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Difference in Chemotype Composition of *Fusarium graminearum* Populations Isolated from Durum Wheat in Adjacent Areas Separated by the Apennines in Northern-Central Italy

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(Received on June 24, 2011; Revised on October 13, 2011; Accepted on October 16, 2011)

Chemotype composition of *Fusarium graminearum* strains, isolated from durum wheat kernels from naturally FHB infected fields in Northern and Central Italy, was investigated by multiplex PCR. The different climatic and environmental conditions of the two examined areas separated by the Apennines affected the composition of chemotypes. 15Ac-DON chemotype was predominant in both the sub areas. Nivalneol chemotype was more frequent in the warmer sub area.

Keywords : chemotype, durum wheat, *Fusarium graminearum*, Fusarium head blight

Fusarium graminearum (teleomorph *Gibberella zeae* (Schwein.) Petch.) is the major pathogen responsible of Fusarium head blight (FHB), an economically crucial and complex etiology disease on cereal crops worldwide (Goswami and Kistler, 2004). FHB has received more attention because of its effect on yield (Parry et al., 1995), grain quality (Liggitt et al., 1997) and grain contamination by mycotoxins (Visconti et al., 2000). FHB resistant cultivars are considered one of the solutions for the problem related to mycotoxin contamination in wheat grains (Lemmens et al., 2004), but unfortunately to date no resistant cultivar is commercially available for durum wheat. Most attention in the analysis of grain affected by FHB was placed on contamination by deoxynivalenol (DON), acetylated forms of DON (3Ac-DON and 15Ac-DON) and nivalenol (NIV).

DON is a mycotoxin that disturbs and/or inhibits DNA, RNA and protein synthesis by binding to the ribosomal peptidyltransferase site leading to a decrease of cell pro-

liferation (Shifrin and Anderson, 1999). It is also known as vomitoxin and it is responsible for hemorrhagic and anorexic syndromes, neurotoxic and immunotoxic effects in mammals (Visconti et al., 2004). DON contamination levels in food and feed were found to be much higher than those observed for NIV (Boutigny et al., 2011), being probably the most negligible toxin due to the small amount usually detected (Yazar and Omurtag, 2008), but with consistent levels in many Asian countries, New Zealand and Brazil (Placinta et al., 1999). However, NIV has shown to have a higher toxicity than DON, for example, in human blood cells (Minervini et al., 2004). This toxin has also shown higher activity than DON in inhibiting DNA, and it may account for various toxic phenomena such as induction of cell death (Poapolathep et al., 2002).

Based on the production of type B trichothecenes, DON and NIV, three *F. graminearum* chemotypes have been described: 3Ac-DON, 15Ac-DON and NIV. Chemotype identification is broadly used to characterize *F. graminearum* for its toxigenic potential (Pasquali et al., 2009). There was a correlation between the presence of *F. graminearum* chemotype and wheat grain mycotoxin content. DON and NIV contents can be successfully predicted by quantifying the fungal biomass of DON and NIV producers with a real-time PCR quantification method (Brandfass and Karlovsky, 2006; Burlakoti et al., 2007; Pasquali et al., 2009).

The dominant chemotypes of *F. graminearum* vary within geographical areas, i.e. different countries and/or continents (Prodi et al., 2009b), but some host preference may affect the composition of chemotypes of *F. graminearum* population. Within the same country, the different geographical areas (i.e. plain, mountains, valleys, etc.) and distances from the sea, may also affect the composition of chemotypes of a fungal population. Mountains may play an importance role as natural obstacles by inhibiting the

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diffusion of *F. graminearum* to other areas and preventing the genetic exchange among populations. The sea may provide humidity and enhance the suitable conditions for *F. graminearum* infection. Different locations have different environmental and climatic conditions, therefore also *F. graminearum* populations should be adapted to the environment under the pressure of natural selection.

The aim of this study was to investigate the chemotype differences of *F. graminearum* populations identified from different regions of Northern-Central Italy, separated by the Apennine mountains.

Durum wheat kernels from different cultivars were collected from naturally FHB infected fields within three

Italian regions (Emilia Romagna, The Marche and Umbria) of Northern-Central Italy during the years 2009–2010 (Table 1). Emilia-Romagna and The Marche are separated from Umbria by the Apennines (Fig. 1) and, therefore, we considered two sub areas: Emilia-Romagna and The Marche merged in the sub area 1 and Umbria in sub area 2. Four hundreds kernels from each of 63 samples of wheat collected were washed in sterile water, disinfected in a 2% sodium hypochlorite solution for 2 min, then placed in Petri dishes containing potato dextrose agar (Difco, USA) with 0.3 g L⁻¹ streptomycin and neomycin sulphate. The Petri dishes were incubated at 22°C in the dark for seven days (Pancaldi et al., 2010). *F. graminearum* was identified, from

Table 1. Chemotypes of *F. graminearum* isolated in the three different examined regions

No. isolates	Field localities	Regions	Cultivars	Chemotypes
Sub area 1				
ER 1	Baricella	Emilia Romagna	Neolatino	NIV
ER 2	Baricella	Emilia Romagna	Normanno	15Ac-DON
ER 3	Budrio	Emilia Romagna	Iride	15Ac-DON
ER 4	Busseto	Emilia Romagna	PR22D89	15Ac-DON
ER 5	Cadriano	Emilia-Romagna	Bologna	15Ac-DON
ER 6	Conselice	Emilia-Romagna	Tiziana	15Ac-DON
ER 7	Crespellano	Emilia Romagna	Normanno	15Ac-DON
ER 8	Crevalcore	Emilia Romagna	Dupri	15Ac-DON
ER 9	Faenza	Emilia-Romagna	Saragolla	15Ac-DON
ER 10	Ferrara	Emilia-Romagna	PR22D66	15Ac-DON
ER 11	Ferrara	Emilia Romagna	Levante	15Ac-DON
ER 12	Fiorenzuola d'Arda	Emilia-Romagna	Simeto	15Ac-DON
ER 13	Malalbergo	Emilia Romagna	Saragolla	NIV
ER 14	Mezzolara	Emilia Romagna	Iride	15Ac-DON
ER 15	Molinella	Emilia Romagna	Normanno	15Ac-DON
ER 16	Molinella	Emilia-Romagna	Saragolla	15Ac-DON
ER 17	Noceto	Emilia Romagna	22D78/874928	15Ac-DON
ER 18	Noceto	Emilia-Romagna	22D89/875015	15Ac-DON
ER 19	Noceto	Emilia-Romagna	22D40/875011	15Ac-DON
ER 20	Ostellato	Emilia-Romagna	Hathor	15Ac-DON
ER 21	Parma	Emilia Romagna	Simeto	3Ac-DON
ER 22	Ravenna	Emilia-Romagna	Saragolla	15Ac-DON
ER 23	Ravenna	Emilia-Romagna	Orobel	15Ac-DON
ER 24	Ravenna	Emilia Romagna	S. Carlo	15Ac-DON
ER 25	S. Agostino	Emilia-Romagna	Svevo	15Ac-DON
ER 26	S. Giorgio di Piano	Emilia Romagna	Neolatino	3Ac-DON
ER 27	S. Pietro Capofiume	Emilia Romagna	S. Carlo	3Ac-DON
ER 28	S. Pietro in Casale	Emilia-Romagna	Alcione	15Ac-DON
ER 29	Selva Malvezzi	Emilia Romagna	Anco Marzio	15Ac-DON
ER 30	Copparo	Emilia-Romagna	Iride	15Ac-DON
ER 31	Copparo	Emilia-Romagna	Iride	15Ac-DON
ER 32	Tamara	Emilia-Romagna	Iride	15Ac-DON
ER 33	Tamara	Emilia-Romagna	Iride	15Ac-DON

Table 1. Continued

No. isolates	Field localities	Regions	Cultivars	Chemotypes
Sub area 1				
M 1	Ancona	The Marche	Saragolla	15Ac-DON
M 2	Ancona	The Marche	Rusticano	15Ac-DON
M 4	Castelfidardo	The Marche	Claudio	15Ac-DON
M 5	Jesi	The Marche	Liberdur	15Ac-DON
M 6	Jesi	The Marche	Svevo	15Ac-DON
M 7	Jesi	The Marche	San Carlo	15Ac-DON
M 8	Jesi	The Marche	Ancomarzio	15Ac-DON
M 9	Jesi	The Marche	Flaminio	15Ac-DON
M 10	Jesi	The Marche	Levante	15Ac-DON
M 11	Jesi	The Marche	Saragolla	15Ac-DON
M 12	Jesi	The Marche	Normanno	3Ac-DON
M 13	Jesi	The Marche	Colosseo	15Ac-DON
M 14	Jesi	The Marche	Colosseo	NIV
M 15	Jesi	The Marche	Dylan	15Ac-DON
M 16	Jesi	The Marche	Ciccio	15Ac-DON
M 17	Macerata	The Marche	S.Carlo	15Ac-DON
M 18	Montefano	The Marche	Achille	15Ac-DON
M 19	Montefano	The Marche	San Carlo	NIV
M 20	San Severino Marche	The Marche	San Carlo	15Ac-DON
M 21	Treia	The Marche	Dorato	15Ac-DON
Sub area 2				
U 1	Perugia	Umbria	Avispa	15Ac-DON
U 2	Perugia	Umbria	Saragolla	15Ac-DON
U 3	Perugia	Umbria	Grecale	15Ac-DON
U 4	Perugia	Umbria	Avispa	NIV
U 5	Acquasparta	Umbria	Iride	NIV
U 6	Capanne	Umbria	Unknown	NIV
U 7	Casalina	Umbria	Latinur	15Ac-DON
U 8	Casalina	Umbria	Latinur	3Ac-DON
U 9	Casalina	Umbria	Latinur	3Ac-DON
U 10	Clitunno	Umbria	Grecale	15Ac-DON
U 11	Colombella	Umbria	Avispa	15Ac-DON
U 12	Corciano	Umbria	Claudio	15Ac-DON
U 13	Foligno	Umbria	Sorrento	15Ac-DON
U 14	Spello	Umbria	Unknown	NIV
U 15	Magione	Umbria	Unknown	NIV
U 16	Marsciano	Umbria	Unknown	15Ac-DON
U 17	Panicarola	Umbria	Ermocolle	NIV
U 18	Castiglioni del lago	Umbria	Unknown	15Ac-DON
U 19	Pozzuolo	Umbria	Duilio	15Ac-DON
U 20	S. Fatucchio	Umbria	Latinur	NIV
U 21	Marsciano	Umbria	Saragolla	15Ac-DON
U 22	Pozzuolo	Umbria	Quadrato	3Ac-DON

the single spore cultures obtained, according to morphological criteria described by Leslie and Summerell (2006). DNA from 75 *F. graminearum* strains, morphologically

identified, was extracted using a CTAB method (Suarez et al., 2005), modified in this work using seven days fungal mycelium (100–200 mg) and adding 1 µl of proteinase K



Fig. 1. Map of Northern-Central Italy, indicating the two sub areas studied (Sub area 1: Emilia-Romagna and The Marche; sub area 2: Umbria).

(20 mg/ml) to CTAB-grinding buffer shortly before the use. *F. graminearum* DNA was analyzed with specific primers Fg16F/Fg16R under the conditions described by Nicholson et al. (1998), to determine the identity of all the strains.

F. graminearum was isolated from all the samples, no more than two isolates of *F. graminearum* per sample were chosen, and 75 isolates were used for further studies (Table 1). The molecular results confirmed the microscopic identifications, based on macroconidia characteristics, with the presence of the expected band of 410 bp.

The potential capacity of *F. graminearum* isolates to produce trichothecenes was evaluated using a multiplex PCR version (Starkey et al., 2007) as described by Prodi et al. (2009b). Primers, designed in the region of the *Tri12* gene located in the terminal gene cluster for trichothecene biosynthesis, can distinguish three subgroups depending on the type of β -trichothecene produced. One primer is common to all chemotypes (12CON) and the others are chemotype-specific for 15Ac-DON (12-15F), 3Ac-DON (12-3F) and NIV (12NF) (Starkey et al., 2007).

All three chemotypes were found in both sub areas at each side of the Apennines (Table 1) and verified with the presence of the expected band of 670 bp for 15Ac-DON chemotype, of 410 bp for 3Ac-DON and of 840 bp for NIV. Sixty-four strains were found to be DON producers (57 strains 15Ac-DON and 7 3Ac-DON). Eleven strains showed to be NIV chemotype. Although in both areas the 15Ac-DON chemotype was dominant, the percentage of 15Ac-DON was variable. In Emilia Romagna and The Marche (sub area 1), the 15Ac-DON chemotype was accounting to 84% followed by 3Ac-DON and NIV at 8% (Fig. 2). In Umbria (sub area 2) 15Ac-DON chemotype was accounting to 54%. NIV was accounting to 32% and 3Ac-DON to 14% (Fig. 2).

This study is consistent with previous studies and reveals that *F. graminearum* 15Ac-DON chemotype is prevalent in

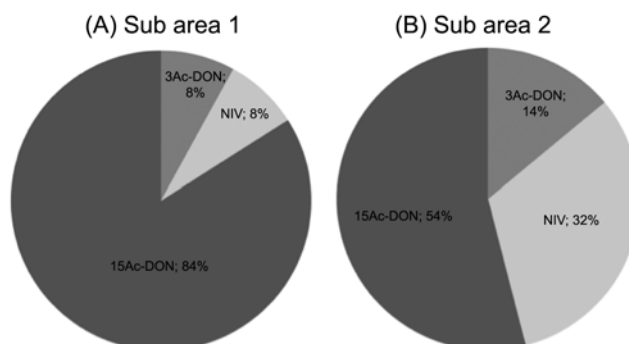


Fig. 2. Frequency of the three *F. graminearum* chemotypes in sub area 1 (A) and sub area 2 (B).

Italy, while 3Ac-DON and NIV chemotypes are much less frequently detected (Gale et al., 2007; Prodi et al., 2009a). Prodi et al. (2009b) found only two isolates of NIV from a total of 74 isolates of *F. graminearum* in 43 fields of a restricted area of Emilia Romagna.

This is the first study that provides data suggesting a different chemotype composition of *F. graminearum* within two adjacent geographical areas separated by natural obstacles. Emilia Romagna and The Marche are separated from Umbria by the Apennine mountains and their climate is classified as humid subtropical while Umbria has a Mediterranean climate (Peel et al., 2007). Humid subtropical climate is characterized by cold winters with average temperatures around 0/1 °C (winters usually colder than those of some countries at higher latitudes), hot summers (average temperature 22/24 °C), high precipitations and absence of a dry season. Mediterranean climate has hot and dry summer, mild winter and rainy autumn. In Umbria, the Apennines act as a barrier to the influence of the Adriatic sea and to the North-east cold air but, several changes in altitude and geographical position can generate a huge variety of climates. The geographical differences in the two sub areas create a specific different climate and environment that may affect the fitness of some chemotypes (Ward et al., 2002). Jennings et al. (2004) proposed that environmental favorable conditions explain the distribution of *F. culmorum* chemotypes in different geographical areas. We suppose that this hypothesis may also be valid in the case of *F. graminearum*. In China, Zhang et al. (2007) reported how temperature affects the different distribution of *F. graminearum* chemotypes and they showed that DON producers were present in cooler regions with the annual average temperatures of 15 °C or lower. On the other hand, NIV chemotype and the new subpopulation of 15Ac-DON producers are mostly present in warmer regions where the annual average temperatures are above 15 °C. This finding somehow supports our results on the different chemotype composition between the two sub areas located on different

sides of the Apennines. In the warmer region with Mediterranean climate (Umbria) *F. graminearum* NIV producers are present three fold more than in cooler regions (Emilia Romagna and The Marche) where we observed a high percentage of DON producers (total 92%; 15Ac-DON=84% and 3Ac-DON=8%).

However, our results do not show a clear cut like reported by Zhang et al. (2007). In Umbria, the warmest region, the percentage of DON producers was still high (total 68%; 54% for 15Ac-DON and 14% 3Ac-DON). In our experiment this could be explained with two hypothesis: first, *F. graminearum* NIV chemotype, mentioned by Zhang et al. (2007), is in lineage 6, so in some way they may behave differently from *F. graminearum* population of Northern-Central Italy (lineage 7), exclusively isolated from durum wheat. On rice Lee et al. (2009), observed that the different ecological ability between lineage 6 isolates and lineage 7 isolates were not directly related to the mechanism of pathogenicity; second, it is possible that some chemotypes of *F. graminearum* could be transported to other locations by seed shipping and long-distance spore transportation influencing chemotype composition (Guo et al., 2008). In fact in Western Canada, between 1998 and 2004, it was detected an increase of more than 14-fold of *F. graminearum* 3Ac-DON producers: that was linked to an introduction of a 3Ac-DON pathogenic population that was more toxigenic and vigorous, with a selective advantage above 15Ac-DON chemotype (Ward et al., 2008).

We can conclude that the different climatic and environmental conditions of the two examined areas separated by the Apennines affected the chemotypes composition of *F. graminearum*. 15Ac-DON was predominant in both the sub areas while NIV chemotype was more frequently detected in Umbria, the warmest sub area.

Acknowledgments

We wish to thank Dr. M. Montanari and Dr. A. Sommovigo (INRAN – ex ENSE sect. Bologna) for kindly supplying several grain samples and Dr. I. Alberti (INRAN – ex ENSE sect. Verona) for her scientific advice. The work was supported by Agenzia Servizi Settore Agroalimentare delle Marche (ASSAM) with the Project “Studi di carattere epidemiologico, monitoraggi e lotta ad alcune malattie fungine delle colture agrarie della Regione Marche”. Witoon Purahong was founded by an Erasmus Mundus Scholarship (IMaHS) from the University of Bologna.

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