



Mining and Activity Characterization of Maize Bundle Sheath Cell-Specific Promoters for Photosynthetic Engineering and Synthetic Biology

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Abstract

Maize (*Zea mays* L.) is a representative C₄ crop in which carbon assimilation through the Calvin cycle mainly occurs in bundle sheath cells (BSCs). Developing precise regulatory elements that enable cell-type-specific gene expression is essential for understanding C₄ photosynthetic regulation and advancing crop genetic engineering. However, systematic identification and functional characterization of BSC-associated regulatory sequences remain limited. In this study, we established an integrated framework combining single-cell transcriptomics, cis-regulatory element analysis, and quantitative functional assays to identify and evaluate BSC-associated regulatory sequences. Ten upstream regulatory regions of reported BSC marker genes were cloned and characterized using dual-luciferase assays in maize protoplasts. The results revealed distinct transcriptional activities among candidate regions, with *PCK1* and *SHBP1* showing strong activation capacity, while GUS-based tissue analysis demonstrated that *PRK2* exhibited highly specific expression in BSCs. Furthermore, integration of three published leaf single-cell transcriptomic datasets identified 81 conserved candidate genes. Developmental expression analysis and transient reporter assays further confirmed several candidates with strong and stable transcriptional activity. In addition, conserved cis-regulatory motifs and transcription factor binding patterns were analyzed to establish a weighted scoring model, enabling the prediction of high-confidence BSC-associated candidates. This study provides a comprehensive strategy for discovering cell-type-specific regulatory elements and offers valuable genetic resources for improving photosynthetic efficiency through plant synthetic biology approaches.

Keywords: cis-regulatory elements; genetic engineering; photosynthesis; single-cell transcriptomics; transcriptional regulation