Thesis for the Degree of Ph.D

Identification and molecular characterization of rice promoters conferring microspore-preferred expression

School of Applied Biosciences, Major in Agronomy
The Graduate School

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Nguyen Tien Dung

Identification and characterization of microspore-preferred promoters in rice (*Oryza sativa* L.)

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(Abstract)

Tissue-specific promoters are a very useful tool for manipulating gene expression in a target tissue or organ; however, their range of applications in other plant species has not been determined, to date. In this study, I identified ten *rice microspore-preferred* (*RMP1* to *RMP10*) promoters via meta-anatomical expression analysis. I then investigated the expression of those promoters in transgenic rice (a homologous system) and *Arabidopsis* (a heterologous system) using GFP-GUS reporter genes. As expected from microarray data analysis, all of the ten *RMP* promoters directed similar GUS expression pattern in anthers, GUS signals were detected from the microspore stage throughout the all stages of pollen development. However, while four promoters, *RMP2, RMP7, RMP9* and *RMP10* did not direct GUS expressed in vegetative tissues such as leaf, stem, root at seedling stage, the other six promoters conferred GUS activity in those of seedlings. These results suggest that *RMP* promoters could be expressed preferentially in microspore in rice. In contrast, *RMP* promoters directed GUS gene showing distinct expression patterns in *Arabidopsis.* In inflorescence, the *RMP2, RMP3* and *RMP8* promoters directed GUS expression in young buds but not in mature flowers. GUS signals were observed only at uni-cellular and bi-cellular stages of pollen development. On the other hand, 2 promoters, *RMP9* and *RMP10,* exhibited GUS expression in mature flowers at late-pollen stages, tri-cellular and mature pollen. Whereas, the other five promoters, including *RMP1, RMP4, RMP5, RMP6,* and *RMP7* conferred GUS expression at all stages of pollen development, from uni-cellular throughout mature pollen. The activity of these promoters was further examined in T_2 seedlings. As a result, seven promoters, except for *RMP1, RMP2* and *RMP10,* showed GUS signals in shoot apical region or root tissues of seedlings. In addition, analyzing promoter sequence revealed that the six most abundant CREs detected in *RMP* promoter regions such as ACGTATERD1, ARR1AT, CAATBOX1, GATABOX, MYBCORE, and DOFCOREZM. Moreover, eleven CREs related to organs/tissues preferred expression. Of them, anther or pollen specific CREs such as GTGANTG10, POLLEN1LELAT52, SITEIIATCYTC, 5659BOXLELAT5659 were identified.

Furthermore, to verify the activity of promoters in microspore I carried out a functional demonstration by performing a complementation analysis using a *sidecar pollen* (*scp*) mutant that displays developmental defects at the microspore stage. Five promoters including the *RMP1, RMP2, RMP3, RMP6* and *OsLPS10* (rice late pollen specific promoter), which showed microspore expression in *Arabidopsis*, were also verified. I found evidence that the *OsLPS10, RMP1, RMP2, RMP3* and *RMP6* promoters, which can be

an applied promoter in *Arabidopsis*, are useful for directing gene expression in the early stages of pollen development. The results indicate that those promoters can direct the expression of target genes during the stages of pollen development in rice, including early and late stages.