Doctoral thesis/dissertation Digest Form

Thesis/dissertation Title: Genetic and morphological dissection of sexual differentiation processes in the liverwort *Marchantia polymorpha*

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Understanding the processes of sexual differentiation in plants is important not only to obtain basic biological knowledge, but also to establish intellectual bases for crop breeding. While plant life cycles alternate between sporophytic and gametophytic generations, plant sexual morphogenesis has mostly been studied using the sporophyte-dominant angiosperm models, thus little is known about the gametophytic sexual morphogenesis including gamete differentiation. The liverwort *Marchantia polymorpha* has been recently acknowledged as an ideal model system to study gametophytic sexual differentiation. Cellular details of the sexual dimorphism, especially those leading to distinct gametangiophore morphologies, however, have not explicitly described. Here, I revealed the cellular patterning processes underlying the sex-specific gametangiophore morphogenesis, and identified genes potentially functioning in female sexual differentiation in *M. polymorpha*.

Gametangiophore primordia emerge and develop at one of the two apical notches in each bifurcated thallus after induction of reproductive development by supplemental FR irradiation. I found that the germline marker Mp*BONOBO* (Mp*BNB*) was already expressed at the convex protrusions emerging in the apical notch region, the earliest detectable process of gametangiophore morphogenesis, suggesting that germline specification and initiation of sexual differentiation are coupled in *M. polymorpha*. Subsequently, male and female primordia developed a similar dome-like morphology but with distinct spatial distribution patterns of germline lineages. In later stage, distinct sexual morphologies gradually formed with male and female receptacles developing a flat morphology and small finger-like rays, respectively. Cell volume analysis suggested that distinct cell proliferation and elongation may underlie the sex-specific gametangiophore morphogenesis, in addition to the distinct spatial distribution patterns of the germline cells.

FEMALE GAMETOPHYTE MYB gene encoding an R2R3 MYB-type transcription factor (Mp*FGMYB*) has been identified as a key regulator of female sexual differentiation in *M. polymorpha*. When Mp*FGMYB* is knocked out in females, their sexual morphologies were masculinized in multiple aspects, including gametangiophore

morphologies, gametangium differentiation, and gamete formation. Nevertheless, expression patterns and contribution of Mp*FGMYB* in these steps have not been studied. Accordingly, I investigated the expression patterns of Mp*FGMYB* during the gametangiophore morphogenesis, and found that the Mp*FGMYB* expression initiates in early stages where germline positioning is established. To understand what genetic networks act downstream of Mp*FGMYB* to achieve female sexual differentiation, I performed a comparative transcriptome analysis, and provided a list of genes up-regulated by Mp*FGMYB* overexpression and preferentially expressed in wild-type female primordia. Expression analyses of selected genes revealed their specific and distinct expression patterns in female gametangia and gametes, demonstrating that my study provided a valuable list of the genes for future functional analyses.

Together, my study revealed cellular bases of distinct gametophytic sexual morphogenesis, and provided a list of genes potentially acting in the female sexual differentiation in *M. polymorpha*.