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# Description of methods and experience of 'in vitro' multiplication of potatoes in the Netherlands

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**Abstract.** Multiplication and storage 'in vitro' of potato plantlets are described, including the factors influencing growth and storage, and tuber formation in soil after multiplication 'in vitro'.
*Key-words:* potatoes, in vitro, multiplication, storage, tuber formation

**Introduction.** Multiplication 'in vitro' is used in seed potato production in the Netherlands to replace diseased stocks rapidly and to accelerate the introduction of new varieties. During multiplication it is essential that characteristics of the varieties are maintained. A review of multiplication is given including the final tuber production in soil.

**Stages of the multiplication procedure.** In the multiplication activities three stages can be distinguished.

1. *Testing.* Before the start of every multiplication seed tubers are tested for bacterial diseases. Healthy tubers are cut into pieces with one bud, which are planted in a glasshouse. Stems growing from the buds are tested 2 or 3 times for virus diseases (e.g. ELISA test).
2. *Multiplication.* Healthy stems are cut into pieces, which are multiplied 'in vitro' by making repeatedly micro-cuttings until the planned number of plantlets has been obtained.
3. *Tuber formation.* Finally, the plantlets are transferred into small pots with a poor mixture of pot soil and sand to promote rooting. After delivery to the breeders the plants are planted in larger containers or in the field for tuber production.

Only stages 2 and 3 will be discussed in this paper.

**Method of 'in vitro' multiplication.** Healthy stem pieces with one axillary bud are sterilized for 20 minutes (1 % NaOCl) and after rinsing 3 times with sterile water transferred into test tubes (150 mm × 24 mm). To the tubes 5 ml of nutrient medium is added containing Murashige & Skoog (1963) macroelements and trace elements supplemented with thiamide-HCl 0.4 mg $l^{-1}$, inositol 100 mg $l^{-1}$, sucrose 2.5-3 % and Difco agar 0.6-0.9 %. The pH is adjusted to pH 6 with NaOH 1 mol $l^{-1}$. The tubes are placed at 20 °C, 16 hours daylength under fluorescent tubes (light intensity 4.5 W $m^{-2}$). After 4-6 weeks the stems grown from the buds are cut into new single bud pieces, which are transferred into tubes with fresh medium, etc.

Tuberization was promoted by adding 8 % sucrose to the nutrient medium.

There are indications for interactions between varieties, temperature and light conditions with respect to optimal rate of tuberization.

**Growth conditions and multiplication rate 'in vitro'.** Growth occurs at various temperatures, daylengths, light intensities and light sources. Growth rate and phenotype of the plantlets are influenced markedly by these conditions: for instance low light intensities, especially in combination with high temperatures cause poor leaf development and weak, elongated stems.

The multiplication rate under the same controlled conditions is constant within a variety, but the number of cuttings varies from 3-10 between varieties. Evidently from every generation of cuttings 3-10 new cuttings per plantlet can be made. Plantlets of varieties with a low multiplication rate may form long internodes, which is not improved by adding the growth retardant daminozide (B9). A too compact growth may also occur. In these cases 2.5 mg $l^{-1}$ gibberellic acid added to the medium had a favourable effect on length of the internodes.

The number of cuttings could not be increased by extending the period of 6 weeks, because the basal buds showed poor or no growth at all after transfer into tubes.

**Storage of 'in vitro' material.** To avoid the laborious repeated testing of the original plant material, healthy sterile plant material should be stored and used as a basis for the next multiplication. In experiments plantlets were stored at 5 °C in complete darkness and in light (light intensity 4 W $M^{-2}$). In darkness the plantlets died too rapidly. After one year of storage in light many plantlets seemed to be dead, but after transfer to 20 °C in light a part showed regrowth. However, the number of surviving plantlets and the number of cuttings that could be made from these plantlets varied widely between varieties and within a variety. Therefore we suggest that 'in vitro tubers' would be more suitable for storage purposes.

In an experiment with cv. Bintje plantlets were stored at 5 °C and 10 and 16 hours daylength. Spontaneous tuber formation occurred at both daylengths. After transferring the tubers into tubes with fresh medium and to 20 °C in light, stems grew only from tubers obtained at 10 hours storage, tubers from 16 hours storage were still dormant.

**Tuber formation in soil.** Tuber yields are influenced by conditions during growth 'in vitro'; at a high light intensity strong plantlets with large leaves were obtained, while at a low light intensity weak plantlets with elongated stems and poor leaf development occurred. After transfer into soil the strong plantlets showed most vigorous growth and produced the highest numbers and yields of tubers.

Plants were grown in a glasshouse in containers (90 cm × 70 cm × 40 cm) filled with a mixture of sand and peat (50/50 vol. perc.) until 15 cm below the top of the containers. At a stem length of 30-40 cm the containers were filled to the top with the same soil mixture. In this way the number of tubers in cv. Bintje increased at least 38 %.

In another glasshouse experiment plantlets (cv. Kennebec) were grown both in

pots (18 cm × 18 cm) and in the above-mentioned containers (in the same way) at 2 plant densities (15 and 30 plants $m^{-2}$). A foliage spray with daminozide (3 g $l^{-1}$) was applied too at stem lengths of about 60 cm. A larger number of tubers per plant was obtained in the containers than in the pots, especially at 15 plants $m^{-2}$ (70 % more). Generally, the daminozide spray did not clearly affect the number of tubers. Only in the containers at 30 plants $m^{-2}$ almost 25 % more tubers were harvested than in the controls.

**Final remarks.** In the Netherlands multiplication 'in vitro' takes place in a well equipped laboratory, but this method can also be used under less favourable conditions (Nguyen Van Uyen, 1984).

Rapid multiplication 'in vitro' can be started with all tissues with meristems, such as sprouts, not only with stem pieces (Nozeran et al., 1977); tubers can be delivered to the customers instead of plantlets (T. D. Hall and C. J. Jeffries, Scotland, personal communication, 1984); larger glasswork with more than one plantlet can be used (Hussey & Stacey, 1984); storage of plantlets at a high temperature is possible, if growth retardants are added to the nutrient medium, but losses may occur (Westcott, 1981).

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