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COMPARATIVE STUDIES ON THE AGRONOMIC VALUE OF in vitro AND CONVENTIONALLY **PROPAGATED STRAWBERRY** (Fragaria × ananassa Duch.) **PLANTS**

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Abstract. In principle, in vitro propagation results in uniform batches of plants, which grow, flower and fruit normally. However, phenotypic changes often observed in the field performance of tissue culture-produced strawberry plants might affect their agronomic value. So, it is of utmost importance to assess the field performance of *in vitro* propagated plants to verify their fidelity to conventional propagated plants. In this study, the agronomic value of the strawberry plants derived from cultivars 'Filon' and 'Teresa' via in vitro propagation and their first vegetative progeny was compared to conventional plants. During the field experiments, agronomic traits such as plant vigor, abundance of flowering and yield components were evaluated. The results showed the different field response of cultivars tested to in vitro propagation. In spite of the phenotypic changes observed in in vitro derived plants, their agronomic value was equal or superior in comparison with conventional plants. In conclusion, it should be stated that in vitro propagation method can be safely recommended for the reproduction of these strawberry cultivars.

Key words: micropropagation, microplants, phenotypic uniformity, runner seedlings, tissue culture

INTRODUCTION

In conventional cultivation, the strawberry (Fragaria × ananassa Duch.) is a species vegetatively propagated via runner seedlings. Nevertheless, such seedlings due to their weakness and susceptibility to pathological agents are not always suitable for this cultivation. Nowadays, the advantageous alternative to this conventional method seems to be the use of micropropagated plants for cultivation. Micropropagation of strawberry has

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been applied on a large scale in commercial production since mid 1970s. First applied to strawberry meristems in vitro techniques have been amplified to an efficient method of mass propagation [Boxus et al. 1977, Boxus 1992]. The use of a genetically stable meristematic tissue for mass in vitro clonal propagation is the best tool to obtain healthy and genetically unified plant material. The possibility of obtaining disease-free plants through meristem culture was demonstrated by Mahajan et al. [2001], Sowik et al. [2001], Hammerschlag et al. [2006]. Production of the tissue culture-propagated strawberry plants has been introduced to prevent most of plant and soil transmissible diseases. Several improvement of the technology have been proposed by authors working with strawberry [Passey et al. 2003, Debnath 2005, 2006, Lucyszyn et al. 2006]. In spite of an unquestionable advantages resulted from the use of a meristematic tissue culture for producing of high quality strawberry seedlings, some difficulties concerning the use of micropropagation as a commercial mass production method have arisen over the years. Phenotypic changes and variation in performance of tissue culture-produced strawberry plants have been widely reported [Borkowska 2001, Graham 2005]. Some reports note an increase in fruiting linked to enhanced crown proliferation and to flower bud differentiation and a marked fruit quality decline in comparison with standard propagated plants from the same clone [Boxus et al. 2000].

Such abnormalities are due to epigenetic changes caused by modifications in the expression of the information in the DNA brought about by alterations in DNA methylation, in histones, or in both. These modifications may influence a gene transcription [Miguel and Marum 2011]. Epigenetic changes are often temporary and plants may revert to the normal phenotype relatively easily but some can be long lasting and may even be transferred during sexual propagation [Kaeppler et al. 2000]. Field performance of micropropagated plants is a method used for evaluating their phenotypic uniformity to conventional plants obtained via standard runner propagation of cultivars. In this study, on the basis of such comparison, an agronomic value of the microplants directly obtained via micropropagation and their first vegetative progeny was assessed in cultivars 'Filon' and 'Teresa'.

MATERIAL AND METHODS

The plant material was two june-bearing strawberry cultivars 'Filon' and 'Teresa' grown in the field at the Experimental Station, University of Life Sciences in Lublin $(51^{\circ}13'59'' \phi N, 22^{\circ}34'0'' \lambda E$, elevation: 225.48 m) located in the south-east region of Poland. Both cultivars were micropropagated via runner tips which were derived from donor plants in their second year of vegetation. Runner tips were first rinsed under running tap water for 1 h and then surface-sterilized by immersing and shaking them in 70% ethanol for 1 min, and followed by immersing and shaking in sodium hypochlorite sterile distilled water solution (4.9 v/v) for 10 minutes. Finally, the explants were rinsed with sterile distilled water three to five times. The sterilized tips were cultured in 250 cm³ Erlenmeyer flasks containing 40 cm³ of Murashige and Skoog (MS) medium [Murashige and Skoog 1962] supplemented with indole-3-acetic acid (IAA) 1 mg·dm⁻³, 6-benzylaminopurine (BAP) 1 mg·dm⁻³, gibberellic acid (GA₃) 0.01 mg·dm⁻³, 2% su-

crose and solidified with 0.6% agar. The medium was autoclaved for 20 minutes at 121°C and 0.1 MPa, pH of the medium was adjusted to 5.7 before autoclaving. Each cultivar was represented by 30 explants. The culture was performed in the phytotron at 21°C under a 16 h photoperiod and proliferated twice, every 8 weeks. After two subcultures, the obtained microshoots were rooted on the MS medium without growth regulators. After rooting, the microplants were acclimatized to the field conditions.

The field experiment was conducted in two cycles. The first cycle was carried out in 2008–2010 years and the second one in 2010–2012 years. In the first cycle, the microplants (M) directly obtained via micropropagation from cvs. 'Filon' and 'Teresa' were compared with conventionally propagated (CP) plants derived from these cultivars via runner seedlings, which were used as control plants in this experiment. In the second cycle, the first vegetative progeny of microplants (VPM₁) derived via runners was compared with control plants. All tested plants were planted in the field in three replicates in each cycle. Each replicate included 20 microplants (in the 1st cycle) or 20 plants of the first vegetative progeny of microplants (in the 2nd cycle) and 10 control plants planted in spacing 80×30 cm in three rows, 10 plants per row. In the first cycle, the microplants and control plants were planted in autumn of 2008 year and evaluated in 2010 year, in the second cycle the first vegetative progeny of microplants and control plants were planted in the autumn of 2010 year and evaluated in 2012 year. In each cycle the field evaluation of plants was performed in the second year of their vegetation. According to typical agronomic procedures recommended for strawberry plantations [Żurawicz et al. 2005], the mineral soil fertilization was used in doses of 30 kg N, 40 kg P2O5 and 120 kg K_2O per ha throughout the study. Plants were irrigated when needed, whereas the foliar fertilization were not applied. During the field experiment, the phenotypic expression of such agronomic traits as plant vigour, abundance of flowering, yield and its components were assessed in all tested plants. According to European Council Regulation of 22 October 2007, No 1234/2007 [Official Journal of EU, 2007] the following fruit size categories i.e.: large ($\phi > 25$ mm, weight above 10 g), medium (ϕ 18–25 mm, weight 5–10 g), small ($\phi < 18$ mm, weight below 5 g) were separated from the total fruit yield. The large and medium fruit categories formed the marketable yield, whereas the small fruit category formed the non-marketable yield.

The agronomic value of *in vitro* propagated plants was assessed on the basis of comparison to conventionally propagated plants. Results were statistically evaluated by analysis of variance, using Duncan's multiple range test for determining significant differences between the means at a level of P = 0.05.

RESULTS

Field-grown plants were evaluated in 2010 (first cycle of experiment) and 2012 (second cycle of experiment) for the expression of agronomic traits. Among them, generative characteristics i.e. the number of inflorescences and flowers per plant as well as the number of flowers per inflorescence were assessed (tab. 1). *In vitro* propagated plants of cv. 'Filon' produced insignificantly more inflorescences than did conventional plants (CP). While in the second test cycle, the first vegetative progeny of microplants

(VPM₁) formed significantly fewer inflorescences, compared to CP plants. In 2010 micropropagated plants of cv. 'Teresa' developed insignificantly more inflorescences than CP plants, however, in the second cycle of studies, an equal number of inflorescences in daughter and CP plants was estimated. The number of flowers per plant was insignificantly higher in microplants of both cvs. 'Filon' and 'Teresa' when compared to CP plants, but VPM₁ of cv. 'Filon' developed significantly lower number of flowers per plant in comparison with CP plants. On the other hand, in VPM₁ of cv. 'Teresa' was observed an equal number of flowers per plant when compared to CP plants. Micropropagated and standard propagated strawberry plants of cv. 'Filon' demonstrated no differences in the number of flowers per inflorescence. Whereas in cv. 'Teresa', in the case of this trait, traditionally propagated plants were significantly superior to *in vitro* propagated plants in the first cycle of experiment. In the second one, VPM₁ of cv. 'Teresa' produced an equal number of flowers per inflorescence in comparison with CP plants.

 Table 1. Differences in the number of inflorescences, flowers per inflorescence and the number of flowers per plant between micropropagated and conventionally propagated strawberry plants of cultivars 'Filon' and 'Teresa'

Cultivar	Year of evaluation	The origin of plants and differences between them	Number of inflorescences per plant	Number of flowers per plant	Number of flowers per inflorescence
Filon	2010	microplants (M)	18.00	83.00	4.61
		conventionally propagated plants (CP)	15.00	78.00	5.20
		differences (M-CP)	3.00 ns	5.00 ns	-0.59 ns
	2012	vegetative progeny of microplants (VPM ₁)	12.00	37.00	3.08
		conventionally propagated plants (CP)	19.00	54.00	2.84
		differences (VPM ₁ -CP)	-7.00*	-17.00*	0.24 ns
Teresa	2010	microplants (M)	13.00	90.00	6.92
		conventionally propagated plants (CP)	9.00	87.00	9.66
		differences (M-CP)	4.00 ns	3.00 ns	-2.74*
	2012	vegetative progeny of microplants (VPM ₁)	10.00	45.00	4.50
		conventionally propagated plants (CP)	10.00	45.00	4.50
		differences (VPM ₁ -CP)	0.00 ns	0.00 ns	0.00 ns

ns – differences not significant LSD = 5.00, LSD = 15.00, LSD = 1.00

* – differences significant at P = 0.05

In the case of cv. 'Filon', microplants (M) as well as their first vegetative progeny (VPM_1) were characterized by an insignificantly lower mean weight of leaves per plant in comparison with conventional plants (CP) (tab. 2). Micropropagated plants of cv. 'Teresa' were characterized by higher weight of leaves per plant than the control plants in both test cycles, but these differences were not significant. In the first cycle of experiment, microplants of cv. 'Filon' produced the insignificantly higher yield when

compared to CP plants. On the contrary conventional propagated plants yielded insignificantly better than VPM1 in the second cycle of studies. The yielding of cv. 'Teresa' microplants and their first vegetative progeny was insignificantly higher that produced by standard propagated plants as was shown in Table 2. In the case of cv. 'Filon' differences in the mean weight of fruit between microplants as well as their VPM₁ and CP plants were insignificant. The weight of berries picked from micropropagated plants of cv. 'Teresa' and their vegetative offspring was significantly higher in comparison with CP plants.

Table 2.	Differences in the weight of plant foliage, fruit yield and average weight of fruit
	between micropropagated and conventionally propagated strawberry plants of cultivars
	'Filon' and 'Teresa'

Cultivar	Year of evaluation	The origin of plants and differences between them	Mean weight of fresh leaves (F.W.) per plant (g)	Yield per plant (g)	Mean weight of fruit (g)
Filon	2010	microplants (M)	76.11	320.74	6.07
		conventionally propagated plants (CP)	112.50	278.05	6.64
		differences (M-CP)	-36.39 ns	42.70 ns	-0.57 ns
	2012	vegetative progeny of microplants (VPM_1)	71.27	88.17	7.82
		conventionally propagated plants (CP)	95.72	170.17	7.54
		differences (VPM ₁ -CP)	-24.25 ns	-82.00 ns	0.33 ns
Teresa	2010	microplants (M)	166.55	325.44	6.68
		conventionally propagated plants (CP)	159.29	251.57	5.09
		differences (M-CP)	7.26 ns	73.87 ns	1.59*
	2012	vegetative progeny of microplants (VPM $_1$)	73.86	126.26	7.51
		conventionally propagated plants (CP)	67.32	98.11	5.73
		differences (VPM ₁ -CP)	6.54 ns	28.15 ns	1.78*

ns - differences not significant LSD = 40.20, LSD = 85.30, LSD = 1.07

* – differences significant at P = 0.05

As was shown in Table 3, in 2010 the large fruits collected from in vitro-derived plants of cv. 'Filon' were insignificantly bigger in contrast to control berries of CP plants. Whereas the mean weight of medium and small fruits was insignificantly higher in the case of plants conventionally propagated by runners. Such phenomena had diametrically changed in the second cycle of investigations (2012 year). Generally, in all size categories, fruits collected from microplants (M) of cv. 'Teresa' and their progeny (VPM_1) were slightly bigger in comparison with those gathered from conventional plants (CP). Should be emphasized, that in this cultivar the largest but insignificant differences occurred in the case of the biggest fruits.

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Cultivar	Year of evaluation	The origin of plants and differences between them	Mean weight of large fruits (g)	Mean weight of medium fruits (g)	Mean weight of small fruits (g)
Filon ·	2010	microplants (M)	12.86	6.37	2.87
		conventionally propagated plants (CP)	12.28	6.97	3.30
		differences (M-CP)	0.58 ns	-0.60 ns	-0.43 ns
	2012	vegetative progeny of microplants (VPM1)	13.58	7.68	3.36
		conventionally propagated plants (CP)	13.91	7.14	3.21
		differences (VPM ₁ -CP)	-0.34 ns	0.54 ns	0.15 ns
Teresa -	2010	microplants (M)	10.90	6.64	3.40
		conventionally propagated plants (CP)	10.67	6.21	3.02
		differences (M-CP)	0.23 ns	0.43 ns	0.38 ns
	2012	vegetative progeny of microplants (VPM1)	14.21	6.97	3.47
		conventionally propagated plants (CP)	12.94	6.65	3.37
		differences (VPM ₁ -CP)	1.27 ns	0.32 ns	0.10 ns

Table 3. Differences in the mean weight of large (> 10 g), medium (5–10 g) and small (< 5 g) fruits between micropropagated and conventionally propagated strawberry plants of cultivars 'Filon' and 'Teresa'

ns - differences not significant LSD = 1.43, LSD = 1.12, LSD = 0.51

* – differences significant at P = 0.05

Table 4. Differences in the percentage of large, medium and small fruits in the yield between micropropagated and conventionally propagated strawberry plants of cultivars 'Filon' and 'Teresa'

Cultivar	Year of evaluation	The origin of plants and differences between them	Contribution of large fruits in the yield (%)	Contribution of medium fruits in the yield (%)	Contribution of small fruits in the yield (%)
Filon	2010	microplants (M)	32.12	38.30	25.41
		conventionally propagated plants (CP)	42.84	31.32	20.37
		differences (M-CP)	-10.71 ns	6.97 ns	5.04 ns
	2012	vegetative progeny of microplants (VPM $_l$)	32.84	44.71	13.48
		conventionally propagated plants (CP)	40.41	37.89	15.09
		differences (VPM ₁ -CP)	-7.57 ns	6.82 ns	-1.61 ns
Teresa	2010	microplants (M)	29.06	55.85	13.37
		conventionally propagated plants (CP)	18.55	46.77	33.08
		differences (M-CP)	10.51 ns	9.08 ns	-19.71*
	2012	vegetative progeny of microplants (VPM $_l$)	39.40	34.72	16.56
		conventionally propagated plants (CP)	14.30	39.40	32.92
		differences (VPM ₁ -CP)	25.10*	-4.68 ns	-16.36*

ns – differences not significant LSD = 13.47, LSD = 11.06, LSD = 10.68

* – differences significant at P = 0.05

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Contribution of large fruits in the yield of conventional propagated plants in cv. 'Filon' was insignificantly higher than in the case of *in vitro* plants and their vegetative offspring (tab. 4). It was found an insignificant increase in the proportion of medium fruits in the yield of M and VPM₁ plants in relation to the CP plants in both rounds of research. Besides, no significant differences between the contribution of small fruits in the yield of microplants as well as their VPM₁ and standard propagated plants in this cultivar was observed. The yield of advantageous structure was produced by *in vitro* derived plants of cv. 'Teresa' in both cycles of experiment. Microplants of this cultivar produced the yield with an insignificantly higher contribution of large fruits, whereas in their first vegetative progeny a significantly higher contribution of large fruits in the yield when compared to CP plants was estimated. On the other hand, distinct differences occurred in this cultivar between tissue culture plants and standard propagated plants in terms of the percentage of small fruit in the yield. In both series of research the contribution of small fruits in the yield of M and VPM₁ plants was significantly lower in comparison with CP plants.

DISCUSSION

Micropropagation results in uniform batches of plants, which grow, flower and fruit normally. Although, it can be expected that eventually plants multiplied *in vitro* will be equivalent or superior to those propagated by conventional techniques. So, it is of utmost importance to assess the field performance of micropropagated plantlets to ensure their fidelity or superiority to conventional propagated plants. Numerous studies have been published regarding field behavior of micropropagated strawberry [Boxus et al. 2000, Borkowska 2001, Szczygieł et al. 2002, Litwińczuk 2004]. Such plants more or less often exhibit characteristics like: leaf-color variants, stem fasciations, intensified vigor, hyper-runnering, dwarf plants and abnormal flowering (hyper-flowering) accompanied by increased production of smaller fruits. In our study micropropagated and conventionally propagated plants of strawberry cvs. 'Filon' and 'Teresa' were transferred to the similar field conditions and growth stage to evaluate for their vigor, flowering and fruiting ability. Presented study revealed a higher agronomic value of plants obtained directly via micropropagation compared to the conventionally propagated plants. This phenomenon was particularly evident in cultivar 'Teresa' considering the mean weight of single fruit. Also for the mean weight of leaves per plant and total yield per plant the same tendency was observed, but differences between micropropagated and conventional plants were insignificant. It should be emphasized, that these traits were stable, because they also occurred in the first vegetative progeny of microplants (VPM₁).

Increased branching and vigorous vegetative growth are often noted in plants produced through *in vitro* culture. As was stated by Boxus et al. [2000] tissue culturederived strawberry plants grow more vigorously producing more crowns and runners and increased petiole length, yield per area and number of inflorescences per crown than conventionally propagated plants. Litwińczuk [2004] compared strawberry plants of cv. 'Senga Sengana' obtained *in vitro* from axillary and adventitious shoots with their runner progeny and with standard runner (control) plants under field conditions. In the planting year, *in vitro* obtained plants developed significantly more crowns and runners when compared to other groups. Such differences, especially in runners' number were not observed in the next two years. In the planting year, all *in vitro* propagated plants and about 80% their runner progeny flowered contrary to control. Every year tissue culture plants developed significantly more inflorescences than other groups studied. Plants obtained *in vitro* produced bigger fruits and higher yield than other groups in the first two years. However, a reduction of berry yield for tissue culture plants in contrast with control was observed in third year only.

Similar abnormalities in *in vitro* derived plants were observed in our study. Presented results revealed the insignificantly higher number of inflorescences per plant as well as number of flowers per plant in microplants of cv. 'Filon' in comparison with plants propagated through planting of runners. While in the second cycle of investigation the values of these characteristics were significantly lower in VPM₁. This phenomenon may indicate a genetic liability of this trait expression in this cultivar.

Some authors revealed that hyper flowering was appreciable reduced in the first generation of micropropagated plants obtained traditionally, by runners [Litwińczuk 2004]. Results of the present study confirmed such phenomenon. In cultivar 'Teresa' flowering-related traits, such as: numbers of inflorescences per plant, number of flowers per plant as well as number of flowers per inflorescence were normalized in the first runner progeny of microplants. Existing differences in these quantitative characteristics appeared to be epigenetic in nature, i.e., disappearing after the first vegetative multiplication, and were not indicative that mutations had occurred. Yield and its components are essential, because they constitute one of the most important traits determining an agronomic value of micropropagated plants. Szczygieł et al. [2002] determined runner plant production and fruit yield from micropropagated strawberry plantlets, taken directly from micropropagation and after their first and second reproduction cycles by runners, in comparison with conventionally propagated plants. The effect of micropropagation on fruit yield and quality was usually smaller than on reproduction rate and was limited mainly to plantlets coming directly from micropropagation; their total yield was higher than that of standard plantlets only in one cycle, but average fruit size was usually smaller. Similar results were obtained by Karhu and Hakala [2002]. Due to the more abundant flowering, but without effect on berry size, microplants of cv. 'Senga Sengana' produced higher marketable crop than runner plants in the first cropping year. During the second cropping year the larger number of berries led to decreased in berry size, and no differences in marketable yield between plant types was evident. However, cropping was equal for both plant types of cv. 'Zephyr'. Whereas in our study, cultivar 'Teresa' produced a yield of advantageous structure; the significantly higher contribution of large fruits in the yield was observed in VPM_1 when compared to CP. Simultaneously, in the case of this cultivar, microplants and their vegetative progeny showed a significantly lower contribution of small fruits in the yield. On the other hand, the mean weight of fruits in cv. 'Filon' was not affected by methods of propagation. It follows that the behaviour of specific characteristics considerably depends on strawberry cultivars.

At present no single identity verification step exists and laboratories must use a combination of field evaluation of plant and molecular techniques to check their culture systems. A comparative study was conducted by Gantait et al. [2010] based on morphological parameters as well as genetic assessments. The *in vitro* generated strawberry plants exhibited significantly vigorous morphological growth and earlier flower induction when compared to the plants propagated through planting of runners. Genetic analysis showed no polymorphism in banding pattern and thus it was revealed that there was no significant variation between micropropagated and conventional propagated plants at molecular level.

In research of Adel El-Sawy [2007], meristem tips of three strawberry cultivars, namely 'Chandler', 'Sweet Charlie' and 'Gaviota' were excised and cultured for 5 weeks in *in vitro* conditions. *In vitro*-derived plantlets and standard propagated plants were analyzed to detect possible drift in genetic stability of micro-propagated plants. It was concluded that mass propagation via meristem tip culture is reliable in producing genetically similar plants to the mother ones.

Stable phenotypic variation in *in vitro* propagated strawberry plants was clearly observed by Biswas et al. [2009] in the leaf morphology, flower cluster branching and fruit shape. They were able to co-relate these phenotypic changes with changes in the DNA banding pattern as differences were observed in the selected three clonal lines. Three of the stable selections were distinct from each other in terms of fruit and other horticultural characters, and have potential for commercial cultivation.

CONCLUSIONS

Obtained results confirm some of the earlier statements that micropropagation can be safely recommended for strawberry reproduction. Micropropagated plants of the cultivars examined produced a high quality yield and grew without appreciable, undesirable alterations in flowering or in yield-forming habit. In general, the agronomic value of *in vitro* propagated plants was equal or higher when compared to conventional plants. However, in our study the different response of cultivars tested to *in vitro* propagation was exhibited. Differences observed between micropropagated and conventional plants were probably epigenetic in nature, because they were unstable and disappeared after the first runner propagation of microplants. This phenomenon was particularly evident in cv. 'Teresa' regarding the flowering related traits. Therefore the success in applying of meristematic tissue culture in commercial cultivation of strawberry is dependent on the genetic stability of the cultivar and its individual response to *in vitro* propagation.

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ANALIZA PORÓWNAWCZA WARTOŚCI UŻYTKOWEJ ROŚLIN TRUSKAWKI (Fragaria × ananassa Duch.) UŻYSKANYCH IN VITRO ORAZ METODĄ KONWENCJONALNĄ

Streszczenie. Z reguły rośliny uzyskane w wyniku propagacji *in vitro* rosną, kwitną i plonują prawidłowo, jednakże fenotypowe zmiany często obserwowane w uprawie polowej u roślin truskawki pochodzących z kultur tkankowych mogą wpływać na ich wartość agronomiczną. Bardzo ważna jest więc ocena wierności takich roślin w stosunku do uzyskanych metodą konwencjonalną. W przeprowadzonych badaniach oszacowano wartość użytkową roślin truskawki odmian 'Filon' i 'Teresa' uzyskanych w kulturze *in vitro* oraz ich pierwszego wegetatywnego potomstwa, porównując je z roślinami uzyskanymi konwencjonalnie. W obserwacjach polowych oceniono takie cechy agronomiczne, jak wigor roślin, obfitość kwitnienia oraz komponenty plonowania. Uzyskane wyniki wykazały różną polową reakcję testowanych odmian na propagację *in vitro*. Pomimo obserwowanych w ocenie polowej fenotypowych zmian u roślin uzyskanych *in vitro*, ich agronomiczna wartość utrzymywała się na tym samym lub wyższym poziomie w porównaniu z roślinami konwencjonalnymi. Podsumowując, należy stwierdzić, iż metoda rozmnażania *in vitro* może być bezpiecznie stosowana do reprodukcji tych odmian truskawki.

Słowa kluczowe: mikrorozmnażanie, mikrorośliny, jednolitość fenotypowa, sadzonki rozłogowe, kultura tkankowa

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