ISSN: 1125-4718

Italian Journal of Agronomy
Rivista di Agronomia

An International Journal of Agroecosystem Management

July-September 2008 Vol. 3, No. 3 supplement

Rivista trimestrale registrata dal Tribunale di Udine con atto n.3/97 del 12/02/1997
Direttori responsabili: Carlo Giupponi e Michele Perniola
Direzione e Redazione: Centre for Environmental Economics and Management, Dipartimento di Scienze Economiche, Università Cà Foscari di Venezia, San Giobbe, 873 - 30121 Venezia; Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università della Basilicata, Viale dell'Ateneo Lucano, 10 - 85100 Potenza
Proprietario: Società Italiana di Agronomia
Editore: Forum, Editrice Universitaria Udinese S.r.l., Via Palladio, 8 - 33100 Udine
Stampa: Grafiche Tielle, Sequals (Pn).

In caso di mancata consegna, inviare all'Ufficio di Udine Ferrovia per la restituzione al mittente, che si impegna a versare la dovuta tassa.

10th Congress of the European Society for Agronomy
15-19 September 2008, Bologna, Italy

Multi-functional Agriculture
Agriculture as a Resource for Energy and Environmental Preservation

edited by
Paola Rossi Pisa

The Official Journal of the
Italian Society of Agronomy

Restriction Analysis of the *dsp*E Gene of Virulent Strains of *Erwinia amylovora* from Different Host Plants in the Po Valley

Paola Minardi[1], Sara Mucini[2], Carla Lucchese[2] and Umberto Mazzucchi[2]

[1]*Dep. of Veterinary Morphophysiology and Animal Production - DIMORFIPA, Univ. Bologna, Italy, paola.minardi@unibo.it*

[2]*Dep. of Agro-Environmental Sciences and Technologies - DISTA, Univ. Bologna, Italy*

Introduction

In the Po Valley (Northern Italy) the primary fire blight *foci* caused by *Erwinia amylovora* (Ea) were certified on pear plants in 1994. In the following years Ea was progressively spread in that valley. In 1997, a severe epidemic of fire blight occurred in the Emilia-Romagna region. The AFLP genomic profiles of Ea populations associated with this epidemic revealed that the isolates belong to the same clone [1]. From 1994 to 2001 systematic monitoring showed that the infection frequency was high and prevalent on pear plants (87.2%) whereas it was low in hawthorn shrubs (9.14%) and very low in apple trees (1.68%) [2]. Recently, an increasing infection frequency on apple trees was evidenced beside a widespread presence of the pathogen in hawthorn. The Phytosanitary Services assumed the occurrence of strains with different virulence. The pear clonal populations might have adapted to different host plants or new strains might have been introduced in the Po Valley. These hypotheses were tested studying the genomic profiles and the virulence either of the wild populations currently present in the area and of the experimental populations obtained through successive passages on different host plants. Here we report the results of the restriction fragment analysis of the virulence *dsp*E gene in wild Ea populations from different host plants using the 1994 clone as reference strain.

Methodology

Bacterial cultures. 7 Ea isolates from apple (4386), pear (3605, 3963), hawthorn (4378, 4366, 4368/1) and photinia (4395) were used. The strains were isolated in the Emilia-Romagna and Veneto regions during 2000-2001. Ea OMP-BO 1077/7 virulent strain, isolated from pear in 1994, kept lyophilized at 4°C, was used as reference strain. The cultures were grown on YDC agar for 24 h at 27 °C.

Virulence assessment. This was carried out on pear fruitlets [1] and apple leaves of clonal M9 plantlets grown in single pots in the greenhouse. A standard inoculum dose was inoculated through transversal cuts at the leaf apex. After 21 days, the percentage of the infected area was evaluated using the APS Assess software [3]. For each strain the mean of the percentage infected areas of 10 leaves was used to calculate the virulence index (I).

***dsp*E gene analysis.** Oligonucleotides EF_2 *(forward primer*: 5'-CGG TTG CAG AGA ATT GCA-3') and ER_1 (*reverse primer*: 5'-TTC ATT TCC AGC CCT TCC TT-3') based on the *dsp*E gene of Ea321 strain [4] were designed [5] and used as primers in the PCR with the total DNA of Ea 1077/7 as template. The PCR product, analyzed on an 0,9% agarose gel, was digested overnight with *Eco*RI, *Alu*I, *Hae*II, *Msp*I, *Hha*I, *Mse*I and *Rsa*I. The fragments were separated in a 1.5 % agarose gel.

Results

In all the 7 Ea strains, the virulence index was higher (1.8) than that of 1077/7 (0). On apple leaves the infected area was highly variable even within the same plantlets inoculated with the same strain. The virulence index of the 7 strains varied between -0,464 and 0,847. The amplicon (≈ 5.5kb) obtained in the reference strain by PCR using primer EF_2 and ER_1 was similar in size to *dsp*E gene [4]. In the reference strain, the restriction fragment profiles of the *dsp*E gene showed distinct and reproducible fragments within the range of 5,000 and 100 bp showed in Fig.1: *Eco*RI (3 bands); *Mse*I (13); *Rsa*I (13); *Hha*I (11); *Hae*II (14); *Msp*I (14) and *Alu*I (12). The same profile was obtained by each enzyme in the 7 *Ea* isolates.

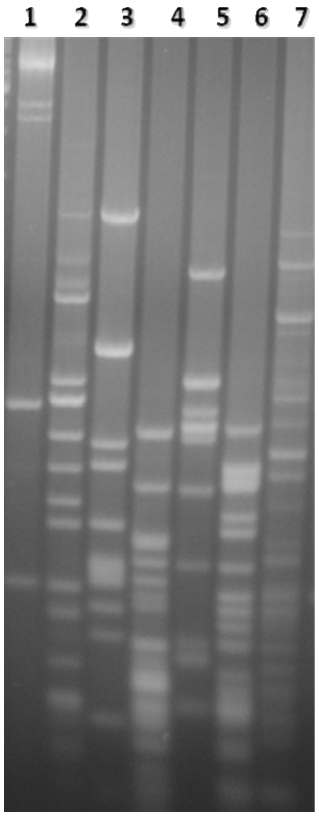

Figure 1. Restriction fragment analysis of the *dsp*E gene of the Ea reference strain. Lanes 1 to 7: restriction fragment profiles by *Eco*RI; *Mse*I; *Rsa*I; *Hha*I; *Hae*II; *Msp*I and *Alu*I respectively.

Conclusions

The Ea virulent strains chosen for the restriction fragments analysis of the *dsp*E gene were initially selected for their high virulence index on immature pear fruits within a wide collection of strains isolated from different host plants and locations in the Po Valley [1]. Therefore, they represented the more virulent strains of Ea existing in the Po Valley in 2001-2002. The virulence analysis on M9 apple leaves evidenced a high intra-strain variability in the percentage of infected areas and a high inter-strain variability in the virulence indexes. As a consequence, the statistical analysis did not revealed any significant differences in the virulence between the 7 Ea strains and the reference one. These more virulent strains had restriction profiles of *dsp*E gene indistinguishable from those of the reference strain. These results agree with the hypothesis according to which the Padanian Ea strains belong to the same clone evidenced in the previous AFLP genomic analysis [1]. The product encoded by the *dsp*E gene is injected into the plant host cells [6, 7] and can be subjected to a selective pressure from the host species becoming a key factor in the Ea adaptation to a plant species other than that of origin. The above restriction analysis of the *dsp*E gene did not support the hypothesis on the adaptation of the pear original clone to new hosts in the Po Valley. Our results concur with those of Giorgi and Scortichini [8] even if they analyzed only a portion of the *dsp*E gene taken from a wide collection of strains isolated from different host plants around the world.

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Acknowledgements

Financial support for this was provided by the Emilia-Romagna and Veneto regions.