Scientific Bulletin. Series F. Biotechnologies, Vol. XXVI, No. 1, 2022
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

COMPARATIVE STUDY OF TWO VARIETIES OF PURPLE FLASH POTATO GROWN *IN VITRO*

Alexandra-Mihaela NAGY[1], Paula BOBOC (OROS)[3], Corina CĂTANĂ[3], Maria-Mihaela ANTOFIE[2], Camelia SAVA SAND[1, 2]

[1]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Doctoral School of Agricultural Engineering, 3-5 Calea Mănăștur, Cluj-Napoca, Romania
[2]"Lucian Blaga" University of Sibiu, Faculty of Agricultural Science, Food Industry and Environmental Protection, 7 Dr. Ion Rațiu, Sibiu, Romania
[3]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Centre for Biodiversity and Conservation, Department of Horticulture and Landscaping, 3-5 Calea Mănăștur, Cluj-Napoca, Romania

Corresponding author emails: corina.catana@usamvcluj.ro, alexandra.nagy@ulbsibiu.ro

Abstract

Solanum tuberosum L. is considered a major food/feed source since ancient times for both humans and animals. Potato cultivation is important at the global level to its extraordinary yield per unit area, being cultivated in over 120 countries worldwide. Over time, the consumption tendencies regarding potato have changed, and new varieties including purple flesh, are gradually growing in popularity. Purple flash potatoes are native to Peru but during the past decades became popular in Europe as well as in Romania. Among the main varieties of popular purple flash potatoes grown in Romania are 'Salad Blue' and 'Violet Negretin' both being rich in nutritional substances including anthocyanins. The scope of this study is to analyse the in vitro initiation and micropropagation of these cultivars. In this regard the best cultivation media is Murashige & Skoog (1962, MS62) for micropropagation. By adding Gamborg vitamins to MS62 minerals we also tested the effects of chitosan (2 mg/l) and/or active charcoal (2 g/l). Visible positive effects on micropropagation only on the culture medium supplemented with active charcoal were obtained.

Key words*: charcoal, chitosan, micropropagation, purple potato.*

INTRODUCTION

Purple flash potatoes are rich source of anthocyanins (Zhang *et al.,* 2018), which is why there has been a growing interest in introducing them in food consuming in recent years (Nemś *et al*., 2015). Purple pigmentation is due to the accumulation and storage of anthocyanins in the parenchymal tissue of potato tubers (Smeriglio *et al.,* 2016).

Anthocyanins are currently being studied extensively (Chen *et al.*, 2020; Oancea *et al.,* 2021), an essential role being attributed to their presence in the daily diet (Chen *et al.*, 2019). Potatoes rich in anthocyanin have proven antioxidant and anti-inflammatory properties (Henriques *et al*., 2020), while also possessing beneficial effects in preventing many human diseases (Mishra *et al*., 2020).

The colour of the potato flesh is a very clear indication of the presence of anthocyanins, therefor the ones with purple or red flesh have a high concentration of them (from 61.5 to 573.5 mg/kg cyanidin). Also, they have an antioxidant action of 4 to 5 times higher compared to yellow or white potato tubers (Hamouz *et al*., 2011).

In addition to cyanidin, other anthocyanins have been identified. They belong to the following groups of anthocyanidins: petunidin (Tang & Giusti, 2020), pelargonidin (Sampaio *et al*., 2021), delphinidin (Makori *et al*., 2022), peonidin (Sigurdson *et al.*, 2019) and malvidin (Pino *et al.*, 2021).

For this study, two varieties of purple potato are studied, `Salad Blue` and `Violet Negretin`, cultivated in Romania as well. Multiplication *in vitro* is a complex process material influenced by the mineral composition of culture media, vitamins formula, hormone balance as well as other chemical factors that can be supplemented to the culture media (Murashige & Skoog, 1962).

The purpose of this article is to test the effect of vitamins, chitosan, and active charcoal on the *in*

vitro development two purple potato varieties as following.

MATERIALS AND METHODS

Plant material

Two varieties of purple flash potatoes (*Solanum tuberosum* L.) as certified seed tubers were used in this study.

`Salad Blue` variety was provided by the National Research and Development Institute for Potato and Sugar Beet from Brașov, Romania. The second variety, `Violet Negretin` was provided by a nationally recognized chain of stores. This is cultivated, and distributed by Agro Brava Farm from Constanța, Romania.

The `Salad Blue` potato is originally from Scotland. Contrary to its name, due to its floury pulp, it is suitable for baking, frying, pureeing or even for making chips. It is an early variety, with flowers in shades of blue to purple. The shape of the tubers is oval, and their size is average. The epidermis has a dark blue shade, the flesh is purple to blue with white insertions (Pęksa *et al.*, 2013).

`Violet Negretin` variety, also called `Vitelotte Noire` or `Truffe de Chine` is a late potato variety of French origin. Potatoes of this variety have a dark blue peel, almost black, and the flesh is dark blue or dark purple. The shape of tubers is elongated, has a thick shell and sunken mesh (Ombra *et al*., 2015).

***In vitro* culture initiation and multiplication**

Starting material was represented by meristematic apexes taken from the sprouts of purple potato tubers in the sterile hood. The clean tubers were maintained in humidity chambers at the room temperature in jars, filter paper and soaked with distilled water.

Sterilization and initiation. The potatoes sprouts were sterilized in the sterile hood in 5% sodium hypochlorite solution for 15 minutes, followed by 3-5 times rinsing in sterile water and inoculated into test tubes with MS62 medium.

The multiplication. The first and second multiplication of the plant material was done by micro cuttings and transfer on MS62 medium without hormones (Table 1), 4 weeks after their initiation. 5 micro cuttings were transferred to each culture jar.

Micropropagation. On each culture medium variant (Table 1), micro cuttings were transferred in jars for each of the two potatoes cultivars (Figure 1) 4 weeks after their multiplication. Each jar contains 5 micro cuttings with two leaves.

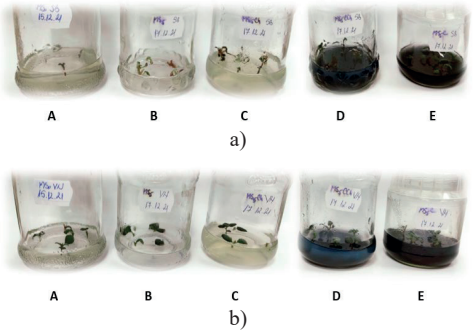

Figure 1. **a)** Transfer of the `Salad Blue` variety on culture medium variants; **b)** Transfer of the `Violet Negretin` variety on culture medium variants (**A** = MS62 medium; **B** = MS62 medium with B5 vitamins; **C** = MS62 medium with B5 vitamins and chitosan; **D** = MS62 medium with B5 vitamins, chitosan, and active charcoal; **E** = MS62 medium with B5 vitamins and active charcoal)

Culture and growth room conditions

For meristem initiation test tubes with a volume of 50 ml, height 14.5 cm, Ø of 2.5 cm, with 5 ml culture medium were used.

Jars used for multiplication and plant transfer of 200 ml, 8 cm in height, a diameter of 6 cm and filled with 50 ml of culture medium.

Culture medium was sterilized by autoclaving at 121°C for 20 minutes for test tubes and 30 minutes for the rest of the culture jars.

The culture jars were placed in a growth room with fluorescent tubes (2000 lx), at 23°C ± 2°C for night/day, with a photoperiod of 16 hours light and 8 hours darkness.

Culture media

The culture medium used for initiation was preparate according to the formula of Murashige & Skoog, 1962 medium (MS62), with 20 g sucrose and 3 g of Gelrite (Duchefa). pH before adding the Gelrite was 5.7. The pH was adjusted with NaOH 1 N and/or HCl 1 N.

The first and second multiplication were performed on MS62 without hormones.

The third multiplication was dedicated to the following experiments.

Table 1. Culture media used for studying multiplication of `Salad Blue` and `Violet Negretin` cultivars

	1	2	3	4	5
	MS62 minerals	MS62 vitamins	B5 vitamins	Chitosan	Active charcoal
Initiation and multiplication					
Initiation	X	x			
1st and 2nd multiplication	X	x			
1st experiment regarding the vitamins effect					
Control	X	x			
MS62 with B5	X		x		
2nd experiment regarding the chemicals effect					
Control	X		x		
Chitosan	X		x	x	
Active charcoal	X		x		x
Chitosan and active charcoal	X		x	x	x

1 - minerals in according to the original recipe of MS62; **2** - vitamins in according to the original recipe of MS62; **3** - B5 vitamins according to Gamborg B5 medium instead of regular vitamins; **4** - 2 mg/l chitosan; **5** - 2 g/l active charcoal.

Micro cuttings were transferred on two types of culture medium: MS62 (full formula of minerals and vitamins according to Murashige & Skoog, 1962) and a modified MS62 medium where the MS62 vitamins were replaced with B5 vitamins of Gamborg medium.

Second experiment was conducted by using the modified MS62 medium (vitamins replaced with B5 vitamins) and testing the effects of chitosan (2 mg/l) and/or active charcoal (2 g/l).

Chitosan is a biomaterial obtained more than 50 years ago through the extraction of polysaccharides substance from the exoskeleton of crustaceans (crabs, lobsters, and shrimps) (Periayah *et al.,* 2016). This biomaterial has been of interest to plant biotechnologies for over 20 years, as well as today (Hirano, 1995; Struszczyk *et al*., 2002; Li *et al*., 2020).

Active charcoal is commonly used in tissue culture media. The addition of it to the culture medium may promote or inhibit *in vitro* growth, depending on the species and tissues used (Pan & Staden, 1998).

The active charcoal used was produced by Duchefa and solubilized in the culture medium.

The chitosan was produced by Sigma-Aldrich and was solubilized with 3-4 drops of glacial acetic acid, then added to the composition of the culture medium before boiling.

All these types of media were solidified by Gelrite (Duchefa) after checking the pH and bringing it to 5.7.

Data analysing

Morphometry. The number of shoots obtained per explant as well as the heigh of plantlets were monitored.

Data were collected after 4 weeks and analysed by applying the Polifact statistical program, performing bifactorial analyses, using the Duncan test, where the first factor was the variety, and the second factor was the type of culture medium. Graphs were obtained using Excel from the Microsoft Office suite.

RESULTS AND DISCUSSIONS

Initiation and multiplication

As the plant material used for initiation comes from the natural environment conditions, it was sterilized to minimize the risk of infection and to achieve the highest rate of success of the crop.

The success rate of the initiation was 60-70%. Plantlets resulting after initiation and first multiplication were used as stock plant material for the experiments discussed in this paper.

The effect of vitamins

Each purple potato variety was grown on two types of culture medium (MS62 and MS62 with addition of B5 vitamins) after the second multiplication for studying the influence of vitamins on shooting.

The results represent an average of the values from 10 plantlets of each variety.

The analysis of the results of this experiment shows that the vitamins from the composition of the culture media doesn't have a significative influence at the shooting process for either of `Salad Blue` and `Violet Negretin` potato varieties.

The highest average shoots` number was 2.03 for the `Violet Negretin` variety grown on the MS62. This was followed by the `Salad Blue` variety grown on the same type of medium, with an average up to 1.97 shoots/explant. For the culture medium supplemented by B5 vitamins, in case of `Salad Blue` variety was obtained a higher average value (1.67) compared to the `Violet Negretin` variety (1.50) of shoots number.

However, even if different values were recorded (Figure 2), there are no statistical difference.

It is possible that multiple shoots will be generated by the stress of injury of the explants (Levshin *et al*., 2019).

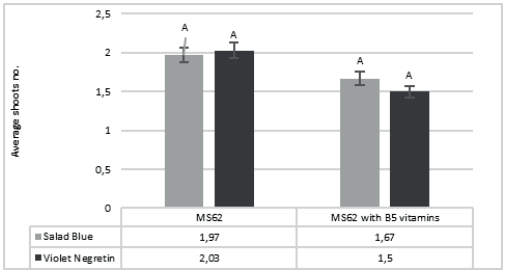

Figure 2. The effect of vitamins on shooting
(A-A - no statistical difference according Duncan test)

Another indicator followed in this experiment was the shoots heigh induced on the two types of culture medium. MS, and MS with addition of B5 vitamins. The results represent the average of the 10 length shoots per variant. The obtained results as well as the statistical approach show that vitamins influence the growth of plantlets (Figure 3).

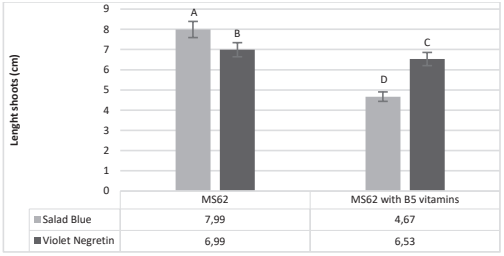

Figure 3. The effect of vitamins in the growth shoots
(A-D - significant statistical difference
according Duncan test)

The plantlets grown on the MS62 medium have a uniform growth, the average length of the shoots being insignificant between the two varieties of potato.
On the MS62 medium supplemented by B5 vitamins, the `Violet Negretin` variety (6.53 cm) registered an approximately equal increase compared to the control sample (6.99 cm), while for the `Salad Blue` variety there is a significant reduction of the growth in length of the shoots. On the MS62 medium the average of length shoots was 7.99 cm compared to that cultivated on the medium supplemented by B5 vitamins, where the average of length shoots was 4.67 cm.

The effect of the active charcoal and chitosan
Due to a noticed reduction in the shoots` height, we continued to study the effect of active charcoal and chitosan on shoots development.

The growth of the shoots for `Salad Blue` and `Violet Negretin` purple potato varieties were studied in four types of culture media (Table 1). The results represent the average of the 10 length shoots of each variant.
From the analysis of the data presented in Figure 4, it is observed that the type of culture medium influences the elongation of the shoots.

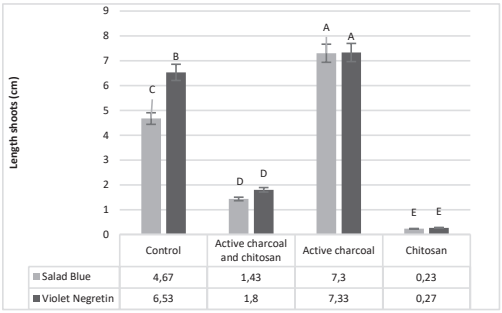

Figure 4. The effect of culture medium on the length shoots (A-E - significant statistical difference according Duncan test)

Significant differences have been observed between potato plants grown on the two-culture media regardless of the variety.
Considering the results of the second experiment, we can appraise that the effect of adding active charcoal together with chitosan addition in the culture medium could decrease the inhibitory effects of chitosan.
Compared to the control, the best results were observed for the culture medium supplemented by active charcoal. Regarding the culture medium that contains only chitosan, the plantlets stopped growing, remaining at the stage they were transferred on the culture medium.
Asghari-Zakaria *et al.* (2009) showed that chitosan, as a growth promoter and stimulator of plant protection mechanism, could alleviate the stress caused by *in vitro* culture conditions and acclimatization. The addition of chitosan into the culture medium at a concentration of 500 mg/l mainly influenced the increase in the production of greenhouse and minitubers. The increase in the yield and the number of minitubers after the application of 500 mg/l of chitosan was 12.6% and 36.3%. The results of that study indicate that chitosan can also be successfully incorporated into artificial seeds (Asghari-Zakaria *et al*., 2009).

In some species, active charcoal promotes tissue growth for apex and anthers cultures, as it has been found that charcoal powder inactivates toxic compounds produced and secreted into the culture medium (Ngomuo *et al*., 2014).

CONCLUSIONS

The initiation and multiplication of the plant material consisting of the two varieties of purple flash potato (`Salad Blue` and `Violet Negretin`) was successful.

The general formulas of the culture media used, MS62 and MS62 supplemented by B5 vitamin do not greatly influence the development processed in potato.

The culture medium that favoured a uniform and constant growth for purple potato plantlets, regardless of variety, was MS62.

Regarding the MS supplemented by B5 vitamins, the growth was negatively influenced. From the analysis of the results of these experiments it can be considered that the addition of chitosan to the MS62, also supplemented by B5 vitamins culture medium completely inhibits the growth.

On the other hand, supplementing the culture medium with active charcoal stimulates the growth compared to the control variant in both potatoes' cultivars.

In a future experiment it is desirable to monitor the effect of the chitosan on the micro-tuberization process.

ACKNOWLEDGEMENTS

This study was realized in the Centre of Biodiversity and Conservation of the University of Agricultural Sciences and Veterinary Medicine Cluj Napoca, Romania during the PhD training program.

REFERENCES

Asghari-Zakaria, R., Maleki-Zanjani, B., & Sedghi, E. (2009). Effect of in vitro chitosan application on growth and minituber yield of Solanum tuberosum L. *Plant soil environ*, *55*(6), 252-256.

Chen, C. C., Lin, C., Chen, M. H., & Chiang, P. Y. (2019). Stability and quality of anthocyanin in purple sweet potato extracts. *Foods*, *8*(9), 393.

Chen, S., Wu, M., Lu, P., Gao, L., Yan, S., & Wang, S. (2020). Development of pH indicator and antimicrobial cellulose nanofibre packaging film based on purple sweet potato anthocyanin and oregano essential oil. *International Journal of Biological Macromolecules*, *149*, 271-280.

Hamouz, K., Lachman, J., Pazderů, K., Tomášek, J., Hejtmánková, K., & Pivec, V. (2011). Differences in anthocyanin content and antioxidant activity of potato tubers with different flesh colour. *Plant, Soil and Environment*, *57*(10), 478-485.

Henriques, J. F., Serra, D., Dinis, T. C., & Almeida, L. M. (2020). The anti-neuroinflammatory role of anthocyanins and their metabolites for the prevention and treatment of brain disorders. *International Journal of Molecular Sciences*, *21*(22), 8653.

Hirano, S. (1995). Some ecologically friendly applications of chitin and chitosan in biotechnology. *Industrial Biotechnology Polymers*, 189-203.

Levshin, A. G., Gasparyan, I., Bitsoev, B., & Shchigolev, S. (2019). Constructive features of the device to remove the apical shoots of potatoes. In *18th International Scientific Conference``Engineering for Rural Development,* 532-537.

Li, Q., Dunn, E. T., Grandmaison, E. W., & Goosen, M. F. (2020). Applications and properties of chitosan. In *Applications of chitin and chitosan* (pp. 3-29). CRC Press.

Makori, S. I., Mu, T. H., & Sun, H. N. (2022). Profiling of Polyphenols, Flavonoids and Anthocyanins in Potato Peel and Flesh from Four Potato Varieties. *Potato Research*, *65*(1), 193-208.

Mishra, T., Raigond, P., Thakur, N., Dutt, S., & Singh, B. (2020). Recent updates on healthy phytoconstituents in potato: a nutritional depository. *Potato Research*, *63*(3), 323-343.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3), 473-497.

Nemś, A., Pęksa, A., Kucharska, A. Z., Sokół-Łętowska, A., Kita, A., Drożdż, W., & Hamouz, K. (2015). Anthocyanin and antioxidant activity of snacks with coloured potato. *Food Chemistry*, *172*, 175-182.

Ngomuo, M., Mneney, E., & Ndakidemi, P. A. (2014). The in vitro propagation techniques for producing banana using shoot tip cultures. *American Journal of Plant Sciences*, *2014*.

Oancea, S., Perju, M., Coman, D., & Olosutean, H. (2021). Optimization of conventional and ultrasound-assisted extraction of *Paeonia officinalis* anthocyanins, as natural alternative for a green technology of cotton dyeing. *Romanian Biotechnological Letters*, *26*(2), 2527-2534.

Ombra, M. N., Fratianni, F., Granese, T., Cardinale, F., Cozzolino, A., & Nazzaro, F. (2015). In vitro antioxidant, antimicrobial and anti-proliferative activities of purple potato extracts (*Solanum tuberosum* cv Vitelotte noire) following simulated gastro-intestinal digestion. *Natural product research*, *29*(11), 1087-1091.

Pan, M. J., & Staden, J. V. (1998). The use of charcoal in in vitro culture–A review. *Plant growth regulation*, *26*(3), 155-163.

Pęksa, A., Kita, A., Kułakowska, K., Aniołowska, M., Hamouz, K., & Nemś, A. (2013). The quality of

protein of coloured fleshed potatoes. *Food Chemistry*, *141*(3), 2960-2966.

Periayah, M. H., Halim, A. S., & Saad, A. Z. M. (2016). Chitosan: A promising marine polysaccharide for biomedical research. *Pharmacognosy reviews*, *10*(19), 39.

Pino, M. T., & Vergara, C. (2021). Red and Purple Flesh Potatoes a Healthy and Attractive Alternative Associated with New Market Trends. In *Solanum tuberosum-a Promising Crop for Starvation Problem*. IntechOpen.

Sampaio, S. L., Lonchamp, J., Dias, M. I., Liddle, C., Petropoulos, S. A., Glamočlija, J., ... & Barros, L. (2021). Anthocyanin-rich extracts from purple and red potatoes as natural colourants: Bioactive properties, application in a soft drink formulation and sensory analysis. *Food Chemistry*, *342*, 128526.

Sigurdson, G. T., Robbins, R. J., Collins, T. M., & Giusti, M. M. (2019). Molar absorptivities (ε) and spectral and colorimetric characteristics of purple sweet potato anthocyanins. *Food chemistry*, *271*, 497-504.

Smeriglio, A., Barreca, D., Bellocco, E., & Trombetta, D. (2016). Chemistry, pharmacology, and health benefits of Anthocyanins. *Phytotherapy Research*. 30, 1265-1286.

Struszczyk, M. H., Pośpieszny, H., & Gamzazade, A. (2002). Chitin and chitosan. *Polimery*, *47*(5), 316-325.

Tang, P., & Giusti, M. M. (2020). Metal chelates of petunidin derivatives exhibit enhanced color and stability. *Foods*, *9*(10), 1426.

Zhang, L., Zhao, L., Bian, X., Guo, K., Zhou, L., & Wei, C. (2018). Characterization and comparative study of starches from seven purple sweet potatoes. *Food hydrocolloids*, *80*, 168-176.