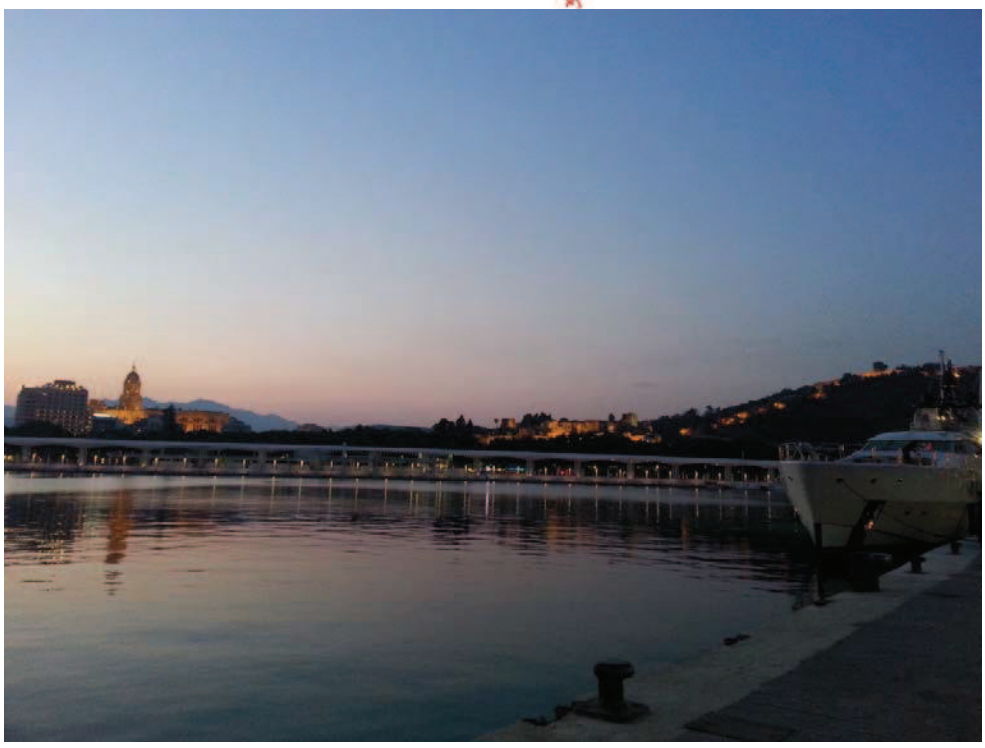
9th International Conference on *Pseudomonas syringae* and Related Pathogens

Málaga, 2nd-5th June 2015



P12. Over-expression of virulence inhibiting peptides in *Nicotiana tabacum* plants as a tool to control *Pseudomonas syringae* pv. *tabaci*

Matteo Cerboneschi[1], Patrizia Bogani[2], Stefano Biricolti[1], Carola Biancalani[1], Sofia Macconi[1], Maria Vittoria Ortenzi[1], Stefania Tegli[1]

[1]Department of AgriFood Production and Environmental Sciences. Molecular Plant Pathology Laboratory - University of Florence. Via della Lastruccia 10, 50019 Sesto Fiorentino (Firenze) Italy
[2]Department of Biology – Plant Genetics Laboratory - University of Florence. Via Madonna del Piano 6, 50019 Sesto Fiorentino (Firenze) Italy

e-mail: matteo.cerboneschi@unifi.it

The use of antimicrobial peptides (AMPs) to control phytopathogenic bacteria and fungi has been suggested as a potential alternative to conventional pesticides and antibiotics (Montesinos, 2007). Transgenic plants expressing AMPs could represent a good strategy to fight pathogens in both animal and plant hosts, although some concern still exist about several their intrinsic drawbacks (Keymanesh et al., 2009). Conversely, the design and use of innovative oligopeptides targeting Type Three Secretion System (T3SS) of *Pseudomonas savastanoi* pv. *nerii* has been previously showed to inhibit the development of the disease in its host *Nerium oleander*, without any toxic impact on plants (Cerboneschi et al., 2012). In this work, we describe the stable transformation and expression in *Nicotiana tabacum* plants of two of these short peptides, 17 and 27 aminoacids long, named AP17 and LI27, respectively. The transformation of Tobacco plants was performed using *Agrobacterium* EHA105, carrying the pCambia 1305.2Δ*gus* binary vector, containing AP17 or LI27 sequences downstream the signal peptide GRP to target peptide delivery to apoplast (Biancalani et al., 2014). Transgenic lines were scored for antibiotic resistance on Linsmaier and Skoog medium containing 50 mg/l hygromycin, and then for AP17 or Li27 expression. Transgenic lines expressing high levels of each peptide were selected, and then *in vitro* inoculated with *P. syringae* pv *tabaci* ATCC 11528 (*Pstab*). While control untrans-formed plants were severely affected by *Pstab* infection, AP17 and Li27 transgenic plants showed faint or no symptoms, and the bacterial growth was severely impaired to reach zero level in few days after inoculation. This data demonstrated that constitutively expressed virulence inhibiting peptides could represent a promising strategy to control bacterial diseases of plants. Prelimary experiments are ongoing about the stable transformation and expression of *Actinidia chinensis* with peptides targeting T3SS of *Pseudomonas syringae* pv. *actinidiae*.

Key Words: AMPs, *Nicotiana tabacum*, transgenic plants, T3SS, *Pseudomonas syringae*.

REFERENCES

BIANCALANI, C.; CERBONESCHI, M.; MACCONI, S.; MONCELLI, M.R.; SMEAZZETTO, S.; BIRICOLTI, S.; BOGANI, P.; TEGLI, S. (2014): Peptides as inhibitors of type three secretion system for ecofriendly control of bacterial diseases of plants. Environmentally loyal plant protection: from nano- to field-scale, XX SIPAV Congress, p. 37, Pisa, September 22-24, 2014.
CERBONESCHI, M.; BRITTOLI, A.; ARRIGONI, E.; CAPARROTTA, S.; CIOLLI MATTIOLI, C.; COLOMBI, E.; BIANCALANI, C.; MACCONI, S.; TEGLI, S. (2012): Protein-protein interaction model: new strategies for bacterial diseases control. Proceedings of the 3rd International Workshop on Expression, Structure and Function of Membrane Proteins, p. 24, Florence, September 23-27, 2012.
KEYMANESH, K.; SOLTANI, S.; SARDARI, S. (2009): Application of antimicrobial peptides in agriculture and food industry. World Journal of Microbiology and Biotechnology, 25 (6), 933-944.
MONTESINOS, E. (2007): Antimicrobial peptides and plant disease control. FEMS Micro-biology Letters, 270 (1), 1-11.

ACKNOWLEDGMENTS

Project funded by EU, LIFE12 ENV/IT/336 "AFTER Cu"