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US-1 and US-8 genotypes of *Phytophthora infestans* differentially affect local, proximal and distal gene expression of phenylalanine ammonia-lyase and 3-hydroxy, 3-methylglutaryl CoA reductase in potato leaves

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Abstract

Differential expression of *pal1* and *hmgr2* was investigated using northern blot analysis in two potato cultivars (Russet Burbank (RB), susceptible and Kennebec (KB), moderately tolerant) after inoculation with two *Phytophthora infestans* isolates from the formerly (US-1) and currently predominant genotypes (US-8). The accumulation of *pal1* transcripts was weaker in response to US-8 as compared to US-1 and occurred earlier in KB than in RB. The stronger expression of *pal1* in response to US-1, as compared to US-8, is suggested to be due to defense gene suppression by the latter. No apparent strong accumulation of *hmgr2* transcripts was recorded in RB as compared to KB inoculated with either US-1 or US-8. The induction of *pal1* and *hmgr2* was first observed in un-inoculated (proximal) close to the inoculated leaflets, then in un-inoculated (distal) leaflets of leaves adjacent to the inoculated leaf, and finally in local inoculated leaflets. The stronger expression of the two genes in proximal and distal leaflets, as compared to the local site of inoculation suggests the translocation of signal(s) from this site to healthy parts of the plant.

Keywords: *Solanum tuberosum* L. (potato); *Phytophthora infestans* (Mont.) de Bary; Phenylalanine ammonia-lyase (PAL); 3-Hydroxy 3-methylgluatryl CoA reductase (HMGR); Differential induction; Local, proximal and distal expression; Northern blot

1. Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most devastating diseases on potato worldwide [18]. It is famous for the epidemics that destroyed potato crops in Europe in the 1840s and led to the Irish potato famine [2,18]. Ever since, it caused losses in both potato and tomato crops worldwide. However, it is over the last 10–15 years that late blight has re-emerged as one of the most important diseases on potatoes [17]. Concurrently to this re-emergence, populations of *P. infestans* have considerably changed. Surveys conducted in Canada during 1994–2000 showed that the previously predominant *P. infestans* US-1 genotype (A1 mating type) has been gradually replaced by the US-8 genotype (A2 mating type) [10,12–14,49,50]. An extensive literature, including epidemiological and genetic analyses, is available, describing the shifts in the clonal lineages of *P. infestans* populations in North America and worldwide [12,16,17,20,21,41]. However, relatively few studies thoroughly investigated differential potato interactions with isolates from different groups such as A1 and A2 mating types, or specifically with US-1 and US-8 genotypes, the formerly and currently predominant genotypes of the pathogen, respectively [13,17,38,43]. Isolates of the US-8 genotype were reported to have shorter latent periods, to cause larger lesions, and to produce more sporangia on detached potato leaflets [30] and to rotten tubers substantially faster [38] than their US-1 counterparts.

Abbreviations: PAL, phenylalanine ammonia-lyase; HMGR, 3-hydroxy 3-methylglutaryl-CoA reductase; RB, cv.Russet Burbank; KB, cv. Kennebec; h.p.i., hours post-inoculation.

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The mechanisms, by which such differences occur, either from the pathogen or the plant side, are still not fully understood. Biochemical and molecular bases for the much higher pathogenic success of the new *P. infestans* genotype in potato could be searched either on the pathogen side [4,7], because that is where the most apparent changes recently occurred, but also on the plant side [8,27,51], where consequent differential responses might have been developed. In fact, several of the studies published on host–pathogen interactions in the potato-late blight system were probably carried out using isolates from the old *P. infestans* population and only a few of them have included isolates from the new lineage [34]. Strains from different *P. infestans* genotypes (i.e. US-1 and US-8) constitute a material of choice toward characterizing the mechanisms that differentially regulate the activation of potato defense genes in response to the infection by *P. infestans*, and thus understanding potato defense limitations toward this oomycete. To date, however, no studies have been dedicated to the comparison of biochemical and/or molecular aspects of potato reaction specifically to US-1 and US-8.

PAL and HMGR are the key enzymes in the phenylpropanoid and terpenoid pathways, respectively (Fig. 1). They were both reported to play a role in the resistance mechanisms of many plants [31,32,48,54]. PAL was shown to be associated with the early induction of resistance in potato–*P. infestans* system during an incompatible interaction but not during a compatible interaction [11]. Similarly, HMGR catalyzes the first step in the sesquiterpenoid phytoalexins production [33], and has been reported to play an important role in induced resistance of both potato and sweet potato tubers [24,35,39,40,45].

The objectives of this study were: (i) to assess the accumulation of mRNA transcripts of both PAL and HMGR in potato leaves in response to inoculation with US-1 and US-8 genotypes of *P. infestans*, (ii) to follow such gene expression over time at three levels of the plant tissues: (a) leaflets inoculated with either genotype (local), (b) healthy leaflets from the inoculated leaf (proximal), and (c) leaflets from healthy leaves adjacent to the inoculated leaves (distal), and (iii) to conduct these studies on two potato cultivars with two levels of response to late blight: Russet Burbank (RB, susceptible), and Kennebec (KB, moderately tolerant).

2. Materials and methods

2.1. Plant materials

Potato plants (*Solanum tuberosum* L.) were produced from high quality seed tubers planted in clay pots containing soil–sand–peat–perlite mixture (4:4:4:1) and kept in a growth room at 20 ± 2 °C and 16 h photoperiod. Six weeks-old plants from RB and KB were used for inoculations. RB is a widely used commercial cultivar and is considered to be susceptible to late blight while KB is known to be a moderately tolerant cultivar. Leaves were collected from three different parts of healthy or inoculated potato plants; local leaflets, which represent the primary leaflets inoculated with *P. infestans*; proximal leaflets, which are the non-inoculated secondary leaflets of the inoculated primary leaf; and distal leaflets in reference to those from the leaf adjacent to the inoculated leaf. All leaf samples were frozen in liquid nitrogen after harvest and stored immediately at −80 °C until used. Additional inoculated and non-inoculated plants were kept as controls for the whole periods of experiments.

2.2. Pathogens and inoculations

Two *P. infestans* isolates were used in this study. The isolate FA1 (US-1) was provided by Dr P. Audy (AAFC, Fredericton, NB, Canada) while the isolate D1901 (US-8) was collected in Manitoba in 2001. Genotyping of these and other isolates was performed previously (unpublished results). Both *P. infestans* isolates were grown on rye B

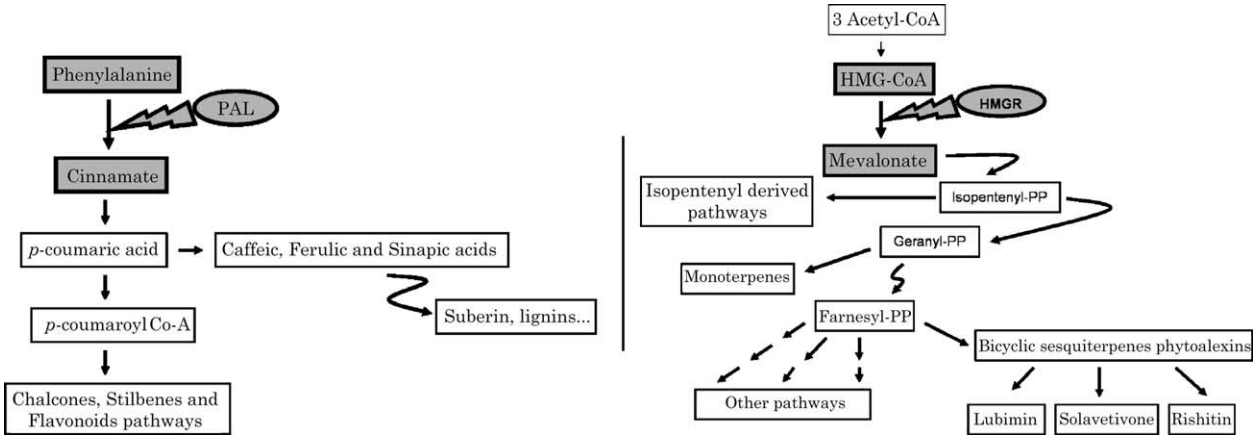

Fig. 1. Diagram showing PAL and HMGR as key enzymes of phenylpropanoid and isoprenoid biosynthesis pathways, respectively.

medium [20] at 20±2 °C. Virulence of the two isolates was maintained by inoculating them on potato leaves and re-isolating them every 3–4 months.

For the inoculation, 10–14 days old *P. infestans* cultures grown on rye B in Petri dishes (US-1 and US-8) were smashed down using sterile distilled water and sporangia were harvested the following day by flooding the plates with sterile distilled water. Inoculations of whole plants of RB and KB were performed by spraying on 100 µl of sporangia suspension adjusted at 5×10^6 sporangia ml^{-1} on the primary leaflet of the third or fourth leaf. The inoculum was deposited as multiple tiny droplets using a micropipette to prevent inoculum run off. To maintain the humidity required for infection, the inoculated potato plants were kept for 48 h in a misting chamber at 100% relative humidity. Three pots, containing two potato stems each, were considered per treatment (healthy controls RB and KB; US-1×RB, US-1×KB; US8×RB, US-8×KB) and per time point (0, 6, 12, 24, 48, 72, 96 and 120 hours post-inoculation (h.p.i.) in a randomized complete block design and the whole experiment was repeated twice overtime.

2.3. Physiologic race determination

The physiological races of the two *P. infestans* isolates (FA1 and D1901) were determined by their performance on a differential set of potato cultivars harboring late blight single resistance genes *R-1* to *R-11* (R0- Bintje, R1, Craig's Royal; R2, 1512 C16; R3, Pentland Ace; R4, 1563 C14; R5, 3053-18; R6, 2424a5; R7, 2182ef7; R8, 2424a5; R9, 2573(2); R10, 36581ad1; R11, 5008ab6; Platt, AAFC, PEI, Canada). For the inoculation, detached leaflets were used as described above. Physiological races were determined based on the presence or the absence of the hypersensitive reaction, and the extension of the infection lesion with or without sporulation on the leaf of each differential cultivar [14].

2.4. Differential pathogenicity of the two isolates on detached leaves and on whole plant

In parallel to the inoculated whole plants described above, leaflets collected from the third or the fourth leaf were incubated in vitro and inoculated with the two *P. infestans* isolates as an additional control for the pathogenicity testing. The leaflets were surface-sterilized using 70% ethanol, placed in Petri plates containing a humidified filter paper, and inoculated with 20 µl of each sporangia suspension adjusted at 5×10^6 sporangia ml^{-1}. After inoculation, the leaflets were incubated in a culture chamber maintained at 20±2 °C and 16 h photoperiod. Five detached leaflets were considered per treatment and the experiment was repeated independently two times. Infected leaflets of cultivars RB and KB from both types of inoculation (whole plant and detached leaflets) were harvested over time (0, 6, 12, 24, 48, 72, 96 and 120 h.p.i.). They were then scanned using a Microtek digital scanner Model 4800 (Microtek, Inc., Belleville, Ont., Canada) and the disease severity was assessed as percentage of diseased area using the image analysis software ASSESS developed in our department [36].

2.5. Preparation of total RNA

RNA was extracted only from the leaflets that were growing on the whole plant. These leaflets represent three strata: inoculated leaflets (inoculation site, local), un-inoculated leaflets from the same leaf where the main leaflet was inoculated (proximal), and un-inoculated leaflets from leaves adjacent to the inoculated leaves (distal). The samples were collected at 0, 6, 12, 24, 48, 72, 96 and 120 h.p.i. The harvested leaves were ground to a fine powder in a mortar pre-cooled with liquid nitrogen. Total RNA was isolated from 100 mg fresh weight following the method of Verwoerd et al. [55] and the absorbance at 260 nm and the ratio A_{260}/A_{280} were used to determine RNA concentration and purity. Leaf samples harvested from the two separate sets of inoculations were used separately for RNA extractions, gel blot or dot blot. However, within each experiment, leaflets collected from two stems grown in the same pot were pooled to ensure sufficient material for RNA extraction.

2.6. RNA dot blot and gel blot analysis

RNA analyses were carried out using both dot and gel blots. RNA dot blot hybridization, performed after loading the RNA on the membranes directly, without size separation, is a commonly used technique for gene expression assays to quantify the amount of transcripts. Northern gel blotting, performed by agarose gel electrophoresis of RNA, followed by its transfer onto a porous solid support (nylon or nitrocellulose membranes), is more suitable for size determination of transcripts. RNA dot blot analysis was carried out by depositing 10 µg of denatured total RNA of each sample on a Hybond–N^+ membrane (Hoffmann–La Roche Ltd, Mississauga, Ont., Canada). RNA gel blot analysis was performed on the same amount of RNA samples separated by electrophoresis under denaturing conditions. RNA was then transferred on a Hybond–N^+ membrane (Hoffmann–La Roche Ltd, Mississauga, Ont., Canada) and a pre-hybridization was performed for 3–4 h in DIG Easy high solution at 50 °C. The hybridization was conducted in the same solution at 50 °C for 18 h. Following hybridization, the membranes were washed in 2× SSC containing 0.1% SDS for 15 min at room temperature then in 0.5% SSC added with 0.1% SDS for 15 min at 68 °C.

2.7. Probes for hybridization

For probes synthesis, a 1.2 kb fragment of potato *pal1* genomic clone (Genebank no. X63103; [26]), representing the 3′ conserved region of *pal* [26], a 1.5 kb fragment of *hmgr2* genomic DNA clone (Genebank no. AB041031) that contains the highly conserved active site of the enzyme [9,44], and a 686 bp fragment of 18s rDNA (Genebank no. 67238) were used as DNA templates for PCR amplification. The probes were synthesized and labeled using a PCR DIG labeling kit (Hoffmann–La Roche Ltd, Mississauga, Ont., Canada) following the manufacturer's recommendations. The forward and reverse primers used for *pal1, hmgr2*, and 18s rDNA probe synthesis were designed using OligoPerfect™ Designer software (Invitrogen™ Life Science Software, Invitrogen, Inc., Ont., Canada). The primer sequences were

pal1-F	5′-GCGATTTTCGCTGAAGTG-3′
pal1-R	5′-TGTGCTTCGGCACTCTGA-3′
hmgr2-F	5′-TGACGCAATGGGAATGAA-3′
hmgr2-R	5′-ATGATGGCAAGGACCTCC-3′
18s-F	TAGATAAAAGGTCGACGCGG-3′
18s-R	5′-TCATTACTCCGATCCCGAAG-3′

The expected size of probes for *pal1*, *hmgr2* and 18s rDNA were 596, 530, and 686 bp, respectively. For each PCR reaction, a labeling mixture of 25 μl was preheated at 95 °C for 2 min followed by 25 cycles of 30 s at 95 °C, 30 s at 55 °C and 45 s at 72 °C using a programmed thermocycler (Techne Flexigene, Inc., Canada).

2.8. Image analysis

The data of *pal1* and *hmgr2* expression were based on three independent replicates of dot blots per set of experiment. Five micrograms 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 for image development to avoid the possible variation that may be caused by the slightly different exposure duration. The films were recorded using a digital camera at 1200 dpi resolution (Sony Ltd, Toronto, Ont., Canada) and the dots on the films were analyzed using ImageJ 1.32 software for Windows (http://rsb.info.nih.gov/ij/download.html). Background hybridization was measured by sampling membrane areas outside of the loaded lanes and the radiation counts were subtracted using ImageJ 1.32 software. After background subtraction the net signal from each lane was normalized for the amount of total leaf RNA in each lane. An initial normalization was performed with two sets of RNA blots, containing 15 and 1.5 μg of total 18s RNA in order to obtain a highly reproducible hybridization intensities among experiments. Corrections of slight differences in loading were made for each gel by normalizing the results against the constitutively expressed 18s rDNA gene. The intensity of hybridization signal represented by the integrated density ($\times 10^3$) was calculated from each dot. The average of three replicates ($\pm$SE) from each dot was considered to construct the final expression profiles of *pal1* and *hmgr2* transcripts.

3. Results

3.1. Differential pathogenicity of the two US-1 and US-8 isolates on potato detached leaflets (in vitro)

Pathogenicity of the US-1 and US-8 isolates was assessed on both potato cultivars (RB and KB). Small lesion spots were observed on most inoculated leaflets 36 h.p.i. On KB leaflets inoculated with US-1, lesions were of a dark black color, initially localized and developed later into a larger diseased area. In counterpart, RB leaflets inoculated with either US-1 or US-8 isolate, and KB leaflets inoculated with US-8 isolate showed more typical late blight lesions with a brown dark necrotic spot surrounded by a chlorotic ring (Fig. 2).

Fig. 3(a) shows the disease progress on detached leaflets during a period of 5 days post-inoculation (d.p.i.). No visible lesions were visible during the first 24 h.p.i. on either cultivar. However, after the first symptom appeared approximately 36 h.p.i., visible lesions then developed quickly on both cultivars. Entire RB leaflets inoculated with US-8 were destroyed 72 h.p.i. Comparatively, on KB detached leaflets, in spite of their apparent tolerance to both *P. infestans* genotypes, over 90% of the leaf area had late blight symptoms 5 days after inoculation with US-1 or US-8.

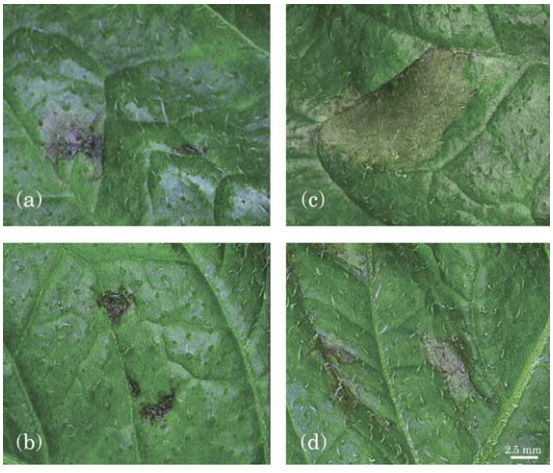

Fig. 2. Symptoms of late blight observed 72 h after inoculation on whole plants. RB (a) and KB (b) leaves inoculated with *P. infestans* US-1; RB (c) and KB (d) leaves inoculated with *P. infestans* US-8. Similar types of lesions were observed on inoculated detached leaves in vitro. The pictures show only a section of the leaflets with the typical lesions observed.

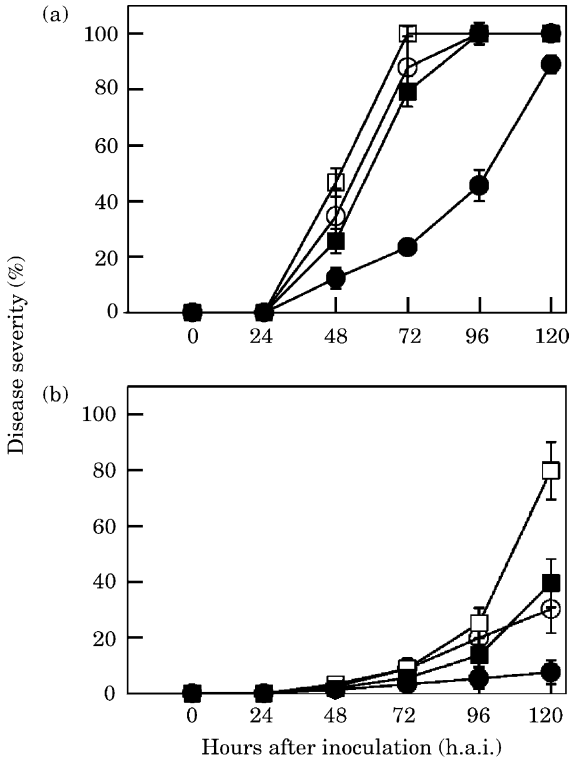

Fig. 3. Disease severity (% of necrotic leaf-surface area) on detached or whole plant (b) leaves. RB (–○–) or KB (–●–) inoculated with *P. infestans* US-1; RB (–□–) or KB (–■–) inoculated with US-8.

3.2. Differential pathogenicity of the two US-1 and US-8 isolates on whole potato plants

No symptoms were visible within the first 48 h.p.i. on either cultivar and only small lesions became apparent 48–72 h.p.i. Leaflets of RB inoculated with either US-1 or US-8 and KB inoculated with US-8 all had a chlorotic ring surrounding the necrotic lesion. A distinct localized necrotic lesion was only observed on KB inoculated with US-1, but later developed into a larger lesion (Fig. 2).

Disease progress on inoculated plants is shown in Fig. 3(b). Cultivar RB inoculated with US-8 had the highest disease severity (83%) 5 d.p.i. whereas KB inoculated with the US-1 isolate exhibited the lowest percent of diseased leaf area (8%). In general, disease severity was higher on RB than on KB inoculated with either US-1 or US-8. The US-8 isolate was more aggressive than US-1 on both cultivars.

3.3. Physiologic race determination

Physiologic races of the two isolates were different but both isolates had virulence factors to overcome at least five out of the 11 known *R* genes (Table 1). The physiologic race of the US-1 isolate was less complex than that of the US-8 isolate. The US-1 race was 1, 2, 4, 7, 9 while the US-8 isolate one was 1–4, 6–10.

3.4. Differential accumulation of *pal1* transcripts in potato leaflets inoculated with *P. infestans* US-1 or US-8

No noticeable difference in the induction of *pal1* was recorded in healthy leaflets (local, proximal and distal) of both RB and KB sampled over the experimental period of 5 days (Fig. 4). In inoculated samples, *pal1* was detected before the lesions became visible (Figs. 4–6).

3.4.1. Local leaflets

In RB leaflets inoculated with US-1 (local), the accumulation of *pal1* transcripts was noticeable 24–48 h.p.i. In contrast, the induction of *pal1* started 12 h.p.i. in KB leaflets inoculated with US-1 (local). Thereafter, the level of *pal1* transcripts was maintained at just about the detectable level in both cases until 120 h.p.i. In RB and KB leaflets inoculated with US-8 (local), mainly a baseline accumulation of *pal1* transcripts was detected with a slight increase in KB around 24 h.p.i. and in RB around 120 h.p.i. (Fig. 4).

3.4.2. Proximal leaflets

In RB leaflets un-inoculated, but adjacent to the ones inoculated with US-1 (proximal), a baseline of *pal1* expression was observed since 6 h.p.i. with its highest level recorded 24–72 h.p.i. Contrarily, in proximal KB leaflets from leaves inoculated with US-1, a rapid increase in the accumulation of *pal1* transcripts was observed and the highest induction was recorded 12 h.p.i. No accumulation of *pal1* transcripts above the basal level was observed in proximal leaflets of RB plants inoculated with US-8 while a slight accumulation was detectable in their KB counterparts around 72 h.p.i. (Fig. 5).

3.4.3. Distal leaflets

In un-inoculated (distal) leaflets of RB from leaves adjacent to the ones inoculated with US-1, a baseline of *pal1* induction was perceptible at 6 h.p.i., but a great

Table 1
Physiological races of *P. infestans* isolates FA-1 (US-1) and D1901 (US-8)

Isolate	d.p.i.	Potato *R* gene										
		1	2	3	4	5	6	7	8	9	10	11
FA-1	5	+	+	−	+	−	−	+	−	−	−	−
	10	+	+	−	+	−	−	+	−	+	−	−
D1901	5	+	+	+	+	−	+	+	+	+	+	−
	10	+	+	+	+	−	+	+	+	+	+	−

d.p.i., Days post-inoculation; +/−, presence/absence of visible lesion area on the inoculated leaves.

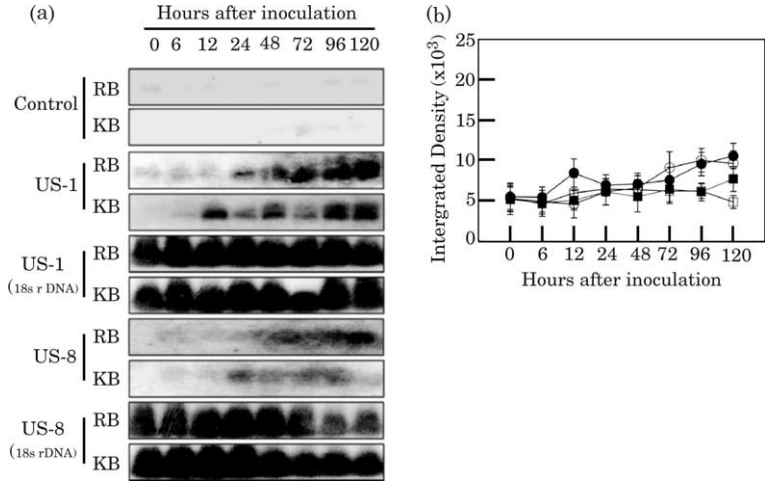

Fig. 4. Northern gel blots showing *pal1* transcripts (a) and their relative abundance on dot blots (b) (means ± SE) in local leaflets of two potato cultivars (KB and RB) healthy (control) or inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. 18s rDNA probe was used as internal control in the northern blot (a) to normalize the expression of *pal1*. No significant difference was observed in the accumulation of 18s rDNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (–○–) RB × US-1; (–●–) KB × US1; (–□–) RB × US-8; (–■–) KB × US-8.

accumulation was recorded 72 h.p.i. Comparatively, in distal leaflets of KB plants inoculated with US-1, the *pal1* transcripts accumulated to a detectable level since 12 h.p.i. with a maximum recorded 48 h.p.i. There was no detectable accumulation of *pal1* transcripts in distal leaflets of RB plants inoculated with US-8, whereas an accumulation was detected since 24 h.p.i in their KB counterparts with a maximum recorded 72 h.p.i. (Fig. 6).

3.4.4. Spatio-temporal accumulation of pal1 transcripts

The earliest accumulation of *pal1* transcripts was observed in proximal leaflets followed by local and distal leaflets. The induction of *pal1* in proximal leaflets was stronger than in local and distal leaflets. The pattern of *pal1* transcripts accumulation was also different in the two cultivars tested. Furthermore, the two isolates of *P. infestans* had different effects on the timing of *pal1* transcripts accumulation, which occurred earlier in response to the US-1 isolate than to the US-8 one (Figs. 4–6).

3.5. Differential accumulation of hmgr2 transcripts in potato leaflets inoculated with P. infestans US-1 or US-8

No accumulation of *hmgr2* transcripts was observed overtime in healthy leaflets (local, proximal and distal) from RB, whereas a baseline accumulation was recorded in KB (Fig. 7). In inoculated plants, the accumulation of *hmgr2* transcripts preceded the development of visible lesions. In general, the pattern of accumulation was different from that of *pal1* (Figs. 7–9).

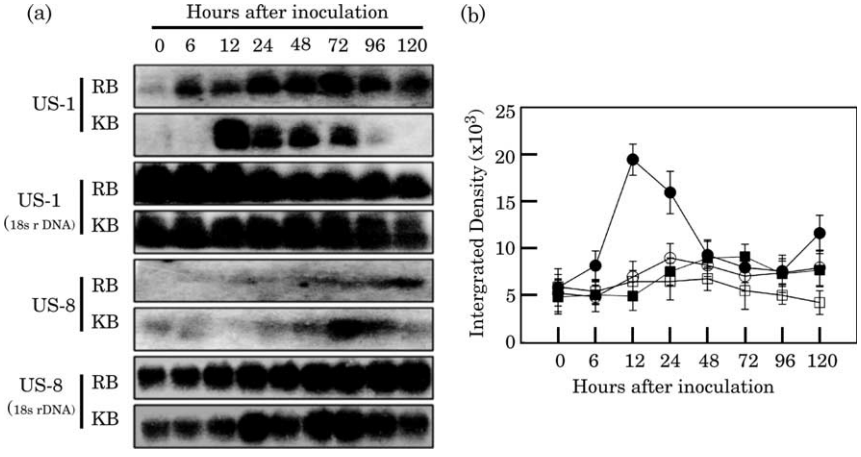

Fig. 5. Northern gel blots showing *pal1* transcripts (a) and their relative abundance on dot blots (b) (means ± SE) in proximal leaflets of two potato cultivars (KB and RB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. 18s rDNA probe was used as internal control in the northern blot (a) to normalize the expression of *pal1*. No significant difference was observed in the accumulation of 18s rDNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (–○–) RB × US-1; (–●–) KB × US1; (–□–) RB × US-8; (–■–) KB × US-8.

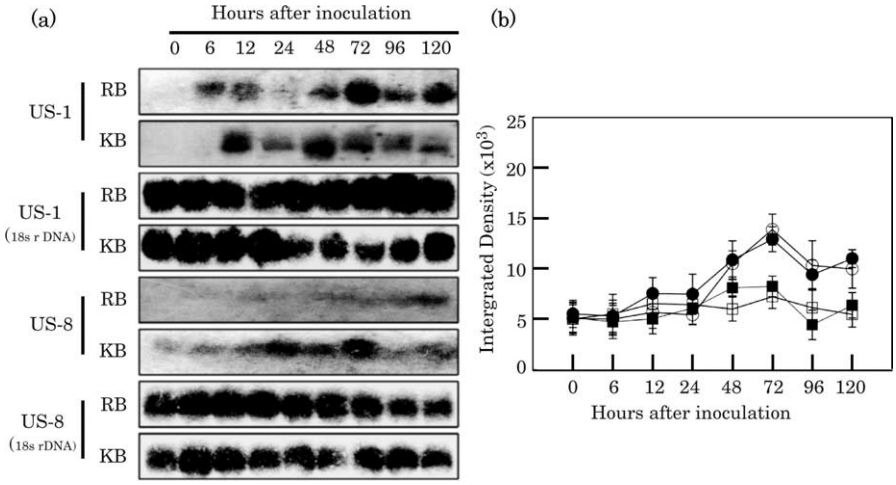

Fig. 6. Northern gel blots showing *pal1* transcripts (a) and their relative abundance on dot blots (b) (means ± SE) in distal leaflets of two potato cultivars (KB and RB) plants inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. 18s rDNA probe was used as internal control in the northern blot (a) to normalize the expression of *pal1*. No significant difference was observed in the accumulation of 18s rDNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (–○–) RB × US-1; (–●–) KB × US1; (–□–) RB × US-8; (–■–) KB × US-8.

3.5.1. Local leaflets

There was no accumulation of *hmgr2* mRNA transcripts in local leaflets of RB plants inoculated with US-1. In local leaflets inoculated with US-8, a slight induction was recorded 120 h.p.i. In KB leaflets only a baseline accumulation was observed overtime in both cases (Fig. 7).

3.5.2. Proximal leaflets

A strong transient accumulation of *hmgr2* transcripts was recorded 24–72 h.p.i. in proximal leaflets of KB plants inoculated with US-1. In contrast, no noticeable accumulation was observed in their RB counterparts. No noticeable accumulation of *hmgr2* mRNA transcripts was observed in proximal leaflets of either RB or KB plants inoculated with US-8, where only a baseline was perceptible (Fig. 8).

3.5.3. Distal leaflets

No visible accumulation of *hmgr2* transcripts was observed in distal leaflets of RB plants inoculated with either US-1 or US-8 except for a weak accumulation 120 h.p.i. in response to US-1. However, a strong accumulation of *hmgr2* transcripts was observed 48 h.p.i. in distal leaflets of KB plants inoculated with US-1. The accumulation level remained high until 120 h.p.i. A transient

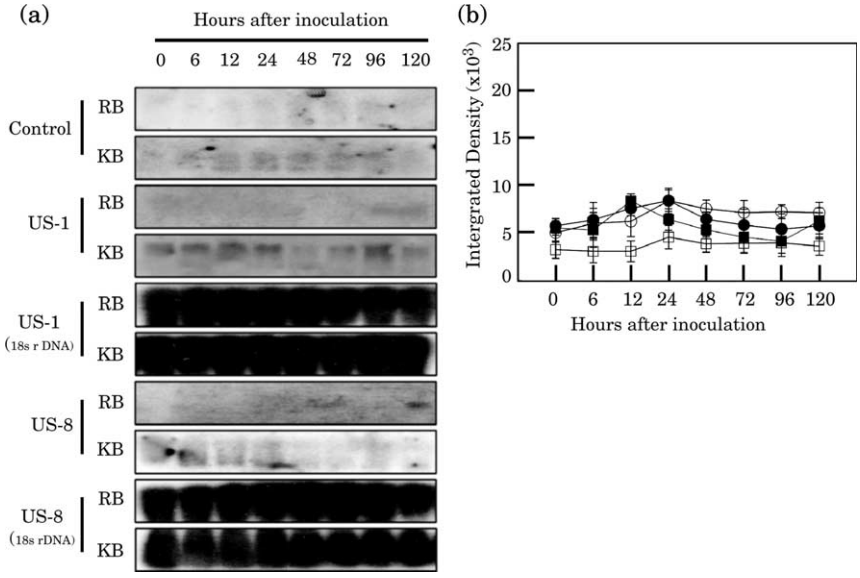

Fig. 7. Northern gel blots showing *hmgr2* transcripts (a) and their relative abundance on dot blots (b) (means ± SE) in local leaflets of two potato cultivars (KB and RB) healthy (control) or inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. 18s rDNA probe was used as internal control in the northern blot (a) to normalize the expression of *hmgr2*. No significant difference was observed in the accumulation of 18s rDNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (–○–) RB × US-1; (–●–) KB × US1; (–□–) RB × US-8; (–■–) KB × US-8.

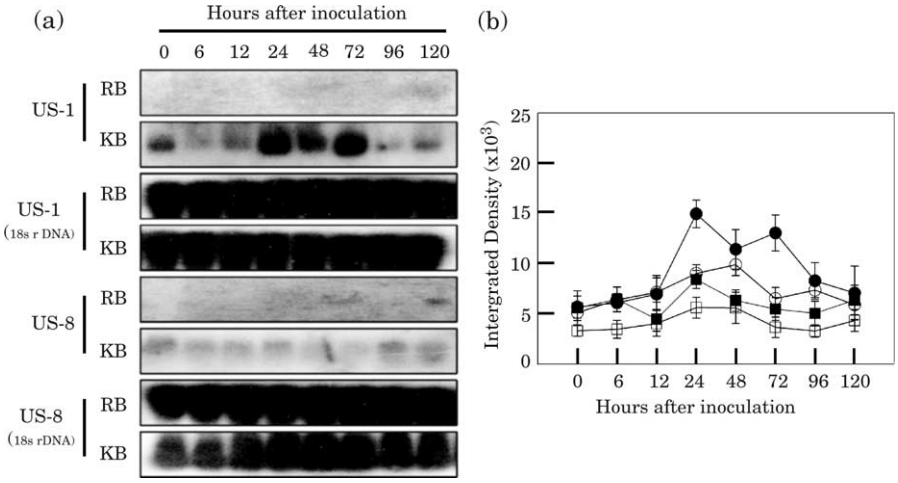

Fig. 8. Northern gel blots showing *hmgr2* transcripts (a) and their relative abundance on dot blots (b) (means ± SE) in proximal leaflets of two potato cultivars (KB and RB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. 18s rDNA probe was used as internal control in the northern blot (a) to normalize the expression of *hmgr2*. No significant difference was observed in the accumulation of 18s rDNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (–○–) RB × US-1; (–●–) KB × US1; (–□–) RB × US-8; (–■–) KB × US-8.

accumulation of *hmgr2* transcripts was observed also in distal leaflets of KB plants inoculated with US-8 48–72 h.p.i., but not as strong as with US-1 (Fig. 9).

3.5.4. Spatio-temporal accumulation of hmgr2 transcripts

The *hmgr2* transcripts accumulated early in proximal leaflets, then in distal leaflets of KB plants inoculated with US-1, whereas there was no induction of *hmgr2* in local leaflets of RB or KB, in response to US-1 or US-8, over the period of 120 h.p.i. The level of *hmgr2* transcripts observed in distal leaflets was relatively stronger than in proximal leaflets. Comparison between the two tested cultivars showed differences in the patterns of accumulation of *hmgr2* transcripts with no perceptible spatio-temporal accumulation in RB leaflets either in response to US-1 or US-8 and a strong transient accumulation in KB leaflets especially in response to US-1. Induction of *hmgr2* was greater in response to US-1 than to US-8, especially in KB (Figs. 7–9).

4. Discussion

The US-8 isolate used in this study was more aggressive than the US-1 isolate on both cultivars. The necrotic lesions observed on KB leaflets infected with US-1 initially looked

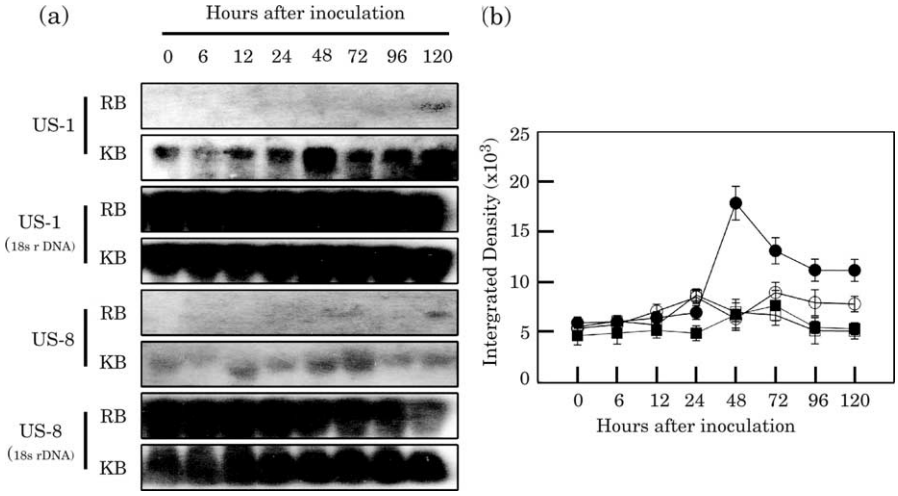

Fig. 9. Northern gel blots showing *hmgr2* transcripts (a) and their relative abundance on dot blots (b) (means ± SE) in distal leaflets of two potato cultivars (KB and RB) inoculated with two isolates of *P. infestans* from two genotypes: US-1 and US-8. 18s rDNA probe was used as internal control in the northern blot (a) to normalize the expression of *hmgr2*. No significant difference was observed in the accumulation of 18s rDNA transcripts in most samples. Where slight differences were observed, the amount of RNA samples loaded were adjusted accordingly. (–○–) RB × US-1; (–●–) KB × US1; (–□–) RB × US-8; (–■–) KB × US-8.

like HR lesions but their progression expanded overtime to the rest of the leaflet surface. The differential pathogenicity of the two isolates was observed in both detached leaflets and whole plants, with a slower progression of US-1 in the latter case. The faster disease progression on detached leaflets may be due, at least in part, to their 'surviving' state as nutrients supply from the original plant is removed.

Recent advances in genetic, biochemical and cytological characterization of disease resistance have shown constant relation between hypersensitive response (HR) and all forms of resistance to *Phytophthora* species [27,28,56]. The HR follows recognition between specific plant receptors encoded by resistance (*R*) genes and pathogen signal molecules encoded by avirulence genes (*Avr*) [15,22,37,53]. Cultivar KB harbors resistance gene *R-1* and was shown to be resistant to some US-1 isolates [34]. However, in the present study, the US-1 isolate was shown to be virulent on both KB and the potato differential line carrying *R-1* resistance gene as did the US-8 isolate. Therefore, differential reactions observed here toward the two isolates account under race-non-specific resistance.

Evidence suggesting that *pal* and *hmgr* genes are related to potato resistance against *P. infestans* has been reported by many authors. Cuypers and Hahlbrock [11] early reported that the accumulation of *pal* mRNA and proteins was faster during an incompatible potato×*P. infestans* interaction than during a compatible interaction. Similarly, Yoshioka et al. [57] has found that levels of *hmgr* mRNA and enzyme activity were higher during an incompatible than during a compatible interaction. Plant defense related phenolic compounds and sesquiterpenoid phytoalexins have been also shown to accumulate more rapidly in incompatible than in compatible interactions [11]. In the present study, we compare data in two compatible interactions, in both cultivars, where the two isolates have different levels of aggressiveness. Transcripts of *pal1* and *hmgr2* increased in both RB and KB leaflets inoculated with US-1. In response to this isolate, the accumulation of either gene's transcripts occurred at an earlier stage of infection than in response to US-8, and remained at a level above the expression baseline. In KB in particular, the increase in *pal1* and *hmgr2* transcripts in presence of the US-8 isolate was slower and much weaker, as compared to the relatively strong and early induction of these transcripts after inoculation with US-1. This was in line with the differential disease responses of cultivars KB and RB to the two isolates.

We also investigated the accumulation of *pal1* and *hmgr2* transcripts in leaflets from different parts of potato plants. In KB leaflets, there was a 12–24 h delay in induction of *pal1* and *hmgr2* mRNA in distal than in proximal leaflets. Similarly, Beligni et al. [5] showed that a GRAPDHc (glyceraldehydes-3-phosphate dehydrogenase) was induced 12 h earlier in un-inoculated short distance leaves (proximal) than in long distance leaves (distal). It might be reasonable to assume that the delay in *pal1* and *hmgr2* induction between proximal and distal leaflets is related to the time required for a signal(s) movement from inoculated leaflets to distant leaflets. However, more information is needed before we speculate on the nature of the translocated signal(s) during this interaction. Systemic plant responses have been described for several decades [23], and salicylic acid (SA), one of the potential signal molecules, has been previously shown to move from its production site in the inoculated leaves to remote leaves by the phloem [52].

There was no or a weak and late induction of *pal1* and *hmgr2* in local leaflets inoculated with either US-1 or US-8 while a noticeable early induction was observed in proximal and distal leaflets. This result is in agreement with previous findings by Choi et al. [9] and Yoshioka et al. [57] who reported the suppression of *hmgr* and *pal* mRNA accumulation in potato leaves infected by *P. infestans*, respectively, and with other findings reporting different suppressors of potato defense response against *P. infestans* [1,46,47]. Inhibition of *pal* expression was also found in other plant–pathogen interactions. Lee et al. [42] have reported that there was a substantial suppression, in presence of the pathogen, of mRNA levels in susceptible tomato lines as compared to resistant ones. All these findings indicate that the differential expression and inhibition of *pal* and *hmgr* in response to US-1 and US-8 may be directly related to the potato defense suppressors, previously reported in *P. infestans* [9,57]. The nature and the mode of action of these suppressors are still unclear. Andreu et al. [1] showed an inhibition of the accumulation of sesquiterpene phytoalexins in potato tubers by glucans produced by virulent isolates of *P. infestans* as compared to less virulent ones. Ozeretskovskaya et al. [47] observed that a β-glucan caused a local and race-specific suppression of the plant defense response. Under race-specific interaction, Ordoñez et al. [46] reported the presence of dominant specific suppressors of *R* gene function that were elicited by specific isolates of the fungus and that segregated in the host populations independently. One original finding in the present study is that despite the potential suppression at the site of inoculation, especially with the US-8 isolate, *pal1* and *hmgr2* expression were still detected in tissues remote from the inoculation site. This suggests the possible translocation of signal(s) escaping the initial suppression by the pathogen to healthy parts of the plant. Another hypothesis that conforms with our finding is that the effect of some *P. infestans* suppressors is only local, as suggested for β-glucans by Ozereskovskaya et al. [47].

The level of expression of *pal1* and *hmgr2* was different in potato plants inoculated with US-1 and US-8 isolates. This differential expression of the two genes is possibly regulated at the transcription level as shown for *pal* on French bean cell suspension [6] and for *hmgr* in eukaryotes in general [19,25]. Since we did not examine the enzymatic activity of PAL and HMGR in the present study, a post-transcriptional regulation mechanism of

these two proteins could not be excluded. With this regard, it has been reported that HMGR activity is controlled both at the translation and the transcription levels in aged potato tubers [57]. Few researches have suggested also that the binding activities of promoters *trans*-binding factors are responsible for the activation of genes involved in the phenlypropanoid pathway [3,29]. Although no information is available on the mechanism responsible for the initiation of *pal1* and *hmgr2* genes' translations, they seemed to be coordinately induced and/or slightly suppressed by US-1 and highly suppressed in the presence of the US-8 isolate. This suggestion is in agreement with the findings reported by Andreu et al. [1], who showed that the accumulation of sequiterpene phytoalexins, initially controlled by the activity of HMGR, was not affected by glucans produced by less virulent isolates of *P. infestans* while it was highly suppressed by those produced by highly virulent isolates.

Clearly, much more studies are necessary to dissect and understand the complex mechanisms involved in the gene regulation of potato defense responses to pathogens like *P. infestans*. However, in the light of the present findings, it may be suggested that the differential responses of KB to US-1 and US-8 isolates might be partially due to the timing and the level of induction of potato defense genes such as *pal1* and *hmgr2*. A suppression of induction of plant defense genes by the US-8 isolate at the local site of infection and a translocation of a signal to other healthy parts of the plant might be also hypothesized.

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