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Manipulation of genetic recombination by editing the transcriptional regulatory regions of a meiotic gene in hybrid rice

Dear Editor,

Genetic breeding involves the recombination and selection of various valuable genes. Meiotic crossover (CO) promotes the generation of new allelic combinations on chromosomes, which is essential for breeding elite varieties (Wijnker and de Jong, 2008). An increase in CO promotes genetic diversity, whereas a decrease can rapidly stabilize excellent traits (Mercier et al., 2015). Furthermore, the complete elimination of CO facilitates heterotic fixation during apomixis (Wang et al., 2019). However, the number and distribution of COs are tightly restrained in crops, severely hindering agricultural breeding (Crismani et al., 2012). To date, many meiotic genes involved in CO formation have been identified in different organisms. Unfortunately, null mutations in these genes usually cause infertility, thus preventing direct use of the mutants in crop breeding.

In this study, we investigated whether COs could be increased or decreased while preserving fertility through the artificial manipulation of a single meiotic gene. *HumanEnhancer ofInvasion 10* (*HEI10*) is a meiotic gene that is highly conserved among animals and plants. Multiple studies have shown that knockout of *HEI10* significantly reduces the number of COs and nearly eliminates the fertility of male and female gametes (Wang et al., 2012; Qiao et al., 2014). Here, we selected *HEI10* as a candidate gene for genome editing.

First, we predicted the promoter and 5′ UTR of *HEI10* and identified 50 core regulatory elements using the PlantCARE website (Figure 1A). We then constructed two eight-target knockout vectors, one targeting the promoter (P1–P8) and the other targeting the 5′ UTR (U1–U8) (Figure 1B; Supplemental Table 1). Chunyou84 (CY84), an *indica–japonica* hybrid variety, was used for genetic transformation, and 56 T_0 plants were obtained. Through Sanger sequencing, 10 mutants harboring homozygous or biallelic mutations, including five promoter-edited mutants (PRO series) and five 5′ UTR-edited mutants (UTR series), were selected for subsequent studies (Supplemental Figure 1A and 1B).

Next, we performed qRT–PCR experiments to detect *HEI10* expression levels. Using transgene-free CY84 as the control, *HEI10* expression was reduced by 22.05%–85.19% in PRO series mutants. Conversely, *HEI10* expression was elevated by 16.56%–73.71% in UTR series mutants (Figure 1C). We found no obvious differences in vegetative growth or pollen fertility among these mutants (Figure 1D and Supplemental Figure 2). Notably, the seed setting rate of CY84 was 81.30% ± 3.31%, whereas that of PRO series mutants ranged from 40.03% ± 5.35% to 63.48% ± 1.77%, and that of the UTR series mutants ranged from 45.25% ± 1.58% to 74.89% ± 0.66% (Figure 1E). This result indicates that seed setting rate can be partially retained by modulating the expression of *HEI10* in rice.

We then used the promoter-edited PRO-24 mutant and the 5′ UTR-edited UTR-56 mutant, which showed the lowest and highest *HEI10* expression, respectively, to observe chromosome behaviors during meiosis. Compared with CY84, no obvious defects were found in all stages of both mutants (Supplemental Figure 3). COs were then calculated based on the morphology of metaphase I chromosomes (Figure 1F), with rod- and ring-shaped bivalent chromosomes considered to contain one and two COs, respectively (Wang et al., 2012). The mean CO number of CY84 was 16.34 ± 3.44 (n = 108) per cell, which deviated from a Poisson distribution ($\chi_{[23]}^2$ = 57.76, $p < 0.01$), showing that COs were not randomly distributed among cells. The mean CO number of the PRO-24 mutant was 14.91 ± 2.91 (n = 130) per cell, which was lower than that of CY84 and deviated from a Poisson distribution ($\chi_{[21]}^2$ = 36.21, $p < 0.05$). The mean CO number of the UTR-56 mutant was 17.53 ± 3.35 (n = 116) per cell, which exceeded that of CY84 and also deviated from a Poisson distribution ($\chi_{[23]}^2$ = 107.16, $p < 0.01$) (Figure 1G).

Changes in cytological recombination do not necessarily cause changes in genetic recombination (Wang et al., 2015). We next investigated genetic recombination in each T_0 hybrid mutant by genotyping their self-fertilized segregating offspring (equivalent to an F_2 population). Based on the two parental genomes of the CY84 hybrid, 130 SNP markers distributed on 12 pairs of chromosomes were designed and used for genetic analysis (Supplemental Figure 4; Supplemental Table 2). By analyzing 93 offspring of the PRO series mutants, we found that the recombination frequencies of all mutants were lower than that of CY84. The PRO-24 mutant displayed the greatest reduction in mean CO frequency (reduced from 0.85 ± 0.33 to 0.61 ± 0.21 per chromosome) (Supplemental Table 3). In the 118 intervals generated by the 130 SNP markers, 77.97% in the PRO-24 mutant had lower CO frequencies than those of CY84 and higher CO frequencies than those of other mutants (Figure 1H and Supplemental Figure 5). These results suggest that decreased recombination is likely correlated with reduced *HEI10* expression in the mutant.

To test whether editing the 5′ UTR region of *HEI10* could also bring about changes in genetic recombination, we analyzed 93 offspring of each hybrid mutant and found that the recombination frequencies of four mutants were increased relative to CY84. The

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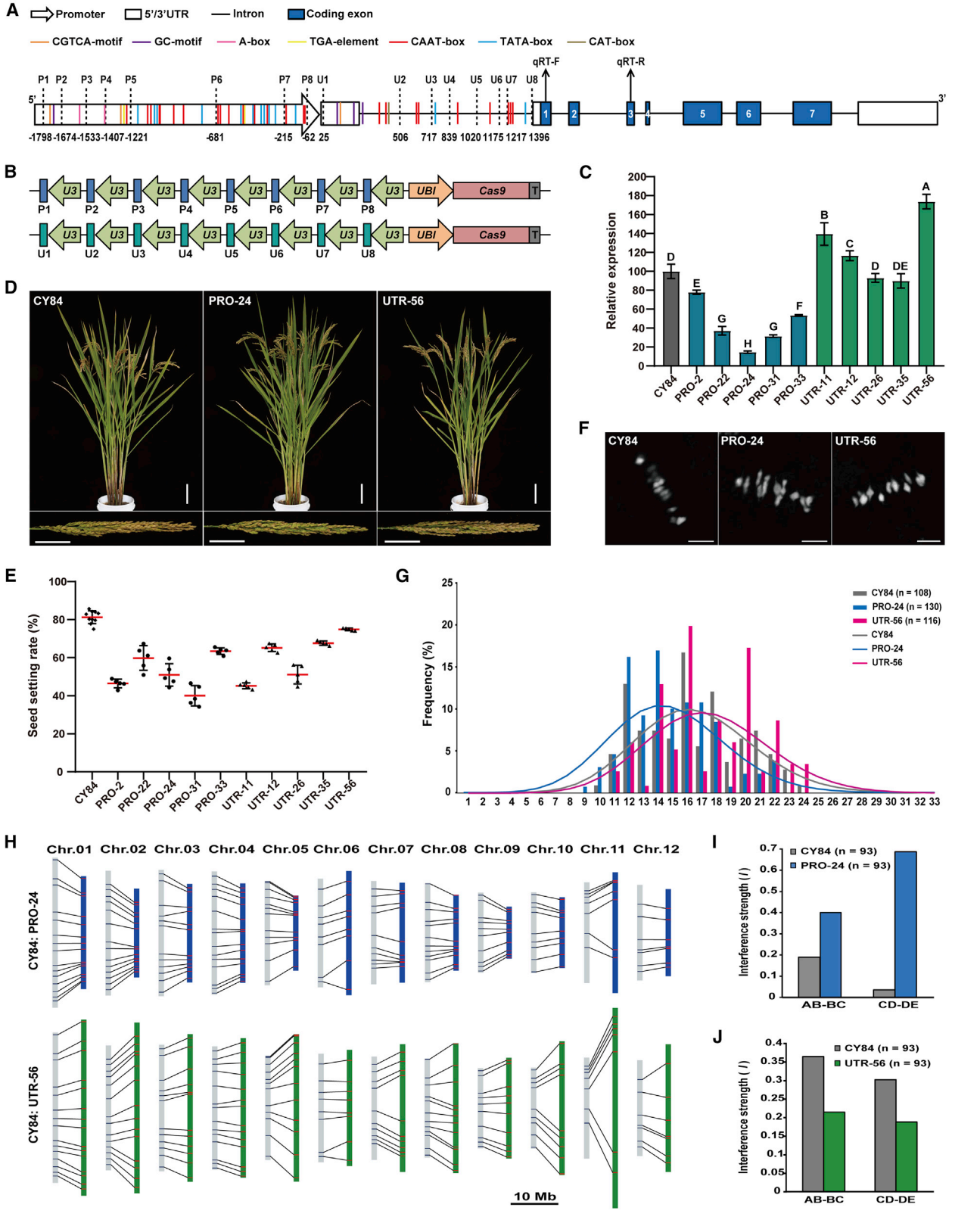

Figure 1. Using CRISPR–Cas9 technology to edit the transcriptional regulatory regions of *HEI10* can artificially control the increase or decrease in COs.

(A) Structure of *HEI10*. P1–P8, target sites in the promoter region; U1–U8, target sites in the 5′ UTR region.

(B) Structure of CRISPR–Cas9 vectors targeting the transcriptional regulatory regions of *HEI10*.

(legend continued on next page)

UTR-56 mutant displayed the greatest increase in mean CO frequency (increased from 0.80 ± 0.32 to 0.96 ± 0.35 per chromosome) (Supplemental Table 4). Notably, 67.80% of the intervals in the UTR-56 mutant had increased CO frequencies relative to those of CY84 and were also higher than those of the other mutants (Figure 1H and Supplemental Figure 6). This result suggests that increased recombination may be related to elevated *HEI10* expression in the mutant.

To explore whether genetic interference was affected in the mutants, we used a coefficient analysis to calculate the interference strength between adjacent interval pairs (Libuda et al., 2013). Chromosome 5, which exhibited significant changes in genetic recombination in both PRO-24 and UTR-56 mutants, was chosen for interference analysis. We divided the chromosome into four large intervals and found that the interference strength of the PRO-24 mutant ranged from 0.40 to 0.69, higher than that of CY84 (0.04–0.19) (Figure 1I and Supplemental Figure 7A and 7B). In the UTR-56 mutants, the interference strength of 0.19–0.21 was lower than that of CY84 (0.30–0.36) (Figure 1J and Supplemental Figure 7A and 7C). The opposite changes in interference strength between the mutants indicate that genetic interference may be negatively correlated with recombination frequency when *HEI10*expression is manipulated.

In summary, this study demonstrated that editing the transcriptional regulatory regions of *HEI10* can generate different types of mutants with retained gene functions. The ability to increase or decrease CO frequency while maintaining a certain seed setting rate indicates that recombination changes can be artificially manipulated by editing only one meiotic gene. As many meiotic genes are involved in recombination, we propose that recombination can be stacked by editing more genes. The method used in this research may be applied in various fields of crop breeding to obtain more excellent varieties.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.

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AUTHOR CONTRIBUTIONS

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(C) *HEI10* expression levels of the T_0 generation. Bars, SD (n = 3). Letters, Duncan's test ($p < 0.01$).
(D) Plant and panicle types. Plant, bar: 10 cm. Panicle, bar: 5 cm.
(E) Seed setting rate of the T_0 generation. Bars, SD ($n \geq 5$). Black spots, numerical values of seed setting rate. Red lines, mean values.
(F) Arrangement of bivalent chromosomes on the equatorial plate. Bar: 5 μm.
(G) The distribution of CO numbers in meiotic cells. Abscissa, the number of COs. Bars, the observed distributions. Curves, the predicted Poisson distributions.
(H) Genetic maps of the PRO-24 and UTR-56 mutants compared with CY84. CY84 is shown on the left (gray), and mutants are shown on the right (blue and green) in each map; markers are connected between the maps. CO frequency, COs per Mb.
(I and J) Interference strength (*I*) of the PRO-24 **(I)** and UTR-56 **(J)** mutants compared with CY84. Fisher's exact test (n = 93). Interference strength (*I*) = 1 − observed number (O)/expected number (E).