ABSTRACT

The *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE* (*SPL*) family of transcription factors is functionally diverse in controlling a number of fundamental aspects of plant growth and development. Since the *SPL* genes exist amongst plants only, hence, it is imperative to understand their functions amongst basal lineages of land plants, to gain understanding of their evolution. The first part of my thesis presents the phylogenetic relationships between *SPL* family members from representatives of all lineages of bryophytes: two hornworts, *Anthoceros agrestis* and *A. punctatus,* liverwort *Marchantia polymorpha,* and moss *Physcomitrium patens,* and angiosperms representatives, *Arabidopsis thaliana*. The phylogenetic analysis classified SPL proteins in four phylogenetic groups. We found that the *SPL* family members within the same group share similar gene structures and protein domains which might hint towards the possible overlap in their putative functions. Moreover, there were no *SPL* genes identified in the hornwort lineage when we started our analysis and our results established that a minimal set of *SPL* genes is present in hornworts of Anthoceros lineage, which is similar to liverwort, *M. polymorpha*.

In the second part of presented thesis several molecular genetic tools were applied to characterize the function of Mp*SPL3* and Mp*SPL4* genes from model liverwort species, *M polymorpha*. First, combining *in planta* promoter activity using GUS reporter gene together with RT-qPCR analysis we have shown that both Mp*SPL3* and Mp*SPL4* genes are ubiquitously expressed during the vegetative as well as reproductive phases of Marchantia's life cycle. Next, to obtain knockout plants for each gene, CRISPR/Cas9 approach was used. The obtained two loss-of-function Mp*SPL3* plants displayed reduced thalli with delayed growth in comparison to wild-type plants. On the other hand, Mp*SPL4* loss-of-function plants displayed more severe phenotype resembling a prothallus-like stage with no production of gemma cups. As in the case of both genes' loss-of-function mutations caused very strong effect on Marchantia development, we applied artificial miRNA approach to knockdown the expression of Mp*SPL3* and Mp*SPL4* genes. The obtained knockdown plants for both genes displayed growth retardation during their vegetative growth. Moreover, gametangiophores production was completely abolished in Mp*spl3-kd* lines, while Mp*spl4-kd* plants exhibited delayed archegoniophores production which additionally showed morphological distortions. Therefore, proper level of Mp*SPL3* and Mp*SPL4* genes expression is also indispensable for Marchantia sexual organs development. In third

approach, we have prepared overexpression lines of both genes to study how the MpSPL3 and MpSPL4 protein excess will influence Marchantia development. The preliminary phenotypic analysis for gain-of-function Mp*SPL3* transgenic plants displayed no significant changes in phenotype during vegetative stage of growth as compared to wild-type plants. On the other hand, the plants overexpressing MpSPL4 protein displayed smaller and narrower thalli with bigger gemma cups in comparison to wild-type plants.

Taken together, the presented results provide significant insights into the basic functions of Mp*SPL3* and Mp*SPL4* genes from the *SPL* TF family from liverwort *M. polymorpha*, which are crucial players in controlling proper growth and development of both vegetative thallus and reproductive organs.