1   *Phytophthora* × *pelgrandis* **causes root and collar rot of** *Lavandula stoechas* **in Italy**

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15

16   **ABSTRACT**

17   In 2007, *Phytophthora* isolates with atypical morphological and biological characteristics

18   were found associated with root and collar rot of potted plants Stoechas lavender (*Lavandula*

19   *stoechas* L.) in an ornamental nursery in Italy. A polyphasic approach, including

20   morphological and cultural observations, sequencing the ITS-rDNA region, the *Pheca* and the

21   mitochondrial *cox*I genes, multiplex PCRs with primers specific for *P. nicotianae* or *P.*

22   *cactorum*, as well as RAPD-PCR, was used to characterize these isolates. On the basis of

23   morpho-cultural and molecular analyses the isolates from Stoechas lavender were identified

24   as *Phytophthora* × *pelgrandis,* a natural hybrid of *P. nicotianae* x *P. cactorum* previously

25   reported in other European countries, Americas and Taiwan, as a pathogen of ornamentals and

26 loquat plants. In pathogenicity tests using potted plants of Stoechas lavender the *P.* ×

27 *pelgrandis* isolates, similarly to the parental species *P. nicotianae*, induced the symptoms

28 observed on plants with natural infections and were reisolated only from artificially

29 inoculated plants. Dispersal of *P.* x *pelgrandis* on this host could exacerbate the damage

30 caused by Phytophthora root and collar rot of which main causal agent presently is *P.*

31 *nicotianae* on lavender in Europe. Application of hygienic measures are important to reduce

32 the proliferation and spread of the *Phytophthora* hybrids.

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35   Lavender (*Lavandula* spp., family Lamiaceae) is widely cultivated in Italy as an

36 ornamental and aromatic plant. It is estimated that lavender plants constitute about 30% of

37 approximately 20 millions potted aromatic plants produced each year in Liguria, the leading

38 producing Italian region (28). In Italy the most common disease of potted lavender in

39 nurseries is Phytophthora root and collar rot. *P. nicotianae* is the prevalent species causing

40 this disease. The pathogen was first reported on *Lavandula* spp. in Greece (33) and

41 subsequently on English lavender (*Lavandula angustifolia* Mill.) in several other countries,

42 including the USA (34), Italy (6,27), Spain (1) and Bulgaria (29). *P. palmivora* is another

43 species quite common on lavender; it was recorded both on French lavender (*L. dentata* L.) in

44 Spain (32) and English lavender in southern Italy (10) and Turkey (11). Another species

45 infecting lavender is *P. cinnamomi* that was found to be the causal agent of shoot and root rot

46 of English lavender in greenhouse beds and container-grown nursery in Poland (31). Hybrid

47 isolates of *P. nicotianae* and *P. cactorum,* formally described as *Phytophthora* × *pelgrandis*

48 W.F. Gerlach, Nirenberg & Gräfenhan nothosp. nov. (30) from *Pelargonium grandiflorum*

49 (Andr.) Willd., were also recovered from *Lavandula* sp. plants affected by root rot and basal

50 stem necrosis in greenhouse in The Netherlands (4) and more recently in Hungary (35).

51   In October 2007, symptoms of leaf chlorosis, wilting associated with root and basal stem

52   necrosis, and final collapse of the whole plant were observed on 4 to 6-month-old potted

53   plants of Stoechas lavender (*Lavandula stoechas* L.) in an ornamental nursery in Liguria,

54   northern Italy (lat 44°3'43'' N, long 8°11'5'' E;). Disease incidence was about 30% with 15%

55   dead plants in a nursery stock of 24,000 plants. Isolations from necrotic tissues of roots and

56   basal stem consistently yielded *Phytophthora* colonies exhibiting morphological features

57   resembling those reported for *Phytophthora* x *pelgrandis*. The aim of this study was to

58   identify and characterize the atypical *Phytophthora* isolates from Stoechas lavender using a

59   polyphasic approach, comprising morphological and cultural observations, sequencing of

60   nuclear (ITS-rDNA and *Pheca*) and mitochondrial (*cox*I) DNA regions as well as two

61   species-specific multiplex PCR and RAPD-PCR assays. In addition, pathogenicity tests were

62   performed to fulfill Koch's postulates.

63

Materials and methods

65   **Isolation and morpho-cultural characterization.** Isolations were made from 20

66   symptomatic Stoechas lavender plants with different disease severity. Basal stems and roots

67   were washed with tap water, rinsed in sterile H_2O and blotted dry with a filter paper, before

68   being cut into 5-mm pieces. Stem and root pieces were plated onto *Phytophthora* selective

69   medium PARP (16) in 9-cm-diameter Petri dishes (5 pieces per dish). Petri dishes were

70   incubated for five days at 24±1°C in the dark. Hyphal tips of colonies resembling the

71   morphology of *Phytophthora* spp. were subcultured on potato dextrose agar (PDA, Oxoid

72   Ltd, Basingstoke, UK) to obtain pure single-hyphae cultures. Isolates were then grown in 9-

73   cm-diameter Petri dishes containing 20 ml of either PDA or V8A (Campbell V8 juice agar) at

74   24±1°C for 14 days in darkness to determine the colony morphology. Temperature-growth

75   relationships of isolates were determined by transferring 5-mm-diameter plugs taken from the

76   margins of 7-day-old actively growing colonies at 24±1°C onto both PDA and V8A and

77   incubating at temperatures from 5 to 35°C with intervals of 5±1°C, in the dark. Colony

78   diameters were measured in two orthogonal directions five days after incubation and the mean

79   daily radial growth rate (mm day^{-1}) at each temperature was calculated. Maximum

80   temperature for growth was tested between 33 and 36°C with 1±0.2°C increments following

81   the method of Brasier *et al.* (5). Carrot agar (CA) plates started with three 4 mm actively

82   growing mycelial plugs were immediately transferred to the test temperatures and the extent

83   of mycelial growth was recorded daily for 3 days. Sporangia production was induced by

84   transferring 5-mm-plugs of 7-day-old mycelium grown on V8A into Petri dishes containing

85   sterile H$_2$O. Plates were incubated at room temperature (18 to 24°C) for 5-10 days under

86   natural light on the bench and features of sporangia were recorded for each isolate performing

87   70 measurements. Similarly, a total of 50 measurements of oogonia, oospores and antheridia

88   produced on V8A at 24±1°C after 14 days of incubation, were made for each isolate. A total

89   of five representative isolates (953/07, 956/07, 957/07 and STLV8 from roots, and STLV11

90   from stem) were characterized and each experiment included three replicate dishes per isolate

91   and culture medium. Each experiment was repeated twice. All isolates were deposited at the

92   culture collection of the Department of Agri-food and Environmental Systems Management,

93   Plant Pathology Section, University of Catania.

94   **DNA isolation.** Genomic DNA was extracted from two representative isolates (956/07 and

95   957/07) of the putative hybrid of *P. nicotianae* x *P. cactorum* recovered from Stoechas

96   lavender. Reference isolates of both putative parental species, i.e. *P. nicotianae* isolate IMI

97   398853 from blue Mediterranean fan palm (13) and *P. cactorum* isolate IRF14 from oregano

98   were included in this study. DNA was extracted from mycelium of 7-day-old colonies grown

99   in Petri dishes on V8A at 24°C by using DNeasy Plant Mini Kit according to the

100 manufacturer's instructions (Qiagen GmbH, Hilden, Germany). DNA concentration and

101   quality were determined using the Quant-it dsDNA BR assay kit and Qubit fluorometer

102   (Invitrogen). DNA samples were stored at -20°C before PCR amplification.

103   **ITS-rDNA, *cox*I and *Pheca* sequencing.** ITS regions of the rDNA repeat units of isolates

104   were amplified with the universal primers ITS6 and ITS4 according to the protocol described

105   by Cooke *et al.* (9). Sequencing of the cytochrome c oxidase subunit I (*cox*I) gene was

106   performed to identify the donor parental species of the mitochondrial genome. Templates of

107   the *cox*I gene cluster were amplified by using the primers FM 85 and the reverse complement

108   of FM 80 (FM80RC) (25). PCR reactions were done in 25 μl and contained 10 ng of DNA,

109   0.5 μM of each primer, 2.5 μl of 10x buffer, 100 μM of each dNTP, 2 mM $MgCl_2$, and 1 unit

110   of *Taq* DNA Polymerase (Invitrogen, Life Technology Corporation, Carlsbad, CA, USA).

111   Thermocycler parameters were 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C

112   for 30 s, 72°C for 90 s with a final extension at 72°C for 5 min.

113   Sequence analysis of a portion of phenol acid decarboxylase (*Pheca*) gene was performed to

114   establish the allelic state of this gene in the putative *P. nicotianae* x *cactorum* hybrid isolates.

115   Specific primers (PhecaF: 5'-GAAGCCGATCATGGTGGT-3' and PhecaR: 5'-

116   TGACCTCCACCTGCTGTACG-3') were designed by aligning available *Pheca* sequences

117   (*P. nicotianae*, FJ459730; *P. cactorum*, FJ459731; *P. nicotianae* x *cactorum*, FJ459732 and

118   FJ459733) (15) with ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and chemically

119   synthesized by Invitrogen (Invitrogen, Life Technology Corporation, Carlsbad, CA, USA).

120   Each PCR reaction mix contained 2.5 μl of 10x buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP,

121   0.5 μM of each primer, 10 ng DNA template, and PCR grade water to a total volume of 25 μl.

122   The PCR thermocycling conditions were as follows: 2 min at 95°C, and 35 cycles of 95°C for

123   20 s, 60°C for 30 s, 72°C for 45 s, followed by a final extension at 72°C for 5 min. The

124   experiment was repeated three times under the same conditions using different DNA extracts.

125   In all experiments, the presence of PCR products was confirmed by electrophoresis on 1.5%

126   agarose gel and used directly for sequence determination following treatment with ExoSAP-

127   IT (United States Biochemical, Cleveland). DNA sequencing was performed in both

128   directions from PCR products by BMR-Genomics (www.bmr-genomics.it) with the Big Dye

129   Terminator ver. 3.1 (Applera Corporation, Foster City, California, USA) on a 96-capillary

130   Applied Biosystems 3730xl DNA Analyzer.

131   **Species-specific multiplex-PCR and RAPD-PCR assays.** Multiplex ITS-PCR assay was

132   performed according to the protocol described by Bonants *et al.* (4) using the primer pairs

133   NICF1/NICR2.1 and CACTF1/CACTR1, specific for *P. nicotianae* and *P. cactorum*,

134   respectively. In addition, a different multiplex PCR assay (multiplex PN/PC-PCR) was also

135   performed by using the primer pair PNF/PNR designed by Kong *et al.* (17) on the basis of *P.*

136   *nicotianae*-specific sequences of the elicitin gene (*parA1*), and the SCAR primer pair

137   PC1/PC2 for the detection of *P. cactorum* (7). The multiplex amplifications were carried out

138   in 25 μl reaction mixtures as described for *Pheca* amplification and subjected to thermal

139   cycling (3 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 62°C and 1 min at 72°C, and a final

140   step of 10 min at 72°C) with a Perkin-Elmer Cetus (Norwalk, CT, USA) Gene Amp PCR

141   System 9600. A negative control using water instead of template DNA was included in all

142   PCR reactions. Amplicons were analyzed by electrophoresis in 1.5% agarose gel containing

143   SYBR Safe DNA gel stain (Invitrogen, Life Technology Corporation, Carlsbad, CA, USA) in

144   Tris-acetate-EDTA (TAE) buffer. To confirm the parental origin of the specific PCR products

145   obtained in multiplex ITS-PCR amplicons were directly sequenced as described above.

146   For RAPD-PCR, a total of 5 decanucleotide primers (OPB-12, OPB-15, OPB-16, OPE-18,

147   OPE-20) acquired from Operon Technologies Inc. (Alameda, CA, USA) were used.

148   Amplification reactions and post-PCR processes were performed according to the protocol

149   described by Faedda *et al.* (12).

150   **Pathogenicity tests.** Pathogenicity trials were conducted using 6-month-old plants of both

151   Stoechas lavender and English lavender cv Rosea that is known to be very susceptible to *P.*

152   *nicotianae* root rot (28). *P. nicotianae* reference isolates IMI 398853 and TL8VP from

153   lavender (21), one reference isolate of *P. cactorum* (IRF14), and two representative hybrid

154   isolates (956/07 and 957/07) from Stoechas lavender were tested. Inoculum was produced on

155   a mixture of vermiculite and autoclaved oat seeds (10). Plants were transplanted to pots filled

156   with a mixture of soil (sand/lime/peat 1:1:1) and 4% (vol/vol) inoculum. . Control plants

157   were grown in pots containing noninfested soil. After transplanting, all pots were flooded for

158   24 h by plugging the drain hole. Plants were grown in a greenhouse at 24±4°C. The test was

159   performed twice.

160

161   **Results**

162   **Morphological and cultural characterization.** Isolates with uniform colony morphology

163   were obtained from both basal stems and roots of all the 20 symptomatic Stoechas lavender

164   plants with a mean frequency of 30 and 70%, respectively. On PDA, cultures formed whitish,

165   tender cottony colonies with flattened mycelium and irregular stoloniferous margins. On

166   V8A, colonies showed a chrysanthemum pattern and abundant aerial mycelium (Fig. 1A, B).

167   Optimum growth temperature was 30±1°C and mean (± S.D.) radial growth rate of the 5

168   isolates were 10±0.5 and 14±1 mm day^{-1} on PDA and V8A, respectively. The maximum

169   temperature at which continuous growth occurred was 34 ±0.2°C. No growth was observed at

170   5 and 35°C.

171   Colonies developed on V8A were homothallic and produced smooth-walled oogonia

172   measuring 33.3±2.8 μm (mean of five isolates ± S.D.) diameter (range 26.9 to 38.5 μm);

173   oospores were aplerotic with a mean diameter of 28.5±2.1 μm (range 22.5 to 34.2 μm);

174   antheridia were predominantly amphigynous (Fig. 1E, F). A low proportion of abortive

175 oogonia (less than 20%) were also observed in all isolates. Sporangia produced in water were

176 subglobose with a prominent papilla, both caducous with a short pedicel (2-3 μm in length)

177 and persistent, sometimes with a lateral insertion in the sporangiophore. The average

178 sporangium size was 46.9±4.2 × 36.5±3.2 μm, with a mean length-to-width ratio of 1.28 (Fig.

179 1C, D). Chlamydospores, both terminal and intercalary, were spherical with a diameter

180 ranging from 26.2 to 30.1 μm.

181 **ITS-rDNA, mitochondrial DNA and *Pheca* analyses.** PCR amplification of ITS regions

182 from 956/07 and 957/07 isolates resulted in a single band of 831 bp. Direct sequencing of this

183 DNA fragment did not reveal any ambiguous nucleotide sites, and BLASTn search in

184 GenBank showed that sequences of these isolates were identical to those of several *P.*

185 *nicotianae* reference isolates (e.g. Accession Nos. GU902246 and HG709173).

186 PCR reactions of *cox*I amplified a DNA fragment of the expected size (654 bp) from all

187 *Phytophthora* isolates examined. The *cox*I sequences obtained from isolates 956/07

188 (Accession No. KC109828) and 957/07 (Accession No. KC109829) also showed 100%

189 identity with sequences of *P. nicotianae* isolates available in GenBank database (e.g.

190 Accession No. GU945494), including the sequence of the isolate IMI 398853 (Accession No.

191 KC109827).

192 PCR amplification of a portion of *Pheca* gene of all *Phytophthora* isolates using primers

193 PhecaF and PhecaR produced DNA fragments of the expected size (271 bp). Consensus

194 sequences of isolates 956/07 (Accession No. KC109825) and 957/07 (Accession No.

195 KC109826) revealed double bases at 12 positions, whereas both *P. nicotianae* IMI 398853

196 (Accession No. KC109823) and *P. cactorum* IRF14 (Accession No. KC109824) contained

197 whole sequence with single bases, as expected. Multiple sequence alignment showed 10

198 identical double bases at the same positions between sequences of isolates from Stoechas

199 lavender and loquat trees (*P. nicotianae* x *cactorum* 95023: Accession No. FJ459732, and

200   LT2852: Accession No. FJ459733) (15). In addition, Stoechas lavender isolates exhibited two

201   additional heterozygous sites at positions 136 and 178 as compared to those of loquat isolates

202   (Table 1). *Pheca* sequences of *P. nicotianae* IMI 398853 and *P. cactorum* IRF14 were 100%

203   identical to those of *P. nicotianae* LT215 (FJ459730) and *P. cactorum* LT198 (FJ459731)

204   isolates available in GenBank (15) (Table 1). In the hybrids examined in this study, all the

205   nucleotide differences were localized at positions wherein the sequences of *P. nicotianae* and

206   *P. cactorum* diverged.

207   **Multiplex and RAPD PCR analyses.** Multiplex ITS-PCR using primers specific for *P.*

208   *nicotianae* (NICF1 and NICR2.1) and *P. cactorum* (CACTF1 and CACTR1) produced a

209   single DNA fragment of the expected size for *P. nicotianae* IMI 398853 (700 bp) and *P.*

210   *cactorum* IRF14 (365 bp) reference isolates, while both products were amplified

211   simultaneously for the putative hybrid isolates from Stoechas lavender (Fig. 2). BLASTn

212   analysis in GenBank database showed that the 700 bp and 365 bp consensus sequences were

213   identical to those of *P. nicotianae* and *P. cactorum*, respectively. The alignment between

214   these sequences revealed altogether 7 heterozygous bases only at those positions wherein the

215   365 bp portion of ITS sequences of *P. nicotianae* and *P. cactorum* diverged. Therefore,

216   species-specific ITS-PCR assay followed by sequencing highlighted the coexistence of ITS

217   sequences of both *P. nicotianae* and *P. cactorum* in Stoechas lavender isolates, unlike the

218   direct sequencing of ITS amplicons with universal primers which did not reveal any

219   heterozygous sites. Likewise, in the multiplex PN/PC-PCR experiments with the primer pairs

220   PNF/PNR and PC1/PC2 both isolates from Stoechas lavender showed two bands of 230 bp

221   and 450 bp specific for *P. nicotianae* and *P. cactorum,* respectively. As expected, isolates IMI

222   398853 of *P. nicotianae* and IRF14 of *P. cactorum* gave a single PCR product of the

223   predicted size for each species (Fig. 3).

224   Highly reproducible RAPD profiles were obtained from isolates with all selected random

225   primers. Three primers out of five generated monomorphic bands specific for each isolate of

226   parental species and some of these species-specific bands were also observed in the patterns

227   of Stoechas lavender isolates (Fig. 4). These intermediate profiles were compatible with the

228   hybrid origin of isolates from Stoechas lavender with *P. nicotianae* and *P. cactorum* as

229   progenitors.

230   **Pathogenicity tests.** Three months after transplanting all plants grown in pots containing

231   soil infested with 956/07 and 957/07 hybrid isolates as well as with IMI 398853 and TL8VP

232   of *P. nicotianae* showed dieback symptoms and extensive root necrosis on both *Lavandula*

233   species tested. Plants grown in pots containing soil infested with the *P. cactorum* isolate

234   showed no aerial symptom and only few necrotic roots, suggesting that this species is weakly

235   pathogenic on lavender. Control plants remained healthy. *P. nicotianae* and *P. cactorum* as

236   well as both hybrid isolates were reisolated solely from roots of inoculated plants. The

237   identity of each species was confirmed by both morphological and cultural characteristics and

238   multiplex PCR analyses with species-specific primers, thus fulfilling the postulates of Koch.

239

240   **Discussion**

241   The putative hybrid nature of the *Phytophthora* isolates recovered from Stoechas lavender

242   in Italy was suspected on the basis of morphological traits, namely the stoloniferous colony

243   morphology on PDA peculiar of *P. nicotianae* and homothallism, which is by contrast

244   atypical for the latter, and was confirmed by the molecular analyses. It could be inferred from

245   DNA analysis that *P. nicotianae* and *P. cactorum* are the parental species of these hybrids as

246   in their genome the *Pheca* gene is in a heterozygous condition and each allelic form

247   corresponds to that of these two species. This conclusion is supported by the results of both

248   multiplex and RAPD PCR analyses showing the DNA of the isolates from Stoechas lavender

249   comprised regions from both *P. nicotianae* and *P. cactorum*. Analysis of *cox*I gene suggests

250   that these interspecific hybrids inherited the mitochondrial genome from *P. nicotianae* as

251   hitherto has been found in all these hybrids to date (15,24). Both morphological

252   characteristics and sequences of *Pheca* gene of the Italian hybrid isolates from Stoechas

253   lavender correspond to those of *P. nicotianae* x *cactorum* hybrids recovered from *P.*

254   *grandiflorum* and formally described by Nirenberg *et al.* (30) as *Phytophthora* x *pelgrandis*.

255   Natural hybrids between *P. nicotianae* and *P. cactorum* were first reported on various

256   ornamental plants, including *Lavandula* sp., grown in greenhouses in The Netherlands (4,24).

257   Later, two *Phytophthora* isolates from loquat trees (*Eryobotria japonica*) collected in central

258   Taiwan in 1995, which were earlier referred to as atypical strains of *P. nicotianae* (8), were

259   identified as hybrids of *P. nicotianae* and *P. cactorum* (22). Conceivably, the homothallic

260   *Phytophthora* isolates from loquat described by Weltzien and Schwinn (37) in Lebanon were

261   highly likely hybrids of the same species as their morphological characteristics were very

262   similar to those of isolates from Taiwan. More recently, hybrids originating from *P.*

263   *nicotianae* and *P. cactorum* were recovered from loquat in Peru (2). After natural hybrids of

264   *P. nicotianae* and *P. cactorum* were recovered from *P. grandiflorum* in Germany and

265   formally described as *Phytophthora* x *pelgrandis* (30), they were also reported with the same

266   name in the USA (20) as well as on common box, lavender and Port-Orford-cedar in different

267   ornamental nurseries in Hungary (35). In this study, *Phytophthora* x *pelgrandis* is reported for

268   the first time in Italy. *Phytophthora* hybrids generally show numerous abortive oospores,

269   heterozygous profiles for *Mdh-2*, *Mdhp* or *Gpi* isoenzymes, and ITS sequences of both

270   parental species (23); however in some instances a molecular polyphasic approach is required

271   to ascertain the hybrid nature of the isolates as direct sequencing of ITS-rDNA does not

272   always reveal intra-isolate heterogeneity. In fact, ITS sequencing of PCR products obtained

273   with universal primers ITS6 and ITS4 failed to discriminate the Stoechas lavender isolates of

274   *P.* x *pelgrandis* from *P. nicotianae* in this study and similar results were already reported by

275   Nirenberg *et al.* (30) for *P. grandiflorum* hybrids from Germany. However, amplification of

276   ITS region with specific primer pairs NICF1/NICR2.1 and CACTF1/CACTR1 revealed the

277   heterozygous condition of this region in the lavender Stoechas isolates. The inability of

278   generic primers to reveal the heterozygosity within the ITS-rDNA with direct sequencing may

279   be explained assuming that they contain a number of copies from the parental *P. nicotianae*

280   species larger than those of the other donor species. Moreover, it can be supposed that the

281   high proportion of G-C in *P. cactorum* ITS sequences might have favoured the amplification

282   of the allele of the other parental species *P. nicotianae* as demonstrated by Walsh et al. (37)

283   for other genes in humans. The heterozygous condition of ITS regions in lavender Stoechas

284   isolates was confirmed by the multiplex ITS-PCR assay. Likewise, multiplex PN/PC-PCR

285   provides an additional molecular tool to further demonstrate the hybrid nature of the Italian

286   Stoechas lavender isolates. Interestingly, the nucleotide differences at two positions in the

287   sequence of the *Pheca* gene between Italian isolates from Stoechas lavender and isolates from

288   loquat trees found in Taiwan and Peru (2) suggest these hybrids originated from independent

289   hybridization events involving parental species with intraspecific mutations.

290   Like the parental species *P. nicotianae*, *P. x pelgrandis* seems a quite common pathogen of

291   lavender as it was recovered from lavender plants affected by root rot and basal stem necrosis

292   in ornamental nurseries in several European countries, including The Netherlands (4),

293   Hungary (35) and now Italy. By contrast, *P. cactorum*, the other parental species, is an

294   occasional and weak root pathogen on this host. Pathogenicity tests indicate that natural

295   hybrids of these two species are as aggressive as the parental *P. nicotiane*, from which they

296   also inherited other ecological traits, such as a high optimum temperature and partly

297   amphigynous antheridia, and perhaps their persistent sporangia. Conversely, these hybrids

298   share with *P. cactorum* homothallism, partly paragynous antheridia and sporangium caducity.

299   It can be hypothesized that this novel combination of biological and ecological characteristics

300   may improve the fitness of the interspecific hybrids of *P. nicotianae* and *P. cactorum* and

301   their adaptability to the environmental conditions in nurseries of ornamentals. A high

302   optimum temperature, comparable to the optimum of the thermophilic parental species *P.*

303   *nicotianae*, conceivably favours their activity in greenhouse and during summer months,

304   while a minimum temperature higher than the minimum of some isolates of *P. nicotianae* (30)

305   may be conducive to early infections in late winter thus widening the favourable period for

306   the infections by hybrids. In addition, these hybrids can produce resting structures such as

307   chlamydospores that allow them to survive during winter or dry periods in soil or within

308   infected host tissues. Moreover the rapid spread of these hybrids in nurseries is favoured by

309   the production of caducous sporangia which are disseminated more easily than persistent

310   sporangia by irrigation water. So far only natural hybrids of few *Phytophthora* species have

311   been identified and described (19,23). Recently *Phytophthora andina* has also been identified

312   as a natural hybrid of *P. infestans* and a hitherto unknown other parent (14). The wide

313   diffusion of natural hybrids of *P. nicotianae* and *P. cactorum* and the differences in the

314   sequences of the *Pheca* gene between isolates, recovered from Stoechas lavender in Italy and

315   isolates from loquat trees found in Taiwan and Peru, indicate that breeding events between

316   these two *Phytophthora* species occur frequently even though *P. nicotianae* and *P. cactorum*

317   are in different ITS subclades (3,9,18), thus suggesting a higher affinity than inferred on the

318   basis of ITS sequences alone. Both parents evolved allopatric and hence may not have

319   developed premating barriers. By contrast, it can be supposed that the close similarity

320   between *P.* x *pelgrandis* isolates recovered from lavender in Hungary, Italy and Netherlands

321   would suggest these hybrids propagate clonally and are spreading in nursery of ornamentals

322   through global trade following commercial routes. Horticultural centres are known to be the

323   main propagators of pathogen dispersion (26). High-resolution whole genome DNA profiling

324 analysis (e.g. AFLP or microsatellites) would be required to determine whether the lavender

325 strains are really identical. As demonstrated by AFLP analysis, the three hybrid strains from

326 lavender in the Netherlands originated from at least two independent hybridization events (4).

327 The diffusion of *P.* x *pelgrandis* pathogenic isolates in cultivations of lavender could

328 exacerbate the damage caused by Phytophthora root and collar rot whose main causal agent in

329 Europe presently is *P. nicotianae*, one of the parental strains of these hybrids. Hygienic

330 measures such as presymptomatic testing of plants may reduce the proliferation and spread of

331 the *Phytophthora* hybrids.

332

333 **Acknowledgments**

340

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445

446 Table 1. Comparison of variable nucleotide positions identified in a 271 bp fragment of the Phenol acid

447 decarboxylase (*Pheca*) gene between *Phytophthora* hybrids and their parental species. R = G/A; S = C/G. *Pheca*

448 sequences FJ459733, FJ459732, FJ459730 and FJ459731 deposited in GenBank by Hurtado-Gonzales *et al.*

449 (2009) were used as references.

Accession No.	Isolate	Species	Nucleotide position											
			19	34	40	46	49	91	100	136	178	220	229	235
KC109825	956/07	*P.* x *pelgrandis*	R	R	R	R	S	S	S	R	R	R	R	R
KC109826	957/07	*P.* x *pelgrandis*	R	R	R	R	S	S	S	R	R	R	R	R
FJ459733	95023	*P.* x *pelgrandis*	R	R	R	R	S	S	S	A	A	R	R	R
FJ459732	LT2852	*P.* x *pelgrandis*	R	R	R	R	S	S	S	A	A	R	R	R
KC109823	IMI 398853	*P. nicotianae*	G	G	A	G	C	C	C	A	A	A	A	G
FJ459730	LT215	*P. nicotianae*	G	G	A	G	C	C	C	A	A	A	A	G
KC109824	IRF14	*P. cactorum*	A	A	G	A	G	G	G	G	G	G	G	A
FJ459731	LT198	*P. cactorum*	A	A	G	A	G	G	G	G	G	G	G	A

450 R = G/A; S = C/G. *Pheca* sequences FJ459733, FJ459732, FJ459730 and FJ459731 deposited in GenBank by

451 Hurtado-Gonzales *et al.* (2009) were used as references.

452

453

454 Table 2. Comparison of variable nucleotide positions identified in a 365

455 bp fragment of the ITS1-5.8S-ITS2 regions between *Phytophthora*

456 hybrids and their parental species.

Isolate	Species	Nucleotide position						
		1	15	27	47	48	55	363
956/07	*P.* x *pelgrandis*	R	W	R	K	Y	Y	Y
957/07	*P.* x *pelgrandis*	R	W	R	K	Y	Y	Y
IMI 398853	*P. nicotianae*	A	A	A	T	T	T	C
IRF14	*P. cactorum*	G	T	G	G	C	C	T

457 R = G/A; W = A/T; K = G/T; Y = C/T.

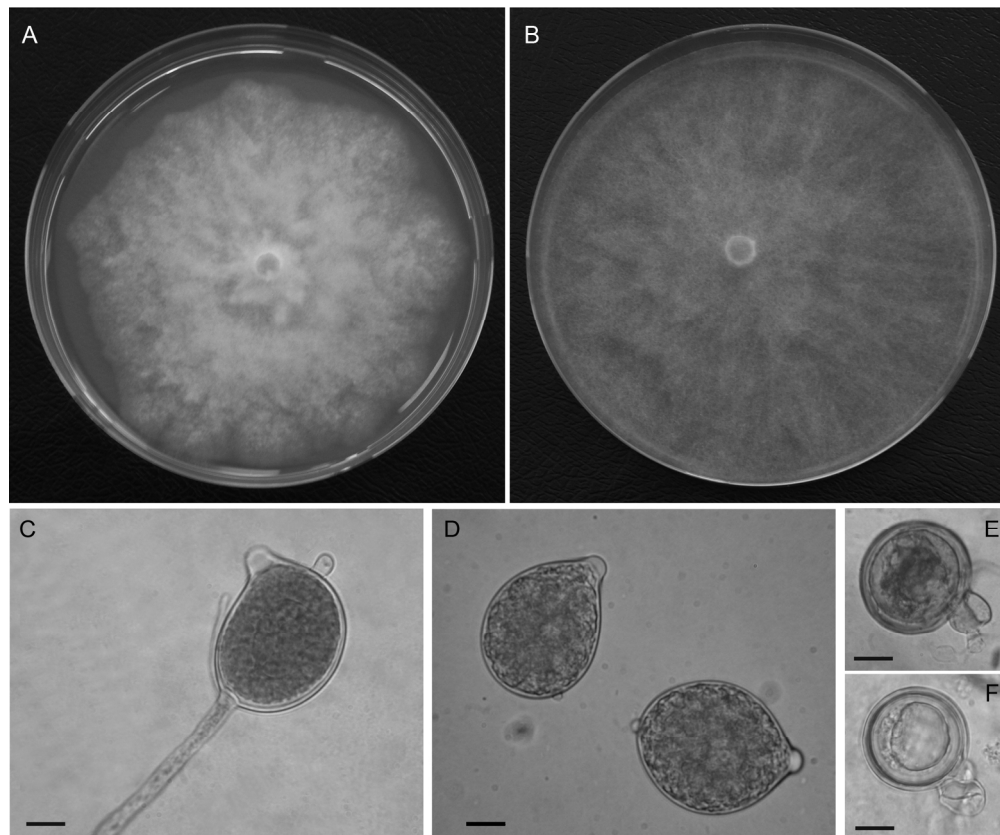458

459   Fig. 1. A and B, Ten-days old colonies of a *Phytophthora × pelgrandis* isolate (956/07) from

460   lavender on PDA and V8A, respectively. C, Persistent papillate sporangium of isolate 956/07.

461   Note the unipodial ramification at the base of the intercalary sporangium. D, Subspherical,

462   prominently papillate, caducous sporangia with a short pedicel of isolate 956/07. E and F,

463   Gametangia and oospores of isolate 956/07 produced in a single culture: paragynous and

464   anphigynous antheridia, respectively. Bar in C, D, E and F represents 10 μm.

465

466   Fig. 2. ITS-rDNA products amplified in multiplex PCR, using primer-pairs NICF1/NICR2.1

467   and CACTF1/CACTR1, from *Phytophthora nicotiane* IMI 398853, *P. cactorum* IRF14 and

468   one (956/07) of the two putative hybrid isolates from Stoechas lavender. Size of marker bands

469   is indicated in bp.

470

471   Fig. 3. Multiplex PCR with primer pairs PNF/PNR and PC1/PC2 specific for *Phytophthora*

472   *nicotianae* and *P. cactorum*, respectively, of DNA from *P. nicotianae* IMI 398853, *P.*

473   *cactorum* IRF14 and putative hybrid isolates 957/07 and 956/07 from Stoechas lavender. Size
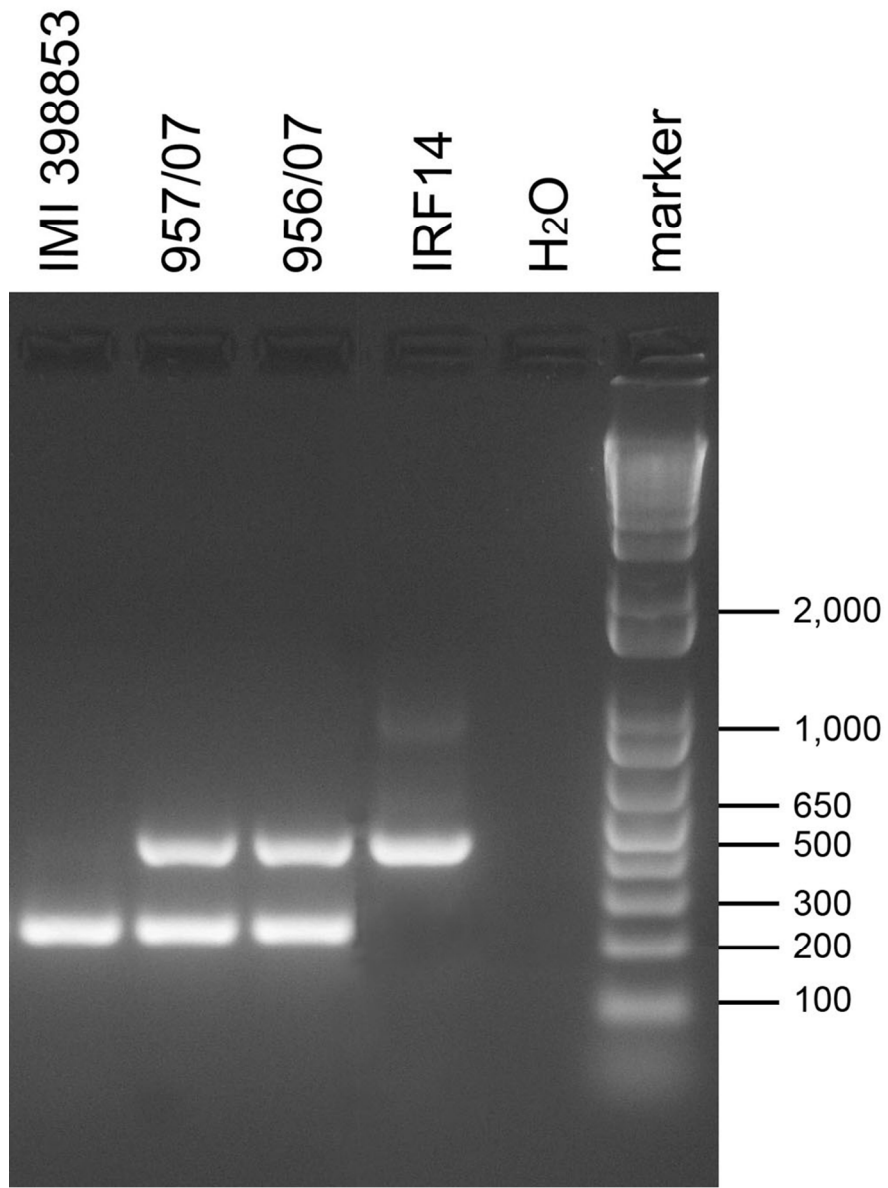
474   of marker bands is indicated in bp.

475

476   Fig. 4. RAPD profiles of *Phytophthora nicotianae* IMI 398853, *P. cactorum* IRF14 and one

477   putative hybrid isolate 956/07 from Stoechas lavender obtained using two universal decamer
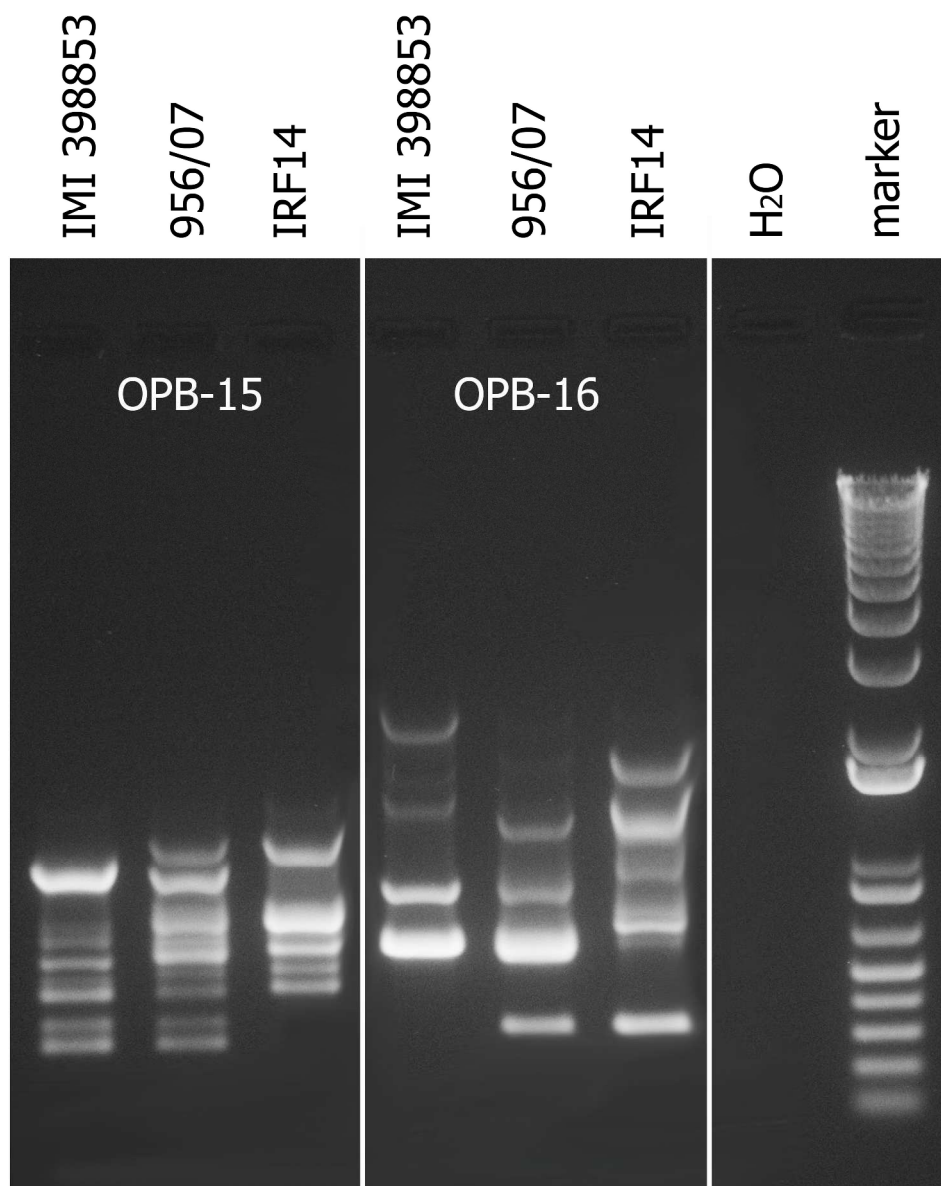
478   primers OPB-15 and OPB-16.

A and B, Ten-days old colonies of a *Phytophthora × pelgrandis* isolate (956/07) from lavender on PDA and V8A, respectively. C, Persistent papillate sporangium of isolate 956/07. Note the unipodial ramification at the base of the intercalary sporangium. D, Subspherical, prominently papillate, caducous sporangia with a short pedicel of isolate 956/07. E and F, Gametangia and oospores of isolate 956/07 produced in a single culture: paragynous and anphigynous antheridia, respectively. Bar in C, D, E and F represents 10 μm.
177x148mm (300 x 300 DPI)
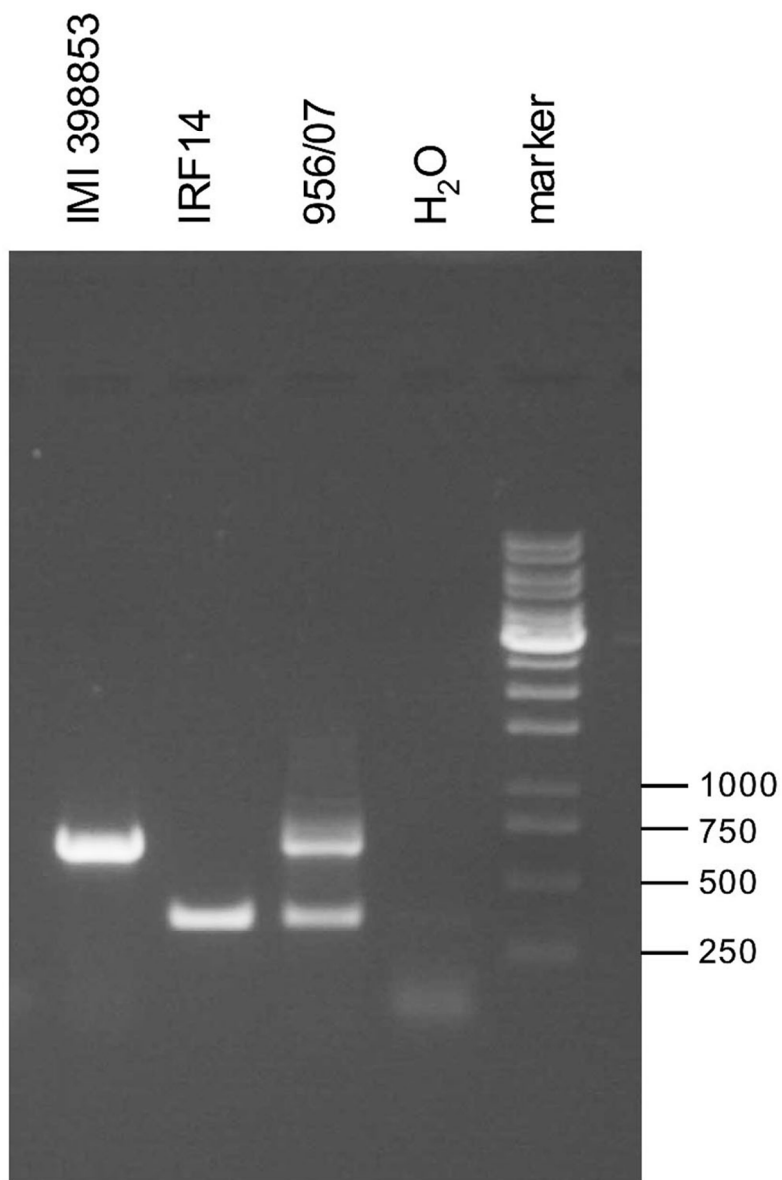
ITS-rDNA products amplified in multiplex PCR, using primer-pairs NICF1/NICR2.1 and CACTF1/CACTR1, from *Phytophthora nicotiane* IMI 398853, *P. cactorum* IRF14 and one (956/07) of the two putative hybrid isolates from Stoechas lavender. Size of marker bands is indicated in bp.
85x129mm (300 x 300 DPI)

Multiplex PCR with primer pairs PNF/PNR and PC1/PC2 specific for *Phytophthora nicotianae* and *P. cactorum*, respectively, of DNA from P. nicotianae IMI 398853, P. cactorum IRF14 and putative hybrid isolates 957/07 and 956/07 from Stoechas lavender. Size of marker bands is indicated in bp.
85x115mm (300 x 300 DPI)

RAPD profiles of *Phytophthora nicotianae* IMI 398853, *P. cactorum* IRF14 and one putative hybrid isolate 956/07 from Stoechas lavender obtained using two universal decamer primers OPB-15 and OPB-16.
271x344mm (300 x 300 DPI)