COST Action FA0807 Integrated Management of Phytoplasma Epidemics in Different Crop Systems Short-term Scientific Mission (STSM) Report

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Purpose of the visit
The topics of this visit were: A. to introduce infected shoots of grapevines to *in vitro* culture in order to start nurse cultures of phytoplasma *in vitro*. B. to detect phytoplasma in plant phloem using FISH staining.

Description and results of the work:

Nurse culture: Shoots with yellows symptom from grapevines cv. Chardonnay, Pinot noir and Barbera were used for the experiment. The shoots were collected from vineyards in Oltrepò pavese (Pavia province) area ca. 70 km south of Milan and kept in plastic bags and in ice. In the lab, the shoots were surface sterilized and nodal sections of one bud and no leaves were planted in Woody Plant Medium (WPM) supplemented with activated charcoal and PPM ®. From each variety 15-20 nodes were planted. No contamination was observed in the tubes. After few days the shoots started to sprout. Each shoot will be further sub-cultured separately as a clone and will be tested for phytoplasma presence.

Detection of phytoplasma using FISH staining: in order to transfer phytoplasma from an infected plant to a healthy plant two kinds of micrografing were performed *in vitro*. The experiment was performed using plants of grapevines cv. Chardonnay and Cabernet-Sauvignon. The source plantlets *in vitro* were taken from a nurse culture clone PCR positive to GY, whereas the source plants from the vineyard were heavily symptomatic to GY phytoplasma: 1. Nodal section with one bud (no leaves) served as a scion and was grafted into a solid callus. The scion was taken from an infected plantlet and the callus was grown from a healthy plantlet. 2. Nodal section with one bud (no leaves) from a healthy plantlet served as a scion and was grafted on a nodal stem section (with no bud) from an infected plant. The grafts were grown on WPM supplemented with activated charcoal and PPM® in a controlled growth room under 16/8h day/night photoperiod and 25^{O}C. After 2 and 3 weeks of growth (stem grafting

and callus grafting respectively) the samples were hand sectioned longitudinally and fixated in 95% ethanol: 5% acetic and kept in 100% ethanol until staining. In the host lab, thinner hand and microtome sections of longitudinal and transverse direction were further performed. The FISH staining was performed according to the protocol of Bulgari et al. (2011), using probes for stolbur type phytoplasma. The stained samples were observed under confocal microscopy. The presence of phytoplasma was confirmed only when cells were fluorescent under a FISH beam and not under a DAPI beam. Using bright field microscopy analysis, we observed vascular contact between scion and callus as a stock (fig. 1a). We also detected phytoplasma cells in infected scion sections (fig 1b) and in the vascular area in the callus tissues (fig 1c). Previous study showed that vascular cells including xylem and phloem differentiate in a callus following micrografting in vitro. However, we could not detect phytoplasma in the scion of grafts made between the nodal stem sections. We hypothesize that the growth period following micro-grafting and fixation was too short.

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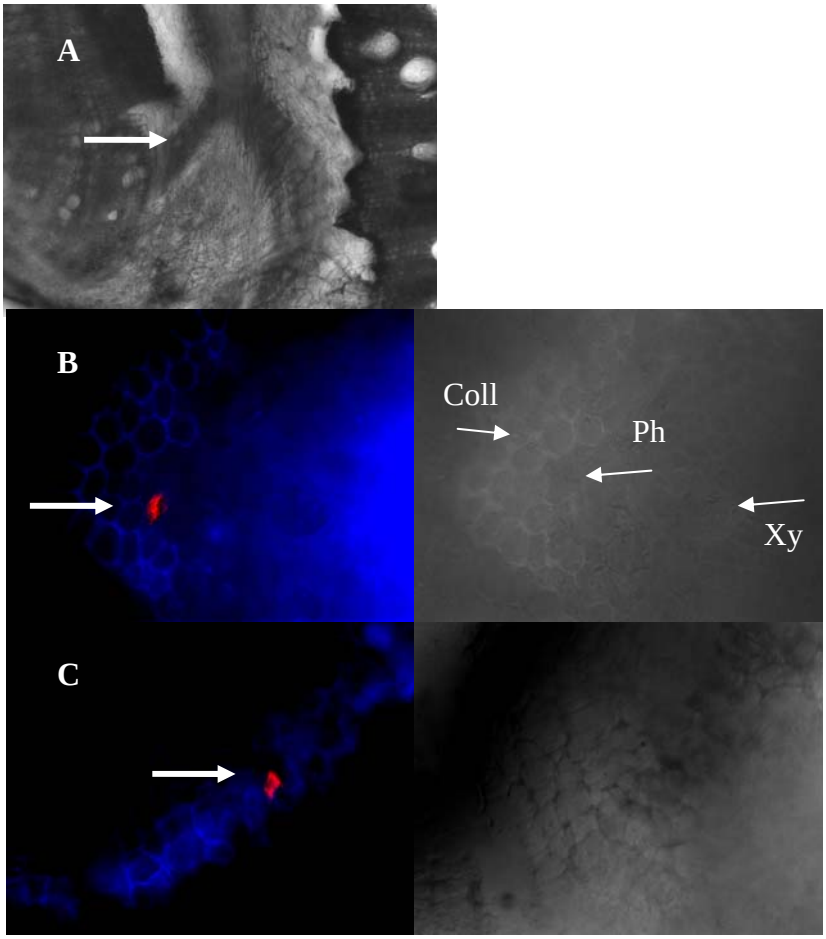

Figure 1. Transverse hand sections from micro-grafted plant X40. Phytoplasma is seen as red fluorescence under confocal microscopy (blue- autoflurescnce of thick cell walls. A. Graft zone between callus and scion (arrow points at the connection of the

vascular tissue between callus and scion. B. phytoplasma in a phloem cell in a stock, (left-confocal microscopy, right - brightfield microscopy); ph-phloem, Coll-colenchyma. Xy-xylem C. phytoplasma in callus tissue, (left-confocal microscopy, right - brightfield microscopy).