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Environmental and Experimental Botany 57 (2006) 70–79
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Local and distal gene expression of *pr-1* and *pr-5* in potato leaves inoculated with isolates from the old (US-1) and the new (US-8) genotypes of *Phytophthora infestans* (Mont.) de Bary

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Received 19 November 2004; accepted 15 April 2005

Abstract

The time-course and the spatial accumulation of PR-proteins *pr-1* and *pr-5* gene transcripts were investigated in two potato cultivars differing in their levels of susceptibility to late blight, caused by *Phytophthora infestans* (Mont.) de Bary. Cultivars Russet Burbank (RB, susceptible) and Kennebec (KB, moderately tolerant) were inoculated with either *P. infestans* genotype US-1 (old lineage) or US-8 (new lineage). A strong induction of both genes was detected in both cultivars inoculated with either *P. infestans* genotype, as compared to the healthy-controls. The accumulation of transcripts from both genes occurred earlier in KB than in RB leaflets. By comparing the two *P. infestans* isolates tested, a stronger and earlier induction of both PR genes was recorded in response to US-1 as compared to US-8. The spatio-temporal profiling of *pr-1* and *pr-5* genes expression showed a strong and early accumulation of transcripts at the local infection site, a late and intermediate level of induction at the proximal site, and no or very weak induction at a distal site remote from the infection site. These results show that *pr-1* and *pr-5* genes both are related to the defense mechanisms of potato to late blight, and that the higher infection success of *P. infestans* US-8 as compared to US-1 might be due to the late and/or the weak induction of these defense genes.

Keywords: Potato (*Solanum tuberosum*); Late blight; *Phytophthora infestans* (Mont.) de Bary; PR-proteins (PR-1; PR-5); Defense genes

1. Introduction

During a potato–pathogen interaction, compatibility is constitutively expressed as a disease gradient among potato plants depending on their level of susceptibility to the pathogen. Incompatibility occurs through an active hypersensitive-like reaction, resulting from a specific recognition between a receptor of the host and a corresponding elicitor of the pathogen (Michelmore and Meyers, 1998; Lebrun-Garcia et al., 1999; Kawchuk et al., 2001), followed by the activation of defense genes and the production of antimicrobial substances (Yoshioka et al., 1999). This leads to an early localized cell death around the infection site that often limits the pathogen progression. During this process, up- and down-regulations of many genes, including those encoding pathogenesis-related (PR) proteins are observed (Choi et al., 1992; van Loon and van Strien, 1999).

Pathogenesis-related proteins accumulate rapidly at the intra- or extra-cellular level under various biotic and abiotic stimuli, including fungal, elicitor, and physical or chemical treatments (van Kan et al., 1992; van Loon and van Strien, 1999; Kim and Hwang, 2000; Graham et al., 2003; Przymusiński et al., 2004). In case of induction under pathological conditions, the importance of these proteins to plant defense has been related to: (i) their rapid and early accumulation (van Loon and van Strien, 1999), often associated with incompatibility (Linthorst, 1991), (ii) their antimicrobial activity (Woloshuk et al., 1991; Ponstein et al., 1994; Beerhues and Kombrink, 1994; Vleeshouwers et al., 2000; Fung et al., 2002; Velazhahan and Muthukrishnan, 2003), and (iii) their ability to reduce symptoms development (Niderman et al., 1995; Liu et al., 1994). Many studies have reported a correlation between the timing of PR gene expression and

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doi:10.1016/j.envexpbot.2005.04.006

the initiation and the duration of systemic acquired resistance (SAR; Vleeshouwers et al., 2000; McGee et al., 2001), although the causal–effect relationship between the expression of PR-proteins and SAR has never been thoroughly proven. PR proteins were initially isolated from tobacco showing a hypersensitive reaction against tobacco mosaic virus (van Loon and van Kammen, 1970). They have thereafter been found in a wide range of both monocotyledonous and dicotyledonous plant species (van Loon et al., 1994). These proteins are determined by the type of interaction between the host and the pathogen even though their induction is not specific to one particular pathogen (Cuypers and Hahlbrock, 1988). In potato leaves challenged by *Phytophthora infestans*, induced PR proteins differ from those isolated from tobacco by their numbers and by their function during the infection process (Friends, 1991). In many cases, this function is related to their antifungal activity. For example, members of the PR-2 family are known for their β-1,3-glucanase activity (Beerhues and Kombrink, 1994; Ji and Kuć, 1996; Borkowska et al., 1998), PR-3 for their chitinolytic activity (van Loon et al., 1994; van Loon and van Strien, 1999), and PR-5 for their osmo-permeabilization of fungal plasma membranes (van Loon et al., 1994; Abad et al., 1996). The mode of action of members of the PR-1 family (Bowles, 1990; van Loon and van Strien, 1999) remains unclear, except that in tobacco and tomato such proteins inhibit zoospore germination and mycelial growth of *P. infestans* (Niderman et al., 1995).

P. infestans is the oomycete causing late blight (LB), and is a major constraint to potato production around the world (Fry et al., 1993; Duncan, 1999). Disease management has mostly relied on integrated management strategies based on disease monitoring, some cultural practices, and heavy use of chemicals (Daayf and Platt, 2000). The population structure of this pathogen has changed during the last two decades, including an increase in aggressiveness and insensitivity to chemical fungicides (Daayf and Platt, 2000; Daayf et al., 2001). In North America, the old US-1 genotype has been displaced by US-8, which is more aggressive on most potato cultivars. This genotype causes larger lesions, produces higher numbers of sporangia, and has shorter latent periods (Kato et al., 1997).

In the potato–*P. infestans* system, many aspects of this interaction have been studied (Cuypers and Hahlbrock, 1988; Freytag et al., 1994; Vleeshouwers et al., 2000). However, no data is available about the differential responses of potato to the old *versus* the new genotypes of this pathogen. The objectives of the present study were: (i) to assess the differential accumulation of *pr-1* and *pr-5* transcripts in two potato cultivars 'Russet Burbank, RB' (susceptible) and 'Kennebec, KB' (moderately tolerant), in response to inoculations with the old US-1 versus the new US-8 genotypes, and (ii) to follow such gene expression both overtime: time after inoculation, and space: local inoculation site, proximal (leaflets adjacent to the inoculated leaflet) and distal leaflets (from adjacent leaves) remote from the inoculation site.

2. Materials and methods

2.1. Plant materials

High quality tuber seeds of potato cultivars 'Russet Burbank, RB' and 'Kennebec, KB' were used to generate plants under controlled conditions. The choice of these two cultivars was directed by the fact that RB is susceptible to LB and widely used as a commercial cultivar while KB is known to be moderately tolerant to this disease. Plants were grown in clay pots containing soil–sand–peat–perlite mixture (4:4:4:1) in a growth room maintained at $20 \pm 2\,^{\circ}C$ with 16 h photoperiod.

2.2. Pathogens

One isolate of *P. infestans* (FA1) belonging to the old lineage US-1 and one (D1901) belonging to the new lineage US-8 were used in this study. The physiological races of the two isolates were previously determined by their reactions on a differential set of potato cultivars harboring specific resistance genes *R-1* to *R-11*. The physiologic race of FA1 isolate was 1, 2, 4, 7, 9 while that of the D1901 isolate was found to be 1, 2, 3, 4, 6, 7, 8, 9, 10 (Wang et al., 2005). Both isolates were maintained on rye B medium at $20 \pm 2\,^{\circ}C$ as described by Goodwin et al. (1995) with repetitive inoculations followed by re-isolations from potato leaves every 3–4 months in order to maintain their virulence.

2.3. Inoculation procedure

For inoculations, plants of 5–6 weeks were used. Inoculum was prepared from both US-1 and US-8 isolates by washing 10–14 days old *P. infestans* cultures using distilled water. Sporangia were then harvested 24 h later in sterile distilled water and the suspensions were adjusted at 5×10^6 sporangia per ml. Inoculations were performed on the primary leaflet of the third or fourth leaves of potato plants by placing many droplets for a total of 100 μl on their surface. Inoculated potato plants were kept for 48 h in a moisture chamber with 100% relative humidity. All the experimental design schemes were randomized complete blocks with three blocks and three replicates per each treatment. The whole experiment was repeated independently two times.

2.4. Disease assessment

The inoculated leaves from both cultivars were collected over time (0, 24, 48, 72, 96 and 120 h post-inoculation (h.p.i.)) and the percentage of leaf diseased area was estimated using the image analysis software ASSESS (Lamari, 2002). The relative area under the disease progress curve (relative AUDPC) was then calculated and used to compare either the tolerance of potato cultivars or the aggressiveness of the *P. infestans* isolates: $\text{AUDPC} = \sum_{i=1}^{n-1}[((y_i + y_{i+1})/2)\,(t_{i+1} - t_i)]$, where y_i is the percentage of diseased leaf area at the time t_i.

2.5. Leaves harvesting and total RNA preparation

Three types of leaf material were collected: inoculated primary leaflets (local), secondary non-inoculated leaflets from the same inoculated leaf (proximal), and leaflets from the leaves adjacent to the inoculated leaf (distal). The time-course of harvest was 0, 3, 6, 12, 24, 48, 72, 96, and 120 h.p.i. All the harvested leaf samples were frozen in liquid nitrogen and immediately stored at −80 °C until further use. For total RNA extraction, 100 mg FW generated from six leaf samples of each type (local, proximal and distal) were used. They were then ground to a fine powder within a pre-cooled mortar in liquid nitrogen. Total RNA was isolated following the method of Verwoerd et al. (1989) and quantified at A_{260}. Two separate RNA extractions were performed from each independent experiment.

2.6. RNA dot and gel blots analysis

Dot blots were performed by depositing 10 μg of denatured total RNA of each sample on the Hybond–N^+ membrane (Hoffmann-La Roche Ltd., Mississauga, Ont., Canada) while gel blots were carried out using 10 μg of RNA pre-separated by electrophoresis under denaturing conditions and transferred onto Hybond–N^+ membrane. The prehybridizations were performed in DIG Easy high solution at 50 °C for 3–4 h. The same solution was used for the hybridization at 50 °C for 18 h and the membranes were washed at room temperature using 2× SSC containing 0.1% SDS for 15 min. A second washing of the membranes was performed using 0.5% SSC containing 0.1% SDS at 68 °C for 15 min.

2.7. Probes for hybridization

For probes generation from DNA templates, the following fragments were used: 589 bp of *pr-1* gene, 380 bp of *pr-5* gene and 686 bp fragment of 18s RNA (Genebank no. 67238). The probes were then labeled using a PCR DIG labeling kit (Hoffmann-La Roche Ltd., Mississauga, Ont., Canada) following the manufacturer's recommendations. The primers were designed using OligoPerfect™ Designer software (Invitrogen™ Life Science Software, Invitrogen Inc., Ont., Canada) and the primers' sequences used for *pr-1*, *pr-5*, and 18s RNA probe synthesis were: *pr-1*-F 5′-TCACTCTTGTGATGCCCAAA-3′/*pr-1*-R 5′-AGTGGAA-ACAAGAAGATGCA-3′, *pr-5*-F 5′-TAAAGCTTCCGGC-GTATTTG-3′/*pr*-5-R 5′-AATCGGTAGGACCACATGGA-3′, and 18S-F TAGATAAAAGGTCGACGCGG-3′/18S-R 5′-TCATTACTCCGATCCCGAAG-3′.

2.8. Image analysis

Dot blots of the *pr-1* and *pr-5* transcripts were repeated three times. In order to avoid possible variation in image development due to slightly different exposure times, 5 ng of DIG labeled control DNA (Hoffmann-La Roche Ltd., Mississauga, Ont., Canada) was loaded on the right corner of each membrane and used as a control. The dots on films were photographed using a digital camera at 1200 dpi resolution (Sony Ltd., Toronto, Ont., Canada) and analyzed using ImageJ 1.32 (http://rsb.info.nih.gov/ij/download.html). The expression profiles of the *pr-1* and *pr-5* transcripts were calculated by integration of the density of each dot and an average of three replicates was considered in the final construction. Corrections of slight differences in loading were made by normalizing the results against the constitutively expressed 18s RNA gene.

3. Results

3.1. Disease progress

The highest disease severity was observed on cultivar RB inoculated with the US-8 isolate, as more than 83% of the leaf surface area was destroyed 120 h.p.i. (Fig. 1). Inversely, cultivar KB inoculated with the US-1 isolate showed the lowest percent of diseased area (less than 8%). Intermediate severity levels were recorded on KB inoculated with US-8 and on RB challenged with US-1. In general, disease severity was higher on RB than on KB inoculated with either US-1 or US-8 isolates and the US-8 isolate was more aggressive than US-1 on both cultivars.

3.2. Spatio-temporal accumulation of pr-1 transcripts

3.2.1. Local leaflets

No accumulation of *pr-1* transcripts was observed in healthy-control local leaflets of both tested cultivars over the experimental period of 5 days (Fig. 2). However, a transient strong accumulation of *pr-1* mRNA transcripts was observed 48 h.p.i. in RB and KB leaflets inoculated with either US-1 or US-8. This accumulation was recorded prior to the appearance of lesions (72 h.p.i.) and occurred at a higher level in

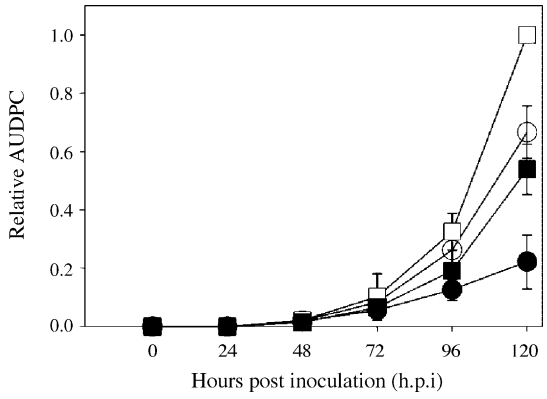

Fig. 1. Relative AUDPC scored on cultivars Russet Burbank (RB) and Kennebec (KB) inoculated with *P. infestans* US-1 and US-8 isolates. (-◯-) RB × US-1; (-●-) KB × US-1; (-□-) RB × US-8; (-■-) KB × US-8.

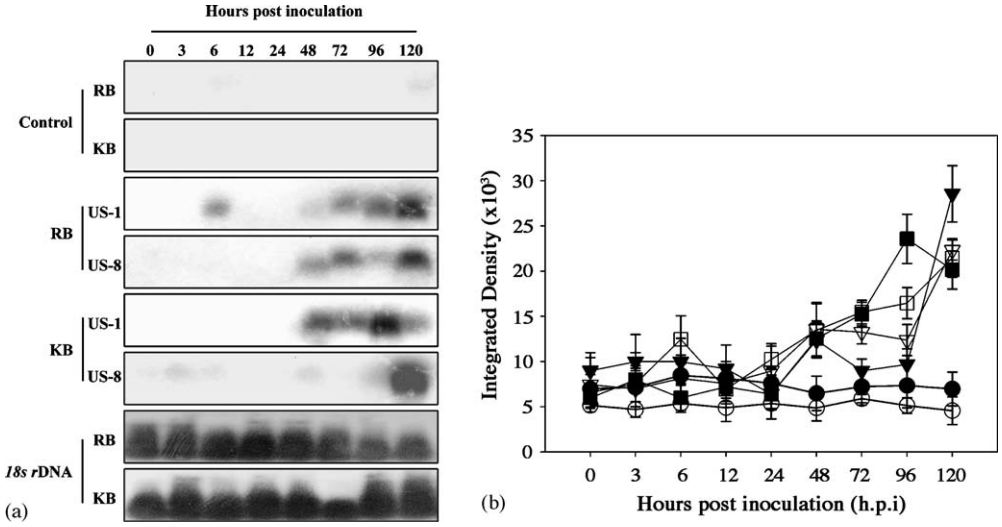

Fig. 2. Northern gel blots showing *pr-1* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-1* mRNA (b) on dot blots (means ± SE) in local leaflets of two potato cultivars (KB and RB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. 18s *r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-1*. No significant difference was observed in the accumulation of 18s *r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-◯-) RB control; (-●-) KB control; (-□-) RB × US-1; (-■-) KB × US-1; (▽) RB × US-8; (▼) KB × US-8.

KB than in RB leaflets. No difference in accumulation of *pr-1* transcripts was observed in RB leaflets inoculated with either US-1 or US-8. The strongest accumulation was recorded 120 h.p.i. in both cases. A weak and early accumulation of *pr-1* mRNA was observed 6 h.p.i. in response to US-1 and not to US-8. In KB leaflets, *pr-1* transcripts accumulated in response to US-1 earlier than to US-8. The highest level of *pr-1* transcripts accumulation was recorded 96 h.p.i. with US-1 and 120 h.p.i. with US-8.

3.2.2. *Proximal leaflets*

As observed in local leaflets, there was no accumulation of *pr-1* transcripts in RB and KB healthy-control proximal leaflets (Fig. 3). The accumulation of *pr-1* mRNA in RB leaflets inoculated with either US-1 or US-8 was weak except at 72 h.p.i. in response to US-8. A weak and early accumulation of *pr-1* mRNA was observed 6 h.p.i. in response to US-1 and not to US-8. In KB leaflets, *pr-1* transcripts accumulated strongly in response to US-1 as compared to US-8.

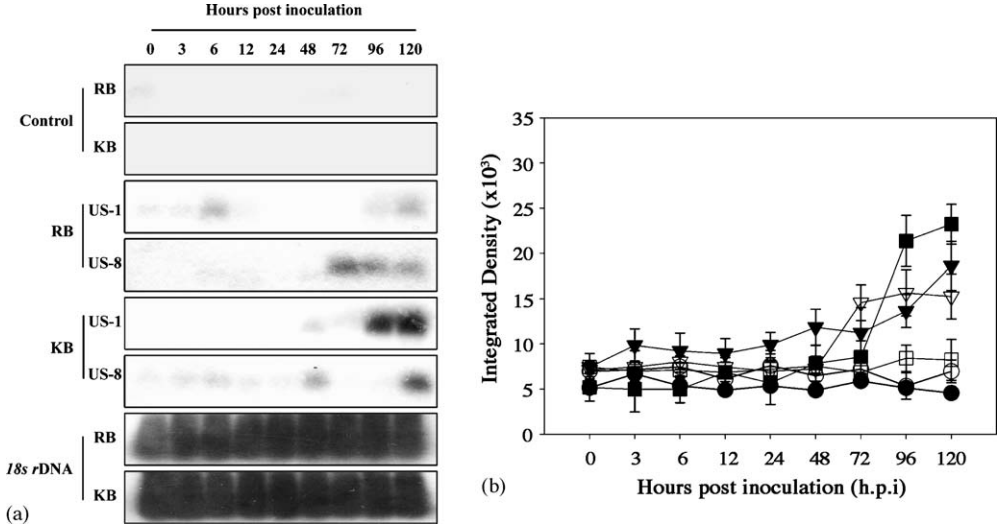

Fig. 3. Northern gel blots showing *pr-1* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-1* mRNA (b) on dot blots (means ± SE) in proximal leaflets of two potato cultivars (KB and RB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-1*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-◯-) RB control; (-●-) KB control; (-□-) RB × US-1; (-■-) KB × US-1; (▽) RB × US-8; (▼) KB × US-8.

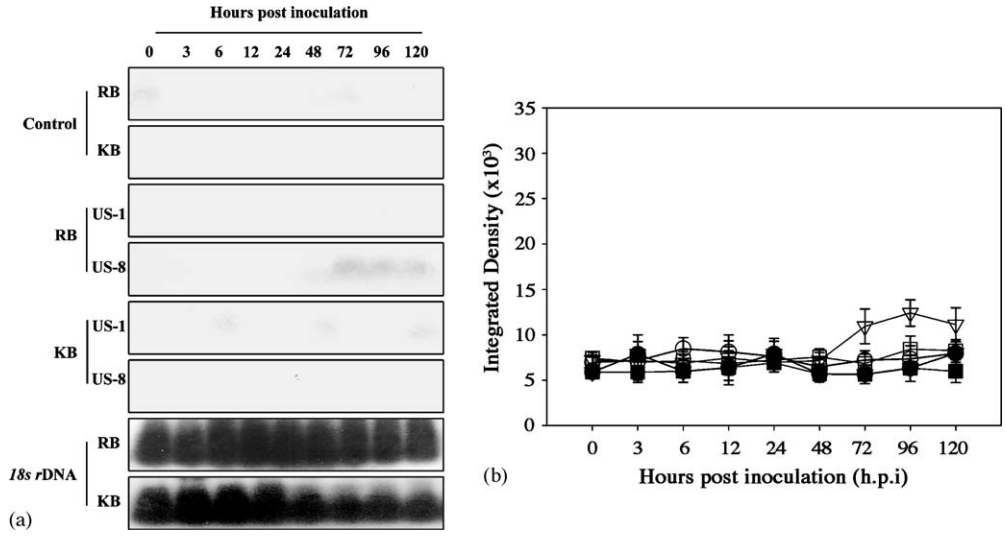

Fig. 4. Northern gel blots showing *pr-1* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-1* mRNA (b) on dot blots (means ± SE) in distal leaflets of two potato cultivars (KB and RB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-1*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-◯-) RB control; (-●-) KB control; (-□-) RB × US-1; (-■-) KB x US-1; (▽) RB × US-8; (▼) KB × US-8.

This accumulation occurred also earlier with US-1 (96 h.p.i) versus US-8 (120 h.p.i.).

3.2.3. Distal leaflets

There was no accumulation of *pr-1* mRNA transcripts in healthy–control distal leaflets originating from RB or KB (Fig. 4). No accumulation of these transcripts was observed also in both inoculated cultivars with either US-1 or US-8 except a very weak accumulation in RB leaflets in response to US-8 starting at 72 h.p.i.

3.3. Spatio-temporal accumulation of pr-5 transcripts

3.3.1. Local leaflets

No accumulation of *pr-5* mRNA transcripts was observed in RB or KB healthy-control local leaflets (Fig. 5). However, these transcripts accumulated differentially in both RB and KB leaflets inoculated with either US-1 or US-8. The earliest strong accumulation of *pr-5* transcripts was observed in KB leaflets inoculated with US-1 48 h.p.i. No noticeable accumulation of *pr-5* transcripts was observed before 120 h.p.i. in

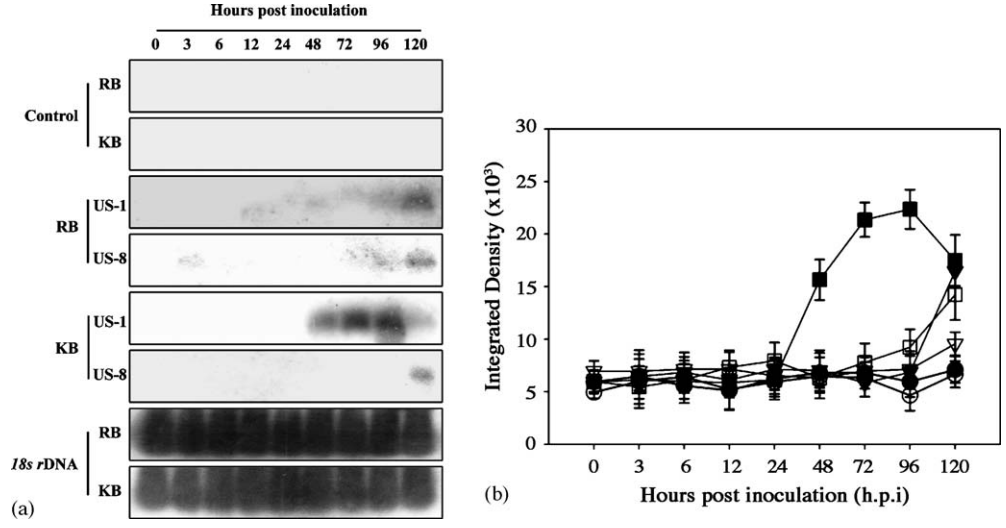

Fig. 5. Northern gel blots showing *pr-5* transcripts accumulation (a) and densitometric analysis the abundance of *pr-5* mRNA (b) on dot blots (means ± SE) in local leaflets of two potato cultivars (KB and RB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-5*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-◯-) RB control; (-●-) KB control; (-□-) RB × US-1; (-■-) KB × US-1; (▽) RB × US-8; (▼) KB × US-8.

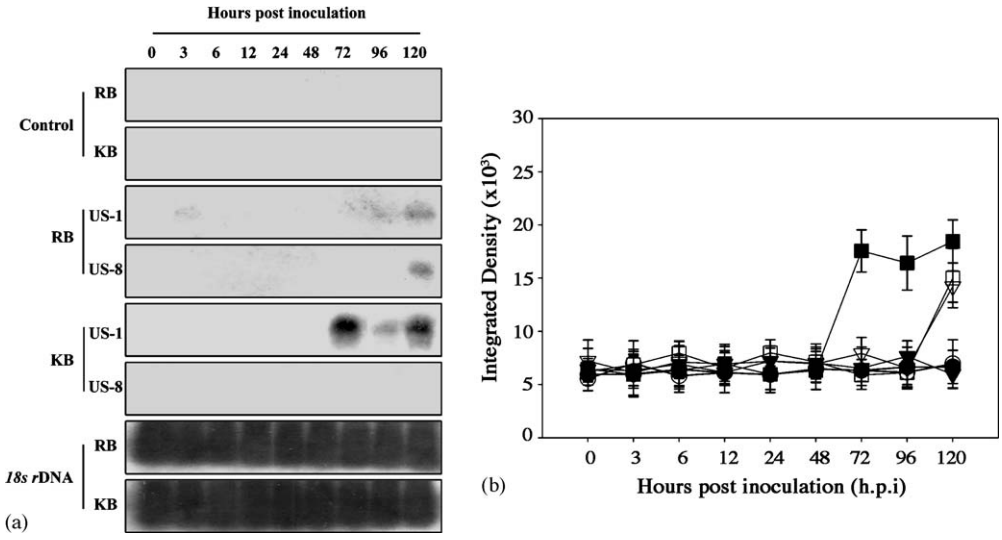

Fig. 6. Northern gel blots showing *pr-5* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-5* mRNA (b) on dot blots (means ± SE) in proximal leaflets of two potato cultivars (KB and RB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-5*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-◯-) RB control; (-●-) KB control; (-□-) RB × US-1; (-■-) KB × US-1; (▽) RB × US-8; (▼) KB × US-8.

RB leaflets inoculated with either US-1 or US-8 and in KB leaflets inoculated with US-8.

3.3.2. Proximal leaflets

There was no accumulation of *pr-5* transcripts in RB and KB healthy–control proximal leaflets (Fig. 6). The strongest and earliest accumulation of *pr-5* transcripts occurred 72 h.p.i. in KB leaflets in response to US-1. A weak accumulation of *pr-5* transcripts was also perceptible 120 h.p.i. in RB leaflets in response to both US-1 and US-8, whereas no accumulation was recorded in KB leaflets in response to US-8.

3.3.3. Distal leaflets

There was no accumulation of *pr-5* transcripts in RB and KB healthy–control distal leaflets (Fig. 7). As well, no accumulation of *pr-5* mRNA was observed in RB or KB leaflets in response to US-1 or US-8 except for a very weak perceptible accumulation 120 h.p.i. in RB challenged by either US-1 or US-8.

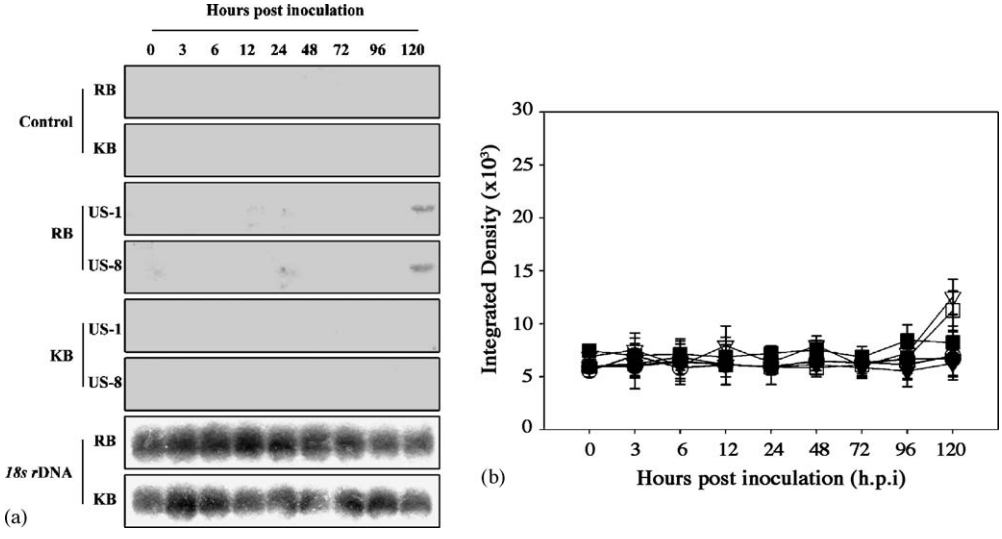

Fig. 7. Northern gel blots showing *pr-5* transcripts accumulation (a) and densitometric analysis of the abundance of *pr-5* mRNA (b) on dot blots (means ± SE) in distal leaflets of two potato cultivars (KB and RB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. *18s r*DNA probe was used as internal control in the northern blot (a) to normalize the expression of *pr-5*. No significant difference was observed in the accumulation of *18s r*DNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (-◯-) RB control; (-●-) KB control; (-□-) RB × US-1; (-■-) KB × US-1; (▽) RB × US-8; (▼) KB × US-8.

3.4. Comparison between the spatio-temporal patterns of *pr-1* and *pr-5*

The comparison of the accumulation of *pr-1* and *pr-5* transcripts over all the treatments analyzed testified an earlier and stronger induction of *pr-1* and *pr-5* genes in both tested cultivars in response to US-1 than to US-8. Accumulation of both genes' transcripts occurred strongly and earlier in KB than in RB leaflets. The spatial profiling of *pr-1* and *pr-5* genes showed a strong and early accumulation of transcripts at the local infection site, then an intermediate level of induction at the proximal site, and no or very weak induction at the distal site.

4. Discussion

The potato–*P. infestans* system has been used to study various biochemical aspects of host–pathogen interactions (Niderman et al., 1995; Andreu et al., 1998; Bos et al., 2003). Although a number of studies have been dedicated to profile the expression of *pr* genes during the infectious process (Niderman et al., 1995; Vleeshouwers et al., 2000; Hoegen et al., 2002), no one has compared potato reactions to the old (US-1) versus the new lineages of *P. infestans* (US-8) at the molecular level.

In the present study, a strong and rapid accumulation of *pr-1* transcripts was observed *prior* to the appearance of the lesions on both susceptible (RB) and moderately tolerant (KB) cultivars inoculated with US-1. In comparison, such accumulation was rather late in response to US-8. This result shows that *pr-1* gene can be quickly induced after the infection and concur other findings showing an accumulation of *pr-1* transcripts 12 h.p.i. in detached potato leaflets inoculated with *P. infestans*, and in pepper leaves challenged with an incompatible pathogen (Kim and Hwang, 2000; Hoegen et al., 2002). Interestingly, an early induction of *pr-1* was observed on RB inoculated with US-1 both in local and proximal leaflets. However, no hypothesis is available to explain such an early expression, except that it might be related to the successive alterations in *P. infestans* structure during early stages of infection as shown by Birch and Whisson (2001) and Avrova et al. (2003). Indeed, these authors have shown that *P. infestans* produces several cell types *prior* to and during the early stages of infection (formation and encytement of zoospores, production of germ tube, and development of appresoria, hyphae, haustoria, and sporangiophores). Each of these developmental stages is under the control of specific genes that might generate a specific interaction with those of the host. Transcripts of *pr-5* accumulated also strongly and earlier in KB leaves inoculated with US-1 as compared to US-8. These findings suggest that the level and the time-course accumulation of *pr-1* and *pr-5* transcripts might be related to the level of tolerance of KB to *P. infestans* genotype US-1. The non-sufficient and/or the late accumulation of these transcripts might also be related to the higher infection success of US-8 on both cultivars as compared to US-1. Given the low efficiency of the Northern blot technique in detecting differences between low levels of transcripts, these findings should be confirmed using a more accurate and sensitive method such as the real-time quantitative PCR.

Recent studies on *pr-1* and *pr-5* genes, have revealed that these genes were strongly expressed following the infection of various plant species by diverse pathogens (Ponstein et al., 1994; Hoegen et al., 2002; Park et al., 2002) and that they have antifungal activity (Ponstein et al., 1994; Niderman et al., 1995; Abad et al., 1996; Hoegen et al., 2002). The induction of *pr-1* and *pr-5* observed here in potato cultivars RB and KB inoculated with either *P. infestans* US-1 or US-8 isolates corroborated the possible involvement of these genes in the differential disease responses of the two cultivars to each genotype, and of each cultivar to the two genotypes.

The fungal growth of *P. infestans* US-1 seems to be inhibited to some extent on KB leaflets where the reaction reminds of previous observations within a 'true' incompatible interaction (Lazarovits and Higgins, 1976). The connection between the fungal growth inhibition and the induced expression of PR genes suggests the involvement of one or several of these proteins in successful defense reactions against the pathogen. Indeed, many PR-proteins are known for their demonstrated anti-microbial activity in vitro or after overexpression in transgenic plants (Woloshuk et al., 1991; Beerhues and Kombrink, 1994; Liu et al., 1994; Niderman et al., 1995; Vleeshouwers et al., 2000). The overexpression of osmotin (PR-5) derived from tobacco has led to a reduction of *P. infestans* in potato (Liu et al., 1994), most likely via a permiabilization of the fungal plasma membrane (Abad et al., 1996). Elsewhere, the over-expression of PR-1a or PR-1b in tobacco to control Tobacco Mosaic Virus (Cutt et al., 1989; Linthorst et al., 1989) has been shown to enhance significantly the resistance levels to two oomycetes (Alexender et al., 1993). The antifungal effect of potato PR-1 on *P. infestans* through inhibition of both zoopore germination and mycelial growth have also been pointed out (Niderman et al., 1995) even though the mechanism of such effect is still unknown.

The local and distal accumulation of PR-1 and PR-5 mRNA transcripts observed in the present study were different depending on the potato cultivar, the pathogen genotype and the stratum of leaf examined. The transcripts accumulated earlier in local, then in proximal leaves with a delay of 24–72 h. However, a very weak or no accumulation at all was recorded in distal leaflets, suggesting the activation of *pr*' genes mostly in tissues close to the infection site. This concurs with other findings suggesting the antifungal activity of PR-proteins acting at the local and to some extent at a close level of the infection site (Park et al., 2002; Graham et al., 2003). Furthermore, it has been reported that PR-proteins targeted to the vacuole or outside of the cell are less involved as components of the front line defense action. However, they probably have major effects after the decompartimentaliza-

tion has occurred, particularly against biotrophic pathogens (Hammond-Kosak and Jones, 1996). However, PR-proteins localized in the cytoplasm such as PR-1 and PR-5 rapidly accumulate after elicitor treatments or pathogen inoculations and might be involved in the front line of defense response (Somssich et al., 1989; Hahbrock et al., 1995). The present findings, showing a delay in PR-1 and PR-5 accumulations by 24–72 h in proximal leaflets as compared to locals, corroborate the suggestion that these two proteins are involved in the front line defense mechanisms of potato against *P. infestans*.

Plants exhibit generally localized and/or systemic acquired resistances (LAR and SAR) against invading pathogens. LAR takes place in a ring of living cells surrounding the lesion and exhibiting an activation of host defense-related genes including the PRs (Costet et al., 2002). LAR provides a locally highly uncongenial setting for the invading pathogen (Heath, 1998). The importance of PRs in the plant defense mechanisms in this case has been related, among other reasons, to their ability to reduce the development of symptoms in the area surrounding the lesions (Niderman et al., 1995; Liu et al., 1994). SAR develops, however, beyond tissues exhibiting LAR (Ross, 1961a,b) and provides a low, though significant, level of resistance against a subsequent infection (Ryals et al., 1996; Sticher et al., 1997). It occurs in the primary infected parts of the plant and throughout the host. A strong expression of a wide range of defense responses occurs in LAR including the accumulation of antimicrobial proteins such as PRs (Fritig et al., 1998). During SAR, messenger RNA coding for acidic and basic PR-1, PR-2, PR-3, and PR-5 proteins were shown, in many pathosystems, to accumulate in high amounts (Brederode et al., 1991; Ward et al., 1991). The timing of their expression has been related to the initiation and the duration of SAR without any causal-effect relationship thoroughly proven (Vleeshouwers et al., 2000; McGee et al., 2001). Our results show that *pr-1*and *pr-5* were early expressed in local leaves and that might be related to the LAR. However, an expression of the same genes in proximal and distal leaves is in accordance with a SAR-related induction.

In the light of the present findings, it may be concluded that PR-proteins, particularly PR-1 and PR-5 are a component of the defense mechanism 'puzzle' of potato against *P. infestans*. Their precise function is still unknown for the limitation of the infectious process in some cultivars such as Kennebec, and further studies are still needed to confirm whether they are components of the front or the back lines of defense. Our future investigations will be carried out in this way using real-time quantitative PCR as a more sensitive method for detection and quantification of gene expression.

Acknowledgements

This work was supported by a grant of the Natural Sciences and Engineering Research Council (NSERC-Canada) to Dr. Fouad Daayf. We would like to acknowledge Dr. P. Audy (AAFC, Fredericton, New Brunswick, Canada) for providing the *P. infestans* US-1 isolate.

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