

Reactions of Local Maize Cultivars to *Fusarium verticillioides* Based on Disease Severity and Production of Pectolytic Enzymes and Zearalenone Toxin*

Orhan BÜYÜK** Nuray ÖZER***

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** Plant Protection Central Research Institute, Food, Agricultural and Livestock Ministry, Ankara 06172 Turkey

*** Department of Plant Protection, Faculty of Agriculture, Namık Kemal University, Tekirdağ 59030, Turkey

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ABSTRACT

Eleven local maize cultivars from the West Black Sea Region of Turkey were analyzed for reactions to *Fusarium verticillioides*, the causal agent of ear rot in maize. Tests were based on disease severity, associated quantitative and qualitative polygalacturonase (PG) and pectate lyase (PL) activities, and zearalenone (ZEA) concentration after kernel inoculation by pathogen. The kernels of same cultivars were also tested for the presence of ZEA before inoculation. The pathogen caused low disease severity and exhibited low PG and no PL activity in the cultivars Bartın22 and Düzce50. ZEA concentration in all cultivars except two of them were below the recommended limits before inoculation of the pathogen and increased at very low rates in the cultivars Bartın22, Düzce50 and Düzce97 after inoculation. PG and PL activities were positively correlated with disease development. This study suggests the importance of pectolytic enzyme activity produced by *F. verticillioides* in maize kernels at the early phases of pathogenesis and it can be possible use in monitoring resistance.

Keywords: *Fusarium verticillioides*, cultivar reactions, pectate lyase, polygalacturonase, zearalenone toxin

INTRODUCTION

Maize (*Zea mays L.*) is one of the most important agricultural crop in hot and temperate regions around the world. Turkey is the world's 21st producer with an average production of about 4.5 million tons in the last year (FAOSTAT 2010). The crop is currently the third cereal being cultivated after wheat and barley in the country. However ear rot caused by *Fusarium verticillioides* (Sacc.) Nirenberg (FV) (Synonym *F. moniliforme* Sheldon), (Teleomorph, *Gibberella moniliformis*) is a limiting factor in maize production. Typical symptoms of the disease are individual or groups of infected kernels scattered randomly on the entire ear. Whitish-pink fungal growth can be seen on infected kernels and/or silks. The pathogen is also known with a “starburst symptom” of white or pink streaks radiating from silk insertion of the kernel or from the base (Payne 1999). Additionally it is able to grow in kernels without causing visible symptoms, as a seedborne endophyte (Munkvold et al. 1997)

Pectolytic enzymes produced by this pathogen could play a role in tissue colonization. Endopolygalacturonase of FV is expressed during maize seedling infection and may be necessary for fungal penetration even in monocotyledons (Daroda et al. 2001). Inoculation of the roots of tomato and cauliflower with FV isolated from mangrove plants showed that the pathogen produced PG and PL in hypocotyls and roots of tomato and cauliflower (Niture and Pant 2007; Niture et al. 2008). PG and PL isoforms of the pathogen were generally detected under *in vitro* conditions (Rao et al. 1996; Posada et al. 2001; Niture et al. 2001; Niture and Pant 2004). A few studies reported that the pathogen produced PG isoforms during infection of tomato (Niture and Pant 2004) and maize seedling (Daroda et al. 2001); but the role of PGs and PLs in determining pathogenesis in maize kernels remains unclear.

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FV is known as a producer of fumonisins (FUM) and studies have focused on this toxin in the pathogen (Presello et al. 2007; Presello et al. 2008; Blandino et al. 2009; Löffler et al. 2010a; Miedaner et al. 2010; Mukanga et al. 2010). However, deoxynivalenol (DON), nivenol (NIV) and zearalenone (ZEA) were also detected in the ears and kernels of maize from which FV was commonly isolated (Cvetnić et al. 2005; Adejumo et al. 2007a; Adejumo et al. 2007b). Previously Büyük (Büyük O., 2009, unpublished data) analyzed the presence of *Fusarium* spp and their mycotoxins in the kernels of local cultivars from growers' fields in the West Black Sea Region of Turkey. He determined that FV was the most commonly isolated fungus (71%). Samples were analyzed for the mycotoxins FUMB1, DON and ZEA using the technique of LC/MS/MS. Thirty-eight per cent of these samples exceeded the recommended limit for FUM and sixty-one for ZEA. DON was not detected in the samples. It is well known that ZEA is dangerous for animals and humans. It causes reproductive disorders of farm animals and may cause premature thelarche in humans (Pitt 2000; Zöllner et al. 2002). To minimize the risk of human exposure to this mycotoxin, the European Union and Turkey released limits for ZEA (300 ppb) in unprocessed maize for use indirectly as human food in 2007 (EU Commission 2007). Results from the Büyük study showed that the kernels of 11 local cultivars did not contain any *Fusarium* species, thus providing a possibility for their natural resistance to FV.

The mechanism of maize resistance to FV is complex (Presello et al. 2004). Evaluations associated with kernel resistance to this pathogen are based on pericarp thickness and wax content (Hoenisch and Davis 1994; Sampietro et al. 2009), presence of dominant resistance genes, defence related genes and enzymes (Clement et al. 2004; Presello et al. 2004; Lanubile et al. 2010; Lanubile et al. 2012), disease severity level and FUM concentration (Presello et al. 2007; Presello et al. 2008; Schjøth et al. 2008; Löffler et al. 2010 a; Löffler et al. 2011).

Production of pectolytic enzymes and toxins by the pathogen during the early stages of kernel germination may represent a reliable indicator of resistance. This study was conducted to assess the resistance of local cultivars, previously found to be free of any *Fusarium* species (Büyük O., 2009, unpublished data), to infection by FV based on production of pectolytic enzymes and ZEA during kernel germination after artificial inoculation of the pathogen.

MATERIALS AND METHODS

Plant Material

Eleven local maize cultivars (Bartın22, Bartın43, Bartın47, Bolu74, Bolu84, Düzce50, Düzce72, Düzce97, Zonguldak3, Zonguldak8 and Zonguldak14) collected from the West Black Sea Region of Turkey were assayed. Ears of each cultivar were dried at room temperature for one week and shelled. These cultivars were previously characterized for the absence of any *Fusarium* spp. placing the kernels of each cultivar on different agar media (Potato Dextrose Agar, Synthetic Nutrient Agar, Pepton PCNB Agar, Water Agar) and sterile filter paper (Blotter method) moistened with sterile distilled water in 9 cm petri dishes. Kernel samples were stored at 4°C until used in assays.

Culture of the fungus

Isolate FV61* was obtained from naturally infected maize growing in the West Black Sea Region of Turkey and used to produce the inoculum. This isolate was selected for its high level of aggressiveness in preliminary tests (data not shown) and it was grown in Potato Dextrose Agar (PDA, Oxoid; Unipath Ltd., Basingtone, UK).

For enzyme preparation, the isolate was surface cultured in Czapek's liquid medium (pH 5.0) containing NaNO₃ (2 g/l), KH₂PO₄ (1 g/l), MgSO₄.7H₂O (0.5 g/l), KCl (0.5 g/l), FeSO₄.7H₂O (0.01g/l), ZnSO₄.7H₂O (0.01g/l) and 10 g/l citrus pectin as the sole carbon source. The inoculum was one agar disc (6 mm diameter) cut from the edge of a 7-day-old-culture on PDA. Cultures were grown for 7 days at 25°C in 250-ml Erlenmeyer flasks containing 50 ml of medium in an incubator (Binder KB240; GmbH, Tuttingen, Germany).

* This isolate was identified by Prof. Dr. B. Tunalı (Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayıs University)

Kernel inoculation

Kernels from each cultivar were surface sterilized by immersing them in a 1% solution of sodium hypochlorite for 5 min, rinsing in sterile distilled water and air drying on sterile filter paper. Five kernels were placed in a petri dish containing PDA. A total of forty petri dishes were prepared from each cultivar. Agar discs (1 cm) from the edges of 7-day-old cultures on PDA were placed on each kernel (Sneh and Ichielevich-Auster 1998). The same number of kernels without inoculation was prepared as control for each cultivar. All petri dishes were incubated in the dark at 25°C for 7 days.

Disease assessment

Germinating kernels were rated according to the following 0 to 3 scale: 0, no damage; 1, undeveloped root and infected root-tips; 3, no germination, kernels completely colonized. The percentage of disease severity (DS) was calculated from the following equation (Unterstenhöfer 1963).

$$DS = \frac{\sum \text{ratings of each germinating kernel}}{\text{Total number of germinating kernels rated X the high score}} \times 100$$

Enzyme extraction from fungus culture and inoculated maize kernels

After 7 d of growth on Czapek liquid medium, FV isolate mycelial mats from three flasks were gently removed. The culture filtrates were centrifuged at 15.000xg/rpm for 15 min at 4°C. Supernatants were dialysed against several changes of distilled water at 4°C. Enzyme preparations from kernels were obtained by grinding infected tissues in an ice-cooled mortar in 0.05 M Tris-HCL buffer pH 7.8 (1 g tissue/ml buffer) containing 0.1 M KCl, 0.5% (mass/v) cysteine, and 1% (mass/v) insoluble polyvinylpyrrolidone (Sigma Chemical Co., St. Louis, MO, USA). The mixture was then strained through three layers of cheesecloth, centrifuged at 15.000xg/rpm for 20 min at 4°C, and dialysed several times against distilled water. The same procedures were applied to control kernels.

Pectolytic enzyme assays

PG activity was determined as the increase of reducing end-groups over time according to Nelson's method (Nelson 1944), slightly modified, using D-galacturonic acid (Sigma Chemical Co.) as a standard. Activity was expressed as reducing units (RU). One RU was defined as the amount of enzyme producing 1 µmol/min of reducing groups from 0.25% (mass/v) polygalacturonic acid (Sigma Chemical Co.) in sodium acetate buffer (0.1 M, pH 5.0) at 35°C. PL activity was assayed spectrophotometrically by measuring the increase in absorbance at 235 nm. An increase in absorbance of 1.73 indicated the formation of 1 µmol of unsaturated uronide (Zucker and Hankin 1970). One unit of enzyme activity catalyzed the formation of 1 µmol/min of unsaturated uronide from 0.25% (mass/v) polygalacturonic acid in Tris-HCl buffer (0.1 M, pH 8) at 35°C. The experiments were conducted twice.

Isoenzyme identification

Isoenzyme separation by isoelectric focusing (IEF) was realized horizontally with a Mini IEF cell apparatus (Bio Rad, Milano, Italy) using 0.4 mm thick polyacrylamide gels containing 5% (v/v) ampholyte (Sigma Chemical Co.) and covering a pH range of 3.5-10.0. The gels were run at 200 V, 450 V, 600 V and 950 V for 15, 30, 20 and 25 minutes, respectively. After IEF, the gels were overlaid with ultrathin (0.4 mm) agarose gels for PL and PG isoenzyme detection, prepared as described by Ried and Collmer (1985). For PL isoenzyme detection, a 1% (mass/v) agarose (Sigma Chemical Co.) gel contained 0.1% (mass/v) polygalacturonic acid buffered at pH 8.0 with 50 mM Tris-HCl; for PG isoenzyme detection, a 1% (mass/v) agarose gel contained 0.1% (mass/v) polygalacturonic acid in 50 mM sodium acetate buffer, pH 5.0. The runs were conducted twice.

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IEF polyacrylamide gels overlaid with ultrathin agarose gels were incubated at 100% relative humidity for 120 min at 35°C. Activity bands were visualized by staining the agarose overlay for 30 min in 0.05% (mass/v) ruthenium red (Sigma Chemical Co.), followed by rinsing in distilled water. PL and PG isoenzymes appeared as white bands. The isoelectric point (pI) values of pectolytic isoenzymes were estimated from a regression equation of standard protein vs. the distance migrated.

Zearalenone Analysis

Samples (50 g) from each local cultivar were ground to fine powder in a mill (Retsch ZM 200 GmbH Co. Kg., Haan, Germany) at 230V, 50/60Hz, 1100W, 12A with 20 mm mesh screen; 25 g were taken for toxin analysis. The toxin was extracted in 100 ml methanol:water (80:20 v/v) containing 4 g sodium chloride using a reciprocating shaker (Johann Otto, GmbH, Germany) at 230V, 50/60 Hz, 0,16A for 1 hour. The mixture was separated through Whatman filter no.4 paper.

ZEA toxin standard was from Sigma-Aldrich (Germany). The analysis of ZEA was carried out using HPLC after sample clean up by immunoaffinity column following the method described by Visconti and Girolamo (2005). The filtrate (10 ml) was mixed with 40 ml distilled water and filtered through glass microfiber. The mixture (20 ml) was rapidly passed through the immunoaffinity column at a flow rate of one or two drops per sec. The column was washed with 20 ml of distilled water (two drops per sec) and dried by flushing air through the column. The Zearalenone was eluted by passing 1.5 ml HPLC grade acetonitrile through the column. The eluate was mixed with deionized water (1.5 ml) and passed through a filter (0.2 µm), then transferred to an autosampler vial. HPLC equipment of the Agilent 1100 (Agilent, USA) series was used. The stationary phase was ZORBAX EclipseXDB, C18 column (4.6X150 mmX5 µm, Agilent) and the eluent was acetonitrile:water (1:1, v/v); the flow rate was adjusted to 1.0 ml/min. For detection a Fluorescence Detector was used with wavelengths set at λ_{ex} 232 nm and λ_{em} 440 nm. Detection limits of the method ranged between 50 and 800 ppb. The linearity (R) of the standard curve was 0.99927. The recovery rates ranged from 83% to 104 %. The limit of quantification (LOQ) for the toxin was 300 ppb on maize.

Data analysis

Quantitative data on PG, PL, and DS (%) were statistically evaluated by analysis of variance (one-way ANOVA). Statistical significance of mean differences was estimated according to the Duncan multiple range test (P=0.05). The increase of ZEA after inoculation was calculated as increase % [= (ZEA concentration of kernel after inoculation (Z1) – that of kernel before inoculation)/Z1 X 100]. The Pearson coefficient of simple correlation (r) was calculated between DS and PG, PL, and ZEA production for various infected cultivars. Data statistics were performed using SPSS 15.0 for Windows (Statistical Package for Social Sciences, Inc., 2001, Chicago).

RESULTS

Disease severity of the cultivars

Kernels of eleven local maize cultivars inoculated with FV isolate were monitored 7 days after inoculation along with uninoculated kernels for the development of visible symptoms (Figure 1). Disease severity differed significantly among the cultivars. The cultivars Bartın22 and Düzce50 showed very low disease severity. Maximum disease (91.5%) was observed in the kernels of cv. Zonguldak14 followed by Bartın47, Bolu84 and Bartın43, and they were statistically different from other cultivars.

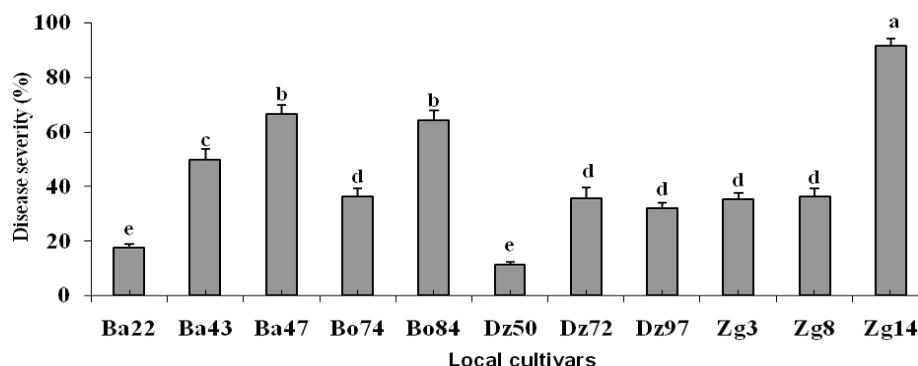


Figure 1. Disease severity (\pm SE) caused by *Fusarium verticillioides* on the kernels of different cultivars. Ba: Bartın, Bo: Bolu, Dz: Düzce, Zg: Zonguldak. Bars topped by the same letters do not differ significantly, according to the Duncan Multiple Range Test ($p < 0.05$).

Pectolytic enzymes of FV in liquid culture and during kernel colonization of different cultivars

During the 7-day growth period in liquid culture, FV61 produced a higher amount of PG and PL enzyme activities than infected kernels (Table 1). When FV61 was able to colonize maize kernels of different cultivars, production of two pectolytic enzymes (PG and PL) were found depending on the cultivar. The pathogen produced PG in all cultivars whereas PL was found in only three of them. Significant differences in PG and PL production were found among the cultivars tested, being significantly low RU and absent in cultivars Düzce50, Bartın22 and Bolu74, respectively; medium RU and absent in Bartın 43, Düzce97 and Zonguldak8, respectively. However, PG production by pathogen was significantly high in Bolu84, Zonguldak3 and Zonguldak14, compared with other cultivars.

Table 1. Polygalacturonase (PG) and pectate lyase (PL) activity from liquid culture and from kernels of local maize cultivars inoculated with *Fusarium verticillioides*

Local Cultivars	PG (RU)	PL (U)
Bartın22	0.46 \pm 0.03 ef	0.00 \pm 0 c
Bartın43	0.63 \pm 0.06 de	0.00 \pm 0 c
Bartın47	0.83 \pm 0.06 bc	0.00 \pm 0 c
Bolu74	0.47 \pm 0.01 e	0.00 \pm 0 c
Bolu84	1.37 \pm 0.08 a	0.13 \pm 0.003 a
Düzce50	0.42 \pm 0.02 f	0.00 \pm 0 c
Düzce72	0.73 \pm 0.03 cd	0.10 \pm 0.006 b
Düzce97	0.60 \pm 0.06 de	0.00 \pm 0 c
Zonguldak3	0.94 \pm 0.06 b	0.00 \pm 0 c
Zonguldak8	0.58 \pm 0.05 def	0.00 \pm 0 c
Zonguldak14	0.95 \pm 0.08 b	0.12 \pm 0.003 a
Liquid culture	2.30 \pm 0.05	0.35 \pm 0.01

PG activity is expressed as reducing units (RU), PL activity as units (U). Means (\pm SE) in each column followed by the same letters do not differ significantly, according to the Duncan Multiple Range Test ($p < 0.05$).

Enzyme extracts of the isolate from culture filtrates and infected kernel tissues were separated by IEF on thin layer polyacrylamide gels and evaluated for their PG and PL isoenzyme patterns. Three PG isoenzyme forms, PG1 (pI 4.7), PG4 (pI 6.6), PG5 (pI 7.2) were observed from liquid culture (Figure 2). No PG bands were resolved from extracts of cultivars Bartın22, Bartın43, Düzce50, Düzce72, Zonguldak97 and Zonguldak8 inoculated with the pathogen. Cultivars Bartın47, Zonguldak3 and Zonguldak14 were characterized by the presence of PG4; Bartın47 by one acidic and two basic extra-bands at pI 5.5 (PG2-very faint), pI 8.1 (PG6-faint), and pI 8.5 (PG7); Bolu84 by one acidic extra-band at pI 5.9 (PG3-faint), and PG6 and PG7; and Bolu74 by PG3.

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One acidic and five alkaline PL isoenzyme patterns, PL1 (pI 6.6), PL2 (pI 7.2), PL3 (pI 7.4), PL4 (pI 8.1), PL5 (pI 8.5) and PL7 (pI 9.3) were detected from the culture filtrate of the pathogen (Fig. 3). PL3 and PL4 were observed from the cultivars Bolu84 and Zonguldak14, respectively. Extra bands PL6 (faint-pI 8.7) and PL7 (very faint) were expressed in the cultivars Bolu84, Düzce72 and Zonguldak14. The pathogen did not exhibit any PL isoenzyme pattern in kernels of cultivars Bartın22, Bartın43, Bartın47, Bolu74, Düzce50, Düzce97, Zonguldak3 and Zonguldak8.

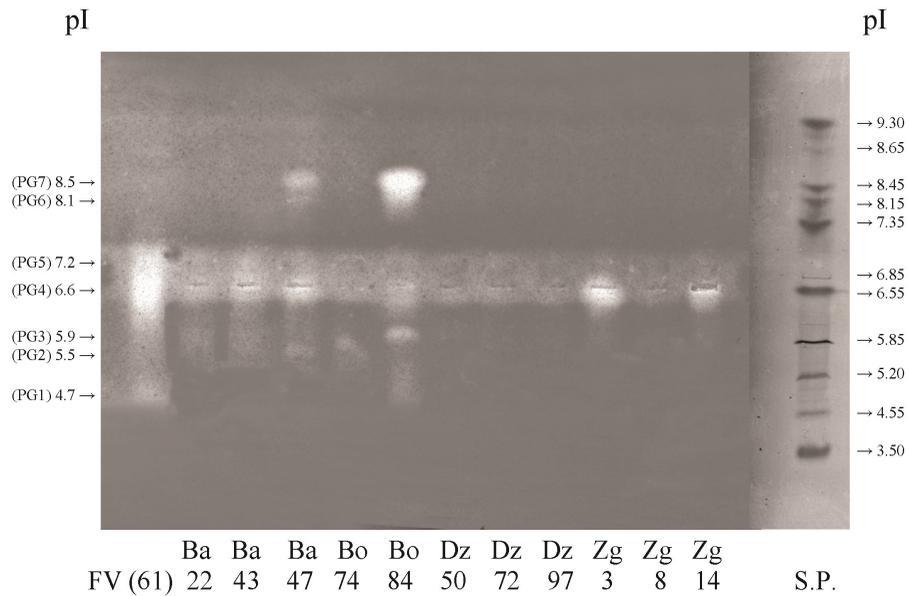


Figure 2. Polygalacturonase isoenzyme patterns (white bands) from liquid culture and from kernels of different local maize cultivars inoculated with *Fusarium verticillioides*. Ba: Bartın, Bo: Bolu, Dz: Düzce, Zg: Zonguldak. Estimated pIs are indicated on the left, pIs of standard proteins (S.P) on the right.

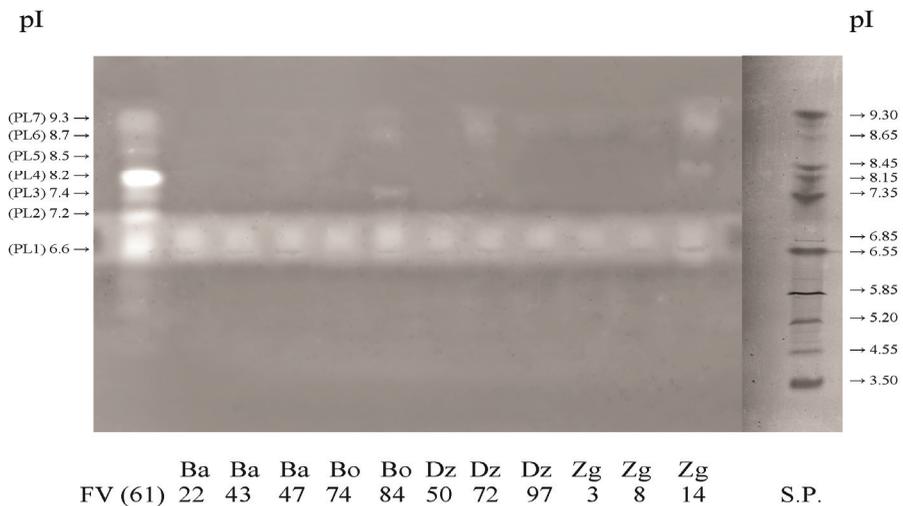


Figure 3. Pectate lyase isoenzyme patterns (white bands) from liquid culture and from kernels of different local maize cultivars inoculated with *Fusarium verticillioides*. Ba: Bartın, Bo: Bolu, Dz: Düzce, Zg: Zonguldak. Estimated pIs are indicated on the left, pIs of standard proteins (S.P) on the right.

ZEA concentration

Kernels of the local cultivars, which did not contain any *Fusarium* spp., were analyzed for ZEA before and after inoculation of the pathogen (Table 2). Among the cultivars, Bolu84 and Zonguldak3 had ZEA concentrations higher than EU and Turkey limits before and after pathogen inoculation, although the increase in concentration was low in cultivar Bolu84 after inoculation. ZEA concentrations in other cultivars did not exceed the maximum limits in either treatment. The lowest increase was detected in the local cultivar Düzce97, followed by Düzce72, Düzce50 and Bartın22.

Table 2. Zearalenone (ZEA) concentration in local maize cultivars (*Fusarium* spp-free) before and after inoculation with *Fusarium verticillioides*, and increase in toxin concentration

Local Cultivar	ZEA concentration (ppb)		Increase in toxin concentration (%)
	Before inoculation	After inoculation	
Bartın22	286.40	293.10	2.28
Bartın43	76.40	108.20	23.39
Bartın47	129.10	163.70	21.13
Bolu74	124.30	136.70	9.07
Bolu84	343.40	356.40	3.64
Düzce50	106.27	107.63	1.26
Düzce72	250.70	253.00	0.90
Düzce97	276.30	278.40	0.75
Zonguldak3	307.60	376.30	18.25
Zonguldak8	272.81	286.40	4.74
Zonguldak14	220.10	236.70	7.01

Correlations between disease severity and pectolytic enzymes and ZEA production

A linear correlation existed between disease severity of the cultivars tested and PG ($r=0.68$, $p<0.05$) and PL ($r=0.61$, $p<0.05$) activities detected from diseased tissues upon inoculation with the pathogen. There was no correlation between disease severity and ZEA production.

DISCUSSION

Some ear and kernel characteristics of specific cultivars provide chemical and mechanical barriers to ear rot infection caused by FV (Hoenisch and Davis 1994; Munkvold, 2003; Sampietro et al. 2009; Lanubile et al. 2010; Lanubile et al. 2012). Maize genotypes or cultivars from different countries are currently characterized for their resistance to infection by FV and also to FUM accumulation in kernels using silk channel inoculation of spore suspension (Presello et al. 2007; Presello et al. 2008; Miedaner et al. 2010; Löffler et al. 2010 a; Löffler et al. 2010b; Löffler et al. 2011) and side needle inoculation (Lanubile et al. 2010) under field conditions. Ears and kernels of maize infected with FV can contain other mycotoxins such as DON, NIV and ZEA (Cvetnić et al. 2005; Adejumo et al. 2007b). In one of the project (Büyük, O., 2009, unpublished data), it was determined that the rate of ZEA-contaminated kernels exceeding the recommended limit was higher than FUM-contaminated kernels. However information on the effect of ZEA on resistance to FV in maize kernels is lacking. In addition, *Fusarium* spp. perforates the seed coat and invades the endosperm producing pectolytic enzymes as well as mycotoxins (Kikot et al. 2009). No studies have addressed the role of pectolytic enzymes in resistance to the same disease. However, these enzymes are thought to play a role in the infection by FV of tomato, cauliflower and maize seedling (Daroda et al. 2001; Niture and Pant 2004; Niture and Pant 2007; Niture et al. 2008). In this study we evaluated disease severity as well as production of pectolytic enzymes and ZEA by FV to determine resistance to ear rot of maize cultivars, and we examined the relationships among them. Eleven local pathogen-free cultivars from the Black Sea Region of Turkey were used in the study.

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Virulence and production of PG and PL pectolytic enzymes by the pathogen varied greatly depending on cultivar. Our results demonstrated different degrees of susceptibility among the cultivars; “Düzce50” was the most resistant based on the disease severity, the production of PG and PL by pathogen; “Bolu84” was a susceptible cultivar in which the pathogen exhibited the highest PG and PL activity; another susceptible cultivar, “Zonguldak14”, had the highest disease severity and the pathogen produced high PG and PL enzyme activity. The high resolving capacity and sensitivity of the staining technique for pectolytic activity after IEF permitted the detection of a number of isoenzymes of PG and PL. Three PG isoenzyme forms (PG1, PG4 and PG5) were expressed in the extracts from liquid culture; among them one PG form (PG4, pI 6.6) appeared during pathogenesis in susceptible maize cultivars. This suggests that this form may be important for colonizing the pathogen into the kernel tissues. Daroda et al (2001) determined that a commercial preparation of FV produced four endo PG isoforms (38.0, 41.5, 45.0 and 48.5 kDa) *in vitro* and in infected maize seedlings. In our experiments extra PG acidic and alkaline isoforms, which were not expressed in liquid culture, were detected in the extracts from infected kernels of some cultivars. Among those, an alkaline PG isoenzyme focusing at pI 8.1 during infection of kernels of sensitive cultivars Bartın47 and Bolu84 was also previously reported in liquid culture of the pathogen and during infection of tomato tissue (Niture et al. 2001; Niture and Pant 2004).

This study showed that during growth on pectin as a sole carbon source, FV produced PL activity and six PL isoforms, although Rao et al. (1996) detected the presence of a PL isoenzyme form (pI 9.1) in the extracts from liquid culture. Low levels of PL activity and four isoenzyme forms as faint bands were identified during infection of local cultivars Bolu84, Zonguldak14 and Düzce72. Studies reporting PL isoforms produced by FV during maize kernel or seedling infection are not available in the literature.

It has been shown in various plant-fungal pathogen interactions that the ability to produce symptoms parallels the level of PG isolated from infected tissues (Baayen et al., 1997; Le Cam et al. 1997; Garcia-Maceira et al. 2001; Roncero et al. 2003). Positive correlations between disease development and PG and PL activity by the pathogen in the infected maize kernels are suggestive of a possible causal relationship. The IEF analysis did not show isoenzyme forms of PG and PL from local cultivars Bartın22, Bartın43, Düzce50, Düzce97, which also had low disease severity.

FV can produce FUM in kernels without visible disease symptoms (Munkvold et al. 1997). Our results also demonstrated the presence of ZEA in symptomless kernels of different local cultivars. Interestingly, no *Fusarium* spp. could be found in these cultivars. It is well known that sporulation and mycotoxin production in *Fusarium* are both regulated by G protein signaling pathways which commonly regulate fungal development, stress response and expression of virulence. However fungal development is also influenced by external factors such as lipids and, in particular, oxylipin signals in maize kernels which have the potential to elicit profound changes in sporulation in the fungus (Brodhagen and Keller 2006). Although amounts of oxylipin in maize kernels were not determined in the current study, the results based on presence of ZEA in *Fusarium* spp-free kernels indicates that sporulation of *Fusarium* spp, including FV, seems to be affected by oxylipin content of the kernels. This study clearly showed that laboratory inspection of maize kernels alone for the presence of FV is not a reliable predictor for ZEA.

ZEA concentrations tested in nine cultivars before and after inoculation were under the recommended maximum limits. The least increase in concentration after inoculation was recorded in cultivar Düzce97, followed by Düzce72, Düzce50 and Bartın22. These cultivars showed low disease severity, but ZEA concentration was not significantly correlated with disease severity. In previous studies, no correlation or a negative association between disease severity and FUM concentration for FV was observed (Presello et al. 2007; Presello et al. 2008; Covarelli et al. 2012). Conversely, in another study a positive correlation between disease severity and FUM concentration was recorded (Löffler et al. 2011). Therefore we hypothesize that ZEA production depends on the given *Fusarium* isolate, the substrate and the environment.

In conclusion, for this set of cultivars and experimental conditions, local cultivars Bartın22 and Düzce50 were determined as resistant to FV. Cultivar Düzce 97 showed low disease severity and had also the lowest increase in ZEA concentration after inoculation with FV. The enzyme activity tests described in this study can be used as

additional tools to evaluate resistance mechanisms to FV as well as contributing to a better understanding of the infection ability of this pathogen.

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ÖZET

HASTALIK ŞİDDETİ, PEKTOLİTİK ENZİM VE ZEARELENONE ÜRETİMİ AÇISINDAN YEREL MISIR ÇEŞİTLERİNİN *Fusarium verticillioides* 'E KARŞI REAKSİYONLARI

Türkiye'nin Batı Karadeniz Bölgesinden toplanan 11 yerel mısır çeşidinin mısırdaki koçan çürüklüğü etmeni *Fusarium verticillioides*'e karşı reaksiyonları belirlenmiştir. Testlerde, etmenin mısır tanelerine inokulasyonundan sonra oluşan hastalık şiddeti, kalitatif ve kantitatif poligalakturonaz (PG) ve pektat liyaz (PL) enzim aktiviteleri, ve zearalenone (ZEA) üretimi dikkate alınmıştır. Aynı çeşitlerin tanelerinde inokulasyondan önceki ZEA miktarı da tespit edilmiştir. Patojen Bartın22, Düzce50 çeşitlerinde düşük hastalık şiddetine neden olmuş, az miktarda PG sergilemiş, PL üretmemiştir. İki çeşit hariç, tüm çeşitlerde inokulasyondan önceki ZEA konsantrasyonu sınırların altında olmuş, inokulasyondan sonra ise Bartın22, Düzce50 ve Düzce97 çeşitlerinde çok düşük oranlarda artış göstermiştir. PG ve PL aktiviteleri ve hastalık oluşumu arasında pozitif bir ilişki olduğu belirlenmiştir. Bu çalışma sonuçlarına göre mısır tanelerinde hastalık oluşumunun başlangıcında *F. verticillioides* tarafından üretilen pektolitik enzimlerin önemli olduğu ve dayanıklılığı kontrol etmek için kullanılabilmesi ileri sürülebilir.

Anahtar kelimeler: *Fusarium verticillioides*, çeşit reaksiyonu, pektat liyaz, poligalak-turonaz, zearalenone toksini

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