

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

STSM Grantee: Christina Mortada , christina.mortada@hotmail.com

Department of plant protection at Lebanese Agricultural Research Institute (IRAL),
Talamara, Rayak, Bekaa, Lebanon.

STSM Host: Dr. Xavier Foissac, foissac@bordeaux.inra.fr

UMR1332 Biologie du fruit et Pathologie

INRA and University of Bordeaux (France)

STSM Topic: Survey of bois noir epidemics: Analysis of the genetic diversity of bois noir phytoplasma isolates

Reference codes: STSM: COST-STSM-FA0807-10012

Grant letter: COST-STSM-ECOST-STSM-FA0807-050312-015565

STSM dates: 05 March 2012 to 30 March 2012

Purpose of the visit

The topic of my visit to INRA was the molecular characterization of phytoplasmas infecting vineyards in Bekaa. I was aiming to acquire competence in the techniques used for molecular characterization, such as Nested PCR and RFLP analysis, and to learn the use of bioinformatics tools for sequencing and phylogenetic analysis.

Description of the work

DNA was extracted from 220 samples, of which 51 were collected in 2010 and 169 were collected in 2011 from grapevine in the Bekaa region and samples were shipped to the INRA laboratories. All previously proved to display Bois Noir symptoms. Reference isolates were from the Collection of INRA-Bordeaux

Tab.1: Samples collected in 2010

| running nb. | extraction date | running nb. | extraction date |
|-------------|-----------------|-------------|-----------------|
| G1 | 2010 | G32 | 2010 |
| G10 | 2010 | G33 | 2010 |
| G11 | 2010 | G34 | 2010 |
| G12 | 2010 | G35 | 2010 |
| G13 | 2010 | G36 | 2010 |
| G14 | 2010 | G37 | 2010 |
| G15 | 2010 | G38 | 2010 |
| G16 | 2010 | G39 | 2010 |
| G17 | 2010 | G4 | 2010 |
| G18 | 2010 | G40 | 2010 |
| G19 | 2010 | G41 | 2010 |
| G2 | 2010 | G42 | 2010 |
| G20 | 2010 | G43 | 2010 |
| G21 | 2010 | G44 | 2010 |
| G22 | 2010 | G45 | 2010 |
| G23 | 2010 | G46 | 2010 |
| G24 | 2010 | G47 | 2010 |
| G25 | 2010 | G48 | 2010 |
| G26 | 2010 | G49 | 2010 |
| G27 | 2010 | G5 | 2010 |

| | | | |
|-----|------|-----|------|
| G28 | 2010 | G50 | 2010 |
| G29 | 2010 | G51 | 2010 |
| G3 | 2010 | G6 | 2010 |
| G30 | 2010 | G7 | 2010 |
| G31 | 2010 | G8 | 2010 |
| | | G9 | 2010 |

Tab.2: Samples collected in 2012

| running nb. | Isolate code | extraction date | sampling site | running nb. | Isolate code | extraction date | sampling site |
|-------------|--------------|-----------------|---------------|-------------|--------------|-----------------|---------------|
| 1 | F16 P3 | 6.10.2011. | TalDnoub | 84 | F7 P30 | 25.10.2011 | TalDnoub |
| 2 | F15 P15 | 6.10.2011. | TalDnoub | 85 | F7 P17 | 25.10.2011 | TalDnoub |
| 3 | F19 P112 | 6.10.2011. | TalDnoub | 86 | F5 P2 | 25.10.2011 | TalDnoub |
| 4 | F21 P5 | 6.10.2011. | TalDnoub | 87 | F5 PP26 | 25.10.2011 | TalDnoub |
| 5 | F17 P181 | 6.10.2011. | TalDnoub | 88 | F9 P7 | 25.10.2011 | TalDnoub |
| 6 | F16 P178 | 6.10.2011. | TalDnoub | 89 | F11 P68 | 25.10.2011 | TalDnoub |
| 7 | F16 P149 | 6.10.2011. | TalDnoub | 90 | F12 P152 | 25.10.2011 | TalDnoub |
| 8 | F22 P40 | 6.10.2011. | TalDnoub | 91 | F9 P14 | 26.10.2011 | TalDnoub |
| 9 | F71 P20 | 6.10.2011. | Kanafar | 92 | F9 P40 | 26.10.2011 | TalDnoub |
| 10 | F22 P191 | 6.10.2011. | TalDnoub | 93 | F12 P70 | 26.10.2011 | TalDnoub |
| 11 | F15 P35 | 12.10.2011 | TalDnoub | 94 | F14 P17 | 26.10.2011 | TalDnoub |
| 12 | F17 P191 | 12.10.2011 | TalDnoub | 95 | F67 P47 | 26.10.2011 | Kanafar |
| 13 | F17 P14 | 12.10.2011 | TalDnoub | 96 | F2 P170 | 26.10.2011 | TalDnoub |
| 14 | F13 P186 | 12.10.2011 | TalDnoub | 97 | F6 P145 | 26.10.2011 | TalDnoub |
| 15 | F17 P4 | 12.10.2011 | TalDnoub | 98 | F15 P188 | 26.10.2011 | TalDnoub |
| 16 | F19 P113 | 12.10.2011 | TalDnoub | 99 | F3 P38 | 26.10.2011 | TalDnoub |
| 17 | F17 P13 | 12.10.2011 | TalDnoub | 100 | F3 P18 | 26.10.2011 | TalDnoub |
| 18 | F14 P73 | 12.10.2011 | TalDnoub | 101 | F1 P2 | 27.10.2011 | TalDnoub |
| 19 | F23 P13 | 12.10.2011 | TalDnoub | 102 | F5 P41 | 27.10.2011 | TalDnoub |
| 20 | F17 P7 | 12.10.2011 | TalDnoub | 103 | F5 P107 | 27.10.2011 | TalDnoub |
| 21 | F24 P116 | 13.10.2011 | TalDnoub | 104 | F3 P4 | 27.10.2011 | TalDnoub |
| 22 | F15 P188 | 13.10.2011 | TalDnoub | 105 | F83 P1 | 27.10.2011 | Kanafar |
| 23 | F2 P154 | 13.10.2011 | TalDnoub | 106 | F3P7 | 27.10.2011 | TalDnoub |
| 24 | F24 P95 | 13.10.2011 | TalDnoub | 107 | F13 P5 | 27.10.2011 | TalDnoub |
| 25 | F24 P15 | 13.10.2011 | TalDnoub | 108 | F3 P11 | 27.10.2011 | TalDnoub |
| 26 | F18 P131 | 13.10.2011 | TalDnoub | 109 | F1 P60 | 27.10.2011 | TalDnoub |
| 27 | F13 P86 | 13.10.2011 | TalDnoub | 110 | F15 P25 | 27.10.2011 | TalDnoub |
| 28 | F13 P122 | 13.10.2011 | TalDnoub | 111 | F3 P150 | 27.10.2011 | TalDnoub |
| 29 | F14 P33 | 13.10.2011 | TalDnoub | 112 | F3 P49 | 27.10.2011 | TalDnoub |
| 30 | F20 P8 | 13.10.2011 | TalDnoub | 113 | F8 P145 | 27.10.2011 | TalDnoub |
| 31 | | 17.10.2011 | ? | 114 | F13 P113 | 27.10.2011 | TalDnoub |
| 32 | F65 P70 | 17.10.2011 | Kanafar | 115 | F19 P13 | 27.10.2011 | TalDnoub |
| 33 | F2 P160 | 17.10.2011 | TalDnoub | 116 | F17 P59 | 27.10.2011 | TalDnoub |
| 34 | F2 P146 | 17.10.2011 | TalDnoub | 117 | F13 P44 | 27.10.2011 | TalDnoub |
| 35 | F9 P118 | 17.10.2011 | Kanafar | 118 | F15 P151 | 27.10.2011 | TalDnoub |
| 36 | F1 P99 | 17.10.2011 | TalDnoub | 119 | F18 P33 | 27.10.2011 | TalDnoub |
| 37 | F17 P25 | 17.10.2011 | TalDnoub | 120 | F22 P178 | 27.10.2011 | TalDnoub |
| 38 | F67 P47 | 17.10.2011 | Kanafar | 121 | F13 P25 | 28.10.2011 | TalDnoub |

| | | | | | | | |
|----|----------|------------|----------|-----|----------|------------|----------|
| 39 | F69 P19 | 17.10.2011 | Kanafar | 122 | F17 P117 | 28.10.2011 | TalDnoub |
| 40 | F66 P88 | 17.10.2011 | Kanafar | 123 | F5 P17 | 28.10.2011 | TalDnoub |
| 41 | F66 P101 | 19.10.2011 | Kanafar | 124 | F1 P79 | 28.10.2011 | TalDnoub |
| 42 | F65 P5 | 19.10.2011 | Kanafar | 125 | F13 P190 | 28.10.2011 | TalDnoub |
| 43 | F11 P25 | 19.10.2011 | TalDnoub | 126 | F23 P93 | 28.10.2011 | TalDnoub |
| 44 | F79 P22 | 19.10.2011 | Kanafar | 127 | F15 P88 | 28.10.2011 | TalDnoub |
| 45 | F78 P32 | 19.10.2011 | Kanafar | 128 | F13 P41 | 28.10.2011 | TalDnoub |
| 46 | F78 P24 | 19.10.2011 | Kanafar | 129 | F4 P30 | 28.10.2011 | TalDnoub |
| 47 | F77 P44 | 19.10.2011 | Kanafar | 130 | F3 P73 | 28.10.2011 | TalDnoub |
| 48 | F72 P33 | 19.10.2011 | Kanafar | 131 | F733 P3 | 29.10.2011 | Kanafar |
| 49 | F72 P85 | 19.10.2011 | Kanafar | 132 | F80 P90 | 29.10.2011 | Kanafar |
| 50 | F72 P97 | 19.10.2011 | Kanafar | 133 | F78 P6 | 29.10.2011 | Kanafar |
| 51 | F72 P67 | 20.10.2011 | Kanafar | 134 | F78 P30 | 29.10.2011 | Kanafar |
| 52 | F70 P5 | 20.10.2011 | Kanafar | 135 | F73 P70 | 29.10.2011 | Kanafar |
| 53 | F67 P18 | 20.10.2011 | Kanafar | 136 | F73 P21 | 29.10.2011 | Kanafar |
| 54 | F65 P61 | 20.10.2011 | Kanafar | 137 | F66 P88 | 29.10.2011 | Kanafar |
| 55 | F76 P51 | 20.10.2011 | Kanafar | 138 | F79 P2 | 29.10.2011 | Kanafar |
| 56 | F76 P51 | 20.10.2011 | Kanafar | 139 | F76 P31 | 29.10.2011 | Kanafar |
| 57 | F80 P12 | 20.10.2011 | Kanafar | 140 | F73 P20 | 29.10.2011 | Kanafar |
| 58 | F65 P16 | 20.10.2011 | Kanafar | 141 | F66 P30 | 31.10.2011 | Kanafar |
| 59 | F75 P69 | 20.10.2011 | Kanafar | 142 | F66 P35 | 31.10.2011 | Kanafar |
| 60 | F75 P28 | 20.10.2011 | Kanafar | 143 | F77 P3 | 31.10.2011 | Kanafar |
| 61 | F70 P8 | 22.10.2011 | Kanafar | 144 | F67 P4 | 31.10.2011 | Kanafar |
| 62 | F78 P16 | 22.10.2011 | Kanafar | 145 | F78 P12 | 31.10.2011 | Kanafar |
| 63 | F78 P25 | 22.10.2011 | Kanafar | 146 | F21 P124 | 31.10.2011 | TalDnoub |
| 64 | F84 P6 | 22.10.2011 | Kanafar | 147 | F82 P23 | 31.10.2011 | Kanafar |
| 65 | F83 P72 | 22.10.2011 | Kanafar | 148 | F18 P180 | 31.10.2011 | TalDnoub |
| 66 | F84 P82 | 22.10.2011 | Kanafar | 149 | F81 P6 | 31.10.2011 | Kanafar |
| 67 | F74 P81 | 22.10.2011 | Kanafar | 150 | F81 P3 | 31.10.2011 | Kanafar |
| 68 | F80 P34 | 22.10.2011 | Kanafar | 151 | F24 P183 | 1.11.2011 | TalDnoub |
| 69 | F70 P55 | 22.10.2011 | Kanafar | 152 | F83 P91 | 1.11.2011 | Kanafar |
| 70 | F68 P47 | 22.10.2011 | Kanafar | 153 | F84 P17 | 1.11.2011 | Kanafar |
| 71 | F65 P26 | 24.10.2011 | Kanafar | 154 | F68 P102 | 1.11.2011 | Kanafar |
| 72 | F84 P77 | 24.10.2011 | Kanafar | 155 | F83 P89 | 1.11.2011 | Kanafar |
| 73 | F70 P70 | 24.10.2011 | Kanafar | 156 | F84 P86 | 1.11.2011 | Kanafar |
| 74 | F77 P64 | 24.10.2011 | Kanafar | 157 | F69 P70 | 1.11.2011 | Kanafar |
| 75 | F84 P56 | 24.10.2011 | Kanafar | 158 | F70 P31 | 1.11.2011 | Kanafar |
| 76 | F67 P17 | 24.10.2011 | Kanafar | 159 | F71 P96 | 1.11.2011 | Kanafar |
| 77 | F67 P63 | 24.10.2011 | Kanafar | 160 | F72 P95 | 1.11.2011 | Kanafar |
| 78 | F84 P44 | 24.10.2011 | Kanafar | 161 | F81 P47 | 1.11.2011 | Kanafar |
| 79 | F82 P47 | 24.10.2011 | Kanafar | 162 | F72 P34 | 1.11.2011 | Kanafar |
| 80 | F65 P75 | 24.10.2011 | Kanafar | 163 | F71 P40 | 1.11.2011 | Kanafar |
| 81 | F66 P27 | 25.10.2011 | Kanafar | 164 | F72 P57 | 1.11.2011 | Kanafar |
| 82 | F71 P38 | 25.10.2011 | Kanafar | 165 | F70 P90 | 1.11.2011 | Kanafar |
| 83 | F71 P24 | 25.10.2011 | Kanafar | 167 | F69 P48 | 1.11.2011 | Kanafar |
| | | | | 169 | F71 P1 | 1.11.2011 | Kanafar |
| | | | | 171 | F69 P77 | 1.11.2011 | Kanafar |
| | | | | 172 | F68 P21 | 1.11.2011 | Kanafar |

First of all, the presence of phytoplasma infection was checked with Nested PCR with STAMP (stamp F/R0 and stamp F1/R1) (Fabre *et al.*, 2011). Stamp is a gene encoding the antigenic membrane protein of stolbur phytoplasma.

Tab.3: Results of samples with nested-PCR with stamp

| | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| | | + | | + | | + | + | + | | | |
| G13 | G14 | G15 | G16 | G17 | G18 | G19 | G20 | G21 | G22 | G23 | G24 |
| + | | | | | + | | | | | | |
| G25 | G26 | G27 | G28 | G29 | G30 | G31 | G32 | G33 | G34 | G35 | G36 |
| + | | | | | | | | | | | |
| G37 | G38 | G39 | G40 | G41 | G42 | G43 | G44 | G45 | G46 | G47 | G48 |
| | | | | | | + | | | | | |
| G49 | G50 | G51 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| | | | | + | + | + | + | | + | + | + |
| 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| + | + | + | + | + | + | + | + | + | + | + | + |
| 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 |
| + | + | + | + | | + | + | | + | + | | + |
| 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 |
| + | + | + | + | + | + | + | | | + | | |
| 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 |
| + | | | | | + | | | + | + | | + |
| 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 |
| | + | | | | + | | + | | | | + |
| 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 |
| + | + | | + | + | | + | | | + | + | + |
| 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 |
| | | + | + | | + | | + | + | + | | + |
| 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | 103 | 104 | 105 |
| + | + | | + | | + | + | + | + | + | + | |
| 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 |
| | + | + | | | + | | | + | + | + | + |
| 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 |
| | + | | + | + | + | + | + | + | | + | |
| 130 | 131 | 132 | 133 | 134 | 135 | 136 | 137 | 138 | 139 | 140 | 141 |
| + | | + | + | + | + | | + | | | | + |
| 142 | 143 | 144 | 145 | 146 | 147 | 148 | 149 | 150 | 151 | 152 | 153 |
| | + | + | + | | | | + | + | | + | |
| 154 | 155 | 156 | 157 | 158 | 159 | 160 | 161 | 162 | 163 | 164 | 165 |
| + | + | + | + | | + | | + | | | + | + |
| 167 | 169 | 171 | 172 | O1 | O2 | O2 | PS | LG | H2O | | |
| + | + | | + | + | | | | ++ | | | |

Once we had positive results, molecular characterization of the isolates was performed on 4 different non-ribosomal genes: *tuf*, *secY*, *vmp1* (Cimerman *et al.*, 2009; Fialova *et al.*, 2009), and *vmp3* (Foissac, unpublished). Amplification of the four non-ribosomal genes was carried out by nested PCR with the primers described in table 4.

Tab.4: Primer sets for the amplification and sequencing of *vmp1*, *secY*, *tuf* and *vmp3*

| Gene | | Primer sets | Annealing temperature for nested- PCR condition |
|-------|------------|---|---|
| Stamp | 1st PCR | StampF / StampR0 (Fabre et al., 2011) | 52°C/30s |
| | Nested PCR | StampF1/ StampR1 (Fabre et al., 2011) | |
| Vmp1 | 1st PCR | STOLH10F1 / STOLH10R1 (Foissac et al, unpublished) | 55°C/30s |
| | Nested PCR | TYPH10F / TYPH10R (Foissac et al, unpublished) | |
| SecY | 1st PCR | PosecF1 / PosecR1 (Fialova et al., 2009) | 52°C/30s |
| | Nested PCR | Posec N2 / Posec R3 (Foissac et al., unpublished) | |
| Tuf | 1st PCR | ftuf1 / rtuf1 () | 55°C/30s |
| | Nested PCR | ftufAY / rtufstol | |
| | | (Langer and Maixner, 2004; Foissac and Fabre unpublished) | |
| Vmp3 | 1st PCR | vmp3-F5 /vmp3-R3 | 57°C/30s |
| | Nested PCR | vmp3-R4 /vmp3-R3 (Salar and Foissac, unpublished) | |

Description of the main results:

Samples that tested positives for stamp nested-PCR were tested with *vmp1*. All sample gave the middle size PCR product, except the sample 114 which gave a smaller PCR product.

RsaI-RFLP analysis was performed for samples that were positives with *Vmp1*-nested PCR.

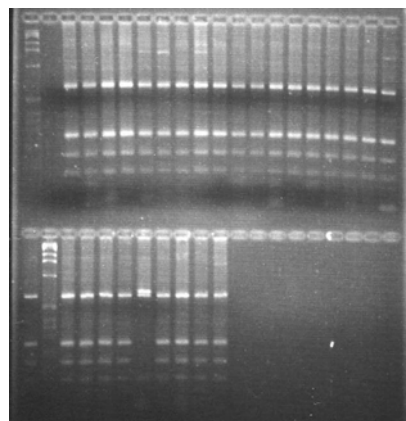


Fig.1: *RsaI*-RFLP of *Vmp1* PCR products

All sample except sample 20 had a RFLP profile identical to that of the reference isolate P7 from Lebanon (profile V15TH). Sample 20 had a PCR profile (V16) different to any of the yet recorded profiles.

Then *vmp1* positive samples were tested with *secY* nested-PCR (Fialova *et al.*, 2009).

Finally *stamp*, *secY* and *vmp1* PCR products of a set of 22 samples were send for sequencing.

These samples were also tested with *tuf*-RFLP and *vmp3* nested-PCR. RFLP analysis of *tuf* with restriction enzyme *HpaII* (Langer and Maixner, 2004) gave a pattern corresponding to *tufB* for all the samples analyzed.

Vmp3 is a variable membrane protein gene with a C-terminal part containing collagen-like (GXY) repeats, the number of which varies from one strain to another: The samples were divided into three different groups based on the size of the *vmp3* PCR product (Fig.2)

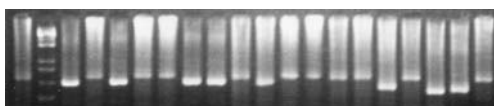


Fig.2: example of *vmp3* nested -PCR

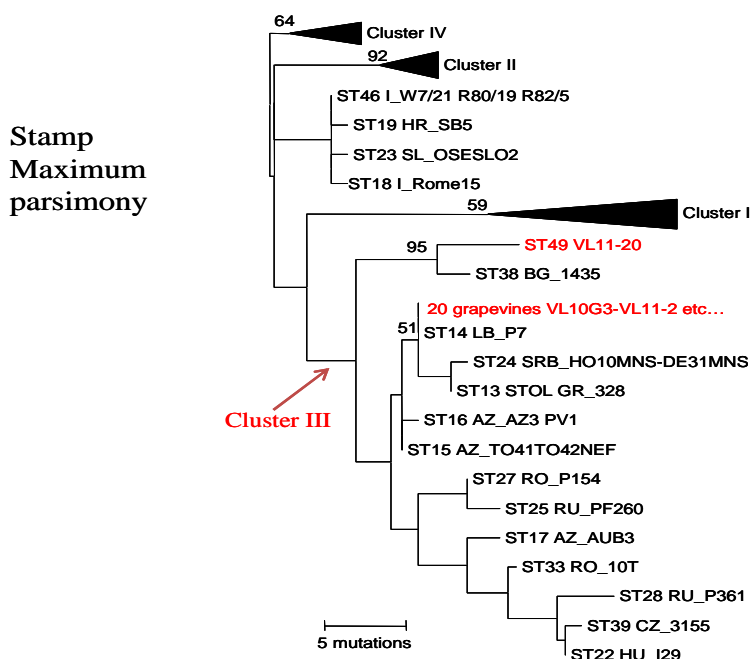
A phylogenetic tree including Lebanese selected samples that were sequenced and reference sequences was generated for each gene. The sequences obtained were assembled and edited with the Staden package (Pregap4 and Gap4) or the Phred-Phrap-Consed package. Alignment of the sequences and reconstruction of phylogenetic trees (maximum parsimony or UPGMA methods) were performed with the Mega5 software.

Stamp:

The phylogenetic tree generated with the maximum parsimony method (fig. 4) grouped the Lebanese isolates in the same cluster III.

All samples had the same stamp sequence as the Lebanese reference isolate P7 (genotype ST14), except the sample 20 which differed from the Bulgarian BG_1435, ST38 genotype and was a new genotype designated ST49.

Fig. 3 Phylogenetic tree generated using maximum parsimony method with Stamp sequences (Fabre *et al.*, 2011).



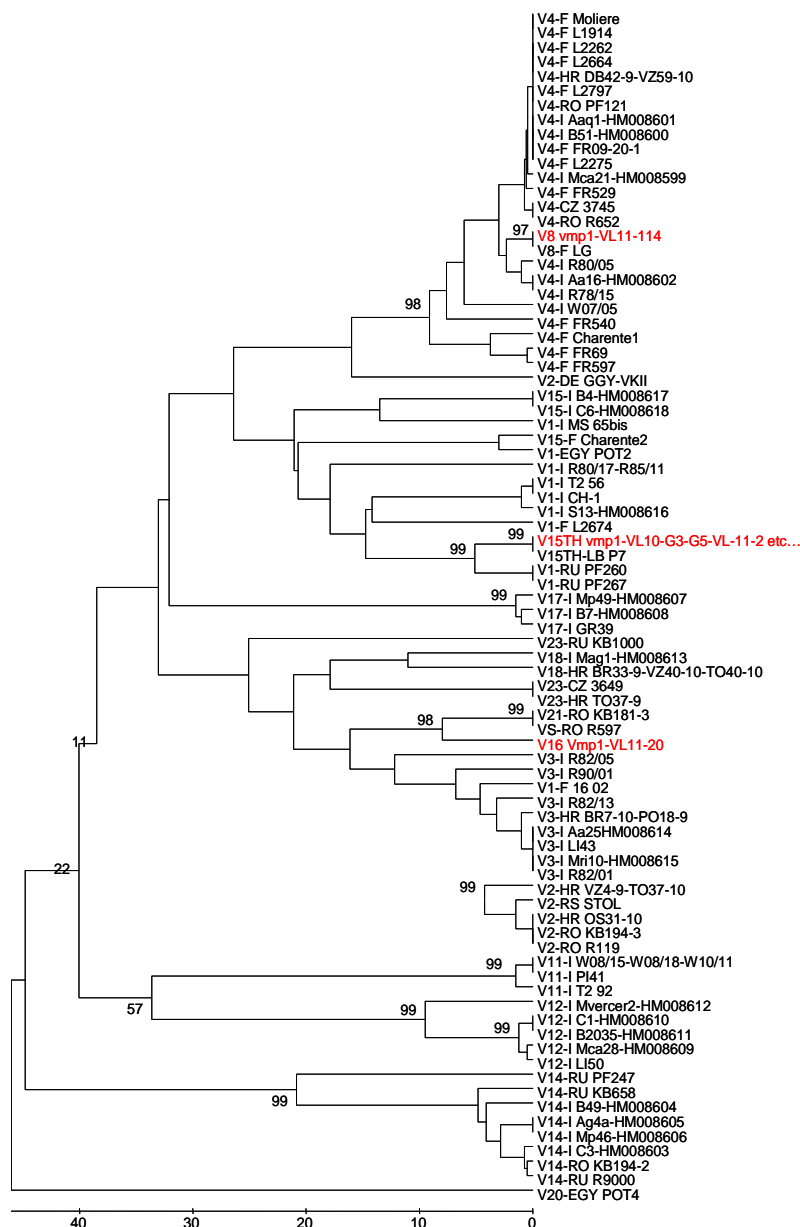
***secY*:**

Twenty *secY* PCR product sequences corresponded to the *secY* S3 genotype (reference isolate P7 from Lebanon), whereas samples 20 and 114 had a sequence corresponding to the *secY* genotype S4.

Vmp1:

The phylogenetic tree generated with the maximum parsimony method (fig. 4) grouped the Lebanese BN isolates into three different genetic clusters corresponding to genotype V15TH (20 samples), V8 (smaller PCR product with sequence identical to the French reference isolate Lot-et-Garonne) and V16, a new genotype according to its sequence and its *RsaI* RFLP profile.

Fig. 4 Phylogenetic tree generated using UPGMA method for *vmp1* sequences.



Summary:

In conclusion, among 22 BN isolates, 5 different genotypes were found.

The two most common genotypes were T2-V15TH-S3-ST14-M9 (11 cases), T2-V15TH-S3-ST14-M5 (8 cases), T2-V15TH-S3-ST14-M4 (1 case), T2-V16-S4-ST49-M5 (1 case) and T2-V8-S4-ST14b-M9 (1 case). No significant difference was found regarding the relative prevalence of the major genotypes between 2010 and 2011.

As expected, the use of the vmp3 genetic marker allows better differentiation of Lebanese BN isolates from the Bekaa Valley.

| Plant position code | Extra. nb (lab code) | extraction date | sampling site | plant species | tuf | vmp1 | secY | stamp | Vmp3 |
|---------------------|----------------------|-----------------|---------------|----------------|-----|-------------|------|------------------------------|------|
| F15 P15 | 2 | 6.10.2011. | TalDnoub | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| F19 P112 | 3 | 6.10.2011. | TalDnoub | Vitis vinifera | T2 | V15TH | S3 | ST14 | M5 |
| F21 P5 | 4 | 6.10.2011. | TalDnoub | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| F17 P181 | 5 | 6.10.2011. | TalDnoub | Vitis vinifera | T2 | V15TH | S3 | ST14 | M5 |
| F22 P40 | 8 | 6.10.2011. | TalDnoub | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| F71 P20 | 9 | 6.10.2011. | Kanafar | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| F22 P191 | 10 | 6.10.2011. | TalDnoub | Vitis vinifera | T2 | V15TH | S3 | ST14 | M5 |
| F17 P7 | 20 | 12.10.2011 | TalDnoub | Vitis vinifera | T2 | V16 | S4 | ST49 | M5 |
| F2 P160 | 33 | 17.10.2011 | TalDnoub | Vitis vinifera | T2 | V15TH mixed | S3 | ST14 | M9 |
| F9 P118 | 35 | 17.10.2011 | TalDnoub | Vitis vinifera | T2 | V15TH mixed | S3 | ST14 | M5 |
| F11 P25 | 43 | 19.10.2011 | TalDnoub | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| F13 P113 | 114 | 27.10.2011 | TalDnoub | Vitis vinifera | T2 | V8 | S4 | ST14 + 4 ambiguous positions | M9 |
| F67 P4 | 144 | 31.10.2011 | Kanafar | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| | G13 | 2010 | ? | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| | G18 | 2010 | ? | Vitis vinifera | T2 | V15TH | S3 | ST14 | M5 |
| | G25 | 2010 | ? | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| | G3 | 2010 | ? | Vitis vinifera | T2 | V15TH | S3 | ST14 | M4 |
| | G43 | 2010 | ? | Vitis vinifera | T2 | V15TH | S3 | ST14 | M5 |
| | G5 | 2010 | ? | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| | G7 | 2010 | ? | Vitis vinifera | T2 | V15TH | S3 | ST14 | M9 |
| | G8 | 2010 | ? | Vitis vinifera | T2 | V15TH | S3 | ST14 | M5 |
| | G9 | 2010 | ? | Vitis vinifera | T2 | V15TH | S3 | ST14 | M5 |

Future collaboration with host institution:

The results obtained from this scientific mission allowed the molecular characterization of phytoplasmas infecting two vineyards in the Bekaa valley in Lebanon. Up to this study, only the genotype T2-V15TH-S3-ST14 could be confirmed in this region. The confirmation of the existence of genetic diversity opens the way for a future collaboration on the time course variation of genotypes occurring in the vineyards of Bekaa and on the investigation of plant reservoirs for stolbur-BN in Lebanon.