Dominant and Pleiotropic Effects of a *GAI* Gene in Wheat Results from a Lack of Interaction between DELLA and GID1[1][C][W][OA]

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Dominance, semidominance, and recessiveness are important modes of Mendelian inheritance. The phytohormone gibberellin (GA) regulates many plant growth and developmental processes. The previously cloned semidominant *GA-insensitive* (*GAI*) genes *Reduced height1* (*Rht1*) and *Rht2* in wheat (*Triticum aestivum*) were the basis of the Green Revolution. However, no completely dominant *GAI* gene has been cloned. Here, we report the molecular characterization of *Rht-B1c*, a dominant *GAI* allele in wheat that confers more extreme characteristics than its incompletely dominant alleles. *Rht-B1c* is caused by a terminal repeat retrotransposons in miniature insertion in the DELLA domain. Yeast two-hybrid assays showed that Rht-B1c protein fails to interact with GA-INSENSITIVE DWARF1 (GID1), thereby blocking GA responses and resulting in extreme dwarfism and pleiotropic effects. By contrast, Rht-B1b protein only reduces interaction with GID1. Furthermore, we analyzed its functions using near-isogenic lines and examined its molecular mechanisms in transgenic rice. These results indicated that the affinity between GID1 and DELLA proteins is key to regulation of the stability of DELLA proteins, and differential interactions determine dominant and semidominant gene responses to GA.

Reduced height (*Rht*) genes in wheat (*Triticum aestivum*) are used to increase lodging resistance and harvest index (Gale and Youssefian, 1985). Twenty-one genes with major effects on plant height in wheat have been reported and assigned designations from *Rht1* to *Rht21* (McIntosh et al., 2008). Two of these genes, *Rht1* (semidominant) and *Rht3* (completely dominant), are allelic at the renamed *Rht-B1* locus (Börner et al., 1996; McIntosh et al., 2008). Norin 10, carrying *Rht1* and *Rht2*, is an important semidwarf source that contributed to increased wheat production in the 1960s and 1970s and events known as the Green Revolution (Borlaug, 1983; Gale and Youssefian, 1985; Peng et al., 1999).

In 1999, an *Rht* gene from Norin 10 wheat was isolated and shown to be an ortholog of an Arabidopsis GA-INSENSITIVE (GAI) gene (Peng et al., 1999). It encoded a DELLA protein. A base substitution difference, C for T, between the sequences of the *Rht-B1a* tall wild type and the *Rht-B1b* semidwarf allele led to a stop codon and the termination of translation within the DELLA region, resulting in a truncated Rht protein lacking part of the DELLA domain (Peng et al., 1999). The DELLA protein belongs to a plant-specific family of transcription factors known as GRAS (for GAI, REPRESSOR OF GA1-3 [RGA], and SCARECROW [SCR]) and possesses a variable N-terminal region and a considerably conserved C-terminal GRAS domain (Pysh et al., 1999). Because of its variable N terminus, DELLA has a special function in the GA signal transduction pathway. A DELLA box located at the N terminus acts as the GA signal for repressors of GA signaling (Dill et al., 2001; Silverstone et al., 2001; Itoh et al., 2002; Fu and Harberd, 2003; Jiang and Fu, 2007). Another important factor in GA signaling is the GA receptor GA-INSENSITIVE DWARF1 (GID1), which was first isolated through the analysis of a rice (*Oryza sativa*) mutant. GID1 binds to SLENDER RICE1 (SLR1), a rice DELLA protein, in a GA-dependent manner in yeast cells (Ueguchi-Tanaka et al., 2005).

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Later papers reported that GA-dependent interactions between GID1 and DELLA protein also occur in Arabidopsis (*Arabidopsis thaliana*; Griffiths et al., 2006; Nakajima et al., 2006; Willige et al., 2007). They concluded that the DELLA domain of RGA and GAI is necessary for interaction with AtGID1a (Griffiths et al., 2006; Willige et al., 2007). Meanwhile, in rice, Ueguchi-Tanaka et al. (2007), using yeast two-hybrid (Y2H), gel filtration, and bimolecular fluorescence complementation methods, confirmed that DELLA regions are essential and sufficient for GA-dependent GID1, with a DELLA protein-SLR1 interaction. However, GA is not always necessary for the interaction between GID1 and DELLA proteins (Yamamoto et al., 2010). Whereas there has been clear evidence for GID1-mediated GA signaling in Arabidopsis, rice, and nonflowering plants (mosses and ferns; Ueguchi-Tanaka et al., 2005, 2007; Griffiths et al., 2006; Nakajima et al., 2006; Hirano et al., 2007; Willige et al., 2007; Yasumura et al., 2007; Yamamoto et al., 2010), there has been no report about any interaction between GID1 and DELLA proteins in wheat.

Tom Thumb, a well-known wheat landrace originally from Tibet, China, is characterized by several specific characters: (1) strongly reduced plant height of about 30 cm, much shorter than other dwarf sources in wheat except for Aibian 1; (2) resistance to preharvest sprouting, a serious problem in many wheat-producing regions of the world; (3) late maturity; (4) shriveled seed with reduced fecundity; and (5) larger tiller angle and looser plant type (Morris et al., 1972; Flintham and Gale 1982, 1983). Genetic analysis showed that Tom Thumb possessed a single completely dominant reduced-height and GAI gene, initially named *Rht3*, but later revised to *Rht-B1c* (Morris et al., 1972; Gale and Marshall, 1973; Börner et al., 1996; McIntosh et al., 2008). *Rht-B1c* was located on the short arm of chromosome 4B at a distance of less than 15 centimorgan from the centromere and at the same locus (or closely linked) as *Rht-B1b* (Gale and Marshall, 1976; McVittie et al., 1978).

As introduced above, several partially dominant genes confer a dwarf phenotype in different plant species. However, the molecular characteristic of *Rht-B1c*, a dominant and pleiotropic *GAI* allele, has not yet been reported, and the molecular mechanisms of this GA-insensitive growth response still remain unclear. In this study, we isolated a completely dominant allele from a multiallelic gene in wheat, deciphered its mode of action, and discovered differences between complete and partial dominance.

RESULTS

A Genomic Segment with Large Effects on Multiple Traits

To better understand the effect of *Rht-B1c* on agronomic traits, we developed a pair of near-isogenic lines (NILs) using Yanzhan 1 (YZ1), a commercial variety that carries *Rht-B1a*, as a recurrent parent, and Tom Thumb (*Rht-B1c*) as the donor parent (Fig. 1A). Field examination under natural long-day (Hebei; 115°41′E, 41°41′N) and short-day (Yunnan; 101°41′E, 25°42′N) conditions showed that five agronomic traits (plant height, resistance to preharvest sprouting, tiller angle, fertility and fecundity, and heading date) were significantly affected by the presence of *Rht-B1c* (Fig. 1). The plant height of the NIL-*Rht-B1c* was 30 cm, or about 50% ($P \leq 0.01$) shorter than NIL-*Rht-B1a* (YZ1; Fig. 1, A and B). NIL-*Rht-B1c* had more shriveled seed and lower setting percentage than NIL-*Rht-B1a* (Fig. 1C).

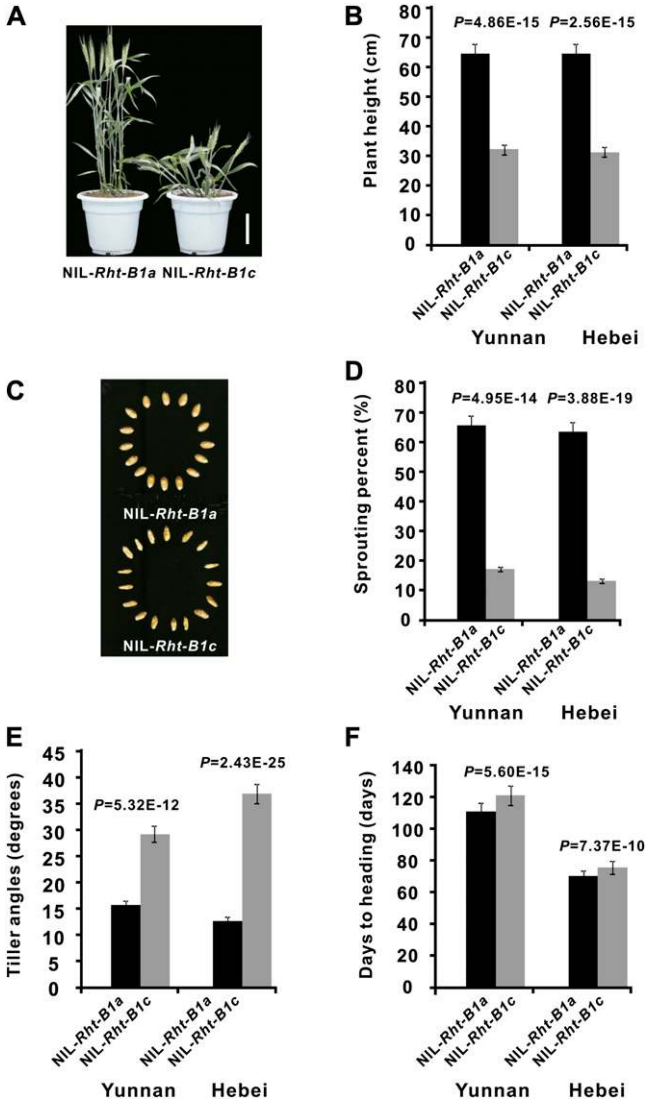

Figure 1. The phenotypes of NIL-*Rht-B1c* plants. A, Photographs of plants with NIL-*Rht-B1a* and NIL-*Rht-B1c*. Bar = 20 cm. B to F, Comparison of agronomic traits: plant height (B); seeds of plants with NIL-*Rht-B1a* and NIL-*Rht-B1c* (C); preharvest sprouting (D); tiller angle (E); and heading date (F). Student's *t* test was used to generate *P* values. [See online article for color version of this figure.]

The level of preharvest sprouting of NIL-*Rht-B1c* was 13.7% and 17.8% in Hebei and Yunnan, respectively, significantly lower than NIL-*Rht-B1a* (64.7% and 66.7%, respectively; $P \leq 0.01$; Fig. 1D). The mean tiller angle of NIL-*Rht-B1c* plants was obviously larger than that of NIL-*Rht-B1a* plants (Fig. 1E). There were clear differences in heading date between NIL-*Rht-B1a* and NIL-*Rht-B1c*, the latter being 10 d later than NIL-*Rht-B1a* in Yunnan and 5 d later in Hebei (Fig. 1F). All five traits were investigated in two segregating populations. One was a fifth backcross (Tom Thumb/6*YZ1) F2 population, the other was an F2 population derived from a recombinant inbred line (RIL; *Rht-B1c*) crossed with Yumai 18, which lacks *Rht-B1c*. These results showed that all traits cosegregated; that is, all short plants showed late maturity, resistance to preharvest sprouting, and displayed larger tiller angles (Supplemental Table S1), obviously reflecting pleiotropic effects of the same dwarfing gene, namely *Rht-B1c*.

A New Terminal Repeat Retrotransposons in Miniature Element Is Inserted in the DELLA Domain of *Rht-B1a*

Assuming an allelic series at the *Rht-B1* locus (Gale and Marshall, 1976), we set out to clone *Rht-B1c* based on the known sequences of *Rht-B1a* and *Rht-B1b*. We amplified template DNAs extracted from Chinese Spring (*Rht-B1a*), Norin 10 (*Rht-B1b*), and Tom Thumb (*Rht-B1c*). We obtained the *Rht-B1a* and *Rht-B1b* sequences, which were as expected (i.e. a T-for-C substitution in *Rht-B1b*; Supplemental Fig. S1). However, we could not get the full-length *Rht-B1c* sequence from Tom Thumb using this normal PCR procedure.

We next modified the PCR amplification system, such as increasing denaturizing temperature (94°C–98°C) and prolonging extension time (2–4 min), and used a new PCR enzyme (Kod Fx). We obtained a larger PCR-amplified fragment from Tom Thumb. Strikingly, we found that the fragment was about 4 kb, which was approximately twice of the length of the fragment among other alleles (Fig. 2A). Furthermore, a pair of *Rht-B1* primers was designed to amplify the full-length cDNA of *Rht-B1c*, and the PCR products were sequenced. Compared with *Rht-B1a*, we found that there was a 2,026-bp insertion at position 147 bp numbered from the ATG start code in *Rht-B1c* (Fig. 2B; Supplemental Fig. S1). In the process of transcription, the N-terminal DELLA domain of *Rht-B1c* exists as a 90-bp insertion, which completely matched the 3′ end of the genomic 2,026-bp insertion fragment (Fig. 2B). Interestingly, this is consistent with other alleles in the C-terminal GRAS domain. *Rht-B1a* (and *Rht-B1b*) is an intronless gene, whereas *Rht-B1c* consists of two exons and one intron.

At the posttranslational level, this insertion fragment resulted in an additional 30 amino acid residues in the DELLA domain of the predicted Rht-B1c protein (Fig. 3A). Further detailed sequence alignment analysis showed that the 30 amino acids were inserted between residues Lys (K) and Val (V) of DELLAALGYKV, which function as a GA receptor recognition sequence (Murase et al., 2008).

To assess whether this 30-amino acid insertion affected three-dimensional (3D) protein structures of Rht-B1c, we then used the swissmodel workspace (http://swissmodel.expasy.org/) to construct homology protein structure models of Rht-B1a and Rht-B1c based on the template of Arabidopsis GA_3-GID1A-DELLA (Fig. 3B). As predicted, the DELLA domain of Rht-B1a formed five α-helices (αA, αB, αC, αD, and αE), and αA, αB, αC, and αD were consistent with Arabidopsis GAI. However, Rht-B1c possessed an additional β-sheet, which resulted from the 30-amino acid insertion. In addition, αB of Rht-B1c was shorter than that of Rht-B1a. Thus, the 3D protein structure of Rht-B1c was severely damaged by the supererogatory β-sheet. We also constructed a 3D model based on template GA_4-GID1A-DELLA and found that there was another α-helix (αF) caused by the 30-amino acid insertion located within loop A-B of the DELLA domain

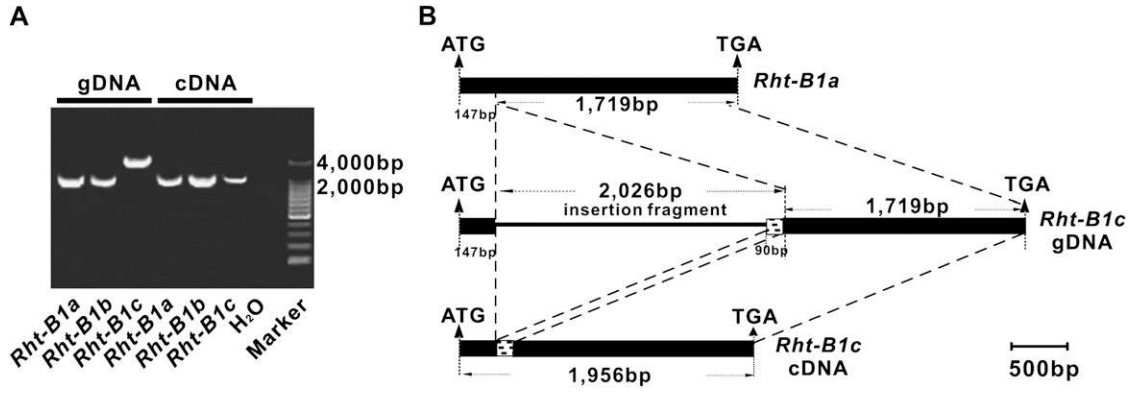

Figure 2. Homology cloning of *Rht-B1c*. A, Amplification of the full lengths of *Rht-B1a, Rht-B1b,* and *Rht-B1c*. B, Comparison of gene structures of *Rht-B1a* and *Rht-B1c*. The thick black bars represent the coding sequence with the predicted translation start site (ATG) and stop code (TGA); the thin line represents the intron; and the dashed boxes represent the 90-bp insertion at the transcriptional level. gDNA, Genomic DNA.

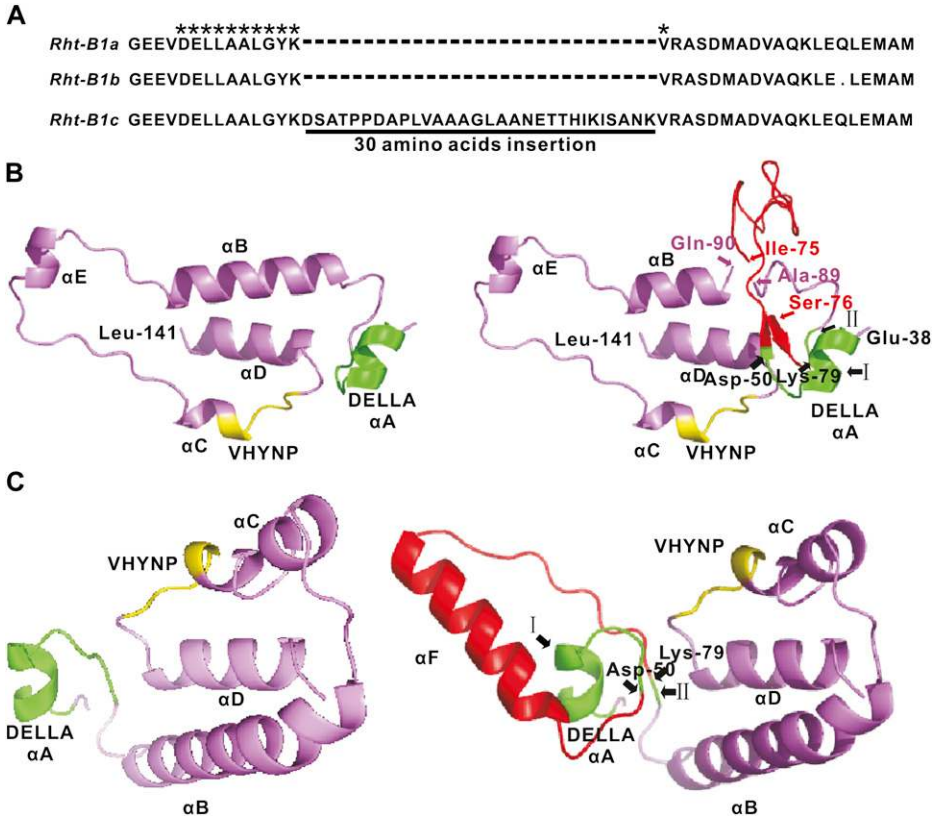

Figure 3. Architecture of DELLA domains of Rht-B1a and Rht-B1c. A, Comparison of amino acid sequences of DELLA domains among Rht-B1a, Rht-B1b, and Rht-B1c. The translated Rht-B1c protein contains an additional 30-amino acid insertion in the DELLAALGYKV motif. Asterisks represent the GID1 recognition sequence. B, A 3D model based on template 2ZSH (GA_3-GID1A-DELLA). C, A 3D model based on template 2ZSI (GA_4-GID1A-DELLA). In B and C, Rht-B1a is shown at left and Rht-B1c is shown at right. The DELLA motif of Rht-B1c formed three parts, residues 38 to 75, 76 to 89, and 90 to 141. αA, α, αC, αD, and αE represent five α-helices. DELLAALGYKV (green) and VHYNP (yellow) motifs are essential for GID1 binding; the insertion α-helix (αF) of Rht-B1c is located within loop A-B, which is highlighted in red. [See online article for color version of this figure.]

of Rht-B1c (Fig. 3C). These data suggested that the DELLAALGYKV motif was split into regions I (DELLAALGYK) and II (V) by insertion of a β-sheet or an α-helix, and this insertion disrupts the DELLA domain structure.

More detailed analysis of the insertion sequence revealed flanking 5-bp nucleotides containing AG (located upstream of the 5′ insertion sequence) and GTG (located downstream of the 3′ insertion sequence) to form intact terminal repeat retrotransposons in miniature (TRIM), for which the total length is 2,031 bp. This TRIM element contains a conserved 503-bp terminal direct repeat sequence with no substitution or deletion and flanks a 1,015-nucleotide internal domain containing a 19-bp primer-binding site and a 15-bp polypurine tract. The primer-binding site is located immediately downstream of the 3′ terminal direct repeat complementary to Met tRNA, and the polypurine tract is located immediately upstream of the 5′ terminal direct repeat (Fig. 4A). None of the elements characterized to date encodes mobility-related proteins in the internal domain (Fig. 4A). The intact element is flanked by 5-bp direct repeats AGGTG, representing the target site duplications generated after insertion. BLASTed against the Triticeae Repeat Sequence Database (release 10; http://wheat.pw.usda.gov/ITMI/Repeats/), there was low similarity with known elements. Therefore, we deduced that the insertion sequence was a new nonautonomous long terminal repeat retrotransposon known as TRIM, designated as CAAS-TRIM. To further investigate the origin of the inserted TRIM fragment, we searched the draft genome sequence database of Chinese Spring (http://www.cerealsdb.uk.net/search_reads.htm). The homologous sequence obtained from the Chinese Spring database was assembled for about 5,000 bp (Fig. 4A), and the TRIM region of 2,026 bp was identical to the TRIM sequence found in Tom Thumb, implying a high degree of conservation. To map the TRIM in the genome of Chinese Spring, a pair of primers (TRIM-F/TRIM-R) spanning TRIM was designed based on the TRIM flanking sequences (Fig. 4A) and used to amplify the target fragments in Lumai 14 and Hanxuan 10, parents of a doubled haploid mapping population. A fragment with a length close to the expected 2,862 bp was detected in Lumai 14, confirming that there was a TRIM insertion in the target region. A fragment with a length close to 836 bp was detected in Hanxuan 10 (Fig. 4B). Sequencing results confirmed that there was no TRIM insertion in the target region of Hanxuan 10. The insertion/deletion polymorphic marker was used to genotype the Hanxuan 10/Lumai 14 doubled haploid population. Using available mapping data for the same population, linkage analysis showed that TRIM was located on chromosome 6B and flanked by simple sequence repeat markers Xwmc341 and Xwmc182 at 7.9 and 7.5 centimorgan, respectively (Fig. 4C). To detect the distribution of CAAS-TRIM-6B in wheat germplasm, 150 diverse wheat accessions were screened with the

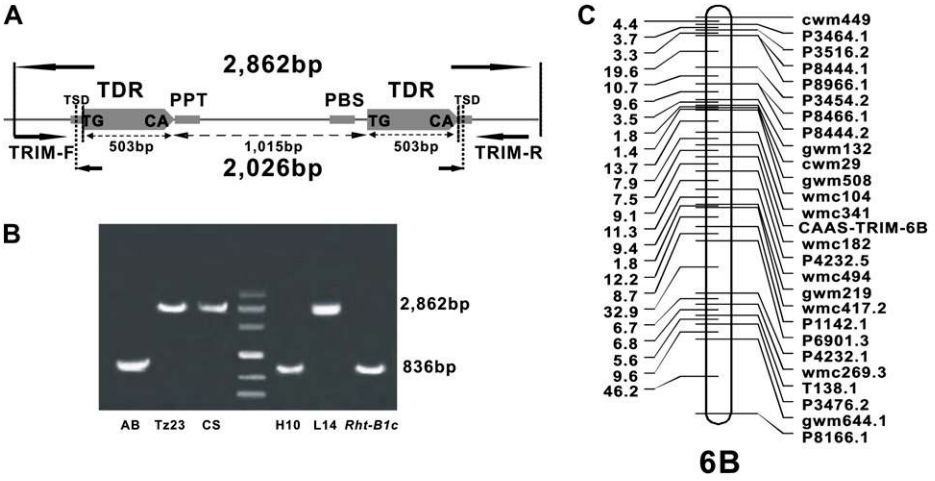

Figure 4. Structure of new TRIM. A, Schematic diagram of the structure of CAAS-TRIM-6B. PBS, Primer-binding site; PPT, polypurine tract; TDR, terminal direct repeat; TSD, target site duplication. B, Identification of TRIMs in different wheat cultivars using PCR. AB, *T. durum* (cv Langdon); Tz23, Taizhong 23; CS, Chinese Spring; H10, Hanxuan 10; L14, Lumai 14. C, Linkage map of CAAS-TRIM-6B and its surrounding markers on wheat chromosome 6B. [See online article for color version of this figure.]

CAAS-TRIM-6B marker (Supplemental Table S2). Nine lines, including Chinese Spring, Lumai 14, and Taizhong 23, carried the CAAS-TRIM-6B element (Fig. 4B). However, in most tested lines without a CAAS-TRIM on chromosome 6B, including Langdon (tetraploid durum wheat [*Triticum durum*]) and Tom Thumb, the element was apparently present on another chromosome, such as 4B in Tom Thumb. TRIM appears to be an active element in the wheat genome capable of random movement to other locations.

Verification of the Pleiotropic Effects of *Rht-B1c* in Wheat NILs and Transgenic Rice

We developed a specific PCR-based marker (*Rht-B1c*M) to detect the presence of the 2,026-bp insertion in *Rht-B1c*, allowing it to be distinguished from other *Rht-B1* alleles. Its forward and reverse primers span the 2,026-bp insertion and produced different PCR-amplified fragments with different alleles (Supplemental Fig. S2A). Tom Thumb, which contains the *Rht-B1c* allele, gave an amplification product of the expected size (2,300 bp) with the *Rht-B1c*M-specific primer, whereas the amplified fragment from Chinese Spring without *Rht-B1c* was about 250 bp (Supplemental Fig. S2B). PCR results showed that *Rht-B1c*M could be used to detect *Rht-B1c* in wheat. *Rht-B1c*M was then employed to test the segregating populations (fifth backcross [Tom Thumb/6*YZ1] F2 population and another F2 population derived from RIL [*Rht-B1c*] crossed with Yumai 18). The results showed that *Rht-B1c*M was cosegregated with the target traits (dwarfing, later flowing, larger tiller angles, etc.) for Tom Thumb and also confirmed that *Rht-B1c* conferred a number of morphological traits.

To further confirm the function of *Rht-B1c*, we generated constructs with the full-length *Rht-B1b* and *Rht-B1c* genes in the vector pCAMBIA1300 under the control of their own wheat promoters. We introduced the constructs into rice cv Nipponbare, which is tall and has small tiller angles, by *Agrobacterium tumefaciens*-mediated transformation. We obtained 25 independent transgenic T0 rice plants with *Rht-B1b* constructs; all were shorter in stature than Nipponbare. We also obtained approximately 70 transgenic T0 plants with *Rht-B1c*; most of them showed the expected dwarf phenotypes (Fig. 5; Table I). Compared with Nipponbare, the average height of *pRht-B1b*:*Rht-B1b* heterozygous transgenic plants was reduced by 23.8%, whereas the average height of *pRht-B1c*:*Rht-B1c* T0 transgenics at 8.6 cm was reduced by 80.9% (Table I). This effect of *Rht-B1c* was more extreme in transgenic rice than in wheat, where the reduction was about 50%. In order to answer whether GA responses were correlated with the dwarfism, we analyzed the GA response in those transgenic rice plants expressing *Rht-B1b* and *Rht-B1c* (Supplemental Table S3). The plant height of Nipponbare treated with exogenous GA was significantly higher (4.76 $\pm$ 1.34 cm; $P \leq 0.01$) than that of the nontreated control. Conversely, those transgenic rice plants expressing *Rht-B1b* and *Rht-B1c* showed GA-insensitive responses (*Rht-B1b*, 0.72 $\pm$ 0.18 cm; *Rht-B1c*, 0.68 $\pm$ 0.21 cm).

Furthermore, *pRht-B1c*:*Rht-B1c* transgenic plants also showed increased tiller spread with wider tiller angles, whereas *pRht-B1b*:*Rht-B1b* transgenics, like Nipponbare, had a compact plant architecture with narrower tiller angles (Fig. 5). *pRht-B1c*:*Rht-B1c* transgenics had delayed heading dates, whereas *pRht-B1b*:*Rht-B1b* transgenics and Nipponbare had similar heading dates. These results confirmed the pleiotropic functions of *Rht-B1c* in reducing plant height, increasing tiller angle,

Table I. *Comparison of plant heights of Rht-B1c transgenic rice plants with those having Rht-B1b*

Genotype	No. of T0 Lines	Average Height	Relative Reduction	SD
		cm	%	
pRht-B1b:*Rht-B1b*	25	34.3	23.8	3.4
pRht-B1c:*Rht-B1c*	72	8.6	80.9	0.8
Nipponbare	33	45		

and delaying heading date. The above results also confirmed that the cloned gene was *Rht-B1c*. Reverse transcription-PCR analysis revealed no significant difference in transcription level between *Rht-B1b* and *Rht-B1c* (Supplemental Fig. S2C), suggesting that the difference in plant height between *Rht-B1b* and *Rht-B1c* was not due to a difference in expression level.

Comparative Responses of *Rht-B1b* and *Rht-B1c* to Exogenous GA_3

Rht-B1b and *Rht-B1c* have different mutations leading to different of functions. In order to explore the differences, we compared the physiological effects of GA_3, α-amylase activity, and TaGID1 protein interaction with Rht-B1b and Rht-B1c.

The seedling length responses of lines with *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* to exogenous GA_3 treatment were assessed on 10-d-old seedlings sprayed with 50 μL L^{-1} exogenous GA_3 after germination (Fig. 6A). The average length of NIL-*Rht-B1a* seedlings to such treatment was significantly higher (30%; $P \leq 0.01$) than that of the nontreated control. Seedlings with *Rht-B1b* differed from the control at $P \leq 0.05$, with an increase of 11%. GA treatment of *Rht-B1c* seedlings resulted in a 5% increase that was not statistically significant (Fig. 6B). The GA responses of the three genotypes were significantly different at $P \leq 0.01$ (Fig. 6C). These results indicate that *Rht-B1a* has a normal GA response, *Rht-B1b* has a reduced GA response, and *Rht-B1c* is GA insensitive.

The seed aleurone layer provides an effective and convenient system for the study of molecular mechanisms involved in GA-regulated gene expression, and exogenous GA_3 causes rapid increases in α-amylase expression in isolated aleurone layers (Gubler et al., 1995). The differences in response to GA_3 in terms of α-amylase activity among aleurones of *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* individuals were determined by an agar plate assay using embryoless seed halves (Fig. 6, D and E). Controls with *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* produced scarcely any α-amylase in the absence of exogenous GA_3 (Fig. 6D). Embryoless half-seeds from *Rht-B1c* genotypes produced a very small amount of α-amylase on the GA_3 plates, whereas those from *Rht-B1a* and *Rht-B1b* plants secreted the enzyme, as evidenced by the clear zones on the plates containing GA_3; the clear zones of *Rht-B1a* were larger than those for *Rht-B1b*, indicating higher activity (Fig. 6E). These results indicated that the α-amylase activity in wild-type (*Rht-B1a*) plants was higher than those in the *Rht-B1b* and *Rht-B1c* variants. Because *Rht-B1c* genotypes were more insensitive than *Rht-B1b* to GA_3, much less α-amylase was produced in the seeds, resulting in further resistance to preharvest sprouting.

In order to detect the levels of interaction between TaGID1 protein with Rht-B1a, Rht-B1b, and Rht-B1c, we used full-length *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* cDNA as prey constructs, and TaGID1 full-length cDNA was amplified from Chinese Spring cDNA as the bait construct. Interaction activity between Rht-B1a and Rht-B1b with TaGID1 protein in both plate and liquid assays showed that GA-dependent interaction occurred between the proteins (Fig. 6F). Interestingly, positive colonies of Rht-B1b on fresh synthetic dextrose/−Trp/−Leu/−His/−Ade master plates grew for longer times than those of Rht-B1a before colonies appeared. Positive colonies of Rht-B1a were observed on plates after about 36 h, whereas for Rht-B1b, the corresponding time was more than 60 h. Liquid assays showed similar results to the plate assays; the highest activity was detected with Rht-B1a-TaGID1, which was about twice that for Rht-B1b-TaGID1 in the presence of GA (Fig. 6F, right). We next examined the biochemical characteristics of the DELLA protein containing the 30-amino acid insert in the Lys-49 codon (*Rht-B1c*) in terms of its interaction with TaGID1 protein. In agreement with the absence of colonies produced by Rht-B1c-TaGID1 on the same plates even after much prolonged periods (Fig. 6F), the liquid assay also showed that GA-dependent interaction between TaGID1 and Rht-B1c was abolished. These results thus showed that a nucleotide substitution in *Rht-B1a* to generate *Rht-B1b* led to a partial decrease in the binding activity, whereas the 90-bp insertion into *Rht-B1a* to produce *Rht-B1c* resulted in a drastic decrease in the presence of GA.

Figure 5. Gross morphology of transgenic rice plants. A transgenic Nipponbare plant (right) expressing *Rht-B1b* (middle) and *Rht-B1c* (left and top left corner) under the control its native promoter has semidwarf and extreme dwarf stature, respectively. Bar = 10 cm; bar in top left corner = 2.5 cm. [See online article for color version of this figure.]

DISCUSSION

Dominance/Recessiveness of *GAI* Depends on the Level of Interaction between GAI and GID1

The previous studies have shown that the semidominant *GAI* genes *Rht1* and *Rht2* in wheat were the

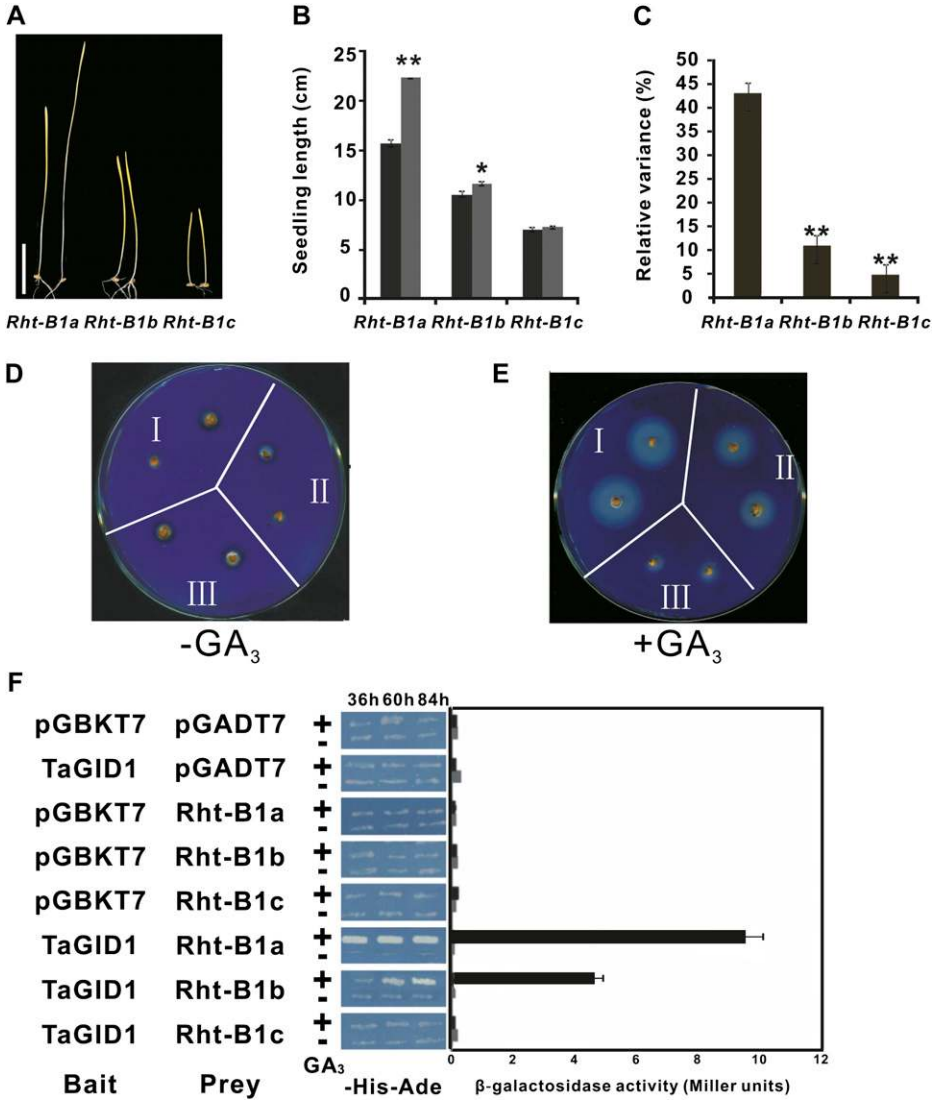

Figure 6. Loss of interaction activity with TaGID1 results in GA insensitivity. A, Morphologies of 10-d-old *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* wheat seedlings treated with 50 μL L^{-1} GA_3 (right) or without GA_3 (left). Bar = 5 cm. B, Comparison of seedling lengths of 10-d-old *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* plants treated with (gray bars) or without (black bars) 50 μL L^{-1} GA_3. Data were measured as means of 30 seedlings at each time with three replicates. Significance is as follows: * $P \leq 0.05$, ** $P \leq 0.01$. C, Relative variances of *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* seedlings. The formula for variance is as follows: relative variance = (length of seedling with GA_3 − length of seedling without GA_3)/length of seedling without GA_3 × 100%. Significance is as follows: ** $P \leq 0.01$. D and E, GA-mediated induction of α-amylase activity in *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* seeds. A concentration of 1 μM GA_3 was applied to the plates. Sector I, *Rht-B1a*; sector II, *Rht-B1b*; sector III, *Rht-B1c*. F, Interaction between TaGID1 and Rht-B1. Y2H assays were performed using TaGID1 as bait and Rht-B1a, Rht-B1b, and Rht-B1c as prey. Left, growth of yeast strain AH109 transformants on −His−Ade plates; right, β-galactosidase activity detected in liquid assay with yeast strain AH109 transformants. To measure relative lacZ activity, three independent experiments were carried out. Five individual transformants were used each time. [See online article for color version of this figure.]

molecular basis of the Green Revolution (Peng et al., 1999). However, the completely dominant *GAI* gene has not yet been cloned. In this study, we demonstrated that the completely dominant *Rht-B1c*, an ortholog of Arabidopsis *GAI*, was caused by a 30-amino acid insertion in the DELLA domain and disrupted the GA-dependent interaction with TaGID1. According to the "relief-of-restraint" model, DELLA proteins restrain plant growth while GA relieves DELLA repression by overcoming DELLA-mediated growth restraint (Dill and Sun, 2001; Richards et al., 2001; Silverstone et al., 2001; Harberd, 2003; Jiang and Fu, 2007; Hou et al., 2010; Gao et al., 2011). Binding of bioactive GA to GID1 promotes the interaction of GID1 with the DELLA proteins, the subsequent polyubiquitination of the DELLAs via the E3 ubiquitin-ligase $SCF^{SLY1/GID2}$, and the eventual destruction of DELLA proteins in the 26S proteasome (McGinnis et al., 2003; Sasaki et al., 2003; Dill et al., 2004; Fu et al., 2004; Ueguchi-Tanaka et al., 2005; Griffiths et al., 2006; Jiang and Fu, 2007; Murase et al., 2008; Harberd et al., 2009; Hou et al., 2010). Thus, DELLA proteins restrain plant growth while GA promotes growth by targeting the DELLA proteins for degradation (Silverstone et al., 2001; Fu et al., 2002; Gao et al., 2011).

Previous studies have suggested that the DeLLaΦ-LxYxV motif is a GA receptor GID1 recognition sequence (Murase et al., 2008), and the deletion of this motif results in a GA-insensitive dwarfism, such as in *gai* and *rga-Δ17* (Peng et al., 1997; Dill et al., 2001; Itoh et al., 2002). In our experiments, we found that there were no, weak, and strong interactions between TaGID1 and Rht-B1c, Rht-B1b, and Rht-B1a, respectively. Furthermore, we found that the 30-amino acid insertion into the DELLA domain causes the reduction of affinity between GID1 and Rht-B1c, which in turn abolished the GA-mediated degradation of DELLA protein and consequently resulted in DELLA protein accumulation and GA-insensitive dwarfism. These observations suggested that Rht-B1c might not form

the Rht-B1c-TaGID1 complex. Therefore, the affinity of the interaction between DELLA and GID1 controls DELLA protein accumulation, which determines completely insensitive, semi-insensitive, and sensitive responses to GA in *Rht-B1c*, *Rht-B1b*, and *Rht-B1a*, respectively.

Pleiotropism of *GAI* Is Associated with Its Dominance

Our results showed that *Rht-B1c* affects several traits, including strongly reduced plant height, preharvest sprouting tolerance, extreme prostrate habit, late flowering, and partial male sterility. However, *Rht-B1b*, a semidominant *GAI*, affects only plant height. GA acts as a major hormone in plants and regulates many processes of growth and productivity. It has been reported that GA controls plant height (Richards et al., 2001; Jiang and Fu, 2007; Davière et al., 2008; Gao et al., 2008; Shimada et al., 2008), seed germination and dormancy (Peng and Harberd, 2002; Finch-Savage and Leubner-Metzger, 2006; Razem et al., 2006; Seo et al., 2006), fertility and flowering (Achard et al., 2004, 2007; Cheng et al., 2004; Kaneko et al., 2004; Chhun et al., 2007; Lin et al., 2011), and prostrate habit (Cui et al., 2005; Appleford et al., 2007; Wolbang et al., 2007). Our results showed that plants with *Rht-B1c* are completely insensitive to GA, whereas those with *Rht-B1b* and *Rht-B1a* are weakly insensitive and sensitive, respectively. We hypothesized that responses to endogenous GA for different traits are different. For example, plant height might be more sensitive than other traits, such that small reductions in endogenous GAs result in reduced height without significantly affecting the other traits. Thus, only dramatic reductions in endogenous GAs lead to multiple phenotypic changes. Further studies are required to examine these differential responses to GA.

Structural Characteristics and Function of CAAS-TRIM-6B

In this work, we determined that the *Rht-B1c* mutation resulted from an insertion of a highly conserved TRIM element, CAAS-TRIM, belonging to the long terminal repeat retrotransposon class. TRIM is believed to be constantly active (Witte et al., 2001). Sabot et al. (2006) suggested that TRIM elements were the most active retrotransposons in the wheat genome based on an activity index (Sabot et al., 2006). According to Witte et al. (2001), TRIM elements have significant functions in the reorganization of plant genomes by affecting the promoter, coding region, and intron-exon structure of genes (Witte et al., 2001). In this report, a TRIM-CAAS insertion in the DELLA domain of *Rht-B1a* resulted in a structural change of the gene. TRIM elements also have positive roles in creating new functions in the *Brassica* genome (Yang et al., 2007). High conservatism of terminal direct repeat sequences showed that CAAS-TRIM is a recently inserted element. However, we have not been able to determine the mobilization mechanism of CAAS-TRIM, because it does not appear to encode its own proteins, such as gag, pol, and env. The retrotransposons Morgane and LARDs similarly lack all or most of their open reading frames, indicating that they are probably parasitic on autonomous retrotransposon families that in turn are parasitic on the genome (Witte et al., 2001; Kalendar et al., 2004; Antonius-Klemola et al., 2006; Sabot et al., 2006; Tanskanen et al., 2007). We detected CAAS-TRIM in 150 wheat accessions and found that it acts as a highly active element capable of being inserted in different locations of the wheat genome. When it is inserted into a gene, such as *Rht-B1a*, the gene structure and function may be altered. The function is similar to that of rice retrotransposon Tos17 and maize (*Zea mays*) transposable elements Ac/Ds and En/Spm (Walbot, 1992; Wisman et al., 1998; Parinov et al., 1999; Speulman et al., 1999; Tissier et al., 1999; Hirochika, 2001). Therefore, it can be used as a tool to study wheat gene function by the detection of genes in which it is inserted. Among 150 accessions in which the element was detected in this study, its position was located on chromosome 6B in only nine. Detection of the insertion position in the other accessions may lead to the discovery of genes with altered functions. We assume that insertion is random and, therefore, that its insertion into *Rht-B1a* was an extremely rare event, probably explaining why Tom Thumb is a unique short variant among hundreds of thousands of wheat accessions in the world.

Potential Application of *Rht-B1c* in Crop Improvement

The cloning of *Rht-B1c* will accelerate its application in agriculture. Our work confirms that *Rht-B1c* is a GAI gene. GAI genes are highly conserved across plant species, including Arabidopsis (*GAI*), *Brassica rapa* (*dwf2*), grape (*Vitis vinifera*; *VvGAI1*), rice (*SLR1*), maize (*D8*), barley (*Hordeum vulgare*; *SLN1*), and wheat (*Rht-B1b* and *Rht-D1b*; Peng et al., 1999; Dill and Sun, 2001; Ikeda et al., 2001; Boss and Thomas, 2002; Chandler et al., 2002; Muangprom and Osborn, 2004). We transferred *Rht-B1c* to rice and proved that it functions in that species, where its effect was even more extreme than in wheat. Hence, it should function in other species. Its ability to confer large reductions in plant height could be used in tall plants, such as rye (*Secale cereale*), maize, and sorghum (*Sorghum bicolor*). Its ability to reduce height and increase tiller angles and to delay heading date might be useful in turf grass improvement. Among its several pleiotropic functions, some are advantageous for plant improvement and some are disadvantageous. To utilize favorable functions and avoid unfavorable ones, tissue-specific promoters may offer opportunities. For example, resistance to sprouting is an important attribute of *Rht-B1c*, but to exploit this function and avoid unwanted functions, an endosperm-specific promoter could be used when constructing a vector for transformation.

MATERIALS AND METHODS

Plant Materials, Growth Conditions, and Field Trials

Materials included wheat (*Triticum aestivum*) lines Tom Thumb, Norin 10, and Chinese Spring. For developing NILs for the *Rht-B1c* allele, a cross was made between cv YZ1 with genotype *Rht-B1a*/*Rht-D1b* as a recurrent parent and Tom Thumb with genotype *Rht-B1c* as donor. A segregating population of 408 F2 plants derived from a cross between Yumai 18 and *Rht-B1c*-RIL (selected from a RIL population derived from a cross between Tom Thumb and YZ1), a previously mapped random set of 150 Hanxuan 10 × Lumai 14 doubled haploid lines, and 150 diverse wheat accessions from different countries (Supplemental Table S2) were also used. The NILs and parents were planted in long-day (Hebei; 115°41′E, 41°41′N) and short-day (Yunnan; 101°41′E, 25°42′N) environments. The F2 segregating population, doubled haploid mapping population, and 150 diverse wheat accessions were planted in Beijing (116°21′E, 39°54′N). Plant heights were measured as means of five individuals in each plot after flowering. Tiller angles were measured between the vertical and most extreme intact tiller on each plant at the mature stage. One hundred visually undamaged seeds per wheat genotype were used in germination tests with two replicates 10 d before harvest.

Isolation of *Rht-B1c* Using PCR of Tom Thumb Genomic DNA and cDNA, Protein Structure Prediction, and Analysis

Genomic DNA was isolated from wheat tissues as described by Sharp et al. (1989). Total RNA was obtained using TRIzol reagent (Invitrogen). cDNA was synthesized using SuperScript reverse transcriptase (Invitrogen). The *Rht-B1c* genomic DNA was amplified with primers Rht-B1cp (5′-ATGCCGTCTACAACTACTACGCTG-3′ and 5′-AGTCCGGCCCGTGCTTATTTTG-3′) at 98°C for 10 min, followed by 32 cycles at 98°C for 45 s, 63°C for 45 s, 72°C for 4 min, and an extension at 72°C for 10 min. Entire Rht-B1c cDNA was amplified with primers Rht-B1c-cDNAp (5′-ATGAAGCGCGAGTACCAGGA-3′ and 5′-TCACGGCGCGGCCAGGCGCCATGC-3′) at 98°C for 10 min, followed by 32 cycles at 98°C for 45 s, 63°C for 45 s, 72°C for 2 min, and an extension at 72°C for 10 min. For mapping TRIM, we used primers 5′-CCGGACAAATGGAGGAGA-3′ and 5′-CGCGCATCATCCCGAGTAT-3′ at 95°C for 5 min, followed by 32 cycles at 95°C for 45 s, 55°C for 45 s, 72°C for 2.5 min, and an extension at 72°C for 10 min. Selected NILs were amplified with primers Rht-B1cM (5′-ATGCCGTCTACAACTACTACGCTG-3′ and 5′-TAGTGCACGGTGTCCGTGGCGA-3′) at 98°C for 10 min, followed by 32 cycles at 98°C for 45 s, 63°C for 45 s, 72°C for 2 min 30 s, and an extension at 72°C for 10 min. The full-length TaGID1 cDNA was amplified using the primers 5′-ATGGCCGGCAGCGACGAGGT-3′ and 5′-CTACAGGAGGTTAGCTCGGA-3′ at 94°C for 5 min, followed by 32 cycles at 94°C for 45 s, 63°C for 45 s, 72°C for 1 min 30 s, and an extension at 72°C for 10 min. All PCR products were separated on agarose gels and visualized after ethidium bromide staining using standard procedures and cloning of these fragments in competent cells following the pGEM-T protocol (Promega). Clones used Big-Dye terminator chemistry on an ABI 3730 automated capillary sequencer (Applied Biosystems). The sequence reads were assembled used lasergene software (http://www.dnastar.com/t-products-lasergene.aspx) and aligned using ClustalW (http://www.genome.jp/tools/clustalw/) with default parameters. The 3D structure of a protein was constructed using swissmodel workspace (http://swissmodel.expasy.org/) and PyMol software (http://www.pymol.org/).

GA Treatment Used in Seedlings and Assays of α-Amylase Activity

For GA sensitivity, seeds of lines with *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* were germinated in water. Seedlings were sprayed daily with an aqueous solution containing or lacking 50 μL L^{-1} GA_3 for 10 d. For assays of α-amylase activity, embryoless half-seeds of wheat were surface sterilized for 30 min with 3% NaClO and washed several times with sterile distilled water. The half-seeds were placed on an agar medium (pH 5.3) containing 0.2% starch, 2% agarose, 10 mM sodium acetate, and 2 mM $CaCl_2$ with or without 1 μM GA_3. The plates were incubated for 60 to 72 h at 30°C in darkness, following which they were exposed to iodine vapor to detect whether starch had been digested by α-amylase.

Plasmid Construction and Rice Transformation

Full-length *Rht-B1b* and *Rht-B1c* cDNAs were produced by reverse transcription-PCR from total RNA from Norin 10 and Tom Thumb. For transgenesis, full-length *Rht-B1b* and *Rht-B1c* cDNAs, including about 4 kb of the promoter and NOS terminator regions, were inserted into binary vector pCAMBIA1300 to generate the plasmids *pRht-B1b*:*Rht-B1b* and *pRht-B1c*:*Rht-B1c*.

The binary vectors were introduced into *Agrobacterium tumefaciens* strain EHA105 by electroporation. Rice transformation was performed as described by Hiei et al. (1994). Transgenic rice plants were selected on medium containing 50 mg L^{-1} hygromycin. Transgenic rice plants were grown in a greenhouse in Beijing (116°21′E, 39°54′N) and in the field in Hainan (110°0′E, 18°24′N).

Yeast Two-Hybrid System

For the Y2H assay, full-length *Rht-B1a*, *Rht-B1b*, and *Rht-B1c* cDNAs were amplified using primers 5′-GGAATTCCATATGATGAAGCGCGAGTACCAGGA-3′ and 5′-CGGAATTCTCACGGCGCGGCCAGGCGCC-3′ and inserted into the *Nde*I-*Eco*RI sites of the pGADT7 (Clontech) vector. The full-length TaGID1 cDNA was amplified using primers 5′-GGAATTCCATATGATGGCCGGCAGCGACGAGGT-3′ and 5′-CGGAATTCCTACAGGAGGTTAGCTCGGA-3′ and inserted into the pGBKT7 (Clontech) vector as an *Nde*I-*Eco*RI fragment. pGBKT7-TaGID1 served as the bait and pGADT7-Rht-B1a, pGADT7-Rht-B1b, and pGADT7-Rht-B1c served as the prey. Yeast strain AH109 was used in detection plate (−His−Ade) and β-galactosidase liquid assays, both performed according to the manufacturer's protocols (Yeast Protocols Handbook PT3024-1; Clontech). In both assays, the medium either contained 10^{-4} M GA_3 or did not.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers JN857970 (gDNA of *Rht-B1c*) and JN857971 (cDNA of *Rht-B1c*).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Nucleotide sequences of *Rht-B1a*, *Rht-B1b*, and *Rht-B1c*.

Supplemental Figure S2. Specific marker for *Rht-B1c* in wheat and mRNA levels for *Rht-B1b* and *Rht-B1c* in transgenic rice.

Supplemental Table S1. Agronomic traits of NILs and the F2 segregating population planted under natural conditions.

Supplemental Table S2. One hundred fifty accessions representing a broad range of wheat germplasm.

Supplemental Table S3. Increasing heights of different transgenic rice plants treated with GA_3.

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LITERATURE CITED

Achard P, Baghour M, Chapple A, Hedden P, Van Der Straeten D, Genschik P, Moritz T, Harberd NP (2007) The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. Proc Natl Acad Sci USA **104:** 6484–6489

Achard P, Herr A, Baulcombe DC, Harberd NP (2004) Modulation of floral development by a gibberellin-regulated microRNA. Development **131:** 3357–3365

Antonius-Klemola K, Kalendar R, Schulman AH (2006) TRIM retrotransposons occur in apple and are polymorphic between varieties but not sports. Theor Appl Genet **112:** 999–1008

Appleford NEJ, Wilkinson MD, Ma Q, Evans DJ, Stone MC, Pearce SP, Powers SJ, Thomas SG, Jones HD, Phillips AL, et al (2007) Decreased shoot stature and grain α-amylase activity following ectopic expression of a gibberellin 2-oxidase gene in transgenic wheat. J Exp Bot **58:** 3213–3226

Borlaug NE (1983) Contributions of conventional plant breeding to food production. Science **219:** 689–693

Börner A, Plaschke J, Korzun V, Worland AJ (1996) The relationship between the dwarfing genes of wheat and rye. Euphytica **89:** 69–75

Boss PK, Thomas MR (2002) Association of dwarfism and floral induction with a grape 'Green Revolution' mutation. Nature **416:** 847–850

Chandler PM, Marion-Poll A, Ellis M, Gubler F (2002) Mutants at the *Slender1* locus of barley cv Himalaya: molecular and physiological characterization. Plant Physiol **129:** 181–190

Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J (2004) Gibberellin regulates *Arabidopsis* floral development via suppression of DELLA protein function. Development **131:** 1055–1064

Chhun T, Aya K, Asano K, Yamamoto E, Morinaka Y, Watanabe M, Kitano H, Ashikari M, Matsuoka M, Ueguchi-Tanaka M (2007) Gibberellin regulates pollen viability and pollen tube growth in rice. Plant Cell **19:** 3876–3888

Cui D, Neill SJ, Tang Z, Cai W (2005) Gibberellin-regulated XET is differentially induced by auxin in rice leaf sheath bases during gravitropic bending. J Exp Bot **56:** 1327–1334

Davière JM, de Lucas M, Prat S (2008) Transcriptional factor interaction: a central step in DELLA function. Curr Opin Genet Dev **18:** 295–303

Dill A, Jung HS, Sun TP (2001) The DELLA motif is essential for gibberellin-induced degradation of RGA. Proc Natl Acad Sci USA **98:** 14162–14167

Dill A, Sun TP (2001) Synergistic derepression of gibberellin signaling by removing RGA and GAI function in *Arabidopsis thaliana*. Genetics **159:** 777–785

Dill A, Thomas SG, Hu J, Steber CM, Sun TP (2004) The *Arabidopsis* F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell **16:** 1392–1405

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol **171:** 501–523

Flintham JE, Gale MD (1982) The Tom Thumb dwarfing gene, *Rht3* in wheat. I. Reduced pre-harvest damage to breadmaking quality. Theor Appl Genet **62:** 121–126

Flintham JE, Gale MD (1983) The Tom Thumb dwarfing gene, *Rht3* in wheat. II. Effects on height, yield and grain quality. Theor Appl Genet **66:** 249–256

Fu X, Harberd NP (2003) Auxin promotes Arabidopsis root growth by modulating gibberellin response. Nature **421:** 740–743

Fu X, Richards DE, Ait-Ali T, Hynes LW, Ougham H, Peng J, Harberd NP (2002) Gibberellin-mediated proteasome-dependent degradation of the barley DELLA protein SLN1 repressor. Plant Cell **14:** 3191–3200

Fu X, Richards DE, Fleck B, Xie D, Burton N, Harberd NP (2004) The *Arabidopsis* mutant sleepy1$^{gar2\text{-}1}$ protein promotes plant growth by increasing the affinity of the SCF^{SLY1} E3 ubiquitin ligase for DELLA protein substrates. Plant Cell **16:** 1406–1418

Gale MD, Marshall GA (1973) Insensitivity to gibberellin in dwarf wheats. Ann Bot (Lond) **37:** 729–735

Gale MD, Marshall GA (1976) The chromosomal location of *Gai1* and *Rht1*, genes for gibberellin insensitivity and semi-dwarfism, in a derivative of Norin 10 wheat. Heredity **37:** 283–289

Gale MD, Youssefian S (1985) Dwarfing genes in wheat. *In* GE Russell, ed, Progress in Plant Breeding. Butterworth, London, pp 1–35

Gao XH, Huang XZ, Xiao SL, Fu XD (2008) Evolutionarily conserved DELLA-mediated gibberellin signaling in plants. J Integr Plant Biol **50:** 825–834

Gao XH, Xiao SL, Yao QF, Wang YJ, Fu XD (2011) An updated GA signaling 'relief of repression' regulatory model. Mol Plant **4:** 601–606

Griffiths J, Murase K, Rieu I, Zentella R, Zhang ZL, Powers SJ, Gong F, Phillips AL, Hedden P, Sun TP, et al (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in *Arabidopsis*. Plant Cell **18:** 3399–3414

Gubler F, Kalla R, Roberts JK, Jacobsen JV (1995) Gibberellin-regulated expression of a myb gene in barley aleurone cells: evidence for Myb transactivation of a high-pI α-amylase gene promoter. Plant Cell **7:** 1879–1891

Harberd NP (2003) Relieving DELLA restraint. Science **299:** 1853–1854

Harberd NP, Belfield E, Yasumura Y (2009) The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: how an "inhibitor of an inhibitor" enables flexible response to fluctuating environments. Plant Cell **21:** 1328–1339

Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. Plant J **6:** 271–282

Hirano K, Nakajima M, Asano K, Nishiyama T, Sakakibara H, Kojima M, Katoh E, Xiang H, Tanahashi T, Hasebe M, et al (2007) The GID1-mediated gibberellin perception mechanism is conserved in the lycophyte *Selaginella moellendorffii* but not in the bryophyte *Physcomitrella patens*. Plant Cell **19:** 3058–3079

Hirochika H (2001) Contribution of the Tos17 retrotransposon to rice functional genomics. Curr Opin Plant Biol **4:** 118–122

Hou X, Lee LYC, Xia K, Yan Y, Yu H (2010) DELLAs modulate jasmonate signaling via competitive binding to JAZs. Dev Cell **19:** 884–894

Ikeda A, Ueguchi-Tanaka M, Sonoda Y, Kitano H, Koshioka M, Futsuhara Y, Matsuoka M, Yamaguchi J (2001) slender rice, a constitutive gibberellin response mutant, is caused by a null mutation of the SLR1 gene, an ortholog of the height-regulating gene GAI/RGA/RHT/D8. Plant Cell **13:** 999–1010

Itoh H, Ueguchi-Tanaka M, Sato Y, Ashikari M, Matsuoka M (2002) The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. Plant Cell **14:** 57–70

Jiang C, Fu X (2007) GA action: turning on de-DELLA repressing signaling. Curr Opin Plant Biol **10:** 461–465

Kalendar R, Vicient CM, Peleg O, Anamthawat-Jonsson K, Bolshoy A, Schulman AH (2004) Large retrotransposon derivatives: abundant, conserved but nonautonomous retroelements of barley and related genomes. Genetics **166:** 1437–1450

Kaneko M, Inukai Y, Ueguchi-Tanaka M, Itoh H, Izawa T, Kobayashi Y, Hattori T, Miyao A, Hirochika H, Ashikari M, et al (2004) Loss-of-function mutations of the rice GAMYB gene impair α-amylase expression in aleurone and flower development. Plant Cell **16:** 33–44

Lin CC, Chu CF, Liu PH, Lin HH, Liang SC, Hsu WE, Lin JS, Wang HM, Chang LL, Chien CT, et al (2011) Expression of an Oncidium gene encoding a patatin-like protein delays flowering in Arabidopsis by reducing gibberellin synthesis. Plant Cell Physiol **52:** 421–435

McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP, Steber CM (2003) The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell **15:** 1120–1130

McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers J, Morris C, Somers DJ, Appels R, Devos KM (2008) Catalogue of gene symbols for wheat. *In* Proceedings of the 11th International Wheat Genetics Symposium. Sydney University Press, Sydney, pp 30–35

McVittie JA, Gale MD, Marshall GA, Westcott B (1978) The intra-chromosomal mapping of the Norin 10 and Tom Thumb genes. Heredity **40:** 67–70

Morris R, Schmidt JW, Johnson VA (1972) Chromosomal location of a dwarfing gene in 'Tom Thumb' wheat derivative by monosomic analysis. Crop Sci **12:** 247–249

Muangprom A, Osborn TC (2004) Characterization of a dwarf gene in *Brassica rapa*, including the identification of a candidate gene. Theor Appl Genet **108:** 1378–1384

Murase K, Hirano Y, Sun TP, Hakoshima T (2008) Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature **456:** 459–463

Nakajima M, Shimada A, Takashi Y, Kim YC, Park SH, Ueguchi-Tanaka M, Suzuki H, Katoh E, Iuchi S, Kobayashi M, et al (2006) Identification and characterization of *Arabidopsis* gibberellin receptors. Plant J **46:** 880–889

Parinov S, Sevugan M, Ye D, Yang WC, Kumaran M, Sundaresan V (1999) Analysis of flanking sequences from dissociation insertion lines: a database for reverse genetics in *Arabidopsis*. Plant Cell **11:** 2263–2270

Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP (1997) The *Arabidopsis GAI* gene defines a signaling pathway that negatively regulates gibberellin responses. Genes Dev **11:** 3194–3205

Peng J, Harberd NP (2002) The role of GA-mediated signalling in the control of seed germination. Curr Opin Plant Biol **5:** 376–381

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE,

Beales J, Fish LJ, Worland AJ, Pelica F, et al (1999) 'Green Revolution' genes encode mutant gibberellin response modulators. Nature **400:** 256–261

Pysh LD, Wysocka-Diller JW, Camilleri C, Bouchez D, Benfey PN (1999) The GRAS gene family in *Arabidopsis*: sequence characterization and basic expression analysis of the SCARECROW-LIKE genes. Plant J **18:** 111–119

Razem FA, Baron K, Hill RD (2006) Turning on gibberellin and abscisic acid signaling. Curr Opin Plant Biol **9:** 454–459

Richards DE, King KE, Ait-Ali T, Harberd NP (2001) How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling. Annu Rev Plant Physiol Plant Mol Biol **52:** 67–88

Sabot F, Sourdille P, Chantret N, Bernard M (2006) Morgane, a new LTR retrotransposon group, and its subfamilies in wheats. Genetica **128:** 439–447

Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong DH, An G, Kitano H, Ashikari M, et al (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science **299:** 1896–1898

Seo M, Hanada A, Kuwahara A, Endo A, Okamoto M, Yamauchi Y, North H, Marion-Poll A, Sun TP, Koshiba T, et al (2006) Regulation of hormone metabolism in *Arabidopsis* seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. Plant J **48:** 354–366

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet **78:** 342–348

Shimada A, Ueguchi-Tanaka M, Nakatsu T, Nakajima M, Naoe Y, Ohmiya H, Kato H, Matsuoka M (2008) Structural basis for gibberellin recognition by its receptor GID1. Nature **456:** 520–523

Silverstone AL, Jung HS, Dill A, Kawaide H, Kamiya Y, Sun TP (2001) Repressing a repressor: gibberellin-induced rapid reduction of the RGA protein in *Arabidopsis*. Plant Cell **13:** 1555–1566

Speulman E, Metz PL, van Arkel G, te Lintel Hekkert B, Stiekema WJ, Pereira A (1999) A two-component enhancer-inhibitor transposon mutagenesis system for functional analysis of the *Arabidopsis* genome. Plant Cell **11:** 1853–1866

Tanskanen JA, Sabot F, Vicient C, Schulman AH (2007) Life without GAG: the BARE-2 retrotransposon as a parasite's parasite. Gene **390:** 166–174

Tissier AF, Marillonnet S, Klimyuk V, Patel K, Torres MA, Murphy G, Jones JD (1999) Multiple independent defective suppressor-mutator transposon insertions in *Arabidopsis*: a tool for functional genomics. Plant Cell **11:** 1841–1852

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YI, Kitano H, Yamaguchi I, et al (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature **437:** 693–698

Ueguchi-Tanaka M, Nakajima M, Katoh E, Ohmiya H, Asano K, Saji S, Hongyu X, Ashikari M, Kitano H, Yamaguchi I, et al (2007) Molecular interactions of a soluble gibberellin receptor, GID1, with a rice DELLA protein, SLR1, and gibberellin. Plant Cell **19:** 2140–2155

Walbot V (1992) Strategies for mutagenesis and gene cloning using transposon tagging and T-DNA insertional mutagenesis. Annu Rev Plant Physiol Plant Mol Biol **43:** 49–82

Willige BC, Ghosh S, Nill C, Zourelidou M, Dohmann EMN, Maier A, Schwechheimer C (2007) The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of *Arabidopsis*. Plant Cell **19:** 1209–1220

Wisman E, Hartmann U, Sagasser M, Baumann E, Palme K, Hahlbrock K, Saedler H, Weisshaar B (1998) Knock-out mutants from an En-1 mutagenized *Arabidopsis thaliana* population generate phenylpropanoid biosynthesis phenotypes. Proc Natl Acad Sci USA **95:** 12432–12437

Witte CP, Le QH, Bureau T, Kumar A (2001) Terminal-repeat retrotransposons in miniature (TRIM) are involved in restructuring plant genomes. Proc Natl Acad Sci USA **98:** 13778–13783

Wolbang CM, Davies NW, Taylor SA, Ross JJ (2007) Gravistimulation leads to asymmetry of both auxin and gibberellin levels in barley pulvini. Physiol Plant **131:** 140–148

Yamamoto Y, Hirai T, Yamamoto E, Kawamura M, Sato T, Kitano H, Matsuoka M, Ueguchi-Tanaka M (2010) A rice gid1 suppressor mutant reveals that gibberellin is not always required for interaction between its receptor, GID1, and DELLA proteins. Plant Cell **22:** 3589–3602

Yang TJ, Kwon SJ, Choi BS, Kim JS, Jin M, Lim KB, Park JY, Kim JA, Lim MH, Kim HI, et al (2007) Characterization of terminal-repeat retrotransposon in miniature (TRIM) in Brassica relatives. Theor Appl Genet **114:** 627–636

Yasumura Y, Crumpton-Taylor M, Fuentes S, Harberd NP (2007) Step-by-step acquisition of the gibberellin-DELLA growth-regulatory mechanism during land-plant evolution. Curr Biol **17:** 1225–1230