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IDIAM –PROJECT- Analysis of genetic variability and identification of genes involved in rootworm damage tolerance in maize.*

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Western corn rootworm (WCR) (*Diabrotica virgifera virgifera*), is a devastating maize pest in North America and recently in Europe. The major damage to maize plants is caused by larval feeding on roots; the adult stage can cause silk clipping with low fertility of the ear and reduced production. Plants resistant to insects lead to a reduction in production losses, a decrease of the costs of insecticide treatments and improved food safety for animal feed and human. Among the prevention and containment measures appear effective the use of some agricultural practices such as hybrid selection, crop rotation, sowing early, good availability irrigation, earthing up and insecticide treatments (Hibbard B.E. et al., 1999. Maydica 44: 133-139; Eubanks M.W., 2002. pp. 2544-2550. *In:* Proc. NSF Design Iowa State University, Ames, IA; Kiss et al., 2011. Proceedings of XXI IWGO Conference, 27 Oct- 3 Nov, Legnaro, Italy, 29-37). However, these strategies of control and prevention, appear poorly effective in containing the pest damage, in addition to high costs and negative effects on the environment and the ability of the parasite to evolve individuals tolerant to different active ingredients and their host plants.

Therefore,in addition to these strategies, the use of resistant maize varieties by classical plant breeding, or transgenic approaches have been the most important methods to control this pest (Punja ZK, 2001. Can. J. Plant Pathol., 23, 216-235). Maize expressing *Bacillus thuringiensis* (bt) toxins or the *Caryophyllene synthase* gene (Degenhardt et al. 2009. Proc. Natl. Acad. Sci. 106: 13213-13218), responsible for (E)-β-Caryophyllene production in maize, were used as protection from pests. The identification of genes and molecules underlying the defensive plant response against the corn rootworm is of primary importance for the establishment of plants tolerant to the damage caused by rootworm larvae.

The main topics of our research involve: i) analysis of genetic variability and identification of hybrids with reduced radical damage; ii) identification of genes underlying the plant response to damage by corn rootworm; iii) validation of candidate genes and polymorphisms mapping.

In our laboratory, experiments of artificial WCR eggs infestation tests on maize B73x Mo17 hybrid roots were set up in order to obtain root samples for differential gene expression analysis in comparison with not-infested controls. The method implied that diabrotica eggs hatch in contact with corn seeds germinated in Magenta boxes with soil, so that the newborn larvae could feed corn roots. The development of corn plantlets (hybrid B73 x Mo17) infested (or not as control) with artificial corn rootworm larvae was performed in a containment greenhouse. The phenotypic observation of roots and above ground foliage of the control and infested plants, 30 Days After Infestation (DAI), indicated that the artificial infestation conditions adopted, allowed a significant difference in the structure of the root system of infested plants, resulting smaller than control plants. At this stage, samples of roots (control and infestation) were collected for microarray analysis in order to identify differential gene expression. The damage inflicted by larvae on the roots, was even more evident when the plantlets were transferred from Magenta boxes into pots for additional 15 days. At this stage (45 DAI) not only the root system was severely damaged, but also the leaves suffered a severe stress after infestation.

A preliminary experiment was set up comparing, in addition to the experimental hybrid B73 x Mo17, the response of two different commercial hybrids (Eleonora, PR32G44) to diabrotica eggs infestation. The test (30 replicates for each hybrid and treatment) was conducted under infestation

conditions previously described. At 21 DAI phenotypic structure of root systems was observed. Root system of Eleonora hybrid appeared to be the less damaged after infestation; plant height of both genotypes was significantly affected by the infestation. Similarly, data about biomass of leaves and roots, indicated clearly, for all hybrids, a significant effect of reduction by treatment with diabrotica eggs, confirming the reliability and reproducibility of the method of infestation previously set up. The comparison between the not-infested hybrids, showed that root weight of B73xMo17 was significantly different from PR32G44 and Eleonora . The artificial WCR eggs infestation tests will be used for the analysis of a broad spectrum of genotypes for the identification of 50 hybrids (commercial and experimental) with reduced radical damage. The data will be compared with the response of the same commercial hybrids tested in agronomical trials performed in two years (2010-2011) at 20 different locations, representative of the maize Italian areas, in the frame of WCR monitoring programme (by Pherocon AM traps). Preliminary data indicated that, in all monitored areas, the total mean WCR adults capture was higher in 2011 than in 2010.

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