SOME POSSIBILITIES OF VEGETATIVE PROPAGATION OF STRAWBERRY CULTIVARS *IN VITRO*

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Abstract
This work presents the production technology of five strawberry cultivars by micropropagation. Starting materials were taken in May or June from the stolons that were in the air and meristems of 0.5mm were isolated in the laboratory. The isolated strawberry meristems were cultivated in a medium containing macro and micro elements and phytohormones in different concentrations. The highest percentage of initiated culture was recorded by the cultivar Senga Sengana (81.48%) and the lowest by the cultivar Marmolada (48.00%). Optimal hormone balance for initiating culture strawberries was 1.0 mg / l BAP (Benzylaminopurine), 0,1 mg / l IBA (indole-3 -butyric acid) and 0.1 mg / l GA_3 (gibberellic acid). After 50 days of cultivation in the culture, the formed shoots were transferred to medium for multiplication. The multiplication of isolated buds was achieved on the medium with phytohormones BAP and IBA. The highest multiplication index was recorded by the cultivar Senga Sengana (8.77) and the lowest by the cultivar Marmolada (5.42). When planlets reached a height of 10-12mm they were transferred to the rooting medium. The optimal concentration of phytohormones IBA for rooting of the strawberry cultivars was about 5 mg / l. Number of roots ranged from 5.09 for the cultivar Idea to 6.11 for the cultivar Senga Sengana. After two months the plants with developed roots were transplanted into peat briquettes. Adjustment by external conditions was conducted in greenhouses under a mist system. The reception was submitted well by the planted strawberries (90%). The results presented in this paper indicate that micropropagation can speed up the process of getting healthy and HIGH quality planting material of strawberries. The procedure for obtaining strawberries by micropropagation can be accelerated by proper selection of the combination and concentrations of phytohormones.

Keywords: micropopagation, multiplication, phytohormones, adjustment, greenhouse.

Introduction

The district of Jablanica is known for the production of berry fruits. The most represented berry fruits are raspberries, strawberries and blackberries. By building the processing capacities of the cold storages in Leskovac, Vucje, Bojnik and Lebane, the task for improving the production of berry fruit was set, with a special emphasis on continuous improvement of technological usability and quality of the fruits. Special emphasis is given on the biological and pomological properties of strawberry cultivars for a particular region, as well as the use of varietally pure and healthy planting material. In practice, there are several ways of producing strawberry planting material. Regardless of the way, planting material should be high quality, healthy and monovarietal. The condition of planting material in the Republic of Serbia is in an unenviable situation. Strawberry producers, for economic reasons, mostly use planting material from production plants. On that occasion, rosettes are often mixed with seedlings, and very often the real name of the cultivar or degree of infection diseases and pests is unknown, contributing mostly to the low yield and poor quality fruit. In order to improve this situation, it is necessary to introduce newer and more fertile cultivars, and for the production of strawberry runner plants, for obtaining healthy planting material, the micropropagation methods should be used. Micropropagation of plants implies the production of plants from the cells of the

meristematic tissue. This vegetative propagation is provided only under laboratory conditions. Micropropagation is perfomed on a special medium, which contains a larger number of nutrients, especially minerals, sucrose and growth hormones. Modern production of strawberry runner plants consists of the production of selected and virus free planting material. The strawberries can be freed from the virus by the method of micropropagation. This method is based on the fact that viruses are not widespread throughout the plant. The apical bud of stolon does not contain viruses, even when the whole cluster is infected. On the other hand, meristematic tissue can be easily isolated from the plant under sterile conditions, and the whole plant can easily regenerate on the nutrient medium.

Material and methods

The research used five strawberry cultivars: Senga Sengana, Cortina, Idea, Marmolada and Humel's constantly giving birth. Explants of apical buds were used as starting material for the induction of callus, organogenesis and regeneration of plants of studied strawberry cultivars. The starting materials were taken in May or June from stolons that were still in the air. Preparation of the starting material and Murashige and Skoog's medium (Murashige and Skoog, 1962) was carried out in the biology laboratory "Zdravlje" Leskovac, and isolation in the microbiological laboratory.The explants were kept under controlled conditions, at a temperature 20-25 ^{o}C, with light intensity of 2000 – 2500 lx and the length of brightness of 16 hours per day and 8 hours per night. Adjustment to environmental conditions was carried out in the greenhouses under the a ''mist'' system, while adaptation to external conditions was carried out on the plots of "Porecje" Vučje. Strawberries were kept there for three years.

Results and discussion

Stolons of the runner plants of the following strawberry cultivars: Senga Sengana, Cortina, Idea, Marmolada and Humel's constantly giving birth were used for the isolation of meristems. Plant materials were collected during May or June. Meristems size of 0.5-1mm were isolated in the laboratory from the stolons of strawberries. The isolated strawberry meristems were cultured on Murashige and Skoog's medium containing macro and micro elements, sugar and agar. Phytohormones BAP, IBA and GA3 at different concentrations were added to this substrate. From the applied concentrations, the optimal hormone balance for the culture of primary explants was: 1.0 mg / l BAP, 0.1 mg / l IBA and 0.1 mg / l GA3. The influence of these hormone concentrations on the initiation of cultures varieties is shown in Table 1.

Table 1. The beginning of the development of various sorts of strawberry cultivars

Cultivar	The number of meristems present in the culture	Uninfected cultures		Activated cultures		% of success
		Number	%	Number	%	
Senga Sengana	30	27	90.00	22	81.48	73.33
Cortina	30	25	83.33	12	72.00	60.00
Marmolada	30	25	83.33	18	48.00	40.00
Idea	30	26	86.67	17	65.38	56.67
Humel's constantly giving birth	30	19	63.33	11	57.90	36.67

The highest success in culture initiation was achieved with the Senga Sengana cultivar (81.48%), and the lowest with the Marmolada cultivar (48%). The highest percentage of success was recorded in the Senga Sengana cultivar (73.33%), and the lowest in the Humel's constantly giving birth (36.67%). Milosavljevic S. (1999) achieved the highest success in culture initiation with the Selena cultivar (70%), Nikolic at al (2004) achieved a high percentage of culture initiation with the Selena cultivar

(76.67%). After six weeks of cultivation in a culture, formed scions size 10-20mm were transferred to the medium for multiplication. The multiplication of isolated buds was achieved at Murashige and Skoog's medium with phytohormones BAP and IBA. In our research, the influence of different concentrations of BAP hormone on the reproduction and extinction of shoots in all tested cultivars was also monitored. The optimal hormone balance for continual multiplication of strawberies is 1mg / l BAP and 1 mg / l IBA. Without the presence of BAP hormones in the nutrient medium there was no multiplication, while the omission of hormone IBA reduced the multiplication. For the most favorable concentrations of phytohormones, multiplication in the tested cultivars is shown in Table 2.

Table 2. The multiplication of various strawberry cultivars

Cultivar	Number of plants in culture	The number of produced plants per row	Index of multiplication
Senga Sengana	22	193	8.77
Cortina	18	111	6.17
Marmolada	12	65	5.42
Idea	17	96	5.65
Humel's constantly giving birth	11	47	4.27

The highest multiplication index was recorded in the Senga Sengana cultivar (8.77) and the lowest in the Humel's constantly giving birth cultivar (4.27). In his experiments, Milosavljević S. (1999), reached the highest index of multiplication with the Careca cultivar (6.00), and the lowest with the Sena cultivar (4.1). The presence of BAP hormone in the nutrient medium effectively prevented the rooting of strawberries. The omission of the hormone IBA resulted in a reduction in multiplication, but not in the omission of reproduction. By eliminating BAP hormones, multiplication is absent. When obtained in the multiplication and elongation phase strawberry planlets are distinguished by the fact that they have no roots. When the plantlets reached a height of 10-20 mm, they were transferred to the rooting medium. The composition of this medium includes ½ MS macro and micro salts, sucrose, agar and inizitol, and hormones, IBA hormones at different concentrations. The optimal concentration of IBA hormone for the rooting of strawberry cultivars is 0.5 mg/l. At a higher concentration of IBA hormone (1mg/l) the larger mass of callus was formed at the basal part of the scion, but at a lower concentration of hormone (0,1mg/l IBA), the number of rooted scions was quite limited. The results of rooting of strawberry cultivars with a concentration of 0.5 mg / l IBA are given in Table 3.

Table 3. The rooting of various strawberry cultivars after 60 days

Cultivar	Number of plants in culture	Number of rooting plants	% of rooting	The average number of root/culture
Senga Sengana	30	26	86.67	6.11
Cortina	30	27	90.00	6.04
Marmolada	30	25	83.33	5.96
Idea	30	22	73.33	5.09
Humel's constantly giving birth	30	20	66.67	4.95

The first root initials could be spotted after 15 days, and after 4-5 weeks the veins reached a length of about 40 mm. The roots were slender and long, and on the cutting site, there was a little plant with little callus. The number of roots per plant ranged from 4.95 in the cultivar Humel's constantly giving birth, to 6.11 in the cultivar Senga Sengana. The highest percentage of rooting is in the Cortina cultivar, and the lowest in the Senga Sengana cultivar. Petrovic D. (1990) recorded the highest

success in culture initiation in the Selena cultivar (70%), and the lowest in the Sena cultivar (10%). Nikolic et al. (2004) achieved a high percentage of the initiation of the cultures with the cultivar Selena (76.67%). Petrović et.al (1990) achieved rooting of 80.00% in the Senga Sengana cultivar. After rooting the most convenient moment for transplanting the obtained plantlets is after two months of incubation of scions on agrarian 1/2 Murashige and Skoog's medium when the rootlets have reached the length of 30-50 mm. Then the roots of young plants were carefully rinsed with water to remove the agar, in order to avoid harmful settled microflora. Thus washed, young plants were placed into sterile plastic dishes with a sterile mixture of vermiculite, peat, and sand, in a ratio of 1:1:1. 1/2 Murashige and Skoog's mineral solution was added to this mixture. The courts were placed in plastic tubs with water and covered with jar. Thus planted plants were kept for a month under plastic sheets, and after that they were transplanted into pots with soil. The results of adaptation of strawberry cultivars are presented in the following table.

Table 4. The adaptation of various strawberry cultivars to the outdoor conditions

Cultivar	The number of planted plants	The number of plants that survived	% of survived plants
Senga sengana	40	31	77.50
Cortina	40	34	85.00
Marmolada	40	32	80.00
Idea	40	29	72.50
Humel's constantly giving birth	40	27	67.50

The highest number of adapted plants was recorded in the Cortina cultivar (85.00%), and the lowest in the Humel's constantly giving birth cultivar (67.50%).

Conclusions

Considering the conducted analyses, we can conclude that vegetative multiplication of five strawberry cultivars is possible in *in vitro* conditions. The optimal hormone concentration balance for strawberry cultivars is 1mg / l BAP, 0.1mg / l IBA and 0.1mg / l GA3. The highest success in the initiation of cultivars is in the Senga Sengana cultivar (84.48%), and the lowest in the cultivar Marmolada (48%). For the multiplication of strawberries, the hormone BAP plays the most important role. The best strawberry multiplication was achieved by a combination of phytohormones 1.0 mg / l BAP and 1 mg / l IBA. The highest multiplication index is with the Senga Sengana cultivar (8.77), and the lowest with the Humel's constantly giving birth cultivar (4.27). The optimum concentration of BAP phytohormone for rooting of strawberries is 0.5 mg / l. The highest percentage of rooting is found in the Cortina cultivar (90%), and the lowest in the Humel's constantly giving birth cultivar (66.75%). The highest number of roots was recorded in the Senga Sengana cultivar (6.11), and the lowest in the Humel's constantly giving birth cultivar (4.95). When adapting to external conditions, the largest number of plants have been adapted with the Cortina cultivar (85.00%), and the lowest with the Humel's constantly giving birth cultivar (67.50%).

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