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SUMMARY OF DOCTORAL THESIS

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Title: Targeted expression of *bgl23-D* a dominant-negative allele of *ATCSLD5* and expression analysis of plant intracellular Ras-group related leucine-rich repeat (LRR) proteins (PIRLs) in *Arabidopsis thaliana*

（シロイヌナズナにおける*ATCSLD5*のドミナントネガティブアレル*bgl23-D*の標的発現と植物細胞内Rasグループ関連ロイシンリッチリピート (LRR) タンパク質 (PIRLs) の発現解析）

Arabidopsis thaliana has been the most used plant model system for decades. The current significance of *Arabidopsis* research reflects scientists growing recognition that this simple angiosperm may serve as a useful model for addressing fundamental concerns of biological structure and function common to all eukaryotes as well as plant biology. *A. thaliana* is helpful to researchers for genetic mapping and sequencing because of its small genome size. Apart from these, its short life cycle, self-pollinating nature and ability to produce many seeds make *A. thaliana* a valued genetic model plant to the scientific community. The research described in this thesis aimed to improve our understanding of the use of novel dominant-negative mutations as a genetic tool to suppress the function of a specific gene in the target tissue. The development of a compatible genetic tool to modify the target tissue or organ is highly required to understand the role of specific genes. However, tissue-specific expression analysis is also required to understand the physiological functions of specific genes.

In the Chapter 1, the author provides a general introduction to this work. The **Chapter 2** presents the identification of a new dominant-negative mutation, *bgl23-D,* in the *A. thaliana cellulose synthase-like D5* (*ATCSLD5*) gene that was reported to function in the division of guard mother cells. In plant cells, cellulose is an important component of cell wall and is synthesized by the cellulose synthases known as CESA proteins. The *CSLD* gene family is highly related to *CESA* because of sequence similarity. *ATCSLD5* was a direct target of SPEECHLESS and was involved in cell plate formation, including stomata lineage cells. A novel dominant-negative mutation showing bagel-shaped stomata has been successfully identified in *ATCSLD5* from ethylmethanesulfonate (EMS) mutagenized seeds of *A. thaliana* L*er* by next-generation sequencing and Mitsucal computer system using genomic DNA of bulk populations. The construct of own promoter-driven *ATCSLD5* cDNA containing C3220T (C2989T in the cDNA) substitution was introduced into WT-Col and found that the transgenic plants showed bagel-shaped stomata phenotype similar to the *bgl23-D* mutant, revealing that *ATCSLD5* is the responsible gene for the *bgl23-D* mutant. To assess the efficacy of the *bgl23-D* mutant in suppressing ATCSLD5 functions in target tissues or cells, two types of targets were chosen: (1) stomata: the region showing phenotype in the *bgl23-D* mutant, and (2) pollen and root: regions not showing phenotype in the *bgl23-D* mutant. The binary construct was prepared to express *bgl23-D* cDNA with the promoter of stomata-lineage specific genes. *ProSDD1:bgl23-D*, *ProMUTE:bgl23-D*, and *ProFAMA:bgl23-D* showed incompletely divided GCs that observed in the *bgl23-D,* as expected due to its dominant nature. The *ProFAMA:bgl23-D* plants showed highest percentage of abnormal bagel-shaped stomata. In addition, The *ProFAMA:bgl23-D* plants exhibited a wide range of severe cytokinesis defects, ranging from donut-shaped SGC having a single pore at the middle of the cell to the most severely affected spherical SGC without a pore. Moreover, bagel-shaped stomata were found on the abaxial and adaxial surfaces of the

leaves of *ProFIL:bgl23-D* plants with different frequencies. Tapetum plays an important role in the proper development of pollen and exine patterning by providing nutrients to the developing pollen grains. Expression of *bgl23-D* cDNA produced new phenotypes with promoter of tapetum- or anther-specific genes. The study revealed that *ProSP11:bgl23-D* showed slightly shrunken pollen grains, while *ProATSP146:bgl23-D* exhibited a combination of slightly shrunken and severely shrunken pollen grains. The normal reticulate structure of exine was lost in *ProSP11:bgl23-D* and *ProATSP146:bgl23-D*, respectively. However, microspores with no cellular content in *ProATSP146:bgl23-D* after pollen mitosis I (PMI) at the bicellular stage suggested that *bgl23-D* was expressed in microspores and disturbed cell division to some extent. Since the promoter activity of *ATSP146* was found in both the anther and the QC, root and QC phenotypes of *ProATSP146:bgl23-D* were analyzed. *ProATSP146:bgl23-D* exhibited a normal QC pattern and well-organized cell layer in the root apical region, as observed in WT. Additionally, *bgl23-D*, *ProSDD1:bgl23-D*, *ProMUTE:bgl23-D*, and *ProFAMA:bgl23-D* showed increased rosette size and larger epidermal cells, which indicate that *bgl23-D* has an effect on plant growth and development. Thus, this work suggests that *bgl23-D* could efficiently alter pollen shape and exine patterning as well as enhance plant growth, which makes it suitable to use as a genetic tool to modify the target tissue or cell.

To understand the physiological roles of specific genes, it is essential to know their expression patterns. After gaining insightful knowledge about the effect of *bgl23-D* on pollen shape and exine structure, it is essential to know the expression analysis of genes that have a role in reproductive organ development. For these reasons, expression analysis of plant intracellular Ras-group related leucine-rich repeat (LRR) proteins (PIRLs) was studied and presented in the **Chapter 3.** Plants contain a tiny group of proteins that are structurally similar to Ras-group LRRs named plant intracellular Ras-group LRR proteins (PIRLs). These PIRLs are involved in the growth and development of male and female reproductive organs in plants. Promoter:GUS assay revealed that *PIRLs* are expressed throughout the vegetative and reproductive organs. Concerning *PIRL1*, GUS expression was observed in the junction between the root and hypocotyl, the primary root tip, while *PIRL2* showed unique expression in root hairs and strong expression in the stipule, which indicate their specific functions in this region. *PIRL3*, *PIRL4*, *PIRL7*, and *PIRL9* showed expression in root but not in the aboveground organs. Concerning reproductive tissues, for *PIRL1*, GUS was slightly expressed in petals and filaments but was strongly expressed in anthers and pollen. Pollen tube-specific expression was observed for *PIRL1*, *PIRL6*, and *PIRL7.* Distinct expression of *PIRL3* was found in anthers and pollen but not in pollen tube. *PIRL5* was detected very weakly and only in anthers. Heat map analysis using microarray datasets of *PIRL* genes sourced from the Arabidopsis eFP browser revealed that *PIRL6* was specifically expressed in pollen, while *PIRL1* and *PIRL3* were expressed strongly in pollen and moderately in vegetative organs, and that *PIRL5* was expressed in pollen and roots. Moreover, a microarray study using visual expression of *PIRL* genes during developmental stages revealed that *PIRL1* and *PIRL3* were significantly expressed in floral organs, specifically in mature pollen rather than vegetative organs.

In the Chapter 4, the author provides proposed conclusions and remarks and highlights the key findings presented in this study.

In a nutshell, next-generation sequencing and Mitsucal computer system could successfully identify the novel dominant-negative mutation, *bgl23-D,* in *ATCSLD5.* The expression of *bgl23-D* cDNA with cell- or tissue-specific promoters inhibited ATCSLD5 functions in target tissues while stimulating plant growth. A detailed expression patterns of nine *PIRLs* were analyzed by promoter:GUS assay. These findings will open the way to use *bgl23-D* as a tool for functional analysis of ATCSLDs and other genes in specific tissues and organs and manipulate plant growth.