Proceedings of the 50th Italian Society of Agricultural Genetics Annual Congress
Ischia, Italy – 10/14 September, 2006
ISBN **88-900622-7-4**

Poster Abstract – C.33

CONSTRUCTION OF PEAR PLANTS DISPLYING FIRE BLIGHT TOLLERANCE AND DEVELOPMENT OF MOLECULAR TOOLS TO EVALUATE THE BACTERIAL INVASION

M. NESTA*, M. CACCAVELLO*, H. ABDOLLAHI**, D. MIANO*, R. LUPI*, A. DI BIASIO*, E. RUGINI*, M. RUZZI***, R. MULEO*

*) Department of Crop Production, Woody Plant Molecular Ecophysiology Laboratory, Agricultural Facolty, Tuscia University, Via S.Camillo de Lellis s.n.c., 01100 Viterbo, Italy – muleo@unitus.it
**) Horticultural Research Department, Seed and Plant Improvement Inst. Karaj, Iran
***) Department of Agrobiology and Agrochemistry, University of Tuscia, Via S.Camillo de Lellis snc, 01100 Viterbo, Italy

Gox gene, fire blight, Pyrus communis, stress tolerance, diagnostic test

Fire blight is one of major bacterial pandemic disease, which attacks many woody fruit crop species. Recently, we have isolated a few bacterial RNA expressed by *E. amylovora* during the early stage of plant tissue invasion. Specific primers were designed on the sequence of those bacterial genes to tests their suitability to be use as molecular marker, for detecting the presence of bacteria in infected asymptomatic plants of pear cultivars. Moreover, in this work we also report results on transgenic plants of the cvs Conference and Dargazi, expressing the *Aspergillus niger gox* gene, codifying for the glucose oxidase (GOX) enzyme which increases the production of H_2O_2 in plant tissue. The hydrogen peroxidase is supposed to increase the tolerance to biotic stress in plant. Transgenic plants have been obtained by using *A. tumefaciens*, strain EHA 105, delivering the plasmid PBI121 armed with *gox* and *nptII* genes, both under 35S promoter and the NOS terminator. Regenerated clones were selected on Kanamycin enriched medium, while gene insertions and expressions were confirmed by PCR tests, using specific primers. To exclude any accidental presence of *A. tumefaciens* we also search the presence of *Agrobacterium* tryptophan repressor gene in transgenic plant tissues, using specific primers in PCR protocols. One-month-old wild type in vitro plantlets of cvs Harrow Sweet, Williams, Conference and Dargazi, together with *gox*-transgenic plantlets of cvs Conference and Dargazi, grown *in vitro* conditions, were used in the *E. amylovora* artificial infection trials. *E. amylovora* was cultured in LB medium and 0.600-culture density (OD_{600}) was used to infect the pears plants. Every six hours, starting from the inoculation, the appearance of symptoms was monitored on the stem and leaves of wt and transgenic pears plants. The rapid progression of bacterial invasion through the xylem tissue, from the bottom to the top of plants, was also checked by molecular tests. Results suggest that the cv Harrow Sweet is the most tolerant cultivar, while among the others the degree of tolerance is cv Dargazi > Williams > Conference. The *gox* gene increased the resistance in Conference and Dargazi plants. 72 hours after the bacterial inoculation the necrosis were diffused on 100% of Conference and Williams of wt plant tissues; under the same conditions the necrosis were only diffused on 59% of wt Dargazi tissues and on 55% of wt Harrow Sweet tissues. Instead, the diffusion of necrosis on tissues of *gox*-transformed Conference and Dargazi plants was 35 and 12%, respectively. Moreover, we observed a 12-hours delay in the appearance of the symptom disease in transgenic plants *vs* wt plants.

Molecular diagnostic tests carried out on a 4 mm section of the stem ascertained that *E. amylovora* migrated into the xylem tissue at the same speed in both wt and transgenic plants; after 24 hours from the inoculation *E. amylovora* caught up the apices of plantlets, in asymptomatic plants.

Our results indicate that: a) *gox* gene from *Aspergillus niger* can help the pear plant to contrast the invasion of *E. amylovora,* b) the isolated bacterial genes are good molecular tools to identify the presence of *E. amylovora* in asymptomatic plants during the early stage of invasion.

The work was financial supported by University of Tuscia