

# The effectiveness of flow-through substrateless hydroponics in the adaptation of strawberry microplants

Sergey Kornatskiy\*, Milana Kuzmina, Zhanna Serebryakova, and Anastasia Kudrina

RUDN University, 117198 Moscow, Russia

**Abstract** A new approach to the adaptation of test-tube strawberry microplants under flow-through hydroponics conditions was tested. The study was carried out on strawberry cultivars “Asia”, “Florence”, “Kimberly”. Murashige-Skoog medium was used for in vitro cultivation. During propagation,  $1.0 \text{ mg}\cdot\text{L}^{-1}$  6-BAP was added to the medium, during elongation -  $0.05 \text{ mg}\cdot\text{L}^{-1}$  6-BAP, and during rooting -  $1.0 \text{ mg}\cdot\text{L}^{-1}$  IBA. Plants were grown in vitro in a light room at a temperature of  $23\pm 1^\circ\text{C}$ , a 16-hour photoperiod and a light intensity of 5-6 klx. Flow hydroponics was used to adapt microplants. Plastic containers with dimensions of  $0.6 \times 0.4 \times 0.04 \text{ m}$  (volume 9 l), specially prepared for working in a hydroponic system, were taken. They were covered on top with non-woven polymer material, perforated with planting holes with a diameter of 3-4 mm according to a 4 x 4 cm pattern (150 pcs/box), into which plants were planted. This reduced labor costs for adaptation and ensured the efficiency of the operation to 86-100% within 1 month.

## 1 Introduction

The importance of strawberries as a valuable berry crop is often mentioned in scientific and specialized literature all over the world. This interest is due to the high consumer properties of its berries and the wide spread of the crop in different soil and climatic conditions. Accordingly, a lot of efforts are directed to the development of effective cultivation technologies not only for commercial plantations, but also for the cultivation of planting material for their establishment. New opportunities in solving these problems have opened up with the development of biotechnological methods in crop production. Strawberries turned out to be a successful model crop, with which the elements of the method of clonal micropropagation were tested. A great deal of experimental experience and quite a lot of information concerning the success of cloning of strawberry varieties and species, the peculiarities of cultivation and subsequent growth of test tube plants in the open ground have been accumulated so far [1-4]. In many respects, the achievements are explained by the good evolutionary adaptation of the ancestral species and plant forms to vegetative propagation, of which clonal micropropagation is essentially a variant. Moreover, aseptic conditions allow for a fuller disclosure of the biological potential of the crop in terms of reproduction rate. The

---

\* Corresponding author: [kornatskiy\\_sa@pfur.ru](mailto:kornatskiy_sa@pfur.ru)

most active work on strawberry micropropagation has been carried out to obtain and accelerate the multiplication of virus-free material [5]. Also, clonal micropropagation is considered to be the fastest and most efficient way to propagate new and promising cultivars [6-8]. If we evaluate the level of manufacturability of the method, we can conclude that, as a rule, the stage of strawberry introduction into culture has been successfully solved in all known cases. The micropropagation proper, for the most part, does not present any problems either. However, in the course of long-term subcultivation, according to reports of a number of researchers, some varieties show specific requirements to environmental factors, mineral and hormonal composition of nutrient media [9-12]. Emerging problems are also solved by selecting the duration of passages, taking preventive measures to overcome vitrification, etc. [13]. In micropropagation of strawberries, the stage of shoot elongation was very useful, when it is possible to obtain microrosettes more suitable for subsequent rooting. However, the issue of using auxins at the stage of rhizogenesis in strawberry remains debatable, since spontaneous and quite effective root formation in microplants directly at the proliferation stage, even in the presence of cytokinins, is evident [14-16]. This indicates that the use of auxins at this stage is not necessary, although rooting starts and proceeds faster in their presence. However, the qualitative parameters of roots induced by exogenous auxin are physiologically much inferior to those formed spontaneously or on a hormone-free medium. It should be taken into account that the condition and structure of the root system largely determines the possibility of successful subsequent adaptation of microplants to non-sterile conditions. The known traditional adaptation schemes are very labor-intensive, as they imply preliminary sterilization of large volumes of soil substrate for planting microplants. In addition, preventive measures against the spread of soil pathogens are necessary, as well as maintaining air humidity in the leaf area close to 100%. When attempting to adapt relatively large numbers of microplants, these parameters are difficult to maintain and this often leads to very large losses of plants due to their death [17]. Therefore, research focused on dramatically increasing the viability of microplants during adaptation is very relevant.

The aim of the study was to investigate the possibilities of using flow hydroponics to improve the processability and efficiency of adaptation of strawberry microplants.

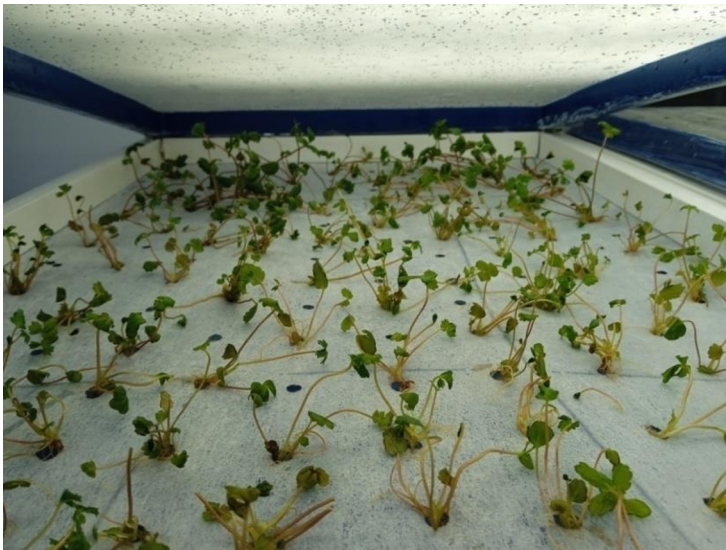
## 2 Materials and methods

The objects of research were modern and high-yielding strawberry varieties of European selection: Kimberly, Asia, Florence. In the process of micropropagation we implemented the basic elements of the *in vitro* method, partially modernized in the future taking into account the varietal specificity. Nutrient media for the stages of explants proliferation, elongation and rooting of micrografts were prepared according to Murashige-Skoog's prescription [18]. In the first two cases, benzylaminopurine (6-BAP) was used as growth regulators at concentrations of 1.0 mg/L and 0.05 mg/L, respectively. At the rhizogenesis stage, indolylbutyric acid ( $\beta$ -IBA) was added to the nutrient medium composition at a concentration of 1.0 mg/L instead of benzylaminopurine. A 16-hour photoperiod, illumination within 5-6 thousand lux at the level of the surface of culture vessels and plants, and temperature +20...+22°C in light rooms were maintained. Plastic boxes with dimensions 0.6 x 0.4 m x 0.04 m (volume 9 L) specially prepared for hydroponic system were used for adaptation of microplants. Plastic frames covered with nonwoven polymeric material (Lutrasil, 17 g/m<sup>2</sup>) were placed on top of them. Before planting the plants, the surface of the material was perforated with planting holes 3-4 mm in diameter with a planting pattern of 4 x 4 cm (150 pieces/box). Microplants were removed from the tubes and prepared for planting by washing the roots from agarized nutrient medium. The roots of the microplants were passed through the planting holes using fine tweezers so that the microrosettes remained on the surface of the nonwoven material. The leaves were periodically moistened with distilled water using a

sprayer to maintain leaf turgor during planting. After planting was completed, the frames with plants were placed on boxes and covered with transparent polyethylene film hoods to maintain light and humidity regimes. Adaptation was carried out during 1 month, during the last week of which the film hoods were partially lifted for airing and reducing air humidity. The significance of the differences in the experiments was evaluated using the LSD test at a 5% significance level [19].

### 3 Results and discussion

Given the natural ability of easy rooting of strawberry runners and rapid development of rosettes in contact with moist soil or substrate surface in natural conditions, as well as the fact that the method of clonal micropropagation is realized using a nutrient medium consisting of almost 96% water, we developed a scheme of adaptation of microplants on flowing hydroponics. This approach allows us to simultaneously remove a number of problems accompanying this process. When planting on a water solution, the substrate on which rooting takes place practically does not change. It is the same distilled water with mineral salts, which does not have only such components as growth regulators, carbohydrates and agar. But at the same time, the water is enriched with oxygen during circulation, which is impossible during *in vitro* cultivation. As observations have shown, this has a positive effect on the activation of the development of new roots, which, unlike the existing ones, visually have a completely different structure. Moreover, the use of aqueous solution practically does not require preventive measures against the spread of pathogenic microflora, which progressively develops in the case of using organic and organic-mineral substrates during the adaptation period and is the cause of mass death of microplants. The air humidity maintenance regime is also provided equally with *in vitro* conditions. Since the stem part of the plants is placed over a fully humid surface, it does not require additional moistening of the leaves by spraying, which simplifies care. The beginning of the adaptation process is illustrated in Figures 1 and 2.



**Fig. 1.** Strawberry microplants during planting.



**Fig. 2.** Microplants 10 days after planting.

As observations of plants planted for adaptation at flowing hydroponics have shown, initial morphometric indices of microplants are essential and determine the rate of their subsequent development. The most important indicator turned out to be the diameter of the stem base. Due to the larger base area, new roots developed faster and more efficiently, which contributed to a corresponding increase in plant height and root length (Table 1).

**Table 1.** Development of strawberry microplants in the process of adaptation to non-sterile conditions using flow hydroponics\*

Strawberries cultivar	Morphometric indicators of microplants	Stem base diameter, mm	Length roots, cm	Plant height, cm	Safety of plants during adaptation, %
<i>Asia</i>	when planting on hydroponics	1.3 <sup>a</sup> **	2.4 <sup>a</sup>	3.7 <sup>a</sup>	-
		3.2 <sup>b</sup> ***	2.6 <sup>a</sup>	3.8 <sup>a</sup>	-
	after 1 month of adaptation	1.8 <sup>a</sup>	4.7 <sup>ab</sup>	6.4 <sup>b</sup>	100
		4.3 <sup>b</sup>	6.7 <sup>b</sup>	9.5 <sup>c</sup>	100
<i>Florence</i>	when planting on hydroponics	1.2 <sup>a</sup> **	2.7 <sup>a</sup>	4.1 <sup>a</sup>	-
		3.1 <sup>b</sup> ***	2.8 <sup>a</sup>	4.0 <sup>a</sup>	-
	after 1 month of adaptation	1.6 <sup>a</sup>	4.3 <sup>ab</sup>	6.7 <sup>b</sup>	86
		3.9 <sup>b</sup>	6.5 <sup>b</sup>	9.9 <sup>c</sup>	100
<i>Kimberly</i>	when planting on hydroponics	1.2 <sup>a</sup> **	2.3 <sup>a</sup>	3.6 <sup>a</sup>	-
		3.2 <sup>b</sup> ***	2.6 <sup>a</sup>	3.9 <sup>a</sup>	-
	after 1 month of adaptation	2.0 <sup>a</sup>	4.1 <sup>ab</sup>	6.8 <sup>b</sup>	93
		4.2 <sup>b</sup>	6.2 <sup>b</sup>	9.6 <sup>c</sup>	100

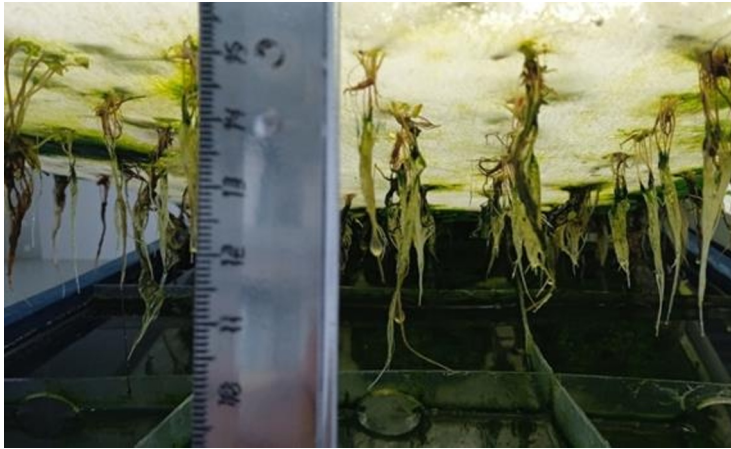
\*Means followed by different letters in the column differ from Duncan's multiple range test ( $P < 0.05$ ).

\*\* - a group of plants with a stem base diameter of 1-2 mm

\*\*\* - a group of plants with a stem base diameter of 3-4 mm

Based on the results of the experiment presented in Table 1, we can conclude that there is no reason to talk about any varietal differences in the dynamics of plant development during adaptation, which was quite similar. Significant differences in morphometric parameters of plants 1 month after planting for adaptation in all cases compared to the initial ones are evident. In addition, the parameters of microplants that had a heart base diameter of 3-4 mm at planting significantly exceeded those of the other group with a starting diameter

of 1-2 mm. However, the most significant result can be considered the high preservation of microplants during adaptation, which was in the range of 86-100%, which is little achievable in mass adaptation on substrate, based on personal and literature data [20]. Figure 3 shows the result of active development of new roots during adaptation, which stand out with a lighter tone and less thickness, while Figure 4 shows the general condition of plants that have undergone adaptation.



**Fig. 3.** Root system of microplants after 1 month of adaptation.



**Fig. 4.** Microplants adapted to hydroponics: *a* - cultivar *Florence* and *b* - cultivar *Kimberly*

## 4 Conclusions

Based on the conducted studies, there is every reason to believe that the developed approach to adaptation of *in vitro* microplants of strawberry can be successfully used to increase the efficiency of clonal micropropagation of this crop. An obvious reduction in the labor intensity of the process of adaptation of microplants to non-sterile conditions is achieved by avoiding the use of any solid substrate. In this case, respectively, there is no need for preliminary energy-intensive preparation of it by high-temperature disinfection from pathogenic microflora. Planting microplants, which initially requires sufficiently qualified personnel, is also greatly simplified. High viability of microplants during the adaptation period is ensured by the fact that the roots of plants are partially immersed in water containing the necessary mineral salts, which circulates with a certain periodicity in a closed system.

The publication has been prepared with the support of the Ministry of Science and Higher Education of the Russian Federation (project number FSSF-2023-0014)

## References

1. P. Toppo, G. Sharma, P. Singh, H. Panigrahi, L. Verma, *Pharma Inn. J.* **12**, 5 (2023)
2. K. Moradi, M. Otroshy, M. Azimi, *J. Agric.Tech.* **7**, 6 (2011)
3. M. Ashrafuzzaman, M. Faisal, D. Yadav, D. Khanam, F. Raihan, *Bangladesh J. Agric. Res.* **38**, 3 (2013)
4. R. Cappelletti, S. Sabbadini, B. Mezzetti, *Sci. Hortic. (Amsterdam)* **207** (2016)
5. C.-Y. Ko, A. Al-Abdulkarim, S. Al-Jowid, A. Al-Baiz, *African Journal of Biotechnology* **8**, 11 (2009)
6. M. Hasan, S. Nigar, M. Rabbi, S. Mizan, M. Rahman, *Int. J. Sustain. Crop Prod.* **5**, 4 (2010)
7. S. Nunes, D. Sousa, V. Pereira, S. Correia, L. Marum, C. Santos and M. Dias, *In Vitro Cell. Dev. Biol. Plant* **54**, 2 (2018)
8. I. Iliev, A. Gajdoov, G. Libiakov, S. Jain, *Plant Cell Culture: Essential Methods* (Wiley-Blackwell, Chichester, 2010)
9. M. Munir, S. Iqbal, J. Baloch and A. Khakwani, *J. Appl. Hortic.* **17**, 3 (2015)
10. H. Sharma, *Int. J. Adv. Res.* **5** (2017)
11. S. El-Sayed, A. El-Sawy and S. Taha, *J. Plant Breed.* **21** (2017)
12. D. Baladeh, B. Kaviani, *Int. J. Fruit Sci.* **21**, 1 (2021)
13. S. Sabbadinia, M. Marcellini, B. Mezzetti, F. Capocasa, *Acta Hortic.* **1309** (2021)
14. M. Dhukate, M. Kher, A. Vadawale, P. Giri, *Environmental and Experimental Biology* **19** (2021)
15. A. Kadhimi, A. Alhasnawi, A. Mohamad, W. Yusoff, C. Binti, C. Zain, *Life Sci. J.* **11**, 8 (2014)
16. F. Capocasa, F. Balducci, M. Marcellini, D. Bernardini, O. Navacchi and B. Mezzetti, *Plant Cell Tissue Organ Cult* **136**, 1, (2019)
17. A. Batukaev, S. Kornatskiy, M. Gaplaev, *IOP Conf. Series: Earth and Environmental Science* **624**, (2021)
18. T. Murashige, F. Skoog, *Physiol. Plant.* **15** (1962)
19. R. Steel, J. Torrie, *Principle of statistics. A biometrical approach 2<sup>nd</sup>* (McGraw-Hill Kogakusha, 1980)
20. S. Kornatskiy, *E3S Web Conf.* **486** (2024)