Features of the development of strawberry microplants in vitro

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Abstract. This article presents the results of a study of the effect of different sterilizers and nutrient media of different composition on the characteristics of the growth and development of garden strawberry (Fragaria x ananassa) microplants in vitro. To introduce a plant into a cell and tissue culture, two very important conditions must be met - to choose the right sterilizing solution, its concentration and processing time, and to choose the right balanced nutrient medium suitable for a particular culture. The studies were carried out on two varieties of garden strawberries: Favorit and White Swede. Sterilization of plant material was carried out with two sterilizers - 5% sodium hypochlorite solution and 1% silver nitrate solution. Studies have shown that the largest number of sterile explants was obtained by sterilizing the material with silver nitrate. Subsequently, viable meristems were planted on three nutrient media of different composition. According to the data obtained, it can be concluded that the MS nutrient medium with the addition of cytokinin 6-BAP at a concentration of 1 mg/L turned out to be the best in terms of the multiplication factor, the percentage of survival and the height of microplants..

1. Introduction

Strawberry (Fragaria x ananassa) belongs to the widespread berry crops, both in our country and around the world.

Depending on the variety and place of growth, strawberries contain%: water - 80-90; sugar - 4.5-10; acids - 1-1.6; nitrogenous substances - 0.9-1.7; tannins - 0.16-0.25; proteins - 0.8-1; oils - 0.6. Berries also contain folic acid (vitamin B 9) 0.5-0.6 mg%, essential oils, pectin, phenolic compounds, anthocyanins, trace elements, dry matter - 5-24 mg; magnesium - 1218 mg; calcium compounds - 28-42 mg; iron - 0.6-10.9 mg; phosphorus - 25-29 mg; copper - 0.01-0.03 mg; potassium - 161 mg; sodium - 18 mg [1].

Strawberries are suitable for growing in various soil and climatic conditions, it bears fruit in the year of planting or the next year, ripens earlier than most other fruit and berry crops, and gives high yields.

Garden strawberries in modern realities are one of the highly profitable and productive horticultural crops. Various countries of the world (more than 75 countries) are engaged in the production of garden strawberries. At present, the following countries are the main producers of berries of this crop: the USA, China and Spain [2-4].

Today, 6% of the world strawberry production is grown in the Russian Federation, and 95% of this volume is grown by the population [5]. The area under strawberries in our country is 33.8 thousand hectares and there is a tendency to increase the share of this crop to 30-40% of the area occupied by all berry growers [6].

Over the past 10-15 years, there has been a decrease in the yield of this crop due to the loss of resistance to diseases and pests, the spread of viral diseases. Therefore, the system for the production of healthy strawberry planting material using the micropropagation method is becoming increasingly important [7].

Micropropagation makes it possible to obtain a huge number of homogeneous healthy plants in a short time. The terms of creating the introduction of new varieties into production are reduced.

An important role in the recovery and replication of plant material on an industrial scale is played by biotechnological methods, thanks to which the modern assortment of garden strawberries is maintained [8-9].

Rehabilitated strawberry plants provide a high and stable yield, increase the vigor of growth and the ability to vegetative reproduction, and are used for laying queen cells.

One of the important factors that affects the processes of morphogenesis of plant organs and tissues in vitro is the composition of the nutrient medium.

The aim of our study is to select a nutrient medium for growing two varieties of garden strawberry plants in vitro.

2. Materials and methods

The studies were carried out at the Micropropagation Laboratory of the FGBOU HE SPbSAU in 2022. The objects of the study were two varieties of garden strawberries (Fragaria x ananassa) Bely Swede and Favorit.

Variety White Swede early ripe, winter-hardy, well tolerated by heat. This variety has compact bushes up to 20 cm high, with vertically directed dark green leaves. The mustache variety does not produce much. One plant produces up to ten flower stalks. The berries have a blunt-conical shape, weigh 20-22 g, are sweet with a well-noticeable aftertaste of pineapple or mulberry. White color is combined with a pink tint. Due to the lack of red pigment, White Swede garden strawberries are considered dietary, they are consumed by people who cannot tolerate red varieties, including due to allergies. With the right agricultural technology, 0.6-0.8 kg of berries are harvested from a bush. Not subject to storage and transportation.

Variety Favorit universal large-fruited mid-season variety. The plant is low, medium sprawling. Usage is moderate. The leaves are very large, medium-ribbed, light green in color with a glossy sheen. The flowers are bisexual, white, collected in compact multi-flowered inflorescences. Peduncles of medium thickness, located at the level of the leaves. The berries of Favorit are medium and large in size 30-35 g, beautiful, regular wide-conical shape of bright red color. The yield is average, about 500-700 grams of berries per bush, depending on the intensity of agricultural technology.

The sterilization of the starting material was carried out according to the scheme:

- Rinsing with running water - 60 minutes.
- Flushing with 70% ethanol solution - 15 seconds.
- Three times washing with autoclaved distilled water - 9 minutes.
- Treatment with the main sterilizer - 2-10 minutes.
- Washing five times with autoclaved distilled water - 15 minutes.

5% sodium hypochlorite solution and 1% silver nitrate solution were used as sterilizers.

At the stage of introduction into culture in vitro, MS nutrient medium was used with the addition of cytokinin 6-BAP at a concentration of 1 mg/l. At this stage, the infection and viability of objects were taken into account.

The actual micropropagation was carried out on MS nutrient medium with different composition of hormonal preparations (table 1).

Table 1. The composition of the nutrient medium during microclonal propagation of garden strawberry.

Nutrient medium components, mg/l	1	2	3
Agar-agar, g/l	7	7	6
Sucrose, g/l	20	20	30
mesoinositis	100	100	100
Glycine	200	-	200
6-BAP	1	0.2	1
Gibberelin	-	0.2	-
IUK	-	0.1	0.2
Salts and vitamins according to MS	+	+	+

Laboratory studies were performed in accordance with the guidelines for obtaining regenerated fruit and berry plants in in vitro culture [10–14].

The cultivation of microplants was carried out in glass jars with a volume of 100 ml on phytotron installations with a controlled temperature regime of 21-23 °C at an irradiation power of cold white light of 62.0 ± 3.1 μmol/m^2/s and a photoperiod of 16 h, relative air humidity 75%.

3. Results

Table 2 presents data on the effectiveness of using two different sterilizers when introducing garden strawberry into in vitro culture, while considering the infection and viability of explants.

Table 3 presents data on the percentage of survival of microplants during microclonal propagation, depending on the composition of the nutrient medium.

Table 4 presents morphometric parameters. At the end of the third passage, the number of microplants in conglomerates and their height were measured.

Table 2. The effectiveness of the use of various sterilizers when introducing garden strawberry into in vitro culture 2022, %.

Sterilizer		Varieties of strawberries	
		White Swede	Favorite
Sodium hypochlorite	infection	81.3	83.3
	viability	18.7	16.7
Silver nitrate	infection	62.5	58.3
	viability	37.5	41.7

Table 3. Survival rate of initial microplants of garden strawberry depending on the composition of the nutrient medium in 2022, %.

Variety	Nutrient medium		
	1	2	3
White Swede	98.0	83.0	59.0
Favorite	95.0	70.0	35.0

Table 4. Multiplication rate and plant height at passage 3, 2022.

Variety	Nutrient medium					
	1		2		3	
	Plant height, mm	Multiplication factor, pcs.	Plant height, mm	Multiplication factor, pcs.	Plant height, mm	Multiplication factor, pcs.
White Swede	15.0±0.8	30.0±1.8	13.0±0.8	23.0±0.9	7.0±0.2	12.0±0.8
Favorite	11.0±0.6	28.0±1.2	10.0±0.3	17.0±0.8	6.0±0.2	9.0±0.4

4. Discussion

The main factors of introduction into culture in vitro is the choice of a sterilizer and the selection of a nutrient medium.

Sterility of plant material is a necessary condition for obtaining healthy material and developing explants.

Most biotechnology laboratories working with in vitro cultures use agar media as a nutrient medium. Generally media contain about 6-8 g/l of agar. The whole process and efficiency of micropropagation depends on the correct choice of nutrient medium.

The basis of nutrient media for growing strawberries, as well as other crops, includes the main nutrients necessary for the growth of cells, tissues and plant organs: nitrogen, potassium, calcium, magnesium salts, phosphorus, sulfur, trace elements, carbohydrates, some amino acids, vitamins, phytohormones and others. Currently, various recipes of nutrient media for specific plants and tissue types have been developed. To obtain plants from isolated apexes and micropropagation processes, the recipes of Gautre, White, Morel, Heller, Murashige-Skoog are most often used [15].

According to the research results, the largest number of sterile explants was obtained by sterilizing the material with silver nitrate. The viability of explants obtained by sterilization with silver nitrate in the Favorit variety was 41.7%, and in the White Swede variety it was 37.5%, which is 2-2.5 times more than in the variants with the use of sodium hypochlorite.

Subsequently, the obtained microplants were planted on MS nutrient media differing in composition. According to table 3, the survival rate of microplants of both varieties was higher when growing microplants on nutrient medium No. 1 containing cytokinin 6-BAP in an amount of 1 mg/l, and amounted to 95-98%. On nutrient medium No. 2 containing a minimum amount of hormones (0.2 mg/l 6-BAP, 0.2 mg/l gibberellin and 0.1 mg/l IAA), the survival rate of microplants was 70-83%. The lowest percentage of plant survival was observed on nutrient medium No. 3 containing 1 mg/l of 6-BAP and 0.2 mg/l of IAA - 35-59%.

At the end of the third passage, on average, the conglomerate contained from 12 to 30 pieces. microplants of the White Swede variety and from 9 to 28 pcs. microplants of the Favorit variety. The maximum value of the multiplication factor in the studied varieties was observed on nutrient medium No. 1 - MS + 6-BAP with a concentration of 1 mg/l.

Plant height, when measured at the 3rd passage, ranged from 6-15 mm. The maximum values for the height of microplants were observed during cultivation on nutrient medium No. 1 and amounted to 11-15 mm, depending on the variety.

5. Conclusion

Based on the results of the study, the following conclusions can be drawn:

- When introduced into in vitro culture to obtain sanitized material, the choice of the main sterilizer, its exposure and concentration play an important role. According to studies, the best results were obtained when using a 1% solution of silver nitrate as a sterilizer for 9 minutes.
- On the studied varieties of garden strawberry during microclonal propagation, more intensive growth and development of microplants differed in variants with the use of a nutrient medium MS + 6-BAP with a concentration of 1 mg/l.

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