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COMPARATIVE ASSESSMENT OF THE EFFICIENCY OF PRAPOGATION OF STRAWBERRY VARIETIES UNDER *IN VITRO* CONDITIONS*

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The article presents the results of evaluating the efficiency of propagation of garden strawberry varieties under *in vitro* conditions. The paper compared the strawberry varieties of the Italian selection Asia, Cleri, Quicky and the varieties of the selection of the Federal State Budgetary Scientific Institution NCFSCHVW Nelli, Taira and Kemia. At the stage of introduction into *in vitro* culture, the Nelly varieties have a high level of regeneration – 80 %, Kemia

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СРАВНИТЕЛЬНАЯ ОЦЕНКА ЭФФЕКТИВНОСТИ РАЗМНОЖЕНИЯ СОРТОВ ЗЕМЛЯНИКИ В УСЛОВИЯХ *IN VITRO**

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В статье представлены результаты по оценке эффективности размножения сортов земляники садовой в условиях *in vitro*. В работе сравнили сорта земляники итальянской селекции Азия, Клери, Квики и сорта селекции ФГБНУ СКФНЦСВВ Нелли, Таира и Кемия. На этапе введения в культуру *in vitro* высокий уровень регенерации имеют сорта Нелли – 80 %, Кемия и Азия – 73 %, Таира – 70 %, Клери – 62 %.

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and Asia – 73 %, Taira – 70 %, Clery – 62 %. The survival rate of explants Quicky variety was 34 %. A gradual increase in the concentration of 6-BAP during strawberry micropropagation increases the formation of strawberry shoots with each passage. An increase in the concentration of 6-BAP to 0.75 mg/l at the second passage contributes to an increase in the number of shoots by 1.5-2.7 times, depending on the genotype, and is 3.5-10.2 shoots. Increasing the concentration of 6-BAP to 1.0 mg/l at the third passage increases the number of shoots formed by another 8.9-31.4 % compared with the second passage and amounts to 5.1-11.3 shoots, depending on the variety. Thus, the varieties Taira, Nelli and Asia have a high breeding efficiency under aseptic conditions, on media with different hormonal composition, in total 22.7-26.0 microshoots per 3 passages, the average – varieties Clery and Kemiya – 13.7-14.8 shoots, low – variety Quicky – 10.7. The efficiency of rhizogenesis of regenerated plants of the studied strawberry varieties on a hormone-free Murashige-Skoog medium with half the content of macrosalts is 95-98 %. The successful rate of adaptation of microplants of the studied varieties ranges from 87 to 95 %.

Key words: CLONAL MICROPROPAGATION, STRAWBERRY, *IN VITRO*, REPRODUCTION RATE, 6-BENZYLAMINOPURINE (BAP)

У сорта Квики приживаемость эксплантов составила 34 %. Поэтапное увеличение концентрации 6-БАП при микроразмножении земляники, с каждым пассажем повышает образование побегов земляники. Увеличение концентрации 6-БАП до 0,75 мг/л на втором пассаже способствует увеличению количества побегов в 1,5-2,7 раза в зависимости от генотипа и составляет 3,5-10,2 побегов. Повышение концентрации 6-БАП до 1,0 мг/л на третьем пассаже увеличивает количество образующихся побегов еще на 8,9-31,4 % по сравнению со вторым пассажем и составляет 5,1-11,3 побегов, в зависимости от сорта. Таким образом, высокую эффективность размножения в асептических условиях, на средах с разным гормональным составом имеют сорта Таира, Нелли и Азия суммарно 22,7-26,0 микропобегов за 3 пассажа, среднюю – сорта Клери и Кемия 13,7-14,8 побегов, низкую – сорт Квики 10,7. Эффективность ризогенеза растений-регенерантов, исследуемых сортов земляники, на безгормональной среде Мурасиге-Скуга с половинным содержанием макросолей составляет 95-98 %. Успешность адаптации микрорастений исследуемых сортов – от 87 до 95 %.

Ключевые слова: КЛОНАЛЬНОЕ МИКРОРАЗМНОЖЕНИЕ, ЗЕМЛЯНИКА, *IN ITRO*, КОЭФФИЦИЕНТ РАЗМНОЖЕНИЯ, 6-БЕНЗИЛАМИНОПУРИН (БАП)

Introduction. Micropropagation is one of the important tools in the development of modern nursery. This is especially relevant within the framework of the import substitution program, when the demand for healthy, high-quality planting material has grown. The use of biotechnological methods is one of the best and most effective ways to recover from harmful organisms and propagate plants, including strawberries, after which the plant's potential is most fully realized [1-3]. In addition, plants can be propagated all year round and obtain the required amount

of material in a short period of time [4–6]. Studies show that characteristics such as yield, flowering intensity, bush habit, and mother plant productivity were much higher in micropropagated plants than in conventionally propagated plants [7].

Although, in general, clonal micropropagation technologies have been developed for most cultures, nevertheless, an individual approach is required for each culture and even variety. Since the assortment of garden strawberries is constantly updated, it becomes necessary to study the variety specificity of the development of strawberry plants at all stages of micropropagation.

In this regard, the purpose of our research is to evaluate the efficiency of propagation of garden strawberry varieties under *in vitro* conditions at various stages of micropropagation.

Materials and methods. The material for research was selected in the collection plantations (genetic collection) of the Central Collective Use Center "North Caucasian Federal Scientific Center of Horticulture, Viticulture, Winemaking" (NCFSCHVW), the studies were carried out in the laboratory of virology in 2020-2022. according to the methodological recommendations of Dzhigadlo [8]. The objects of research are the garden strawberry varieties Taira, Kemiya, Nelly (NCFSCHVW breeding (Krasnodar)) and varieties Asia, Clery, Quicky (Italian breeding). For introduction into *in vitro* culture, we used the apex shoots of garden strawberry, selected in the period from mid-June to early July (the period of active shoot formation). The apexes were washed with soapy water, then in running water for an hour. The main sterilization was carried out with a 0.5 % OKA-TAB solution with explants exposed for 7 minutes, followed by three washings of the explants.

For the cultivation of explants, a nutrient medium was used according to the prescription of Murashige and Skoog (1962) [9]. 6-BAP was used as cytokinin in concentrations: 0.5; 0.75; and 1.0 mg/l. Control was a hormone-free medium.

Conditions for plant cultivation: photoperiod 16/8, illumination – 2-3 thousand lux, air temperature +24±2 ºC.

Mathematical data processing, was carried out using the MO Excel program – calculation of average values, standard errors.

Discussion of results. At the stage of introduction into *in vitro* culture, an important point is to obtain a sterile culture capable of successful regeneration on a nutrient medium. The efficiency of the stage depends on the type of sterilizing agent, regime of the sterilization, the plant genotype, the season of isolation, and the physiological state of the explant [5].

Initiation into *in vitro* culture during the period of active formation of strawberries runners showed a different level of regeneration of explants. In variety Nelly, 80 % of explants were regenerated, in varieties Kemiya and Asia – 73 %, in Taira variety – 70 %, in Clery variety – 62 %. The lowest percentage of regeneration was in Quicky variety – 34 % (Fig. 1).

The level of contamination ranged from 10 to 22 % of the total number of explants. Quicky variety had the highest level of explant necrosis – 46 %, while the other varieties were in the range of 8-17 %. Explants of Quicky variety were more sensitive to the disinfectant used, which had a strong phytotoxic effect.

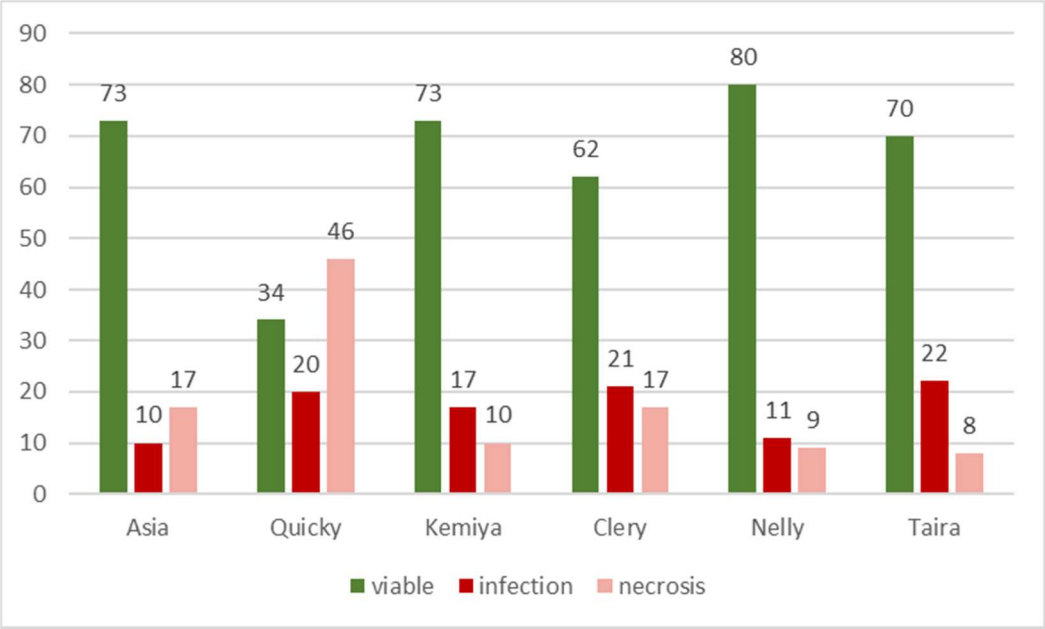

Fig. 1. Survival of strawberry explants at the stage of initiation into *in vitro* culture

At the stage of micropropagation, the genotype of the propagated plant, the composition of the nutrient medium, the concentration of cytokinin, and the physical conditions of cultivation (temperature, lighting) play an important role.

Most often, for active shoot formation of strawberries, 6-BAP is used as a growth regulator of a cytokinin nature [10-15].

The study of the effect of 6-BAP on the reproduction of strawberry genotypes showed that the multiplication factor of strawberry shoots varies from 2 shoots to 11. Variety specificity is traced (tab.).

Influence of 6-BAP concentration on shoot formation of garden strawberry

Strawberry variety	Multiplication factor		
	I passage 6-BAP – 0.5 mg/l	II passage 6-BAP – 0.75 mg/l	III passage 6-BAP – 1.0 mg/l
Asia	4,3±0,21	10,2±0,3	11,2±0,37
Quicky	2,1±0,13	3,5±0,17	5,1±0,16
Clery	2,1±0,15	5,6±0,19	7,1±0,24
Kemiya	3,0±0,22	4,5±0,18	6,2±0,19
Nelly	5,4±0,23	9,3±0,28	11,3±0,40
Taira	4,2±0,2	8,1±0,27	10,4±0,39

After the first passage on the medium with 6-BAP – 0.5 mg/l, the multiplication factor was 2-5 shoots. In varieties Asia, Taira and Nelly a conglomerate of 4-5 shoots was formed at a concentration of 6-BAP of 0.5 mg/l after the first passage, in variety Kemiya – 3 shoots, while in the Italian varieties Quicky and Clery the multiplication factor was two times lower – 1:2.

At the second passage, the concentration of cytokinin was increased to 0.75 mg/g, which led to an increase in the formation of adventitious shoots. In varieties Clery, Asia, Nelly and Taira, the number of shoots increased almost 2 times to 5.6-10.2 pieces (Fig. 2). In variety Asia, this indicator was the maximum – 10.2 pieces per explant. In Kemiya variety, the multiplication factor was 1:4.5, in the Quicky variety – 1:3.5.

Fig. 2. Strawberry microshoots of the Nelly variety (6-BAP – 0.75 mg / l)

At the third passage, the concentration of 6-BAP was increased up to 1.0 mg/l. The number of adventitious shoots increased by 27.4 % in variety Kemiya,, in variety Nelly, Clery and Taira – by 17.7-22.1 % and amounted up to 11.3 pcs/exp. For variety Nelly, 10.4 pcs/exp. for Taira variety and 7.1 pcs/exp. for Clery variety.

In Quicky variety, with an increase in the amount of 6-BAP in the medium to 1.0 mg/l, it contributed to an increase in shoot formation by 31.4 %, on average, up to 5.1 shoots from one explant. In variety Asia, the average number of shoots formed after the 3rd passage was 11.2 pieces/exp.

The increase in the concentration of 6-BAP over 1.0 mg/l was not carried out, because, based on our previous studies, this leads to the vitrification of strawberry shoots, in which only single shoots are suitable for further reproduction [16].

The results obtained confirm the variety-specific behavior of strawberry plants of different varieties in *in vitro* culture, their response to changes in the concentration of phytohormones. After the first passage, varieties Clery and Quicky formed the smallest number of shoots 2.1 pcs/exp. Other varieties have 3-5.4 pieces/exp. An increase in the concentration of 6-BAP to 0.75 mg/l at the second passage contributed to an increase in the number of shoots by 1.5-1.9 times. In varieties Quicky and Clery – 2.4-2.7 times. The addition of 6-BAP in the amount of 1.0 mg/l at the third passage increased the number of shoots formed in the varieties Clery, Kemiya, Nelly and Taira by 17.7-27.4 % more compared to the second passage. Quicky variety has a maximum increase of 31.4 %. The variety Asia, on the contrary, has the smallest increase – 8.9 %.

Thus, the varieties such as Taira, Nelly and Asia showed high breeding efficiency on media with different hormonal composition. The total coefficient for 3 passages is from 22.7 to 26.0 microshoots. The average efficiency was noted in the varieties Clery and Kemiya – 13.7-14.8 shoots per 3 passages, low – in the Quicky variety – 10.7 pcs/exp.

Unlike many plant species propagated *in vitro* culture, strawberry microshoots root easily. Already at the stage of micropropagation, some microshoots and even conglomerates were able to produce some roots [14]. Even on a hormone-free nutrient medium, the formation of roots on strawberry microshoots was by 93-100 % [17].

The stage of rooting strawberry microplants was carried out on the Murashige-Skoog medium with half the content of macrosalts, with the addition of sucrose in the amount of 20 g/l, without the use of root formation inducers. The first roots began to appear after 10-12 days. After 3-4 weeks, rooted plants were planted in non-sterile conditions (Fig. 3). Root formation was 98-100 % in microshoots of all varieties.

Fig. 3. Root formation of strawberries
on a hormone-free nutrient medium ½ MS, Taira variety

For plant adaptation, a substrate was used consisting of a mixture of soil: perlite: vermiculite in a ratio of 3:1:1. The height of the planted rosettes was from 2 to 3.5 cm. During the period of adaptation, the humidity was maintained at 95 %.

In general, the survival rate of microplants in all varieties was high from 87 to 95 % and amounted to 87 % for Quicky, 89 % for Clery, 91 % for Kemiya, 93 % for Nelly, 94 % for Taira, and 95 % for Asia (Fig. 4).

Fig. 4. Adapted strawberry plants

Within three years, 12 varieties of strawberries of domestic and foreign breeding were propagated on Murashige-Skoog nutrient medium with different hormonal composition. This planting material was used for further reproduction, in order to lay strawberry mother plantations.

Conclusions:

1. At the stage of introduction into *in vitro* culture, a high level of regeneration showed varieties such as Nelly – 80 %, Kemiya and Asia – 73 %, Taira – 70 %, Clery – 62 %. In Quicky variety the survival rate of explants was 34%.

2. During the micropropagation of garden strawberry, a gradual increase in the concentration of 6-BAP with each passage increased the formation of adventitious shoots of strawberries.

3. An increase in the concentration of 6-BAP up to 0.75 mg/l at the second passage contributes to an increase in the number of shoots by 1.5-2.7 times, depending on the genotype.

4. Increasing the concentration of 6-BAP up to 1.0 mg/l at the third passage increased the number of formed shoots, depending on the variety, by 8.9-31.4 % compared with the second passage.

5. Varieties Taira, Nelly and Asia showed the highest breeding efficiency under aseptic conditions on media with different hormonal composition. The total coefficient of these varieties for 3 passages was 22.7-26.0 microshoots from one explant. The average breeding efficiency in Clery and Kemiya varieties was 13.7-14.8 shoots, for Quicky variety was low – 10.7 shoots.

6. The efficiency of rhizogenesis of studied regenerated plants of garden strawberry varieties on a hormone-free Murashige-Skoog medium with half the content of macrosalts was 95-98 %.

7. The success of adaptation of microplants of the studied varieties ranged from 87 to 95 %.

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