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Identification of Spanish isolates of *Rhizoctonia solani* from potato by anastomosis grouping, ITS-RFLP and RAMS-fingerprinting

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Summary Anastomosis grouping, restriction fragment length polymorphism (RFLP) of the ITS regions including the 5.85 rDNA, and random amplified microsatellites (RAMS) were used to characterize isolates of *Rhizoctonia solani* collected from Spain and Finland. There was a high similarity between the results obtained with the three techniques. RAMS markers revealed more genetic variation among isolates of *R. solani* than RFLP. The anastomosis group (AG)–3 isolates were clearly separated from isolates belonging to other AGs by RAMS, RFLPs and anastomosis grouping. Almost all the isolates sampled from potato belonged to AG–3. No differences were observed between Spanish and Finnish AG–3 isolates.

Key words: black scurf, *Rhizoctonia* canker, rDNA-ITS, RFLP.

Introduction

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* [Frank Donk]), the causal agent of Rhizoctonia canker and black scurf of potato (*Solanum tuberosum* L.), causes reductions in quality and yield all over the world. Either or both diseases may occur in an individual potato plant crops (Banville *et al*., 1997).

Rhizoctonia solani is a heterogeneous species comprising a number of independent populations (Sneh *et al*., 1991). The species has been divided into 14 anastomosis groups (AGs) designated as AG–1 to 13 and a bridging isolate group, AG–BI (Carling *et al*., 2002). However, it has recently been suggested that the bridging isolates are a subset of AG–2 (Carling *et al*., 2002). AGs are identified by pairing unknown isolates with tester strains and observing the hyphal anastomoses (Parameter *et al*., 1969). Isolates of some groups may anastomose with members of other AGs, such as AG–BI, AG–8, AG–6, AG–3 and AG–2. However most isolates, including members of AG–1, AG–4, AG–5, AG–7 and AG–9 anastomose only with members of their own group (Kuninaga *et al*., 1979; Carling, 1996).

Isolates of *R. solani* have also been divided into pectic zymogram groups (ZGs) on the basis of the pectic enzyme produced during growth on pectin (MacNish *et al*., 1994). Genetic variation is possible between isolates from the same AG, e.g. AG–8 is divided into four ZGs (MacNish *et al*., 1994). More recently, molecular techniques have made it possible to study the genetic and taxonomic relationships between groups of *Rhizoctonia* isolates (Duncan and O'Brien, 1993; Kuninaga *et al*., 1997; Carling *et al*., 2002).

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Isolates of AG–3 have been identified as a serious pathogen of potato (Carling *et al*., 1989). However other AGs have also been isolated from potatoes: AG–4 (Anguiz and Martin, 1989), AG–5 (Bandy *et al*., 1984), AG–1, AG–2 and AG–9 (Chand and Logan, 1983; Carling and Leiner, 1986).

The main objective of this study was to characterise isolates of *R. solani* from Spanish potato plants using three different methods: anastomosis grouping, restriction fragment length polymorphism (RFLP) of the internal transcribed spacer and random amplified microsatellites (RAMS).

Materials and methods

Collection and isolation

The study material included 40 *R. solani* isolates from northeastern Spain (Catalonia). Of these, 36 were isolated from potato and one each from: onion (*Allium cepa* L.), carnation (*Dianthus caryophyllus* L.), common bean (*Phaseolus vulgaris* L.) and radish (*Raphanus sativus* L.). Four isolates from potato in Finland were included to observe geographical differences (Table 1).

Roots, stolons or tubers from diseased plants were washed under tap water, surface sterilized with 1% sodium hypochlorite for 30 s and blotted dry on absorbent paper. For isolation, small pieces of necrotic tissue were excised from the roots or stolons and placed on potato-dextrose agar (PDA, 39 g l^{-1}, Biolife, Milano, Italy). Visible sclerotia or the mature mycelia found on tubers with the aid of a binocular microscope were also placed on PDA. Plates were incubated at room temperature (27ºC). Reisolation from the edge of each colony was done according to Korhonen and Hintikka (1980) and the isolated tip cells were placed on PDA containing 50 mg l^{-1} streptomycin sulfate and neomycin sulfate (Sigma Chemicals, St Louis, MO, USA). The nuclear condition of purified *Rhizoctonia*-like isolates was checked as described by El Bakali and Martín (2000). The isolates were stored in dried cereal grains (Sneh *et al.,* 1991).

Anastomosis grouping

Forty-four multinucleate isolates were paired with testers representing the anastomosis groups: AG–1–1A, AG–2–1, AG–2–2, AG–3, AG–4, AG–5, AG–6GV, AG–7, and AG–10, supplied by S. Jabaji-Hare (McGill University, Canada) according to the procedure of Parmeter *et al*. (1969). PDA plugs (5 mm in diameter) were cut from the margins of one-week-old colonies and placed on opposite edges of 9-cm Petri dishes containing 2% water agar (WA, Difco, Detroit, USA). Converging colonies were checked for hyphal anastomoses under a light microscope (100×) on 10 microscope fields after incubation at room temperature (27ºC) for 24h and 48h.

The isolates from potato R24, R28, R30, 9133, 9421, 9422, 24017 and 28754, which did not anastomose with the testers used in this study, were sent to D. Carling (Alaska) who kindly made a complete anastomosis evaluation.

DNA isolation

DNA isolation was carried out as described by Vainio *et al*. (1998). Mycelia were collected from the surface of culture medium with a sterile scalpel and disrupted in an extraction buffer (50 mM Tris-HCl at pH 7.2, 50 mM EDTA, 3% SDS and 1% 2-mercaptoethanol). A microtube containing the mixture was incubated at 65ºC for 1 h and centrifuged at 14,000 rpm (10,000 × *g*) for 30 min. The aqueous phase was purified three times with phenol-chloroform-isoamyl alcohol (25:24:1, v:v:v) and once with chloroform-isoamyl alcohol (24:1, v:v). DNA was pelleted by polyethylene glycol (20% PEG and 2.5 M NaCl) precipitation. The resulting solution was incubated on ice for 20 min and DNA was collected by centrifugation for 20 min at 14,000 rpm. The pellet was washed with 70% ethanol, dried under vacuum and resuspended in TE (10 mM Tris-HCl at pH 7.2, 1 mM EDTA).

ITS-RFLP analyses

The pzYmer pair ITS1 and ITS4 (White *et al.,* 1990) was used to amplify the Internal Transcribed Spacer (ITS) regions including 5.8S from ribosomal DNA. PCR reactions were set up using Dynazyme II DNA polymerase (Finnzymes Ltd, Espoo, Finland) in 50 µl volume using buffer conditions recommended by the manufacturer. PCR cycles were conducted on an automated thermocycler (Perkin Elmer Cetus, Norwalk, CT, USA). The following parameters were used: initial denaturation at 94ºC for 10 min, followed by 35 cycles of 94ºC for 1 min, 50ºC for 45 s, 72ºC for 1 min and finally 72ºC for 10 min. Control tubes without DNA template were included in each experiment and at least two independent polymerase chain reaction

Table 1. Isolates of *Rhizoctonia solani* studied.

Isolate code[a]	AG type	Host	Infected part[b]	Geographic origin[c]	Source[d]
R24	4	*Solanum tuberosum*	R	Barcelonès, S	1
R28	4	*Solanum tuberosum*	R	Barcelonès, S	1
R30	4	*Solanum tuberosum*	R	Barcelonès, S	1
R32	-	*Solanum tuberosum*	R	Barcelonès, S	1
R34	-	*Solanum tuberosum*	R	Barcelonès, S	1
R36	-	*Solanum tuberosum*	R	Barcelonès, S	1
T12	3	*Solanum tuberosum*	T	Barcelonès, S	1
T17 (IMI385795)	3	*Solanum tuberosum*	T	Barcelonès, S	1
T32	3	*Solanum tuberosum*	T	Barcelonès, S	1
T36 (IMI385796)	3	*Solanum tuberosum*	T	Barcelonès, S	1
T39 (IMI385798)	3	*Solanum tuberosum*	T	Barcelonès, S	1
P32	3	*Solanum tuberosum*	T	Baix Camp, S	1
A730	-	*Solanum tuberosum*	S	Baix Camp, S	1
9133	4	*Solanum tuberosum*	R	Baix Camp, S	2
289612	-	*Allium cepa*	R	Barcelonès, S	2
9421	4	*Solanum tuberosum*	S	Tarragonès, S	2
9422	4	*Solanum tuberosum*	S	Tarragonès, S	2
F1	3	*Solanum tuberosum*	T	Tikkurila, F	3
F2	3	*Solanum tuberosum*	T	Tikkurila, F	3
F11	3	*Solanum tuberosum*	T	Tikkurila, F	3
F22	3	*Solanum tuberosum*	T	Tikkurila, F	3
R0297	3	*Solanum tuberosum*	T	Baix Camp, S	1
R2297	3	*Solanum tuberosum*	T	Baix Camp, S	1
R0397	3	*Solanum tuberosum*	T	Barcelonès, S	1
R1397	3	*Solanum tuberosum*	T	Barcelonès, S	1
R0497	3	*Solanum tuberosum*	T	Barcelonès, S	1
R1497	3	*Solanum tuberosum*	T	Barcelonès, S	1
R2497	3	*Solanum tuberosum*	T	Barcelonès, S	1
R3497	3	*Solanum tuberosum*	T	Barcelonès, S	1
R1697	3	*Solanum tuberosum*	T	Alt Emporda, S	1
R2697	3	*Solanum tuberosum*	T	Alt Emporda, S	1
24017	-	*Phaseolus vulgaris*	R	Barcelonès, S	1
28754	-	*Dianthus caryophyllus*	R	Barcelonès, S	1
TC1	3	*Solanum tuberosum*	T	Alt Emporda, S	1
TC2 (IMI385799)	3	*Solanum tuberosum*	T	Alt Emporda, S	1
TC3 (IMI385800)	3	*Solanum tuberosum*	T	Alt Emporda, S	1
R198	3	*Solanum tuberosum*	R	Barcelonès, S	1
R398	3	*Solanum tuberosum*	R	Barcelonès, S	1
R698	3	*Solanum tuberosum*	R	Barcelonès, S	1
R998	3	*Solanum tuberosum*	R	Alt Emporda, S	1
R1098	3	*Solanum tuberosum*	R	Barcelonès, S	1
R1398	3	*Solanum tuberosum*	R	Alt Emporda, S	1
RV	4	*Raphanus sativus*	R	Unknow, S	5
RH4	4	*Solanum tuberosum*	R	Baix Camp, S	1

[a] Isolate name provided by the collector, between bracket ID number.
[b] Infected part: Root (R), Stolon (S), Tuber (T).
[c] Country of isolates, S, Spain; F, Finland.
[d] Author of strains, 1, M.A. El Bakali, 2, F. García-Figueres, 3, A. Lilja, 5, M. Nadal.

amplifications were performed on each isolate. After amplification, 5 µl from each sample was subjected to electrophoresis in 1.5% agarose gels (Boehringer Mannheim Gmbh, Mannheim, Germany). The size of the amplified fragments was determined using a 100 bp DNA Ladder (Gibco BRL, Gaithersburg, MD, USA). Gels were stained with ethidium bromide and photographed on an UV transilluminator. Prior to RFLP analyses, amplified PCR products were precipitated for 30 min in 3 M NaCl and 95% EtOH on ice, washed with 70% EtOH, dried under vacuum and resuspended in TE buffer.

Restriction enzyme digestions were carried out using *Hinf*I, *Eco*RI, *Msp*I and *Taq*I enzymes in conditions recommended by the manufacturer (Boehringer). Restriction fragments were separated by electrophoresis in gels containing SynerGel (Diversified Biotech, Boston, MA, USA) and agarose (FMC BioProducts, Rockland, ME, USA) at concentrations of 0.9 and 0.9% (w:v) respectively (Vainio *et al.,* 1998). The gels were run in TAE-buffer (40 mM Tris/acetate, pH 8.0, 1 mM EDTA), stained using ethidium bromide and visualized by UV transillumination. In order to determine the size of the restriction fragments, a 100 bp DNA Ladder (Gibco BRL) was used as a molecular weight standard.

RAMS analysis

In random amplified microsatellite (RAMS) analysis the DNA between the distal ends of two closely located microsatellites is amplified and the resulting PCR products are separated electrophoretically in an agarose gel (Zietkiewicz *et al*., 1994; Hantula *et al*., 1996). Fungal DNA was amplified in reaction mixtures of 50 µl containing 200 µM of dNTP, 5 µl of 10× reaction buffer (100 mM Tris-HCl pH 8.8, 15 mM $MgCl_2$, 500 mM KCl, 1% Triton X-100), 1 U of Dynazyme II DNA polymerase (Finnzymes Ltd) and 2 µM of a RAMS-primer. The RAMS-primers used were: GT-primer, 5'YHY$(GT)_7$G; and TCG-primer, 5'DHB$(TCG)_5$, where D=G/A/T, H=A/T/C, B=G/T/C and Y=G/A/C (Hantula *et al*., 1996). The samples were denatured at 95ºC for 10 min, after which 35 (TCG-primer) or 37 (GT-primer) cycles of amplification were carried out (denaturation at 95ºC for 30 s, annealing at a primer-dependent temperature for 45 s and extension at 72ºC for 2 min). After the last cycle a final extension at 72ºC was carried out for 7 min. The annealing temperatures used were 61ºC (TCG-primer) and 58ºC (GT-primer). At least two independent polymerase chain reaction amplifications were performed from each isolate. Amplification products were analysed by electrophoresis in gels as mentioned above.

Data analyses

The absence or presence of bands from the RFLP and RAMS analyses was scored, and only clear and reproducible bands were included in the analyses. When the relative intensity of a band varied among the isolates, appropriate dilutions of the amplification products were compared in a single gel.

Data were analysed using the TREECON programe (Van de Peer and De Wachter, 1994). The genetic distance (GDxy) or genetic dissimilarity was calculated according to Nei and Li (1979) as Gdxy=1– 2Nxy/[Nx+Ny]), where Nxy is the number of fragments (bands) shared by isolates x and y, Nx the number of fragments in isolate x, and Ny the number of fragments in isolate y.

The total variation was divided into a "within anastomosis group" population and "between anastomosis groups" components.

Results

Anastomosis grouping

Of the forty multinucleate *R. solani* isolates from potato, twenty-nine belonged to AG–3 and one to AG–4 (RH4). The isolate from *Raphanus sativus* also belonged to AG–4. Ten multinucleate isolates from potato as well as the isolates from *Allium cepa*, *Dianthus caryophyllus* and *Phaseolus vulgaris* did not anastomose with the testers used in this study. According to D. Carling, isolates R24, R28, R30, 9133, 9421 and 9422 were identified as members of AG–4. Isolates 24017 and 28754 were degenerated and did not grow properly.

RFLP

RFLPs obtained after digestion of amplified ITS differed depending on the restriction enzyme and isolate used. All AG–3 isolates showed a similar RFLP pattern. The digestion of PCR products with *Hinf*I gave two major bands of 390 and 375 bp (Fig. 1). *Eco*RI digestion produced a double band of 380 bp, and *Taq*I cut the ITS in two bands of 370 and 350 bp. *Msp*I did not cut the amplified rDNA in

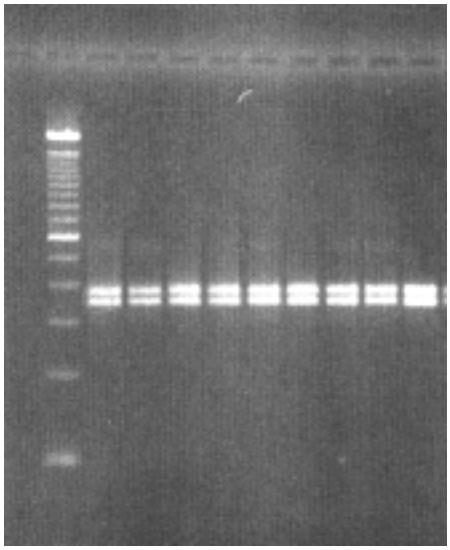

Fig. 1. Fragment lengths (bp) after endonuclease digestion with *Hinf*I of the ITS regions including the 5.8S rDNA of *Rhizoctonia solani* isolates. From left to right: 100 bp DNA Ladder, R0297, R2297, R0397, R1397, R0497, R1497, R2497, R1697, TC1.

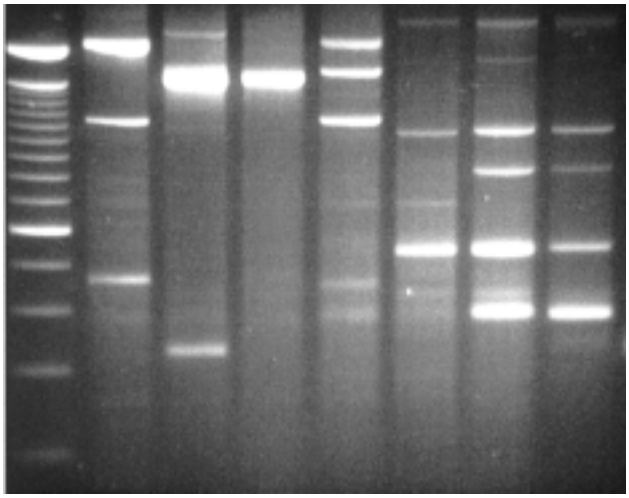

Fig. 2. Fragment lengths (bp) obtained after amplification with GT-primer. From left to right: 100 bp DNA Ladder, A730, 9133, 289612, 9421, T36, F1, AG–3.

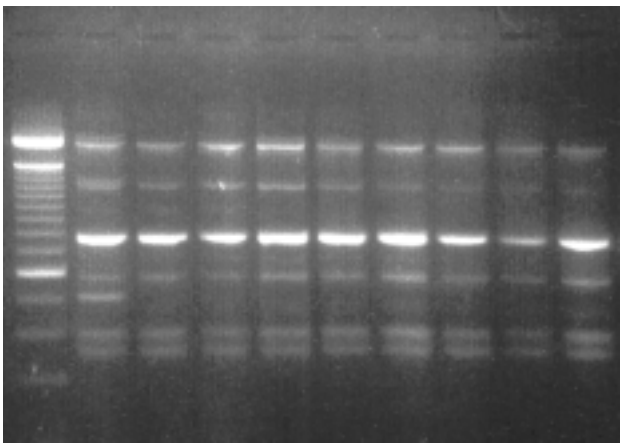

Fig. 3. Fragment lengths (bp) obtained after amplification with TCG-primer. From left to right: 100 bp DNA Ladder, T12, T17, T32, T36, T39, F1, F2, F11, F22, AG–3.

any of the isolates. Testers of the other AGs as well as a few field isolates showed different patterns.

In the dendrogram constructed from the PCR-RFLP data (Fig. 4), AG–3 isolates formed a separate clade. The group closest to the AG–3 isolates consisted of four unidentified AG isolates from potato (9421, 9422, A730 and R32). The difference between these two groups was obtained after digestion with *Eco*RI and *Taq*I. With the first enzyme, AG–3 isolates showed two bands above 380 bp, whereas the other four isolates gave three bands around 390, 290 and 80 bp. After *Taq*I digestion, all the isolates showed two bands: 370 and 350 bp (AG–3, 9421 and 9422), 370 and 345 bp (A730), and 380 and 345 bp (R32).

RAMS markers

The analysis of *R. solani* isolates with RAMS-primers GT and TCG revealed variable banding patterns (Fig. 2 and 3). In total, 56 markers were scored and named after the approximate length (in bp) of the respective amplification product and the primer used.

The number of amplified fragments with GT-primer –32- was higher than that of TCG-primer –24-. In addition, considerable variation was observed in the number of markers produced by the two primers from single isolates using GT and TCG primers: 1–8 per isolate with the GT primer, 3–12 with the TCG primer. All the RAMS patterns obtained from AG–3 isolates were grouped in the same cluster (Fig. 5).

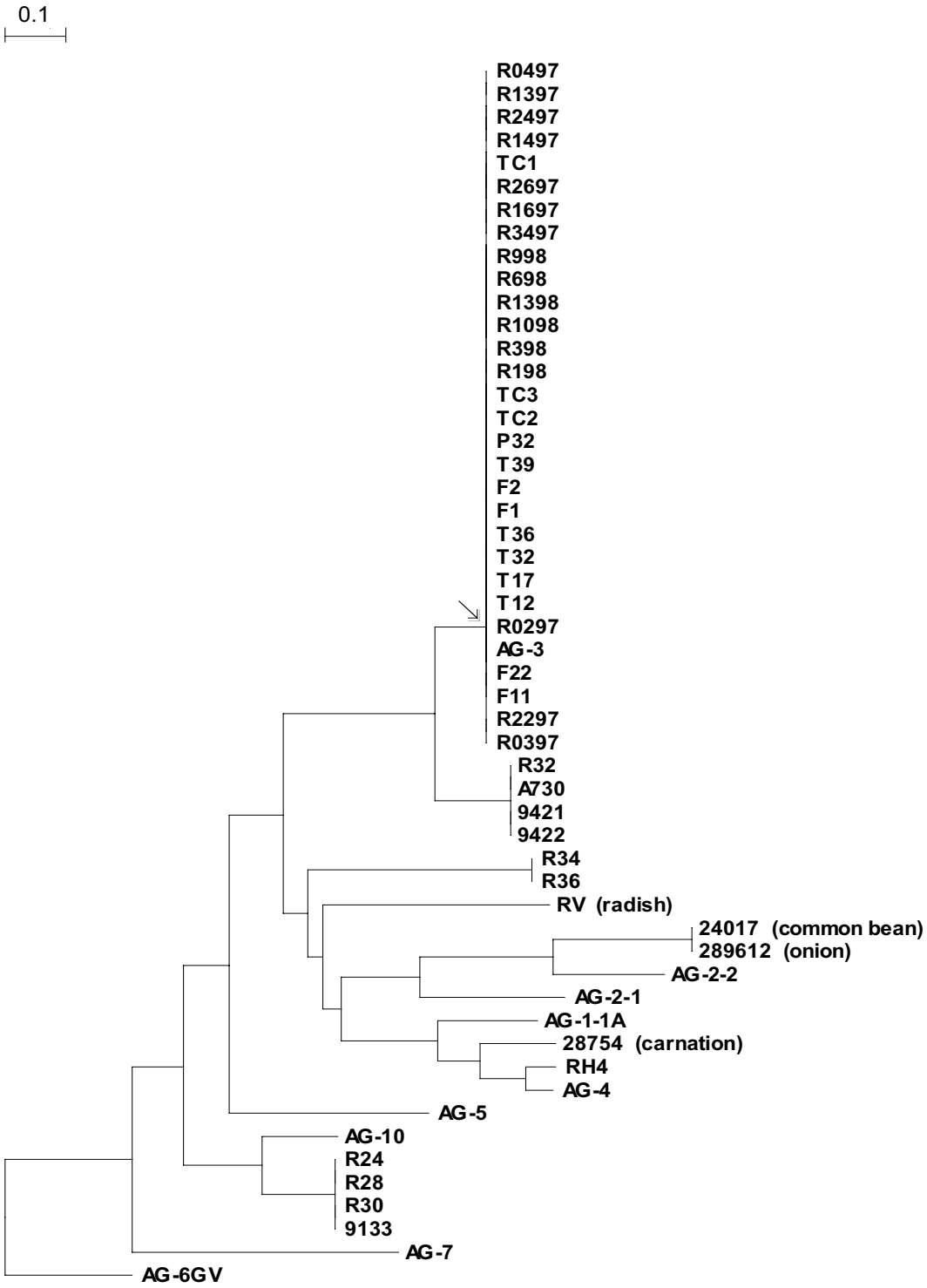

Fig. 4. Dendrogram constructed from PCR-RFLP data of ITS1–5.8S–ITS2 of *Rhizoctonia solani* isolates using the neighbour-joining method from the distance matrix calculated and analysed as described in the text. For isolates codes and sources see Table 1.

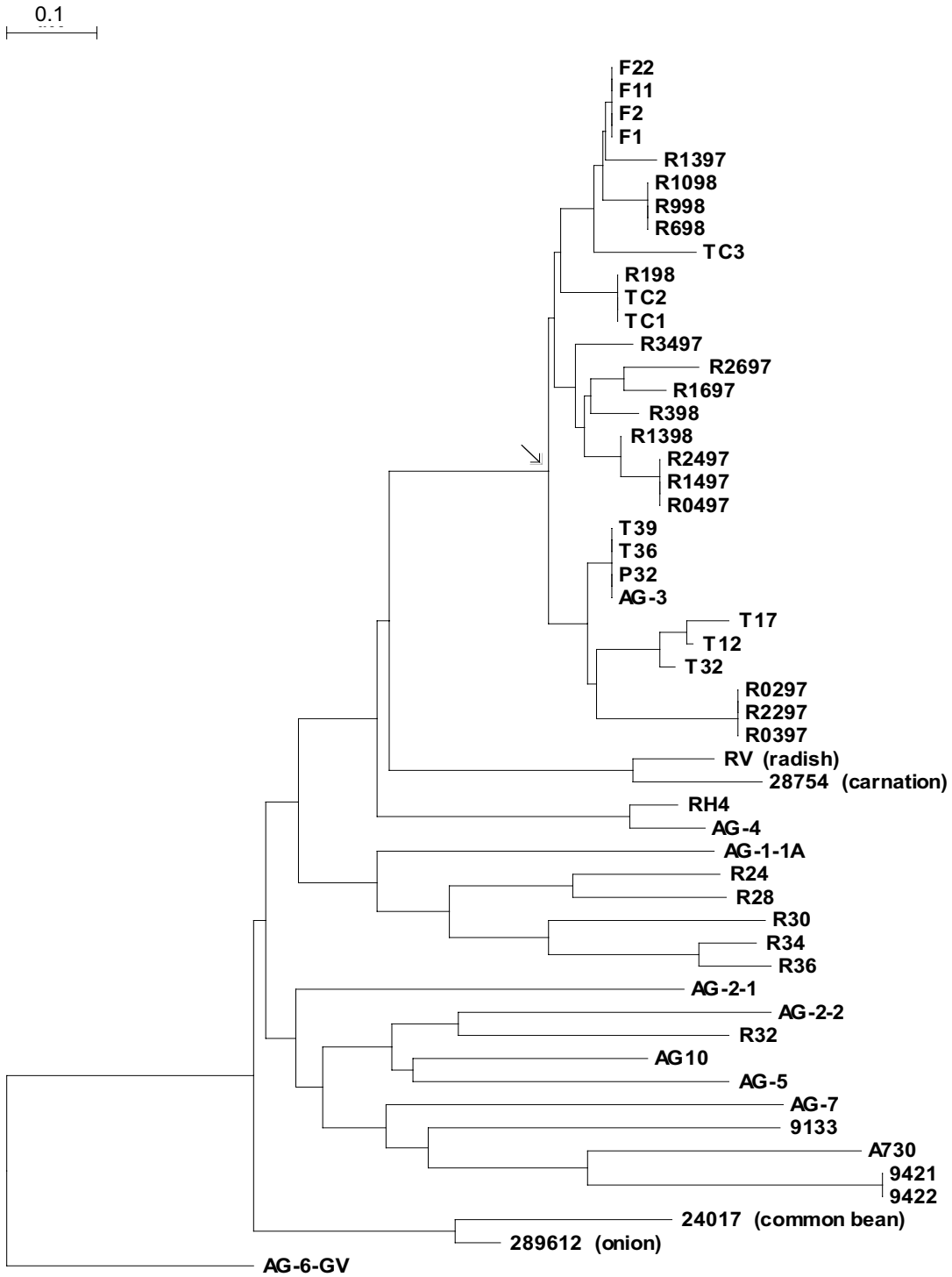

Fig. 5. Dendrogram constructed from RAMS patterns of *Rhizoctonia solani* isolates using the neighbour-joining method from the distance matrix calculated and analysed as described in the text. Isolates codes and sources of the isolates are given in Table 1.

Discussion

The aim of this study was to identify *R. solani* isolates from potato plants by three different methods. According to anastomosis grouping, 25 *R. solani* isolates recovered from root lesions and sclerotia on potato tubers in Spain belonged to AG–3. The isolates from potato tubers in Finland also belonged to this anastomosis group. The two DNA-based molecular techniques, ITS-RFLP and RAMS-analysis, gave results which were in agreement with the anastomosis grouping. ITS-RFLP grouped the isolates in the same cluster/clade as the tester representing AG–3.

According to Banville *et al.* (1996), AG–3 is most frequently associated with *Rhizoctonia* disease of potato, although it has also been isolated from other crops, including tomato, pepper, sugar beet, bean and tobacco (Sneh *et al.*, 1991). El Bakali *et al.* (2000) was the first to report AG–3 on potato in Catalonia. In potato cultures, *Rhizoctonia* diseases comprise two phases: *Rhizoctonia* canker, infecting growing plants (roots and stolons), and black scurf on tubers (Banville *et al.*, 1996). Most *Rhizoctonia* isolates from potato tubers belong to AG–3, and recently a specific PCR assay has been developed for the detection and identification of AG–3 on potato (Lees *et al.*, 2002). In this study, isolates of AG–3 were collected from both plants and tubers.

Representatives of other anastomosis groups of *R. solani* were identified as belonging to AG–4, obtained from roots or stolons of potato in Spain. Another isolate from this AG–4 was collected from a root of *Rhaphanus sativus*. AG–4 is known to be pathogenic to potato, but it causes serious damage only in warm temperatures (Anguiz and Martin, 1989). Other AGs, AG–1, AG–2 (subgroups 1 and 2), AG–4, AG–5, AG–8 and AG–9, have also been found on potato in nature and in growth-chamber studies (Chand and Logan, 1983; Bandy *et al.*, 1984; Carling and Leiner, 1986; Anguiz and Martin, 1989). Both AG–5 and AG–3 attack the roots, stems and stolons of potato, but AG–5 is less aggressive than AG–3 (Bandy *et al.,* 1988).

Three multinucleate *Rhizoctonia* sp. isolates from potato roots and one from potato stolons did not anastomose with the testers used in this study. AG–1, AG–2, AG–3, AG–4, AG–6, AG–8 and AG–9 have divided into subgroups (Sheh *et al.*, 1991). Probing of various restriction digests (Jabaji *et al.*, 1990) with cloned rDNA revealed that isolates from AG–1, AG–2, AG–3 and AG–6 had one or more unique patterns, whereas isolates of AG–4, AG–5, AG–7 and AG–9 produced a variety of patterns, some of which were shared among these AGs. AG–3 is a host-specific group. In pathogenicity tests and in fatty acid analysis AG–3 isolates from potato were distinct from AG–3 isolates from tobacco (Stevens Johnk *et al.,* 1993). There are sequence variations in the rDNA-ITS regions between AG–3 populations on potato, tomato and tobacco (Kuninaga *et al.*, 2000, Gonzalez *et al.*, 2001). No differences among isolates were found by ITS-RFLP in our study. The analysis of RAMS patterns showed two groups, which, however, were not related to the host, as all our isolates of AG–3 came from potato. Thus, as expected, the RAMS technique was more effective to find genetical differences among the AG–3 isolates than RFLP. The RAMS results demonstrated a high degree of diversity in AG–3 populations from different fields. This indicated that AG–3 isolates on potato in Spain and Finland were not clonal (or that RAMS-markers are instable). Genetic diversity has also recently been found among field populations of *R. solani* AG–3 in North Carolina (Ceresini *et al.,* 2002).

Traditionally, AG–4 has been defined as an anamorph of *Thanatephorus practicola* (Kotila) Flentje, and analysis of rDNA-ITS sequences has provided new data, which give additional support for AG–4 as a distinct species (Anderson, 1982; Mordue *et al.,* 1989; Gonzalez *et al.*, 2001). The subgroups found in AG–4, based on DNA/DNA hybridization, fatty acid analysis and sclerotial morphology (Kuninaga and Yokosawa, 1984; Vigalys, 1988; Sneh *et al.*, 1991; Stevens Johnk and Jones, 2001) do not necessarily represent separate species (Gonzales *et al.,* 2001). Matsumoto *et al.* (1996) reported a close relationship between AG–3 and AG–4, as shown by RFLP analysis of 28S rDNA. In our study both DNA based molecular techniques placed the AG–4 isolate from potato in different clades/clusters and they not appear in closer proximity to the tester representing AG–4. The other isolate anastomosing with the AG–4 tester was from *R. sativus* (isolate RV), and seemed to be very different from the isolates from potato.

The ITS-RLFP and RAMS techniques gave similar results for the distinction between AG–3 and other AGs. The two RAMS primers (TCG-primer,

GT-primer) amplified 56 fragments ranging in size from 140–1800 bp, of which 42 were polymorphic. Specific RFLP patterns for each anastomosis group have also been obtained from products of RAPD–PCR using six different primers (Duncan and O'Brien, 1993) and the ITS of AG–1/AG–10 and AG–BI (Liu *et al.,* 1992, 1993).

In conclusion, almost all isolates sampled from potato cultures in Spain and four isolates sampled from Finland belonged to AG–3. The RAMS technique was suitable for the study of *R. solani* and may help to clarify the taxonomy and population biology of this heterogeneous species. We can not arise clear conclusions about AG–4 since we will need to include more isolates from AG–4, as well from other AGs.

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Literature cited

Anderson N.A., 1982. The genetics and pathology of *Rhizoctonia solani*. *Annual Review of Phytopathology* 20, 329–347.

Anguiz R. and C. Martin, 1989. Anastomosis groups, pathogenicity, and other characteristics of *Rhizoctonia solani* isolated from potatoes in Peru. *Plant Disease* 73, 199–201.

Bandy B.P., S.S. Leach and S.M. Tavantzis, 1988. Anastomosis group 3 is the major cause of Rhizoctonia disease of potato in Maine. *Plant Disease* 72, 596–598.

Bandy B.P., D.H. Zanzinger and S.M. Tavantzis, 1984. Isolation of anastomosis group 5 of *Rhizoctonia solani* from potato field soils in Maine. *Phytopathology* 74, 1220–1224.

Banville G., D. Carling and B. Otrysko, 1996. *Rhizoctonia* in potato. In: Rhizoctonia *species*, *Taxonomy, Molecular Biology, Ecology, Pathogenicity and Disease Control*. (B. Sneh, S. Jabaji-hare, S. Neate, G. Djist, ed.), Kluwer Academic Publisher, Dordrecht, Netherlands, 321–330.

Carling, D.E., 1996. Grouping of *Rhizoctonia solani* by hyphal anastomosis. In: *Rhizoctonia species*, *Taxonomy, Molecular Biology, Ecology, Pathogenicity and Disease Control*. (B. Sneh, S. Jabaji-hare, S. Neate, G. Djist, ed.), Kluwer Academic Publisher, Dordrecht, Netherlands, 37–47.

Carling D.E. and R.H. Leiner, 1986. Isolation and characterization of *Rhizoctonia solani* and binucleate *R. solani*-like fungi from aerial stems and subterranean organs of potato plants. *Phytopathology* 76, 725–729.

Carling D.E., R.H. Leiner and P.C. Westphale, 1989. Symptoms, signs, and yield reduction associated with Rhizoctonia disease of potato induced by tuberborne inoculum of *Rhizoctonia solani* AG–3. *American Potato Journal* 66, 693–702.

Carling D.E., R.E. Baird, S. Kuninaga and R.D. Gitaitis, 1999. Characterization of anastomosis group AG–13 of *Rhizoctonia solani*. *Phytopathology* 89, 511 (abstract).

Carling D.E., R.E. Baird, R.D. Gitaitis, K.A. Brainard and Kuninaga S., 2002. Characterization of AG–13 a Newly Reported Anastomosis Group of *Rhizoctonia solani*. *Phytopathology* 92, 893–899.

Chand T. and C. Logan, 1983. Cultural and pathogenic variation in potato isolates of *Rhizoctonia solani* in Northern Ireland. *Transactions of the British Mycological Society* 81, 585–589.

Ceresini P.C., H.D. Shew, R.J. Vigalys and M.A. Cubeta, 2002. Genetic diversity of *Rhizoctonia solani* AG–3 from potato and tobacco in North Carolina. *Mycologia* 94, 437–449.

Duncan B.J., J.E. Barton and P.A. O'Brien, 1993. Analysis of variation in isolates of *Rhizoctonia solani* by random amplified polymorphic DNA assay. *Mycological Research* 97, 1075–1082.

El Bakali M.A. and M.P. Martín, 2000. Morphological, molecular and pathological characteristics of *Rhizoctonia*-like fungi from Spanish forest nurseries. *Finnish Forest Research Institute, Research Papers* 781, 139–149.

El Bakali M.A., M.P. Martín, F.F. García, R.C. Montón, B.A. Moret and P.M. Nadal, 2000. First report of *Rhizoctonia solani* AG–3 on potato in Catalonia, NE Spain. *Plant Disease* 84, 806.

Gonzalez D., D.E. Carling, S. Kuninaga, R. Vigalys and M.A. Cubeta, 2001. Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorphs. *Mycologia* 98, 1138–1150.

Hantula J., M. Dusabenyagasani and R.C. Hamelin, 1996. Random amplified microsatellites (RAMS) - a novel method for characterising genetic variation within fungi. *European Journal of Forest Pathology* 26, 159–166.

Jabaji S.H., Y. Meller, S. Gill and P.M. Charest, 1990. Investigation of genetic relatedness among anastomosis groups of *Rhizoctonia solani* using cloned DNA probes. *Canadian Journal of Plant Pathology* 12, 393–404.

Korhonen K. and V. Hintikka, 1980. Simple isolation and inoculation methods for fungal cultures. *Karstenia* 20, 19–22.

Kuninaga S. and R. Yokosawa, 1984. DNA base sequence homology in *Rhizoctonia solani* Kühn. IV. Genetic re-

latedness within AG–4. *Annals of the Phytopathological Society of Japan* 50, 322–330.

Kuninaga S., and R. Yokosawa and A. Ogoshi, 1979. Some properties of anastomosis group 6 and BI in *Rhizoctonia solani* Kühn. *Annals of the Phytopathological Society of Japan* 45, 207–214.

Kuninaga S., T. Natsuaki and R. Yokosawa, 1997. Sequence variation of the rDNA ITS region within and between anastomosis groups in *Rhizoctonia solani*. *Current Genetics* 32, 237–243.

Kuninaga S., D.E. Carling, T. Takeuchi and R. Yokosawa, 2000. Comparison of rDNA-ITS sequences between potato and tobacco strains in *Rhizoctonia solani* AG–3. *Journal of Genetical Plant Pathology* 66, 2–11.

Lees A.K., D.W. Cullen, L. Sullivan and M.J. Nicholson, 2002. Development of conventional and quantitative real-time PCR assays for the detection and identification of *Rhizoctonia solani* AG–3 in potato and soil. *Plant Pathology* 51, 293–302.

Liu Z.L., D.L. Nickrent and J.B. Sinclair, 1993. Genetic relationship among isolates of *Rhizoctonia solani* anastomosis group 2 based on isozyme analysis. *Canadian Journal of Plant Pathology* 12, 376–382.

Liu Z.L., J.B. Sinclair and W. Chen, 1992. Genetic diversity of *Rhizoctonia solani* anastomosis group 2. *Phytopathology* 82, 778–787.

MacNish G.C., D.E. Carling, M.W. Sweetingham and K.A. Brainard, 1994. Anastomosis groups (AG) affinity of pectic isozyme (zymogram) groups (ZG) of *Rhizoctonia solani* from the Western Australian cereal-belt. *Mycological Research* 98, 1369–1375.

Matsumoto M., N. Furuya, Y. Takanami and N. Matsuyama, 1996. RFLP analysis of the PCR-amplified 28S rDNA in *Rhizoctonia solani*. *Mycoscience* 37, 351–356.

Mordue J.E.M., R.S. Currah and P.D. Bridge, 1989. An integrated approach to *Rhizoctonia* taxonomy: cultural, biochemical and numerical techniques. *Mycological Research* 92, 78–90.

Nei M. and W. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences, USA* 76, 5269–5273.

Parmeter J.P., R.T. Sherwood Jr. and W.D. Platt, 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. *Phytopathology* 59, 1270–1278.

Sneh B., L. Burpee and A. Ogoshi, 1991. *Identification of Rhizoctonia species*. APS Press Inc., St Paul, Minnesota, MO, USA, 133 pp.

Stevens Johnk J., R.K. Jones, H.D. Shew and D.E. Carling, 1993. Characterization of populations of *Rhizoctonia solani* AG 3 from potato and tobacco. *Phytopathology* 83, 854–858.

Stevens Johnk J. and R.K. Jones, 2001. Differentiation of three homogeneous groups of *Rhizoctonia solani* anastomosis group 4 by analysis of fatty acids. *Phytopathology* 91, 821–830.

Vainio E.J., K. Korhonen and J. Hantula, 1998. Genetic variation in *Phlebiopsis gigantea* as detected with random amplified microsatellite RAMS markers. *Mycological Research* 102, 187–192.

Van de Peer Y. and R. De Wachter, 1994. TREECON for Windows, a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Compendium of Applied Bioscience* 10, 569–570.

Vigalys R. 1988. Genetic relatedness among anastomosis groups in *Rhizoctonia* as measured by DNA/DNA hybridization. *Phytopathology* 78, 698–702.

Virgen-Calleros G., V. Olade-Portugal and D.E. Carling, 2000. Anastomosis groups of *Rhizoctonia solani* on potato in Central Mexico and potential for biological control. *American Journal of Potato Research* 77, 219–224.

White T.J., T. Burns, S. Lee and J. Taylor, 1990. Amplifications and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocol. A Guide to Methods and Amplifications* (M.A. Innis, ed.), Academic Press, Inc, San Diego, CA, USA, 315–322.

Zietkiewicz Z.E., Rafalski A. and Labuda D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20, 176–183.

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