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THE EFFECT OF CULTURE SUBSTRATE AND WATER STRESS ON TUBER DEVELOPMENT IN THREE SWEET POTATO CULTIVARS ACCLIMATIZED IN THE GREENHOUSE

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Abstract

Starting from the micropropagation and selection of sweet potato varieties that responded best to in vitro water stress conditions, this study aims to identify the most effective methods of plantlets acclimatization in protected space. The three-factor experiment was carried out by combining the following factors, analyzed in several gradations, as follows: experimental factor A-variety, experimental factor B-substrate, experimental factor C-irrigation. Regarding the influence of the culture substrate and the irrigation variant on the tuberization of the three varieties of sweet potato (CD/1, CD/3, CD/4), the CD/1 genotype is noted on the normal irrigation variant and the use of the culture substrate containing: red peat, black and perlite (2: 1: 1), with a very significant positive difference (5.00) at the average number of tubers/pot. On the same culture substrate, but in a deficient level of irrigation, the CD/3 variety recorded at the average mass and the number of tubers a distinctly negative difference (-131.63 g) and a negative significant difference (-3.25), compared to the control variety (CD/4).

Key words: *acclimatization, culture substrate, protectedspace, sweet potato, water stress.*

INTRODUCTION

The sweet potato crop grows well in marginal soils having a good yield with little demand for fertilizer or water. Although sweet potato is relatively drought tolerant, can provide good ground cover and can be grown without pesticides (Ewell, 1990), its productivity is limited by a large number of biotic and abiotic stresses (Alam et al., 2010). The sweet potato is the target of a large number of pathogenic organisms. This category includes viral, fungal and bacterial diseases and those caused by nematodes. Among the 20 viruses that have been identified, the most widespread sweet potato virus found worldwide is Sweet Potato Feathery Mottle Virus (SPFMV, genus *Potyvirus*, family *Potyviridae*) is widespread in all areas of sweet potato cultivation while the others are located in one or more geographical areas (Moyer & Salazar, 1989; Kreuze et al.,2000). In mixed infections with Sweet Potato Chlorotic Stunt Virus (SPCSV), SPFMV is associated with severe sweet potato virus disease (SPVD), which causes significant damagein many regions of the globe and can lead to a 98% yield loss in production. Viral epidemics of sweet potato were, in many cases, followed by the disappearance of an elite variety (Gibson et al., 1997; Gibson et al., 1998). Other viruses are: Sweet Potato Mild Mottle Virus (SPMMV), Sweet Potato Chlorotic Flecks Virus (SPCFV), Sweet Potato Virus G (SPVG), Sweet Potato Leaf Curl Virus (SPLCV). A regular supply of clean planting material is therefore necessary for sustainable production (Wang & Valkonen, 2008). Biotechnological approaches using different tissue culture techniques can circumvent these problems by producing a large number of plants of different species, through already proven studies. Plant tissue culture is an efficient and reliable method for large-scale production of high-quality, disease-free plants in a short period of time in a small space and providing elite planting material to farmers worldwide (Butt et al., 2015; Mukhopadhyay et

al., 2016; Mukhopadhyay et al., 2019; Behera et al. 2019; Behera, 2022). Rapid propagation is based on the use of the *in vitro* propagation technique in aseptic conditions, in special culture vessels, the product obtained is named with the prefix "micro" (microplants, microtubers, microtuberization). The nutritional substrate consists of microelements, macroelements and vitamins considered as basic culture medium (Murashige & Skoog, 1962) with the addition of growth hormones depending on the culture phase (initiation, multiplication, tuberization) (Chiru & Antofie, 1997). Addition of cysteine to the culture medium in the pre-acclimation phase can further support the success of *de novo* shoot development for acclimation (Sand & Antofie, 2022). Microplants require special growing methods and cannot be directly planted in the field (Wiersema et al., 1987). Plantlets are very small plants obtained in completely sterile conditions and transplants are produced by *in vivo* planting of seedlings in non-sterile conditions (Struik & Wiersema, 1999), through a protected space (greenhouse, tunnel) preferably insect-proof, to avoid contamination with aphids where the seedlings are acclimatized. The success of feeding depends on a series of factors, among which we mention: the state of health and the size at transplantation, the choice of the culture substrate, the prevention of diseases and the control of some physical parameters. The choice of the substrate, which must be of good quality, with good permeability, well aerated with a suitable pH (Ourèye, 2013).

MATERIALS AND METHODS

Plant material

The origin of the plant material used consists of glass plates regenerated from shoots from three sweet potato (*Ipomoea batatas*) genotypes: CD/1, CD/3 and CD/4. The three varieties were obtained from the Research-Development Station for Agricultural Plants on Sands Dabuleni, and the country of origin of the varieties is Korea. Some of the morphological and production characteristics of these varieties grown in the experimental fields from Dabuleni are presented in Table 1.

Table 1. Characterization of the varieties used in the study

Genotype	Flesh color	Tuber number/ plant	Tuber weight/ plant	Tuber average weight	Production t/ha
DK 19/1	White	8	1673	210	32
DCh 19/3	Purple	5	767	105	25
DK 19/4	Yellow	5.33	1233	232	36

In vitro culture condition

For culture initiation and *in vitro* multiplication, the culture medium Murashige & Skoog (1962) is used, to which growth regulators (auxins), agar, sucrose and the antimicrobial agent PPM (Plant Preservation Mixture) are added. Sterilization of the culture medium is carried out at 121°C and a pressure of 1.1-1.2 atmospheres. The explants are inoculated inside the hood with laminar air flow. Incubation is carried out in the growth chamber, at a temperature of 25°C, with a light intensity of 3000 lux and a photoperiod of 16 hours of light, alternating with 8 hours of darkness.

The *in vitro* evolution of these varieties had superior results in terms of shoot proliferation and elongation, rooting and a very good tolerance of induced water stress. After eight weeks, the glass plants were prepared for their transfer to a protected space.

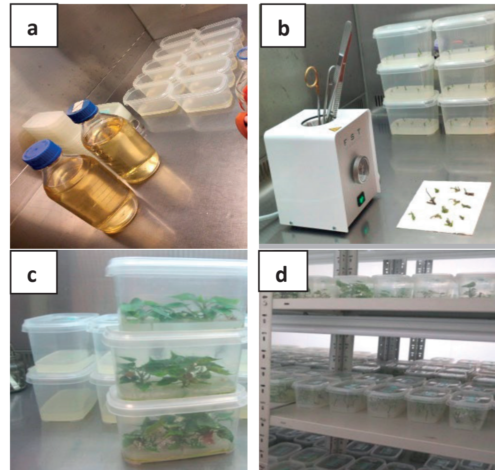


Figure 1. Distribution of the nutrient medium in the culture containers (a); Initiation of sweet potato *in vitro* culture (b); Multiplication by successive subcultures of varieties (c); Incubation of the culture containers in the growth chamber (d)

Experimental variants

The experiment was carried out in the greenhouse of the research laboratory for tissue culture from INCDCSZ Brasov. On the work tables in the greenhouse, the pots containing perlite (Pe) and substrate (T) containing: red peat, black peat and perlite (2:1:1), in which the three-threes were planted sweet potato varieties. The substrates used must be sterilized, permeable in order not to retain excess water, have a suitable pH. Irrigation of plants developed on the T substrate as well as on the substrate on according to their necessity, but there was also conditional irrigation on the same substrats (T stress, Pe stress). The temperature in the greenhouse oscillates from 15-20°C (in the morning) and can reach up to 40-43°C (in the afternoon) and the humidity is between values of 70-80% and 92.9%, depending on the registered temperature. Maintaining plants in an optimal phytosanitary state required the application of prophylactic treatments with fungicides and insecticides. For the growth and development of plants, both incorporable fertilizers were used in the culture substrate (Osmocote Exact) and foliage (Cropmax, Razormin, Agroleaf Power). All culture conditions have been controlled (temperature, humidity, bacterial, fungal, nematodes aphids). After about 120 days after planting the tubers were harvested and an assessment of the number of tubers and their mass, on each variety, type of culture and irrigation variant were made.

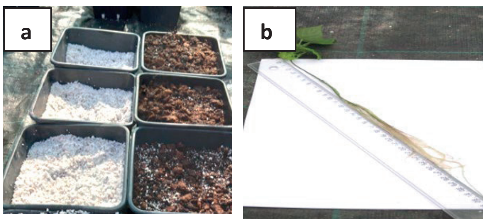


Figure 2. Studied substance (a); The length of the root of a sweet potato plant (b)

Data analysis

The analyzed factors were the variety (A) with three gradations, the substrate (B) with two gradations and the irrigation (C) with two gradations. The statistical analysis was carried out by the Duncan test and by the analysis of variation.

RESULTS AND DISCUSSIONS

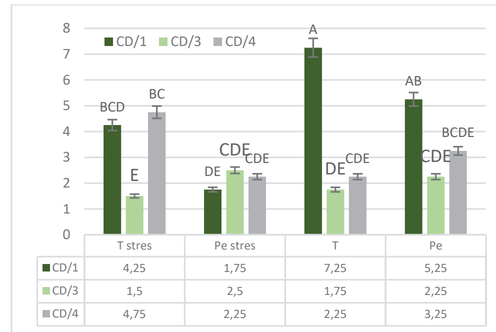


Figure 3. Average number of tubers/plant obtained in the experiment

*The means in the column inside the table followed by different letters are significant according to Duncan's MR test ($p < 0.05$).
** SX = 0.78 DS value: 2.27-2.67.

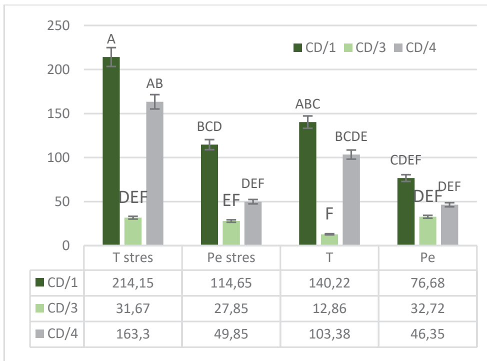
From the analysis of the average number of tubers obtained, the best results were recorded for the CD/1 variety (7.25), on the T culture substrate with better results than the control, and the CD/3 genotype shows a low productivity (1.5) on the T stress culture substrate, compared to the pots in this control variety CD/1.

From the data entered in Table 2 where the results of the influence between the variety and the culture substrate are presented, it follows that the variety CD/3 planted on the culture substrate T stress and Pe stress significantly negative differences (-3.25) compared to the control variety CD/4.The variety CD/1 surpassed the control variety, presenting very significant positive differences (5.00) in the culture substrate T, on the normal irrigation variant. Insignificant values were recorded for the other types of variants.

Table 2. The influence of the sweet potato variety in obtaining tubers for each culture substrate

Variety	Culture substrate	Avg. nr of tubers	%	Diff.	Sigf.
CD/4 (Ct)	T stress	4.75	100.0	0.00	Ct
CD/1	T stress	4.25	89.5	-0.50	ns
CD/3	T stress	1.50	31.6	-3.25	0
CD/4 (Ct)	Pe stres	2.25	100.0	0.00	Ct
CD/1	Pe stress	1.75	77.8	-0.50	ns
CD/3	Pe stress	2.50	111.1	0.25	ns
CD/4 (Ct)	T	2.25	100.0	0.00	Ct
CD/1	T	7.25	322.2	5.00	***
CD/3	T	1.75	77.8	-0.50	ns
CD/4 (Ct)	Pe	3.25	100.0	0.00	Ct
CD/1	Pe	5.25	161.5	2.00	ns
CD/3	Pe	2.25	69.2	-1.00	ns

*LSD 5%=2.45; 1%=3.43; 0.1%=4.90



	T stres	Pe stres	T	Pe
CD/1	214,15	114,65	140,22	76,68
CD/3	31,67	27,85	12,86	32,72
CD/4	163,3	49,85	103,38	46,35

Figure 4. Mass (g) of harvested tubers/plant

* The means in the column inside the table followed by different letters are significant according to Duncan's MR test ($p < 0.05$).
** SX = 25.99 DS value: 75.51-88.77.

In the case of the analysis of the weight of the tubers obtained per plant, it can be seen in Figure 4 that the three varieties responded better to the T stress variant the total weight oscillating between 214.15-163.3-31.67 g compared to the T variant where the total weight of the tubers is lower, respectively 140.22-103.38-12.86 g, in the varieties analyzed (CD/1-CD/4-CD/3). In the Pe stress variant, only two varieties CD/1 and CD/4 recorded a higher weight of the harvested tubers, 114.65-49.85g, compared to the Pe variant 76.68-46.35g

Table 3. The average mass of tubers influenced by the sweet potato variety and the culture substrate

Variety	Culture substrate	Avg. nr of tubers	%	Diff.	Signf.
CD/4	T stress	163.30	100.0	0.00	Ct
CD/1	T stress	214.15	131.1	50.85	ns
CD/3	T stress	31.68	19.4	-131.63	00
CD/4	Pe stresss	49.85	100.0	0.00	Ct
CD/1	Pe stress	114.65	230.0	64.80	ns
CD/3	Pe stress	27.85	55.9	-22.00	ns
CD/4	T	103.38	100.0	0.00	Ct
CD/1	T	140.23	135.6	36.85	ns
CD/3	T	12.86	12.4	-90.52	0
CD/4	Pe	46.35	100.0	0.00	Ct
CD/1	Pe	76.68	165.4	30.33	ns
CD/3	Pe	32.72	70.6	-13.63	ns

*LSD 5%=85.42; 1%=120.69; 0.1%=174.18.

From the data presented in Table 3, where the results of the influence on each substrate of the same type are showedit results that the variety CD/3 planted on the substrate (T stress) has a distinctly significant negative difference (-131.63 g), and on the culture substrate variant T, the same variety registers a negative difference (-90.52 g) compared to the control variety CD/4.

CONCLUSIONS

Starting from the micropropagation method of some sweet potato genotypes to the acclimatization in protected spaces under conditions of water stress, this study constitutes a beginning in order to produce a planting material with high biological value and the identification of varieties with resistance to drought.

At the same time, the use of two culture substrates was experimented: a. T which contains in the composition: red peat, black peat and perlite (2:1:1); b. Pe on which it contains only perlite (industrial substrate). The acclimatization method was largely conditioned by the choice of the substrate for the transfer of the sweet potato vitroplants into pots, but also by the control of the factors specific to the *ex vitro* environment, the survival percentage of the seedlings was 98%.

The data regarding the evaluation of the weight of tubers obtained per plant show that the three varieties studied, CD/1, CD/3 and CD/4, responded better to the T stress variant compared to the T variant where the total weight of the tubers/plant was more small, there were distinctly significant negative differences in the variety CD/3 (-131.63 g) compared to the control variety CD/4. In the Pe stress variant, only two varieties CD/1 and CD/4 recorded a higher weight of the harvested tubers, compared to the Pe variant, but with insignificant differences.

Acclimatization of sweet potato seedlings in protected areas has been successful, developing and producing tubers that can be used as efficiently as acclimatized material in field culture.

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