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OPTIMIZATION OF NUTRIENT MEDIUM COMPOSITION FOR CLONAL MICROPROPAGATION OF FRAGARIA ANANASSA

The article presents the results of research on optimization of the composition of nutrient medium for effective replication of garden strawberry (Fragaria × ananassa) microplants. Garden strawberry is a highly demanded berry crop due to its high flavor and rich vitamin composition. Biological features of this crop create limiting factors for accelerated multiplication of high-yielding varieties and their introduction into production. The method of clonal micropropagation allows overcoming many problems of vegetatively propagated crops, including those related to stunting and disease incidence. In this study, the effect of agar-agar as a part of nutrient medium MS and dosage of BAP preparation on the dynamics of development of strawberry plants under in vitro conditions was investigated. The results of the study showed that for induction of strawberry (Fragaria × ananassa) morphogenesis under in vitro conditions, the use of liquid nutrient medium MS with the addition of BAP preparation at a dosage of 0.5 mg/L is the most effective, since the plant height growth, leaf and root formation are higher. Thus, using liquid nutrient medium with BAP preparation at a dosage of 0.5 mg/l, it is possible to obtain fully formed plants with well-developed root system and developed leaf apparatus in a short period of time. This is of great practical importance to accelerate the growth of strawberry starter material of valuable varieties, suitable for further propagation in production.

Key words: strawberry, nutrient medium, development, in vitro, height, root formation.

Introduction

Garden strawberries are known for their juicy and sweet flavor and are a valuable source of vitamins, antioxidants and other nutrients. It contains a large amount of vitamin C (583 mg/kg fresh berry weight), as well as vitamins B, A, K, E, beta-carotene, minerals valuable for the human body: iron, calcium, magnesium, zinc, potassium, fluorine, phosphorus and other valuable substances. Garden strawberries are a popular and demanded agricultural crop. In 2023, the collection of garden strawberries in Russia amounted to more than 1.75 million tons, of which more than 80 % of the berries were obtained in the North-Caucasus and Southern Federal Districts. High demand for garden strawberries in the market causes the need to improve the technology of its cultivation [1, p. 45; 2, p. 35–40].

Both in Russia and Kazakhstan nowadays the issue of planting industrial plantations with certified high-yielding material in sufficient quantity is acute [3, p. 74]. The use of basic certified planting material in mother and industrial plantations of fruit and berry crops increases the production increment by 1.5...4.0 as compared to the use of row material [4, p. 28–30].

The main obstacle in the promotion of new and valuable healthy varieties is the duration of strawberry propagation by the traditional method and the high cost of seedlings. Since its seeds are slow-germinating, producers face big problems with germination when using them. Traditionally, garden strawberries are propagated vegetatively by means of whiskers, but, for example, for remontant strawberries this method of propagation is ineffective, because during the growing season it forms 1-2 whiskers per rosette [4, p. 28]. In addition, the vegetative method of propagation is more conducive to the transmission from the mother plant and accumulation of fungal and viral pathogens in seedlings [3, p. 74–75].

In overcoming these difficulties, the use of the method of clonal micropropagation in vitro, which allows not only to obtain plants recovered from phytopathogens, viruses and other infections, but also significantly accelerate the process of reproduction (up to 3 million seedlings per year from one initial plant), which is of particular value for garden strawberry varieties that are poorly propagated vegetatively because of low runners-forming ability [5, p. 42–45].

Many researchers have developed approaches to use the method of clonal micropropagation for berry crops, as in this case the potential of plant organism for reproduction is most fully realized. Under *in vitro* conditions, it is most possible to eliminate phytopathogens and obtain healthy planting material for further propagation. High reproduction rate of regenerants in in vitro culture directly depends on the use of optimal growing conditions [6, p. 60–63].

Under *in vitro* conditions, the reaction of genotypes is stronger than in traditional methods of propagation, and the influence of physiological,

hormonal and physical factors, which must be taken into account for successful propagation, is more significant [4, p. 32–34; 5, p. 45–49; 6, p. 62–63].

Despite the large number of studies in the direction of improving the efficiency of the technology of clonal micropropagation of strawberry *in vitro*, the problems remain, which requires further study [7; 8; 9; 10].

The aim of the study was to investigate the effect of agar-agar as a part of nutrient medium MS and the dosage of BAP preparation on the dynamics of strawberry (Fragaria × ananassa) plant development under *in vitro* conditions to accelerate the growth of strawberry starter material suitable for further *in vivo* propagation.

Materials and methods

The research was conducted in the laboratory of NCJSC «Toraighyrov University» (October 2023 – February 2024). The object of the study were cultured plants of strawberry variety Rujana (Czech variety of alpine strawberry).

Work with tissue culture was carried out in a laminar box Sentinel™ Gold Microprocessor Control System according to the generally accepted methodology [2, p. 35-36]. Strawberry regenerant plants obtained in the laboratory of NCJSC «Toraigyrov University» served as an object of study.

PB-16 tubes with cotton-gauze plugs were used for plant cultivation. Nutrient media were prepared on the basis of the mineral basis of MS medium (Murashige Skooga) with 3-times increased concentration of iron chelate, 6-BAP at a concentration of 0.5 mg/L, IBA at a concentration of 1.0 mg/L, casein hydrolysate 120 mg/L, sucrose 20 g/L. After planting in nutrient medium, tubes with explants were placed in a phytocamera, where they were cultured at 26±2 °C. White spectrum fluorescent lamps were used as a light source and the illumination was 3000 Lx. A 16-h photoperiod was used. The relative humidity level was maintained within 70 %.

During the experiment, the work was carried out according to the generally accepted protocols of clonal micropropagation of plants [2, p. 35–36].

During the experiment, 4 variants of cultivation were studied.

In each variant, 10 plants were studied in 3-fold repetition. When analyzing the root system development, a 3-point system was used, and the following was considered: 1 point – the number of roots no more than two hairs; 2 points – the number of roots 3–4 hairs; 3 points – the number of roots 5 or more hairs.

When analyzing leaf formation, the number of leaves in pieces was taken into account.

Research options:

To study the dynamics of development of strawberry regenerant plants, the following variants of Murashige-Skoog medium composition were included in the study:

1 variant MS + 1 mg/l IBA + 0.5 mg/l BAP + agar-agar;

2 variant MS + 1 mg/l IBA + 1 mg/l BAP + agar-agar;

3 variant MS + 1 mg/l IBA + 0.5 mg/l BAP;

4 variant MS + 1 mg/l IBA + 1 mg/l BAP.

The following parameters were measured regularly during cultivation: number of leaves, root development and plant height.

Subsequently, these indicators were processed using the program STATISTICA for Windows, 6.0 (StatSoft, Inc. 1984–2001).

The experiment to study the dynamics of development of strawberry regenerant plants on media with and without agar-agar addition, as well as with the use of different dosages of cytokinin preparation BAP lasted about 4 months Strawberry explants were transplanted into PB–16 tubes (Figure 1).

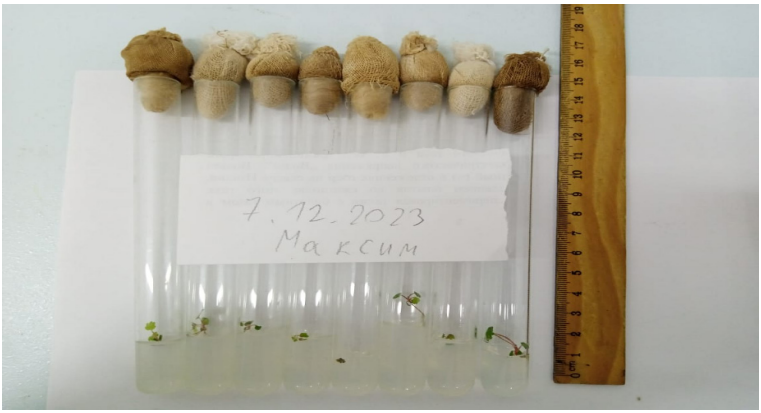

Figure 1 – Regenerant plants at the beginning of the experiment

Results and discussion

During the experiment, results were observed for the following indicators:

- height of plant stems in cm;
- number of developed roots in points;
- leaf formation in pcs.

The results of measurements were recorded in Table 2.

Table 2 – Indicators of strawberry regenerant plant development

Variant	Date of measurement	Average development indicators		
		Number of leaves, pcs	Plant height, cm	Root development, points
1 variant	21.12.2023	4,8±0,3	1,0±0,3	1,9
	28.12.2023	5,1±0,2	1,3±0,2	2,7
	12.01.2024	8,3±0,3	2,2±0,4	3,0
	22.01.2024	9,6±0,4	3,1±0,3	3,0
2 variant	21.12.2023	4,4±0,4	1,2±0,2	1,7
	28.12.2023	4,9±0,3	1,4±0,3	2,3
	12.01.2024	7,8±0,2	2,4±0,3	2,6
	22.01.2024	9,8±0,4	3,0±0,2	2,8
3 variant	21.12.2023	5,1±0,2	1,4±0,2	2,2
	28.12.2023	5,9±0,3	1,9±0,3	2,6
	12.01.2024	9,5±0,5	2,6±0,4	2,9
	22.01.2024	12,6±0,4	4,0±0,3	3,0
4 variant	21.12.2023	5,0±0,3	1,0±0,3	2,0
	28.12.2023	5,8±0,3	1,2±0,4	2,4
	12.01.2024	9,3±0,4	2,4±0,4	2,6
	22.01.2024	12,0±0,3	3,7±0,3	2,8

During the experiment, it was noted that the aggregate state of the nutrient medium had a greater effect on plant development than the dosage of BAP preparation. When comparing plants with similar composition of nutrient medium (for example 1 and 3 variants), it can be noted that plants on agarized medium are significantly inferior in development to plants grown on liquid medium (Figure 2). Apparently, this is due to the higher dissociation rate in liquid solution and greater availability of nutrient ions.

Figure 2 – Strawberry plants 15 days after planting in in vitro conditions:
1) solid agar-agar medium; 2) on liquid medium

When comparing the average development indicators, it can be concluded that the increased dose of BAP preparation had a restraining effect on the development of the root system. At the same time, this effect was observed in variants with agarized medium and without agar-agar.

When comparing leaf formation and height indicators, the data were obtained indicating that the dosage of BAP preparation - 1 mg/l had some suppressive effect on the development of strawberry regenerant plants and it was observed both on liquid medium and agar-agarized medium, this confirms the conclusions made by Tashmatova L.V. in work with blackberry culture [6, p. 61–63].

If we compare the indicators of plant development on agarized medium with a similar composition, they were lower than those on liquid medium. Thus, in variants with dosage of BAP – 0.5 mg/l, the average number of leaves in the variant with liquid medium was 31 % higher and the average height of plants was 29 % higher than in solid medium, in the variant with dosage of BAP – 1.0 mg/l, the number of leaves in the variant with liquid medium was 22.5 % higher and the average height of plants was 29 % higher than in solid medium (Figure 3).

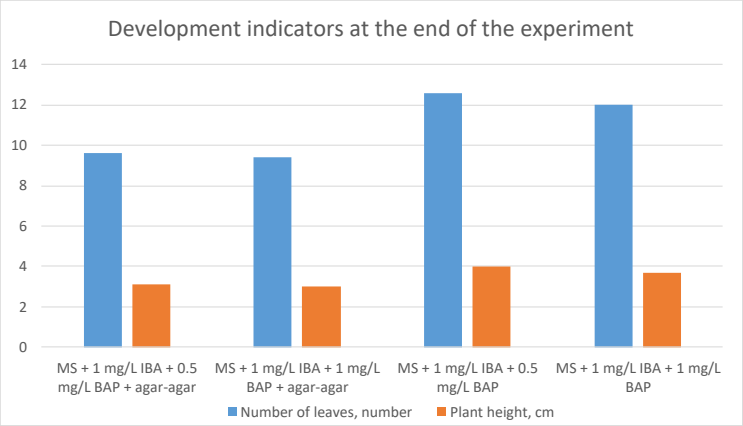

Figure 3 – Comparative characterization of strawberry plants development indicators in vitro by experiment variants

Conclusion

Thus, in the course of the conducted studies it was revealed that for induction of strawberry (Fragaria × ananassa) morphogenesis in in vitro conditions, the use of liquid nutrient medium MS with the addition of BAP preparation in the dosage of 0.5 mg/l is the most effective, since in this case the dynamics of plant development is more optimal, i.e. in a short period of time it is possible to obtain fully formed plants with well-developed root system and developed leaf apparatus.

The results of the conducted research allowed us to draw the following conclusions: when comparing the indicators of plant development (Fragaria × ananassa) in in vitro conditions on agarized medium in variants with the dosage of BAP preparation – 0.5 mg/l, the average number of leaves in the variant with liquid medium by 31 % was more and the average height of plants more by 29 % than on solid medium, in the variant with the dosage of BAP preparation – 1.0 mg/l, the number of leaves in the variant with liquid medium by 22.5 % was more and the average height of plants more by 29 % than on solid medium.

The use of liquid modification of MS medium with the addition of IBA 1 mg/l and BAP 0.5 mg/l is reasonable to use in production conditions to increase the planting material of valuable strawberry varieties *(Fragaria × ananassa)* for subsequent transplanting into the soil or hydroponics.

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ҚОРЕКТІК ОРТАНЫҢ ҚҰРАМЫН ЖАҚСАРТУ ЖОЛДАРЫ FRAGARIA ANANASSA КЛОНДЫҚ МИКРОКӨБЕЮ ҮШІН

Мақалада бақша құлпынайын (Fragaria × ananassa) микротарату үшін қоректік ортаның құрамын тиімді оңтайландыру бойынша зерттеу нәтижелері берілген. Дәмдік сапасы мен витаминдік құрамы жоғары болғандықтан, бақша құлпынайы кең сұранысқа ие жидек мәдениеті болып табылады. Бұл дақылдың биологиялық ерекшеліктері жоғары өнімді сорттарды жедел көбейту мен оларды өндіріске енгізуге шектеу қойып отыр. Клондық микрокөбею әдісі вегетативті жолмен көбейетін дақылдардағы қиындықтарды, соның ішінде өсірудегі кедергілер мен ауруларға қарсы осалдық мәселелерін шешуге мүмкіндік береді. Осы зерттеуде агар-агардың МС қоректік ортасындағы құрамы және БАП препаратының дозировкасы құлпынай өсімдіктерінің in vitro жағдайындағы даму динамикасына әсері зерттелді. Зерттеу нәтижелері көрсеткендей, құлпынай дақылының (Fragaria × ananassa) in vitro жағдайындағы морфогенезін индукциялау үшін МС сұйық қоректік ортасын БАП препаратының 0,5 мг/л дозировкасымен пайдалану ең тиімді болып табылады, өйткені бұл жағдайда өсімдік биіктігі, жапырақ түзу және тамыр

түзу көрсеткіштері жоғарылайды. Осылайша, БАП препаратының 0,5 мг/л дозасы бар сұйық қоректік ортаны қолдану арқылы қысқа мерзім ішінде жақсы дамыған тамыр жүйесі мен жапырақ аппараты бар толыққанды өсімдіктерді алуға болады. Бұл бағалы сорттардың бастапқы материалын өндірісте әрі қарай көбейтуге жарамды етіп тезірек жинақтау үшін үлкен практикалық маңызға ие.

Кілтті сөздер: құлпынай, қоректік орта, даму, in vitro, биіктік, тамыр түзу.

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ОПТИМИЗАЦИЯ СОСТАВА ПИТАТЕЛЬНОЙ СРЕДЫ ДЛЯ КЛОНАЛЬНОГО МИКРОРАЗМНОЖЕНИЯ FRAGARIA ANANASSA

В статье представлены результаты исследований по оптимизации состава питательной среды для эффективного тиражирования микрорастений земляники садовой (Fragaria × ananassa). Земляника садовая в связи с высокими вкусовыми качествами и богатым витаминным составом является высоковостребованной ягодной культурой. Биологические особенности данной культуры создают ограничивающие факторы для ускоренного размножения высокоурожайных сортов и внедрения их в производство. Метод клонального микроразмножения позволяет преодолевать многие проблемы вегетативно размножаемых культур, в том числе связанные с туговсожестью и поражаемостью болезнями. В данном исследовании изучено влияние агар-агара в составе питательной среды МС и дозировки препарата БАП на динамику развития растений земляники в условиях in vitro. Результаты исследований показали, что для индукции морфогенеза культуры земляники (Fragaria × ananassa) в условиях in vitro использование жидкой питательной среды МС с добавлением препарата БАП в дозировке 0,5 мг/л наиболее эффективно, так как при этом показатели прироста высоты растений, листообразование и корнеобразование более высокие. Таким образом, используя жидкую питательную

среду с препаратом БАП в дозировке 0,5 мг/л, с за короткий срок можно получить полноценные сформированные растения с хорошо развитой корневой системой и развитым листовым аппаратом. Это имеет большое практическое значение для ускорения наращивания стартового материала земляники ценных сортов, пригодного для дальнейшего размножения в производстве.

Ключевые слова: земляника, питательная среда, развитие, in vitro, высота, корнеобразование.

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