



Identification and Characterization of White Mold Disease (*Sclerotinia sclerotiorum*) in Globe Artichoke

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ARTICLE INFO

Article history:

Received date: 31.08.2020

Accepted date: 17.11.2020

Edited by:

Murat KARACA; Selcuk University, Turkey

Keywords:

Cynara scolymus

White Mold

Identification

Characterization

ABSTRACT

Globe artichoke (*Cynara scolymus* L.) is an economically important plant that is cultivated over 30 countries in the world. In 2017, wilting and lodging symptoms were observed on globe artichoke plants in three locations (Serik-Gebiz, Serik-Karadayı and Gazipaşa-Bakılar) in the Western Mediterranean Region of Turkey. To determine the cause of the symptoms, samples from necrotic tissues were cultured in vitro. Based on the morphological and ITS sequences, the causal agent was identified as *Sclerotinia sclerotiorum* (Lib.) de Bary. Pathogenicity test was conducted using cv. Bayrampaşa in the greenhouse. Three isolates (Ser3, Ser4 and Gzp3) of *S. sclerotiorum* were characterized on the basis of mycelial compatibility, colony radial growth, sclerotia formation, sclerotia size, sclerotia weight and sclerotia number. Virulence of the isolates was also determined using detached leaf technique. No mycelial compatibility was detected among the isolates. Significant ($P < 0.01$) differences were found in the examined morphological features and virulence of the isolates. Gzp3 was the most virulent isolate forming average 8.60 cm lesion length. However, lesion lengths formed by the other isolates, Ser3 and Ser4, were average 4.16 and 1.93 cm, respectively. This is the first detailed characterization of *S. sclerotiorum* causing crown and stalk rot in globe artichoke.

1. Introduction

Globe artichoke (*Cynara scolymus* L.) is a herbaceous plant from Asteraceae family. It is mostly grown in the Mediterranean Basin and has various bioactive phenolic compounds, inulin, fibre, minerals and cynarin. Therefore, it is a functional food source and constitutes significant part of the Mediterranean cuisine (Lattanzio et al 2009). With 39477 t annual globe artichoke production, Turkey is the 11th producer in the world (FAO 2020).

Globe artichoke production is negatively affected by fungal pathogens. One example of which is *Sclerotinia sclerotiorum* (Lib.) de Bary. Usually called as white mold, it is a necrotrophic fungus infecting over 400 plant species (Allan et al 2019). Infection of the fungus appears as whitish, cottony and dense mycelial mats on host tissue. The fungus can infect miscellaneous parts of plants depending on germination of sclerotia. Thus, the disease caused by *S. sclerotiorum* might be designated differently. In addition to white mold, it is also called as stem rot, watery

soft rot, cottony rot, white blight, root rot, stem break, stalk rot or white canker (Kapatia et al 2016).

Sclerotia (aggregates of hyphae containing thick melanin) are essential inoculum sources and long-term survival structures of the fungus. They can survive up to 8 years in soil (Bolton et al 2006). Depending on the environmental conditions, sclerotia could germinate carpogenically or myceliogenically (Smolińska & Kowalska 2018). If myceliogenic germination occurs, sclerotia form hyphae that infect root, crown and stalks of host plants, which is called basal infection of the fungus (Lane et al 2018). In this infection phenomenon, the fungus could form appressoria on host surface or directly penetrate cuticle through its infective hyphae at basal stem of host. Ensuing penetration, hyphae develop inter and intracellularly in cells by excreting cell wall degrading enzymes, results in tissue collapse of host. The fungus maintains its colonization on dead tissue of host, which is seen as white fluffy mycelial mats (Davar et al 2012).

In addition to basal infection, if the carpogenic germination of sclerotia occurs, the fungus induces rots on different parts of host (e.g. pod rot on bush bean, fruit rot on garden pea, head rot on cauliflower and blossom rot on salvia) (Rahman et al 2020). All of

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these indicate destructiveness of the fungus on many agricultural crops. As regards this, Grabowski & Malvick (2017) reported that *S. sclerotiorum* caused stem and crown rot and consequently wilt and death of a wide range of annual flowering plants.

Due to difficulty in controlling of *S. sclerotiorum*, there is a steady increase in losses stem from the fungus in horticultural crops in the world (Smolińska & Kowalska 2018). To give some examples, it was reported that *S. sclerotiorum* led to substantial yield and quality losses in bean (*Phaseolus vulgaris*), oilseed rape (*Brassica napus* and *B. juncea*), lettuce (*Lactuca sativa*), sunflower (*Helianthus annuus*), perilla (*Perilla frutescens*) and cabbage (*Brassica oleracea*) production in Brazil, Australia, UK, China, Korea and Sri Lanka, respectively (Meinhardt et al 2002; Li et al 2006; Clarkson et al 2014; Liu et al 2018; Afroz et al 2019; Mahalingam et al 2020). With regard to globe artichoke, too little is known about *S. sclerotiorum* on globe artichoke.

The aims of the study were to i) identify causal agent causing crown and stalk rot on globe artichoke in the Western Mediterranean Region of Turkey, ii) characterize isolates of white mold based on morphologic and genetic features, and iii) determine virulence of isolates of *S. sclerotiorum* on globe artichoke.

2. Materials and Methods

2.1. Isolation

Disease symptoms were observed in globe artichoke growing fields of Serik-Karadayı, Serik-Gebiz and Gazipaşa-Bakılar locations of the Western Mediterranean Region of Turkey in 2017. Samples from crown and stalk parts of lodging plants were taken and tagged in paper bags. They were taken to the Mycology Laboratory of Batı Akdeniz Agricultural Research Institute. Initially, the samples were washed under tap water and then necrotic tissues were cut into small pieces. They were exposed to NaOCl (2%) for two min and rinsed with sterile distilled water. Afterwards, they were put on sterile filter papers for drying for nearly 45 min and then transferred on potato dextrose agar (PDA). Petri dishes were kept at 25 °C at 6 days. Fungal colonies developing on PDA were subcultured by taking tips of hyphae of each colony.

2.2. Identification

Three isolates of *S. sclerotiorum* were designated as Ser3, Ser4 and Gzp3.

2.2.1. Morphological identification

Morphological features (structure of mycelium, colony color and pattern) were examined using an Olympus BX43 microscope with SC100 digital color camera. Sclerotia numbers of the isolates were calculated by counting sclerotia in each petri. Size and weight of sclerotia were determined using a caliper and precision scales (Bolton et al 2006).

2.2.2. Molecular identification

DNA of each isolate was extracted using purification protocol of Promega. Following DNA extraction, rDNA fragments were amplified using primer pairs ITS-1 (5' TCC GTA GGT GAA CCT GCGG 3') and ITS-4 (5' TCC TCC GCT TAT TGA TATGC 3') (White et al 1990). Amplifications were performed in a SimpliAmp Thermocycler (Applied Biosystems, USA) and consisted of 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, annealing temperature at 58.5°C for 1 min, 72°C for 1 min, with a final extension at 72°C for 7 min. PCR products were separated in 2% agarose gels, stained with safe DNA dye and visualized under UV light. Sequence analysis was done by GENOKS (Çankaya-Ankara). The ITS sequences of the isolates, Ser3, Ser4 and Gzp3, were deposited at GenBank (<http://www.ncbi.nlm.nih.gov>) with the accession numbers of MH593866, MH593867 and MH593868, respectively.

In addition, a phylogenetic tree was constructed using neighbour-joining method in MEGA version 7.0 program to compare relatedness of our isolates with the other isolates of *S. sclerotiorum* in the genbank.

2.3. Pathogenicity test

Two month-old seedling of globe artichoke (cv. Bayrampaşa) was planted per pot containing autoclaved soil and vermiculites (1:1). Mycelial plugs (0.5 cm) were taken from the edges of 7 day-old colonies of each isolate. These mycelial plugs were attached to just below the crown of seedlings. A total of 4 seedlings were inoculated for each isolate. In the controls, only agar plugs were used. Fifteen days after inoculation, wilting symptoms were observed on inoculated seedlings, while no symptom was seen on the control seedlings. Samples from the necrotic tissues of the inoculated seedlings were taken and each isolate of *S. sclerotiorum* was re-isolated separately.

2.4. Colony radial growth and sclerotia formation

Mycelia plugs (0.5 cm) of each isolate were put on the center of petri dishes (9 cm) containing PDA. One plug was used per petri and incubated at 25 °C for 5 days. Colony radial growth of each isolate was measured daily. The experiment was carried out according to completely randomized design with four replicates. Experimental unit consisted of one petri dish. Sclerotia formation, sclerotia size, sclerotia weight and sclerotia number per petri were recorded (Mert-Türk et al 2007).

2.5. Mycelial compatibility groupings (MCGs)

One mycelial plug (0.5 cm) of each isolate was put nearly 2 cm away from the edge of Petri dish containing PDA with red food coloring (six drops/L). Each pairing was performed with four replications. In addition, self-pairings for each isolate were done as aforementioned (Kohn et al 1990; Schafer & Kohn 2006).

2.6. Virulence of the isolates of *S. sclerotiorum*

Virulence of each isolate was determined using detached leaf technique. One mycelial plug (0.5 cm) of each isolate was put on the center of two-month-old leaf of globe artichoke seedling (cv. Bayrampařa) placed on moist sterile filter paper per Petri dish. The experiment was conducted using completely randomized design with three replications. In the controls, only agar plugs were put on the leaves. Four days after inoculation, lesion lengths on the detached leaves were measured. Mean lesion length of each isolate was compared using variance analysis (Zanatta et al 2019; Rahman et al 2020).

2.7. Statistical analysis

For variance analysis, SAS 9.1 software program (SAS Institute Inc., Cary, NC, USA) was used. Means of colony radial growth, sclerotia size, sclerotia weight, sclerotia number and lesion lengths lesions were calculated using $LSD_{0.01}$.

3. Results and Discussion

3.1. Symptoms of white mold on globe artichoke in the field

Plants infected by white mold (*S. sclerotiorum*) initially showed wilting symptoms. Then, rotted areas were observed on the stalks. The fungus progressed from crown to upper parts of the stalks. As a result of entire rotting of the stalks, plants lodged and fell over the ground. When the infected stalks were cut longitudinally, sclerotia were observed inside of those stalks (Figure 1).



Figure 1
Wilting, crown and stalk rot symptoms caused by *S. sclerotiorum* on globe artichoke plants (A) and sclerotia of *S. sclerotiorum* in stalks of globe artichoke (B) (Gazipařa, Antalya).

3.2. Pathogenicity of the isolates of *S. sclerotiorum*

Fifteen days after inoculation, all the isolates (Ser3, Ser4 and Gzp3) initially led to wilting with necrotic tissues and then rot on crown and stems of the globe artichoke seedlings (Figure 2). The isolates were re-isolated from those necrotic tissues and their pathogenicity to globe artichoke plants was confirmed. In the pathogenicity test, globe artichoke seedlings were severely affected by infections by the isolates of *S. sclerotiorum* (Figure 2).



Figure 2
Wilting symptoms (death of lower shoots with red arrows) of seedling of cv. Bayrampařa inoculated with Gzp3 isolate of *S. sclerotiorum* (left) and non-inoculated (control) seedling (right).

3.3. Morphological features of isolates of *S. sclerotiorum*

Hyphae of all isolates of *S. sclerotiorum* were septate, branched and transparent in color (Figure 3). These are typical features of hyphae of *S. sclerotiorum* described by Bolton et al (2006).

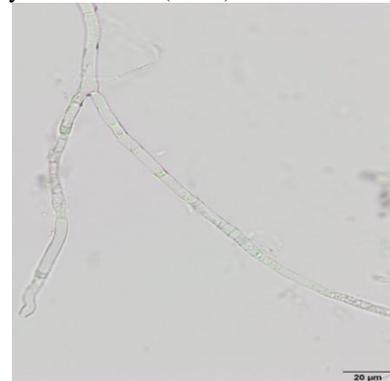


Figure 3
Hyphae of Ser3 isolate of *S. sclerotiorum*

Colonies of the isolates were white to off-white. Colony pattern of Ser3 and Ser4 isolates were similar in forming loose mycelial growth. However, Gzp3 isolate displayed dense velvety aerial mycelia in the center of the petri. Five-six days later, initially mycelium mats appeared as drop like and then became rigid and darkish-black in color. Shapes of the sclerotia of the isolates were irregular to globe (Figure 4).

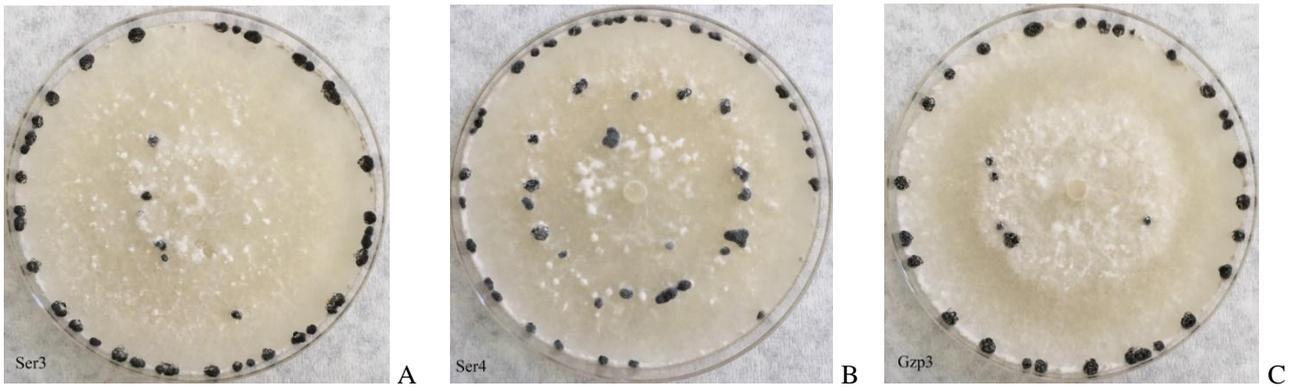


Figure 4

Colony development and sclerotia formation of isolate Ser3 (A), Ser4 (B) and Gzp3 (C) on PDA

Significant differences were found in colony radial growth, sclerotia size, sclerotia weight and sclerotia number per petri (Table 1).

Colony radial growths of Gzp3 and Ser3 isolates were not different statistically ($P < 0.01$). However, the least colony radial growth, 37.42 mm/48 h, was detected in Ser4 isolate. Mean of sclerotia size of Gzp3 isolate was the largest (2.62 mm), while mean of sclerotia sizes of the other isolates were not different statistically

($P < 0.01$). Similarly, mean of sclerotia weight of Gzp3 isolate was 18.63 mg, whereas mean of sclerotia weights of the other isolates were not different statistically ($P < 0.01$). As for sclerotia number, differences in sclerotia numbers of all the isolates were significant ($P < 0.01$). Mean of sclerotia numbers of Gzp3, Ser4 and Ser3 isolates were 23.25, 40.25, 50.25, respectively (Table 2).

Table 1

Variance analysis of colony radial growth, sclerotia size, sclerotia weight and sclerotia number per petri

Source	Degree of freedom	Colony radial growth (mean of squares)	Sclerotia size (mean of squares)	Sclerotia weight (mean of squares)	Sclerotia number per petri (mean of squares)
Isolate	2	100.053**	1.618**	104.513**	745.333**
Error	9	0.293	0.049	2.896	18.694
Total	11	11	11	11	11
CV(%)		1.25	11.69	13.29	11.40

**Significant at $P < 0.01$

Table 2

Colony radial growth, sclerotia size, sclerotia weight and sclerotia number of *S. sclerotiorum* isolates

Isolates	Colony radial growth (mm/48 h)	Sclerotia size (mm)	Sclerotia weight (mg)	Sclerotia number per petri
Gzp3	46.62 a	2.62 a	18.63 a	23.25 c
Ser3	45.42 a	1.43 b	9.11 b	50.25 a
Ser4	37.42 b	1.64 b	10.65 b	40.25 b
LSD	1.24	0.51	3.91	9.93

Values within each column above are means of four replications and the same letters in each column did not differ statistically (1%)

3.4. Mycelial compatibility groupings (MCGs)

In mycelial compatibility, there is no formation of either a distinctive barrier or aerial mycelial development in the interaction zone of colonies. In the assessment of mycelial compatibility, PDA containing red food coloring was used to distinguish compatibility. As a result of the pairings, distinct barriers composed of aerial mycelial developments occurred in the interaction zones of the isolates. Thus, there was no mycelial compatibility between each isolate, which was also confirmed with emergence of red distinct line in the interaction zones (Figure 5).

3.5. Phylogenetic analysis

ITS sequence sizes of the isolates [(MH593866; Ser3), (MH593867; Ser4) and (MH593868; Gzp3)] were 478, 460, 470 bp, respectively. The phylogenetic tree was constructed using other *S. sclerotiorum* isolates displaying 100% homology in the genbank with our isolates. In the phylogenetic tree, all of our isolates (MH593866, MH593867 and MH593868) of *S. sclerotinia* were on the different clades (Figure 6), which indicated genetic variability of the isolates obtained from globe artichoke growing locations of the Western Mediterranean Region of Turkey.

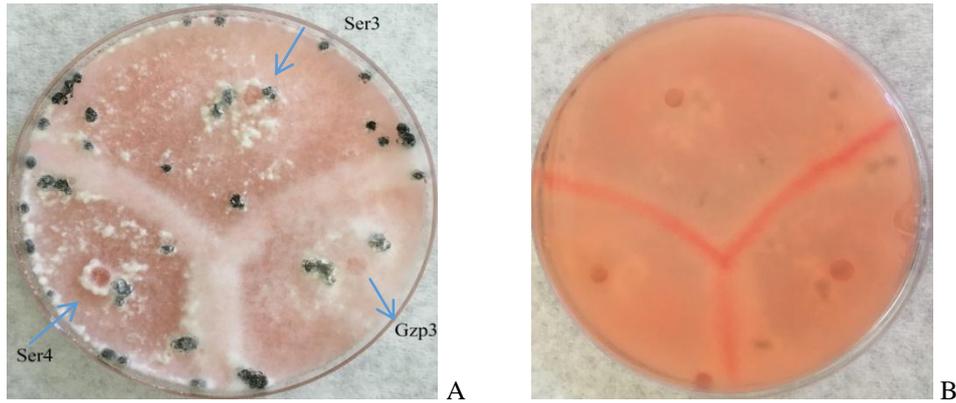


Figure 5 Mycelial compatibility grouping of the isolates of *S. sclerotiorum*, surface side (A) and reverse side (B)

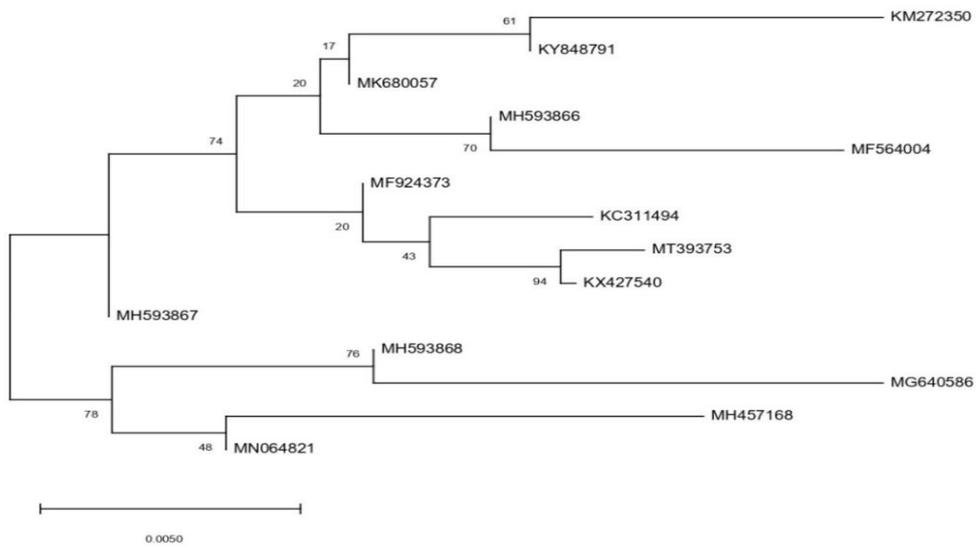


Figure 6 Phylogenetic tree constructed with isolates of *S. sclerotiorum* showing 100% homology in the genbank with our isolates (MH593866, MH593867 and MH593868)

3.6. Virulence of the isolates of *S. sclerotiorum*

From the point of contact of mycelial plugs of each isolate, lesions (necrotic tissues) occurred on the detached leaves in petri plates. Lesion lengths varied depending on each isolate (Figure 7).

Significant ($P < 0.01$) differences occurred in lesion lengths of the isolates on the detached leaves of globe artichoke (Table 3).



Figure 7

Lesion lengths caused by the isolates [Ser4 (A), Ser3 (B) and Gzp3 (C)] of *S. sclerotiorum* on leaves of globe artichoke (cv. Bayrampařa) and no lesion development on the control (D)

Table 3

Variance analysis of lesion lengths caused by the isolates of *S. sclerotiorum* on detached leaves in petri

Source	DF	Mean of squares	F value	P>F
Isolate	2	34.543**	661.47	<0.01
Error	6	0.052		
Total	8			
CV(%)	4.6			

**Significant at P<0.01

Mean lesion length caused by each isolate on the detached leaves was significantly (P<0.01) different from each other, indicating difference in virulence of each isolate. Based on this comparison, it was concluded that the most virulent isolate was Gzp3 forming 8.60 cm mean lesion length. However, the other isolates, Ser3 and Ser4, caused 4.16 and 1.93 cm mean lesion lengths, respectively (Table 4).

Table 4

Comparison of mean lesion lengths caused by each isolate of *S. sclerotiorum* on detached leaves of cv. Bayrampařa

Isolates	Mean lesion lengths on detached leaves in petri
Gzp3	8.60 a
Ser3	4.16 b
Ser4	1.93 c

LSD_{0.01}: 0.69

If sclerotia of *S. sclerotiorum* germinate myceliogenically, they form hyphae that infect root, crown and stalks of host plants, which is called basal infection (Lane et al 2018). In our study, this type of infection of *S. sclerotiorum* was detected on globe artichoke plants in the field. Similarly, Brosten & Sands (1986) found that *S. sclerotiorum* was pathogenic to thistle (*Cirsium arvense* L.; Asteraceae), causing crown and root rot and death of shoots of thistle in Canada. These findings might indicate that *S. sclerotiorum* may be prone to induce basal infection on hosts from asteraceae. However, it may not be

associated with the host. Because, when sclerotia of *S. sclerotiorum* germinate carpogenically, they produce ascospores that act as an airborne manner by infecting above ground parts of host (Foley et al 2016). For example, in this way, the fungus causes pod rot on bush bean, fruit rot on garden pea, head rot on cauliflower, blossom rot on *Salvia* (Rahman et al 2020). In this context, environmental conditions in particular moisture and temperature are primary factors affecting carpogenic or myceliogenic germination of sclerotia (Smolińska & Kowalska 2018).

Mycelial compatibility is the capability of two fungi to constitute one continuous colony with anastomoses. If mycelia of two fungi don not anastomose, a distinct reaction line occurs in the interaction zone of the two fungi, which implies incompatible reaction (Schafer & Kohn 2006). Likewise, in our study, there were distinct reaction lines among three isolates of *S. sclerotiorum*. This indicates mycelial incompatibility of all the isolates. This is also means that there is a genetic variability in our isolates. Because, mycelial compatibility/incompatibility is a way of establishment of genetic variability of *S. sclerotiorum* isolates (Kohn et al 1990). Moreover, in our study, in the phylogenetic tree, all the isolates are on different clades, indicating genetic variability of the *S. sclerotiorum* isolates in globe artichoke. Similarly, Mert-Türk et al (2007) found a high genetic diversity among *S. sclerotiorum* isolates obtained from oilseed rape fields in the Çanakkale Province of Turkey. Yanar & Onaran (2011) also detected a wide range of mycelial incom-

patibility groups among *S. sclerotiorum* isolates from cucumber in Kumluca, Finike and Demre locations of Antalya Province of Turkey. These are supported our findings. In addition, genetic diversity of *S. sclerotiorum* was reported on various crops and regions around world (e.g. Barari et al 2012; Liu et al 2018; Mahalingam et al 2020). Outcrossing and evolutionary potential of the fungus might play a significant role in its genetic diversity (Mert-Türk et al 2007). Genetic diversity of *S. sclerotiorum* isolates might be also associated with coinfection of the fungus. In this regard, Sexton et al (2006) reported that even a single canola stem could be infected by multiple isolates of *S. sclerotiorum*, inducing outcrossing/recombination and consequently emergence of new genotypes.

In our study, based on mean lesion length caused by each isolate, virulence levels of the isolates of *S. sclerotiorum* were determined. Difference in virulence of each isolate was significant ($P < 0.01$). Oxalic acid, cell wall-degrading enzymes and other secreted proteins have primary role in virulence of *S. sclerotiorum* (McCaghey et al 2019). There was a significant positive correlation between the lesion length caused by *S. sclerotiorum* on sunflower leaves and oxalic acid production of the fungus (Liu et al 2018). These justified the method we used in determining virulence of the isolates. In the detached leaf technique, measurement of lesion length can be done easily. Moreover, comparison on the basis of this measurement gives accurate results in determining virulence of *S. sclerotiorum* isolates and evaluation of host reactions as well. Apart from this, there are numerous studies reporting virulence differences of *S. sclerotiorum* isolates in various crops (soybean, cucumber, canola, drybean, cabbage, pear, rapeseed, sunflower, dry bean, pinto bean and tomato) (Kull et al 2004; Davar et al 2011; Otto-Hanson et al 2011; Yanar & Onaran 2011; Barari et al 2012; Karimi et al 2012). *S. sclerotiorum* has a broad-range of pathogenicity mechanisms enabling infection of a great number of host (Sexton et al 2006). In addition to soilborne and airborne nature, that *S. sclerotiorum* could be spread to long distance through sunflower seeds, indicating seedborne nature of the fungus as well (Zancan et al 2015). Therefore, seeds might also harbor mycelium of *S. sclerotiorum* and constitute a risk factor in spreading of the fungus.

4. Conclusions

Limited information is present about *S. sclerotiorum* on globe artichoke. With our study, isolates of the fungus were characterized in detail for the first time.

Sclerotinia sclerotiorum is a fungus which infects numerous agricultural crops. However, management of the fungus through either cultural or chemical way is hard in any agricultural crop. Because, the fungus has multiple pathogenesis mechanisms to overcome its host and long term survival structures (sclerotia). Therefore, using host resistance is the only plausible approach in

the management of the fungus. Selecting virulent isolates of *S. sclerotiorum* is important for evaluation of globe artichoke genotypes for resistance to white mold disease. In this regard, virulent Gzp3 isolate can be used as inoculum source for screening globe artichoke genotypes against *S. sclerotiorum* in further studies..

5. Acknowledgements

This study was carried out within the scope of the project (TAGEM-BS-15/09-10/02-10) and supported by General Directorate of Agricultural Research and Policies, Turkey.

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