

**ANALYSIS OF TRANSGENIC
WILD RICE: MOLECULAR CONFIRMATION
OF TRANSGENE PRESENCE AND RISK ASSESSMENT
FOR POLLEN VIABILITY**

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**DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF
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ABSTRACT

Rice is one of the major sources of food for the population of the world, and almost 50% of the world population depends on it. Malaysian national rice production was estimated to provide just 60-65% of the domestic requirement, so the strategies that can help to increase rice yield are necessary for the agricultural sector of Malaysia. Towards this, one useful research approach is to reduce or knock down expression of a rice gene using RNA interference (RNAi) with the goal of obtaining a phenotype that is suggestive of its function.

This project is based on the analysis of RNAi “knock down” transgenic rice which has been previously developed to determine function of the putative *CLAVATA1* (*CLV1*) receptor kinase-like protein gene from Malaysian wild rice *Oryza rufipogon* rice (IRGC105491). Putative transgenic plants from the T1 and T2 generations were analyzed in two main respects. The first was the determination of the segregation analysis in T1 plants by PCR detection and Southern hybridization, and the second was the assessment of pollen viability using staining and microscopy towards establishment of biosafety data for these plants.

The segregation analysis of T1 plants revealed that most of the transgenic lines showed 3:1 Mendel’s segregation ratio whereas there was only one line that deviated from this ratio. The optimization of the Southern blot hybridization, for detection of transgene copy number, was performed successfully for the positive control however this was not reproducible for the plant samples with only one time that two bands were observed on the nylon membrane after detection step. After several attempts, the southern blotting procedure was not attempted further because of the time limitation of

the project and also the lack of support for the necessary extra expenditure for supplying new materials.

This study also concluded that pollen viability did not vary with the presence of this transgene, as the control untransformed plants and transgenic plants showed the same attributes from the pollen viability point of view. This study has established the groundwork for further studies with transgenic *O. rufipogon*, an important wild rice variety in Malaysia.

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TABLE OF CONTENTS

	Page
Preface	
Title page	
Abstract	ii
Acknowledgment	iv
Table of Contents	v
List of Figures	ix
List of Tables	xi
List of Symbols and Abbreviations	xii
Chapter 1 Introduction	1
Chapter 2 Literature Review	5
2.1 Rice	5
2.1.1 Wild rice	5
2.1.2 Genetically modified rice	6
2.2 Functional Genomics	7
2.2.1 Functional genomics based on reverse genetics in rice	8
2.3 Quantitative Trait Loci (QTLs)	10
2.3.1 Yield-Related QTL in Rice	12
2.3.2 Yield-Related Genes in Rice	14

Continue Table of Contents

2.4	Receptor Like Kinase (RLK)/ Pelle Family	16
2.4.1	CLAVATA genes involving in plant growth and development	19
2.5	RNA interference	21
2.5.1	The Biology and Mechanism of RNA interference	22
2.5.2	Hairpin RNA (hpRNA)–mediated gene silencing in plants	25
2.6	Biosafety of Transgenic Plants	26
Chapter 3 Materials and Methods		30
3.1	Plant Materials	30
3.2	Plant Growth Conditions	31
3.3	DNA extraction	32
3.4	DNA quantity and quality measurements	32
3.5	Polymerase Chain Reaction (PCR)	32
3.6	Analysis by Agarose Gel Electrophoresis	34
3.6.1	TBE buffer preparation	34
3.6.2	Agarose gel preparation	34
3.6.3	Preparation of DNA samples	35
3.6.4	PCR products	35
3.7	Purification of PCR products	35
3.8	Gel extraction	36
3.9	Sequencing	36
3.9.1	Analysis of sequences	36
3.10	Southern blot analysis	36
3.10.1	Conventional DNA extraction	36

Continue Table of Contents

3.10.2	Probe synthesis and labelling	37
3.10.3	Probe sequence analysis	38
3.10.4	Genomic and plasmid DNA digestion	38
3.10.5	Purification and precipitation of digested genomic DNA	40
3.10.6	Gel treatment and blotting procedure	41
3.10.7	Hybridization and Visualisation of Genomic DNA Blot	41
3.11	Pollen viability tests	42
3.11.1	In vitro pollen tube germination	43
3.11.2	Iodine potassium iodide Staining	43
Chapter 4	Results	44
4.1	DNA extraction	44
4.2	DNA quantity and quality measurements	46
4.3	PCR amplification of DNA samples obtained from T1 plants	47
4.4	Germination of T1 plant's seeds	48
4.5	PCR amplification of DNA samples obtained from T2 plants	49
4.6	Sequencing analysis of Gus sequences cloned from transformed rice plants	51
4.7	Southern Blotting	51
4.8	Pollen viability tests	56
4.8.1	Iodine-potassium iodide staining	56
4.8.2	In vitro pollen tube germination	57

Continue Table of Contents

Chapter 5	Discussion	59
5.1	Plant growth	59
5.2	DNA extraction	60
5.3	PCR screening of plants in two generations	60
5.4	Segregation analysis of T2 plants	61
5.5	Southern blotting analysis	63
5.6	Pollen fertility tests for biosafety considerations	67
	5.6.1 Pollen staining for viability	68
	5.6.2 Pollen tube germination	69
Chapter 6	Conclusion	70
Chapter 7	References	71
Appendixes		
Appendix A	Recipe for CTAB buffer	91
Appendix B	PCR amplification of DNA samples obtained from T2 plants	92
Appendix C	Sequencing analysis of Gus sequences	94
Appendix D	Restriction Summary results	109
Appendix E	Schematic map of pANDA vector	113
Appendix F	Pollen tube germination percentage of plants at different time intervals after anther dehiscence	114

LIST OF FIGURES

<u>Figure No.</u>	<u>Title of Figure</u>	<u>Page No.</u>
Figure 2.2	From genome sequence to gene function.	8
Figure 2.4	Common themes in plant and animal receptor protein kinases.	16
Figure 2.4.1	<i>CLV1</i> -mediated signaling pathway involves the activation of <i>CLV1</i> by the ligand <i>CLV3</i> and also the interference of <i>CLV2</i> with unknown exact role.	21
Figure 2.5.1	Core features of siRNA and miRNA silencing.	24
Figure 4.1	Agarose electrophoresis (1%) of total genomic DNA extracted from eight A9 line T1 plants (A) and twenty-one A3.2 line T2 plants (B).	46
Figure 4.3.1	PCR screening of A5 events with Gus linker primer (A and B).	47
Figure 4.3.2	PCR screening of Ci line as control untransformed plants with Gus linker primers.	48
Figure 4.5.1	PCR screening of A2.2 (a) and A.3.2 (b) lines with <i>Gus</i> linker primers.	50
Figure 4.5.2	PCR screening of Cii line control untransformed plants with Gus linker primers.	50
Figure 4.7.1	Agarose electrophoresis (1%) of total genomic DNA extracted from eight T1 transgenic plants.	52
Figure 4.7.2	Gus linker probe synthesised by PCR amplification of 636 bp fragment of transgene.	53
Figure 4.7.3	The optimized restriction digestion of transgenic DNA sample by three different restriction enzymes.	53
Figure 4.7.4	Southern-blot analysis for the detection of <i>Gus</i> linker sequence of pANDA vector to use as control positive of southern blotting of transgenic samples.	55
Figure 4.7.5	Southern-blot analysis of A1.1 DNA sample while <i>HindIII</i> was used to cut.	55

Continue List of Figures

Figure 4.8.1	Pollen grains of rice stained by I ₂ -KI under light microscope (40X).	57
Figure 4.8.2.1	Anther dehiscence of a transgenic <i>Oryza rufipogon</i> (IRGC105491) rice plant.	57
Figure 4.8.2.3	Pollen tube germination of transgenic plants (A1.1, A2.4 and A3.7) and non-transgenic plants (Ci3, Ci6 and Ci8).	58

LIST OF TABLES

<u>Table No.</u>	<u>Title of Table</u>	<u>Page No.</u>
Table 2.3.1	Chromosome-wise distribution of yield-related QTL and related traits from wild species of rice	14
Table 3.1	All the transgenic and non transgenic control plants involved in the project.	31
Table 3.5.1	The primers sequences for Polymerase Chain Reaction	33
Table 3.5.2	The reaction mixture for Polymerase Chain Reaction	33
Table 3.10.4.1	Restriction digestion of genomic DNA by <i>SacI</i> restriction enzyme	39
Table 3.10.4.2	Restriction digestion of genomic DNA by <i>SpeI</i> restriction enzyme	39
Table 3.10.4.3	Restriction digestion of genomic DNA by <i>HindIII</i> restriction enzyme	40
Table 3.10.4.4	Restriction digestion of plasmid DNA by <i>HindIII</i> restriction enzyme	40
Table 4.1.1	The extracted DNA samples from T1 plants	44
Table 4.1.2	The extracted DNA samples from T2 plants	45
Table 4.4	Germination percentage of T1 seeds and control non-transformed seeds (Ci6 and Cii)	49
Table 4.5	Segregation ratio of transgene presence (<i>Gus</i> linker) in transgenic T1 rice seeds	51
Table 4.8.1	Fertility percentage of pollen from transgenic and non-transgenic plants subjected to I ₂ -KI staining	56

LIST OF SYMBOLS AND ABBREVIATIONS

<u>Symbols/Abbreviations</u>	<u>Descriptions</u>
β	Beta
$^{\circ}\text{C}$	Degree Celsius
μg	Microgram
μl	Microliter
mg	Milligram
ml	Milliliter
%	Percentage
~	Approximate
A_{230}	Absorption at 230 nanometer
A_{260}	Absorption at 260 nanometer
A_{280}	Absorption at 280 nanometer
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
cDNA	Complementary deoxyribonucleic acid
<i>CLVI</i> gene	<i>CLAVATA1</i> gene
DNA	Deoxyribonucleic acid
dH ₂ O	Distilled water
dNTP	Deoxyribonucleic acid
ddH ₂ O	Double distilled water
dsDNA	Double-Stranded DNA
<i>E.coli</i>	<i>Escherichia coli</i>
EDTA	Ethylenediamine tetra-acetic acid

Continue List of Symbols and Abbreviations

<i>et al</i>	et alia
Etbr	Ethidium bromide
g	Gram
<i>GUS</i>	Beta-glucuronidase
hpRNA	hairpin RNA
hr	Hour
IR	Inverted repeat
I ₂ -KI	Iodine-potassium iodide
kb	Kilobase pair
L	Liter
LE	Low electroendosmosis
M	Molar
MgCl ₂	Magnesium chloride
mRNA	Messenger RNA
NBT	Nitro blue tetrazolium chloride
PCR	Polymerase Chain Reaction
rpm	Revolutions per minute
RdRp	RNA dependent RNA polymerase
RE	Restriction enzymes
RISC	RNA-induced silencing complex
RKN	Receptor-like kinase
RLK	Receptor-like protein kinase
RNAi	RNA interference
siRNA	Short-interfering RNA
SRF	Stained round fertile

Continue List of Symbols and Abbreviations

ssRNA	Single-stranded RNA
Taq	<i>Thermus Aquaticus</i>
TBE	Tris-borate EDTA
UV	Ultraviolet
w/v	Weight per Volume