Innovative techniques in seed potato production in the Netherlands

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Voorwoord

In de Sovjet Unie wordt er jaarlijks een bijeenkomst georganiseerd waarin vertegenwoordigers van verschillende onderzoeksinstituten rapporteren over de resultaten van bepaald onderzoek.

Dr. Van der Zaag bezocht in 1988 verschillende instituten in de Sovjet Unie en op zijn voorstel werd besloten om in 1989 een internationaal seminar te organiseren met deelname van onderzoekers van West Europa. Het thema van het seminar in 1989 werd "Toepassingen van biotechnologie in de aardappelveredeling en pootgoedproduktie". Met Boris Dorozhkin ("Siberian Research Institute of Agriculture", Omsk), A. Kuchko (directeur van het "Ukrainian Potato Research Institute") en Yuri Gleba (directeur van "The Cell Biology and Engineering Division of the Ukrainian Academy of Science") werd besloten om als vier buitenlandse gastsprekers de volgende personen uit te nodigen: Gerhard Wenzel (Institute for Resistance Genetics, Gruenbach, Bondsrepubliek Duitsland), George Mackay (Scottish Crop Research Institute, Dundee, Schotland), Lidwine Dellaert (SVP) en Anton Haverkort (CABO). Van der Zaag was verhinderd om zelf deel te kunnen nemen. Het Seminar vond plaats op 5, 6 en 7 juli 1989 op het aardappelonderzoeks-instituut bij Kiev.

De uitnodiging verliep via Michael Y. Veselovski, chef van de afdeling internationale betrekkingen van de "V.I. Lenin All-Union Academy of Agricultural Sciences" (VASKHNIL), Moskou. Met deze uitnodiging hoopt men de wetenschappelijke contacten met West-Europa te versterken onder andere door samenwerking en/of uitwisselingsprogramma's. Overeenkomstig de contacten van Nederlandse onderzoeksinstellingen met andere oostbloklanden is ook vanuit de Sovjet Unie veel belangstelling voor korte studiereizen en post-doc trainingen van 6 maanden tot 1 jaar. Voor Nederlandse onderzoekers lijken met name korte studiereizen gecombineerd met het bijwonen van een (internationaal) congres interessant. Kennis en faciliteiten zijn niet voldoende voor een langer verblijf.

Bijgaand artikel "Innovative techniques in seed potato production in the Netherlands" is gebaseerd op de voordracht te Kiev gehouden.

A.J. Haverkort

Summary

During the last decade, the use of micro propagation techniques has played an increasingly important role in seed potato production in the Netherlands. Already 25 % of the basic seed stocks are derived from in vitro plantlets. Presently, research is being carried out on the production of mini-tubers, derived from in vitro plantlets which are transplanted to beds in the greenhouse, and on micro-tubers, grown on plantlets in vitro. This paper discusses these various uses of in vitro techniques, the research carried out to optimally utilize in vitro plantlets, mini- or micro-tubers and their potential role for the improvement of seed potato production in the Netherlands.

Introduction

In 1987, 170 000 ha of potatoes were grown in the Netherlands. This represents 22 % of the total available arable land. Production totalled 7 million tonnes. One third of this production is processed in the starch industry, 12 % is marketed as fresh ware potatoes on the domestic market, 21 % is processed domestically in the food industry (pommes frites, chips and frozen products) and 5 % is used as seed potatoes for planting in the Netherlands. Of total production, two thirds are exported either as seed potatoes, fresh consumer potatoes or processed. Such data emphasis the importance of potato production for the Netherlands. Trends in exports are shown in Fig. 1. Although potato occupies less than one quarter of the

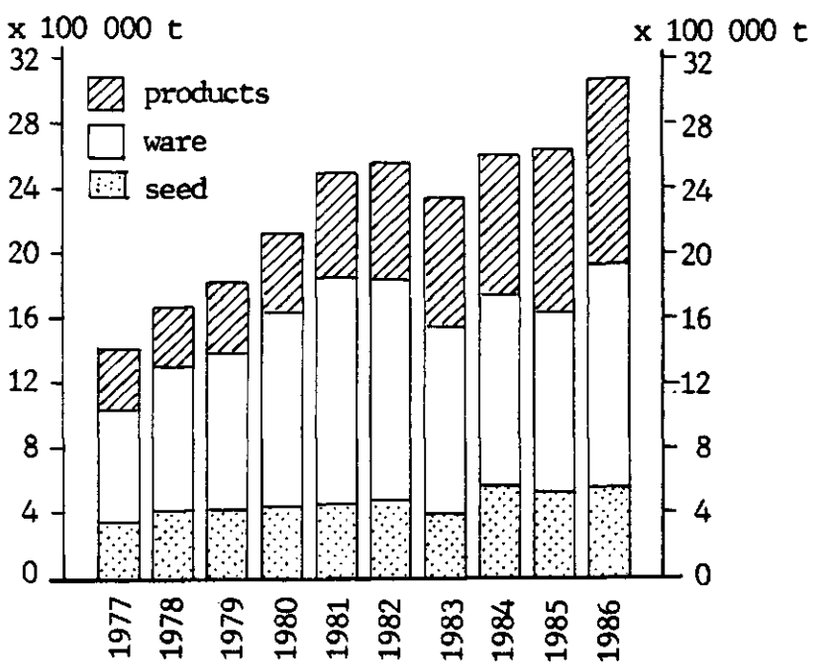

Figure 1. Netherlands exports of seed and ware potatoes and potato products based on fresh potatoes; (data from 'Produktschap voor aardappelen, 1987).

total arable land hectareage in the Netherlands, because of its profitability, it represents about half of the income of arable farmers. A further increase of production is limited because soil pathogens restrict a further narrowing of rotations (Van Loon, 1988; Van der Zaag, 1988c).
It appears from these data that slightly over 13 % of the total production in the Netherlands is sold as seed potatoes, almost one million tonnes, of

which 60 % is exported. Because of the higher prices fetched for seed potatoes, relative farm income from seed production is higher, about 25 %. The complete national seed potato programme consists of three stages: the production of pre-basic seed, basic seed and certified seed (Van der Zaag, 1987). The production of pre-basic seed, or stock seed, usually takes place on specialized seed farms. The number of multiplications or generations of pre-basic seed, each multiplied in the field, is about 6. In addition, about three multiplications are needed to produce certified seed, a total of about nine multiplications (Fig. 2). A major proportion of seed potatoes

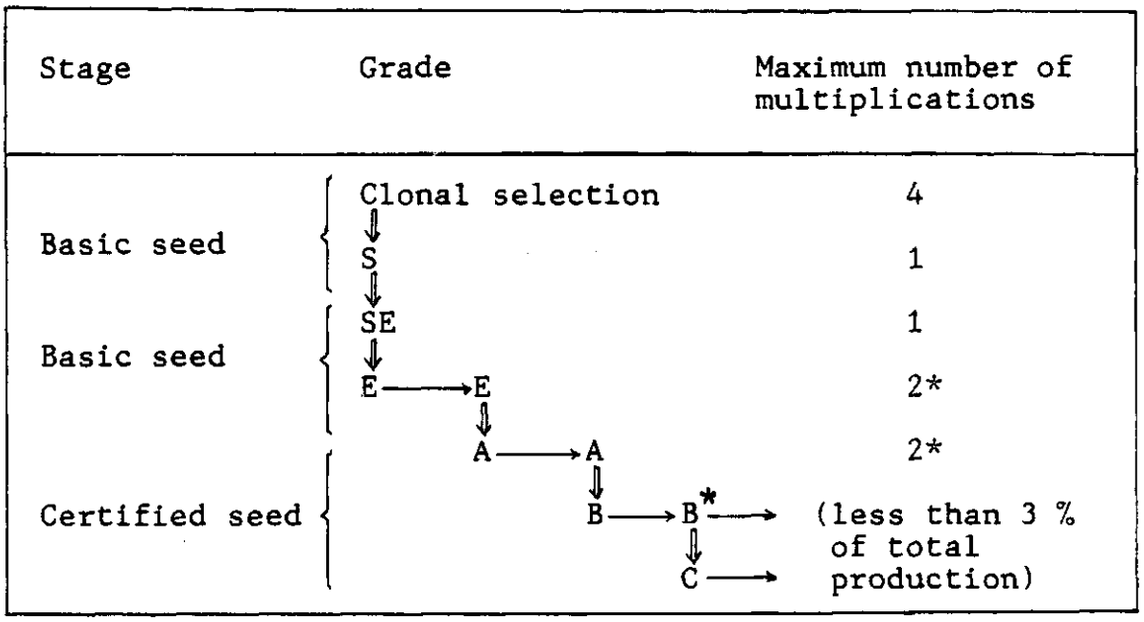

Figure 2. Seed potato production scheme in the Netherlands.

in the Netherlands (75 %) is still derived from traditional clonal selection procedures. Class E seed tubers have then been multiplied 6 to 8 times in the field, class A seed 7 to 10 times. With each multiplication in the field, there is a chance of infection with virus, fungal and bacterial diseases. Sometimes infection levels may decrease, depending on pathogen and growing conditions. For virus diseases such as PVX and PVY, a reduction of the number of field multiplications is an important means to keep infection levels low. With bacterial diseases, infection levels increase with each growing season as well. These are the reasons why the lowering of certification grades is proportional to the number of multiplications in the field. Some bacterial diseases such as *Erwinia carotovora* var. *atroseptica* which causes black leg is important in temperate climates and

Erwinia chrysanthemi which causes stem rot and is more important in warmer regions, receive special attention because of their potential danger for seed potatoes exports.

Micro propagation techniques have mainly been developed for two purposes:

- to rapidly bulk newly bred cultivars, or cultivars for which a sudden demand arises. Due to the export oriented seed potato production and the rapidly shifting markets, certain cultivars occasionally have to be bulked more rapidly than is possible through field planting;
- to replace basic plants in the traditional clonal selection system by in vitro plantlets to improve the standard of health of the pre-basic seed or even to elininate quarantine diseases.

Recently developed new micro propagation techniques producing tuberlets, offer possibilities to reduce the number of multiplications in the field. This is an advantage in seed potato production; exposing seed potatoes for less seasons to diseases, especially soil-borne diseases, will reduce their accumulation and infection levels.

Several rapid multiplication techniques exist:

- in vivo techniques based on cuttings from plant parts (leaf buds, stems, sprouts) which produce stolons and underground tubers when planted with at least one leaf bud under soil level, or areal tubers (Marinus, 1987) when planted with only a part of the stem rooting in the soil and with the leaf bud well above ground (Fig. 3)

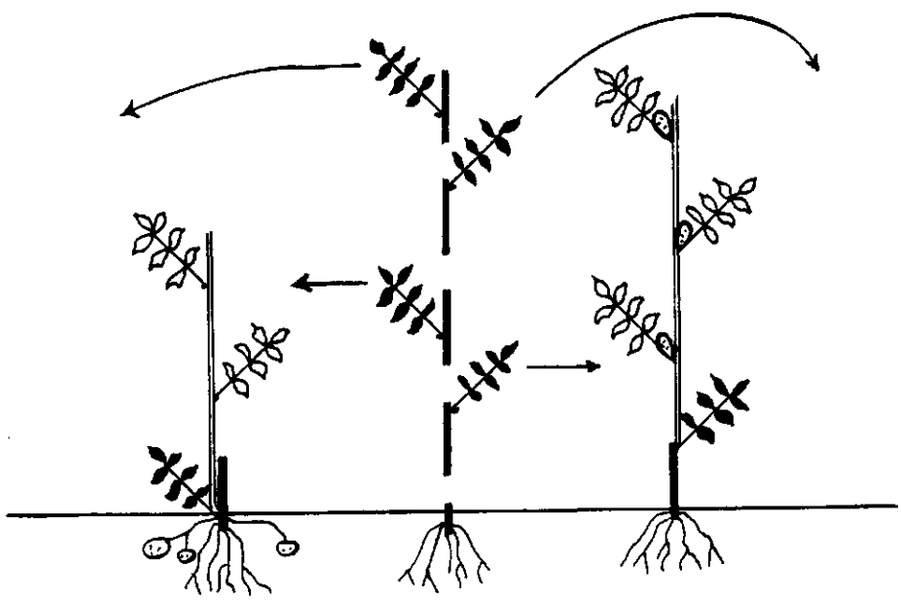

Figure 3. In vivo stem-cutting techniques leading to under-ground (left) or above-ground (right) tubers.

- in vitro based cutting techniques. These techniques, which will be discussed in more detail in the following section, make use of in vitro production of plantlets under sterile conditions. In seed potato production, these plantlets produce almost normal sized tubers when grown in the field, beds or pots if allowed to grow to full maturity. When in vitro plantlets are transplanted to beds at high densities, and if the same plants are harvested several times in succession, they yield small so called mini-tubers. Given time and tuber-inducing conditions, in vitro plantlets may also produce tubers in vitro which are called micro-tubers or in vitro tuberlets.

Production and utilization of in vitro produced material

In vitro plantlets

In vitro production of plantlets in the Netherlands is centrally organized by the Foundation for the Supervision of Rapid Multiplication (FSRM), an organization of the seed potato industry with seed potato specialists as advisors. In vitro plantlets are produced by the NAK, (Netherlands Seed Certification Service) in Slootdorp. Farmers who wish to replace part of the basic plants of which the progeny produces first year clones by vitro plantlets, make a contract with FSRM and send 25 very healthy tubers, preferrably tubers of second year clone to this laboratory

Table 1. Illustrative example of increase rates of clonal material in the field with and without the use of in vitro plantlets.

Year	Traditional	Clonal selection combined with clonal selection the use of vitro plantlets
0	say 10 basic plants	say 500 plantlets yield 2000 tubers
1	10 x 15 plants	2000 plants
2	10 x 200 plants	15 000 plants
3*	10 x 2000 plants	150 000 plants
4*	10 x 20 000 plants	1 500 000 plants

* Usually part of this production is already delivered as S material, particularly for the clonal selection combined with in vitro plantlets

in autumn. After checking these tubers for diseases, sprouts are brought in vitro and multiplied through single node cuttings (Marinus, 1985). In the next spring the farmers grow the plantlets in pots or on beds in a screenhouse. Thus they gain one year and the tubers produced from the in vitro plantlets are further treated as first year clones but are much more numerous (Table 1). Micro propagation does not mean a replacement of the clonal selection procedures, but a valuable addition.

Mini-tubers

Several groups in countries such as Denmark, Hungary, the Netherlands the USSR and Peru (Dodds, 1988; Horvath, 1987; Jones, 1988; Mastenbroek, 1987; Van der Zaag, 1988a) are presently working on in vivo production of high numbers of small tubers derived from in vitro produced plantlets. These mini-tubers are produced in screen- and greenhouses to allow careful handling and to protect them from (vectors of) diseases. The general principles of this method are schematically represented in Fig. 4.

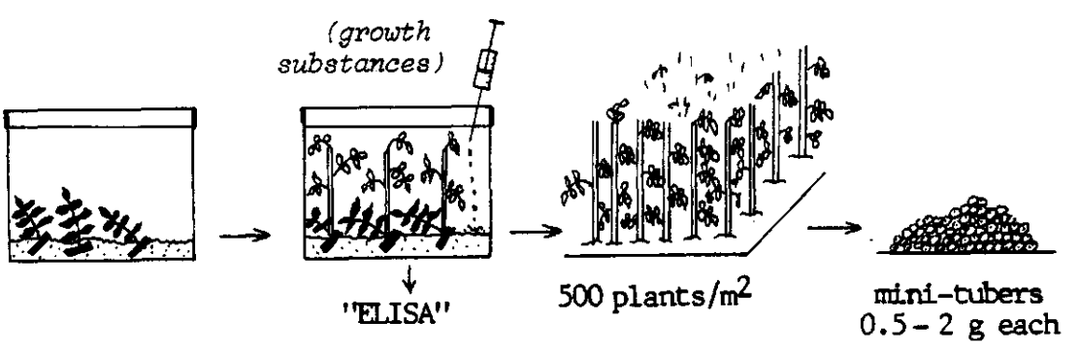

Figure 4. Schematical representation of mini-tuber production.

Plantlets are allowed to grow in small containers on a nutrient medium in sterile conditions. When appropriate plant sizes and numbers are reached, substances may be added which disfavour haulm growth and/or favour tuberization. Plant growth regulators known to influence tuber induction (Stallknecht, 1985) or other substances (Struik et al., 1987) may be involved. A subsequent presence of tuber inducing substances (Hungarian method viz. Van der Zaag, 1988a) may be detected whereupon the plantlets are transplanted to a screen- or a greenhouse. The planting density in a perlite/soil mixture is high, up to 500 plantlets per m^2. The tuberlets that are formed are removed after a few weeks and the plants are replanted.

By repeating this process 2 to 3 times, thousands of mini-tubers are harvested per m^2. When the same space in the greenhouse is replanted 2 to 3 times per year, between 5000 and 10 000 mini-tubers can be harvested per m^2 per year. Their weight varies from 0.5 to over 2 grammes. Their potential role in a seed production programme will be discussed later, along with that of the micro-tubers.

Micro-tubers

Micro-tubers are small tuberlets formed on plants in vitro. Their weight is mostly less than one gramme. They are produced by allowing in vitro plantlets to grow in tubes or containers under tuber-inducing conditions. These conditions may consist of a higher sugar concentration in the medium (8 %) than used for in vitro growth of the plantlets, shorter daylengths (e.g. 12 hrs) and, eventually, the addition of tuber inducing and/or haulm growth inhibiting substances (Fig. 5). Taiwanese researchers

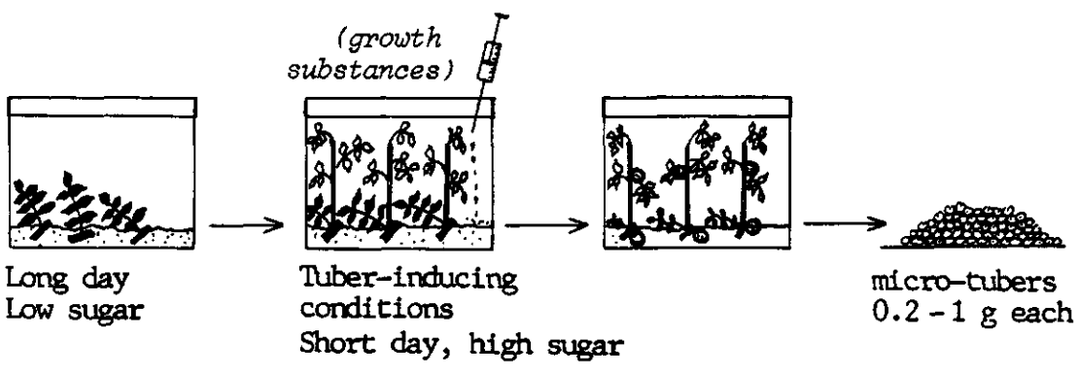

Figure 5. Schematical representation of micro-tuber production.

(Wang & Hu, 1982) reported that they produced 36 300 micro-tubers on 10 m^2 in four months time. After 3 multiplications in the field, 1800 t of seed tubers were derived from them; these sufficiently covered national needs. Plant Biotech Industries Ltd. in Ashrat (Israel) claim to be able to produce 50 in vitro plantlets in 10 cm x 10 cm containers (Van der Zaag, 1988a). In two months these plants produce about 100 micro-tubers: i.e. 10 000 micro-tubers per m^2 per harvest, resulting in about 50 000 microtubers per m^2 per year. This process is largely automated and yields tubers with weights varying from 0.2 to over 1 gramme. USSR scientists in Kiev reportedly (Van der Zaag, 1988a) produce 10 000 micro-tubers per m^2 per year. Planting 45 000 of these per hectare yield 500 000 seed tubers of about 60 g each.

The potential role of mini- and micro-tubers

The future use of mini- and micro-tubers will depend on a number of factors (Van der Zaag: 1988c):

- the possibilities to reduce the number of multiplications in the field;
- the effect of a reduced number of multiplications on the standard of health;
- their yielding ability under various weather conditions;
- their production costs.

The number of multiplications in the field can only be drastically reduced (from 7 or 8 to 3 or 4) with a massive input of mini- or micro-tubers. For the situation in the Netherlands, this ideally would be an annual planting of 1000 ha with mini- or micro-tubers with yields, multiplication rates, domestic utilization and exports as illustrated in Fig. 6. According to

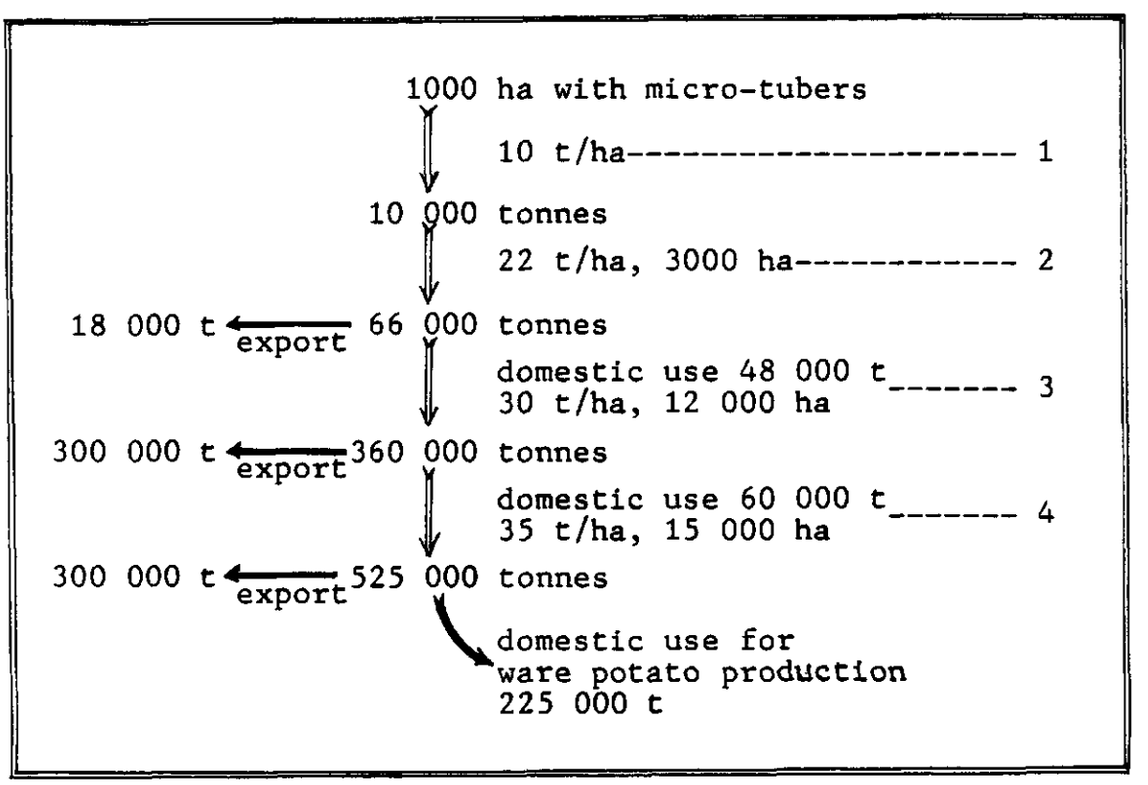

Figure 6. Illustrative example of a seed potato production programme based on 1000 ha planted with micro-tubers (after Van der Zaag, 1989).

this scheme, the total demand of seed potatoes can be met with an annual planting of 60 million mini- or micro-tubers (1000 ha with 60 000 plants per ha). The scheme in Fig. 6 shows that over half the amount of seed tubers that are replanted for seed production in the Netherlands or abroad,

Table 2. Yields from small field grown tubers, planted on 29 April and harvested on 13 July, 1988. Data from ir C.B. Bus, Research Station for Arable Farming and Field Production of Vegetables, Lelystad Three replicates of 9 m^2 per plot, planting distances 75 cm x 20 cm.

Cultivar	Seed tuber weight (g)	Number of tubers per plant	Yield (t/ha)
Bintje	0.44	5.6	9
Bintje	1.49	8.4	16
Ukama	0.77	3.4	12
Ukama	2.12	4.6	20

have only been planted three times in the field. None of all seed potatoes produced will then have passed more than four multiplications in the field. Although it has not yet been researched, it is generally assumed that, when mini- or micro-tubers will be planted on specialized farms (those presently carrying out clonal selection), infection levels of seed potatoes will decrease strongly (Van der Zaag, 1989).

Yields of seed potato crops grown from mini- or micro-tubers are likely to be much lower than those from crops planted from normal (35 to 45 mm) seed tubers, especially when micro-propagated material is not allowed to grow to maturity. Wattimena et al. (1983) observed mainly single stemmed plants, which branched vigourously. At an early harvest both weight and number of tubers from plants grown with micro-tubers were inferior to those from crops grown from normal sized seed tubers. At maturity, however, the number of tubers per micro-tuber propagated plant, was superior.

In the example of Fig. 6, the yield from mini- or micro-tubers is estimated at 10 t/ha. In 1988 two preliminary field trials were carried out that justify this assumption. Table 2 shows yields of crops planted with very small tubers (comparable to mini-tubers) picked up from the field at the previous commercial seed crop harvest. Yields varied from 9 t/ha with the cultivar Bintje of wich seed potatoes of less than 0.5 g were planted, to 20 t/ha with the cultivar Ukama of which the mean seed tuber weight was just over 2 g. This trial was carried out on a clay soil and spanned a normal seed production cropping season period from late April till mid-July. In the same year a field trial (Table 3) was carried out on a sandy soil with the two cultivars Gloria and Morene from late May to early

Table 3. Yields from different propagation techniques in the field. Planted on 26 May, harvested on 8 September, 1988. Two replicates of 7.5 m^2, planting distance 75 cm x 30 cm.

Cultivar	Propagation technique	Tuber fresh weight g/plant	Number of tubers per plant
Gloria	Direct planting of micro-tubers	342	7.35
	Transplanted micro-tuber plants	344	7.98
	In vitro plantlets	147	6.68
	Seed tubers (28-35 mm)	870	10.55
Morene	Direct planting of micro-tubers	452	5.57
	Transplanted micro-tuber plants	697	9.73
	In vitro plantlets	655	7.07
	Seed tubers (28-35 mm)	978	8.36

September. Here several sources of propagation material were tested, including micro-tubers and in vitro-plantlets that were allowed to grow on soil in a greenhouse to plants of about 15 cm, before being transferred to the field on May 26. Yields from micro-tubers were about half as high as those from seed tubers, while the number of tubers produced reached about two thirds. Transplanting plants from micro- tubers rather than direct planting, substantially increased both yields and number of tubers with the late cultivar Morene. Due to risks of nightfrosts, which are more hazardous for plants growing from micro-tubers, planting is likely to be a few weeks later (early May). Haulm killing of basic seed crops is usually between July 15 and 20. The special efforts to obtain yields as high as possible are likely to increase the per hectare costs of production. The influence of production costs and retail prices of mini- or micro-tubers on production costs of seed potatoes, which are produced after a few multiplications, is best illustrated with the aid of a calculation example. This is done for micro-tubers for which a farmer would have to pay Fl 0.24 or 0.16 each, and for regular seed potato tubers which cost Fl 0.08 each. It is assumed that starting in the first season, 50 000 micro-tubers (M) or S-grade seed tubers (S) are planted. They yield 10 and 25 t/ha respectively. In the second year 3 t/ha M- progeny and 4 t/ha S-progeny are planted yielding 22 and 25 t/ha respectively. In the third year both 4 t/ha M- and S-progeny from the second year are planted, both yielding 30 t/ha.

These two seed lots are replanted in the fourth year and yield 35 t/ha. The yield increases are due to later haulm killing dates associated with lower seed grades. It is further assumed that total production costs (less the costs of seed tubers) are Fl 12 000.- per ha for a crop from seed tubers and Fl 14 000.- when micro-tubers are planted. It appears that the production costs of 100 kg of seed tubers after three multiplications from micro-tubers costing Fl 0.24 each, is Fl 52.00 and Fl 47.76 when the scheme started with S-grade seed tubers costing Fl 0.08 each. After 4 multiplications these amounts are Fl 40.23 and 39.74 respectively (Fig. 7).

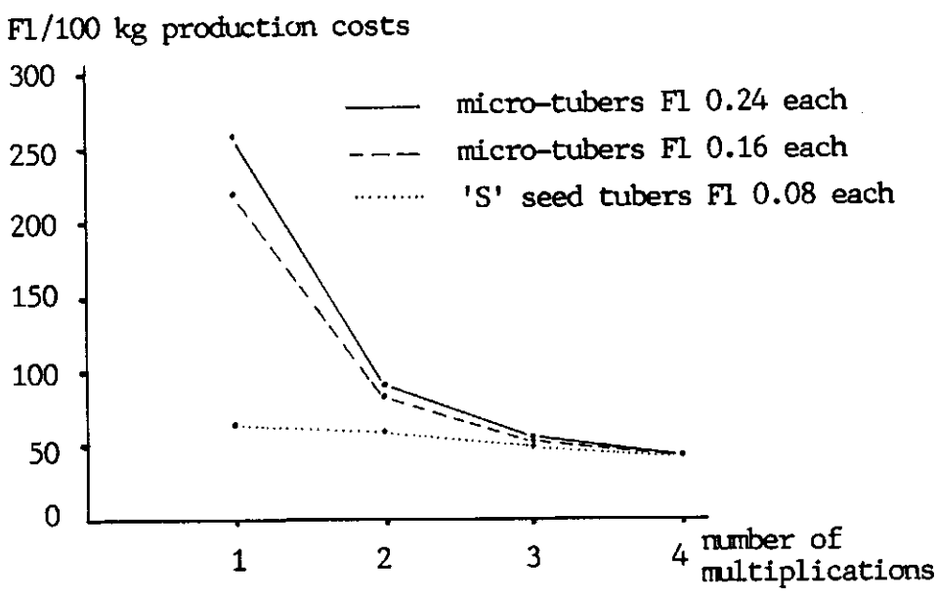

Figure 7. Estimated production costs of 100 kg of seed tubers starting with S-grade seed potatoes and micro-tubers with different prices (after Van der Zaag, 1989).

Thus, production costs of certified seed derived from mini- or micro-tubers after 3 to 4 multiplications are only slightly higher than that of seed derived from pre-basic seed.

Research on the production and utilization of micro-propagated material is still on-going in the Netherlands. Several groups at various institutions divide the different tasks needed to further exploit the possibilities that lay ahead. Research (Table 4) presently focuses on the detection of tuber inducing growth substances and optimizing mini- and

Table 4. Research groups and research themes on the subject of production and utilization of micro-propagated potato material in the Netherlands.

Research group	Themes
Agricultural University Wageningen	- Department of Plant Physiology
Detection of tuber inducing substances	- Department of Crops and Grassland
Mini-tuber production	Science Utilization of mini- and micro-tubers
Centre for Agrobiological Research Wageningen	Micro-tuber production (CABO)
	Utilization of micro-tubers
National Seed Certification Service (NAK) Slootdorp	Production of in vitro plantlets
	Utilization of in vitro plantlets
Research Station for Arable Farming (PAGV) Lelystad	Utilization of in vitro plantlets, mini- and micro-tubers
Institute of Plant Protection (IPO) Wageningen	Study degeneration rates (*)
Institute of Mechanization (IMAG) Wageningen	Mechanization of handling of micro-propagated material (*)

* These themes have as yet to be developed.

micro-tuber production and utilization. Research on mechanization of (trans)planting and on the increased standards of health still need to be formulated. Besides state research institutions mentioned in Table 4, a number of private firms are carrying out development work in order to turn the technology into a profitable part of their enterprises.

Discussion

The incorporation of micro-propagated material in a seed potato production programme has already started in the Netherlands. In vitro plantlets are playing an increasingly important role. Although almost 25 of basic seed production is based on in vitro plantlets, there are no indications that the health status of seed stocks on a whole has improved. This means that the traditional method still fulfills its task. It is likely though, that the use of in vitro plantlets enables some farmers,who had problems with

bacterial diseases, to continue to grow seed potatoes. When mini- and/or micro-tubers become available in sufficiently large quantities, they are very likely to take the place of in vitro plantlets because they are easier to store and handle than small plants. Plantlets in tubes, containers or potted are bulky and difficult to transport while mini- and micro-tubers take very little space. At this moment it is not clear which type of tuberlet is likely to become more important: mini- or micro-tuber. An advantage of mini-tubers is that they are somewhat larger than micro-tubers. Micro-tubers, however, are likely to be cheaper because their production may be fully automated and thus involve less expensive labour. Furthermore, micro-tubers are grown under sterile conditions which ensures complete freedom from diseases.

Once the production of mini- or micro-tubers is possible in great quantities and at reasonable costs, additional research is needed to make full use of this material. The advantage, healthwise, of the strong reduction of the number of generations in the field, still has to be confirmed by research. Equipment to plant the material and to take care of it during the first weeks of growth still has to be developed. And finally, it is important to speed up growth of the plants from mini-or micro-tubers which are planted in the field. Preliminary field trials have already shown that these types of material lead to a much later arrival at 100 % ground cover. With the relatively cold springs and the early haulm killing because of the arrival of aphids, means to improve the early ground cover of the crops are needed. Presently experiments with plastic sheets covering the soil, to warm it up after planting, are in place. A more expensive means, because it involves more labour, is planting the tuberlets in small plastic pots and transplanting them to the field when they are 10 to 12 cm long. This process too can be automated. It may also be considered to use micro-tubers as propagation material in mini-tuber production. The advantage of such a system is is the easier handling of the micro-tubers versus in vitro plantlets.

Finally, as was shown before, the additional costs to plant one hectare with tuberlets should be considered as over-head costs for the production of subsequent crops. After 3 to 4 multiplications, these additional costs seem almost negligible, while the quality of the produce may have improved. Such an improvement, however, still needs to be confirmed by research and practice.

Acknowledgements

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