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Uneven distribution of stolbur phytoplasma in Italian grapevines as revealed by nested-PCR

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Abstract

The distribution and persistence of stolbur phytoplasmas were investigated in grapevines affected by "bois noir" (BN). The phytoplasma was detected using nested-PCR from different plant tissues. In summer it was more frequently detected in symptomatic leaf samples (96.8%), whereas only a low proportion of positive samples was obtained from symptomless leaf samples (12.5%). The analysis of phloem samples from symptomatic and asymptomatic shoots, only revealed a positive reaction (11.1%) with the former. In the same BN-affected grapevines checked during winter, only a low proportion of dormant cane (19%), cordon (8.3%) and root (9%) samples were PCR-positive. Moreover, the pathogen was never detected in trunk or in leaf samples collected from recovered grapevines. The present data indicate an uneven distribution of stolbur in infected grapevines and that the phytoplasma seldom persistently infect plants from year to year.

Key words: Stolbur phytoplasma, PCR detection, grapevine, distribution, persistence.

Introduction

"Bois noir" (BN), one of the most important grapevine yellows in Europe, is induced by a phytoplasma belonging to the stolbur (16SrXII-A) ribosomal group (Lee *et al*., 1998). The phytoplasma is vectored by the polyphagous planthopper *Hyalesthes obsoletus* Signoret from vineyard weeds to grapevines (Maixner *et al.,* 1995; Sforza *et al*., 1998).

BN occurs, often with high incidence and severe economic problems, in all Italian viticultural areas including those of Emilia-Romagna (Credi *et al*., 2001). Symptoms are evident in summer-fall and affected grapevines may display a systemic or local distribution. Persistent disease expression year after year has been observed, but symptom remission can also occur in *Vitis vinifera* cultivars (Credi, 1989).

Little is known about most of the host-pathogen interactions due to lack of investigation. For example, phytoplasma distribution and persistence in symptomatic and recovered grapevines. For this reason, a specific study was setup. The current results are reported here and their significance discussed.

Materials and methods

For the study, 23 grapevines of cultivars Ancellotta, Lambrusco Salamino, Sangiovese and Trebbiano Romagnolo, from different vineyards located in Emilia-Romagna (northern Italy), were examined. All selected grapevines had a recorded BN history over the 2003-2005 period. On sampling, 17 plants were diseased showing local or systemic symptom presence. Six plants were classified as recovered and had been stolbur-positive the previous summer. Grapevines were trained to the cordon system with Sylvoz or spur pruning and their age ranged from 4 and 35 years.

To determine phytoplasma location within grapevines during the summer and the subsequent winter, the canopy of each plant was usually divided into sections and leaf, shoot, dormant cane and cordon samples were taken. Ten grapevines were also sampled from root and trunk tissues. Vascular tissue was randomly collected from these single plant parts and pooled into one sample. Stolbur distribution was investigated in more detail using 5 grapevine plants that showed BN symptoms with a typical local distribution. These plants had also exhibited BN during the previous two vegetative seasons. In September, 2 to 4 shoots samples were selected from each cordon, alternatively with and without leaf symptoms. Similarly, leaf samples collected from 3 single shoots of each recovered grapevine was tested. Grapevines with a systemic distribution of the disease were assessed with leaf sample from some shoots with obvious symptoms.

Total DNA was extracted from leaf veins and petioles, scrapings of vascular tissue from shoots, dormant canes, roots, cordons and trunks, according to Marzachì *et al*. (1999). A separate sterile scalpel blade was used for each sample to avoid contamination. Samples were then screened using the universal primer pair P1/P7 in direct PCR, and the primer pair fStol/rStol in nested PCR to amplify a specific target sequence of the stolbur phytoplasma (Maixner *et al.,* 1995). After amplification each sample was electrophoresed in a 1% agarose gel; products were then stained with ethidium bromide and visualized by UV transillumination.

Results

In the BN-diseased grapevines selected for stolbur distribution and persistence studies, the phytoplasma was successfully detected by the nested-PCR technique. However, not all plants were positive in every sampling period. For example, symptomatic leaf samples of all the 23 grapevines tested in September were stolbur-positive. On the contrary, just a low proportion of grapevines were positive, 6/12, in the January sampling.

Four of these contained the phytoplasma in a dormant cane, whereas the fifth and the sixth appeared to be infected at the root and cordon levels, respectively.

None of the grapevines showing typical BN symptoms were phytoplasma-positive in all tissue types at the same sampling period. For instance, a very high proportion (31/32) of symptomatic leaf samples were positive, whereas only a very low proportion was obtained (2/16) with leaves collected from asymptomatic shoots.

Results of the molecular assays performed on the 5 grapevines with a local distribution of the disease along the cordons were as follows: all leaf samples collected from single symptomatic shoots were positive (9/9), whereas none of 8 leaf samples from single asymptomatic shoots was found to be infected. When phloem samples were analysed from the same shoots, a positive reaction was only obtained from the symptomatic ones. None of the symptomless shoots appeared to be infected by the phytoplasma.

In January, a very low proportion of stolbur-positive samples was obtained from dormant canes (4/21), cordons (1/12) and roots (1/11). On the other hand, the phytoplasma was never detected in samples from trunk (0/5) tissues.

Finally, all the recovered grapevines employed to verify the presence of stolbur phytoplasmas were found to be phytoplasma-free. Thus, none of the 18 leaf samples collected in September from separate shoots showed a positive reaction.

Discussion

Nested-PCR assays for stolbur phytoplasmas were used to investigate its distribution and persistence in grapevines affected by BN.

At summer sampling, the highest proportion of phytoplasma presence was revealed amongst the symptomatic leaf samples. Only a few symptomless leaf samples tested positive. In shoots from the same BN-diseased grapevines, the pathogen was only detected in a phloem sample from an obviously affected shoot.

At winter sampling, the proportion of positive reactions markedly decreased: stolbur was only detected in some dormant canes in a root and in a cordon sample. In addition, phytoplasma infection was not demonstrated in other tissue types such as trunks.

Molecular analyses performed on some recovered grapevines severely affected and PCR-positive the previous summer, showed that none of the leaf samples contain the phytoplasma.

These results, although preliminary, strongly suggest that stolbur is unevenly distributed, seldom spreading systemically throughout grapevines and rarely infecting them persistently from year to year. This is in agreement with the low percentage of BN transmission by grafting verified using dormant cuttings from diseased grapevines (Osler *et al*., 2002; Credi *et al*., unpublished). Hovewer the symptoms remission may be due related to the disappearance of the phytoplasma from grapevines.

Seasonal distribution of grapevine phytoplasmas has been also studied in Australia (Constable *et al*., 2003). However, the results obtained there appear to differ from our observations. A possible explanation is that different experimental conditions may influence the mechanism behind the movement of these pathogens in infected plants. The detectable levels of phytoplasma in different tissues of individual grapevines may also fluctuate throughout the year and from season to season. In conclusion, further work needs to be done to improve our knowledge of these important aspects.

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References

CONSTABLE F. E., GIBB K. S., SYMONS R. H., 2003.- Seasonal distribution of phytoplasmas in Australian grapevines.- *Plant Pathology*, 52: 267-276.

CREDI R., 1989.- Flavescenza dorata della vite in Emilia Romagna: evoluzione della malattia nelle piante e suoi effetti sulla produzione e sullo sviluppo vegetativo.- *Phytopatologia Mediterranea*, 28: 113-121.

CREDI R., TERLIZZI F., BISSANI R., POGGI POLLINI C., 2001.- Presenza e diffusione dei fitoplasmi del legno nero e della flavescenza dorata della vite in Emilia-Romagna.- *Vignevini*, 28 (12): 107-110.

LEE I.-M., GUNDERSEN D. E., DAVIS R. E., BARTOSZYK I. M., 1998.- Revised classification scheme of phytoplasma based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences.- *International Journal of Systemic Bacteriology*, 48: 1153-1169.

MARZACHÌ C., ALMA A., D’ACQUILIO M., MINUTO G., BOCCARDO G., 1999.- Detection and identification of phytoplasmas infecting cultivated and wild plants in Liguria (Italian Riviera).- *Journal of Plant Pathology*, 81 (2): 127-136.

MAIXNER M., AHRENS U., SEEMÜLLER E., 1995.- Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure.- *European Journal of Plant Pathology*, 101 (3): 241-250.

OSLER R., ZUCCHETTO C., CARRARO L., FRAUSIN C., PAVAN F., VETTORELLO G., GIROLAMI V., 2002.- Trasmissione di flavescenza dorata e legno nero e comportamento delle viti infette.- *L’Informatore Agrario*, 58 (19): 61-65.

SFORZA R., CLAIR D., DAIRE X., BOUDON-PADIEU E., 1998.- The role of *Hyalesthes obsoletus* (Hemiptera: Cixiidae) in the occurrence of bois noir of grapevine in France.- *Journal of Phytopathology*, 146: 549-556.

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