The Potato micropropagation

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Some characteristics of the potato multiplication

- **Vegetative reproduction:**

  ⇒Degeneration by infection of different pests (viruses, bacteria, fungi,…)

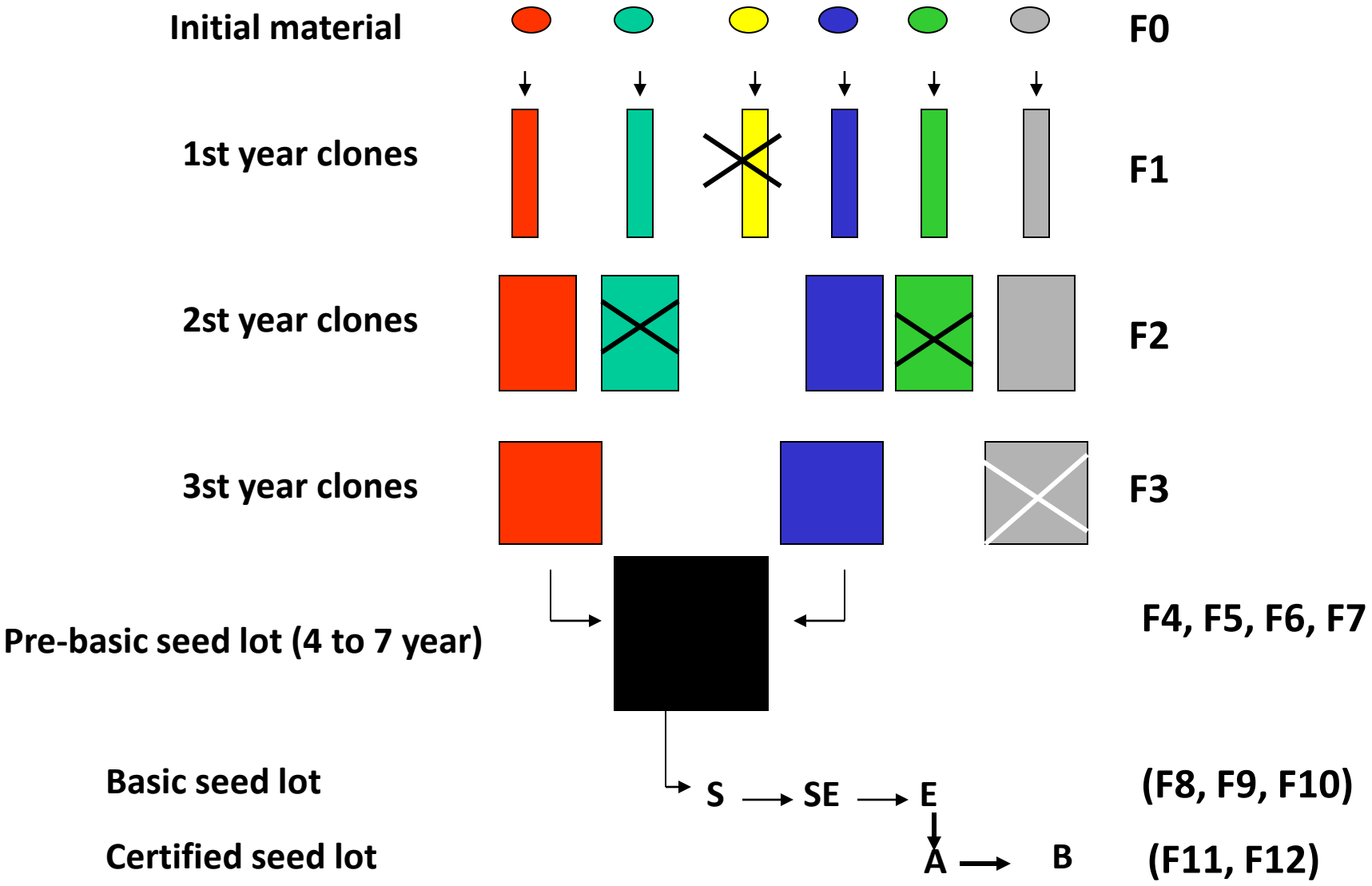
  ⇒ The propagation material needs to be regularly replaced: by injection at the top of the chain of very high quality material (free of diseases infections)

- **How to replace the propagation material?**

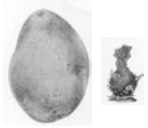
  ⇒ traditionally, by applying clonal and genealogical selection with, as departure point, some tubers that have been selected in field and checked in laboratory

  ⇒or, applying genealogical selection assisted by the *in vitro* micropropagation techniques: more easy, rapid, flexible and give more security for the phytosanitary quality.

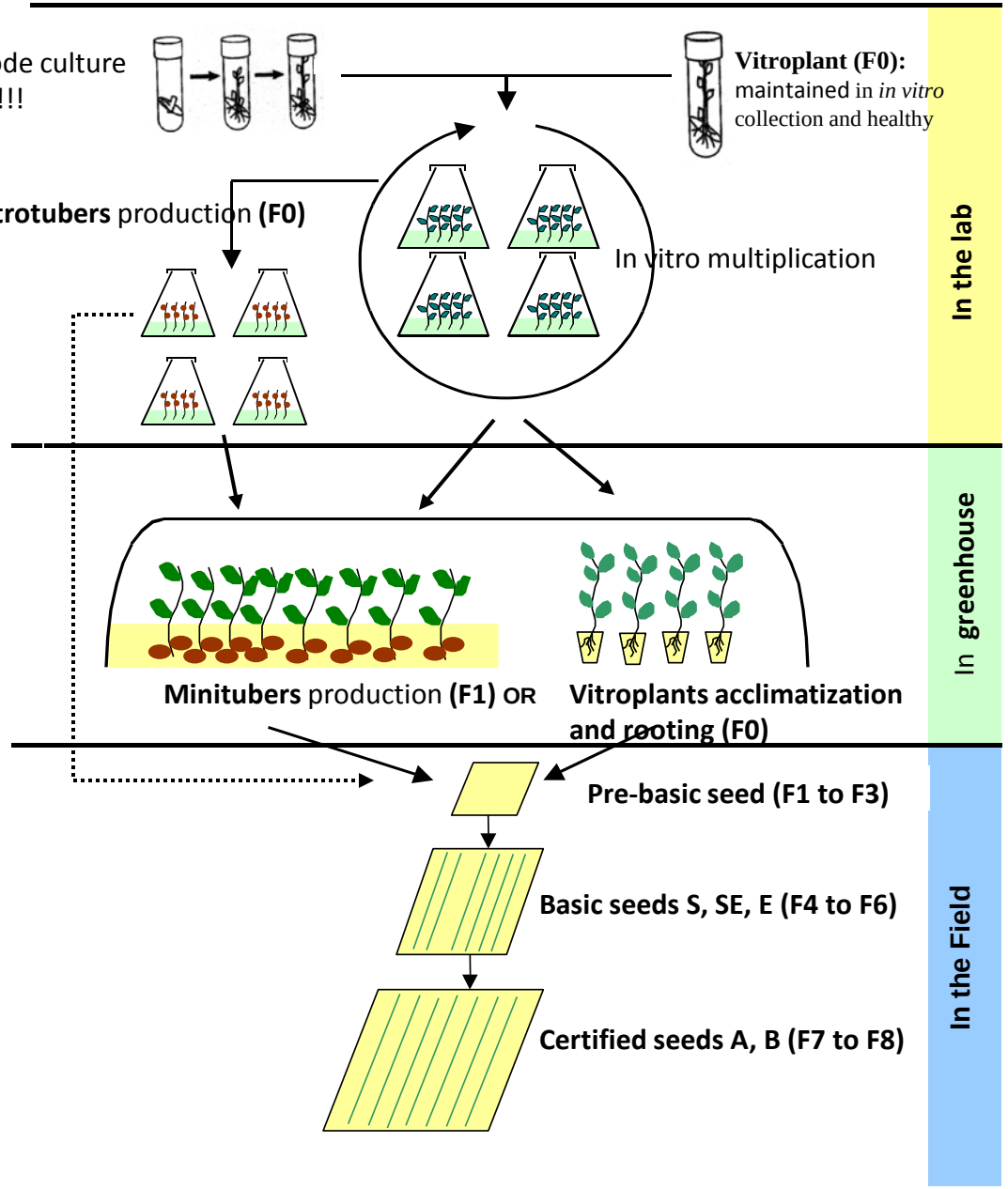
Example of traditionnal way for the production of seed potato material



Genealogical Selection assisted by *in vitro* Micropropagation techniques



Initial material: candidate tuber , indexed or not, sprout node excision and culturing



Advantages given by the genealogical selection assisted by the micropropagation techniques

⇒Saving of open field seeds generations

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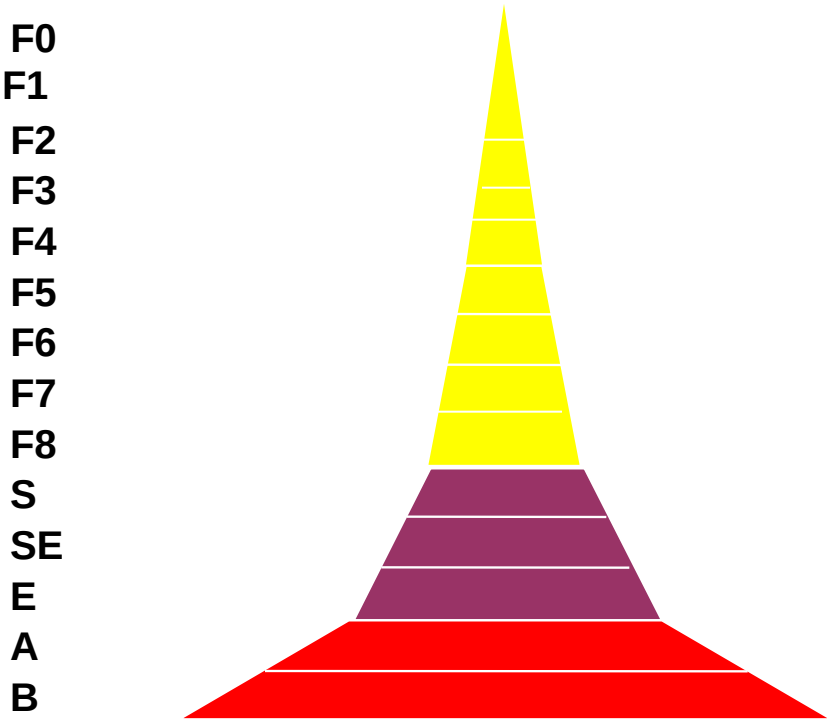
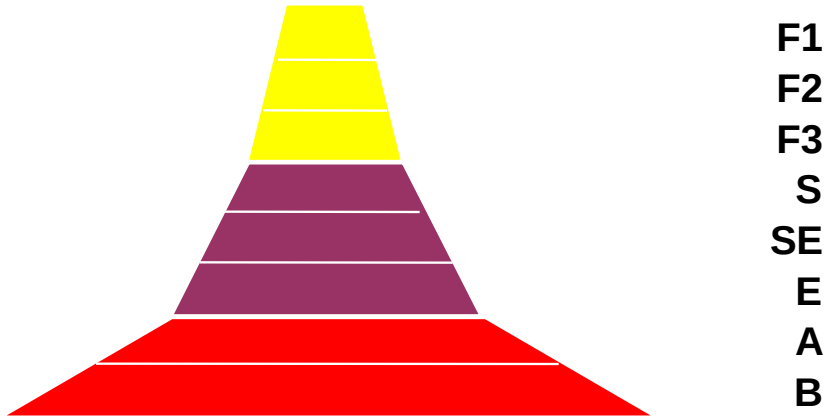
phytosanitary safety

Classical genealogical selection



In Vitro assisted genealogical selection



Advantages given by the genealogical selection assisted by the micropropagation techniques

⇒ **multiplication power**

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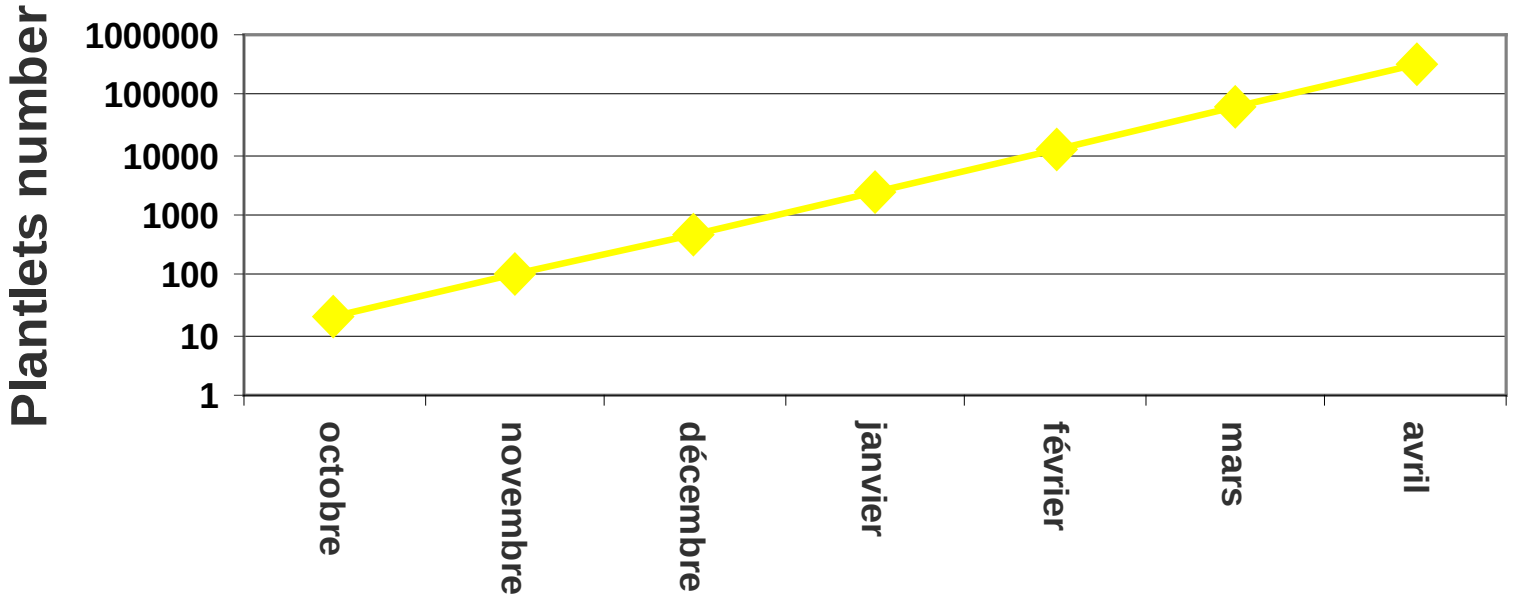
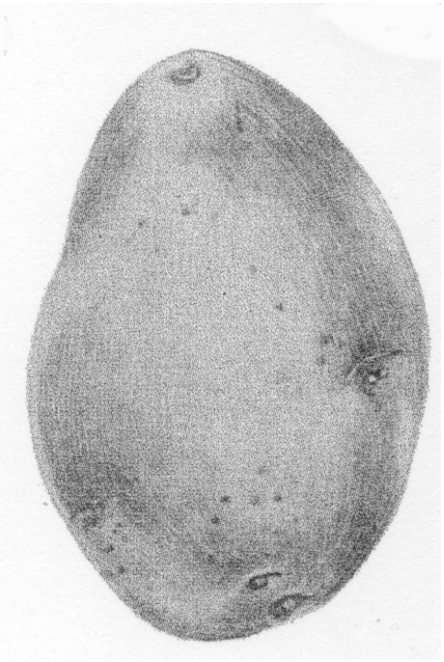
Production flexibility , rapid adaptation to the market opportunities

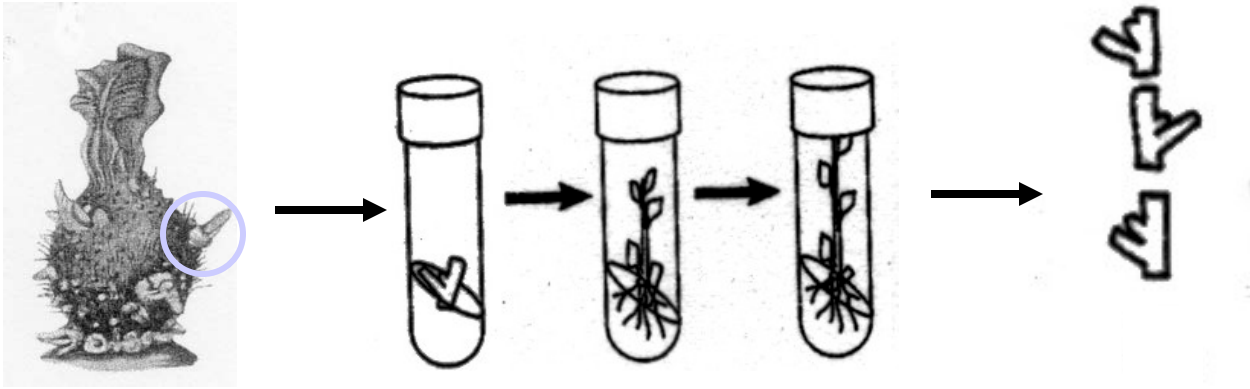
In Vitro multiplication power



Micropropagation techniques

The *in vitro* introduction of a potato variety





Micropropagation techniques

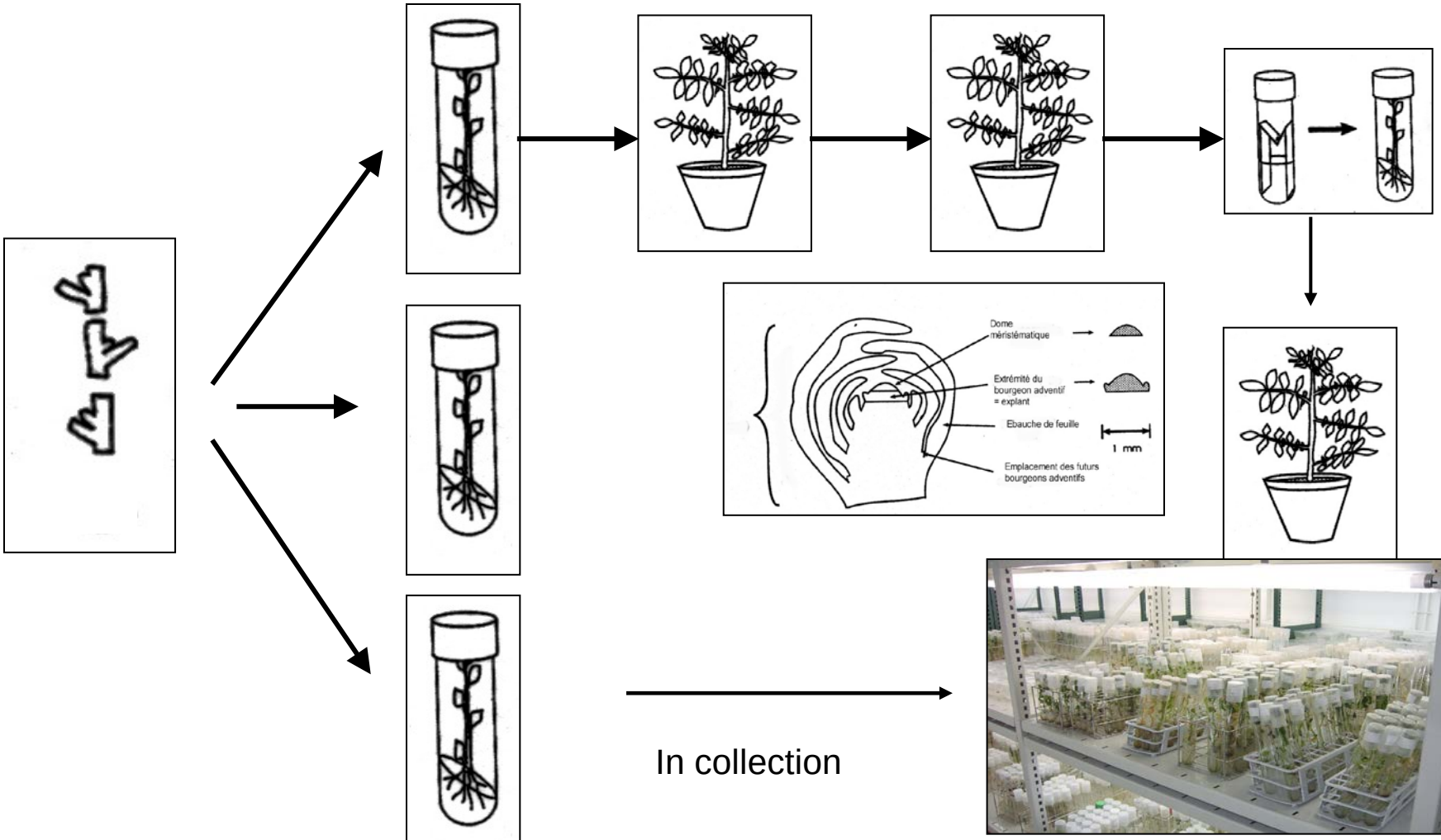
- **<u>Potato variety *in vitro* introduction</u>**

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



Micropropagation techniques

- **<u>Phytosanitary control operated when introducing potato explant in *in vitro* collection</u>**

- 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 separatly. 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 spottled 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 (reniewing): 3 à 4 x/an

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

- System of tracability and documentation operational.

Micropropagation techniques

- **<u>Fast multiplication in the lab (production).</u>**

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

Production objective				
X	**X-1**	**X-2**	**X-3**	**X-4**
10000	2000	400	80	15
October	**September**	**August**	**July**	**June**

Micropropagation techniques

- **<u>Field transfer of the *in vitro* micropropagated material</u>**

  - 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

- **<u>Field transfer of the micropropagated material</u>**

  - Direct sowing of microtubers in field

Micropropagation techniques

- **<u>Field transfer of the *in vitro* micropropagated material</u>**

  - **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!

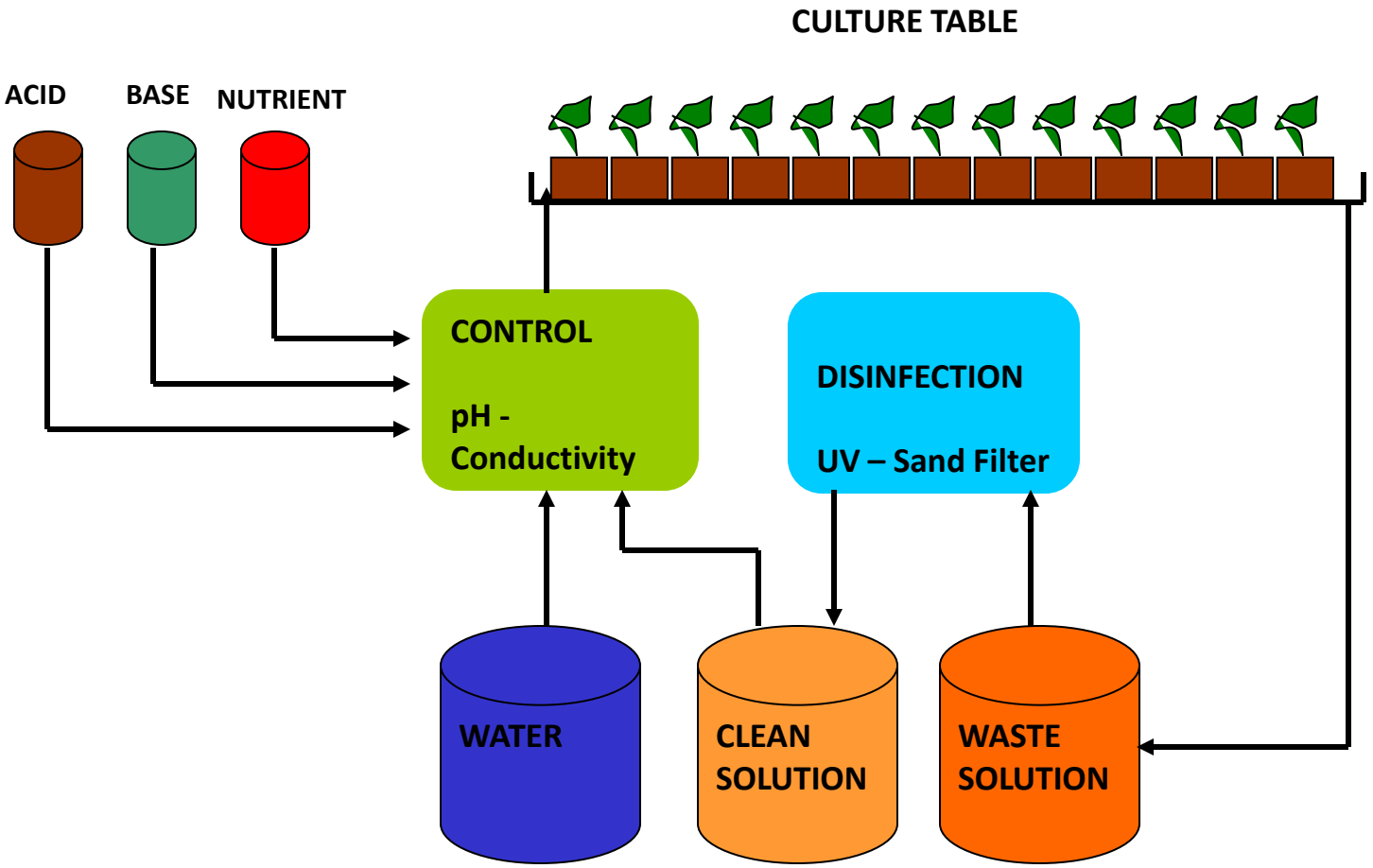
Micropropagation techniques

- **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
    - lenght 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 bacterias after production and before use

Micropropagation techniques

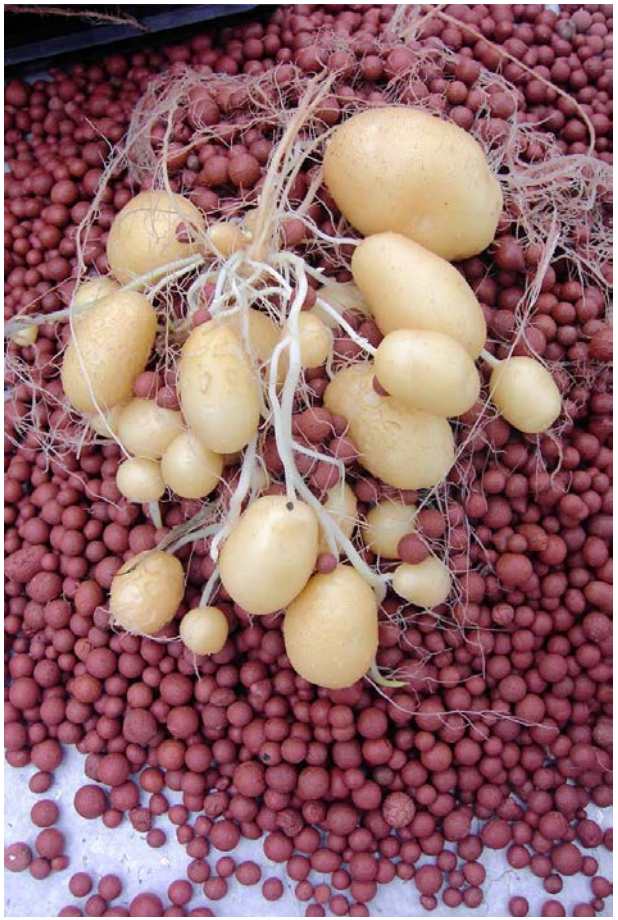
Hydroponic unit for minitubers production



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 transfered

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

Micropropagation techniques

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

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)

a. Microtubers	7/10	0.12 x 0.75 (110833 /ha)
b. Minitubers	10/15	0.12 x 0.75 (110833 /ha)
c. Minitubers	15/20	0.15 x 0.75 (88666 /ha)
d. Minitubers	20/25	0.20 x 0.75 (66500 /ha)

Micropropagation techniques

- **Productivity: comparative study**

Influence of the tuber size on growth and production

ii. Measured variables.

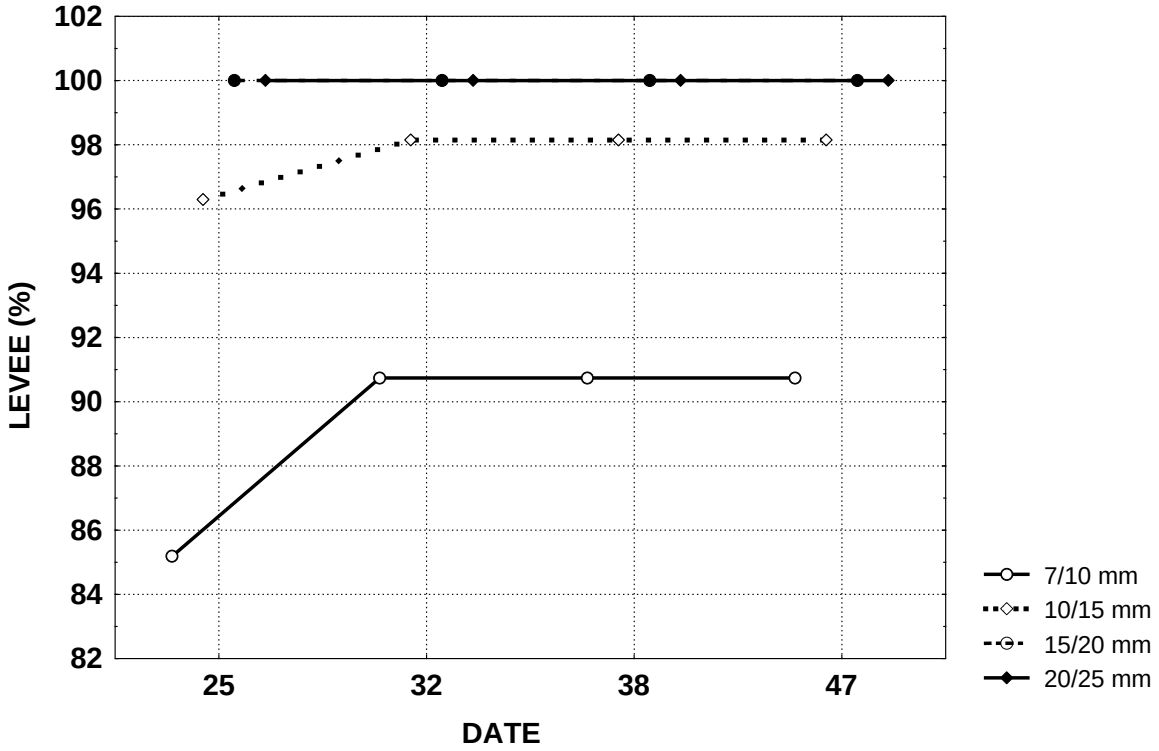
a. Total emergence (%)
b. Plants vigour (Development in cm)
c. Productivity (kg /1,8m of row – kg / ha)
d. Multiplication rate (Tubers number / 1,8m of row – Tubers number / ha)

Micropropagation techniques

- **Productivity: comparative study**

Effect of the tuber size on growth and productivity

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

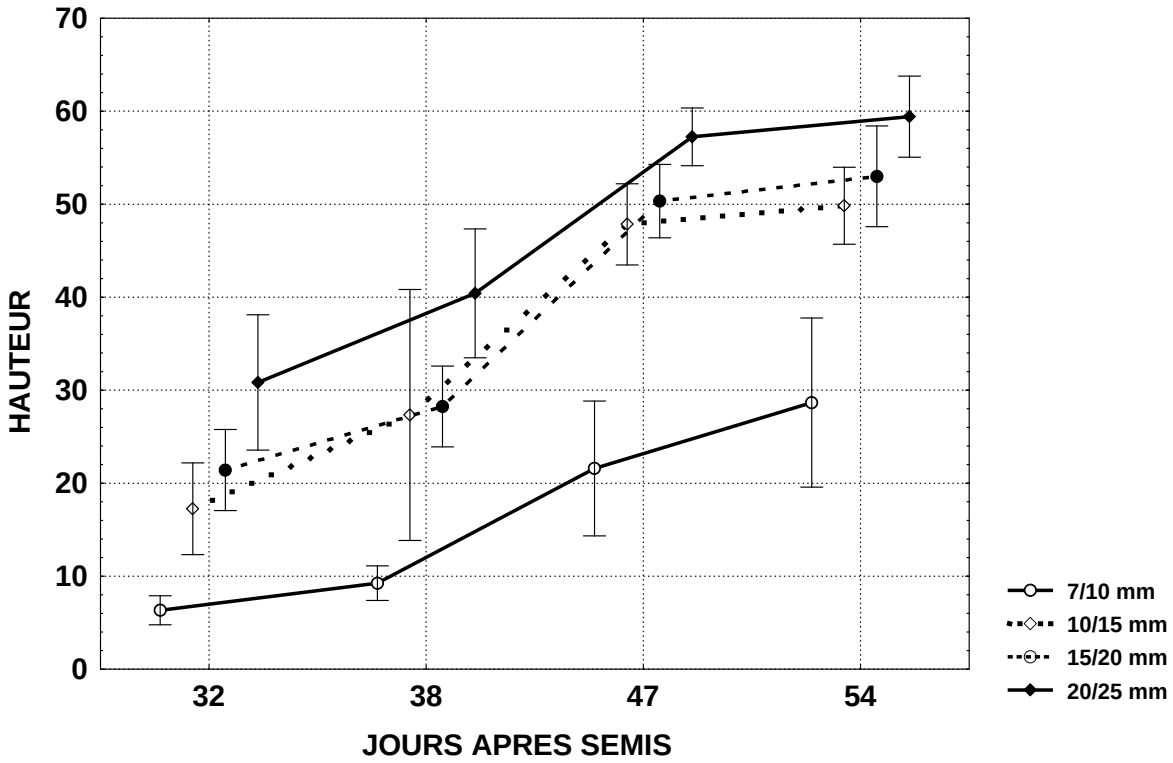


Micropropagation techniques

- **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)

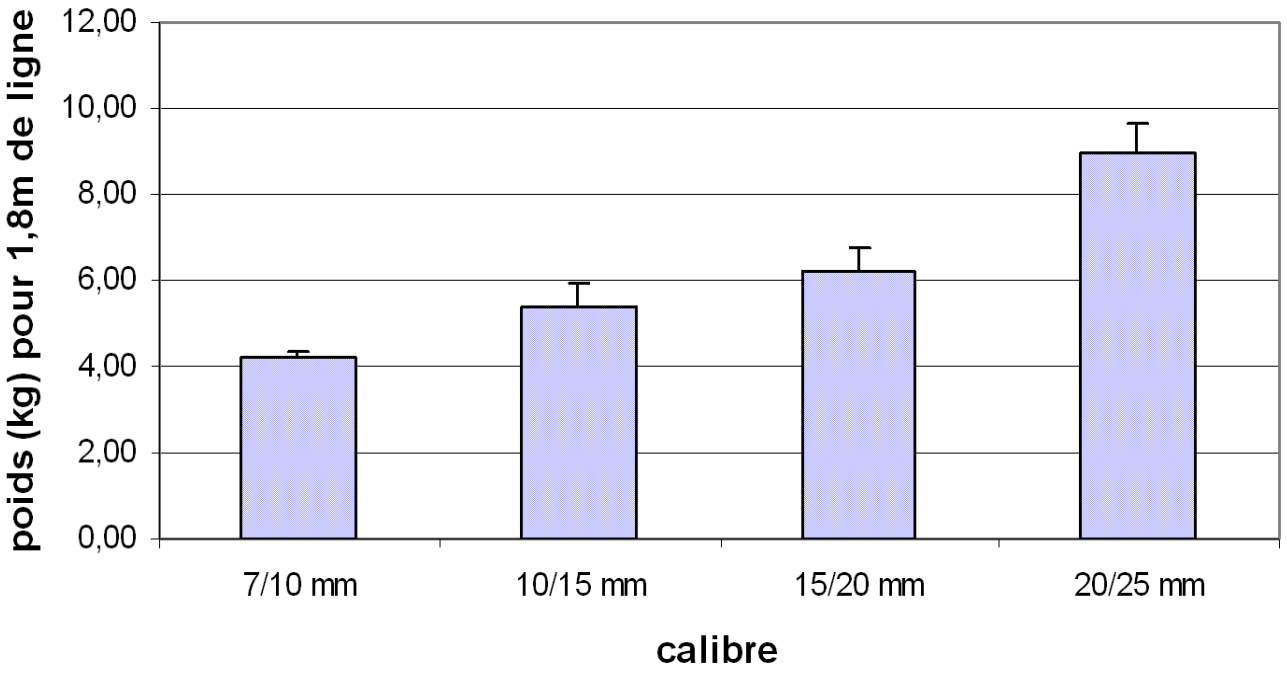
Size	Plantation Distance	Density plants / ha	Tubers weight For 1,8m row (Kg)	Weight / plant (kg)	Weight / ha (kg)
7/10mm	0.12 x 0.75	110833	4,21	0.281	31144
10/15 mm	0.12 x 0.75	110833	5,38	0,359	39752
15/20 mm	0.15 x 0.75	88666	6,18	0,515	45663
20/25 mm	0.20 x 0.75	66500	8,96	0.995	66204

Micropropagation techniques

- **Productivity: comparative study**

Effect of the tuber size on growth and productivity

c. Productivity (Yield)



Micropropagation techniques

• Productivity: comparative study

Effect of the tuber size on growth and productivity

d. Multiplication rate

Calibre	Ecartement	Nombre de plantes / ha	Nombre total de tubercules / 1,8m	Nombre total de tubercules >28mm / 1,8m	Nombre total de tubercules >28mm /ha
7/10 mm	0.12 x 0.75	110833	115.67	67.33	497434
10/15 mm	0.12 x 0.75	110833	95.67	77.33	571314
15/20 mm	0.15 x 0.75	88666	145.33	100.67	743750
20/25 mm	0.20 x 0.75	66500	124.33	107.33	792954

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.

Type matériel	Ecartement	Nombre plantes / ha	Taux de réussite	Nombre total tubercules / ligne 1.8m	Nombre total tubercules >28mm / ligne 1.8m	Nombre total tubercules >28mm / 1 ha
Mini 20/25	0.20 x 0.75	66500	100%	124.33	107.33	792954
Plantules	0.20 x 0.75	66500	100%	157.33	101.70	751360