

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

Nguyen, Tien-Dung

December 2015

The Graduate School
Kyungpook National University

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

Nguyen, Tien-Dung

School of Applied Biosciences, Major in Agronomy
The Graduate School

Supervised by Professor Lee, Jeong-Dong

Approved as a qualified thesis of Nguyen, Tien-Dung
for the degree of Ph.D
by the Evaluation Committee

December 2015

Chairman _____

Prof. Song, Jong-Tae

Prof. Park, Soon-Ki

Prof. Jung, Ki-Hong

Prof. Park, Dong-Soo

Prof. Lee, Jeong-Dong

The Graduate School Council, Kyungpook National University

TABLE OF CONTENTS

LIST OF TABLES	II
LIST OF FIGURES	III
LIST OF APPENDIX	V
ACKNOWLEDGEMENT	VI
CHAPTER 1. GENERAL INTRODUCTION	1
CHAPTER 2. IDENTIFICATION AND CHARACTERIZATION OF MICROSPORE-PREFERRED GENES	19
INTRODUCTION	19
MATERIALS AND METHODS.....	20
RESULTS	27
DISCUSSION.....	56
CHAPTER 3. FUNCTIONAL VERIFICATION OF MICROSPORE- PREFERRED PROMOTERS ACTIVITY IN MICROSPORE	67
INTRODUCTION	67
MATERIALS AND METHODS.....	68
RESULTS	71
DISCUSSION.....	85
CHAPTER 4. GENERAL DISCUSSION	87
REFERENCES	93

LIST OF TABLES

Table 1. Candidate genes exhibiting microspore-preferred expression	30
Table 2. The most abundant CREs in <i>RMP</i> promoter region.....	34
Table 3. Organ specific-CREs in <i>RMP</i> promoter region	35
Table 4. Unique CREs in <i>RMP</i> promoter region	36

LIST OF FIGURES

Figure 1. Diagram of rice (<i>Oryza sativa</i> L.) floral structure.....	3
Figure 2. Schematic diagram of the male gametophyte development in <i>Arabidopsis</i>	8
Figure 3. Female gametophyte development in <i>Arabidopsis</i>	9
Figure 4. Heat map analysis of expression patterns for <i>rice microspore-preferred (RMP)</i> genes	28
Figure 5. Schematic diagram of destination vectors used for plant transformation .	31
Figure 6. Frequency of the most abundant CREs in <i>RMP</i> promoter region	39
Figure 7. Frequency of organ specific-CREs in <i>RMP</i> promoter region.....	40
Figure 8. Frequency of unique CREs in <i>RMP</i> promoter region	41
Figure 9. Gus expression driven by <i>RMP</i> promoters during pollen development in rice	46
Figure 10. Confirmation T-DNA insertion in T2 rice transgenic plants.....	47
Figure 11. Gus expression driven by the <i>RMP</i> promoters in vegetative organs in rice.....	48
Figure 12. GUS expression driven by the <i>RMP</i> promoters during pollen developmental stages in <i>Arabidopsis</i>	53
Figure 13. GUS expression driven by the <i>RMP</i> promoters in <i>Arabidopsis</i> at seedling stage	54
Figure 14. The schematic diagram of proOsLSP10/RMP-SCP:dHA vectors used for genetic complementation analysis.....	73
Figure 15. The percentage of aberrant pollen grains from non-transformed <i>scp-2</i> homozygotes (control) and transformed <i>scp-2</i> hm harboring the proOsLSP10-SCP:dHA.....	74
Figure 16. Complementation analysis of <i>scp-2</i> homozygotes	75

Figure 17. The percentage of aberrant pollen grains from non-transformed <i>scp-2</i> homozygotes (control) and transformed <i>scp-2</i> hm harboring the proRMP1-SCP:dHA.....	79
Figure 18. The percentage of aberrant pollen grains from non-transformed <i>scp-2</i> homozygotes (control) and transformed <i>scp-2</i> hm harboring the proRMP2-SCP:dHA.....	80
Figure 19. The percentage of aberrant pollen grains from non-transformed <i>scp-2</i> homozygotes (control) and transformed <i>scp-2</i> hm harboring the proRMP3-SCP:dHA.....	81
Figure 20. The percentage of aberrant pollen grains from non-transformed <i>scp-2</i> homozygotes (control) and transformed <i>scp-2</i> hm harboring the proRMP6-SCP:dHA	82
Figure 21. Silique production in complementing lines (transformed <i>scp-2</i> hm) compared with <i>scp-2</i> hm mutant background (control) and wild type plants.....	83
Figure 22. Confirmation of T-DNA insertion in the proRMP-SCP:dHA lines	84

LIST OF APPENDIX

Appendix 1. Primers used in this study.....	120
Appendix 2. Medium composition used for rice transformation	122
Appendix 3. Nucleotide sequences of the <i>OsLPS10</i> gene.	125
Appendix 4. Nucleotide and protein sequences of the <i>RMP1 (Os01g0533400)</i> gene.....	129
Appendix 5. Nucleotide and protein sequences of the <i>RMP2 (Os01g0899100)</i> gene.....	131
Appendix 6. Nucleotide and protein sequences of the <i>RMP3 (Os03g0381000)</i> gene.....	133
Appendix 7. Nucleotide and protein sequences of the <i>RMP4 (Os04g0561900)</i> gene.....	135
Appendix 8. Nucleotide and protein sequences of the <i>RMP5 (Os04g0650200)</i> gene.....	137
Appendix 9. Nucleotide and protein sequences of the <i>RMP6 (Os06g0681100)</i> gene.....	139
Appendix 10. Nucleotide and protein sequences of the <i>RMP7 (Os07g0664600)</i> gene.....	141
Appendix 11. Nucleotide and protein sequences of the <i>RMP8 (Os12g0605900)</i> gene.....	144
Appendix 12. Nucleotide and protein sequences of the <i>RMP9 (Os12g0637100)</i> gene.....	147
Appendix 13. Nucleotide and protein sequences of the <i>RMP10 (Os12g0637900)</i> gene.....	149

ACKNOWLEDGEMENT

First and foremost I would like to express my sincere gratitude to Prof. Soon-Ki Park and Dr. Sung-Aeong Oh for all the guidance, helpful advice during the whole period.

I would like to give a big thank to Prof. Ki-Hong Jung in Kuyng Hee University for kindly provided microarray data profile, suggestions for my thesis.

I also would like to thank Prof. Jong-Tae Song, chairman of my advisory committee, Prof. Jeong-Dong Lee, and Prof. Dong-Soo Park for their valuable suggestions and critical review of my thesis.

In addition, I would like to thank all members in Sexual Plant Reproduction Laboratory for their help.

Last, but not least, I wish to thank my wife, son, and family for their support, understanding and encouragement during all this time.

Nguyen Tien Dung

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

Nguyen, Tien-Dung

*School of Applied Biosciences
The Graduate School, Kyungpook National University
Daegu, Korea
(Supervised by Professor Lee, Jeong-Dong)*

(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 T₂ 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.