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Functional characterization of two banana *NPR1* genes for pathogen defense response in *Arabidopsis*

By

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Department of Plant Science

In the

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DECLARATION

I, Rosita Endah Yocgo declare that the thesis which I hereby submit for the degree Philosophiae Doctor at the University of Pretoria, is my original work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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ABSTRACT

Functional characterization of two banana *NPR1* genes for pathogen defense response in *Arabidopsis*

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The *Non-expressor of pathogenesis-related1* gene *(NPR1*) mediates the induction of *pathogenesis-related* (*PR*) gene products, vital for resistance in plants. In this study, the role of two previously isolated Cavendish banana *NPR1*-like genes (*MNPR1A and MNPR1B*) has been characterized in protection against *Xanthomonas campestris*, *Hylaperonospora arabidopsidis*, *Botrytis cinerea* and *Pseudomonas syringae* pathogens. The specific aim was to investigate if sequence differences in both genes are responsible for differential activity against pathogens because in a previous expression study, *MNPR1A* and not *MNPR1B* had been more responsive to the banana necrotrophic pathogen *Fusarium oxysporum*. By challenging *Fusarium*-tolerant GCTCV-218 and susceptible Grand Naine Cavendish banana plants (which had been used in a previous characterization study) with the hemi-biotrophic *Xanthomonas* pathogen (a very important economical pathogen of banana), the two *MNPR1, PR-1 and PR-3* genes were found

to be sequentially expressed. Expression of these genes was more pronounced in the tolerant GCTCV-218 banana cultivar than in the sensitive Grand Naine cultivar. Comparative sequence analysis further showed that these two banana NPR1-like coding sequences had dissimilarities even within conserved functional domains; they grouped closely with other defense-related NPR1-like sequences and harboured defense *cis*-regulatory elements. Transformation of the coding sequences of both genes under the control of the 35S CaMV promoter/terminator sequences into *npr1-2 Arabidopsis* mutant complimented the phenotype of this mutant following infection with distinct classes of pathogens (biotrophic *Hyaloperonospora*, necrotrophic *Botrytis* and hemi-biotrophic *Pseudomonas* pathogens). These Infected-*MNPR1*-expressing plants had higher *PR-1* transcript amounts with more reduced pathogen growth compared to non-transgenic *npr1-2 Arabidopsis* mutant plants. However, the difference in the two banana coding sequences did not translate into a differential pattern of response against the three different classes of pathogens used in this study. Further detailed studies are suggested to investigate the role of the *MNPR1* promoter-coding sequences in the differential response to pathogens using a banana-pathogen system. This study also addressed the question of whether cystosolic glutathione (GSH) is necessary for *NPR1* transcription during systemic acquired resistance. Using *Arabidopsis* mutants (*clt1clt2clt3*) defective in cytosolic GSH biosynthesis and following infection with either *Pseudomonas* or *Botrytis*, *NPR1* and *PR-1* transcription was much reduced rendering the mutants more sensitive to pathogens compared to infected-wild-type *Arabidopsis* plants. Results from this study therefore implicate cytosolic glutathione as an essential antioxidant for the establishment of an effective defense response cascade.

Thesis composition

Chapter 1 of this thesis provides a summary of plant defense responses and an up-to-date review of the NPR1 defense co-transcription factor. Various elicitors required for *NPR1* activation, establishment of systemic acquired resistance and induction of *pathogenesis-related* gene products is reviewed. The rational, aim and objectives for carrying out this study is further outlined at the end of the introduction. In **Chapter 2**, the first objective to determine the expression pattern of two banana *NPR1*-like (*MNPR1*) genes and the subsequent response of downstream *PR-1 and PR-3* gene expression in response to a hemi-biotroph is addressed. Using quantitative realtime-polymerase chain reaction, the expression profiles of these genes are measured at specific time points in *Xanthomonas campestris* pv. *musacearum*-infected banana plants. In **Chapter 3** comparative sequence analysis tools such as multiple sequence aligment and phylogenetics are used to campare the two banana NPR1-like coding sequences with 39 already identified and/or characterized plant NPR1-like sequences from genbank. *Cis*-regulatory elements within these two banana *NPR1*-like sequences are also identified and described in relation to their role in defense. **Chapter 4** describes the procees of stably transforming *Arabipdosis npr1-2* mutant plants with the two *MNPR1* coding sequences under the control of the 35S cauliflower mosaic virus promoter and terminator sequences. The basal transcript amounts of the *MNPR1* coding sequences and of the *Arabidopsis PR-1* gene are further determined in homozyous transgenic lines expressing the *MNPR1* coding sequences. In **Chapter 5**, the response of the plants expressing the two banana *NPR1* coding sequences to pathogen is evaluated in greater detail with specific emphasis on whether the difference in coding sequence within these two genes leads to differential response to various classes of pathogens (necrotroph,

biotroph and hemi-biotroph). The role of cystosolic glutathione in *NPR1* transcription which mediates *PR-1* gene induction and the establishment of systemic acquired resistance is addressed in **chapter 6** using *Arabidopsis* mutants that are deficient in cytosolic gluthatione. **Chapter 7** summarises novel results generated from this work with special focus on how this study has contributed to an advanced understanding of the banana *NPR1*-like genes in defense response to pathogens. It further highlights the important role of the two banana genes in conferring resistance against a broad spectrum of pathogens. The chapter also outlines new research activities that can be applied to further our knowledge of the two *NPR1*-like genes in banana. This is followed by a **reference** list of citations used in this dissertation.

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ABBREVIATIONS AND SYMBOLS

^{o}C	Degree Celcius
%	Percentage
µg	Microgram
µL	Microlitre
bp	Base pair
cDNA	Complimentary DNA
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
dNTP	Deoxynucleoside triphosphate
E. coli	*Escherichia coli*
EDTA	Ethylenediamine tetra acetic acid
g	Grams
h	hours
H_2O	Water
L	Litre
LB	Luria broth
M	Molar
mM	Millimolar
mL	Millilitres
NaCl	Sodium chloride
NaOH	Sodium hydroxide

NaAC	Sodium acetate
ng	Nanogram
PCR	Polymerase chain reaction
DNase	Deoxyribonuclease
rpm	Revolutions per minutes
min	minute
s	Second
wk	week (s)
sd H_2O	Sterile distilled water
UV	Ultraviolet

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