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IDIAM –Project: Identification of genetic variability and genes for the selection of genotypes tolerant to rootworm damage in maize.*

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Recently, *Diabrotica virgifera virgifera*, commonly referred to as the corn rootworm, spread in the Italian areas devoted to corn cultivation. Estimates indicate that the area affected by rootworm has been for 2009 of approximately 165 000 hectares, of which 135 000 in Lombardia, with a major impact in the corn-livestock industry with losses amounting to 100 million euros per year. Among the prevention and containment measures appear effective the use of some agricultural practices (hybrid selection, crop rotation, sowing early, good availability irrigation, earthing up and insecticide treatments). 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 of different active ingredients and their host plants.

One of the most promising strategies to deal with infestations of pests is given by the cultivation of resistant varieties. The establishment of plants that can produce their own insecticide is proposed as an effective strategy, safe from an ecological point of view to counter the spread of insects. 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.

Studies aimed at evaluating the germplasm of maize and its ancestors, allowed i) to identify plants with tolerance against rootworm larvae, useful in breeding programs (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), ii) to set up methods to test the susceptibility of plants (Moellenbeck et al., 1995 J. Econ. Entomol. 88: 1801-1803.; Knutson et al., 1999 J. Econ. Entomol. 92: 714-722.), iii) to elucidate the mechanisms of plant resistance to insect damage (Assabgui et al., 1995 J. Econ. Entomol. 88: 1482-1493. ; Prischmann et al ., 2007 J. Appl. Entomol. 131: 406-415.). This research has also revealed a wide range of varieties and genetic variability in inbred lines showing a complex quantitative genetic basis, making difficult and expensive the selection of tolerant genotypes tolerant. Moreover, investigations of the genetic variability in corn hybrids have shown that indirect selection for large root systems can support the development of secondary roots, reducing larval damage (Simic et al., 2007 Maydica. 52: 425-430.; Tollefson, 2007 Maydica 52: 311-318., Marton et al., 2008 in Hungary Tagung der Vereinigung der Pflanzenzuchter und Saatgutkaufleute Osterreichs 77-80., Ivezic et al., 2009 *In:* Proc. XXIst IWGO). A promising strategy for biological control has recently been proposed by Degenhardt et al. 2009. Proc. Natl. Acad. Sci. 106: 13213-13218). These authors found that the recent maize crops have lost the ability to emit a volatile - (E)-β-caryophyllene, which attracts specific nematodes (*Heterorhabditis megidis*), enemies of the larvae of corn rootworm. To restore the signal insect-radical damage, the corn was engineered by introducing in his genome, a gene that promotes oregano emissions (E)-β-caryophyllene. The results show that maize plants that form (E)-β-caryophyllene have a decrease (60%) of the number of rootworm larvae than non-transformed plants. Another promising strategy for biological control has been proposed by Meissl et al. (2009 Appl. & Environ. Microbiol., 75: 3937-3943). These authors found that the fungus *Metarhizium anisopliae* on maize plants can reduce the growth of larvae of corn rootworm.

It is therefore clear that the identification of genes and molecules underlying the defensive response of the plant against the corn rootworm products are of primary importance for the establishment of plants tolerant to the damage caused by rootworm larvae. To speed up the selection of plants with

damage tolerance of larvae of corn rootworm, research has been recently addressed to clarify the genetic basis of character, with investigations aimed at identifying specific genetic or biochemical compounds and QTL underlying tolerance (Gray et al., 2009 Annu. Rev. Entomol. 54: 303-321). The main objective of this research aims to: i) detect and identify varieties and genes useful for the development of maize genotypes to reduce the damage caused by rootworm larvae on corn production through the development of plants with tolerance to insect infestations; ii) identify genetic factors to speed up the selection of genotypes able to counteract the damage to the larvae of corn rootworm infestation; iii) develop analytical technologies capable of allowing identification of genotypes tolerant to the development of lines and hybrids to be used in areas north-central Italy with the risk of infestation.
It is ,therefore, in progress studies to analyze the genetic variation to estimate the damage caused by rootworm tolerance of about 25 commercial maize hybrids from different maturity classes (FAO 500-700); the hybrids tested were tested in 20 locations representative of the maize Italian areas, using a randomized block experimental design with three replications. The basic plot consists of 4 rows, distant 75 cm and long 10 m. The following observations and the standard agronomic measurements were performed: Grain humidity (%) - Yield (t/ha) – Grain Density - Plant height - Ear height – Percentage plants with split stalk - Percentage lodged plants.
The findings relate to production and agronomic traits (flowering time, moisture at harvest, flattened crop production). At each location of agronomic trials was also determined the frequency of catches of adult insects by traps (Pherocon AM) to correlate the production level and the insect spread in the locations. For two of the hybrids tested at two locations were carried out regular sampling to assess the response of plants to attack the larvae of *D. v. virgifera* determining the extent of the damage with the index node injury scale (Oleson et al., 2005 J. Econ. Entomol. 90: 1-8), frequency of active roots and dimensions of the root (weight biomass), was also estimated the degree of larval infestation by collection of roots and soil, and extraction of larvae by flotation in water and then drying the roots in Berlese funnels. These data will be integrated with other data (radical damage and larval infestation) obtained from other agronomic trials.

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