In vitro micrografting of cherry

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* Rootstock and Scion Production
  - In vitro establishment
  - Multiplication

* In vitro migrografting
  - Scion preparation
  - rootstock rooting induction
  - Micrografting
  - Plant transfer and acclimatisation

* Histology

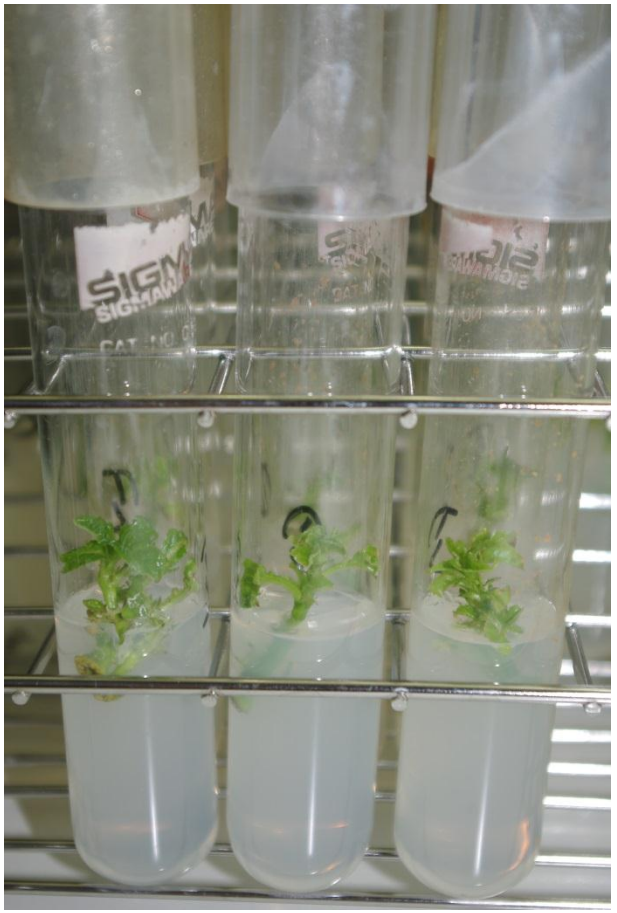
In vitro ESTABLISHMENT

Establishment of *in vitro* shoot cultures as a source of scions and rootstocks

- **Mother plants** : Scions or cuttings (virus and disease free)
- **Initial material** : Newly emerged sprouts developped under greenhouse

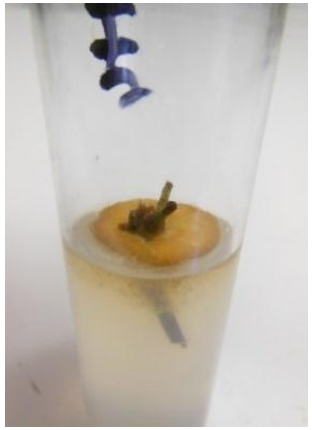
- Actively growing shoots
- Terminal or axillary buds

DISINFECTION PROTOCOLE

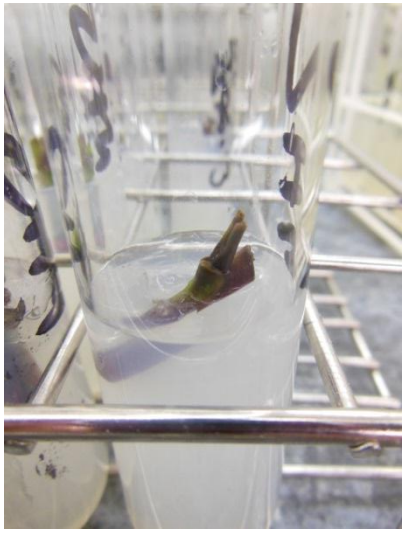


- Explants surface-sterilised
- 20 minutes in commercial bleach 0,6% active chlorine
- Three rinses with sterilised distilled water
- Excised shoot tips individually inoculated in tube with shoot-tip proliferation medium

ESTABLISHMENT CONTROL



Contaminated explant



Necrotic explant

Explant visually sterile

- Visual inspection
- Control on a bacterial growth medium :
  (explant base, old leaves, waste are inoculated in test tube and incubated in growth room)

Healthy test

Contaminated test

SHOOT MULTIPLICATION

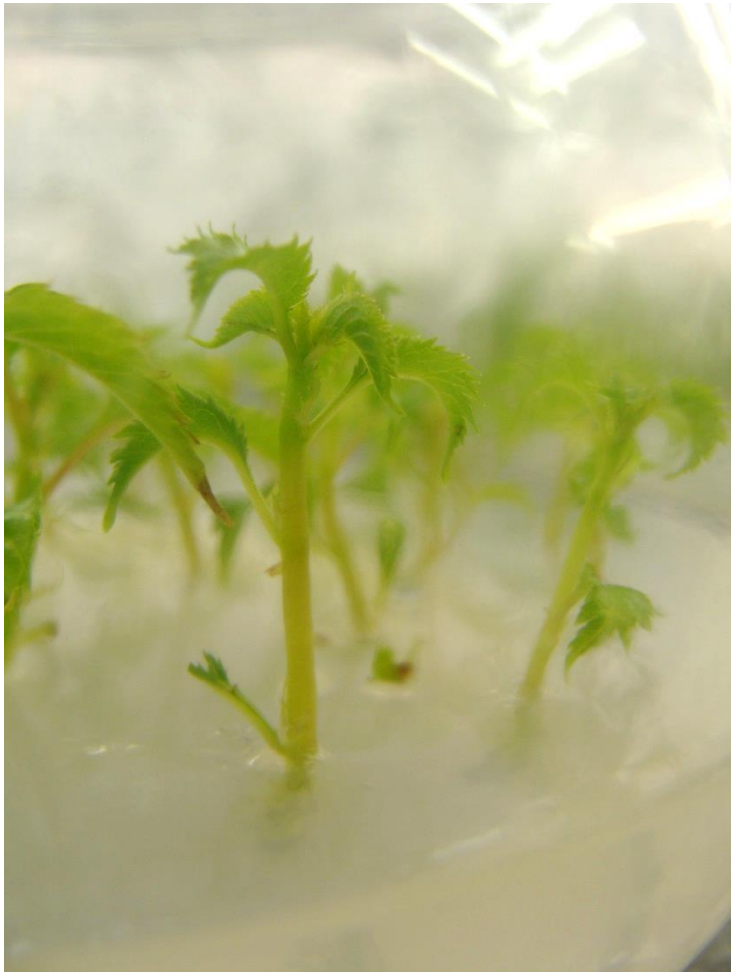
- Subculture : 3-4 weeks
- Culture conditions
  - 16-hour photopériode
  - 23°C ± 2°C
  - 40 µmol/s/m^2
- Medium : Quoirin-Lepoivre – DKW
  - BA : 0,2 – 0,6 mg/l
  - IBA + GA_3

Rootstock

Variety

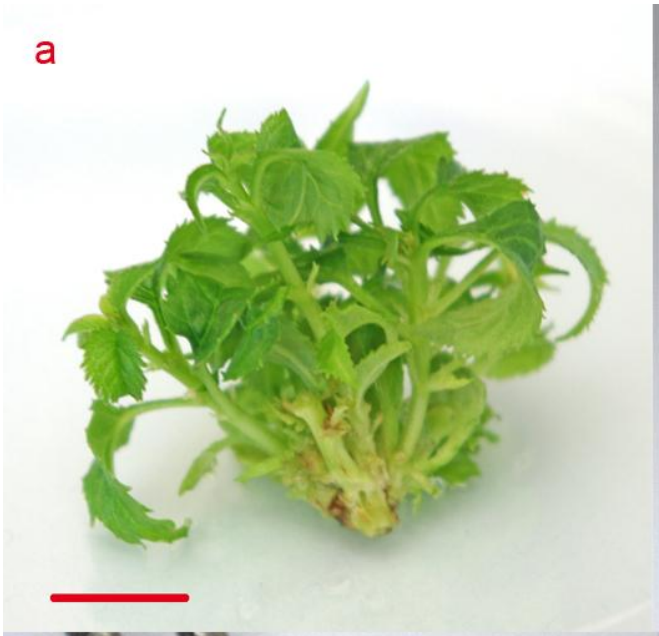
Rooting induction

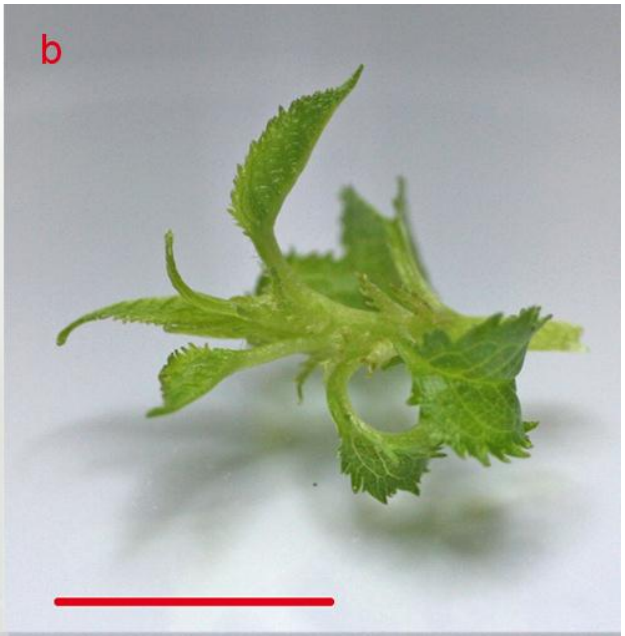
- End of multiplication stage
- 3-4 cm-long shoot
- Induction medium :
  BdR (Ctifl medium for rooting)
  - 1 mg/l IBA
  - 7 days in darkness
  - 23°C ± 2°C



SCION PREPARATION

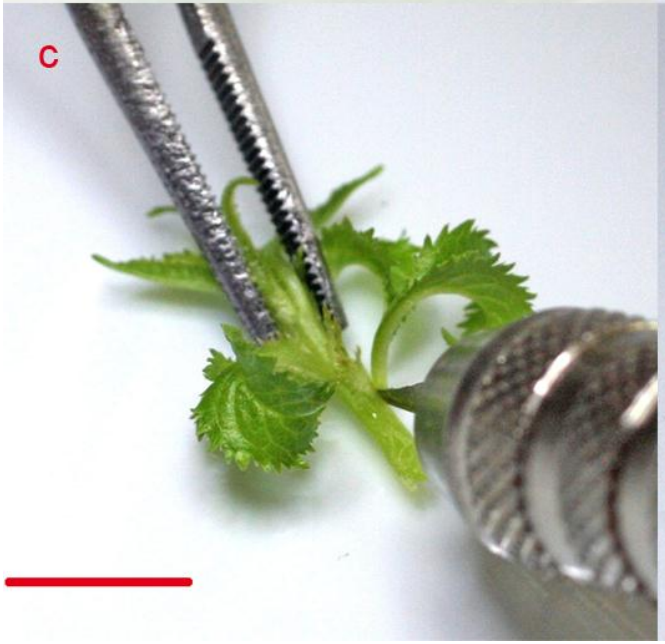
Clump

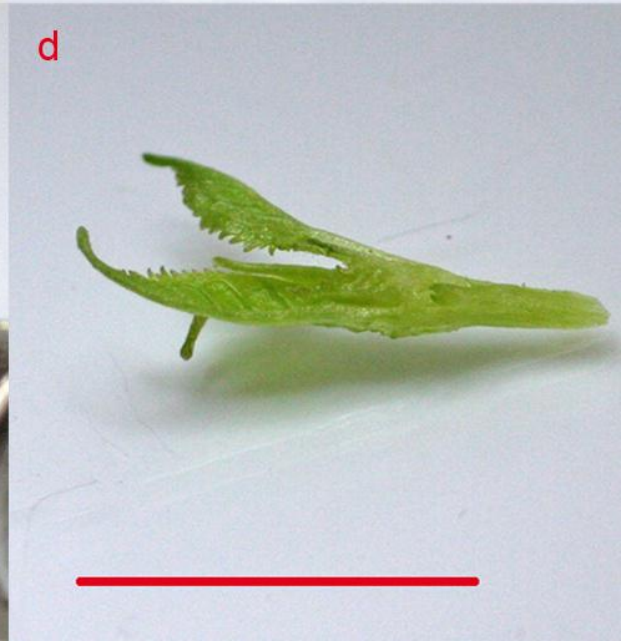




Apex

Developped leaves are removed with a stainless steel razor blade





Scion base cut in a V-shape

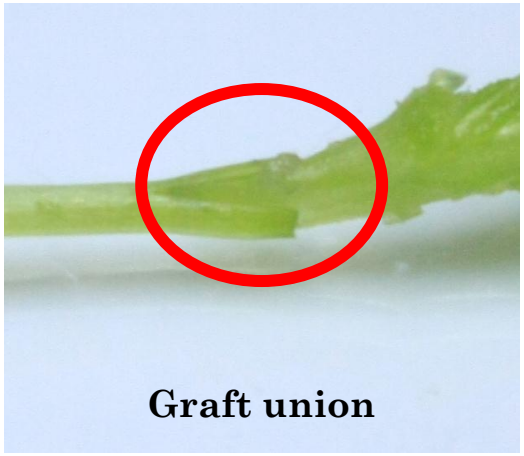
ROOTSTOCK PREPARATION

Clump

- **7 days rootstock induced for rooting**
- **Decapitation of stem and removal of leaves**
- **Vertical slit at the top**

SLIT MICROGRAFTING

Insertion



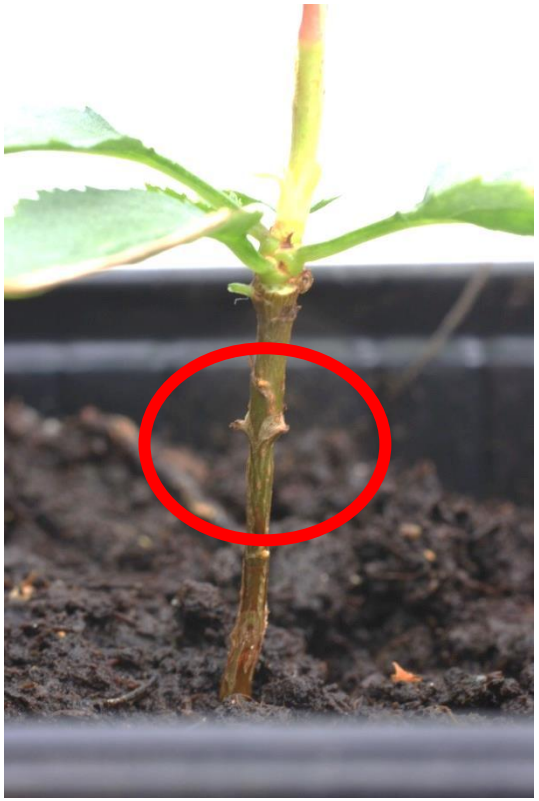
Graft union

In vitro development of scion

Rooting expression medium with vermiculite

MICROGRAFTING

End of in vitro rooting

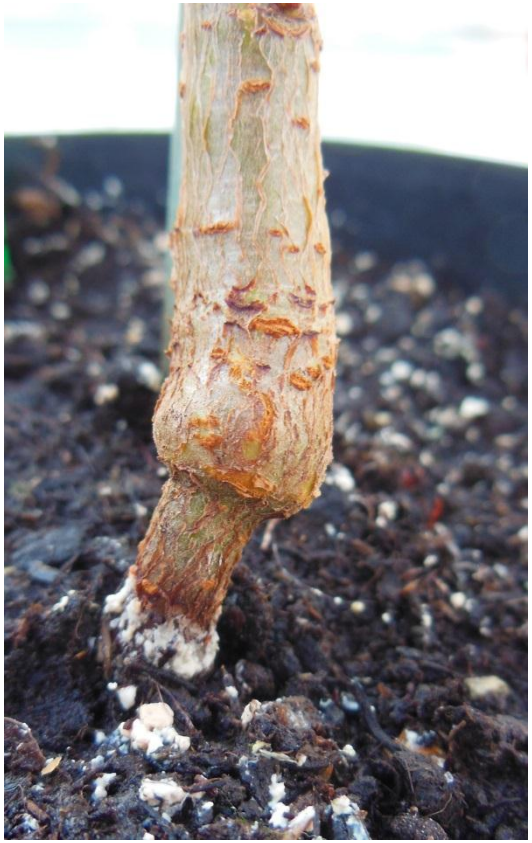


One month of acclimatization

Two month of acclimatization

Behaviour during growth

Normal - Compatible



Abnormal ? – Incompatible ?

RESULTS

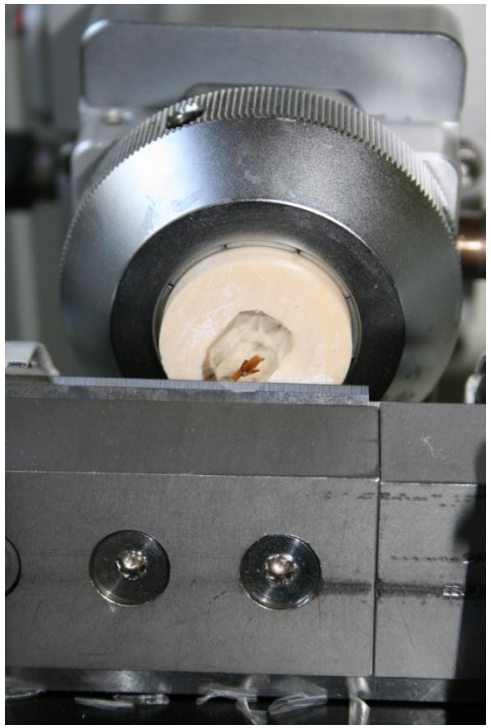
- The aim of the study was to provide a reliable, reproducible and highly efficient protocole which can be used as a tool to facilitate early diagnosis of incompatibility between new cherry varieties and new rootstocks

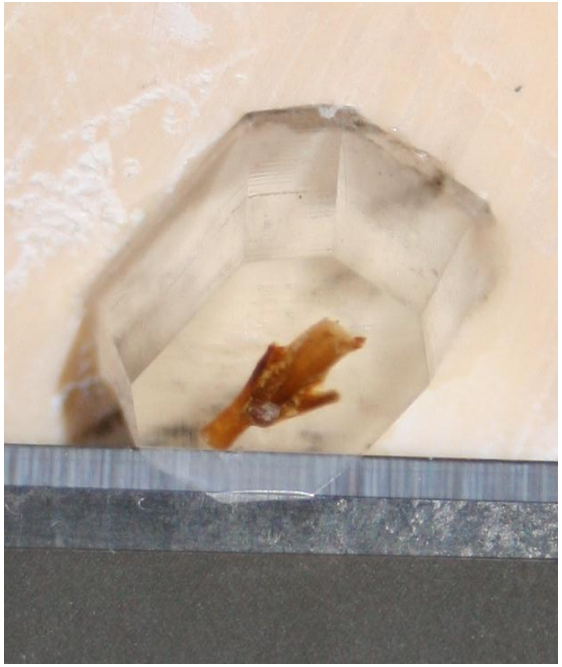
- 4 varieties : Folfer$_{(COV)}$, Ferdiva$_{(COV)}$, Regina et Burlat

- 9 rootstocks : Sainte Lucie 64, Sainte Lucie 35, Sainte Lucie 1960, Maxma Delbard® 14 Brokforest, Maxma Delbard® 60 Broksec, Piku 1®, Furtos, Weiroot® 158 and Gisela

HISTOLOGY

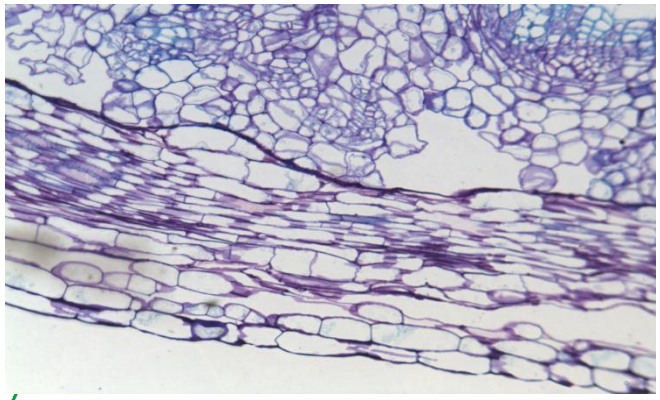
At the end of in vitro stage before acclimatization

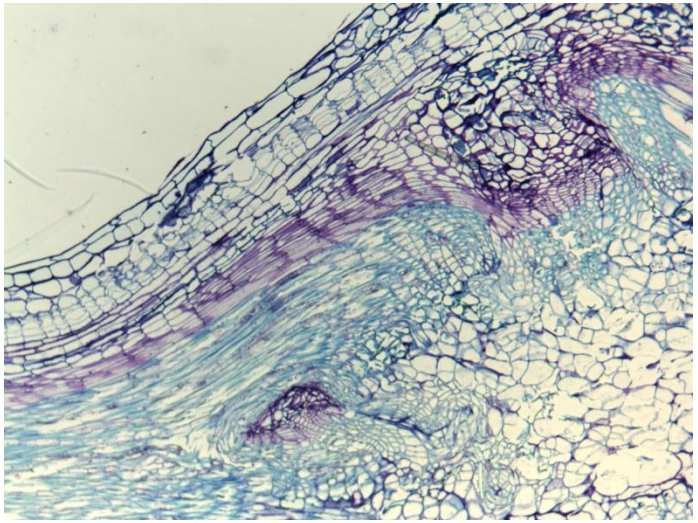




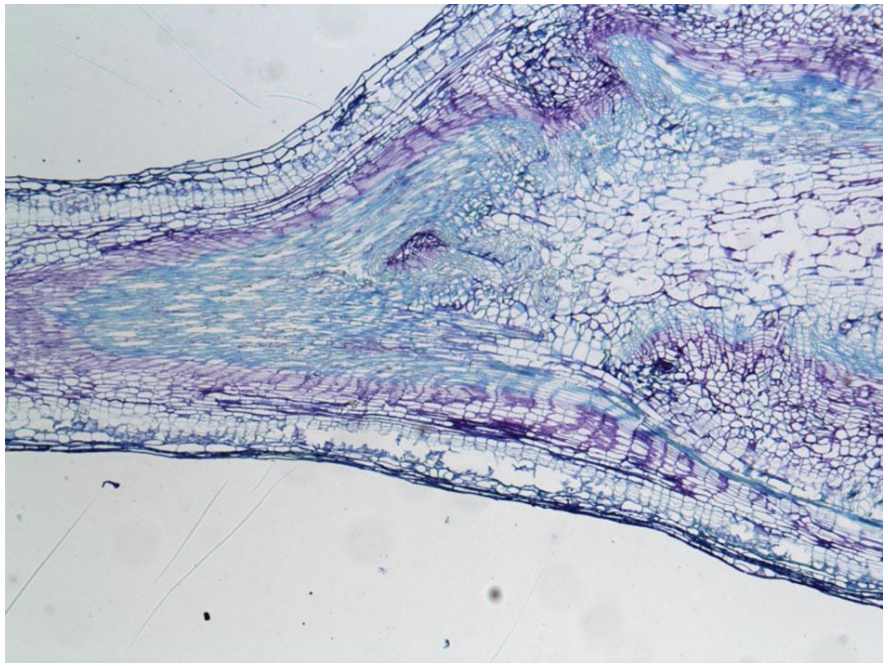
HISTOLOGY

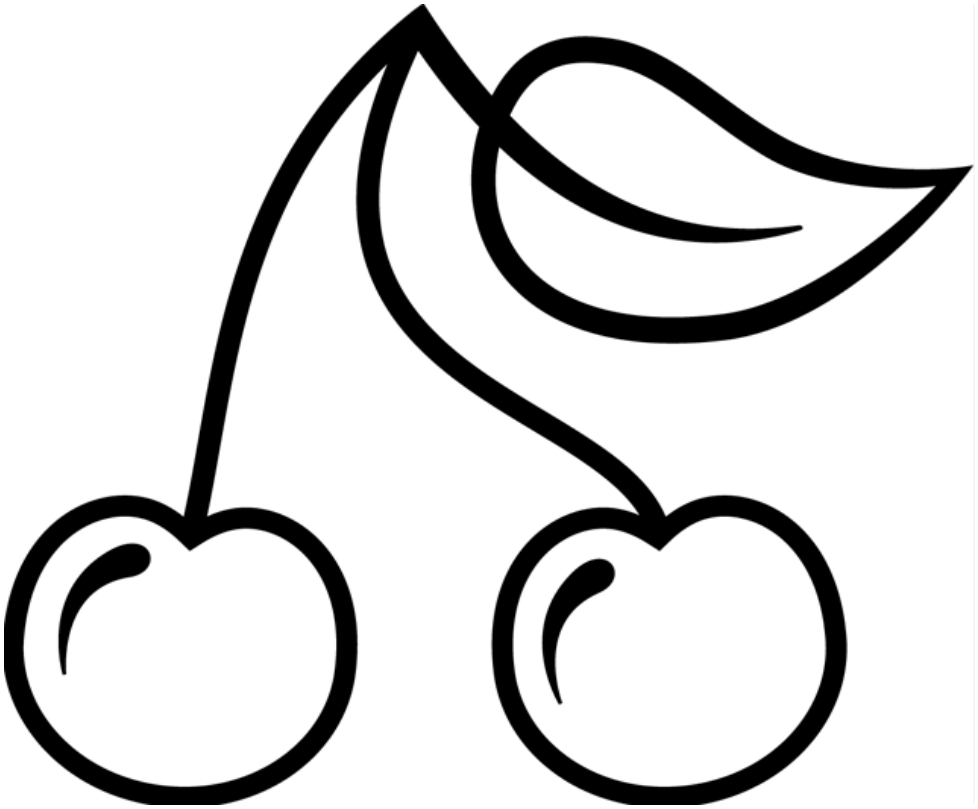
Incompatible rootstock (SL1960)





Compatible rootstock





THANK YOU