How to produce large sized microtubers of potato cv. Desiree

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Summary: *In vitro* tuberization was induced on explants with different number of nodes layered on a medium with high sucrose (8%) content: 30, 15, 10, 7 and 6 explants per jar were cultured containing 1, 2, 3, 4 or 5 nodes, respectively. Microtubers developed were graded by their smallest diameter, and the number of tubers per jar, their size distribution, their fresh weight and the multiplication rate were recorded. The highest multiplication rate (1.98) was obtained for explants with 5 nodes. The size distribution of tubers was markedly affected by treatments. The majority of microtubers (49.4%) were 6–8 mm in the case of the smallest explants (with 1 node). When explants with 2 to 5 nodes were used, the most microtubers were 8–10 mm but with an increase of explant size, more and more microtubers were produced with larger diameter up to 16 mm and average fresh weight of tubers also increased with the increase of explant size. For the microtuber production of Desiree the use of explants with two nodes can be suggested because in this treatment the average fresh weight of microtubers was high enough (250 mg) and the number of large sized microtubers was very high (79% was larger than 6 mm and 53% was larger than 8 mm).

Key words: *in vitro*, potato, explant size, tuber size

Introduction

The final products of potato micropropagation are either plantlets or microtubers. The use of microtubers has several advantages both in the storage of germplasm and in their use in seed-potato production *(Hussey & Stacy,* 1981, *Tovar* et al., 1985, *Seabrook* et al., 1993, *Ranalli* et al., 1994) because microtubers can be stored longer, handled and transported easier than plantlets *(Struik & Lommen,* 1991).

Microtubers larger than 2 mm could be further propagated but only microtubers larger than 4 mm are suitable for long-term storage. However, it is necessary to increase the tuber size because the larger is the microtuber the less is the loss during storage *(Tábori* et al., 1999) and the greater is its early vigour, emergence and performance *(Wiersema* et al., 1987, *Ranalli* et al., 1994).

The size of microtubers can be increased by applying an adequate photoperiod regime *(Seabrook* et al., 1993, *Dobránszki,* 1996), culture density *(Forti* et al., 1991, *Tábori* et al., 2000) type of explants *(Nowak & Colborne,* 1989, *Leclerc* et al., 1994) or proper nitrogen and sucrose concentrations in the medium *(Stallknecht & Farnsworth,* 1982, *Garner & Blake,* 1989, *Slimmon* et al., 1989, *Perl* et al., 1991, *Charles* et al., 1992) and so on. As a results of above mentioned manipulations in the composition of the media or in the environmental factors, a part of tubers developed was larger sized but their final size seldom or no exceeded 1 cm *(Tovar* et al., 1985, *Slimmon* et al., 1989, *Struik & Lommen,* 1991, *Charles* et al., 1992, *Seabrook* et al., 1993).

The aim of our research work was to increase of the size of *in vitro* tubers and find a method for production large-sized microtubers, where the majority (at least 50 per cent) of microtubers exceed 6 mm in diameter. In experiments described here, the effects of explant size on the size of microtubers produced on hormone-free medium with high sucrose content were studied.

Material and method

Five-week-old shoot cultures of *Solanum tuberosum* L. cv. *Desiree* grown in Kilner jar (400 ml, 75×85 mm) on 40 ml of the medium with Murashige-Skoog (MS) salts and vitamins (1962) supplemented with 3% sucrose and 0.8% agar-agar at long day conditions (16 h) and at 22 °C temperature, were used as initial explants.

Tuberization was induced on fully developed potato plantlets by pouring of 8% sucrose solution onto the cultures according to the method described by *Dobránszki* et al. (1999) in the control treatment or in the other five treatments explants with different number of nodes were layered on a medium containing MS salts and vitamins supplemented with 8% sucrose and 0.8% agar-agar. The total number of nodes per jar were about the same for each treatment (28–30) thus 30, 15, 10, 7 and 6 explants per jar were cultured containing 1, 2, 3, 4 or 5 nodes, respectively. The cultures were exposed to short days (8 h) for 2 weeks, then to total darkness for further 11 weeks *(Dobránszki* et al., 1999).

At the end of experiments microtubers were harvested and graded by their smallest diameter, and the number of tubers per jar, their size distribution, their fresh weight and the multiplication rate defined as number of microtubers per explant were recorded. Fifteen jars were observed in each treatment and experiments were repeated three times. Data were analysed by ANOVA followed by Tukey's test using SPSS 7.5 for *Windows* programme.

Results

The different treatments caused statistically significant differences in the number, size and weight of microtubers. Microtubers originated from the different treatments can be seen in the *Figure 1*.

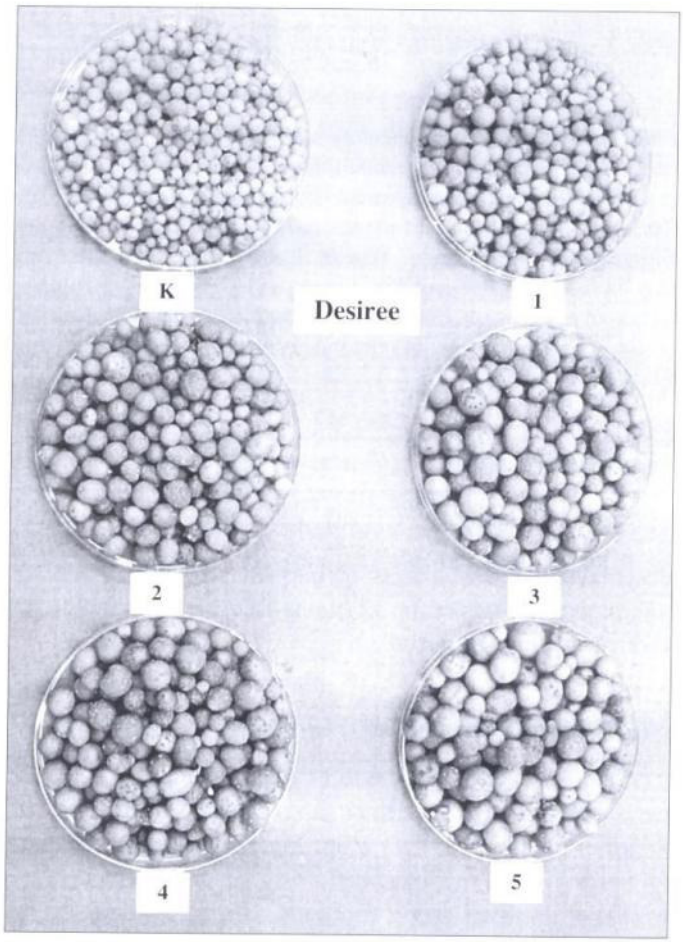

Figure 1 Microtubers originated from the different treatments. 'K' means control treatment and the numbers mean the explant size (=number of nodes) when explants were cultured on high-sucrose-medium.

Number and size of microtubers

The multiplication rate was markedly influenced by treatments as can be seen in *Table 1*. The size of explant had a consistent effect on the number of microtubers per explant: the larger was the explant the higher was the multiplication rate. The highest multiplication rate (1.98 microtubers per explant) was obtained for explants with 5 nodes cultured on medium with high sucrose content. Explants with 1 node showed the lowest multiplication rate: below 1 microtuber per explants, while multiplication rate of control hardly exceeded 1 microtubers per explant.

Comparing the total number of microtubers per jar, the most microtubers were produced by control treatments. On medium with high sucrose content, the number of microtubers decreased as the size of explant increased. However, the number of large sized microtubers (larger than 6 mm) was the highest when explants with 1 node were cultured on medium with high sucrose content and much more large sized microtubers per jar developed than in the control treatment: 16.2 instead of 9.2 microtubers per jar. Considering the microtubers larger than 8 mm, we could observe, that the most microtubers per jar developed when explants with 2 nodes were grown on medium with high sucrose content (9.5 microtubers per jar) *(Table 1)*.

The size distribution of microtubers was also markedly affected by treatments *(Figure 2)*. Normal distribution of

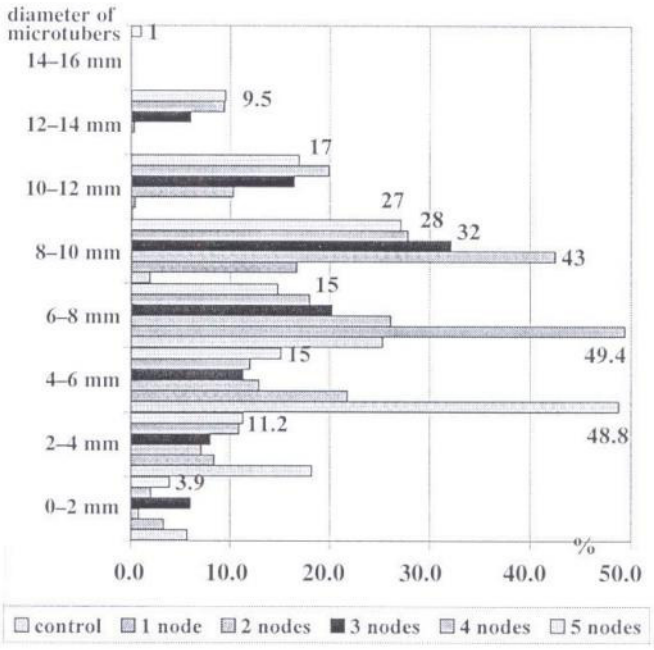

Figure 2 Size distribution of microtubers in the different treatments

Table 1 Effect of explant size on the multiplication rate, on the total number of microtubers per jar and on the number of large sized microtubers per jar*

Treatments	Multiplication rate Tuber number		Total number of microtubers per jar	Number of large sized microtubers per jar	
	per explant	per node		> 6 mm	> 8 mm
Control	1.13 b	1.13 c	34.1 d	9.2	6.8
Explant with 1 node	0.81 a	0.81 b	24.4 c	16.2	4.1
Explant with 2 nodes	1.19 bc	0.59 ab	17.9 b	14.2	9.5
Explant with 3 nodes	1.37 cd	0.46 a	13.7 a	10.5	7.6
Explant with 4 nodes	1.56 d	0.39 a	12.4 a	9.3	7.1
Explant with 5 nodes	1.98 e	0.40 a	11.9 a	8.3	6.5

*: Means within columns followed by the same letter were not significantly different at $p < 0.01$

microtubers could be observed for control treatment and the majority of microtubers (48.8%) were 4–6 mm in their diameter. The size distribution of microtubers produced on medium with high sucrose content showed a slight skew of data towards larger size categories, especially when explants with two and three nodes were cultured. Size distribution of microtubers developed on explants with four and five nodes was similar again to the normal distribution. The majority of microtubers (49.4%) were 6–8 mm in their diameter in the case of the smallest explants (with 1 node). In each other treatment the most microtubers were 8–10 mm but with an increase of explant size, the rate of this size category decreased from 43 to 27% because more and more microtubers were produced with larger diameter up to 16 mm. In the best treatment (explants with 5 nodes), more than half of microtubers (54.7%) were larger than 8 mm in their diameter.

Fresh weight of microtubers

The total yield of microtubers per jar were significantly higher for medium with high sucrose content compared to the control treatment *(Table 2)*. Moreover, if the number of nodes increased from 1 to 2, significantly higher yield was detected. However, further increase in explant size did not affect the total yield per jar.

Both factors examined, the way of sucrose support and the size of explants, affected the average fresh weight of microtubers *(Table 2)*. When high sucrose concentration was applied in the medium, the fresh weight of microtubers were significantly larger than in the control treatments. Moreover, the size of explants had a consistent effect on the average fresh weight of microtubers: it increased significantly as the size of explants increased. Average fresh weight of microtubers was the highest, if explants with 5 nodes were used. It was more than two fold higher, compared to microtubers developed on the explants with 1 node, and about five fold higher compared to the control treatment. The smallest tubers developed in control treatment *(Figure 1)*.

The fresh weight of microtubers varied between treatments even though they belonged to the same size category *(Table 2)*. When the size of microtubers was under 6 mm, no any significant differences could be detected between the treatments (data not presented here). In the size category of 6–8 mm, the lightest tubers developed in the control treatment and the heaviest ones on explants with 4 nodes. Considering the size category of 8–10 mm, the control treatment and explants with one node yielded the lightest microtubers. The fresh weight of microtubers increased significantly with increase of the explant size if the size was larger than 10 mm and 12 mm, respectively.

Discussion

In the present work the production of microtubers occurred on hormone-free medium by different treatments in which different way of sucrose support and different type of explant were applied.

High sucrose concentration (8%) applied in the tuberization medium or poured onto the fully developed plantlets is necessary for induction of tuberization process *in vitro* as described earlier by others *(Stallknecht & Farnsworth,* 1982, *Perl* et al., 1991, *Charles* et al., 1992, *Levy* et al., 1993).

The main aim of present work was to study the effect of explant size on the tuber size, and yield compared to the effect of the method (= control treatment) applied earlier in our laboratory *(Dobránszki* et al., 1999). The type and size of explant markedly influenced both the number and the size of microtubers developed. When the position of plantlets was vertical in the control treatment, the highest number of tubers per jar were produced. The relatively high number of tubers (1.13 tubers per plantlet) in this treatment may be explained by the use of fully developed plantlets with high leaf area for perception of environmental stimuli, such as photoperiod, and with well-balanced hormone state of whole plantlet. However, the size and weight of microtubres was low. Changing of orientation of shoots by 90° caused production of lower number of tubers per jar and the number of tubers decreased as the size of explants increased. However, the size and weight of microtubers increased as the size of layered shoots increased, which may be caused by more sufficient nutrition of buds because of better contact of shoots with the medium. Although, the highest multiplication rate was observed on explants with 5 nodes, it is not true if we consider multiplication rate as tuber number per node. In this case the multiplication rate decreased as the size of explant increased. Cutting of plantlets into nodal cuttings (= explants with 1 node) resulted in the disruption of their hormonale balance, the cessation of correlative inhibition among buds *(Levy* et al., 1993), promoting induction and initiation of more microtubers than on

Table 2 Effect of explant size on the yield and on the fresh weight of microtubers with different size*

Treatments	Total yield per jar (mg)	Average fresh weight (FW) of microtubers (mg)	Fresh weight (FW) of microtubers in size category of			
			6–8 mm	8–10 mm	10–12 mm	12–14 mm
Control	2465 a	73 a	128 a	240 a	450 a	–
Explant with 1 node	3438 b	139 b	145 ab	281 a	480 ab	–
Explant with 2 nodes	4440 c	250 c	149 ab	339 b	535 b	–
Explant with 3 nodes	4127 bc	303 d	146 ab	325 b	557 bc	808 a
Explant with 4 nodes	4058 bc	327 de	156 b	334 b	577 bc	862 a
Explant with 5 nodes	4232 bc	362 e	134 ab	334 b	649 c	973 b

*: Means within columns followed by the same letter were not significantly different at $p < 0.01$

explants with 2–5 nodes, but the size of microtubers was lower compared to the microtubers developed on layered explants with more nodes. This result is similar to the finding of *Leclerc* et al. (1994).

A reliable method for *in vitro* tuberization, which allows high number of large sized tubers would be very important for storage of germplasm and further propagation of virus-free potato. Several authors investigated in the increasing of size of microtubers but the finally size of tubers developed rarely exceed the 1 cm and the average weight of microtubers varied between 19–375 mg on agar-solidified medium *(Tovar* et al., 1985, *Slimmon* et al., 1989, *Chandra* et al., 1992, *Seabrook* et al., 1993, *Anjum & Villiers,* 1997). According to the study of *Liu & Xie* (2001), the main factor influencing the capacity of tubers to act as sinks, is the increase in cell number during growth. Microtuber size could be improved by manipulation of physical or chemical factors promoting cell division. The reason, why microtubers are generally lower than 1 cm in diameter, is the insufficient development of perimedullary region of microtubers *(Struik* et al., 1999). In our experiments the final size of tubers reached 16 mm in diameter and the highest average weight was 362 mg, the average fresh weight of 12–14 mm size category was 973 mg. In the best treatments more than half of tubers exceeded 8 mm in diameter.

Moreover, we could detect differences in the fresh weight of microtubers with the same size categories, which might be caused by differences in dry matter of microtubers or in their physiological age at harvest or their interactions.

Our results suggest that size of microtubers produced can be increased significantly on medium with high sucrose content by combining with an adequate explant size and using a proper photoperiod regime. We can conclude that several factors, which can play role in microtuber yield, are affected by orientation and size of explant.

We suggest production of Desiree microtubers on explants with two nodes on medium with high sucrose content. In this treatment the average fresh weight of microtubers was high enough (250 mg) and the number of large sized microtubers was very high (79% was larger than 6 mm and 53% was larger than 8 mm).

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