for genes related to starch metabolism in barley. Starch is the major reserve of plants and serves as primary carbohydrate component in human and livestock diets and has also numerous industrial applications. Mutants for biosynthetic or regulatory genes of starch metabolism often produce starch granules with abnormal morphological and molecular features that could be of interest also for technological applications. Molecular screening of TILLMore for mutations has already been completed for five starch-related genes (*Limit dextrinase1*, GBSSI, Bmy1, SSI and SSII) with a total number of 20 mutants identified. Almost all the mutations detected were CG-TA transitions and several (ca. 60%) implied a change in amino acid sequence and therefore possible effects on phenotype. In four cases, we identified non-sense or splice junction mutations which affect drastically the protein function. A more detailed description of the mutants identified so far will be presented and discussed.

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[P-P&F.71]

High cytotoxic activity of a bifunctional chimeric protein containing a ribosome inactivating protein (RIP) and a serine protease inhibitor (WSCI)

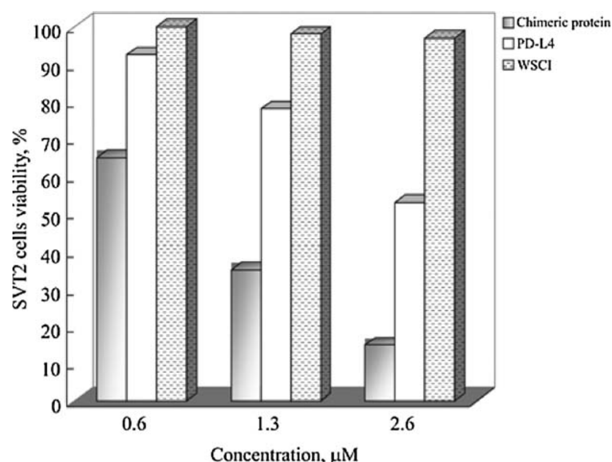
R. Tamburino [1], E. Pizzo [2], A. Di Maro [1], F. Tedeschi [3,*], A.G. Ficca [3], E. Poerio [3]

[1] *Seconda Università di Napoli, Italy*
[2] *Università di Napoli Federico II, Italy*
[3] *Università della Tuscia (VT), Italy*
Keywords: Cell toxicity; Ribosome inactivating proteins; Protease inhibitors

Introduction: Plants have their own networks of defence tools to protect against pathogens; these tools include a vast array of proteins: pathogenesis-related proteins, defensins, ribosome-inactivating proteins, lipid-transfer proteins, killer proteins, protease inhibitors, etc. (Chandrashekar and Satyanarayana, 2006). Recombinant DNA technology is currently used in agriculture to create genetically modified plants with an increased resistance to phytopathogens. In order to provide a more effective control of phytophagous insects, a bifunctional chimeric protein, potentially able to act as insecticide, has been designed and expressed in *E. coli* cells. The N-terminal domain corresponds to the toxic/antiviral protein PD-L4 type 1 RIP, firstly isolated from *P. dioica* L. leaves (Di Maro et al., 1999). The second domain corresponds to the wheat inhibitor WSCI, which is able to interfere with digestive proteases of mammals and insects (Poerio et al., 2003; Di Gennaro et al., 2005).

Materials and methods: The chimeric construct, *pd-l4-cDNA-oligonucleotide linker- wsci-cDNA*, was cloned in the expression vector pET22b and employed in transforming *E. coli* (strain BL21-DE3) (Capuzzi et al., 2009). Cytotoxicity assays were carried out using Simian-virus-40-transformed mouse fibroblasts (SVT2 cells). The cells were plated at a density of 2.5×10^3 cells per well in 100 μL of medium. The cytotoxicity was determined in presence of increasing concentrations of PD-L4, WSCI and PD-L4/WSCI chimera. Cell survival was determined after 72 hours by means of MTT reduction assay.

Results and discussion: The protein chimera PD-L4/WSCI was expressed in *E. coli* and recovered from the inclusion bodies. Both tandem domains (PD-L4 and WSCI) retained their original activities (Di Maro et al., 1999; Poerio et al., 2003). Characterization of the chimera was performed by electrophoretic, chromatographic and N-terminal sequence analyses. Cytotoxicity assays revealed that the chimeric protein strongly affected the viability of SVT2 cells; surprisingly, the recorded levels of toxicity were greater than those observed for PD-L4 (see figure). Somehow, the presence of the non-toxic C-terminal domain WSCI contributed to enhance cytotoxicity of the bifunctional chimeric product.



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[P-P&F.72]

The *Medicago Sativa Hem*L gene as an innovative plant-derived selectable marker for durum wheat transformation

Angelica Giancaspro [1,*], Agata Gadaleta [1], Daniele Rosellini [2], Antonio Blanco [1]

[1] *Università degli Studi di Bari Aldo Moro, Italy*
[2] *Università di Perugia, Italy*
Keywords: HemL; Durum wheat; Transformation; Marker

The production of transgenic plants relies on the delivery into the target tissues of selectable marker genes together with the useful genes, to allow only the cells that have integrated and express the foreign DNA to regenerate into a plant. The most efficient selectable markers for plant transformation are bacterial genes conferring resistance to antibiotics or herbicides. The presence of such genes in transgenic crop plants has become a matter of concern, thus stimulating the scientific community to test alternative selection systems based on genes conferring resistance to phytotoxic chemicals other than antibiotics or herbicides. A recent alternative is the *hem*L gene from *Synechococcus* strain GR6 coding for a mutant form of GSA-AT (glutamate 1-semialdehyde aminotransferase) enzyme which is insensitive to the phytotoxin gabaculine (Gough et al. 2000; Rosellini et al. 2007). The GSA-AT cDNA from *Medicago sativa* was cloned and point-mutated to reproduce the gabaculine-resistance mutation of the *Synechococcus hem*L gene, and it has been shown to be an efficient marker in alfalfa and tobacco transformation (Rosellini et al., unpublished).

In the present experiment we compared the conventional *bar* marker gene from *S. Hygroscopicus* conferring resistance to Bialaphos herbicide, with the newly developed plant-derived *hem*L marker in durum wheat transformation. A co-transformation experiment was carried out on wheat calli by delivering plasmids pAHC20 and pAPCK-GSA carrying the *bar* and the mutant GSA-AT