

Development of molecular tools based on barley proteinase inhibitors for pests control



Rim Hamza*, Doctorate in Biotechnology, Polytechnic University of Valencia

Directors: Luis Cañas Clemente*, Jose Pío Beltrán Porter*

Tutor: Alejandro Atarès Huerta*

* Instituto de Biología Molecular y Celular de Plantas

Introduction

Pests are representing a threat to agriculture worldwide. Losses due to weeds, pathogens and animal pests are estimated between 26–29% for soybean, wheat and cotton, and 31, 37 and 40% for maize, rice and potatoes, respectively (Oerke, 2006). Insecticides and chemicals use have been the major tool for crop protection. In the last years, less polluting approaches have been investigated. In order to improve plant resistance to pests, attention have been focused on the use of anti-metabolic molecules such as proteinase inhibitors (PIs). These proteins are naturally expressed in plants in response to injury by herbivores and are capable of disturbing insects digestive enzymes.

General objective

Investigate the potential use of two barley proteinase inhibitors (PIs), one serine proteinase inhibitor (SPI) and one cysteine proteinase inhibitor (CPI) for the improvement of plant resistance toward pests.

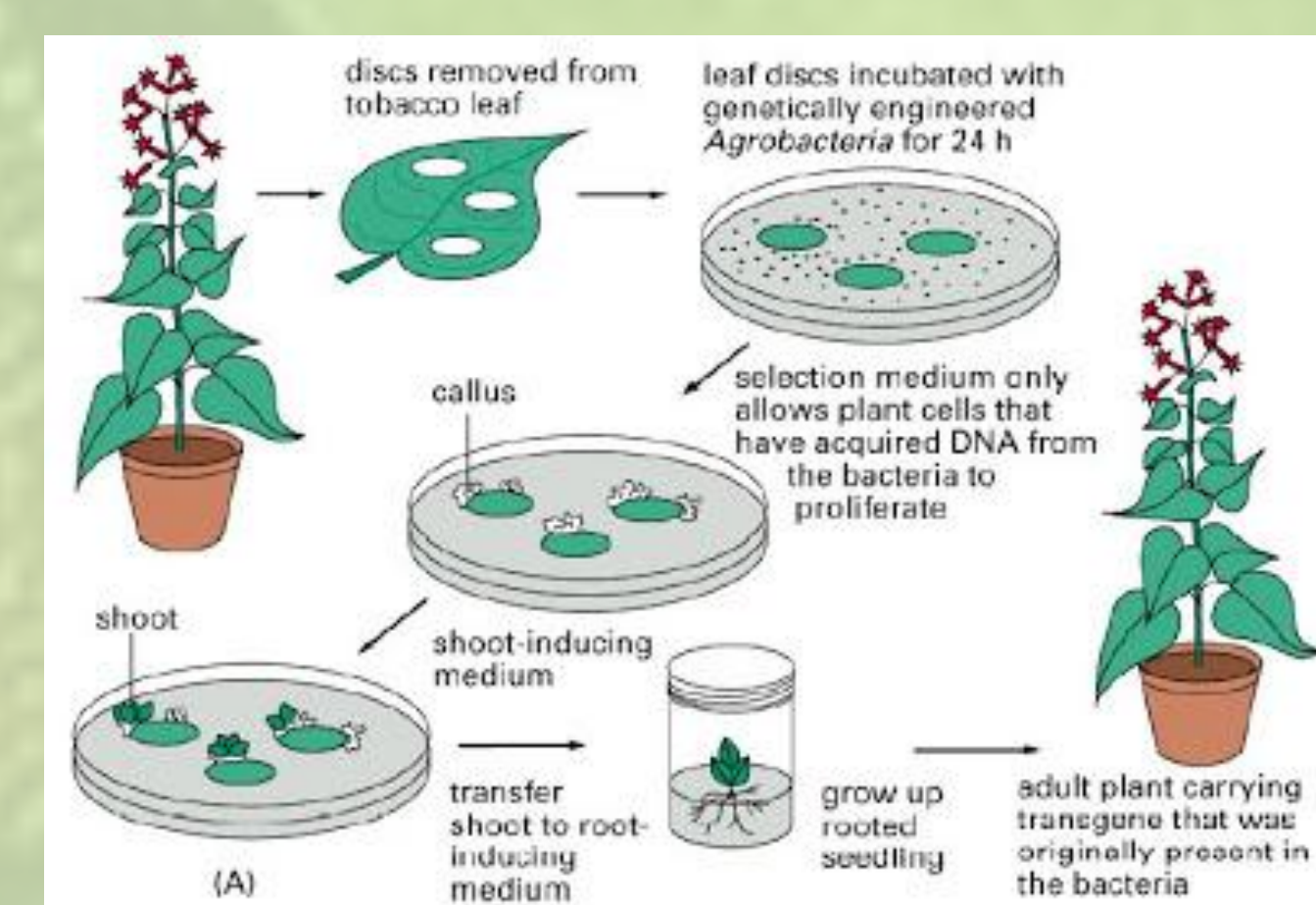
Specific objectives

- Evaluating the *in-vitro* activity of the PIs toward pests and beneficial insects
- Testing the *in-vivo* activity of PIs against pests
- Investigate the effect of co-expression of two PIs on the insects' ability to adapt to PIs

Principal investigation steps

- PI genes' cloning and expression in heterologous system
- Site directed mutagenesis intending to improve the PI activity.
- *In-vitro* activity assays against phytophagous and beneficial insects' digestive enzymes

- Genetic transformation of tomato mediated by *Agrobacterium tumefaciens*; to express a SPI/ CPI/ SPI+CPI



- *In-vivo* activity assays against insects



Expected results

- Assessing the insecticidal potential of barley PIs against phytophagous pests
 - Demonstrating the inactivity of barley PIs toward beneficial insects' enzymes
 - Preventing insects' adaptation to PIs through co-expression of two PIs of two different families.
- ➡ Barley's PIs as a molecular tool for the control of plant pests