# IDENTIFICATION OF GENES ASSOCIATED WITH TOLERANCE IN THE CAVENDISH BANANA SELECTION, GCTCV-218, AGAINST *FUSARIUM OXYSPORUM* F. SP. *CUBENSE*, 'SUBTROPICAL' RACE 4

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## DECLARATION

I hereby certify that this research, unless specifically indicated to the contrary in the text, is the result of my own investigation and that no part of this thesis has been submitted to any other university.

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**People who make their own rules when they know they're right…**
**People who get a special pleasure out of doing something well (even if only for themselves)…**
**People who know there's more to this whole living thing than meets the eye: they'll be with Jonathan all the way.**

**Jonathan Livingstone Seagull**
**(Richard Bach)**

# PREFACE

*Musa acuminata* Colla (banana) is one of the most important food crops in the world and provides a staple food and source of income in many households, especially in Africa. However, bananas worldwide are under serious threat by *Fusarium oxysporum* Schlect. f.sp. *cubense* (E.F. Smith) Snyder & Hansen (*Foc*). There exists no control strategy against the pathogen and control involves the use of resistant cultivars and cultural practices that prevent the introduction and spread of the disease into disease-free areas. Natural disease resistance exists in wild-type bananas and a few hybrids, but these bananas are not acceptable to the Cavendish market and the search for a new tolerant or resistant Cavendish banana is underway. Conventional breeding strategies are however hindered by the fact that Cavendish bananas are sterile and do not produce seed. Therefore, non-conventional strategies such as transformation are more realistic and could be more successful. Unfortunately, very few banana genes have been isolated and characterised up to date and the banana-*Foc* interaction has not yet been studied extensively, if at all, on the molecular level. This leads to a lack of knowledge in understanding disease resistance mechanisms in banana and complicates the matter of transforming susceptible bananas with resistance genes.

This thesis firstly aims to evaluate the disease tolerance of a Cavendish banana, GCTCV-218, infected with *Foc* and secondly to isolate the disease resistance genes expressed early in the banana-*Foc* interaction. Fusarium wilt is a root pathogen and few molecular studies have been done on the plant response in roots to pathogens. This is, so far, known the first molecular study on the Cavendish banana-*Foc* interaction.

**Chapter 1** provides the reader with a short review of banana, and the pathogen, *Foc*. The chapter then gives a broad overview of disease resistance in plants and further provides information on Fusarium resistance in banana and other crops with reference to the type of resistance (i.e. constitutive or actively induced chemical or structural resistance).

The Cavendish banana, GCTCV-218, is a somaclonal variety selected by researchers at the Taiwan Banana Research Institute (TBRI) in Taiwan and showed promising results in disease resistance trials against 'tropical' race 4 (VCG 0121). **Chapter 2** evaluates the disease tolerance of GCTCV-218, under South African conditions against *Foc* 'subtropical' race 4 (VCG 0120). Literature has reported that phenolic compounds may be involved in resistance against *Foc* and this chapter will also study the different phenolic compounds in GCTCV-218 compared to the susceptible Williams at different time intervals after *Foc* infection.

**Chapter 3** describes the construction of a banana cDNA library containing gene fragments that are differentially expressed in GCTCV-218 in response to *Foc* compared to the susceptible Williams cultivar. A highly effective PCR-based technique, termed Suppression Subtractive Hybridisation (SSH), was applied in this chapter.

**Chapter 4** reports on the development of a high-throughput screening method of the banana SSH cDNA library using DNA microarray analysis. This is a novel approach in screening SSH libraries for false positives that have escaped the subtraction process and has been published in **Bio Techniques (2004) 37: 818-824**.

Seventy-nine gene fragments were selected for sequencing after screening the library. In **Chapter 5**, the selected gene fragments were sequenced and subjected to BLASTX, BLASTN and DBEST searches. A table containing non-redundant gene fragments was compiled and some of these gene fragments were subjected to alignments with known corresponding genes from the NCBI database. The expression profile of four defence related genes was further investigated by quantitative Reverse Transcriptase-PCR.

# ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| α | Alpha |
| AC | Acetate |
| ADP | Adenosine diphosphate |
| AFLP | Amplified Fragment Length Polymorphism |
| AIR | Alcohol insoluble residue |
| AMV | Avian Myeloblastosis Virus |
| AOS | Active oxygen species |
| APX | Ascorbate peroxidase |
| ATP | Adenosine triphosphate |
| Avr | Avirulence |
| β | Beta |
| BLAST | Basic Local Alignment Search Tool |
| BLASTX | BLAST algorithm to compare the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database. |
| BLASTN | BLAST algorithm to compare a nucleotide query sequence against a nucleotide sequence database. |
| bp | base pairs |
| BTH | benzo-(1, 2, 3) thiadiazole-7-carbothioic acid S-methyl ester |
| $Ca^{2+}$ | Calcium (II) ions |
| cADPR | Cyclic ADP ribose |
| CAV | Culture collection, Altus Viljoen |
| $Ca(NO_3)_2.H_20$ | Calcium nitrate |
| °C | Degrees Celsius |
| CDPK | Calcium-dependant protein kinase |
| cDNA | complementary Deoxyribonucleic Acid |
| CIRAD-FLHOR | Centre de Coopération Internationale en Recherche Agronomique pour le Développement - Département des productions fruitières et horticoles |
| cm | centimetres |
| Ct | Cycle number at which the fluorescence signal crosses a fixed |

| | |
|---|---|
| | threshold |
| cv | cultivar |
| DIG-dUTP | Digoxygenin deoxyuridine triphosphate |
| DNA | Deoxyribonucleic acid |
| dATP | Deoxyadenosine triphosphate |
| dCTP | Deoxycytosine triphosphate |
| dGTP | Deoxyguanidine triphosphate |
| dITP | Deoxyinosinetriphosphate |
| DIG | Digoxygenin |
| DNase | Deoxyribonuclease |
| dNTP | Deoxyribonucleotide triphosphate |
| DTT | Dithiothreitol |
| dTTP | Deoxythymidine triphosphate |
| dUTP | Deoxyuridine triphosphate |
| EDTA | Ethylenediamine tetraacetic acid |
| EMBRAPA-CNPMF | Empresa Brasileira de Pesquisa Agropecuária – Mandioca e Fruticultura Tropical |
| EF1 | Elongation factor 1 |
| ER | Enrichment ratio |
| EST | Expressed sequence tags |
| ET | Ethylene |
| FABI | Forestry and Agricultural Biotechnology Institute |
| FHIA | Fundación Hondurereña de Investigacón Agrícola |
| f.sp. | Formae speciales |
| *Foc* | *Fusarium oxysporum* f. sp. *cubense* |
| *Fod* | *Fusarium oxysporum* f. sp. *dianthi* |
| *Fol* | *Fusarium oxysporum* f.sp. *lycopersici* |
| g | gram |
| g/l | gram per litre |
| GMP | Guanosine monophosphate |
| GSII | 1,3-β-glucan synthase |
| h | Hour |
| HCl | Hydrochloric acid |

| | |
|---|---|
| hpi | Hours post inoculation |
| $H_2O$ | Water |
| $H_2O_2$ | Hydrogen peroxide |
| HR | Hypersensitive Response |
| H | Hour |
| hrs | Hours |
| $H^+$ | Hydrogen |
| IAA | Indole acetic acid |
| IAEA | International Agricultural Exchange Association |
| IITA | International Institute for Tropical Agriculture |
| IMP | Inosine 5' monophosphate |
| IPTG | Isopropyl-β-D-thiogalactopyranoside |
| IR | Induced resistance |
| ISR | Induced systemic resistance |
| ITS | Internal transcribed spaces |
| JA | Jasmonic acid |
| JME | Jasmonic methyl ester |
| $K^+$ | Potassium |
| KCl | Potassium chloride |
| LAR | Local acquired resistance |
| LB | Luria-Bertani |
| LMP | Low melting point |
| LRR | Leucine-rich repeats |
| M | Molarity |
| MAPK | Mitogen-activated protein kinases |
| MeJA | Methyl jasmonate |
| MeOH | Methyl hydroxide |
| min | Minutes |
| mg | Milligrams |
| $MgCl_2$ | Magnesium Chloride |
| ml | Millilitres |
| $ml^{-1}$ | Per millilitre |
| ml/l | millilitre per litre |

| | |
|---|---|
| mm | Millimetres |
| mM | millimolar |
| mRNA | messenger Ribonucleic Acid |
| $N_2$ | Nitrogen |
| $NaCO_3$ | Sodium carbonate |
| NADH | Nicotinamide adenine dinucleotide |
| NaOH | Sodium hydroxide |
| NBS | Nucleotide-Binding Site |
| NCBI | National Centre for Biotechnology Information |
| ηg | Nanogram |
| nm | nanometre |
| No. | Number |
| NO | Nitric oxide |
| NOS | NO synthase |
| $O_2^-$ | Super oxide |
| OH | Hydroxyl radical |
| P | Proline |
| PAL | Phenylalanine ammonia-lyase |
| PCR | Polymerase Chain Reaction |
| PDA | Potato dextrose agar |
| pH | Log hydrogen ion concentration |
| pI | Isoelectric point |
| POD | Peroxidase |
| PPO | Polyphenol oxidase |
| PR | Pathogenesis-related |
| *R* | Resistance |
| $R^2$ | Correlation coefficient |
| RDA | Representational difference analysis |
| RNA | Ribonucleic acid |
| RNase | Ribonuclease |
| ROS | Reactive oxygen species |
| rpm | revolutions per minute |
| rRNA | ribosomal Ribonucleic Acid |

| | |
|---|---|
| RT | Reverse transcriptase |
| SA | Salicylic acid |
| SAGE | Serial analysis of gene expression |
| SD | Standard Deviation |
| SAR | Systemic acquired resistance |
| SDS | Sodium Dodecyl Sulfate |
| SDW | Sterile distilled water |
| SNP | Sodium nitroprusside |
| spp | species |
| SSC | Sodium Saline Citrate |
| SSH | Suppression Subtractive Hybridisation |
| ST | Subtracted tester |
| TAE | Tris-acetate tetraacetic acid |
| TBRI | Taiwan Banana Research Institute |
| TFA | Triflouroacetic acid |
| Tm | Melting temperature |
| TMV | Tobacco mosaic virus |
| Tris-HCl | 2-amino-2-(hydroxymethyl)-1,3-propandiol chloride |
| tRNA | Total ribonucleic acid |
| UD | Unsubtracted driver |
| UDP | Uridine diphosphate |
| µg | Micrograms |
| µl | Micro litre |
| µM | Micro molar |
| UT | Unsubtracted tester |
| UV | Ultra violet |
| VCG | Vegetative Compatibility Group |
| v/v | Volume per volume |
| v/v/v | Volume per volume per volume |
| w/v | Weight per volume |
| X-gal | 5-bromo-4-chloro-3-indolyl-β-D-galactoside |
| λ | Lambda (wavelength) |
| % | Percentage |

| | |
|---|---|
| Σ | Sum of |