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Molecular characterization of 'bois noir' phytoplasma populations from North-Eastern Italy

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Abstract

Genetic diversity of 'bois noir' phytoplasma populations in two vineyards of Verona province (Veneto region, North-Eastern Italy) was investigated by the use of multilocus analysis of gene sequences amplified in the polymerase chain reaction. Based on results from restriction fragment length polymorphism profiling and nucleotide sequence alignments of *16S rRNA*, *tuf*, *hlyC*, *trxA-truB*, *cbiQ-glyA*, and *rplS-csdB* genes, 'bois noir' phytoplasma strains were grouped in five SNP lineages. The data suggest that strain composition of BN phytoplasma populations may be modified by ecological relationships in vineyards.

Key words: multiple gene analysis, PCR, RFLP, stolbur.

Introduction

'Bois noir' (BN) is a grapevine yellows disease associated with infection by stolbur phytoplasma. Its biological complexity, indicated by the existence of numerous plant hosts and diverse insect vectors, has stimulated studies of molecular markers useful for researching genetic diversity among BN phytoplasma strains. Recently, an impressive diversity of BN phytoplasma strains was revealed by characterization of *vmp1* and *secY* genes (Filippin *et al*., 2009; Pacifico *et al*., 2009).

In the present work, we explored the genetic diversity of BN phytoplasma populations in two vineyards of North-Eastern Italy by use of nucleotide sequence and RFLP analyses of *16S rRNA*, *tuf*, *hlyC*, *trxA-truB*, *cbiQ-glyA*, and *rplS-csdB* genes amplified in polymerase chain reaction (PCR) assays.

Materials and methods

Leaf samples were collected from 20 symptomatic grapevine plants in two vineyards [San Pietro di Lavagno (1) and Ronco all'Adige (2)] of Veneto region, North-Eastern Italy (table 1).

Nested-PCR assays were carried out for amplifying the genes *16S rRNA* [primers R16F2n/R2 (Gundersen and Lee, 1996)], *tuf* [primers fTufAY/rTufAY (Schneider *et al*., 1997)], *hlyC*, *trxA-truB*, *cbiQ-glyA*, and *rplS-csdB* [designed on the basis of phytoplasma sequences deposited in GenBank by Cimerman *et al.* (2006)].

PCR products were digested by using restriction enzymes having recognition sites that included SNPs previously identified (Quaglino *et al*., 2009), distinguishing among BN phytoplasma strains. The enzymes used (and gene analyzed) were: *Alu*I, *Bfa*I, *Bst*UI, and *Mse*I (*16S rDNA*); *Hpa*II (*tuf*); *Ssp*I (*hlyC*), *Bsa*HI (*trxA-truB*), *Hpy*188I (*cbiQ-glyA*), and *Hpy*CH4V (*rplS-csdB*).

BN phytoplasma SNP genetic lineages were identified by comparisons of collective RFLP patterns.

Results and discussion

RFLP patterns of 16S rDNA amplicons indicated that all plants were infected by BN phytoplasma strains of subgroup 16SrXII-A.

For each of the other gene amplicons it was possible to identify two distinct RFLP profiles, previously reported also for BN phytoplasma strains in Lombardy region, north-western Italy (Quaglino *et al*., 2010). Strain types based on RFLP patterns of gene *tuf* were consistent with those based on the gene *hlyC*. RFLP patterns of the other genes were not consistent with *tuf-hlyC* strain types.

Based on collective RFLP patterns from all the analyzed genes, BN phytoplasma strains were grouped in five SNP genetic lineages, named BN4, BN6, BN8, BN9, and BN10 (table 1). Lineages BN4 and BN6 were identified also within BN phytoplasma populations in North-Western Italy (Quaglino *et al*., 2010); lineages BN8, BN9 and BN10 were identified exclusively in North-Eastern Italy. Lineages BN8 (1 strain) and BN9 (one strain) were identified only in the vineyard of San Pietro di Lavagno, while lineage BN10 (five strains) were observed only in the vineyard of Ronco all'Adige. Lineages BN6 (11 strains) and BN4 (two strains) were present in both vineyards (table 1).

Table 1. SNP lineages in multiple genetic *loci* of BN phytoplasma strains from vineyards in north-eastern Italy.

Strain	Vineyard	Grapevine cultivar	16S rDNA *Mse*I	*Tuf*-type *Hpa*II	*hlyC*-type *Ssp*I	*cbiQ-glyA** *Hpy*188I	*trxA-truB** *Bsa*HI	*rplS-csdB** *Hpy*CH4V	Lineage of strain
VR456	1	Chardonnay	XII-A	tuf-a	hlyC-a	B	A	A	BN8
VR460			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR461			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR462			XII-A	tuf-b	hlyC-b	A	B	A	BN9
VR464			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR466			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR475			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR477			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR481			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR501			XII-A	tuf-b	hlyC-b	A	B	B	BN4
VR502	2	Chardonnay	XII-A	tuf-a	hlyC-a	B	A	A	BN10
VR503			XII-A	tuf-a	hlyC-a	B	A	A	BN10
VR507			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR509			XII-A	tuf-b	hlyC-b	A	B	B	BN4
VR510			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR511			XII-A	tuf-a	hlyC-a	B	B	A	BN6
VR512			XII-A	tuf-a	hlyC-a	B	A	A	BN10
VR513			XII-A	tuf-a	hlyC-a	B	A	A	BN10
VR514			XII-A	tuf-a	hlyC-a	B	A	A	BN10
VR516			XII-A	tuf-a	hlyC-a	B	B	A	BN6

*, identical letter = identical profile.

Conclusions

Presence of certain lineages in one and not another vineyard is possibly explained by differences in the ecology of vineyards and/or surrounding areas that influence the composition of BN phytoplasma populations through strain selection. Future research, involving larger sample sizes, will focus on genetic diversity of BN phytoplasma populations from several geographic regions, as well as on BN lineages in grapevines, insects, and weeds in vineyards. The new knowledge should aid understanding of BN epidemics and open new avenues for developing innovative approaches for BN disease management.

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