European Journal of Plant Pathology **104:** 85–91, 1998.

Detection of *Erwinia herbicola* pv. *gypsophilae* in gypsophila plants by PCR

Shulamit Manulis, Nina Kogan, Lea Valinsky, Orit Dror and Frida Kleitman
Department of Plant Pathology, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel (Fax: 972 3-9683543)

Accepted 29 October 1997

Key words: Bio-PCR, diagnosis, galls, nested-PCR

Abstract

Three PCR primer pairs, based on the cytokinins (etz) or IAA biosynthetic genes, were used for detecting *Erwinia herbicola* pv. *gypsophilae* in *Gypsophila paniculata* plants. The primers were specific to all gall-forming *E. herbicola* strains and distinguished them from saprophytic strains associated with gypsophila plants or from other gall-forming bacteria. In pure culture of the pathogen, less than one bacterial cell was detected with nested PCR using the *etz* primers - an increase of 100-fold in sensitivity as compared with single-round PCR. In the presence of plant extract a reduction of tenfold in sensitivity was observed by nested PCR. When cells were grown on a semi-selective medium prior to PCR (Bio-PCR), five cells from pure culture of the pathogen were detected. The bacteria could be detected by nested-PCR or Bio-PCR in symptomless gypsophila cuttings after 7 days. The Bio-PCR procedure described in this study can be used to establish disease-free nuclear stock of mother plants of gypsophila.

Introduction

Erwinia herbicola pv. *gypsophilae* is the most destructive pathogen of *Gypsophila paniculata* L. (Brown, 1934; Clark et al., 1989; Cooksey, 1986). Gypsophila (Baby's breath) is an ornamental used in commercial cut-flower production in Israel, California, Florida and some European countries (Cooksey, 1986; Shilo, 1985). The pathogen induces gall formation at wound sites mainly in the crown region of the stem. The main damage occurs during the development of rooted cuttings in the nurseries (Vigodsky-Haas et al., 1981; Volcani, 1985). The gall weakens the plant, causes defoliation, and ultimately, death of the whole plant (Volcani, 1985). Neither resistant clones nor effective chemical treatments are available. The only control measure is by the production of pathogen-free planting material through culture indexing which requires a zero tolerance level rating, followed by strict sanitation practices. This procedure allows the production of clean mother plants which are vegetatively propagated. The host range of *E. herbicola* pv. *gypsophilae* is restricted to *G. paniculata*, whereas another pathovar of *E. herbicola* is pathogenic on table beet as well as on gypsophila (Burr et al., 1991; Volcani, 1985).

Detection of the pathogen in Israel is currently based on the ELISA procedure (Manulis et al., 1991; Manulis, 1992; Maoz and Vigodsky-Haas, 1983). However, problems related to the specificity of the antiserum have been encountered due to the presence of several serotypes of the pathogen. Moreover, saprophytic strains of *E. herbicola* which are widespread in nature as an epiphyte on plants (Star, 1982) can not be distinguished from pathogenic strains by serological methods. Often time-consuming pathogenicity tests are necessary to confirm the presence of pathogenic strains whenever a positive result is obtained by the ELISA procedure. To overcome these problems we isolated a DNA probe and used it for the detection of *E. herbicola* pv. *gypsophilae* by colony hybridization (Manulis et al., 1991; Manulis, 1992). The DNA probe was taken from a unique plasmid of approximately 150 kb which was detected only in pathogenic strains of *E. herbicola* from different serotypes. This probe contains the genes for indole acetic acid (IAA) biosynthesis through the indole acetamide pathway (Clark

et al., 1993; Manulis, 1992) and 110 bp of the 5′ end of the cytokinin biosynthetic genes (Lichter et al., 1995). By colony hybridizaton we could distinguish between pathogenic and non-pathogenic strains, in all known serotypes. However, the latter technique was limited to 10^4 CFU per ml (Manulis, 1992). To avoid the labor-intensive procedure of colony hybridization and to increase the sensitivity of detection, we examined the use of PCR for detection of the pathogen. In the present study we describe a sensitive, specific and useful PCR-based procedure for detection of *E. herbicola* pv. *gypsophilae* in gypsophila plants. This procedure is simple and allows us to replace the colony hybridization for confirming positive results obtained by the serological method. It is also applicable for use in the large number of samples necessary for the establishment of clean nuclear stock of gypsophila mother plants.

Materials and methods

Bacterial strains and pathogenicity tests

Erwinia herbicola strains used in this study are listed in Table 1. All the Israeli strains have been identified by biochemical and immunological procedures as previously described (Manulis et al., 1991). Strains Eh1188, Eh2188, and Eh4188 were isolated from table beet by T. Burr (Cornell University, Geneva, NY, USA). Strains PD713, PD128 were obtained from J. Janse (Wageningen, the Netherlands). All the other *E. herbicola* strains were isolated from *G. paniculata* plants in Israel. *Pseudomonas syringae* pv. *savastanoi* strains ITM317, PBQ225 and ITM519 were obtained from N.S. Iacobellis (Bari, Italy). *Agrobacterium tumefaciens* strains 13B, IL2 and G114 were obtained from J. Haas (ARO, The Volcani Center, Israel). *E. herbicola* and *A. tumefaciens* strains were maintained on nutrient agar and *P. syringae* pv. *savastanoi* strains were maintained on KB agar. For Bio-PCR, *E. herbicola* strains were grown on a semi-selective medium as described by Manulis et al. (1991). To isolate naturally occurring saprophytic bacteria associated with the gypsophila plant, two leaves were removed from each of ten healthy cuttings 10 days after rooting initiation. The leaves were washed with sterile water and blended for 3 min in a Stomacher Lab-Blender (Seward, UK) with 5 ml of sterile saline solution. Fifty μl from each cutting were spread on LB agar plate. The plates were incubated for 2 days at 28 °C. The total bacteria growing on the plates was collected, diluted 10 and 100, times and used for PCR tests. In addition, 20 individual colonies were isolated in pure culture and tested by PCR. Pathogenicity tests were performed by dipping cuttings of *G. paniculata* var. perfecta (Danziger Ltd. Israel) into an *E. herbicola* suspension before rooting (Manulis et al., 1991).

Table 1. Specificity of the PCR reaction for pathogenic strains of *Erwinia herbicola*

E. herbicola strain	Gall Formation[1]	Serotype[2]	PCR products[3]
824-1	+	SI	+
3-1a	+	SI	+
24-2	+	SI	+
6-2	+	SI	+
13-2	+	SI	+
29-3	+	SI	+
420	+	SI	+
53	+	SI	+
PD713	+	SI	+
PD128	+	SI	+
350-1	+	SII	+
615	+	SII	+
611	+	SII	+
441	+	SII	+
102	+	SIII	+
Eh1188[4]	+	SIV	+
Eh2188[4]	+	SIV	+
Eh4188[4]	+	SIV	+
3-1	-	SI	-
717-2	-	SI	-
1-10	-	SI	-
135	-	SI	-
23-9	-	SI	-
24-8	-	SI	-
163-5	-	SII	-

[1] Pathogenicty tests were carried out on *Gypsophila paniculata* cuttings. + = formation of gall.
[2] SI, SII, SIII, SIV correspond to serotypes 1, 2, 3 and 4, respectively.
[3] PCR reactions were carried out with iaaH, etzI and etzII primers. The presence or absence of PCR products (443bp, 607bp and 522bp) is indicated by + and -, respectively.
[4] Strains were isolated from table beet.

Primers and PCR conditions

Three pairs of primers were designed according to the sequence of the indole acetamide hydrolase gene (*iaa*H) and the cytokinin biosynthesis gene (*etz*). Accession numbers in the EMBL data bank are L33866

for *iaa*H and Z46375 for *etz*. The sequences of the iaaH primers were 5′-TCCGTGATGGCGATGCAG, 5′-CCAACGACCTGTGGTCGG; for etzI 5′- GCAAAA-GAACGCGGCTGG, 5′- GGGTCTCTTGTTCCT-GCC; and for etzII 5′- GTGATAGCTCTGGACAGG, 5′- TCTTCTCCTGGTCGGTTG. Primers were synthesized by Biotechnology General, Nes Ziyona, Israel. Primers iaaH and etzI were used in single- and first-round PCR. For nested PCR, the etzII primers were used as internal primers of etzI.

PCR amplifications were carried out in a 25 μl reaction mixture which contained 0.5 mM $MgCl_2$, 0.2 μm of each primer, 50 μm of each dNTP, 0.05 units Taq DNA polymerase (Appligene, France), template DNA and buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100, 0.2 mg per ml BSA). Amplification was performed in a thermal cycler (Hybaid, Omn-E, UK). Denaturation was done at 94 °C for 1 min followed by 35 cycles of 1 min at 94 °C, 1 min at 58 °C, and 2 min at 72 °C; and a final extension for 5 min at 72 °C. Annealing temperature with primers etzII was 60 °C. The confirmation of the specificity of the PCR products was performed by Southern hybridization with DNA probes for etz (Lichter et al., 1995) and iaaH (Manulis, 1992).

Nested PCR and Bio-PCR

Tenfold serial dilutions of *E. herbicola* pv. *gypsophilae* (strain 824-1) culture grown overnight, were used as template DNA for first- or single-round PCR. The cells (5 μl) were frozen and after thawing were added directly to the PCR reaction mixture. For positive control, total DNA was used as the template. DNA was isolated as described by Manulis et al. 1991. For nested PCR, 1 μl from the first reaction was used as the template with primers etzII.

For Bio-PCR, 10 μl of cell suspension or plant extract were spotted on semi-selective agar plates. On one plate 35 drops were spotted. After incubation for 2 days at 28°C, the cells were collected with a toothpick and suspended in 500 μl of distilled water. Five μl of this suspension were frozen and thawed before being subjected to single-round PCR with etzI primers. PCR reaction products were detected by electrophoresis through 1.2% agarose gels.

Detection of E. herbicola *pv. gypsophilae in the presence of plant extract and in gypsophila plants*

Plant extract was obtained by removing two leaves from each of ten healthy cuttings 10 days after rooting initiation. The leaves were washed with sterile water and blended for 3 min in a Stomacher Lab-Blender with 20 ml of sterile water. Tenfold serial dilutions of *E. herbicola* pv. *gypsophilae* strain 824-1 were added to 1 ml aliquots of the extracts giving a final concentration of 10^5 to 10^1 cells per ml. For the first round of PCR, 5 μl of each dilution were added to the PCR reaction mixture.

For detection of *E. herbicola* pv. *gypsophilae* in gypsophila plants, three cuttings were dipped into each concentration of bacterial suspension as described previously (Manulis et al., 1991). After 7, 14 or 21 days, two leaves from each cutting were removed, washed with sterile water and then blended in 2 ml of sterile water. Five μl of this extract were subjected to PCR. For Bio-PCR, 10 μl were spotted on semi-selective medium as described above. The experiments were repeated four times.

Results and discussion

Specificity of PCR for identification of E. herbicola *pv.* gypsophilae

The PCR products obtained with primers iaaH, etzI and etzII were in the expected size of 443, 607 and 522 bp, respectively (Figure 1). Since other gall-forming bacteria contained the phytohormone's genes (Morris, 1986), we examined the specificity of these primers to E. *herbicola* pv. *gypsophilae.* No PCR products were formed when amplification reactions were carried out with strains of *Pseudomonas syringae* pv. *savastanoi* (ITM317) or *Agrobacterium tumefaciens* (13B)(Figure 1). Four other strains of *P. s. savastanoi* (ITM519, PBQ225) and *A. tumefaciens* (G114, IL2) were also negative in PCR reaction (results not shown). For further testing the specificity of the primers to *E. herbicola* pv. *gypsophilae,* amplification reactions were performed with various strains of *E. herbicola*. Representative strains are listed in Table 1. It constitutes pathogenic strains from the known serotypes as well as non-pathogenic strains of *E. herbicola*. All the pathogenic strains produced the expected products with primers iaaH and etzI. No specific products were obtained with non-pathogenic strains (Table 1).

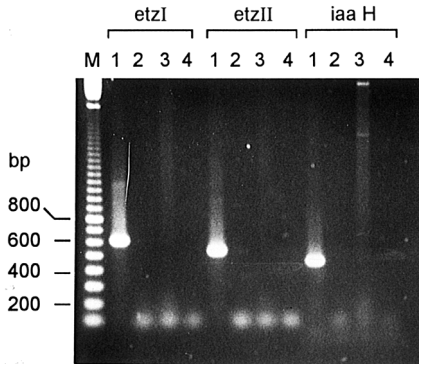

Figure 1. Single-round PCR using etzI, etzII and iaaH primers with *Erwinia herbicola*, *Agrobacterium tumefaciens* and *Pseudomonas syringae* pv. *savastanoi* templates. PCRs were carried out with 10^5cells in a reaction mixture. Lanes: M, 100bp ladder; 1 to 3, *E. herbicola* pv. *gypsophilae*, *A. tumefaciens* and *P. savastanoi* cells, respectively; 4, a PCR reaction without cells.

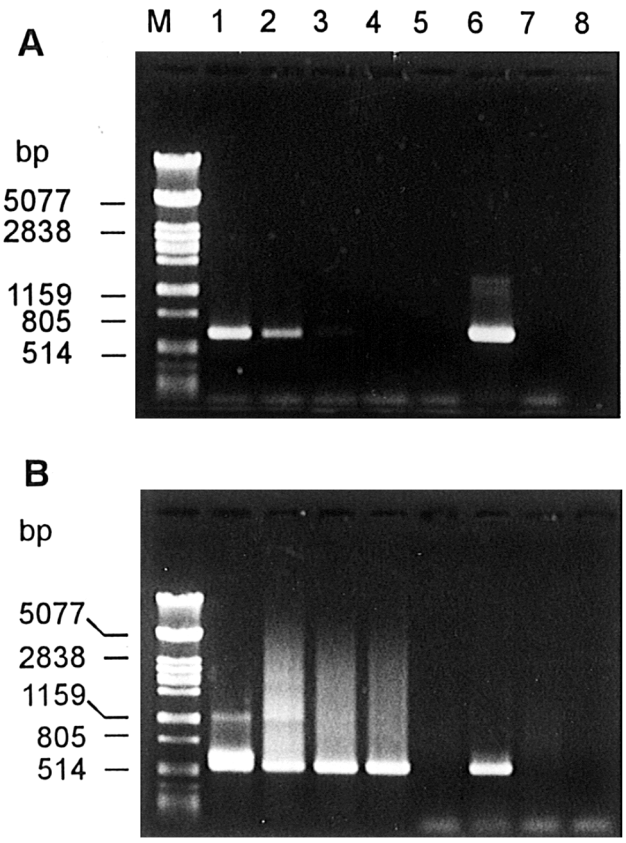

Figure 2. Detection limit of *Erwinia herbicola* pv. *gypsophilae* by first- and second-round of PCR. Tenfold serial dilutions of *E. herbicola* pv. *gypsophilae* culture were used and 5 μl of each dilution was added directly to the PCR mixture. A - first-round of PCR with primers etzI. B - Nested-PCR of amplification products of the first round, with primers etzII. Lanes: M-Lambda DNA digested by *Pst*I; 1 to 5, 10^5 to 10^1 cells per ml, respectively; 6, 10 ng of DNA of *E. herbicola* pv. *gypsophilae*; 7, control PCR without cells; 8, control of nested PCR.

Amplification reactions were also carried out with saprophytic bacteria isolated from gypsophila plants. Twenty purified colonies of saprophytes as well as ten pooled samples were examined. None of the bacteria associated with gypsophila plants showed the specific amplified PCR products.

Although the PCR primers were selected from genes present in other bacteria, they retained their specificity towards *E. herbicola* pv. *gypsophilae*. This was achieved by choosing non-conserved sequences of the IAA and cytokinin biosynthetic genes. The fact that the cytokinin gene of *E. herbicola* pv. *gypsophilae* had the lowest G+C content among all other cytokinin biosynthetic genes (Lichter et al., 1995) facilitated the selection of the primers. Comparison of the primer sequences in the GeneBank database did not show any homology to other phytohormone genes.

The use of virulence genes as a source for primers selection corroborates their reliability for identification of the pathogen. In case of *E. herbicola* pv. *gypsophilae*, we demonstrated previously the significance of the phytohormone genes as virulence determinants (Clark et al., 1993; Lichter et al., 1995). PCR primers based on plasmid sequences could be misleading if the plasmid is conjugative. However, in the case of the pPATH of *E. herbicola* pv. *gypsophilae*, we could not demonstrate transmissibility of the plasmid even to related non-pathogeneic strains (Manulis, unpublished data).

All primers were specific and suitable for performing the PCR reactions with *E. herbicola* strains. However, since preliminary data have shown that the selected nested primers from iaaH gene produced a smaller DNA fragment than the primers based on etz, further work was carried out only with the latter primers.

Sensitivity of the nested-PCR and Bio-PCR

The sensitivity of each procedure was first examined with tenfold dilutions of the pathogen, starting with 500 cells per reaction (Figure 2). Fifty cells were detected following the first round of PCR (Figure 2A), whereas the second round of PCR, using nested primers, increased the sensitivity by 100-fold (Figure 2B).

The effect of plant extract on the sensitivity of the detection limit was further examined by adding tenfold dilutions of the pathogen to crude extract of gypsophi-

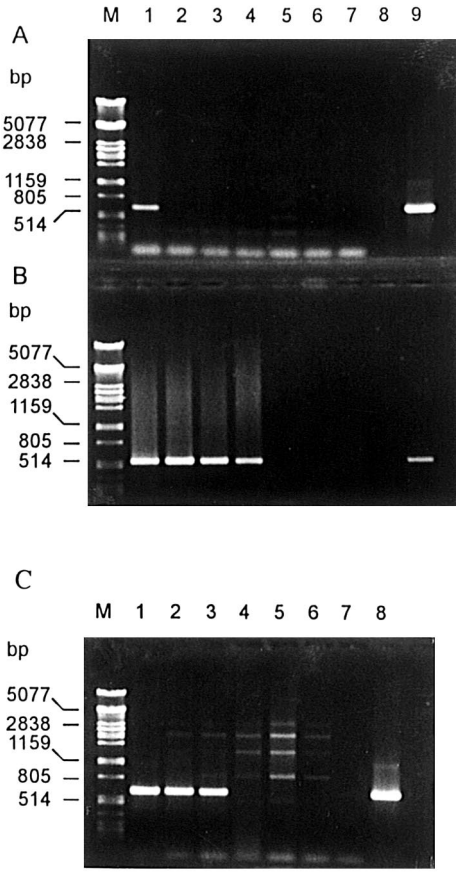

Figure 3. PCR reactions carried out in the presence of plant extract. Tenfold serial dilutions of *Erwinia herbicola* pv. *gypsophilae* culture were added to gypsophila extract; 5 μl of each dilution was added to the PCR mixture. A - first-round of PCR with primers etzI. B-Nested-PCR of amplification products of the first-round of PCR, with primers etzII. C - Bio-PCR with primers etzI. Lanes in A and B: M, molecular weight markers; 1 to 5, 10^5 to 10^1 cells per ml, respectively; 6, control of plant extract; 7, water; 8, control of nested-PCR; 9, 10 ng of DNA of *E. herbicola* pv. *gypsophilae*. Lanes in C: 1 to 7 as above; 8, PCR with 10 ng DNA.

Table 2. Detection of *Erwinia herbicola* pv. *gypsophilae* in inoculated gypsophila cuttings by three PCR-based methods

Cells/ml	Galls[1]	PCR I[2]	N-PCR[3]	Bio-PCR[4]	Days
10^5	-	+[5]	+	+	7
10^4	-	+	+	+	
10^3	-	-	+	+	
10^2	-	-	-	-	
10^1	-	-	-	-	
10^5	+	+	+	+	14
10^4	+	+	+	+	
10^3	-	-	+	+	
10^2	-	-	+	+	
10^1	-	-	-	-	
10^5	+	+	+	+	21
10^4	+	+	+	+	
10^3	-	-	+	+	
10^2	-	-	+	+	
10^1	-	-	-	-	

[1] Pathogenicity tests were conducted by dipping three cuttings of *Gypsophila paniculata* into each concentration of bacterial cell suspension. The experiments were repeated four times.
[2] First round of PCR with primers etz I.
[3] Nested PCR with primers etz II.
[4] Cultures were grown for 2 days on selective medium followed by, the first round of PCR.
[5] The presence or absence of PCR products (607 or 522 bp) is indicated by + and -, respectively.

la (Figure 3). Plant material reduced the sensitivity of the first-round PCR by tenfold (Figure 3A). However, using nested primers, less than one cell could be detected (Figure 3B). Thus, the detection threshold achieved in the presence of plant extract was as sensitive as in water (Figure 2B). This result can be explained by dilution of PCR inhibitors present in gypsophila extracts during the second-round PCR. Numerous reports have indicated that plant tissues contain inhibitors of PCR (Demeke and Adams, 1992; Hartung et al., 1996; McManus and Jones, 1995; Minsavage et al., 1994; Schaad et al., 1995). Several procedures have been suggested to overcome this problem, like GeneReleaser (Levy et al., 1994), Immunocapture PCR (Hartung et al., 1996; Kapperud et al., 1993; Morgan et al., 1991) and Bio-PCR (Schaad et al., 1995). In this study we used the Bio-PCR procedure since it is simple to perform, detects only culturable cells, and is less expensive than the other procedures. Bio-PCR using the etzI primers could detect five cells in the reaction mixture in the presence of gypsophila extract (Figure 3C). The sensitivity threshold was less than with nested-PCR, since only culturable cells could be detected.

Detection of E. herbicola *pv.* gypsophilae *in gypsophila plants*

Gypsophila cuttings were dipped in various concentrations of *E. herbicola* pv. *gypsophilae* culture; 7, 14 and 21 days later, symptoms production was recorded and PCR reactions were performed (Table 2). After 7 days no galls were formed at any concentration. However, with the first round of PCR we could detect the pathogen in cuttings that were inoculated with 10^4cells per ml. With nested PCR or Bio-PCR, the pathogen was

detected even in cuttings inoculated with 10^3cells per ml. The latter inoculum is the lower limit of bacterial cells that can cause tumors. No galls were formed at lower bacterial concentration up to 60 days. After 14 or 21 days galls were formed at 10^4cells per ml. With first-round PCR no increase in sensitivity of the detection limit was observed (Table 2). However, with nested PCR or Bio-PCR the pathogen was detected in cuttings inoculated with 10^2 cells per ml. These results demonstrate that by employing the PCR procedure for detecting the pathogen in gypsophila plants, an increase of 100-fold is obtained, compared with the previously used colony hybridization technique (Manulis, 1992).

The similar sensitivity of the Bio-PCR and nested PCR for detecting cuttings infected with a low level of the pathogen (Table 2) may suggest that no dead bacteria were present in cuttings up to 21 days. Outbreaks of the disease sometimes occur in greenhouses in spite of the intensive culture indexing. These could be explained by low levels of epiphytic populations of the pathogen which were not detected by serological methods or the colony hybridization technique.

Routine detection of the pathogen in mother plants is usually done by extracting a pool of 40 leaves taken from 20 different mother plants. Therefore it was of interest to examine if the above mentioned procedures enabled detection of one infected cutting in a mixture with 19 healthy cuttings. For this experiment cuttings of gypsophila were inoculated with 10^2, 10^3 and 10^4 cells per ml and Bio-PCR was performed after 2 weeks. Detection of the pathogen was achieved in all extracts regardless of the bacterial concentration used (results not shown).

The use of culture indexing to obtain disease-free propagation material is considered the preferred method for controlling bacterial diseases in ornamentals. Gypsophila production is based on establishing nuclear stock of mother plants used for propagation (Manulis et al., 1991; Maoz and Vigodskky-Haas, 1983; Volcani, 1985). Because of the abundance of saprophytic *E. herbicola* strains in nature (Star, 1982), including on gypsophila plants (Manulis, 1992), it was necessary to develop a molecular method for detecting the pathogenic strains. The probe we had developed previously (Manulis et al., 1991) solved the problem of specificity. However, the PCR-based method has the advantage of increased sensitivity and ease of use. Although PCR-based detection methods have been described for several plant pathogens (Henson and French, 1993), in each case it is important to optimize the reaction conditions and establish the most efficient protocol for implementing the method for routine large-scale screening of mother plants. In the present work we demonstrated that detecting low populations of the pathogen can be done by either nested-PCR or Bio-PCR. Each of the methods has its own disadvantages. Nested PCR is sensitive to cross-contamination among samples, detects also dead cells, and is relatively expensive for routine application. Although Bio-PCR requires 2 days of growth, it is the preferable method for detecting *E. herbicola* pv. *gypsophilae* in gypsophila plants since it detects culturable cells and is suitable for screening a large number of samples. It is also less expensive, since it requires single round PCR. The Bio-PCR procedure described in this study is now being used in Israel for confirmation of positive results obtained by the currently used ELISA procedure and for establishing clean nuclear stock of mother plants.

Acknowledgement

This work is a contribution 2080-E from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel

References

Brown NA (1934) A gall similar to crown gall produced on *Gypsophila* by a new bacterium. J. Agric. Res. 48: 1099–1112

Burr TJ, Katz BH, Abawi GS and Crosier DC (1991) Comparison of tumorigenic strains of *Erwinia herbicola* isolated from table beet with *E.h. gypsophila*. Plant Dis. 75: 855–858

Clark E, Vigodsky-Haas H and Gafni Y (1989) Characteristics in tissue culture of hyperplasias induced by *Erwinia herbicola* pathovar gypsophilae. Physiol. Mol. Plant Pathol. 35: 383–390

Clark E, Manulis S, Ophir Y, Barash I and Gafni Y (1993) Cloning and characterization of *iaa*M and *iaa*H from *Erwinia herbicola* pathover *gypsophilae*. Phytopathology 83: 234–240

Cooksey DA (1986) Galls of *Gypsophila paniculata* caused by *Erwinia herbicola*. Plant Dis. 70: 464–468

Demeke T and Adams RP (1992) The effects of plant polysaccharides and buffer additives of PCR. Biotechniques 12: 332–334

Hartung JS, Pruvost OP, Villemot I and Alvarez A (1996) Rapid and sensitive colorimetric detection of *Xanthomonas axonopodis* pv. citri by immunocapture and a nested-polymerase chain reaction assay. Phytopathology 86: 95–101

Henson JM and French R (1993) The polymerase chain reaction and plant disease diagnosis. Annu. Rev. Phytopathol. 31: 81–109

Kapperud G, Vardund T, Skjerve E, Hornes E and T. Michaelson T (1993) Detection of pathogenic *Yersinia enterocolitica* in foods and water by immunomagnetic separation, nested polymerase chain reactions and colorimetric detection of amplified DNA. Appl. Environ. Microbiol. 59: 2938–2944

Levy L, Lee I-M and Hadidi A (1994) Simple and rapid preparation of infected plant tissue extracts for PCR amplification of virus, viroid, and MLO nucleic acids. J. Virol. Methods 49: 295–304

Lichter, A, Barash I, Valinsky L and Manulis S (1995) The genes involved in cytokinin biosynthesis in *Erwinia herbicola* pv. *gypsophilae*: characterization and role in gall formation. J. Bacteriol. 177: 4457–4465

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 (1992) Evaluation of a DNA probe for the detection of *Erwinia herbicola* strains pathogenic on *Gypsophila paniculata*. Plant Pathol. 41: 342–347

Maoz A and Vigodsky-Haas H (1983) Development of a serodiagnostic method for gall-forming bacteria in gypsophila. (Abstr.) Phytoparasitica 11: 239–240

McManus PS and Jones AL (1995) Detection of *Erwinia amylovora* by nested PCR and PCR-dot-blot and reverse-blot hybridizations. Phytopathology 85: 618–623

Minsavage GV, Thompson CM, Hopkins DL, Leite RMVB and Stall RE (1994) Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissure. Phytopathology 84: 456–461

Morgan JAW, Winstanley C, Pickup RW and Saunders JR (1991) Rapid immunocapture of *Pseudomonas putida* cells from lake water by using bacterial flagella. Appl. Environ. Microbiol. 57: 503–509

Morris RO (1986) Genes specifying auxin and cytokinin biosynthesis in phytopathogens. Annu. Rev. Plant Physiol. 37: 509–538

Schaad NW, Cheong SS, Tamaki S, Hatziloukas E and Panopoulos NJ (1995) A combined biological and enzymatic amplification (BIO-PCR) technique to detect *Pseudomonas syringae* pv. phaseolicola in bean seed extracts. Phytopathology 85: 243–248

Shilo R (1985) *Gypsophila paniculata*. In: Halevy A (ed) Handbook of Flowering (pp.83–87) CRC Press, Boca Raton, Fl.

Star MP (1982) The genus *Erwinia*. In: Star MP, Stolp H, Truper HG, Balows A and Schlegel HG (eds) The Prokaryotes: A Handbook on Habitats. Isolation and Identification of Bacteria. Vol. 2 (pp.1260–1271) Springer-Verlag, New York

Vigodsky-Haas H, Sharoni M, Zutra D, Farkas E and Kirshner B (1981) Crown gall of *Gypsophila paniculata*. (Abstr.). Phytoparasitica 9: 253

Volcani Z (1985) Bacterial Diseases of Plants in Israel. Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel 388 pp.(in Hebrew)