(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	SUN, Rui
Thesis Title	Exploring the biosynthesis and physiological function of gibberellin-related compounds in the liverwort *Marchantia polymorpha* (苔類ゼニゴケにおけるジベレリン関連化合物の生合成と生理機能に関する研究)		

(Thesis Summary)

The emergence of land plants from streptophyte algae brought about many evolutionary innovations, including the biosynthesis of several plant hormones. Gibberellins (GAs), a group of diterpenoid hormones, have likely evolved within the land plant lineage. Despite the structural diversity of GA-related compounds, vascular plants synthesize a few bioactive GAs through a conserved pathway. Bryophytes, the sister lineage of vascular plants, possess only the genes to produce GA precursors such as *ent*-kaurenoic acid (KA) and GA_{12}. The moss *Physcomitrium patens* uses KA as a precursor of a novel hormone that regulates plant development. However, in the other bryophytes, the function of the GAs remained to be determined.

The liverwort *Marchantia polymorpha* has four types of GA biosynthesis enzymes: *ent*-copalyl diphosphate synthase (CPS), *ent*-kaurene synthase (KS), *ent*-kaurene oxidase (KO) and *ent*-kaurenoic acid oxidase (KAO). In this thesis, the author resolved the evolutionary history of land plant KO and KAO homologs through phylogenetic analysis. Enzymatic assays indicated that MpKOL1 and MpKAOL1 exhibit the catalytic activities to produce KA and GA_{12}, respectively. Disruption of the Mp*CPS* gene resulted in only mild change in thallus morphology under regular culture conditions. However, it completely inhibited FR-dependent hyponastic and slender growth of the thalli. Instead, it drastically promoted thallus growth under FR-enriched light conditions. Furthermore, this Mp*cps* mutant showed delays in FR-induced reproductive development and distorted morphology of sexual branches (gametangiophores). Transcriptomic analysis showed that more than half of the FR-induced transcriptional changes, especially those related to stress responses and secondary metabolism, depended on GA_{Mp}. Together, these observations suggest that the downstream product of MpCPS plays an inhibitory role in regulating thallus growth.

Next, the bioactivity of several GA-related compounds was evaluated in wild-type and Mp*cps* mutant plants. Application of KA, but not any canonical GAs, fully rescued the phenotypes of Mp*cps*, but none of these compounds evoked significant morphological changes in wild-type plants. Further, the genetic mutations of Mp*KS,* Mp*KOL1*, or Mp*KAOL1* confirmed that KA is critical as a biosynthetic intermediate. While the GA_{12} biosynthesis was lost in all these mutants, the Mp*kaol1* mutation mildly impacted reproductive growth, suggesting that it is not absolutely required for reproductive development in *M. polymorpha*.

In conclusion, it was suggested that *M. polymorpha* is produces one or more bioactive compounds from KA, referred to as GA_{Mp}, which is distinctive to canonical bioactive GAs of vascular plants. GA_{Mp} acts as a plant hormone to regulate thallus growth and the responses to FR enrichment. Future studies will reveal the perception mechanism of GA_{Mp} in *M. polymorpha*, which lacks the canonical GA receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1).

(Form 2)

（Thesis Evaluation Summary）

Land plants evolved from streptophyte algae with many evolutionary innovations, including the invention of plant hormone biosynthesis. GAs are likely to have evolved within the land plant lineage. Vascular plants synthesize bioactive GAs through a conserved pathway. Bryophytes are the sister lineage of vascular plants, and possess only the enzymes that produce GA precursors such as KA and GA_{12}. In the moss *P. patens*, KA is used to produce a novel hormone to regulate development. Yet the function of GA precursors in other bryophytes is still unclear. This thesis primarily used molecular genetic approaches to describe the biosynthesis and physiological function of gibberellin-like compounds in the liverwort *M. polymorpha.*

The PhD candidate showed that the *M. polymorpha* genome encodes four orthologs presumably involved in the GA biosynthesis pathway, CPS, KS, KO and KAO, and conducted detailed phylogenetic analysis to clarify the evolutionary history of land plant KO and KAO homologs. She also performed enzymatic assays to show that MpKOL1 and MpKAOL1 exhibited catalytic activities to produce KA and GA_{12}, respectively. She further showed the physiological function of this biosynthesis pathway. Disruption of Mp*CPS* inhibited the hyponastic and slender thallus growth under FR enrichment conditions. The Mp*cps* mutation promoted vegetative growth and inhibited reproductive growth under FR-enriched light conditions, suggesting the growth inhibition and reproduction promotion activities of the downstream product of MpCPS. Application of KA, but not any canonical GAs, fully rescued the phenotypes of the mutants of Mp*CPS*, Mp*KS*, Mp*KO*, and Mp*KAOL1.* She concluded that the bioactive compound distinct from GAs in vascular plants, named GA_{Mp}, acts as a novel plant hormone to facilitate the responses to FR enrichment in *M. polymorpha*.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of plant molecular genetics and genomics, and presents new discoveries and concepts that contribute to the profound understanding and further development of plant hormone research. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On October 2nd, 2023, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary (Form 1), and thesis evaluation summary (Form 2) will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date below that the thesis can be published.

Publication date of the thesis summary (Form 1) and thesis evaluation summary (Form 2) : mm dd , yyyy