The Potato micropropagation

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Some characteristics of the potato multiplication

- **Vegetative reproduction:**
  - Degeneration by infection of different pests (viruses, bacteria, fungi,...)
  - The propagation material needs to be regularly replaced: by injection at the top of the chain of very high quality material (free of diseases infections)

- **How to replace the propagation material?**
  - Traditionally, by applying clonal and genealogical selection with, as departure point, some tubers that have been selected in field and checked in laboratory
  - Or, applying genealogical selection assisted by the *in vitro* micropropagation techniques: more easy, rapid, flexible and give more security for the phytosanitary quality.
Example of traditionnal way for the production of seed potato material

Initial material

1st year clones

2nd year clones

3rd year clones

Pre-basic seed lot (4 to 7 year)

Basic seed lot

Certified seed lot

F0

F1

F2

F3

F4, F5, F6, F7

F8, F9, F10

(F11, F12)
Initial material: candidate tuber, indexed or not, sprout node excision and culturing

Vitroplant (F0): from sprout node culture
Has to be checked for diseases!!!

Genealogical Selection assisted by in vitro Micropropagation techniques

Vitrotubers production (F0)

In vitro multiplication

Minitubers production (F1) OR

Vitroplants acclimatization and rooting (F0)

Pre-basic seed (F1 to F3)

Basic seeds S, SE, E (F4 to F6)

Certified seeds A, B (F7 to F8)
Advantages given by the genealogical selection assisted by the micropropagation techniques

⇒ Saving of open field seeds generations

= phytosanitary safety

Classical genealogical selection  In Vitro assisted genealogical selection
Advantages given by the genealogical selection assisted by the micropropagation techniques

\[ \Rightarrow \text{multiplication power} \]

= 

Production flexibility, rapid adaptation to the market opportunities

In Vitro multiplication power
Micropropagation techniques

The *in vitro* introduction of a potato variety
Micropropagation techniques

- **Potato variety in vitro introduction**

Main conditions:
- the candidate tuber: to verify the variety
- to get sprouts
- to cut the sprouts, disinfect them with alcohol and cut them between nodes (= explant to be introduced in in vitro culture)
- disinfection of the sprouts nodes = in Na hypochlorite, 5 à 8’ + and rising them in 3 successive bads of sterile H2O, 5’ – 10’ and 5’
- culture media = MS + Sugar (20g/l) + Agar (6g/l), pH 5,9
- culture conditions: 16h/8h , 18 to 22 °C, 4000 to 6000 lux
Micropropagation techniques

Phytosanitary control scheme

In collection
Phytosanitary control operated when introducing potato explant in in vitro collection

- Departure point: one or several tubers, each of them furnishing several sprout nodes: 1 node = 1 clone, then several clones / tuber, each of them having his own code (reference number)

- Each clone (node) is introduced in vitro and multiplied separately. Each clone will be fully tested.

- After being tested, only one clone among the diseases free clones will be choosen and introduced in the in vitro collection.

- A system of tracability must be operationnal in the micropropgation lab: each work and each analyse and results on the selected clone must be listed in a appropriate system of documentation.
Micropropagation techniques

• Phytosanitary control operated when introducing potato explant in *in vitro* collection

Pathogens to be tested on the vegetal material entering in the *in vitro* collection

**BACTERIA**

*Clavibacter michiganensis subsp sepedonicus*
*Ralstonia solanacearum*
*Dickeya sp and Pectobacterium sp.*

**VIRUSES**

*Potato virus X*
*Potato virus S*
*Potato virus Y*
*Potato virus A*
*Potato virus M*
*Potato Leaf roll virus*
*Tobacco rattle virus*
*Potato mop top virus*
*Potato virus V*
*Potato virus T*
*Andean potato latent virus*
*Andean potato mottle virus*
*Arracacha virus*
*Potato black ringspot virus*
*Tomato spotted wilt virus*

**VIROID**

*Potato spindle tuber viroid*
Micropropagation techniques

• Maintenance of the selected clones in the lab

- Medium: MS + 20g/l sucre + 6g/l agar + (max 3% mannitol), pH 5.9
- Temperature: 18-20°C
- Photoperiod: 16h/8h
- Light intensity: 4000-6000 lux

- Maintenance (renewing): 3 à 4 x/an

- True to type control: 1x/2 ans (au champ)

- System of tracability and documentation operational.
Micropropagation techniques

• Fast multiplication in the lab (production).
Micropropagation techniques

- **Fast multiplication in the lab (production).**

- Phytosanitary control on the material selected for the micropropagation:
  - viruses (main: PLRV, PVY, PVA, PVS, PVX, PVM)
  - bacteria (quarantine: Ralstonia, Clavibacter – common ones: Dickeya, Pectbact.)

- Conditions of multiplication:
  - Media: MS + 20g/l sugar + 6g/l agar, pH 5.9
  - Temperature: 20-22 C
  - Photoperiod: 16h/8h
  - Light intensity: 4000-6000 lux

- Monthly multiplication factor: 5

<table>
<thead>
<tr>
<th>Production objective</th>
<th>X</th>
<th>X-1</th>
<th>X-2</th>
<th>X-3</th>
<th>X-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>10000</td>
<td>2000</td>
<td>400</td>
<td>80</td>
<td>15</td>
</tr>
<tr>
<td>September</td>
<td>October</td>
<td></td>
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<tr>
<td>August</td>
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<tr>
<td>July</td>
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<tr>
<td>June</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Micropropagation techniques

- Field transfer of the *in vitro* micropropagated material
  - Intermediate steps are necessary

- 3 possible ways:
  - *in vitro* microtubers production (vitrotubers)
  - minitubers production
  - acclimatization and rooting of the vitroplantlets
Micropropagation techniques

- **Field transfer of the *in vitro* micropropagated material**

  - **Microtubers production:**
    - in the lab, then in a dark room (6 weeks + 4 months + 1 month)
    - first step: *in vitro* vegetative multiplication,
      - Medium MS normal
      - Photoperiod 16h/8h
      - Temperature: 20-22 C
    - second step: microtuberization (6 weeks)
      - Medium: MS/2 + sugar (80 g/l) + coumarine (50 mg/l) + kinetin (4 mg/l)
      - Photoperiod: in the dark 24h/24h
      - Temperature: 20-22 C
Micropropagation techniques

- Field transfer of the *in vitro* micropropagated material

  - Microtubers production:

    - production of 1 to 1.5 microtubers size 5/10mm / vitroplant
    - manually harvested, rinsing in water, disinfection thiabendazol solution, drying, sorting (<5, 5/7, 7/10mm)
    - cold storage (minimum 3 months, 2 to 3 C)
    - presprouting (1 month at 18/20 C, 16h/8h photoperiod)
    - sowing directly in field or minituber production in greenhouse
Micropropagation techniques

- **Field transfer of the micropropagated material**
  - Direct sowing of microtubers in field
Micropropagation techniques

• **Field transfer of the *in vitro* micropropagated material**

  ➢ Microtubers production:

  ▪ **advantages:**
    - Easy to produce, no costly (no greenhouse)
    - No risks of contamination during the production cycle (in the lab)
    - Easy to sow (mechanically)
    - Easy to send (by airplane)

  ▪ **disadvantages:**
    - In field vigour due to the small size: need hot and humid soil, and need to be presprouted before sowing,
    - Long production process (6 weeks + 3 months + 1 month): not flexible!
Micropropagation techniques

• Field transfer of the *in vitro* micropropagated material

➢ Minitubers production:
  ▪ in greenhouses or screenhouses (insectproof), by cultivation of the microplantlets or microtubers
  ▪ classical way: by transfer of vitroplantlets in peat soil
  ▪ other ways: without soil, in hydroponic or aeroponic production method
  ▪ length of production: 3 to 4 months in green or screenhouses + 3 to 6 months of cold storage, + 1 month of pre sprouting
  ▪ harvest made by hand, sorting (10 to 50 mm)
  ▪ needs to be checked on viruses and bacteria after production and before use
Micropropagation techniques

Hydroponic unit for minitubers production

ACID → BASE → NUTRIENT → CONTROL

CONTROL
pH - Conductivity

DISINFECTION
UV – Sand Filter

WATER → CLEAN SOLUTION → WASTE SOLUTION

CULTURE TABLE
Micropropagation techniques

- Field transfer of the *in vitro* micropropagated material
  - Minitubers production
Micropropagation techniques

• Field transfer of the *in vitro* micropropagated material

  ➢ Minitubers production

  ▪ advantages:
    - Size near the usual size of the potato seeds
    - Good productivity in field (vigour and multiplication rate)

  ▪ disadvantages:
    - Long production process (3-4 months + 3 months + 1 month)
    - Flexibility!
    - Expensive (infrastructures – greenhouses)
    - Sanitary risks through the contamination of the substrate, or introduction of diseases vectors in the greenhouse
Micropropagation techniques

• Field transfer of the micropropagated material

➢ Acclimatized and rooted vitroplants production:
  ▪ in greenhouse by transfer of vitroplants in cubes of peat
  ▪ 4 weeks of acclimatization and rooting in greenhouse
  ▪ transfer in open field mechanically or manually
  ▪ virus and bacteria control before the transfer to the open field
Micropropagation techniques

• Field transfer of the *in vitro* micropropagated material

  ➢ Acclimatized and rooted vitroplants

    ▪ advantages:
      - flexibility, rapid production (4 weeks of acclimatization)
      - can be directly protected against diseases in field as soon
        they have been transferred

    ▪ disadvantages:
      - needs rainfall/irrigations for a good recovery in field
      - expensive (greenhouse) but less than minituber
      - risk of infection before being transferred to the field
# Micropropagation techniques

<table>
<thead>
<tr>
<th></th>
<th>Minitubers</th>
<th>Microtubers</th>
<th>Rooted Plantlets</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size</strong></td>
<td>10/15, 15/20, 20/25 &gt;25 mm</td>
<td>5/7 et 7/10</td>
<td>—</td>
</tr>
<tr>
<td><strong>Soil preparation</strong></td>
<td>normal</td>
<td>Fine</td>
<td>Fine Plantation in preformed ridges, or not</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sowing on flatted ridges</td>
<td></td>
</tr>
<tr>
<td><strong>Seeds preparation</strong></td>
<td>Presprouting</td>
<td>Presprouting needed!</td>
<td>—</td>
</tr>
<tr>
<td><strong>Plantation</strong></td>
<td>Mechanically or By hand</td>
<td>Mechanically with pneumatic sowing machine</td>
<td>Mecahnically or by hand</td>
</tr>
<tr>
<td><strong>Plantation date</strong></td>
<td>April-May</td>
<td>April-May</td>
<td>After the last frosts</td>
</tr>
<tr>
<td><strong>Plantation depth.</strong></td>
<td>The height of the ridge needs to be adapted on the tuber size</td>
<td>4-5 cm in the ridge</td>
<td>Rooted plant is transplanted in the soil until its last foliar floor</td>
</tr>
<tr>
<td><strong>Density</strong></td>
<td>Depends on the tuber size 12-15 cm (110.000 à 88.000)</td>
<td>Depends on the microtuber size (10 to 12 cm) (133.000 à 110.000)</td>
<td>20 cm (66.500)</td>
</tr>
<tr>
<td><strong>Herbicide</strong></td>
<td>Before emergence</td>
<td>Before emergence</td>
<td>Before plantation with a light dosis of metribuzine</td>
</tr>
</tbody>
</table>

**Micropropagation techniques**
**Micropropagation techniques**

- **Productivity: comparative study mini, micro, rooted plantlets in Belgian conditions.**

**Influence of the tuber size on growth and production**

i Objects (variety Bintje)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Size</th>
<th>Dimensions (mm)</th>
<th>Yield per hectare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtubers</td>
<td>7/10</td>
<td>0.12 x 0.75</td>
<td>110833 /ha</td>
</tr>
<tr>
<td>Minitubers</td>
<td>10/15</td>
<td>0.12 x 0.75</td>
<td>110833 /ha</td>
</tr>
<tr>
<td>Minitubers</td>
<td>15/20</td>
<td>0.15 x 0.75</td>
<td>88666  /ha</td>
</tr>
<tr>
<td>Minitubers</td>
<td>20/25</td>
<td>0.20 x 0.75</td>
<td>66500  /ha</td>
</tr>
</tbody>
</table>
Micropropagation techniques

• **Productivity: comparative study**

Influence of the tuber size on growth and production

ii. Measured variables.
   a. Total emergence (%)  
   b. Plants vigour (Development in cm)  
   c. Productivity (kg / 1.8 m of row – kg / ha)  
   d. Multiplication rate (Tubers number / 1.8 m of row – Tubers number / ha)
Micropropagation techniques

- **Productivity: comparative study**

Effect of the tuber size on growth and productivity

a. Total emergence (in % of the quantity used for plantation).

![Chart showing the effect of tuber size on levee percentage over dates.](chart.png)
Micropropagation techniques

- **Productivity: comparative study**

Effect of the tuber size on growth and productivity

b. Growth vigour
Micropropagation techniques

- **Productivity: comparative study**

Effect of the tuber size on growth and productivity

c. Productivity (Yield)

<table>
<thead>
<tr>
<th>Size</th>
<th>Plantation Distance</th>
<th>Density plants / ha</th>
<th>Tubers weight For 1,8m row (Kg)</th>
<th>Weight / plant (Kg)</th>
<th>Weight / ha (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/10mm</td>
<td>0.12 x 0.75</td>
<td>110833</td>
<td>4.21</td>
<td>0.281</td>
<td>31144</td>
</tr>
<tr>
<td>10/15 mm</td>
<td>0.12 x 0.75</td>
<td>110833</td>
<td>5.38</td>
<td>0.359</td>
<td>39752</td>
</tr>
<tr>
<td>15/20 mm</td>
<td>0.15 x 0.75</td>
<td>88666</td>
<td>6.18</td>
<td>0.515</td>
<td>45663</td>
</tr>
<tr>
<td>20/25 mm</td>
<td>0.20 x 0.75</td>
<td>66500</td>
<td>8.96</td>
<td>0.995</td>
<td>66204</td>
</tr>
</tbody>
</table>
Micropropagation techniques

- Productivity: comparative study

Effect of the tuber size on growth and productivity

c. Productivity (Yield)

![Graph showing the effect of tuber size on productivity (Yield)]
Micropropagation techniques

- **Productivity: comparative study**

Effect of the tuber size on growth and productivity

d. Multiplication rate

<table>
<thead>
<tr>
<th>Calibre</th>
<th>Ecartement</th>
<th>Nombre de plantes / ha</th>
<th>Nombre total de tubercules / 1,8m</th>
<th>Nombre total de tubercules &gt;28mm / 1,8m</th>
<th>Nombre total de tubercules &gt;28mm / ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/10 mm</td>
<td>0.12 x 0.75</td>
<td>110833</td>
<td>115.67</td>
<td>67.33</td>
<td>497434</td>
</tr>
<tr>
<td>10/15 mm</td>
<td>0.12 x 0.75</td>
<td>110833</td>
<td>95.67</td>
<td>77.33</td>
<td>571314</td>
</tr>
<tr>
<td>15/20 mm</td>
<td>0.15 x 0.75</td>
<td>88666</td>
<td>145.33</td>
<td>100.67</td>
<td>743750</td>
</tr>
<tr>
<td>20/25 mm</td>
<td>0.20 x 0.75</td>
<td>66500</td>
<td>124.33</td>
<td>107.33</td>
<td>792954</td>
</tr>
</tbody>
</table>
Micropropagation techniques

• **Productivity: comparative study**

Effect of the tuber size on growth and productivity

e. **Conclusions.**

- Significative effect of the tuber size on the yield (from 31 to 66 to/ha)

-Significative effect of the tuber size on the multiplication rate:
  - 7/10mm – 10/15mm: 497500 >> 571300 tubers / ha (size > 28mm)
  - 15/20mm – 20/25mm: 744000 >> 793000 tubers / ha
## Micropropagation techniques

- **Productivity: comparative study**

Comparison between minitubers 20/25 and rooted plantlets.

<table>
<thead>
<tr>
<th>Type matériel</th>
<th>Ecartement</th>
<th>Nombre plantes / ha</th>
<th>Taux de réussite</th>
<th>Nombre total tubercules / ligne 1.8m</th>
<th>Nombre total tubercules &gt;28mm / ligne 1.8m</th>
<th>Nombre total tubercules &gt;28mm / 1 ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini 20/25</td>
<td>0.20 x 0.75</td>
<td>66500</td>
<td>100%</td>
<td>124.33</td>
<td>107.33</td>
<td>792954</td>
</tr>
<tr>
<td>Plantules</td>
<td>0.20 x 0.75</td>
<td>66500</td>
<td>100%</td>
<td>157.33</td>
<td>101.70</td>
<td>751360</td>
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</tbody>
</table>