

## ***In vitro* SANITATION OF *Prunus* ROOTSTOCKS FOR USE AS HEALTHY GERMPLASM**

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Several stone fruit species (*Prunus* sp) are severely affected by diseases associated with phytoplasmas. A survey was carried out in the germplasm collections of the CITA, where different *Prunus* genotypes, including several interspecific hybrids used as rootstocks, have shown symptoms of these diseases, such as leaf roll in summer, off-season growth in winter and die-back. The distribution of the phytoplasma along the plant throughout the vegetative period will affect selection of 'Candidatus Phytoplasma prunorum' free plant material. Two different plant explants, hard cuttings in winter and young explants in spring were studied through *in vitro* culture combined with thermotherapy in order to obtain healthy plants from infected rootstocks.

Surface sterilization of selected budwood in winter and axenic cultures were established on appropriate culture media. Three weeks after subculture *in vitro* shoots were transferred to a special growth chamber and grown at an increased temperature regime for 14 to 21 days with a treatment at 37 °C during the day and 36° C during the night. Following pathogen elimination by heat treatment, apical meristems of 2 mm in size were excised immediately after heat treatment and grown on regeneration media. Regeneration of small plantlets occurred within a few weeks. Afterwards, molecular tests for the presence or absence of pathogen were carried out with a nested PCR with universal primers (P1/P7) and specific primers of the 'AP' group. PCR products were further analysed by RFLP by enzymatic digestion and the resulting 'fingerprints' confirmed the identity of the amplified products used to identify the 'Candidatus Phytoplasma prunorum'. The presence of 'Candidatus Phytoplasma prunorum' was compared in plants before and after thermotherapy, showing that this method can be efficiently utilized with the aim of maintaining healthy material in a germplasm collection used as parent material in a rootstock breeding programme.