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DEVELOPMENT OF TRANSGENIC WHEAT PLANTS FREE OF HERBICIDE MARKER GENES AND PLASMID BACKBONE SEQUENCES THROUGH "CLEAN GENE" TECHNOLOGY AND POSITIVE SELECTION

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Pmi, bar, phosphomannose isomerase, marker gene, clean gene

The development of novel biotechnologies for direct gene transfer has accelerated plant improvement programs, especially for monocotyledonus species such as wheat that have been traditionally recalcitrant to transformation. Identification of transgenic plants in important crop species, which have relatively low transformation efficiencies, requires the use of selectable marker genes to minimize regeneration of non-transformed plants.

The most frequently used selectable markers are genes conferring resistance to herbicides or antibiotics. Moreover the routine generation of transgenic plants involves transformation with foreign DNA carried on plasmids, and causes the integration of vector backbone sequences into the genome along with any transgenes. Integrated vector DNA has been detected in transgenic plants generated by *Agrobacterium*-mediated transformation and direct delivery procedures such as particle bombardment. However, in both plant and animal systems, vector backbone sequences may exert undesirable negative effects on transgene or endogenous genes expression and can promote transgene rearrangements. The use of minimal expression vectors comprising linear DNA fragments containing only promoter, transgene coding region and terminator/polyadenylation sites shows significant advantages in reducing these events. In the present work durum wheat transformation of cv. Svevo was carried out in parallel experiments (simultaneously) by using either whole plasmids containing suitable gene constructs, or the minimal gene cassettes, which were linear DNA fragments lacking vector sequences excised from the plasmid. Trasformation experiments were carried out using as target genes two wheat sequences encoding the Dx5 and Dy10 HMW glutenin subunits and the *phosphomannose isomerase (pmi)* gene as the selectable gene and mannose as the selective agent. The integration and expression of genes in T_1 generation was confirmed by PCR analysis with specific primers and chlorophenol red assay.

The average biolistic transformation frequencies obtained using the plasmids and linear DNA were approximately the same: 1.14% in bombardment with entire plasmids and 1.50% with linear DNA.

An efficient selection method was established in durum wheat transformation, without the use of herbicide or antibiotic resistance genes.