Journal of Agricultural Extension and Rural Development Vol. 4(9), pp. 181-183, 14 May, 2012
Available online http:// academicjournals.org/JAERD
DOI: 10.5897/JAERD12.045
ISSN 2141-2154 ©2012 Academic Journals

Full Length Research Paper

Frequency of virus in some *Diplodia pinea* and *Gremmeniella abietina* isolates originated from Turkey

Aday A. G.[1]*, Lehtijarvi A.[2] and Doğmuş- Lehtijarvi H.T.[2]

[1]Yenişar Bademli Vocational School, Suleyman Demirel University, Isparta- Turkey.
[2]Faculty of Forestry, Süleyman Demirel Üniversity, 32260 Isparta- Turkey.

Accepted 30 November, 2011

***Diplodia pinea* and *Gremmeniella abietina* are common pathogens causing shoot blight and dieback of pine all over the world. *D. pinea* is one of the main causal agents of shoot blight of Calabrian pines in the Mediterranean countries including Turkey. *G. abietina* has been recently observed on saplings and seedlings of *Pinus nigra*, which remain under snow cover during winter dormancy in Dedegül Mountain in the Mediterranean Region of Turkey. The presence of viruses in fungi has been known for many years. An accumulating number of cloned and sequenced viral genomes have enabled us to detect virus in increasing number of fungal species in the recent years. *D. pinea* and *G. abietina* are known to contain members of the virus families *Narnaviridae*, *Totiviridae* and *Partitiviridae,* which can infect single fungal isolates. Viral dispersal in fungi mainly occurs via anastomosis. Some *Diplodia* and *G. abietina* isolates have different characteristics, such as reduced virulence and growth rate, lack of pigmentation, altered colony morphology, and reduction in conidial production due to presence of viral particles. In this study, 18 *D. pinea* and 6 *G. abietina* isolates were investigated for the presence of dsRNA. Double-stranded RNA was isolated using a commercial RNA extraction kit and visualized in agarose gel electrophoresis. Isolates containing dsRNA were also investigated for their *in vitro* growth rate and ability to produce conidia. Three (50%) *G. abietina* and ten (56%) *D. pinea* isolates contained dsRNA that had an approximate molecular size of 1.6 kb.**

Key words: Scleroderris canker, calabrian pine, dsRNA.

INTRODUCTION

Diplodia pinea Dezmaz J. Kickx (*Sphaeropsis sapinea* (Fr.) Dyko & Sutton) and *Gremmeniella abietina* (Lagerb) Morelet are common pathogens causing shoot blight and dieback of coniferous tree species all around the world (Adams et al., 2002; Tuomivirta and Hantula, 2003a). *D. pinea* is one of the main fungal agents of shoot blight of Calabrian pines in the Mediterranean countries including Turkey. *G. abietina* was recently detected on *Pinus nigra* Arnold ssp. *pallasiana* (Lamb.) Holmboe most likely causing damage on saplings and seedlings at low temperatures under snow cover in Dedegül Mountain of Mediterranean Region of Turkey (Lehtijärvi et al., 2010).

*Corresponding author. E-mail: guldenaday@orman.sdu.edu.tr.

Mycoviruses with double stranded RNA genomes are widely spread in many major groups of plant pathogenic fungi. More than a hundred fungal species are known to be host for mycoviruses with most of these consisting of dsRNA (Buck, 1986). The main fungal mycovirus families are *Chrysoviridae*, *Hypoviridae*, *Partitiviridae* and *Totiviridae*. Double stranded RNA genomes can spread between mycelia via hyphal anastomosis, conidia, basidiospores, ascospores from yeasts and rarely through ascospores from filamentous ascomycetes (Buck, 1986). In general, mycoviruses do not change the host phenotype (Ghabrial, 1994), but there are several cases where virus infection results in marked structural or physiological changes. An accumulating number of cloned and sequenced viral genomes have enabled us to detect virus in increasing number of fungal species in recent years. *D. pinea* and *G. abietina* are known to con-

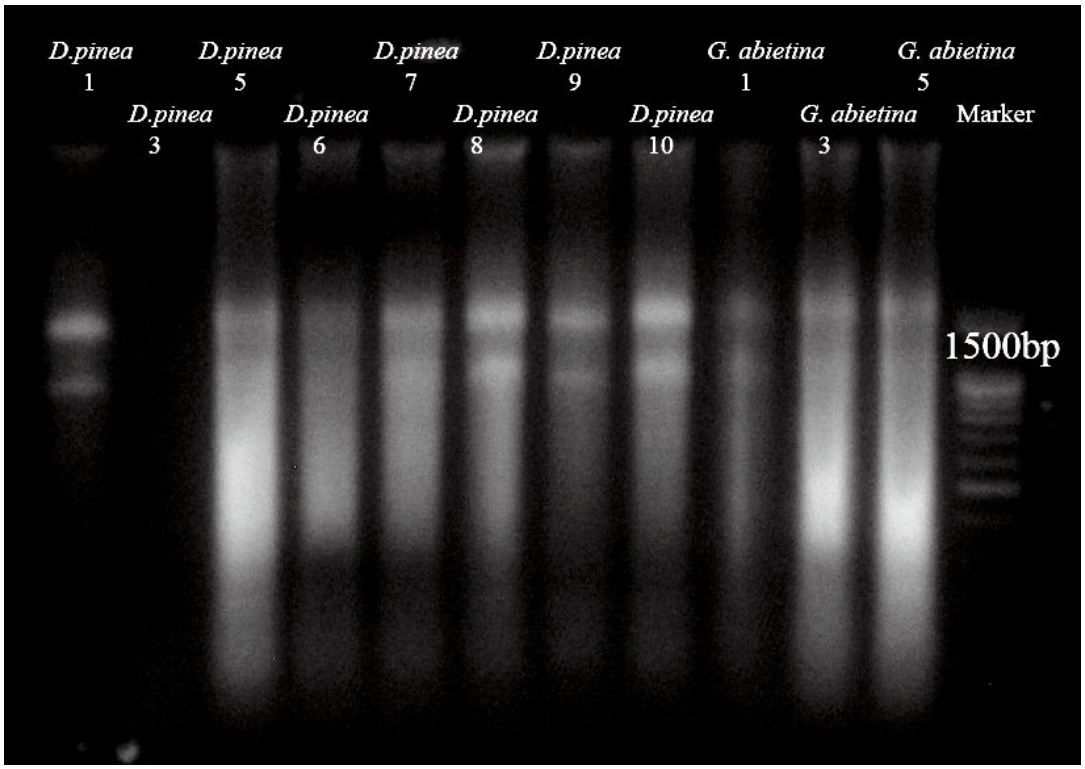

Figure 1. Banding pattern of dsRNA genomes in *D. pinea* and *G. abietina* isolates.

tain members of the virus families *Narnaviridae, Totiviridae* and *Partitiviridae* (Wu et al., 1989; Preisig et al., 1998; Steenkamp et al., 1998; De Wet et al., 2001; Adams et al., 2002; Tuomivirta and Hantula, 2003a,b), which can infect single fungal isolates. Some *Diplodia* and *G. abietina* isolates have different characteristics, such as reduced virulence and growth rate, lack of pigmentation, altered colony morphology, and reduction in conidial production due to presence of viral particles.

Several dsRNA genomes ranging from 600 to 7000 bp in size have been reported from single *Diplodia* isolates (Wu et al., 1989; Preisig et al., 1998; Steenkamp et al., 1998; De Wet et al., 2001; Adams et al., 2002). Two of these elements have been characterized and are known as *Sphaeropsis sapinea* RNA virus 1 and 2 (SsRV1 and SsRV2) (Preisig et al., 1998). Tuomivirta and Hantula (2003a,b) found two unrelated dsRNA patterns in *G. abietina* type A and they described *Totivirus, Partitivirus* and *Mitovirus* genera that are common in A type of *G. abietina* isolates. The aim of this study was to investigate Turkish *D. pinea* and *G. abietina* isolates for the presence of dsRNA.

MATERIALS AND METHODS

Fungal material

In this study, a total of 18 *D. pinea* and 6 *G. abietina* isolates were investigated for the presence of dsRNA. *D. pinea* isolates were obtained from *Pinus brutia.* Ten plantation site in Aşağı Gökdere in Isparta province which was as homogenous as possible regarding to tree size and shoot blight symptoms.

All *G. abietina* isolates were isolated from diseased shoots of *P. nigra* ssp. *pallasiana* in Dedegül Mountain in Yenişarbademli-Isparta province. *D. pinea* and *G. abietina* isolates were grown on potato dextrose agar (PDA) and modified orange serum agar (MOS–agar) respectively at 20°C.

RNA extraction

D. pinea and *G. abietina* isolates were grown on PDA and MOS–agar plates covered with cellophane membrane at 20°C for 10 days. The mycelium was then scraped off, placed into mortar, and ground with liquid nitrogen. Total RNA was isolated using RNA isolation kit following the instructions of the manufacturer and all isolates were screened in agarose gel electrophoresis. Isolates containing dsRNA were also checked for the growth rate and production of conidia.

RESULTS AND DISCUSSION

In visual inspection of the gels containing the total RNA of the fungal isolate fragments of approximate length of 1.6 kb were detected (Figure 1). Owing to the size of these fragments, they were regarded to be putative viral dsRNAs. Totally three (50%) *G. abietina* and ten (56%) *D. pinea* isolates contained these dsRNA fragments (Figure 1).

The size of the putative dsRNA in this study, present in

both *D. pinea* and *G. abietina*, were well within the 1 to 12 kbp range of known dsRNA genomes of mycoviruses (Ghabrial, 1994). In earlier studies, putative viruses of families; *Narnaviridae, Partitiviridae* and *Totiviridae* were detected in *G. abietina* (Tuomivirta and Hantula, 2003a, b) and *D. pinea* (De Wet et al., 2008). Therefore, it is likely that the putative dsRNA molecules found in this study also belong to these virus families. However, other possibilities cannot be excluded until we have determined and compared the sequences of the dsRNAs found in the present work with those in the viral genome database. The number of dsRNA genomes was not investigated in our study. It is common for mycelia to contain more than one dsRNA molecule (Ghabrial, 1998).

Some dsRNA virus molecules reduce the growth rate and spore production of a fungal pathogen like in *Cryphonectria parasitica* (Murr.) Barr (Ihrmark et al., 2001). In our study, the growth rate of *D. pinea* and *G. abietina* were 60 and 40 mm respectively and the dsRNA molecules were found to affect the spore production of only *D. pinea* isolates. These sizes were similar to those of the dsRNA molecules previously reported for viruses of *G. abietina* type A (Tuomivirta and Hantula, 2003a, b).

Conclusions

The results of this study showed that dsRNA fragments were common in Turkish *D. pinea* and *G. abietina* isolates. As the sample size was small, the result indicates that the frequency of the dsRNA in natural *D. pinea* and *G. abietina* populations is high. The dsRNAs from both fungi should be at least partially sequenced for sequence comparison with known viral sequences. In addition, the effect of the dsRNAs on the physiology of the fungal isolates should be investigated.

REFERENCES

Adams GC, Wu NT, Eisenberg BE (2002). Virulence and double-stranded RNA in *Sphaeropsis sapinea*. For. Path. 2: 309-329.

Buck KW (1986). Fungal virology- an overview. In: Fungal Virology. Ed by Buck KW, Boca Raton FL: CRC Pres. pp. 1-84.

De Wet J, Preisig O, Wingfield BD, Wingfield MJ (2008). Pattern of multiple virus infections in the conifer pathogenic fungi, *Diplodia pinea* and *D. scrobiculata*. J. Phytopathol. 156: 725-771.

De Wet J, Wingfield MJ, Preisig O, Wingfield BD (2001). Occurence of dsRNA elements in isolates of the pine pathogen, *Sphaeropsis sapinea* from diverse geographical regions. Phytopathology 91: S22.

Ghabrial SA (1994). New developments in fungal virology. Adv. Virus Res. 43: 303-388.

Ghabrial SA (1998). Origin, adaptation and evolutionary pathways of fungal viruses. Virus Genes. 16: 119-131.

Ihrmark K, Zheng J, Stenström E, Stenlid J (2001). Presence of double-stranded RNA in *Heterobasidion annosum*. For. Path. 31: 387-394.

Lehtijärvi A, Oskay F, Aday AG, Doğmuş Lehtijärvi HT (2010). Isparta-Yenişarbademli İlçesi Konifer Ormanlarında İbre ve Sürgün Hastalıklarına Neden Olan Fungal Etmenlerin Tespiti. (Occurrence of Fungal Pathogens ın Conifer Forests of Isparta Yenişarbademli) 3. Ulusal Karadeniz Ormancılık Kongresi, 20–22 Mayıs 2010, Artvin. Cilt IV, sayfa: 1420-1430.

Preisig O, Wingfield BD, Wingfield MJ (1998). Coinfection of a fungal pathogen by two distinct double-stranded RNA viruses. Virology. 252: 399-406.

Tuomivirta TT, Hantula J (2003a). Two unrelated double-stranded RNA molecule patterns in *Gremmeniella abietina* type a code for putative viruses of the families *Totiviridae* and *Partitiviridae.* Arch Virol. 148: 2293-2305.

Tuomivirta TT, Hantula J (2003b). *Gremmeniella abietina* mitochondrial RNA virus S1 is phylogenetically related to the members of the genus Mitovirus. Arch. Virol. 148: 2429-2436.

Wu NT, Palmer M, Adam G (1989). The relationship of dsRNA to virulence and morphology in type A and type B *Sphaeropsis sapinea*. Phytopathology, 79: 1143.