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Analysis of *S1RR22* gene and its role in seed
wound response and manipulation of gene
expression

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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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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 |
| μ g | microgram |
| μ L | microliter |
| μ M | 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 (SIRR22) (Solyc11g071630)* and four other *SIRRs* which are *SIRR1 (Solyc05g054390)*, *SIRR8 (Solyc10g079700)*, *SIRR10 (Solyc11g066220)*, and *SIRR16 (Solyc06g048930)*. The finding is important as it indicate that the mechanism during wounding is conserved between dry and fleshy fruit types.

SIRR22 has 60% of amino acid similarity and is located within the same group with *ARR22* in phylogenetic tree. RT-PCR analysis indicated that *SIRR22* 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 *SIRR22* is a functional orthologue to *ARR22* or not. This was performed by generated transgenic *Arabidopsis* plant in *ARR22KO* background containing transgene *ARR22* and *SIRR22* 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 *SIRR22* and thus cannot confirm if *SIRR22* is orthologue to *ARR22*. Even though, based on the genomic and gene expression analysis, *SIRR22* did have similarity to *ARR22* and thus further study is needed to confirm the hypothesis.