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

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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 a 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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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols/Abbreviations	Descriptions
ß	Beta
^{o}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
CLV1 gene	*CLAVATA1* gene
DNA	Deoxyribonucleic acid
dH_2O	Distilled water
dNTP	Deoxyribonucleic acid
ddH_2O	Double distilled water
dsDNA	Double-Stranded DNA
E.coli	*Escherichia coli*
EDTA	Ethylenediamine tetra-acetic acid

Continue List of Symbols and Abbreviations

et al	et alia
Etbr	Ethidium bromide
g	Gram
GUS	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	*Thermus Aquatiqus*
TBE	Tris-borate EDTA
UV	Ultraviolet
w/v	Weight per Volume