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Analysis of *SlRR22* gene and its role in seed wound response and manipulation of gene expression

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Thesis submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy

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March 2015

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Declaration

I confirm that the work presented in this thesis is my own and that the use of all the literature from other sources has been properly and fully acknowledge

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March, 2015

Acknowledgement

I thank Allah for the blessing.

First of all, I would like to express my sincere gratitude to my supervisors, Dr. Carol Wagstaff and Prof. Jerry Roberts for their conscientious supervision, and their belief in my ability. I am immensely grateful to the two of them for always believing in me, showing me that nothing is impossible and for providing me a beautiful rewarding learning process.

I thank the Department of Food and Nutritional Sciences at University of Reading and University of Nottingham at Sutton Bonington campus for allowing me to conduct my research, especially Prof. Zoe Wilson and Dr. Alison Ferguson for the guidance and advice during my visits to University of Nottingham. Special thanks to my Wagstaff group members for insightful and stimulating comments.

I dedicate this thesis to my beloved family, who has shown tremendous support and unconditional love, especially my husband, Hafizulazrin, my son, Harraz, and my mother, Wan Kelsom. I am forever indebted to my dearest friends especially Kak Faiz, Kak Naila, Kak Yati, Ika, and Ainul for their continuous help and support.

Last but not least, I would like to thanks to my employer, Universiti Malaysia Terengganu and my sponsor, Ministry of Education Malaysia.

Abbreviations

Asp	Aspartate
bp	Base pair
cDNA	Complementary deoxyribonucleic acid
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
GUS	β-glucuronidase
His	Histidine
HPt	Histidine phosphotransfer
L	Liter
LB	Luria-bertani
Mb	Megabase
Mg	Miligram
mM	Milimolar
ng	Nanogram
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
TAE	Tris-acetate-EDTA
v/v	volume/volume
w/v	weight/volume
µg	microgram
µL	microliter
µM	micromolar

Abstract

Environmental changes such as light, temperature, nutrients level, and pathogen attack are threatening factors to plants. However, the plant has a system to adapt with these changes, known as a two-component system (TCS). The system consists of histidine kinase (HK) that can sense the environmental signal and a response regulator (RR) that respond to the signal. This study was conducted to analyse the genes encoded for response regulator. *Arabidopsis response regulator 22* (*ARR22*) (*At3g04280*) is one type of response regulator that was found in seed and flower and involved in wound signalling. The *ARR22* can regulate the expression of seed storage proteins and proteolysis in wound induction. However, the role of *ARR22* during wounding that mimic the pathogen attack is still remains unclear. This study had identified a potential orthologue to *ARR22* in fleshy fruit of tomato known as *Solanum lycopersicum response regulator 22* (*SlRR22*) (*Solyc11g071630*) and four other *SlRRs* which are *SlRR1* (*Solyc05g054390*), *SlRR8* (*Solyc10g079700*), *SlRR10* (*Solyc11g066220*), and *SlRR16* (*Solyc06g048930*). The finding is important as it indicate that the mechanism during wounding is conserved between dry and fleshy fruit types.

SlRR22 has 60% of amino acid similarity and is located within the same group with *ARR22* in phylogenetic tree. RT-PCR analysis indicated that *SlRR22* was only been expressed in seed at 0 to 20 days after flowering (DAF). The gene also involved in wound signalling as it was down-regulated in 90 minutes post-wounding in seed. Wounding also caused down-regulated of *SSP2* (*Solyc08g080490*) in 10 DAF wounded seed while *SSP1* (*Solyc07g064210*) was degraded after 90 minutes of wounding in seeds at stage 20 and 40

DAF, and breaker. Meanwhile, the expression of *Cyclin A1* (*Solyc11g005090*) was only been up-regulated at later stage (40 DAF, breaker, and ripen) following wounded seeds. I hypothesise that proteolytic enzyme degraded the storage proteins in order to prevent uptake of resources into a non-viable seed during wound induction. Further analysis was conducted to identify whether *SlRR22* is a functional orthologue to *ARR22* or not. This was performed by generated transgenic *Arabidopsis* plant in *ARR22KO* background containing transgene *ARR22* and *SlRR22* to see if they would complement and rescue the molecular phenotype in wound induction. However, this analysis was unable to detect the expression of transgene *ARR22* and *SlRR22* and thus cannot confirm if *SlRR22* is orthologue to *ARR22*. Even though, based on the genomic and gene expression analysis, *SlRR22* did have similarity to *ARR22* and thus further study is needed to confirm the hypothesis.