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**“Protease inhibitor gene transfer as a tool for an ecological and
sustainable plant defence strategy”**

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ABSTRACT

Losses of agricultural production due to pests and diseases have been estimated around 37% in the past years. Worldwide, over 200 plant diseases are known to be transmitted by insects, mites, and nematodes. At present, crop protection relies predominantly on the use of environmentally toxic agrochemicals deleterious to human health. Instead of using chemical pesticides on a large scale, other alternatives need to be explored that are beneficial in terms of sustained agriculture. Such sustainable systems should decrease inputs of energy and chemicals, and should not generate harmful outputs such as pesticide residues. To achieve this objective, it is necessary to enhance the resistance of plants to pests and pathogens through Integrated Pest Management (IPM) programs comprising a combination of control strategies including the judicious use of pesticides, crop rotation, field sanitation, and above all exploitation of inherently resistant plant varieties.

The use of transgenic crops, expressing foreign insecticidal genes, could give a significant contribution to sustainable agriculture and should be considered as an important component of IPM. Current control relies predominantly on the use of transgenic *Bacillus thuringiensis* (Bt) crops. A different alternative in increasing the plant resistance to herbivores is the plant genetic

transformation with genes coding for enzyme inhibitors. Among them Proteinase Inhibitors (PIs) are natural, defense-related proteins often induced in tissues of certain plant species by herbivore injuries. Their effectiveness is related to the inhibition of digestive enzymes present in the insect guts (amylases, proteases) hampering the use of nutrients (carbohydrates, proteins) for the insect growth. The strategy of transferring PI genes in plants should not, at least in principle, have harmful effects on human health for the specific target of these proteins and for the biological origin of the inhibitor. For the environment the risk is uncertain and it may be correlated to the level of PI expressed in plants and to the insect targets.

In our laboratory, genes coding for several different proteinase inhibitors have been identified or produced by mutagenesis. The first of them (mti-2) was identified on the mustard genome and codes for a 63 amino acid polypeptide (MTI-2) highly active in inhibiting trypsin like serine proteinases. Transformation of tobacco, arabidopsis and rapeseed by mti-2 produces transgenic plants highly resistant to several insects belonging to Lepidopteran species as *Spodoptera littoralis*, *Mamestra brassicae* and *Plutella xylostella*, indicating the basic potential of this inhibitor in hampering amino acid availability for the insect growth. Interestingly, in a long term bioassay, when *S.littoralis* larvae were fed on plants expressing high levels of MTI-2, the number of eggs per female decreased significantly.

The results of these investigations demonstrate that the use of this PI would have a mild and reversible effect on the environment. Indeed its presence in plant tissues induce the insect fertility and delays their growth without killing all of them. Consequently, their presence in the field would be restored as soon as the cultivation of that transgenic plant is stopped.

A second inhibitor, CHYMO8 obtained by mutagenesis of mti-2 gene, is highly active against nymphs of aphids affecting pea cultivations. Finally, an inhibitor of glutamic proteases (RTI-II) identified on the rapeseed genome is potentially active against pathogens of bacterial origin.

The commercial relapse of these research products will not require further relevant financial efforts but could be activated once that EU Directives will be changed.