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A Strigolactone Biosynthesis Gene Contributed to the Green Revolution in Rice

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ABSTRACT

Plant architecture is a complex agronomic trait and a major factor of crop yield, which is affected by several important hormones. Strigolactones (SLs) are identified as a new class hormoneinhibiting branching in many plant species and have been shown to be involved in various developmental processes. Genetical and chemical modulation of the SL pathway is recognized as a promising approach to modify plant architecture. However, whether and how the genes involved in the SL pathway could be utilized in breeding still remain elusive. Here, we demonstrate that a partial loss-of-function allele of the SL biosynthesis gene, *HIGH TILLERING AND DWARF 1/DWARF17* (*HTD1/D17*), which encodes CAROTENOID CLEAVAGE DIOXYGENASE 7 (CCD7), increases tiller number and improves grain yield in rice. We found that the *HTD1* gene had been widely utilized and co-selected with *Semidwarf 1* (*SD1*), both contributing to the improvement of plant architecture in modern rice varieties since the Green Revolution in the 1960s. Understanding how phytohormone pathway genes regulate plant architecture and how they have been utilized and selected in breeding will lay the foundation for developing the rational approaches toward improving crop yield.

Key words: rice, strigolactones, tiller number, Green Revolution

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INTRODUCTION

Plant architecture is a complex trait and a major factor influnces crop yield (Wang et al., 2018) and also is the most important agronomic trait for rice yield, which is determined by plant height, tiller number, and tiller angle. Manipulating plant architecture such as plant height, shoot branching, and spikelet number can significantly boost grain yield (Sakamoto and Matsuoka, 2004). By introducing specific beneficial alleles of the genes regulating gibberellin (GA) synthesis (Sasaki et al., 2002) or signaling (Peng et al., 1999), breeders had successfully developed semi-dwarf crops and doubled grain yield in a few decades. These phytohormone genes controlling plant architecture were recognized as ''Green Revolution genes'' (Peng et al., 1999; Sasaki et al., 2002; Nagano et al., 2005).

Strigolactone (SL) is a class of hormones inhibiting branching in plants (Gomez-Roldan et al., 2008; Umehara et al., 2008).

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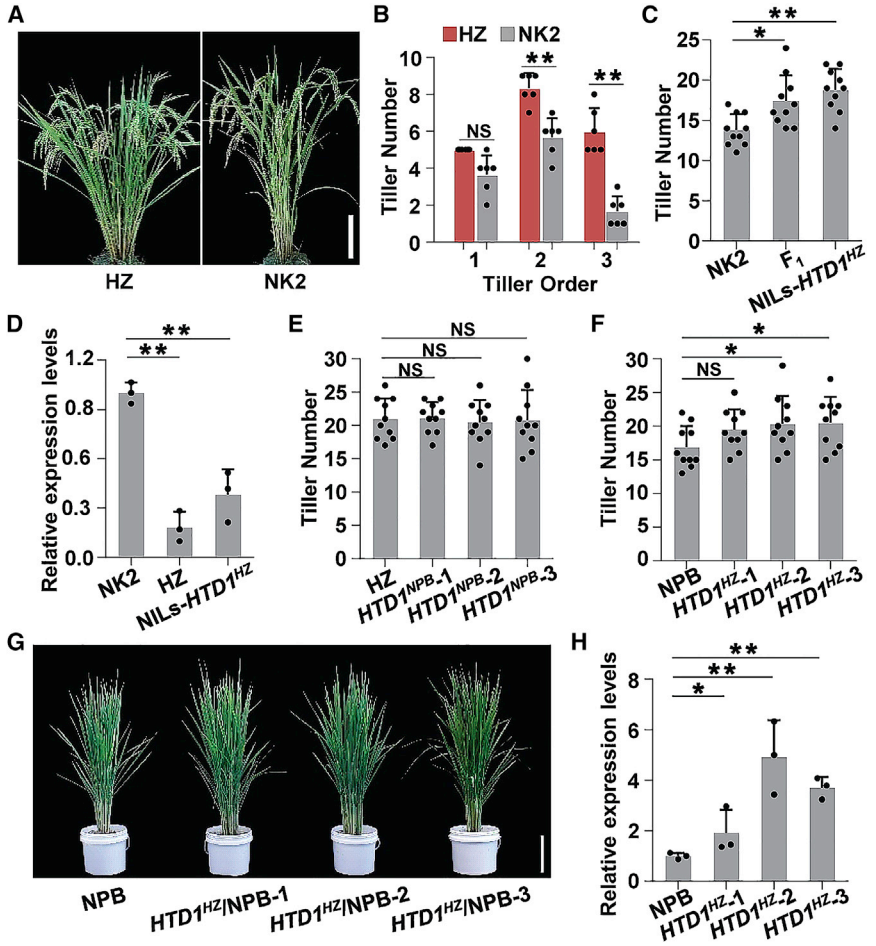


Figure 1. *HTD1*^{HZ} Regulates Tiller Number in a Dominant Manner.

(A) Morphologies of rice HZ and NK2 plants at the mature stage.

(B) Tiller number of different orders in HZ and NK2.

(C) Tiller number of NK2, F_1, and NILs-*HTD1*^{HZ}.

(D) Relative expression levels of *HTD1* in stems of NK2, HZ, and NILs-*HTD1*^{HZ} plants.

(E) Tiller number of HZ and *HTD1*^{NPB}/HZ transgenic lines.

(F) Tiller number of *HTD1*^{HZ}/NPB and transgenic lines.

(G) Plants of NPB, *HTD1*^{HZ}/NPB transgenic lines.

(H) Relative expression levels in NPB and *HTD1*^{HZ}/NPB transgenic lines.

Scale bar in **(A) and (G)**, 20 cm in and . Relative expression levels in **(D)** and **(H)** were normalized to *Ubiquitin* levels. Data in **(B) to (F)** and **(H)** are presented as means ± SD. $n = 6$ in **(B)**; $n = 10$ in **(C)**, **(E)**, and **(F)**; $n = 3$ in **(D)** and **(H)**. NS, not significant; $^*P < 0.05$, $^{**}P < 0.01$, Student's *t*-test.

Genetically or chemically modifying the SL pathway can significantly alter tiller number (Waters et al., 2017), a critical target for rice breeding (Wang et al., 2018). SL is derived from β-carotene (Gomez-Roldan et al., 2008; Umehara et al., 2008), which is subjected to the sequential act of three biosynthetic enzymes, *DWARF27* (*D27*) (Lin et al., 2009), *HIGH TILLERING AND DWARF 1/DWARF17 (HTD1/D17)* (Zou et al., 2006; Umehara et al., 2008), and *DWARF10* (*D10*) (Arite et al., 2007), to produce carlactone (Alder et al., 2012), which is further modified by MORE AXILLARY GROWTH 1 (MAX1) into an active SL in rice (Zhang et al., 2014). There are five homologs of MAX1 in rice, and these cytochrome P450 genes catalyze different steps in SL biosynthesis (Zhang et al., 2014). In the absence of SL, DWARF53 (D53) proteins may interact with transcription co-repressor TPR to regulate downstream gene expression. However, in the presence of SL, D53 is ubiquitinated by SCF^{D3} and degraded by 26S proteasome, which may release the inhibition of the expression of downstream genes (Jiang et al., 2013; Zhou et al., 2013). The disruption of the SL biosynthesis or signaling pathway leads to the accumulation of D53 and release of tiller bud outgrowth from inhibition (Ishikawa et al., 2005; Arite et al., 2009; Jiang et al., 2013; Zhou et al., 2013).

In this study, we show that a beneficial allele of the SL biosynthesis gene, *HTD1*, is the major contributor to high tillering and high yield in the elite rice variety Huazhan (HZ) cultivated widely in China. Furthermore, the beneficial allele of *HTD1* is originally from the milestone variety of the rice Green Revolution, IR8, and its parent Peta, and exists in most IR8-derived varieties. Our findings demonstrate that increased lodging resistance by pyramiding the GA pathway via *Semidwarf 1 (SD1)* and high yield with high tillering by modulating the SL pathway via *HTD1* together contribute to the success of the rice Green Revolution in breeding IR8 and its derived elite varieties.

RESULTS

HTD1^{HZ} Regulates Tiller Number in a Dominant Manner

HZ is a stable high-yield *indica* (Xian) restorer line developed in the 2000s with high productive tiller number, moderate plant height, and high adaptability for different cultivation regions (Figure 1A and 1B). Hybrid rice varieties using HZ as the restorer line had been developed to be the most widely cultivated varieties in southern China in the past decade. To dissect the genetic basis of the plant architecture of HZ, we generated a recombinant inbred line (RIL) population of 120 individual lines by crossing HZ with Nekken 2 (NK2), a relatively less tillering *japonica* (Geng) cultivar, which has fewer high-order tillers than HZ (Figure 1B and Supplemental Figure 1). Quantitative trait loci (QTL) analysis identified a major locus controlling plant height on chromosome 1 and a major locus controlling tiller number on chromosome 4 (Supplemental Figure 2A and 2B).

The genetic analysis suggested that the corresponding generegulating tiller number from HZ is dominant (Figure 1C; Supplemental Figure 2C and 2D), which was further fine mapped to a 107-kb interval between markers

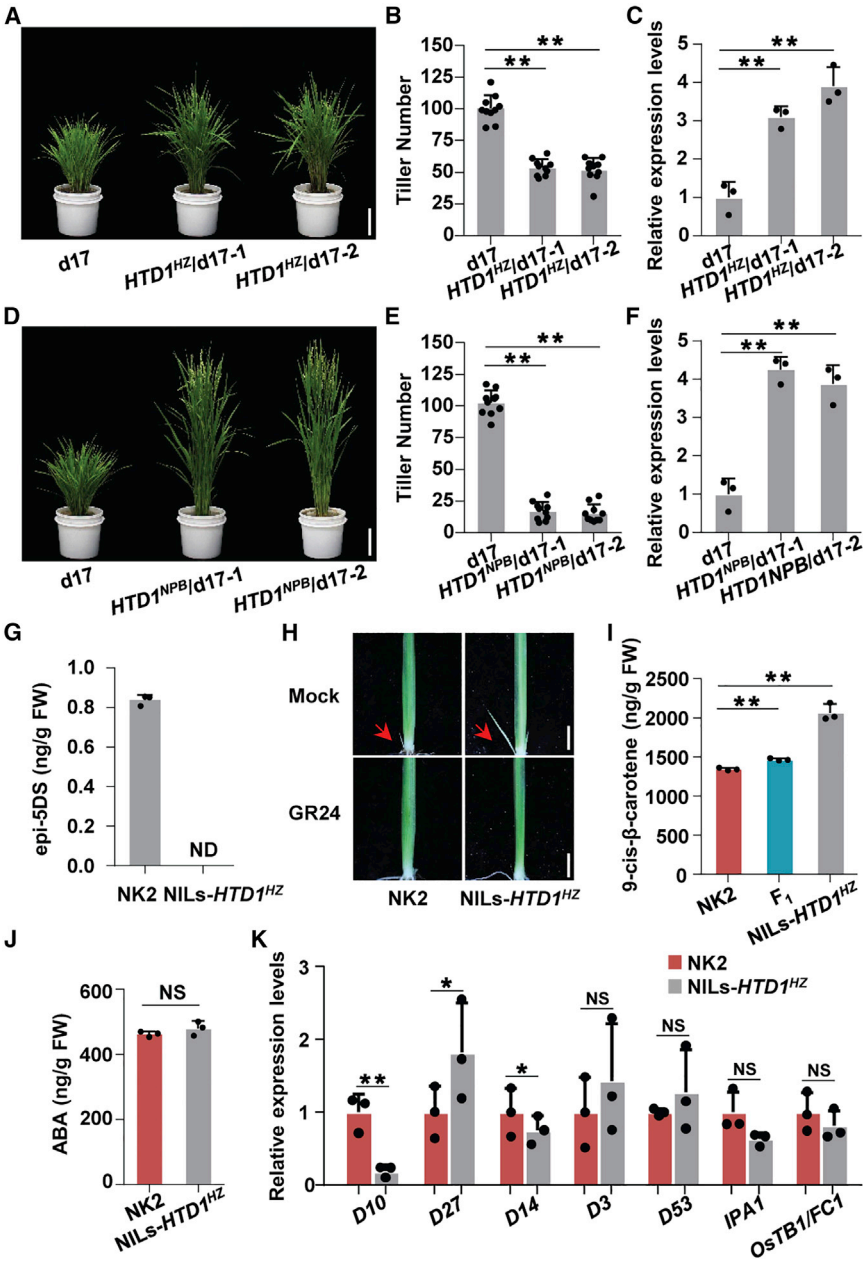


Figure 2. Effect of the Partial Loss-of-Function Allele of *HTD1*on SL Biosynthesis.

(A) The *d17* mutant and *HTD1*^{HZ} transgenic plants in *d17* background.

(B) Tiller number of *d17* and *HTD1*^{HZ}/*d17* transgenic plants.

(C) Relative expression levels of *HTD1* in *d17* and *HTD1*^{HZ} transgenic plants in *d17* background.

(D) *d17* and *HTD1*^{NPB} transgenic plants in *d17* background.

(E) Tiller number of *d17* and *HTD1*^{NPB}/*d17* plants.

(F) Relative expression levels of *HTD1* in *d17* and *HTD1*^{NPB} transgenic plants in *d17* background.

(G) The *epi-5DS* contents in NK2 and NILs-*HTD1*^{HZ} root exudates; g FW, per gram fresh weight; ND, not detected.

(H) Length of the second tiller bud (red arrowhead) of NK2 and NILs-*HTD1*^{HZ} treated with 1 μM GR24 for 2 weeks.

(I) Content of 9-*cis*-β-carotene in NILs-*HTD1*^{HZ}, F_1 (NK2/NILs-*HTD1*^{HZ}), and NK2.

(J) ABA content in NK2 and NILs-*HTD1*^{HZ}.

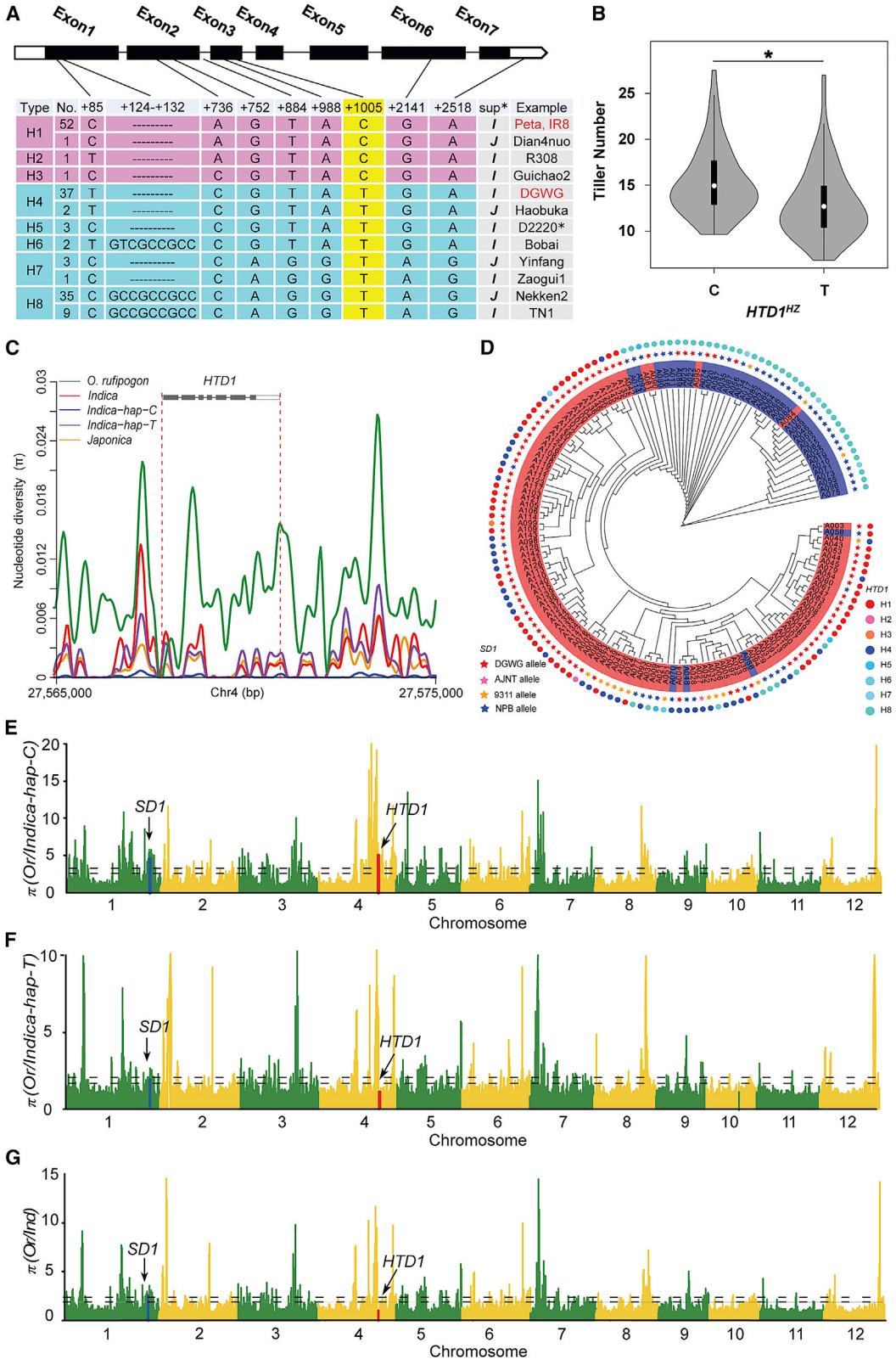
(K) Relative expression levels of *D10*, *D27*, *D14*, *D3*, *D53*, *IPA1*, and *OsTB1/FC1* in stems of NK2 and NILs-*HTD1*^{HZ} plants.

Scale bars, 10 cm **(A and D)** and 1.0 cm **(H)**. Relative expression levels in **(C)**, **(F)**, and **(K)** were normalized to *Ubiquitin* levels. Data in **(B)**, **(C)**, **(E)**, **(F)**, **(G)**, **(I)**, **(J)**, and **(K)** are presented as means ± SD. $n = 10$ in **(B)** and **(E)**; $n = 3$ in **(C)**, **(F)**, **(G)**, **(I)**, **(J)**, and **(K)**. NS, not significant; $^*P < 0.05$, $^{**}P < 0.01$, Student's *t*-test.

RM3288 and RM7187. This region contains *HTD1/D17* (Supplemental Figure 3A) encoding the SL biosynthesis pathway enzyme CAROTENOID CLEAVAGE DIOXYGENASE 7 (CCD7) (Zou et al., 2006; Umehara et al., 2008), a homolog of *Arabidopsis* MORE AXILLARY GROWTH 3 (MAX3) (Booker et al., 2004; Umehara et al., 2008). We found that the coding region sequence of *HTD1* from NK2 is identical to that of Nipponbare (NPB), containing one InDel and five single-nucleotide polymorphism (SNP) variations compared with that of HZ (Supplemental Figure 3B). Since the expression level of *HTD1* in NK2 was higher than that in NILs-*HTD1*^{HZ} (near isogenic lines, Figure 1D), we introduced *HTD1*^{NPB} into the HZ background and found that increases in the *HTD1* expression levels in the transgenic lines had little effect on the tiller number (Figure 1E and Supplemental Figure 3C), indicating that the lower expression levels in NILs-*HTD1*^{HZ} are not responsible for its high-tillering phenotype. However, the tiller numbers in *HTD1*^{HZ} highly expressed transgenic plants were significantly increased compared with NPB (Figure 1F–1H). These results suggested that *HTD1*^{HZ} regulates tiller number in a dominant manner.

HTD1^{HZ} Is a Partial Loss-of-Function Allele and Is Defective in SL Biosynthesis

We further performed *in situ* analysis of *HTD1* and found that *HTD1* is mainly expressed in tiller bud and that *HTD1*^{HZ} showed no obvious difference from *HTD1*^{NPB} (Supplemental Figure 4A). The transient expression in rice protoplasts showed that the subcellular localization of HTD1^{HZ} is similar to that of HTD1^{NPB}, mainly localized in chloroplasts (Supplemental Figure 4B). To determine whether NILs-*HTD1*^{HZ} is deficient in SL biosynthesis, we measured its *epi-5DS* content and found that NILs-*HTD1*^{HZ} has a lower *epi-5DS* level than NK2 (Figure 2G). Exogenous application of GR24, an SL artificial analog, inhibited the tiller bud outgrowth in NILs-*HTD1*^{HZ} (Figure 2H). Both SL and abscisic acid (ABA) are derived from the biosynthesis of β-carotene. The content of ABA showed no significant difference between NK2 and NILs-*HTD1*^{HZ} (Figure 2J). Since 9-*cis*-β-carotene is the substrate of CCD7 (Alder et al., 2012), disrupting the function of *HTD1* (CCD7 in rice) would result in the accumulation of 9-*cis*-β-carotene. We found that the



Type	No.	+85	+124-+132	+736	+752	+884	+988	+1005	+2141	+2518	sup*	Example
H1	52	C	----------	A	G	T	A	C	G	A	*I*	Peta, IR8
	1	C	----------	A	G	T	A	C	G	A	*J*	Dian4nuo
H2	1	T	----------	A	G	T	A	C	G	A	*I*	R308
H3	1	C	----------	C	G	T	A	C	G	A	*I*	Guichao2
H4	37	T	----------	C	G	T	A	T	G	A	*I*	DGWG
	2	T	----------	C	G	T	A	T	G	A	*J*	Haobuka
H5	3	C	----------	C	G	T	A	T	G	A	*I*	D2220*
H6	2	T	GTCGCCGCC	C	G	T	A	T	G	A	*I*	Bobai
H7	3	C	----------	C	A	G	G	T	A	G	*J*	Yinfang
	1	C	----------	C	A	G	G	T	A	G	*I*	Zaogui1
H8	35	C	GCCGCCGCC	C	A	G	G	T	A	G	*J*	Nekken2
	9	C	GCCGCCGCC	C	A	G	G	T	A	G	*I*	TN1

Figure 3. Selection and Utilization of *HTD1* and Co-selection of Beneficial Alleles of *HTD1* and *SD1*in Rice Breeding.

(A) Different haplotypes of *HTD1* in the 147 breeding varieties. "No." indicates the number of accessions, and numbers indicate the position of InDel or SNPs. sup, subspecies; *I*, *indica*; *J*, *japonica*.

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content of 9-*cis*-β-carotene in NILs-*HTD1*^{HZ} was significantly higher than in NK2 (Figure 2I). Meanwhile, the content of 9-*cis*-β-carotene in F_1 plant of *HTD1*^{HZ}/NK2 was also higher than in NK2 (Figure 2I). Compared with NK2, the expression of *D10* was strongly downregulated in *HTD1*^{HZ}/NK2, whereas the expression of *D27* and *D14* was slightly changed in *HTD1*^{HZ}/NK2 (Figure 2K). *d17* is a previously identified *HTD1* loss-of-function mutant in the NPB background (Jiang et al., 2013), the introduction of *HTD1*^{HZ} partially rescued its high-tillering phenotype (Figure 2A–2F), suggesting that *HTD1*^{HZ} is compromised in SL biosynthesis but still has leaky activity. Taken together, these results demonstrate that *HTD1*^{HZ} is a partial loss-of-function allele, leading to defective SL biosynthesis in rice.

Roles of Different Alleles of *HTD1* in Affecting Grain Yield

To investigate the role of *HTD1*^{HZ} in modulating plant architecture, we compared *HTD1*^{HZ} with another recessive allele, *cong'ai2* (*CL2*), a classic high-tillering and dwarf *indica* cultivar. We crossed *CL2* with the wild-type NPB to generate near isogenic lines (NILs) (Wang et al., 2012), and found that different *HTD1* alleles in the NPB background showed diverse effects on plant architecture (Supplemental Figures 3B and 5A). We further found that the D53 protein level in *d17* and NILs-*HTD1*^{CL2} were significantly higher than that in NPB, but had no significant change in the *HTD1*^{HZ}/NPB transgenic plants (Supplemental Figure 5B). The *d17* and NILs-*HTD1*^{CL2} have dramatically increased tiller number but reduced seed-setting rate compared with NPB, whereas NILs-*HTD1*^{HZ} has significantly increased tiller number without compromising other agronomic traits (Supplemental Figure 5C–5J). These results indicate that *HTD1*^{HZ} has leaky activity of CCD7, which is able to maintain SL at a level that could partially release the repression of tiller bud outgrowth but still sustain normal panicle development, thus leading to a significant increase in grain yield.

Selection and Utilization of *HTD1* Beneficial Allele in Rice Breeding

To further understand the *HTD1* function in regulating tiller number, we identified eight haplotypes of *HTD1* in 147 accessions (Figure 3A and Supplemental Table 1). Association analysis showed that the SNP C1005T between HZ and NK2, which resulted in the conserved amino acid T-to-M substitution, was correlated with the tiller number (Figure 3B and Supplemental Figure 3B), and the 147 accessions could be grouped into the *HTD1* C-type and T-type (Figure 3A).Nucleotide diversity analysis suggested that the *HTD1* locus had experienced strong artificial selection (Figure 3C and 3D). Haplotype analysis showed that the diversity of haplotypes is likely derived from Hap_5 (Supplemental Figure 3D and Supplemental Table 1).

To further understand the function of the SNP C1005T between HZ and NK2, we tried to change the 1005T into C by a CRISPR-mediated base-editing approach. We failed to obtain the plants with T to C at position 1005, but generated plants with T to C at position 999 (Supplemental Figure 3B), which resulted in the conserved amino acid L changed to P (Supplemental Figure 6). We found that tiller number increased and the content of *epi-5DS* decreased in the base-edited plants compared with the wild type (Supplemental Figure 7). Consistent with *HTD1*^{HZ}, we found that tiller number of the F_1 between the wild-type and base-edited plants also increased (Supplemental Figure 7A and 7B). These results indicated that changing the T to C at position 999 also created a partial loss-of-function allele of *HTD1* that could increase tiller number. This work provided an effective approach for increasing tiller number and grain yield by genetic modification.

Semi-dwarf rice varieties had been developed by Chinese breeders in the 1950s and by breeders of the International Rice Research Institute (IRRI) in the 1960s (Xie et al., 2015). The cross of a semi-dwarf cultivar "Dee-geo-woo-gen" (DGWG) (Sasaki et al., 2002) and high-tillering cultivar Peta led to the successful breeding of the "miracle rice" IR8, which was first released by the IRRI and considered as a milestone of the Green Revolution in rice (Peng and Khush, 2003). A large number of modern *indica* varieties were derived from a few elite varieties released in the early stage of the Green Revolution of rice (McNally et al., 2009; Xie et al., 2015; Huang et al., 2018). It has been shown that at least four *SD1* alleles had been used in the development of modern rice varieties, among which the *SD1*^{DGWG} has been widely used around the world (Asano et al., 2007). Sequence analysis showed that HZ contains *SD1*^{DGWG} (Supplemental Figure 8A and Supplemental Table 2). Analysis of the nucleotide diversity of *SD1* in these accessions showed that the *SD1* locus had experienced artificial selection (Supplemental Figure 8B).

To our surprise, the haplotype of *HTD1*^{HZ} is present in IR8 and Peta, but not in DGWG (Figure 3A and Supplemental Table 1). We found that the C-type *HTD1* is present not only in IR8-derived elite varieties such as IR36, IR56, and IR64 developed by IRRI breeders but also in Chinese major *indica* varieties such as MH63, Shuanggui36, and Guichao2 (Supplemental Table 1).

(B) Association of the SNP at the site 1005 of the *HTD1* coding region and the tiller number in rice accessions. A box plot and a kernel density plot were generated as violin plots for different groups of 147 accessions (Supplemental Table 1). Tukey's honestly significant difference test, $^*P < 0.05$.
(C) Nucleotide diversity analysis of *HTD1*. Different-colored lines represent samples of different groups including 23 accessions of *O. rufipogon* (green), 54 accessions of *HTD1*-haplotype-C group (*Indica-hap-C*, orange), 52 accessions of *HTD1*-haplotype-T group (*Indica-hap-T*, purple), and 41 accessions of *japonica* (blue).
(D) Distribution of haplotypes of *SD1* and *HTD1* (Supplemental Tables 1 and 2). The phylogenetic tree is based on the SNPs of genomes of 147 accessions. Circles with different colors indicate different haplotypes of *HTD1*, and pentacles with different colors represent different alleles of *SD1*.
(E–G) Beneficial allele of *HTD1*, showing a co-selection pattern with *SD1* in *indica* accessions. The π values between *O. rufipogon* and different groups of *O. sativa* accessions were compared. **(E)** *HTD1*-haplotype-C group *indica* (*Indica-hap-C*) and *O. rufipogon* (*Or*). **(F)** *HTD1*-haplotype-T group *indica* (*Indica-hap-T*) and *O. rufipogon* (*Or*). **(G)** All analyzed *indica* and *O. rufipogon* (*Or*). The upper and lower dashed horizontal lines indicate the genome-wide threshold (top 5% and top 10% of the genome) of the selection signals. The red lines denote the *HTD1* and the blue lines indicate *SD1* gene.

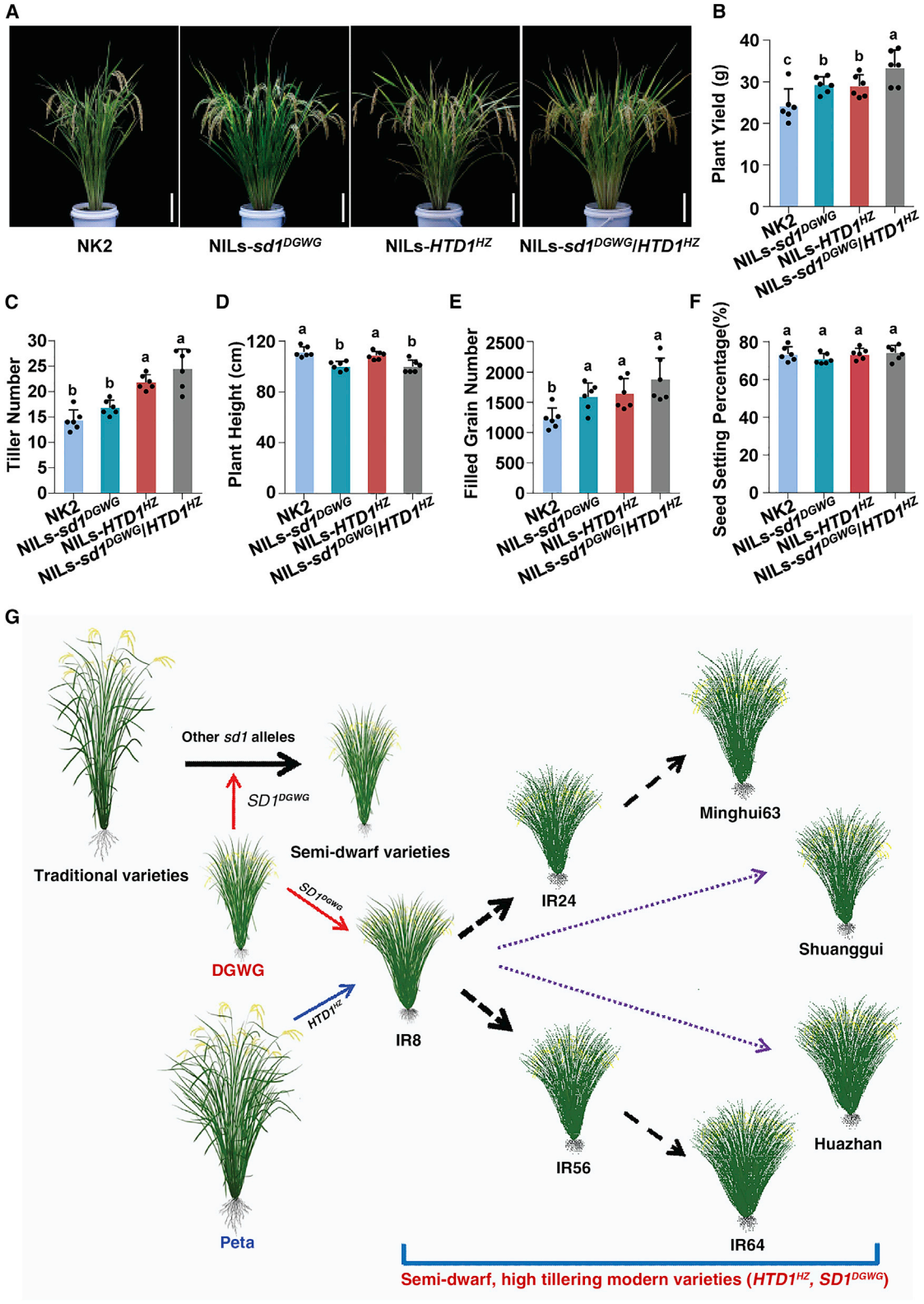

Figure 4. Pyramiding Beneficial Alleles of *HTD1* and *SD1* Contributes to Rice Yield.

(A–F) Pyramiding beneficial alleles of *HTD1* and *SD1* significantly improves grain yield. **(A)** Plant morphologies of NK2, NILs-*sd1*^{DGWG}, NILs-*HTD1*^{HZ}, and NILs-*sd1*^{DGWG}/*HTD1*^{HZ}. **(B)** Plant yields. **(C)** Tiller number. **(D)** Plant height. **(E)** Filled grain number. **(F)** Seed-setting percentage of NK2, NILs-*sd1*^{DGWG}, NILs-*HTD1*^{HZ}, and NILs-*sd1*^{DGWG}/*HTD1*^{HZ}.

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Breeding history and the pedigree information (McNally et al., 2009; Xie et al., 2015; Huang et al., 2018) strongly indicate that introduction of the *SD1* allele from DGWG into IR8 greatly contributed to the improved yield of modern rice varieties. Given that the haplotype of $HTD1^{HZ}$ is present in both IR8 and Peta, it seems that $SD1^{DGWG}$ and $HTD1^{HZ}$ introgressed into IR8 and passed together to their derived elite varieties released in the early stage of the Green Revolution (Xie et al., 2015), from which these beneficial alleles have been passed to and conserved in most modern *indica* varieties developed by IRRI and Chinese breeders.

Co-selection of Beneficial Alleles of *SD1* and *HTD1* in Rice

To determine whether the beneficial alleles of *HTD1* and *SD1* were selected in modern rice accessions, we compared the distribution of haplotypes of *HTD1* and *SD1* (Supplemental Figure 9A; Supplemental Tables 1 and 2) and found that the presence of C-type *HTD1* was closely associated with $SD1^{DGWG}$ and present in modern rice varieties but not in the early cultivated landraces (Supplemental Figure 9A; Supplemental Tables 1 and 2). Haplotype network analysis supports the possibility that the beneficial alleles of $HTD1^{HZ}$ and $SD1^{DGWG}$ have been likely introgressed together into modern *indica* accessions (Supplemental Figure 9B and Supplemental Table 3). Pedigree analysis has succeeded in identifying preserved multiple-yield related inheritance blocks in descendants derived from IR8 and MH63 (Huang et al., 2018). Inheritance blocks retained in progenies are likely to have been targeted by artificial selection (Huang et al., 2018). We then determined the inheritance blocks from DGWG and Peta in 147 accessions (Supplemental Figure 10A) and found that the *HTD1* block from Peta showed a correlation pattern with the *SD1* block from DGWG (Supplemental Figure 10B). The non-random distribution and coincidence of introgressions of the *SD1* and *HTD1* inheritance blocks indicate that both of them were under selection during rice breeding.

To find out whether the $HTD1^{HZ}$ and $SD1^{DGWG}$ were subject to co-selection during rice breeding, we further analyzed the genome-wide differentially selected regions of the *indica* accessions and found that both *HTD1* and *SD1* loci were significantly subject to selection compared with the *Oryza rufipogon* and *C*-type *HTD1* group (Figure 3E), but not significantly selected compared with the *O. rufipogon* to T-type *HTD1* group or with all 106 *indica* accessions (Figure 3F and 3G). Two other different computational methods, *Fst* and XP-CLR (cross-population composite likelihood ratio test), showed similar results (Supplemental Figure 11). The co-appearance of $SD1^{DGWG}$ with *HTD1* 1005C is significantly higher than whole-genome random regions ($P < 0.001$) (Supplemental Figure 12A). Moreover, the beneficial alleles of *HTD1* are significantly enriched in modern Green Revolution varieties compared with pre-Green Revolution landraces in public data of 4726 rice varieties (Xie et al., 2015; Zhao et al., 2015) (Supplemental Figure 12B). The nucleotide diversity surrounding the *HTD1* exhibited a significant decrease in post-Green Revolution varieties relative to pre-Green Revolution landrace varieties (Supplemental Figure 13A and 13B). Tajima's *D* test suggested that *HTD1* deviated significantly from the neutral expectation ($P < 0.01$) in the *indica II*-hap-C group (Supplemental Figure 13C). Given the fact that *SD1* and *HTD1* are located in different chromosomes, these results suggest that $HTD1^{HZ}$ and $SD1^{DGWG}$ have been co-selected in developing modern *indica* varieties.

Pyramiding of Beneficial Alleles of *HTD1* and *SD1* in *Japonica* Varieties

The natural variations of the GA (Asano et al., 2007) and SL (Cardoso et al., 2014) pathway genes associated with plant height and tiller number have great potential in shaping plant architecture to improve grain yield. Co-selection of the beneficial alleles of GA and SL biosynthesis genes resulted in an optimal plant height and tiller number, and thus contributed to the increase in yield. We identified the QTLs that affect the yield using RILs of HZ and NK2, whereby *SD1* and *HTD1* are the top two QTLs that contribute the grain yield of the RILs (Supplemental Figure 14A). Furthermore, we generated the NILs of $SD1^{DGWG}$ and $HTD1^{HZ}$ in NK2 background and compared their effect on grain yield (Figure 4A). The grain yields of NILs-$HTD1^{HZ}$, NILs-$SD1^{DGWG}$, and NILs-$SD1^{DGWG}$/$HTD1^{HZ}$ are 19.5%, 20.6%, and 38.3% higher, respectively, than that of NK2 (Figure 4B), demonstrating that pyramiding $SD1^{DGWG}$ and $HTD1^{HZ}$ will have great potential in shaping plant architecture without affecting seed-setting percentage and in improving grain yield of *japonica* varieties (Figure 4A–4F and Supplemental Figure 14B–14D), as selected and utilized in *indica* varieties by Chinese and IRRI breeders.

DISCUSSION

The Green Revolution in the 1960s was well-known by the success of breeding semi-dwarf varieties, which increased lodging resistance and promoted yield. The milestone of the Green Revolution in rice is the breeding of IR8, which is the basis of modern rice varieties. We showed that the pyramiding of the beneficial alleles of both GA and SL biosynthesis genes had contributed to the Green Revolution in rice and shaped the plant architecture of modern *indica* varieties. It had been revealed that the utilization of the recessive allele of the semi-dwarf trait of IR8 resulted from the introduction of the allele of a biosynthesis of GA gene *sd1* from its one parent cultivar DGWG (Sasaki et al., 2002; Asano et al., 2007). Here, we showed that IR8 contains a beneficial allele of SL biosynthesis gene $HTD1^{HZ}$, which can promote tiller number without compromising setting rate and thus increase yield, was introduced from Peta, which is the other parent cultivar of IR8. Moreover, the favorable allele of *sd1* from DGWG and $HTD1^{HZ}$ from Peta were co-selected into IR varieties.

(G) Schematic diagram showing the introgression of beneficial alleles of *SD1* and *HTD1* during the breeding of modern rice varieties. On one hand, the different alleles of *SD1* had been utilized by breeders to make traditional cultivars for obtaining the semi-dwarf trait and improved grain yield. On the other hand, simultaneous introduction of the *HTD1* allele from Peta and the *SD1* allele from DGWG into IR8 and its descendant elite varieties were released at the early stage of the Green Revolution, from which breeders of IRRI and of China co-selected both of the beneficial alleles into modern *indica* varieties. All data are presented as means ± SD, $n = 6$. Student's *t*-test. Different letters indicate significant differences between lines ($P < 0.05$), Student's *t*-test.

By the wide application of IR8 and its derivatives worldwide, and the introduction of the IR series to China, $HTD1^{HZ}$ and $sd1^{DGWF}$ were introduced into Shuanggui, Minghui 63, and Huazhan by breeders (Figure 4G). Our results show that the favorable allele of the GA pathway can enhance the lodging resistance of rice through *sd1*, and the favorable allele of the SL pathway can increase the tiller number of rice through $HTD1^{HZ}$, which jointly promote the success of the Green Revolution in rice and shape the plant architecture of modern rice *indica* varieties. Since GA could inhibit the biosynthesis of SL and partially rescue the deficiencies of tiller bud outgrowth and plant height of SL-deficient mutants (Ito et al., 2017, 2018), further elucidation of the molecular mechanism underlying crosstalk of the GA and SL pathways will facilitate the optimization of plant architecture and improve crop yield by rational design (Zeng et al., 2017).

In summary, our results demonstrate that $HTD1^{HZ}$ increased tiller number without compromising other important agronomic traits and improved grain yield, and that natural variation of *HTD1* was utilized and selected during the Green Revolution and contributed to the breeding of modern rice varieties. We also showed that pyramiding $SD1^{DGWG}$ and $HTD1^{HZ}$ has great potential in improving grain yield of *japonica* varieties. Our work highlights that understanding the molecular mechanisms underlying the regulation of plant architecture by phytohormones will lay the foundation for further improving crop yield by molecular-design breeding.

METHODS

Plant Materials

Rice (*Oryza sativa* L.) plants were cultivated at the Experimental Stations of the China National Rice Research Institute in Hangzhou, China, during the natural growing season and under field conditions with an interplant spacing of 16.7 × 23.3 cm for transplanting. The 120 RIL population was derived by single-seed descents from the cross between the *indica* rice restorer Huazhan (HZ) and a *japonica* cultivar Nekken 2 (NK2). NILs-$sd1^{DGWG}$/$HTD1^{HZ}$ was developed by selecting the progeny of the repetitive backcross with the parent NK2, and the tiller numbers of 164 BC_6F_2 individuals were measured. NILs-$HTD1^{HZ}$ was developed by repetitive backcrossing to NK2 with marker-assisted selection of the loci of $HTD1^{HZ}$, and NILs-$HTD1^{CL2}$ by repetitive backcrossing of *indica* varieties *cong'ai2* (*CL2*) (Wang et al., 2012) to NPB with marker-assisted selection. The *d17* is a mutant of *HTD1* in the NPB background (Jiang et al., 2013).

Measurement of Traits

Plant height was measured at the maturation stage, tiller number at the late tillering stage, 1000-grain weight using fully filled grains, and yield with six or 10 plants from three completely randomized blocks in the field under normal conditions (one block with 6 × 6 plants).

Linkage Map Construction and QTL Mapping

Reads were aligned to the Nipponbare version 7 reference genome (http://rice.plantbiology.msu.edu/) using BWA-MEM version 0.7.10 (Li and Durbin, 2009). SNP calling and filtration were carried out with SAMtools version 1.6 (Li et al., 2009). A circular ideogram displaying whole-genome variation information was drawn by Circos version 0.67 (Krzywinski et al., 2009). The variation sites among RIL population obtained from HZ and NK2 were compared, and their genotypes were determined using a hidden Markov model approach (Xie et al., 2010). Consecutive SNP sites with the same genotype were lumped into blocks of which less than 100 kb were filtered out. R/QTL (Arends et al., 2010) was used to locate some QTLs controlling plant height and tiller number.

Map-Based Cloning

QTLs were primarily mapped by resequencing the 120 RIL population and phenotyping plant architecture traits. The *HTD1* locus was fine mapped using plants from the segregation population and new markers were developed (Supplemental Table 4). The genomic DNA fragments corresponding to the candidate gene in NK2, NPB, and HZ were sequenced and analyzed.

Conventional Molecular Biology Methods

Total RNA was extracted from various plant tissues from NK2, HZ, NILs-$HTD1^{HZ}$, and the transgenic lines using the AxyPrep Multisource Total RNA Miniprep kit (Axygen). Quantitative RT–PCR was carried out using SYBR Green QPCR mix (Bio-Rad) with *Ubiquitin* as a control. The $HTD1^{HZ}$ complementary vector was constructed by amplifying its coding region from HZ and cloned into the binary pCAMBIA1300. All primers used are listed in Supplemental Table 5. The D53 protein was detected by immunoblot using the polyclonal anti-D53 antibodies (Jiang et al., 2013). The *Agrobacterium tumefaciens*-mediated rice transformation was carried out with the transformation vectors as described previously (Hiei et al., 1994).

Sample Preparation and Sequencing

Genomic DNA of HZ, NK2, 120 RIL population, and 145 accessions were extracted from young leaves using the CTAB method (Allen et al., 2006). The barcode multiplex sequencing libraries were constructed by following the manufacturer's instructions (Illumina), and paired-end sequencing was conducted using the Illumina X-Ten sequencer with 10× sequencing depth for 120 RIL population and 50× sequencing depth for the RIL parents (HZ and NK2) and 145 rice accessions. The resequencing data of the 147 accessions and the 120 RIL population have been deposited in the NCBI Sequence Read Archive under accession BioProject ID PRJNA522896 and PRJNA522923, respectively.

Phylogenetic Analysis

The variations in the coding region of *HTD1* and its upstream 2 kb were acquired from the Sanger sequencing data of 147 accessions (Supplemental Table 1). A neighbor-joining variety tree was constructed using MEGA version 7 (Kumar et al., 2008). After SNP calling, 195 327 evenly distributed SNPs were obtained by randomly choosing two SNPs at most from every 3.8 kb in the genome and were used for constructing the neighbor-joining tree with PHYLIP software (Shimada and Nishida, 2017). The phylogenetic tree was visualized and annotated using Evolview version 2 (He et al., 2016). A panel of 147 accessions was used to construct a minimum spanning tree for *HTD1* and *SD1*. DnaSP software was applied to define haplotypes (Rozas et al., 2017) and calculate the minimum spanning tree drawn by PopART software (Leigh and Bryant, 2015).

Inference of Pedigree Blocks from DGWG and Peta

A total of 147 rice accessions were sequenced to determine the inheritance blocks from DGWG and Peta on the Illumina X-Ten Platform with SNPs filtered with the following criteria: (1) DP <5 and genotype quality <30; (2) non-biallelic; (3) located in annotated transposable element regions. In total, 873 661 SNP markers were identified between DGWG and Peta, and used to infer blocks in 145 varieties. Consecutive genotypes from the same ancestor were used to identify the initial blocks in offspring, and blocks were inferred if more than 75% of markers were from the same ancestor (Huang et al., 2018). A sliding-window method was used to calculate the genotype ratio from one parent in a 100-kb win and 10-kb step. Windows with more than 20 reliable markers and genotype ratio greater than 75% were retained, and others were identified as unknown. Consecutive windows with the same genotype were lumped into one block.

Differentially Selected Regions in Whole Genome of Rice Accessions

Resequencing data of 23 *O. rufipogon* accessions were downloaded from previous studies (Ohyanagi et al., 2016; Zhao et al., 2018). Two haplotype groups (54 *Indica*-hap-C and 52 *Indica*-hap-T) were separated according to the +1005 position of ATG in the *HTD1* gene. Three methods, genetic diversity (π), differentiation statistic (F_{ST}), and XP-CLR, using a 100-kb window were used to identify candidate selective sweeps in three *indica* groups, 54 *indica*-hap-C and 52 *indica*-hap-T, and 106 *indica* from the 147 accessions. The value of π was computed with a custom perl script and the ratios of diversity (π_{or}/π_{indica}, $\pi_{or}/\pi_{indica\text{-hap-C}}$, and $\pi_{or}/\pi_{indica\text{-hap-T}}$) were calculated for each window. F_{ST} was calculated using the R/Hierfstat package (Goudet, 2005). An XP-CLR program with parameters "-w1 0.005 100 100 1" was used to calculate XP-CLR scores (Chen et al., 2010). For each method, the 100-kb windows with either of π ratio, XP-CLR, and F_{ST} ranking top 10% were taken as selective sweeps.

Evaluation of Artificial Selection

The genomic regions of *HTD1* and *SD1* were used to estimate the effects of artificial selection. The π and Tajima's *D* values were calculated in different groups of rice accessions including *indica*, *japonica*, *O. rufipogon*, *indica II*-hap-C, *indica II*-hap-T, *indica III*, and their precise physical locations were determined with default parameters of DnaSP version 6 software.

SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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AUTHOR CONTRIBUTIONS

Q.Q., J.L., and X.Z. conceived the project. Q.Q., J.L., G.X., and Y.W. designed the experiments. Y.W., L.Z., J.H., S.L., Y.R., J.C., X.M., L.W., P.H., J.Y., X.H., Z.G., X.Z., S.N., S.K., T.W., Q.W., and B.W. performed experiments. Y.W., L.S., H.Y., H.L., M.Q., H.C., D.Z., L.G., G.X., Q.Q., and J.L. analyzed data. G.X., Y.W., Q.Q., and J.L. wrote the manuscript.

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