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A Core Regulatory Pathway Controlling Rice Tiller Angle Mediated by the *LAZY1*-Dependent Asymmetric Distribution of Auxin[OPEN]

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Tiller angle in cereals is a key shoot architecture trait that strongly influences grain yield. Studies in rice (*Oryza sativa*) have implicated shoot gravitropism in the regulation of tiller angle. However, the functional link between shoot gravitropism and tiller angle is unknown. Here, we conducted a large-scale transcriptome analysis of rice shoots in response to gravistimulation and identified two new nodes of a shoot gravitropism regulatory gene network that also controls rice tiller angle. We demonstrate that HEAT STRESS TRANSCRIPTION FACTOR 2D (HSFA2D) is an upstream positive regulator of the LAZY1-mediated asymmetric auxin distribution pathway. We also show that two functionally redundant transcription factor genes, *WUSCHEL RELATED HOMEOBOX6* (*WOX6*) and *WOX11*, are expressed asymmetrically in response to auxin to connect gravitropism responses with the control of rice tiller angle. These findings define upstream and downstream genetic components that link shoot gravitropism, asymmetric auxin distribution, and rice tiller angle. The results highlight the power of the high-temporal-resolution RNA-seq data set and its use to explore further genetic components controlling tiller angle. Collectively, these approaches will identify genes to improve grain yields by facilitating the optimization of plant architecture.

INTRODUCTION

In rice (*Oryza sativa*), tiller angle is a key agronomic trait determining rice plant architecture and grain yield per unit area. Rice tiller angle directly affects light interception and photosynthetic efficiency and so influences planting density. Rice plants with large tiller angles have a spreading habit and may therefore experience less pressure for some diseases, and less competition for resources, but such plants occupy a large space, reducing yields per unit area. Alternatively, extremely compact plants with small tiller angles are less efficient at harvesting light and are more susceptible to pathogen attack, though theoretically enable higher yields within a given area. In practice, a compromise is adopted to enable high density of planting while retaining efficient light interception and minimal competition between neighboring plants to achieve optimum rice grain production (Xu et al., 1998; Wang and Li, 2008, 2011; Ikeda et al., 2013). Because of its pronounced influence on yield, tiller angle has long attracted the attention of breeders and plant biologists, and the elucidation of the mechanism(s) controlling tiller angle could enable the development of high-yielding rice varieties with ideal plant architecture suited to different environments and practices.

Research over the past several decades has identified quantitative trait loci (QTL) that regulate rice tiller angle (Xu et al., 1995; Li et al., 1999, 2006; Yan et al., 1999; Qian et al., 2001; Thomson et al., 2003; Yu et al., 2007; Dong et al., 2016; He et al., 2017). The major QTL *TILLER ANGLE CONTROL1* (*TAC1*) is a key regulator responsible for the different tiller angles that are characteristic of *japonica* versus *indica* rice (Yu et al., 2007). A mutation in the 3′-splicing site of its fourth intron decreases the expression level of *TAC1* and results in the compact plant architecture of *japonica* rice, while a high expression level of *TAC1* results in the larger tiller angles of *indica* rice. Moreover, it has been reported that TAC1 has conserved function in regulating tiller or branch angle in peach (*Prunus persica*) trees, *Arabidopsis thaliana*, wheat (*Triticum aestivum*), and *Miscanthus sinensis* (Dardick et al., 2013; Zhao et al., 2014; Cao et al., 2017), and

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leaf angle in maize (*Zea mays*) (Ku et al., 2011). Two other major QTLs, *TAC3* and *DWARF2* (*D2*), were recently identified through genome-wide association studies (Dong et al., 2016). However, previous approaches to identify QTLs that determine tiller angle have been influenced by the environmental conditions and mapping populations employed. Thus, such QTLs have been difficult to characterize and are of limited use in rice breeding. Several key genetic regulators of rice tiller angle have been identified via map-based cloning, including *LAZY1* (*LA1*), *PROSTRATE GROWTH1* (*PROG1*), and *LOOSE PLANT ARCHITECTURE1* (*LPA1*) (Li et al., 2007; Yoshihara and Iino, 2007; Jin et al., 2008; Tan et al., 2008; Wu et al., 2013). The first identified gene that controls rice tiller angle is *LA1*. LA1 regulates rice shoot gravitropism and tiller angle through an asymmetric auxin pathway, which affects auxin transport leading to the asymmetric distribution of auxin (Li et al., 2007; Yoshihara and Iino, 2007). Later studies demonstrated the conserved function of LA1 in maize and Arabidopsis (Dong et al., 2013; Yoshihara et al., 2013).

PROG1 encodes a C2H2 zinc-finger protein and might act as a transcriptional factor. When *PROG1* was disrupted in cultivated varieties, the rice plant became erect and led to more grains and higher grain yield, suggesting that *PROG1* has pleiotropic effects on tiller angle, tiller number, grain number, and ultimately grain yield (Jin et al., 2008; Tan et al., 2008). *LPA1*, encoding the plant-specific INDETERMINATE DOMAIN (IDD) protein, an ortholog of Arabidopsis IDD15/SHOOT GRAVITROPISM5 (SGR5), regulates shoot gravitropism and tiller angle by affecting the rate of amyloplast sedimentation (Wu et al., 2013). Reverse genetics studies have also shown that ADP-GLUCOSE PYROPHOSPHORYLASE1 (AGPL1), PIN-FORMED2 (PIN2), *Oryza sativa* LEAF AND TILLER ANGLE INCREASED CONTROLLER (OsLIC), and α-1,3-FUCOSYLTRANSFERASE (FUCT) are regulators of rice tiller angle (Wang et al., 2008; Chen et al., 2012; Okamura et al., 2013; Harmoko et al., 2016). However, the molecular mechanism underlying the control of tiller angle and the functional relationships among known regulatory genes remain to be elucidated.

Previous studies have indicated that rice tiller angle is strongly related to plant gravitropic responses (Abe and Suge, 1993; Li et al., 2007; Wu et al., 2013; Okamura et al., 2015; Roychoudhry and Kepinski, 2015; Harmoko et al., 2016). In plants, gravitropism involves the perception of gravity and signal transduction cascades that lead to an asymmetric distribution of auxin and differential growth between the upper and lower sides of responding organs (Strohm et al., 2012). Genetic studies in Arabidopsis have identified several genes involved in shoot gravitropism such as *SGR* genes and *PHOSPHOGLUCOMUTASE* genes (Hashiguchi et al., 2013, 2014; Kim et al., 2016; Kolesnikov et al., 2016). Although other studies have used "omics" techniques and identified gravitropism-related genes or proteins in several species (Moseyko et al., 2002; Kimbrough et al., 2004; Hu et al., 2013, 2015; Schenck et al., 2013; Taniguchi et al., 2014; Gerttula et al., 2015; Schüler et al., 2015), the direct link between tiller or branch angle and gravitropism has not yet been established.

In the field, tiller angle is susceptible to environment conditions and exhibits variation between plants. Therefore, it is more difficult to characterize tiller angle than other architectural features of agronomic importance, such as plant height and tiller number. Although genetic approaches to identify genes controlling tiller angle in rice have been successful, they have identified relatively few genes so far and are unlikely to identify genes with small or moderate effects on tiller angle. Therefore, high-throughput and high-resolution methods would be more helpful to identify genes involved in controlling tiller angle. Here, we performed a time-course study of changes in the transcriptomes of rice shoots in response to gravistimulation under light, which largely mimics the natural condition in the field, to screen for more rice tiller angle genes. This approach enabled us to identify genes with distinct temporal expression patterns and to uncover a regulatory network involving genes and hormones that controls rice tiller angle. Specifically, we showed that *HEAT STRESS TRANSCRIPTION FACTOR 2D* (*HSFA2D*) responds to gravistimulation and induces the transcription of *LA1*, thereby initiating the asymmetric distribution of auxin. Subsequently, auxin induces the asymmetric expression of *WUSCHEL RELATED HOMEOBOX6* (*WOX6*) and *WOX11*, two transcription factors that specify tiller angle. These results reveal a core regulatory pathway underlying rice tiller angle.

RESULTS

High-Resolution Transcriptomic Changes Induced by Gravistimulation

We subjected rice seedlings to gravistimulation under light by gently rotating the seedlings 90°, from the vertical to a horizontal position. To characterize the dynamic morphological responses, we first used time-lapse photography to capture images and observed that an upward growth phenotype was visible in seedlings after 8 h gravistimulation (Figure 1A; Supplemental Figure 1). Next, we selected 12 time points within this 8-h period (0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 h) and used RNA-seq to profile the dynamic changes in transcriptome in the basal 1.5 cm of the gravistimulated shoot, compared with control shoots that were not stimulated (Figure 1B). Principle component analysis (Figure 1C) showed clear differentiation of the control and treatment groups and identified clustering of samples from the various time points. Up to 2 h, the samples from the control group and treatment group were not clearly differentiated; by 3 h, however, the two groups were distinct. According to this result, we divided the transcriptome time course into two stages: an early stage (0, 0.25, 0.5, 1, 1.5, and 2 h) and a late stage (3, 4, 5, 6, 7, and 8 h) (Figures 1C and 1D). To further identify the genes involved in the transcriptional regulation of gravistimulation, we compared the expression of all genes between treatment and control samples at each time point. Differentially expressed genes (DEGs) were identified from the 22,522 genes with RPKM > 1 in at least one sample. The genes with an empirical P value < 0.005 by permutation test were considered to show a significant difference at a certain time point. As our data were generated from multiple samples harvested at short time intervals, it allowed us to further filter these genes, and only the genes that showed a significant difference in at least two consecutive time points were ultimately regarded as DEGs. Through this method, we identified a total of 4204 DEGs that showed

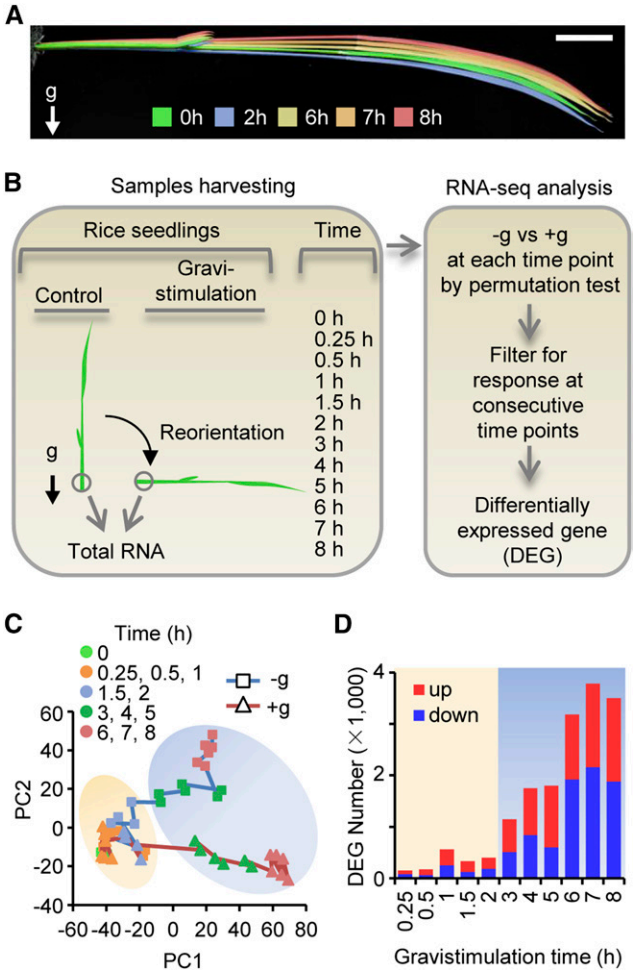

Figure 1. Overview of Transcriptome of Rice Shoot upon Gravistimulation under Light.

(A) Displacement of the rice shoot during the 8-h period upon gravistimulation under light. Bar = 1.2 cm. The arrow marked with "g" indicates the direction of gravity.
(B) RNA-seq strategy. Circles indicate that 1.5-cm shoot bases were harvested for total RNA extraction. The arrow marked "g" indicates the direction of gravity. –g, control; +g, gravistimulation under light.
(C) Principle component analysis of controls and treatment samples. The orange background indicates early stage samples and blue indicates late stage samples. –g, control; +g, gravistimulation under light.
(D) Number of DEGs at different time points upon gravistimulation under light.

a significant difference in at least two consecutive time points (Figure 1D; Supplemental Data Set 1).

Regulatory Network of Genes Responsive to Gravistimulation

To explore the possible functions of DEGs, we grouped them into 98 early DEGs, which showed differential expression patterns only at the early stage; 3246 late DEGs, which showed differential expression patterns only at the late stage; 860 dual DEGs, which showed consecutive differential expression patterns at both stages (Figure 2A). These three groups of genes were further clustered into two early clusters (ECs), four dual clusters (DCs), and four late clusters (LCs) according to their expression profiles (Figure 2A; Supplemental Data Set 1). EC1, DC1, DC2, LC1, and LC3 displayed activated expression patterns, while EC2, DC3, DC4, LC2, and LC4 were repressed upon gravistimulation in the light. Each of the clusters was subjected to both KEGG pathway enrichment and Gene Ontology (GO) term enrichment analyses (Supplemental Tables 1 and 2). DEGs in EC1 displayed a rapid activation after gravistimulation. KEGG pathway enrichment analysis showed that the EC1 DEGs were significantly enriched for "protein processing in endoplasmic reticulum," and GO term enrichment analysis showed enrichment of "unfolded protein binding" (Supplemental Figures 2A and 2B), suggesting that protein processing may be involved in the response of rice shoots to gravistimulation under light. Genes for diverse pathways were enriched in DCs including "plant hormone signal transduction" and "transcription regulator activity" (Supplemental Figures 2A and 2B). More GO terms and KEGG pathways were enriched in LCs than in ECs and DCs (Supplemental Figures 2A and 2B), implying that the late response to gravistimulation might be a consequence of multiple biological processes.

We then predicted the relationships among different clusters. The clusters are connected based on the information from RiceNet (Lee et al., 2011), which can preferentially connect functionally related genes. The result implied that these clusters may be functionally related and formed a complex regulatory network (Figure 2B; Supplemental Data Set 2). As different gene clusters show early or late responses to gravitropism in a sequential pattern, we further inferred that these functionally related clusters are likely to be upstream and downstream, directly or indirectly. Based on this analysis, the responsive clusters were separated into three layers and displayed a hierarchical relationship (Figure 2B). The EC1 cluster in the upper layer directly connected with all DCs in the middle layer and with LC1 in the third layer. These results suggest the possibility that EC1 might positively affect DC1, DC2, and LC1, but negatively affect DC3 and DC4 upon gravistimulation. The LC2 and LC4 clusters were related to DC3, and LC3 was related to DC2. Consistent with this, LC3 and DC2 showed similar upregulation expression patterns, while LC2, LC4, and DC3 displayed similar downregulation patterns. Since "protein processing in endoplasmic reticulum" in EC1 was the earliest activated pathway (Figure 2B; Supplemental Figure 2A), it could potentially influence downstream responsive genes, including transcription factor (TF) genes in DCs that may play key roles in hormone signal transduction upon gravistimulation (Figure 2B; Supplemental Figures 2A and 2B). Further experiments will be required to establish any causal relationship between them.

Auxin-Related DEGs Are Enriched in Response to Gravistimulation

It is well known that auxin is critical for plant gravitropic responses (Žádníková et al., 2015). In the RiceXPro database,

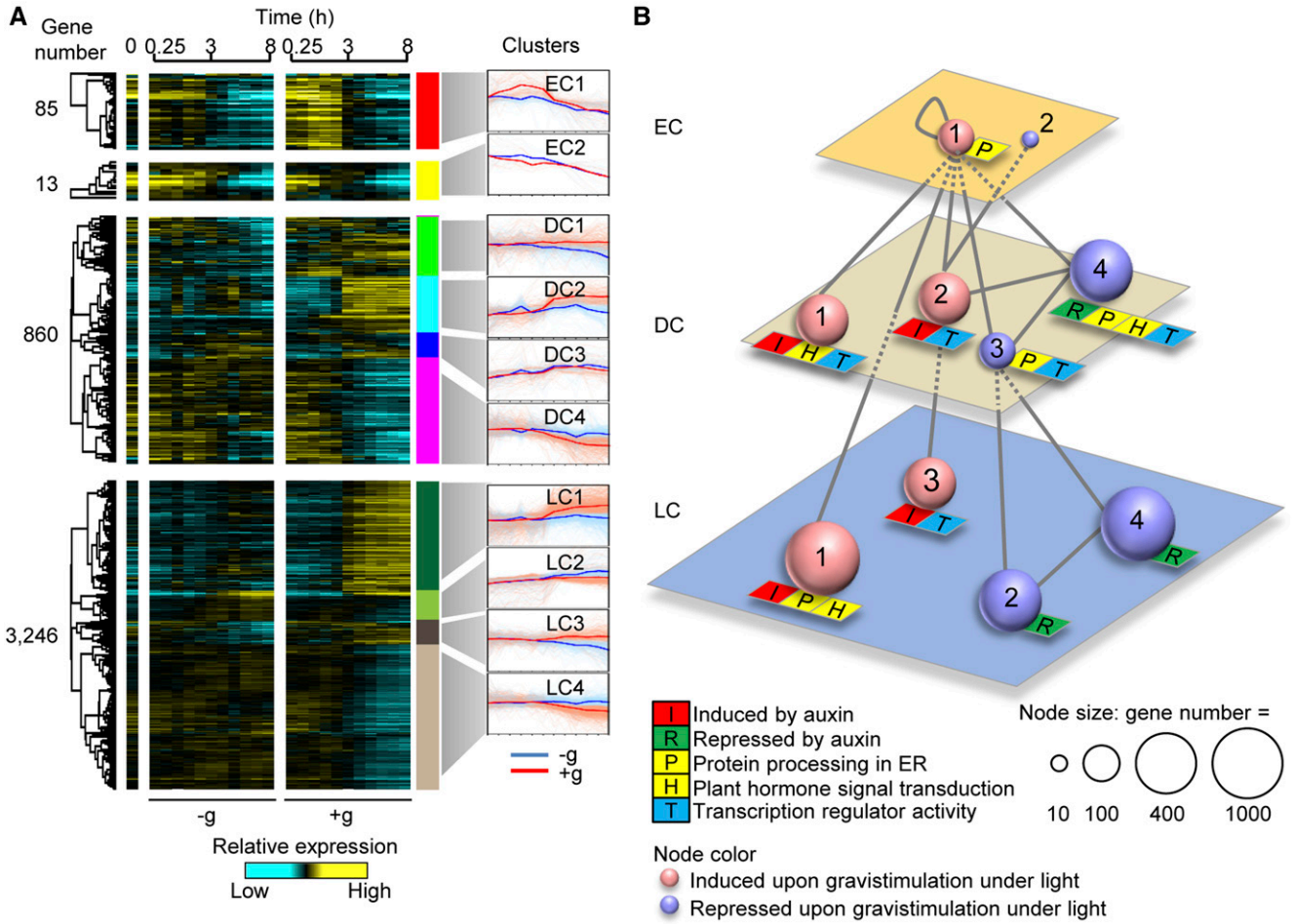

Figure 2. Temporal Expression Patterns and Relationship of Responsive Clusters.

(A) Temporal expression pattern of responsive DEGs and clusters upon gravistimulation under light. The gray shaded markings indicate the corresponding relationship between the left and right panels. –g, control; +g, gravistimulation under light.

(B) Relationship analysis of responsive clusters based on RiceNet. Each node represents different cluster of DEGs. Each edge connecting two nodes indicates that the DEGs in these two clusters are significantly connected in RiceNet (empirical P value < 0.001).

there are multiple transcriptome data sets (Sato et al., 2013). We used the "shoot gene expression profile in response to auxin" microarray data set (ID: RXP-1008) to perform in silico analysis in our study. Gravistimulation-induced clusters DC1, DC2, LC1, and LC3 were significantly enriched in auxin-activated genes, while gravistimulation-repressed clusters DC4, LC2, and LC4 were enriched in auxin-repressed genes (Figure 2B; Supplemental Figure 2C). However, no auxin-responsive genes showed significant enrichment in ECs. This observation strongly suggests that auxin may not participate in the early response to gravistimulation.

To further investigate the involvement of auxin-related genes in response to gravistimulation under light, we analyzed the temporal expression patterns of DEGs for auxin biosynthesis (annotated in KEGG pathway dosa00380: tryptophan metabolism) and signaling (annotated in KEGG pathway dosa04075: plant hormone signal transduction) (Figure 3). The auxin biosynthetic genes *YUCCA3* and *YUCCA7* were present among downregulated DEGs, while *YUCCA6* was gradually induced upon gravistimulation under light. In addition, three *ALDEHYDE DEHYDROGENASE* genes and *INDOLE-3-ACETALDEHYDE OXIDASE3*, which are annotated as auxin biosynthetic genes in KEGG (pathway dosa00380), were among the DEGs (Figures 3A and 3B). *SMALL AUXIN UP RNA* (*SAUR*) and *AUXIN/INDOLE ACETIC ACID* family genes are early auxin-responsive genes (Hagen and Guilfoyle, 2002), and our DEG analysis revealed that the *SAUR* family genes (excepting *SAUR8*) were among the upregulated DEGs following gravistimulation under light. Five *IAA* genes were activated upon gravistimulation under light, while *IAA25* was a downregulated DEG. The *AUXIN RESPONSE FACTOR* genes *ARF2* and *ARF9* were downregulated DEGs, but *ARF7* displayed weak upregulation (Figures 3A and 3B). These results suggest the possibility that auxin biosynthesis and signaling could play a role in the response of rice shoots to gravistimulation under light.

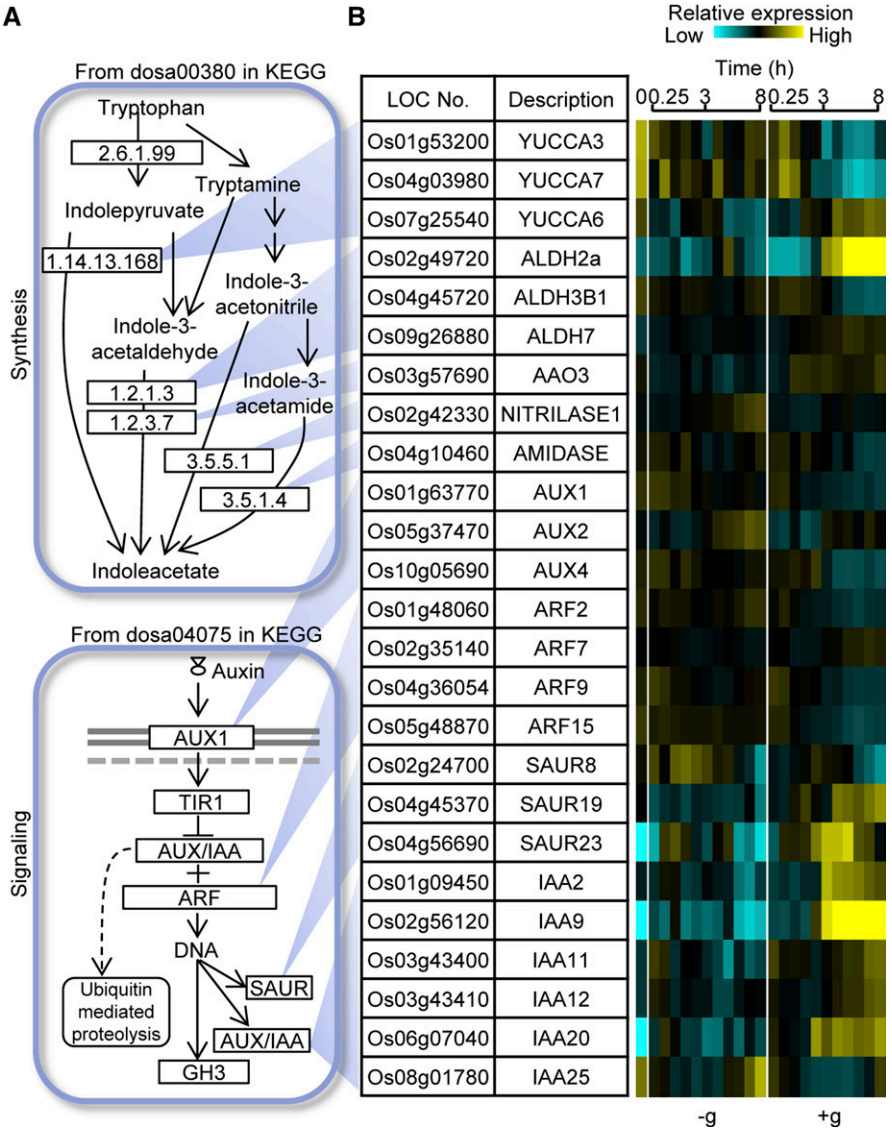

Figure 3. Auxin Biosynthesis and Signaling Pathway-Related Genes of Rice Shoot upon Gravistimulation under Light.

(A) Auxin biosynthesis and signaling pathway related genes from KEGG database.
(B) Heat map showing the normalized time course expression patterns of auxin biosynthesis and signaling pathway related genes in response to gravistimulation under light of rice shoot. –g, control; +g, gravistimulation under light.

HSFA2D Acts Upstream of the *LA1*-Dependent Asymmetric Auxin Pathway

According to their early responsive expression profile, the genes in EC1 are likely to function upstream of auxin in gravitropism. We found that three TF genes of EC1 showed rapid induction of expression (0.25, 0.5, and 1 h time points) upon gravistimulation under light: *HSFA2D*, *MADS57*, and *EARLY PHYTOCHROME RESPONSIVE1* (*EPR1*) (Figure 4A). The expression patterns of these TF genes were validated by RT-qPCR (Figure 4B; Supplemental Figure 3). We then focused on *HSFA2D* and obtained a knockout mutant *hsfa2d* from the Rice Mutant Database (RMD) (Supplemental Figures 4A to 4C). The field-grown adult plants of the *hsfa2d* mutant plants had significantly larger tiller angles than did wild-type plants and the phenotype of *hsfa2d* could be rescued by transforming with *ProHSFA2D:HSFA2D-3×Flag* and *ProHSFA2D:HSFA2D-eGFP* constructs (Figures 4C and 4D; Supplemental Figure 4D), which confirms that *HSFA2D* is responsible for the tiller angle phenotype of *hsfa2d*. Examination of the *hsfa2d* seedlings also revealed that they exhibited a reduced shoot gravitropic curvature under light and in the dark compared with wild-type seedlings (Figures 4E and 4F), and transgenic complementation of *hsfa2d* also rescued the reduced shoot gravitropic response of *hsfa2d* (Supplemental

Figure 4E), indicating that *HSFA2D* is involved in the regulation of tiller angle through mediating the rice shoot gravitropic response.

Assuming a role for auxin, given the large number of auxin-related genes among the DEGs, and considering that *HSFA2D* expression is rapidly induced, we wondered whether *HSFA2D* functions upstream or downstream of auxin in response to gravistimulation. Treatment of wild-type plants with the synthetic auxin analog naphthaleneacetic acid (NAA) did not induce *HSFA2D* expression, supporting the idea that *HSFA2D* controls tiller angle upstream of auxin action (Figure 4G). We therefore tested whether *HSFA2D* influenced the distribution of auxin. We delivered ^{3}H-IAA to the apical end of coleoptile sections and then positioned them horizontally for 2.5 h before using a scalpel to divide the coleoptile sections into upper and lower portions. The lower portions from wild-type coleoptiles had obvious preferential accumulation of ^{3}H, but this asymmetric distribution was much less pronounced for the *hsfa2d* coleoptiles (Figure 4H), indicating that lateral auxin transport (LAT) was attenuated in *hsfa2d.* Consistent with the reduced LAT in the mutant, RT-qPCR assays of gravistimulation of seedlings under light revealed that, compared with the wild type, the lower portion of *hsfa2d* shoot bases had reduced expression of the auxin-responsive marker gene *IAA20* (Figure 4I; Supplemental Figure 5).

Previous work showed that *LA1* also functions upstream of auxin and that the *LA1*-dependent asymmetric auxin pathway controls shoot gravitropism and tiller angle in rice (Li et al., 2007; Yoshihara and Iino, 2007). Our finding that *HSFA2D* also functions upstream of auxin and regulates auxin asymmetric distribution prompted us to examine the relationship between *HSFA2D* and *LA1*. We first examined the expression of *LA1* in *hsfa2d* shoot bases and found that disruption of *HSFA2D* resulted in a decreased expression of *LA1* (Figure 5A). Nevertheless, *HSFA2D* expression levels were comparable in the *la1* mutant and wild-type shoot bases (Figure 5B). These results suggested that *LA1* acts downstream of *HSFA2D*. Moreover, in the field, the *la1* single mutant and *hsfa2d la1* double mutant plants exhibited similar large tiller angle phenotypes (Figures 5C and 5D), and overexpression of *LA1* rescued the tiller angle phenotype of *hsfa2d* mutant plants (Figures 5E and 5F). Collectively, these results show that *HSFA2D* is a positive regulator of the *LA1*-dependent asymmetric auxin pathway in controlling tiller angle.

WOX6 and WOX11 Are Downstream Regulators of the *LA1*-Dependent Asymmetric Auxin Pathway to Control Tiller Angle

Previous work has shown the essential role of *LA1* in the asymmetric distribution of auxin in tiller-angle-related gravitropic responses (Li et al., 2007; Yoshihara and Iino, 2007). However, how auxin gradients trigger downstream tiller-angle-related processes is unknown. Considering that auxin-responsive genes were enriched among the DEGs in response to gravistimulation under light, we focused on the auxin-activated TF genes in DC1 and DC2 and auxin-repressed TF genes in DC4 and identified 17 auxin-responsive TF genes (Supplemental Figure 6). By analyzing auxin-responsive TF genes, we found that *WOX6* and *WOX11* were remarkably responsive to gravistimulation (Figures 6A and 6B; Supplemental Figure 6). We thus treated wild-type plants with NAA and found that the expression of both *WOX6* and *WOX11* was induced in response to auxin (Figure 6C). We then examined the expression levels of *WOX6* and *WOX11* in the upper and lower sides of seedling shoot bases upon gravistimulation under light and found that both genes had asymmetrical expression patterns, which was similar to the pattern of the auxin-responsive marker gene *IAA20* (Figure 6D). Collectively, these results suggest that the expression of *WOX6* and *WOX11* is induced by auxin and that the asymmetric distribution of auxin in rice shoots is responsible for the asymmetric expression of these two TFs.

To investigate the possible functional roles of *WOX6* and *WOX11* in controlling tiller angle, we used CRISPR/Cas9 (CR) to interrupt each of these two genes and also generated *wox6 wox11* double mutants (Figure 6E; Supplemental Figure 7). In the field, neither the *CR-wox6* single mutant nor the *CR-wox11* single mutant displayed an abnormal tiller angle phenotype (Figures 6E and 6F; Supplemental Figures 7E and 7F). Neither of the single mutants differed in their shoot curvature response from the wild type upon gravistimulation under light (Figure 6G). However, the *wox6 wox11* double mutant plants had increased tiller angles compared with the wild type in the field (Figures 6E and 6F; Supplemental Figures 7E and 7F), and the double mutant seedling curvature response was less sensitive to gravistimulation under light than the wild type (Figure 6G). These results show that *WOX6* and *WOX11* function downstream of auxin in responses to gravistimulation and act redundantly to control rice tiller angle.

Since *HSFA2D* and *LA1* function to redistribute auxin in response to gravistimulation, we further explored the relationships among *HSFA2D*, *LA1*, *WOX6*, and *WOX11*. We examined the expression of *WOX6* and *WOX11* upon gravistimulation under light in *hsfa2d* and *la1* mutant plants. Compared with that of wild-type plants, the extent of the induction of *WOX6* and *WOX11* expression in the lower side of shoot bases was significantly reduced in both *hsfa2d* and *la1* mutant plants (Figure 7), suggesting that *HSFA2D* and *LA1* regulate rice tiller angle via the auxin-mediated asymmetric expression of *WOX6* and *WOX11*.

DISCUSSION

Rice tiller angle is an important agronomic trait that contributes to plant architecture and grain yield by determining the compactness of the plants. Several genes that determine rice tiller angle have been cloned and characterized (Li et al., 2007; Yoshihara and Iino, 2007; Yu et al., 2007; Jin et al., 2008; Tan et al., 2008; Wang et al., 2008; Okamura et al., 2013; Wu et al., 2013; Lu et al., 2015a; Dong et al., 2016; Harmoko et al., 2016); however, the regulatory network or pathway of these genes involved in the control of rice tiller angle is largely unknown. Moreover, progress in identifying new genes has been limited, which limits applications in improving rice architecture and elucidation of the molecular mechanisms controlling tiller angle. In this study, we established a core regulatory network of rice tiller angle that directly links tiller angle, auxin, and genes responding to gravistimulation. We propose a core pathway, comprising

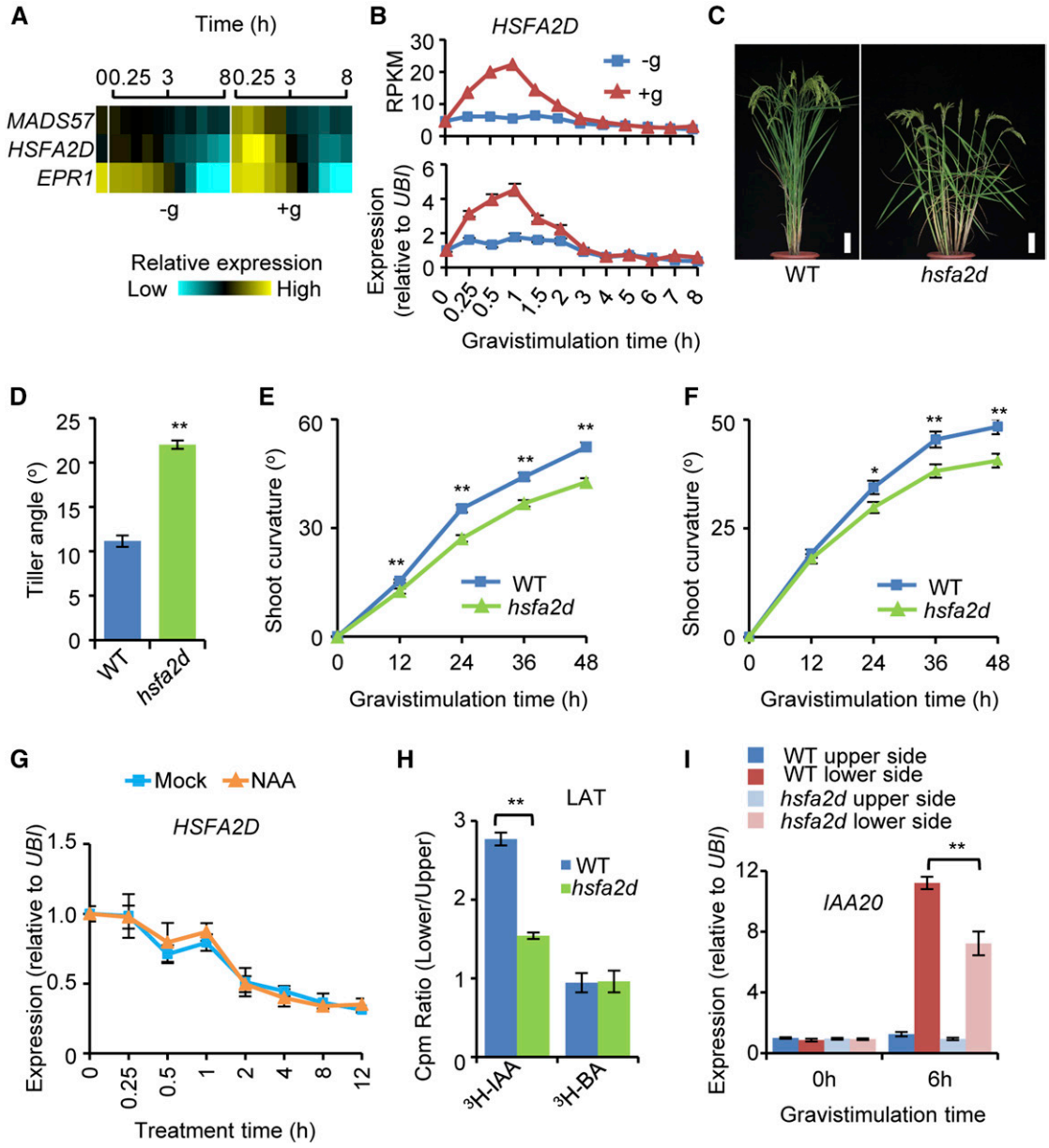

Figure 4. *HSFA2D* Is an Early Responsive Transcriptional Factor upon Gravistimulation under Light and Regulates Rice Tiller Angle through Affecting Auxin Asymmetric Distribution.

(A) Heat map showing the normalized time course expression patterns of *MADS57*, *HSFA2D*, and *EPR1* in rice shoot upon gravistimulation under light.
(B) qRT-PCR (lower panel) validation of RNA-seq results (upper panel) of *HSFA2D*. –g, control; +g, gravistimulation under light. Values are mean ± SE (n = 3).
(C) Phenotypes of the wild type and *hsfa2d* at the mature stage. Bars = 10 cm.
(D) Tiller angle of the wild type and *hsfa2d* at the mature stage. Values are mean ± SE (n = 10).
(E) and **(F)** Shoot curvature of the wild type and *hsfa2d* upon 48-h gravistimulation under light **(E)** and in the dark **(F)**. Values are mean ± SE (n = 20).
(G) Time-course response of *HSFA2D* upon NAA Treatment. The expression levels were determined by RT-qPCR. Values are mean ± SE (n = 3).
(H) Lateral distribution of ^{3}H-IAA in wild type and *hsfa2d* coleoptiles laid horizontally. ^{3}H-benzyl adenine (BA) distribution was analyzed for comparison as control. The ratio indicates the radioactivity of the lower side to that of the upper side of coleoptiles upon gravistimulation (n = 5).
(I) Expression levels of *IAA20* at the lower and upper sides of the shoot base in the wild type and *hsfa2d* seedlings upon gravistimulation under light for 0 and 6 h revealed by RT-qPCR. Values are mean ± SE (n = 3). Significance is determined by two-sided Student's t test, *P < 0.05 and **P < 0.01 (Supplemental File 1).

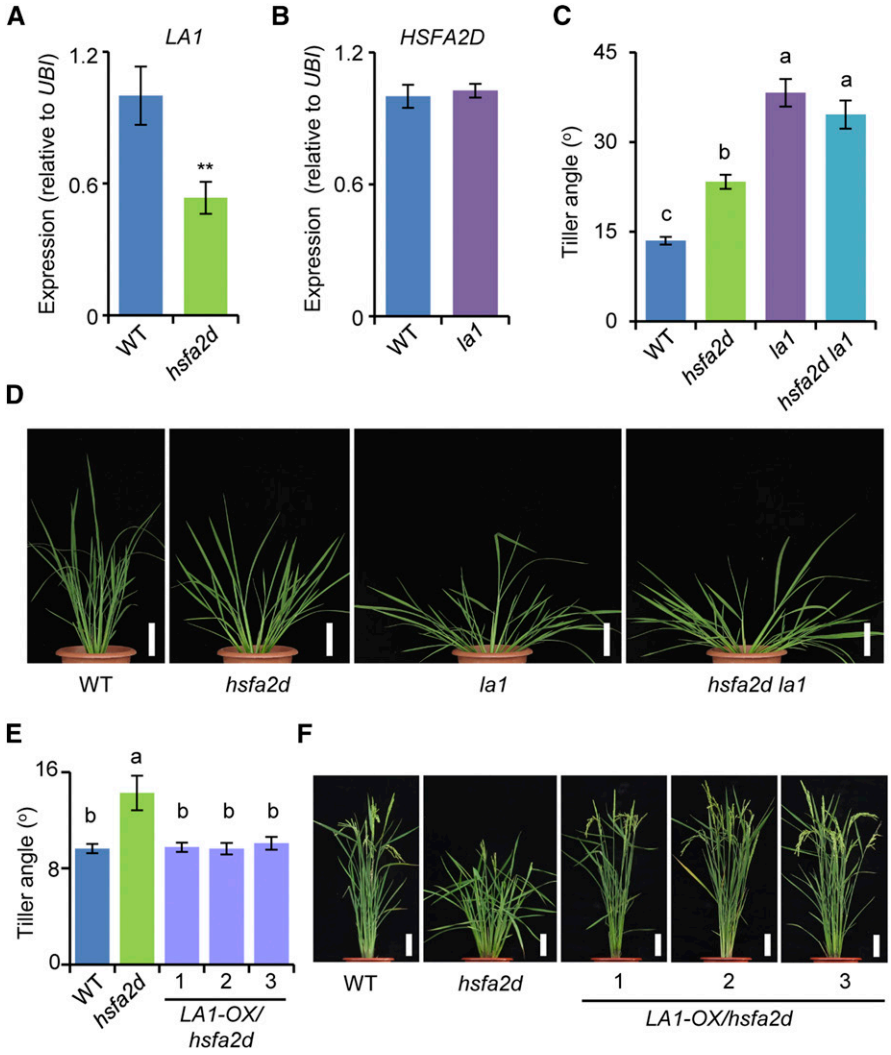

Figure 5. *HSFA2D* and *LA1* May Regulate Rice Tiller Angle through the Same Genetic Pathway.

(A) Expression levels of *LA1* in the wild type and *hsfa2d* revealed by qRT-PCR. Values are mean ± SE (n = 3). Significance is determined by two-sided Student's t test, **P < 0.01 (Supplemental File 1).
(B) Expression levels of *HSFA2D* in the wild type and *la1* revealed by qRT-PCR. Values are mean ± SE (n = 3).
(C) Tiller angle of the wild type, *hsfa2d*, *la1*, and *hsfa2d la1* at the tillering stage. Values are mean ± SE (n = 10). Means with different letters are significantly different (P < 0.05; ANOVA and Tukey's honest significant difference; Supplemental File 1).
(D) Phenotypes of the wild type, *hsfa2d*, *la1*, and *hsfa2d la1* at the tillering stage. Bars = 10 cm.
(E) Tiller angle of the wild type, *hsfa2d*, and *LA1-OX*/*hsfa2d* at the mature stage. Values are mean ± SE (n = 10). Means with different letters are significantly different (P < 0.05; ANOVA and Tukey's honest significant difference).
(F) Phenotypes of the wild type, *hsfa2d*, and *LA1-OX*/*hsfa2d* at the mature stage. Bars = 10 cm.

HSFA2D, *LA1*, *WOX6*, and *WOX11*, to explain how gravitropism and, hence, rice tiller angle is regulated. In this model, the early gravistimulation-responsive gene *HSFA2D* controls the expression of *LA1* and leads to the asymmetric distribution of auxin. Consequently, two key TF genes, *WOX6* and *WOX11*, are induced by auxin and are expressed asymmetrically after gravistimulation in the rice shoot base and thus altering rice tiller angle (Figure 8).

Dynamic Transcriptome of Rice Shoot upon Gravistimulation under Light Provides a Gene Resource for Dissecting Molecular Control of Rice Tiller Angle

A previous study identified gravitropic response genes by microarray analysis and comparison of upper and lower sides of rice shoot bases upon gravitropic stimulation in the dark at two time points (0.5 and 6 h) (Hu et al., 2013). Other "omics"

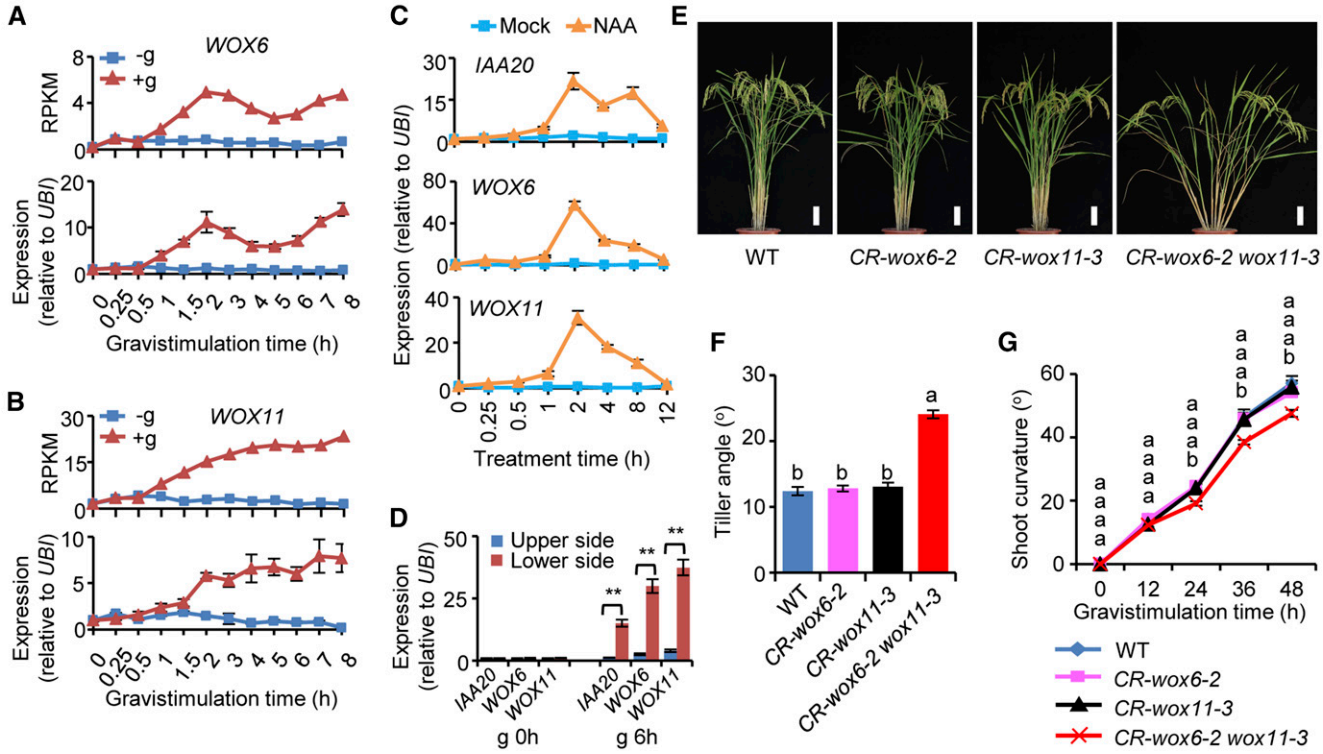

Figure 6. *WOX6* and *WOX11* Are Induced by Auxin Asymmetric Distribution and Act Redundantly to Regulate Tiller Angle.

(A) and **(B)** qRT-PCR (lower panel) validation of RNA-seq results (upper panel) of *WOX6* **(A)** and *WOX11* **(B)**. Values are mean ± SE (n = 3).
(C) Expression levels of *IAA20*, *WOX6* and *WOX11* upon NAA treatment. Analysis by qRT-PCR. Values are mean ± SE (n = 3).
(D) Expression levels of *IAA20*, *WOX6* and *WOX11* at the lower and upper sides of the shoot base upon gravistimulation under light for 0 and 6 h revealed by qRT-PCR. g, gravistimulation under light. Values are mean ± SE (n = 3). –g, control; +g, gravistimulation under light. Significance is determined by two-sided Student's t test, **P < 0.01.
(E) Phenotypes of the wild type, *CR-wox6*, *CR-wox11*, and *CR-wox6 wox11* at the mature stage. Bars = 10 cm.
(F) Tiller angle of the wild type, *CR-wox6*, *CR-wox11*, and *CR-wox6 wox11* at the mature stage. Values are mean ± SE (n = 10).
(G) Shoot curvature of the wild type, *CR-wox6*, *CR-wox11*, and *CR-wox6 wox11* upon 48-h gravistimulation under light. Values are mean ± SE (n = 20). Multiple comparisons are performed at each time point. Means with different letters are significantly different (P < 0.05; ANOVA and Tukey's honest significant difference).

approaches have also been used to identify gravitropism-related genes or proteins in Arabidopsis. However, few of these studies used a strategy with multiple closely-spaced time points (Moseyko et al., 2002; Schenck et al., 2013; Taniguchi et al., 2014). Another study has reported transcriptome profiling of Arabidopsis root apex gravitropism at multiple consecutive time points (0, 2, 5, 15, 30, and 60 min) but analyzed gene expression only at each single time point and did not consider DEGs at consecutive time points (Kimbrough et al., 2004). In our study, the data were generated from samples harvested at multiple time points, allowing genes to be further filtered so that only genes showing a significant difference in at least two consecutive time points were finally regarded as DEGs. In this way, we identified 4204 DEGs in response to gravistimulation (Supplemental Data Set 1). A previous study using microarray data of the rice shoot gravitropic response also found that *WOX6* and *WOX11* were gravistimulation responsive, but not *HSFA2D* (Hu et al., 2013). This is most likely because *HSFA2D* is not differentially expressed between the upper side and lower side of the rice shoot base upon gravistimulation, as is also the case for other reported rice tiller angle genes (Supplemental Figure 8). These results indicate that our more rigorous approach has a greater capability for detecting gravitropism and rice tiller angle genes.

In addition, the expression of some gravitropically responsive genes is light dependent. For example, the promoter region of *LA1* in maize contains 14 light-responsive elements, and the transcription of *LA1* is repressed by light (Dong et al., 2013), which implies that light may also participate in regulating shoot gravitropism and tiller angle. Therefore, the dynamic changes in the transcriptome upon shoot gravistimulation under light provides greater potential for discovering new genes involved in the regulation of rice tiller angle, which will facilitate dissection of molecular mechanisms underlying the regulation of rice tiller angle.

It was well known that auxin plays a central role in gravitropism (Žádníková et al., 2015); however, which auxin-related genes are

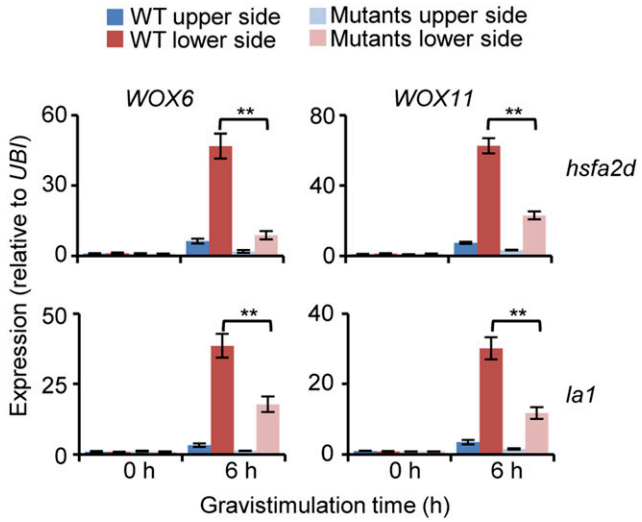

Figure 7. *HSFA2D* and *LA1* Regulate *WOX6* and *WOX11* Asymmetric Expression upon Gravistimulation under Light.

Expression levels of *WOX6* and *WOX11* at the lower and upper sides of the shoot base in the wild type, *hsfa2d*, and *la1* seedlings upon gravistimulation under light for 0 and 6 h revealed by qRT-PCR. Values are mean ± SE (n = 3). Significance is determined by two-sided Student's t test, **P < 0.01.

specifically involved in gravitropism and tiller angle are poorly understood. Our study shows that a set of auxin biosynthesis and signaling genes could respond to gravistimulation (Figure 3). Additional auxin-related genes were revealed through another set of microarray data in the dark (Hu et al., 2013). Further characterization of auxin-related genes from these two sets of data will provide clues to understand the roles of auxin-related genes specifically involved in controlling shoot gravitropism and for determining whether these genes act as key components in the control of tiller angle.

HSFA2D-Regulated *LA1* May Represent an Early Molecular Event Controlling Rice Tiller Angle

Although auxin asymmetric distribution has long been considered to be the prerequisite for asymmetric organ curvature in response to gravity, how the gravity signal induces an auxin gradient is still not well understood. According to our transcriptome data, genes in early responsive clusters may function upstream of auxin and direct auxin redistribution upon gravistimulation. We verified that *HSFA2D* could regulate tiller angle through controlling auxin asymmetric distribution. Furthermore, we showed that *HSFA2D* is required for activation of the *LA1* gene (Figures 5A and 5B). *HSFA2D* belongs to the heat shock factor (HSF) family, which is grouped into three classes, *HSFA*, *HSFB*, and *HSFC* (Nover et al., 2001). *HSFA2D* is not only able to respond to various abiotic stress conditions, but is also highly expressed in rice panicle and seed (Chauhan et al., 2011), implying that *HSFA2D* may have diverse functions. In the gravitropic response, Ca^{2+} is thought to be a second messenger of signal transduction in gravitropism (Singh et al., 2017). The endoplasmic reticulum serves as an important Ca^{2+} holding organelle in plants (Persson and Harper, 2006) and might be involved in gravity perception and signal transduction in the gravitropic response (Zheng and Staehelin, 2001; Leitz et al., 2009). It was proposed that during plant reorientation, the amyloplast sedimentation triggers the release of Ca^{2+}, which may further mediate downstream responses (Sievers and Busch, 1992). A previous study showed that the HSF was activated in a concentration- and time-dependent manner by Ca^{2+} in HeLa cell cytoplasmic extracts (Mosser et al., 1990). In addition, 13 rice *HSF* genes, including *HSFA2D*, are highly induced by 15-min short-term treatments of $CaCl_2$ (Chauhan et al., 2011). It is likely that *HSFA2D* might be activated by Ca^{2+} and subsequently transduces the gravistimulation signal to mediate downstream responses. Further study will be necessary to uncover the action of HSFA2D involved in Ca^{2+}-mediated gravity signal transduction and to elucidate the

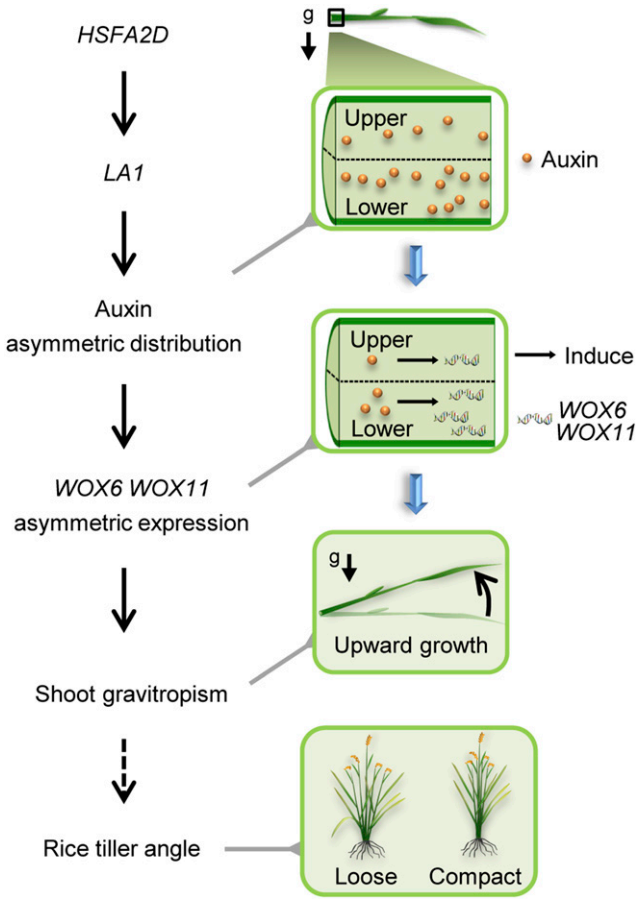

Figure 8. A Regulatory Pathway of Rice Tiller Angle via LA1-Mediated Auxin Asymmetric Distribution.

HSFA2D may regulate auxin asymmetric distribution by regulating expression level of *LA1*, which in turn modulates the asymmetric expression of auxin downstream regulators *WOX6* and *WOX11* and thus modulates rice shoot gravitropism and tiller angle. The arrow marked with g indicates the direction of gravity.

molecular mechanism of how the gravity signal induces auxin gradients and hence regulates rice tiller angle.

WOX6 and *WOX11* Are Downstream Targets of Auxin in Determining Rice Tiller Angle

How an auxin gradient triggers asymmetric growth of a responding organ is still a challenging question. In this study, we identified two gravity-responsive TF genes, *WOX6* and *WOX11*, which function downstream of auxin in shoot gravitropism. Previous studies showed that the rice *WOX* gene family plays diverse roles in shoot, root, trichome, and leaf development (Li et al., 2012; Lu et al., 2015b; Tanaka et al., 2015; Wang et al., 2017a; Zhou et al., 2017). In Arabidopsis, AtWOX11 and AtWOX12, orthologs of rice WOX6 and WOX11, respectively, are reported to play roles in root development (Liu et al., 2014; Hu and Xu, 2016; Sheng et al., 2017). We found that both *WOX6* and *WOX11* contain auxin response elements, TGTCNC (Ponomarenko and Ponomarenko, 2015), in their promoter regions (Supplemental Figure 9). We further verified that *WOX6* and *WOX11* were not only responsive to NAA treatment, but also displayed an asymmetrical expression pattern like that of *IAA20* during shoot gravitropism under light (Figures 6C and 6D). Notably, *WOX6* and *WOX11* showed asymmetric expression levels upon gravity stimulation in the wild type and reduced upregulation of expression in the lower side of shoot bases of *hsfa2d* and *la1*. Unlike *WOX6* and *WOX11*, other genes reported to be involved in regulating rice tiller angle did not show asymmetric expression in shoot bases upon gravistimulation (Supplemental Figure 8). Taking these data together, *WOX6* and *WOX11* may be the downstream targets of auxin in shoot gravitropism and likely act as core regulators of rice tiller angle. Further identification of *WOX6* and *WOX11* downstream transcriptional targets will provide detailed insight into how the auxin gradient triggers asymmetric growth of the shoot base to control rice tiller angle.

METHODS

Plant Materials and Growth Conditions

Zhonghua 11 (ZH11), a *japonica* rice (*Oryza sativa*), was the genetic background of the *hsfa2d*, *la1*, and CR transgenic plants. For field experiments, the plants were grown at spacings of 30 cm in paddy fields under natural conditions. For RNA-seq and time-lapse photography, germinated ZH11 seeds were grown on 0.4% (w/v) agar plates at 28°C for 3 d under a 16-h-light/8-h-dark photoperiod, and then seedlings with uniform growth were transferred into soil and grown under controlled greenhouse conditions (16 h light and 8 h dark at 28°C, 150–200 μmol photons m^{-2} s^{-1}) for 11 d prior to the gravistimulation treatments.

RNA-Seq Analysis of Rice Shoots upon Gravistimulation under Light

ZH11 seedlings were gravistimulated by reorienting plants 90° in the greenhouse. Shoot bases (1.5 cm in length) were harvested at 12 time points (0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 h) after the start of gravistimulation and were frozen immediately in liquid nitrogen. The shoot bases from at least 16 seedlings were pooled to form one biological replicate, and two biological replicates were generated for each sample. Equal numbers of plants were kept vertical as the control, and the same part of the shoot bases from the control plants were harvested at each time point. RNA was extracted using a TRIzol kit according to the user manual (Invitrogen). A total of 2.5 μg RNA per sample was processed into cDNA libraries with an Illumina TruSeq RNA sample preparation kit according to the manufacturer's instructions (Illumina part no. 15026495 Rev. D), and single-end 100-base sequencing was performed on an Illumina HiSeq 2000 instrument. The Bioanalyzer 2100 (Agilent) was used to quantify and assess the quality of the RNA samples and cDNA libraries. The RNA-seq raw reads were mapped to the rice genome (MSU v7) (Kawahara et al., 2013) using TopHat (Trapnell et al., 2009). The read counts for each gene with a rice genome annotation (MSU v7) were obtained using cufflinks (Trapnell et al., 2012). The 22,522 genes with RPKM > 1 in at least one sample were used for further analysis. Principle component analysis was performed using log-transformed RPKM values for all samples using the prcomp function in R version 3.0 with the following parameters: "center = TRUE, scale = FALSE."

Identification of DEGs

For each gene at each time point, an F-statistic was calculated as the variance of averaged RPKM values between the treatment and the control group for a given time point divided by the sum of the variances of all groups across two replicates. "Empirical P values" for each gene at each time point were calculated by shuffling the RPKM among all samples 10,000 times and then comparing the real F-statistic with the shuffled F-statistic. The genes with an empirical P value < 0.005 were regarded as DEGs for a given time point. We further filtered the DEGs, and only the DEGs that showed a significant difference in at least two consecutive time points were further analyzed. We used log-transformed RPKM values to perform the hierarchical clustering algorithm with the hclust function in R version 3.0.

Shoot Gravitropism Assay

The rice seeds were dehusked, surface sterilized with 2.5% (w/v)NaClO for 45 min, and then washed five times with sterile distilled water. The seeds were grown on 0.4% (w/v) agar at 28°C for 3 d (16 h light and 8 h dark at 28°C, 150–200 μmol photons m^{-2} s^{-1}) and were subsequently reoriented by 90° from the vertical for 2 d (gravistimulation treatment); the shoot curvature was recorded every 12 h during treatment.

Quantitative Real-Time PCR

For quantitative real-time PCR (qPCR) assays, 1.5-cm shoot base or the upper and lower portions of the 1.5-cm shoot base of each rice seedling was collected. At least 16 seedlings were pooled for each biological replicate, with three biological replicates for each sample. For all samples assayed by RT-qPCR, reverse transcription reactions were performed with Superscript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. RT-qPCR experiments were performed using a SsoFast EvaGreen supermix kit (Bio-Rad) on a CFX96 real-time system (Bio-Rad) following the manufacturer's instructions. Three technical replicates for each of the three biological replicates were performed for each gene. The expression levels were normalized to the expression of the rice *UBIQUITIN* gene (LOC_Os03g13170). The gene-specific primers used are listed in Supplemental Table 3.

In Silico Analysis of Auxin-Related Genes and Rice TFs

A microarray data set of rice shoot response to auxin treatment was obtained from the "shoot gene expression profile in response to auxin" data set (ID: RXP-1008) in the RiceXPro database (Sato et al., 2013).

Auxin-responsive genes were identified using the analysis tools in RiceXPro (Sato et al., 2013) with a cutoff P value < 0.01 and fold change > 2 for "auxin induced genes" and a P value < 0.01 and fold change < 0.5 for "auxin repressed genes." The enrichment of auxin-responsive genes in different gene clusters responding to gravistimulation was tested by Fisher's exact test with Bonferroni correction. Definition of auxin biosynthesis genes (annotated in KEGG pathway dosa00380: tryptophan metabolism) and auxin signaling genes (annotated in KEGG pathway dosa04075: plant hormone signal transduction) were derived from the KEGG database (http://www.kegg.jp/). The rice transcription factor list was obtained from PlantTFDB 3.0 (Jin et al., 2014).

GO and KEGG Pathway Enrichment Analysis

GO and KEGG pathway enrichment analysis were performed using PlantGSEA (Yi et al., 2013). All enriched GO terms and KEGG pathways are listed in Supplemental Tables 1 and 2. Significantly enriched KEGG pathways or GO terms were selected with cutoff false discovery rate < 0.001, and any gene sets containing more than 1000 genes or fewer than 10 genes were filtered out. The significant GO terms with no child node and all KEGG pathways are shown in Supplemental Figure 2.

RiceNet Analysis

The gene-gene relationship analysis we performed used the Gold Standard Set with positive functional gene associations in RiceNet v2 (Lee et al., 2011). To test whether any of two clusters are functionally related, we tested whether the genes in two clusters are significantly associated or connected in RiceNet. The number of connections between the genes of each two clusters was calculated using RiceNet v2 (Lee et al., 2011). Empirical P value of the connection number of two clusters was calculated by comparing the connection number between two permutated gene sets with the same number of genes in these two clusters (1000 times permutation). Two clusters with empirical P value < 0.001 were considered functionally related. If two clusters are related and show the same activation or inhibition pattern upon gravity stimulation, we inferred that the early cluster might positively regulate the downstream cluster. If two clusters are related, but the opposite expression pattern was observed upon gravity stimulation, we inferred that early cluster might negatively regulate the downstream cluster.

Identification of the T-DNA Insertion Mutant Line

The T-DNA insertion mutant *hsfa2d* (RMD_TTL-03Z11CE69_NTLB5-2) was obtained from the RMD database (Zhang et al., 2006). Homozygous plants for T-DNA insertions were identified by PCR-based genotyping. The primer sequences used for genotyping and RT-PCR are listed in Supplemental Table 4.

Exogenous NAA Treatment

Germinated rice seeds were grown in hydroponic solution. Ten-day-old ZH11 seedlings were transferred to hydroponic solution containing 1 μM NAA in DMSO, or DMSO as the control. A 1.5-cm-long piece of the rice seedling shoot base was harvested for RNA extraction after 0, 0.25, 0.5, 1, 2, 4, 8, and 12 h of NAA treatment.

LAT Assay

LAT was assayed as previously described, with some modifications (Sang et al., 2014). Briefly, 4-d-old dark-grown coleoptiles (1.5 cm) were harvested and deprived of endogenous IAA by preincubation in 0.5× MS (pH 5.8) liquid medium for 2 h. The coleoptiles were laid horizontally with their apical ends inserted into agar blocks that contained 500 nM ^{3}H-IAA or 500 nM benzyl adenine. After transport at 28°C for 2.5 h in the dark, 0.5-cm segments from the nonsubmerged ends were harvested and split evenly with a scalpel into upper and lower side samples. After 18 h of incubation in 2 mL of scintillation fluid, the radioactivity of each side was measured using a liquid scintillation counter (1450 MicroBeta TriLux; Perkin-Elmer).

Constructs for Genetic Transformation

To construct the LA1 overexpression plasmid, the full-length coding sequence (CDS) of *LA1* was amplified by primers attB1_LA1_F and attB2_LA1_R from the cDNA prepared from ZH11 seedlings and cloned into the pMBb7Fm21GW-UBIL vector using a PCR-based Gateway system. The phenotypes were examined in the T1 transgenic plants. To construct the ProHSFA2D:HSFA2D-3×Flag plasmid, a ProHSFA2D:HSFA2D-3×Flag fragment, containing a 2282-bp 5′-upstream sequence, the *HSFA2D* CDS without stop codon, and a 3×FLAG sequence was introduced into the binary vector gateway-pCAMBIA3300 using a PCR-based Gateway system. The pCAMBIA3300 was modified to a gateway-pCAMBIA3300 vector by inserting a fragment containing *att*R1, *ccd*B, and *att*R2 followed by a NOS terminator sequence. The CDS of *HSFA2D* was amplified by primers HSFA2D_BamHI_F and HSFA2D_KpnI_R from the cDNA prepared from ZH11 seedling RNA and cloned into the SC-3×FLAG (SF) vector (Xu et al., 2012) digested with *Bam*HI and *Kpn*I to create SF-HSFA2D plasmid. The 2282-bp *HSFA2D* promoter sequence was amplified by the primers HSFA2D_pro_F and HSFA2D_pro_R from ZH11 genomic DNA, and this sequence is the first fragment. The HSFA2D-3×FLAG sequence was amplified by primers HSFA2D_ATG_F and SC_R from SF-HSFA2D plasmid, and this sequence is the second fragment. The ProHSFA2D:HSFA2D-3×FLAG fragment was amplified with the primers HSFA2D_pro_F and SC_R from the mixture of DNA of the first and second fragments and cloned into the gateway-pCAMBIA3300 vector. The enhanced GFP (eGFP) sequence was added to the 3′ end of the CDS of *HSFA2D* and then the same strategy was used to construct the ProHSFA2D:HSFA2D-eGFP plasmid. The primers are listed in Supplemental Table 5.

Generation of CRISPR/Cas9 Transgenic Lines

Single-guide RNAs (sgRNAs) were designed to target the exon of each gene to generate various mutations within coding sequences (see Supplemental Table 6 for a list of sgRNAs). To generate the *wox6* and *wox11* single mutants, sgRNAs were cloned into a CRISPR/Cas9 vector: either VK005-1 (ViewSolid Biotech) or pYLCRISPR/Cas9P$_{ubi}$-H (Ma et al., 2015). To generate the *wox6 wox11* double mutant, sgRNAs of both genes were cloned into the CRISPR/Cas9 vector pYLCRISPR/Cas9P$_{ubi}$-H. These constructs were introduced into *Agrobacterium tumefaciens* EHA105, and ZH11 was transformed as previously reported (Hiei et al., 1994). The CRISPR/Cas9 transgenic lines were genotyped for mutations using a pair of primers to amplify a region spanning the sgRNA target sequence (Supplemental Table 7). The homozygous T2 or T3 progeny was used for further analysis.

Accession Numbers

The raw RNA-seq data reported in this article have been deposited in the Genome Sequence Archive (Wang et al., 2017b) in the BIG Data Center (BIG Data Center Members, 2017), Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession number CRA000335, accessible at http://bigd.big.ac.cn/gsa. The gene sequence data from this article can be found in the Rice MSU Genome Annotation Release 7 under the following accession numbers: *AGPL1* (LOC_Os05g50380), *CRCT* (LOC_Os05g51690), *D2* (LOC_Os01g10040), *EPR1* (LOC_Os06g51260),

FUCT (LOC_Os08g36840), *HSFA2D* (LOC_Os03g06630), *IAA20* (LOC_Os06g07040), *LAZY1* (LOC_Os11g29840), *LPA1* (LOC_Os03g13400), *MADS57* (LOC_Os02g49840), *ONAC106* (LOC_Os08g33670), *OsLIC1* (LOC_Os06g49080), *PIN2* (LOC_Os06g44970), *PROG1* (LOC_Os07g05900), *TAC1* (LOC_Os09g35980), *TAC3* (LOC_Os03g51660), *TIL1* (LOC_Os04g38720), *WOX6* (LOC_Os03g20910), *WOX11* (LOC_Os07g48560), and *UBI* (LOC_Os03g13170).

Supplemental Data

Supplemental Figure 1. Visualized Morphological Changes of a Rice Seedling upon Gravistimulation under Light.

Supplemental Figure 2. Functional Analysis of DEGs in Response to Gravistimulation under Light.

Supplemental Figure 3. RNA-Seq Results and RT-qPCR Validation of Early TF Genes in EC1.

Supplemental Figure 4. Genotyping of the Wild Type and *hsfa2d* Mutant and Phenotypes of the Wild Type, *hsfa2d*, and Complemented *hsfa2d* Transgenic Plants.

Supplemental Figure 5. Illustration of the Upper and Lower Sides of the Shoot Base of a Rice Seedling upon Gravistimulation.

Supplemental Figure 6. Identification of Auxin-Responsive TF Genes in DCs upon Gravistimulation under Light.

Supplemental Figure 7. Generation of CR-Engineered Mutants of *WOX6* and *WOX11*.

Supplemental Figure 8. Expression Levels of Rice Tiller Angle Genes at the Lower and Upper Sides of the Rice Seedling Shoot Base upon Gravistimulation under Light Revealed by RT-qPCR.

Supplemental Figure 9. Schematic Representation of the *WOX6* and *WOX11* Loci with Predicted AuxREs within 1 kb Upstream of the ATG Site.

Supplemental Table 1. Enriched GO Terms in Clusters.

Supplemental Table 2. Enriched KEGG pathways in Clusters.

Supplemental Table 3. Primer Sequences Used for RT-qPCR Analysis.

Supplemental Table 4. Primer Sequences Used for Genotyping of T-DNA Mutants and RT-PCR.

Supplemental Table 5. Primer Sequences Used for Transgene Constructs.

Supplemental Table 6. Single-Guide RNA Sequences.

Supplemental Table 7. Genotyping Primers for the CR Transgenic Mutants.

Supplemental File 1. ANOVA Tables.

Supplemental Data Set 1. List and RPKM of DEGs in RNA-Seq Data.

Supplemental Data Set 2. Connections between Different Clusters in RiceNet Golden Standard Set.

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AUTHOR CONTRIBUTIONS

N.Z. and Hong Yu designed research, analyzed data, and wrote the article. N.Z. and Hao Yu performed experiments. Y.C., L.H., C.X., G.X., X.M., J.W., H.C., G.L., Y.J., Y.Y., Y.L., and S.L. performed some of the experiments. S.M.S. and J.L. analyzed data and wrote the article. Y.W. supervised the project, designed research, analyzed data, and wrote the article.

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