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Analysis of Soil mycoflora in Phytophthora Infested and Non-Infested Fields

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ABSTRACT

Composition of fungal communities in three microhabitats such as soil, rhizosphere and rhizoplane were studied to understand the root environment of healthy and diseased plants in *Phytophthora* non-infested and infested fields, respectively. Samples were collected from the tomato- and red pepper-growing greenhouses in Kyungsang-Nam Province on April, 1999. Twenty-five species were isolated from each vegetation field using the dilution plate technique. There were a greater variety of species in infested fields than non-infested and in soils than in both rhizospheres and rhizoplanes. The number of species isolated were varied amongst the different microhabitats. A *Trichoderma* species was isolated only from non-infested fields.

Key Words : Soil fungi, Qualitative, Tomato, Red pepper, *Phytophthora* blight.

Introduction

The soil microflora exerts considerable influence upon the fertility of soil and consequently on the growth and development of plants. In most soils, fungi are the major component of the soil microflora, which comprise of saprophytes, mycorrhizal symbionts and parasites.

There have been various mycological characterizations of vegetation and soil conditions. Rhizosphere mycoflora of virus infected plants (Isamil and Elwy, 1996), of powdery mildew infected ornamental plants (Ajay *et al.*, 1994), of nematode infected chickpea (Rao and Krishnappa, 1996), and mycoflora of tomato fields infested with *Fusarium oxysporum* f. sp. *lycopersici* (Abdul Wahid *et al.*, 1997) revealed a strong variety of the mycoflora at the scale of microhabitats.

Soil-borne plant pathogenic fungus *Phytophthora* capsici has been a major concern of plant pathologists in Korea because of its severe damage to its host plants such as tomato and red pepper and financial losses as well. Yang *et al.* (1991) and Lee *et al.* (1993) quantitatively analyzed microbial population of bacteria, actinomycetes and fungi in *Phytophthora*-infested red pepper fields. Although these studies gave information about general biological estimation, neither of them were focused on individual fungal inhabitants in soils. No studies have dealt with the soil mycoflora of tomato fields in respect of healthy and diseased conditions.

The better we understand biological atmosphere of the root-soil zone with its chemistry and physics, the better we have a chance of controlling soil-borne root diseases. As a

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fourth paper of a proposed series which was mainly focused on the bacterial community, this study was conducted to understand the subterranean atmosphere of healthy and diseased tomato and red pepper plants on behalf of fungi in *Phytophthora* non-infested and infested fields.

Materials and Methods

Soils and roots were collected from *Phytophthora*-infested and *Phytophthora*-free tomato- and red pepper-growing greenhouses in Jinju-City, Kyungsang-Nam Province on April, 1999. Dilution plate technique was adopted for isolation of fungi. Initial dilutions of rhizoplanes, rhizospheres and soils were prepared following Kim *et al.* (1999). The dilutes of 1 ml were spread onto plates which consists of Bacto ® Rose Bengal Agar Base 16 g/500 ml. All plates were maintained at 25℃ until discrete colonies appeared. The plates were examined under a dissecting microscope until no additional species were encountered. All the fungal isolates were attempted to be identified to the level of species if possible. The isolates which could not be identified to the species by authors were named by descriptive languages or as something-likes. Relative abundance of each identified species was not quantified. Statistical analyses were not applied due to the lack of enough replicates.

Results

A total of twenty-five species was recorded from the red

Table 1. List of fungi isolated from *Phytophthora*-infested or *Phytophthora*-free red pepper filelds

Isolates	*Phytophthora*-infested			*Phytophthora*-free		
	Rhizoplane	Rhizosphere	Soil	Rhizoplane	Rhizosphere	Soil
Aspergillus sp. 1	+		+			
Aspergillus sp. 2		+	+			+
Aspergillus sp. 3						+
Aspergillus niger		+				
Botrytis - like		+				
Cephalosporium - like		+				
Cladosporium sp.	+	+				
*Chaetomium globosum**			+			
Curvularia lunata	+		+	+		
Cylind/dry/green		+	+			
Dematiaceous imperfectus			+			+
Humicola-like						+
Imperfectus						+
Multi/drk/chain/macro			+			
Penicillium sp. 1	+	+	+			
Penicillium sp. 2			+	+	+	+
Penicillium sp. 3			+			
Penicillium - like						+
Sclerotium - like			+			+
Sterile hyphae 1		+			+	
Sterile hyphae 2	+	+	+	+		
Sterile hyphae 3		+				
Sympodial/hyaline		+				
Trichoderma sp.				+	+	+
*Verticillium chamydosporium**	+					
Verticillium - like	+					
Total	7	11	12	4	3	9

* Deposited in the culture collection center of NIAST (KACC)

Table 2. List of fungi isolated from *Phytophthora*-infested or *Phytophthora*-free tomato filelds

Isolates	*Phytophthora*-infested			*Phytophthora*-free		
	Rhizoplane	Rhizosphere	Soil	Rhizoplane	Rhizosphere	Soil
Alternaria ap.		+		+		
Aspergillus sp. 1	+					
Aspergillus sp. 2						+
Aspergillus sp. 3			+			+
Aspergillus - like			+			
Botrytis - like	+					
Cephalosporium - like				+		
Cladosporium sp.			+			+
*Chaetomium globosum**	+		+			
Cladosporium	+	+		+		
Cladosporium - like	+		+	+	+	+
*Doratomyces microsporus**	+					
Fusarium sp.		+				
*Gliocladium solani**			+			
Imm/drk brwn						+
Imm/drk brwn/chain	+					
Imm/sgl/ormented					+	
Imperfectus	+	+	+	+	+	+
Penicillium sp. 1						+
Penicillium sp. 2						+
Penicillium sp. 3						
Phialophora - like			+			
Stachybotry - like			+			
Sterile hyphate 1			+		+	
Sterile hyphate 2	+	+	+	+		+
Trichoderma sp.				+		+
Total	9	5	11	7	4	10

* Deposited in the culture collection center of NIAST (KACC)

pepper fields (Table 1). Out of the total number, 14 species were found in *Phytophthora*-infested, 5 in *Phytophthora*-free, and 6 species were common to both. A higher number of species were found in both rhizosphere and soil of *Phytophthora*-infested and in soil of *Phytophthora*-free samples. Among microhabitats there were no significant patterns of species distribution except for a *Trichoderma* species which was exclusively found in *Phytophthora*-free field.

Twenty-five species were isolated from tomato field samples (Table 2). Eleven species were found in *Phytophthora*-infested field, 8 in *Phytophthora*-free, and 6 species were found to be common in both fields. A higher number of species were isolated in soil than in both rhizoplane and rhizosphere. Rhizoplane showed more variety of species than rhizosphere, which was opposite to the red pepper fields.

Discussions

Most of the species isolated in this study using dilution plate technique are saprophytic soil fungi which are known to have the decomposition capabilities for pectin,starch, xylan, cellulose, etc. and whose role in soil have been appreciated by many soil microbiologists. Generally speaking, the delimitation between saprophytic and parasitic fungi is by no means sharp and a continuum from saprophytic species to minor pathogens in the soil microflora was also reported by Salt (1979). Even if there are no well-defined plant pathogens isolated in this study, the

presence of some notorious pathogenic groups such as *Fusarium*, *Gliocladium* and *Verticillium* species can be possibles for disease infestation in future.

Saprophytic species in *Phytophthora*-infested fields were richer than non-infested fields of tomato and red pepper. The same result was reported by Abdul Wahid et al. (1997) in the *Fusarium*-infested tomato fields with a slight increase of fungal populations. They interpreted this phenomenon as a result of the contribution from the death or degradation of plants roots infected with *Fusarium*, which provide additional food source for the growth of saprophytic fungi referring to White (1989). The same explanation can be applied in the *Phytophthora*-infested fields where the increase of fungal populations may be occurred after the disease has been well established.

If we consider the contribution of death or degrading plant roots as post-disease establishment condition for the richness of saprophytic fungi, there also can be pre-disease condition. Domsch and Gams (1968) tested in vitro the majority of commonly isolated saprophytic soil fungi and found that they affected plant growth adversely rather than positively. Even though more complex mutual interactions and activities are expected in nature, a hostile environment generated by saprophytic fungi may play a part in infestation of *Phytophthora*. The subterranean environment is subject to the physical condition of plants which again affects the fungal population composition in the soil. The saprophytic soil fungi are known to be remarkably little affected by various agricultural practices, including fertilization, certain crop plants and plant protection chemicals (Swift and Heal, 1986). Even though we don't know which one gets first, the establishement of disease or the colonization of saprophytic fungi, once the saprophytic fungal population is established for various reasons, their mycofloral construction seems to be stable and to act adversely to plant development. A large amount of thorough experiments on soil mycoflora is required to clarify the role of saprophytic fungi in relation with disease development of plants.

Members of the genus *Trichoderma* have been practically applied in the fields because of their antagonistic activities against various soil-borne pathogens including species of *Pythium* and *Phytophthora*. In this study *Trichoderma* species were only isolated from *Phytophthora*-free fields, which may be therefore inferred for them to bring unfavorable environmental conditions for infestation of *Phytophthora* in the fields.

It is generally understood that results of soil-fungal analyses mainly depend on the methods used. Warcup (1955) pointed out that 90% of the isolates obtained in dilution plate techniques originated from resting stages such as spores, chlamydospores and sclerotia which are practically inactive in soil, while fungal hyphae represent the active phase. However, there were no satisfiable techniques isolating active hyphae from soil. Even though the dilution plate technique can give an overall or average picture of fungal composition in soil (Gams, 1992), the application of other techniques such as soil plate technique, baiting and soil washing is recommended to get broader spectrum of soil mycoflora in further study.

The rhizosphere phenomenon coined by Hiltner (1904), an increased microbial density around living roots, was not clearly observed in this study. This result may be attributed to the insufficient number of samplings. The primary biological fact of the rhizosphere or zone of root influence is known as the greater number and activity of soil microorganisms in this region than in soil which does not have any contact with roots (Katznelson, 1965). It is also reported that soil from root surface or rhizosphere has an even higher number of nematodes, actinomycetes, and fungi in comparison with soils (Bruehl, 1987). Other studies using enough numbers of samplings showed the significant differences of microbial distribution between root-regions and root-free soils. Subba-Rao (1977) found considerable variations in the rhizospheres of resistant and susceptible varieties. Abdul Wahid *et al.* (1997) found significant

differences between root-free soil versus both rhizosphere and rhizoplane even though they could not find differences between non-infested and infested status of the three microhabitats.

Due to the lack of enough samplings or replicates, the comparative study among microhabitats was not completed but the significant differences of fungal inhabitants between healthy and diseased, *Phytophthora*-free and *Phytophthora*-infested, respectively, were observed in this study.

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역병의 감염 여부에 따른 토양 내 진균 분포

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불건전 토양과 건전 토양의 환경을 이해하기 위해 자연적으로 역병에 감염되거나 감염되지 않은 고추와 토마토 시설재배지의 비근권토양, 근권토양, 근면을 채취하고 연구하였다. 시료는 경상남도의 시설재배지에서 1999년 4월에 채집되었으며, 진균의 분리는 희석평판법을 이용하였다. 각 작물 별 재배지에서 25 종의 진균이 분리되었으며 건전 시료보다는 감염 시료에서, 근권이나 근면토양보다 비근권토양에서 더 많은 수의 종이 분리되었다. 역병이 감염되지 않은 시료에서만 *Trichoderma* sp.가 분리되었으며 각 작물의 서식지별로 분리된 진균의 수는 다양하였다.

Key Words : Soil fungi, Qualitative, Tomato, Red pepper, *Phytophthora* blight.

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