Genetic diversity in putative effector genes among FDp and FDp-related strains from northern Italy

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INTRODUCTION

Flavescence dorée (FD) is the most important leafhopper-transmitted grapevine disease associated with a quarantine pathogen (FD phytoplasma, FDp). Recent studies demonstrated the presence of many FDp genotypes that differ in their epidemiological cycle, suggesting genotype-specific biological behaviour (Malembic-Maher *et al*., 2020; Rigamonti *et al*., 2023; Rizzoli *et al*., 2021). The recent availability of full FDp genome (Deboneville *et al*., 2022) can allow a deeper understanding of the dynamics that regulate this devastating disease, considering that, to date, phytoplasmas cannot be isolated in a pure culture *in vitro*, so they can be studied only at molecular level. The knowledge of genetic variability of putative effector genes in different FDp strains and how this variability can influence the disease development and severity will be useful to develop more targeted management strategies in agroecosystems. This study reports first results obtained by molecular typing of different FDp strains, identified in North Italy, based on sequence analyses of putative effector genes.

MATERIALS AND METHODS

Twenty-six 16SrV phytoplasma strains, previously identified in grapevine and other plant hosts in northern Italy, have been selected: 23 FDp strains (14 M54, 5 M51, 4 M50), and three FDp-related strains (2 M43, 1 M39) (Table 1). According to the FDp genome annotation by Deboneville *et al*. (2022), two putative effector genes (*hp1*, locus tag M6G77_00200; *hp4*, locus tag M6G77_02130), and a gene encoding the Bax inhibitor 1 (*baxI*, locus tags M6G77_00035 and M6G77_00935) have been selected for molecular typing. Primer pairs for each gene have been designed for direct and nested-PCR amplification carried out as follows: 94°C for 5 minutes followed by 35 cycles at 94°C for 1 min, 50°C (52°C in nested-PCR) for 1 min, 72°C for 2 min, and final elongation at 72°C for 7 minutes. PCR products have been sequenced in both directions. Nucleotide sequences and *in-silico* translated proteins have been compared to identify mutations within the analyzed FDp strains.

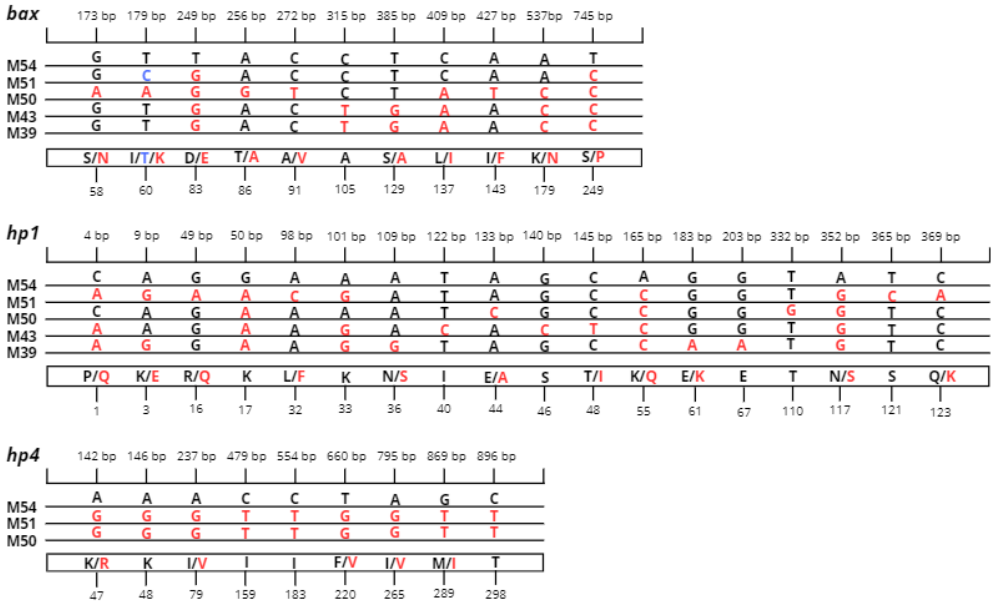
RESULTS AND DISCUSSION

Most of the 16SrV phytoplasma strains were positive to *baxI* gene amplification (25 out of 26), while 20 strains out of 22 and 18 out of 22 were positive to *hp1* and *hp4* amplification, respectively (Table 1). FDp and FDp-related strains belonging to the same *map*-genotype share identical *baxI*, *hp1*, and *hp4* nucleotide gene sequences, while several SNPs (11 in *baxI*, 18 in *hp1*, 9 in *hp4*) were found distinguishing strains of distinct *map*-genotypes. Most of such SNPs (10 in *baxI*, 11 in *hp1*, 5 in *hp4*) were non-synonymous mutations, determining amino acidic variation in the *in-silico* translated proteins (Figure 1). None of the SNPs produced stop-codon interfering with the protein functionality. Differences observed in the putative effector genes and *in-silico* translated proteins, distinguishing FDp *map*-genotypes, could influence their interaction with hosts and determine their specific ecological niches. Further studies will be conducted to evaluate the genetic diversity of FDp and FDp-related *map*-genotypes in other putative effector genes, and to investigate and clarify the functional role of these putative effectors.

Table 1. Results of PCR-based amplification of *baxI*, *hp1*, and *hp4* genes from 16SrV phytoplasma strains

N. of 16SrV phytoplasma strains	Plant species	*map*-genotype	*baxI*	*hp1*	*hp4*
5	*Vitis vinifera*	M54	+	+	+
1	*Hedera helix*	M54	+	n.d.	n.d.
1	*Robinia pseudoacacia*	M54	+	n.d.	n.d.
1	*Convolvulus arvensis*	M54	+	n.d.	n.d.
3	*Euonymus spp.*	M54	+	-	+
2	*Acer spp.*	M54	+	+	+
1	*Ailanthus altissima*	M54	-	+	-
3	*Ailanthus altissima*	M51	+	+	+
1	*Euonymus spp.*	M51	+	+	+
1	*Quercus spp.*	M51	+	+	+
1	*Alnus glutinosa*	M50	+	-	-
3	*Ailanthus altissima*	M50	+	+	+
1	*Ailanthus altissima*	M43	+	+	-
1	*Alnus glutinosa*	M43	+	+	-
1	*Alnus glutinosa*	M39	+	+	-

Figure 1. Single nucleotide polymorphisms and amino acidic variations in *baxI*, *hp1*, and *hp4* genes within analyzed FDp and FDp-related *map*-genotypes



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