STUDY ON "IN VITRO" MICROTUBERIZATION OF POTATO (*SOLANUM TUBEROSUM* L.) GENOTYPES DERIVED FROM TRUE POTATO SEED

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Abstract. Within this study a protocol was developed to obtain "in vitro" microtubers starting from botanical potato seed. The study was carried out between June and September 2023, in the Research Laboratory for Plant Tissue Cultures belonging to National Institute of Research and Development for Potato and Sugar Beet (NIRDPSB) Brasov. For the initiation of microtuberization, it was started from healthy (virus-free) potato plantlets, used as a source of uninodal explants. Fragments approximately 1.5 cm in size, containing a node, associated leaf and portions of neighboring internodes, were inoculated on Murashige-Skoog culture medium supplemented with sucrose, growth regulators and agar, specific to each stage of the microtuberization process. 15 explants were inoculated in each culture vessel, which were subsequently incubated in the climatic chamber, at a temperature of 20±2 °C, a photoperiod of 16 hours of light and 8 hours of darkness, for 4 weeks. When the potato microplants have reached a height of about 7-8 cm, the tuberization medium, liquid (100 ml/jar) is added to the culture vessels and then the cultures are incubated in the growth chamber, at 17±2 °C, in the dark, for a period of 3 months (permanent immersion method). Under these conditions, after about 120 days potato microtubers were formed and evaluated in terms of number, weight and calibration classes. The biological material tested for microtuberization capacity was represented by 2 potato genotypes derived from true seed (GIL 19-03-07, GIL 19-03-29) and 3 potato varieties created at NIRDPSB Brasov (Ervant, Castrum, Marvis). The best results in terms of average weight/jar of potato microtubers (10.89 g), percentage of microtubers > 10 mm (23%) and rate of microtubers in the calibration class 7-10 mm (41%) were recorded at the genotype GIL 19-03-29. The highest values in terms of the average number of microtubers/jar (26.8) was recorded at the genotype GIL 19-03-07.

Keywords: potato, plant tissue culture, true potato seed, microtuberization, permanent immersion

INTRODUCTION

The potato (*Solanum tuberosum* L.), along with wheat and rice, plays an essential role in the human diet. The main problem in potato production is the availability of good quality planting material. Viruses are the major cause of seed potato degeneration and lead to significant yield losses [20]. Countries around the world have increased their production annually due to its nutritional value and the possibility of obtaining high yields per unit area in a short cultivation cycle [12, 6]. However, the sustainability of potato crops depends in part on the use and availability of high phytosanitary quality starting material [23, 6]. Potato yields and storage capacity tend to decrease with each subsequent planting. Globally, losses caused by viral infections amount to 90 million tons, and production decreases by 40-50%;

losses during storage can reach 15-20% [1, 14, 13]. The main methods of multiplication of material used in the production of seed potatoes are: improvement of seed material by tissue culture and selection of virus-free lines; clonal propagation of microplants in the laboratory; obtaining microtubers in "in vitro" conditions, producing disease-free minitubers in a protected space or in hydroponic/aeroponic systems; selection of healthy clones in the field based on visual assessment and in the laboratory by specific testing methods for viral and bacterial infections [13].

Microtubers are small potato tubers obtained through tissue culture. They can be kept for a few months and then planted in protected areas or, more rarely, directly in the field. The **microtubers** obtained "in vitro" are used in the production of seed potatoes. Moreover, microtubers can be used for germplasm conservation [16]. Plant tissue culture techniques are used worldwide to produce virus-free seed potatoes (Prebase category) and by means of microtubers. Microtubers are planted in a protected space to obtain minitubers. They enter in the production chain of certified potato seed to be sold to farmers [20]. Although tubers obtained "in vitro" (microtubers) were described in the middle of the 20th century [5, 17] there is a long list of factors influencing microplant development, induction and growth of microtubers. Some of these variables interact with each other and with genotype. The most critical decisions involve the choice of explants for culture initiation, microtuberization-inducing agents, including media and culture media components [7].

This study was carried out to identify a protocol for obtaining "in vitro" microtubers for potato genotypes derived from true seed.

MATERIAL AND METHOD

For this study, two potato genotypes obtained from botanical seed (GIL 19-03-07, GIL 19-03-29) and three varieties (Marvis, Castrum, Ervant) were chosen. The genotypes GIL 19-03-07 and GIL 19-03-29 obtained the best results in terms of tolerance to induced water stress "in vitro", and the three varieties are highly appreciated by potato growers and consumers due to their high capacity production and outstanding culinary qualities. For the initiation of cultures, started from meristems in the case of the Marvis, Castrum and Ervant varieties, and for the two genotypes, true potato seed was used. Plants regenerated from seeds and meristems respectively were tested for PVS, PVX, PVA, PLRV, PVY and PVM viruses. The results obtained from ELISA testing showed that the plants are healthy. For micropropagation we used explants about 1.5 cm long containing an axillary bud with the associated leaf and half of the neighboring internodes. 15 explants were inoculated in each culture vessel. Uninodal fragments were inoculated on Murashige and Skoog (MS) medium [19] supplemented with 20 g/L sucrose, 0.5 mg/L NAA, and 9 g/L agar, for solidification (Table 1).

Table 1

Composition of the propagation and tuberization medium

Micropropagation medium		Tuberization medium	
Components	**Amount/1 L medium**	**Components**	**Amount/1 L medium**
MS medium salts (g)	4.4	MS medium salts (g)	2.2
Sucrose (g)	20	Sucrose (g)	80

Agar (g)	9	Kinetin (ml)	1.25
NAA (mg)	0.5	Coumarin (g)	0.05
PPM (ml)	3	PPM (ml)	3

RESULTS AND DISCUSSIONS

Microtubercles number

After approximately 120 days, during which the potato plantlets were kept in the climatic chamber, in the dark and under conditions of permanent immersion in liquid tuberization medium, the microtubers formed in the culture vessels were harvested (Figure 1) and evaluated in terms of number, weight and size fraction.

Figure 1. Harvesting of microtubers obtained after approximately 120 days (GIL 19-03-07)

Table 2

The influence of the genotype on the average number of microtubers/jar

Genotype	Average	Difference	Signification
MARVIS	14.67	6.00	ns
ERVANT	17.67	9.00	ns
GIL 19-03-07	26.8	18.13	*
GIL 19-03-29	26.4	17.73	*
CASTRUM (Ct)	8.67	-	-

LSD 5%: 13.58; LSD 1%: 22.47; LSD 0.1%: 42.06

The potato genotypes behaved differently in terms of "in vitro"microtuberization capacity. Regarding the average number of microtubers/jar, the results highlight the performances of the two potato genotypes from botanical seed. Thus, GIL 19-03-07 obtained the maximum number of microtubers/jar (26.8), followed by GIL 19-03-29 (26.4), while the Castrum variety recorded the lowest number of microtubers/jar (8.67). Compared to the control, the differences are significantly positive (Table 2), both for the genotype GIL 19-03-07 (18.13) and for GIL 19-03-29 (17.73).

Microtuber weight

Regarding the weight of microtubers, the highest values were also recorded for potato genotypes from botanical seed, where the average weight of microtubers/jar was 10.89 g for GIL 19-03-29 and 7.13 g, respectively for GIL 03-

19-07. The lowest values in terms of the average weight of microtubers/jar was recorded at the Castrum variety (1.52 g). Compared to the control, GIL 19-03-29 registered a distinctly significant positive difference (9.37 g), and GIL 19-03-07 a significant positive difference (5.61 g), while in the varieties Marvis and Ervant the differences (2.62 g and 2.52 g, respectively) were insignificant (Table 3).

Table 3

Average weight of microtubers/jar depending on the genotype

Genotype	Average	Difference	Signification
MARVIS	4.14	2.62	ns
ERVANT	4.04	2.52	ns
GIL 19-03-07	7.13	5.61	*
GIL 19-03-29	10.89	9.37	**
CASTRUM (Ct)	1.52	-	-

LSD 5%: 3.41 g; LSD 1%: 5.64 g; LSD 0.1%: 10.55 g

Calibration of microtubercles by size fractions

Along with the number and weight of microtubers, their caliber was also determined (Figure 2). The size of the microtubers is an important aspect, because they will be planted in a protected space (greenhouse or tunnel "insect-proof") to obtain the minitubers. The caliber of microtubers obtained "in vitro" is very important in the stage of their transfer from "in vitro" conditions to protected spaces. The larger the size of the microtubers, the more vigorous the potato plants will be and the greater the production of minitubers.

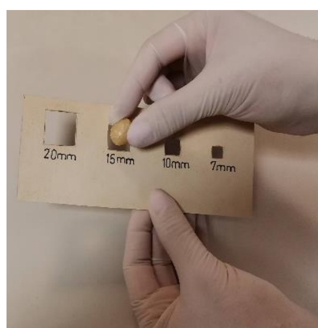

Figure 2. Distribution of microtubers on different calibration classes (GIL 19-03-07)

Following the percentage distribution of microtubercles by calibration classes, it can be seen that the obtained microtubers mainly fell into the size fraction < 7 mm, with the exception of the genotype GIL 19-03-29, which had the highest percentage of microtubercles from the 7-10 mm size fraction (Figure 3).

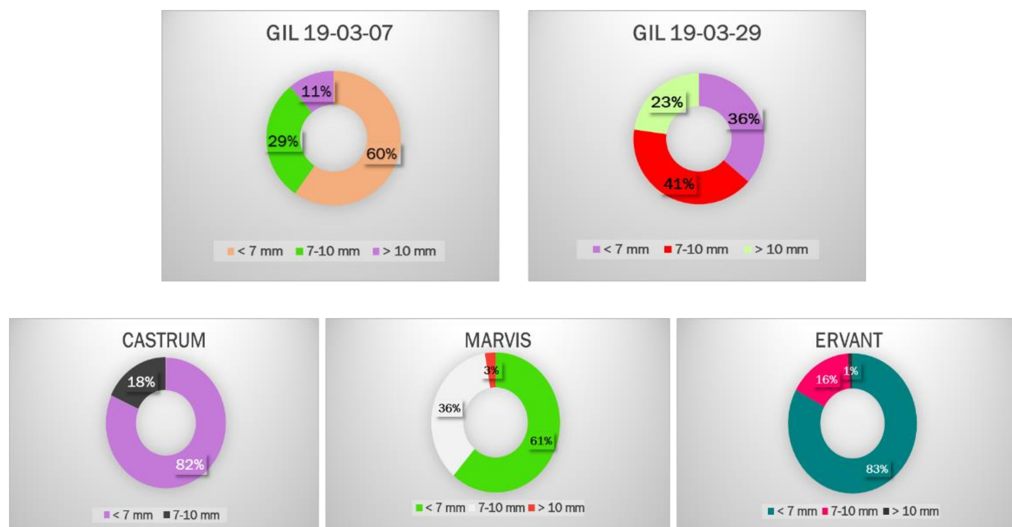

Figure 3. Distribution percentage of microtubers by calibration classes, depending on the genotype

Also, the percentage of microtubers in the size fraction > 10 mm had higher values in the genotypes derived from botanical seed (11 - 23%), compared to the studied varieties, where the percentage of microtubers > 10 mm was 1 - 3%.

Figure 4. Obtaining potato minitubers in protected space starting from botanical seed

After the germination period, the microtubers obtained "in vitro" can be planted in a protected space, where at the end of the vegetation period, potato minitubers are obtained. The scheme above shows the method of obtaining potato minitubers in a protected space using the botanical potato seed as initial material and having as an intermediate stage the "in vitro" production of seedlings and microtubers (Figure 4).

CONCLUSIONS

Potato cultivars have potentially different ”in vitro” microtuberization capacity [4, 9, 18, 22] aspect confirmed and the results obtained in this study. Microtuberization is influenced by several factors, including genotype, explant type, culture medium, and particular cultivation conditions like sucrose, light, temperature [15]. Scientific opinions on the influence of growth regulators on microtuberization in potato are different. The presence of kinetin in the culture medium determined a redistribution of endogenous cytokinins in tissues, in favor of underground organs, stimulating the formation of microtubers [3], but other results suggest a fundamental role of this cytokinin in cell division, carbohydrate metabolism and other mechanisms that require the presence of endogenous cytokinins [2].

The role of kinetin on tuber initiation has also been demonstrated, thus increasing their number [21, 8]. The results show that phytohormones can produce strong effects on tuberization parameters, but these effects depend considerably on sucrose concentration and genotype [21].

To improve the number and size of microtubers produced "in vitro", it would be useful to carry out additional tests, in which the independent effects and interactions of several factors, including genotype, explants, photoperiod, temperature, sucrose concentration, presence or absence of growth regulators. The variability of the results due to the interaction between the genotype and the particular "in vitro" cultivation conditions indicates the need to develop specific protocols for each potato genotype in order to improve the microtubers production techniques as a source of initial material with superior phytosanitary quality [11, 10, 22, 8].

The results obtained in this study demonstrate that the permanent immersion method allows obtaining a large number of microtubers in "in vitro" conditions. However, further research is needed to optimize the cultivation conditions and the composition of the tuberization medium in order to obtain better results in terms of weight and size of microtubers.

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