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IN VITRO PROPAGATION OF SWEET POTATO VARIETIES USING AXIAL SHOOTS AS A SOURSCE OF EXPALNTS AND THEIR ANALYSIS BY COMPARING SOME GROWTH PARAMETERS

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Abstract

In order to obtain the shoots in laboratory conditions, it was necessary to prepare the sweet potato tubers and plant them, ensuring favorable conditions of temperature, light and humidity. Axial shoots from sweet potato varieties: KSP1, DK 19/1, DK 19/2, DCh 19/3, DK 19/4 and DK 19/5 were used as a source of explants for the initiation of in vitro culture. Through a single factor experiment in three repetitions, the analyzed factor was the sweet potato variety, with 6 graduations, and as a control the average values for the studied elements were established. Determinations were made on the following parameters: the formation of shoots, the number of leaves, the height of the plantlets and the weight of the fresh root. The results presented in this study highlight the possibility of obtaining sweet potato shoots and in laboratory conditions, throughout the year, with minimal costs. allowing in vitro cultivation technique to obtain in a short time a stock of healthy starting material, which can be used as planting material.

Key words*: sweet potato shoots, in vitro, plantles.*

INTRODUCTION

Sweet potato crops play an important role in agriculture and facilitate food security in many underdeveloped countries. From recent statistical studies, worldwide, in 2017, 494.6 million tons of sweet potatoes were obtained (FAOSTAT, 2019). The consumption of sweet potatoes for the majority of the global population is on average per capita of 19.4 kg / year (2013-2015) and an increase of 21.0 kg / year is expected in the future, until 2025 (OECD-FAO, 2016).

This crop is widespread in the world, being used fresh, cooked, or as a raw material for industrial processing: starch, alcohol, coloring agent, liqueur, juices, rich in beta-carotene and alpha-tocopherol (vit E) (Benisheikh et al., 2013).

It contains almost all macro- and micro-nutrients, substantial amounts of vitamin C, moderate amounts of vitamin B complex (vitamins B1, B2, B5 and B6) and folic acid, as well as satisfactory amounts of vitamin E. This species is appreciated due to nutritional value of thickened roots (tubers), rich in starch, mineral salts of calcium, potassium, phosphorus, magnesium, various organic acids and beta-carotene. Beta-carotene is converted by the human body into vitamin A, with beneficial effects on increasing immunity, health of the skin and membranes covering the nose, lungs and intestines. The high beta-carotene content also helps to alleviate joint pain, having an anti-inflammatory effect. It tends to become a staple food in many parts of the world. This crop is vital for small farmers with limited land, low labor and small capital. One of its major advantages is the ability to be harvested gradually for family consumption, or income generation (Tumwegamire et al., 2004). Because the sweet potato has a high degree of heterozygotes, the seeds are used only for propagation and creation of new varieties and lines (Gaba and Singer, 2009), used in breeding programs. There are a number of advantages to using in vitro micropropagation methods over conventional vegetative propagation. The micropropagation process has been used on a commercial scale, for the production of qualitative planting material, of several horticultural species. Meristems and nodal explants have become popular in sweet potato

micropropagation. The effectiveness of micropropagation techniques depends on a variety of factors, including: genotype, stock plant physiology, season, culture medium composition, growth regulators, light source (type and intensity), photoperiod, gelling agents and carbon sources (George et al., 2008).

MATERIALS AND METHODS

For this purpose, the biological material (sweet potato tubers) was provided by the Research and Development Station for Agricultural Plants on Sands Dăbuleni (SCDCPN), Dolj County. Six Korean sweet potato varieties were studied and used to obtain the donor (mother) plant., respectively: KSP 1 (control), DK 19/1, DK 19/2, DCh 19/3, DK 19/4 and DK 19/5. The tubers were planted in plastic boxes measuring 57.5 x 29 x 6 cm. This type of perforated box is used for direct sowing, using as substrates fine peat, perlite, or others and provides easy mobility, and can optionally be used together with a tray from which the plants can absorb water or nutrient solution.

Watering and fertilizing were carried out as needed throughout the emergence and growth period. Optimal conditions of temperature (20-24°C) and light were ensured. Until the buds appeared, the boxes were kept in the dark, and after the appearance of the first buds, they were provided with optimal lighting conditions (photoperiod of sixteen hours of light and eight hours of darkness). Under these conditions, after about a month, the tubers began to sprout.

Healthy donor plants, grown for two weeks, were treated preventively to prevent insect infestation and the development of microorganisms (fungi and bacteria). The young explants, with vigorous shoots, had all the chances to develop successfully in laboratory conditions.

One week before the process of obtaining the explants, the plants were sprayed with Ridomil Gold MZ, which has a systemic and contact action. Metalaxyl-M (mefenoxam) is systemic and is rapidly absorbed by the green parts of plants, distributed upwards (acropetally) throughout the plant and in new growths after treatment. Mancozeb has a contact action, forming a protective barrier on the surface of the plants, preventing the germination of spores of any kind.

After the laboratory production of sweet potato shoots, they were used as a source of explants to initiate the in vitro culture of the six sweet potato varieties. When the shoots were about 20-25 cm long, they were detached from the mother plant and fragmented into stem cuttings. Both axillary buds and buds can be used apical (terminal). Cut knots from the same stem, leaving 5-10 mm of stem above and below the buds. The explants are rinsed several times with running water to remove impurities. Immersion in 1% sodium hypochlorite solution and 2-3 drops of Twen 20 (R) for 15 minutes, 70% alcohol immersion, 3 minutes, rinsing. with double-distilled water several times. Remove the explants on a sterilized paper towel to remove the water. Using the instrument from the hood, periodically sterilized in the oven, then each time it is manipulated by the flame, the explant is placed on the propagation medium; 18 x 150 mm sterilized test tubes are used, at a temperature of 1800°C, for 3 hours, in the oven; the test tubes contain the culture medium (5 ml/test tube). Nutrient Murashige-Skoog is used in the cultivation of many plant species commonly used for new species (Table 1). The Murashige-Skoog environment is characterized by a higher nitrogen concentration than that used in previous studies, namely: 5 times higher than that established by Miller (1956), quoted by Badr, 2011, 15 times higher than the one developed by Hildebrandt (1946) and 19 times larger than the one issued by White (1943) (George and Sherrington, 1993/1996, cited by Badr, 2011). MS salts 4.4 g/l, sucrose 30 g/l are used, as gelling agent agar 9 g/l is used, the pH being corrected to 5.8, before autoclaving. Two-distilled water is used, ie distilled water subjected to an autoclave sterilization process. The test tubes containing culture medium are sterilized in an autoclave (20 minutes, 1200°C and a pressure of 1.1-1.2 atmospheres). Chemicals added to the Murashige - Skoog culture medium for the introduction and in vitro multiplication of sweet potatoes (Table 2).

Table 1. Chemical composition of Murashige-Skoog culture medium (mg/l and molar concentrations) (after Robert N. Trigiano, Dennis J. Gray, 2005)

Compounds	Culture medium: Murashige-Skoog	
	mg/l	Molar concentrations
Macronutrients (mg/l or mM)		
NH_4NO_3	1650	20.6
$CaCl_2$	440	3.0
$MgSO_4 \cdot 7H_2O$	370	1.5
KNO_3	1900	18.8
KH_2PO_4	170	1.3
Micronutrients (mg/l or µM)		
H_3BO_3	6.2	100
$CoCl_2 \cdot 6H_2O$	0.025	0.1
$CuSO_4 \cdot 5H_2O$	0.025	0.1
Na_2EDTA	37.3	100
$FeSO_4 \cdot 7H_2O$	27.8	100
$MnSO_4 \cdot H_2O$	16.9	100
KI	0.83	5
$Na_2MoO_4 \cdot 2H_2O$	0.25	1
$ZnSO_4 \cdot 7H_2O$	8.6	30
Organic compounds (mg/l or µM)		
Inositol	100	550
Glycine	2	26.6
Nicotinic acid	0.5	4.1
Pyridoxine HCl	0.5	2.4
Thiamine HCl	0.1	0.3

Table 2. Hormone supplement added to the Murashige-Skoog culture medium

Chemicals	Quantity (g/l)
Ascorbic acid	0.1
Gibberellic acid	0.02
Calcium pantothenate	0.002
Calcium nitrate	0.1
L-arginine	0.1
Putrescine (HCl)	0.02

At the end, each stand is labeled with the name of the variety, the date of initiation and the name of the laboratory that performed the work; after 4-5 weeks of in vitro culture, the plants are visually evaluated to see their growth and development; plants with abnormal or contaminated growth are removed; plants with normal development are used in another in vitro propagation cycle.

Figure 1. Sweet potato shoots

Figure 2. Sterilization of sweet potato shoots

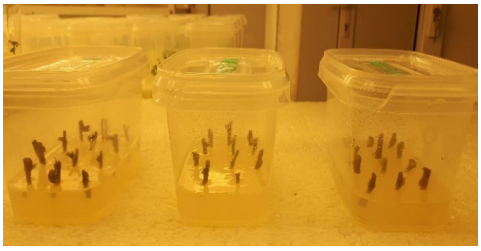

Figure 3. Inoculation of sweet potato cuttings

Figure 4. Incubation of cultures in the growth chamber

RESULTS AND DISCUSSIONS

A photoperiod of 11.5 hours of light/day, or less, stimulates flowering, while 13.5 hours of light/day stops flowering, but does not affect tuber production. In short day conditions and low light intensity, root development is stimulated. The multiplication ratio for the use of stem shoots is estimated at 1:15 to 1:20 (Fuentes and Mwanga, 2011).

During 2020 in the Laboratory of Vegetable Tissue Cultures, NIRDPSB Braşov was initiated a single factor experiment in three repetitions, the analyzed factor being the sweet potato variety, with 6 graduations: KSP 1, DK 19/1, DK 19/2, DCh 19/3, DK 19/4 and DK19/5. Determinations were made on the following parameters: the formation of shoots, the number of leaves, the height of the plantlets and the weight of the fresh root.

Comparing the determinations made weekly (over 6 weeks) for the 6 sweet potato varieties (Figure 5), in terms of shoot formation, it can be said that the DK 19/1 variety performed better than the other varieties during the 5 weeks (from the second week until the end of the study).

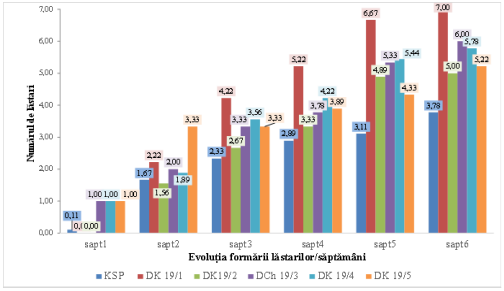

Figure 5. Formation of shoots in the studied varieties / 6 weeks

Regarding the formation of leaves, the same variety of sweet potato DK 19/1 (Figure 6) is highlighted with a high number of leaves from the third week until the end of the determinations (from 4.56 leaves in the third week to 8.00 leaves in the sixth week).

Determinations performed for two other parameters: average plantlet height (cm) and root weight/variety (g) (six weeks after in vitro inoculation show for DCh variety/3 a good development both regarding the average height of the plantlet 5.47 cm and for the weight of the root (0.0826 g) (Figure 7).

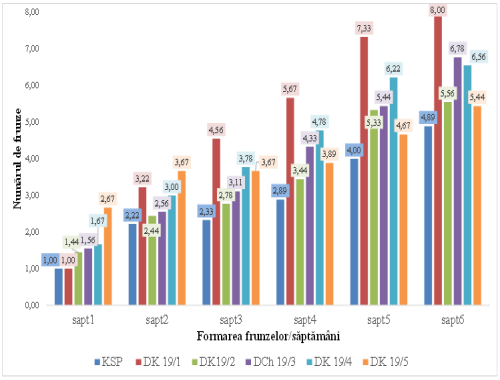

Figure 6. Leaf formation in the studied varieties/6 weeks

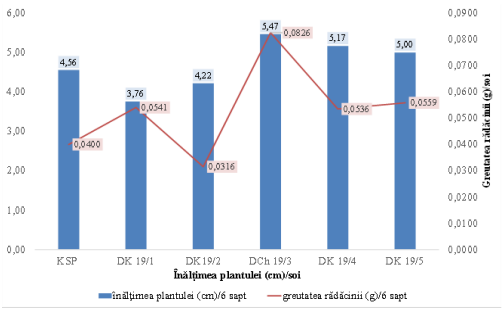

Figure 7. Average plantlet height and root / variety weight, 6 weeks after inoculation

By comparing the differences of the studied variants with the control variant (average values) with LSD of 5, 1 and 0.1%, it appears that the KSP 1 variety registered inferiority in the formation of shoots (at 6 weeks after inoculation), with a significant negative difference of -1.76 shoots. The DK 19/1 variety, although showing a high value of the number of shoots (7.00), does not register a significant difference (Table 3).

Table 3. The influence of sweet potato varieties on the formation of shoots, 6 weeks after inoculation

Variety	The weight of the fresh root (g)	Diff. (g)	Significance
KSP 1	0.040	-0.013	ns
DK 19/1	0.053	0.001	ns
DK19/2	0.033	-0.019	ns
DCh 19/3	0.083	0.031	*
DK 19/4	0.053	0.001	ns
DK 19/5	0.053	0.001	ns
Average (Ct)	0.053	-	-

LSD 5% = 1.67 shoots; 1%= 2.37 shoots; 0.1% = 3.43 shoots.

Regarding the average number of leaves/ plantlet (Table 4), at which the average of the

values of the number of leaves / plantlet (6.24 leaves) was chosen as a control, a positive result was obtained for the sweet potato variety DK 19/1 (at 6 weeks from inoculation), with a significant difference (1.76 leaves).

Table 4. The influence of sweet potato varieties on leaf formation, 6 weeks after inoculation

Variety	Number of leaves	Diff. (no. leaves)	Significance
KSP 1	4.89	-1.35	ns
DK 19/1	8.00	1.76	*
DK19/2	5.55	-0.68	ns
DCh 19/3	6.78	0.55	ns
DK 19/4	6.56	0.32	ns
DK 19/5	5.63	-0.60	ns
Average (Ct)	6.24	-	-

LSD 5% = 1.67 leaves; 1%= 2.37 leaves; 0.5% = 3.43 leaves.

The analysis of plantlet height (Table 5) belonging to the 6 genotypes studied, compared to their average (4.70 cm), shows the DK 19/1 variety with a lower behavior, registering a significant negative difference (-0.94 cm). The Dch 19/3 variety obtained the highest plantlet height (5.47 cm), but with an insignificant difference.

The analysis of the weight (Table 6) of the sweet potato root, freshly sampled, shows the variety DCh 19/3, with a significant positive difference (0.083 g).

Table 5. Influence of sweet potato varieties on plantlet height (cm), 6 weeks after inoculation

Variety	Number of shoots	Diff. (no. Shoots)	Significance
KSP 1	3.78	-1.76	o
DK 19/1	7.00	1.46	ns
DK19/2	5.00	-0.54	ns
DCh 19/3	6.00	0.46	ns
DK 19/4	5.78	0.24	ns
DK 19/5	5.67	0.13	ns
Average (Ct)	5.54	-	-

LSD 5% = 0.028 g; 1%= 0.039 g; 0.1%= 0.057 g.

Table 6. Influence of sweet potato varieties on fresh plantlet root weight (g), 6 weeks after inoculation

Variety	Plantlets height (cm)	Diff. (cm)	Significance
KSP 1	4.56	-0.14	ns
DK 19/1	3.76	-0.94	o
DK19/2	4.22	-0.47	ns
DCh 19/3	5.47	0.77	ns
DK 19/4	5.17	0.47	ns
DK 19/5	5.00	0.31	ns
Average (Ct)	4.70	-	-

LSD 5% = 0.93 cm; 1%= 1.32 cm; 0.1%= 1.90 cm.

CONCLUSIONS

The results presented in this study highlight the possibility of obtaining sweet potato shoots and in laboratory conditions, throughout the year, with minimal costs. By the conventional method from a shoot of 30-35 cm a single rooted plant is obtained, by the micropropagation method 7-8 plantlets can be obtained.

The *in vitro* cultivation technique can be successfully applied in the case of sweet potatoes, using shoots as a source of explants, allowing to obtain in a short time a stock of initially healthy material, which can be used as planting material.

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