

TABLE OF CONTENTS

TITLE PAGE	I
ORIGINAL LITERARY WORK DECLARATION	II
ABSTRACT	III
ABSTRAK	IV
ACKNOWLEDGEMENT	V
TABLE OF CONTENTS	VI
LIST OF FIGURES	XII
LIST OF TABLE	XVI
LIST OF SYMBOLS AND ABBREVIATIONS	XVIII
CHAPTER 1.0: INTRODUCTION	1
CHAPTER 2.0: LITERATURE REVIEW	4
2.1 Rice	4
2.1.1 Cultivated Rice	4
2.1.2 Rice Domestication	5
2.1.3 Rice Genome Sequencing Projects	7
2.2 Quantitative Trait Loci (QTLs)	11
2.2.1 Yield-Related QTL in Rice	12
2.2.2 Advance Backcross QTL	15
2.2.3 Yield-Related Genes in Rice QTL	16
2.3 Receptor-Like Kinase (RLK)/<i>Pelle</i> Protein Kinase Family	17

2.4 RNA Silencing	22
2.4.1 The Biogenesis and Mechanism siRNA and miRNA	23
2.1.2 RNAi Technology in Plant Systems	26
CHAPTER 3.0: MATERIALS AND METHODS	28
3.1 Plant Materials	28
3.2 Growth Conditions	28
3.3 Plasmid Vector and Bacterial Strains	31
3.4 Primer Design	31
3.5 DNA Extraction	34
3.6 Total RNA Extraction	35
3.7 First Strand cDNA Synthesis from Deoxyribonuclease I Treated	36
Total RNA	
3.7.1 Deoxyribonuclease I Treatment	36
3.7.2 First Strand cDNA Synthesis	36
3.8 Polymerase Chain Reaction (PCR)	37
3.9 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)	37
3.10 Gel Extraction	38
3.11 Gene Cloning and Sequencing	39
3.11.1 Preparation of Competent Cells	39
3.11.2 Preparation of Ligation Reactions	40
3.11.3 Transformation of <i>E. coli</i>	41
3.11.4 Plasmid DNA Preparation	41

3.11.5 Sequencing Analysis	42
3.12 Rapid Amplification of cDNA Ends (RACE) of Putative <i>RPKI</i>	42
3.13 Gene Sequence and Structure Analysis	43
3.14 Base Substitution Mutation Analysis	44
3.15 Phylogenetic Analysis	45
3.16 Southern Hybridization Analysis	48
3.16.1 Labeling of Hybridization Probes	48
3.16.2 Preparation of Genomic DNA Blot	48
3.16.3 Hybridization and Visualisation of Genomic DNA Blot	49
3.17 Quantification of Gene Expression	50
3.17.1 Real Time Quantitative Reverse Transcriptase PCR (qRT-PCR) Amplification Efficiency	50
3.17.2 Housekeeping Genes Selection	51
3.17.3 Real Time Quantitative Reverse Transcriptase PCR (qRT-PCR)	52
3.17.4 Statistical Analysis of Real Time qRT-PCR	53
3.18 Construction of RNAi Vectors for Knockdown of Gene Expression	53
3.18.1 Subcloning of Gene of Interest into pENTR/D-TOPO Cloning Vector	54
3.18.2 Cloning of pENTR/D-TOPO Cloning Vector Containing Gene of Interest into pANDA Vector	54
3.19 Plant Transformation	55

3.19.1 Preparation of <i>Agrobacterium tumefaciens</i> Competent Cells	55
3.19.2 Transformation of pANDA RNAi Construct Containing the Required Sequences into <i>Agrobacterium tumefaciens</i> Strain EHA105	56
3.19.3 Transformation and Selection of Hygromycin Resistant Plants	57
 CHAPTER 4: RESULTS	 58
4.1 Characterization and Identification of Full Length cDNA of Putative <i>RPK1</i>	58
4.1.1 Isolation of Full Length cDNA and Sequence Analysis of Putative <i>RPK1</i>	58
4.1.2 Structure Analysis of Putative RPK1	71
4.2 Characterization and Identification of Full Length cDNA of Putative <i>CLV1</i>	73
4.2.1 Isolation of Full Length cDNA and Sequence Analysis of Putative <i>CLV1</i>	73
4.2.2 Structure Analysis of Putative CLV1	80
4.3 Characterization of Kinase of Putative RPK1 and Putative CLV1	83
4.4 Phylogenetic Analysis	86
4.5 Gene Expression Study of Putative <i>RPK1</i> and Putative <i>CLV1</i>	90
4.5.1 Validation of Comparative C _T Method Real Time qRT-PCR	90
4.5.2 Expression Profiles of Putative <i>RPK1</i>	96

4.5.3 Expression Profiles of Putative <i>CLVI</i>	100
4.6 Southern Hybridization Analysis	104
4.6.1 Gene Structure of Putative <i>RPK1</i>	104
4.6.2 Gene Structure of Putative <i>CLVI</i>	106
4.7 Construction of RNAi Vectors for Knockdown of Gene Expression	107
4.7.1 Cloning of Putative <i>RPK1</i> and Putative <i>CLVI</i> Sequences into pANDA Vector	107
4.7.2 Plant Transformation	112
 CHAPTER 5.0: DISCUSSION	 115
5.1 Gene Structure of Putative <i>RPK1</i> and Putative <i>CLVI</i>	116
5.2 Phylogenetic Analysis	122
5.3 Potential Effects of Putative <i>RPK1</i> and Putative <i>CLVI</i> Alleles on Yield	123
 CHAPTER 6.0: CONCLUSION	 126
6.1 Future Work	128
 APPENDICES	 129
APPENDIX A: Solutions	129
APPENDIX B: Amino Acid Sequences of Putative <i>RPK1</i> and Putative <i>CLVI</i>	130
APPENDIX C: Gblocks Cleaned of Orthologous <i>RPK1</i> Amino Acid	132

Sequences	
APPENDIX D: Gblocks Cleaned of Orthologous CLV1 Amino Acid	135
Sequences	
APPENDIX E: ClustalW Alignment of Gblocks Cleaned of	138
Orthologous RPK1 Amino Acid Sequences	
APPENDIX F: ClustalW Alignment of Gblocks Cleaned of	140
Orthologous RPK1 Amino Acid Sequences	
APPENDIX G: DNA Sequences of <i>pANDA_CLV1</i> and <i>pANDA_RPK1</i>	143
Inserts	
APPENDIX H: Preliminary Yield Components	144
APPENDIX I : Gene Sequences of Putative <i>RPK1</i> and Putative <i>CLV1</i>	145
REFERENCES	149

LIST OF FIGURES

<u>Figures</u>	<u>Titles of Figures</u>	<u>Pages</u>
Figure 2.1:	The three general RLK class structures in the plant RLK/ <i>Pelle</i> family, the transmembrane receptor kinase, the receptor-like cytoplasmic kinase (RLCK) and the receptor like protein (RLP).	19
Figure 2.2:	The general classification scheme for plant RLK/ <i>Pelle</i> family.	20
Figure 3.1:	Schematic representation of advanced backcross progenies (<i>Oryza rufipogon</i> (IRGC105491) × <i>Oryza sativa</i> ssp. <i>indica</i> cv. MR219) under national breeding program (Sabu <i>et al.</i> , 2006).	29
Figure 4.1:	RT-PCR amplification of putative <i>RPK1</i> .	59
Figure 4.2:	Gel extraction products of (A) <i>Oruf_RPK1</i> and (B) <i>OsI_RPK1</i> .	60
Figure 4.3:	ClustalW ORF sequence alignment of <i>Oruf_RPK1</i> , <i>OsI_RPK1</i> and <i>OsJ_RPK1</i> (gi:18677097).	61
Figure 4.4:	Base substitution of partial putative <i>RPK1</i> sequences revealed by sequencing from (A) <i>Oryza rufipogon</i> , (B) <i>Oryza sativa</i> ssp. <i>indica</i> cv. MR219, (C) BC ₂ F ₇ line 7 and (D) BC ₂ F ₇ line 23.	65
Figure 4.5:	The inner 5' and 3' RLM-RACE PCR amplification of <i>Oruf_RPK1</i> .	67

Figure 4.6:	The inner 5' and 3' RLM-RACE PCR amplification of <i>OsI_RPK1</i> .	68
Figure 4.7:	Predicted 5' and 3' splice sites of putative <i>RPK1</i> .	70
Figure 4.8:	ClustalW alignment of amino acid sequence and gene structure prediction of Oruf_RPK1, OsI_RPK1 and OsJ_RPK1 (gi:18677097).	72
Figure 4.9:	ClustalW amino acid sequence alignment of OsJ_CLV1 (gi:125602183) and RKN patent sequence (WO/2000/004761).	74
Figure 4.10:	RT-PCR amplification of putative <i>CLV1</i> .	76
Figure 4.11:	ClustalW gene sequence alignment of <i>Oruf_CLV1</i> , <i>OsI_CLV1</i> and <i>OsJ_CLV1</i> (gi:125602183; 2640-3006).	78
Figure 4.12:	Base substitution of partial putative <i>CLV1</i> sequences revealed by sequencing from (A) <i>Oryza rufipogon</i> , (B) <i>Oryza sativa</i> ssp. <i>indica</i> cv. MR219, (C) BC ₂ F ₇ line 7 and (D) BC ₂ F ₇ line 23.	79
Figure 4.13:	ClustalW alignment of amino acid sequence and gene structure prediction of Oruf_CLV1, OsI_CLV1 and OsJ_CLV1 (gi:125602183).	81
Figure 4.14:	ClustalW amino acid sequence alignment of kinase domains with known RLKs.	85
Figure 4.15:	Phylogenetic analysis of orthologous RPK1 amino acid sequences from <i>Arabidopsis thaliana</i> , <i>Oryza sativa</i> ,	87

Oryza rufipogon and *Zea mays*.

Figure 4.16:	Phylogenetic analysis of orthologous CLV1 amino acid sequences from <i>Arabidopsis thaliana</i> , <i>Oryza sativa</i> , <i>Glycine max</i> , <i>Medicago truncatula</i> , <i>Picea glauca</i> , <i>Pisum sativum</i> and <i>Vitis vinifera</i> .	89
Figure 4.17:	Agarose gel electrophoresis of (A) total RNA and (B) DNaseI treated total RNA of <i>Oryza rufipogon</i> (1), <i>Oryza sativa</i> ssp. <i>indica</i> cv. MR219 (2), BC ₂ F ₇ line 7 (3) and BC ₂ F ₇ line 23 (4) at different stages.	91
Figure 4.18:	Selection of housekeeping genes by geNORM v3.4.	92
Figure 4.19:	PCR efficiency of targets and housekeeping genes.	94
Figure 4.20:	Comparative gene expression of putative <i>RPK1</i> between vegetative, reproductive and ripening phase.	97
Figure 4.21:	Comparative gene expression of putative <i>CLV1</i> between vegetative, reproductive and ripening phase.	101
Figure 4.22:	Southern hybridization analysis of putative <i>RPK1</i> .	105
Figure 4.23:	Southern hybridization analysis of putative <i>CLV1</i> .	106
Figure 4.24:	RT-PCR amplification of genes of interest.	108
Figure 4.25:	PCR confirmation of pENTR/D-TOPO cloning vector.	109
Figure 4.26:	The sense orientation and antisense orientation of genes of interest for pANDA construct.	111
Figure 4.27:	PCR screening of <i>Agrobacterium</i> colonies with Gus linker primer.	113

Figure 4.28:	PCR screening of hygromycin resistant plants with Gus linker primers.	114
Figure A.1:	ClustalW alignment of Gblocks cleaned of orthologous RPK1 amino acid sequences.	138
Figure A.2:	ClustalW alignment of Gblocks cleaned of orthologous CLV1 amino acid sequences.	140

LIST OF TABLES

<u>Table</u>	<u>Titles of Tables</u>	<u>Pages</u>
Table 2.1:	Chromosome-wise distributions of yield-related traits of QTL from wild rice species.	13
Table 3.1:	List of rice samples used in this study. Samples were collected at the listed developmental stages from <i>Oryza rufipogon</i> , <i>Oryza sativa</i> ssp. <i>indica</i> cv. MR219, BC ₂ F ₇ line 7 and BC ₂ F ₇ line 23.	30
Table 3.2:	List of plasmid vectors and bacterial strains.	31
Table 3.3:	Primers sequences used to synthesize probes for Southern hybridization.	32
Table 3.4:	Primers sequences used to amplify ORF and RLM-RACE cDNA sequence.	32
Table 3.5:	Primer sequences used for real time qRT-PCR analysis.	33
Table 3.6:	Primer sequences used for amplification for cloning into RNAi vector, pANDA.	33
Table 3.7:	List of orthologous RPK1 amino acid sequences.	46
Table 3.8	List of orthologous CLV1 amino acid sequences.	47
Table 4.1:	Comparative sequence analysis of <i>Oruf_RPK1</i> and <i>OsI_RPK1</i> with <i>OsJ_RPK1</i> (gi:18677097) cDNA.	69
Table 4.2:	Data of rescaled normalized expression level of putative <i>RPK1</i> and standard error of rescaled normalized expression level of putative <i>RPK1</i> of <i>Oryza</i>	98

rufipogon (OR), *Oryza sativa* ssp. *indica* cv. MR219 (MR), BC₂F₇ line 7 and BC₂F₇ line 23 at different developmental stages.

Table 4.3:	Statistical analysis using two-way ANOVA with putative <i>RPKI</i> profiles of <i>Oryza rufipogon</i> , <i>Oryza sativa</i> ssp. <i>indica</i> cv. MR219, BC ₂ F ₇ line 7 and BC ₂ F ₇ line 23 collected at different developmental stages.	99
Table 4.4:	Data of rescaled normalized expression level of putative <i>CLVI</i> and standard error of rescaled normalized expression level of putative <i>CLVI</i> of <i>Oryza rufipogon</i> (OR), <i>Oryza sativa</i> ssp. <i>indica</i> cv. MR219 (MR), BC ₂ F ₇ line 7 and BC ₂ F ₇ line 23 at different developmental stages.	102
Table 4.5:	Statistical analysis using two-way ANOVA with and putative <i>CLVI</i> profiles of <i>Oryza rufipogon</i> , <i>Oryza sativa</i> ssp. <i>indica</i> cv. MR219, BC ₂ F ₇ line 7 and BC ₂ F ₇ line 23 collected at different developmental stages.	103
Table A.1:	Preliminary yield components of <i>Oryza rufipogon</i> , <i>Oryza sativa</i> ssp. <i>indica</i> cv. MR219, BC ₂ F ₇ line 7 and BC ₂ F ₇ line 23.	144

LIST OF SYMBOLS AND ABBREVIATIONS

A	Alpha
AB	Advance backcross
ABA	Abscisic acid
AP	Alkaline phosphatase
<i>At</i>	<i>Arabidopsis thaliana</i>
β	Beta
BAC	Bacterial artificial chromosome
BLAST	Basic Local Alignment Search Tool
bp	Base pair(s)
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
<i>CLV1</i>	<i>CLAVATA1 Receptor Kinase</i>
CTAB	Cetyl Trimethyl Ammonium Bromide
cv.	Cultivar
DCL1	DICER-like1
DEPC	Diethyl pyrocarbonate
dH ₂ O	Distilled water
DIG	Digoxigenin
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide 5' triphosphate

dsRNA	Double-stranded RNA
DTT	Dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	Ethylenediamine tetra-acetic acid
<i>eEF-1α</i>	Eukaryotic elongation factor 1-alpha
<i>et al</i>	<i>et alii/</i> and others
EST	Expressed sequence tag
<i>FLS2</i>	Flagellin Sensitive2
g	Gram
gi	NCBI GenInfo Identifier
GUS	Beta-glucuronidase
hr	Hour
<i>hpt</i>	Hygromycin phosphotransferase
hpRNA	Hairpin RNA
<i>i.e.</i>	<i>id est/</i> that is
IPTG	Isopropyl β -D-1-thiogalactopyranoside
IR	Inverted repeat
kb	Kilobase pairs
L	Litre
LB	Luria-Bertani
LRR	Leucine-rich repeat
M	Molar
MAS	Marker-assisted selection

Mb	Megabase pairs
MCS	Multiple cloning site
mg	Milligram
μl	Microlitre
μg	Microgram
M	Mutant
min	Minute
miRNA	Micro RNA
ml	Millilitre
mM	Millimolar
mRNA	Messenger RNA
MS	Murashige and Skoog
NERICA	New Rice for Africa
ng	Nanogram
no.	Number
NOST	<i>Nopaline synthase</i> terminator
<i>nptII</i>	Neomycin Phosphotransferase
nt	Nucleotide
OD	Optical density
ORF	Open reading frame
<i>OsI</i>	<i>Oryza sativa</i> ssp. <i>indica</i>
<i>OsJ</i>	<i>Oryza sativa</i> ssp. <i>japonica</i>
<i>Oruf</i>	<i>Oryza rufipogon</i>

PCI	Phenol: chloroform: isoamyl alcohol
PCL	Physical Containment Level
PCR	Polymerase chain reaction
PTGS	Post-transcriptional gene silencing
PVP	Polyvinylpyrrolidone
QTL	Quantitative trait locus
RAP-DB	Rice Annotation Project Database
RDR	RNA dependent RNA polymerase
RE	Restriction enzymes
RFLP	Restriction fragment length polymorphism
RISC	RNA-induced silencing complex
RKN	Receptor-like kinase
RLK	Receptor-like protein kinase
RLM-RACE	RNA Ligase Mediated Rapid Amplification of cDNA Ends
RM	Rice marker
RNA	Ribonucleic acid
RNAi	RNA interference
<i>RPKI</i>	Receptor-Like Protein Kinase1
rpm	Revolutions per minute
RT-PCR	Reverse transcription polymerase chain reaction
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
SDS	Sodium dodecyl sulfate
sec	Second(s)

siRNA	Short-interfering RNA
SMART	Simple Modular Architecture Research Tool
SNP	Single nucleotide polymorphism
ssp.	Subspecies
SSR	Simple sequence repeat
ssRNA	Single-stranded RNA
<i>SRK</i>	<i>S</i> -locus receptor kinase
ta-siRNA	Trans-acting siRNA
TBE	Tris-EDTA buffer
TIGR	The Institute for Genomic Research
TILLING	Targeting induced local lesions in genomes
Tris	Tris-hydroxymethyl amino methane
U	Weiss unit
UBQ	Ubiquitin
UTR	Untranslated region
UV	Ultraviolet
VIGS	Virus-induced gene silencing
v/v	Volume per volume
w/v	Weight per volume
× g	Times gravity
°C	Degrees Celsius
%	Percentage
#	Line