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# THE INFLUENCE OF DIFFERENT SUCROSE CONCENTRATIONS AND GENOTYPES OVER PLANTLETS GROWING PARAMETERS

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***Abstract***

*The objective of this study was to determine the effect of sucrose concentrations (2 and 3%) added to nutritive culture medium over growing parameters: plantlets length, number of leaves/plantlets, root length, fresh plantlet weight, fresh root weight. The experience is bifactorial (2 x 3), on 3 repetitions, with next factors: experimental factor A - the sucrose concentration on nutrient medium with two graduations (2, as control and 3%) and experimental factor B: variety, with 3 graduations (Marvis, considered as control, Castrum and Ervant). The use of 3% sucrose concentration has a positive influence in vitro, for leaves number, fresh plantlet weight and fresh root weight. Castrum variety has a significant positive and a distinctly significant positive differences (1.69 cm and 3.44 cm) for the length of the plantlets, respectively the number of leaves/plantlets. Also, Ervant variety had a good in vitro behaviour with a distinctly significant positive (2.50 cm) and significant positive differences (2.11 leaves and 0.052 g) for three of the analysed parameters (plantlet length, number of leaves/plantlet and fresh plantlet weight).*

***Key words****: potato, genotypes, plantlets, in vitro, sucrose concentrations.*

## INTRODUCTION

By cultures of plant tissues and cells, respectively by cultures of explants, it is understood their growth *in vitro*, for different purposes, on artificial culture media having complex chemical compositions (Cachiţă-Cosma D. and Sand C., 2000). Recently, plant tissue culture technology has become very popular and has a visible impact on the production of virus-free seed potatoes (Sadek et al., 2017). Micropropagation becomes the alternative to conventional propagation of potatoes and includes two main steps: multiplication and acclimatization of the *in vitro* obtained plants to growing under field conditions (Salem and Hassanein, 2017). Tissue culture has been applied to improve production of potato by germplasm conservation, pathogen free potato plants and micropropagation (Khalafalla, 2001). *In* vitro propagation of potato using single nodal cutting is the best method of rapid multiplication brates with maximum genetic stability (Chandra and Naik, 1993). The quality potato seed is one of the major constraints in potato production, especially in developing countries where the cost of seed potatoes alone accounts for about 40-60% of the total production cost (Shekhawat et al., 1997). The nutritional composition varied depending on the type of cells, tissues, organs, protoplasts and the plant species used (Badr, 2011). Plant cells, tissue or organ is requiring carbon source (George, 1993; Gopal, 2004). The concentration of sucrose is one of the factors controlling the induction and growth of *in vitro* shoots (Gibson, 2000; Gurel and Gulsen, 1998, cited by Ndagijimana et al., 2014). The optimum sucrose level for shoot development may vary among species and genotypes (Nowak et al., 2004, cited by Ndagijimana et al., 2014). According to the study approached by Durnikin et colab. (2019), The addition of sucrose in the concentration of 3-5% contributed to the formation of more internodes. Good development of plants from cuttings is the main criterion for evaluation of micropropagation efficiency. The main factors influencing the parameters of growth and development of microplants are variety features

and composition of the nutrient medium (Ryabtseva et al., 2017, cited by Durnikin et colab., 2019). Factors of nutrient environment is one of the important parameters that should be optimized to obtain successful plant regeneration (Durnikin et al., 2019). Plants produce sucrose through photosynthesis and can transport this molecule through the vascular system (Taiz and Zeiger, 2006). Carbohydrate is supplemented as a carbon source to maintain carbon supply as well as to maintain osmotic potential of cells. Sucrose is widely used in plant tissue culture due to its most favourable effect on growth and relatively low cost (Sumaryono et al., 2012). Sucrose in concentrations between 2-4% is usually added into culture medium and is a major component (George and Sherrington, 1993/1996). Sucrose also supports the maintenance of osmotic potential and the conservation of water in cells (Gago et al., 2014). Sucrose in culture media is usually hydrolysed totally, or partially, into the component monosaccharides glucose and fructose (George, 1993).

## MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory of National Institute of Research and Development for Potato and Sugar Beet Brasov during the period from December 2020-January 2021. This study is part of the research activity from ADER 5.1.2. project *Research on the production of minitubers under specific isolation conditions*. The main objective of the laboratory activity is to obtain a biological material with a high value, healthy, free of diseases and viruses. The obtaining methodology for this material, corresponding from phytosanitary point of view, is based on the *in vitro* multiplication technology of the potato, starting from the meristem culture. *In vitro* technology uses as first material the potato tubers sprout (Figure 1), which reached the desired length of 2-3cm are detached and disinfected (Figure 2) for meristematic sampling (Figure 3). In order to obtain a virus-free material, in addition to the meristem culture, chemotherapy was applied with 4 antiviral agents: acyclovir, ribavirin, 5-bromouracil and 2-thiouracil (all of them in 2 concentrations: 15 and 30 mg/l). After 6-8 months, depending by genotypes, from meristematic explants plantlets are developed.

The phytosanitary quality of the plantlets is determined by the ELISA test. All plantlets on medium with 30 mg/l ribavirin were virus free.

Plantlets were subjected to fragmentation in order to obtain minicuttings (Figure 4).

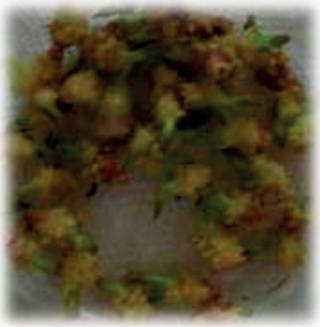

Figure 1. Potato sprouts

Figure 2. Sterilisation of potato sprouts

Figure 3. Meristematic prelevation

Figure 4. Minicuttings

Each minicutting contained a single node with the corresponding leaf. The minicuttings were placed in a culture medium in an upright position, respecting their polarity. The nutrient medium Murashige-Skoog (1962) (MS) was agarized and alpha naphthylacetic acid (NAA) was added as a growth medium regulator at a concentration of 0.5 mg/l. After inoculation, the inoculated tubes were placed in the growth chamber at 22°C with a photoperiod of 16 hours of light and 8 hours of darkness. Murashige-Skoog (1962) was supplemented with 20 g/l sucrose and 30 g/l sucrose respectively and 0.9% phyto-agar. The goal is to improve *in vitro* plantlets development and to determine the beneficial sucrose concentration for plantlets. The pH was reduced to 5.6-5.8. The medium was sterilized in an autoclave for 15'.

Marvis, Castrum and Ervant, Romanian genotypes created at National Institute of Research and Development for Potato and Sugar Beet Brasov were analysed to determine the regeneration of minicuttings produced under micropropagation conditions and plantlets development. At four weeks after inoculation of minicuttings, measurements were made for: plantlets height, number of leaves, root length, weight of the fresh plantlets and the weight of the fresh root. The research consisted on a bifactorial experience (2 x 3), on 3 repetitions, comprising the following factors: experimental factor A - the sucrose concentration on nutrient medium with two graduations: a1-MS control medium, to which 2% sucrose was added; a2- MS medium, to which 3% sucrose was added; experimental factor B: variety, with 3 graduations: b1-Marvis, considered as control; b2- Castrum; b3- Ervant. The obtained results were processed by the analysis of variance (Săulescu N.A & Săulescu N.N., 1967).

## RESULTS AND DISCUSSIONS

The statistical interpretation of the number of leaves/plantlets shows that the difference for 3% sucrose concentration reported to control (concentration of 2%) is significantly positive (1.85 leaves). Regarding the fresh weight of the plantlets (g) and the fresh root (g), the differences for the concentration of 3% are distinctly significant positive (0.05 g/0.07 g), compared to the control (Table 1).

In the case of variety influence (Table 2), it can be seen that the differences for plantlets length (cm) and number of leaves/plantlets are big compared to the Marvis variety control. Thus, the Castrum variety registers significant positive and distinctly significant positive differences (1.69 cm and 3.44 cm) for the length of the plantlets, respectively the number of leaves/plantlets.

The Ervant variety shows distinctly significant positive (2.50 cm) and significant positive (2.11 leaves and 0.052 g) differences for three of the analysed parameters (plantlet length, number of leaves/plantlet and fresh plantlet weight). Regarding the root length, the Ervant variety has an opposite behaviour, presenting a significant negative difference (-2.89 cm, with a value of 3.58 cm.

Research on the combined influence of sucrose concentration and genotype on plantlet length (Table 3) shows that at a concentration of 2% sucrose, the Castrum variety obtains a high value (8.28 cm) and a significant positive difference (2.61 cm).

The concentration of 3% sucrose determined for the Ervant variety a distinctly significant positive difference (3.11 cm), with a value of plantlet length by 10.39 cm. Thus, this concentration (3%) determined for the Ervant variety the development of a bigger plantlet. As greater plant height (Table 3) and number of leaves (Table 4) are higher, the multiplication coefficient is bigger. The concentration of 3% (compared to 2%) had a beneficial effect for the Ervant variety, standing out with a significant positive difference (2.83 cm).

From the examination of the differences obtained between the 2 sucrose concentrations, a larger value for plantlet length it is ascertained (7.28 cm) for the Marvis variety, by applying 3% sucrose in culture medium, but the difference relative to 2% sucrose, although it is positive, is insignificant (1.61 cm).

Table 1. Influence of sucrose concentration in the nutrient medium on the elements studied: plantlet length (cm), leaves number/ plantlet, root length (cm), fresh plantlet weight (g), fresh root weight (g)

| Sucrose conc. (%) (a) | Plantlets length (cm) | Dif. (cm) | Sign. | Leaves no. | Dif. | Sign. | Root length (cm) | Dif. (cm) | Sign. | Fresh plantlet weight (g) | Dif. (g) | Sign. | Fresh root weight (g) | Dif. (g) | Sign. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 (Ct) | 7.17 | - | - | 9.48 | - | - | 5.56 | - | - | 0.11 | - | - | 0.05 | - | |
| 3 | 8.57 | 1.41 | ns | 11.33 | 1.85 | * | 6.04 | 0.48 | ns | 0.16 | 0.05 | ** | 0.12 | 0.07 | ** |

LSD 5%=2.07 cm; LSD 5%=1.84 leaves; LSD 5%=1.90 cm; LSD 5%=0.02 g; LSD 5%=0.01 g;
LSD 1%=4.78 cm; LSD 1%=4.24 leaves; LSD 1%=4.39 cm; LSD 1%=0.04 g; LSD 1%=0.03 g;
LSD 0.1%=15.21 cm. LSD 0.1%=13.50 leaves. LSD 0.1%=13.98 cm. LSD 0.1%=0.13 g. LSD 0.1%=0.09 g.

Table 2. The influence of the varieties on studied the elements

| Varieties(b) | Plantlets length (cm) | Dif. (cm) | Sign. | Leaves no. | Dif. | Sign. | Root length (cm) | Dif. (cm) | Sign. | Fresh plantlet weight (g) | Dif. (g) | Sig n. | Fresh root weight (g) | Dif. (g) | Sign |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Marvis (Mt) | 6.47 | - | - | 8.56 | - | - | 6.47 | - | - | 0.103 | - | - | 0.08 | - | - |
| Castrum | 8.17 | 1.69 | * | 12.00 | 3.44 | ** | 7.33 | 0.86 | ns | 0.142 | 0.039 | ns | 0.10 | 0.02 | ns |
| Ervant | 8.97 | 2.50 | ** | 10.67 | 2.11 | * | 3.58 | -2.89 | ooo | 0.154 | 0.052 | * | 0.08 | 0.00 | ns |

LSD 5%=1.40 cm; LSD 5%=2.02 leaves; LSD 5%=0.91 cm; LSD 5%=0.045 g; LSD 5%=0.05 g;
LSD 1%=2.04 cm; LSD 1%=2.93 leaves; LSD 1%=1.33 cm; LSD 1%=0.066 g; LSD 1%=0.07 g;
LSD 0.1%=3.06 cm. LSD 0.1%=4.40 leaves. LSD 0.1%=1.99 cm. LSD 0.1%=0.099 g. LSD 0.1%=0.10 g.

Table 3. Combined influence of sucrose concentration and variety on plantlet length (cm)

| Conc. (%) (a)/ Variety (b) | 2% ($a_1$) | Dif. (cm) | Sign | 3% ($a_2$) | Dif. (cm) | Sign | $a_2b_1$-$a_1b_1$ (cm) | Sign |
|---|---|---|---|---|---|---|---|---|
| Marvis (Ct) | 5.67 | - | - | 7.28 | - | - | 1.61 | ns |
| Castrum | 8.28 | 2.61 | * | 8.06 | 0.78 | ns | -0.22 | ns |
| Ervant | 7.56 | 1.89 | Ns | 10.39 | 3.11 | ** | 2.83 | * |

LSD 5% =1.98 cm; LSD 5% =2.51 cm;
LSD 1% = 2.88 cm; LSD 1% = 4.79 cm;
LSD 0.1% = 4.33 cm. LSD 0.1% = 12.11 cm.

The combined influence of sucrose concentration and variety on the number of leaves/plantlet (Table 4) confirms the superiority of the Castrum variety, with a value of 14.00 leaves and a distinctly significant positive difference 5.44 leaves, followed by the Ervant variety, with a value of 11.44 leaves (with a significant positive difference of 2.89 leaves) for the concentration of 3% sucrose. Analysing the two concentrations of sucrose, the superiority in the production of the number of leaves of the concentration of 3% is highlighted, for the Castrum variety, with a significant positive difference (4 leaves).

Table 4. The combined influence of sucrose concentration and variety on the number of leaves

| Conc. (%) (a)/ Variety (b) | 2% ($a_1$) | Dif. (g) | Sign. | 3% ($a_2$) | Dif. (g) | Sign. | $a_2b_1$-$a_1b_1$ (g) | Sign. |
|---|---|---|---|---|---|---|---|---|
| Marvis (Ct) | 0.08 | - | - | 0.12 | - | - | 0.04 | ns |
| Castrum | 0.13 | 0.05 | ns | 0.15 | 0.03 | ns | 0.02 | ns |
| Ervant | 0.11 | 0.03 | ns | 0.20 | 0.08 | * | 0.09 | * |

LSD 5% =2.85; LSD 5% =2.86;
LSD 1% = 4.15; LSD 1% = 7.86;
LSD 0.1% = 6.22. LSD 0.1% = 22.42

When examining the combined influence of sucrose concentration and variety on root length (Table 5) it is observed that the difference obtained compared to the control (Marvis variety) is significantly positive for the Castrum variety (1.83 g), with a value of 7.44 cm. Ervant variety had an opposite behaviour, with a value of root length by 3.61 cm and a distinctly significant negative difference (-2.00 cm). Regarding the concentration of sucrose in the culture medium, the experience with 3% sucrose determined the obtaining for the Marvis variety of a root with a positive but insignificant difference (1.72 cm).

Table 5. Combined influence of sucrose concentration and variety on root length (cm)

| Conc. (%) (a)/ Variety (b) | 2% ($a_1$) | Dif. (cm) | Sign. | 3% ($a_2$) | Dif. (cm) | Sign. | $a_2b_1$-$a_1b_1$ (cm) | Sign. |
|---|---|---|---|---|---|---|---|---|
| Marvis (Ct) | 5.61 | - | - | 7.33 | - | - | 1.72 | ns |
| Castrum | 7.44 | 1.83 | * | 7.22 | -0.11 | ns | -0.22 | ns |
| Ervant | 3.61 | -2.00 | oo | 3.56 | -3.78 | ooo | -0.06 | ns |

LSD 5% =1.29 cm; LSD 5% =2.08 cm;
LSD 1% = 1.88 cm; LSD 1% = 3.01 cm;
LSD 0.1% = 2.82 cm. LSD 0.1% = 6.76 cm.

If we examine the fresh plantlet weight of the three genotypes for the two sucrose variants (Table 6), it is observed that by using the concentration of 3%, the Ervant variety achieves a vigorous plantlet, with an average plantlet weight of 0.20 g and a significant positive difference (0.08 g). By comparing the concentration of 3% to 2%, the first one determines for the same variety (Ervant) a significant positive difference (0.09 g).

Table 6. Combined influence of sucrose concentration and variety on plantlet weight (g)

| Conc. (%) (a)/ Variety (b) | 2% ($a_1$) | Dif. | Sign. | 3% ($a_2$) | Dif. | Sign. | $a_2b_1$-$a_1b_1$ | Sign. |
|---|---|---|---|---|---|---|---|---|
| Marvis (Ct) | 8.56 | - | - | 8.56 | - | - | 0 | ns |
| Castrum | 10.00 | 1.44 | ns | 14.00 | 5.44 | ** | 4.00 | * |
| Ervant | 9.89 | 1.33 | ns | 11.44 | 2.89 | * | 1.56 | ns |

LSD 5% =0.06 g; LSD 5% =0.05 g;
LSD 1% = 0.09 g; LSD 1% = 0.21 g;
LSD 0.1% = 0.14 g. LSD 0.1% = 0.65 g.

The statistical interpretation on the fresh root of the plantlets shows that the combined influence of the sucrose concentration and genotype resulted in insignificant differences for the Castrum and Ervant genotypes, at a concentration of 2% and with low values (0.08 and 0.02 g).

The concentration of 3% in the culture medium, determined for Ervant variety obtaining a root with a higher average weight value (0.14). The difference registered between the 2 sucrose concentrations detaches 3% sucrose for Ervant variety, with a significant positive difference (0.11 g) (Table 7).

Table 7. Combined influence of sucrose concentration and variety on root weight (g)

| Conc. (%) (a)/ Variety (b) | 2% ($a_1$) | Dif. (g) | Sign. | 3% ($a_2$) | Dif. (g) | Sign. | $a_2b_1$-$a_1b_1$ (g) | Sign. |
|---|---|---|---|---|---|---|---|---|
| Marvis (Ct) | 0.05 | - | - | 0.10 | - | - | 0.05 | ns |
| Castrum | 0.08 | 0.02 | ns | 0.12 | 0.01 | ns | 0.04 | ns |
| Ervant | 0.02 | -0.03 | ns | 0.14 | 0.03 | ns | 0.11 | * |

LSD 5% = 0.07 g; LSD 5% = 0.06 g;
LSD 1% = 0.10 g; LSD 1% = 0.23 g;
LSD 0.1% = 0.15 g. LSD 0.1% = 0.72 g.

## CONCLUSIONS

In the experimentation of the two sucrose concentrations for the analysed genotypes: Marvis, Castrum and Ervant, they behaved differently for all parameters.

The use of 3% sucrose concentration has a positive influence *in vitro*, for leaves number, fresh plantlet weight and fresh root weight.

The reaction of the varieties analysed shows the superiority of Castrum variety which had a significant positive and a distinctly significant positive differences (1.69 cm and 3.44 cm) for the length of the plantlets, respectively the number of leaves/plantlets. Also, Ervant variety had a good *in vitro* behaviour with a distinctly significant positive (2.50 cm) and significant positive differences (2.11 leaves and 0.052 g) for three of the analysed parameters (plantlet length, number of leaves/plantlet and fresh plantlet weight).

The plantlet height was between 10.39 cm and 7.28 cm for Ervant and Marvis varieties for 3% sucrose. Also, for the same concentration, Castrum variety had a superior number of leaves/plantlet (14.00), followed by Ervant variety (11.44).

Root length oscillated between 7.44 cm and 3.61 cm (for Castrum and Ervant varieties), but for 2% sucrose concentration. For the second concentration no significant differences were obtained (for the two varieties, compared to control) for root length.

The plantlet weight (g) was 0.20 g for Ervant variety and 0.12 g for Marvis variety, when it was used 3% sucrose.

At the analysis of the variety's behaviour at the concentration of 3%, compared to that of 2% it was found Ervant superiority with a significant positive difference (0.11 g).

Following this research, we recommend using in the culture medium of sucrose in a concentration of 3%.

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