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Influence of Genotype on Microtuber Production

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Abstract

In vitro microtuberization represents the transitory phase of *in vitro* multiplication of a healthy material and on field multiplication. Microtubers production is an efficient method for obtaining a healthy material, thus leading to a reduction of the potato production process with 3-4 years. The microtubers of Romanian varieties were obtained from potato micro-cuttings cultured on Murashige-Skoog medium enriched with Cumarin and Kinetin. Sucrose was found to be the most important stimulus for inducing the microtubers. The cultures were maintained in darkness, at 18-20°C for 8-10 weeks for inducing and growing microtubers. The microtubers are important as they can be produced at any time of the year, they are easy to be transported and deposited.

Keywords: microtubers, conservation, storage, tuberization, plantlets and dormancy

Introduction

Microtubers are the first generation of potato seed from tissue culture: they are used to solve the problems of transplanting the plantlets from *in vitro* to in vivo conditions.

The microtubers have a lot of advantages, thanks to their small size and reduced weight, upon storage, transport and mechanization. They can be planted on the soil and can be produced at any time of the year. They have similar morphology and biochemical features with traditional tubers. *In vitro* microtuber production is very important for the production and storage of a potato valuable stock. Potato microtubers obtained through *in vitro* culture from single-node cuttings are convenient for handling, storage and exchange of healthy germplasme.

Microtubers are produced in the laboratory from the axially part of leaves. Darkness and warmth in the room represents an environment similar to the one of potato cultivation. *In vitro* plantlets (Fig. 1) develop microtubers in the next 6 days.

The important factors during the tuberisation are: the sucrose concentration from medium (most favourable condition: 8%); the content of nitrogen; the temperature (the best is 18-20°C); the light conditions (microtuberization can take place in the darkness or within a photoperiod of 8 h).

Plant tissue culture is the only technique that can eliminate approximately 100% viruses in seed production programs and microtuber is one of the strategies in this perspective. Because of their small size and weight, microtubers have tremendous advantages in terms of storage, transportation and mechanization. They can be directly sown into the soil and can be produced in bulk in any season. They have the similar morphological and biochemical characteristics to field produced tubers. Therefore, mass production of potato microtuber is likely to revolutionize the world potato production (Kanwal *et al.,* 2006).

Microtubers can be used to produce minitubers or used for screening genotypes for all of the important tuber characteristics such as color, shape, yield and average weight (Gopal and Minocha, 1997, 1998). Microtubers are easier to transport and handle than plantlets and are less delicate, so requires less after care, when planted in a greenhouse or screenhouse (Hoque *et al.*, 1996). For more detailed information about microtubers, the reader is referred to a recent comprehensive review article by Donnelly *et al.* (2003).

Microtubers reduce the time taken to produce seed tubers, reduce the number of field generations required and hence result in higher quality seed tubers.

Wherever microtuber and minituber production technologies have been implemented, they have halved the field time necessary to supply commercial growers (3 or 4 years compared with 7 or more years), and greatly improved seed tuber quality (fewer viral, bacterial, fungal problems).

Materials and methods

In vitro microtuberisation technique-experimental schedule

In vitro microtuberisation of potato constitutes the transitory phase, between *in vitro* multiplications of

healthy material and multiplication in the field. Microtubers production represents an efficient method for obtaining a healthy material, thus reducing the process of production with 3-4 years.

Microtubers or vitrotubers are small size tubers (3-10 mm diameter), spherical or elongated in shape, with 0.05 to 2 g weight. Microtubers have a content of proteinic nitrogen 2.5 bigger than normal tubers. Under normal culture conditions, microtubers produce prebasic tubers which can be used for basic plants production.

In this study, we establish a working method for obtaining microtubers.

The microtuberization medium is liquid and contains the same substances as medium Murashige-Shook (1962) but half the amount, coumarin, kinetin, sucrose (80-90 g/l). The stock solution of coumarone and kinetin were prepared as follows:

Coumarin: 500 mg in a 200 ml recipient; 25 ml ethanol are added; the solution is completed with distilled water.

Kinetin: 200 mg in a 50 ml recipient; 20 ml NaOH 1N are added; the solution is completed with distilled water.

For 1 l medium we add 20 ml stock solution of coumarin and 12.5 ml stock solution of kinetin. The microtuberisation medium doesn't contain agar. In every plastic recipient we poured 45 ml medium and then we placed the recipients in dark conditions for 8 weeks, at 20°C. We harvested the microtubers separately according to variety. We tested 3 Romanian variety: 'Christian', 'Roclas', 'Ostara'.

After the tuberization period (7-8 weeks of darkness), plantlets were extracted from cultures recipients, and harvested microtubers were washed, to avoid the subsequent infections, which can appear during their storage. Then, microtubers were calibrated, counted and placed for conservation in refrigerators at 4°C, in the darkness. This conservation can be extended for 1 year. At the moment of harvesting, most of the microtubers are in vegetative repose and thus cannot to sprout. The period of vegetative repose is very variable from one tuber to another and this constitutes an important handicap in the moment of planting. In order to solve this problem, the tubers can be treated with Rindite.

Often, the microtubers had a very special role in the production schedule of seeding potatoes. Using microtubers presents few advantages compared to plantlets: they can be produced in all yearround and is not necessary to be produced immediately before they are used; they are easy to transport and store for a couple of months.

Results and discussion

There were significant differences between the varieties, related to size, number and weight of microtubers obtained.

Observations were conducted over all material obtained from 'Ostara', 'Christian' and 'Roclas' (Fig. 2-6). In order to determine the weight of microtubers, the microtubers were individually weighted, microtuber by microtuber.

Tab. 1. Microtubers weight

Variety	Calibre	Microtubers weight
"Ostara"	>10 mm	0 b
	5-10 mm	0.13 ab
	< 5 mm	0.05 b
"Christian"	>10 mm	0.28 a
	5-10 mm	0.14 ab
	< 5 mm	0.06 b
"Roclas"	>10 mm	0.29 a
	5-10 mm	0.14 ab
	< 5 mm	0.05 b

LSD=0.1851 for alpha =0.05

Tab. 2. Number of microtubers

Variety	Calibre	Number of microtubers	Duncan test
"Ostara"	>10 mm	0	a
	5-10 mm	4.31	b
	< 5 mm	13.68	c
"Christian"	>10 mm	0.9	a
	5-10 mm	8.17	b
	< 5 mm	6.86	c
"Roclas"	>10 mm	0.45	a
	5-10 mm	7.68	b
	< 5 mm	20	c
Std. Error of Mean		1.2356	
Std. Deviation		6.4205	
Minimum		.00	
Maximum		21.00	
Mean		6.8944	

Tab. 3. Number of microtubes

Variety	Subset for alpha=0.05	
	1	2
'Christian'	1026 b	
'Rustic'	1137 b	
'Rodas'		1632 a
Sig.	0.118	1.000

Means for groups in homogeneous subsets are displayed; a uses harmonic mean sample size=3.000

For 2009, Fig. 7 shows, that: the weight of microtubers for the three varieties of potato/three calibers varies according to variety. 'Roclas', a semi-early variety, with resistance to viruses, had the highest weight (0,29 g) from the maxim size class (>10mm). For the next size class (5-10 mm) 'Christian' and 'Roclas' had the same weight (0,14g). In last class of size, the 'Christian' variety had the highest weight (0,06 g).

'Christian' variety had the highest capacity of production microtubers/plantlets (1,12) while the 'Ostara' variety presented the lowest number of microtubers/plantlets (0,68) (Fig. 8).

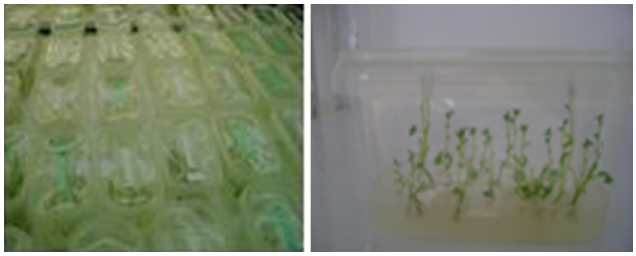

Fig. 1. Photo from growing room; regenereted plantlets before tuberisation

Fig. 2. Microtuberization of 'Christian' variety

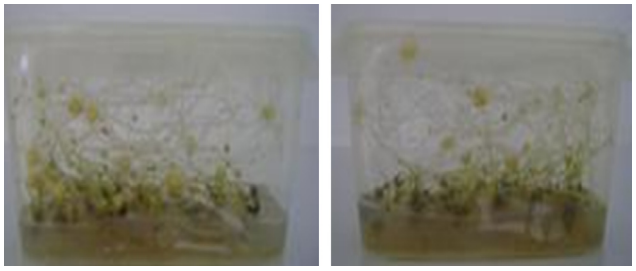

Fig. 3. Microtuberization of 'Roclas' variety

Fig. 4. Microtubers of 'Cristian' variety

Fig. 5. Microtubers of 'Roclas' variety

Fig. 9 shows that the highest number of microtubers/

Fig. 6. Microtuberization in incubator conditions

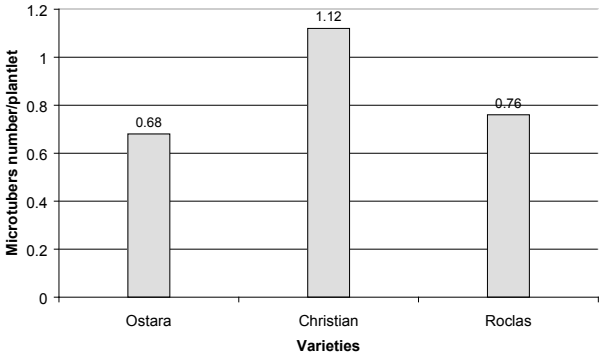

Fig. 7. Variation of microtubers weight in function of variety and caliber

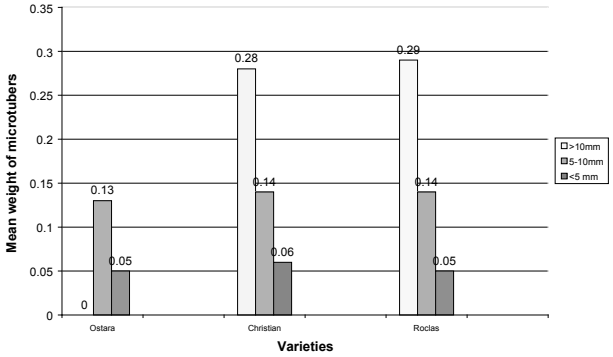

Fig. 8. Variation of microtubers number/plant

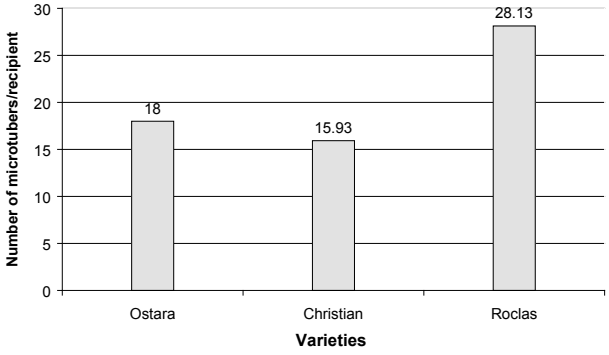

Fig. 9. Variation of number of microtubers/recipients

recipient is obtained from the 'Roclas' variety (28,13), followed by 'Ostara' (18 microtubers/recipient).

Studying the behavior of the three potato varieties in terms of microtubers production/recipient, accord-

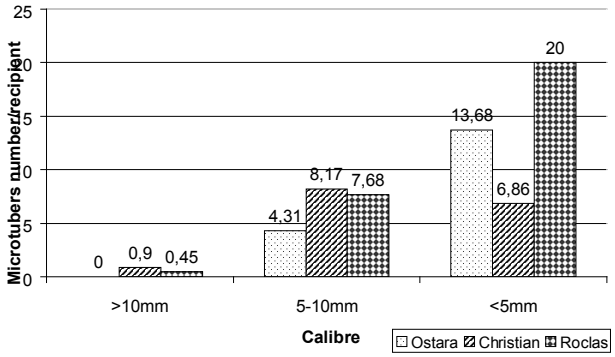

Fig. 10. Variation of microtubers/recipient /calibre

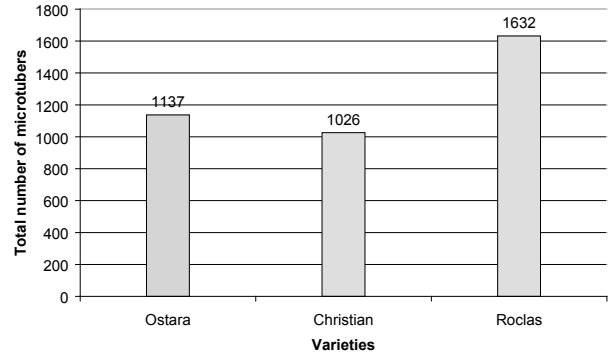

Fig. 11. Variation of total number of microtubers

ing to 3 size classes, we may establish that the 'Christian' variety, had the highest number of microtubers from the maximum size class/recipient (0,9). In the next class (5-10 mm), it was also the 'Christian' variety that presented the highest number of microtuber/recipient (8,17), followed by 'Roclas' and 'Ostara' varieties. In the last class of size (<5mm), the 'Roclas' variety produced the highest number of microtubers/plantlets (20).

Microtubers were harvested from 58 recipients (for every varietiy), each recipient containing 25 plantltets.

By analyzing the total number of microtubers we may remark the high capacity of production for the 'Roclas' variety (1632), followed by 'Ostara'.

Conclusions

We may conclude that the number of microtubers and their size are influenced by genotype. The 'Christian' variety had the highest capacity of production microtubers/plantlets (1,12), followed by 'Roclas' (0.76 microtuber/plantlet). For the maxim size class, the biggest number of microtuber/recipient is produced by the 'Christian' variety (0.9 microtuber/recipient >10 mm), while in the small size class, the biggest number of microtuber/recipient is produced by the 'Roclas' variety (20 microtubers/recipient <5mm). 'Roclas' produced the biggest number of microtubers (1632), followed by 'Ostara' (1137).

There is a similitude between the production capacity of tubers *in vitro* from maxim and middle class of size and field production.

References

Donnelly, D. J., W. K. Coleman and S. E. Coleman (2003). Potato microtuber and performance: A review. Am J Potato Res. 80:103-115.

Kanwal Amina, A. A. and K. Shoaib (2006). *In Vitro* Microtuberization of Potato (*Solanum tuberosum* L.) Cultivar Kuroda-A New Variety in Pakistan. International Journal of Agriculture and Biology 08(3):337-340.

Gopal, J and J. L. Minocha (1997). Effectiveness of selection at microtuber crop level in potato. Plant Breed. 116:293-295.

Gopal, J and J. L. Minocha (1998). Effectiveness of in vitro selection for agronomic characters in potato. Euphytica 103: 67-74.

Hoque, M. I., N. B. Mila, M. D. S. Khan and R. H. Sarker (1996). Shoot regeneration and in vitro microtuber formation in potato (*Solanum tuberosum* L.). Bangladesh J Bot. 25:87-93.

Murashige, T. and F. Skoog, (1962). A revised medium for rapid growth and bioassay with tobacco cultures. Physiol. Plant. 15:473-497.