Kor. Turfgrass Sci., 20(1), 65~76, 2006

Genetic and Physiological Discrepancies from Isolates of *Sclerotinia homoeocarpa* causing Zoysiagrass Dollar Spot Disease

Dae-Sup Park[1]*, Kyung-Duck Kim[1], Joon-yeong Kihl[2], and Jae-Ho Pyee[2]

[1]*Turfgrass & Environment Research Institute, Samsung Everland Inc., Gunpo 435-737, Korea*
[2]*Department of Molecular Biology, Dankook University, Seoul 140-714, Korea*

한국잔디에 발생하는 동전마름병 원인균의 유전 및 생리적 특성차이

박대섭[1]*, 김경덕[1], 길준영[2], 피재호[2]

[1]삼성에버랜드 잔디환경연구소, [2]단국대학교 분자생물학과

ABSTRACT

Scz1, an isolate of *Sclerotinia homoeocarpa*, was recently reported as a novel pathogen responsible for dollar spot disease in Zoysiagrass, a warm season turfgrass. Scz1 possessed different characteristics on mycelial pigment, mycelial affinity and host pathogenecity compared to those of Scb1, a typical isolate, obtained from creeping bentgrass, a cool season turfgrass. In this study, only three isolates, Scz1, Scz2(another analogous isolate of *Sclerotinia homoeocarpa* from zoysiagrass), and Scb1, were examined at the molecular level using the internal transcribed spacer(ITS) and random amplified polymorphic DNA(RAPD) assays to verify their identification and genetic variation. As a result of ITS assay, partial ITS sequences of three isolates showed 94-97% similarity with a standardized ITS sequence of *S. homoeocarpa* registered on BLAST. In the analysis of RAPD, range value through similarity matrix was 0.167 between Scz1 and Scb1, 0.139 between Scz2 and Scb1, and 0.713 between Scz1 and Scz2, respectively. Furthermore, tendegram analysis indicated that Scz1 and Scz2, unlike Scb1, were clustered together as accompanying a high genetic similarity. In *in vitro* fungicide bioassay, EC_{50} value representing the sensitivity degree to propiconazole, a well-known fungicide for dollar spot disease, was 0.012 μg/mℓ for Scz1, 0.003 μg/mℓ for Scz2, and 0.030 μg/mℓ for Scb1. From all data taken, we concluded that both Scz1 and Scz2 belonged to one group of *S. homoeocarpa*, since they exhibit the same host range and high level of genetic similarity, whereas their chemical competences to a fungicide

*Corresponding author. Tel : 031-460-3407
E-mail : daesup.park@samsung.com

were different. This study would provide further approach for assessing genetic diversity of *S. homoeocarpa* isolates as well as characterizing individual isolate against chemical exposure.

Key words : dollar spot disease, *Sclerotinia homoeocarpa*, zoysia dollar spot

INTRODUCTION

Dollar spot is a ubiquitously problematic disease of turfgrass, which is infectious to all turf species including Kentucky bluegrass, creeping bentgrass, ryegrass, tall fascue, and even zoysiagrass, and which is distributed in many countries including U.S.A. and European countries(Smiley et al., 1992; Vargas, 1981; Walsh et al., 1999). This disease named from a coin-shaped symptom is known to be caused by *Sclerotinia homoeocarpa,* which is exceptionally present within *Sclerotinia spp.* because of absence of apothecial and conidial stages(Bennett, 1937; Kohn, 1979). Therefore, the pathogen has been often classified as *Lanzia, Moellerodiscus,* or *Rutstroemia*(Carbone and Kohn, 1993; Couch, 1985). It is generally known that the fungus survives as stroma in soils, thatch layers, and clipping residues in the unfavorable conditions(Couch, 1995).

Both incidence and progress of the disease are affected by external factors such as humidity and temperature. In particular, temperature is a vital factor on the disease progress. The causal fungus, *Sclerotinia homoeocarpa,* thrives at 22~28℃ but ceased at 30℃ above(Bennett, 1937). It is grounded on the fact that dollar spot disease is an epidemic with two-seasonal occurrences in disease cycle; spring and fall. Also, the disease incidence and severity depends on nitrogen fertilization but not soil pH(Couch and Bloom, 1960; Davis and Dernoeden, 2002; Couch ,1995; Smiley et al., 1992).

In cool season turfgrasses, disease symptoms of dollar spot appeared on the leaves of turfgrass as yellow spots. The yellow spots turned brown and the spots spread rapidly to the whole leave under the favorable conditions. Later, leaf turned white and the browned leaves and stems formed big circles(Couch, 1995). Sometimes, the these symptoms on the putting green area are considerably confused with those of *Pythium* blight disease, appearing similar symptoms composing of small sized patches and leaf blight.

In warm season turfgrasses, small yellow spots were appeared on the infected leaf blades and then spread to a whole leaf. The bleached leaf turned white and blighted. Small patches in diameters of 5 cm formed enlarged patches and then merged gradually each other under a favorable condition(Park et al. 2005). Occasionally, the

disease is likely to be misunderstood as *Rhizoctnia* leaf blight due to similar leaf spot symptoms. In Korea, this disease mostly shown on creeping bentgrass occurs from early June to late September(Shim et al., 2000). Since the first report on zoysiagrass(*Zoysia japonoca*) dollar spot in 2000 and second report on efficacy of selected fungicides for disease control in 2001(Shim et al., 2001), few report has been published so far.

Recently, we obtained a novel pathogen from golf courses established with Anyang Joongi, a cultivar of zoysiagrass, at which small circular patches were severely distributed from early summer to late fall of 2005(Park et al., 2005). In the previous study, a newly introduced isolate, Sczl, compared with Scbl, a representative isolate harvested from creeping bentgrass, was readily identified as *S. homoeocarpa* on the basis of morphologic and phenotypic features. On the other hand, two isolates were distinguished by other conventional assays such as mycelial pigment, vegetative compatibility, and host pathogenecity(Park et al., 2005). In pathogenecity test, Sczl isolate produced spotting and blight lesions on the leaves of zoysiagrass. In contrast, Sczl did not invoke any symptoms and lesions on creeping bentgrass and showed weak symptoms on Kentucky bluegrass. It was also observed that zoysiagrass infected with Sczl prolonged the duration of the symptom and the degree of disease severity as compared with those of creeping bentgrass and Kentucky bluegrass.

In the mean time, two molecular techniques for identifying and classifying fungi and bacteria have been widely used; ITS DNA analysis and RAPD(Randomly Amplified Polymorphic DNA)/AFLP(Analysis by Amplified Fragment Length Polymorphism) analysis(Carbone and Kohn, 1993; Hsiang et al., 2000; Clarridge, 2004; Viji et al., 2004). The results from these assays might be useful to make an understanding of correct identification as different strains in either host cultivars or species. Recently, isolates of *S. homoeocarpa* had been identified and classified by using two methods. Carbone and Kohn(1993) examined numerous genera of *Sclerotiniaceae* using ITS analysis as well. They suggested hereby that *S. homoeocarpa* is reclassified as *Rutstroemia spp*. Powell(2001) identified the isolates collected at two different seasons by ITS sequence analysis and vegetative affinity testing. Hsiang et al.(2000) conducted RAPD assay with many isolates of *S. homoeocarpa* from cool season turfgrasses in USA and Canada. Viji et al.(2004) also used AFLP to analyze genetic variations of isolates of *S. homoeocarpa* from cool season turfgrasses, which were distributed in several states in North America.

Many studies had proved that sensitivity of *S. homoeocarpa* is decreased by frequent application of fungicides(Burpee, 1997; Detweiler, 1983; De Waard et al.,

1993; Hsiang et al., 1998; Staub, 1991). Vargas et al.(1992) first reported that *S. homoeocarpa* was resistant to DMI(dimethylation inhibition) fungicide groups. Later, Burpee(1997) observed that there were distinct differences in the degree of sensitivity of two resistant isolates of *S. homoeocarpa* to several fungicides including DMIs. Thereof, it is imperative to examine the degree of fungicide sensitivity from collected isolates and to know whether their observation measured *in vitro* is parallel to the progress and fitness of disease in the field. Hsiang et al.(1998) denied that growth rate of the isolates attributed virulence of isolates influencing disease severity in field, and that the degree of resistance of isolates was able to be estimated by *in vitro* bioassay, representing growth inhibition rates of isolates. However, Miller et al.(2002) suggested that, in the greenhouse experiment, the degree of sensitivity measured from isolates varying responses to propiconazole was closely related to the disease development.

The objective of this study was to confirm a new collected isolate as *S. homoeocarpa* using molecular techniques, to investigate the genetic discrepancies between two isolates, Scz1 and Scb1, revealing different host specificities, and to test the degree of sensitivity of two analogous strains, Scz1 and Scz2, through the fungicide bioassay.

MATERIALS AND METHODS

Isolation and maintenance of fungal isolates Infected stems and leaves of zoysiagrass were harvested at fairways of golf courses in the Gyeonggi province of Korea. The infected tissues cut into 5 mm length were surface-sterilized with 70 % ethanol for 1 minute twice then placed on the potato dextrose agar(PDA) medium at 25℃. Young mycelia grown on the medium were transferred to new PDA plates for consecutive subculturing. The agar holes containing fungal mycelia were obtained using a cork borer(6 mm) for fungicide bioassay. Mycelia grown in an Erlenmeyer flask containing PD broth was harvested for DNA extraction.

DNA extraction of isolates Scz1, Scz2, and Scb1 were grown at 28℃ on PDA medium for three days and then lyophilized. The genomic DNA of mycelia was extracted and purified with genomic DNA extraction kit(cat number A1120, Promega, U.S.A.). These DNA samples extracted were used for ITS sequencing and RAPD analysis.

ITS DNA sequence analysis Identification of three isolates was examined by sequencing their partial ITS regions. The assay was accomplished by amplifying two ITS regions between 18S and 28S and one ITS region in 5.8S region using PCR(White et al, 1990). For the sequences of ITS regions from three isolates, primers used were as follow; ITS1(5'-TCCGTAGGTGAACCTGCGG-3'), ITS4(5'-TCCTCCGCTTATTG ATATGC-3'), ITS2(5'-GCTGCGTTCTTCATCGATGC-3'), ITS3(5'-GCATCGATGAAGAA CGCAGC-3'). Analysis for ITS sequence was performed by ABI 3730 DNA analyzer(Applied Biosystem Inc., USA). Both identification and alignment of the partial sequences were assessed by using NCBI BLAST(Basic Local Alignment Search Tool) through the internet(http://www.ncbi.nlm.nih.gov/BLAST/). As a reference, 18S rDNA sequence of *S. homoeocarpa*(accession number AF067640) was used.

RAPD analysis The RAPD analysis was carried out using the following mixture: genomic DNA(10 ng/$\mu\ell$) 2 $\mu\ell$, 10-mer random primer(10 pmol/$\mu\ell$) 1 $\mu\ell$, 10 mM dNTP 0.6 $\mu\ell$, Taq-polymerase(5 U/$\mu\ell$) 0.4 $\mu\ell$, 10X buffer 2 $\mu\ell$, 25 mM $MgCl_2$ 1.6 $\mu\ell$, distilled water 12.4 $\mu\ell$, for a total of 20 $\mu\ell$ reaction mixture. Twenty random primers(OPERON Technologies Inc., U.S.A.) were used for the analysis. Amplification reactions were carried out on the GeneAmp PCR System 2400(Perkin-Elmer) programmed for 5 min at 95℃ followed by 40 cycles of 1 min at 95℃(denaturation), 1 min at 37℃(annealing) and 2 min at 72℃(extension) and a final stage of 10 min at 72℃. Amplification products were analyzed by electrophoresis on 1.5% agarose gel(FMC Bioproduct, Rockland, ME, USA) in 1X TAE buffer and detected by ethidium bromide staining under UV lights. Only clear and distinct bands were scored both in agarose gels, attributing '1' to the presence and '0' to the absence of a band. The NTSYSpc(Ver 2.02k) software was used for statistical analysis of data. UPGMA dendrogram was generated by using Dice similarity index with NTSYS-pc(ver 2.11, Rohlf FJ 2000).

Fungicide bioassay The assay was conducted to determine the sensitivity degree of three isolates, against propiconazole, a representative fungicide for dollar spot. Mycelia of Scb1, Scz1, and Scz2 were incubated for 24 and 48 hours on PDA plates containing various concentrations of propiconazole; 0.3 $\mu g/m\ell$, 0.03 $\mu g/m\ell$, 0.012 $\mu g/m\ell$, and 0.003 $\mu g/m\ell$. EC_{50} value indicating an effective concentration of a fungicide to 50 % inhibition of fungal growth was measured on the basis of relative mycelial growth of fungal isolates on PDA plate unamended with propiconazole as control. The procedure was followed by Detweiler et al.(1983) used. The data obtained from three repeated experiments were determined by one-way ANOVA test.

RESULTS AND DISSICUSSION

ITS DNA analysis BLAST search through ITS sequencing is currently a common method for fungal or bacterial identification(Clarridge, 2004). In this experiment, ITS sequencing was accomplished by using two sequencing primers, which were constructed from two ITS regions between 18S and 28S, and also from one ITS region in 5.8S region(White et al., 1990). ITS regions from three isolates were investigated by the partial sequencing rather than the entire sequencing. Alignment of ITS sequences of the isolates was fulfilled by using BLAST search program(Fig. 1).

```
Reff. CGGGTATCCCTACCTGATCCGAGGTCAACCAGAGAAAATGAGGGGGGTTTTAC GGCGGGAGCGCGCGGGG-ACCC
Scb1  CGGGTATCCCTACCTGATCCGAGGTCAACCAGAGAAAATGAGGGGGGTTTTACGGCGGGAGCGCGCGGGG-ACCC
Scz1  --------CCTACCTGATCCGAGGTCAACCAGCGAAAATGAGGGGGGTTTTACGGCGGGCGCGCGCGGGG-ACCC
Scz2  --GGGATCCCTACCTGATCCGAGGTCAACCAGCGAAAATGAGGGGGGTTTTACGGCGGGCGCGCGCGGGGCACCC

Reff. TGTAACGAGAGGTATGTGTTACTACGTTCAGGACCCAGCGGCGCCGCCACTGACTTTAAGGCCCGCCGTGTGACC
Scb1  TGTAACGAGAGGTATGTGTTACTACGTTCAGGACCCAGCGGCGCCGCCACTGACTTTAAGGCCCGCCGTGTGACC
Scz1  TGTAACGAGAGGTATGTGTTACTACGTTCAGGACCCAGCGGCACCGCCACTGACTTTGAGGCCCGCCGTGTGACC
Scz2  TGTAACGAGAGGTATGTGTTACTACGTTCAGGACCCAGCGGCACCGCCACTGACTTTGAGGCCCGCCGTGTGACC

Reff. GGCGGAGGCCCAATACCAAGCAGAGAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCCCCGGAATACCA
Scb1  GGCGGAGGCCCAATACCAAGCAGAGAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCCCCGGAATACCA
Scz1  GGCGCAGGCCCAAGACCAAGCAGAG--CTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCCCCGGAATACCA
Scz2  GGCGCAGGCCCAAGACCAAGCAGAG--CTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCCCCGGAATACCA

Reff. AGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTG
Scb1  AGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAA-TCACATTACTTATCGCATTTCGCTG
Scz1  AGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTG
Scz2  AGGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTG

Reff. CGTTCTTCATCGATGCCAGAACCAAGAGATCCCGTTGTTGAAAGTTTTAACTATTAGATAGTCACTCAGACGACA
Scb1  CGTTCTTCATCGATGCCAGAACCAAGAGAT--CGTTGTTGAG---TTTTACTATTAGATAGTCACTCAGACGACA
Scz1  CGTTCTTCATCGATGCCAGAACCAAGAGATC-CGTTGTTGAG---TTTTACTATTAGATAGTCACTCAGACGACA
Scz2  CGTTCTTCATCGATGCCAGAACCAAGAGAT--CGTTGTTGAG---TTTTACTATTAGATAGTCACTCAGACGACA

Reff.. CTGACAATTCAGAGTTGTGATTTTCCTCCGGCAGGCGACTCTCCGGCCCCGGAGGGCGCTGAGGCTGTCCCCGGA
Scb1  CTGACAATTCAGAGTTGTGATTTTCCTCCGGCAGGCGACTCTCCGGCCCCGGAGGGCGCTGAGGCTGTCCCCGGA
Scz1  CTGACAATTCAGAGTTGTGGTTTTCCTCCGGCCGGCGACTCTCGGGCCCCGGAGGGCGCTGAGGCTGTCCCCCGA
Scz2  CTGACAATTCAGAGTTGTGGTTTTCCTCCGGCCGGCGACTCTCGGGCCCCGGAGGGCGCTGAGGCTGTCCCCCGA

Reff. AGGGTCGAGCAGCCTGCCAAAGCAACATGGTAGAGATACACAAGGGTTGGAGGTCTACCCGTGAGGGCGTGAACT
Scb1  AGGGTCGAGCAGCCTGCCAAAGCAACATGGTAGAGATACACAAGGGTTGGAGGTCTACCCGTGAGGGCGTGAACT
Scz1  AGGGTCGAGCAGCCGGCCAAAGCAACATGGTAGAGATACACAAGGGTTGGAGGTCTACCCGTGAGGGC-------
Scz2  AGGGTCGAGCAGCCGGCCAAAGCAACATGGTAGAGATACACAAGGGTTGGAGGTCTACCCGTGAGGGCGTGAACT
Reff. CGGTAATGATCCTTCCGCAGGTTCACCTACGGA-
Scb1  CGGTAATGATCCTTCCGCAGGTTCACCTACGGAA
Scz1  ----------------------------------
Scz2  CGGTAATGATCCTTCCGCAGGTTCACCTACGGAA
```

Fig. 1. Alignment of ITS nucleotide sequences of *Sclerotinia homoeocarpa* isolates.
Ref.1 means ITS sequence of *S. homoeocarpa*(GeneBank accession no. AF067640) as a reference.

Three isolates, Scz1, Scz2, and Scb1, yielded each ITS fragment of 509, 553, and 557 base pairs(Table 1). As compared to ITS sequence of reference *(standardized

sequence) of *S. homoeocarpa* registered in BLAST/Gene Bank, Scb1 showed 99.3 % homology but each Scz1 and Scz2 revealed 96,7 % and 96.6 % homology. Moreover, by co-alignment analysis among three isolates, a close relationship was observed between isolates, Scz1 and Scz2, belonging to one group of *S. homoeocarpa*(data not shown); 99.6 % homology between Scz1 and Scz2, 97.4 % between Scz1 and Scb1 and 97.1 % homology between Scz2 and Scb1. Thus, it was assured that all 3 isolates were identified as *S. homoeocarpa* and both Scz1 and Scz2, unlike Scb1, were almost analogous, suggesting two isolates were clustered as a group of *S. homoeocarpa*.

Table 1. Identification and genetic homology from three isolates of *Sclerotinia homoeocarpa*.

Isolate name	Identication	Homology
Scz-1	*Sclerotinia Homoeocarpa*	96.7%*
Scz-2	*Sclerotinia Homoeocarpa*	96.6%
Scb-1	*Sclerotinia Homoeocarpa*	99.3%

*Percentage indicates average homology of isolates as aligned with a referential fungus of *S. homoeocarpa*(GeneBank accession no. AF067640)

RAPD analysis Genomic DNA of three isolates amplified using twenty random 10-mer primers was analyzed by electrophoresis(Fig. 2). Based on the statistical analysis of data scoring bands on the gels scored as '1' to the presence and '0' to the absence of a band, the unweighted pair-group method using arithmetic average

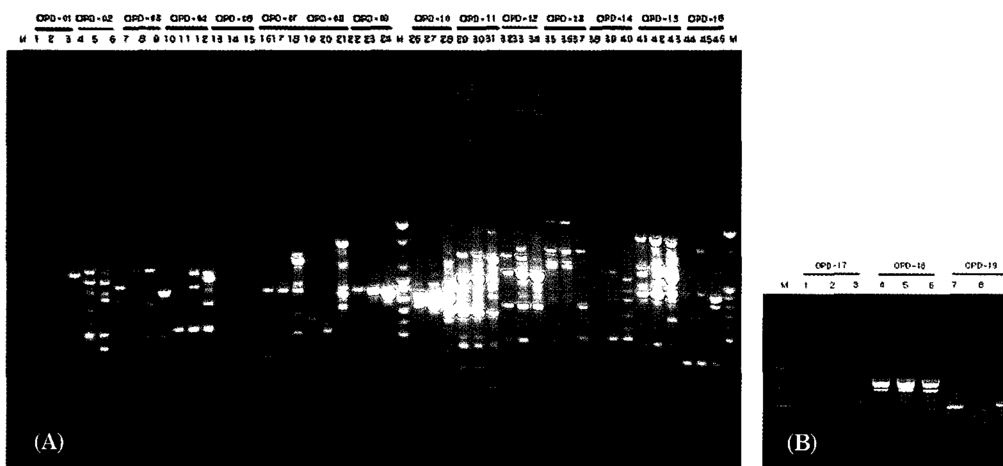

Fig. 2. Genomic DNA amplified from *Sclerotinia homoeocarpa* isolates using the primer OPD-01~20 (A) Lanes M, DNA molecular weight marker; 1, 4, 7, 10, 13, 16, 19, 22, 26, 29, 32, 35, 38, 41, 44, Scz1; 2, 5, 8, 11, 14, 17, 20, 23, 27, 30, 33, 36, 39, 42, 45, Scz-2; 3, 6, 9, 12, 15, 18, 21, 24, 28, 31, 34, 37, 40, 43, 46, Scb1.(B) Lanes M; DNA molecular weight marker, Scz1; 1, 4, 7, 10, Scz2; 2, 5, 8, 11, Scb1; 3, 6, 9, 12.

(UPGMA) was used to construct the genetic similarity matrix(Table 2). Similarity matrix analysis using Jaccard's similarity showed that the datum was almost consistent with the result from ITS analysis. Thus, similarity value of Scb1 was significantly different those of Scz1 and Scz2.

Table 2. Similarity matrix of three isolates of *Sclerotinia homoeocarpa* using Jaccard's similarity.

Isolate	Scz-1	Scz-2	Scb-1
Scz-1	1.000*		
Scz-2	0.713	1.000	
Scb-1	0.167	0.139	1.000

*Range of value was from 0 to 1, with values closer to 1 indication increasing similarity.

Dendrogram indicated that there are two major groups among three isolates; Scz1 and Scz2 were included in a group of S. *Homoeocarpa* and Scb1 was in other group(Fig. 3) From all our previous data, it was well explained that Scz1and Scz2, two isolates analogous to host specificity and general characters such as pigment and mycelial affinity also revealed the high genetic similarity analyzed by ITS and UPGMA.

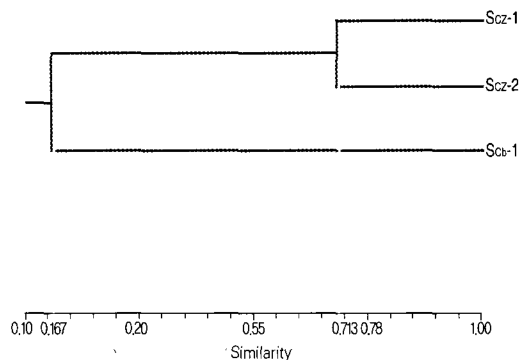

Fig. 3. Dendrogram of *Sclerotinia homoeocarpa* isolates based on the UPGMA clustering method using NTSYS program

The fungicide bioassay The bioassay is to measure the sensitivity degree of isolates of *S. homoeocarpa* as well as another determinant to distinguish among several analogous strains(Burpee, 1997; Detweiler, 1983; Hsiang et al, 1997; Hsiang et al., 1998). Burpee(1997) observed that two similar isolates collected through different appealed different resistance to specific chemicals. Propiconazole is one of most efficacious DMI(demethylation inhibitors) fungicides for control of dollar spot disease(Hsiang et al., 1998; Miller et al., 2002). It have been already reported that *S. homoeocarpa* is disposed to be resistant to the systemic and DMI chemicals(Burpee 1997; Golembiewski et al., 1995).

Table 3 indicated that EC_{50} values for propiconazole were different among three isolates; 0.030 ㎍/㎖ of Scb1, 0.012 ㎍/㎖ of Scz1, and 0.003 ㎍/㎖ of Scz2. Similar observations had been made in two research papers ; the EC_{50} values of isolates were

either ranged from 0.002 μg/mℓ to 0.103 μg/mℓ(Golembiewski et al., 1995) or ranged from 0.003 μg/mℓ to 0.069 μg/mℓ(Hsiang et al., 1998). The result stated that two isolates, Scz1 and Scz2, were more sensitive to propiconazole than Scb1 was. It is likely to explain that frequent exposure of *S. homoeocarpa* to various fungicides resulted in a decrease in fungicide sensitivity(Golembiewski et al., 1995; Vargas et al.1992). Namely, it means that Scb1, an isolate harvested from putting green seemed to be exposed more frequently to chemicals than Scz1 and Scz2 isolated from fairways. In addition, Scz1 and Scz2 showed different responses to propiconazole, implying the fact that Scz1 was relatively much more exposed to the fungicides including propiconazole than Scz2 was.

Table 3. EC50 value for propiconazole from 3 different isolates of *Sclerotinia homoeocarpa*.

Isolate	Location	Year collected	EC50 value ((μg/mℓ)
Scb1	Yeoju	2005	0.030[z]
Scz1	Yongin	2005	0.012
Scz2	Ansung	2005	0.003

[z]denote a significant difference at p<0.05, as determined by one-way ANOVA test.

In conclusion, this study is based on the fundamental observation on the dollar spot and fungal pathogens. Unfortunately, in this study, use of limited sample numbers collected does not seem to provide a full understanding of the relationship between genetic diversity of pathogen population and their characteristics such as virulence and fungicide sensitivity. Further study should be hopefully provided not only for better understanding of the disease occurrence and pathogen population but also for stimulating of further national wide research regarding the disease frequency. In near future, dollar spot disease may be a newly emerging problem in warm season turfgrass as well as cool season turfgrass on golf courses in Korea. Although further studies have yet to be in motion so far, it should be more concerning about the disease and control management strategy.

국문요약

난지형 잔디인 한국 안양잔디에서 달라스팟의 병원균인 *Sclerotinia homoeocarpa*의 isolate, Scz1이 최근 새롭게 동정되었다. Scz1은 한지형 잔디인 크리핑 벤트그래스에서 분리된 표준 균주인 Scb1과는 다른 균사의 색상, 균사간의 친밀도 그리고 병 기주 특이성을 가지는 것으로 알려졌다.

본 연구에서는, Scz1, Scz2(난지형 잔디에서 분리한 또 다른 달라스팟 병원균) 그리고 Scb1을

분자생물학적인 연구, internal transcribed spacer(ITS) 와 random amplified polymorphic DNA(RAPD) assays를 이용하여 동정 및 유전자적 차이를 알아보았다.

ITS 실험의 결과, 3개의 isolates가 ITS 부분적 염기 서열 비교 BLAST에 등록되어 있는 *S. homoeocarpa* 의ITS 염기 서열과 94~ 97%의 동일성을 지니는 것으로 밝혀졌다. RAPD 실험 결과로는, Scz1과 Scb1의 similarity matrix 범위는 0.167이였고, Scz2와 Scb1은 0.139 그리고, Scz1과 Scz2은 0.713이였다. 계통수(系統樹) 결과는 Scb1과는 달리 Scz1과 Scz2는 유전적으로 높은 동일성을 지니고 있어, 같은 분류에 속한다는 것을 알 수 있었다. 달라스팟 병원균 억제에 효과적인 농약인 프로피코나졸에 대한 EC_{50}은 Scz1은 0.012 μg/mℓ, Scz2은 0.003 μg/mℓ 그리고 Scb1은 0.030 μg/mℓ이었다. 상기 결과로, 동일 병원 기주성과 유사한 유전적 친밀성을 보인 Scz1과 Scz2는 *S. homoeocarpa*의 동일 그룹에 속하였으나 농약 민감도에서는 차이점을 보였다는 것을 알 수 있었다. 향후, 보다 더 많은 한지형과 난지형 잔디에서 분리된 병원균들을 이용하여 유전적 다양성을 밝히는 연구가 진행되어야 할 것이다.

주요어 : 달라스팟 병, *Sclerotinia homoeocarpa,* 한국잔디 달라스팟 병,

ACKNOWLEDGEMENT

This research was supported, in part, by funding from the ministry of Commerce, Industry and Energy in Korea.

REFERENCES

1. Bennett, F.T. 1937. Dollar spot disease of turf and its causal organism *Sclerotinia homoeocarpa* n. sp. Ann. Appl. Biol. 24:236-257.
2. Burpee, L.L. 1997. Control of Dollar Spot of Creeping Bentgrass caused by an Isolate of *Sclerotinia homoeocarpa* resistant to Benzimidazole and demethylation-inhibitor Fungicides. Plant Dis. 81:1259-1263.
3. Carbone, I. and Kohn, L.M. 1993. Ribosomal DNA sequence divergence within internal transcribed spacer 1 of the *sclerotiniaceae.* Mycologies 85:415-427.
4. Clarridge, J.E. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clinic. Microbiol. Rev.1&:4 840-862.
5. Couch H.B. 1995. Diseases of turfgrasses : 3rd edition Krieger Publishing company Malabar. Florida.

6. Couch, H.B. 1985. Common names of plant diseases. Turfgrass(several cultivated spp.) Plant Dis. 69:672-675.
7. Couch, H.B. and Bloom, J.R. 1960. Influence of soil moisture stresses on the development of the root knot nematode. Phytopathol. 50: 319-321.
8. Davis, J.G. and Dernoeden, P.H. 2002. Dollar Spot severity, tissue nitrogen, and soil microbial Activity in Bentgrass as influenced by nitrogen source. Crop Sci. :271-282.
9. De Waard, M.A., Georgopoulos, S.G., Holloman, D.W., Ishii, H, Leroux, P., Ragsdale, N.N. and Schwinn F.J. 1993. Chemical Control of Plant Diseases: problems and prospects. Annu. Rev. Phytopathol. 31: 403-431.
10. Detweiler, A.R. 1983. Resistance of *Sclerotinia homoeocarpa* to Iprodione and Benomyl. Plant Dis. 67:6 627-629.
11. Golembiewski, R., Vargas, J.M., Jones, Jr.A.L. and Detweiler, A.R. 1995. Detection of demethylation inhibitor(DMI) resistance in *Sclerotinia homoeocarpa* populations. Plant Dis. 79:491-493.
12. Hsiang, T., Barton, W. and Yang, L. 1997. Baseline sensitivity and cross-resistance to dimethylation-inhibiting fungicides in Ontario isolates of *Sclerotinia homoeocarpa*. Euro. J. Plant Pathol. 103:409 416.
13. Hsiang, T., Ma, X.L., Yang, L. and Cook, S. 2000. Analysis of RAPD data for detection of host specialization on *Sclerotinia homoeocarpa*. Plant Pathol. 49:269-275.
14. Hsiang, T., Yang, L. and Barton, W. 1998. Relative virulence of isolates of *Sclerotinia homoeocarpa* with varying sensitivity to propiconazole. Euro. J. Plant Pathol. 104:163-169.
15. Kohn, L.M. 1979. Delimitation of the economically important plant pathogenic *Sclerotinia* species. Phytopathol. 69:881-886.
16. Miller, L.G., Stevenson, L.K. and Burpee, L.L. 2002. Sensitivity of *Sclerotinia homoeocarpa* Isolates to Propiconazole and Impact on Control of Dollar Spot. Plant Dis. 86:1240-1246.
17. Park, D.S., Kim, K.D., Yeom, J.R., Oh, B.S. and Park, B.S. 2005. Identification and Characteristics of *Sclerotinia homoeocarpa* Causing Dollar spot Disease in *Zoysiagrass*. Kor. Turfgrass Sci. 19(2), 85-94.
18. Powell, J.F. 2001. Vegetative compatibility and seasonal variation among isolates of *Sclerotinia homoeocarpa*. Plant Dis. 85:4 377-381.
19. Shim, G.Y., Min, G.Y., Shin, H.D. and Lee, H.J. 2000. Occurrence dollar spot caused by *Sclerotinia homoeocarpa* in turfgrass of golf course in Korea. Kor.

Turfgrass Sci.14(1), 241-150.

20. Shim, G.Y., Min, G.Y., Shin, H.D. and Lee, H.J. 2001. Occurrence of Chemical Resistance and Control of Dollar Spot Caused by *Sclerotinia homoeocarpa* in Turfgrass of Golf Course. Kor. Turfgrass Sci. 15(1), 1-8.
21. Smiley, R.W., Dernoeden P.H. and Clarke B.B. 1992. Compendium of turfgrass diseases. The American Phyopathological society, St. Paul, MN.
22. Staub, T. 1991. Fungicide resistance: practical experience with antiresistance strategies and the role of integrated use. Annu. Rev. Phytopathol 29:421-442.
23. Vargas, J.M. 1981. Management of Turfgrass disease. Burgess Publishing Co., Minneapolis, MN.204 pp.
24. Vargas, J.M., Golembiewski, R., and Detweiler, A.R. 1992. Dollar spot resistance to DMI fungicides. Golf course Management 60(3):50-54.
25. Viji, G., Uddin. W., O'Neill, N. R., Mischke, S. and Saunders, J. A. 2004. Genetic Diversity of *Sclerotinia homoeocarpa* Isolates from Turfgrasses from Various Regions in North America. Plant Dis. 88:1269-1276.
26. Walsh, B., Ikeda, S.S. and Boland, G.J. 1999. Biology and management spot(*Sclerotinia homoeocarpa*) : an important disease of turgrass. HortSci. 34:13-21.
27. White, T.J., Bruns, T., Lee, S. and Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M. A., Gelfand D. H., Sninsky, J. J., and White, T. J., Academic Press, Inc., New York.