UDC 602:57.085.2:634.75 https://doi.org/10.21498/2518-1017.16.3.2020.214925

In vivo adaptation of regenerant plants of *Fragaria vesca* L. cultivars

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Purpose. The adaptation of regenerant plants to environmental conditions is the final stage of micropropagation. According to a number of authors, when *in vitro* plants are transferred to *in vivo* non-sterile conditions, a significant percentage of mortality is recorded. In a previous publication, the regenerative capacity of strawberry (*Fragaria vesca* L.) *in vitro* tissues on MS culture medium (Murashige & Skoog, 1962) and a regenerants was obtained (Chornobrov O. Yu., 2019). The objective of the study is to develop an optimal protocol of acclimation of *in vitro F. vesca* plants to *in vivo* conditions. **Methods.** Biotechnological and statistical methods of research were applied. For the research 'Ruiana' and 'Zhovte Dyvo' cultivars were used with *in vitro* cultivation cycle of 30–35 days. Prepared plants were planted in 0.33 L plastic containers, one piece in a mixture of coconut substrate and perlite (3:1). Plants were kept under high relative humidity (85–90%) conditions for 3–5 days, 6–8 days and 10–14 days. The studies were carried out in the Plant Biotechnology Laboratory of SS of NULES of Ukraine "BFRS" during 2019–2020. **Results.** The duration of *Fragaria vesca* regenerant plants exposure in conditions of high relative humidity significantly affected adaptation efficiency. The proportion of 'Ruiana' and 'Zhovte Dyvo' plants adapted to the greenhouse conditions were 47.6 ± 2.5% and 60.0 ± 1.7%, respectively, when the plants were kept for 10–14 days. A significant efficiency of plant adaptation (more than 70%) was obtained under condition of preliminarily exposure the roots of the plants in an auxin solution for 25–30 minutes with daily application of 30% solution of glycerine as foliar spray. The plants adapted to the greenhouse conditions had pigmentation characteristic of the variety, without signs of chlorosis and vitrification. **Conclusions.** An optimal protocol for in vitro adaptation of *F. vesca* cultivars to *in vivo* conditions was developed and viable plants were obtained. Further research will be aimed at studying the growth and development of *F. vesca* regenerant plants in open ground.

Keywords: *wild strawberry; in vitro plant tissue culture; microclonal propagation; ex vitro acclimation; plant viability.*

Introduction

Micropropagation is widely used to obtain a healthy genetically homogeneous planting material for berry plants, in particular, strawberries (*Fragaria vesca* L.) to create high-quality plant nurseries [1–9]. Traditional plant propagation in a vegetative way spreads viral, bacterial and fungal microbiota. In addition, such reproduction makes it impossible to obtain healthy plants and does not always meet the modern requirements of consumers [4, 5].

The final stage of micropropagation is the adaptation of regenerant plants to environmental conditions (indoor and outdoor). According to the research results, when *in vitro* plants are transferred to non-sterile conditions of a closed ground, a significant percentage of their mortality is recorded [1, 3, 10, 11]. In the case of replanting, the plants are subjected to a sharp change in the relative humidity. Plants are especially sensitive to dehydration immediately after opening the culture tubes, which is associated with many anatomical features: thin cuticle, which contains little wax and wax-like substances; a small amount of mechanical tissues with thin leaves; poorly developed vascular bundles; limited stomata function [1, 12].

In vitro plant water loss occurs mainly through stomata, which do not function for 10–14 days after replaning [13]. Many plant species require gradual changes in environmental conditions to avoid dehydration. In particular, for *Fragaria ananassa* Duch ('Nikte') regenerants two-stage adaptation, consisted of a previous 20-day acclimatization at 25 ± 1 °C and 60% relative humidity (RH), followed by

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holding in a greenhouse under a protective mesh with a 75% degree shading and watering three times a week with nutrient solution, was effective (Hoagland & Arnon, 1950). At the first stage of adaptation, the plants replanted in a mixture of peat, perlite, and vermiculite (1: 1: 1) were covered on top with transparent 1.0 liter plastic containers. Under such conditions, plant survival was 91.9% [4].

Providing appropriate levels of plant nutrition – mineral, air and water regimes, and compliance with a gradual change in temperature and relative humidity of the air is important during the adaptation of regenerat plants to environmental conditions [1]. The authors recommend to adapt the 4-week-old regenerant plants of the strawberry cultivar 'Alba' obtained on the MS nutrient medium (Murashige & Skoog, 1962) [14] with 0.25–0.35 mg · l^{-1} BA (6-benzylaminopurine). The indicated BA concentrations did not cause anomalies and ensured the production of plants genetically identical to donors. The regenerants were planted in 100 ml containers in a substrate of peat (80%) and perlite (20%), followed by 40-day acclimatization in a greenhouse [15].

The condition of the root system is of great importance for the plants adaptation [10]; the place of roots laying affects the viability of rooted plants *in vitro* [2]. To prevent dehydration of regenerants, it is recommended to spray them with a 50% solution of glycerin in diethyl ether [16].

The duration and effectiveness of regenerant plants adaptation is determined by a number of factors, in particular, the characteristics of the genotype, therefore, a separate protocol is developed for each variety. In the previous publication [17], the regenerative capacity of strawberry plants *in vitro* tissues on a nutrient medium according to the MS prescription was indicated and regenerants were obtained.

The aim of the study was to develop an optimal protocol for the adaptation of regenerated plants of *F. vesca* cultivars to *in vivo* conditions.

Materials and methods

In vitro plants of 'Ruiana' and 'Zhovte Dyvo' strawberry varieties were obtained using biotechnological methods (*in vitro* plant tissue culture, microclonal reproduction) by various types of *in vitro* morphogenesis (activation of growth of existing meristem explants and direct morphogenesis). For the research, we used 30–35 day old regenerant plants obtained on modified MS nutrient (Fig. 1 a, b) according to the generally accepted method [12]. Plants were removed from test tubes with tweezers, the root system was washed from the remains of the nutrient medium in tap water, followed by transfer to 0.001% $KMnO_4$ (up to 1 min). The plants were planted in 0.33 L plastic containers, 1 pc. into a mixture of coconut substrate and perlite (3: 1). Regenerants were fed once every 30 days with a solution of macro- and micro-salts according to MS. Plants were kept in conditions of high RH (85–90%) for 3–5 days, 6–8 days and 10–14 days. RH was determined using a digital thermo-hygrometer. To maintain given conditions, containers with plants were covered with transparent plastic containers (Fig. 1 c). The high RH was reduced to 60–70% gradually by artificial ventilation. Plants were sprayed with water when signs of wilting appeared. Plants were grown in an adaptation room under Osram Fluora phytolamps (lighting 3.0–4.0 CLA, 16-h photoperiod) at a temperature of 21 ± 2 °C. Plant survival was recorded after the appearance of new leaves (22–30 days of adaptation).

To increase the number of rooted plants, the root system before planting in the soil mixture was kept for 25–30 min in a solution of auxins (1.0 mg · L^{-1} 3-IAA (β-indolyl-3-acetic acid), 1.0 mg · L^{-1} NAA (α-naphthylacetic acid), 1.0 mg · L^{-1} BCI (3-indolylbutyric acid). To prevent wilting, the leaves were daily sprayed with 30% glycerin solution. Plants that grew in the usual adaptation mode (not treated auxin and glycerin).

The experimental data were processed using the MS Excel software. The repeatability of each variant of the experiment was 5-fold (10–20 plants were used in the experiment). The studies were carried out in the research laboratory of plant biotechnology, Plant Biotechnology Laboratory of SS of NULES of Ukraine "BFRS" during 2019–2020.

Results

It was found that the duration of exposure of regenerants in conditions of high RH significantly influenced the proportion of viable plants (Tables 1, 2).

Table 1

The efficiency of *F. vesca* regenerant plants adaptation to indoor conditions on the 30th day of cultivation

Variety	The share of adapted plants with different duration of cultivation in conditions of high RH, %		
	3–5	6–8	10–14
'Ruiana'	–	25.2 ± 1.8*	47.6 ± 2.5
'Zhovte Dyvo'	–	34.6 ± 1.6	60.0 ± 1.7

* Mean ± standard error.

Table 2

Results of one-way analysis of variance

Analysis of variance						
Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	$F_{crit.}$
Between groups	1254.4	1	1254.4	53.3787234	8.33966E-05	5.317655072
In the middle of groups	188	8	23,5	–	–	–
Total	1442.4	9	–	–	–	–

Note. *SS* – sum of squares; *df* – number of degrees of freedom; *MS* – dispersion; *F* – the calculated value of the Fisher criterion; *P-values* – calculated value of minimum significance; $F_{crit.}$ – the critical value of the Fisher criterion.

Plants exposure in conditions of high RH for 3–5 days caused their death on 7–10 days of cultivation. The proportion of viable plants can be reliably increased in the case of an increase in the duration of plants exposure from 6–8 days to 10–14 days. Under these conditions, the effectiveness of adaptation was: 'Ruiana' – 47.6 ± 2.5%, 'Zhovte Dyvo' – 60.0 ± 1.7%.

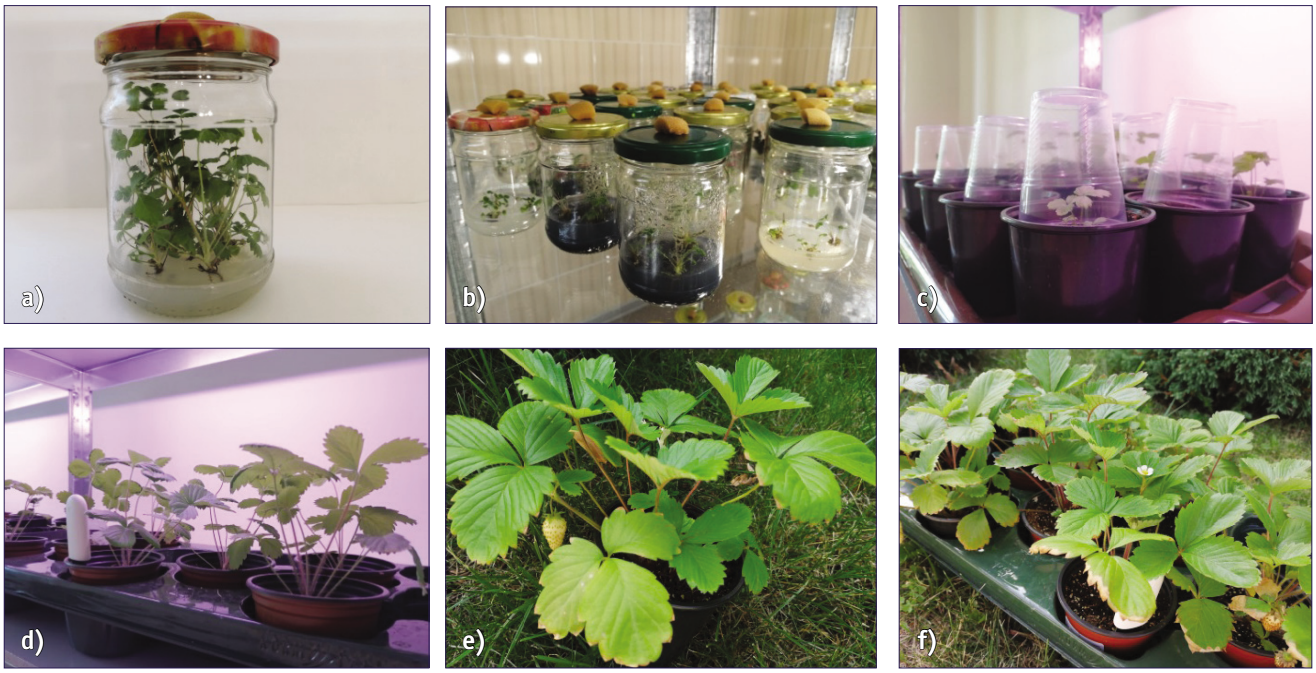

Fig. 1. The sequence of stages of *in vitro F. vesca* plants adaptation to open field conditions:
a) 30-day-old regenerated plants 'Ruiana' on a MS nutrient medium; b) *Fragaria* plants in the culture room; c) regenerants in conditions of high relative humidity under a plastic canopy; d) plants adapted to indoor conditions; e) and f) container culture of 'Zhovte Dyvo' and 'Ruiana' varieties in open field conditions

The effectiveness of gradual adaptation of *in vitro* strawberries to open field conditions was studied by a group of authors [4] and was consistent with the results of our research.

According to the results of I. V. Knyazeva's research [10], the degree of plants rooting is of great importance in adapting plants to non-sterile conditions, which in turn directly affects their survival rate. At the same time, it is important to maintain an optimal water balance, so glycerin was used to prevent dehydration. The characteristics of strawberry varieties growth with the use of auxins (IVC, NOC and IMC) with daily application of 30% glycerin solution as a foliar spray are given in table 3.

Exposure of regenerants root systems in a solution of auxins followed by daily application of 30% glycerin solution as a foliar spray significantly increased plant survival in relation to control, which is consistent with the results of other authors studies [16]. Under these conditions, the emergence of new photosynthetic leaves on the 22nd day with characteristic pigmentation was recorded. In general, the proportion of viable plants is significantly higher in the variety 'Zhovte Dyvo' than in 'Ruiana'. The difference in the rates of plant survival in different varieties was statistically significant ($F_{rated.}$ = 116.0360; $F_{crit.}$ = 5.3177; $F_{rated.} > F_{crit.}$). It was found that plants of 'Zhovte dyvo' variety were characterized by active growth. In par-

Table 3

Growth characteristics of *F. vesca* regenerant plants under conditions of closed ground, 30th day

Variety	Plant rooting under greenhouse conditions, %	The beginning of new photosynthetic leaves formation, days	Linear growth**	Plants pigmentation	Other signs
'Ruiana'	71.6 ± 2.7*	28–30	+++	green	no signs of chlorosis and vitrification were found
'Zhovte Dyvo'	82.8 ± 3.2	22–25	++	–	–

* Mean value ± standard error;

** Linear growth: (+++) – active (more than 2.0 cm); (++) – medium (1.0–1.9 cm); (+) – weak (less than 0.9 cm); (–) – absent.

ticular, on the 30th day of adaptation they were 6.0 ± 0.4 cm long (Fig. 1 d), while in 'Ruiana' – 4.6 ± 0.2 cm.

In the summer-autumn period, the container culture of plants was kept for 8–9 hours in open ground conditions with subsequent transfer to the adaptation room (Fig. 1 e). Under such adaptation conditions, the regenerants began to bloom on 8th–10th week. The beginning of fruiting in the 'Zhovte Dyvo' plants was recorded on 12th–13th week of cultivation, which is a week earlier than in 'Ruiana'. The regenerants adapted to indoor conditions had pigmentation characteristic of the species, without signs of chlorosis and vitrification (Fig. 1 f). The fruits of the regenerated plants had identical to the donors color, aroma and taste characteristics.

Conclusions

An optimal protocol for *in vitro* adaptation of *F. vesca* plants to *in vivo* conditions were developed and viable plants were obtained. It was found that the duration of regenerant plants exposure under conditions of high relative humidity significantly influenced the effectiveness of adaptation. The largest share of plants adapted to indoor conditions was obtained by holding under conditions of 85–90% RH for 10–14 days on a mixture of coconut substrate and perlite: 'Ruiana' – 47.6 ± 2.5%, 'Zhovte Dyvo' – 60.0 ± 1.7%. In the case of soaking the root system in auxin for 25–30 minutes, followed by daily spraying of the leaves with a solution of 30% glycerin, plant survival was more than 70%. Further research will be aimed at studying the growth and development of *F. vesca* plant varieties in open field conditions.

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УДК 602:57.085.2:634.75

Чорнобров О. Ю.*, **Ткачова О. Е.** Адаптація рослин-регенерантів сортів *Fragaria vesca* L. до умов *in vivo*. *Plant Varieties Studying and Protection*. 2020. Т. 16, № 3. С. 248–252. https://doi.org/10.21498/2518-1017.16.3.2020.214925

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Мета. Адаптація рослин-регенерантів до умов довкілля – заключний етап мікроклонального розмноження. За даними низки авторів, при перенесені рослин *in vitro* в нестерильні умови закритого ґрунту фіксують значний відсоток відпаду. У попередній публікації досліджено регенераційну здатність тканин рослин суниці (*Fragaria vesca* L.) *in vitro* на живильному середовищі MS та одержано регенеранти (Чорнобров О. Ю., 2019). Мета дослідження – розроблення оптимального протоколу адаптації рослин-регенерантів сортів *F. vesca* до умов *in vivo*. **Методи.** Для досліджень використовували рослини суниці сортів 'Руяна' і 'Жовте диво' із циклом культивування *in vitro* 30–35 діб. Рослини висаджували в пластикові контейнери (об'єм – 0,33 л) по 1 шт. у суміш кокосового субстрату та перліту (3:1). Рослини витримували в умовах високої відносної вологості повітря (85–90%) упродовж 3–5 діб, 6–8 діб і 10–14 діб. Дослідження проводили в науково-дослідній лабораторії біотехнології рослин ВП НУБіП України «Боярська ЛДС» упродовж 2019–2020 рр. **Результати.** Тривалість витримування рослин-регенерантів *F. vesca* в умовах високої ВВП достовірно впливала на ефективність адаптації. За витримування упродовж 10–14 діб частка адаптованих до умов закритого ґрунту рослин становила для сорту 'Руяна' 47,6 ± 2,5% і 60,0 ± 1,7% для сорту 'Жовте диво'. Значну приживлюваність рослин (понад 70%) одержано за умов попереднього витримування кореневої системи в розчині ауксинів упродовж 25–30 хв із щоденним обприскуванням листків 30%-м гліцерином. Адаптовані до умов закритого ґрунту регенеранти мали характерну для сорту пігментацію, без ознак хлорозу та вітрифікації. **Висновки.** Розроблено оптимальний протокол адаптації сортів *F. vesca in vitro* до умов *in vivo* та одержано життєздатні рослини. Подальші дослідження будуть спрямовані на вивчення росту й розвитку рослин-регенерантів *F. vesca* в умовах відкритого ґрунту.

Ключові слова: *суниця лісова; культура in vitro; мікроклональне розмноження; акліматизація ex vitro; приживлюваність рослин.*

Надійшла 12.08.2020
Погоджено до друку 21.09.2020