Scientific Papers. Series A. Agronomy, Vol. LXV, No. 2, 2022
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

OBTAINING POTATO MICROTUBERS UNDER THE INFLUENCE OF OSMOTIC AGENTS IN DIFFERENT CONCENTRATIONS

Andreea TICAN[1], Mihaela CIOLOCA[1], Carmen Liliana BĂDĂRĂU[1, 2], Nina BĂRĂSCU[1], Monica POPA[1]

[1]National Institute of Research and Development for Potato and Sugar Beet of Brasov, Brasov County, Romania
[2]Transilvanya University of Brasov, Faculty of Food and Tourism, 148 Castelului Street, 500014 Brasov County, Romania

Corresponding author email: mihaela.cioloca@potato.ro

Abstract

In order to investigate the effect of osmotic agents on the induction of microtubers, a study was carried out in the Laboratory of Vegetable Tissue Cultures of NIRDPSB Braşov. Bifactorial experience included the following factors: experimental factor A, variety, with three graduations: a1 - Marvis; a2 - Castrum; a3 - Ervant (as a control, the average of the values obtained for the three varieties was taken into account) and experimental factor B, microtuberization medium (with 4 graduations): b1 - classical microtuberization medium without osmotic agent (control medium); b2 - microtuberization medium with mannitol; b3 - microtuberization medium with sorbitol; b4 - microtuberization medium with PEG. The determinations were made for number of microtuber/plantlets and weight of microtubers/plantlets, in function of experimental factors. Compared to the control medium, osmotic agents added to nutritive medium had a positive effect on studied parameters Therefore, osmotic agents in low concentrations can be introduced as a stimulator of microtuberization.

Key words*: potato, genotype, osmotic agents, microtubers.*

INTRODUCTION

Microtubers have become an important mode of rapid multiplication for pre-basic stock in seed tuber multiplication as well as germplasm exchange (Chandra et al., 1992).

Microtubers are particularly convenient for handling, storage and transport of germplasm. Also, unlike *in vitro* propagated plants, they do not need a hardening period in a greenhouse and may be adapted to some form of large-scale mechanized planting (Ranalli et al., 1994).

According to the study by Naik and Karihaloo (2007), microtubers are small tubers (average weight being 100-150 mg) formed *in vitro* respecting the conditions of microtuberization.

In addition to growth hormones, type of media is also important for microtuberization efficiency (Kumlay, 2014; Yagiz et al., 2020, cited by Astarini, 2021).

Sucrose is the decisive factor in the formation of tubers *in vitro* (Wang and Hu, 1982; Abbot and Belcher, 1986, cited by Donnelly et al., 2003). Sucrose is a source of energy, at higher concentrations, favouring the formation of microtubers (Perl et al., 1991; Simko, 1994; Struik and Wiersema, 1999, cited by Donnelly et al., 2003).

Dodd's et al. (1992) showed that for the microtubers formation the optimal concentration of sucrose was between 60-80 g/l.

Sucrose has a dual role in microtubers development. First, it could be a suitable carbon source, easily assimilated by microplants and transformed into starch and developing microtubers. Also, sucrose, with a concentration of 80 g/l, offers a favorable osmolarity for microtubers formation (Khuri & Moorby, 1995; Yu et al., 2000).

Sugars such as mannitol and sorbitol have been shown to induce *in vitro* regeneration, further studies revealed that mannitol mainly acts as an osmoticum rather than be uptaked or metabolized as an energy or carbon source (Altindal and Karado, 2010, cited by Motallebi-Azar, 2012).

Inorganic salt and sugar solutions, components of the culture environment besides the fact that they having a purely nutritious effect, they also

influence the growth of plant cells through their osmotic properties (George, 1993).
At slight stress conditions, potato plants utilize the strategy to generate more tuber. Therefore, the lower concentrations of PEG can be introduced as a stimulator of microtubers (Jamshid et al., 2020).

MATERIALS AND METHODS

In order to investigate the effect of osmotic agents on microtubers induction, a study was carried out within the Laboratory of Vegetable Tissue Cultures of NIRDPSB Brasov. The technique of obtaining microtubers consisted in applying the liquid microtuberization medium in special recipients, which contain developed potato plantlets (Figure 1). The mini-cuttings from the uninodal segmentation of plantlets were inoculated on the propagation medium, 15 segments with a single node and a single leaf / culture vessel. The recipents were transferred to the growth chamber, where they had a growth and development regime of 22-25^{0}C, with a photoperiod of 16 h, and in the culture vessels with these plantlets the microtuberization medium was applied.
The recipients were incubated in dark conditions, at a temperature of 18^{0}C for 90 days.
The harvested microtubers (Figure 2 are sterilized) to prevent possible injections, exposed to drying, afterwhich they are kept in cold conditions (4-5^{0}C) for a good preservation.

Figure 1. Applying liquid medium in recipients with developed potato plantlets

In this study, two factors were analyzed: the variety and the microtuberization medium (the classic microtuberization medium, without osmotic agents considered as the control medium) and in addition to this, three osmotic agents considered as the control medium) and in addition to this, three osmotic agents were added: mannitol, sorbitol, PEG (in two concentrations 1 and 1.5%, for each one).

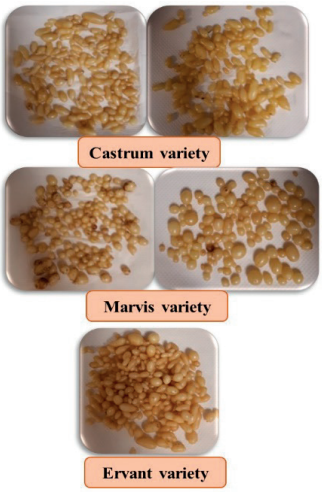

Figure 2. Microtubers harvested

To identify the osmotic agent with a higher efficiency in microtuberization, the values obtained for the two concentrations were averaged for each element studied. Thus, the bifactorial experience (3 x 4), on 3 repetitions had the following factors: experimental factor A - variety, with three graduations: a1 - Marvis; a2 - Castrum; a3 - Ervant (for this factor, as control we reported to mean values obtained for the studied parameters) and experimental factor B - microtuberization medium (with 4 graduations): b1 - classical microtuberization medium (without osmotic agent); b2 - medium with mannitol added; b3 - medium with sorbitol added; b4 - medium with PEG. The number of microtubers/plantlet and microtubers weight of/plantlets were determined at harvest.
In this study, results were processed by analysis of variance, used to establish the significance of the differences between the variants.

RESULTS AND DISCUSSIONS

From the analysis of variety influence on microtubers production, Ervant variety (Table 1) is distinguished, with a high microtuberization capacity, determining obtaining of a distinctly significant positive difference (0.221) and a value of 1.308 microtubers/pl. At the opposite pole is Marvis variety, with a low rate

of microtuberization (0.95 microtubers) and a distinctly significant negative difference (-0.137 microtubers), followed by the Castrum variety which recorded a significant negative difference (-0.084). For the second parameter taken into analysis, microtubers weight/plantlets, the values obtained for the three varieties were very close, without any significant differences, compared to all values mean (considered control), the highest value being obtained for the variety Ervant (0.33 g).

By comparing the experimental differences with the calculated limit differences over influence of the microtuberization medium on the number of microtubers obtained/plantlet (Table 2), the beneficial effect of PEG and mannitol is noted, which led to statistically assured results with very significant positive differences (0.281 and 0.215). Also, sorbitol addition to microtuberization medium had a positive influence, leading to a distinctly significant positive difference (0.207).

Examination results on microtubers weight/plantlet shows the positive influence of sorbitol and PEG, expressed by very significant positive differences (0.162 and 0.127 g). Also, for microtubers weight, the sugar alcohol, mannitol, is notiable, which led to a distinctly significant positive difference (0.091 g) compared to the control medium.

Using these osmotic agents strongly influences the microtubers formation for Castrum variety, whose very significant positive differences stand out from the control medium (0.62, 0.59 and 0.49 for mannitol, PEG and sorbitol). Applying PEG in the microtuberization medium positively influenced the formation of microtubers for the Ervant variety, causing a significant positive difference (0.26 g) and a value of 1.43 microtubers/pl. (Table 3).

The combined influence of variety and microtuberization media on microtubers weight/plantlets (Table 4) highlights Castrum variety for all osmotic agents, with very significant positive differences (0.42; 0.36; 0.36 microtubers for sorbitol, mannitol and PEG). The Ervant variety is distinguished with a high value of microtubers weight (0.402 g) by using sorbitol, leading a significant positive difference (0.133 g).

Table 1. Variety influence on microtubers obtained/plantlet and on their weight (g)/plantlet

Variety (a)	Microtubers number/pl.	Diff./Sign.	Microtubers weight/pl.(g)	Diff. (g)/Sign.
Castrum	1.003	-0.084 o	0.31	0.00 ns
Marvis	0.950	-0.137 oo	0.30	-0.01 ns
Ervant	1.308	0.221 **	0.33	0.02 ns
Mean (Ct)	1.087		0.31	

LSD 5% = 0.08; 1% = 0.133; 0.1% = 0.248. LSD 5% =0.05 g; 1%= 0.08 g; 0.1% = 0.15 g.

Table 2. Influence of the microtuberization medium on microtubers number and on their weight/plantlet (g)

Microtuberization medium (b)	Microtubers number /pl.	Diff./Sign.	Microtubers weight/pl.(g))	Diff. (g)/Sign.
Classical microtuberization medium (Ct)	0.911	-	0.216	-
Medium with mannitol	1.126	0.215 ***	0.307	0.091 **
Medium with sorbitol	1.119	0.207 **	0.377	0.162 ***
Medium with PEG	1.193	0.281 ***	0.342	0.127 ***

LSD 5% = 0.112; 1% = 0.153; 0.1% = 0.209. LSD 5% = 0.057 g; 1%= 0.078 g; 0.1% = 0.106 g.

Table 3. Combined influence of the variety and microtuberization medium on microtubers number/plantlet

Variety (a)/ Microtuberization medium (b)	Castrum (a_1)		Marvis (a_2)		Ervant (a_3)		a_1-a_3/ Sign.	a_2-a_3/ Sign.
	Microtubers number/pl.	Diff./Sign.	Microtubers number/pl.	Diff./Sign.	Microtubers number/pl.	Diff./Sign.		
Classical microtuberization medium (Ct)	0.58	-	0.98	-	1.18	-	-0.60 ooo	-0.20 ns
Medium with mannitol	1.20	0.62 ***	0.87	-0.11 ns	1.31	0.13 ns	-0.11 ns	-0.44 ooo
Medium with sorbitol	1.07	0.49 ***	0.98	0.00 ns	1.31	0.13 ns	-0.24 o	-0.33 oo
Medium with PEG	1.17	0.59 ***	0.98	0.00 ns	1.43	0.26 *	-0.27 o	-0.46 ooo

By studing the influence of the microtuberization medium on the number of microtubers/ plantlet, the beneficial effect of mannitol in low concentration (1%) is noticed, followed by sorbitol (1.5%) and PEG (in both concentrations) obtaining higher values of the number of microtubers (1.25, 1.24, 1.23 and 1.16) compared to the control medium (0.91). When using as osmotic agents 1.5% mannitol and 1% sorbitol were equal values (1.00), lower than those mentioned above, but higher than the control medium (Figure 3).

Microtubers weight mean values/plantlet examination showed a positive influence of sorbitol at both concentrations, with the highest microtubers weight (0.38 g). It is also noted that 1% mannitol in the microtuberization medium determining a high value obtaining (compared to the control medium) of microtubers weight (0.36 g). This value is followed by the results obtained when using PEG (0.35 g and 0.34 g both concentrations). The lowest value of the microtubers weight for all osmotic agents was when applying 1.5% mannitol, but this value is higher than the value of the microtubers weight obtained for the control medium (0.22 g) (Figure 3).

Table 4. Combined influence of the variety microtuberization medium microtubers weight / plantlet (g)

Variety (a)/ Microtuberization medium (b)	Castrum (a_1)		Marvis (a_2)		Ervant (a_3)		a_1-a_3/ Sign.	a_2-a_3/ Sign.
	Microtubers weight/pl. (g)	Diff./Sign.	Microtubers weight/pl. (g)	Diff./Sign.	Microtubers weight/pl. (g)	Diff./Sign.		
Classical microtuberization medium (Ct)	0.10	-	0.276	-	0.270	-	-0.17 o	0.01 ns
Medium with mannitol	0.36	0.26 ***	0.243	-0.034 ns	0.320	0.051 ns	0.04 ns	-0.08 ns
Medium with sorbitol	0.42	0.32 ***	0.308	0.032 ns	0.402	0.133 *	0.02 ns	-0.09 ns
Medium with PEG	0.36	0.26 ***	0.355	0.079 ns	0.312	0.043 ns	0.05 ns	0.04 ns

LSD 5% = 0.10; 1%= 0.14; 0.1% = 0.18. LSD 5% = 0.11; 1%= 0.16; 0.1% = 0.23.

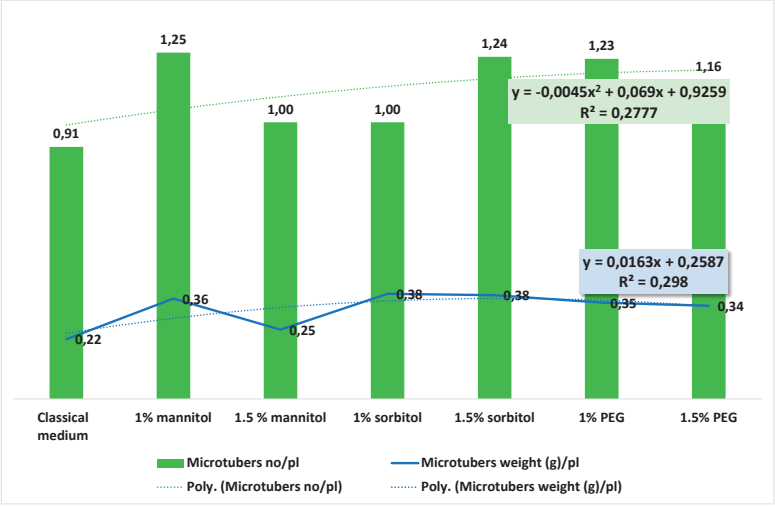

Figure 3. Microtubers number and weight in function of osmotic agents and their concentration

Examination of results regarding the number of microtubers / plant suggests the high capacity of Ervant variety for microtubers production, the highest value being recorded when 1.5% sorbitol is used in the microtuber medium (1.53 microtubers). This is followed by the Castrum variety, by using 1% mannitol (1.44), a value equal to that obtained by the Ervant variety, when 1% PEG was used (Figure 4).

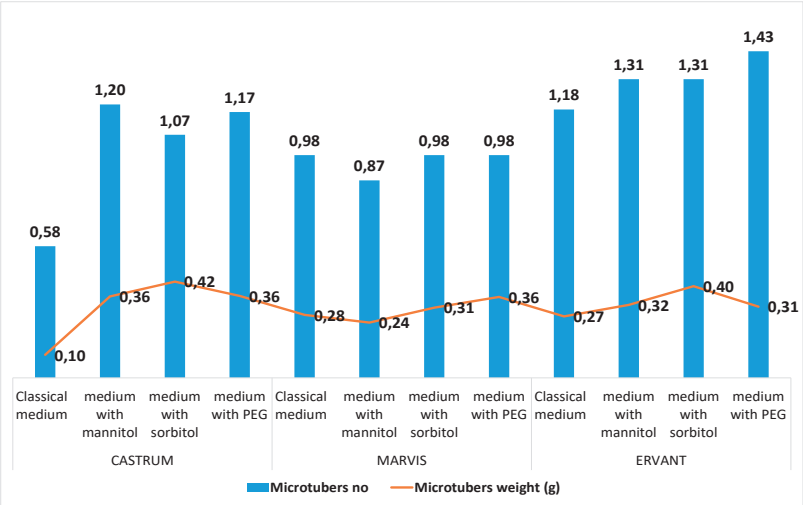

Figure 4. Microtubers number and weight in function of varity and osmotic agents

Regarding the weight of the microtubercles, the highest values are obtained for the Castrum variety, by using 1% mannitol (0.44 g) and 1.5% sorbitol (0.43 g).

CONCLUSIONS

From this study it can be concluded that by using lows concentration of sugar alcohols and PEG can be an effective method of producing microtubers. PEG and mannitol introduction into medium strongly influenced the microtuberization process for numbers (1.193 and 1.126), leading to very significant positive differences. Sorbitol also showed a beneficial effect in microtubers production (1.119), causing a distinctly significant positive difference.

The beneficial effect of mannitol in low concentration (1%) was noticed, followed by sorbitol (1.5%) and PEG (in both concentrations) obtaining higher values of microtubers number of microtubers (1.25, 1.24, 1.23 and 1.16) compared to the control medium (0.91).

The Ervant variety showed a superior behaviour to the other varieties in microtubers production, the highest value being registered when the sorbitol 1.5% (1.53 microtubercles) was used in the microtuberization medium. This is followed by the Castrum variety, by using 1% mannitol (1.44), a value equal to that obtained by the Ervant variety, by applying 1% PEG.

In microtubers formation as a number, PEG introduction in medium had a pronounced positive influence, obtaining the highest value.

For the second parameter studied, the highest values were obtained by applying sorbitol and PEG in the medium (0.377 g and 0.332 g) with very significant positive differences

Mannitol utilisation, also improved the microtubers weight/plant with a high value (0.307 g) compared to the control medium (0.216 g) and resulted in a distinctly significant positive difference.

Regarding the microtubers weight, the positive influence of sorbitol was observed for both concentrations, obtaining the highest value of microtubers weight (0.38 g).

For microtubers weight, the highest values were recorded for the Castrum variety, using 1% mannitol (0.44 g) and 1.5% sorbitol (0.43 g).

Supplementation with sugar alcohols and PEG has enhanced the microtuberization process. Under conditions of slight osmotic stress, the potato plantlets produced a higher number of microtubers, compared to the classical environment.

ACKNOWLEDGEMENTS

This research work was carried out with the support of National Institute of Research and Development for Potato and Sugar Beet Brasov and also was financed from ADER 5.1.2.

project Research on the production of minitubers under specific isolation conditions.

REFERENCES

Astarini, I.A. Febryanti, N., Miller, J. (2021). Influence of media composition and genotype on potato (*Solanum tuberosum* L.) microtuberization. *J. Hort. Indonesia*, *12*(1), 51–58.

Chandra, R., Randhawa, G.J., Chaudhari, D.R., & Upadhya, M.D. (1992). Efficacy of triazoles for *in vitro* microtuber production in potato. *Potato Research*, *35*, 339–341.

Dodds, J.H., Silva-Rodrigues, D., & Tovar, P. (1992). Micropropagation of potato (*Solanum tuberosum* L.). *Biotechnology in Agriculture and Forestry*, *19*, 91–106. Bajaj YPS (ed), Springer Verlag, Berlin.

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

George, E.F. (1993): Plant propagation by tissue culture. Part 1. *The technology Exegetics England. Potato. Phd Thesis*, Punjab Agricultural University Ludhiana (Pb) India.

Khuri, S. & Moorby, J. (1995). Investigations into the role of sucrose in potato cv. Estigma microtuber production in vitro. *Ann Bot.*, *75*, 295–303.

Jamshid, B., Jahromi, M.G., & Mousavi, A. (2020). Microtuberization efficiency of three potato (*Solanum tuberosum* L.) cultivars under osmotic stress *in vitro*. *Journal of plant productions*, available Online from 20 June 2020, doi: 10.22055/PPD.2020.30032.1782, ISSN 2588-543X

Naik, S. P., Karihaloo, J. L. (2007). Micropropagation for production of quality potato seed in Asia-Pacific. *Asia-Pacific Consortium on Agricultural Biotechnology*.

Ranalli, P., Bassi, F., Ruaro, G., Del Re P., Candilo Di, M., & Mandolino, G. (1994). Microtuber and minituber production performance compared with normal tubers. *Potato Research*, *37*, 383–391.

Yu, W.C., Joyce, P.J., Cameron, D.C., McCown B.H. (2000): Sucrose utilization during potato microtuber growth in bioreactors. *Plant Cell Rep.*, *19*, 407–413.