

**CRA-MAC****Unità di Ricerca per la Maiscoltura****Via Stezzano 24, 24126 Bergamo (Italy)****Screening of Italian maize inbred lines for nutritional quality, resistance to *Fusarium verticillioides* and differential gene expression in resistant and susceptible genotypes \***

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The introduction of hybrids into cultivation brought up a significant increase in maize (*Zea mays* L.) grain yield and a better resistance to pathogens. On the other hand, the possibility to help maintaining a good health through a convenient diet have recently focused the interest in food plants on the nutritional quality issue. The worldwide maize (*Zea mays* L.) germplasm has been shown to possess a large genetic variability for the main components of the grain. In particular, in Italy the availability of a large number of populations and ecotypes represents an interesting starting point for the identification of genotypes with good nutritional value and safety characteristics (Berardo et al., J. Agric. Food Chem. 57: 2378-2384, 2009). Mycotoxin contamination in maize grain is a global threat to both safety of human food and animal feed (Balconi et al., World Mycotoxin Journal 3: 239-250, 2010; Berardo et al., Food Additives and Contaminants: Part B., 4: 116–124 2011). With the aim to find new sources of genetic variability to improve the nutritional quality of maize hybrids and their resistance to pathogens, a set of 35 traditional Italian inbred lines and six public inbred lines (**TABLE 1**) was evaluated in 2009 and 2010 in Bergamo (Northern Italy). A preliminary survey was realized to test their response to the fungal pathogen *Fusarium verticillioides*, the causal agent of ear rot in most maize-growing areas of Southern Europe. This test implied: i) artificial inoculation of the ears in field trials at 15 DAP (Days After Pollination) through the KIA (Kernel Inoculation Assay) with a spore suspension obtained from a mix of two toxigenic *F. verticillioides* strains isolated in Northern Italy; ii) visual rating of the infected ears; iii) evaluation of the internal infection of the kernels; iv) quantification (ELISA) of the mycotoxins (fumonisins) present in the grain. Controls were not inoculated or inoculated with sterile H<sub>2</sub>O ears. Most of the inbred lines (around 60%) both in 2009 and in 2010 showed, after artificial inoculation, a low susceptibility to *F. verticillioides*, as deduced by visual rating evaluation. Both during 2009 and 2010, artificial inoculation induced an evident increase in fumonisin content. Large variability was observed between the genotypes. During both years, around 15% of the tested inbred lines showed fumonisin content  $\geq 10^5$   $\mu\text{g}/\text{kg}$  after artificial inoculation; the other genotypes were almost equally distributed in two groups depending on fumonisin content, respectively:  $\leq 10^4$   $\mu\text{g}/\text{kg}$  and  $10^4 \geq 10^5$   $\mu\text{g}/\text{kg}$ .

The inbred lines were also characterized by NIRS in terms of grain chemical composition (protein, lipid and starch content). Crude protein and lipid contents, expressed on a dry matter basis, were determined during both years on ears harvested after different treatments. Inoculation with *F. verticillioides* in 2009 appeared to slightly reduce the nutritional compounds as compared to the control inoculations, whereas in 2010 the lowest content of protein and lipid was found in the not inoculated ears.

In order to address the study of differential gene expression in resistant and susceptible genotypes, two of the inbred lines under study were selected: i) Lo 186, exhibiting abundant mycelium growth and high level of fumonisins; ii) Lo 435 with a far more resistant phenotype and low fumonisin content. Material was collected at two time points after inoculation; total RNA was then isolated from each of the collected samples in order to prepare hybridization

probes, which were subsequently used to hybridize an Affymetrix maize array. Gene expression data analyses were conducted in R language using the Limma package from Bioconductor, comparing expression profiles of sterile H<sub>2</sub>O-inoculated (control) and *F. verticillioides*-inoculated samples. Upon comparison, around 500 genes (P-value  $\leq$  0.05) identified in Lo186 kernels after fungal inoculation were differentially expressed. The classification in GO (Gene Ontology) functional categories, showed their relative involvement mainly in i) the response to biotic stimuli (for example mechanisms related to response to fungus); ii) anatomical development processes.

In summary, results from the research indicated that i) most of the inbred lines both in 2009 and in 2010 showed a low-medium susceptibility to *F. verticillioides*, as deduced by visual rating evaluation; ii) during both years, around 15% of the tested inbred lines showed a fumonisin content  $\geq 10^5$   $\mu\text{g}/\text{kg}$  after artificial inoculation; the other genotypes could be almost equally partitioned into two groups depending on fumonisin content; iii) plant defence against *F. verticillioides* infection involved the expression change of several genes; the knowledge of their association with main functional pathways might disclose important information regarding those molecular processes active during fungal infection.

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**TABLE 1-Maize genotypes under study**

Italian inbred lines	Source	Italian inbred lines	Source
Lo 002	Nostrano dell'Isola	Lo 491	Nostrano dell'Isola Finardi
Lo 003	Nostrano dell'Isola	Lo 514	Dente di cavallo
Lo 005	Nostrano dell'Isola	Lo 520	ICAR 54
Lo 017	Nostrano dell'Isola	Lo 589	Nostrano dell'Isola
Lo 018	Nostrano dell'Isola	Lo 241	Lo3 x Lo38
Lo 020	Nostrano dell'Isola	Lo 295	70 x 110
Lo 021	Nostrano dell'Isola	Lo 352	Lo32xLo18
Lo 033	Isola basso	Lo 446	Lo80 x Lo71
Lo 043	Scagliolo	Lo 452	Lo5 <sup>2</sup> x Lo19
Lo 051	Bianco Oderzo	Lo 457	Lo43 x Lo58
Lo 058cmsC	Marano	Lo 1264	P3394 (Cecilia)
Lo 067	Scagliolino G.V	Lo 577	N.I. maranizzato
Lo 093	Scagliolino G.V. precoce	Lo 578	N.I. maranizzato
Lo 186	Marano x Isola basso		
Lo 249	Scagliolo Marne		<b>Public inbred lines</b>
Lo 309	King Ko (Foggia)		A632
Lo 387	Cinquantino San Fermo		B73
Lo 404	Sacra Famiglia 43		DSP1771
Lo 434	Cinquantino Bianchi		F2
Lo 435	Cinquantino Bianchi		MBS847
Lo 441	Scagliolo Marne		W117
Lo 465	Nostrano dell'Isola Finardi		