Optimization of stages of clonal micropropagation of garden strawberry varieties Nelly and Kemiya breeding of NCFSCHVW

M.V. Karpushina*, and *M.V.* Vinter

Federal State Budget Scientific Institution «North Caucasian Federal Scientific Center of Horticulture, Viticulture, Wine-making», 40 Let Pobedy str., 39, 350901 Krasnodar, Russia

Abstract. The production of strawberry planting material using the method of clonal micropropagation of plants is promising, modern and already widely used. This paper presents the results of assessing the breeding potential of garden strawberry varieties Nelly and Kemiya (selection of FSBSI NCFSCHVW) in in vitro culture. Apexes from growing rosettes of strawberries served as the starting material for the initiation. The best survival rate of explants was noted during the initiation in the second decade of August, which were in Nelly variety - 90%, Kemiya - 83.3%. At the stage of proliferation, 6-Benzylaminopurine (6-BAP) was injected into the culture medium at various concentrations (0; 0.5; 1.0 and 1.5 mg / L). To increase the efficiency of micropropagation of strawberries Nelly and Kemiya varieties, it is advisable to add 6-BAP into the culture medium with the amount of 1.0 mg / l. In this variant, 10.6 shoots were formed in the explants in Nelly variety, 5.9 in Kemiya variety. With an increase of the concentration of 6-BAP to 1.5 mg / l, the number of vitrified shoots were raised. According to the results of three subcultures, it was noted that the Nelly variety has a higher reproduction potential compared to the Kemiya variety. At the stage of rhizogenesis, the addition of auxins to the medium was not required, since the roots of regenerated plants formed independently.

1 Introduction

Strawberry is the most popular berry crop in the world due to its taste, aroma, antioxidant capacity, high availability of anthocyanins, vitamins, flavonoids and other nutrients [1, 2]. Cultivation of strawberry has significantly increased in the recent years, due to its profitability in production and high consumer demand.

In the North Caucasus region, the total area of strawberry plantations is more than 15 thousand hectares [3]. The largest areas of strawberry planting are in the republics of Kabardino-Balkaria (542.5 hectares), Adygea (56.7 hectares), as well as in Krasnodar region (167.6 hectares), and Stavropol region (96.9 hectares) [4]. Currently the main obstacle in the expansion of strawberry production is the lack of high-quality domestic strawberry planting material in the required amount.

* Corresponding author: markarp@yandex.ru

Garden strawberry is a hybrid of two species, Frageria chiloensis and Frageria virginiana. All modern strawberry varieties are octaploid (2n = 56) and are well adapted to a wide range of climatic conditions [5].

Traditionally, strawberry planting material is obtained in a vegetative way, by rooted rosettes. The usage of the *in vitro* clonal micropropagation method provides an alternative opportunity to increase the production of strawberry planting material, which is potentially free from harmful organisms transmitted from mother plant during vegetative propagation by the rosettes [6, 7].

The improvement of the method of clonal micropropagation of garden strawberries *in vitro* has been carried out for many years. In the literature there is a fairly large amount of data on the composition of culture media, the effect of growth regulators on the efficiency of reproduction, protocols for sterilization of explants, etc. [2, 6, 8-13] However, the assortment of garden strawberry is constantly updating, new varieties are created, the genotypic reactions of which *in vitro* conditions are not known yet.

In this regard, the goal of our research was to optimize the stages of clonal micropropagation of garden strawberry varieties of NCFSICHVW and to assess their reproduction potential under *in vitro* conditions.

2 Materials and methods

The studies were carried out in the laboratory of virology of the FSBSI NCFSCHVW in 2019-2020. Objects of the research: garden strawberry varieties Nelly and Kemiya, selected by FSBSI NCFSCHVW.

Nelly is a medium late ripening variety with a yield of 15-20 t / ha. The advantages of the variety are a high level of adaptation to unfavorable abiotic and biotic environmental factors, resistance to powdery mildew and verticillary wilt, winter hardiness, and high commercial qualities of berries.

Kemiya is a late ripening variety that combines high adaptability, large fruit size, attractive berries, manufacturability, and high resistance to fungal diseases. Productivity 15-20 t / ha [5]. Explants from growing rosettes of strawberries were used as a starting material.

Sterilization of the plant material was carried out according to the scheme: preliminary preparation and basic processing. First, the prepared segments were rinsed under running tap water for 30 minutes. The main processing of explants was carried out with a solution of NaOCl, with a concentration of 1.5% for 5 minutes, followed by 3 times washing with bidistilled water for 5 minutes. The explants were isolated under aseptic conditions in the laminar boxes of the BAVnp-01-Laminar-S -1.2 brand and planted in the test tubes with a culture medium. The initiation to the culture was carried out in three terms: in the second decades of June, July and August. As the basis for the culture medium, we used a medium according to the prescription of Murashige and Skoog (MS) [14]. At the stage of initiation: MS medium without hormonal, at the proliferation stage with the addition of 6-BAP (0.5; 1.0; 1.5 mg / L), at the stage of rooting, ½ MS medium with the addition of sucrose 20 g / L, without auxins. After the introduction into the culture, the explants were placed in the dark for 3-5 days. The plants were cultivated under a 16-hour photoperiod (light / dark 16/8 hours) at a temperature of 25 ± 2 ° C and illumination of 2500-3000 lux.

The rooted plants were adapted to ex vitro conditions after 4-6 weeks of rooting. The plants were planted in mini-greenhouses containing a sterilized soil-peat mixture, perlite, and vermiculite (3: 1: 1), watered with a ½ solution of MC macro salts, and kept in a phytotron for a 16-hour photoperiod, 22 ± 2 ° C at 6000 lux. The percentage of plant survival was recorded 20 days after transplanting.

3 Results and discussion

To determine the most favorable period for the introduction of strawberries of varieties Nelly and Kemiya into in vitro culture, initiation was carried out in three periods: June, July and August (Fig. 1).

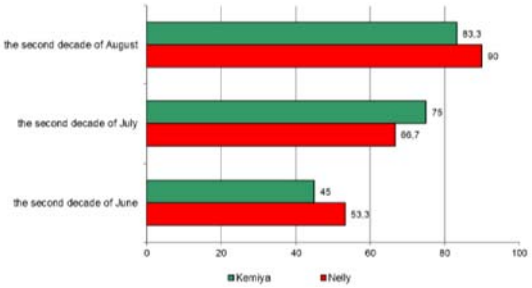

Fig. 1. Efficiency of in vitro introduction of strawberry explants in terms of initiation time

According to the results of the experiment, the late summer period is the best time for initiationg the strawberry varieties Nelly and Kemiya into *in vitro* culture. The highest *in vitro* survival rate of explants in both varieties was noted when introduced in the second decade of August. The maximum amount of regenerated explants was 90% for the Nelly variety, and 83.3% for the Kemiya variety.

To determine the optimal concentration of cytokinins at the stage of strawberry multiplication, shoots were transplanted onto Murashige-Skoog (MS) medium with different content of 6-BAP: 0; 0.5; 1.0 and 1.5 mg / l. (Table 1, Fig. 2).

After the first passage, the largest number of shoots on average in both varieties was observed on the medium with the highest amount of 6-BAP (1.5 mg / l), 11.6 shoots in variety Nelly; 9.8 - in the Kemiya variety. However, only a few shoots were suitable for further propagation, since most of them were vitrified.

The most optimal concentration of 6-BAP in the culture medium for the Kemiya variety is 0.5 - 0.1 mg / l, at which the largest number of viable microshoots was formed 4.95 and 5.9, respectively, while in the control variant, without adding 6 -BAP, on average 2.05 shoots were formed.Variety Nelly had a higher breeding potential compared to Kemiya variety. On a hormone-free environment, 3.1 new shoots were formed per explant. The best option for effective multiplication of shoots for this variety is also a medium with 6-BAP - 1.0 mg / l, on which from one micro-shoot to 10.6 new ones were formed. In the variant with 6-BAP - 0.5 mg / l, an average of 8.3 shoots were formed.

Sequential subculturing of strawberries during three passages showed that at the stage of proliferation, the Nelly variety had a higher regenerative capacity. On a medium supplemented with 6-BAP in an amount of 1.0 mg / l, an average of 10.6 shoots per explant were formed in the first passage, and 12.6 in the third passage; in the variety Kemiya on the same medium, an average of 5.9 shoots were formed in the first passage; 9.2 - on the second.

Table 1. The multiplication factors of Kemiya and Nelly strawberries, depending on the number of subcultures

Variety	Concentration, mg / l	Passage number		
		1	2	3
Kemiya	6-БАП 0	2,05	2,9	2,45
	6-БАП 0,5	4,95	6,3	7,9
	6-БАП 1,0	5,9	9,2	8,7
	6-БАП 1,5	9,8	10,7	12,7

	LSD	*0,8074*	*0,8830*	*0,8082*
Nelly				
	6-БАП 0	3,1	3,9	3,3
	6-БАП 0,5	8,3	10,1	9,8
	6-БАП 1,0	**10,6**	**11,4**	**12,6**
	6-БАП 1,5	11,5	11,1	10,8
	LSD	*0,9335*	*0,8616*	*0,8279*

One-way ANOVA
Kemiya 2 passage
RESULTS

Groups	Score	Sum	Average	Varian
control	20	58	2,9	0,621053
BAP-0,5	20	126	6,3	2,010526
BAP-1,0	20	184	9,2	2,063158
BAP-1,5	20	214	10,7	3,168421

ANOVA

ce of varia	SS	df	MS	F	P value	value critic
Between	710,55	3	236,85	120,4859	8,35E-29	2,724944
Within gro	149,4	76	1,965789			
Total	859,95	79				
	LSD0,05	0,883052				

One-way ANOVA
Kemiya 3 passage
RESULTS

Groups	Score	Sum	Average	Varian
control	20	49	2,45	0,365789
BAP-0,5	20	158	7,9	1,463158
BAP-1,0	20	174	8,7	1,063158
BAP-1,5	20	254	12,7	3,694737

ANOVA

ce of varia	SS	df	MS	F	value critic	value critical
Between g	1067,558	3	355,8458	216,095	4,15E-37	2,724944
Within gro	125,15	76	1,646711			
Total	1192,688	79				
	LSD 0,05	0,808214				

One-way ANOVA
Kemiya 1 passage
RESULTS

Groups	Score	Sum	Average	Varian
control	20	41	2,05	0,786842
BAP-0,5	20	99	4,95	1,944737
BAP-1,0	20	118	5,9	1,357895
BAP-1,5	20	196	9,8	2,484211

ANOVA

ce of varia	SS	df	MS	F	P value	value critic
Between	614,65	3	204,8833	124,6668	2,86E-29	2,724944
Within gro	124,9	76	1,643421			
Total	739,55	79				
	LSD0,05	0,807407				

Concentration, 6-BAP 0,5 mg / l

Concentration, 6-BAP 1,0 mg / l

Concentration, 6-BAP 1,5 mg / l

Fig. 2. Influence of 6-BAP concentration on the reproduction of strawberries, Kemiya variety

According to the authors of the studied varieties, V.V. Yakovenko and others, the variety Nelly in the conditions of the mother plant has a high adaptive capacity (more than 50 rosettes from the mother bush), the Kemiya variety has an average (no more than 30 rosettes). This is probably explains why, in addition to the concentration of cytokinin and the number of subcultures, the varietal characteristics of strawberries affect the level of shoot proliferation under *in vitro* conditions. According to O.V. Matushkina, I.N. Pronina (2012) under *in vitro* conditions at the first passages high shoot formation was shown by the strawberry varieties Sudarushka (12.8-13.2 pcs. / Explant), Elsanta (9.3-11.8 pcs. / Explant), Kamarossa (7, 2-9.6 pcs / explant) [5, 15].

Rooting of strawberry microplants was carried out on Murashige-Skoog medium with half the content of macrosalts, adding sucrose in an amount of 20 g / l. The use of auxins at

the stage of rhizogenesis of varieties Kemiya and Nelly was not required, because after 8-10 days the plants began to form roots and after 3-4 weeks on a hormone-free rooting medium, the plants were ready for transplantation into non-sterile conditions (Fig. 3). In the Nelly variety, the percentage of root formation was 95%, in the Kemiya variety - 98%.

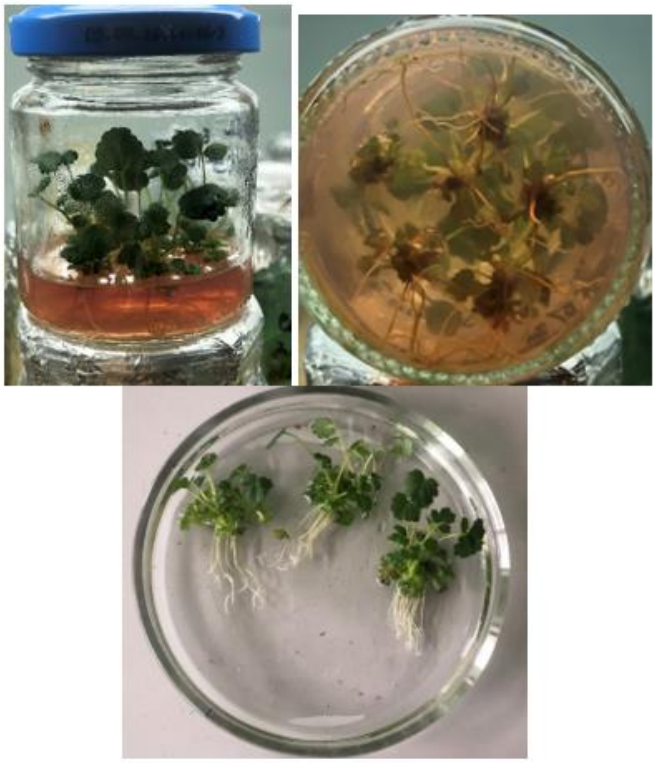

Fig. 3. Rooting of strawberries on a hormone-free culture medium MS, Nelly variety

The transfer of plants from *in vitro* conditions to non-sterile conditions is one of the crucial stages in the process of clonal micropropagation. However, strawberry is one of those crops that is highly adaptable and stress-resistant when transferred from *in vitro* to *ex situ* conditions.

To transplant regenerant plants, a soil-peat mixture, perlite and vermiculite in a ratio of 3:1:1 were used. The adaptation was carried out in mini-greenhouses. Plants were kept in a phytotron with a 16-hour photoperiod, temperatures of 22 ± 2 ° C and illumination of 6000 lux. The average percentage of adapted plants is on average 80 - 95%. (Fig. 4).

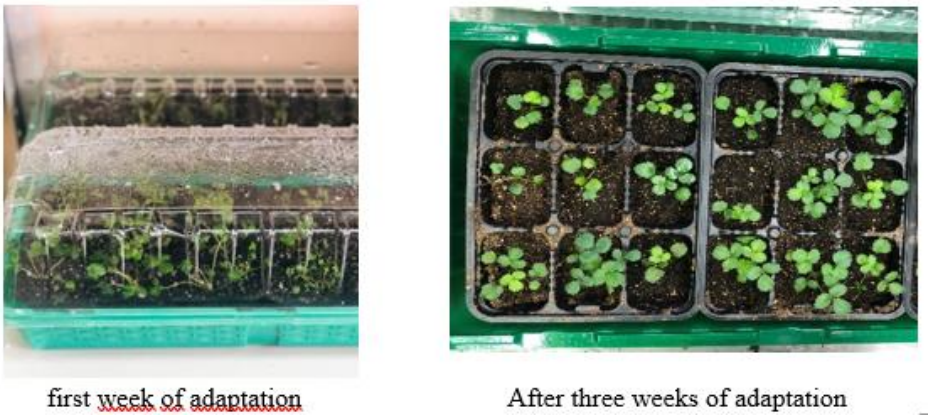

Fig. 4. Adaptation of strawberry microplants, Nelly variety

After 1-1/5 months, the adapted plants were transplanted into containers with a volume of 150-200 ml (Figure 5a). After another two months of growing in the greenhouse, the strawberry new plant's rosettes began to formulate (Figure 5.b).

Fig. 5. Strawberry plants after 1/5 month of adaptation, Nelly variety

4 Conclusions

The introduction of explants of garden strawberry Nelly and Kemiya varieties into *in vitro* culture is more effective in the late summer period, in the second decade of August. Then the survival rate of explants is on average 83.3% for the Kemiya variety, and 90% for Nelly. At the stage of proliferation, the most effective amount of 6-BAP into the medium culture was 1.0 mg / L. In this variant, 5.9 shoots were formed in the explants of the Kemiya variety, and 10.6 in the Nelli variety. With the increase of concentration of 6-BAP to 1.5 mg / l, the number of vitrified shoots increased. In general, the Nelly variety had a higher reproductive potential compared to the Kemiya variety. At the stage of rhizogenesis in regenerant plants, the formation of roots took place on a half-medium Murashige-Skoog, without the addition of auxins. Roots were formed in 95-98% of the plants. The rooted plants successfully adapted in a substrate consisting of a soil-peat mixture, perlite and vermiculite in a ratio of 3: 1: 1.

References

1. Global consumption of strawberries increases annually (2018) https://www.freshplaza.com
2. O.V. Matsneva, L.V. Tashmatova, N.YU. Orlova, V.V. Shakhov, Selection and cultivation of garden crops, **4(1-2),** 93 (2017)
3. V.Yakovenko V.I. Lapshin, Scientific works SKFNTSSVV, **14**, 147 (2018)
4. I. Koziy, Yagody Rossii, **1**, 3 (2020)
5. FGBSO "North Caucasian Region Research Institute of Horticulture and Viticulture", https://kubansad.ru
6. K. Moradi, M. Otroshy, M.R. Azimi, Journal of Agricultural Technology, **7(6),** 1755 (2011)
7. K. Mahmoud Ben, A.Najar, E. Jedid, Jema, A. Jemmali, Journal of new sciences, Agroculture and Biotechnology, **47(2),** 2564 (2017)
8. T. Ara, R. Karim, M.R.Karim, Sh. Ahmad, R. Islam, International Journal of Biosciences, **10(1),** 86 (2012)
9. K. T. Oo, K. S. Oo, Y.Y. Mon, Journal of Scientific and Innovative Research, **7(3),** 70 (2018).
10. Rattanpal, Harinder& Gill, Manav&Sangwan, Anil, 149 (2011)
11. Ionela, Rusea&Popescu, Aurel& Valentina, Isac& Dorel, Hoza, **24,** 218 (2020)
12. A. Jan, K. M. Bhat, Bhat, S. J. A., M. A. Mir1, M. A. Bhat, Imtiyaz A.,Wani, J. A. Rather, *Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for in vitro culture*, 12(39), 5749 (2013).
13. Jhajhra, Sunita & Dashora, L.K. & Singh, Jitendra & Bhatnagar, Prerak & Kumar, Ashok & Arya, International Journal of Current Microbiology and Applied Sciences, **7,** 3030 (2018)
14. T. Murashige, F.A. Skoog, Physiol Plant, **15(3),** 473 (1962)
15. O.V. Matushkina, I.N. Pronina, *Clonal micro-freezing technologies for strawberries*, 20 (2012)