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Correlation between polygalacturonase activity of some *Fusarium* species *in vitro* and aggressiveness on wheat

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Abstract

Fusarium head blight (FHB) has reemerged as one of the most significant problems affecting wheat worldwide. Polygalacturonase (PG) is an important pectolytic enzyme produced by *Fusarium*species during the infection and colonistation in wheat plants. In this work, the correlationbetween *in vitro*polygalacturonase (PG) activityand aggressiveness of 15 *Fusarium* isolates (6 *F.culmorum*, 6 *F. verticillioides* and 3 *F. solani*) was investigated. Isolatesaggressivenesswereevaluated as the *percentage*of symptomatic spikelets *per*spike. In addition, isolates ability to produce *in vitro*PG activities were compared when grown ona solid medium. Aggressive isolates released more PGthanweakly aggressive isolates. Correlation analysis revealed a significant relationship (r = 0.57, r = 0.60, r = 0.64; P< 0.01; and r = 0.51, r = 0.64, r = 0.81; P< 0.01) between the PG produced by *F. culmorum,F. verticillioides*and *F. solani* isolates and theiraggressiveness on the two wheat cultivars Cham4 and Hourani, respectively. On the other hand, no correlation was found between the PG production and the origin of the isolates. The datademonstrate that production of PGcould influence the aggressiveness of *Fusarium* spp. isolates towards wheat heads.

Keywords: *Fusarium* spp, aggressiveness, polygalacturonase, *in vitro.*

1. Introduction

Fusarium is one of the most important pathogens attacking wheat and other plants that are the basis of human and animal nutrition. FHB is a devastating fungal disease of wheat globaly thatis caused bya number of *Fusarium* species, except *F. acuminatum* and *F. equiseti* [11]. It can cause yield losses up to 70% under favourable climate conditions [8]. Yield losses are mostly caused by mycotoxins produced by *Fusarium*, which causes significant grain yield and quality reductions [27].

The mechanism and aggressiveness of FHBsymptom induction by *Fusarium* speciesare poorly understood despite ultrastructural, biochemical and genetic studies [1, 3, 6]. Pectinases are one the most important virulence factor in plant fungal pathogens,which are responsible for fungi to decompose pectin in plant cell wall.

Pectin hydrolysis weakens the host cell wall to facilitate penetration and colonization and provides fungus with carbon sources for its growth [19].

Polygalacturonase (PG, E.C.3.2.1.15) is one of the major members of pectinases which cleaves α-1, 4-glycosidic of D-galacturonic acid in pectin, and it is classified into endo- and exo-polygalacturonase based on of the manner of eliminating galacturonic acid [5], and ithas been reported that PGwasthe first enzyme produced during infection by*Fusarium* spp., that plays a functional role during penetration and colonization in planttissue [9, 12].

Degradation of wheat tissues generally begins withproduction of *Fusarium* (PG) enzyme thathydrolyze pectin, a constituent of the middle lamellaand primary cell walls that often cause wheattissue maceration and cell death [16].

However, there is large evidence that several species use pectic enzymes as aggressiveness factors, which was demonstrated for the species *Colletotrichum gloeosporioides* [7],

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Verticillium dahliae and *Fusarium oxysporum* f. sp. *Vasinfectum* [18], *Alternaria alternata* and *Colletotrichum nicotianae* [28].

Additionally, in wheat plants expressing a bean PG-inhibiting protein, the demonstration of reduced foliar symptoms by the fungus*Bipolaris sorokiniana* supported a function of PG as a "pathogenic factor" in [23].

Considering the advantages of PG enzyme in aggressiveness of fungal isolates, the currentwork was undertaken to investigate whether or not a relationship between PG production *in vitro* and FHB severity existsinsixteen isolates of three *Fusarium* spp. (*F.culmorum*, *F. verticillioides* and *F. solani*) differing in their aggressiveness.

2. Materials and Methods

2.1.Fungal isolates

Fifteenfungal isolates representing three *Fusarium* species; *F. culmorum*, *F. verticillioides* and *F. solani* were collected from wheat heads displaying observable FHB symptoms from Syrian crop production areas.

All isolates were morphologically identified on the basis of macroscopic features such as pigmentations and growth rates over the surface of Potato Dextrose Agar (PDA, DIFCO, Detroit, MI. USA) in 9-cm Petri dishes, as well as their microscopic characteristics involving the size of macroconidia, presence of microconidia and chlamydospores [2, 22]. The *Fusarium* spp. isolates are listed in Table1.

Table 1. Origin of 15 isolates *Fusarium* species used in this study

Isolate	Host	Location	Year of collection	Colony colour
F.culmorum				
1	wheat seeds	north-west	2005	yellow mycelia -red base
2	"	"	2004	"
3	"	"	2005	yellow mycelia -brown base
6	"	"	2005	"
9	"	"	2005	yellow mycelia -red base
12	"	"	2005	yellow mycelia -brown base
F.verticillioides				
15	wheat seeds	north-west	2004	red
16	"	middle region	2004	brown
19	"	"	2004	red
27	"	"	2005	white mycelia -brown base
29	"	"	2004	red
31	"	"	2003	"
F.solani				
7	wheat root	middle region	2003	cream
11	"	north-west	2005	white
20	"	"	2004	cream

2.2. Enzyme production

PG production by the *Fusarium* spp. isolates was carried out in 250 ml Erlenmeyer flasks containing 5 g of solid substrate and nutrients (based on 100 ml of liquid medium) plus distilled water to adjust the moisture content to 75%.

Fresh fungal spores were used as inocula and 1mL spore suspension (containing around 10^6 spores/mL) was added to the sterilized medium and incubated at 30°Cfor 5 days in a rotary shaker (120 r.p.m). PG was extracted by adding distilled water containing 0.1% Tritonx100 in a total volume of 100 mL.

The flasks contents were stirred for 1.5 hours on a magnetic stirrer. The clear supernatant obtained by centrifugation (5000 *xg* for 15 min) followed by filtration (Whatman no. 1. paper)and was used as an enzyme source.

2.3.Pectinolytic Activity

PG activity was evaluated by determining the release of reducing groups from citrus pectin as described by Miller (1959) [21]. The reaction mixture containing 0.8 ml of 1% citric pectin with 67% of metoxilation in 0.2M citrate-phosphate, pH 6.0 buffer and 0.2 ml of culture supernatant, was incubated at 40ºC for 10 min. One unit of enzymatic activity (U) was expressedas the amount of enzyme which produced one µmole of galacturonic acid per minute.

2.4.Aggressiveness test

The aggressiveness of the 15 isolates was conducted under growth room conditions using two local cultivars (Cham4 and Hourani) of wheat, were chosen for their different reaction [2]. Seeds were surface-sterilized with 5% sodium hypochlorite solution for 5 min and then washed three times in sterile distilled water. They were sown into plastic pots (15-cm) filled with sterilized peatmoss, and arranged in a randomized complete block design with three replicates. Each experimental unit consisted of five pots of 20 seedlings per genotype. A full replicate consisted of the plots of two cultivars; this full test was repeated three times. Pots were placedin a growth chamber at temperatures at 23-25°C during the day and 18-20°C at night. Supplemental light was provided by 300-W metal halide lamps to ensure a 16 h photoperiod and a minimum intensity of 350 $\mu molm^{-2}ms^{-1}$. Following emergence, plants were thinned to three per pot and fertilized with a 1% solution of 20-20-20 (N-P-K) once a week starting 5 weeks after planting.

Inoculation was performed at 10-14 d after heading as described by Bekele (1987) [10] using a little piece of cotton soaked with *Fusarium* suspension was placed between the glumes of a spikelet in contact with the anthers and the stigma of a floret. The size of the cotton was about one fifth of a glume and the amount of the soaked inoculums about 16 µl. The growth chamber was operated at 25°C with a 12-h photoperiod and 80-90% RH. Disease severity was estimated after 21 d, when plants were at the soft dough stage as described by Xue et al. (2004) [29].

The disease incidence was measured by counting the number of heads with typical FHB symptoms (i.e. fully or partially bleached heads).

Disease incidence was expressed as the percentage of symptomatic wheat heads using a scale of Xue et al. (2004) [29] where; 0 (no visible FHB symptoms) to 9 (severely diseased, spike dead). Each head was assessed separately in all experiments. The experiment was repeated twice.

2.5.Statistical analysis

Data was subjected to analysis of variance using the STAT-ITCF statistical programme [4] to determine whether there was a significant test × genotype interaction. Correlations between data for *in vitro* enzyme production and aggressiveness were calculated with the SAS General Linear Models Procedure (SAS Institute; Cary, NC, USA).

3. Results and Discussion

All the 15 isolates of *Fusarium* species caused disease symptoms on the wheat cultivars, but the there was a large variation in aggressiveness (data not shown) and that neither of the two tested cultivars was immune from FHB. The analysis of variance of the combined results of wheat tests demonstrated a significant ($P< 0.05$) test × genotype interaction for each isolate.

On the other hand, significant differences ($P< 0.05$) in the mean PG yield values were detected among isolates, with high values being consistently higher in the isolates *F. culmorum* F6 and F12. Whereas, the lowest enzyme production was detected for *F. verticillioides* F16 and F19 (Fig 1).No correlation was found between PG production and the origin of the isolates. A significant relationship was found ($r = 0.57$, $r = 0.60$, $r = 0.64$; $P< 0.01$; and $r = 0.51$, $r = 0.64$, $r = 0.81$; $P< 0.01$) between the PG produced by *F. culmorum,F. verticillioides* and *F. solani* isolates and aggressiveness on the two wheat cultivars Cham4 and Hourani, respectively (Fig. 2). This gives us reason to believe that the ability of *Fusarium* spp. to produce PG may be of importance in the aggressiveness of this fungus towards wheat heads. However, enzyme and immunogold-labelling investigations confirmed involvement of extracellular enzymes that is pectinases, in degradation of cell wall components in *F. culmorum*-infected wheat spikes [17].

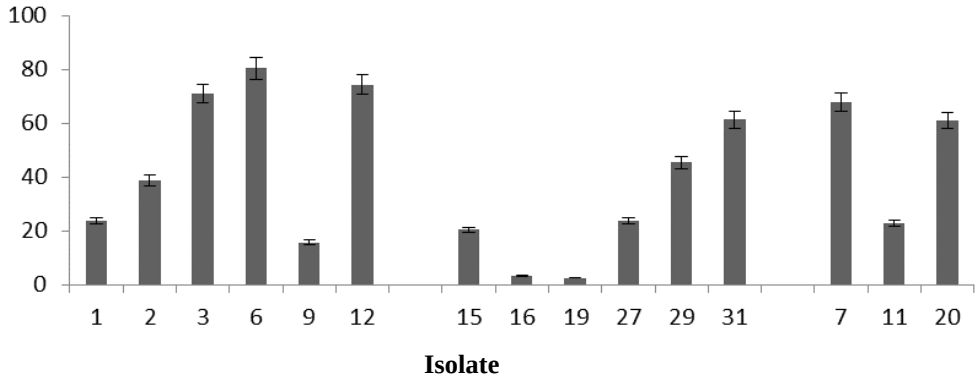

Figure 1. Polygalacturonase activity of 15 isolates ofthree *Fusarium* speciesused in this study

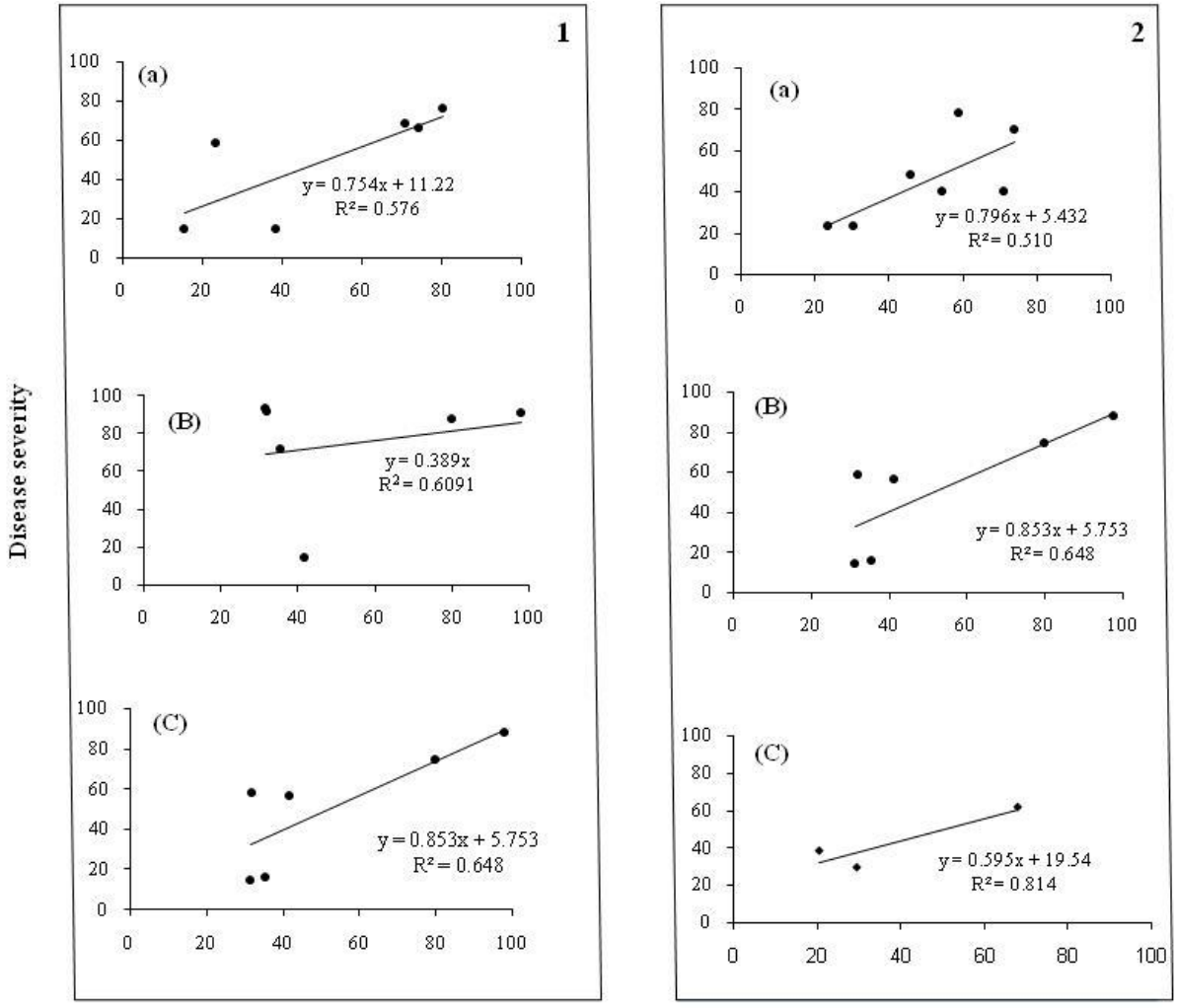

Figure 2. Correlative analysis between disease severity of 15*Fusarium* isolates (a: *F. culmorum;* b:*F. verticillioides* and c: *F. solani*) on two wheat cultivars, Horani (1) and Cham4 (2), and their Polygalacturonase activities *in vitro*. Disease severity was assessed as a percentage of each head which is diseased and calculate the average severities for all the heads (include the zero ratings as well) according to Xue et al.(2004) [29].

Our data demonstrated that there is no correlation between the aggressiveness of the *Fusarium* species isolates and the geographical origin of the isolate, this result is in agreement with those reported by Sidaoui et al. (2018) [26]. In addition, Brennan et al. (2003) [13] reported that *Fusarium* species isolated from some parts of Europe were not able to cause the same infection levels under different agroecological conditions.

However, since the aggressiveness has been documented to beevaluated by different factors and variables, several criteria can be utilized to determine the infectivity of the inoculum obtained from isolates. For this reason, the PG enzyme was selected in this work, according to the relevance of its function in the infective process. It is well known that pectinases are crucial to start the infection process, allowing the action of other enzymes [24]. However, although PG has been widely studied, it would act to a certain extenst in the prior degradation of the external cuticle. Since this current work analyzes simultaneously the *in vitro* PG enzymatic activity and as estimative of isolates aggressiveness, the criterion proposed for characterization and selection of isolates would result in a novel approach.

The *Fusarium* spp. isolates studied here produced significant levels of PG activity *invitro*, andthat the production of PG influences the isolates aggressiveness towards wheat heads. Kang and Buchenauer (2000) [17] reported that *Fusarium culmorum* infects the wheat ovary usually through thejunctions between the epidermal cell walls,allowing a quicker establishment of infection. The sameepisode was reported by Clay et al. (1997) [14] for *Cochliobolus sativus*, which infects barley leaf tissuethrough the pectin-rich junction between the epidermalcell wall. Liu et al. (2018) [19] reported that PG production by fungal pathogen is critical for their success and survival during plant infection. It has been confirmed that the absence of a polygalacturonase gene in some fungal pathogens would result in decreased pathogenicity [20]. Shieh et al. (1997) [25] mentioned that a polygalacturonase gene is *associated* to the infection of *Aspergillus flavus* in cotton bolls. In addition, Have et al. (1998) [15] found that the endopolygalacturonase gene *Bcpg1* was crucial for pathogenicity of *Botrytis cinerea*.

In the current work, our data suggest that PG is likely to be key determinants of aggressiveness in *Fusarium* species. The results also confirm the variability of PG activities produced *in vitro* among these species. The an earlier characterization of *Fusarium* speciesisolates regarding to aggressiveness components such as PG enzyme production would be useful as selection criteria for further investigation tending to help disease control. However, efforts to increase plant host resistance might be aided by new information on aggressiveness determinants. Moreover, in the future, attempts to elucidate the role of PG in the aggressiveness of *Fusarium* and an increased focus on the chemotype of the isolates used will undoubtedly be of value.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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