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In vitro MEDIUM TERM CONSERVATION OF SWEET POTATO GENOTYPES USING MANNITOL AND SORBITOL

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ABSTRACT

The study is aimed at establishing an efficient protocol for *"in vitro"* medium term conservation of sweet potato. Three medium variants and three different temperatures were used for this purpose. In this experiment 6 varieties of sweet potato were tested (Juhwangmi, KSC1, Yulmi, KSP1, Hayanmi and VSP1). Uninodal segments of fully developed sweet potato plants already established *"in vitro"* were cultured on MS basal medium with and without osmotic regulators (mannitol and sorbitol). The cultures were incubated at three different temperatures: 24±2ºC, 20±2ºC and 16±2ºC. After 140 days of *"in vitro"* conservation shoots height, number of leaves, number of roots and survival rate (%) were measured. Culture condition variants V1 (MS, 24±2ºC) and V2 (MS + 10g/l mannitol + 10 g/l sorbitol, 20±2ºC) ensured the best results regarding shoots height, number of leaves and number of roots. The highest survival rate (87.78% and 84.44%, respectively) were observed in the V2 and V3 (MS + 15g/l mannitol + 15 g/l sorbitol, 16±2ºC) culture condition variants. The slow growth technique allowed the successful *"in vitro"* medium term conservation of sweet potato genotypes.

Keywords: sweet potato, *in vitro* conservation, mannitol, sorbitol, low temperature.

INTRODUCTION

In vitro plant culture is an useful and efficient method for the conservation of germplasm, especially vegetatively propagated species, such as the sweet potato. It facilitates the availability of planting materials at any time and the exchange of germplasm, avoids major pests, pathogens and other risks that occur in the field, makes posible virus eradication through meristem culture and reduces costs (Roca et al., 1979; George, 1993; Golmirzaie and Toledo, 1999; Arrigoni-Blank et al., 2014; Vettorazzi et al., 2017; Withers and Williams, 1998; Cid, 2001). *In vitro* conservation is less expensive than cryopreservation of field-grown clonal materials (Florkowski and Jarret, 1990).

In vitro plantlets exhaust the nutrients from the medium in 2-3 months. Therefore, *in vitro* plantlets have to be transferred frequently to fresh medium. The interval between subcultures can be extended through growth rate reduction by modifications to the environment or changes in some media components (Golmirzaie and Toledo, 1999). The purpose of slow growth *in vitro* conservation technique is to maximize the period of subculture or extend it for as long as possible. To achieve this aim, changes are made in the cultivation environment to slow down the growth of cells and tissues (Arrigoni-Blank et al., 2014).

Osmoticums such as mannitol or sorbitol reduce mineral uptake by cells through differences in osmotic pressures thereby retarding plant growth (Dodds and Roberts, 1985; Thompson et al., 1986). The use of sorbitol as an osmoticum is applied to many crops without any physiological changes such as callus formation or vitrification. Reducing temperature from growth room several degrees below normal can also minimize the growth rate in many crops (Dodds and Roberts, 1985; George and Sherrington, 1984). *In vitro* plantlets growing in closed culture vessels have low concentrations of CO_2. Carbon absorbtion is maintained by

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supplementing the medium with sugar. Reducing light intensity also affects growth rate by reducing photosynthetic requirements and therefore metabolism (Hughes, 1981). A combination of osmoticums, low temperature and low light intensity has been the most effective in lengthening periods between subcultures (Golmirzaie and Toledo, 1999; Tahtamouni et al., 2001; Islam et al., 2003; Sarwar and Siddiqui, 2004; Divakaran et al., 2006; Vettorazzi et al., 2017).

A number of problems prevent the long term conservation of sweet potato. Many attempts to establish an efficient slow growth medium have failed due to a strong genotypic response to the modified culture media, low survival percentage under restrictive growth conditions, or the formation of callus and vitrification during storage (Golmirzaie and Toledo, 1999).

Because of its many uses (human consumption, feeding animals, biomass in biofuel production, raw material in industrial processes for the aweets, flour, flakes and starch production) the sweet potato is considered a species of great commercial interest (Arrigoni-Blank et al., 2014; Ferrari et al., 2013; Wang et al., 2013; Zhang et al., 2013; Vettorazzi et al., 2017). Therefore the conservation of sweet potato germplasm is necessary and useful. The basis of all genetic improvement lies in genetic diversity, which is reflected in the creation of plants with resistance/tolerance to various biotic and abiotic factors, ensuring increased productivity (Arrigoni-Blank et al., 2014). Some factors like modernization of agriculture (which leads to rural exodus and contributes to the loss of genetic diversity of crops that were traditionally cultivated by small farmers) and the change in the eating habits of populations caused the replacement of landraces with commercial varieties. To prevent this loss of diversity it is essential to preserve the germplasm (Vettorazzi et al., 2017).

MATERIAL AND METHODS

Six sweet potato genotypes provided by Research and Development Station for Plant Growing on Sands (RDSPGS) Dăbuleni, Dolj County were used as plant material. The study was carried out at the National Institute of Research and Development for Potato and Sugar Beet (NIRDPSB) Braşov, Research Laboratory for Plant Tissue Culture.

The explants used in this experiment were uninodal segments of fully developed sweet potato plants already established *"in vitro"*. The experimental design was completely randomized with a 6x3 factorial arrangement, testing six genotypes (Yulmi, KSC1, KSP1, Juhwangmi, VSP1 and Hayanmi), three different variants of culture medium at three temperatures (24±2ºC, 20±2ºC and 16±2ºC) (Table 1).

For a more effective control of microbial contamination, a broad-spectrum product PPM - Plant Preservation Mixture (Plant Cell Technology) that inhibits the growth of pathogens in plant tissue cultures was added to the medium. After inoculation, the tubes were covered with aluminum foil.

All three culture medium variants contain 4.4 g/l Murashige-Skoog (MS) salts and vitamins (Murashige and Skoog, 1962). Culture condition V1 variant contains explants inoculated on MS medium added with 30 g/l sucrose, 9 g/l agar, 3 ml/l PPM, incubated at 24±2ºC, and the test tubes are not sealed with parafilm. The other two culture medium variants contain explants inoculated on MS medium added with 10 g/l, respectively 15 g/l mannitol and sorbitol, incubated at 20±2ºC and 16±2ºC, respectively, and the tubes are sealed with parafilm. The quantities of sucrose, agar and PPM are identical to all three culture medium variants.

The pH was adjusted to 5.7 for all culture medium variants followed by autoclave sterilization (temperature of 121ºC for 20 minutes).

After inoculation, the material was kept in three different growth rooms (to ensure three different temperatures: 24±2ºC, 20±2ºC and 16±2ºC, respectively), under a photoperiod of 16:8 hours of light:darkness for over four months without subcultivation.

After four months of culture the sweet potato plantlets were removed from the test tubes and a series of measurements were

made regarding: the height of the shoots, the number of leaves, the number of roots and the survival rate (%) was also quantified.

Statistical analysis. All of the data were subjected to analysis of variance (Săulescu and Săulescu, 1967).

Table 1. Culture condition variants for *"in vitro"* medium term conservation

Culture condition variants	Temperature (ºC)	Mannitol (g/l)	Sorbitol (g/l)	Sucrose (g/l)	Agar (g/l)	PPM (ml/l)	Parafilm
V1	24±2	-	-	30	9	3	No
V2	20±2	10	10	30	9	3	Yes
V3	16±2	15	15	30	9	3	Yes

RESULTS AND DISCUSSION

1. Effect of different culture medium variants and different temperatures on the height of the shoots. The results revealed culture conditions V2 (MS + 10 g/l mannitol + 10 g/l sorbitol, 20±2ºC) as having the highest mean values for shoot height (6.45 cm). This was followed by culture conditions V1 (MS, 24±2ºC) with a mean value for shoot height of 6.09 cm. From the analysis of the culture medium and temperature influence on the height of the shoots it can be observed that on the culture conditions variant in which the highest concentration of mannitol and sorbitol (15 g/l) was used and the plantlets were incubated at the lowest temperature (16±2ºC), there was a very significant negative difference (-2.92 cm) from the average of all values of the culture conditions used in this study (Table 2).

Table 2. The influence of the culture medium and the temperature on the height of the shoots

Culture condition variants	Shoots height (cm)	Diff. (cm)	Sign.
V1 - Murashige and Skoog including vitamins (MS) - Temperature: 24±2ºC	6.09	1.28	***
V2 - MS + Mannitol 10 g/l + Sorbitol 10 g/l - Temperature: 20±2ºC	6.45	1.64	***
V3 - MS + Mannitol 15 g/l + Sorbitol 15 g/l - Temperature: 16±2ºC	1.89	-2.92	ooo
Mean (Ct)	4.81	-	-

LSD 5% = 0.39 cm; 1% = 0.65 cm; 0.1% = 1.22 cm

The value of the average shoots height (1.89 cm) obtained on the culture medium variant with 15 g/l mannitol, 15 g/l sorbitol and incubation of the cultures at a temperature of 16±2ºC, represented an advantage in the *"in vitro"* medium term conservation of sweet potato. The slowdown of growth allows the transfer of explants to new containers and fresh medium (sub culturing) to longer intervals. In this way, the costs for both the preparation of the culture medium and the labor are reduced.

Table 3. The influence of the variety on the height of the shoots

Variety	Shoots height (cm)	Diff. (cm)	Sign.
Juhwangmi	8.84	4.03	***
KSC1	4.60	-0.21	ns
Yulmi	4.27	-0.54	ns
KSP1	4.29	-0.52	ns
Hayanmi	3.45	-1.36	o
VSP1	3.43	-1.38	o
Mean (Ct)	4.81	-	-

LSD 5% = 1.17 cm; 1% = 1.57 cm; 0.1% = 2.09 cm

The shoots height differed significantly among the cultivars. As shown in Table 3 Juhwangmi produced the longest shoots (8.84 cm). The varieties Hayanmi and VSP1 recorded the lowest values of the shoots height (3.45 cm and 3.43 cm, respectively). The varieties KSC1 (4.60 cm), Yulmi (4.27 cm) and KSP1 (4.29 cm) showed insignificant differences from the average of all varieties, as well (Table 3). The varieties that show a slower growth of shoots height are suitable for an *"in vitro"* medium term conservation.

When interpreting the combined influence of culture conditions and variety on the height of shoots it can be observed that variety Juhwangmi recorded a significant difference (2.59 cm) in culture conditions V1 (MS, 24±2ºC) and a very significant difference (8.25 cm) in culture conditions V2 (MS + 10 g/l mannitol + 10 g/l sorbitol, 20±2ºC) (Table 4).

Table 4. The influence of the culture medium, temperature and variety on the height of the shoots

Variety	V1	Diff. (cm)	Sign.	V2	Diff. (cm)	Sign.	V3	Diff. (cm)	Sign.	Dif. a_2-a_1 (cm)	Sign.	Dif. a_3-a_1 (cm)	Sign.
Juhwangmi	8.68	2.59	*	14.71	8.25	***	3.14	1.25	ns	6.03	***	-5.54	ooo
KSC1	7.41	1.32	ns	4.48	-1.97	ns	1.92	0.03	ns	-2.93	oo	-5.49	ooo
Yulmi	6.54	0.45	ns	5.24	-1.21	ns	1.02	-0.87	ns	-1.30	ns	-5.52	ooo
KSP1	4.93	-1.17	ns	5.74	-0.71	ns	2.20	0.31	ns	0.81	ns	-2.73	oo
Hayanmi	5.03	-1.07	ns	4.09	-2.36	o	1.23	-0.66	ns	-0.94	ns	-3.79	ooo
VSP1	3.97	-2.12	o	4.47	-1.98	ns	1.85	-0.04	ns	0.49	ns	-2.12	o
Mean (Ct)	6.09	-	-	6.45	-	-	1.89	-	-	0.36	-	-4.20	-

LSD 5% = 2.02 cm; 1% = 2.72 cm; 0.1% = 3.61 cm DL 5% = 1.88 cm; 1% = 2.55 cm; 0.1% = 3.45 cm

Using MS medium and a temperature of 24±2ºC a significant negative difference for variety VSP1 (-2.12 cm) was observed and using culture medium with 10 g/l mannitol and 10 g/l sorbitol and a temperature of 20±2ºC a significant negative difference for Hayanmi variety (-2.36 cm) was recorded. On culture conditions V3 (MS + 15 g/l mannitol + 15 g/l sorbitol, 16±2ºC) all the analyzed varieties showed an insignificant difference compared to the average regarding the shoots height. When differentiating the type of culture conditions applied, respectively, the culture medium with the highest concentration of mannitol (15 g/l) and sorbitol (15 g/l) and the lowest temperature (16±2ºC) and the MS culture medium without osmotic regulators and at the highest temperature (24±2ºC), the analyzed varieties recorded significant differences, -2.12 cm for the variety VSP1, distinctly significant -2.73 cm for the variety KSP1 and very significant -5.54 cm, -5.49 cm, -5.52 cm and -3.79 cm, respectively for the Juhwangmi, KSC1, Yulmi and Hayanmi varieties (Table 4).

2. Effect of different culture medium variants and different temperatures on the number of leaves. The MS medium associated with a temperature of 24±2ºC (V1) provided the highest number of leaves (9.44 leaves) with a distinctly significant difference from the average (2.17 leaves). This was followed by culture conditions V2 (MS + 10 g/l mannitol + 10 g/l sorbitol, 20±2ºC) with a significant difference (1.11 leaves). Under growth conditions provided by V3 variant (MS + 15 g/l mannitol + 15 g/l sorbitol, 16±2ºC) the sweet potato plants produced the lowest number of leaves (3.98) with a very significant negative difference from the average (Table 5).

Plants of variety Juhwangmi exhibited the greatest number of leaves (8.62) with a significant difference (1.35 leaves) from the average. In contrast, the VSP1 variety recorded the lowest number of leaves (5.02) with a very significant negative difference from the average (-2.25 leaves), as shown in Table 6. The other four varieties showed insignificant differences from the average regarding the number of leaves (Table 6).

Table 5. The influence of the culture medium and the temperature on the number of leaves

Culture condition variants	Number of leaves	Diff. (leaves)	Sign.
V1 - Murashige and Skoog including vitamins (MS) - Temperature: 24±2ºC	9.44	2.17	**
V2 - MS + Mannitol 10 g/l + Sorbitol 10 g/l - Temperature: 20±2ºC	8.38	1.11	*
V3 - MS + Mannitol 15 g/l + Sorbitol 15 g/l - Temperature: 16±2ºC	3.98	-3.29	ooo
Mean (Ct)	7.27	-	-

LSD 5% = 0.83 leaves; 1% = 1.37 leaves; 0.1% = 2.56 leaves

When referring to the number of leaves, it also includes the aged leaves (dried, yellow leaves). The measurements were made after four months of *"in vitro"* cultivation, without transfer to fresh medium. After this time, the surviving plants had both aged and green leaves. In Figure 1 it can be observed the appearance of sweet potato plantlets belonging to the Yulmi variety, in the three cultivation conditions after 70, 100 and 140 days respectively of *"in vitro"* conservation.

Table 6. The influence of the variety on the number of leaves

Variety	Number of leaves	Diff. (leaves)	Sign.
Juhwangmi	8.62	1.35	*
KSC1	7.29	0.02	ns
Yulmi	7.84	0.57	ns
KSP1	7.89	0.62	ns
Hayanmi	6.93	-0.34	ns
VSP1	5.02	-2.25	ooo
Mean (Ct)	7.27	-	-

LSD 5% = 1.09 leaves; 1% = 1.47 leaves; 0.1% = 1.95 leaves

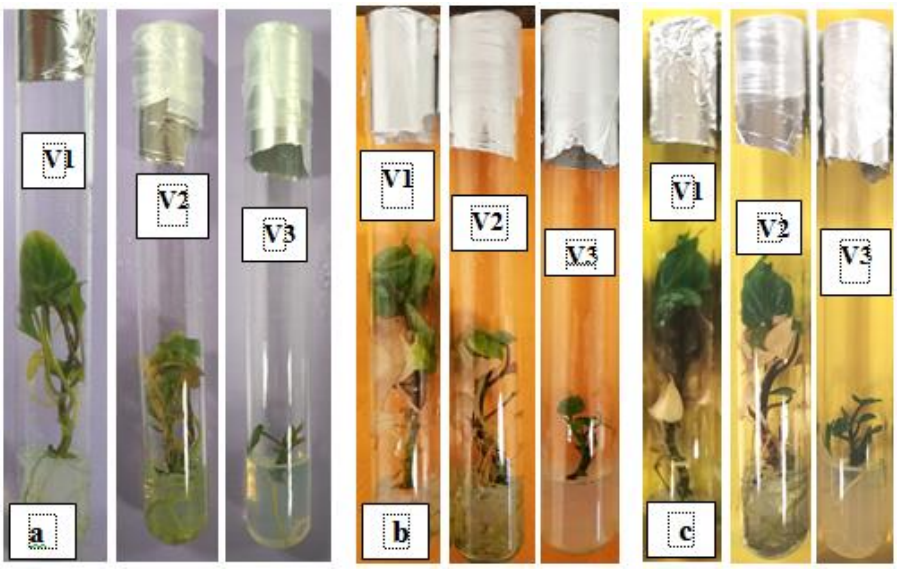

Figure 1. The aspect of sweet potato plantlets in the cultivation condition variants - Yulmi variety (a - 70 days after initiation; b - 100 days after initiation; c - 140 days after initiation)

Using V1 cultivation conditions, the highest number of leaves was obtained for the Hayanmi variety with a very significant difference (3.56 leaves) compared to the average, followed by the Yulmi variety with a significant difference (2.29 leaves). The lowest number of leaves was obtained by the variety VSP1, with a distinctly significant negative difference (-2.71), followed by the Juhwangmi variety, with a significantly negative difference (-2.44 leaves) compared to the average (Table 7). V2 culture conditions variant provided a greater number of leaves for variety Juhwangmi (14.33), as opposed to varieties Hayanmi and VSP1, which in the same conditions obtained the lowest number of leaves (5.60 and 5.73, respectively). On V3 culture conditions variant the KSP1 variety was noted as having the highest number of leaves (7.40) with a very significant positive difference (3.42 leaves) from the average (Table 7).

Table 7. The influence of the culture medium, temperature and variety on the number of leaves

Variety	V1	Diff. (leaves)	Sign.	V2	Diff. (leaves)	Sign.	V3	Diff. (leaves)	Sign.	Dif. a_2-a_1 (leaves)	Sign.	Dif. a_3-a_1 (leaves)	Sign.
Juhwangmi	7.00	-2.44	o	14.33	5.95	***	4.53	0.55	ns	7.33	***	-2.47	o
KSC1	10.27	0.83	ns	7.27	-1.11	ns	4.33	0.35	ns	-3.00	oo	-5.93	ooo
Yulmi	11.73	2.29	*	9.00	0.62	ns	2.80	-1.18	ns	-2.73	oo	-8.93	ooo
KSP1	7.93	-1.51	ns	8.33	-0.05	ns	7.40	3.42	***	0.40	ns	-0.53	ns
Hayanmi	13.00	3.56	***	5.60	-2.78	oo	2.20	-1.78	ns	-7.40	ooo	-10.80	ooo
VSP1	6.73	-2.71	oo	5.73	-2.65	oo	2.60	-1.38	ns	-1.00	ns	-4.13	ooo
Mean (Ct)	9.44	-	-	8.38	-	-	3.98	-	-	-1.07	-	-5.47	-

LSD 5% = 1.88 leaves; 1% = 2.54 leaves; 0.1% = 3.37 leaves LSD 5% = 1.90 leaves; 1% = 2.64 leaves; 0.1% = 3.75 leaves

3. Effect of different culture medium variants and different temperatures on the number of roots. The MS medium associated with a temperature of 24±2ºC (V1) provided the highest number of roots (6.12) with a very significant difference from the average (1.74). This was followed by culture conditions V2 (MS + 10 g/l mannitol + 10 g/l sorbitol, 20±2ºC) with a distinctly significant difference (1.16). Under growth conditions provided by V3 variant (MS + 15 g/l mannitol + 15 g/l sorbitol, 16±2ºC) the sweet potato plants produced the lowest number of roots (1.47) with a very significant negative difference from the average (Table 8).

Table 8. The influence of the culture medium and the temperature on the number of roots

Culture condition variants	Number of roots	Diff. (roots)	Sign.
V1 - Murashige and Skoog including vitamins (MS) - Temperature: 24±2ºC	6.12	1.74	***
V2 - MS + Mannitol 10 g/l + Sorbitol 10 g/l - Temperature: 20±2ºC	5.54	1.16	**
V3 - MS + Mannitol 15 g/l + Sorbitol 15 g/l - Temperature: 16±2ºC	1.47	-2.91	ooo
Mean (Ct)	4.38	-	-

LSD 5% = 0.53 roots; 1% = 0.87 roots; 0.1% = 1.63 roots

The number of roots differed significantly among the cultivars. As shown in Table 9 Juhwangmi displayed a greater number of roots (6.49). The varieties Hayanmi and VSP1 recorded the lowest values of the roots number (2.96 and 2.76, respectively). The varieties KSC1, Yulmi and KSP1 showed insignificant differences from the average (Table 9).

Table 9. The influence of the variety on the number of roots

Variety	Number of roots	Diff. (roots)	Sign.
Juhwangmi	6.49	2.11	**
KSC1	5.53	1.15	ns
Yulmi	3.87	-0.51	ns
KSP1	4.67	0.29	ns
Hayanmi	2.96	-1.42	o
VSP1	2.76	-1.62	o
Mean (Ct)	4.38	-	-

LSD 5% = 1.29 roots; 1% = 1.74 roots; 0.1% = 2.30 roots

Table 10. The influence of the culture medium, temperature and variety on the number of roots

Variety	V1	Diff. (roots)	Sign.	V2	Diff. (roots)	Sign.	V3	Diff. (roots)	Sign.	Dif. a_2-a_1 (roots)	Sign.	Dif. a_3-a_1 (roots)	Sign.
Juhwangmi	6.60	0.48	ns	10.80	5.26	***	2.07	0.60	ns	4.20	***	-4.53	ooo
KSC1	9.67	3.55	**	5.80	0.26	ns	1.13	-0.34	ns	-3.87	oo	-8.53	ooo
Yulmi	6.73	0.61	ns	4.33	-1.21	ns	0.53	-0.94	ns	-2.40	o	-6.20	ooo
KSP1	5.33	-0.79	ns	5.40	-0.14	ns	3.27	1.80	ns	0.07	ns	-2.07	ns
Hayanmi	5.00	-1.12	ns	2.73	-2.81	o	1.13	-0.34	ns	-2.27	o	-3.87	oo
VSP1	3.40	-2.72	o	4.20	-1.34	ns	0.67	-0.80	ns	0.80	ns	-2.73	o
Mean (Ct)	6.12	-	-	5.54	-	-	1.47	-	-	-0.58	-	-4.66	-

LSD 5% = 2.23 roots; 1% = 3.01 roots; 0.1% = 3.99 roots LSD 5% = 2.10 roots; 1% = 2.86 roots; 0.1% = 3.88 roots

When interpreting the combined influence of culture conditions and variety on the number of roots (Table 10), using V1 cultivation conditions, the highest number of roots was obtained for the KSC1 variety with a distinctly significant difference (3.55 roots) compared to the average and the lowest number of roots was obtained for the variety VSP1, with a significant negative difference (-2.72 roots). V2 culture conditions variant

provided a greater number of roots for variety Juhwangmi (10.80), as opposed to varieties Hayanmi, which in the same conditions obtained the lowest number of roots (2.73). On V3 culture conditions variant the varieties did not show significant differences from the average (Table 10).

When differentiating the types of culture conditions applied, respectively, the culture medium with the highest concentration of mannitol (15 g/l) and sorbitol (15 g/l) and the lowest temperature (16±2ºC) and the MS culture medium with no osmotic regulators and at the highest temperature (24±2ºC), the analyzed varieties recorded significant differences (-2.73 roots) for the variety VSP1, distinctly significant (-3.87 roots) for the variety Hayanmi and very significant (-4.53 roots, -6.20 roots and -8.53 roots) for the Juhwangmi, Yulmi and KSC1 varieties respectively (Table 10).

4. Effect of different culture medium variants and different temperatures on the survival of sweet poatato plants after 140 days of "in vitro" conservation. Variety Yulmi (86.66%) showed a higher survival rate using MS medium associated with a temperature of 24±2ºC (V1) with a significant difference (22.96%) compared to the average (Table 11).

Table 11. The influence of the culture medium, temperature and variety on the survival of sweet potato plantlets (%) after 140 days of *"in vitro"* conservation

Variety	V1	Diff.	Sign.	V2	Diff.	Sign.	V3	Diff.	Sign.	Dif. a_2-a_1	Sign.	Dif. a_3-a_1	Sign.
Juhwangmi	53.34	-10.36	ns	93.33	5.55	ns	73.33	-11.11	ns	40.00	**	19.99	ns
KSC1	53.33	-10.37	ns	93.33	5.55	ns	80.00	-4.44	ns	40.00	**	26.67	*
Yulmi	86.66	22.96	*	100.00	12.22	ns	93.33	8.89	ns	13.34	ns	6.67	ns
KSP1	73.33	9.63	ns	86.67	-1.11	ns	100.00	15.56	ns	13.33	ns	26.67	*
Hayanmi	60.00	-3.70	ns	80.00	-7.78	ns	93.33	8.89	ns	20.00	ns	33.33	**
VSP1	60.00	-3.70	ns	73.33	-14.45	ns	66.67	-17.78	ns	13.33	ns	6.66	ns
Mean (Ct)	64.44	-	-	87.78	-	-	84.44	-	-	23.33	-	20.00	-
LSD 5% = 21.22%; 1% = 28.60%; 0.1% = 37.97%										LSD 5% = 21.73%; 1% = 30.42%; 0.1% = 43.67%			

Adding mannitol and sorbitol (10 g/l) to the culture medium and incubating the cultures at a temperature of 20±2ºC provided a greater survival rate for microplants of varieties Yulmi (100%), Juhwangmi and KSC1 (93.33%) and KSP1 (86.67%), while for the Hayanmi and VSP1 varieties the survival rate was 80% and 73.33% respectively. After 140 days on the culture conditions variant in which the highest concentration of mannitol and sorbitol (15 g/l) was used and the microplants were incubated at the lowest temperature (16±2ºC), the survival rate was 100% for KSP1, 93.33% for Yulmi and Hayanmi and 80% for KSC1. The lowest survival rate being recorded in the VSP1 variety (66.67%). When differentiating the type of culture conditions, respectively, the culture medium with mannitol and sorbitol (10 g/l) combined with a temperature of 20±2ºC and MS culture medium associated with the highest temperature 24±2ºC the Juhwangmi and KSC1 varieties recorded a distinctly significant differences (40%) compared to the average. By differentiating culture medium variant with the highest concentration of mannitol (15 g/l) and sorbitol (15 g/l) and the lowest temperature (16±2ºC) and the MS medium with no osmotic regulators and the highest temperature (24±2ºC) Hayanmi variety recorded a distinctly significant difference (33,33%); KSC1 and KSP1 varieties recorded a significant difference (26.67%) compared to the average (Table 11). This different response of the varieties regardind survival rate after 140 days of *"in vitro"* conservation in three different conditions may be related to the genetic characteristics of each plant because different varieties were used.

There are several raports in the literature highlighting the effect of osmotic active compounds on the *"in vitro"* conservation of different plants: potato (*Solanum tuberosum)* (Muñoz et al., 2019; Nasiruddin and Islam, 2018), date palm (*Phoenix dactylifera*) (El-Bahra et al., 2016), indian ipecac (*Tylophora indica*) (Haque and Grosh, 2013), orchid (*Epidendrum chlorocorymbos*) (Lopez-Puc, 2013), globe artichoke (*Cynara scolymus*) (Bekhett and Usama, 2007), rose coloured leadwort (*Plumbago indica*) (Chaeoensub and Phansiri, 2004), deutzia (*Deutzia scabra*) (Gabr and Sayed, 2010), garlic (*Allium sativum*) (Hassan et al., 2007), grape (*Vitis vinifera*) (Tehrim and Sajid, 2011).

CONCLUSIONS

In choosing the best treatment for *"in vitro"* conservation of sweet potato, all of the variables and their effects on variety conservation should be considered together. The addition of osmotic regulators in the culture medium, increasing their concentration as well as reducing the temperature in the growth chamber determined the decrease of microplants growth regarding the shoots height, number of leaves and number of roots. Sweet potato develops better in regions where the average temperature exceeds 24ºC and in its natural environment it does not withstand low temperatures well, maintaining this characteristic also under *"in vitro"* conditions (Silva et al., 2004; Vettorazzi et al., 2017). However, regarding the survival percentage of sweet potato plants after 140 days of *"in vitro"* conservation, the highest survival rate was recorded on the culture medium variants to which mannitol and sorbitol (10 g/l and 15 g/l) were added and the plantlets were incubated at a low temperature of 20±2ºC and 16±2ºC, respectively.

During *"in vitro"* conservation regular monitoring of cultures is recommended. It is mandatory that contaminated cultures be removed as soon as they have been detected in order to limit the spread of microbial infection to healthy plants.

The varieties Yulmi, KSP1, KSC1 and Juhwangmi had good results regarding the shoots height, number of leaves, number of roots, and the varieties Hayanmy and VSP1 got lower results on these parameters. The highest survival rate was recorded in the varieties: Yulmi and KSP1, followed by Hayanmi, KSC1, Juhwangmi and VSP1.

An efficient strategy for obtaining good results regarding conservation of sweet potato germplasm is the combination of several factors, such as lowering the temperature to reduce the metabolism of the plant and adding osmotic regulators to reduce the osmotic potential of the medium, which in turn reduces the absorption of nutrients by the plant. With these treatments, it was possible to reduce plant growth and maintain green leaves, allowing the subsequent regeneration and multiplication of the sweet potato varieties.

The results of the present study indicate that it is possible to extend the period between subculturing of sweet potato plants to more than 140 days, thereby reducing the working time and the amount of compounds utilized in the preparation of the growth medium, which will ultimately result in cost reduction for *"in vitro"* conservation of sweet potato germplasm. This method allows the genetic diversity of the sweet potato to be maintained in many regions of the world.

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