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Characterization of a naturally occurring early-flowering rice mutant resulting from a novel variation in the *Ghd7* locus

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Abstract: Rice is one of the main staple food crops and is consumed by more than half of the global population. Optimizing the flowering time may help to maximize grain production. In this study, we characterized a rice mutant, Zixiangnuo early-flowering mutant (ZXN-E). Compared with the wild-type variety, Zixiangnuo (ZXN), the mutant plants flowered earlier, were shorter and produced smaller panicles. Bulked segregant analysis revealed a 7-bp deletion in the first exon of the *Grain number, plant height, and heading date 7* (*Ghd7*) locus. The allele with the 7-bp deletion was not detected in the rice varieties included in the 3 000 Rice Genomes Project. Thus, the 7-bp deletion identified in the naturally occurring mutant ZXN-E is a novel variation. Our findings suggest that ZXN-E may be a useful germplasm for breeding new rice varieties.

Keywords: bulked segregant analysis; flowering time; *Ghd7*; rice

Flowering time is one of the important limiting factors influencing rice grain yield and is strongly regulated by environmental factors such as day length and temperature (Hori et al. 2016). Multiple genes associated with flowering time have been isolated (http://www.ricedata.cn/gene/; Hori et al. 2016). For example, *Grain number, plant height and heading date 7* (*Ghd7*), the important gene for adapting rice variates to the northern climates, encodes one of the repressors of *Early heading date 1* (*Ehd1*) expression that delays flowering (Zheng et al. 2016; Cai et al. 2019). A previous study confirmed the existence of dozens of diverse variants in the *Ghd7* sequence (Lu et al. 2012). Rice varieties with an allele that encodes a functional Ghd7 can extend the growing season in tropical and subtropical regions, whereas an allele encoding a partially functional or nonfunctional Ghd7 can shorten the rice life cycle, thereby enabling rice to adapt to a relatively cool summer in temperate regions (Xue et al. 2008).

Zixiangnuo (ZXN) is a rice variety with a purple pericarp as well as fragrant and glutinous grains and usually flowers in about 120 days after sowing in Jinan (36°43'N, 117°05'E), Shandong province, China. In 2015, one early-flowering plant, approximately

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20 days earlier, was identified in a paddy field in Jinan. This mutant was named Zixiangnuo early-flowering mutant (ZXN-E). The early-flowering trait is stably inherited to the next generation through an analysis of agronomic traits in three consecutive years (Figure 1A). These results implied that the

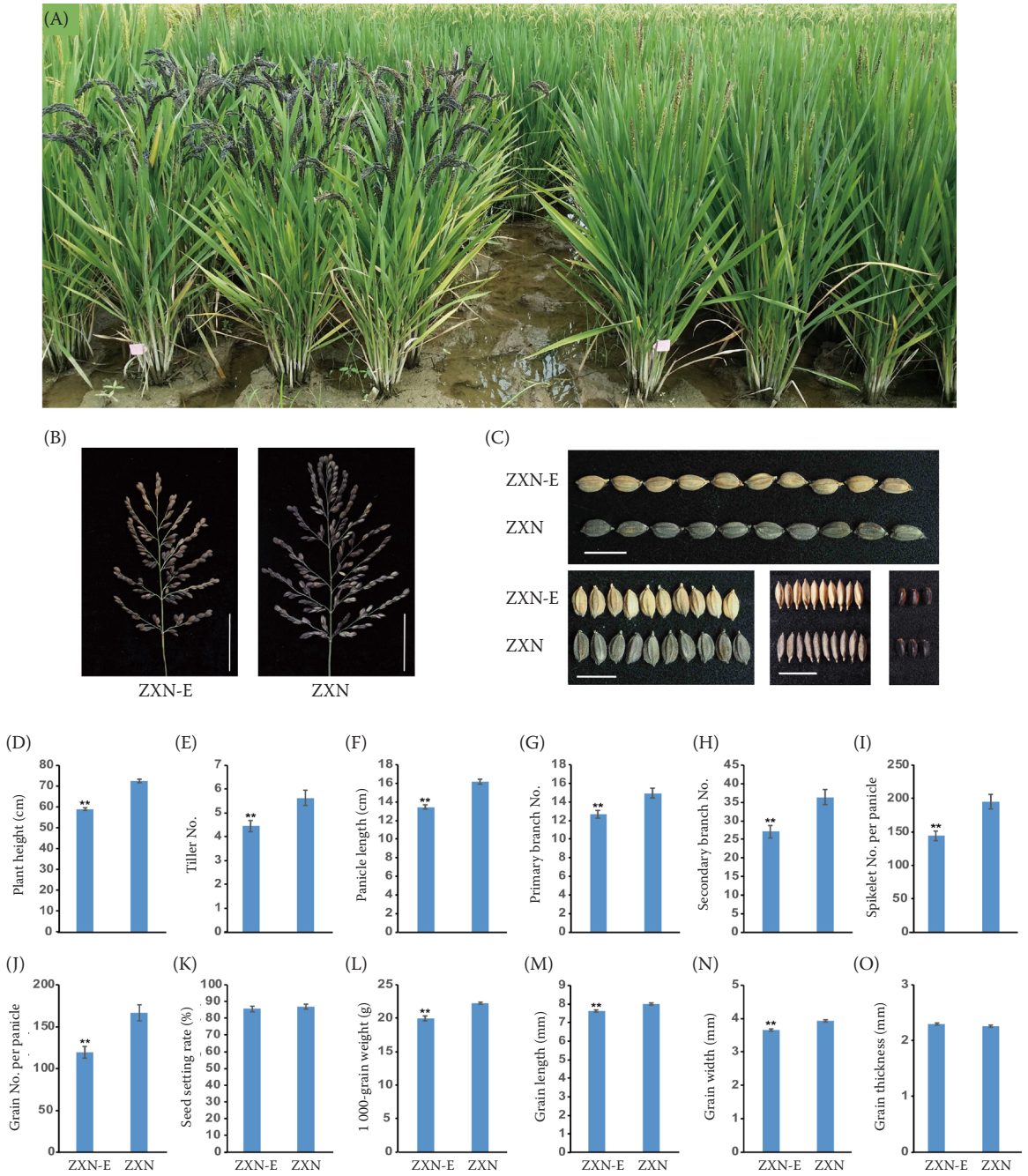

Figure 1. Agronomic traits of Zixiangnuo (ZXN) and Zixiangnuo early-flowering mutant (ZXN-E) rice plants: ZXN-E (left) and ZXN (right) in a paddy field (A), phenotypes of the main panicles of ZXN-E and ZXN plants, scale bar 5 cm (B), comparison of the length, width, thickness, and brown rice of ZXN-E and ZXN rice grains, scale bar 1 cm (C), the following traits were compared between ZXN and ZXN-E: plant height (D), tiller number (E), panicle length (F), number of primary branches per main panicle (G), number of secondary branches per main panicle (H), number of spikelet per main panicle (I), number of filled grains per main panicle (J), seed-setting rate (K), 1 000-grain weight (L), grain length (M), grain width (N), and grain thickness (O); data are presented as the mean ± SE; $^{**}P < 0.01$ compared with ZXN (Student's *t*-test)

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early-flowering trait of the mutant is controlled by a homozygous allele.

Compared with ZXN, ZXN-E plants were much shorter, had considerably fewer effective tillers, a shorter main panicle, less primary branches and secondary branches. The seed-setting rates of ZXN and ZXN-E were similar. Furthermore, the ZXN-E rice grain was shorter and narrower than the ZXN rice grain. However, the thickness of the ZXN-E and ZXN rice grains were very similar. The 1 000-grain weight was 22.2 g for ZXN and 20.0 g for ZXN-E (Figure 1B–O). Unfortunately, the yield has also decreased. These results indicated that the natural genetic variation in ZXN-E may have a pleiotropic effect on plant growth.

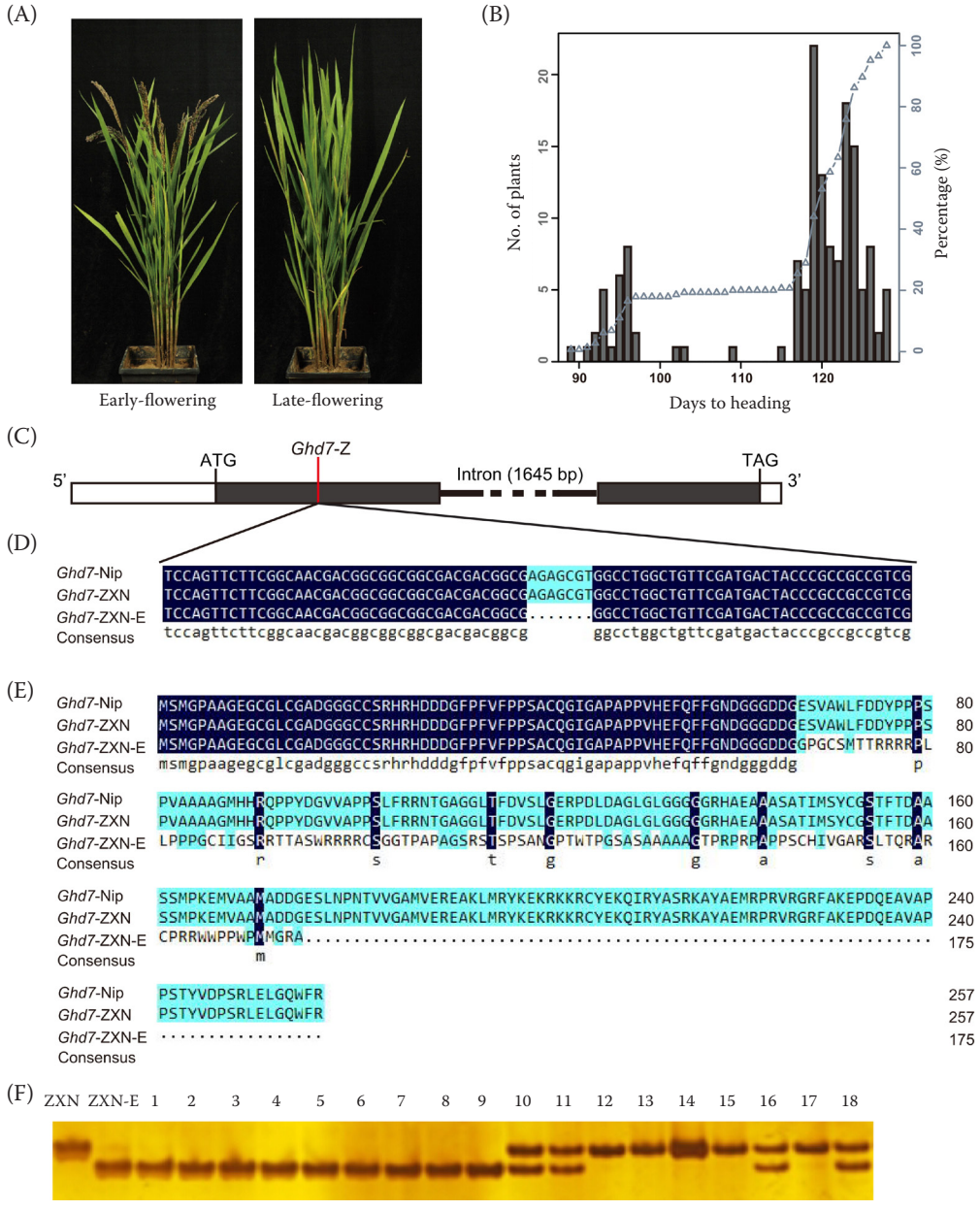

Figure 2. Polymorphism of *Ghd7* in Zixiangnuo early-flowering mutant (ZXN-E): early- and late-flowering plants of the F_2 population (A), days-to-flowering of the plants in the F_2 population (B), structure of the *Ghd7* gene (C); the position of the 7-bp deletion (*Ghd7-Z*) is indicated in red; boxes represent exons and the black bar represents an intron; alignment of the *Ghd7-Z* sequences in Nipponbare (Nip), Zixiangnuo (ZXN), and ZXN-E (D), alignment of the Ghd7 protein sequences in Nip, ZXN, and ZXN-E (E), representative gel electrophoresis patterns of *Ghd7* in ZXN, ZXN-E, and the F_2 population (F); 1–9: early-flowering plants in the F_2 population; 10–18: late-flowering plants in the F_2 population

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To clarify the genetic basis of the early-flowering trait, ZXN-E was crossed with ZXN to generate F_2 progeny. All of the F_1 individuals exhibited a wild-type phenotype, with a flowering time of about 120 days after sowing. The ratio of late-flowering to early-flowering plants in the F_2 population was 116 : 29 (Figure 2), which was consistent with a 3 : 1 segregation ratio according to a χ^2 test (χ^2 = 1.68, P = 0.20). These results suggested that the early-flowering trait in the ZXN-E mutant is controlled by a single recessive mutation.

Bulked segregant analysis was used to identify the canididate variants (Wu et al. 2019). 62 candidate variants were identified in the two extreme DNA pools (Table S1 in Electronic Supplementary Material (ESM)) by bulked segregant analysis. Among them, only one variant was located in the exon region and resulted in a frameshift. The 7-bp deletion (5'-AGAGCGT-3') in the *Ghd7* coding sequence (CDS) introduced a premature stop codon. Using one reported primer pair (F: 5'-CATCGCCACGATGATGATGG-3'; R: 5'-GCGGCGGGTAGTCATCGAAC-3') (Li et al. 2015), the *Ghd7* genotypes in the two parents and in all 145 F_2 plants were examined by polyacrylamide gel electrophoresis. Both ZXN-E and the early-flowering individuals in the F_2 population carried this homozygous mutation (Figure 2). Besides, this is a novel allele that was not reported in the 3 000 Rice Genomes Project (Wang et al. 2018) (Figure S1 in ESM), and we named it *Ghd7*-Z.

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