In vitro preservation of traditional sweet cherry varieties of Emilia-Romagna region, Italy

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The Fruit Research Unit of Forlì (CRA-FRF) has recently started a programme to preserve the traditional sweet cherry varieties of Emilia-Romagna region (Southern Po Valley, Italy) from erosion, as well as their valuable agronomic and adaptive traits.


'Gemella'

In vitro and *in vivo* preservation of the most valuable varieties

- ✓ ***In vivo.*** Accessions of 9 traditional varieties were grafted onto Colt (3 trees/plots) and are maintained at the Heritage fruit tree collection of CRA-FRF, where they are undergoing characterization.
- ✓ ***In vitro***. Protocols of micropropagation are being optimized for the varieties **'Gemella'** and **'Durone di Cesena',** with the final aim to replicate *in vitro* the entire pool. In addition, to minimize subculturing and contamination risks, trials of application of the *slow growth* technique were carried out on the two varieties.

Multiplication culture medium:

- ✓ **Mineral basis: DKW (Driver & Kuniyuki, 1984)**
- ✓ **Sucrose: 25 g/l**
- ✓ **Agar: 4,5 g/l**
- ✓ **Growth hormones** → **IBA: 0,01 mg/l**; **0,4 mg/l< BAP <0,6 mg/l depending on the variety**

Growth chamber :

- ✓ **Temperature: 23 ± 2°C**
- ✓ **16 h photoperiod**
- ✓ **150 µEm $^{-2}$ s $^{-1}$ light intensity**

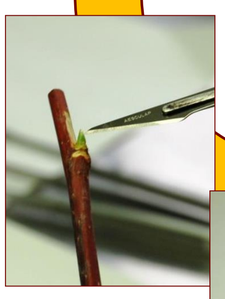
Subculturing every 20 days

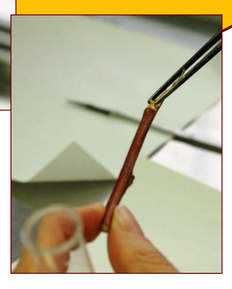
In vitro establishment cultures


'Durone di Cesena'

1 Disinfection of budwoods with 70% ethanol

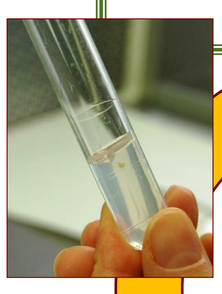
Bud excision from donor plants





2 Removal of bud scales and isolation of the shoot tip under laminar flow cabinet

3 Establishment of the explants in the culture medium



Development and growth of the explant

4 Aseptic transfer of shoots in test tubes

Trials of *slow growth* conservation

Trials to optimise *slow growth* conditions

Optimization of the *slow growth* technique was carried out by modifying some medium characteristics, such as the concentration of the mineral basis, the sugars and hormons. Different containers were also compared.

Each test was repeated 3 times.

Medium:

- ✓ Mineral basis (DKW; DKW ½)
- ✓ Sucrose (30; 40 and 50 g/l)
- ✓ BAP (0; 0,25; 0,4 and 0,5 mg/l)
- ✓ Mannitol (0; 5; 10 and 15 g/l)

Containers:

- ✓ 500 ml glass jars (15 clusters/each)
- ✓ 500 ml plastic trays (15 clusters/each)
- ✓ 50 ml Falcon tubes (2 clusters/each)

Preliminary Results

Shoot cultures performed best with:

- ✓ DKW ½;
- ✓ Sucrose concentration: 40 g/l;
- ✓ BAP concentration: 0,4 mg/l;
- ✓ Without Mannitol

50 ml Falcon tubes proved to be the most adequate containers to preserve the sterility of cultures

Shoots preserved at 4°C

Storage room

After 12 months at 4°C in the darkness, 'Gemella' and 'Durone di Cesena ' shoots showed a high survival rate, and high proliferation capacity when restored to normal growing conditions.

The *in vitro* technology complements efficiently the open-field preservation, offering several advantages for the preservation of the germplasm of interest: costs and spaces reduced; preservation from biotic and abiotic agents.

Activity carried out in the frame of the RGV-FAO project of the Italian Ministry of Agriculture