Elicitor and Fusarium-induced expression of NPR-1 like genes in banana

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Abstract

NPR1 is an essential positive regulator of salicylic acid-induced *PR* gene expression and systemic acquired resistance. Two novel full-length *NPR1*-like genes; *MNPR1A* and *MNPR1B*, were isolated by application of the PCR and RACE techniques. The two identified *MNPR1*-sequences differed greatly in their expression profile using qRT-PCR following either elicitor or *Foc* treatment. *MNPR1A* was greatly expressed after *Foc* treatment with higher and earlier expression in the *Foc*-tolerant cultivar GCTCV-218 than in the sensitive cultivar Grand Naine. In comparison, *MNPR1B* was highly responsive to SA, but not to MeJA treatment, in both the tolerant banana cultivar GCTCV-218 and the more sensitive cultivar Grand Naine. Expression of the *MNPR1* genes further directly related to *PR* gene expression known to be involved in fungal resistance. Reduced sensitivity to *Foc* in GCTCV-218 might be partially attributed to the higher and an earlier expression of both *MNPR1A* and *PR-1* in this cultivar after *Foc* treatment.

Keywords: NPR1; Banana; *Musa*; *Fusarium oxysporum*; systemic acquired resistance; PR proteins

Abbreviations: NPR1, non-expressor of pathogenesis-related genes 1; *MNPR1A*, Musa non-expressor of pathogenesis-related genes 1A; *MNPR1B*, Musa non-expressor of pathogenesis-related genes 1A; SA, Salicylic acid; MeJA, methyl jasmonate; *Foc*, *Fusarium oxysporum* Schlecht f. sp. *cubense* (Smith) Snyd; SAR, systemic acquired resistance; PR proteins, pathogenesis-related proteins

Article Outline

1. Introduction

NPR1 is an essential positive regulator of SA-induced *PR* gene expression and SAR [39]. The PR proteins are expressed in plants in response to infection by pathogens such as fungi or viruses [16, 31, 38]. These include PR-1, whose biological activity is still unknown but seemingly has antifungal activity, and PR-3, which consists of various chitinases and lysozymes. *NPR1* is localized in the cell cytoplasm [21] and exists as an inactive oligomer. This oligomer has to be activated by the perception of salicylic acid [21]. Redox changes in the cytoplasm results in the dissociation of the NPR1 protein into monomeric active forms, which translocates to the nucleus where they interact with members of the TGA family of transcription factors [13]. NPRI-TGA transcription factor complexes are known to bind to SA-responsive elements in the *PR-1* promoter, facilitating *PR* gene expression and the deployment of SAR [27, 39]. The co-regulatory activity of *NPR1* is facilitated by the presence of the Bric-a-Brack Poxvirus and zinc finger (BTB/POZ) domain and the ankyrin repeats found within their protein structure [6, 8, 28]. In addition to its role in regulating SAR, a further function of *NPR1* in cross-communication between SA- and jasmonic acid-dependent defence signalling pathways has been found [24].

Several studies have shown that over-expression of *NPR1* provides resistance to a variety of bacterial and fungal pathogens [11, 12, 19, 40]. Also there is evidence from transgenic plants that SA and *PR-1* are required in *Arabidopsis* for resistance against pathogen infection [34], and that *NPR1* is involved in resistance to Fusarium head blight in wheat [18]. However, transgenic *Oryza sativa* (rice) plants, expressing an *Arabidopsis NPR1* gene displayed a lesion mimic cell death phenotype [14], while

rice plants over-expressing a rice *NPR1* homologue (*NH1*) had increased SA levels and were more sensitive to light resulting in a dwarf phenotype [11].

NPR1 is further functionally conserved in diverse plant species and full length *NPR1* sequences from some of these have been deposited in the Genbank. In the *Arabidopsis* genome six *NPR1*-related genes have been identified [17]. In addition *MpNPR1-1*, has been recently cloned from *Malus domestica* (apple) [19] while in *Brasica juncea*, two copies of the *NPR1* gene have been identified [20]. In rice, three homologous *NPR1*-like genes, *OsNPR1/NH1*, *OsNPR2/NH2* and *OsNPR3*, have been isolated [40]. *OsNPR1* is induced not only after treatment with the rice pathogens bacterial blight *Xanthomonas oryzae* pv. *oryzae*rice and blast *Magnaporthe grisea*, but also by benzothiadiazole, methyl jasmonate (MeJA) and ethylene [40]. Despite these reports, information about existence and expression of *NPR1*-like genes in monocot plants is still very limited.

The aim of this study was therefore to isolate and characterize expression of *NPR1*-like genes from banana following SA, MeJA and Fusarium treatment. Fusarium wilt caused by *Foc* is one of the most destructive diseases known in banana and a major threat to the international banana industry [26]. Results show that the two newly isolated *NPR1*-like genes, *MNPR1A* and *MNPR1B*, greatly differed in their expression due to elicitor and *Foc* treatment in two banana cultivars, a relatively tolerant cultivar GCTCV-218 and a more-sensitive cultivar Grand Naine.

2. Results

2.1. Identification and isolation of NPR1-like genes

PCR amplification using primer pairs designed to amplify the highly conserved ankyrin repeat region of known *NPR1* gene sequences yielded two distinct products of approximately 570bp. By applying a combination of the 3' RACE and 5'-end genome walking techniques, full-length sequences were isolated. *MNPR1A* (GenBank accession no. **DQ925843**) had a full length of 1927 bp and *MNPR1B* (GenBank accession no. **EF137717**) had a full length of 2073 bp. The complete amino acid sequences of the two gene sequences displayed 78% identity. Further, these full length sequences had the highest identity with the rice *NPR1* gene sequence, 63% for *MNPR1A* and 65% for *MNPR1B* (*table I*). A preliminary phylogenetic analysis using bootstrap consensus for neighbour joining, maximum parsimony and maximum likelihood revealed that *MNPR1A* and *MNPR1B* grouped closely with other monocot plants, such as rice and maize, for which *NPR1* gene sequences have already been identified (data not shown).

Detailed analyses of the two MNPR1 sequences revealed that the two sequences harbour a BTB/POZ zinc finger domain and the ankyrin repeat domain (*figure 1*), typical features of *NPR1* genes which are highly conserved across many species. However, there is a relative positional change and some amino acid dissimilarities occur in these domains between the two isolated sequences. The BTB/POZ domain of *MNPR1A* was identified at amino acid positions 58 to136 while the ankyrin repeats were identified at amino acid positions 290 to 365 and 324 to 349, respectively (*figure*

1). In comparison, the BTB/POZ domain of *MNPR1B* occupies amino acid positions 65 to 148 and the ankyrin repeats are found at positions 302 to 377 and 336 to 361.

2.2. Elicitor-induced MNPR1 and PR gene expression

SA and MeJA treatment induced *MNPR1A* and *MNPR1B* gene expression in both the tolerant GCTCV-218 and the sensitive Grand Naine banana cultivars (*figure 2A* and *2B*). However, *MNPR1A* expression was not significantly ($P<0.01$) different from basal levels of expression in both banana cultivars at the start of the treatment (*figure 2A*). *MNPR1B* expression was significantly induced 1.3-fold ($P<0.01$) 12 h after SA treatment in Grand Naine and in GCTCV-218, *MNPR1B* was significantly elevated by 3.2 fold ($P<0.01$) 24 h after treatment (*figure 2B*). In general, MeJA-induced *MNPR1B* expression was much lower than SA-induced *MNPR1B* expression. When plants of both cultivars were treated with MeJA, a 3.2-fold significant induction ($P<0.05$) in *MNPR1A* expression was observed at 12 h after MeJA treatment, followed by a decline in expression in GCTCV-219 when compared to expression at the beginning of the treatment (*figure 2C*). Such a significant increase in expression (3.5-fold) ($P<0.05$) at the same time point followed by a decline in expression was also observed for *MNPR1B* in GCTCV-219 (*figure 2D*). In contrast, in Grand Naine no significant induction of *MNPR1A* (*figure 2C*) and a 2.9-fold induction of *MNPR1B* expression over 48 h (*figure 2D*) were found.

SA and MeJA treatments also induced *PR-1* and *PR-3* gene expression in GCTCV-218 and Grand Naine (*figure 3A* and *3B*). However, in contrast to SA-induced expression in Grand Naine, *PR-1* expression in GCTCV-218 significantly increased ($P<0.01$) at 12 h (1.8-fold), 24 h (5.6-fold) and 48 h (4-fold) after SA

164 treatment. In Grand Naine, no significant increase in *PR-1* expression occurred over
165 48 h. However, *PR-3* expression increased after SA treatment in both banana cultivars
166 and expression was significantly higher ($P<0.05$) in GCTCV-218 at 24 h (1.8-fold)
167 and 48 h (1.5-fold) after SA treatment when compared to Grand Naine (*figure 3B*).

168 When *PR-1* and *PR-3* expression was measured in the two cultivars after MeJA
169 treatment, *PR-1* expression significantly increased (10.9-fold) ($P<0.05$) 12 h post
170 MeJA treatment in GCTCV-218 (*figure 3C*) compared to the expression at 0 h. This
171 was followed by a sharp decline in *PR-1* expression. Such an increase in *PR-1*
172 expression was not found for Grand Naine. In contrast, *PR-3* expression significantly
173 increased following MeJA treatment and expression was 13.9-fold ($P<0.05$) higher at
174 48 h post MeJA treatment when compared to *PR*-3 expression at the beginning of the
175 experiment (*figure 3D*). In GCTCV-218, no increase in *PR3* expression was found
176 after MeJA treatment.

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178 *2.3. Fusarium-induced MNPR1 and PR gene expression*

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180 Expression of both *MNPR1A* and *MNPR1B* was found due to *Foc* treatment in
181 both banana cultivars (*figure 4A* and *4B*). However, increase in *MNPR1A* expression
182 was much higher (1.3-fold) in GCTCV-218 than in Grand Naine. In Grand Naine,
183 *MNPR1A* expression at 24 hours after infection was significantly 1.9-fold higher
184 ($P<0.05$) than *MNPR1B* expression (*figure 4A*) whereas in GCTCV-218 this increase
185 in expression at 12 hours after infection was 14.7–fold higher ($P<0.05$) (*figure 4B*) for
186 *MNPR1A* compared to *MNPR1B.*

187

Both cultivars also expressed *PR-1* and *PR-3* due to *Foc* treatment (*figure 4C* and *4D*). However, Grand Naine expressed significantly more *PR-3* than *PR-1* with a 1.5-fold difference in expression ($P<0.05$) 24 hours after infection (*figure 4C*), whereas GCTCV-218 expressed significantly more *PR-1* than *PR-3* with a 3.9–fold difference in expression ($P<0.05$) (*figure 4D*) 12 hours after infection. However, this increase in *PR-1* expression in GCTCV-218 was followed by a sharp decline to near basal levels.

3. Discussion

This is the first report on the isolation of *NPR1*-like gene sequences from banana and their expression due to elicitor and fungal pathogen treatments. We have isolated two distinct sequences; *MNPR1A* and *MNPR1B*, from Cavendish banana and both have the typical features of other previously described *NPR1*-like gene sequences. This includes two identifiable protein-protein interaction motifs; a zinc finger and ankyrin repeat domains (ARD) [8, 13, 28]. Further, the two sequences share a 78% similarity in their amino acid sequence but vary in their sequence from previously described *NPR1*-like gene sequences [17]. Our preliminary data also show that the two banana sequences also group more closely with other monocot *NPR1* sequences but less with known dicot sequences (Endah, unpublished results).

So far, we have no knowledge of the genomic origin of the two banana sequences. Cultivated banana plants in the genus *Musa*, such as Grand Naine (AAA), are derived from the wild diploid banana species *M. acuminata* and *M. balbisiana* [22] contributing either the A or B genome, respectively. Cultivars resulting from this hybridisation are either diploid (AA, AB, BB), triploid (AAB, AAA, ABB), or tetraploid (AAAB, AABB, ABBB) [22]. In *Brassica juncea*, there is evidence that the two versions of *NPR1* originate from two individual parental genomes (*B. rapa* and *B. nigra*) [20]. However, since Grand Naine (AAA) only contains the A genome, this genome has very likely contributed both *MNPR1* gene sequences. We currently speculate that the two sequences could be part of a greater *NPR1* gene family in banana and are possibly involved in a variety of pathogen defence mechanisms like other *NPR1*-like gene sequences [4, 17]. Alternatively, intra-specific and inter-specific hybridisation of subspecies belonging to the *Musa* genus might have

contributed to the overall genome of Cavendish banana resulting in a very complex genome [5, 10] in which the A genomes are not identical.

This study further showed that *MNPR1A* and *MNPR1B* are expressed in banana after SA and MeJA elicitor treatment. This result is consistent with findings of other research groups that *NPR1* is expressed when plants sense SA, MeJA or pathogen attack [8-9, 18, 25, 40]. However, in comparison to *MNPR1A, MNPR1B* was highly responsive to SA-treatment in both banana cultivars and to a much smaller degree to MeJA treatment. In a previous study with *B. juncea* plants, JA was ineffective in both *NPR1* and *PR-1* expression [20]. However, in a recent study expression of the rice *OsNPR1*SA was found after MeJA treatment by Yuan *et al*. [40]. In general, jasmonic acid pathways have been shown to be activated during herbivore and pathogen attack [32]. Further, there is evidence that *NPR1* is also involved in cross-communication between SA- and jasmonic acid-dependent defense signalling pathways [24].

In our study, both *MNPR1A* and *MNPR1B* expression was associated with greatly increased *PR* gene expression in the more *Foc*-tolerant cultivar GCTVV-218. This increase was either gradual, as a response to SA treatment, or rapid followed by a sharp decline as a response to MeJA treatment. A similar expression profile was observed in the response of *MNPR1B* to MeJA treatment. In contrast, *PR-3* was highly responsive to MeJA treatment but only in the more *Foc*-sensitive cultivar Grand Naine. Future research has therefore to show if *MNPR1B,* in comparison to *MNPR1A*, is more prominently involved in *PR-3* expression.

In this study there was also a clear difference in *MNPR1A* and *MNPR1B* expression following *Foc* treatment. *MNPR1A*, but not *MNPR1B,* was more responsive in both cultivars to treatment with *Foc*. Response to *Foc* treatment was also earlier and of a higher magnitude in the more *Foc*-tolerant cultivar GCTCV-218

than in the more *Foc*-sensitive cultivar Grand Naine. Similar observations were also made on the expression of the two pathogenesis-related genes *PR-1* in GCTCV-218 and *PR-3* in Grand Naine. Less sensitivity to *Foc* in GCTCV-218 might be partially attributed to a higher and an earlier expression of both *MNPR1A* and *PR-1* in this cultivar after *Foc* treatment. This response of *PR-1* has also been reported for GCTCV-218 after treatment with *Foc* [36]. There is evidence that necrotrophic pathogens, such as Fusarium, elicit the jasmonic acid/ethylene-dependent pathway, whereas biotrophic pathogens elicit a SA-dependent pathway [23, 33]. Recent analysis in Arabidopsis further revealed that resistance to *Fusarium oxysporum* requires, besides the ethylene, jasmonic acid, and SA signalling pathways also the *NPR1* gene [7]. Since we did not observe a pronounced response by *MNPR1A* to SA or MeJA treatment in comparison to the high response that was found for *MNPR1B* after SA treatment, we currently speculate that *MNPR1A* might be more responsive to the ethylene dependent pathway when treated with *Foc*.

This study has provided first evidence for the existence of a possible *NPR1* gene family in banana. We have also shown that the two newly identified *MNPR1*-sequences differ greatly in their expression profile following either elicitor or *Foc* treatment. Expression of the two gene sequences further related to the expression of two specific *PR* genes known to be involved in fungal resistance. However, the exact function of the two genes, *MNPR1A* and *MNPR1B*, in plant defence response is yet to be elucidated in further studies. As a first step, we are currently investigating if transformed plants over-expressing either *MNPR1A* or *MNPR1B* are more resistant to *Foc* treatment.

4. Materials and methods

4.1. Isolation of banana NPR1-like gene sequences

For the isolation of *NPR1*-like gene sequences from banana, PCR primers (forward primer 5'-GAGCTTTTGGATCTCGCACTTGCAGA-3'; reverse primer 5'-CCGAGCTCCACTGTTTTGGAGAGTGCT-3') were designed using Primer 3 software based on sequence information available for the rice *NPR1* gene (GenBank accession no. **AY92398**). Double-stranded cDNA synthesized from Cavendish banana (Grand Naine) roots was used as a PCR template. For amplification by PCR, a primer annealing temperature of 55 ˚C was used in a standard PCR reaction.

A combination of both 5' and 3' Rapid Amplification of cDNA Ends (RACE) and genome walking were applied to isolate full-length cDNA clones of *NPR1*-like banana sequences. For isolation of *MNPR1A*, both 5' and 3' RACE was performed using the GeneRacer™ kit according to the manufacturer's instruction (Invitrogen, USA) along with gene-specific primers. Two nested gene-specific forward primers 5'-TGGTGATGACTTGCGGGGAAGATT-3' and 5'-TTGCCATGGACATTGCTCGAGTTG-3' and two reverse nested primers 5'-AATCTTCCCCGCAAGTCATCACCA-3' and 5'-TGCGGGTCTCTTCTTTCAGCTTGC-3' were used to amplify the 3'- and 5'-ends, respectively, of the *MNPR1A* gene. Both ends were joined by amplifying with forward 5'-CGGCGCGATATGGAAGACAA-3' and reverse 5'-GCAGGAGTCAGCAAAAGGAAGC-3' primers that flank the coding region and a portion of un-translated regions (UTRs) of the *MNPR1A* gene. Similarly, 3' RACE was performed to isolate the 3' end of *MNPR1B* using two nested gene specific

293 primers 5'-TGATGGCACATCGGAGTTCACC-3' and 5'-
294 GCATCTGGCACGAATGAGAGCA-3'. The 5' RACE, 5' nested, 3' RACE and 3'
295 nested primers were provided with the GeneRacer™ kit (Invitrogen, USA) that were
296 used together with the gene specific primers. The 5' end of *MNPR1B* was amplified
297 from genomic DNA by genome walking using a series of gene specific and adapter
298 specific primers from a library generated by digestion with different restriction
299 enzymes (*EcoR*V, *Pvu*II, *Sma*I, *Sca*I and *Stu*I) and ligation of adapters according to
300 the method described by Siebert *et al.* (1995). The coding region and portions of
301 UTRs of *MNPR1B* were then amplified from a cDNA template using forward 5'-
302 TTGGACGACGGCGGTACACG-3' and reverse 5'-
303 CAGCATGATCTAGTGGTGTGTCATGG-3' primers. All amplified PCR products
304 were T/A cloned into the pCR4-TOPO cloning vector (Invitrogen) and sequenced
305 using M13 forward and reverse primers.
306
307 4.2. *Sequence analysis*
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309 Sequencing of the inserts was performed by using the BigDye® Terminator
310 Cycle Sequencing FS Ready Reaction Kit, v 3.1(Perkin Elmer, Applied Biosystems,
311 USA) in an ABI PRISM® 3100 automatic DNA-Sequencer (Applied Biosystems).
312 The BLASTN and BLASTP programs [1] were used for gene sequence similarity
313 searches. Amino acid sequences of selected monocot and dicot *NPR1*-like sequences
314 were aligned using Clustal W [35] and ExPASy [15] was utilized for the prediction of
315 amino acid features and identification of conserved domains of *MNPR1A* and
316 *MNPR1B.*
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318 *4.3. Plant material and treatment*

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320 Tissue cultured banana plants (cv Grand Naine and GCTCV-218) were
321 hydroponically grown in 250 mL cups in a green house following the method of Van
322 den Berg *et al*. [36]. Once plants had attained a five leaf stage and had developed a
323 healthy root system, they were challenged with an inoculum of *Foc* (2.5 x 10^3
324 condia/mL), 5 mM SA or 5 µM MeJA. Unless stated otherwise, the entire root system
325 was harvested at time points 0, 12, 24, and 48 h post treatment and flash frozen in
326 liquid nitrogen and stored at -80^{o}C. Three plants were used for each time point for
327 every treatment, and for sample collection roots of the three plants were pooled
328 together. The experiment was repeated once.

329 Pathogen infection of the banana plants was done as described in Van den Berg
330 *et al.* [36]. The entire root system of the control plants was slightly wounded and 2.5
331 mL of sterile distilled water was added to each cup. Control samples were harvested
332 in the same manner as described above.

333 Treatment with SA was performed following a modified method of Anderson *et*
334 *al.* [2]. Both the roots and leaves of each plant were sprayed with a 5 mM salicylic
335 acid salt solution until imminent run-off. Plants were kept in a closed Perspex box
336 until time for collection of samples. Control plants were sprayed in the same way with
337 sterile distilled water. Treatment with MeJA was carried out by taping cotton balls
338 containing 400 µL of a 5 µM MeJA solution in ethanol on the roof of a sealed
339 Perspex box in the which banana plants were kept. All SA and MeJA treated samples
340 were collected and stored as described above.

341

342 *4.4. RNA Extraction and cDNA synthesis*

Total RNA was extracted from root material of Grand Naine and GCTCV-218 using the Qiagen RNeasy plant mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Traces of DNA in the RNA samples were eliminated by treating each RNA sample with DNase 1 (Fermentas Life Sciences, Hanover, MD). First strand cDNA was subsequently synthesized from the DNA-free RNA samples by random hexamer primering (Fermentas Life Sciences, Hanover, MD) using the first strand cDNA synthesis kit according to the manufacturer's instruction (Promega, USA). The quality of the cDNA was verified by amplifying a 170 bp actin fragment (data not shown) with banana actin forward 5'-ACCGAAGCCCCTCTTAACCC-3' and reverse 5'-GTATGGCTGACACCATCACC-3' primers [37].

4.5. Quantitative RT-PCR

Four genes *(MNPR1A, MNPR1B, PR-1* and *PR-3)* were used for expression studies in Cavendish banana plants. The Musa 25s rRNA was used as an endogenous control. Primer 3 was used to design primers from *MNPR1A* and *MNPR1B* gene sequences while primer sequences for the amplification of PR-1, PR-3 and Musa 25s rRNA PCR products were obtained from Van den Berg *et al.* [36]. Primers for *MNPR1A* were 5'-GTCGGCATTGTACCAACACA-3' (forward primer) and 5'-CAGTGCAGGAGTCAGCAAAA-3' (reverse primer); *MNPR1B* 5'-AGGTTTGCCCGAACAAGAAG-3' (forward primer) and 5'-TGAGAGGCAACAACTCAGAGAG-3' (reverse primer).

Quantitative real time PCR (qRT-PCR) was performed using the LightCycler® 480, 384-well PCR plates and the LightCycler® 480 SYBR Green I Master kit

(Roche Diagnosits, Germany) following the manufacturer's instructions. All reactions were conducted in triplicate with each PCR reaction consisting of 1 µL of the diluted template (1/10), 1 µM primers, and 5 µL Lightcycler® 480 SYBR-Green I master mix. The reaction volume was adjusted to 10 µL with nuclease-free water. Non-template control (NTC) reactions contained water instead of cDNA as template. Cycling consisted of an initial denaturation phase of 10 min at 95°C an amplification phase of 45 cycles each consisting of a denaturation step at 94°C for 5 s, annealing at 63°C for 5 s and extension at 72°C for 10 s. Individual PCR products were analysed by melting-point analysis during which samples were heated from 65°C for 10 s to 95°C and the decline in fluorescent signals of each individual sample was assessed.

4.6. Data analysis

QRT-PCR data was analysed as previously described in the Applied Biosystems, User Bulletin No. 2 [3]. The significance of differences for all treatments and between the two cultivars was analysed by One-way ANOVA and the Tukey Highest Square Difference (HSD) test at $p<0.05$ using the Statistica software [30].

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510

511

512 Table I. Homology between MNPR1A and MNPR1B and NPR1-like amino acid
513 sequences (% identity) in plants.

514

	MNPR1A	MNPR1B
Musa NPR1A (MNPR1A)		78
Musa NPR1B (MNPR1B)	78	
Capsicum annum	60	63
Oryza sativa	63	65
Hordeum vulgare	60	62
Nicotiana tabacum	58	62
Lycopersicum esculentum	60	63
Arabidopsis thaliana	47	48
Brassica napus	46	46
Helianthus annuus	40	38

515

Figure legends

Fig. 1. Multiple alignment of *MNPR1A* and *MNPR1B* with selected plant NPR1-like amino acid sequences. Amino acid sequences were aligned by Clustal W multiple alignment software (Thompson *et al*., 1994). Identical amino acids are represented with dots. Vertical rectangles represent conserved cysteine residues and horizontal rectangles represent BTB/POZ domain (filled) conserved ankyrin repeat domain in both MNPR1A and MNPR1B. Accession numbers used in the alignments are *Capsicum annum* (ABG38308.1), *Lycopersicum esculentum* (AAT57637.1), *Nicotiana. tabacum* (ABH04326.1), *Oryza sativa* (NP_001042286.1), *Hordeum. vulgare* (CAJ19095.1), *Arabidopsis thaliana* (AAM65726.1), and *Brassica napus* (AAM88865.2).

Fig. 2. Relative gene expression levels in roots of plants of Cavendish banana cultivars GCTCV-218 and Grand Naine. Gene expression was determined for *MNPR1A* and *MNPR1B* after treatment with 5 mM SA (A) and (B) and 5 µM MeJA (C) and (D), respectively. Samples were collected at 0 h and 12, 24 and 48 hours after treatment with the pathogen or the respective elicitor. The experiment was repeated once; the relative expression was determined by quantitative RT-PCR and expressed relative to a 'calibrator', the expression level at 0 h. The relative expression ratios were plotted on the graph. Results are means ± SEM of six individual plants. *Significant difference at $P<0.05$.

Fig. 3. Relative gene expression levels in roots of plants of Cavendish banana cultivars GCTCV-218 and Grand Naine. Gene expression was determined for *PR-1* and *PR-3* after treatment with 5 mM SA (A) and (B) and 5 µM MeJA (C) and (D), respectively. Samples were collected at 0 h and 12, 24 and 48 hours after treatment with the pathogen or the respective elicitor. The experiment was repeated once; the relative expression was determined by quantitative RT-PCR and expressed relative to a 'calibrator', the expression level at 0 h. The relative expression ratios were plotted on the graph. Results are means ± SEM of six individual plants. *Significant difference at $P<0.05$.

Fig. 4. Relative gene expression of *MNPR1A* and *PR-1* (dark bars) and *MNPR1B* and *PR-3* (white bars) in *Foc*-infected roots of Cavendish banana cultivar Grand Naine and GCTCV-218. Samples were collected for analyses at 0 h and 12, 24 and 48 hours after treatment. Relative gene expression of *MNPR1A* and *MNPR1B* (A and B) and *PR-1* and *PR-3* (C and D) was determined and compared in each of the cultivars. The experiment was repeated once; the relative expression was determined by quantitative RT-PCR and expressed relative to a 'calibrator', the expression level at 0 h. The relative expression ratios obtained from the only wounded control plants at each time point was subtracted from those of the infected and wounded samples to obtain the effect due to infection only. The expression ratios due to infection were plotted on the graph. Results are means ± SEM of six individual plants. *Significant difference at $P<0.05$.

Figure 1

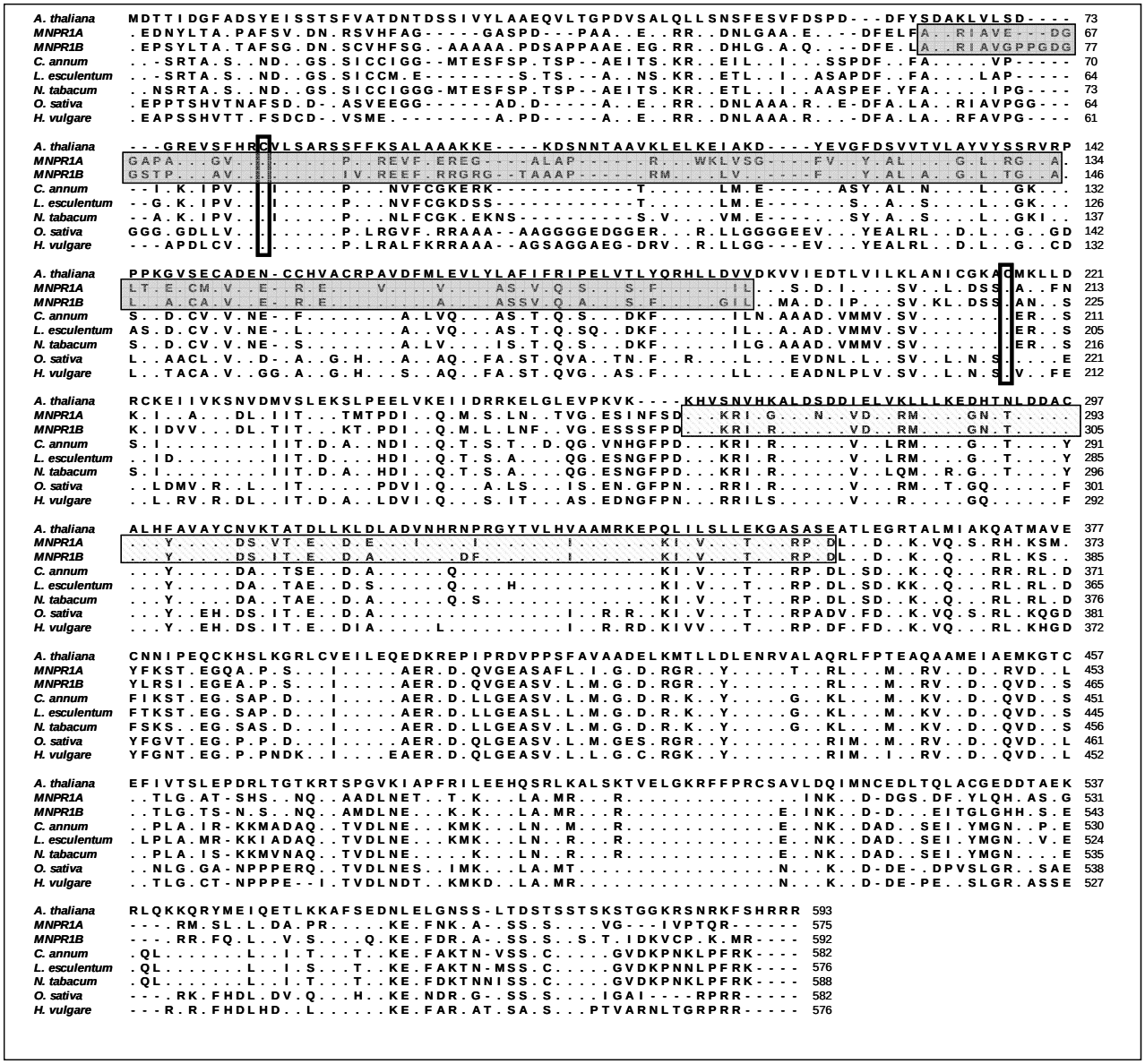

A. thaliana
MNPR1A
MNPR1B
C. annum
L. esculentum
N. tabacum
O. sativa
H. vulgare

Figure 2

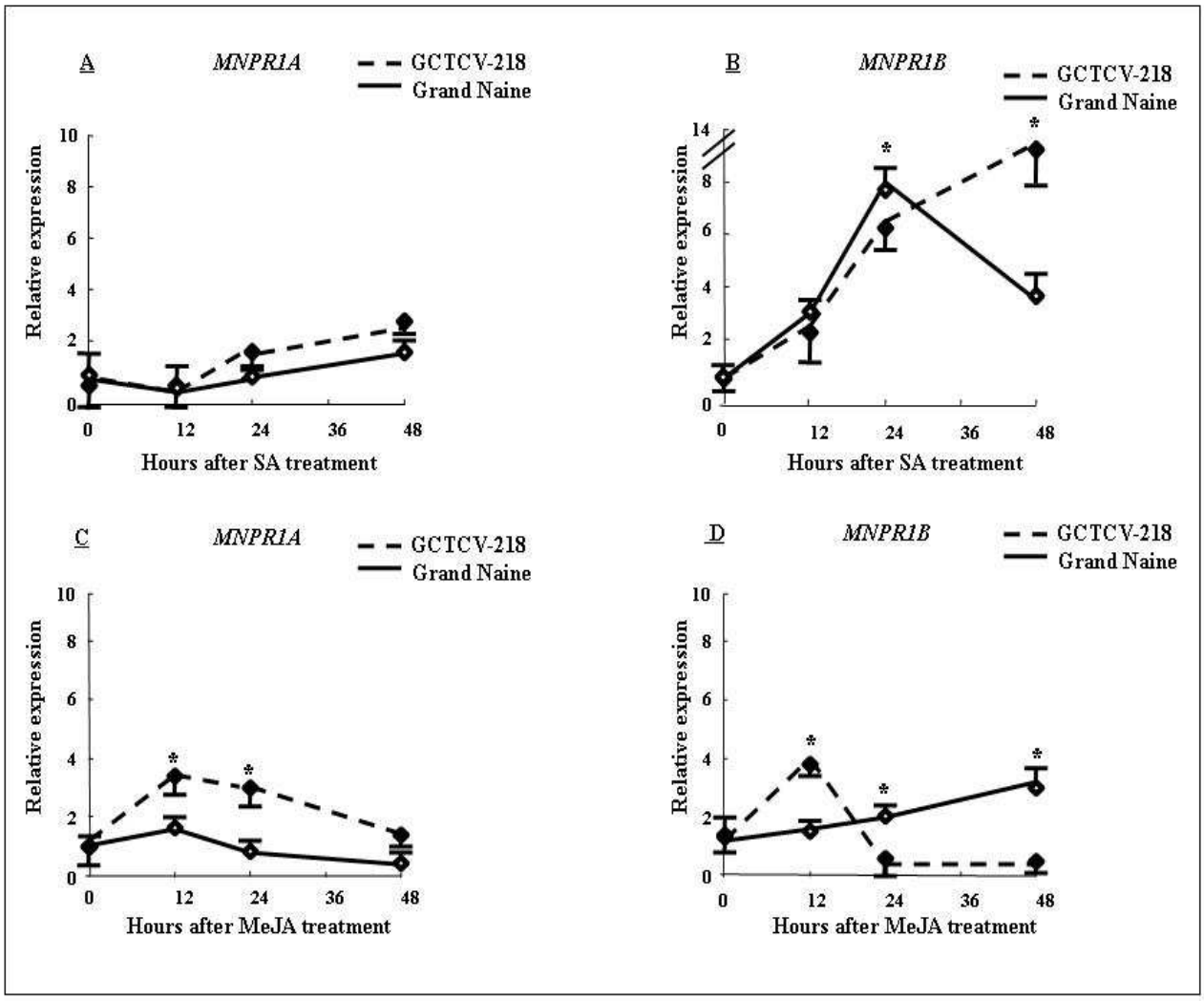

A
MNPR1A
GCTCV-218
Grand Naine
Relative expression
Hours after SA treatment
B
MNPR1B
GCTCV-218
Grand Naine
Relative expression
Hours after SA treatment
C
MNPR1A
GCTCV-218
Grand Naine
Relative expression
Hours after MeJA treatment
D
MNPR1B
GCTCV-218
Grand Naine
Relative expression
Hours after MeJA treatment

Figure 3

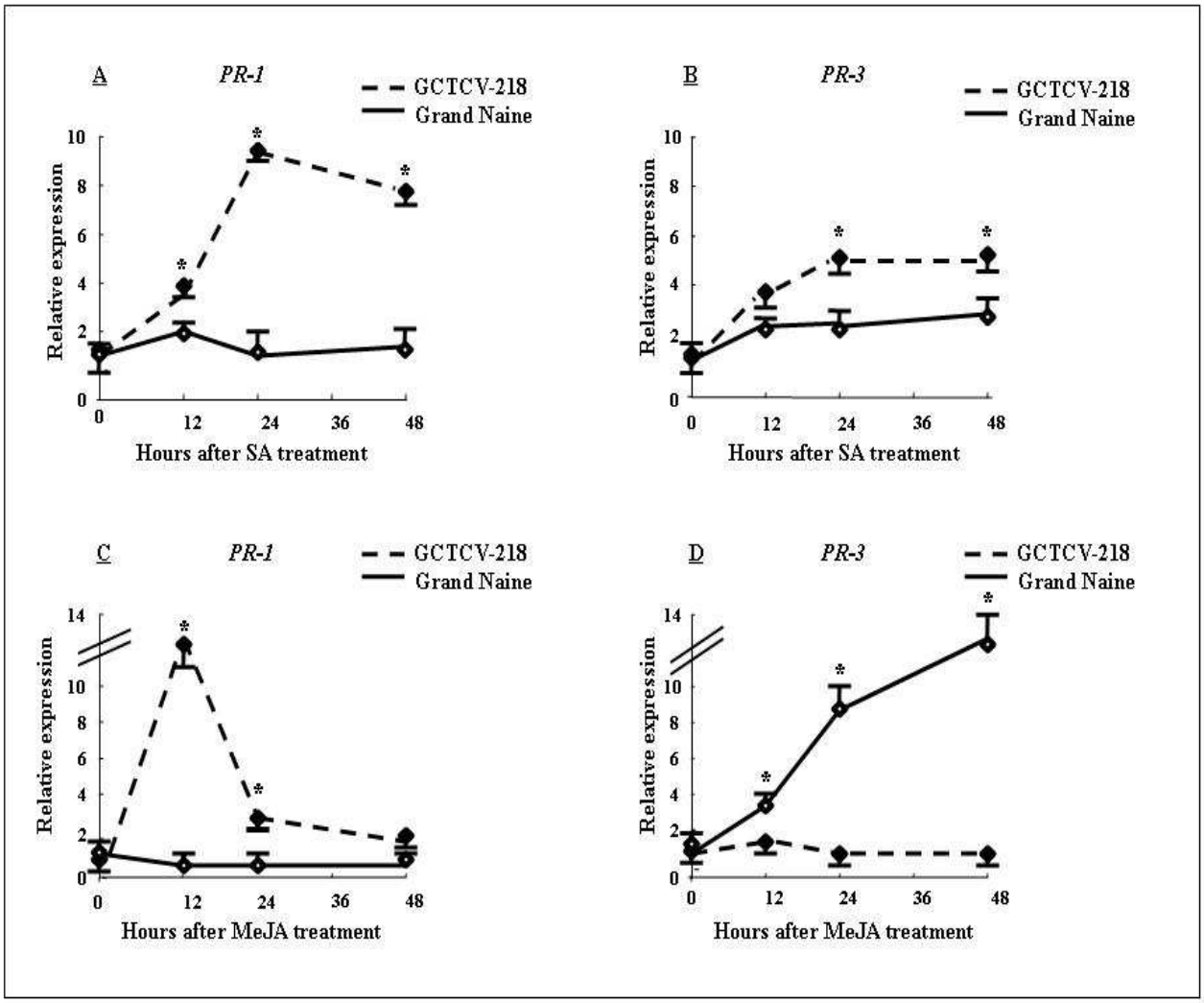
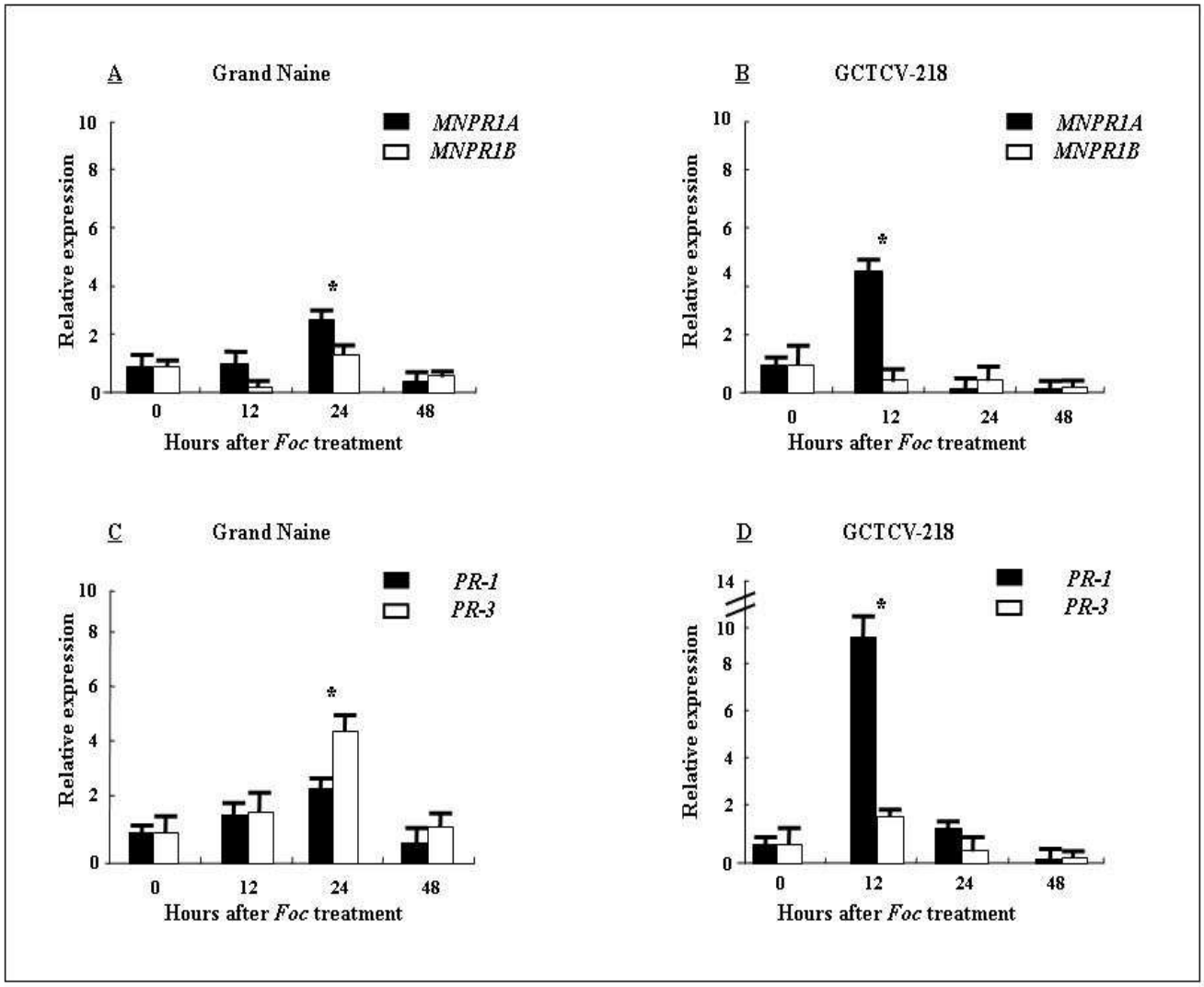

A
PR-1
GCTCV-218
Grand Naine
Relative expression
Hours after SA treatment
B
PR-3
GCTCV-218
Grand Naine
Relative expression
Hours after SA treatment
C
PR-1
GCTCV-218
Grand Naine
Relative expression
Hours after MeJA treatment
D
PR-3
GCTCV-218
Grand Naine
Relative expression
Hours after MeJA treatment

Figure 4


A
Grand Naine
MNPR1A
MNPR1B
Relative expression
Hours after Foc treatment
B
GCTCV-218
MNPR1A
MNPR1B
Relative expression
Hours after Foc treatment
C
Grand Naine
PR-1
PR-3
Relative expression
Hours after Foc treatment
D
GCTCV-218
PR-1
PR-3
Relative expression
Hours after Foc treatment