Expression of defence-related genes against *Phytophthora cinnamomi* in five avocado rootstocks

Avocado (*Persea americana*) – a major fruit crop worldwide – is threatened by root rot caused by *Phytophthora cinnamomi*. This pathogen is known to infect the plant via the feeder roots leading to branch dieback, and eventually tree mortality. While it is known that different avocado rootstocks have varying degrees of susceptibility to *Phytophthora* root rot, little research has been done on the avocado–*Phytophthora* interaction. In this study, transcript abundance levels of defence-related genes coding for phenylalanine ammonia-lyase, lipoygenase, pathogenesis-related protein 5, endochitinase, gluathionine S-transferase and metallothionein were characterised and compared among five rootstocks with varying susceptibility to root rot, after exposure to *P. cinnamomi*. Root samples were collected at 0 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h post-infection and transcript abundance of the defence-related genes was determined using quantitative real-time reverse transcription PCR. The results indicated the involvement of *PR-5* and *endochitinase* in the defence response of all avocado rootstocks to *P. cinnamomi* but these genes could not be directly linked to the observed phenotypic resistance. *PR-5* and *endochitinase* were highly upregulated at 72 h post-infection. Differences in transcript abundance of phenylalanine ammonia-lyase and lipoygenase genes were seen when comparing tolerant and less tolerant rootstocks, which may suggest that transcripts of these genes contribute to resistance. These data provide important insights into plant defence and into how different avocado rootstocks may exhibit increased resistance to infection by *P. cinnamomi*.

**Introduction**

Avocados (*Persea Americana* Mill.) are susceptible to a wide range of pathogens and pests and it is therefore not surprising that *Phytophthora* root rot (PRR) is one of the most damaging diseases, and severely affects commercial production worldwide. The global destructiveness caused by *Phytophthora cinnamomi* Rands has made it one of the most economically important groups of plant pathogens in the world and also one of the most studied. Primary infection by *P. cinnamomi* occurs at the small absorbing avocado feeder roots, resulting in a brownish black and brittle appearance. There is almost no progression into the larger roots. This root rot leads to death of the feeder roots which results in insufficient water and nutrient uptake, which eventually leads to tree mortality.

Although PRR has been studied for more than 60 years, total control remains elusive and economic losses continue to increase. Currently, control is achieved by spraying or injecting trees with phosphite in conjunction with using tolerant rootstocks. However, in 2006, Dobrovolski et al. showed a decreased pathogen sensitivity to phosphite in Australian avocado orchards where phosphite had been used against *P. cinnamomi* for prolonged periods. Although this decreased sensitivity is currently not viewed as a major threat, the potential impact of such a phenomenon on the effectiveness of phosphite for PRR control may be severely negative. In addition to chemical control, much emphasis is currently placed on the selection and use of tolerant avocado rootstocks.

Tolerant rootstocks offer the greatest possibility of a sustainable long-term solution for root rot. Several breeding and selection programmes around the world have identified rootstocks with a high degree of resistance to *P. cinnamomi*. *Duke 7* was discovered by Zentmyer and in 1975 it became the first commercial rootstock with moderate resistance against the pathogen. It has been a highly successful rootstock and is still used worldwide, although several newer varieties have been selected since then. The fact that *Duke 7* can acclimatise to many different environmental conditions makes it a popular rootstock today. Other tolerant varieties include ‘Thomas’, ‘Toro Canyon’, ‘Martin Grande’, ‘Spencer’ and ‘G755’. ‘Dusa’:* (‘Merensky 2’), a new variety selected by Westfalia Technological Services, has surpassed the performance of *Duke 7*, especially under South African climatic conditions.

Although defence responses have been vigorously studied in model plant species, there is a vast amount yet to be discovered in non-model plants in order to comprehend their underlying defence mechanisms. Moreover, defence mechanisms can vary between different plant species and therefore each particular pathosystem of interest should be studied individually. Numerous studies have shown that salicylic acid is more involved in defence responses associated with biotrophic pathogens whereas jasmonic acid and ethylene are more involved against necrotrophic pathogens and insects. Therefore, depending on the lifestyle of a pathogen, different defence mechanisms will be activated. *Phytophthora cinnamomi* is a hemibiotroph – that is, it has both biotrophic and necrotrophic phases which is a great advantage because it is capable of switching to a necrotrophic phase when plant mechanisms are encountered that limit its spread. In such a case, a plant defence mechanism such as the hypersensitive response would be ineffective against *P. cinnamomi* because the pathogen switches to the necrotrophic phase and uses the dead tissue as a nutrient source.

Traditionally, genetic resistance has been classified into two types: qualitative and quantitative resistance. Qualitative resistance is mediated by R-genes and only provides short-lived resistance in the field as new virulent races of the pathogen rapidly overcome resistance encoded by single resistance genes. In contrast, quantitative resistance is controlled by multiple interacting genes that do not prevent infection, but slow down the development of the pathogen and, hence, lasts longer. In the case of *P. cinnamomi* and avocado, R-mediated resistance is unlikely to...
be the mechanism responsible for conferring resistance. A multigenic trait is more likely as various levels of resistance in avocado rootstocks against *P. cinnamomi* have been documented.

When *P. cinnamomi* infects avocado various defence responses are induced, but few molecular studies have elucidated this interaction. In studies by Garcia-Pineda et al.13 on the defence response in avocado roots against *P. cinnamomi*, a reactive oxygen species burst as well as the involvement of catalase, epicatechin and nitric oxide were also highlighted. Müniz et al.14 used a proteomics approach to report on protein induction in response to infection of *P. cinnamomi* in roots of avocado. Proteins that were found to be involved in this interaction included isoamylase reductase, glutathione S-transferase, several asbiscic acid-stress-opening proteins, cinnamyl alcohol dehydrogenase, cinnamyl-CoA reductase, cysteine synthase and quinone reductase. These studies have barely begun to unravel how defence against *P. cinnamomi* is mediated within avocado and a comprehensive analysis of differentially expressed genes could contribute to a better understanding of the molecular processes involved in conferring resistance to PRR. Knowledge of the genetic basis of this observed resistance would not only contribute to the understanding of defence mechanisms but also aid in the development of superior avocado rootstocks.

Therefore, in this study, we elucidated the transcript abundance level of known plant defence-related genes in the response against *P. cinnamomi* in five avocado rootstocks that vary in resistance to PRR. The transcript abundance of these genes was investigated using the quantitative reverse transcription polymerase chain reaction (qRT-PCR) over a time course following inoculation with *P. cinnamomi*.

**Materials and methods**

**Plant material**

A hydroponic system previously used for banana15 was adapted for the avocado–*P. cinnamomi* pathosystem. Five avocado rootstocks were selected based on their resistance level to *P. cinnamomi*. These rootstocks were: R0.06 (highly tolerant), Dusa® (highly tolerant), Duke 7 (tolerant), R0.01 (tolerant) and R0.12 (least tolerant); the rootstocks were obtained from Westfalia Technological Services (Tzaneen, Limpopo, South Africa). The 9-month-old clonally propagated plantlets were obtained from Westfalia Technological Services (Tzaneen, Limpopo, South Africa). The plantlets were removed from their bags and transplanted into 500-mL polystyrene cups filled with water to ensure easy access to the roots.

**Inoculum preparation**

A *P. cinnamomi* isolate provided by Westhala Technological Services was used as the inoculum source. Prior to inoculation, the identity of this isolate was confirmed by amplifying the species-specific LPV3 fragment using LPV3 F (5'- GTGAGCACTGTCGATTTG 3') and LPV3 R (5'- GAACCAACAGGCACGT 3') primers.14 Genomic DNA was extracted from mycelia growing on ½ PDA (10 g potato dextrose, 15 g agar) using PrepMan Ultra Reagent (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer’s instructions. The 20-μL PCR reaction contained 2.5 μL 10X PCR reaction buffer, 2.5 mM MgCl₂, 200 μM dNTP, 0.25 μM of each specific primer, 1 U Taq polymerase (Bioline Ltd, London, UK) and 20–50 ng of template DNA. PCR cycling conditions were: an initial denaturation step at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 58.5 °C for 30 s and 72 °C for 1 min and a final extension step at 72 °C for 7 min. PCR products were separated and examined by electrophoresis on 2% agarose with GelRed (Biolium Inc., Hayward, CA, USA) and visualised under UV light.

**Mycelial suspension**

The *P. cinnamomi* isolate was grown on ½ PDA at 25 °C for 4 days, whereafter a sterile broth (8 g D-glucose, 0.8 g yeast extract, 800 mL distilled water, autoclaved at 121 °C for 15 min) was inoculated with six agar blocks (10 mm x 5 mm) containing mycelia and shaken at 25 °C at 150 rpm for 10–14 days. Mycelial balls were placed on Whatman filter paper and left to dry briefly. Mycelia (3.05 g) were mixed with 1 L sterile water and blended for a few minutes to macerate the mycelial pieces. The solution was mixed with 5 L of sterile water and used for inoculation.

**Zoospore suspension**

*Phytophthora cinnamomi* was first grown on V8 agar plates (50 mL of filtrated V8 juice, 0.5 g CaCO₃, 20 g agar) for 5 days. Small agar blocks (10 mm x 5 mm) containing mycelia were cut at the actively growing margin of the plates and transferred onto empty plates, to which 25 mL of 2% V8 broth (20 mL V8 juice with 0.5 g CaCO₃) was added and left for at least 3 days at room temperature (ca. 25 °C). The broth was removed and agar blocks containing mycelia were rinsed three times with distilled water after which 25 mL filtered stream water was added to each of the plates and incubated for 2–3 days at room temperature under UV light. Sporangia formation was monitored during this incubation step by using a Zeiss Stermi 2000 stereomicroscope (Carl Zeiss Ltd., Munich, Germany). Once sufficient sporangia formation was observed, the cultures were cold shocked by placing them at 4 °C for 45 min after which they were removed immediately and left at room temperature for 1 h to stimulate the release of zoospores. The zoospore suspension was decanted from the plates, pooled together and used for inoculation.

**Inoculation and sample collection**

Avocado roots were suspended in 500-mL polystyrene cups and inoculated with a mixture containing 50 mL macerated mycelia and 1.5 x 10⁸/mL zoospores. Control plants received sterile water instead of inoculum. Root tissue for RNA extraction was collected at 0 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h post-infection (hpi). Because of a limited number of avocado plants, we did not include uninoculated controls at each time point for this study. Root material from three to five plants per avocado rootstock was harvested per time point to form three biological samples representing either one or two avocado plants pooled together. Root tissue was immediately frozen in liquid nitrogen, ground to a fine powder with a homogeniser (IKA A11 United Scientific (Pty) Ltd, San Diego, CA, USA) and stored at -80 °C. After inoculation, plants were evaluated for root rot symptoms. Selected infected plants were transplanted to plastic bags containing perlite and kept for 6 weeks to confirm the development of PRR symptoms.

**Gene expression profiling**

RNA extraction and cDNA synthesis

RNA was extracted from root powder using a modification of the CTAB RNA extraction method developed by Chang et al.16 and stored at -80 °C. It is known that avocados have a high polysaccharides content, which seems to influence the quality of RNA; therefore the number of chloroform extractions and centrifugation steps was increased to four. Concentration of RNA was determined using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies Inc., Monterey, DE, USA). RNA integrity was assessed under non-denaturing conditions as described. DNase treatment of RNA was performed by the addition of 1 U RNase-free DNase (Fermentas Life Sciences, Hanover, MD, USA), 1 μL 10X reaction buffer with MgCl₂, 1 μg RNA and diethylpyrocarbonate-treated water to a final volume of 9 μL. The mixture was incubated at 37 °C for 30 min followed by the addition of 25 mM EDTA and incubation at 65 °C for 10 min. DNase-treated RNA was column purified using the RNeasy MiniElute Cleanup kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions.

First-strand cDNA synthesis was carried out in a total volume of 5 μL which consisted of 0.5 μg random hexamers (Invitrogen Life Sciences, CA, USA), 1 μg RNA from the previous step and RNase free water. The mixture was incubated at 70 °C for 5 min and then chilled on ice for 5 min, followed by the addition of 40 U RNase inhibitor (Fermentas, Ontario, Canada), 0.5 mM dNTPs (Fermentas, Ontario, Canada), 3 mM MgCl₂, 4 μL 5X ImProm-II™ reaction buffer and 1 μL ImProm-II™ reverse transcriptase (Promega Corporation, Madison, WI, USA). Finally the mixture was incubated at 25 °C for 10 min followed by 60 min at 42 °C and 10 min at 70 °C.
The cDNA was analysed for genomic DNA contamination by PCR using the gene-specific primers F3H-F (5’ TCTGATTCCAGGAGACTGCG 3’) and F3H-R (5’ TGATAGCTTGGCACCCTTTT 3’) which flank an intron of the flavanone 3-hydroxylase (F3H) gene. PCR amplifications were carried out using first-strand cDNA as the template. The PCR reaction mixture of 20 μL final volume contained 2.5 μL 10× PCR reaction buffer, 2.5 mM MgCl₂, 200 μM dNTP, 0.25 μM of each specific primer, 1 U Taq polymerase and 1 μL cDNA and water. Amplifications were performed in an Eppendorf MasterCycler gradient (Eppendorf, Hamburg, Germany) under the following initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min. Final extension was carried out at 72 °C for 7 min. PCR products were visualised as before.

Real-time RT-PCR primer design

The transcription abundance of six avocado defence-related genes – phenylalanine ammonia-lyase (PAL), lipoxigenase (LOX), pathogenesis-related protein 5 (PR-5), endochitinase, glutathione S-transferase (GTH) and metallothionein – was investigated. Actin and 18S rRNA were used as endogenous controls. Primers for these genes were designed from sequences obtained from the NCBI database using Primer 3 software and synthesised by either Operon Biotechnologies GmbH (Cologne, Germany) or Inqaba Biotec (Pretoria, South Africa) (Table 1).

Table 1: Primer sequences of defence-related genes studied in avocado roots by quantitative reverse transcription PCR following infection by Phytophthora cinnamomi

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Forward primer sequence (5’ to 3’)</th>
<th>Reverse primer sequence (5’ to 3’)</th>
<th>Product size (bp)</th>
</tr>
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<tbody>
<tr>
<td>PAL</td>
<td>ATCTTGGAAAGGACTCAC</td>
<td>CAGCAATGTTAGGATAAGG</td>
<td>110</td>
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<tr>
<td>LOX</td>
<td>GTAAATCCAGTATTCTCATCAG</td>
<td>TAGGTTCTTCTCAATG</td>
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<td>PR-5</td>
<td>TAAAGATATTGTGTGGAATTAG</td>
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<td>GTH</td>
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<td>ATCCACACTGCTACTATC</td>
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<td>CTGACCAGTACAGTCACAC</td>
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<tr>
<td>18S rRNA</td>
<td>GTACATTGAGGACTCGGCC</td>
<td>TTCCTTTAAGGTTCCAGGCTTG</td>
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<tr>
<td>Endochitinase</td>
<td>ATACCAAAACATCATCAC</td>
<td>CTCTTGTGAAAGGCAATG</td>
<td>83</td>
</tr>
</tbody>
</table>

Gene expression analysis using real-time qRT-PCR

A dilution series was performed and a standard curve was generated for each gene to examine the linearity of amplification over a dynamic range. Quantitative RT-PCR was performed using the Bio-Rad™ CFX 96 instrument (Bio-Rad, Hercules, CA, USA). Serial dilutions (1:10, 1:25, 1:50, 1:100 and 1:1000) were performed using 5 μL of diluted cDNA from a mixture of all treatment samples inoculated with the pathogen to 1:50, 1:100 and 1:1000 (Table 1). Primer pairs were tested for successful amplification of target genes with SYBR No-ROX (Bioline Ltd, London, UK), 1 μL of each of the forward and reverse primers (10 μM), 5 μL diluted (1:5) cDNA template and 3 μL PCR-grade water (Roche Applied Science, Mannheim, Germany). The cycling conditions were as follows: pre-incubation for 10 min at 95 °C (hot start) followed by 44 cycles, each consisting of 10 s denaturing at 95 °C, 10 s annealing at 59 °C, 10 s primer extension at 72 °C and data acquisition at 95 °C. Melting curve analysis of the qRT-PCR products was performed to confirm that the individual qRT-PCR signals corresponded to a single homogenous cDNA product.

Data analyses

Standard regression curves were calculated from amplification data of the serial dilutions as follows: y=mx+b, where b is the y-intercept of the standard curve line (crossing point) and m is the slope of the standard curve line (a function of PCR efficiency). The resulting crossing point values for each input amount of template were plotted as a function of the log10 concentration of input amounts and a linear trend line was imposed on the data. R² is the proportion of variability that the data set is accounted for by a statistical model; thus, R²=1 indicates that the fitted model explains all variability in y, while R²=0 indicates no linear relationship between the response variable and regressors. Statistical significance from the qRT-PCR data was analysed by one-way analysis of variance followed by a Student’s multiple comparison t-test using the software package JMP 9 (SAS Institute Inc., Cary, NC, USA). In all cases significance was evaluated at p<0.05.

Results

Confirmation of P. cinnamomi and symptom evaluation

The identity of the P. cinnamomi isolate was confirmed. The amplification of a region of the LPV3 gene from DNA yielded the expected amplicon size of 450 bp (Figure 1). Avocado plants (five rootstocks) infected with P. cinnamomi developed typical root rot symptoms 6 weeks after infection. Differences between rootstocks were clearly visible 2 weeks after infection (Figure 2). These symptoms included necrotic lesions that developed on the roots and lower stem and some of the feeder roots were black and brittle. Plants showed yellowing and wilting of leaves and, in severe cases, leaf abscission and death. The highly tolerant R0.06 appeared the healthiest when compared to the least tolerant rootstock (R0.12) which showed severe symptoms. The pathogen was re-isolated and Koch’s postulates were proven.
RNA extraction and cDNA synthesis
Avocado root RNA samples were of high quality and the absence of contaminating genomic DNA was confirmed for all cDNA samples. The amplification of a region of the \( F3H \) gene from cDNA yielded the expected 241-bp RNA-derived product, which was clearly distinguishable from the 1221-bp genomic DNA-derived, intron-containing product (Supplementary figure 1 online).

Real-time RT-PCR primer design
\( \text{Actin} \) and 18S rRNA consistently exhibited M values (reference gene stability values) less than 0.50 (0.1543 and 0.2508, respectively) and therefore were selected as endogenous controls for this study. \( \text{Gene stability is expressed as an M value, which is inversely proportional to the variation in expression for a given gene.}^{[17]} \) The lower the M-value, the more stably expressed the reference gene is.

Gene expression profiling
Basal (0 hpi) and early transcript levels (3 hpi, 6 hpi and 12 hpi) of PR-5 were very low and similar in tolerant and less tolerant avocado rootstocks (Figure 3a). The first significant response after \( P. \) cinnamomi infection occurred at 24 hpi in the moderately tolerant Duke 7. By 48 hpi all rootstocks (except Duke 7) had a significant increase in PR-5. The least tolerant R0.12 and highly tolerant R0.06 had the highest transcript abundance at 48 hpi. By 72 hpi PR-5 transcript levels in all rootstocks continued to increase significantly. The highest transcript abundance of

Figure 2: Root symptoms on Dusa\(^\text{®} \) (highly tolerant) and R0.12 (less tolerant) avocado rootstocks after Phytophthora cinnamomi infection: R0.12 roots at (a) 0 days post-infection (dpi) and (b) 14 dpi and Dusa roots at (c) 0 dpi and (d) 14 dpi.
PR-5 was observed at 72 hpi for all rootstocks. R0.06 and Dusa had a 42- and 24-fold increase, respectively, compared with uninfected roots (0 hpi); the least tolerant rootstock (R0.12) also showed a 19-fold increase (Figure 3a).

Basal transcript levels of PAL were significantly higher in the least tolerant R0.12 compared with all rootstocks except Dusa (Figure 3b). By 3 hpi, all rootstocks (except R0.01) showed a decrease in PAL levels (although not significant). A significant increase in PAL was observed in the highly tolerant Dusa at 6 hpi. PAL levels in the less tolerant R0.12 and moderately tolerant Duke 7 continued to decrease significantly at 6 hpi whereas R0.06 and Dusa only decreased at 12 hpi. By 24 hpi, R0.06 and Dusa once again had increased significantly but levels of PAL between the different rootstocks were similar (Figure 3b). At 48 hpi, PAL transcript levels in R0.12 and Duke 7 were significantly lower than in the other rootstocks and remained low at 72 hpi. The levels in all other rootstocks remained similar at 48 hpi when compared with levels at 24 hpi. By 72 hpi, the highly tolerant Dusa also exhibited a significant decrease in PAL levels (Figure 3b). Noteworthy is that transcript levels of PAL in the less tolerant R0.12 continued to decrease over the entire time course.

The constitutive abundance of LOX transcripts was significantly higher in the highly tolerant Dusa when compared with the other rootstocks. LOX levels in Dusa had decreased significantly by 3 hpi (Figure 3c). The levels remained similar in all rootstocks at 6 hpi and 12 hpi. By 24 hpi, Duke 7 and Dusa showed significantly higher levels when compared with the same rootstocks at 12 hpi and when compared with other rootstocks at 24 hpi. LOX levels in R0.01 and less tolerant R0.12 increased significantly from 24 hpi to 48 hpi. At 72 hpi, R0.06 and R0.01 showed a significant increase in LOX, with transcript levels in R0.01 being the highest across all time points and rootstocks (Figure 3c). R0.01 showed an 8-fold increase at 72 hpi compared with 0 hpi.

**Figure 3:** Relative expression levels of defence-associated genes: (a) PR 5, (b) phenylalanine ammonia lyase, (c) lipoxygenase LOX (d) endochitinase, (e) metallothionein-like protein and (f) glutathione S-transferase in avocado rootstocks infected with Phytophthora cinnamomi at 0 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h post-infection (hpi).
Expression of avocado defence genes against Phytophthora cinnamomi

Expression analysis of PAL transcripts after infection with P. cinnamomi showed that the less tolerant R0.12 and Duke 7 rootstocks showed strong downregulation of PAL transcripts. It can be hypothesised that this downregulation of PAL might aid establishment and development of P. cinnamomi in the root tissue. Upon recognition of P. cinnamomi by avocado, a series of signalling pathways is activated. Among these, the phenylpropanoid pathway plays an extremely important role in secondary plant metabolism by producing numerous phenolic propanoids such as phenolic acids and flavonoids that have vital structural- and defence-related functions. Phenylalanine ammonia-lyase is one of the fundamental enzymes in the phenylpropanoid pathway. Therefore it is possible that more phenolic compounds are produced in the highly tolerant rootstocks to combat P. cinnamomi growth whereas the downregulation in the less tolerant rootstocks implies that fewer phenolic compounds are being produced. In pepper (Capsicum annuum L.), an increase in phenolic acids reduced lesion length and invasion upon infection by Phytophthora capsici, whereas susceptible cultivars of C. annuum produced lower amounts of phenolic acids and lesion lengths were not reduced. Inoculation of avocado plants resulted in significant increased levels of LOX transcript levels in Dusa and Duke 7 at 24 h. An increase in LOX has also been reported to occur in other plant–pathogen interactions. Lipoxigenases (EC 1.13.11.12) are a family of enzymes that catalyse the dioxygenation of polyunsaturated fatty acids in lipids. The role of LOX in defence against pathogens is connected to the production of compounds that are involved in signalling, antimicrobial activity, and hypersensitive response development. The fact that R0.12 remained unchanged during the first 24 h indicates a delayed response and therefore subsequent failure to restrict the pathogen by not allowing signalling of other defence responses or by a lack of antimicrobial activity. Tomato plants infected with Phytophthora infestans have been shown to display fungitoxic activities by producing linolenic acid via lipoxigenase. An increase in transcript levels of endochitinase became evident at 24 hpi in all rootstocks with the exception of R0.12. R0.12 was the only rootstock that did not show any significant changes during the first 24 hpi, which suggests that the delay of 24 h allowed establishment of P. cinnamomi in the less tolerant rootstock when compared with other rootstocks that showed significant upregulation at 24 hpi. Endochitinases play a role in plant defence by attacking structural chitin present in the cell wall of fungi. Unlike fungal cell walls, oomycete cell walls are mainly composed of celluloseic compounds and glucans but have been found to contain limited amounts of chitin. Consequently, limited studies have been conducted to investigate the role of endochitinases with oomycetes such as Phytophthora spp. From the present study it is clear that endochitinase was activated upon infection and at 72 hpi it was highly upregulated compared with the uninjected samples (0 hpi) in

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Expression of avocado defence genes against Phytophthora cinnamomi

Expression analysis of PAL transcripts after infection with P. cinnamomi showed that the less tolerant R0.12 and Duke 7 rootstocks showed strong downregulation of PAL transcripts. It can be hypothesised that this downregulation of PAL might aid establishment and development of P. cinnamomi in the root tissue. Upon recognition of P. cinnamomi by avocado, a series of signalling pathways is activated. Among these, the phenylpropanoid pathway plays an extremely important role in secondary plant metabolism by producing numerous phenolic propanoids such as phenolic acids and flavonoids that have vital structural- and defence-related functions. Phenylalanine ammonia-lyase is one of the fundamental enzymes in the phenylpropanoid pathway. Therefore it is possible that more phenolic compounds are produced in the highly tolerant rootstocks to combat P. cinnamomi growth whereas the downregulation in the less tolerant rootstocks implies that fewer phenolic compounds are being produced. In pepper (Capsicum annuum L.), an increase in phenolic acids reduced lesion length and invasion upon infection by Phytophthora capsici, whereas susceptible cultivars of C. annuum produced lower amounts of phenolic acids and lesion lengths were not reduced. Inoculation of avocado plants resulted in significant increased levels of LOX transcript levels in Dusa and Duke 7 at 24 h. An increase in LOX has also been reported to occur in other plant–pathogen interactions. Lipoxigenases (EC 1.13.11.12) are a family of enzymes that catalyse the dioxygenation of polyunsaturated fatty acids in lipids. The role of LOX in defence against pathogens is connected to the production of compounds that are involved in signalling, antimicrobial activity, and hypersensitive response development. The fact that R0.12 remained unchanged during the first 24 h indicates a delayed response and therefore subsequent failure to restrict the pathogen by not allowing signalling of other defence responses or by a lack of antimicrobial activity. Tomato plants infected with Phytophthora infestans have been shown to display fungitoxic activities by producing linolenic acid via lipoxigenase. An increase in transcript levels of endochitinase became evident at 24 hpi in all rootstocks with the exception of R0.12. R0.12 was the only rootstock that did not show any significant changes during the first 24 hpi, which suggests that the delay of 24 h allowed establishment of P. cinnamomi in the less tolerant rootstock when compared with other rootstocks that showed significant upregulation at 24 hpi. Endochitinases play a role in plant defence by attacking structural chitin present in the cell wall of fungi. Unlike fungal cell walls, oomycete cell walls are mainly composed of celluloseic compounds and glucans but have been found to contain limited amounts of chitin. Consequently, limited studies have been conducted to investigate the role of endochitinases with oomycetes such as Phytophthora spp. From the present study it is clear that endochitinase was activated upon infection and at 72 hpi it was highly upregulated compared with the uninjected samples (0 hpi) in...
all rootstock varieties. Similar results were obtained in a study conducted by Mishra et al. who demonstrated by use of Northern blot analysis that transcripts of endochitinase were highly upregulated, reaching the highest expression 36 h after Phytophthora in the leaves and roots of avocado. The focus of this study was to obtain an overview of the expression patterns of selected defense genes during P. cinnamomi infection, with the intention of further understanding the mechanism of resistance observed. Because differences in gene expression are responsible for morphological and phenotypical differences, gene transcript profiles of avocado infected with P. cinnamomi, over a time period, provided evidence of genes involved in resistance and served as a basis for investigating plant–pathogen interactions and gene function.

Conclusion

The high expression 36 h after infection, with the intention of further understanding the mechanism of resistance observed. Because differences in gene expression are responsible for morphological and phenotypical differences, gene transcript profiles of avocado infected with P. cinnamomi, over a time period, provided evidence of genes involved in resistance and served as a basis for investigating plant–pathogen interactions and gene function.

Acknowledgements

This research was funded by the Hans Merensky Foundation and the THRIP programme of the National Research Foundation of South Africa. Plant material was provided by Westfalia Technological Services and infrastructure was provided by the University of Pretoria.

Authors’ contributions

J.E. contributed to the collection and analyses of the data and to the writing of the manuscript. N.v.d.B. was the project leader and assisted in the writing of the manuscript.

References


