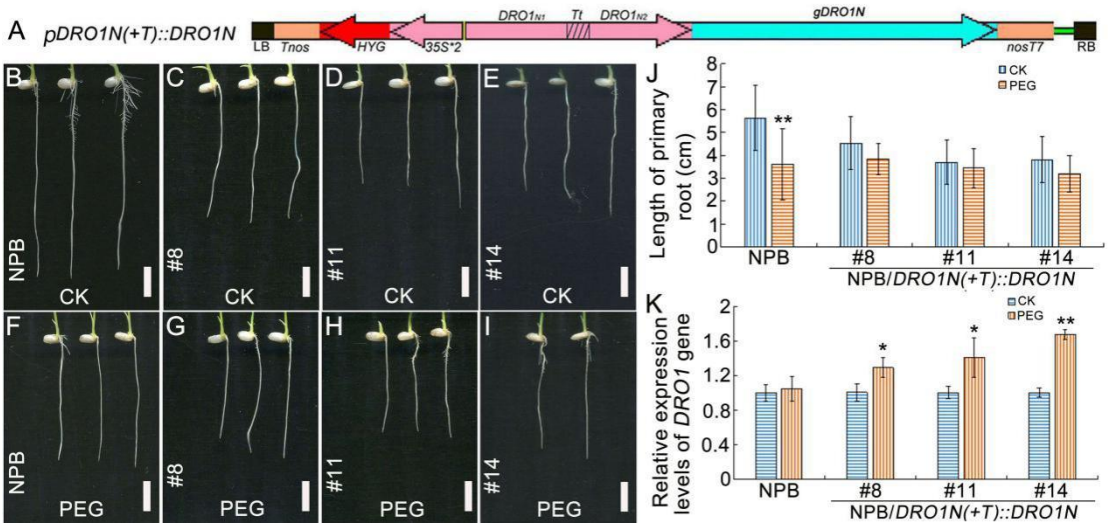


Figure S23. Phenotypes of transgenic Nipponbare rice lines containing the *INDITTO2* transposon in the *DRO1* promoter.

Schematic diagram of the expression construct containing an *INDITTO2* transposon inserted into the Nipponbare *DRO1* promoter (A). Root phenotypes of wild-type Nipponbare (B, F) and T1 transgenic rice lines expressing the *INDITTO2* transposon (C-E, G-I) grown on MS medium with (F-I) or without (B-E) 15% PEG6000 supplementation. Quantification of the primary root lengths (J) (NPB: $n_{CK} = 20$, $n_{PEG} = 24$; #8: $n_{CK} = 14$, $n_{PEG} = 9$; #11: $n_{CK} = 16$, $n_{PEG} = 12$; #14: $n_{CK} = 7$, $n_{PEG} = 10$). The expression levels of *DRO1* (K) in the root meristem region of wild-type and transgenic rice lines. The *OsActin* gene was used as an internal control. Data are means ± SD. * $P < 0.05$, ** $P < 0.01$ (Student's *t*-test for root length, SPSS analysis for gene expression). *pDRO1N (+T)::DRO1N* = the Nipponbare *DRO1* promoter containing the *INDITTO2* transposon and fused with Nipponbare genomic *DRO1* gene. NPB = Nipponbare, CK = rice seedlings grown on MS medium, PEG = rice seedlings grown on MS medium supplemented with 15% PEG6000. *gDRO1N* = Nipponbare genomic *DRO1* gene. Bar = 1 cm.

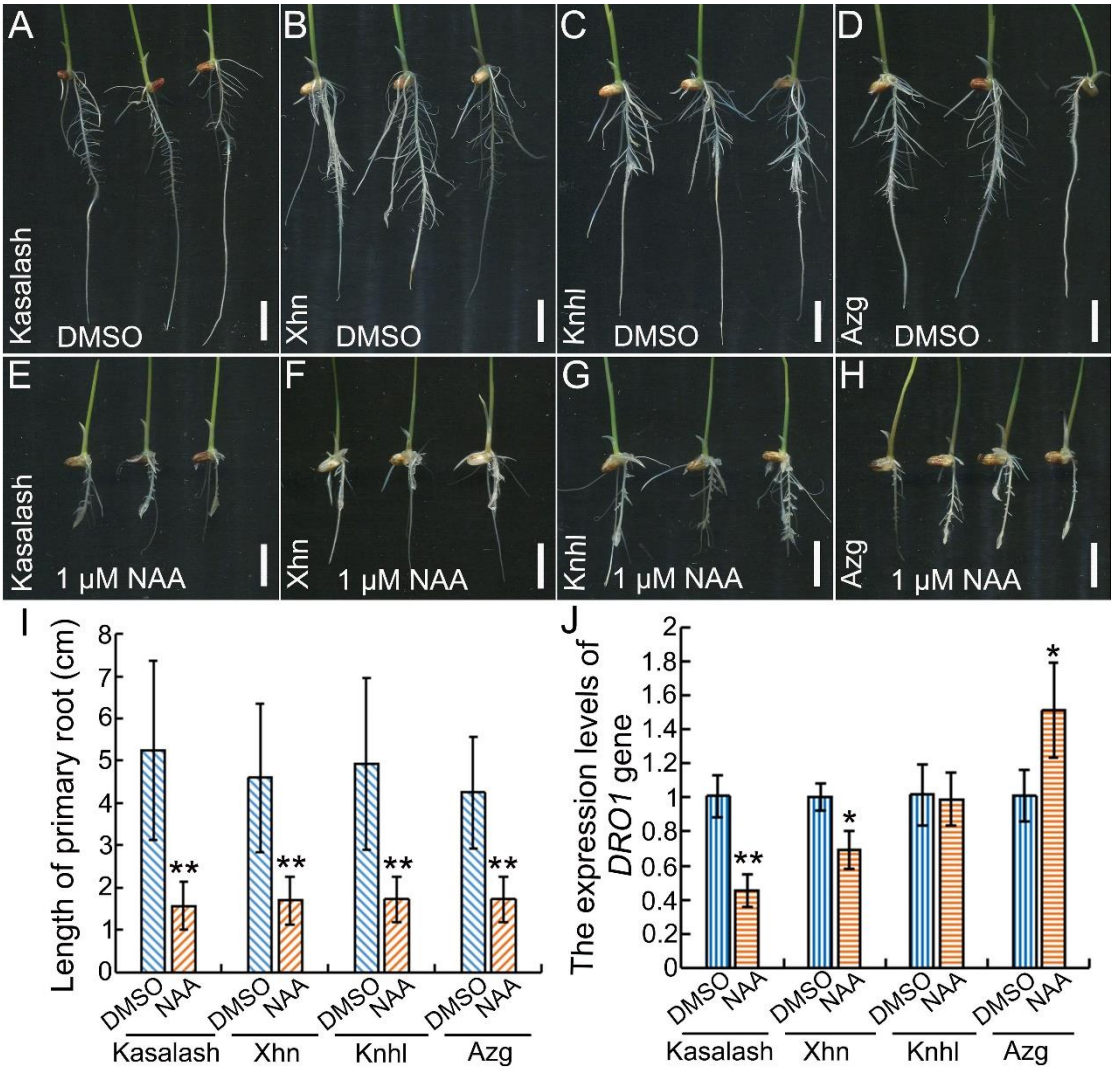
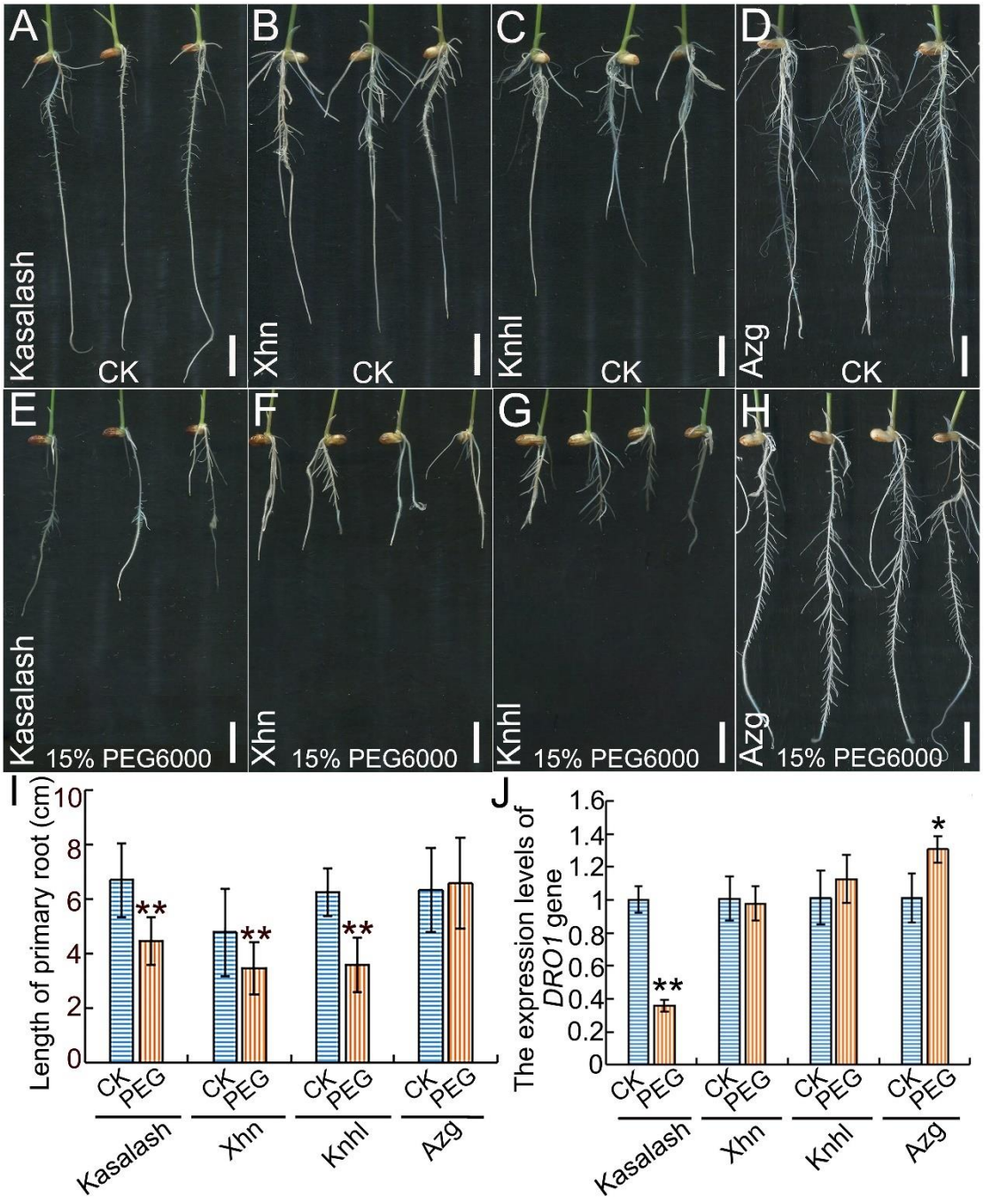


Figure S24. The expression levels of *DRO1* in four rice varieties treated with auxin.

Six-day-old seedlings of Kasalath (A, E), Xhn (B, F), Knhl (C, G) and Azg (D, H) were grown on MS medium supplemented with DMSO (A-D) or with (E-H) 1 μM NAA, and the expression levels of *DRO1* gene in root tip were checked by real-time PCR (C). Seedlings grown on the MS medium containing DMSO was acted as a control. The actin gene was used as an internal control. Data are the means ± SD. * $P < 0.05$, ** $P < 0.01$ (SPSS analysis). Azg = Ai zhe gu, Knhl = Kou ni he lve, Xhn = Xiao hua nuo.



1431

1432 **Figure S25. Root lengths and the levels of *DRO1* in rice varieties grown under**

1433 **drought stress.**

1434 Seedlings of Kasalath (A and E), Xhn (B and F), Knhl (C and G) and Azg (D and H)

1435 grown on MS medium without (A-D) or witht (E-H) 15% PEG6000 supplementation

1436 for 6 days. The primary root lengths were measured and quantified (I) (Kasalath: n_{CK}

1437 = 47, $n_{PEG6000}$ = 43, Xhn: n_{CK} = 42, $n_{PEG6000}$ = 42, Knhl: n_{CK} = 65, $n_{PEG6000}$ = 56, Azg:

n_{CK} = 12, $n_{PEG6000}$ = 12). The expression levels of *DRO1* (J) in the rice root meristem region were determined using real-time PCR. The *OsActin* gene was used as an internal control. Data are means ± SD. * $P < 0.05$, ** $P < 0.01$ (Student's *t*-test for root length analysis, SPSS analysis for gene expression). CK = rice seedlings grown on MS medium, PEG = rice seedlings grown on MS medium supplemented with 15% PEG6000. Azg = Ai zhe gu, Knhl = Kou ni he lve, Xhn = Xiao hua nuo. Bar = 1 cm.

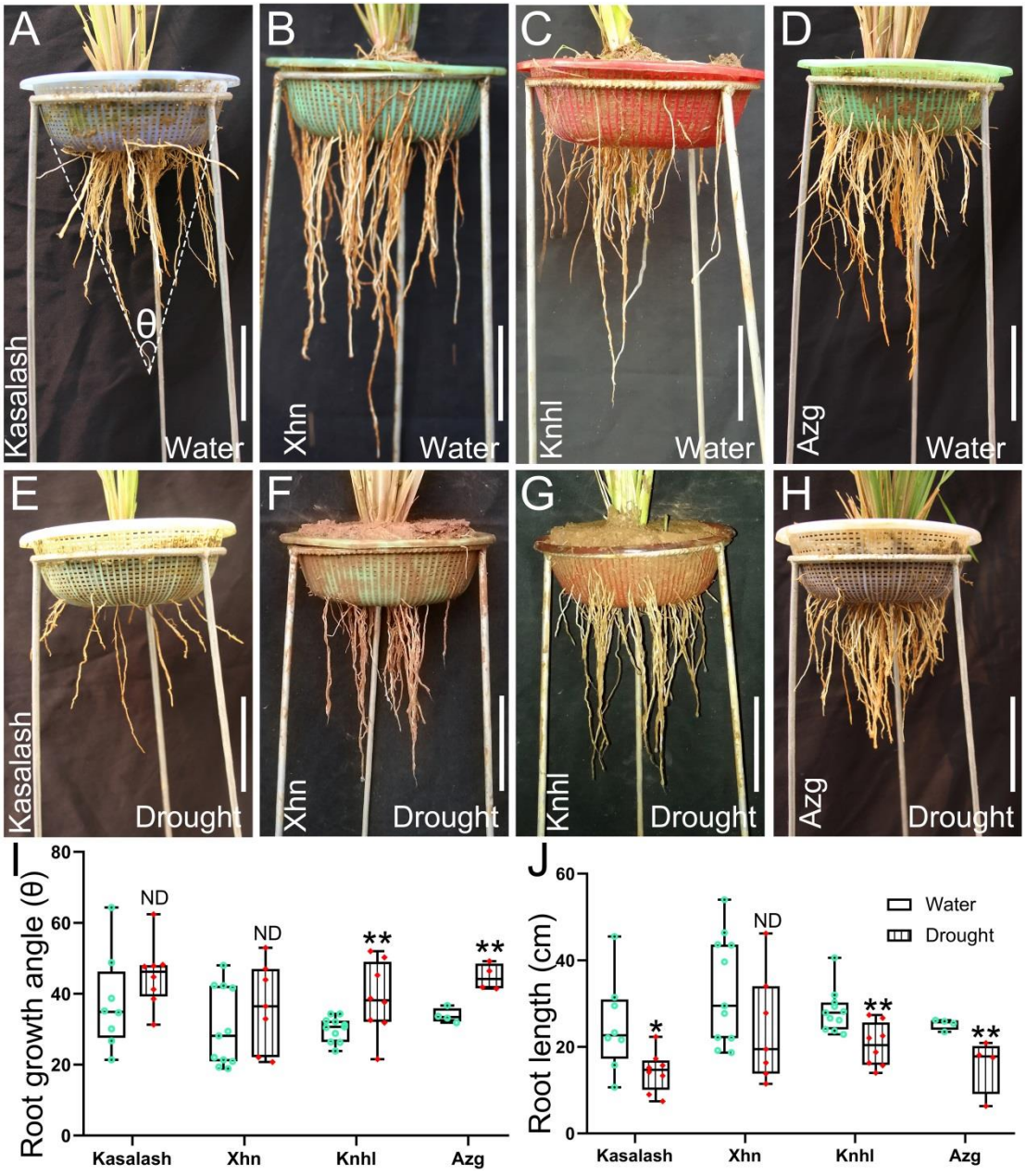
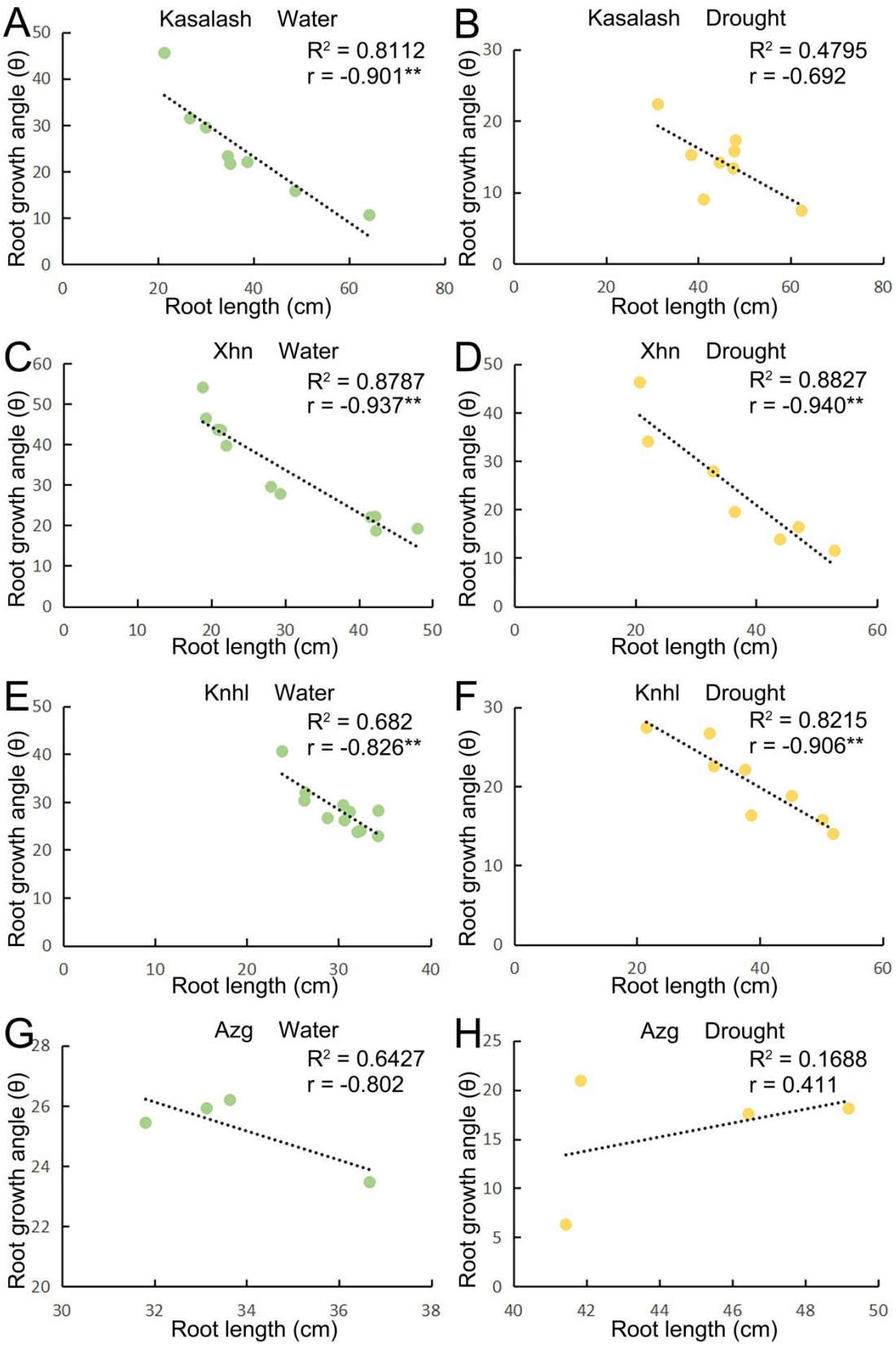


Figure S26. Root architectures of rice varieties grown in the field.

Kasalath (A, E), Xhn (B, F), Knhl (C, G) and Azg (D, H) were grown with irrigation conditions (A-D) or under drought stress (E-H) for 3 months. Quantification of the root growth angles (I) (Kasalath: $n_{water} = 8$, $n_{drought} = 8$, Xhn: $n_{water} = 11$, $n_{drought} = 7$, Knhl: $n_{water} = 11$, $n_{drought} = 8$, Azg: $n_{water} = 4$, $n_{drought} = 4$) and root length (J) (Kasalath: $n_{water} = 8$, $n_{drought} = 8$, Xhn: $n_{water} = 11$, $n_{drought} = 7$, Knhl: $n_{water} = 11$, $n_{drought} = 8$, Azg: $n_{water} = 4$, $n_{drought} = 4$). Data are means ± SD; * $P < 0.05$, ** $P < 0.01$ (Student's *t*-test). Azg = Ai zhe gu, Knhl = Kou ni he lve, Xhn = Xiao hua nuo, ND = no difference, θ =

root growth angle. Bar = 10 cm.



1456

1457 **Figure S27. Correlation analysis of root length and root growth angle in different**

1458 **rice varieties.**

Kasalath (A, B), Xhn (C, D), Knhl (E, F) and Azg (G, H) were grown with irrigation conditions (A, C, E, G) or under drought stress (B, D, F, H) for 3 months. The correlation between root length and root angle was analyzed using SPSS software. Data are means ± SD; ** $P < 0.01$ (double-tailed test) indicates a significant correlation. R^2 = Determination coefficient, r = Pearson's correlation coefficient; r > 0, positive correlation; r < 0, negative correlation. Azg = Ai zhe gu, Knhl = Kou ni he lve, Xhn = Xiao hua nuo.

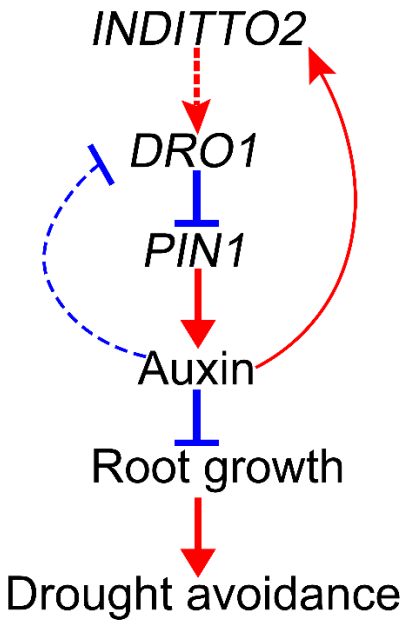


Figure S28. A model of auxin-mediated *DRO1* transcription is conveyed by the *INDITTO2* transposon to enhance rice drought avoidance.

DRO1 is involved in negatively regulating the subcellular localization of the auxin transporter *OsPIN1b* and controls auxin distribution; auxin negatively regulates the expression level of the *DRO1* gene; *INDITTO2* transposon conveys the auxin signal to mediate the *DRO1* transcription and regulate root growth and drought avoidance; thus, providing a mechanism for rice to adapt to environmental drought stress. The dotted line indicates that there may still be undiscovered factors in this pathway requiring elucidation. → = promotion, ⊣ = inhibition.

1478 **Table S1.** Rice landraces collected from the Yuanyang Hani's terraced fields

	Rice variety	Subspecies
1	Acuce	*indica* group
2	Ai jiao gu	*indica* group
3	Ai zhe gu	*indica* group
4	Bai gu	*indica* group
5	Ban jiu gu	*indica* group
6	Che bu	*indica* group
7	Che jia	*indica* group
8	Che zuo	*indica* group
9	Chuan bai gu	*indica* group
10	Da leng shui	*japonica* group
11	Da pi gu	*japonica* group
12	Duo dian	*indica* group
13	Ga niang hong gu	*indica* group
14	Gan di gu	*indica* group
15	Gan tian nuo	*indica* group
16	Hei gu	*japonica* group
17	Hong jiao lao geng	*indica* group
18	Hong yang 1	*indica* group
19	Hua ke nuo	*indica* group
20	Jian shui gu	*indica* group
21	Jiu yue nuo	*indica* group
22	Kou ni he lve	*indica* group
23	Le che che ma	*indica* group
24	Ma xian gu	*indica* group
25	Ma zha nuo	*indica* group
26	Man che hong nuo	*indica* group
27	Mao lai gu	unknown
28	Meng la gu	*indica* group
29	Meng la nuo	*indica* group
30	Qi xian gu	unknown
31	Shi yue bai gu	*indica* group
32	Si ma che	*indica* group
33	Xi bai gu	*indica* group
34	Xiao gu	*indica* group
35	Xiao hua gu	*indica* group
36	Xiao hua nuo	*indica* group
37	Xiao pi gu	*indica* group
38	Ye bai gu	*indica* group
39	Yun hui 290	*indica* group
40	Yun xiang	*japonica* group

1479

1480 **Table S2.** Sequences of gene-specific primers

Target gene	Primer	Sequence of gene-specific primer	Locus
OsDRO1	DRO1-rFP	5’-AATGGAGAAGTTGCTCAAGGCA-3’	Os09g0439800
	DRO1-rRP	5’-TCATCGTCTAGATCACGCAGTG-3’	
OsPIN1b	OsPIN1b-rFP	5’-TCCTGCACGTCGCCATTGT-3’	Os02g0743400
	OsPIN1b-rRP	5’-GATGTAGTAGACGAGGGTGAT-3’	
OsPIN2	OsPIN2-5FP	5’-ACCAACGACCCCTACTCCATGAAC-3’	Os06g0660200
	OsPIN2-5RP	5’-AAGAGGGTGATGGTCCAGTCGAGC-3’	
OsPIN3t	OsPIN3t-FP	5’-TATGTATAGCCTGTTGTCGG-3’	Os01g45550
	OsPIN3t-RP	5’-TTGTACAAATGTCGCAGAGA-3’	
Cas9	Cas9-FP	5’-CACCATCTACCACCTGAGAA-3’	
	Cas9-RP	5’-CGAAGTTGCTCTTGAAGTTG-3’	
gRNA	PUV3-R	5’-CTGGCGAAAGGGGGATGTGCTGCAA-3’	
	gRNA-R5	5’-ACGACCGGGTCACGCTGCACCT-3’	
GUS	GUS-rFP	5’-CGTGATGCGCGTCCAAGGA-3’	AF354045
	GUS-rRP	5’-TGATGGTGATGGTGATGGCTA-3’	
NaActin	NaActin-rFP	5’-GGTCGTACCACCGGTATTGTG-3’	JQ256516
	NaActin-rRP	5’-GTCAAGACGGAGAATGGCATG-3’	
OsActin	OsActin-FP	5’-GAGTATGATGAGTCGGGTCCAG-3’	Os11g0163100
	OsActin-RP	5’-ACACCAACAATCCCAAACAGAG-3’	
ProDRO1A	PrDRO1-FP	5’-CAGTGGTCTCATAGAGAGCTCATATATATTTTGAT-3’	Os09g0439800
	PrDRO1-RP	5’-CAGTGGTCTCAGTTGGGTACCGGCATGTCACTTCC-3’	
ProDRO1N	PrDRO1-FP	5’-CAGTGGTCTCATAGAGAGCTCATATATATTTTGAT-3’	Os09g0439800
	PrDRO1-RP	5’-CAGTGGTCTCAGTTGGGTACCGGCATGTCACTTCC-3’	
INDITTO2	PDI-FP	5’-GCCGCGCTGTACTTTATCTTA-3’	MN650123
	PDI-RP	5’-TTAGGACGAAGGTAGTATATCG-3’	
INDITTO2	Transposon-FP	5’-GAGCACCCGCAATGGTAAAGT-3’	MN650123
	Transposon-RP	5’-TTAGGGCACCCACAATGGTTAT-3’	
INDITTO4	Transposon-FP	5’-GAGCACCCGCAATGGTAAAGT-3’	MN650124
	Transposon-RP	5’-TTAGGGCACCCACAATGGTTAT-3’	

OsDRO1	OsDRO1-ccFP	5’-TCCCCTCAAGGAACAGGGAA-3’	Os09g0439800
	OsDRO1-ccRP	5’-TCGCCAATAGCAGCCACTAC-3’	
OsYUCCA2a	OsYUCCA2a-rFP	5’- TCAGAGAAAGATGGCTTCCCA -3’	Os01g0224700
	OsYUCCA2a-rRP	5’-CATATCGTGCAAACGCTGC-3’	
OsYUCCA5b	OsYUCCA5b-rFP	5’-AACGGATGGAAGGGTGAGT-3’	Os04g0128900
	OsYUCCA5b-rRP	5’-GCACGCCGTGCTCTTCTT-3’	

1481

1482 **Table S3.** Profiles of *INDITTO4* transposon homologues in the Nipponbare genome

Transposon	Chromosome	Start	End	E-value	Identity(%)	Remark	ARE
INDITT04	Chr1	1789089	1789352	3.44E-120	96.226	Os01g04110	-
INDITT04	Chr1	2546416	2546151	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr1	4928955	4928690	2.66E-121	96.255	not align to a gene	-
INDITT04	Chr1	6014561	6014298	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr1	6099685	6099945	7.40E-122	96.947	not align to a gene	-
INDITT04	Chr1	8712415	8712680	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr1	9249970	9250232	1.24E-119	96.212	Os01g16310	-
INDITT04	Chr1	12319012	12318749	7.40E-122	96.604	not align to a gene	-
INDITT04	Chr1	24450817	24450552	1.23E-124	97.004	not align to a gene	-
INDITT04	Chr1	26507044	26506779	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr1	27776411	27776676	2.64E-126	97.378	not align to a gene	-
INDITT04	Chr1	33086212	33086482	2.66E-121	95.956	not align to a gene	-
INDITT04	Chr1	34782540	34782277	7.40E-122	96.604	not align to a gene	-
INDITT04	Chr2	1049	784	2.55E-118	95.506	Os02g16940	-
INDITT04	Chr2	1085	1348	7.09E-119	95.849	Os02g45630	+
INDITT04	Chr2	4536323	4536588	2.66E-121	96.255	not align to a gene	+
INDITT04	Chr2	4589012	4588747	5.72E-123	96.629	not align to a gene	-
INDITT04	Chr2	8611637	8611900	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr2	9454779	9454516	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr2	23533755	23533492	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr2	30575282	30575547	2.66E-121	96.255	Os02g50040	-
INDITT04	Chr2	32356824	32356559	2.66E-121	96.255	not align to a gene	-
INDITT04	Chr2	32636947	32637214	3.44E-120	95.911	not align to a gene	-
INDITT04	Chr2	32742755	32742490	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr3	1927	1664	7.09E-119	95.849	Os03g22590	-
INDITT04	Chr3	8572827	8572608	2.13E-97	95.909	not align to a gene	-
INDITT04	Chr3	9787254	9787519	1.23E-124	97.004	Os03g17590	-
INDITT04	Chr3	11513912	11514175	7.40E-122	96.604	not align to a gene	-
INDITT04	Chr3	12673257	12673522	5.72E-123	96.629	not align to a gene	-
INDITT04	Chr3	12916441	12916706	5.72E-123	96.629	not align to a gene	-
INDITT04	Chr3	16060470	16060735	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr3	21148823	21148558	5.72E-123	96.629	not align to a gene	-
INDITT04	Chr3	22604626	22604871	3.49E-110	95.951	not align to a gene	+
INDITT04	Chr3	27782605	27782342	1.59E-123	96.981	not align to a gene	-
INDITT04	Chr3	32622292	32622027	1.24E-119	95.88	not align to a gene	+
INDITT04	Chr3	34775226	34775491	1.23E-124	97.004	not align to a gene	-
INDITT04	Chr3	34931133	34930886	2.70E-111	95.984	not align to a gene	-
INDITT04	Chr3	35833625	35833890	5.72E-123	96.629	not align to a gene	-
INDITT04	Chr4	7704267	7704532	2.66E-121	96.255	Os04g13810	-
INDITT04	Chr4	28191069	28191334	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr4	29486242	29486505	3.44E-120	96.226	Os04g49410	-
INDITT04	Chr4	30176489	30176752	7.40E-122	96.604	not align to a gene	-

INDITT04	Chr4	31782391	31782655	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr5	334612	334347	1.23E-124	97.004	not align to a gene	-
INDITT04	Chr5	3496882	3497145	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr5	5022414	5022149	5.72E-123	96.629	not align to a gene	-
INDITT04	Chr5	6941578	6941313	2.66E-121	96.255	not align to a gene	-
INDITT04	Chr5	13086371	13086634	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr5	21060271	21060008	3.44E-120	96.226	not align to a gene	+
INDITT04	Chr5	27356360	27356623	3.44E-120	96.226	Os05g47750	-
INDITT04	Chr6	1831660	1831397	7.40E-122	96.604	Os06g04310	-
INDITT04	Chr6	8571622	8571887	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr6	12848628	12848365	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr6	17980600	17980861	4.45E-119	96.198	not align to a gene	-
INDITT04	Chr6	27708100	27708365	1.23E-124	97.004	not align to a gene	-
INDITT04	Chr6	29399341	29399604	1.59E-123	96.981	Os06g48590	-
INDITT04	Chr7	102786	103051	2.66E-121	96.255	not align to a gene	-
INDITT04	Chr7	5672370	5672633	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr7	18837192	18837457	5.72E-123	96.629	not align to a gene	-
INDITT04	Chr7	19283173	19283438	5.72E-123	96.629	Os07g32420	-
INDITT04	Chr7	19447764	19447501	7.40E-122	96.604	not align to a gene	-
INDITT04	Chr7	21757809	21757546	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr7	24097563	24097828	5.72E-123	96.629	not align to a gene	+
INDITT04	Chr8	600564	600299	1.24E-119	95.88	not align to a gene	+
INDITT04	Chr8	5237146	5237409	7.40E-122	96.604	not align to a gene	-
INDITT04	Chr8	5550744	5551007	1.59E-123	96.981	not align to a gene	-
INDITT04	Chr8	7583109	7582846	7.40E-122	96.604	not align to a gene	+
INDITT04	Chr8	21312747	21312482	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr8	21940236	21939973	1.59E-123	96.981	not align to a gene	+
INDITT04	Chr8	23025352	23025087	1.24E-119	95.88	not align to a gene	+
INDITT04	Chr8	25611522	25611259	7.40E-122	96.604	Os08g40490	-
INDITT04	Chr8	27152477	27152214	7.40E-122	96.604	not align to a gene	-
INDITT04	Chr9	12105739	12105474	5.72E-123	96.629	not align to a gene	-
INDITT04	Chr9	13658520	13658783	7.40E-122	96.604	not align to a gene	-
INDITT04	Chr9	15196667	15196402	2.66E-121	96.255	not align to a gene	-
INDITT04	Chr9	18440510	18440245	2.66E-121	96.255	not align to a gene	+
INDITT04	Chr10	94153	93887	3.44E-120	95.896	not align to a gene	-
INDITT04	Chr10	2790768	2791031	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr10	2799992	2800255	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr10	11000532	11000797	2.66E-121	96.255	not align to a gene	-
INDITT04	Chr11	4470796	4470531	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr11	6937844	6937581	7.40E-122	96.604	not align to a gene	-
INDITT04	Chr11	6938110	6937847	3.44E-120	96.226	not align to a gene	-
INDITT04	Chr11	16926034	16925771	7.40E-122	96.604	not align to a gene	-
INDITT04	Chr11	18570203	18570468	2.66E-121	96.255	not align to a gene	-
INDITT04	Chr11	22529778	22530043	1.23E-124	97.004	not align to a gene	-

INDITT04	Chr11	25170925	25171190	2.66E-121	96.255	not align to a gene	-
INDITT04	Chr11	27187297	27187032	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr12	3987	3722	9.18E-118	95.506	Os12g41060	-
INDITT04	Chr12	2917445	2917710	1.23E-124	97.004	not align to a gene	-
INDITT04	Chr12	23973392	23973127	1.24E-119	95.88	not align to a gene	-
INDITT04	Chr12	23985997	23985732	1.23E-124	97.004	not align to a gene	-

Note: Chr = chromosome, + and – means transposon containing with or without ARE, respectively. ARE = Auxin response element

1486

Table S4. Prediction of transcription factor binding sites in the *INDITTO2* transposon

Family	Start	Stop	Strand	p-value	q-value	Matched sequence
C2H2	17	34	-	2.34E-06	0.00106	TAGATAGCACCTTACTTT
BBR-BPC	169	189	+	6.21E-06	0.00188	TTTGCTCTCTCTCTTCATTTA
BBR-BPC	168	191	-	6.44E-06	0.00263	ATTAAATGAAGAGAGAGAGCAAAA
BBR-BPC	165	185	+	9.27E-06	0.00188	TAGTTTTGCTCTCTCTCTTCA
HD-ZIP	183	193	+	1.49E-05	0.00557	TCATTTAATAC
C2H2	198	209	-	1.51E-05	0.00762	TTTTCCTTCTTG
HD-ZIP	183	193	+	2.09E-05	0.00777	TCATTTAATAC
BBR-BPC	167	187	+	3.10E-05	0.00419	GTTTTGCTCTCTCTCTTCATT
bZIP	210	224	-	3.16E-05	0.0159	ATCCATGTCAGCATA
bZIP	210	224	+	3.28E-05	0.0166	TATGCTGACATGGAT
bZIP	210	224	+	3.28E-05	0.0166	TATGCTGACATGGAT
HD-ZIP	183	193	-	6.53E-05	0.0121	GTATTAAATGA
MYB-related	160	173	-	9.70E-05	0.0352	GCAAAACTATCTAC

1487