

EVALUATION OF THE PROTECTION AGAINST *XANTHOMONAS* IN TRANSGENIC *PELARGONIUM* CONTAINING A CHIMAERIC CECROPIN GENE

J.P. Renou, I. Mary, S. Hanteville
INRA - Unité d'amélioration des espèces,
fruitières et ornementales
42 r. G. Morel
49071 Beaucauzé-Cedex
FRANCE

D. Florack
Dpt of Molecular
Biology, DLO
PO Box 16
6700 AA Wageningen
THE NETHERLANDS

J.P. Narcy, A. Diolez
INRA - Pathologie Végétale
Rte de St Cyr
78026 Versailles
FRANCE

Keywords: *Pelargonium*, *Xanthomonas*, cecropin, disease resistance, transgenic plants

1. Introduction

The, phytopathogenic bacterium *Xanthomonas campestris* pv. *pelargonii* causes severe losses in vegetatively propagated *Pelargonium* cultivars. Symptoms are characterized by wilting of the plant, localized water-soaked lesions and stem rot (1) To overcome this problem horticulturists have to produce *Xanthomonas*-free planting material in insect-proof greenhouses after indexing the propagating stocks, which is expensive and time consuming. All commercial cultivars of pelargonium are susceptible to *Xanthomonas* and no source of resistance is known in the compatible *Pelargonium* germplasm. Thus, attempts were initiated to obtain *Xanthomonas* resistant plants by a transgenic approach.

2. Materials and methods

Cecropin B from *Hyalophora cecropia* was tested in vitro and shown to be toxic against *Xanthomonas*. A chimaeric cecropin B gene was excised from pCPOC4 (2) and introduced into a binary vector, giving the plasmid pGP30 (fig. 1). Then pelargoniums were transformed as previously described (3). Insertion of the T-DNA was confirmed by PCR analysis, and expression of the transgene was monitored by RT-PCR and northern-blot. The pathogenicity was tested in three independent experiments, ten plants per clone, with the virulent *Xanthomonas* strain 10342. 5 ml of the bacterial suspension (2.10^5 CFU/ml) was dropped onto a petiole wounding. Wilting was scored using the following scale: 0 = healthy plants, 1 = traces, 2 = up to 20% of plant wilted, 3 = up to 40%, 4 = up to 60%, 5 = up to 80%, 6 = between 80% and 100%, 7 = plant completely wilted.

3. Results and discussion

Six transgenic clones of *Pelargonium X hortorum* were analyzed and inoculated with *Xanthomonas*. PCR analysis confirmed the presence of the chimaeric cecropin gene in all six clones. Northern analysis showed transcription of the transgene in only four of them, presence of the transcript in the clones N° 5 and 6 could only be revealed by RT-PCR analysis, suggesting a low level of transcription in both clones. Inoculations with *Xanthomonas* showed a reduced symptom level in three clones (fig. 2), which is not correlated with the transcription level. The clone N° 5 showed a high tolerance to the

bacterial disease, it is probably a good candidate for testing the inheritance of this resistance in the progeny. More than 30 other transgenic clones of *P. X hortorum* and *P. peltatum* have been obtained with the same construct. They will be multiplied and analyzed in order to confirm the absence of correlation between the levels of transcription and resistance. These first results suggest that this transgenic approach could be a solution to control this disease.

Acknowledgments

This work was supported by the TECHNIGERA Company and the French Ministry of Agriculture

References

- Dougherty D.E., Powell C.C. and Larsen P.O., 1974. - Epidemiology and control of bacterial leaf spot and stem rot of *Pelargonium hortorum*. *Phytopathol.* 64. 1081-1083.
- Florack D., Allefs S., Bollen R., Bosch D., Visser B. and Stiekema W., 1995. - Expression of giant silkworm cecropin B genes in tobacco. *Transgenic res.* 4, 132-141.
- Robichon M.P., Renou J.P. and Jalouzot R., 1995. Genetic transformation of *Pelargonium X hortorum*. *Pl.Cell Rep.* 15, 63-67.

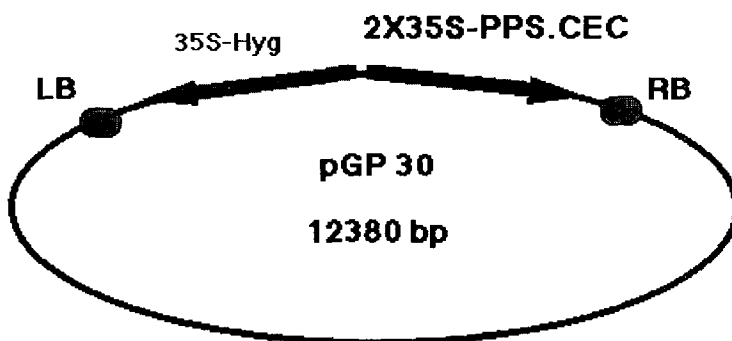


Figure 1 - Schematic diagram of pGP30.

2X35S-PPS.CEC: double enhanced 35S - plant peptide signal and cecropin B coding sequences.

35S-Hyg: 35S-hygromycin phosphotransferase.

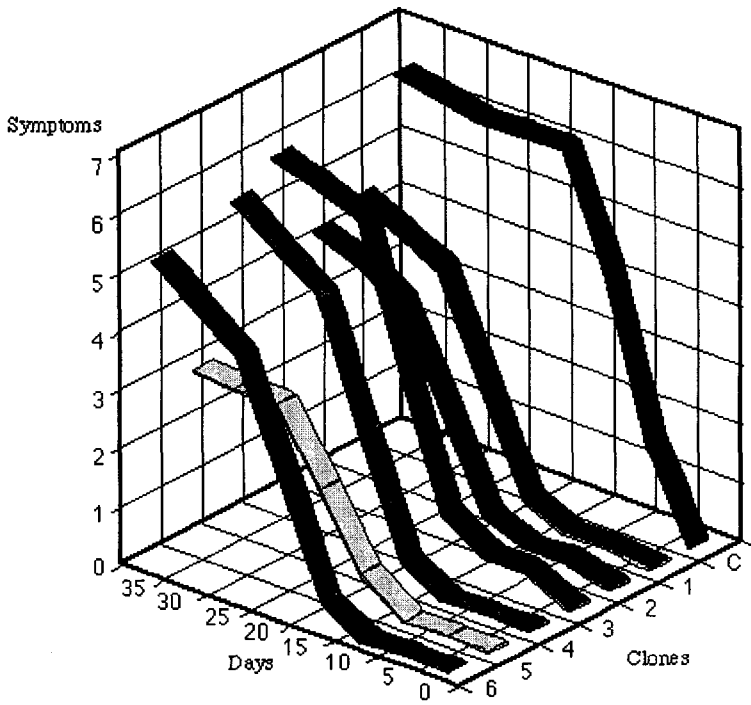


Figure 2 - Symptoms evolution (average grades).
 C: control plant, 1 to 6: transgenic clones