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Impact of genome-edited *PPD-1* promoter mutations on diurnal expression rhythms and heading time in *Triticum aestivum* L.

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Motivation and aim: Heading time in cultivated plants is one of the key factors determining their adaptability to the environment, which, in turn, affects crop yield. A collection of spring bread wheat (*Triticum aestivum* L.) line Velut plants with mutations in the promoter region of *PPD-1* genes was generated using CRISPR/Cas9 genome editing. This mutant plant collection was used to investigate the function of transcription factor binding sites identified in the *PPD-1* promoter.

Materials and methods: To assess heading time, yield-related traits, and *PPD-1* gene expression in plants with different mutations, we cultivated selected T2 plants under long-day field conditions. For this experiment, we selected six lines with different types of mutations, including deletions and insertions of varying lengths, and confirmed their genotypes using PCR with specific primers. To evaluate the expression dynamics of *PPD-1* genes in field-grown plants, four individuals per line at the flag leaf stage were sampled. Tissue collection was performed over a 24-hour cycle at 4-hour intervals. The expression of the target gene was normalized to the reference gene *MetAP1*, and relative expression values were calculated using the Pfaffl method.

Results: The study assessed heading time, plant height, and main spike grain weight across multiple lines. Several lines exhibited delayed heading relative to controls, while plant height and grain weight varied by line. A line harboring a large deletion in *Ppd-D1* and a more extensive *Ppd-B1* deletion compared to other lines showed a statistically significant advance in heading time, suggesting that the combined mutations in both genes may underlie its accelerated heading phenotype.

Analysis of the diurnal expression pattern of *PPD-1* genes revealed distinct trends. In control plants, *Ppd-D1* and *Ppd-B1* expression exhibited two peaks: one after four hours of light exposure and another after twelve hours, with nearly absent expression during the dark period. Lines with a deletion in the BOXIIPCCHS binding site and a 345 bp insertion located below the two CHE binding sites demonstrated similar *Ppd-D1* expression patterns, peaking after four and twelve hours of light exposure, though at slightly reduced levels compared to controls.

In contrast, a line with a large deletion affecting both CHE binding sites exhibited elevated *Ppd-D1* expression, supporting the hypothesis that these CHE sites constitute a core regulatory region necessary for the high-level expression of *Ppd-D1*.

Regarding *Ppd-B1* expression, lines with partial or complete deletions of a single CHE binding site displayed expression patterns similar to those of the control line.

Conclusions: Our findings demonstrate that targeted deletions in transcription factor binding sites, including the CHE motif, modulate gene expression patterns, underscoring the functional role of these cis-regulatory elements in controlling *PPD-1* expression. The engineered wheat lines carrying diverse mutations in the *Ppd-D1* and *Ppd-B1* promoters provide a valuable resource for in-depth analysis of their regulatory mechanisms.

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