Functional characterization of liverwort-specific miRNAs in sexual organ development of Marchantia polymorpha

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ABSTRACT

The developmental processes must be precisely controlled at all stages of a plant's life cycle to ensure proper sexual organ development and reproductive success. These processes rely on complex genetic networks, which remain unknown in non-vascular plants. In liverworts, nine conserved miRNAs common to all land plants and a set of non-conserved (liverwort-specific) miRNAs were identified. However, the functional roles of these non-conserved miRNAs in *Marchantia polymorpha* remain largely unexplored. I investigated the expression pattern of six miRNAs (MpmiR11737a/b, MpmiR11865/11865*, MpmiR11887 and MpmiR11796) and conducted in-depth analyses of two miRNAs (MpmiR11887 and MpmiR11796) to elucidate their roles in Marchantia's sexual reproduction. MpmiR11887 showed accumulation exclusively in antheridiophores. My analyses revealed that Mp*MIR11887* represents an independent transcriptional unit. Its promoter is active predominantly in young antheridia and spermatogenous cells. ∆mp*mir11887*^{ko} plants showed morphological differences in antheridiophore development, with wider antheridial discs and larger mature antheridia, suggesting its role in the male sexual organ development. MpmiR11796 accumulates mainly in archegoniophores and is encoded by an intron-less independent transcriptional unit. Mp*MIR11796* promoter activity was observed in the pegged rhizoids located within digitate rays and stalk of archegoniophores. ∆mp*mir11796*^{ko} male plants produced less rhizoids during gemmae development, while, female ∆mp*mir11796*^{ko} plants showed several defects, including archegonial receptacle size, reduced stalk length, and abnormalities in asymmetrical divisions during egg cell development. Crossing experiments revealed severe impairment in gametangiophores production and gamete development. Collectively, the important role of MpmiR11796 in the overall development of Marchantia, predominantly in sexual organ formation and reproduction is shown. Finally, transcriptome and degradome data analyses identified several potential targets for MpmiR11887 and MpmiR11796 which requires further validation.

Keywords: *Marchantia polymorpha*, miRNA, miRNA differential expression pattern, pri-miRNA, miRNA targets, degradome, vegetative development, reproductive development, functions of selected liverwort-specific miRNAs.