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Trichothecene and beauvericin mycotoxin production and genetic variability in *Fusarium poae* isolated from wheat kernels from northern Italy

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Abstract

The importance and widespread incidence of *Fusarium poae* as a natural contaminant of wheat in different climatic areas warrants investigations into the genetic diversity and toxin profile of a northern Italy population. Eighty-one strains of *F. poae* isolated from durum wheat kernels, identified by species-specific PCR and translation elongation factor-1α gene sequence analysis, were genetically characterized by the Amplified Fragment Length Polymorphism (AFLP) technique and analyzed by High Pressure Liquid Chromatography for their ability to produce the beauvericin (BEA) and trichothecene mycotoxins. A high level of variability was observed by using AFLP analyses, with the lowest level of genetic similarity among the strains approximately 61%. Most of the strains, 95%, produced BEA at < 2,655 µg/g; 88% produced the trichothecene nivalenol at < 865 µg/g and 76% produced the trichothecene fusarenon-X at < 167 µg/g. These data show that *F. poae* can produce high amounts of BEA together with trichothecenes, and can represent a high potential mycotoxin risk in Italy for wheat colonized by this species.

Introduction

Fusarium poae is considered to be a cosmopolitan species with a wide host range (Leslie and Summerell, 2006). Several reports showed that *F. poae* is a common field contaminant of wheat cultivated in a wide range of environmental conditions (Pettersson, 1990; Sugiura et al., 1993; Xu et al., 2005). The occurrence of *F. poae* on wheat is often related to Fusarium Head Blight (FHB), an important and insidious cereal disease caused by a *Fusarium* species complex (Xu et al., 2008). Although *F. graminearum* is the most frequent and virulent species within the FHB complex, *F. poae* is reported to predominate in some regions of Europe and North-America and its importance is increasing, creating great economical problems worldwide (Langseth et al., 1999; Birzele et al., 2002; Leonard and Bushnell, 2003; Xu et al., 2005). *Fusarium poae* is morphologically and genetically related to a recently described species, *F. langsethiae*, and to *F. sporotrichioides*, two highly toxingenic species producing T-2 and HT-2 toxins (Thrane et al., 2004). With respect to the toxin profile, *F. poae* exhibits variation in trichotheces production, according to several often contrasting reports (De Nijs et al., 1996; Langseth et al., 1999; Torp and Langseth, 1999; Thrane at al., 2004). In particular, *F. poae* was reported can produce both type A trichothecenes such as T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), and neosolaniol (NEO) and type B trichothecenes nivalenol (NIV) and fusarenon-X (FX) (Desjardins et al., 2006). In addition also the cyclohesadepsipeptide enniatins (ENNs) and beauvericin (BEA) production has been widely reported (Logrieco et al., 1998; Torp and Langseth, 1999; Uhlig et al., 2006). Since *F. poae* strains may have different mycotoxin profile, it is important to accurately characterize the mycotoxin potential of the populations of this species from different geographic areas. Moreover, in Italy *F. poae* incidence has not been deeply investigated and little information is available on its toxicological and

genetic diversity. A very informative tool for studying genetic variation within and among *Fusarium* species is Amplified Fragment Length Polymorphism (AFLP). This molecular technique allowed to support the description of new species within the genus *Fusarium* (Zeller et al., 2003) and to define genetic relationships within the same species (Leslie et al., 2007).

The objectives of this study were: (1) to determine the molecular intraspecific variability by using AFLP; (2) to characterize trichothecenes and beauvericin production for these strains; and (3) to assess the relationship, if any, between mycotoxin production and molecular variability.

Materials and Methods

Fungal strains and DNA extraction. Eigthy-one strains isolated from wheat kernels randomly collected from 37 fields of farms located in Ferrara Provence, an important area of the northern-eastern Italy for wheat cultivation, during the 2005 crop season. The strains were deposited in the Institute of Science of Food Production Collection (ITEM accession: http://www.ispa.cnr.it/Collection/) and reported as *F. poae* by morphological identification. They were analysed by translation elongation factor-1α (TEF) gene sequencing to confirm the taxonomic identity, by AFLP in order to investigate the genetic intraspecific variability and by a multitoxin extraction method to detect toxin production.

Each strain was grown in 100 ml of liquid medium (glucose 4%, malt extract 0.3%, yeast extract 0.3% and peptone 0.5%) in shaken culture (120rpm) at 25°C for 48 hours. Mycelium was filtered, washed with sterile distilled water, lyophilized and ground to a fine powder. DNA extraction was carried out on 25 mg of lyophilized mycelium with E.Z.N.A. Fungal DNA Miniprep Kit (Omega Bio-tek, Doraville, GA) according to the

manufacturer's protocol. Genomic DNA was checked for quality and quantity by agarose gel electrophoresis (0,8%) and comparison with a DNA standard.

Sequencing analysis. Genomic DNA was used as a template to amplify a portion of the TEF gene by using primer pairs EF1/EF2 and protocol from O'Donnell et al. (1998). The purity and quantity of the products were established by visual inspection on agarose gels stained with ethidium bromide. The PCR products were then purified through filtration by Sephadex G-50 (Sigma) columns and used for sequencing by using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) according manufacturer's instructions. Sequences were analysed by Basic Local Alignment Search Tool (BLAST) and compared with references strains of *F. poae* and related species.

AFLP analysis. AFLP analysis was performed according instructions of AFLP™ Microbial Fingerprint Kit (Applied Biosystems, Fostercity, CA), as described previously (Moretti et al., 2004). Genomic DNA (10ng) was digested with 10U of *EcoRI* and 1U of *MseI* restriction enzymes (New England Biolabs, Hitchin, Hertfordshire, United Kingdom), in a final volume of 11µl containing T_4DNA ligase buffer 1X, NaCl 0,05M, *MseI* adaptor 1µM, *EcoRI* adaptor 1µM, 0,5µg of BSA, 1U of T4 DNA and incubated at room temperature overnight. Four microliters of 1:20 dilution of the described mix were used for preselective amplification. Two selective amplifications were carried out with labelled *EcoRI* primers to allow automated fluorescence analysis. Primer pairs used for AFLP analyses were *Eco RI*-AC/*MseI*-CC and *EcoRI*-AC/*MseI*-CA. One microliter of PCR product diluted 1:20 was mixed to 20µl of deionized formamide and 0.5µl of DNA size standard GS500 ROX (Applied Biosystem), denatured and subjected to electrophoresis. Fragment analysis was performed with ABI 310 GeneScan®3.1.2 software (Applied Biosystem) and a binary matrix was obtained with ABI Genotyper® 2.5.2 software (Applied Biosystem). Cluster analysis by NTSYSpc 2.1 software was carried out on fragments from 100 to 500 bp by

using the unweighted pair group method algorithm (UPGMA) and Dice similarity coefficient.

Growth conditions for toxin detection. All the strains of *F. poae* were tested for toxin production, in particular ENNs, BEA and tricothecenes T-2, HT-2, DAS, NIV, DON, FX, NEO, zearalanone (ZAN), zearalenone (ZON). A small amount of each strain was inoculated on 50 g of autoclaved wheat previously kept to 45% of moisture for one night. Cultures were incubated at 25°C under fluorescent light (12h photoperiod) for four weeks (Leslie and Summerell, 2006), then dried at 48°C for 24 hours and ground to a fine powder. Control wheat was treated in the same way, except that it was not inoculated.

Chemical analysis. A multitoxin analytical method, combining high-performance liquid chromatography (HPLC), atmospheric pressure chemical ionization (APCI) and triplequadrupole tandem mass spectrometry (LC-MS/MS) under the selected reaction monitoring (SRM) mode was used to detect the mycotoxins here reported: namely, BEA, ENNs, NIV, DON, FX, NEO, HT-2, T-2, DAS, ZON, ZAN. Standards of above named toxins were purchased from Sigma-Aldrich (Milan, Italy) and stored at 4°C in the dark. They were individually dissolved in methanol at 1mg mL−1, mixed and conditioned to room temperature before use. Water for LC mobile phase and organic solvents were HPLC grade from Merck (Darmstadt, Germany), Ammonium acetate (MSgrade) was bought from Sigma-Aldrich, while formic acid was obtained from Fluka (Milan, Italy). The extracts were filtrated with Whatman no. 4 type filter papers (Maidstone, England). Strata X C18-E columns were purchased from Phenomenex (Aschaffenburg, Germany) and trichothecene EP columns from R-Biopharm Rhone Ltd (Darmstadt, Germany). Filtek syringe filters (0.22 μm; 25 mm) were provided from Chemtek Analytica (Bologna, Italy). LC analysis was performed using a system consisting of two micropumps (Series 200, PerkinElmer, Waltham, MA, USA). A

Gemini column (3 μmC18 110A, 150 × 4.60 mm; Phenomenex, USA) heated to 50°C was used; the flow rate was set to 0.8 mL min−1, while the injection volume was 20 μL. Mobile phase A consisted of an H_2O/CH_3OH mixture (90:10, v/v) containing 5 mmol L−1 ammonium acetate, while mobile phase B consisted of a CH_3OH/H_2O mixture, also containing 5 mmol L−1 ammonium acetate (90:10, v/v). MS/MS data were obtained using an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada). All analyses were performed with an APCI interface. The declustering potential (DP) and collision energy (CE) were optimized for each compound by direct infusion of standard solutions (10 μgmL−1) into the mass spectrometer at a flow rate of 8 μL min−1, using a Model 11 syringe pump (Harvard Apparatus, Holliston, MA, USA) and a solution of CH_3OH/H_2O 5 mmol ammonium acetate (90 : 10, v/v) as liquid carrier at a flow rate of 200 μL min−1. The acquisition was carried out by selected reaction monitoring (SRM) both in the negative and positive ion mode for each compound. Data acquisition and processing were performed using Analyst software v. 1.4. The simultaneous extraction of the above-mentioned toxins was obtained by adding 50 mL of a CH_3CN/H_2O (84 : 16; v/v) mixture to a vessel containing 10 g of finely grounded wheat sample. The obtained extract phase was paper filtered and 5 mL of the filtrate were recovered. A cleanup with C18 cartridges was used. These cartridges were attached to a vacuum manifold apparatus and, immediately prior to use, they were activated with 3 mL CH_3OH and washed with 3 mL H_2O. A 5 mL aliquot of the dry extract was redissolved in 3 mL H_2O and the resulting clear solution was passed through the pre-conditioned C18 cartridge. The cartridge was washed with 3 mL H_2O and then the fusariotoxins were eluted with 3 mL CH_3OH. The residue was finally evaporated using a centrifugal evaporator. The dry extracts produced using each method were then dissolved in 1 mL of CH_3OH/H_2Omixture (70 : 30, v/v) by vortexing vigorously and finally filtered through a 0.22 μm cellulose filter (Chemtek Analytica,

Bologna, Italy) before LC-S/MS analysis. For each toxin, stock solutions of 1000 µgmL−1 dissolved in methanol were prepared and stored at T=−20°C in the dark. Mixed standard solutions of 10, 20, 50, 100, 250, 500 and 1000 µg L−1 were prepared by diluting the stock solutions with a CH_3OH/H_2O mixture (70:30, v/v). The limits of detection for NIV, DON, FX, NEO, HT-2, T-2, DAS, ZON, ZAN were 0.0033, 0.0005, 0.0015, 0.0033, 0.0033, 0.001, 0.0025, 0.001, 0.00125 and 0.0002 µg/g , respectively.

Results

Sequencing analysis

Twenty-six strains, selected to be representative of the entire genetic variability obtained by AFLP analysis, were sequenced for TEF gene portion to confirm the identification. All the tested strains were identified, according to BLAST analysis, as *F. poae*, in a range of similarity coefficient from 98 to 100%. A comparison with representative sequences of *F. poae*, *F. langsethiae*, *F. sporotrichioides* and *F. kyushuense*, identified by Knutsen et al. (2004) in a phylogenetic analysis based on partial sequences of TEF gene, was made (Figure 1). All the strains sequenced in this paper clustered with the *F. poae* strain VI01250, while sequences belonging to *F. langsethiae*, *F. sporotrichioides* and *F. kyushuense*, represented by VI01268, VI01290 and VI01325 strains, respectively, formed a distinct cluster.

AFLP analysis

Fusarium poae strains were analyzed by AFLPs, in order to obtain information about the genetic intra-specific variability of an Italian population of *F. poae*. Cluster analysis was performed on 90 amplified fragments, 48 obtained by the primer combination *Eco RI*-AC/*MseI*-CC, 42 by the *EcoRI*-AC/*MseI*-CA one. Clear polymorphisms were obtained by using the two primer pairs.which yielded the same results in separate analyses. The AFLP profiles showed an high genetic variability, as demonstrated by the

dendrogram in Figure 2; the population shared 61% of the electrophoretic profile, as lowest coefficient of similarity. It is possible to define about six main groups with values of similarity coefficient between the groups ranging from 65 to 74%. Moreover each group showed also a high degree of internal variability, being the similarity coefficient values within each group in a range from 70 to 76% (Figure 2). In addition six strains (ITEM 9138, 9169, 9178, 9188, 9191 and 9195) resulted isolated in the dendrogram with the level of their similarity coefficient ranging from 61 to 64%, with respect to the *F. poae* population.

Toxin production

In order to investigate the chemical profile of the population, toxin production was tested (Table 1). None of the 81 strains tested produced DON, ENNs, HT-2, T-2, ZAN and ZON. The production of NIV, DAS, NEO and FX was detected for 69 (88%), 49 (63%), 62 (79%) and 59 strains (76%), respectively. Production level was very variable, ranging from 0.2 to 865 µg/g for NIV, from 0.1 to 2.5 µg/g for DAS, from 0.1 to 6.1 µg/g for NEO and from 0.2 to 167 µg/g for FX. Almost all strains (77 on the 81) produced BEA at a range of 2 to 2,655 µg/g (Table 2). Fifty-three strains produced both type A and B trichotecenes as well as BEA. Only 2 strains (ITEM 9125 and 9204) produced only type A trichotecenes and BEA, while 6 strains (ITEM 9174, 9186, 9187, 9188, 9207 and 9209) produced only B trichotecenes and BEA. Finally, 12 strains produced BEA but no thrichotecenes and three strains (ITEM 9181, 9185, 9203) produced thrichotecene but no BEA (Table 1).

Discussion

The wide incidence of *F. poae* as a natural contaminant of wheat in different climatic areas (Xu et al., 2005) warrants careful investigations on its genetic diversity and toxin profile of the populations isolated from wheat worldwide. In this study, we analyzed a

larger population of *F. poae* for mycotoxin production and genetic variability. Recent phylogenetic studies based on multi-gene sequence analyses, identified the "powdery" variant of *F. poae* as a new species, *F. langsethiae* (Knutsen et al., 2004; Torp and Nirenberg, 2004; Wilson et al., 2004; Yli-Mattila et al., 2004). The strong morphological and genetic similarity between these two species has often led to species misidentification, which could result in incorrect mycotoxin risk evaluation since *F. poae* and *F. langsethiae* strains were shown to have a significant diversity in their mycotoxin profile (Thrane et al., 2004). Moreover, the variability in mycotoxin production among isolates of *F. poae* recovered from different parts of the world indicates that future examination of mycotoxin production in this species should be done on a more global level. It is therefore important to have broader genetic and mycotoxin investigations on *F. poae* populations from different geographical areas to define a more reliable biological profile of this species. The genetic analyses carried out on *F. poae* by using phylogeny (Knutsen et al., 2004; Yli-Mattila et al., 2004) and/or AFLP (Schmidt et al., 2004) are few and often related only to a limited number of strains for each investigation. These studies were mainly addressed to emphasize species boundaries between *F. poae* and the closely related species *F. langsethiae* and *F. sporotrichioides* and did not provide data on the intra-specific genetic variability of *F. poae*. In our report, our main goal was to assess the genetic variability of a large population of *F. poae* isolated from wheat in northern Italy, in order to relate the eventual observed variability to other phenotypic traits such as the mycotoxin production. In the AFLP analyses, the occurrence of six groups with the highest similarity coefficient around 76%, showed that this population has a remarkable genetic variability. These groups are not strictly defined and some single strains with lower coefficients of similarity (from 61% to 64%) were out of these groups and they did not cluster among them probably since they were not well represented in the population of

isolates that we examined in this study. We lacked to relate such variability to other well characterized traits of the strains such as mycotoxin profile. According to Leslie and Summerell (2006), strains that share ~ 60% or more of the AFLP bands are in the same species while isolates in different species rarely share more than 40% of the bands. Interestingly, some strains of the population here analyzed are located at the border of the species boundaries defined by AFLP, warranting further studies on their genetic identity. However, the existence of this genetic variability could provide to *F. poae* a wide genetic pool that strains of this species can use for better facing both new and different environmental conditions. On the other hand, this wide genetic pool can provide to *F. poae* the capability of developing multi-gene resistance to fungicides and to overcome the wheat resistance to the *F. poae* colonization. Future genetic analyses could provide more information on the mechanism by which *F. poae* maintains a high level of genetic diversity in the absence of an apparent sexual stage. A similar approach to study genetic variation by using AFLP in *F. graminearum*, a species complex provided with a known sexual stage (Leslie and Summerell, 2006), has shown the existence of a slightly higher genetic variability among the strains analyzed (between 49% and 67% of AFLP similarity; Leslie et al., 2007). The data led the authors to define the existence of sub-species within the *F. graminearum* species complex. If our data on the genetic variation of *F. poae* can represent preliminary evidence for the occurrence of genetic barriers within this species needs further and wider investigations. Toxin production of the population investigated in this report generally agrees with a previous study with respect to the capability of DAS, NIV and FX production observed in almost all the *F. poae* strains analyzed (Thrane et al., 2004). Thrane et al. (2004) reported that *F. poae* strains could produce rarely T-2 and HT-2. Our paper seems to confirm the lack of production of T-2 and HT-2 by *F. poae*, reported also by other authors (Sugiura et al., 1993; Liu et al., 1998), since none of the 81 strains here analyzed produced these

mycotoxins. On the other hand, this is in contrast with other reports showing that this species can produce T-2 and HT-2 (Bocarov-Stancic et al., 2007; Vogelgsang et al., 2008). However, a possible explanation of the contrasting reports on T-2 and HT-2 production by *F. poae* could be due to either to a misidentification of *F. poae* with *F. langsethiae*, a high T-2 and HT-2 producing species, or studies that have been carried out before the description of *F. langsethiae* (Torp and Nirenberg, 1999). A global reassessment of the capability of *F. poae* and related species (*F. sporotrichioides* and *F. langsethiae*) to produce T-2 and HT-2 is warranted since these mycotoxins will be soon regulated by the EU and a correct evaluation and management of the mycotoxin hazard could have a dramatic economical impact on the wheat production worldwide. With the respect to the other trichothecenes, the low levels of DAS production detected in this report (Table 3) confirm previous investigations (Marasas et al., 1984). Low production levels were also observed for NEO, with more than 50% of the strains included in a range from 0.1 to 10 µg/g (Table 3). However, while in our report 79% of strains could produce this mycotoxin, only few strains were reported capable of producing NEO by Thrane et al. (2004). Finally, our data showed a highly variable production of NIV and FX, with high mean values (Table 2), confirming a previous study on *F. poae* isolated from maize in Poland (Grabarkiewicz-Szczesna et al., 1999).

The ability of *F. poae* to produce both type A and type B trichothecenes was found for the first time in Japan (Sugiura et al., 1993). This report confirms such capability and shows that single strains can use two different branches in thrichotecene biosynthesis, since several strains could produce not only DAS and NIV (being the first an intermediate of the second metabolite), but also NEO and NIV, which are toxins that need different branches of the thrichotecene biosynthetic pathway. Moreover, these data warrant a deeper investigation on the correlation between the assessed production of trichothecenes and the specific genetic pathway involved in the trichothecene

production of *F. poae*. Finally, the simultaneous production of NIV, FX, DAS, and NEO by the same strains shows that a high toxigenic risk can be related to the occurrence of *F. poae* on wheat. Low levels of BEA production were previously observed in a few strains of *F. poae* isolated from maize (Logrieco et al., 1998) and wheat (Thrane et al., 2004). This is the first report on BEA production of a relatively large population of *F. poae* from wheat in Italy. Levels of production were variable in the population, but most of the strains showed a high level of production (up to 2,655 µg/g), as demonstrated by the mean value of 197 µg/g (Table 2). These results show that BEA could represent a serious risk for wheat consumption in Italy. Beauvericin can induce apoptosis on murine cell lines (Ojcius et al., 1991) and can be toxic on a wide range of mammalian cell lines (Que et al., 1997; Calò et al., 2004). Moreover, BEA showed negative effects on heart rate and contraction strength in guinea pig hearts (Lemmens-Gruber et al., 2000). Thus, it may be involved in human and animal pathology, representing a serious risk for health when occurring as a contaminant in wheat kernels. Contamination of BEA was frequently found in wheat fields affected by FHB (Jestoi et al., 2004; Uhlig et al., 2006), therefore this compound has been considered in the last decade in Europe as an emerging problem for food safety and was often associated to the occurrence of *F. poae* on wheat (Logrieco et al., 2008).

Finally, the simultaneous production of BEA and type A and B tricothecenes, often at high levels, assessed for the most of *F. poae* strains examined in this report (around 70%), is very important since the co-occurrence of different toxic metabolites could imply additive and/or synergic effects on target organisms (Muller and Schwadorf, 1993). Therefore, this paper warrants further studies on the toxicity of *F. poae* by using a multi-toxin approach on the target organisms. Finally, since *F. poae* is often occurring on wheat ears showing very low if any symptoms (Vogelgsang et al., 2008), its importance as toxigenic species affecting the wheat has been often underestimated. A

deeper knowledge of the ecology, genetic and toxigenicity of *F. poae* and its related species, at a global level, would be an important key to improve and prevent the mycotoxin risk on wheat worldwide.

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Table 1 - Toxin production of *F. poae* strains isolated from wheat kernels in Northern-Italy.

ITEM	Toxin production*					Toxin profile
	NIV	FX	NEO	DAS	BEA	
9122	95.1	144.0	3.3	1.7	46	1
9123	4.3	17.8	ND	0.1	57	1
9124	40.6	26.9	2.1	1.0	12	1
9125	3.5	ND	ND	ND	87	2
9126	ND	ND	ND	ND	124	4
9127	42.3	0.6	TR	TR	48	1
9128	ND	23.8	2.4	0.4	346	1
9129	38.6	40.8	0.4	0.3	45	1
9130	14.5	4.1	0.1	TR	68	1
9131	95.1	167	3.3	2.5	5	1
9132	37.3	26.9	2.1	1.0	78	1
9133	37.7	25.8	0.1	0.9	168	1
9134	2.5	1.6	TR	TR	34	1
9135	TR	0.6	TR	TR	94	1
9136	19.3	36.0	0.1	0.2	31	1
9137	ND	ND	ND	ND	347	4
9138	37.7	25.8	0.1	0.9	16	1
9139	ND	ND	ND	ND	2	4
9140	19.3	36	0.1	0.9	16	1
9141	7.8	22.9	TR	TR	24	1
9142	168.0	13.7	TR	ND	101	1
9143	14.5	4.1	0.1	TR	174	1
9144	2.5	1.6	TR	TR	59	1
9145	8.5	0.1	TR	TR	30	1
9146	ND	ND	ND	ND	101	4
9147	ND	ND	ND	ND	6	4
9148	24.9	22.5	0.5	TR	46	1
9149	0.3	0.7	0.6	1.0	565	1
9150	6.5	ND	TR	0.1	44	1
9151	8.5	0.2	TR	TR	234	1
9152	5.6	9.3	0.1	1.6	64	1
9154	2.2	2.6	0.1	TR	70	1
9155	4.6	2.5	0.1	TR	TR	1
9156	168.0	13.7	TR	ND	22	1
9157	5.3	ND	TR	0.1	175	1
9158	82.4	4.6	0.1	TR	174	1
9159	262.0	8.6	0.2	TR	125	1
9162	82.4	5.6	0.1	0.1	46	1
9163	ND	ND	ND	ND	607	4
9164	4.9	0.6	TR	ND	161	1
9165	3.7	1.1	TR	ND	14	1
9166	4.6	2.5	0.1	TR	57	1
9167	72.5	8.5	0.4	ND	290	1

9168	**NA**	**NA**	**NA**	**NA**	**4**	**-**
9169	**ND**	**ND**	**ND**	**ND**	**10**	**4**
9170	**1.0**	**1.5**	**TR**	**TR**	**86**	**1**
9171	**0.4**	**ND**	**ND**	**TR**	**47**	**1**
9172	**NA**	**NA**	**NA**	**NA**	**81**	**-**
9173	**NA**	**NA**	**NA**	**NA**	**2,655**	**-**
9174	**7.1**	**ND**	**ND**	**ND**	**15**	**3**
9176	**7.4**	**2.5**	**TR**	**ND**	**739**	**1**
9178	**76.1**	**6.5**	**0.1**	**TR**	**122**	**1**
9180	**12.3**	**1.7**	**TR**	**0.6**	**4**	**1**
9181	**76.1**	**6.5**	**0.1**	**TR**	**ND**	**5**
9182	**12.3**	**1.7**	**0.1**	**0.6**	**85**	**1**
9183	**0.2**	**5.7**	**TR**	**ND**	**116**	**1**
9184	**ND**	**ND**	**ND**	**ND**	**84**	**4**
9185	**21.0**	**0.8**	**TR**	**ND**	**ND**	**5**
9186	**2.9**	**ND**	**ND**	**ND**	**462**	**3**
9187	**5.2**	**ND**	**ND**	**ND**	**189**	**3**
9188	**0.5**	**ND**	**ND**	**ND**	**150**	**3**
9189	**ND**	**ND**	**0.1**	**ND**	**35**	**2**
9190	**1.0**	**1.5**	**TR**	**TR**	**468**	**1**
9191	**ND**	**ND**	**ND**	**ND**	**197**	**4**
9192	**126.1**	**8.5**	**2.3**	**1.4**	**376**	**1**
9193	**0.3**	**0.7**	**0.2**	**1.0**	**16**	**1**
9194	**ND**	**ND**	**ND**	**ND**	**347**	**4**
9195	**5.6**	**9.3**	**0.1**	**1.6**	**252**	**1**
9196	**0.4**	**ND**	**ND**	**TR**	**41**	**1**
9197	**ND**	**ND**	**ND**	**ND**	**85**	**4**
9198	**ND**	**ND**	**ND**	**ND**	**ND**	**-**
9199	**ND**	**ND**	**ND**	**ND**	**133**	**4**
9200	**625.0**	**54.8**	**6.1**	**ND**	**183**	**1**
9202	**21.0**	**0.9**	**TR**	**ND**	**66**	**1**
9203	**0.2**	**5.7**	**0.1**	**ND**	**ND**	**5**
9204	**ND**	**ND**	**0.1**	**ND**	**800**	**2**
9205	**7.4**	**1.9**	**TR**	**ND**	**153**	**1**
9207	**2.9**	**ND**	**ND**	**ND**	**107**	**3**
9209	**0.5**	**ND**	**ND**	**ND**	**776**	**3**
9210	**5.3**	**0.6**	**0.1**	**ND**	**561**	**1**
9211	**865.0**	**54.8**	**2.5**	**ND**	**52**	**1**

*= Values are expressed in ppm (µg/g); NIV=nivalenol; FX=fusarenon-X; NEO=neosolaniol; DAS=diacetoxyscirpenol; BEA=beauvericin; ND = not detected; TR = detected in trace levels; NA = not analyzed; 1 = type A and type B trichothecene and beauvericin producer; 2 = type A trichothecene and beauvericin producer; 3 = type B trichothecene and beauvericin producer; 4 = beauvericin producer; 5 = type A and type B trichothecene producer.

Table 2 – Summarizing data about toxin production of *F. poae* strains isolated from wheat kernels in Northern-Italy.

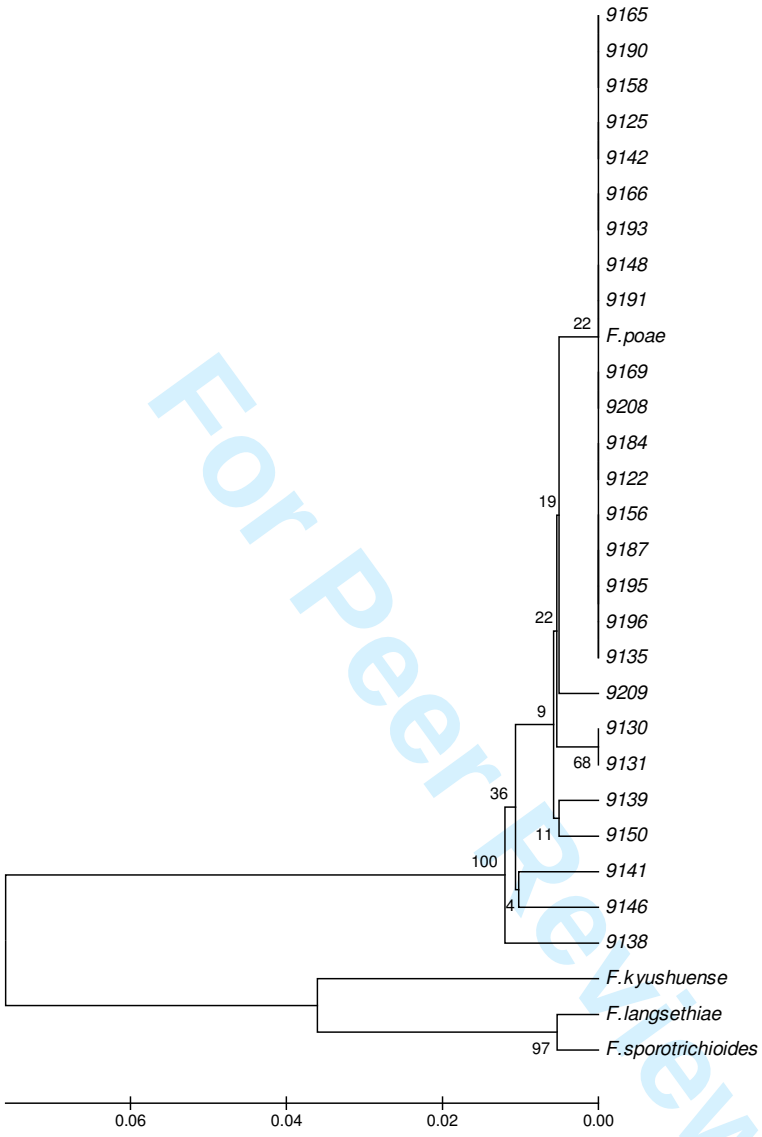
Toxins	Producer strains	Toxin production*		
	(%)	min	max	mean
NIV	**88**	**0.2**	**865**	**54.3**
FX	**76**	**0.2**	**167**	**16.7**
NEO	**79**	**0.1**	**6.1**	**0.8**
DAS	**63**	**0.1**	**2.5**	**0.8**
BEA	**95**	**1.7**	**2,655**	**197**

*= Values are expressed in ppm (µg/g); NIV=nivalenol; FX=fusarenon-X; NEO=neosolaniol; DAS=diacetoxyscirpenol; BEA=beauvericin.

Table 3 – Levels of toxin production of *F. poae* strains isolated from wheat kernels in Northern-Italy.

Toxin production*	N. of strains				
	NIV	FX	NEO	DAS	BEA
ND	16	26	23	36	4
0.01 – 0.1	1	0	17	21	1
0.1 - 1	10	10	30	16	0
1 - 10	23	25	8	5	5
10 - 100	22	15	0	0	36
100 - 1000	6	2	0	0	34
More than 1000	0	0	0	0	1

*= Values are expressed in ppm (µg/g); NIV=nivalenol; FX=fusarenon-X; NEO=neosolaniol; DAS=diacetoxyscirpenol; BEA=beauvericin.



1

2

1

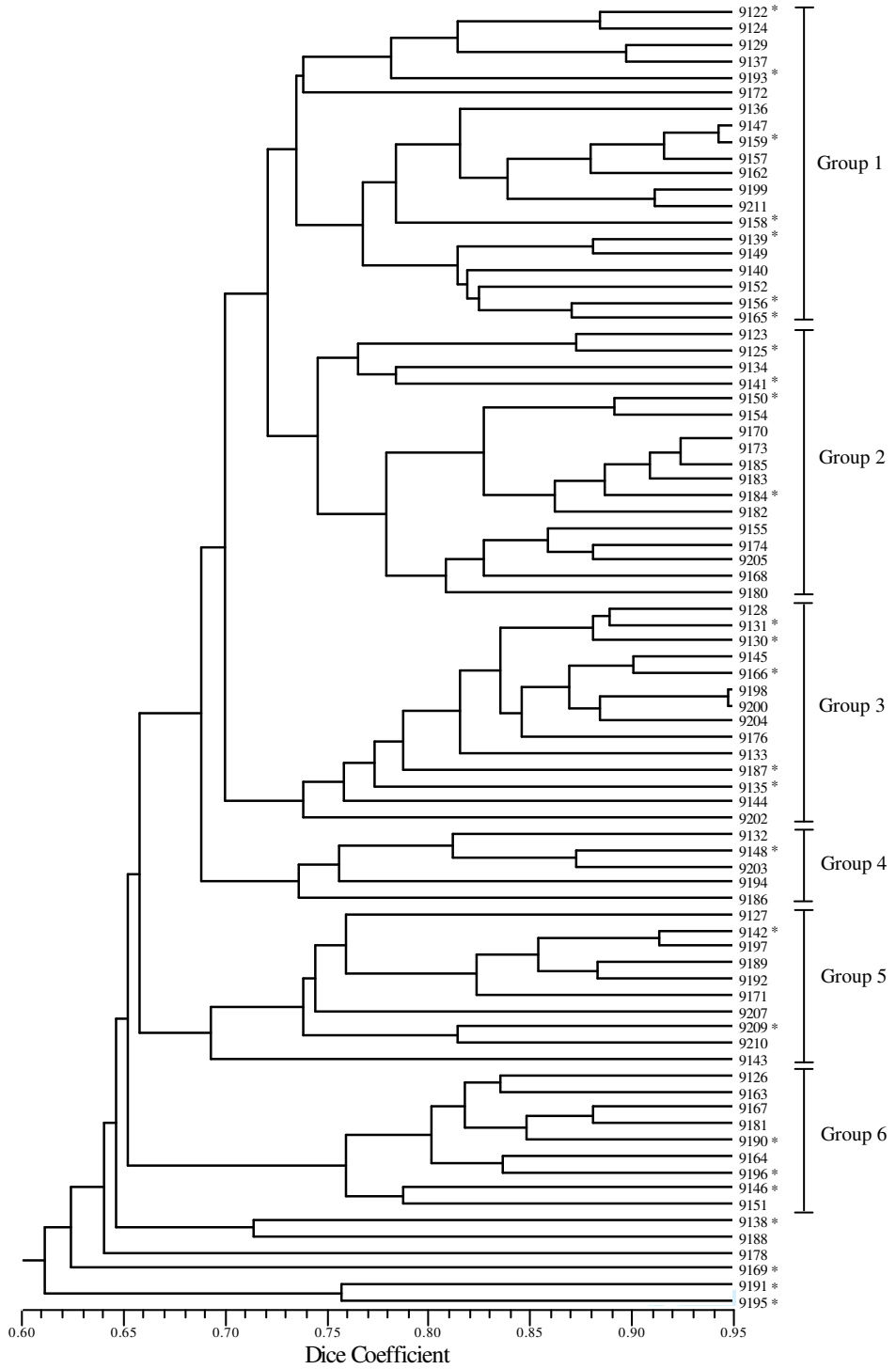

9122 *
9124
9129
9137
9193 *
9172
9136
9147
9159 *
9157
9162
9199
9211
9158 *
9139 *
9149
9140
9152
9156 *
9165 *
Group 1
9123
9125 *
9134
9141 *
9150 *
9154
9170
9173
9185
9183
9184 *
9182
9155
9174
9205
9168
9180
Group 2
9128
9131 *
9130 *
9145
9166 *
9198
9200
9204
9176
9133
9187 *
9135 *
9144
9202
Group 3
9132
9148 *
9203
9194
9186
Group 4
9127
9142 *
9197
9189
9192
9171
9207
9209 *
9210
9143
Group 5
9126
9163
9167
9181
9190 *
9164
9196 *
9146 *
9151
Group 6
9138 *
9188
9178
9169 *
9191 *
9195 *
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95
Dice Coefficient

FIGURE LEGEND

Figure 1 - Phylogenetic tree generated by UPGMA method (Saitou et al., 1987) on translation elongation factor 1-α partial sequences of 24 strains of *Fusarium poae* of this study and 4 sequences identified by Knutsen et al. (2004) as *F. poae*, *F. langsethiae*, *F. sporotrichioides* and *F. kyushuense* (represented by VI01250, VI01268, VI01290 and VI01325 strains, respectively). Branch lengths are in the same units of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances are in the units of the number of base substitutions per site. Numbers at the major branches indicate the percentage occurrence of the cluster to the right of the branch in 500 bootstrapped data sets.

Figure 2 - Dendrogram obtained by AFLP analyses with two primer combinations on a *F. poae* population, by using UPGMA clustering method and Dice similarity coefficient. Strains with asterisk were analyzed by sequencing of translation elongation factor partial gene.