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LETTER TO THE EDITOR

Natural variation in *Ghd7.1* plays an important role in grain yield and adaptation in rice

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Dear Editor,

Flowering adaptability of cultivars to growth conditions should be one of the most important targets in crop domestication and selection. We report here the positional cloning of a major pleiotropic QTL, *Ghd7.1*, which encodes a PSEUDO-RESPONSE REGULATOR 7-like protein. Under long-day conditions, *Ghd7.1* greatly delays rice heading and enhances grain productivity.

Although rice is a short-day species, it has a strong adaptability to photoperiod, which has enabled it to grow widely in tropical, subtropical and temperate regions. Currently, the northern limit for rice-farming areas has spread to latitude 50°N, compared to latitude 28°N of its wild ancestor *O. rufipogon* [1]. More than 16 genes/QTLs have been identified as being involved in the photoperiodic flowering pathway in rice [2, 3]. *Ghd7* and *Ghd8* are key photoperiodic flowering suppressors that increase grain yield and plant height under long-day (LD) conditions [4, 5].

In our previous study, a major QTL for heading date and a major QTL for grain per panicle were coincidently identified on the distal end of chromosome 7 [6]. Near-isogenic lines (NILs), NIL^{TQ}, NIL^{MH} and NIL^{NIP}, for the target region were obtained by consecutive backcrossing with Zhenshan 97 (ZS97) as the recurrent parent and Teqing (TQ), Minghui 63 (MH63) and Nipponbare (NIP) as the donors, respectively. Compared to ZS97, all the NILs showed a delayed heading date, larger rice panicles and increased plant height under LD conditions. Specifically, NIL^{TQ} exhibited a heading delay of 19.5 days, yielded 60% more grains and showed ~27.2 cm increase in plant height (Figure 1A, and Supplementary information, Table S1).

A large NIL F_2 population consisting of 2 278 individuals was used to delineate the QTL to the region from RM22181 to the end of chromosome 7. Parental comparative sequencing of the region detected an 8-bp deletion in *OsPRR37* (Supplementary information, Figure S1), a homolog of *Arabidopsis PSEUDO-RESPONSE REGULATOR 7* (*PRR7*) [7], in ZS97 compared to TQ, MH63 and NIP. The coding sequences of *OsPRR37* in MH63 and TQ are identical. We generated a construct by placing *Ghd7.1* from NIP into the pCAMBIA1301S vector and introduced this construct into ZS97. The resulting transgenic plants displayed an increased plant height, coupled with later heading and a larger panicle, compared to the negative control under LD conditions. In particular, the transgenic plants delayed rice heading for 15 days and yielded 50% more grains than the negative control (Figure 1B, and Supplementary information, Table S1). Thus, *OsPRR37* comprises the pleiotropic QTL, and we renamed the gene as *Ghd7.1* (**g**rain number, plant **h**eight and heading **d**ate). *Ghd7.1* was found to be mainly expressed in the leaves and panicles, and strong expression was found in cells with meristem activity in the young panicles (Supplementary information, Figure S2).

A total of 24 *Ghd7.1* haplotypes (Haps) detected in 178 rice varieties (GP1) of broad genetic diversity were divided into two groups, with *indica* and *japonica* prevailing in each group, respectively (Figure 1C, Supplementary information, Figures S3 and S4). The *Ghd7.1* haplotypes from 47 wild rice (*O. rufipogon*) accessions (GP2) were also clustered into the two groups (Supplementary information, Figure S4). Three major haplotypes (Hap1, Hap2 and Hap3), and six rare haplotypes (Hap12, Hap14, Hap19, Hap21, Hap22 and Hap23) that caused a premature stop codon in *Ghd7.1* were identified in GP1 (Supplementary information, Figure S3). These defective alleles and Hap2 and Hap5 were not found in wild rice, which was further confirmed by the fact that no SNPs causing a stop codon were detected in 588 wild rice accessions (GP3) (Supplementary information, Tables S2 and S3). All five haplotypes (Hap1, Hap3, Hap6, Hap7 and Hap13) in wild rice were also detected in GP1 (Supplementary information, Table S2), indicating that they are pre-existing variants in wild rice. The other haplotypes were likely to have been generated from these five pre-existing haplotypes by mutations (Figure 1C). Therefore, different cultivars have accumulated natural variations in *Ghd7.1*, including the retention of the pre-

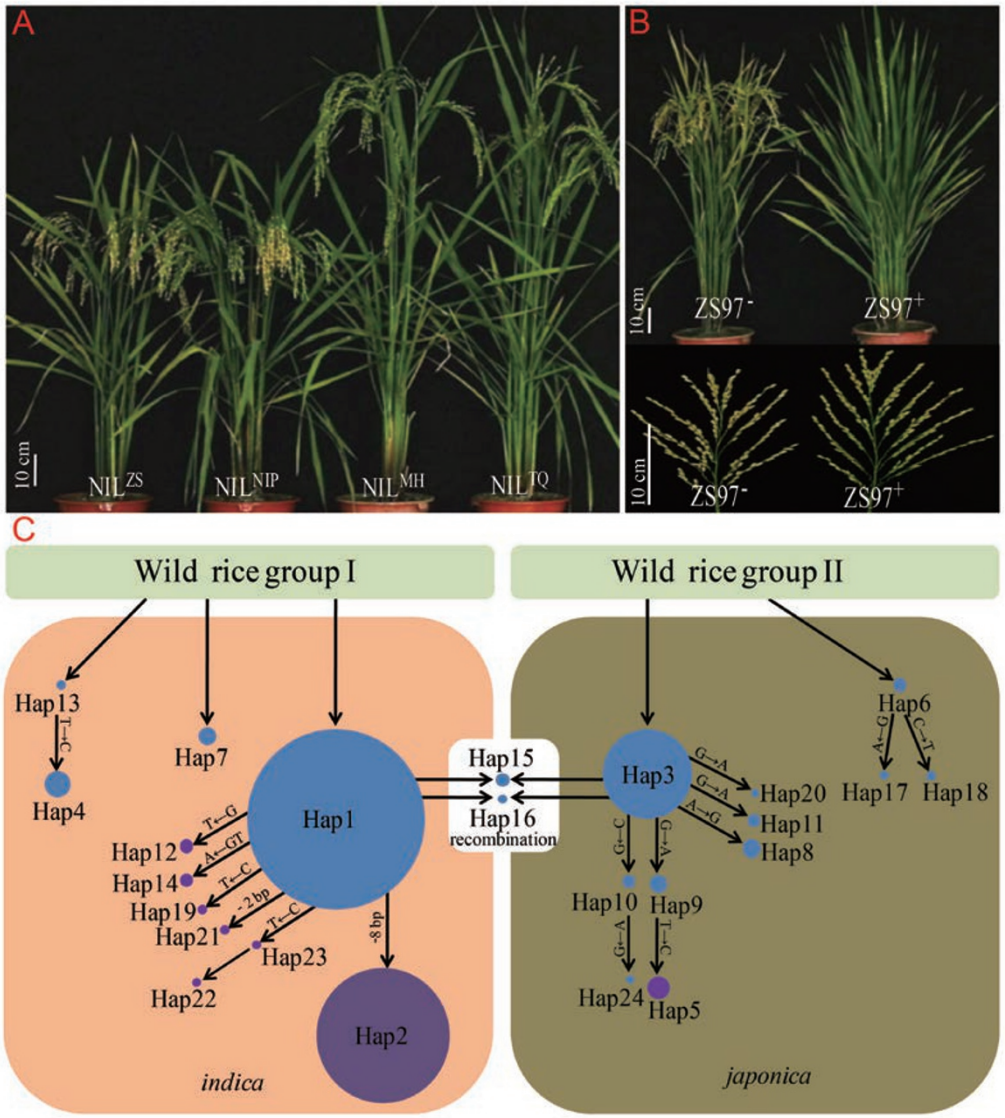

Figure 1 Trait performance of NILs and transgenic plants for *Ghd7.1* and the proposed model for the evolution of all cultivated rice haplotypes from wild rice. **(A)** Phenotypes of several NILs for *Ghd7.1* at the mature stage of NIL^{ZS}. **(B)** Performance of positive transgenic plants. $ZS97^{-}$ and $ZS97^{+}$ are the vector control and positive transgenic plants, respectively; Bar = 10 cm. **(C)** The different *Ghd7.1* alleles originated independently from two wild rice groups as *indica* rice and *japonica* rice. Two wild rice alleles were independently traced in *indica* and *japonica* rice. *Indica*- and *japonica*-specific haplotypes Hap1, Hap3, Hap6, Hap7 and Hap13 were found in wild rice accessions. Other mutant types were then derived from the pre-existing alleles in wild rice. The size of the circle representing each haplotype is proportional to its frequency in GP1.

existing genetic variants in wild rice and acquisition of mutations after domestication.

The strong allele (Hap1) represented by TQ and the non-functional allele (Hap2) represented by ZS97 are widely distributed in cultivars (Figure 1C, Supplementary information, Figure S3 and Table S2). Hap1 has frequently been found in single-cropping rice, which has great yield potential and is grown in central and southern China and Southeast Asia. Hap3 has a sequence very similar to the weak allele of Hap10 carried by NIP and is widely distributed from central to northern China. The newly generated allele Hap2 has been found mainly in the early-season rice accessions of central China and Southeast Asia, cultivars that ensure an early heading to allow sufficient time for the cropping of late rice cultivars (Supplementary information, Figure S5 and Table S2). Hap2 was enriched in GP1 (Supplementary information, Figure S3 and Table S2). The varieties carrying Hap5, which shows a T/C substitution at the S2129 site in the CCT domain, always flower early in GP1 (Supplementary information, Figure S6). Hap5 exists only in temperate *japonica* cultivars grown in Heilongjiang, the northern limit for rice cultivation (Supplementary information, Figure S5 and Table S2). These observations indicate that different *Ghd7.1* alleles have distinct eco-geographical distribution patterns. The wide existence of Hap1, Hap3, Hap2 and Hap5 in modern cultivars indicates that both pre-existing and newly derived alleles could be selected for and enriched after domestication. This finding is different from the case of *HvCEN* alleles in barley, where the adaptability to environments is influenced by the selection and enrichment of pre-existing *HvCEN* genetic

variants rather than the acquisition of mutations [8].

Molecular evolution analysis using OBSM [9] showed that the optimal model for the estimation of ω = Ka/Ks is a three-ratio model. This model suggests that the *PRR37* gene has a very strong functional restriction in most grass species, as the ω value at these branches is far less than 1 ($\omega_1 = 0.2946$, $\omega_2 = 0.0881$). However the ω values at the two cultured rice branches are much larger than 1 ($\omega_3 = 2.3855$), indicating that *Ghd7.1* in cultured rice was under strong positive selection, most likely due to artificial selection (Supplementary information, Figure S7).

Sequence comparison of *OsPRR37* alleles from the two parents for producing NILs for *Hd2* revealed a premature stop codon in the conserved CCT domain [10], and it was reported that rice *OsPRR37* could complement the late-flowering phenotype of the *Arabidopsis prr7* mutant [7]. These results indicated that *OsPRR37* is the gene responsible for *Hd2*. In this study, we confirmed that *Ghd7.1* is the *OsPRR37* gene via a map-based cloning approach; therefore, *Ghd7.1* is allelic with *Hd2*. Because the function of *Ghd7.1* is largely affected by light conditions, we tested the expression of some important photoperiodic regulators, such as *Hd1*, *Ehd1* and *Hd3a* between ZS97 and NIL^{TQ}. The results showed that *Ghd7.1* does not regulate *Hd1* but has a profound impact on the expression of a rice-specific flowering integrator, *Ehd1*, and the rice florigen, *Hd3a*, under LD conditions (Supplementary information, Figure S8).

The *indica* and *japonica* haplotypes of *Ghd7.1* independently originated from different wild rice plants (Figure 1C), consistent with a report that the *indica* and *japonica* haplotypes of *Ghd7*, a key flowering repressor, evolved from two distinct ancestral gene pools [11]. These results indicate that *indica-japonica* differentiation had already occurred in wild rice, a notion that is also supported by the evolutionary analysis of a major reproductive barrier regulator, *S5*, and other studies [12, 13].

Ghd7.1 contributes greatly to regulating rice photoperiodic flowering, plant architecture and grain productivity. The retention of its pre-existing genetic variants in ancestral species and the acquisition of mutations after domestication together have contributed to rice adaptation. The isolation of *Ghd7.1* provides an opportunity to breed high-yield varieties with improved adaptive flexibility for special farming regions.

Detailed methods are described in the Supplementary information, Data S1.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)