Grain Number, Plant Height, and Heading Date7 Is a Central Regulator of Growth, Development, and Stress Response

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Grain number, plant height, and heading date7 (Ghd7) has been regarded as an important regulator of heading date and yield potential in rice (Oryza sativa). In this study, we investigated functions of Ghd7 in rice growth, development, and environmental response. As a long-day dependent negative regulator of heading date, the degree of phenotypic effect of Ghd7 on heading date and yield traits is quantitatively related to the transcript level and is also influenced by both environmental conditions and genetic backgrounds. Ghd7 regulates yield traits through modulating panicle branching independent of heading date. Ghd7 also regulates plasticity of tiller branching by mediating the PHYTOCHROME B-TEOSINTE BRANCHED1 pathway. Drought, abscisic acid, jasmonic acid, and high-temperature stress strongly repressed Ghd7 expression, whereas low temperature enhanced Ghd7 expression. Overexpression of Ghd7 increased drought sensitivity, whereas knock-down of Ghd7 enhanced drought tolerance. Gene chip analysis of expression profiles revealed that Ghd7 was involved in the regulation of multiple processes, including flowering time, hormone metabolism, and biotic and abiotic stresses. This study suggests that Ghd7 functions to integrate the dynamic environmental inputs with phase transition, architecture regulation, and stress response to maximize the reproductive success of the rice plant.

Rice (Oryza sativa) is a main staple food crop that feeds almost half of the world population. Flowering time is one of the most important agronomic traits that determines rice yield. *Grain number, plant height, and heading date7 (Ghd7)* encoding a CCT (CONSTANS, CONSTANS-LIKE, and TIMING OF CHLOROPHYLL A/B BINDING1) domain protein is considered to be a key regulator of the rice-specific flowering pathway and also contributes to rice yield potential (Xue et al., 2008). Ghd7 controls the critical daylength response of *Early heading date1* (Ehd1) and florigen expression through circadian gating and phytochrome action (Itoh et al., 2010; Osugi et al., 2011). Two orthologs of EARLY FLOWERING3 genes, which mediate the circadian and photoperiodic regulation, act as negative regulators of Ghd7 (Zhao et al., 2012; Yang et al., 2013). Rice *Indeterminate1* acts as a master switch for the transition from vegetative to reproductive phase and regulates the expression of Ghd7 independent of the photoperiod (Wu et al., 2008). Ehd3, which contains two plant homeodomain finger motifs and is possibly involved in chromatin state modulation, negatively regulates the transcription of Ghd7 (Matsubara et al., 2011). *Heading date16* (Hd16), a flowering time quantitative trait locus gene, was recently shown to encode a casein kinase I protein that mediates the phosphorylation of GHD7 and enhances the photoperiod response (Hori et al., 2013). Although the complex regulation network of Ghd7 at transcription and post-transcription levels in flowering time control has been extensively studied, the regulation domain of Ghd7 in rice growth, development, and environmental response has not been adequately investigated.

Recent studies suggested that traditional flowering time genes may have roles in plant development and stress response. In rice, two key flowering time genes, *Hd1* and *Ehd1*, also control panicle development (Endo-Higashi and Izawa, 2011). In Arabidopsis (Arabidopsis thaliana), the flowering promoting gene GIGANTEA and the florigen genes FLOWERING LOCUS T (FT) and TWIN SISTER OF FT (TSF) play a central role in floral transition and lateral shoot development (Hiraoka et al., 2013). Molecular evidence revealed that FT and TSF proteins directly interact with BRANCHED1/TEOSINTE BRANCHED1-LIKE1 (BRC1) protein, a homolog of TEOSINTE BRANCHED1 (TBI) (Takeda et al., 2003; Choi et al., 2012), and modulate florigen activity in the axillary buds to prevent premature floral transition of the axillary meristems (Niwa et al., 2013). These findings suggest...
that the regulation of the transition to flowering also plays an important role in the modulation of plant architecture plasticity and environment adaptation.

In this article, we show that the flowering time gene Ghd7 also regulates plant architecture and such regulation is dependent on both genetic background and environmental signaling. Ghd7 responds to various environment signals in addition to daylength to regulate growth, development, and biotic and abiotic stress responses. Our results suggest that Ghd7 may function as a sensor for the plant to adapt to dynamic environmental inputs and that Ghd7 is involved in the plant architecture regulation and stress-response pathways.

RESULTS

The Phenotypic Effect of Ghd7 Is Correlated with Its Expression Level

Ghd7 showed pleiotropic effects on heading date, plant height, and yield traits, and its expression was regulated by light signal and photoperiod (Xue et al., 2008; Itoh et al., 2010). We previously developed a pair of near-isogenic lines, designated NIL(zs7) and NIL(mh7), with almost all of the genetic background of Zhenshan 97 except the introgressed segment, which contained the Ghd7 (Xue et al., 2008). Comparison of the phenotypes of NIL(zs7), NIL(mh7), and their hybrid NIL(het) showed that Ghd7 has a partial-dominant effect on flowering time, plant height, and yield traits (Fig. 1, A and D; Table I), consistent with previous results (Xue et al., 2008). The expression level of Ghd7 in NIL(mh7) is nearly twice that in the heterozygous plants, especially at dawn (Fig. 1G). We examined the relation between the expression level of Ghd7 and the phenotype in transgenic plants, in which the coding sequence of Ghd7 from Minghui 63 driven by the ubiquitin promoter was transformed into Hejiang 19 (HJ19) that has a nonfunctional allele of Ghd7 (Xue et al., 2008). Of the 42 T0 plants, 37 were transgene positive (OX-Ghd7HJ19) and exhibited the expected phenotype (tall with late heading and large panicles; Supplemental Fig. S1). Analysis of

Figure 1. Phenotypes and Ghd7 expression levels of the various genotypes generated in this work. A and D, The whole plants (A) and main panicles (D) of NIL(zs7), NIL(het), and NIL(mh7) under natural long-day conditions in Wuhan taken at maturity. B and E, The whole plants (B) and main panicles (E) of the Ghd7-overexpressor in the HJ19 background under natural long-day conditions in Wuhan. C and F, The whole plants (C) and main panicles (F) of OX-Ghd7 and Ami-Ghd7 in the ZH11 background sown in June in Wuhan. Bar in (A) to (C) = 50 cm; bar in (D) to (F) = 10 cm. (G) Diurnal expression analysis of Ghd7 in leaf blades of the near-isogenic lines. The samples were collected at 40 d after germination under natural long-day conditions in Wuhan and were used for RNA preparation. The numbers below the x axis indicate zeitgeber times (ZTs) of the day. The white bar indicates the light period, and the black bar indicates the dark period. The points and error bars indicate average values and SE, respectively, based on three biological repeats. H and I, Expression levels of Ghd7 in transgenic plants in the HJ19 background (H) and the ZH11 background (I). Leaf blades from plants 30 d after germination were collected at 2 h after dawn and used for RNA preparation. Bars and error bars indicate average values and SE, respectively, based on three biological repeats.
two random T1 families (OX-14 and OX-25) from the T0 plants showed perfect cosegregation between the transgene and the phenotype (Table I). Notably, the amount of the \( \textit{Ghd7} \) transcripts was closely related to the degree of heading delay and yield traits at T1 generations (Fig. 1, B, E, and H). These results indicated that the phenotypic effect of \( \textit{Ghd7} \) is quantitatively related to the abundance of its transcript, and the enhanced transcript level of \( \textit{Ghd7} \) caused delayed flowering, increased plant height, and yield traits.

**Pleiotropic Effects of \( \textit{Ghd7} \) on Traits Vary with Genetic Backgrounds and Environmental Conditions**

It was previously reported that enhancement of \( \textit{Ghd7} \) expression had no effect on plant height and yield traits in the \( \textit{ehd3} \) mutant (Matsubara et al., 2011), and the authors supposed that the function of \( \textit{Ghd7} \) also depended on other cues such as genetic background or environmental conditions. We performed transformation experiments using Zhonghua 11 (ZH11), a variety with a weak-function allele of \( \textit{Ghd7} \) (Xue et al., 2008). We introduced \( \textit{Ghd7} \) overexpression (OX-Ghd7ZH11) and artificial microRNA (amiRNA; Ami-Ghd7) constructs, respectively, into ZH11. Seventeen of the 23 independent OX-Ghd7ZH11 T0 transformants showed delayed heading, and conversely 13 of the 21 independent Ami-Ghd7 T0 transformants showed accelerated flowering (Supplemental Fig. S2, A and B). Analysis of T1 families of OX-Ghd7ZH11 and Ami-Ghd7 transformants showed perfect cosegregation between the transgene and the heading date phenotype (Table I). However, no significant increase in plant height or number of spikelets per panicle was detected in the OX-Ghd7ZH11 plants (seeds were sown May 1 in Wuhan field conditions, as discussed below; Table I; Supplemental Fig. S2C), whereas Ami-Ghd7 plants showed a large

### Table I. Performance of near-isogenic lines and T1 family of transgene-positive and transgene-negative plants in HJ19 and ZH11 backgrounds in Wuhan under natural long-day field conditions

Data presented in the first three rows are from the three near-isogenic lines planted in a randomized complete block design with three replications. Plus and minus signs indicate transgene positive and transgene negative, respectively.

| Genotype       | No. of Plants | Plant Height (cm) | No. of Days to Heading | No. of Spikelets on the Main Panicle |
|----------------|---------------|-------------------|------------------------|--------------------------------------|
| NIL(zs7)       | 20            | 93.3              | 66.3                   | 125.6                                |
| NIL(het)       | 20            | 114.0             | 80.1                   | 194.7                                |
| NIL(mh7)       | 20            | 127.9             | 87.8                   | 230.1                                |
| T1 generation  |               |                   |                        |                                      |
| HJ19           | 10            | 59.3 ± 1.1        | 52.4 ± 1.4             | 56.4 ± 6.9                           |
| OX-Ghd7HJ19    | 10            | 58.7 ± 1.0        | 50.2 ± 1.4             | 57.9 ± 8.2                           |
| OX-Ghd7HJ19-14 | 20            | 73.5 ± 2.0        | 82.1 ± 2.4             | 112.4 ± 10.2                         |
| OX-Ghd7HJ19-25 | 20            | 99.1 ± 3.1        | 103.2 ± 3.1            | 157.1 ± 16.3                         |
| T1 generation  |               |                   |                        |                                      |
| ZH11           | 16            | 103.7 ± 2.0       | 70.8 ± 1.5             | 167.7 ± 10.8                         |
| OX-Ghd7ZH11    | 16            | 105.6 ± 2.3       | 87.8 ± 2.8             | 176.9 ± 15.0                         |
| OX-Ghd7ZH11-14 | 12            | 104.6 ± 1.2       | 68.8 ± 1.9             | 160.6 ± 10.0                         |
| Ami-Ghd7       | 16            | 79.4 ± 3.1        | 57.1 ± 1.7             | 92.3 ± 8.6                           |
| Ami-Ghd7-14    | 11            | 104.8 ± 1.3       | 67.9 ± 1.9             | 163.6 ± 8.4                          |

*Ranking by Duncan test at \( P < 0.01 \).  **Significantly different from footnote a.  "Significantly different from footnote b.

### Table II. Performance of the single-copy transgenic plants of T2 generation of OX-Ghd7ZH11 and Ami-Ghd7 with different sowing times in Wuhan field conditions

| Genotype       | Sowing Time | No. of Plants | Plant Height (cm) | No. of Days to Heading | No. of Spikelets on the Main Panicle |
|----------------|-------------|---------------|-------------------|------------------------|--------------------------------------|
| Wild type      | April 15    | 20            | 106.8 ± 1.5       | 79.7 ± 1.8             | 210.6 ± 13.4                         |
| OX-Ghd7ZH11    | 20          | 102.5 ± 2.7   | 97.1 ± 1.5        | 236.4 ± 15.7          |
| Ami-Ghd7       | 20          | 92.5 ± 2.3    | 71.7 ± 1.7        | 167.6 ± 16.9          |
| Wild type      | May 20      | 20            | 95.0 ± 1.9        | 69.3 ± 1.7             | 174.1 ± 11.2                         |
| OX-Ghd7ZH11    | 20          | 102.5 ± 4.1   | 86.2 ± 1.9        | 176.4 ± 25.3          |
| Ami-Ghd7       | 20          | 74.3 ± 2.6    | 56.6 ± 1.2        | 104.7 ± 15.7          |
| Wild type      | June 22     | 20            | 89.3 ± 2.8        | 63.1 ± 2.0             | 166.5 ± 14.0                         |
| OX-Ghd7ZH11    | 20          | 112.5 ± 3.9   | 85.9 ± 2.4        | 267.5 ± 21.7          |
| Ami-Ghd7       | 20          | 68.7 ± 2.9    | 47.8 ± 1.6        | 62.5 ± 8.1            |

*Statistically significant at \( P < 0.01 \) by \( t \) test.
reduction in all three traits (Table I; Supplemental Fig. S2C). A comparison of these results with those obtained from the transformants of HJ19 suggests that the pleiotropic effects of Ghd7 are dependent on the genetic background, which is similar to previous findings (Xue et al., 2008).

The phenotypic effects of Ghd7 also varied with the environmental conditions. When grown in the Hainan Island winter nursery (natural short-day), the OX-Ghd7ZH11 transgenic plants showed a significant increase in plant height and panicle size as well as delayed heading compared with the wild type (Supplemental Fig. S3; Supplemental Table S1). We subsequently evaluated the extent to which the environment may influence the effects of Ghd7 on phenotype by examining T2 families of single-copy transgenic plants of OX-Ghd7ZH11 and Ami-Ghd7 in three plantings in the summer rice growing season in Wuhan. The first planting sown on April 15 and the second planting sown on May 20 subjected the plants to natural long-day conditions, whereas the third planting sown on June 22 exposed the plants to natural short-day conditions. Compared with the wild-type plants, the Ami-Ghd7 plants in general significantly accelerated heading with decreased panicle branch number and plant height in all three plantings (Table II). The phenotype effect of Ami-Ghd7 plants was much larger in the June 22 planting than in the other two plantings (Fig. 1, C, F; Table II). Conversely, OX-Ghd7ZH11 plants showed delayed heading in all three planting conditions (Table II). However, the effect of the transgene on plant height and spikelet number of the June 22 planting was much more drastic than the other two plantings (Fig. 1, C, F; Table II). It should be noted that the increases in the panicle size and plant height of the June 22 planting were not proportional to the length of delayed heading compared with the other two plantings (Table II). It should also be noted that although no significant change was observed in the number of spikelets per panicle between the OX-Ghd7ZH11 and the control in the May 20 planting, the panicle architecture was changed and showed an increase in the primary branch number in the transgenic plant (Supplemental Table S2). These results suggest that the pleiotropic effects of Ghd7 on the phenotype are influenced by the environment, and that Ghd7 might regulate yield traits through modulating panicle architecture independent of the heading date.

Ghd7 Regulates Branching in a Density-Dependent Manner

Ghd7 increased the panicle branch with a reduced tiller number in NIL(mh7) compared with NIL(zs7) under normal field conditions (Xue et al., 2008). However, we observed that overexpressing Ghd7 in HJ19 increased vegetative branching in pots (Supplemental Fig. S4). We supposed that the enlarged plant size of NIL(mh7) relative to NIL(zs7) brings more competitive pressure, which may promote the shadow avoidance signals. To test such a hypothesis, we planted NIL(mh7) and NIL(zs7) under different density conditions. We found that NIL(mh7) plants had significantly more tillers than NIL(zs7) plants at low-density conditions (Fig. 2), demonstrating that Ghd7 regulates tiller number in a density-dependent manner. Interestingly, there was also a significant increase in secondary branches of the panicles in NIL(mh7) relative to NIL(zs7) at low density, leading to an increased grain number without compromising the number of primary branches (Supplemental Table S3). These results suggest that Ghd7 regulates the plasticity of branch development of the plant to adapt to the neighborhood environment.

TEOSINTE BRANCHED1 (OsTB1) was previously shown to act as a negative regulator of lateral branching in rice (Takeda et al., 2003; Choi et al., 2012). We found that OsTB1 was repressed in the shoot tip region in NIL(mh7) compared with NIL(zs7) (Fig. 3C). Thus, we generated double-stranded RNA interference lines with reduced expression of OsTB1 (OsTB1RNAi) in theZH11 background, which showed more tillers but less panicle branching compared with the control plants, in agreement with previous results (Supplemental Fig. S5; Takeda et al., 2003; Choi et al., 2012). We then crossed the OsTB1RNAi plants to Ami-Ghd7 plants (with reduced tiller number and panicle branching relative to the control plants), and examined the branch phenotype.
of the resulting F1. No significant difference in the tiller number of the Ami-Ghd7/OsTB1RNAi plants was detected compared with OsTB1RNAi plants (Fig. 3, A, D, and F), whereas the flowering time of Ami-Ghd7/ OsTB1RNAi plants was similar to Ami-Ghd7 plants (Fig. 3, D and E). Using quantitative real-time PCR (qRT-PCR) analysis, we found a moderate increase in the OsTB1 transcript level in Ami-Ghd7 plants (Fig. 3B). These results suggest that Ghd7 acts upstream of OsTB1 in regulating branching.

Ghd7 Mediates the PHYTOCHROME B-OsTB1 Pathway

Previous studies revealed that the plant response to shadow signals and control of branching mainly depended on the PHYTOCHROME B (PHYB)-TB1 pathway (Kebrom et al., 2006; Gonzalez-Grandío et al., 2013). The role of phytochromes in photoperiodic flowering in rice was recently elucidated (Osugi et al., 2011). The mRNA levels of both Ghd7 and Ehd1 increased in the phyB mutant relative to the wild type (Osugi et al., 2011). To understand the effect of PHYB on the Ghd7 pathway in flowering time and branch development control, we analyzed a phyB mutant in the ZH11 background. The phyB mutant accelerated the heading date as previously described (Takano et al., 2005) accompanied by a reduction in the tiller number in Wuhan field conditions (Supplemental Fig. S6). However, we found no significant difference in Ghd7 gene expression between the phyB mutant and wild-type plants (Fig. 4B). We prepared an anti-GHD7-specific antibody to compare the GHD7 protein level (Supplemental Fig. S7). In wild-type plants, the GHD7 level started to accumulate in the morning, peaked at noon, gradually decreased in the afternoon until midnight, and reached a very low level before dawn (Fig. 4C). The level of GHD7 was low in the phyB mutant throughout the day under long-day conditions (Fig. 4C). This result suggests that PHYB maintains the protein level of GHD7.

To understand the genetic interaction between PHYB and Ghd7, we generated a phyB/OX-Ghd7 double mutant and compared the phenotypes of the resulting F2 generation. The phyB/OX-Ghd7 double mutant showed a heading date similar to OX-Ghd7 (Fig. 4, A and D). Overexpression of Ghd7 partially rescued the tiller number of the phyB mutant (Fig. 4, A and E). These analyses suggest that Ghd7 works downstream of the PHYB.

GHD7 Represses Transcriptional Activity

It was previously reported that the middle region of CCT domain proteins has transcriptional activation activity (Tiwari et al., 2010; Wu et al., 2013). We thus performed a transcriptional activation assay using the Galactin4 (GAL4) DNA binding domain and the herpes simplex virus protein16 (VP16) activation domain using a transient assay system with luciferase (LUC) as a reporter (Fig. 5A; Jing et al., 2013). As shown in Figure 5B, BD-GHD7 did not activate the transcription of the LUC reporter gene, suggesting that GHD7 has no transactivation activity in the plant cell. A high LUC signal was detected in the transformants of the BD-VP16 construct, as a result of the transcriptional activation by the VP16 domain (Fig. 5B). However, the

Figure 3. Genetic interaction between Ghd7 and the OsTB1 pathway. A and D, Plants of wild-type ZH11 (a), Ami-Ghd7 (b), Ami-Ghd7/OsTB1RNAi (c), and OsTB1RNAi (d) at the vegetative stage (A) and at the reproductive stage (D) under natural long-day conditions in Wuhan. Bar in (A) = 20 cm; bar in (D) = 40 cm. B, Expression levels of OsTB1 in wild-type (a), Ami-Ghd7 (b), Ami-Ghd7/OsTB1RNAi (c), and OsTB1RNAi (d) plants. The samples were collected at 30 d after germination (DAG) under natural long-day conditions in Wuhan and used for RNA preparation. Bars and error bars indicate average values and SE, respectively, based on three biological repeats. C, Expression levels of OsTB1 in NIL(zs7) and NIL(mh7). The samples were collected at 35 DAG under natural long-day conditions in Wuhan and used for RNA preparation. Bars and error bars indicate average values and SE, respectively, based on three biological repeats. E and F, The number of days to heading (E) and the number of tillers (F) of wild-type (a), Ami-Ghd7 (b), Ami-Ghd7/OsTB1RNAi (c), and OsTB1RNAi (d) plants under long-day conditions in Wuhan (n ≥ 15 each). Error bars indicate SE.
activity of LUC was drastically reduced by GHD7 (BD-VP16-GHD7) (Fig. 5B). These results suggest that GHD7 has intrinsic transcriptional repression activity in vivo.

Expression of Ghd7 Is Regulated by Environmental Signals

An analysis of the Ghd7 spatial expression profile revealed that the expression of Ghd7 was mainly detected in the emerged leaf blade, whereas it was virtually absent in other tissues assayed even in the preemerged immature leaf blade surrounded by the leaf sheath (Supplemental Fig. S8A). In the emerged leaf blade, Ghd7 transcripts displayed a gradient with much higher transcript accumulation in the leaf tip than the leaf base (Supplemental Fig. S8B). The Ghd7 transcript levels were relatively constant at vegetative, reproductive, and ripening stages in the leaf blade (Fig. 6A), which was similar to Hd1 (Supplemental Fig. 4).
respectively, based on three biological repeats. The expression of Ghd7 was induced by cold treatments, but was repressed by drought, stress. The accumulation of Ghd7 was low during the first 7 weeks, but increased at reproductive and ripening stages (Fig. 6B; Supplemental Fig. S9B).

We analyzed the DNA sequence of the promoter region of Ghd7 and found a number of cis-elements, including ones involved in stress response, such as the abscisic acid (ABA) response element and the C-repeat binding protein element, and hormone response elements, such as the MYB/MYC recognition site and ABA/jasmonic acid (JA) response elements (Fig. 6C; Finkelstein and Lynch, 2000; Abe et al., 2003; Brown et al., 2003; Simpson et al., 2003; Svensson et al., 2006).

Thus, we assayed Ghd7 expression in rice seedlings treated with different phytohormones and drought stress. The accumulation of Ghd7 mRNA was induced by cold treatments, but was repressed by drought, ABA, JA, and high-temperature treatments (Fig. 6D). The expression of Ghd7 was slightly affected by 1-aminocyclopropane-1-carboxylic acid (ACC) and salicylic acid (SA) treatments (Fig. 6D). These results suggest that Ghd7 is involved in response to various environmental signals in addition to photoperiod.

**Ghd7 Regulates the Transcriptomes of Multiple Processes**

To gain insight into downstream genes regulated by Ghd7, we performed a microarray analysis using Affymetrix rice gene chips. Young leaves (35 d after germination) and developing panicles (0.1 cm) from field-grown OX-Ghd7 plants and wild-type plants were used to isolate RNA for chip analysis. With a cutoff of 2-fold change, a total of 256 and 622 genes were up- and down-regulated, respectively, in the leaves of OX-Ghd7 plants (Fig. 7, A and D; Supplemental Table S4). In the young panicles of OX-Ghd7 plants, 177 genes were up-regulated and 303 were down-regulated compared with the wild type (Fig. 7, B and E; Supplemental Table S5). These analyses support the previous conclusion that Ghd7 plays an inhibitory role in gene expression.

Expression of several flowering-related genes was altered in OX-Ghd7 plants, both in young leaves and in developing panicles. Ehd1 and FT-like genes were down-regulated in leaves of OX-Ghd7 plants, consistent with previous results (Supplemental Table S4; Xue et al., 2008; Itoh et al., 2010). Expression of a large number of MADS box genes appeared to be altered in both leaves and panicles in OX-Ghd7 plants, mostly down-regulated, including OsMADS1, OsMADS14, OsMADS18, and OsMADS34 in leaves, which regulate reproductive transition and panicle architecture (Supplemental Table S4; Lee et al., 2004; Kobayashi et al., 2012). However, OsMADS55, which was considered a negative regulator of flowering associated with ambient temperature, was significantly up-regulated in both leaves and panicles in OX-Ghd7 plants (Supplemental Table S4; Lee et al., 2012).

The expression of many genes involved in hormone metabolism and signaling pathways was affected in OX-Ghd7 plants. The expression of an auxin-inducible gene Osnox1, which regulates the sensitivity of polar auxin transport (Scarpella et al., 2002), increased in OX-Ghd7 plants (Fig. 7C). The cytokinin oxidase gene OsCKX2, which negatively regulates the rice grain number (Ashikari et al., 2005), was down-regulated in OX-Ghd7 plants (Fig. 7C). Ethylene and GA contribute to internode elongation (Iwamoto et al., 2011). The transcript abundance of OsACO1, a key enzyme gene involved in the ethylene synthesis pathway (Iwamoto et al., 2010), was up-regulated in OX-Ghd7 plants (Fig. 7C). The GA2-oxidase gene OsGA2ox6, which controls plant height and tiller number (Lo et al., 2008; Huang et al., 2010), was repressed in OX-Ghd7 plants (Fig. 7C). OsCKX2 and OsACO1 were also consistently down-regulated and up-regulated in Ami-Ghd7 plants, respectively (Fig. 7C). These results suggest that Ghd7 is involved in regulating multiple hormonal pathways.

Many transcription factor (TF) families also appeared to be affected in OX-Ghd7 plants, most notably the APETALA2, basic/helix-loop-helix, myeloblastosis, WRKY, and Zinc finger TFs, both in leaves and panicles (Supplemental Tables S4 and S5). Some TF families, such as CCT domain genes in leaves and TCP and YABBY genes in panicles, are tissue specifically down-regulated in OX-Ghd7 plants (Supplemental Tables S4 and S5). The CCT genes were implicated in flowering time control by photoperiod and circadian pathways (Valverde, 2011). The YABBY and TCP genes were shown to participate in the activities controlling lateral organs as well as the shoot apical meristem (Dai et al., 2007; Martin-Trillo and Cubas, 2010). These results suggest that Ghd7 plays a different role in vegetative and
reproductive organs by regulating various transcription networks.

**Ghd7 Is Involved in Stress-Response Pathways and Reactive Oxygen Species Homeostasis**

Interestingly, we found that many Ghd7-regulated genes are involved in abiotic and biotic stress-response pathways. Among them, OsDREB1A and OsPR4, which play a role in cold and drought stress, respectively (Dubouzet et al., 2003; Wang et al., 2011), were both significantly up-regulated in OX-Ghd7HJ19 plants (Fig. 7F). OsDREB1A was down-regulated in Ami-Ghd7 plants, but not OsPR4 (Fig. 7F). We applied drought stress to OX-Ghd7HJ19 and Ami-Ghd7 plants, and found that Ami-Ghd7 plants showed enhanced drought tolerance, whereas OX-Ghd7HJ19 plants were more sensitive to drought (Fig. 8). These results indicate that Ghd7 is indeed involved in regulation of drought stress response.

Reactive oxygen species (ROS) serve as important signaling molecules that participate in response to both biotic and abiotic stresses (Sagi et al., 2004; Gechev et al., 2006; Miller et al., 2008). OsMT2b is a ROS scavenger and functions as the signal in resistance response (Wong et al., 2004). Our analysis showed that OsMT2b was up-regulated in OX-Ghd7HJ19 plants, but down-regulated in Ami-Ghd7 plants (Fig. 7F). OsrbolHE and RACK1A genes, which are involved in ROS production during the immune response, were down-regulated in OX-Ghd7HJ19 plants (Fig. 7F; Yoshiaki et al., 2005; Nakashima et al., 2008), whereas both genes were up-regulated in Ami-Ghd7 plants (Fig. 7F). Finally, a group of ROS homeostasis-related genes and wall-associated kinase family genes showed at least a 2-fold change in expression in OX-Ghd7HJ19 plants (Supplemental Tables S6 and S7). These data suggest that Ghd7 affects the expression of genes whose proteins might be components in the network of ROS homeostasis and that Ghd7 responds to biotic stresses by changing the cell wall components.

**DISCUSSION**

Unlike animals, plants have a remarkable ability to alter their development in response to myriad exogenous and endogenous signals in the life cycle. We previously cloned the quantitative trait loci gene Ghd7, which acts as an important regulator of heading date and yield potential in rice (Xue et al., 2008). More recent works showed that Ghd7 mainly functions as a flowering repressor under long-day conditions and was regulated by light- and circadian clock-dependent gating (Xue et al., 2008; Itoh et al., 2010; Osugi et al., 2011). In addition to the light signal, another important environmental aspect, temperature, also regulated Ghd7 expression (Song et al., 2012).

In this study, we found that the Ghd7 transcript was regulated by various environmental signals such as...
light, temperature, and abiotic and biotic stresses and the expression level of Ghd7 subsequently regulated the growth and development of the rice plant. ABA is a regulatory molecule involved in drought stress tolerance and JA is involved in the plant response to biotic stresses (Yamaguchi-Shinozaki and Shinozaki, 2006; Robert-Seilaniantz et al., 2007). We showed that ABA, JA, and drought treatments strongly repressed Ghd7 expression, which may be related to the response of the plant to quickly end the life cycle in adverse conditions in order to escape or avoid stresses. Moreover, our results showing that Ghd7 regulates stress-related genes and ROS homeostasis genes suggest that Ghd7 might be involved in these stress pathways as well.

Matsubara et al. (2011) reported that a plant homeodomain finger gene Ehd3 repressed Ghd7 transcription. However, they observed no substantial increase in seed productivity in the ehd3 mutant, despite increased Ghd7 expression (Matsubara et al., 2011). In this study, we found that overexpression of Ghd7 in ZH11 delayed the heading date regardless of the planting conditions, but drastically increased the yield traits in June but not
April or May plantings under natural field conditions in Wuhan. These results imply that a certain combination of environmental conditions may be required for Ghd7 to increase the yield traits of the rice plant. Thus, Ghd7 might function not only as a flowering time regulator, but also as a sensor of the environmental signals for the plant to dynamically regulate growth, development, morphology, architecture, and stress responses (Fig. 9).

Tiller and panicle branches are lateral organs at vegetative and reproductive stages in rice, respectively. Panicle branching is often associated with the flowering time, likely because of longer vegetative periods. Studies have also revealed that some flowering time genes, such as *Hd1* and *Ehd1*, control panicle development in rice, independently of flowering time control (Endo-Higashi and Izawa, 2011). Meanwhile, several genes, such as *Gn1a*, *SP1*, and *DEP1*, exclusively alter the number of panicle branches without simultaneous changes of flowering time or the tiller number in rice (Ashikari et al., 2005; Huang et al., 2009; Li et al., 2009). Tiller branching is modulated by both genetic factors and environmental conditions. The mutations of *MOC1*, *LAX1*, and *LAX2* lead to a reduced number of both tillers and panicle branches (Komatsu et al., 2003; Li et al., 2003; Tabuchi et al., 2011). While in the case of *d* and *OsTB1* mutants, the effect of the genes on tillers and panicle branches is opposite to each other; an increase in the tiller number is accompanied by a decrease in the panicle branches (Takeda et al., 2003; Lin et al., 2009; Choi et al., 2012).

Two florigen genes, *FT* and *TSF*, were recently shown to modulate lateral shoot outgrowth in Arabidopsis (Hiraoka et al., 2013). These results suggest a potential link between flowering time control and branching development. We previously showed that Ghd7 increased panicle branching but decreased tiller branching. These results suggest that Ghd7 positively regulates both tiller and panicle branches in a density-dependent manner, and that Ghd7 is involved in regulating the plasticity of branch development for adaptation to different environmental conditions. This process is partly regulated by PHYB by maintaining the GHD7 protein. Ghd7 then repressed expression of *OsTB1*, partly through GA signaling (Lo et al., 2008), and enhanced the floral transition of the axillary buds in Arabidopsis (Niwa et al., 2013). These results suggest a potential link between flowering time control and branching development.

Figure 8. Response of Ghd7 to drought stress. A, Phenotypes of OX-Ghd7**HJ19** and Ami-Ghd7 under drought stress. Bar = 10 cm. B, Survival rate of OX-Ghd7**HJ19** and Ami-Ghd7 after drought stress (*n* = 30 each). Bars and error bars indicate average values and s, respectively, based on three biological repeats.

Figure 9. A schematic illustration of the Ghd7 functions learned from this study. Ghd7 functions to link the dynamic environmental inputs with phase transition, architecture regulation and stress response to maximize the reproductive success of the rice plant.
were transplanted to the winter nursery in Hainan has relatively low temperatures and short-day conditions, and the rows were 26 cm apart. Field management, including irrigation, fertilizer between plants in a row, and the density experimental groups were grown in Wuhan (Huazhong Agricultural University, lat 114°21'E, long 30°29'N) and Hainan Island (Lingshui County, lat 11°01'E, long 18°30'N), China. The summer rice growing season in Wuhan generally has relatively high temperatures and long-day conditions (unless otherwise specified), whereas the winter nursery in Hainan has relatively low temperatures and short-day conditions. Germinated seeds were sown in the seed beds (late April to late June in Wuhan, and middle to late November in Hainan) and 1-month-old seedlings were transplanted to the fields. The plant density was normally 16.5 cm between plants in a row, and the rows were 26 cm apart. For the density experiment, this normal density was regarded as the high-density condition. In the low-density condition, the plants were 70 cm apart in a row, and the distance between rows was 30 cm. Field management, including irrigation, fertilizer application, and pest control, followed essentially normal agricultural practices. The heading date was the day when the first panicle of the plant emerged. The total number of spikelets on the main panicle of the plant was counted approximately 10 d after heading. Plant height was measured from the ground to the tip of the tallest tiller of the plant.

**Generation of Constructs and Transformation**

To construct the OX-Ghd7 vector, the open reading frame of Ghd7 was amplified by PCR using primers OX-F and OX-R (Supplemental Table S8), containing restriction sites for KpnI and BamHI respectively, for subcloning. Complementary DNA (cDNA) was cloned into the pCAMBIA1301 vector and then transformed into HJ19.

To construct the OX-Ghd7 vector, the Ghd7 promoter region was amplified with PRO-F and PRO-R primers containing KpnI and BamHI respectively, and subcloned into the pCAMBIA1301 vector (Supplemental Table S8). The full-length cDNA of Ghd7 was then amplified by PCR using primers ORF-F and ORF-R containing BamHI and HindIII sites, respectively (Supplemental Table S8), and inserted into the pCAMBIA1301 vector to fuse with the promoter region to generate the OX-Ghd7 vector and then transformed into ZH11.

To construct the Ami-Ghd7 vector, we used a customized version of the original Web MicroRNA Designer platform to design amiRNA sequences (21-mer) based on the TIGR5 rice genome annotation. We selected the most suitable amiRNA candidates suggested by the Web MicroRNA Designer platform that have good hybridization properties to the target mRNAs with a single target in the rice genome, with no off-target effect to other genes. The primary amiRNA construct was amplified with Ami-Ghd7-F, Ami-Ghd7-R, and Ami-Ghd7-IV primers (Supplemental Table S8), which were engineered from pN55 as previously described (Warthmann et al., 2008). The fusion product of 554 bp was cloned into the pGEM-T Vector (Promega), excised with KpnI and BamHI, and cloned into the pCAMBIA1301 vector and then transformed into ZH11.

To construct the OsTB1RNAi-F primer, a 484-bp fragment of OsTB1 was amplified by PCR using primers OsTB1RNAi-F and OsTB1RNAi-R (Supplemental Table S8). The OsTB1RNAi-F primer contained SpeI and KpnI sites and the OsTB1RNAi-R primer contained SacI and BamHI sites, for subcloning into the pDS1301 vector that was a modified version of pCambia1301 (Yuan et al., 2007). All of the constructs were independently introduced into Agrobacterium tumefaciens strain EHA105, and transformation was performed as previously described (Ge et al., 2006).

**RNA Extraction and qRT-PCR**

We isolated total RNA using an RNA extraction kit (TRizol reagent; Invitrogen) according to the manufacturer's instructions. For qRT-PCR, approximately 3 μg of total RNA was reverse transcribed using SuperScript II reverse transcriptase (Invitrogen) in a volume of 100 μL to obtain cDNA. We carried out qRT-PCR in a total volume of 25 μL containing 2 μL of the reverse-transcribed product above, 0.25 μM gene-specific primers, and 12.5 μL SYBR Green Master Mix (Applied Biosystems) on an Applied Biosystems 7500 Real-Time PCR System according to the manufacturer's instructions. Primer pairs for qRT-PCR analysis are listed in Supplemental Table S8. The measurements were obtained using the relative quantitation method (Livak and Schmittgen, 2001).

**Purification of Recombinant Protein**

To construct the recombinant MBP-GHD7 vector, the open reading frame of Ghd7 was amplified with MBP-GHD7-F and MBP-GHD7-R primers containing EcoRI and BamHI sites and subcloned into the pMAL vector. MBP and MBP-GHD7 recombinant fusion proteins were induced by isopropyl β-D-thiogalactoside and purified from Escherichia coli BL21 (DE3) strain. The proteins were then purified by MBP beads according to the manufacturer's instructions.

**Antibody Production and Immunoblotting**

We synthesized the peptide corresponding to amino acids 243 to 257 of GHD7 (CTYVIPDRLKEILDQWFR) conjugated with keyhole limpet hemocyanin, and polyclonal antibody was raised in rabbit. Rice leaf total protein extraction was performed as described (Li et al., 2013). Proteins were boiled in SDS loading buffer, separated by 10% SDS-PAGE gels, and blotted onto polyvinylidene fluoride membranes. The proteins were then incubated with anti-GHD7 (1:200 dilution) or anti-heat shock protein (1:5,000 dilution; Li et al., 2011) and subsequently with the horseradish peroxidase-conjugated goat-antirabbit secondary antibody (Abcam) according to the manufacturer's instructions. The protein bands were visualized by a standard enhanced chemiluminescence kit (Thermo Scientific Pierce) and the signal was exposed with x-ray film.

**LUC Activity Assay**

To determine the transcriptional activation activity of GHD7, the full-length GHD7 fused with the GAL4 DNA binding domain (BD-GHD7) was cotransformed into Arabidopsis (Arabidopsis thaliana) protoplasts with a reporter construct containing the 4× upstream activation sequence region and mini355 promoter sequence fused to LUC cDNA. To analyze the transcriptional repression activity of GHD7, the full-length GHD7 was fused with the GAL4-VP16 domain (BD-VP16-GHD7), which is a widely used transcriptional activator, and cotransformed into Arabidopsis protoplasts with the reporter construct. The LUC activity assay was performed as previously reported (Tang et al., 2012). LUC reporter activity was detected with a luminescence kit using the LUC assay substrate (Promega). Relative reporter gene expression levels are expressed as the ratio of LUC to GUS.

**Stress Treatments of Plant Materials**

To check the expression level of the Ghd7 under various abiotic stresses or phytohormone treatments, rice plants of NIL(mh7) were grown in hydroponic culture medium for approximately 3 weeks in a phytotrons (14-h light/10-h dark at 32°C/26°C). Seedlings at the four-leaf stage were treated with abiotic stresses, including drought (removing the water supply under phytotron conditions, 14-h light/10-h dark at 32°C/26°C), cold (seedlings were transferred to a phytotron at 14-h light/10-h dark at 10°C/10°C), and heat (seedlings were transferred to a phytotron at 14-h light/10-h dark at 42°C/42°C). For phytohormone treatments, 20 μM ABA, 0.5 mM JA, 0.1 mM SA, and 0.1 mM ACC were individually added to the culture medium. The sample was collected at the designated time points (0 min, 30 min, 6 h, and 12 h).
To test the drought stress tolerance of transgenic plants at the seedling stage, transgenic-positive and wild-type plants (30 plants each, three repeats) were grown in a half-and-half manner in barrels filled with sandy soil. Drought stress testing was conducted at the four-leaf stage, following a previously described procedure (Tang et al., 2012).

Microarray Analysis
RNA samples used for microarray analysis were prepared from young leaves in a vegetative stage (35-d-old) and from developing panicles (0.1 cm in length) of OX-Ghd7HJ19 transgenic and wild-type plants grown under normal field conditions with two biological replicates. RNA isolation, purification, and Affymetrix microarray hybridization were carried out using the Affymetrix GeneChip service (CapitalBio) protocol. The microarray analysis was conducted according to a previously described process (Yang et al., 2012).

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number GSE51616.

Supplemental Data
The following materials are available in the online version of this article. Supplemental Figure S1. T0 generation plants of OX-Ghd7HJ19 planted under natural long-day field conditions in Wuhan. Supplemental Figure S2. T0 and T1 generation plants of OX-Ghd7HJ11 and Ami-Ghd7 planted under natural long-day field conditions in Wuhan. Supplemental Figure S3. T2 generation plants of OX-Ghd7HJ11 and Ami-Ghd7 planted under natural short-day field conditions in Hainan Island. Supplemental Figure S4. OX-Ghd7HJ19 plants that showed an increase in the tiller number at the vegetative stage. Supplemental Figure S5. Phenotype of OsTB1RNAi plants in the ZH11 background planted under natural long-day field conditions in Wuhan. Supplemental Figure S6. phyB mutants in the ZH11 background planted under natural long-day field conditions in Wuhan. Supplemental Figure S7. Detection of the GHD7-MBP protein by the GHD7 antibody. Supplemental Figure S8. Expression feature of Ghd7.

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