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To cite this article: Fatih Mehmet Tok, Sibel Derviş & Mehmet Arslan (2016) Analysis of genetic diversity of *Sclerotinia sclerotiorum* from eggplant by mycelial compatibility, random amplification of polymorphic DNA (RAPD) and simple sequence repeat (SSR) analyses, *Biotechnology & Biotechnological Equipment*, 30:5, 921-928, DOI: [10.1080/13102818.2016.1208059](https://doi.org/10.1080/13102818.2016.1208059)

To link to this article: <http://dx.doi.org/10.1080/13102818.2016.1208059>



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Published online: 18 Jul 2016.



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Analysis of genetic diversity of *Sclerotinia sclerotiorum* from eggplant by mycelial compatibility, random amplification of polymorphic DNA (RAPD) and simple sequence repeat (SSR) analyses

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ABSTRACT

The genetic diversity and pathogenicity/virulence among 60 eggplant *Sclerotinia sclerotiorum* isolates collected from six different geographic regions of Turkey were analysed using mycelial compatibility groupings (MCGs), random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) polymorphism. By MCG tests, the isolates were classified into 22 groups. Out of 22 MCGs, 36% were represented each by a single isolate. The isolates showed great variability for virulence regardless of MCG and geographic origin. Based on the results of RAPD and SSR analyses, 60 *S. sclerotiorum* isolates representing 22 MCGs were grouped in 2 and 3 distinct clusters, respectively. Analyses using RAPD and SSR markers illustrated that cluster groupings or genetic distance of *S. sclerotiorum* populations from eggplant were not distinctly relative to the MCG, geographical origin and virulence diversity. The patterns obtained revealed a high heterogeneity of genetic composition and suggested the occurrence of clonal and sexual reproduction of *S. sclerotiorum* on eggplant in the areas surveyed.

ARTICLE HISTORY

Received 1 September 2015
Accepted 28 June 2016

KEYWORDS

MCGs; RAPD; SSR; stem rot

Introduction

Eggplant (*Solanum melongena* L.) is an important solanaceous crop for Turkey, which is the largest producer of eggplant in Europe, averaging a production of 826.941 tons and it ranks as one of the most important vegetable crops in the world with an annual worldwide production of 49.418.212 tons. [1]

Sclerotinia sclerotiorum (Lib.) de Barry, cosmopolitan soilborne necrotrophic plant pathogens, infects more than 400 different plant species worldwide and causes white mold, stem rot and fruit rot diseases.[2] Most of the economically important dicotyledonous plant species are the main hosts of *S. sclerotiorum*, although several economically important monocotyledonous plant species are also reported as host of *S. sclerotiorum*. [2] Recent estimates of annual yield losses caused by *S. sclerotiorum* exceed over several hundred million dollars due to lack of high levels of host resistance and difficulty of management practices. Management of diseases caused by *S. sclerotiorum* was highly difficult due to widespread distribution, sporadic occurrence and the long-term persistence of sclerotia in soil. Chemical and cultural control measures have been the major strategies for combating the diseases caused by *S. sclerotiorum*. [3]

although none of these management measures effectively prevent the infection process alone.

The amount and distribution of genetic variability of plant pathogen populations affect the efficacy of control strategies of plant diseases. Therefore, the studies on the genetic structure of pathogen populations should be an important step when planning management programmes aimed to prevent or control the disease. Differentiation of *S. sclerotiorum* strains based on morphological differences in sclerotia, mycelial growth and ascospores has been reported in previous studies.[4,5]

In Turkey, during the last few years, many *Sclerotinia* isolates have been recovered from host plants such as sunflower, sweet basil, rape seed, tomato, potato and melon, [6,7] and identified based on cultural and morphological characteristics. However, as the *Sclerotinia* collection grew, great variability in these characteristics was noted among isolates belonging to the same *Sclerotinia* sp. The presence of different physiological, ecological, biochemical, pathogenic and molecular variations within *Sclerotinia* isolates has also been reported worldwide.[8–10]

Two different methods, mycelial compatibility groups (MCGs) and molecular markers, were used to determine genetic variation among populations of *S. sclerotiorum*.

[4] Mycelial compatibility (MC) is the ability of two fungal strains hyphae to anastomose and form one integrated colony in filamentous fungi.[4] MCGs tend to be genetically isolated from each other.[4] A number of molecular techniques have been used to investigate genetic diversity of *S. sclerotiorum* including random amplified polymorphic DNA (RAPD),[11] inter simple sequence repeat (ISSR) [12] and simple sequence repeat (SSR).[13] Previous studies indicated that populations of *S. sclerotiorum* were genetically diverse and had predominantly several clonal components with occasional recombination within populations, as well as contemporary population diversification at a local scale.[6,8,13,14]

MCG diversity, genetic variability and pathogenicity relationships of *S. sclerotiorum* populations infesting eggplant production areas have not been extensively reported yet. It is important to understand the epidemiology and genetic diversity of *S. sclerotiorum* to control plant diseases by chemical and cultural methods. Additional insight into *Sclerotinia* problems might be achieved via molecular characterization of local isolates. The purpose of the current study was to investigate the genetic structure within the eggplant host populations of *S. sclerotiorum* in southern provinces of Turkey based on molecular and morphological markers.

Materials and methods

Sclerotia of *S. sclerotiorum* were obtained from stem rot infested plants from commercial eggplant fields and greenhouses in Turkey during 2012, surface-sterilized using 70% ethanol for 1 min, washed three times in sterile distilled water, plated on potato dextrose agar (PDA) (Difco Laboratories, Detroit) containing 75 $\mu\text{L L}^{-1}$ tetracycline and incubated for 72 h at room temperature for three days. A total of 60 isolates were collected on single infected eggplant plants grown in Adana, Antalya, Hatay, Kahramanmaraş and Osmaniye provinces (Table 1). The isolates were stored in a collection maintained at Mustafa Kemal University, Plant Protection Department.

MC grouping was determined as described by Schafer and Kohn.[15] A 6 mm plug was taken from each isolate, 1 cm inside the growing edge of the colony, and placed in PDA (9 cm Petri plate) amended with 75 $\mu\text{L L}^{-1}$ of McCormick's red food colouring. Each isolate was purified by transferring the single hyphal tip onto the fresh medium, and generated sclerotia were stored at -20°C until use.[8]

Each isolate was paired with itself as a control for compatibility. Pairing plates were incubated in 12/12 h light/dark cycle at room temperature. The two sets of isolates were paired in all possible combinations. MC and incompatibility reactions were recorded 7 and 14 days after inoculation. Each experiment was repeated

at least twice. Pairings were scored as mycelially compatible when no reaction line was observed within the interaction zone between paired isolates. Pairings were scored as mycelially incompatible when a thin to wide band of uniform, aerial mycelium or thin red line on the colony surface, reverse or both was observed between adjacent paired isolates.[4]

All 60 *S. sclerotiorum* isolates were tested for pathogenicity assays on stems of eggplant cultivar Halep Karasi. The plants were grown in potting mix in containers for three weeks in a greenhouse under natural light. A cut-stem method of inoculation [14] was employed. The wide ends of 1000 μL sterile pipette tips were pressed into the leading edge of mycelium of four- to five-days-old cultures grown on PDA until having 12 mm length of agar inside the pipette tip. Then, the pipette tips were placed over the ends of freshly cut stems until the cut ends of stems were inserted into the agar. The inoculated eggplants were kept under humid conditions ($\geq 90\%$ relative humidity (RH)) for 36 h, and then transferred in a growth room at $26 \pm 2^{\circ}\text{C}$ under fluorescent lights for a 12 h day^{-1} . Lesion length was recorded four days after inoculation. Pipette tips containing PDA having no inoculum were placed on the control plants.

DNA manipulation

Single sclerotium isolates were incubated at 28°C for three days on Petri dishes containing solid PDA medium. Single 3 mm mycelial plugs excised from the margin of the colonies grown on PDA were introduced into conical flasks containing 50 mL potato dextrose broth and were maintained at 28°C in a shaker at 120 rpm for 10 days. Mycelium was collected by filtration, washed with saline and kept in liquid nitrogen until use. DNA extraction was performed according to the protocol described by Zolan and Pukkila.[16] DNA quantification was determined by spectrophotometry and visualization under ultraviolet light after electrophoresis on 0.8% agarose gels prepared with $1\times$ TBE (Tris-borate-ethylenediaminetetraacetic acid (EDTA)) and staining with ethidium bromide (0.5 $\mu\text{g/mL}$ in water).[17]

RAPD analysis

Purified DNA was amplified with 10 different 10-mer oligonucleotide RAPD primers; OPA-01, OPA-05, OPA-16, OPB-01, OPB-06, OPB-114, OPB-119, OPC-02, OPC-08 and OPC-15 (Table 2). The RAPD reactions were performed in a final volume of 25 μL reaction mixture containing 16.0 μL milliQ-water (MQ-water), 2 μL (50 ng) template DNA, 1.5 μL deoxyribonucleotide triphosphates (dNTPs), 1.5 μL MgCl_2 , 2.5 μL $10\times$ buffer, 0.5 μL (0.5 U) Taq DNA polymerase and 1 μL primer. All of the reagents were obtained

Table 1. *Sclerotinia sclerotiorum* isolates used in this study with indication of geographic origin (province and location), mycelial compatibility grouping (MCG), virulence (lesion length) and pattern of random amplified polymorphic DNA (RAPD) and haplotype of single strand polymorphism.

Isolate code	Location	Province	Lesion length (mm)	MCG	RAPD group	SSR group
Scl1	Ceyhan	Adana	56	1	1	1
Scl2	Ceyhan	Adana	32	1	2	2
Scl3	Ceyhan	Adana	37	1	1	2
Scl4	Ceyhan	Adana	83	1	1	3
Scl5	Center	Adana	66	2	1	1
Scl6	Center	Adana	82	2	1	3
Scl8	Center	Adana	40	2	1	2
Scl9	Center	Adana	90	2	2	3
Scl7	Center	Adana	38	3	2	2
Scl10	Kozan	Adana	62	4	2	1
Scl11	Kozan	Adana	100	4	1	3
Scl12	Kozan	Adana	32	4	1	2
Scl13	Kozan	Adana	91	5	2	3
Scl14	Demre	Antalya	65	6	2	1
Scl16	Demre	Antalya	93	6	1	3
Scl17	Demre	Antalya	63	6	2	1
Scl18	Demre	Antalya	102	6	1	3
Scl15	Demre	Antalya	32	7	1	2
Scl19	Kumluca	Antalya	30	8	2	2
Scl20	Kumluca	Antalya	90	8	1	3
Scl21	Kumluca	Antalya	60	9	1	1
Scl22	Kumluca	Antalya	23	10	2	2
Scl23	Center	Antalya	93	11	1	3
Scl24	Center	Antalya	31	11	2	2
Scl25	Center	Antalya	36	11	1	2
Scl26	Center	Antalya	68	11	1	1
Scl28	Manavgat	Antalya	94	11	1	3
Scl27	Manavgat	Antalya	37	12	2	2
Scl29	Manavgat	Antalya	36	12	1	2
Scl30	Manavgat	Antalya	30	12	2	2
Scl31	Kırıkhan	Hatay	85	13	1	3
Scl32	Center	Hatay	65	14	1	1
Scl33	Center	Hatay	85	14	2	3
Scl34	Center	Hatay	30	14	1	2
Scl35	Center	Hatay	30	14	1	2
Scl36	Center	Hatay	100	14	2	3
Scl37	Reyhanlı	Hatay	40	14	1	2
Scl38	Samandağ	Hatay	62	15	2	1
Scl39	Samandağ	Hatay	86	15	1	3
Scl40	Samandağ	Hatay	23	15	1	2
Scl41	Samandağ	Hatay	32	15	2	2
Scl42	Samandağ	Hatay	97	15	1	3
Scl43	Andırın	Kahramanmaraş	62	17	2	1
Scl44	Andırın	Kahramanmaraş	98	17	1	3
Scl45	Center	Kahramanmaraş	38	17	2	2
Scl46	Center	Kahramanmaraş	52	17	1	1
Scl47	Center	Kahramanmaraş	85	17	1	3
Scl48	Erdemli	Mersin	38	18	2	2
Scl49	Center	Mersin	34	19	1	2
Scl50	Center	Mersin	60	19	2	1
Scl51	Center	Mersin	28	19	1	2
Scl52	Silifke	Mersin	23	19	2	2
Scl53	Silifke	Mersin	39	20	1	2
Scl54	Tarsus	Mersin	85	20	2	3
Scl55	Tarsus	Mersin	70	21	1	1
Scl56	Center	Osmaniye	93	22	2	3
Scl57	Center	Osmaniye	52	22	1	1
Scl58	Center	Osmaniye	100	22	2	3
Scl59	Center	Osmaniye	20	22	1	2
Scl60	Center	Osmaniye	53	22	2	1
LSD (0.05)			3.4			

Note: LSD, least significant difference.

from Fermentas Inc., USA. Amplification was performed in a programmable thermo cycler (Eppendorf Mastercycler) with the following profile: one cycle at 95 °C for 5 min; 35 cycles at 94 °C for 1 min, 45 °C for 1 min and 72 °C for 2 min; one cycle at 72 °C for 5 min. Amplification was

performed in programmable thermo cycler (Eppendorf Mastercycler) with amplification profile. The amplified fragments were electrophoresed on 1.8% (w/v) agarose gel with ethidium bromide (EtBr) stain (0.1 µg µL⁻¹), for 3 h at 80 V in 1× TBE buffer (89 mmol/L Tris, 89 mmol/L boric

Table 2. List of RAPD primers, observed band numbers and polymorphic band numbers.

Primer code	Primer sequence (5'–3')	Band number	Polymorphic band number	Polymorphism (%)
OPA-01	CAGGCCCTTC	3	1	33
OPA-05	AGGGGTCTTG	4	2	50
OPA-18	AGGTGACCGT	5	1	20
OPB-01	GTTTCGCTCC	3	0	0
OPB-06	TGCTCTGCCC	3	1	33
OPB-14	TCCGCTCTGG	2	1	50
OPB-19	ACCCCCGAAG	3	1	33
OPC-02	GTGAGGCGTC	4	2	50
OPC-08	TGGACCGGTG	3	1	33
OPC-15	GACGGATCAG	5	3	60

acid and 2 mmol/L EDTA). Gel was visualized under UV light with Gel Documentation System (Vilbert, Lourmat, Marne La Vallee, France).

SSR analysis

For the SSR reactions, six primer pairs were used. Polymerase chain reaction (PCR) amplifications were performed in 25 µL reaction mixtures solution containing 2.5 µL of 10× standard buffer (15 mmol/L of MgCl₂), 0.25 µmol/L each SSR primer (Table 3), 250 µmol/L each dNTP (Promega), 1.25 U of Taq DNA polymerase, 0.20 µL of stabilizer (1% W-1 (v/v), Life Technologies), ddH₂O and 25 ng template DNA. Amplification was performed in a programmable thermo cycler (Eppendorf Mastercycler) with the following profile: initial denaturation at 95 °C for 8 min, followed by 35 cycles primer annealing at 59 °C (for all microsatellite primers) and extension at 72 °C for 60 s, with a 5 min extension at 72 °C on the final cycle. The SSR amplification products were separated in a vertical denaturing 10% polyacrylamide gel by electrophoresis at 200 V in TBE buffer (500 mmol/L EDTA pH 8.0, 1 mol/L Tris pH 8.0, 89 mmol/L boric acid) for 2 h. After that, the gel was silver nitrate-stained (0.2%) and visualized under UV light with Gel Documentation System (Vilbert, Lourmat, Marne La Vallee, France).

Statistical analysis

Banding patterns were scored as 1 when present or 0 when absent. DNA fragments of the same size, produced by a single primer, were considered to be identical. Each

isolate was identified regarding the presence (1) or absence (0) of the fragment amplified in each isolate for each primer. Similarity matrices were obtained by the un-weighted pair group method with arithmetic mean (UPGMA) using the NTSYSpc2 software, Version 2.1.[18] The pathogenicity data were analysed by one-way analysis of variance using PROC GLM in SAS. Isolate was the treatment and lesion length was the observational units.

Results and discussion

For plant-pathogenic fungi, MCG, a phenotypic marker system controlled by multiple loci, is a useful method to study the distribution of isolates in geographic areas for a fungal species.[19] During the surveys, 60 isolates each from a different commercial field and unique sclerotium were recovered from Adana (3 locations, 13 isolates), Antalya (3 locations, 17 isolates), Hatay (4 locations, 12 isolates), Kahramanmaraş (2 locations, 5 isolates), Mersin (4 locations, 8 isolates) and Osmaniye (1 location, 5 isolates).

S. sclerotiorum isolates obtained from 6 provinces in 16 locations were compared with each other and with isolates in the other localities and 22 MCGs were obtained (Table 1). MCGs 1, 2, 3, 4 and 5 originated exclusively from Adana province. MCG 1 occurred only in Adana-Ceyhan location, and MCGs 4 and 5 were found in Adana-Kozan, while Adana-Center had mostly MCG 2 and rarely MCG 3. In Antalya province, 7 different MCGs, from 6 to 12 were identified. MCGs 6 and 7 were isolated

Table 3. List of SSR primers, observed band numbers and polymorphic band numbers.

Repeat motif	Locus	Primer sequence (5'–3')	Size range (bp)	Band number	Polymorphic band number
(GA)14	7	TTTGCGTATTATGGTGGGC ATGGCGCAACTCTCAATAGG	160–172	3	2
(CA)9	12	CGATAATTCCCTCACTTGC GGAAGTCCTGATATCGTTGAGG	215–225	5	3
(TTA)9	17	TCATAGTGAGTGATGATGCC CAGGGATGACTTTGGAATGG	345–390	3	1
(TACA)10	55	GTTTTCGTTGTGTGCTGG GCTCGTTCAAGCTCAGCAAG	173–221	4	2
(CT)12	92	TCGCCTCAGAAGAATGTGC AGCGGGTTACAAGGAGATGG	374–378	5	2
(TATG)9	110	ATCCCTAACATCCCTAACGC GGAGAATTGAAGAATTGAATGC	362–378	3	2

merely from Demre, MCGs 11 and 12 were isolated from Manavgat, MCGs 8, 9 and 10 from Kumluca and MCG 11 from Antalya-Center. Three MCGs determined in Hatay province were MCGs 13, 14 and 15. The MCG 13 was unique in Kırıkhan. The MCG 14 was found from Hatay-Center and Reyhanlı, while MCG 15 was isolated from Samandağ. MCG 17 was the only MCG at all locations of Kahramanmaraş. MCGs 18, 19, 20 and 21 isolated from Mersin province at Erdemli, Mersin-Center and Silifke locations. MCGs 18 and 21 were unique to Erdemli-Mersin and Tarsus-Mersin, respectively. MCG 20 was found in Tarsus and Silifke. However, the most prevalent group was MCG 19 found in Mersin-Center and Mersin-Silifke province. No MCG diversity was observed among isolates from Osmaniye and Kahramanmaraş. MCGs 17 and 22 were unique to all locations of Kahramanmaraş and Osmaniye, respectively.

Isolates of *S. sclerotiorum* from eggplant fields of Adana and Antalya were heterogeneous mix of MCGs. Consequently, the population of *S. sclerotiorum* isolates affecting eggplants of Adana and Antalya is more diverse than the population affecting eggplants grown in Mersin, Hatay, Osmaniye and Kahramanmaraş. This verifies the previous reports of *S. sclerotiorum* MCG population structures on cucumber,[20] sunflower,[5] soybean [21] and canola.[22] In the present study, some of the isolates collected from the same or rather close location of the same provinces were grouped into two different MCGs, suggesting the existence of a heterogeneous mix of MCGs in that location. Variation in environmental conditions, selection pressure due to introduction of resistant eggplant cultivars can favour the increase of certain MCGs, while recombination and mutations can lead to new MCGs in *S. sclerotiorum* contaminated eggplant fields. In addition, new isolates can be introduced by human activities and contaminated plant materials. The distribution of *S. sclerotiorum* in Adana, Antalya, Mersin, Hatay, Osmaniye and Kahramanmaraş suggests that almost all of the MCGs are more specific for certain locations, showing very limited movement between locations.

To determine pathogenicity/virulence of 60 isolates, lesion lengths were measured. All tested isolates were pathogenic and initial lesion formation was visible 24 h after inoculation. Lesion length of isolates significantly varied between 20 and 102 mm. The lowest and the highest lesion lengths were obtained from Scl59 in Osmaniye-Center and Scl18 in Antalya-Demre locations, respectively (Table 1). Significantly different lesion lengths were obtained in the same MCGs and in the same locations. In terms of virulence, there were significant differences among isolates and groups in all localities. Highly virulent or weakly virulent isolates were found in the same group.

The results of the pathogenicity test showed that all of the tested 60 isolates were pathogenic to eggplant. However, significant differences in virulence were observed within the same location, similar to other reports.[5,10] Isolates Scl11, Scl18, Scl36, Scl58 and Scl44 from Adana, Antalya, Hatay, Osmaniye and Kahramanmaraş, respectively, were consistently the most virulent isolates tested on eggplant. While the isolates Scl59 (Osmaniye), Scl51 and Scl52 (Mersin), Scl40 (Hatay) and Scl22 (Antalya) were the least virulent isolates. In the current study, significant differences in virulence were observed among same MCGs, regardless of the isolates locations. Therefore, MCG did not seem to be a suitable marker for virulence since the variations in virulence of isolates associated with neither MCG, nor geographic origin belongings. Highly virulent or weakly virulent isolates of *S. sclerotiorum* were obtained in the same group and also among the groups. Our findings agree with the previous studies [20,23] in which virulence levels of the isolates were different within and among the MCGs. Results of this study are in line with other published studies on *S. sclerotiorum*, with examples of different levels of MCG diversity in a given geographical area.[11] Significant differences in aggressiveness among MCGs were also reported by Li et al.[5] However, no variation in aggressiveness among isolates from different geographical areas has been noted in a number of studies.[8,24]

S. sclerotiorum isolates evaluated in the current study were characterized using 12 random primers of the OPC and OPE series. The data set of DNA markers obtained from all the isolates with RAPD markers was analysed with UPGMA by the NTSYS 2.0 software, and a dendrogram (Jaccard's coefficient) was constructed (Figure 1). Based on the results of RAPD analysis, 60 *S. sclerotiorum* isolates representing 22 MCGs were grouped in two distinct clusters at 89.2% of distance (Figure 1). Group I consisted of 35 isolates with 93%–95% genetic similarity, whereas, Group II comprised of 25 isolates in distinct locations, and exhibited genetic similarity of 89%–94%. At 93% of genetic similarity coefficient, Group I was divided into four subgroups with 28 isolates in subgroup IA, 4 isolates in subgroup IB, 3 isolates in subgroup IC, 3 isolates in subgroup ID, while Group II was divided into five subgroups, with 3 isolates in subgroup IIA, 13 isolates in subgroup IIB, 2 isolates in subgroup IIC, 4 isolates in subgroup IID and 3 isolates in subgroup IIE at 95% similarity coefficient. There were no strong associations between RAPD clustering and MCG profiles.

The data set of DNA markers obtained from all the isolates with SSR markers was analysed with UPGMA by the NTSYS 2.0 software, and a dendrogram (Jaccard's coefficient) was constructed (Figure 2). All the

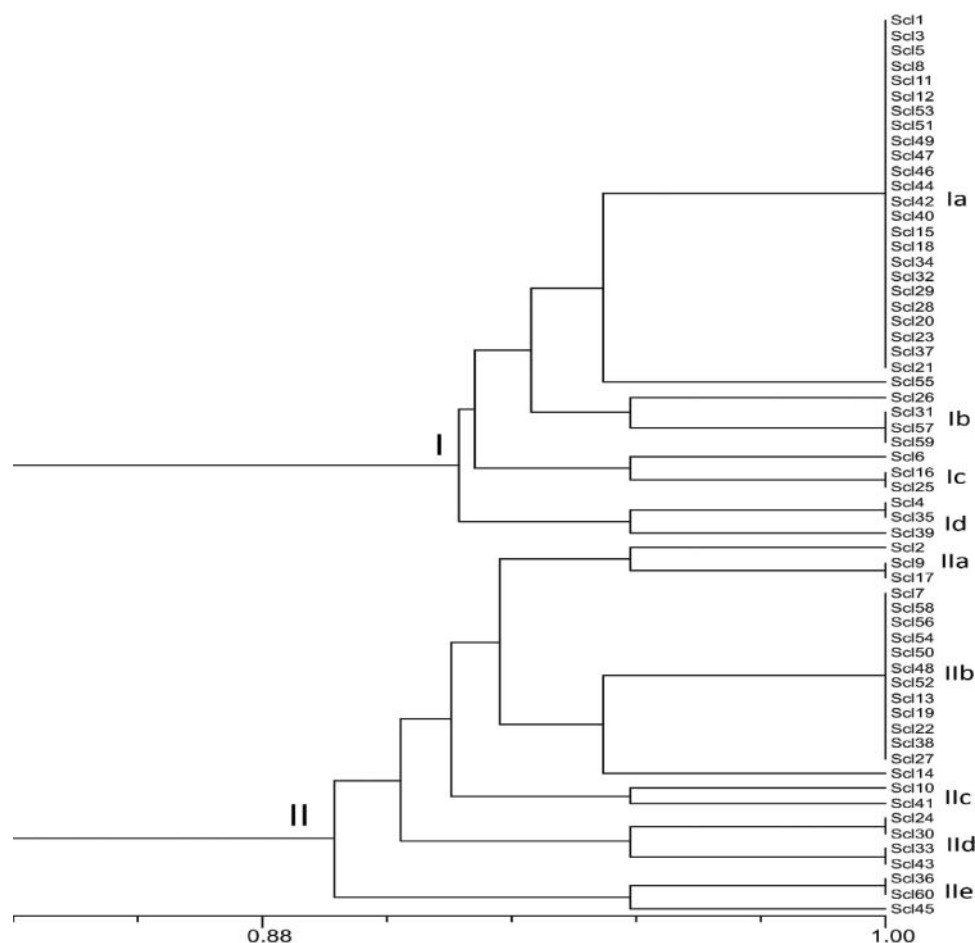


Figure 1. Un-weighted pair group method with arithmetic mean (UPGMA) dendrograms showing the genetic relationships between the 60 isolates of *Sclerotinia sclerotiorum* based on RAPD markers.

isolates were divided into three major groups (Figure 2). Group I consisted of 15 isolates with 89% genetic similarity. Group II comprised of 25 isolates in distinct locations, exhibited genetic similarity of 92%, whereas, Group III comprised of 20 isolates, and had genetic similarity of 91%. At 95% of genetic similarity coefficient, Group I was divided into four subgroups with 3 isolates in subgroup IA, 2 isolates in subgroup IB, 4 isolates in subgroup IC and 4 isolates in subgroup ID. Group II was divided into four subgroups with 5 isolates in subgroup IIA, 12 isolates in subgroup IIB, 3 isolates in subgroup IIC and 5 isolates in subgroup IID, and Group III was divided into four subgroups with 4 isolates in subgroup IIIA, 11 isolates in subgroup IIIB, 3 isolates in subgroup IIIB and 2 isolates in subgroup IIID at 95% similarity coefficient. There were no strong associations between SSR clustering and MCG profiles.

There was no obvious clustering according to geographic location for MCGs. However, MCGs containing isolates from more than one location clustered separately from groups containing isolates from only one

location. Previous studies showed that MCG diversity can vary greatly on a specific host in a given geographical area.[25] The present study confirmed this conclusion, for instance, 17 isolates collected from eggplant fields in Antalya were separated into seven MCGs. Most of the isolates collected from the same province could be grouped together, such as MCG 17 in which the tested isolates were collected from Kahramanmaraş province, MCG 22 mainly contains isolates collected from Osmaniye; also, majority of isolates from Adana province was grouped into MCG 11 and MCG 12 (Table 1). Conversely, some of *S. sclerotiorum* isolates, collected from the same provinces such as Antalya (17 isolates), were grouped into seven different MCGs. The presence of unique MCGs in some of the locations is the evidence of new MCGs emergence and evolution of new genotypes.

The analysis of population dynamics of *S. sclerotiorum* is an essential part of understanding how the underlying mechanisms are involved in pathogen distribution across different geographic regions. Based on molecular

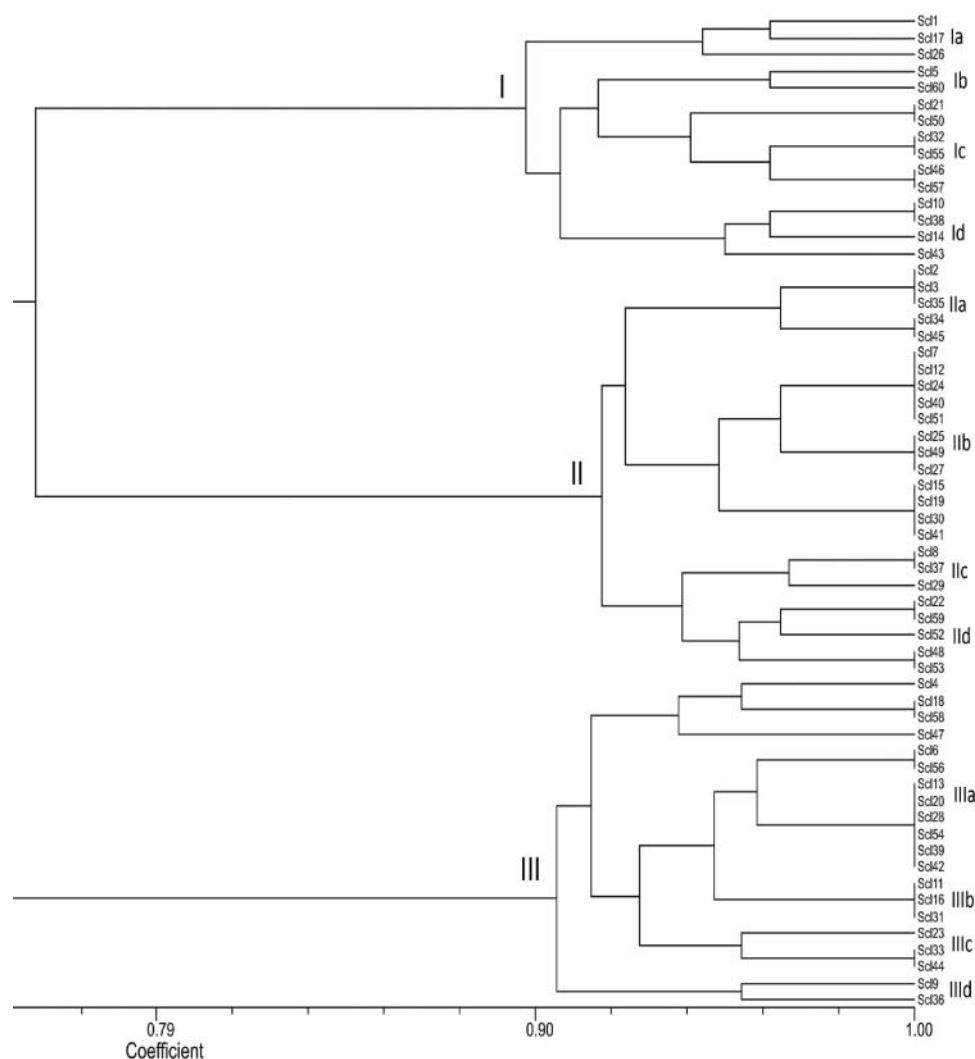


Figure 2. Un-weighted pair group method with arithmetic mean (UPGMA) dendrograms showing the genetic relationships between the 60 isolates of *Sclerotinia sclerotiorum* based on SSR markers.

characterization of *S. sclerotiorum* isolates, it is evident that the isolates from eggplants in Adana, Antalya, Mersin, Hatay, Osmaniye and Kahramanmaraş provinces had a high level of diversity and were grouped in two and three clusters determined by the RAPD and SSR markers, respectively. When clusters of RAPD and SSR were compared with those of MC, there was no strong association between the genetic distance for isolates collected from the different locations and provinces (Figures 1 and 2). The distribution of the isolates indicated no sign of tendency toward the formation of clusters with respect to province, showing that occurrence of specific population distribution restricted to a specific location. Several studies have demonstrated that isolates from the same MCG are genetically more similar than isolates from different MCGs.[4,26,27] Previous studies showed that MCGs or SSR markers were not associated with specific virulence characteristics or ecological adaptations of the pathogen. [8,9,14,25] However, in other studies, a close association

between MCG and cluster grouping was reported [5,21]; SSR markers were found to be more efficient in detecting genetic diversity among the isolates than RAPD. SSR markers exhibited the best polymorphic DNA banding pattern.

Conclusions

The results from this study demonstrate that MCG variations in *S. sclerotiorum* occur within the populations of provinces, as well as populations collected from the same location of each province. The difference in the distribution of MCG among locations and provinces was notable. Pathogen virulence had neither relationship with clusters of RAPD and SSR nor with MCGs. The populations of *S. sclerotiorum* were genetically heterogeneous and that combination of MCG and molecular analysis did not offer a powerful tool for understanding this heterogeneity. Human activities, movement of contaminated plant

materials and soil debris might tend to increase the diversity of MCGs while outbreeding would raise genetic diversity through meiotic recombination that causes to increase MCG numbers. The potential sexual recombination between MCGs indicates the possible rise of diverse genotypes in each province. Therefore, preventive control methods combined with agricultural practices are needed to restrict gene flow between populations and provinces.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by Scientific Research Projects Coordination Unit [project number MKU-BAP 89].

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