Survival and development of strawberry plants on various substrates at the stage of adaptation

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Abstract. At the stage of adaptation during micro clonal propagation of garden strawberries, the substrate plays an important role, acting as a medium for the root system and ensuring balanced growth and development of the entire plant. The research was conducted at the Scientific and Production Center for Fruit and Berry nursery of Stavropol State Agrarian University at the conveyor breeding of strawberries. The experience was two–factorial: factor A - strawberry variety, factor B – substrate. The effectiveness of the substrate at the stage of adaptation of garden strawberries was studied on Albion, Aprica and Clery varieties. Peat was chosen as the main substrate, the properties of which were improved by mixing with perlite, vermiculite and rice husk. The addition of additional components to peat has improved the survival rate and all indicators of growth and development of the root system and the aboveground part of strawberries. When using a mixture of peat with perlite and vermiculite or rice husk in the Experimental substrate, an increase in the percentage of survival of strawberry plants by 5.9-7.5% on average relative to the control was revealed. Depending on the substrate, the height of the rosette of plants increased relative to the control by 1.0-2.1 cm on average according to the experiment, the number of leaves and whiskers increased by 0.5-1.3 and 0.8-1.6 pieces, respectively. The experimental substrate (peat + rice husk + mineral fertilizer), having the advantages of better water and air permeability and a set of nutrients, provided maximum values for all the noted indicators of the root system and rosette of strawberries. The varietal characteristics of strawberries ensured the greatest root growth and survival in the Albion variety, while the height of plants and the size of the leaf apparatus in the Clery variety.

1 Introduction

Strawberries are one of the most popular berries in the world. The widespread distribution of strawberries in various climatic zones is due to their high taste qualities. However, the duration of growing crops in a vegetative manner is limited due to the high susceptibility of plants to various phytopathogens. In this regard, the propagation of strawberries by micro clonal propagation has become widespread [1].

Obtaining healthy strawberry plants with prolonged vegetative propagation is associated with difficulties – subject to strict observance of agrotechnical conditions, over time, an

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accumulation of phytopathogens is observed in the soil, which leads to a decrease in the biological potential of the variety and its degradation [2]. This problem is solved by the method of micro clonal reproduction.

The quality of the planting material of fruit and berry crops is a key factor in the stability and productivity of garden plantings and also determines the economic performance of the industry. The method of obtaining healthy planting material makes it possible to produce high-quality plants in a short time. It makes it possible to produce genetically homogeneous and disease-free plants quickly and on a large scale, starting from the original parent plants, regardless of seasonal restrictions, using small work areas and plant samples [3].

The process of micro clonal reproduction consists of two key stages: in- vitro and ex-vitro. The first stage in vitro is critically important and is carried out in a biotechnological laboratory, its effectiveness depends on the complex of components and especially sterility. After the laboratory stage of micro clonal reproduction, it is necessary to adapt plants to field conditions (ex- vitro). In vitro adaptation of micro plants is performed in greenhouse conditions while controlling the microclimate and creating optimal conditions for the development of the root system. An effective process of acclimatization of plants saves material resources and reduces the cost of producing high-quality planting material [4].

Garden strawberries are cultivated at the ex-vitro stage using a substrate. Therefore, the substrate plays an important role in the processes of survival, acting as a medium for the growth and development of the root system. The substrate must have a set of optimal properties (moisture capacity, breathability, sterility, and others) and promote high absorption of nutrients by the root system [5].

At the ex-vitro stage, a different substrate can be used: organic or mineral. Peat, vermiculite, and perlite are used in practice, the qualitative characteristics of which affect the rooting and growth of seedlings, increasing hydraulic conductivity and promoting the absorption of mineral elements from the nutrient medium. However, these substrates are not optimal when used alone due to changes in the physic-chemical properties and varietal requirements of the crop being grown, so the question remains of studying the effectiveness of substrate combinations in the process of micro clonal reproduction [6].

The aim of the work is to evaluate the effect of the substrate on the adaptation of regenerated strawberry plants to ex vitro conditions.

2 Materials and Methods

The research was conducted at the Scientific and Production Center for Fruit and Berry Nursery of Stavropol State Agrarian University during the conveyor breeding of strawberries in 2022-2024.

The object of research is regenerating strawberry plants. The experience was two–factorial: factor A - strawberry variety, factor B – substrate. After the in-vitro stage, plants were adapted to greenhouse conditions under microclimate control. The plants were grown on different substrates: 1 – peat (control), 2 – peat + perlite (in a ratio of 1:1), 3 – peat + perlite + vermiculite (in a ratio of 1:1:0.5), experimental substrate. The strawberry varieties Albion, Aprica and Clery were studied in the experiment.

Characteristics of peat used in the experiment: riding peat contains a set of nutrients (macronutrients, mg/l: nitrogen (NH4 + NO3) -100, phosphorus (P2O5) – 90 mg/l, potassium (K2O) – 120), pH – 5.5, fractional composition – 0-20 mm. The experimental substrate is developed by scientists of Stavropol State Agrarian University, and consists of peat, mineral fertilizers and rice husks.

The experience scheme included 12 options. The experience is repeated three times. Strawberry plants were transplanted from in- vitro to ex -vitro into a 90-cell cassette with a cell volume of 50 ml. The substrate was moistened before planting. The total period of

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adaptation of plants in the greenhouse was 7 weeks. Conditions in the greenhouse: temperature – 22-25 ° C, air humidity – about 60%, day/night photoperiod – 16/8 hours, the intensity of illumination of plants with additional illumination – was maintained at the level of 15 Kcal during the day.

The condition of the plants was assessed by biometric indicators of the root system and the aboveground part according to generally accepted methods. The experimental data was processed using Statistica 8 and Microsoft Excel software.

3 Results and Discussion

The adaptation of plants obtained at the in-vitro stage to non-sterile conditions refers to the labor-intensive stages of micro clonal reproduction. The main task in ex -vitro is to provide conditions that affect the further growth, development and rooting of plants in conditions as close as possible to natural conditions. At this stage, special attention is paid to the adaptation of plants to external factors, leveling the effects of stress and obtaining environmentally resistant planting material [7].

Plants grown under reference laboratory conditions show increased vulnerability to the influence of adverse factors at the ex -vitro stage, in particular, sensitivity to temperature conditions, changes in conditions for the root system and demonstrate a high degree of transpiration. The adaptation process can be assessed as successful when, after a period of rest, intensive regrowth of the leaf mass and the development of the root system are observed. The temporary dormancy of the plant is caused by a change in the type of nutrition, damage during transplantation and changes in growing conditions.

An important element of the adaptation process is the substrate. When transplanting plants in greenhouse conditions into the substrate, plant growth is suspended, leaves fall off and plants die, which is because the plants do not develop root hairs and, as a result, the absorption of water and nutrients is disrupted. Therefore, the selection of the optimal substrate at the ex -vitro stage plays an important role in the successful adaptation of plants.

When studying the effect of substrates on the process of plant adaptation, it was found that the survival rate varied on average for strawberry varieties from 81.6 to 89.1% (Table 1).

Table 1. The effect of the substrate on the effectiveness of adaptation and development of the root system of regenerated strawberry plants

Variety (factor A)	Peat (factor B)	Survival rate. %	Number of roots. pcs.	Length of roots. mms	Root biomass. gr
Albion	Peat (control)	82.4	4.1	45	0.51
	Peat + perlite	89.2	4.9	52	0.75
	Peat + perlite+ vermiculite	89.7	5.5	59	0.81
	Experimental substrate	90.5	6.0	63	0.89
Aprica	Peat (control)	80.9	3.2	37	0.42
	Peat + perlite	85.7	3.9	42	0.61
	Peat + perlite+ vermiculite	88.1	4.5	45	0.67
	Experimental substrate	89.0	5.1	52	0.74
Clery	Peat (control)	81.4	3.7	42	0.48
	Peat + perlite	87.5	4.2	46	0.71
	Peat + perlite+ vermiculite	87.8	4.9	51	0.79
	Experimental substrate	87.7	5.4	55	0.85

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$LAD_{0.05}$*(A)	1.84	0.31	3.11	0.048
$LAD_{0.05}$*(B)	4.54	0.20	1.85	0.027
$LAD_{0.05}$*(AB)	6.18	0.47	4.84	0.074

*LSD $_{05}$ – the least significant difference for a 5 % significance level

When using peat alone as a substrate, the lowest survival rate was obtained. The addition of additional components to peat has improved the survival rate and all indicators of plant growth and development. Mixing peat with perlite,3

vermiculite or an organic component (rice husk in the Experimental substrate) creates a loose and stable structure and, as a result, increases the degree of aeration of the substrate. The improved structured mixture ensures good drainage and reduces the risk of root system putrefaction. The use of additional components increases the availability and degree of absorption of nutrients for strawberry plants.

With the improvement of peat properties due to the use of perlite and vermiculite, an increase in the survival rate was revealed – by 4.1-7.3% relative to the control. The use of mineral fertilizers and an organic component in the initial composition of the Experimental substrate provided an advantage in the survival rate of strawberry plants – 89.1% on average according to the experience, the difference in the use of peat alone or peat with perlite and vermiculite was at the level of 0.6-7.5%.

The data obtained confirms that the substrate used and its properties play an important role in the survival of plants at the ex-vitro stage. The high percentage of death of clones during transfer from in vitro to ex -vitro is primarily due to the peculiarities of their growth and development in a sterile environment, in particular, the nature of the formation of regenerate roots at the stage of reproduction in -vitro [8].

In general, the process of adaptation and further development of the plant's root system was observed on all the studied substrates. Successful transplantation of test tube plants into non-sterile conditions is due to several factors, of which the condition of the root system plays an important role for strawberries [9]. The survival rate is the main indicator for assessing the success of plant transplantation. The choice of a substrate for transplantation significantly affects the survival of plants [10].

The influence of the substrate on the development of strawberries was reflected in the parameters of the root system development. The use of mineral substrates (perlite, vermiculite) in peat and the use of an improved Experimental substrate showed an increase in the number of roots relative to peat – by 0.7-1.8 pcs. / plant on average according to the experiment. The introduction of mineral elements in fertilizers into the Experimental Substrate provided the necessary set of nutrients, which activated the growth and development of the root system and ensured maximum root growth on the new experimental substrate. The number of strawberry roots on the Experimental substrate exceeded the value of peat, a mixture of peat with perlite and vermiculite by 9.1-32.7%. It should be noted that when comparing the number of roots on a new substrate and a mixture of peat and perlite, no significant differences were found.

Measuring the length of the roots showed significant differences between all the variants of the experiment. The average root length of plants grown on peat in various mixtures differed significantly from the control by 5-15 mm, with the highest value obtained from the Experimental substrate.

The volume of the root system plays a key role in the overall development of the planting material, ensuring the absorption of water, nutrients and influencing the overall growth and adaptation of the plant. The analysis of root biomass showed that the data on the substrate mixtures were statistically significant when compared with the control when only peat was used: the average difference for the studied strawberry varieties was 0.13-0.27 g.

The experimental substrate, having the advantages of better water and air permeability and a set of nutrients, provided the highest values for all the noted indicators of the root system, in particular, the increase in root biomass was 48% on average in the experiment.

The varietal characteristics of strawberries cause differences in the formation of the root system of plants. A comparison of strawberry varieties in terms of root system growth parameters showed that the Albion variety was ahead of the Aprica and Clery varieties. The differences in survival rate between the Aprica and Clery varieties, as well as Albion and Clery, were insignificant. In terms of survivability, the Albion variety stood out.

The effectiveness of the adaptivity of regenerating plants is largely determined by their biometric growth parameters [11].

During the adaptation period (7 weeks), strawberry plants showed an active increase in the aboveground part. When comparing the substrates, the growth-stimulating effect of additional components in the substrates on the biometric parameters of the above-ground part of strawberries was revealed. Depending on the substrate, the height of the rosette of plants increased relative to the control by 1.0-2.1 cm on average according to the experiment (Table 2).

Table 2. The effect of the substrate on the development of the aboveground part of the regenerated strawberry plants

Variety (factor A)	Substrate (factor B)	Rosette height. cms	Quantity of leaves. pcs.	Number of runners. pcs.
Albion	Peat (control)	4.9	6.5	6.4
	Peat + perlite	6.1	6.9	7.2
	Peat + perlite+ vermiculite	6.7	7.2	7.6
	Experimental substrate	7.2	7.4	8.1
Aprica	Peat (control)	4.6	5.9	6.0
	Peat + perlite	5.5	6.3	6.8
	Peat + perlite+ vermiculite	6.0	6.7	7.3
	Experimental substrate	6.7	7.2	7.6
Clery	Peat (control)	5.8	6.9	6.9
	Peat + perlite	6.8	7.6	7.6
	Peat + perlite+ vermiculite	7.4	8.1	7.9
	Experimental substrate	7.7	8.5	8.5
$LAD_{0.05}$*(A)		0.27	0.31	0.29
$LAD_{0.05}$*(B)		0.39	0.48	0.45
$LAD_{0.05}$*(AB)		0.64	0.73	0.74

Not only the height of strawberry plants increased, but also the number of leaves and whiskers. The number of strawberry leaves on peat substrates with additional components exceeded the control by 1.2-1.4 times on average in the experiment, the number of whiskers – by 0.8-1.6 pieces. A significant increase in the number of whisker leaves was found in plants planted on an Experimental substrate.

The increase in biomass of the above-ground part of strawberry varieties differed from the indicators of the root system. The highest plant height and the size of the leaf apparatus were obtained on the Clery variety, which had significant differences in the above-ground indicators compared to the Albion and Aprica varieties.

All the characteristics of strawberry regenerants for the development of the root system and the aboveground part in the studied substrates at the end of adaptation were better than in control, when only peat was used. Improving the properties of the substrate reduces the time for successful transplanting, allowing for higher quality seedlings. The results obtained by comparing substrates show the potential of rooting regenerant plants at the ex -vitro stage to optimize the production of planting material under production conditions, offering economic and practical advantages for nurseries.

4 Conclusions

Studies have shown that mixing peat with additional mineral organic components created a loose and stable composition and improved the survival rate and all indicators of growth and development of strawberry plants.

When using a mixture of peat with perlite and vermiculite or rice husk in the Experimental substrate, an increase in the percentage of survival of strawberry plants by 5.9-7.5% on average relative to the control was revealed. On the studied substrates, an increase in the size of the strawberry root system was obtained relative to the control: as a result, the difference in root biomass was 0.22-0.36 g on average compared to the experience with obtaining an advantage on the Experimental substrate.

When comparing the substrates, the growth-stimulating effect of mixtures of peat, perlite, vermiculite and rice husk on the biometric parameters of the aboveground part of strawberries was revealed. Depending on the substrate, the height of the rosette of plants increased relative to the control by 1.0-2.1 cm on average according to the experiment, the number of leaves and whiskers increased by 0.5-1.3 and 0.8-1.6 pieces, respectively. A mixture of peat with an organic component and a mineral fertilizer (Experimental substrate) contributed to the greatest increase in the parameters of the aboveground part of plants. The experimental substrate, having the advantages of better water and air permeability and a set of nutrients, provided maximum values for all the noted indicators of the root system and rosette of strawberries.

At the stage of adaptation, varietal differences in regenerate plants were revealed. When considering the growth parameters of the strawberry root system, the Albion variety was ahead of the Aprica and Clery varieties. The highest plant height and the size of the leaf apparatus were in the Clery variety.

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References

1. R. Cappelletti, S. Sabbadini, B. Mezzetti, Strawberry (Fragaria × ananassa). In: Wang, K. (eds) Agrobacterium Protocols. Methods in Molecular Biology, vol 1224. NY: Springer, 217–227 (2015) https://doi.org/10.1007/978-1-4939-1658-0_18
2. A.V. Schiavon, T.B. Becker, E. E. Delazeri, G.K. Vignolo, P. Mello-Farias, L.E.C. Antunes, Production and quality of strawberry plants produced from different nutrient solutions in soilless cultivation. Revista Ceres**, 69(3),** 348–357 (2022) https://doi.org/10.1590/0034-737X202269030013
3. R. Pardi, G.N. Basile, G. De Mastro, M.L. Gargano, A. Tagarelli, C. Ruta, Efficient Micropropagation by Ex Vitro Rooting of *Myrtus communis* L. *Horticulturae*. **11(2),** 207 (2025). https://doi.org/10.3390/horticulturae11020207
4. O. Panfilova, N. Ryago, G. Ondrasek, I.V. Knyazeva, I. Kahramanoğlu, O. Vershinina, M. Tsoy, A.Y. Izmailov, A.S. Dorokhov, Optimizing Microclonal Propagation of Red

Currant Cultivars: The Role of Nutrient Media, Sterilizers, and LED Lighting in Plant Adaptation, Horticulturae, **11(2),**149 (2025) https://doi.org/10.3390/horticulturae11020149

5. N. Valasevich, N. Kukharchyk, T. Krasinskaya, Influence of adaptation substrates on morphological development of raspberry plantlets during acclimatization ex vitro, Acta Hortic, **812**, 409–414 (2009) https://doi.org/10.17660/ActaHortic.2009.812.57
6. N.N. Hoang, Y. Kitaya, T. Shibuya, R. Endo, Effects of supporting materials in in vitro acclimatization stage on ex vitro growth of wasabi plants, Scientia Hortic, **261**, 109042 (2020) https://doi.org/10.1016/j.scienta.2019.109042
7. M.A. Yartseva, A.B. Khvostova , L.A. Ivanova, M.V Slukovskaya, Growth and development of regenerant strawberry (fragaria x ananassa (weston) duchesne ex rozier) plants on various vermiculite substrates during transfer from in vitro to ex vitro conditions, Transactions of Karelian Research Centre of Russian academy of science, **7,** 91-101 (2024) https://doi.org/10.17076/eb1915
8. I.D. Borodulina, T.V. Plaksina, Ex vitro adaptation of regenerated strawberry (moscow delicacy variety), Acta Biologica Sibirica, **1(1-2),** 74-84 (2015) https://doi.org/10.14258/abs.v1i1-2.832
9. E.V. Ambros, S.Y. Toluzakova, R.A. Gorodova, E.G. Shapolova, The effect of the siliceous composites of rice husk and green tea on growth and development of Fragaria × ananassa Duch. regenerants during the adaptation to ex vitro conditions, Turczaninowia, **18(3).** 96-102 (2015)
10. M. Welehaweria, D.B. Sbhatu, In vitro micropropagation of Aloe elegans Tod. using offshoot cuttings, BMC Research Notes, **16(1),** 215 (2023) https://doi.org/10.1186/s13104-023-06490-0
11. E. Ambros, O. Kotsupiy, E. Karpova, U. Panova, A. Chernonosov, E. Trofimova B. Goldenberg, A Biostimulant Based on Silicon Chelates Enhances Growth and Modulates Physiological Responses of In-Vitro-Derived Strawberry Plants to In Vivo Conditions. Plants, **12(24),** 4193 (2023) https://doi.org/10.3390/plants12244193