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Endophyte-pathogen continuum in *Alternaria alternata*

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Endophytes are microorganisms that live within healthy plant foliage for at least a part of their life cycle without causing any sign of disease. Thus endophytes are defined by their niche, rather than their function. This means that symptomless and unobtrusive behavior of endophytes may be a transient phenomenon, and that the interacting partners may shift among a wide range of interactions from mutualism to parasitism to saprophytism or exploitation. Here, we aimed at clarifying whether *Alternaria alternata* isolates, formerly introduced by our lab as endophytic fungi of cupressaceous trees, could induce disease in other hosts. Here it is shown that such endophytic *A. alternata* isolates could induce disease on potato leaves. Indeed, by spraying the conidial suspensions of six *A. alternata* isolates on target potato, tomato and wheat seedlings, the symptoms of leaf spot were emerged on leaves of Marfona and Agria potato cultivars, but not on tomato and wheat foliage. The percent of disease intensity (PDI) on potato leaves ranged from 9% to 70%, depending on the fungal isolate. Assessment of disease intensity on the potato cultivars indicated that there were differences among pathogenicity of fungal isolates at Pd"0.01. Also, the host cultivars responded differently to the isolates. In conclusion, host-microbe interactions are often dynamic and one organism can change from one life style to another given the appropriate trigger, in this case: the host plant. Thus, our finding provides new insight into such complexity in relation to endophyte-pathogen continuum.

Keywords: *Alternaria alternata,* Endophyte,pathogenicity

INTRODUCTION

Endophytes are defined as microorganisms that live inter- or intra-cellularly within healthy plant foliage, for at least a part of their life cycle, without causing any apparent symptom of plant disease (Bacon and White, 2000). Endophyte definition is further expanded functionally to include all microorganisms that occupy symptomlessly living internal tissues of the host plant during a long period of their life.

However, a common endophyte which is capable of colonizing multiple host species may not interact with each host in a similar manner, because of functional or ecological specificity (Arnold, 2007). Endophyte surveys occasionally recover microorganisms that are conspecific with known plant or human pathogens (Ganley and Newcombe, 2006; Soltani and Hosseyni Moghaddam, 2014 a, b; Soltani *et al.* 2016; Soltani, 2017). Hence, it is speculated that some foliar endophytes may be latent pathogens, or may exhibit pathogenicity on host plants with which they do not form endophytic associations (Arnold, 2007). On the other hand, some plant pathogens with complex life styles may inhabit alternative host species at different stages of their life cycle and interact with those hosts markedly in different manners (Agrios, 2005). Whether endophytes, conspecific with pathogens, are virulent pathogens of other plants or human species has remained to be evaluated *in vivo*.

It is shown that distinct ascomycetous classes of fungi, from Pezizomycotina, endophytically associate cupressaceous trees (Hoffman and

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Arnold, 2008; Hosseyni Moghaddam, 2013; Soltani and Hosseyni Moghaddam, 2015; Soltani, 2017). Of these, several *Alternaria* species from Dothediomycetes, Ascomycota, are introduced to form symptomless endophytic associations with healthy foliage of *Cupressus arizonica, Cupressus sempervirens* and *Thuja orientalis* from the Cupressaceae plant family (Hoffman and Arnold, 2008; Soltani and Hosseyni Moghaddam, 2014a). Interestingly, endophytic *Alternaria* species from cupressaceous trees were conspecific with known pathogens, among which *Alternaria alternata* (Fr.) Keissler represented the most frequent species with a ubiquitous host range (Soltani & Hosseyni Moghaddam, 2014a). Also, in vivo bioassays indicated significant antiproliferative, growth inhibitory and antibacterial activities for cypress endophytic *A. alternata,* especially against cypress fungal pathogens (Soltani and Hosseyni Moghaddam, 2014a). This raised the speculation that endophytic *Alternaria* species play a protective role in cupressaceous trees against biotic stresses. However, *A. alternata* is mostly reported as a plant pathogen and a human opportunistic pathogen and is a common source of human asthma (Schell, 2000; Thomma, 2003). *Alternaria alternata* is capable of pathogenicity in over 100 plant hosts (Thomma, 2003), causing leaf spots and blights on many plant parts. Blight diseases whose foliar symptoms are often called brown spot are caused by *A. alternata*. Although there is no report of *A. alternata* infection in cypress trees, possibly because of non-host resistance, it could induce disease in solanaceous plants such as potato (*Solanum tuberosum L.*) and tomato (*Solanum lycopersicum L.*), as well as in crops such as wheat (*Triticum aestivum L.*). High humidity, leaf wetness and warm temperatures increase the incidence of infection.

Potato, tomato and wheat are ubiquitously cultivated across Iran, including the provinces in which endophytic *A. alternata* isolates were recovered from cupressaceous trees (Soltani and Hosseyni Moghaddam, 2014a). So, we aimed at elucidating the potential of *A. alternata*, isolated as endophytes from cypress trees, in pathogenicity on putative susceptible hosts such as potato, tomato, and wheat cultivars which are under extensive cultivation in Iran.

MATERIALS AND METHODS

Alternaria alternata isolates

Six *Alternaria alternata* (Fr.) Keissler isolates that were previously recovered, as endophytic fungi, from internal tissues of healthy *Cupressus arizonica, C. sempervirens* and Thuja orientalis at Fars and Hamedan provinces of Iran (Soltani and Hosseyni Moghaddam, 2014a) were used as the infecting fungi. The characteristics of cupressaceous endophytic *A. alternata* isolates, their location of origin, and the host plant species are represented in (Table1).

Target host plant species, and their growth conditions

Potato (*Solanum tuberosum)* cultivars “Marfona” and “Agria”, as well as Tomato (*Solanum lycopersicum*) cultivar Flat CH and Wheat (*Triticum aestivum*) cultivar Pishgam were used as the target host plants.

Fresh potato tubers, seeds of tomato and seeds of wheat were first surface sterilized by Sodium hypochlorite (3%, for 3'), and rinsed in distilled water (2 times). Subsequently, the plant materials were cultivated in pots filled with autoclaved soil, incubated under greenhouse conditions (22-25°C) and let to grow the seedlings for 5 weeks (35 days).

Alternaria alternata inoculum preparations

Fungal conidial suspensions were selected as the method of choice for plant inoculations. Thus, for preparation of conidial suspension, each *A. alternata* isolate was grown on Potato-Carrot-Agar (PCA) medium in 9-cm Petri plates. The *Alternaria* isolates were incubated for 14 days at 22±2°C under alternate fluorescent light and dark (8/16 hrs). Afterwards, 10 mL of distilled water plus 1% Tween 20 was used to harvest the conidia with a brush from each Petri plate (Foolad *et al*. 2000). The conidial suspensions were collected in test tubes, and the conidial concentrations were determined by hemacytometer, and adjusted to 1×10^4 conidia per milliliter.

Pathogenicity assay, experimental design, and statistical analyses

Five-week old seedlings of potato, tomato and wheat were sprayed by conidial suspensions of *A.*

alternata isolates. The control plants were treated by distilled water. The inoculated plants were covered by polyethylene plastic bags for 24 h to provide a moist microclimate under greenhouse condition (Fig. 1). Afterwards the plastic bags were removed and the plants were kept in greenhouse. The plants were inspected daily, after inoculation, for any signs of *A. alternata* leaf spots or blights. Observed symptoms of leaf spots were verified in potato but not in tomato and wheat. The fungus was re-isolated (and re-identified) from infected tissues of potato seedlings. The experiment was performed in a completely randomized blocks design, with three replicates. Data obtained from pathogenicity assay were subjected to analysis of variances (ANOVA) and means were compared by least significant differences (LSD) test, using SAS statistical software (Steel *et al.* 1997). The differences among different treatments were determined at 1% and 5 % levels (P< 0.01and P< 0.05).

Assessment of disease intensity

Disease intensity of inoculated leaves of potato seedlings was assessed after seven weeks of inoculation and categorized using the scale of Pandey and Pandey (2002) with a minor modification as shown in (Table2). The percent of disease intensity (PDI) was calculated using the equation given by FAO (Anonymous, 1967) as follows :

$$PDI = \frac{\Sigma(n \times v)}{N x S} x\ 100$$

where Σ = Summation, N = Number of leaves in each category, V = Numerical value of leaves observed, and S = Maximum numerical value/ grade.

RESULTS AND DISCUSSION

Endophytes are defined as microorganisms that live within healthy plant foliage for at least a part of their life cycle without causing any sign of disease (Bacon and White 2000). Thus endophytes shape a cost-benefit plant association and are defined by their niche, rather than their function (Kusari *et al.* 2012). This means that symptomless and unobtrusive behavior of endophytes may be a transient phenomenon (Kusari and Spiteller, 2012). Thus, a given endophyte that is capable of colonizing multiple plant hosts may interact with each host in a different manner, depending on its interactions. Endophytes conspecific with known pathogens are occasionally isolated (Ganley and Newcombe, 2006). Several studies have also shown that some endophytes could be avirulent strains of pathogenic species , latent pathogens or strains that infect stressed host

Fig.1: The inoculated plants covered by polyethylene plastic bags under greenhouse condition

Fig.2: Symptoms of *Alternaria alternata* on potato leaf

plants (Stanosz *et al*., 2001), poor competitor pathogens (Arnold *et al.* 2009), or strains that are virulent pathogens of other hosts (Arnold, 2007). For example, it is reported that some endophytes are latent pathogens (reviewed in Hyde and Soytong, 2008). Also, the plant pathogens usually can endure within other plants, and such hosts can serve as the reservoirs of the pathogen inocula (Agrios, 2005). Thus, the interacting partners may shift among a wide range of interactions from mutualism to parasitism to saprophytism or exploitation (Millet *et al.* 2010; Zuccaro *et al.* 2011).

Table 1: Characteristics of cypress endophytic*Alternariaalternata* isolates (modified from Soltani and HosseyniMoghaddam, 2014a)

Endophyte species	Isolate	Host cypress species	Plant tissue, and location (Iran)
A. alternata	CAE_{92}	*Cupressusarizonica*	Twig, Fars
	CSE_{165}	*Cupressussempervirens*	Twig, Fars
	CSE_{177}	*C. sempervirens*	Leaf, Fars
	POE_{43}	*Thujaorientalis*	Leaf, Fars
	POE_{72}	*T. orientalis*	Leaf, Fars
	POE_{104}	*T. orientalis*	Twig, Hamedan

Table 2: Assessment scale of disease intensity of *Alternaria* blight of potato (Pandey and Pandey, 2002)

Category	Grade/Numerical value	Leaf area infected (%) Disease free
I	0	
II	1	1-10
III	2	11-25
IV	3	26-50
V	4	51-75
VI	5	=76

Table 3: Analysis of variance (ANOVA) of disease intensity caused by *Alternariaalternata* isolateson the potato cultivars

		Mean squares of disease intensity in potato cultivars	
Source of variation	Degrees of freedom	Agria	Marfona
Repeat	2	1.84ns	2.04ns
Fungal isolates	5	1611.38**	638.85**
Error	10	8.16	10.17
CV%	-	10	9.64

ns: Not significant; **: Significant at Pd”0.01.

Table 4: Assessment of potato leaf area infected (%), according to the Table 2, by *Alternariaalternata* isolates recovered as endophytesof cupressaceous trees.

Potato cultivar	Leaf area infected (%) by *Alternariaalternata* isolates					
	CSE177	POE43	POE72	CAE92	CSE165	POE104
Agria	59.2	37.3	32.7	28.0	24.7	16.7
Marfona	70.0	40.1	24.3	15.9	12.0	9.0

Furthermore, a large body of fungi exhibit multiple ecological functions, such as a human or plant pathogen and a saprotroph (Arnold, 2007). However there is still a lack of sufficient documents whether the same isolate can play each of these roles with equal success.

A. alternata is a weak and usually secondary pathogen in tomato and wheat, but a major leaf pathogen in potato. As seen, medium- to large-size brown spots, typical of *A. alternata*, were on potato leaves (Fig.2). To the best of our knowledge, *A. alternata* is pathogenic to potato, and no endophytic or latent *A. alternata* are reported within potato foliage. Thus, the external spots on infected leaves could be assigned to sprayed *Alternaria*.

All six *A. alternata* isolates, originally recovered as endophytes of cupressaceous trees, were capable of inducing disease on both potato cultivars, from 9 to 70 percent (Table3), according to the scales represented in (Table2).

Analysis of variance (ANOVA) of disease intensity (Table3) indicated that there were differences among pathogenicity of fungal isolates at Pd”0.01.

Data represented in (Table 4) indicates that *A. alternata* CSE177, originally isolated from the leaves of C. sempervirens in Fars province, caused the most severe symptoms on Marfona cultivar with a PDI of 70%. However, *A. alternata* POE104 that was originally isolated from twigs of *T. orientalis* in Hamedan province showed a pathogenicity of 9.0- on 16.7 percent on Marfona an Agria, respectively. *A. alternata* isolates are genetically diverse based on their geographic locations (Bagherabadi *et al.* 2015; Leiminger *et al.* 2013; Odilbekov *et al.* 2016). This may explain the variations in *A. alternata* pathogenicity in our experiments.

Furthermore, there were some degrees of differences between disease intensity observed in two potato cultivars. Indeed, except for the isolates CSE177 and POE43, Agria cultivar showed more susceptibility to the *A. alternata* POE72, CAE92 and CSE165 isolates than Marfona cultivar. This is in accordance with former findings that Agria cultivar was more susceptible to A. alternata than Marfona cultivar (Mirkarimi *et al*. 2014; Nasr Esfahani *et al.* 2017), and that most *A. alternata* isolates, pathogenic to potato in Hamedan province, were recovered from Agria cultivar (Bagherabadi *et al*. 2015).

In conclusion, we show for the first time that the same *A. alternata* isolates that were originally recovered as endophytic fungi from foliage of cupressaceous trees could induce disease on putative hosts such as potato with equal success. We had already shown that those *A. alternata* isolates were highly bioactive in vitro (Soltani and Hosseyni Moghaddam, 2014a). Here, we further confirm their possible deleterious activities on a field crop such as potato. This finding strengthens the hypotheses represented by former researchers (Arnold, 2007; Kusari and Spiteller, 2012), in that some endophytes are cryptic pathogens, or are virulent pathogens of other host plants. We have already isolated some endophytic fungi and bacteria from cupressaceous tress that are conspecific with human pathogens (Hosseyni Moghaddam *et al.* 2013; Hosseyni Moghaddam and Soltani, 2014 a, b; Soltani and Hosseyni Moghaddam, 2014 a, b, 2015; Soltani *et al.* 2016; Soltani, 2017). This indicates the possibility of human disease-inducing capabilities of those species. Moreover, we show that *A. alternata* isolates differ in their pathogenicity on the susceptible host, and that the host responds differently to the isolates. This may be due to the pathogenicity potential of the microorganisms, as well as to the resistance genes that the host harbors.

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