**Phytoplasma strains maintained in micropropagation since 30 years and the EPPO-Qbank collection**

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The Alma Mater Studiorum, University of Bologna, DISTAL is hosting a collection of more than 140 phytoplasma strains maintained mainly in micropropagated *Catharanthus roseus* shoots. The collection was initiated in 1989 and maintained over the years with strains provided from greenhouse collections located in Udine (Italy), Dossenheim (Germany), Bordeaux (France) and other Institutions in Serbia, Chile, and USA. The different strains were insects or dodder transmitted from original host plants by researchers from around the world after the discovery of these pathogens in 1967 in Japan. The shoots were kept in a growth chamber and the presence and identity of phytoplasmas was verified before micropropagation by PCR followed by RFLP and/or sequencing of the 16S rRNA gene. For the micropropagation 1-3 cm long symptomatic shoots are subjected to sterilization in 10% sodium hypochlorite and rinsed in sterile water before their insertion in a MS based solid medium added with low amount of BAP. The shoots are kept under artificial lights (16 h photoperiod) at the constant temperature of 24°C + 2°C and multiplied by microcuttings every 3 to 6 months. When necessary, strains still maintained in vivo under greenhouse conditions are micropropagated again after phytoplasma molecular identity further verification. The strains were all sequenced on 16Sr and *tuf* genes to produce specific barcodes for the quarantine strains and their look-alike strains, under the EU founded project QBol, ended in 2013. The collection was made officially available for general consultation and strain identification through barcode firstly as Q-Bank (Q-Collect EU founded project) and, since May 1 2019, as EPPO-QBank (ttps://qbank.eppo.int/phytoplasmas/). Shoots of other phytoplasma-infected plant species such as cactus pear, tobacco and paulownia are presently maintained in the same collection only for research purposes. Over the time also other phytoplasma-infected species such as hydrangea, jujube, bindweed, *Prunus* spp., apple and grapevine infected by phytoplasmas were micropropagated, however their survival was, in many cases, limited in time and their micropropagation performance very poor, probably because of the strong effect of the pathogens and the reduced suitability of the medium (same for all the strains) used for the micropropagation. Molecular testing was performed on micropropagated shoots to verify presence and genetic identity of phytoplasma strains over the years on several genes. Among the studied genes the 16S rRNA, leucyl-tRNA synthase (*leuS*), *secA* and *tuf* resulted the most reliable in providing results, while *secY* and *rp* only resulted amplifiable in some of the tested strains. The comparison of RFLP profiles obtained from these with the same amplicons from the original strains before the micropropagation showed the presence of different restriction profiles in some of the strains. After about 25 years in micropropagation phytoplasma strains enclosed in groups 16SrII, -III, and -IX exhibited different profiles in amplicons of *rp*, *leuS* and *secA* genes. Moreover, the sequencing of *secA* gene of strain SOYP (16SrII-C) showed the presence of a differential SNP confirming the presence of some selective pressure related to the micropropagation maintenance. Periodic verification is performed by PCR/RFLP analyses on 16S rRNA gene to confirm phytoplasma presence and identity. The strains present in the collection (http://www.ipwgnet.org/collection) are available for sale for diagnostic and research purposes.