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DETERMINATION OF DEOXYNIVALENOL AND NIVALENOL PRODUCING CHEMOTYPES OF *FUSARIUM GRAMINEARUM* ISOLATED FROM DURUM WHEAT IN DIFFERENT ITALIAN REGIONS

ABSTRACT

Durum wheat production in Italy is economically of great importance. *Fusarium graminearum* is the main fusarium head blight (FHB) causal agent in wheat, reducing both yield and grain quality. *F. graminearum* produces several mycotoxins and, among trichothecenes, deoxynivalenol (DON) and nivalenol (NIV) are the most studied for their toxicity towards humans and animals. DON-producing isolates can be further distinguished on the basis of the predominant acetyl-DON derivative in 3-acetyldeoxynivalenol (3-ADON) or 15-acetyldeoxynivalenol (15-ADON). In order to evaluate possible mycotoxin contamination risks in food, it is very important to know which chemotype is the prevalent in a *F. graminearum* population.

F. graminearum sensu stricto strains were collected from symptomatic durum wheat heads and grains of several naturally infected fields located mostly in Emilia – Romagna, The Marche, Lazio, Tuscany and Umbria. A multiplex PCR in the region of genes *Tri*12, located in the terminal gene cluster of trichothecenes, was used to characterize 187 single-spore isolates of *F. graminearum* as NIV, 3-ADON and 15-ADON chemotypes.

All the three chemotypes were present in the *F. graminearum* population studied. The most frequent chemotype was 15-ADON (83.4%), followed by 3-ADON (10.7%) and NIV (5.9%). NIV-producing isolates were found only in Emilia-Romagna (3.5%), Umbria (33.3%) and The Marche (5.7%).

Key words: DON and NIV chemotypes, durum wheat, Fusarium Head Blight, *Tri*12

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INTRODUCTION

Fusarium Head Blight (FHB) of wheat has been reported for the first time in Italy at the beginning of the XX century by Peglion (1900). Since then, it has been constantly present in Italy, especially in northern and central regions, but particularly strong attacks have been recorded from 1995 (Pancaldi *et al*., 1996) to date. The disease is caused by several *Fusarium* species such as *F. graminearum* Schwabe, *F. culmorum* (W. G. Smith) Sacc. and *F. poae* (Peck) Wollenweb. (Parry *et al*., 1995; Shah *et al*., 2005; Pancaldi *et al*., 2010).

FHB incidence and severity are related to year and area of cultivation and to the wheat variety utilized (Pancaldi *et al*., 1996; Balmas *et al*., 2000a; Rossi *et al*., 2006) and its main consequences are low seed quality and mycotoxin accumulation. *F. graminearum* in Emilia-Romagna, from 1995 to 2007, was the most frequently isolated species from blighted heads (average incidence of 32.1%) (Pancaldi *et al*., 2010). *F. graminearum* mainly produces deoxynivalenol (DON), a trichothecene mycotoxin that inhibits DNA, RNA and protein synthesis responsible of hemorrhagic and anorexic syndromes, neurotoxic and immunotoxic effects in mammals (Bottalico and Perrone, 2002) and which is strictly regulated in the EU (CE n. 856/2005 and updated n.1126/2007).

F. graminearum population can be divided into two chemotypes based on the production of the 8-ketotrichothecenes, DON and nivalenol (NIV). DON-producing isolates can be further distinguished on the basis of the predominant acetyl DON derivative that they produce; 3-acetyl DON (3-ADON) or 15-acetyl DON (15-ADON) (Miller *et al*., 1991; Jennings *et al.*, 2004).

In order to evaluate possible mycotoxin contamination risks in food it can be very important to know the prevalent chemotype into a *F. graminearum* population (Quarta *et al.,* 2005). The aim of this study was to map the chemotypes in Italy during investigations conducted from 2006 to 2009 in durum wheat fields.

MATERIALS AND METHODS

Durum wheat kernels and ears, showing the typical symptoms of FHB, of different cultivars were collected from several Italian regions. Mycological analyses were carried out by the methodology of Pancaldi *et al*. (2004). Plates containing potato dextrose agar (PDA) were incubated at 22°C in the dark for 5 days and the developed mycelium was transferred into new PDA plates under near-ultraviolet (NUV) alternating light and dark (12 h photoperiod) for l0 days to induce sporulation. The single spore cultures obtained (Balmas *et al.,* 2000b) were identified as *F. graminearum* according to the morphological criteria proposed by Leslie and Summerell (2006).

The DNA of each strain was extracted using a CTAB (exadecyl-trimethyl-ammonium bromide) method adapted from Lhodi *et al*. (1994) and subjected to PCR reactions to confirm that these strains belonged to *F. graminearum sensu stricto*, using specific primers under the conditions described by Nicholson *et al*. (1998).

The *F. graminearum* chemotype was assigned with a multiplex version (Starkey *et al*., 2007) of a chemotype specific test, previously validated by Ward *et al*. (2002). Primers are designed in the region of genes *Tri*12, located in the terminal gene cluster of trichothecenes and can distinguish three subgroups, depending on the type of β-trichothecenes product. One primer is common to all chemotypes (12CON) and the others are chemotype-specific for 15-ADON (12-15F), 3-ADON (12-3F) and NIV (12NF) (Starkey *et al.,* 2007). PCR was carried out using the protocol reported by Prodi *et al.* (2009).

RESULTS

In total, 187 isolates [140 obtained in this work plus 47 previously characterized by Prodi *et al.* (2009)] were identified as *F. graminearum* using traditional identification techniques and all were confirmed as *F. graminearum* by species-specific PCR assays.

Table 1

***Fusarium graminearum* chemotype (%) presence in kernels and wheat ears collected in the Italian surveyed regions.**

Regions	Number of strains isolated			15- ADON [%]			3- ADON [%]			NIV [%]		
	K	E	T	K	E	T	K	E	T	K	E	T
Emilia Romagna	60	54	114	91.7	83.3	87.7	6.7	11.1	8.8	1.7	5.6	3.5
The Marche	23	12	35	91.3	91.7	91.4	4.3	0.0	2.9	4.3	8.3	5.7
Umbria	15		15	53.3		53.3	13.3		13.3	33.3		33.3
Tuscany	7		7	57.1		57.1	42.9		42.9	0.0		0.0
Sardinia	4		4	100.0		100.0	0.0		0.0	0.0		0.0
Lombardy	4		4	50.0		50.0	50.0		50.0	0.0		0.0
Lazio	2		2	0.0		0.0	100.0		100.0	0.0		0.0
Piedmont	2		2	100.0		100.0	0.0		0.0	0.0		0.0
Campania	1		1	100.0		100.0	0.0		0.0	0.0		0.0
Veneto	1	2	3	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
			187			83.4			10.7			5.9

K – kernels; E – ears; T - total

The percentages of the three chemotypes found in kernels and ears for each region are reported in Table 1. All the three chemotypes were present in the *F. graminearum* population considered in this study . The most frequently isolated chemotype was 15-ADON (83.4%), followed by 3-ADON (10.7%) and NIV (5.9%). 15-ADON-producing isolates were found in all the Italian regions but Lazio, where 2/2 strains were 3-ADON. 3-ADON seemed to be more present in Lombardy and Tuscany than in other regions. NIV-producing isolates were

found only in Emilia-Romagna (3.5%), Umbria (33.3%) and The Marche (5.7%).

DISCUSSION AND CONCLUSIONS

In the recent years, in several wheat growing Italian areas, DON has been constantly found in FHB infected kernels of durum and bread wheat (Lops *et al*., 1998; Pascale *et al*., 2002; Rossi *et al*., 2006). In the 2007-2008 growing season, DON levels in many durum wheat samples exceeded the legal limits in Emilia-Romagna (Rossi, 2008). Knowledge on the distribution of *Fusarium* chemotypes is considered effective on a regional basis to predict a possible mycotoxin contamination (Jennings *et al*., 2004); Pasquali *et al.* (2010) reported that chemotyping is a useful tool for predicting nivalenol contamination in winter wheat.

Our data on chemotype frequency are comparable with those reported by several authors in the world. In different USA areas, Gale *et al*. (2007) identified 15-ADON as the prevailing chemotype of *F. graminearum sensu stricto*, followed by 3-ADON (5.1% of total), and one to NIV chemotype. In southern Russia 90% of the isolates was 15-ADON (Yli-Mattila *et al*., 2008), in England and Wales, Jennings *et al*. (2004) found DON (75%) and NIV (25%) with the predominant 15-ADON chemotype (95%), in Luxembourg the 15-ADON chemotype was the major population (94.3%), the 3-ADON chemotype was not detected and the NIV chemotype was detected sporadically (5.8%) (Pasquali *et al.*,2010). To our knowledge, only another research, performed on a very few *F. graminearum* Italian strains collected from 8 different regions, indicated that 58% of the analyzed isolates were 15-ADON, while both 3-ADON and NIV were present at 21% (Gale *et al*., 2007). Results on NIV chemotypes do not correspond to those obtained in the present study where they averaged at 5.9% and where they showed a high incidence (33.3%) only in Umbria. The results, presented by Ward *et al*. (2008), show how a population of *F. graminearum* with 3 -ADON chemotype replaced the dominant 15-ADON population in western Canada, evidencing a selective advantage. This was confirmed by Guo *et al*. (2008) that showed that in Manitoba, the 15-ADON chemotype remains predominant, but the 3-ADON had an increase trend in the southern part of the examined region. This fact was not due to the capacity of the 3-ADON strains to produce an higher level of DON, because DON production was significantly higher only on the susceptible cultivar and not on moderately or resistant wheat cultivars and, therefore, DON production was not correlated with higher aggressiveness (Ward *et al.*, 2008, von der Ohe *et al.*, 2010).

In China, the situation is much more complicated for the simultaneous presence of *F. graminearum* lineage 7 (*sensu stricto*) and *F. graminearum* lineage 6 (*F. asiaticum*), this last lineage not being present in Europe and USA (Ji *et al*., 2007). Qu *et al*. (2008a) found that *F. asiaticum* was more frequent in warmer

regions while *F. graminearum sensu stricto* was significantly present in cooler regions.

In this study the consistent population of *F. graminearum,* collected in Italian areas exclusively isolated from durum wheat, showed a chemotyping similar to that observed in other geographical areas. The continuous expansion of durum wheat cultivation in Italy will permit to increase the collection of *F. graminearum* strains from this important food crop in order to obtain more accurate information on the frequency and geographical distribution of isolates with different mycotoxin production and to identify the Italian areas exposed to the mycotoxin risk.

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