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STUDY ON THE EFFECT OF ACTIVATED CHARCOAL IN STIMULATING *IN VITRO* RHIZOGENESIS OF SWEET POTATO PLANTLETS

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Abstract

Activated charcoal is recommended to be added to nutrient media, used in crops of plant tissues, to the growth and development of some types of explants, but also its role of absorbing toxic compounds present in aseptic environments, which cause necrosis of inoculated tissues. . The main purpose of this study was to assess the influence of three concentrations of activated carbon (0.5 g/l, 1 g/l, 2 g/l) added to the MS culture medium, on the formation and root development of two varieties of sweet potato (DK 19/1 and DK 19/5).). A bifactorial experience was initiated, the analyzed factors being: variety and culture environment. Determinations were made on the following parameters: root length, fresh root weight and dry root weight. Based on the obtained results, an optimal rooting was observed, without callus formation, ensuring a higher percentage of capacity at acclimatization of sweet potato seedlings from in vitro to in vivo.

Key words*: sweet potato, plant tissue cultures, activated charcoal, rhizogenesis.*

INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam.) is a dicotyledonous vegetable belonging to the family Convolvulaceae. It is the seventh most produced crop worldwide after wheat, rice, maize, potato, barley, and cassava, and the fifth in developing countries (Jung, J.K., 2011). It is recognized as a major tuber crop (Ghasemzadeh et al., 2016) and is ranked third most important tuber after potato (*Solanum tuberosum*) and cassava (*Manihot esculentum*) (FAO, 2003). Sweet potato tubers are rich in dietary fiber, minerals, vitamins, and antioxidants such as phenolic compounds (Lebot et al., 2016; Tang et al., 2015; Teow et al., 2007). Sweet potato has recently been reevaluated as a valuable medicinal plant with anticancer, antidiabetic, and anti-inflammatory properties (Mohanraj and Sivasankar, 2014; Wang et al., 2016). The rhizoderm of the thickened roots (the tuber) has different colors: white, cream, yellow, orange, pink, red to purple, and the pulp has shades of ivory, orange, or purple-lilac (Abubakar et al., 2010; Aywa et al., 2013). Sweet potato leaves also have different shapes and colors, depending on the variety. Regarding the color of the leaves, it is usually green, yellowish-green, or with purple pigmentation. Sweetpotato leaf contains high concentrations of polyphenolics, when compared with the major commercial vegetables such as spinach, broccoli, cabbage, lettuce, and so forth. Therefore, with its tuberous roots, stems and leaves that can be fully consumed, the sweet potato is a crop that can solve food, energy, resource and environmental problems in the 21st century (Islam S., 2006). Sweet potato has received increased attention because the crop can adapt to a wide range of environmental conditions and grow on marginal areas with poor soils of limited fertility and inadequate moisture (Bioethics, 2004). For these reasons, the sweet potato crop is attractive not only to farmers but also to ecologists and economists, who are interested in developing sustainable food production systems in the tropics (Bovell-Benjamin, 2007; Loebenstein, 2009; Mukherjee et al., 2012; Iese et al., 2018). It is a new culture, which through the expansion of cultivation in our country will bring an

important contribution to the diversification of vegetable products. The experimental model carried out by us in this work aims to take cuttings obtained under laboratory conditions from tuberous root shoots of some sweet potato (*Ipomoea batatas*) genotypes, with the aim of defining the optimal factors for establishing an *in vitro* culture and making some preliminary studies, regarding the development of the root system of the seedlings obtained for their good adaptability to the specific conditions of the *ex vitro* environment.

MATERIALS AND METHODS

The plant material used consists of shoots from two varieties of sweet potato provided by the Research-Development Station for Plant Culture on Sands Dăbuleni, Dolj, within the study initiated on the basis of the ADER Sectoral Project 7.3.4. The two Korean varieties of sweet potato were studied: DK 19/1 and DK 19/5. Dk/1 is a white-fleshed sweet potato genotype with a vegetation period of 120 days with the following characteristics:

- rope length - 1 m;
- total weight - 430 g;
- average number of tubers/plant - 8;
- weight of tubers/plant - 1673 g;
- tuber weight -212 g;
- tuber length - 16 cm;
- tuber diameter - 7.43 cm

Genotype DK 19/5 has tubers with butter-colored pulp with pink streaks, which turns yellow when boiled, with a vegetation period of 120 days and the following characteristics:

- rope length - 0.93 m;
- total weight - 433 g;
- average number of tubers/plant - 8;
- weight of tubers/plant - 1000 g;
- tuber weight - 125 g;
- tuber length 18 cm;
- tuber diameter - 4.86 cm;
- the plants show anthocyanin coloring on the leaves, their chlorophyll content being lower (Extract from the Technical Report/2021 - ADER Project 7.3.4).

To obtain the shoots, it was necessary to prepare the sweet potato tubers and plant them, having ensured favorable conditions of temperature, light and humidity. Watering and fertilization were carried out as necessary throughout the emergence and growth period. The pre-sprouting period was 4 weeks, after which the tuberous root buds have began to elongate, forming shoots with a length of approximately 20-25 cm. This process continued for a period of 4 months, the shoots being periodically detached from the mother plant (Figure 1) and fragmented into stem cuttings, used as a source of explants for the initiation of *in vitro* cultures (Figure 2).

Figure 1. The donor plant, source of the shoots

Figure 2. Stem cuttings

Put the cut knots of the same variety in a sterilized glass container and disinfect them with a 25% sodium hypochlorite solution and 2-3 drops of Twen 20 (R) for 15 minutes (Figure 3), operation that took place in the

hood. Then immersion in 70% alcohol, 3 minutes rinsing with double-distilled water several times, after which the explants are removed on a sterilized paper towel to remove the water. These segments were then inoculated, in test tubes containing the culture medium (5 ml/test tube) for the *in vitro* multiplication of the sweet potato (Table 1) and the amounts of activated carbon produced by Duchefa that was directly solubilized in the culture medium. In order to follow the mode of action of charcoal on the sweet potato root, a culture medium was prepared in which three concentrations of activated charcoal were added:

Culture medium: code MS (control medium);

- MS medium with an activated carbon concentration of 0.5 g/l: code MS+C1;
- MS medium with an activated carbon concentration of 1 g/l: code MS+C2;
- MS medium with an activated carbon concentration of 2 g/l: code MS+C3.

Table 1. Composition of the nutrient medium used for the inoculation of sweet potato plants

Chemical components	**Quantity /1 L mediu**
MS culture medium salts *	4.4 (g)
Ascorbic acid	0.1 (g)
Gibberellic acid	0.02 (g)
L-arginine	0.1 (g)
Calcium nitrate	0.1 (g)
Calcium pantothenate	0.002 (g)
Putrescine HCl	0.02 (g)
α-Naphthylacetic acid (NAA)	0.5 (mg)
Sucrose	30 (g)
Agar	8.5 (g)
PPM	3 (ml)

*MS – Murashige & Skoog, 1962

They were sterilized in an autoclave (20 minutes, 120^{0}C and pressure of 1.1-1.2 atmospheres). At the end, the samples (test tubes with explants) are obturated with tinfoil. Insert the racks with test tubes into the growth chamber, at a temperature of 25^{0}C±2^{0}C, with a photoperiod of 16 hours of light and 8 hours of darkness.

Figure 3. Stem cuttings disinfection

RESULTS AND DISCUSSIONS

In the Plant Tissue Culture Laboratory, NIRDPSB Brasov, a bifactorial experiment was initiated, the analyzed factors being: the variety and the culture medium. Determinations were made on the following parameters: root length, fresh root weight and dry root weight. To obtain the results of the dry root mass, the freshly taken roots of the seedlings were kept in a room, in the dark, with a temperature of 21^{0}C, for 48 hours. After 10 weeks after inoculation, the obtained results were analyzed. It is recommended in the literature for its role in absorbing toxic compounds excreted by *in vitro* cultured tissues in the culture medium and causing necrosis of tissues inoculated on aseptic media. Activated charcoal is often used in plant tissue culture to improve cell division and development. It stimulates nitrogen uptake by shoots, the appearance of the nutrient medium is dark, thus promoting rooting *in vitro* (Sharma et al., 2012). Several authors have reported its beneficial effect as a culture medium supplement. This includes absorption of the phenolic complex (Pan and Staden, 1998); it is stimulatory for root formation (George and Ravishankar, 1997); rhizome development (Kim and Lee, 1992); improving seedling development (Choi and Chung, 1989); and absorption of toxic substances present in the culture medium (Fridborg and Eriksson, 1978).

In the analysis of the root length belonging to the two studied varieties, it is observed that the type of culture medium influences the growth and development process of the root. The highest average of 11.8 (cm) is found for the variety DK 19/1, on the culture medium MS+C2.

Figure 4 and Table 2 shows the results regarding the influence of the four culture medium on the growth in length of the roots of sweet potato seedlings. The best growth was recorded in the DK 19/1 variety (11.8 cm), on the MS+C2 growth medium variant.

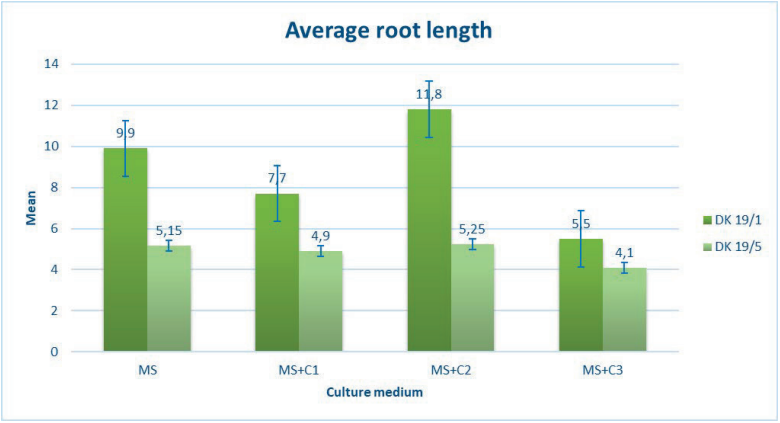

Figure 4. The influence of the culture medium with the three concentrations of activated carbon on root length

Table 2. The increase in length of the roots depending on the culture medium

Variety	Culture medium	Root length (cm)	%	Difference (cm)	Signification
DK 19/1	MS	9.90	100.0	0.00	Ct
	MS+C1	7.70	77.8	-2.20	-
	MS+C2	11.80	119.2	1.90	-
	MS+C3	5.50	55.6	-4.40	0
DK 19/5	MS	5.15	100.0	0.00	Ct
	MS+C1	4.90	95.1	-0.25	-
	MS+C2	5.25	101.9	0.10	-
	MS+C3	4.10	79.6	-1.05	-

DL 5% = 2.49 cm; 1% = 3.36 cm; 0.1% = 4.48 cm

In Figure 5, the determinations made for this studied parameter, 10 weeks after *in vitro* inoculation, show for the DK 19/1 variety, on MS+C2 culture medium, the highest average with a weight of 0.22 (g) .

In Figure 6, the determinations made for this studied parameter, 10 weeks after *in vitro* inoculation, show for the DK 19/1 variety, on the MS+C2 culture medium, the highest average with a weight of 0.22 (g).

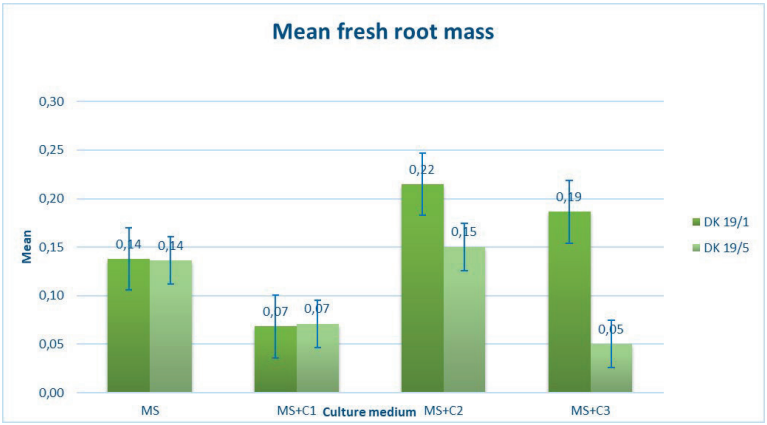

Figure 5. The influence of culture media on fresh root mass

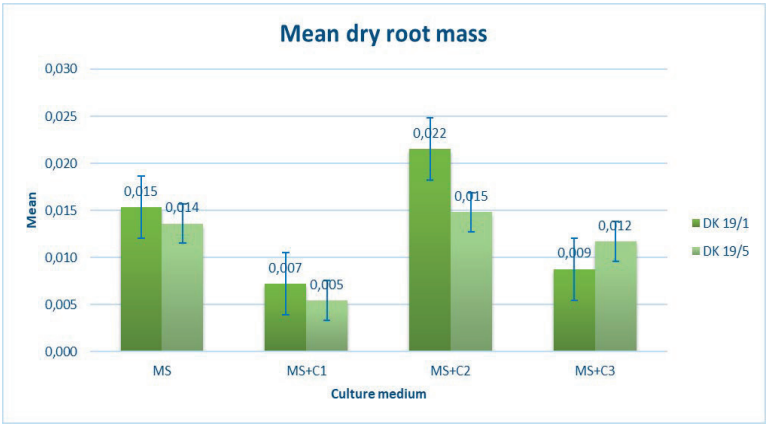

Figure 6. The influence of culture media on dry root mass

CONCLUSIONS

From the analysis of the presented results, it can be deduced that the culture environment influenced the growth and development of the roots in the two analyzed varieties. Even if the obtained values are different, they are not statistically guaranteed differences. Compared to the control sample, the best results were observed for the culture medium MSC_2 (active carbon concentration of 1 g/l), for the three parameters studied, the differentiation being factor 1, the sweet potato variety DK 19/1.

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