Romanian Biotechnological Letters *Vol. 15, No.3, 2010*

ORIGINAL PAPER

Influence of potato genotypes on "in vitro" production of microtubers

Received for publication, August 29, 2009
Accepted, May 10, 2010

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Abstract

"In vitro" microtuberization represents the transitory phase from "in vitro" multiplication of a healthy material to on field growing. Microtuber production is an efficient method for obtaining a healthy material, through which the process of potato production is reduced with 3-4 years. In the same time the microtubers are important because they can be produced in any period of the year, they are easy to be transported and stored..

Microtubers of semi-early Romanian varieties (Christian and Roclas) and of early Dutch variety (Ostara) were obtained from potato micro-cutting cultures on Murashige-Skoog medium enriched with Cumarin and Kinetin. The sucrose was the most important stimulus for inducing the microtubers. Microtuber inducing and growing was achieved in cultures maintained in darkness, at 18-20° C for 8-10 weeks.

Keywords: potato microtubers, conservation, storage, **"*in vitro*"** tuberization, plantlets, dormancy

Introduction

Microtubers ("in vitro" developed tubers) are miniature seed potatoes and they represent an intermediary phase between "in vitro" plantlets and minitubers. Microtubers are the first generation of potato seed from tissue culture, being used to solve the problems of transplanting the plantlets from "in vitro" to "in vivo" conditions.

The microtubers offer a lot of advantages to storage, transport and mechanization due to their little size and reduced weight. They can be planted directly in the soil and they can be produced in any period of the year. They have similar morphology and biochemical features with traditional tubers. Microtuber production "in vitro" is very important for producing and storage of valuable seed potato stocks. Potato microtubers obtained by "in vitro" culture from single-node cuttings are convenient for handling, storage and exchange of a healthy germplasm, representing an important component, along with plantlets and minitubers, for seed potato production programs [1].

"In vitro" microtuberisation of potato constitutes the transitory phase, between "in vitro" multiplication and establishment of cultures in the field. Microtuber production represents in the same time an efficient method for obtaining a healthy material, by which the process of production is reduced with 3-4 years.

Conventional propagation of potato is done vegetatively by using seed tubers, meant to ensure uniformity of the crop in terms of growth and yield, but a common drawback is due to degeneration of the crop because of virus infections, the rate of degeneration varying from place to place and from a cropping season to another. The viruses are transmitted through different ways including the planting of infected tubers. If the seed stock is not properly maintained or frequently replaced with fresh one, the virus infiltration can reach up to 100%

in 3 - 4 successive crop seasons, resulting in reduction to almost half or even one third of the yields. This is the major problem faced by seed producers [2].

Microtubers are produced in laboratory from axially part of ”in vitro” plantlet leaves, the darkness and warmish temperatures representing favorable physical factors that trigger the development of microtubers in the next 6 days.

The most important factors during the tuberisation period are:

- sugar concentration in the culture medium (optimal condition: 8%);
- nitrogen content (there is a clear interaction between sugar and nitrogen concentration);
- temperature (incubation at 18–20 °C is preferable);
- light conditions (incubation can occur in the dark or at low light intensity with a photoperiod of 8 h) [3].

The sucrose is the most critical stimulus for tuber induction [4, 5], as results from the review of EWING and STRUIK (1992), regarding induction, initiation and growth of potato tubers. The high sucrose concentrations are essential for “in vitro” microtuber induction, influencing this process through the osmotic effect [7], and by serving as energy source [8, 9, 10]. For achieving a maximum microtuber induction, the sucrose level must be increased from 2-3%, the concentration usually used for micropropagation, up to 8%.

Generally, microtubers are produced “in vitro”, by varying the medium types, the medium components and the storage periods.

Many interactions between “in vitro” conditions significantly influence the productivity and much of these interactions seem to be genetically specified. The microtubers usually are approximately of the size of a pea seedpod and vary in shape (rounded or elongated), surface (smooth or rough), color (yellowish to greenish), weight (ranging from 24 to 273 mg), diameter (4–7 mm), and length (10–12 mm) [3].

While the temperature of 20-25^{0}C determines the plantlet growing, for microtuber inducing the temperatures are generally lower (15-18^{0}C) [4, 11, 12]. The temperature interactions with the sucrose from the medium and with the growth regulators are also influencing the “in vitro” tuberization [13].

Storing the microtubers for a long period of time is disputable, as is their ability to be planted in the field with the probability to behave as conventional seed tubers. Field samples revealed a low production potential for “in vitro” produced microtubers, comparative with conventional seed tubers.

Nevertheless, the microtubers have some superior qualities and represent an essential tool of research. The microtubers are used for conservation of potato germplasm, the prominent variable affecting this aspect being the dormancy length. This variable depends on genotype, abscisic acid (ABA), and sucrose [3]). ABA decreases both microtuber production and microtuber dormancy, whereas higher concentrations of sucrose (60–80 g l−1) promote biomass production, microtuber production as well as microtuber dry matter content. Microtubers stored under diffuse light have longer dormancy than those kept continuously in the dark [3].

The biologic performances (storage, conservation) depend on the size of microtubers [3] and may be optimized by producing of much more uniform and larger microtubers, by modifying the “in vitro” procedures. The effect of growth regulators on plantlet development and on increasing the microtuber yield was described in literature, with jasmonic acid and gibberellic acid speeding up the increasing of microtuber development, while ABA having an inhibitory effect over this process.

In optimal conditions the “in vitro” techniques lead to production of thousands of microtubers in a short time, which can be stored in a small area (in a humid container at 4 °C) for long periods of time [3].

Materials and method

In vitro propagation methods based on using meristem tips, nodal cuttings and micro tubers are more reliable for maintaining genetic integrity of the multiplied clones. Meristem cultures provide a reproducible and economically viable method for producing pathogen free plants.

The experiment was conducted at the Department of Tissue Culture of National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania.

Potato tubers of Christian, Roclas and Ostara varieties were washed with tap water and kept in craft paper bags. These bags were stored under dark conditions for one and a half month at constant temperature of 25°C for the development of etiolated shoot sprouts [14]). Etiolated shoots were washed in tap water and surface sterilized in 70% alcohol for 1-2 minutes and then for 15 minutes in 10% sodium hypochlorite with the addition of one or two drops of Tween-20. Finally the explants sources were washed with sterile distilled water, three times for 10 minutes each.

Aseptic dissection of meristems is a delicate process and all operations are accomplished in sterile conditions, under a hood with laminar flux of sterile air, by using a stereomicroscope. The meristems were prelevated with the first pair of leaf primordia. After prelevation, the meristematic explants were inoculatated on MS basal medium supplemented with 0.5 mg/l gibberellic acid. After that, the cultures were transferred to the growing room.

After 2-3 months, depending on the genotype, the developed plantlets were transferred to test-tubes with MS medium supplemented with 0.5 mg/l NAA (α-naphthalene acetic acid).

The potato plantlets (figure 1) regenerated from meristems, were fragmented for obtaining uninodal cuttings (figure 2).

Figure 1. Potato plantlet

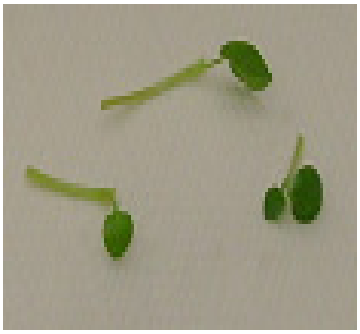

Figure 2. Uninodal cuttings

For microtuberisation, the plantlets developed from 25 uninodal cuttings inoculated in every culture vessel (figure 3) were grown on 45 ml half strength liquid MURASHIGE-SHOOG (MS) basal medium [15], supplemented with coumarin, kinetin and sucrose (80-90g/l).

The stock solution of coumarine and kinetin were prepared as follows:

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

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

For 1 l medium 20 ml stock solution of coumarin solution is added and 1.25 ml stock solution of kinetin. The cultures were maintained in dark conditions for 8 weeks, at 20^0C, and the microtubers were separately harvested, according to the 3 varieties tested: Christian, Roclas and Ostara.

After the tuberization period was completed, (7-8 weeks of darkness), the plantlets were extracted from culture vessels (figures 4, 5), and the microtubers were harvested and

washed to avoid the subsequent infections which can appear during the period of their storage. The microtubers were harvested from 58 vessels (for every variety), each vessel containing 25 plantlets. Then the microtubers were calibrated, counted and stored for conservation in refrigerator at 4^0C, in darkness. Observations were performed over all material obtained from the varieties Ostara, Christian and Roclas. For determination of the microtuber weight, the microtubers were individually weighted, microtuber by microtuber.

The conservation in the above-mentioned storage conditions can be extended for 1 year. In the moment of harvesting, most of the microtubers (figures 6 and 7) are in vegetative repose so they cannot sprout. The period of vegetative repose is very variable from a tuber to another, and this constitutes an important handicap in the moment of planting.

Figure 3. Regenereted plantlets before tuberisation

Figure 4. Microtuberization – Christian variety

Figure 5. Microtuberization – Roclas variety

Figure 6. Microtubers from Cristian variety

Figure 7. Microtubers from Roclas variety

Results and discussions

There were significant differences between the varieties, regarding the size, number and weight of the obtained microtubers **(Table 1) .**

Table 1. Microtuber weight and caliber according to varieties

Variety	Caliber	Microtuber weight (g)
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

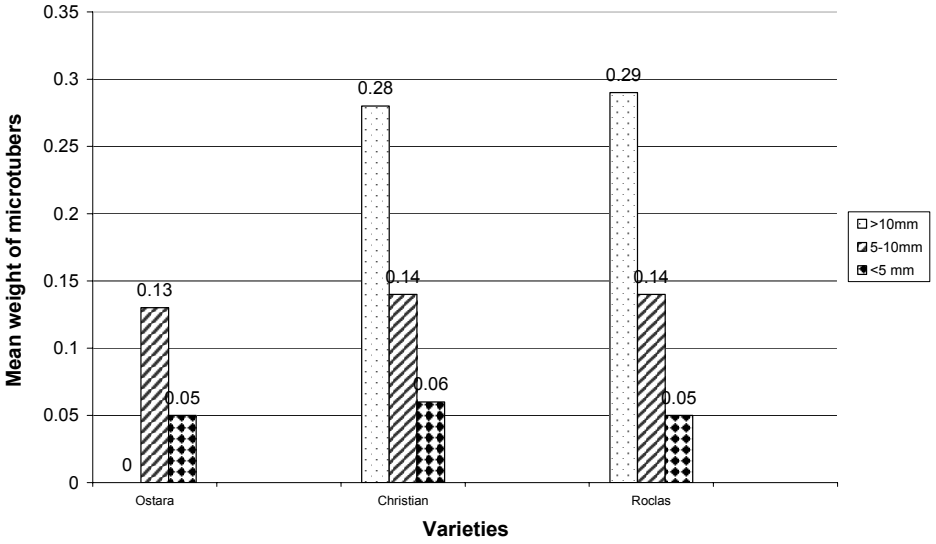

Figure 8. Variation of microtubers weight in function of variety and caliber

The weight of microtubers from the three potato varieties and the calibers vary in function of variety. Roclas, a semi-early variety, with resistance to viruses (PVY and PLRV), had the highest mean weight (0.29 g) from class of maxim size (>10mm) **(figure 8)**. In the next size class (5-10 mm) Christian and Roclas had the same mean weight (0.14g). In the last class of size, Christian variety had the highest mean weight (0.06 g).

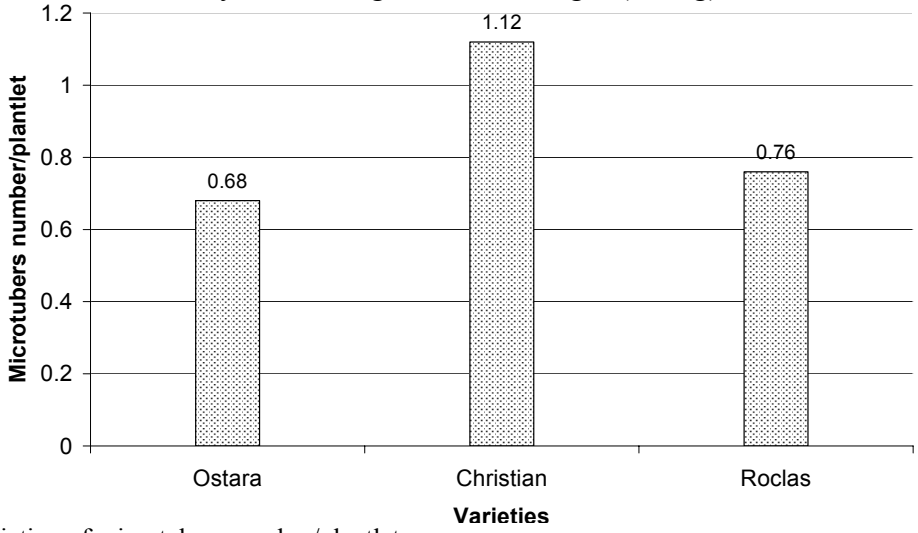

Figure 9. Variation of microtubers number/plantlet

Christian variety had the highest capacity of production microtubers/plantlet (1.12) while Ostara variety, recorded the lowest number of microtubers/plantlet (0.68) **(Figure 9).**

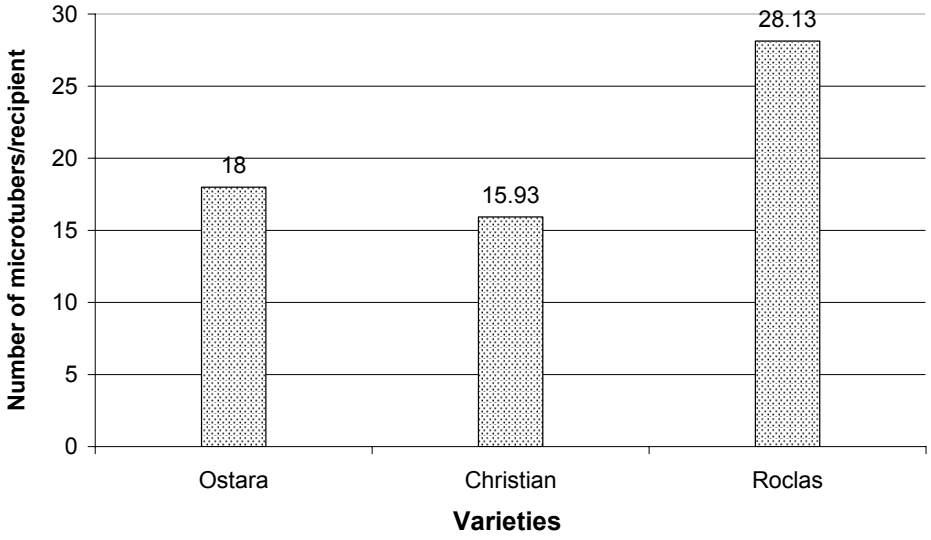

Figure 10. Variation of number of microtubers/culture vessel

The highest number of microtubers/culture vessel was obtained in Roclas variety (28.13), followed by Ostara (18 microtubers/recipient) **(figure 10).**

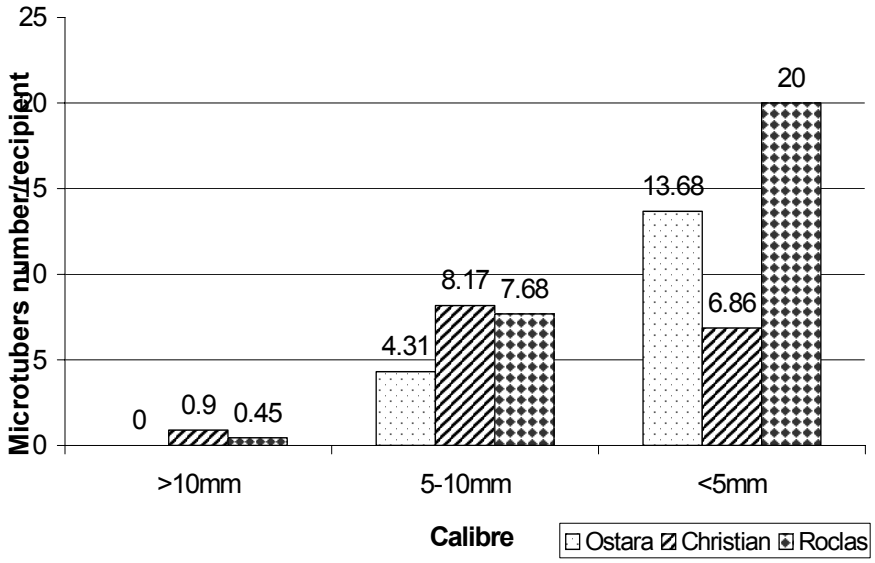

Figure 11. Variation of microtuber caliber/recipient

Table 2. Variation of microtuber number and caliber according to variety

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

Table 3. Statistical data regarding the microtubers/culture vessel

Mean	6.8944
Std. Error of Mean	1.2356
Std. Deviation	6.4205
Minimum	.00
Maximum	21.00

Following the study of the behavior of the three potato varieties regarding the microtuber production/culture vessel and their inclusion in the 3 size classes, it was evident that Christian variety produced the highest number of microtubers framed in maximal class of size/culture vessel (0.9). In the next size class (5-10 mm), also Christian variety presented the highest number of microtubers/culture vessel (8.17), followed by Roclas and Ostara varieties. In the last class of size (<5mm), Roclas variety produced the highest number of microtubers/plantlet (20) **(Figure 11; Table 2)**

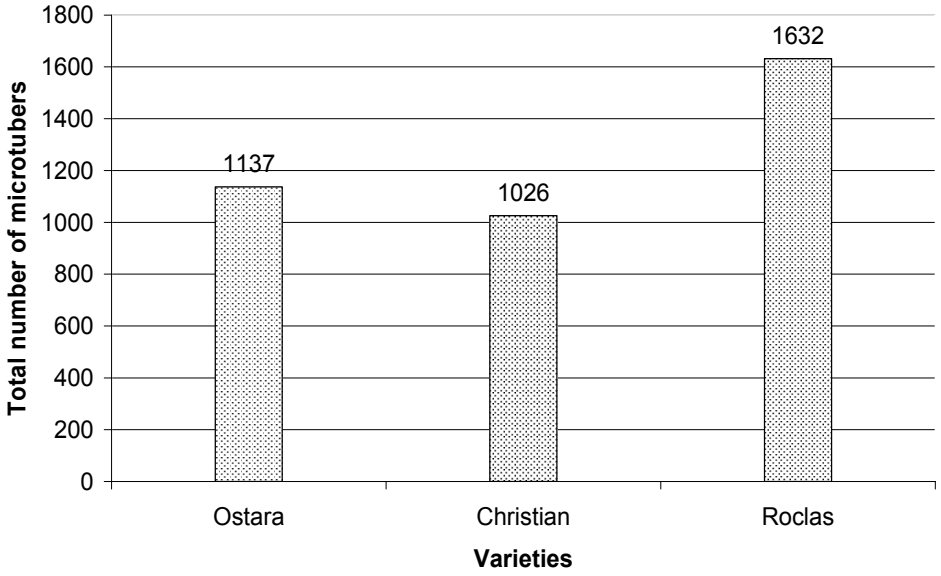

Figure 11. Variation of total number of microtubers according to variety

Table 4.

Number of microtubers

	VARIETY	Subset for alpha = .05 1	2
Duncan[a]	Christian	1026 b	
	Ostara	1137 b	
	Roclas		1632 a
	Sig.	.118	1.000

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000.

Analyzing the total number of microtubers we may remark the high capacity of production of Roclas variety (1632), followed by Ostara (**figure 11, Table 4**).

We may conclude that the number of microtubers and their size are influenced by genotype :

- Christian variety had the highest capacity of production microtubers/plantlets (1.12), followed by Roclas (0.76 microtuber/plantlet);
- In the maximal class of size, the biggest number of microtubers/recipient was recorded in Christian variety (0.9 microtubers/recipient >10 mm), while in the small class of size the highest number of microtubers/recipient was produced by Roclas variety (20 microtubers/recipient <5mm);
- Roclas produced the greatest number of microtubers (1632), followed by Ostara (1137).

There is a correlation between the production capacity of tubers “in vitro” belonging to the maximal and middle class of size and the field performance. Microtuber production is an efficient method for obtaining a healthy material, through which the process of potato production is reduced with 3-4 years.

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