INFLUENCE OF SOME NATURAL AND SYNTHETIC TOXINS CHARACTERISTIC FOR FUSARIUM FUNGUS ON IZOPEROXIDASES IN SOME WHEAT GENOTYPES

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ABSTRACT

Analyses of izoperoxidase activity in seedling stage at three wheat genotypes (Fundulea 4 –sensitive; 201 S-medium resistant and Fundulea 29 – resistant) with different degree of filed susceptibility to Fusarium fungus were performed. The seedlings were grown under laboratory conditions, in media with different DON and NIV concentrations. Other experimental variants included culture filtrates partlally purified on a Florisil column. Electrophoresis in a 7% polyacrylamide gel revealed the outlining of 10 types of enzimograms. The lowest number of izoperoxidases – 5 was recorded in Fundulea 4 seedlings, cultivated on a medium with NIV, while the maximum number of izoenzymes – 9 – resulted in Fundulea 29 and 201 S varieties. Comparing these results with those obtained under filed artificial inoculation applied directly in the ear at anthesis, a good correspondence was obtained with assessment of some yield parameters (reduction of ear weight as well as of seed weight per ear as compared to both the uninoculated check and the fungus *in toto*. Thus, the electrophoretic polymorphism of biochemical parameter investigated using standard or natural toxins, proved to be useful in evaluating genetic diversity of germplasm for resistance to *Fusarium*, in the seedling stage.

Key words: izoperoxidase, natural and synthetic toxins, yield components.

INTRODUCTION

Damage produced by Fusarium in wheat is considerable, both quantitatively and qualitatively. The fungus, which is extremely penetrating and has a high rate of multiplication in the plants, produces a large range of toxic metabolites, among which deoxinivalenol (DON), nivalenol (NIV) and zearalenon (ZEN) were more studied. The effects of host plant-parasite interaction within the Triticum – Fusarium system stirred an increasing interest due to their economic and social implications such as yield decrease and food and forage toxic contaminations (Cristiani, 1992; Hagima and Ittu, 1993; Ittu and Hagima, 1993; Ittu et al., 1995; Moraru et al., 1995). Utilization of standard mycotoxins in breeding for resistance to this pathogen has become in the last year a current procedure, which, as compared to the inoculum by *in toto* fungus, shows important advantages such as physical-chemical stability and a higher reproductibility, along with a quantitative evaluation of resistance. Recent research revealed in genotypes with higher resistance level, lower accumulation of toxic metabolites as compared to susceptible genotypes.

In parallel with the evolution and improvement of suitable analysis methods, from the simple revealing of the presence of the mycotoxins up to their characterization at the molecular level (Yli-Mattila et al., 1995; Procunier et al., 1995) a large amount of data related to the genetics of the resistance has been accumulated and aimed towards the reduction of the impact with this highly damaging pathogen.

Additionally techniques based on fragmentation, amplification and analysis of DNA structure (among which the most widely spread are: polymerase chain reaction, polymorphism of the length of restriction fragments and of DNA amplified with random or specific primers) have opened new perspectives in approaching host plant-parasite relations, with practical applications in breeding for resistance (Ittu, 1994).

In the present paper, izoperoxidases were analysed in three wheat genotypes with different degrees of field susceptibility to artificial inoculation with both standard toxins (DON and NIV) and three partially purified culture filtrates.

MATERIALS AND METHODS

Three common wheat genotypes have been analysed: Fundulea 4 - sensitive, 201 S - medium resistant and Fundulea 29 – resistant. Standard DON and NIV toxins in three concentrations, and three culture filtrates in two different dilutions were used for artificial inoculations. Seedlings were artificially inoculated under laboratory conditions.

Field inoculation was done directly in the ear, at anthesis. Additionally to standard toxins and filtrates, the variant using *in toto* fun-

gus was used (suspension of mycelium and spores on Czapek – Dox liquid medium).

Electrophoresis of extracts obtained from seedlings was performed in a vertical system, in 7% polyacrylamide gel, tris-glicocol buffer, pH 8.3 In order to point out izoenzymes we used as substrate benzidine prepared in acetic acid – sodium acetate buffer, pH 4.7. The yield components considered in field infection were the relative weight of ears and total seed weight/ear.

RESULTS AND DISCUSSIONS

Results obtained under field conditions showed that yield components were significantly influenced by all experimental variants used, as compared to *in toto* fungus (Table 1).

Table 1. The influence of mycotoxins, filtrate and culture treatment of *Fusarium* (difference against average)

Genotypes			Treatment
201 S	Fundulea 29	Fundulea 4	
a) Mean weight of ears			
0.09	0.67^{---}	0.90^{---}	DON 10^{-6}
0.64^{---}	1.08^{---}	2.02^{---}	DON 10^{-12}
0.19	0.15	0.89^{---}	NIV 10^{-6}
0.20	0.41^{--}	0.61^{---}	NIV10^{-12}
0.27^{--}	0.20	0.75^{---}	Filtrate 1
0.04	0.15	0.86^{---}	Filtrate1
0.07	0.65^{---}	0.48^{--}	Filtrate 2
0.04	0.42^{--}	0.63^{---}	Filtrate 2
0.04	0.42^{--}	0.47^{--}	Filtrate 3
0.13	0.31^{-}	0.20	Filtrate 3
1.17^{---}	0.72^{---}	1.60^{---}	*Fusarium* culture (*in toto*)
DL 5% = 0.27			
b. Weight of seeds/ear			
0.15^{-}	0.49^{---}	0.75^{---}	DON 10^{-6}
0.62^{---}	0.90^{---}	1.91^{---}	DON 10^{-12}
0.10	0.09	0.80^{---}	NIV 10^{-6}
0.15^{-}	0.32^{--}	0.68^{---}	NIV10^{-12}
0.56^{---}	0.15^{-}	0.76^{---}	Filtrate 1
0.08	0.10	0.82^{---}	Filtrate 1
0.08	0.48^{---}	0.86^{---}	Filtrate 2
0.31^{---}	0.31^{---}	0.69^{---}	Filtrate 2
0.14	0.31^{---}	0.48^{---}	Filtrate 3
0.20--	0.14	0.33^{---}	Filtrate 3
1.33^{---}	0.82^{---}	1.54^{---}	*Fusarium* culture (*in toto*)
DL 5% = 0.15			

In the sensitive Fundulea 4 genotype all the experimental variants produced a significant reduction of the parameters analysed by values ranging between 0.20-2.02 (relative ear weight) and between 0.33-1.91 (total seed weight/ear). The least affected by the treatmant was 201 S genotype, known to have a higher field resistance level. However, DON 10^{-12} experimental variant nevertheless determined a significant reduction of parameters considered but with lower values than those registered in the other genotypes, respectively 0.64 (relative ear weight) and 0.62 (total seed weight/ear) as compared to 1.08 in Fundulea 29 and 2.02 in Fundulea 4 for relative ear weight and 0.90 in Fundulea 29 and 1.91 in Fundulea 4 for total seed weight/ear.

These results were in general in agreement with he results obtained by inoculation with *in toto* fungus. The least affected genotype was Fundulea 29.

Peroxidase of the analysed material showed a significant variability expressed in the outlining of 10 types of enzymograms (Figure 1). The minimum number of izoperoxidases revealed was five, the maximum number-nine.

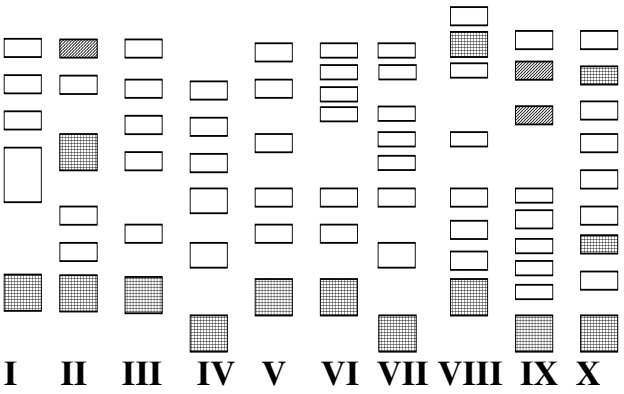

Figure 1. Enzymogram types of peroxidase from seedlings grown on Fusarium toxins media

The results pointed out the specificity of both genotype and mycotoxin treatments, respectively. Table 2 presents the relationship between mycotoxins in different concentrations and the spectra of izoperoxidases revealed. Analysing isoenzymatic spectra of the three wheat varieties it was observed that in Fundulea 4 (sensitive) variety the most reduced number of isoperoxidases was revealed in seedlings treated with the three NIV concentrations. The same treatment applied to 201

S (medium resistant) variety permitted the pointing out of larger number of izoenzymes. As peroxidase implication in increasing resistance to pathogens is already mentioned, the hypothesis may be launched that this multiplicity expresses one aspect of the defence mechanism. As a matter of fact the phytotoxic effect of mycotoxins produced by *Fusarium* in wheat, expressed through the modification of some vital physiological processes, among which protein synthesis, enzymatic activity and other ones, was described earlier (Hagima and Ittu, 1993; Ittu and Hagima, 1993). The same synthetic NIV toxin, determined also in Fundulea 29 variety the establishment of same types of enzymograms (type IX). This observation permits the assumption that toxin nature and not its concentration is more implicated in the *de novo* biosynthesis of some izoenzymes.

Table 2. The relationships between *Fusarium* mycotoxins, wheat genotypes and izoperoxidases

Genotype	Experimental variant	Enzymogram types									
		I	II	III	IV	V	VI	VII	VIII	IX	X
FUNDULEA 4 (sensible)	DON - 10^{-6}				+						
	DON - 10^{-8}			+				+			
	DON - 10^{-12}			+							
	NIV- 10^{-6}	+									
	NIV - 10^{-8}	+									
	NIV - 10^{-12}	+									
	Filtrate 1								+		
	Filtrate 1								+		
	Filtrate 2									+	
	Filtrate 2									+	
	Filtrate 3										+
	Filtrate 3					+					
201 S (medium resistant)	DON - 10^{-6}		+								
	DON - 10^{-8}		+								
	DON - 10^{-12}										+
	NIV- 10^{-6}									+	
	NIV - 10^{-8}									+	
	NIV - 10^{-12}									+	
	Filtrate 1						+				
	Filtrate 1						+				
	Filtrate 2					+					
	Filtrate 2					+					
	Filtrate 3								+		
	Filtrate 3								+		
FUNDULEA 29 (resistant)	DON - 10^{-6}									+	
	DON - 10^{-8}								+		
	DON - 10^{-12}				+						
	NIV- 10^{-6}									+	
	NIV - 10^{-8}									+	
	NIV – 10^{-12}				+						
	Filtrate 1							+			
	Filtrate 1							+			
	Filtrate 2								+		
	Filtrate 2								+		
	Filtrate 3								+		
	Filtrate 3								+		

Among the two toxins tested, a stronger influence on izoenzymatic polymorphism (translated into a large number of molecular forms) was exerted by NIV toxin in case of 201 S and Fundulea 29 varieties.

Concerning the influence of culture filtrates in all varieties analysed an emphasised variability was recorded. In table 2 is shown

the influence of filtrates on the spectra of izoperoxidases which was found depending on both the genotype and filtrate.

As regards the level of resistance, respectively of susceptibility, it was ponted out that in Fundulea 29 genotype (more resistant towards synthetic toxins and filtrates 2 and 3) the polymorphism revealed was higher significant.

Comparing both Fundulea 4 (sensitive) and 201 S (medium resistant) it is evident that NIV toxin (determined in the three concentrations) reveals the presence of 5 izoperoxidases (type I of enzymogram) for the sensitive genotype and 9 izoperoxidases (type IX of enzymogram) in the second one. This difference suggests a possible correlation with the level of resistance, in which a certain role could be played by the number of izoenzymes of peroxidase. In fact is already known that in an organism, increased multiplicity of izoenzymes improves metabolic flexibility, which is expressed by a better resistance.

CONCLUSIONS

Natural *Fusarium* toxins utilised, under two forms: culture filtrates or standard solutions ones, differently influenced the polymorphism of wheat genotypes izoperoxidases, in accordance with field reaction. The sensitive Fundulea 4 variety revealed in NIV treatment the smallest number of izoperoxidases while the highest number was evinced in 201 S and Fundulea 29 varieties (medium resistant and resistant).

Results of the analysis at the izoenzymatic level generally agree with the ones obtained by conventional assessment of the resistance components: relative weight of ears and total seed weight per ear, following field inoculation, within the limits of the differences already observed between infection in seedling adult stages.

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	NIV - 10^{-8}	+									
	NIV - 10^{-12}	+									
	Filtrate 1								+		
	Filtrate 1								+		
	Filtrate 2									+	
	Filtrate 2									+	
	Filtrate 3										+
	Filtrate 3					+					
201 S (resistant)	DON - 10^{-6}		+								
	DON - 10^{-8}		+								
	DON - 10^{-12}										+
	NIV- 10^{-6}									+	
	NIV - 10^{-8}									+	
	NIV - 10^{-12}									+	
	Filtrate 1						+				
	Filtrate 1						+				
	Filtrate 2					+					
	Filtrate 2					+					
	Filtrate 3								+		
	Filtrate 3								+		
FUNDULEA 29 (resistant)	DON - 10^{-6}									+	
	DON - 10^{-8}								+		
	DON - 10^{-12}				+						
	NIV- 10^{-6}									+	
	NIV - 10^{-8}									+	
	NIV – 10^{-12}				+						
	Filtrate 1							+			
	Filtrate 1							+			
	Filtrate 2								+		
	Filtrate 2								+		
	Filtrate 3								+		
	Filtrate 3								+		

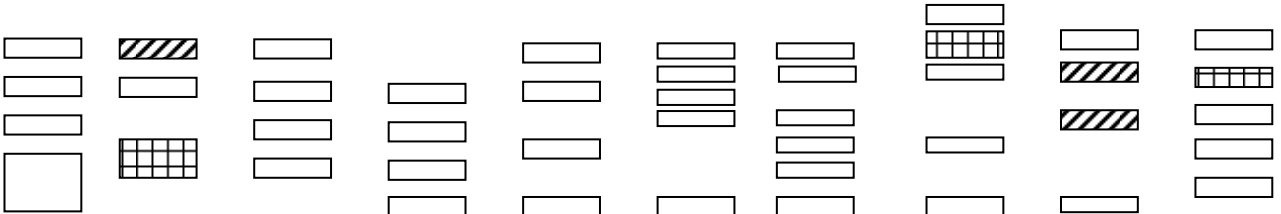

Fig.1. Enzymogram types of perixidase from seedlings grown on Fusarium toxins media