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# **Field performance of potato microtubers as propagation material**

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### **Summary**

In five field trials with the cultivars Gloria (early), Bintje (mid-early) Désirée (mid-late) and Morene (late), crops grown from conventional 28-35 or 35-45 mm seed were compared with crops grown from microtubers, of various sizes and from various sources, that had been produced *in vitro.* The following means to quicken initial crop development from the micropropagated material were tested: large microtubers, plastic soil cover, and transplanting of plantlets grown from microtubers pre-planted in a glasshouse. Crops grown from microtubers weighing less than half a gram yielded much less than crops grown from conventional seed crops but their yields were increased by each treatment. With the later-maturing cultivars, which generally produce few tubers per plant, the yields within seed grades from plants grown from transplanted microtuber plants were comparable with those of conventional crops. Reasons are given, why direct planting of microtubers, with or without plastic foil, is not a practical option.

# **Introduction**

In the Netherlands in 1989, potatoes were grown on 160000 ha, which represented  $21\%$  of the total available arable land. Production totalled 7 million tonnes.

Currently, one third of the crop 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 and  $5\%$  is used as seed potatoes in the Netherlands. Of the total production, two thirds are exported either as seed potatoes, fresh consumer potatoes or processed in the food or starch industry (Produktschap voor Aardappelen, 1990). Because of its profitability, potato production represents about half the net income of Dutch arable farmers. Any potential increase of production by shortening rotations is limited by the hazard of a build up of soil-borne pests and pathogens (Van Loon, 1987). Rapid multiplication techniques under partly controlled diseasefree and vector-free conditions are currently used both to bulk rapidly those cultivars for which a sudden need arises and to hasten the traditional clonal selection process (Haverkort & Van der Zaag, 1989). When there are only a few multiplication stages in the field, there is a correspondingly low risk of accumulating soil-borne pests and pathogens.

Rapid multiplication of potato seed in the Netherlands is usually done with the aid of various *in vitro* procedures (Marinus, 1985). Worldwide, so-called minitubers are

produced by several research groups from plantlets grown *in vitro* that are planted in beds in glass- or screenhouses at densities of about 200-500 plants per  $m<sup>2</sup>$ . These produce thousands of minitubers of about 0.5 to 2 g each (Dodds, 1988, Horvath  $\&$ Foeglein, 1987; Jones, 1988). So-called microtubers are small tubers, generally weighing less than 1 g, that are produced by allowing plantlets to grow and tuberize in glass containers under tuber-inducing conditions, sometimes with the addition of growth regulators. In this way, thousands of microtubers may be produced per  $m<sup>2</sup>$  in 3 to 4 months (Wang & Hu, 1982).

Microtubers need to be multiplied at least once under protected or field conditions, but preferably two to three times, to reduce the costs of production of seed potatoes (Van der Zaag, 1991) or for other uses. The general objective of research on the production and use of minitubers and microtubers is to improve the health status of conventionally sized seed potatoes by reducing the number of multiplications in the field. The foliage of basic seed potatoes in The Netherlands usually has to be destroyed in the second half of July and by then there has to be an acceptable tuber yield at an acceptable cost.

The specific objective of the research described here was to determine the yield potential of microtubers when planted in the field as propagation material for seed potato production. Four cultivars (an early, mid-early, mid-late and late) were grown in three seasons and several cultural techniques to hasten initial crop development were tested; these included the application of transparent plastic foil at planting to preserve soil moisture and to warm the top layer of the soil in which the microtubers were planted, pre-planting the microtubers in a glasshouse and transplanting them to the field when they were about 10cm high, and the use of larger microtubers.

### **Materials and methods**

*General.* Five field trials, laid down as fully randomized complete block designs, were planted in 1988, 1989 and 1990 on the experimental farm, "Droevendaal", of the Centre for Agrobiological Research (CABO-DLO) Wageningen. The trials were intended to compare the growth and yield of seed grades from variously treated plots of plants grown from rnicrotubers of different sizes and sources, with that of plots grown from conventional seed (Dutch class E, well sprouted, size grade 28-35 or 35-45 mm). The cultivars used were chosen to represent four growth categories, early (Gloria), mid-early (Bintie), mid-late (Désirée) and late (Morene).

The experimental details are given in Table I which shows the various planting distances within the rows which were spaced 75 cm apart. Each plot for the final or periodic harvests consisted of 3 or 4 rows of 3 to 9 plants surrounded by two guard plants and one guard row. Prior to planting, NPK fertilizer was broadcast at 100-200- 180 kg ha<sup>-1</sup> which corresponds to the rate recommended locally for seed potato production. Conventional seed tubers were planted about 15 cm deep in hills whereas the microtubers were planted about 3 cm deep in a 5 cm deep planting furrow. Plants grown from microtubers were transplanted also into a 5 cm deep furrow and all plants grown from microtubers were earthed up by hand when they were about 20 cm high. Plants were irrigated once or twice per week until emergence but thereafter according to evapotranspirative requirements so as to avoid drought stress at any time during crop growth.



Table I. Conditions of the five field experiments.

\* Included as a treatment.

*Micro-tuber production.* The microtubers used in Experiments I, 2 and 4 were produced at CABO by allowing plantlets in glass tubes, containing 8% sugar agaragar medium, to tuberize under short day (12 hrs) and low temperature (18  $^{\circ}$ C) conditions, and without growth regulators. The microtubers used in the Experiments 3 and 5 were produced through unrevealed procedures by Plant Biotech Ltd. (Israel) and Small Potato Inc. (Wisconsin, USA) respectively. In all experiments, microtuber masses varied from 0.2 to 0.4 g, except in Experiment 2 where different size grades (0.1-0.2, 0.2-0.3 and 0.3-0.5 g each) were compared. The microtubers used in the first two trials were well-sprouted. They were pre-sprouted in the light for the last month before planting to harden the sprouts which were about 3 mm long at the time of (trans)planting. Microtubers in Experiments 3 and 4 were just sufficiently sprouted at the time of planting but the microtubers used in Experiment 5 had to be treated with Rindite some three weeks prior to planting to break dormancy.

*Planting treatments.* The plots planted with microtubers were given various treatments to enhance the performance of the microtubers as propagation material. Plots of directly planted microtubers were untreated, or covered with a transparent plastic perforated foil (5% perforation) for about four weeks from planting until the plants were about 5 cm high, a treatment designed to increase soil temperature and to preserve soil moisture (the perforations allowed irrigation water to reach the soil). Those plants growing from directly-planted microtubers in Experiment 5, that were unprotected with plastic foil, were killed by a night frost during the first few days of June just after emergence and no further reference will be made to this treatment. For

the transplanting treatments, the microtubers were first planted into small 5 cm diameter 7 cm deep pots placed in a glasshouse with day/night temperatures of 21/15  $°C$ . When the plants were about 15 cm high they were placed outside for 3 to 4 days to harden prior to transplanting to the field.

*Records.* The percentage ground cover was determined twice weekly with a  $75 \times 90$ cm grid split into 100 rectangles and encompassing three or 4 plants. The cumulative amount of solar radiation intercepted by the crop was calculated from this percentage and total weekly solar radiation (e.g. Haverkort & Harris, 1986). At each periodic harvest (Table 1) the masses of fiesh tuber, haulm, and underground parts were determined as well as their percentage dry matter by drying for 24 hours at 105 °C. The number of stems and of tubers (defined as swellings not less than twice stolon thickness) were counted and the tuber size distribution was assessed by grading them into the classes  $\langle 28 \text{ mm}, 28-35 \text{ mm}, 35-45 \text{ mm}$  and  $>45 \text{ mm}$ .

# **Resulls and discussion**

In Experiment 1, plants derived from *in vitro* plantlets and from microtubers had one mainstem, occasionally two (Table 2). The early cv. Gloria yielded less per plant than did the late cv. Morene, whatever the source of propagation material. With cv. Gloria, plants from microtubers (direct and transplanted) yielded less than half of plants from conventional seed tubers, and plants grown from *in 9itro* plantlets yielded less than  $17\%$  of the control. With cv. Morene, directly planted microtubers yielded the least (50% of the control), while transplants from microtubers and *in vitro*  plantlets both yielded about  $70\%$  of the control. A similar performance for these two propagation methods has also been reported by Leclerc & Donelly (1990). With all four cultivars, the number of tubers per plant was not proportional to tuber yield and this resulted in reduced mean tuber masses from the *in vitro* propagated material. The high harvest index of the cv. Gloria suggests that the crop was mature at 101 days after planting

The different planting materials of the cvs Gloria and Morene in the second experiment in 1989 produced plants that covered the ground with green leaves at



Table 2. Results of field trial 1 (experimental details in Table 1). Data indicated with the same letter per column do not differ at the  $5\%$  probability level.



Fig. 1. Ground cover development of crops of cvs Gloria and Morene grown from transplanted microtuber plants (open symbols) and from 28-35 mm conventional seed tubers (closed symbols). Data represent the mean values of the three microtuber sizes used in Experiment 2 in 1989.

different rates (Fig. 1). Within 11 weeks from planting the Gloria control plants had reached maturity and, accordingly, ground cover had decreased greatly whereas at that time the Morene control plants still covered the soil. In contrast the Gloria crops grown from microtubers had then not yet reached  $50%$  ground cover but the Morene crop from microtubers almost fully covered the soil. The prolonged and slow development of the canopy of Gloria growing from transplanted microtubers indicate that this early cultivar partitioned proportionately more dry matter to the tubers than did the late cv. Morene. The failure of these plants of Gloria to become senescent may have been due to the ample availability of light, water and nutrients for which the small plants of Gloria did not compete so strongly as did the large plants of Morene. Yields from microtubers of Gloria (Table 3) were about half that of the Gloria control. The number of tubers per transplant of each cultivar was significantly higher than that of its control as had also been observed with cv. Morene in Experiment 1 (Table 2). The same phenomenon was observed by Levy (1985) when transplanted proliferated shoot cuttings, initially produced *in vitro,* of cvs Idit and Orit were grown in the field, by Wattimena et al. (1983) with plants grown from microtubers and from *in vitro* cuttings of cvs Norland and Red Pontiac, and by Leclerc & Donelly (1990)

Variety	Material	Stems	Tubers	Tubers	Yield	<b>Size</b>	Harvest	
		/plant	/plant	$\sqrt{\text{stem}}$	(g/pl)	(g/tuber)	index	
Gloria	Transplants (mean)	1.55 <sub>b</sub>	13.7b	8.84b	330	24.6a	0.80 <sub>b</sub>	
	Seed tubers (control)	2.03c	9.7a	4.78a	638	67.3 <sub>b</sub>	0.85 <sub>bc</sub>	
Morene	Transplants $0.1 - 0.2$ g	1.17a	15.9b	13.59c	462	29.2a	0.57a	
	$0.2 - 0.3$ g	1.53 <sub>b</sub>	17.6bc	11.50 <sub>bc</sub>	539	30.6a	0.59a	
	$0.3 - 0.5$ g	1.55b	22.8c	14.71c	621	27.6a	0.62a	
	Seed tubers (control)	2.17c	10.8a	4.98a	853	81.2b	0.75 <sub>b</sub>	

Table 3. Results of field trial 2 at 70 days after (trans)planting. Data followed by the same letters in each column do not differ significantly  $(P < 0.05)$ .

with microtubers and ex vitro plantlets of cv. Russet Burbank. Single stemmed plants are often more vigourous and have a higher degree of branching both above and below ground. Such plants seem to form stolons and tubers over a longer period during their development than do conventional crops. The greater sizes of the microtuber of cv. Morene resulted in more stems and more tubers per plant, and a greater tuber yield per plant as welt as a higher harvest index.

In Experiment 3 (Table 1 and Fig. 2) transplants reached full ground cover about four weeks later than did plants of the control but they maintained full cover for only 2 weeks compared with the four weeks of the control. Crops from directly planted microtubers achieved a maximum ground cover of  $70\%$ . The conversion of intercepted solar radiation into total dry matter did not differ between the three crops (Fig. 2b); the mean value of 1.44 g MJ<sup>-1</sup> is similar to those reported by Marshall  $\&$  Taylor



Fig. 2. Ground cover development and relations between yield and cumulative intercepted global solar radiation of cv. Bintje ( $\bullet$  seed tubers,  $\circ$  transplants) in Experiment 3 in 1989. The directly planted microtubers  $(\odot)$  were covered for one month with plastic foil.

(1990) and radiation use efficiencies for tuber dry matter production of 1.36 g  $MJ^{-1}$ were also similar.

The fresh tuber yield, tuber dry mater content and the harvest indices of the three periodic harvests  $(61, 83$  and  $119$  days after planting, Table 4), clearly show that the differences between the control and the micro-propagated material in yield, dry matter content and harvest index decreased as the growing season progressed. For seed potato production, the first harvest on July 24 is of greatest interest. Then, fresh total tuber yield of the transplants was only 22% and of the directly planted material (mean values, with and without plastic) was only 15% of the control. In this experiment (trans)planting took place about three weeks later than usual because of the late availability of the rnicrotubers. The length of a usual seed potato production season is better represented by the second harvest, 83 days after planting. Then, the transplants yielded about 500 g per plant which was close to 50% of the control. Plastic cover did not significantly increase tuber yields, probably because the soil temperatures were sufficiently high in the uncovered soil during the sunny and warm month of May 1989 for optimal emergence and initial plant development. Henceforward, both treatments will be referred to as 'direct planting'.

The number of tubers per plant in the control was high (24.5), all tuber bearing stolons in general bore one tuber. The (often) single-stemmed plants from microtubets (mean, 1.35 stems per plant) produced as many stolons per stem as the control, but these stolons usually bore two tubers. The mean weight of the individual tubers



Table 4. Results of field trial 3. Experimental details and harvest dates in Table I. Data followed by the same letter in each row do not differ significantly ( $P < 0.05$ ).

Observation	<b>Microtubers</b>			Control			Probability		
	Bintie		Gloria Morene Bintje Gloria Morene v					$\mathbf{D}$	$p \times v$
Yield $(g/pl)$	527	279	438	651	812	701	ns.	***	4
Tuber dm $(\%)$	19.9	22.0	19.6	20.8	19.4	18.1	$\sim$	$\ast$	$\ast$
Harvest index	0.68	0.85	0.63	0.70	0.76	0.63	$* *$	$+ +$	***
Tubers per plant	14.7	6.9	9.6	19.6	11.9	12.8	$\ast$	$\ast$	ns
Size $(g/tuber)$	36.3	40.3	45.6	33.6	73.2	54.9	$\ast$	*	ns
Stems per plant	1.25	1.14	1.08	5.39	3.93	3.56	∗.	***	ns
Tubers per mainstem	11.71	6.04	8.98	3.65	3.19	3.60	$\ast$	***	$\star$
Tuber $(m\% < 28$ mm)	11.5	6.7	5.7	8.5	3.9	2.9	$***$	$\ast$	ns.
Tuber (m% $28 - 45$ mm)	82.2	67.1	67.6	87.7	68.5	52.4	**	ns	ns

Table 5. Results of field trial 4 with different propagation materials (p) and varieties (v) at 75 days after (trans)planting. Probability of statistical significance:  $ns = not$  significant, \*  $P < 0.1$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

of plants derived from directly-planted microtubers was lower than that of the control. The transplants had the highest proportion of tubers in the seed grades (28 and 45 mm), namely 91% compared with 81% in the control.

The results of the comparison of crops grown from convential seed, the controls, with those grown from microtubers of cvs Bintje, Gloria and Morene in the growing season of 1990, 75 days after (trans)planting is shown in Table 5. Fresh tuber yields were highest in the Gloria control and lowest in the transplants of Gloria. The early cultivar initiates its tubers early on in growth as becomes apparent, among other phenomena, in the high harvest indices. For the Gloria control this was advantageous because it favoured tuber production over haulm production. Tuber induction on plants grown from microtubers may have started too soon, that is before the plants had produced sufficient foliar mass to sustain adequate tuber production.

Of the three cultivars grown from microtubers, cv. Bintje performed best, yielding 80% of the control. The lateness of cv. Morene is reflected by its low harvest index. The lower yields of cvs Bintje and Morene in this experiment (Experiment 4, Table 5) were about proportional to their lower numbers of tubers per plant, but the reduction was proportionately greater with cv. Gloria. The Bintje control produced the highest number of tubers per plant (19.6) followed by Bintje grown from microtuber transplants (14.7), mainly because whereas the control had the highest number of stems, the transplants had the highest number of tubers per stem of all treatments. This high number of tubers per plant combined with the slightly lower tuber yields of Bintje resulted in the lowest mean individual tuber weight and the highest proportion of tubers in the seed grade (28-45 mm), over  $80\%$  from both Bintie treatments.

As in 1989, the Bintje transplants in Experiment 5 in 1990 reached full ground cover about 4 weeks later than plants from seed tubers and they also reached maturity a few weeks later (Fig. 3). The patterns of ground cover throughout the growing season were similar for cvs Bintje and Désirée both for plants derived from directly planted microtubers and from transplants. There was a clear difference in growth pattern between these two cultivars, the plots of cv. Bintje, growing from directly-planted microtubers and covered with plastic foil never reached full ground cover and obtained a ground cover duration of only 40.6 days whereas all other treatments had



Fig. 3. Ground cover development of crops of cvs Bintje and Désirée in Experiment 5 planted in 1990 with conventional seed ( $\bullet$ , 35-45 mm), with transplanted plants grown from microtubers ( $\circ$ ) and from directly planted microtubers ( $\odot$ ) covered for one month with plastic foil.

values which did not significantly differ from  $65$  days (harvest 3 data in Table 6). The plants grown frorn directly planted rnicrotubers of Bintje reached their highest value of ground cover on August 20 which was nine weeks after planting and two weeks later than Désirée. Solar radiation in mid-August is lower than in mid-June which may explain why the Désirée control, with 63.2 days of ground cover duration, yielded 1132 g per plant at the final harvest but the plants front directly planted microtubers, with 63.8 days of ground cover duration, produced only 849 g per plant (harvest 3 data in Table 6).

The first periodic harvest in Experiment 5 (Table 5) took place 50 days after transplanting or 65 days after planting. The control of cv. Bintje then yielded 407 g per plant and that of Désirée 299 g (Table 6). The transplants yielded less than one third of the controls while the plants from directly planted microtubers had hardly tuberized as is clear from the data on their tuber yield and dry matter concentration. All plants from seed tubers emerged and all transplants survived, but 20-30% of the microtubers planted tinder plastic did not emerge or their plants did not survive the first 2 months. By the second harvest, 76 days after transplanting or 91 days after planting, the yield differences between the plants from seed tubers and microtubers had decreased; the transplants then yielded 60% of the controls. As was to be expected from their poor development of ground cover, the tuber yields from the directly planted microtubers (Fig. 3) was poor, that of Bintje was only 15% and of





 $\frac{1}{24}$  and

Désirée only  $35\%$  of the control. The plants from the directly planted microtubers partitioned the smallest proportion of dry matter to the tubers, and the transplants the next smallest. Again, this is an indication that plants from microtubers lagged greatly in their development. Plants from microtubers in this experiment also produced fewer and smaller tubers on fewer mainstems than did the controls. The control of Désirée produced half the number of tubers per plant compared to the control of Bintje and this resulted in Désirée having the highest mean tuber weights; over  $70\%$ of the tubers were oversized, i.e. larger than 45 mm, and only 29.4% of the total tuber weight comprised tubers within the desired grade (28 to 45 mm). With the transplants of Désirée this last proportion was twice as high resulting in 265 g of seed sized tubers per plant compared with only 216 g per plant of the D6sir6e control. Bintje transplants yielded 419 g of seed sized tubers per plant which is equivalent to a seed production yield of 25 t per ha on the basis of 6 plants per  $m<sup>2</sup>$ . The harvest 3 data (Table 6) shows that when allowing the plants to reach maturity, the differences between the treatments in tuber yield, tuber dry matter concentration and tuber size further decreased.

# **Conclusions**

Direct planting into the field of small microtubers (0.2 to 0.4 g) was hazardous because the vulnerability to night frost of the plants grown from them (Experiment 5) led to complete loss of yield. Although the use of plastic foil reduced this risk, lower emergence and survival rates and increased risks of infection with *Rhizoctonia solani*  and virus (see also Struik & Lommen, 1991) remain. Extrapolated yields from directly planted microtubers after 80 to 90 days from planting were less than 10 tonnes per ha, both in our Experiments 3 and 5 and also from the smallest minitubers used by Struik & Lommen (1991). When directly planted, physiologically older microtubers performed better than younger microtubers (compare Experiments 1 and 2 with Experiments 3, 4 and 5), a result that corroborates the finding of Ogilvy et al. (1990); their partially dormant minitubers in 1988 yielded only 22% of the conventional crop while well-sprouted minitubers yielded up to 89%. Genotypic effects are large. Following the planting of conventional seed, cv. Gloria performed best within 90 days, but its performance following the planting of microtubers was the worst. Plants growing from conventional seed tubers benefit from a partitioning which favours their progeny tubers, but plants growing from microtubers are at disadvantage because initially the foliar mass is insufficient to sustain adequate tuber production. Indeed in Experiments 1 and 2 some of the transplants of cv. Gloria had already initiated some tubers at the time of transplanting but the much later tuber initiation in crops from microtubers of the late cv. Morene led, eventually, to higher tuber yields because of the initial development of adequate foliage. Struik & Lommen (1991), comparing minitubers of less than 0.25 g with minitubers between 0.25 and 0.5 g, found a doubling of tuber yields with cv. Bintje within 80 days from planting. The results of our Experiment 2 are far less striking, perhaps because transplanting reduced the effect of the initial seed weight. The occasional phenomenon of higher numbers of tubers in micro-propagated material than in conventionally planted crops in Experiments 1 and 2 has also been reported elsewhere (Levy, 1985; Leclerc  $\&$ Donelly, 1990) but its cause is not fully understood. Investigation into these matters might suggest ways of increasing the numerical multiplication factor of microtubers. Because this method of propagation is so costly, all tubers however small need to be harvested for future multiplications and a high number of tubers per plant may thus

be more favourable than relatively high yields per plant.

Crops grown from microtubers weighing less than 0.5 g yielded much less than crops grown from conventional seed tubers. The yields of crops grown from larger microtubers, and especially from microtubers pre-grown in a glasshouse and transplanted to the field, were increased by covering the soil with plastic for about one month after planting. Further, when late maturing cultivars, which generally produce few tubers per plant, are grown from transplants, their yields of seed sized progeny tubers may be comparable to those of crops grown from conventional seed tubers.

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