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

Poster Abstract – E.06

A TRANSGENIC WHEAT LINE WITHOUT ANTIBIOTIC AND HERBICIDE MARKER GENES

A. GADALETA*, A. GIANCASPRO*, A. BLECHL**, A. BLANCO*

*) Department of Environmental and Agro-Forestry Biology and Chemistry, University of Bari sect. of Genetics and Plant Breeding, Via Amendola 165/A, 70126 Bari, Italy
**) USDA-ARS Western Regional Research Center, Albany, CA 94710-1105, USA

pmi, phosphomannose isomerase, marker genes, HMW glutenin subunit, clean gene

Genetic engineering of monocots is usually carried out by direct methods such as particle bombardment or protoplast transformation and involves the use of selective agents and marker genes to favour the multiplication of the initially transformed cell. Antibiotics or herbicides are used as selective agents. However, both may have some undesirable effects. It has been argued that there might be a risk, that the antibiotic-resistance gene could be transferred from plants to pathogenic bacteria while herbicide resistance might be transferred to weeds. Moreover the routine production of transgenic plants involves transformation with foreign DNA carried on plasmids, and causes the integration of vector backbone sequences into the genome along with the transgenes.

Several strategies have been developed to avoid the use of antibiotic and/or herbicide marker genes in plant transformation. Among them there are the elimination of marker genes by recombinases or the use of alternative marker genes.

In the present work durum wheat transformation was carried out with minimal gene cassettes, which were linear DNA fragments lacking vector sequences excised from the plasmids. Two transformation experiments were carried out using as target genes two wheat sequences encoding the Dx5 and Dy10 HMW glutenin subunits and *phosphomannose isomerase (pmi)* gene as the selectable marker. Phosphomannose isomerase catalyzes the reversible interconversion of mannose-6-phosphate and fructose-6-phosphate, allowing plant cells to utilize mannose as a carbon source and to grow and differentiate on media containing mannose. PCR assay of T_1 generation identified fifteen transgenic plants of which the line TC-52 was subsequently investigated. A stable integration and expression of Dy10 HMW glutenin subunit gene was confirmed by protein gel analyses of T_1, T_2 and T_3 generations. In order to identify transgenic lines free of marker genes, segregation analysis of *Dy10* and *pmi* genes was conducted in 150 T_3 progenies. The absence of co-segregation of the two genes suggested the localization on two different chromosomes and allowed the identification of transgenic lines completely marker free.