Short communication

Polar auxin transport is essential for gall formation by *Pantoea agglomerans* on gypsophila

LAURA CHALUPOWICZ¹, DAN WEINTHAL¹, VICTOR GABA¹, GUIDO SESSA², ISAAC BARASH² AND SHULAMIT MANULIS-SASSON^{1,*}

¹Department of Plant Pathology and Weed Research, ARO, Volcani Center, Bet Dagan 50250, Israel

²Department of Molecular Biology and Ecology of Plants, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv 61390, Israel

SUMMARY

The virulence of the bacterium *Pantoea agglomerans* pv. *gypsophila* (*Pag*) on *Gypsophila paniculata* depends on a type III secretion system (T3SS) and its effectors. The hypothesis that plant-derived indole-3-acetic acid (IAA) plays a major role in gall formation was examined by disrupting basipetal polar auxin transport with the specific inhibitors 2,3,5-triodobenzoic acid (TIBA) and *N*-1-naphthylphthalamic acid (NPA). On inoculation with *Pag*, galls developed in gypsophila stems above but not below lanolin rings containing TIBA or NPA, whereas, in controls, galls developed above and below the rings. In contrast, TIBA and NPA could not inhibit tumour formation in tomato caused by *Agrobacterium tumefaciens*. The colonization of gypsophila stems by *Pag* was reduced below, but not above, the lanolin–TIBA ring. Following *Pag* inoculation and TIBA treatment, the expression of *hrpL* (a T3SS regulator) and *pagR* (a quorum-sensing transcriptional regulator) decreased four-fold and that of *pthG* (a T3SS effector) two-fold after 24 h. Expression of PIN2 (a putative auxin efflux carrier) increased 35-fold, 24 h after *Pag* inoculation. However, inoculation with a mutant in the T3SS effector *pthG* reduced the expression of PIN2 by two-fold compared with wild-type infection. The results suggest that *pthG* might govern the elevation of PIN2 expression during infection, and that polar auxin transport-derived IAA is essential for gall initiation.

INTRODUCTION

The development of plant tumours or galls is associated with an increase in the number (hyperplasia) and size (hypertrophy) of cells triggered by the phytohormones indole-3-acetic acid (IAA) and cytokinin (CK) (Jameson, 2000). Gall formation caused by phytopathogenic bacteria has been attributed primarily to pathogen-secreted IAA and CKs (Morris, 1995). This notion gained early support from the observations that gall-forming bacteria deficient in IAA or CK production were nonpathogenic. Thus, the causal agent of the olive and oleander knot, *Pseudomonas*

savastanoi pv. *savastanoi*, became nonpathogenic on curing of the pIAA plasmid harbouring the indole-3-acetamide (IAM) pathway for IAA biosynthesis. Virulence could be restored by transformation with the wide-host-range vector, RSF1010, bearing the IAA biosynthesis pathway (Comai and Kosuge, 1980). In addition, removal of the phytohormone biosynthesis genes from the Ti plasmid of *Agrobacterium tumefaciens* abolished tumour production, demonstrating that IAA and CKs are required for tumorous growth (Zupan *et al.*, 2000). Finally, the essential role of CKs in leafy gall disease of numerous hosts caused by *Rhodococcus fascians* was strongly supported by the identification of a CK biosynthetic gene on the *fas* operon on the linear virulence plasmid pFID188 (Stes *et al.*, 2011). In contrast with bacterial-derived phytohormones, the contribution of plant-derived IAA and CK to gall formation by bacteria remains elusive. However, a recent article has suggested that plant-derived auxin plays an accessory role in symptom development of *R. fascians* (Stes *et al.*, 2012).

Pantoea agglomerans pv. *gypsophila* (*Pag*) causes galls on baby's breath (*Gypsophila paniculata* L.) (Cooksey, 1986), generally in the stem crown region. Galls are most common in gypsophila nurseries, preventing root development of cuttings and may limit propagation (Volcani, 1985). The pathogenicity of *Pag* is dependent on an indigenous plasmid, designated pPATH_{*Pag*} (see Barash and Manulis-Sasson, 2009). This plasmid harbours a pathogenicity island containing a hypersensitive response and pathogenicity (*hrp*)/*hrp* conserved (*hrc*) gene cluster encoding a type III secretion system (T3SS), a regulatory cascade activating the Hrp regulon, type III effectors and a gene cluster encoding for IAA and CK biosynthesis (Mor *et al.*, 2001; Nissan *et al.*, 2005). The pathogenicity of *Pag* is *hrp* dependent, as any mutation in the *hrp* gene cluster completely abolishes gall initiation (Mor *et al.*, 2001; Nizan-Koren *et al.*, 2003). By contrast, simultaneous inactivation of the pathways for the biosynthesis of IAA and CKs, including the plasmid-borne IAM pathway and the chromosome-located indole-3-pyruvate (IPyA) route for IAA biosynthesis, reduces gall size by approximately 50% without abolishing gall initiation (Manulis *et al.*, 1998). These findings suggest that gall formation by *Pag* relies primarily on T3SS effectors, which induce host auxin accumulation and lead to gall initiation. However, it could be argued that, in the light of the multiple IAA biosynthetic pathways in

*Correspondence: Email: shulam@volcani.agri.gov.il

bacteria (Spaepen *et al.*, 2007), the production of bacterial IAA *in planta* could be via pathways that are not expressed in culture. To circumvent this possibility and to examine the hypothesis that plant-derived IAA plays a major role in gall formation, the effect of disruption of polar auxin transport (PAT) in gypsophila on tumour induction by *Pag* was investigated.

PAT is mediated by putative auxin efflux carriers (the PIN gene family) (Gälweiler *et al.*, 1998) and putative auxin influx carriers (the AUX/LAX gene family) (Kleine-Vehn and Friml, 2008). PAT is essential for plant differentiation and plays a pivotal role in the regulation of numerous physiological phenomena (Kleine-Vehn and Friml, 2008). Accumulating evidence has suggested that PAT plays a decisive role in the establishment of local maxima of auxin concentration, leading to the stimulation of meristem formation (Kramer, 2004), possibly a prerequisite for organogenesis as well as hyperplasia and gall formation. Knowledge of the importance of PAT and its role in plant development was based initially on pharmacological tools [2,3,5-triiodobenzoic acid (TIBA) and *N*-1-naphthylphthalamic acid (NPA)], later supported by PIN mutants (Dhonukshe *et al.*, 2008; Kleine-Vehn and Friml, 2008). TIBA and NPA disrupt auxin efflux from the cell and prevent the basipetal movement of auxin by blocking PIN carrier cycling. Here, we report the decisive role of PAT in gall formation by *Pag*.

RESULTS

Stems of gypsophila plants were puncture inoculated with *Pag* above and below the lanolin ring containing TIBA, NPA or water, as described. Galls developed above and below the lanolin–water control ring within 3 weeks (Fig. 1A,C). In contrast, gall

formation was completely abolished when stems were inoculated below, but not above, the lanolin–TIBA ring (Fig 1B,D). Similar results were obtained when NPA was employed instead of TIBA (results not shown). These findings suggest that gall formation by *Pag*-inoculated gypsophila plants is dependent on the basipetal polar movement of plant-derived auxin. In contrast with *Pag*, TIBA did not inhibit gall formation by *A. tumefaciens* when inoculation was performed above and below the lanolin–TIBA ring (Fig. 2).

When the effects of TIBA and NPA on the colonization of *Pag* in gypsophila stems were examined, no significant differences in bacterial populations were observed between samples taken above or below the water–lanolin ring or samples taken above the TIBA– or NPA–lanolin ring during 96 h post-inoculation (hpi) (Fig. 3). However, colonization below the TIBA– or NPA–lanolin ring was reduced significantly at 48 hpi and reached an inhibition of 2.5 orders of magnitude at 96 hpi relative to the control (Fig. 3). Neither TIBA nor NPA at a concentration of 100 μ M showed an effect on the multiplication rate of *Pag* *in vitro* when grown on minimal or rich medium (results not shown).

The expression of *pagR* [a transcriptional regulator of the quorum-sensing (QS) system] and *hrpL* (an *hrp* regulatory gene) is stimulated significantly *in planta* by *Pag*-synthesized IAA (Chalupowicz *et al.*, 2009). It was therefore important to examine whether IAA of plant origin had a stimulating effect on the expression of these genes and other *Pag* virulence genes, namely *pthG* (a type III effector) and *iaaH* (encoding an IAA biosynthesis gene) (Barash and Manulis-Sasson, 2009). The expression of these genes was measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) in infected gypsophila stems below and above the water– and TIBA–lanolin rings. The expression of *hrpL*,

Fig. 1 Effect of blocking auxin polar transport on gall formation by *Pantoea agglomerans* pv. *gypsophilae* (*Pag*). Stems of 5-week-old gypsophila plants were puncture inoculated with a suspension of *Pag*824-1 above or below a lanolin ring containing water (A,C) or 2,3,5-triiodobenzoic acid (TIBA) (B,D). IS denotes inoculation site. Gall formation was examined 3 weeks after inoculation (20 plants per treatment). The mean gall size of three independent experiments for treatments (A), (B) and (C) was in the range 150–180 mg fresh weight.

Fig. 2 Effect of auxin polar transport on gall formation by *Agrobacterium tumefaciens*. Three-week-old tomato plants were puncture inoculated with bacterial suspension above or below a lanolin ring containing water (A,C) or 2,3,5-triiodobenzoic acid (TIBA) (B,D). IS denotes inoculation site. Gall formation was scored 4 weeks after inoculation of 20 plants per treatment. The mean gall size of three independent experiments for treatments (A)–(D) was in the range 180–230 mg fresh weight.

Fig. 3 Effect of auxin polar transport inhibitors on the colonization of *Pantoea agglomerans* pv. *gypsophila*. Stems of 5-week-old gypsophila were inoculated with bacterial suspensions above or below the lanolin ring containing 2,3,5-triiodobenzoic acid (TIBA) or *N*-1-naphthylphthalamic acid (NPA). Colonization was measured at intervals after inoculation. Results are the means ($\pm$ SD) of three independent experiments with three repetitions per time point. Bars indicate the standard deviation (SD) of three biological repeats and asterisks denote significant differences ($P < 0.05$) at 48, 72 and 96 h post-inoculation (hpi) for TIBA or NPA compared with other treatments, according to Student's *t*-test. cfu, colony-forming unit.

pagR and *pthG* was reduced significantly only when inoculation was below the TIBA ring (Fig. 4). At 24 and 48 hpi, *hrpL* and *pagR* transcription levels were reduced by approximately four-fold and those of *pthG* by approximately two-fold. In contrast, *iaaH* showed similar expression levels in infected stems independent of the

inoculation site, suggesting that the pathogen's IAA biosynthetic gene expression is not affected by plant-derived IAA. Similar results were obtained when NPA was employed instead of TIBA (results not shown).

To determine whether *Pag* infection exerts any effect on PAT expression, the transcript levels of PINs and AUX1 were examined. Because the gypsophila genome sequence is not available, PIN1, PIN2, PIN3, PIN7 and AUX1 primers were derived from the Arabidopsis genome. PCR products using the DNA template of *G. paniculata* were obtained only for PIN2 and AUX1. The PCR products were sequenced and used for the design of specific primers for qRT-PCR.

The PIN2 transcript levels were measured after inoculation with the wild-type strain and mutants of two type III effectors (*hsvG* and *pthG*), which are important in studies of *Pag* pathogenicity (Barash and Manulis-Sasson, 2009). The relative expression of PIN2 in the absence and presence of TIBA was measured by qRT-PCR at 24 hpi. PIN2 expression without TIBA increased 35-fold following inoculation by *Pag* or *MxhsvG* relative to mock inoculation (Fig. 5). However, expression of PIN2 after *MxpthG* inoculation was reduced significantly (about two-fold) relative to the wild-type strain. A significant reduction in PIN2 expression was observed in the presence of TIBA in all treatments (Fig. 5). In tissues collected from above the lanolin ring containing TIBA, the PIN2 expression level was similar to that of the mock treatment without TIBA (data not shown). PIN2 expression following *Pag*, *MxhsvG* and *MxpthG* inoculation was reduced approximately six-fold relative to the treatments without TIBA. However, *MxpthG* still retained its significant reduction of PIN2 expression relative to the wild-type. The effect of bacterial auxin production on PIN2

Fig. 4 Effect of 2,3,5-triiodobenzoic acid (TIBA) on transcript levels of virulence genes (*hrpL*, *pagR*, *pthG* and *iaaH*) during infection of gypsophila plants by *Pantoea agglomerans* pv. *gypsophila*. Stems of 5-week-old gypsophila plants were inoculated with a bacterial suspension above or below a lanolin ring containing TIBA. cDNA templates were generated from total RNA extracts of inoculated stems at 24 and 48 h post-inoculation (hpi). Gene expression was normalized with *gyrB* and *mdh* as internal references and *P. agglomerans* 824-1 at the time of inoculation as control. Results are means ($\pm$ SD) of three different experiments with three replicates per time. Bars indicate the standard deviation (SD) of three biological repeats and asterisks denote significant differences ($P < 0.05$) in gene expression of treatment 4 relative to treatment 2, according to Student's *t*-test.

Fig. 5 Expression of the auxin efflux carrier PIN2 in gypsophila plants inoculated with *Pantoea agglomerans* pv. *gypsophila* (*Pag*) and its mutants. Stems of 5-week-old gypsophila plants were puncture inoculated with bacterial suspension of *Pag*824-1, *MxhsvG* (mutant in *hsvG*), *MxpthG* (mutant in *pthG*) or water (mock) below a lanolin ring containing water or 2,3,5-triiodobenzoic acid (TIBA). cDNA templates were generated from total RNA extracts at 24 h post-inoculation (hpi). Gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and β -actin as internal references and mock-inoculated plants at zero time as control. Results represent means ($\pm$ SD) of three different experiments, each with three replicates. Asterisks denote significant differences ($P < 0.05$) in gene expression of *MxpthG* relative to *Pag*, according to Student's *t*-test. SD, standard deviation.

expression was also examined using a mutant in the indoleacetamide pathway (*iaaH*) (Manulis *et al.*, 1998), and showed similar levels to the wild-type (data not shown). The relative expression of AUX1 was not affected by inoculation with *Pag* or its mutant strains with or without TIBA (results not shown).

DISCUSSION

PAT-dependent local auxin gradients represent a common module for plant organ formation regardless of their mature morphology or developmental origin (Benková *et al.*, 2003; Friml, 2003). It could be hypothesized that, similar to the initiation of organogenesis, PIN-derived local auxin maxima, meristem precursors, may also be instrumental in the initiation of hyperplasia by *Pag*. The results of this study indicate that gall development incited by *Pag* in gypsophila is dependent on PAT activity (Fig. 1). This phenomenon could be analysed in the light of two hypotheses that are not mutually exclusive: (i) PAT provides a continuous IAA supply for optimal activation of the Hrp regulon, which is mandatory for gall development (Nizan-Koren *et al.*, 2003); (ii) the local auxin maxima generated by PAT might serve as a prerequisite for conferring gall initiation. Support for the first hypothesis is drawn from the observation that PAT inhibition by TIBA or NPA reduced significantly the bacterial colonization (Fig. 3) and expression of QS and *hrp* regulatory genes (Fig. 4). Gall initiation by *Pag* in gypsophila is dependent on *hrp*, and *hrp* genes are also responsible for bacterial multiplication *in planta* (Barash and Manulis-Sasson, 2009). A central role has been demonstrated for the QS system in controlling the *hrp* regulatory genes cascade, namely *hrpXY*, *hrpS* and *hrpL* (Chalupowicz *et al.*, 2009). Thus, disruption of the QS genes, *pagR* (transcriptional regulator) and *PagI* (synthase of the *N*-acyl-L-homoserine lactone signal) (Chalupowicz *et al.*, 2008), substantially reduces the transcription levels of the *hrp* regulatory genes and, consequently, the *hrp* regulon is not properly expressed (Nizan-Koren *et al.*, 2003). IAA appears to act as a signalling molecule for the *Pag* QS genes,

which regulate the expression of the *hrp* gene cluster (Chalupowicz *et al.*, 2009; Panijel *et al.*, 2011). The loss of bacterial-derived IAA biosynthesis reduces the gall size by about 50%, but does not prevent gall induction (Manulis *et al.*, 1998). It can be hypothesized that, although bacterial-derived auxin turns on the *hrp* regulon during the initial infection stages, it is not sufficient for full expression of the type III effectors to complete the disease cycle, and requires a continuous supply of PAT-derived IAA for gall development.

The second hypothesis implies that PAT is not merely a provider of IAA signalling which activates the Hrp regulon, but is also instrumental in attaining local auxin maxima for the initiation of hyperplasia. This hypothesis gains support from the 35-fold increase in PIN2 expression of inoculated gypsophila stems at 24 hpi relative to mock inoculation (Fig. 5). Thus, an elevated PAT threshold could be necessary to obtain local auxin maxima for gall initiation. The pathogen factors responsible for the latter phenomenon are not known. Although mutation in bacterial IAA production did not affect PIN2 expression, it might be speculated that type III effectors are involved. Elevated PIN2 expression by *Pag* can be attributed to PthG and possibly to other effectors, but not HsvG. The involvement of PthG is supported by a previous report in which the IAA level was increased in PthG-transgenic tobacco (Weinthal *et al.*, 2010). It is noteworthy that, on the one hand, PthG is affected by PAT-derived IAA via the *hrp* regulon and, on the other, induces PAT expression as a type III effector, by an as yet unknown mechanism.

Plant-pathogenic bacteria induce galls through various mechanisms and PAT may not always be an obligatory mechanism for gall initiation, as exemplified by *A. tumefaciens* (Fig. 2). The inability of TIBA or NPA to inhibit tumour formation by *A. tumefaciens*, as opposed to *Pag*, could be caused by the different pathogenicity modes of the pathogens: (i) *A. tumefaciens* employs oncogenes specifying IAA and CK with eukaryotic promoters that integrate into the plant genome (Binns and Costantino, 1998), whereas *Pag* employs T3SS effector peptides that must be constantly translocated into host cells (Barash and Manulis-Sasson, 2007); (ii) following infection, the *A. tumefaciens*-induced tumours develop independently of the pathogen (Binns and Costantino, 1998), whereas a continuous presence of *Pag* cells in the plant is necessary for gall development (Barash and Manulis-Sasson, 2007). These different pathogenicity mechanisms are also expressed in morphologically and anatomically distinct galls (Chalupowicz *et al.*, 2006). Because the IAA and CK biosynthetic genes in *A. tumefaciens* are integrated into the host genome via the T-DNA, their effect on gall formation might be equivalent to plant-derived phytohormones. Notably, galls of *A. tumefaciens* can differentiate to produce 'shooty' or 'rooty' galls as a result of T-DNA mutations with different IAA/CK ratios (Binns and Costantino, 1998), suggesting that organogenesis might be obtained either independently of PAT activity or, possibly, through oncogene regulation.

PAT activity affected significantly gall development in the Gram-positive bacterium *R. fascians* (Stes *et al.*, 2012), but was not obligatory as in *Pag*. Thus, an increase in auxin level via the IPyA pathway of *Arabidopsis* induced PAT, whereas CKs encoded by the *R. fascians* linear plasmid inhibited PAT activity (Stes *et al.*, 2011). This pathogen is unique among hyperplasia-inducing bacteria in provoking differentiated leafy galls.

The absolute requirement for PAT function has not yet been reported in any other tumour-inducing phytopathogenic bacteria apart from *Pag*. Among the gall-forming bacteria, only *Pag* and *P. savastanoi* have been reported to be *hrp* dependent (Barash and Manulis-Sasson, 2009; Sisto *et al.*, 2004). It may be of interest to determine whether PAT dependence is a characteristic of the *hrp*-dependent mechanism of gall formation.

EXPERIMENTAL PROCEDURES

The wild-type strain *Pag*824-1 (Manulis *et al.*, 1991), its marker exchange mutants *Pag*MxpH (Ezra *et al.*, 2000), *Pag*MxhsvG (Valinsky *et al.*, 1998) and *Pag*MxlaaH (Manulis *et al.*, 1998), and *A. tumefaciens* strain A7 (laboratory collection) were grown in Luria-Bertani (LB) broth or on agar plates at 28 °C, and maintained as glycerol stocks at –80 °C.

Pag strains and *A. tumefaciens* were used for pathogenicity tests on gypsophila and tomato, respectively. Gall development by *A. tumefaciens* on tomato is markedly faster than on gypsophila. Inoculation was performed on 5-week-old plants of *G. paniculata* cv. *perfecta* (Danzinger Ltd., Bet Dagan, Israel) or 3-week-old tomato plants (*Solanum lycopersicum* L. cv. 1125). Plants were grown in a peat-sand (1:1) mixture using standard glasshouse conditions (i.e. 25 ± 2 °C and a 16-h photoperiod).

Assays with the PAT inhibitors TIBA and NPA (Sigma-Aldrich, Rehovot, Israel) were performed by mixing each compound with lanolin (Sigma-Aldrich) to a final concentration of 100 µM. The control was lanolin mixed with water. Lanolin pastes were applied as a ring around gypsophila or tomato stems approximately 5–6 cm above the soil. Stems were puncture inoculated with bacterial suspensions (10 µL of 10⁸ cells/mL), 1–2 cm above or below the lanolin ring. Gall formation was examined 3 and 4 weeks after inoculation of gypsophila and tomato plants, respectively. Approximately 20 plants were used per treatment.

Bacterial colonization in gypsophila plants was performed by excising 10-mm samples of the inoculated stem above or below the lanolin ring at intervals, with five plants per treatment. Each stem segment was macerated in 1 mL of 10 mM phosphate buffer (pH 7.0) and serial 10-fold dilutions were plated onto LB plates supplemented with rifampicin (150 µg/mL). The results of three independent experiments were used to calculate the average number of colony-forming units (cfu) per gram fresh weight of stem. Statistical analyses were performed using Excel software (Microsoft, Seattle, WA, USA).

Total RNA for qRT-PCR analysis was extracted from gypsophila stems inoculated with *P. agglomerans* or mock-inoculated plants. For each time point and for each treatment (above or below lanolin), two separate pools of three inoculated segments (length, 10 mm) were cut from randomly selected plants and used for RNA extraction. The MasterPure RNA Purification Kit (Epicentre, Madison, WI, USA) was used to isolate total RNA

according to the manufacturer's instructions. RNA quantification was performed with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Epsom, Surrey, UK). To remove residual genomic DNA, the isolated RNA was treated with DNase (Turbo DNA-free; Ambion Inc., Austin, TX, USA). Reverse transcription was carried out with 1 µg of total RNA using a Verso cDNA Kit (Thermo Fisher Scientific) with random hexamer and oligo(dT) primers, respectively, for bacterial and plant cDNA synthesis. Triplicates of cDNA syntheses were performed for 45 min at 42 °C. cDNA samples were subjected to PCR amplification with Power SYBR Green PCR Master Mix (Applied Biosystems, Warrington, Cheshire, UK) in a final volume of 20 µL. Specific primers for *hrpL*, *pagR*, *iaaH* and *gyrB* for real-time PCR were used according to Chalupowicz *et al.* (2009). Primers for the bacterial genes *pthG* and *mdh* (malate-lactate dehydrogenase) and gypsophila *PIN2* were designed for qRT-PCR by the Primer Express 3.0 program (Applied Biosystems). Primer sequences were: *pthG*-Forw, 5'-GCATTGAGCAAGAGC ATTGG-3'; *pthG*-Rev, 5'-TAGCCGGGCTCGTTGTCA-3'; *mdh*-Forw, 5'-ACTCTGCGTAACCATTC-3'; *mdh*-Rev, 5'-GGTCAGATCAGCCTTC-3'; *PIN2*-Forw, 5'-TTACGGCTCCGTCAAATGGT-3'; *PIN2*-Rev, 5'-GCGACGAAA CGGTTTATGC-3'. Real-time detection was performed with a 7300 Real-Time PCR System (Applied Biosystems Instrument, Foster City, CA, USA).

Activation of Taq DNA polymerase was at 95 °C for 10 min, followed by a thermal cycling programme of 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The bacterial genes, *gyrB* and *mdh*, and the gypsophila genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin (Nissan *et al.*, 2012), were used as internal standards for RT-PCR normalization of bacterial and plant gene expression, respectively. The values from inoculated or mock-inoculated plants at inoculation time were used as control for expression of bacterial virulence genes or *PIN2* in gypsophila, respectively. Relative quantification and statistical analysis were performed with the real-time PCR data analysis software (Applied Biosystems Instrument). The results represent the mean of three different experiments with five replications per time point.

REFERENCES

- Barash, I. and Manulis-Sasson, S. (2007) Virulence mechanisms and host specificity of gall-forming *Pantoea agglomerans*. *Trends Microbiol.* **15**, 538–545.
- Barash, I. and Manulis-Sasson, S. (2009) Recent evolution of bacterial pathogens: the gall-forming *Pantoea agglomerans* case. *Annu. Rev. Phytopathol.* **47**, 133–152.
- Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G. and Friml, J. (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell*, **115**, 591–602.
- Binns, A.N. and Costantino, P. (1998) The *Agrobacterium* oncogenes. In: *The Rhizobiaceae, Molecular Biology of Model Plant-Associated Bacteria* (Spaink, H.P., Kondorski, A. and Hooykaas, P.J., eds), pp. 251–266. Dordrecht/Boston/London: Kluwer Academic Publisher.
- Chalupowicz, L., Barash, I., Schwartz, M., Aloni, R. and Manulis, S. (2006) Comparative anatomy of gall development on *Gypsophila paniculata* induced by bacteria with different mechanism of pathogenicity. *Planta*, **224**, 429–437.
- Chalupowicz, L., Manulis-Sasson, S., Itkin, M., Sacher, A., Sessa, G. and Barash, I. (2008) Quorum sensing system affects gall development incited by *Pantoea agglomerans* pv. *gypsophila*. *Mol. Plant-Microbe Interact.* **21**, 1094–1105.
- Chalupowicz, L., Barash, I., Panijel, M., Sessa, G. and Manulis-Sasson, S. (2009) Regulatory interactions between quorum-sensing, auxin, cytokinin and hrp-regulatory genes in relation to gall formation and epiphytic fitness of *Pantoea agglomerans* pv. *gypsophila*. *Mol. Plant-Microbe Interact.* **22**, 849–856.
- Comai, L. and Kosuge, T. (1980) Involvement of plasmid deoxyribonucleic acid in indoleacetic synthesis in *Pseudomonas savastanoi*. *J. Bacteriol.* **143**, 950–957.
- Cooksey, D.A. (1986) Galls of *Gypsophila paniculata* caused by *Erwinia herbicola*. *Plant Dis.* **70**, 464–468.
- Dhonukshe, P., Grigoriev, P., Fischer, R., Tominaga, M., Robinson, D.G., Hasek, J., Paciorek, A.T., Petrusek, J., Seifertova, D., Tejos, R., Meisel, L.A., Zazimalova, E., Gadella, T.W.J. Jr, Stierhof, Y.-D., Ueda, T., Oiwa, K., Akhmanova, A., Brock, R., Spang, A. and Friml, J. (2008) Auxin transport inhibitors impair vesicle motility and actin cytoskeleton dynamics in diverse eukaryotes. *Proc. Natl. Acad. Sci. USA*, **105**, 4489–4494.
- Ezra, D., Barash, I., Valinsky, L. and Manulis, S. (2000) The dual function in virulence and host range restriction of a gene isolated from the pPATH_{ehg} plasmid of *Erwinia herbicola* pv. *gypsophila*. *Mol. Plant-Microbe Interact.* **13**, 683–692.
- Friml, J. (2003) Auxin transport—shaping the plant. *Curr. Opin. Plant Biol.* **6**, 7–12.
- Gälweiler, L., Guan, C., Müller, A., Wisman, E., Mendgen, K., Yephremov, A. and Palme, K. (1998) Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science*, **282**, 2226–2230.
- Jameson, P. (2000) Cytokinin and auxin in plant–pathogen interactions—an overview. *Plant Growth Regul.* **32**, 369–380.
- Kleine-Vehn, J. and Friml, J. (2008) Polar targeting and endocytic recycling in auxin-dependent plant development. *Annu. Rev. Cell Dev. Biol.* **24**, 447–473.
- Kramer, E.M. (2004) PIN and AUX/LAX proteins: their role in auxin accumulation. *Trends Plant Sci.* **9**, 578–582.
- Manulis, S., Gafni, Y., Clark, E., Zutra, D., Ophir, Y. and Barash, I. (1991) Identification of a plasmid DNA probe for detection of strains of *Erwinia herbicola* pathogenic on *Gypsophila paniculata*. *Phytopathology*, **81**, 54–57.
- Manulis, S., Haviv-Chesner, A., Brandl, M.T., Lindow, S.E. and Barash, I. (1998) Differential involvement of indole-3-acetic acid biosynthetic pathways in pathogenicity and epiphytic fitness of *Erwinia herbicola* pv. *gypsophila*. *Mol. Plant-Microbe Interact.* **11**, 634–642.
- Mor, H., Manulis, S., Zuc, M., Nizan, R., Coplin, D.L. and Barash, I. (2001) Genetic organization of the *hrp* gene cluster and dspAE/BF operon in *Erwinia herbicola* pv. *gypsophila*. *Mol. Plant-Microbe Interact.* **14**, 431–436.
- Morris, R.O. (1995) Genes specifying auxin and cytokinin biosynthesis in prokaryotes. In: *Plant Hormones* (Davies, P.J., ed.), pp. 318–339. Dordrecht/Boston/London: Kluwer Academic Publishers.
- Nissan, G., Manulis, S., Weinthal, D., Sessa, G. and Barash, I. (2005) Analysis of promoters recognized by HrpL, an alternative sigma-factor protein from *Pantoea agglomerans* pv. *gypsophila*. *Mol. Plant-Microbe Interact.* **18**, 634–643.
- Nissan, G., Manulis-Sasson, S., Chalupowicz, L., Teper, D., Yehekel, A., Pasmanik-Chor, M., Sessa, G. and Barash, I. (2012) The type III effector HsvG of the gall-forming *Pantoea agglomerans* mediates expression of the host gene *HSVGT*. *Mol. Plant-Microbe Interact.* **25**, 231–240.
- Nizan-Koren, R., Manulis, S., Mor, H., Iraki, N.M. and Barash, I. (2003) The regulatory cascade that activates the Hrp regulon in *Erwinia herbicola* pv. *gypsophila*. *Mol. Plant-Microbe Interact.* **15**, 1249–1260.
- Panijel, M., Chalupowicz, L., Sessa, G., Manulis-Sasson, S. and Barash, I. (2011) Quorum sensing directly controls the Hrp regulatory cascade and the Gac/Rsm signal transduction pathway in the gall forming *Pantoea agglomerans* (Abstract). *Phytopathology*, **101**, S136.
- Sisto, A., Cipriani, M.G. and Morea, M. (2004) Knot formation caused by *Pseudomonas syringae* subsp. *savastanoi* on olive plants is hrp-dependent. *Phytopathology*, **94**, 484–489.
- Spaepen, S., Vanderleyden, J. and Remans, R. (2007) Indole-3-acetic acid in microbial and microorganism–plant signaling. *FEMS Microbiol. Rev.* **31**, 425–448.
- Stes, E., Vandeputte, O., El Jaziri, M., Holsters, M. and Vereecke, D. (2011) A successful bacterial coup d'état: how *Rhodococcus fascians* redirects plant development. *Annu. Rev. Phytopathol.* **49**, 69–86.
- Stes, E., Prinsen, E., Holsters, M. and Vereecke, D. (2012) Plant-derived auxin plays an accessory role in symptom development upon *Rhodococcus fascians* infection. *Plant J.* **70**, 513–527.
- Valinsky, L., Manulis, S., Nizan, R., Ezra, D. and Barash, I. (1998) A pathogenicity gene isolated from the pPATH plasmid of *Erwinia herbicola* pv. *gypsophila* determines host-specificity. *Mol. Plant-Microbe Interact.* **11**, 753–762.
- Volcani, Z. (1985) *Bacterial Diseases of Plants in Israel*. Bet Dagan: Agricultural Research Organization, The Volcani Center.
- Weinthal, D.M., Yablonski, S., Singer, S., Barash, I., Manulis-Sasson, S. and Gaba, V. (2010) The type III effector PthG of *Pantoea agglomerans* pv. *gypsophila* modifies host plant responses to auxin, cytokinin and light. *Eur. J. Plant Pathol.* **128**, 289–302.
- Zupan, J., Muth, T.R., Draper, O. and Zambryski, P. (2000) The transfer of DNA from *Agrobacterium tumefaciens* into plants: a feast of fundamental insights. *Plant J.* **23**, 11–28.