Improvement of stages of adaptation and *ex vitro* pre-growing of red raspberry (*Rubus idaeus* L.) plants

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Abstract. At the adaptation stage, a new multicomponent microbial concentrate obtained by fermentation of substrate from a mixture of peat, humus and compost by bifidobacteria was used for the first time. The microbial preparation is rich in BAS (probiotics, enzymes, amino acids, etc.), due to which it activates plant immunity and improves plant establishment, growth and development. The influence of different modifications of Revitalize liquid preparation (marka U - universal; marka Ya - for berry crops) in preparation of red raspberry microplants for the stage of adaptation to non-sterile conditions was studied. The work demonstrated the positive effect of Revitalize liquid marka Ya at the stage of adaptation for a single treatment with exogenous treatment. At the stage of pre-growing, the effectiveness of a single treatment with fertilization and a double treatment with mixed feeding was revealed.

1 Introduction

Every year in the Russian Federation the variety of raspberry varieties in the State Register of breeding achievements allowed for use is replenished. Therefore, at present there is a need to study new genotypes, to develop techniques to improve the technology of accelerated multiplication of these varieties, including the technology of clonal micropropagation [1, 2, 3]. Many works have been devoted to the improvement of nutrient media composition at various stages of plant cultivation *in vitro*, but the stage of adaptation to non-sterile *ex vitro* conditions of plants is no less significant [4]. Plant losses at this stage can be more than 50 per cent [5, 6, 7, 8]. The method of vegetative propagation has a significant effect on the development indicators of raspberry plantations under open ground conditions, but this aspect requires further study. One of the advantages of clonal micropropagation technology is the increased ability of *ex vitro* plants to vegetatively propagate after cultivation of plants under *in vitro* conditions [9]. However, under the influence of various unfavourable abiotic factors faced by plants planted in the open

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ground, morphometric indices can significantly decrease. In this regard, one of the main tasks is to increase the adaptability of raspberry plants to non-sterile conditions in order to create mother plantations of high quality.

The aim of the research was to study the effect of different modifications of Revitalize liquid in the preparation of red raspberry microplants for the stage of adaptation to non-sterile conditions.

2 Materials and methods

Experiments were carried out at Russian State Agrarian University—Moscow Timiryazev Agricultural Academy, Department of biotechnology and berry crops of Edelstein Educational Scientific and Production Center for Horticulture and Vegetable Growing in 2024.

The *ex vitro* microplants of red raspberry (*Rubus idaeus* L.) cultivar Gusar served as an object of research.

Two passages were performed at the multiplication stage. Raspberry microplants were planted on nutrient medium containing macro- and micronutrients according to Murashige-Skoog (MS) prescription [10], enriched with double iron chelate, vitamins B1,B6,PP - 0.5 mg/L each, 6-BA - 0.5 mg/L, sucrose - 30000 mg/L, agar-agar - 6000 mg/L. The duration of subcultivation was 60 days.

Further, the experimental plants were planted for in vitro rooting stage in nutrient medium for rhizogenesis according to MS prescription diluted twice in macronutrient content with double iron chelate enriched with the following: (mg/L) thiamine hydrochloride (B1), pyridoxine hydrochloride (B6), nicotinamide (PP) - 0.5, IBA - 0.2, sucrose - 15000, agar-agar - 6000. Five microcuttings were placed in each vessel in a laminar box.

After passaging, plants were cultured in 300 ml jars with 25 ml volume of nutrient medium under light room conditions at 2500 lux illumination, +20+22°C and 16/8 h photoperiod.

Microplants were transplanted to the stage of adaptation to non-sterile conditions after 40 days of subcultivation at the rhizogenesis stage (30.03.2024). Twenty-four hours before planting microplants, the substrate was saturated with water and then drenched with Previcur fungicide solution at a concentration of 2 ml/L. The substrate used was a mixture of Pelgorsk peat and perlite in the ratio of 3:1, planting was carried out in plastic cassettes (49 cells, 4 × 4 cm).

Immediately after planting microplants for adaptation and after 14 days of cultivation, single and double exogenous treatments, fertilization and mixed (exogenous + fertilization) fertilization with modifications of Revitalize liquid LLC NPO “Zhivaya Universe” (marka U - universal; marka Ya - for berry crops), control water.

After 30 days of *ex vitro* cultivation of plants at the stage of adaptation (30.04.2024), morphometric parameters of development were measured, and the following were taken into account: average shoot length, average total leaf area, average total root length, average number of roots and their average length.

After counting the variants, *ex vitro* adapted plants were transplanted to 0.5 L plastic pots and transferred to the growing compartment.

Single and double exogenous treatments, fertilization and mixed (exogenous + fertilization) fertilization with modifications of Revitalize liquid (marka U - universal; marka Ya - for berry crops), control water (Table 1).

Table 1. Experiment scheme

Feeding option Revitalize liquid	Adaptation stage		Pre-growth stage	
	Once at planting	Twice at planting and on 14 days	Once at planting	Twice at planting and on 14 days
Exogenous 500 ml:500 ml water (1:1)	+	+	+	+
Fertilization 25 ml:1000 ml water (1:40)	+	+	+	+
Exogenous (1:1) + fertilization (1:40)	+	+	+	+

After 30 and 60 days of pre-growing (30.05. and 30.06.2024), morphometric indices of development were measured, and the following were taken into account: shoot length, total leaf area, total root length, number of roots and their length.

Repetition of experiments was threefold at the stage of adaptation - 7 rooted microplants per repetition, at the stage of pre-growing - 5 *ex vitro* plants. The experimental data were analyzed by two-factor analysis of variance according to Dospekhov B.A. (1985) and A.V. Isachkin (2020) using Microsoft Office Excel 2010 and PAST 4.03 programs [11, 12].

3 Results and discussions

Studies and observations carried out on 30 days of cultivation of experimental microplants at the stage of adaptation to non-sterile conditions to assess the effect of treatments with Revitalize liquid modifications of both studied preparations revealed the advantage of a single exogenous treatment carried out before planting microplants for adaptation.

When studying the aftereffect of application of Revitalize liquid marka U modification, a reliable influence of the feeding variant (factor A), treatment frequency (factor B) and their interaction on the average shoot length of 7.6 ± 0.16 cm vs. 5, 8 ± 0.06 cm in control, on mean total leaf surface area 49.9 ± 0.11 cm^2 vs. 34.4 ± 0.07 cm^2 in control, mean total root length 46.7 ± 0.06 cm vs. 41.5 ± 0.03 cm and mean root length 7.2 ± 0.02 cm vs. 6.6 ± 0.02 cm (Table 2).

Table 2. Determination of the effectiveness of supplementation with Revitalize liquid preparation of "Universal" marka during *ex vitro* adaptation of red raspberry plants of Gusar variety (30^{th} day).

Feeding option	Frequency of treatments (Factor B)		Factor average A
(Factor A)	Single	Double	
	Length of microshoots, cm		$LSD_{05}a$= 0.21
Untreated (control)	5.8 ± 0.06	5.8 ± 0.06	5.8
Exogenous	7.6 ± 0.16 $^{a, b, ab}$	6.7 ± 0.12 ^{a}	7.2
Fertilization	3.0 ± 0.01 ^{b}	2.6 ± 0.04	2.8
Exogenous + fertilization	5.1 ± 0.11 ^{b}	1.5 ± 0.06	3.3
Factor average B	5.4	4.2	×
$LSD_{05}b$ = 0.11			
$LSD_{05}ab$ = 0.33			
	Total sheet surface area, cm^2		$LSD_{05}a$= 0.41
Untreated (control)	34.4 ± 0.07	34.4 ± 0.07	34.4
Exogenous	49.9 ± 0.11 $^{a, b, ab}$	41.9 ± 0.39 ^{a}	45.9
Fertilization	20.5 ± 0.38	20.8 ± 0.09 ^{b}	20.6

Exogenous + fertilization	31.3 ± 0.12 [b]	12.2 ± 0.02	21.7
Factor average B	34.0	27.3	×
$LSD_{05}b = 0.22$			
$LSD_{05}ab = 0.67$			
	Total root length, cm		$LSD_{05}a = 0.22$
Untreated (control)	41.5 ± 0.03	41.5 ± 0.03	41.5
Exogenous	46.7 ± 0.06 [a, b, ab]	46.1 ± 0.25 [a]	46.4
Fertilization	32.6 ± 0.08 [b]	30.2 ± 0.12	31.4
Exogenous + fertilization	40.5 ± 0.06 [b]	21.2 ± 0.07	30.8
Factor average B	40.3	34.7	×
$LSD_{05}b = 0.12$			
$LSD_{05}ab = 0.36$			
	Number of roots, pcs.		$LSD_{05}a = 0.31$
Untreated (control)	4.7 ± 0.12	4.7 ± 0.12	4.7
Exogenous	6.0 ± 0.12 a	6.0 ± 0.18 [a]	6.0
Fertilization	4.9 ± 0.12	5.6 ± 0.[20 a, b, ab]	5.2
Exogenous + fertilization	5.8 ± 0.18 [a, b, ab]	4.0 ± 0.18	4.9
Factor average B	5.3	5.1	×
$LSD_{05}b = 0.16$			
$LSD_{05}ab = 0.50$			
	Length of roots, cm		$LSD_{05}a = 0.05$
Untreated (control)	6.6 ± 0.02	6.6 ± 0.02	6.6
Exogenous	7.2 ± 0.02 [a, b, ab]	6.8 ± 0.01 [a]	7.0
Fertilization	5.1 ± 0.03	5.2 ± 0.02 [b]	5.1
Exogenous + fertilization	6.3 ± 0.03 [b]	4.3 ± 0.03	5.3
Factor average B	6.3	5.7	×
$LSD_{05}b = 0.03$			
$LSD_{05}ab = 0.08$			

When the after-effect of Revitalize liquid marka Ya was taken into account, the advantage of using exogenous treatments on microplants planted at the rooting stage was also revealed, with a significant effect of fertilization variant (factor A), treatment frequency (factor B) and their interaction on the average shoot length of 8, 4 ± 0.05 cm vs. 5.8 ± 0.06 cm in the control, on the average total leaf area 52.6 ± 0.07 cm^2 vs. 34.4 ± 0.25 cm^2 in the control, on the average total root length 50.6 ± 0.15 cm vs. 41.5 ± 0.02 cm and on the average root length 7.7 ± 0.04 cm vs. 6.5 ± 0.04 cm (Table 3).

Table 3. Determination of the effectiveness of supplementation with the preparation Revitalize liquid marka "Yagodny" during *ex vitro* adaptation of red raspberry plants of the variety Gusar (30th day).

Feeding option	Frequency of treatments (Factor B)		Factor average A
(Factor A)	Single	Double	
	Length of microshoots, cm		$LSD_{05}a$= 0.08
Untreated (control)	5.8 ± 0.06	5.8 ± 0.06	5.8
Exogenous	8.4 ± 0.05 [a, b, ab]	6.6 ± 0.03 [a]	7.5
Fertilization	3.3 ± 0.02	4,6 ± 0.02 [b]	3.9
Exogenous + fertilization	4.1 ± 0.04 b	1.3 ± 0.01	2.7
Factor average B	5.4	4.6	×
LSD05b = 0.04			
$LSD_{05}ab$ = 0.13			
	Total sheet surface area, cm^2		$LSD_{05}a$= 0.30
Untreated (control)	34.4 ± 0.25	34.4 ± 0.25	34.4
Exogenous	52.6 ± 0.07 [a, b, ab]	43.6 ± 0.09 [a]	48.1
Fertilization	18.9 ± 0.18	31.4 ± 0.04 [b]	25.1
Exogenous + fertilization	22.7 ± 0.07 [b]	13.7 ± 0.07	18.2
Factor average B	32.1	30.8	×
$LSD_{05}b$ = 0.16			
$LSD_{05}ab$ = 0.49			
	Total root length, cm		$LSD_{05}a$= 0.23
Untreated (control)	41.5 ± 0.02	41.5 ± 0,02	41.5
Exogenous	50.6 ± 0.15 [a, b, ab]	43.7 ± 0,06 [a]	47.1
Fertilization	27.3 ± 0.26	35.5 ± 0.06 [b]	31.4
Exogenous + fertilization	33.3 ± 0.04 [b]	20.1 ± 0.09	26.7
Factor average B	38.2	35.2	×
$LSD_{05}b$ = 0.12			
$LSD_{05}ab$ = 0.38			
	Number of roots, pcs.		$LSD_{05}a$= 0.32
Untreated (control)	4.7 ± 0.12	4.7 ± 0.12	4.7
Exogenous	6.4 ± 0.12 [a, b, ab]	5.8 ± 0.07 [a]	6.1
Fertilization	4.9 ± 0.12	5.1 ± 0.35 [a, b]	5.0
Exogenous + fertilization	5.0 ± 0.12 [b]	4.0 ± 0.12	4.5
Factor average B	5.3	4.9	×
$LSD_{05}b$ = 0.17			
$LSD_{05}ab$ = 0.53			
	Length of roots, cm		$LSD_{05}a$= 0.06
Untreated (control)	6.5 ± 0.04	6.5 ± 0.04	6.5
Exogenous	7.7 ± 0.04 [a, b, ab]	6.8 ± 0.02 [a]	7.2

Fertilization	5.3 ± 0.02	6.4 ± 0.04 ^{b}	5.9
Exogenous + fertilization	5.9 ± 0.02 ^{b}	4.4 ± 0.02	5.1
Factor average B	6.4	6.0	×
$LSD_{05}b = 0.03$			
$LSD_{05}ab = 0.10$			

When comparing the efficacy of both tested preparations, we see that Revitalize liquid marka Ya is more effective in affecting the average shoot length 8.4 ± 0.05 cm vs. 7.6 ± 0.16 cm marka U, on mean total leaf surface area 52.6 ± 0.07 cm^2 vs. 49.9 ± 0.11 cm^2 of marka U, on mean total root length 50.6 ± 0.15 cm vs. 46.7 ± 0.06 cm, on mean root length 7.7 ± 0.04 cm vs. 7.2 ± 0.02 cm (Figure 1).

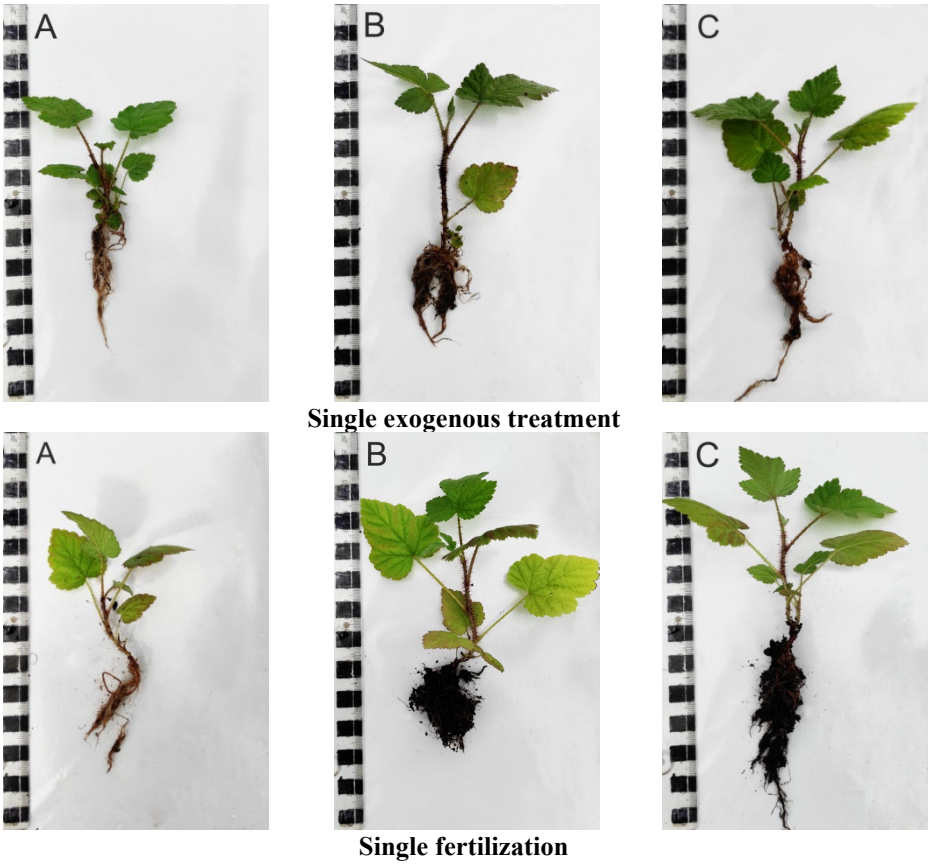

Fig. 1. *Ex vitro* appearance of red raspberry plants of Gusar variety in variants with a single exogenous feeding and fertilization (on the 30th day of adaptation): A - control, B - Revitalize liquid marka Ya; C - Revitalize liquid marka U.

Thus, for preparation of experimental microplants of Gusar variety before planting for adaptation it is recommended to apply Revitalize liquid marka Ya at a concentration of 500 ml: 500 ml of water (1:1) for 5 minutes.

After the conducted studies and observations, the experimental ex vitro plants were transplanted into plastic pots of 0.5 L and transferred to a compartment for pre-growing, where, immediately after transplanting and on 14 days of cultivation, single and double

exogenous fertilization, fertilization and mixed (exogenous fertilization + fertilization) fertilization with the studied preparations Revitalize liquid were carried out.

Accounts and observations carried out on 60 days of cultivation at the stage of growing up, in contrast to the experiments conducted at the stage of adaptation, both studied preparations showed the advantage of fertilization.

The application of Revitalize liquid marka U showed a significant effect of fertilization variant (factor A), treatment frequency (factor B) and their interaction on the average total leaf area of 85 ± 0.05 cm^2 compared to 64.7 ± 0.02 cm^2 in the control and the average root length of 12.4 ± 0.07 cm compared to 11.6 ± 0.02 cm in the control (Table 4).

Table 4. Determination of the effectiveness of supplementation with Revitalize liquid preparation of "Universal" marka during *ex vitro* pre-growing of red raspberry plants of Gusar variety (60^{th} day).

Feeding option	Frequency of treatments (Factor B)		Factor average A
(Factor A)	Single	Double	
	Length of microshoots, cm		$LSD_{05}a$= 0.23
Untreated (control)	5.2 ± 0.14	5.6 ± 0.04	5.39
Exogenous	4.3 ± 0.03	5.4 ± 0.10 ^{b}	4.83
Fertilization	7.4 ± 0.09 ^{a}	7.4 ± 0.19 ^{a}	7.39
Exogenous + fertilization	7.0 ± 0.05 $^{a, b, ab}$	6.7 ± 0.17 ^{a}	6.83
Factor average B	5.96	6.26	×
$LSD_{05}b$ = 0.12			
$LSD_{05}ab$ = 0.38			
	Total sheet surface area, cm^2		$LSD_{05}a$= 0.18
Untreated (control)	64.7 ± 0.02	66.4 ± 0.12	65.58
Exogenous	49.5 ± 0.16	68.6 ± 0.05 $^{a, b, ab}$	59.02
Fertilization	85.0 ± 0.05 $^{a, b, ab}$	83.7 ± 0.11 ^{a}	84.32
Exogenous + fertilization	83.3 ± 0.05 $^{a, b, ab}$	82.5 ± 0.06 ^{a}	82.90
Factor average B	70.63	75.28	×
$LSD_{05}b$ = 0.09			
$LSD_{05}ab$ = 0.29			
	Total root length, cm		$LSD_{05}a$= 0.23
Untreated (control)	168.9 ± 0.27	181.1 ± 0.10	175.01
Exogenous	132.5 ± 0.07	170.8 ± 0.08 ^{b}	151.63
Fertilization	224.1 ± 0.06 ^{a}	230.4 ± 0.05 $^{a, b, ab}$	227.28
Exogenous + fertilization	230.9 ± 0.03 $^{a, b, ab}$	202.3 ± 0.03 ^{a}	216.61
Factor average B	189.10	196.17	×
$LSD_{05}b$ = 0.12			
$LSD_{05}ab$ = 0.37			
	Number of roots, pcs.		$LSD_{05}a$= 0.45
Untreated (control)	14.1 ± 0.41	14.6 ± 0.16	14.33
Exogenous	11.7 ± 0.09	13.3 ± 0.09	12.50

Fertilization	18.0 ± 0.33 [a]	18.3 ± 0.19 [a]	18.13
Exogenous + fertilization	19.5 ± 0.09 [a]	16.3 ± 0.19 [a]	17.93
Factor average B	15.82	15.63	×
$LSD_{05}b$ = Fe<Ft			
$LSD_{05}ab$ = 0.73			
	Length of roots, cm		$LSD_{05}a$= 0.10
Untreated (control)	11.6 ± 0.02	11.8 ± 0.04	11.72
Exogenous	11.1 ± 0.09	11.8 ± 0.04 [b]	11.44
Fertilization	12.4 ± 0.07 [a, b, ab]	12.3 ± 0.02 [a]	12.40
Exogenous + fertilization	12.3 ± 0.02 [a, b, ab]	11.9 ± 0.07	12.10
Factor average B	11.86	11.97	×
$LSD_{05}b$ = 0.06			
$LSD_{05}ab$ = 0.17			

The application of Revitalize liquid marka Ya showed a significant effect of type of fertilization (factor A), frequency of treatments (factor B) and their interaction on average shoot length 8.8 ± 0.06 cm vs. 5.2 ± 0.06 cm in the control, on mean total leaf surface area 96.0 ± 0.02 cm^2 versus 64.7 ± 0.05 cm^2 in control, mean total root length 256.4 ± 0.06 cm versus 169.0 ± 0.16 cm and mean root length 12.7 ± 0.06 cm versus 11.6 ± 0.06 cm in control (Table 5).

Table 5. Determination of the effectiveness of supplementation with the preparation Revitalize liquid of the marka "Yagodny" during *ex vitro* pre-growing of red raspberry plants of the variety Gusar (60^{th} day).

Feeding option	Frequency of treatments (Factor B)		Factor average A
(Factor A)	Single	Double	
	Length of microshoots, cm		$LSD_{05}a$= 0.20
Untreated (control)	5.2 ± 0.06	5.6 ± 0.09	5.39
Exogenous	5.1 ± 0.18	6.5 ± 0.02 [a, b, ab]	5.76
Fertilization	8.8 ± 0.06 [a, b, ab]	6.3 ± 0.12 [a]	7.58
Exogenous + fertilization	5.7 ± 0.13 [a]	8.8 ± 0.06 [a, b, ab]	7.25
Factor average B	6.17	6.82	×
$LSD_{05}b$ = 0.11			
$LSD_{05}ab$ = 0.33			
	Total sheet surface area, cm^2		$LSD_{05}a$= 0.11
Untreated (control)	64.7 ± 0.05	66.5 ± 0.05	65.62
Exogenous	64.1 ± 0.06	80.9 ± 0.04 [a, b, ab]	72.48
Fertilization	96.0 ± 0.02 [a, b, ab]	75.8 ± 0.07 [a]	85.88
Exogenous + fertilization	75.6 ± 0.10 [a]	94.7 ± 0.03 [a, b, ab]	85.12
Factor average B	75.10	79.45	×
$LSD_{05}b$ = 0.06			

$LSD_{05}ab$ = 0.19			
	Total root length, cm		$LSD_{05}a$= 0.21
Untreated (control)	169.0 ± 0.16	181.1 ± 0.07	175.07
Exogenous	151.7 ± 0.09	211.5 ± 0.05 [a, b, ab]	181.58
Fertilization	256.4 ± 0.06 [a, b, ab]	208.0 ± 0.07 [a]	232.18
Exogenous + fertilization	171.9 ± 0.19 [a]	260.6 ± 0.06 [a, b, ab]	216.23
Factor average B	187.24	215.29	×
$LSD_{05}b$ = 0.11			
$LSD_{05}ab$ = 0.35			
	Number of roots, pcs.		$LSD_{05}a$= 0.32
Untreated (control)	14.1 ± 0.09	14.5 ± 0.19	14.27
Exogenous	11.9 ± 0.19	17.5 ± 0.25 [a, b, ab]	14.73
Fertilization	20.9 ± 0.09 [a, b, ab]	16.7 ± 0.19 [a]	18.83
Exogenous + fertilization	14.3 ± 0.09	20.9 ± 0.09 [a, b, ab]	17.57
Factor average B	15.30	17.40	×
$LSD_{05}b$ = 0.17			
$LSD_{05}ab$ = 0.52			
	Length of roots, cm		$LSD_{05}a$= 0.13
Untreated (control)	11.6 ± 0.06	11.8 ± 0.07	11.72
Exogenous	11.4 ± 0.05	12.0 ± 0.03 [a, b, ab]	11.74
Fertilization	12.7 ± 0.06 [a, b, ab]	12.1 ± 0.12 [a]	12.37
Exogenous + fertilization	11.4 ± 0.06	12.8 ± 0.04 [a, b, ab]	12.09
Factor average B	11.79	12.17	×
$LSD_{05}b$ = 0.07			
$LSD_{05}ab$ = 0.21			

When comparing the post-emergence efficacy of both the tested preparations in single treatment during fertilization, we see that Revitalize liquid marka Ya is more effective in affecting the average shoot length 8.8 ± 0.06 cm vs. 7, 4 ± 0.09 cm marka U, on average total leaf surface area 96.0 ± 0.02 cm^2 vs. 85.0 ± 0.05 cm^2 marka U, on average total root length 256.4 ± 0.06 cm vs. 224.1 ± 0.06 cm, on average root length 12.7 ± 0.06 cm vs. 12.4 ± 0.07 cm (Figure 2).

Fig. 2. *Ex vitro* appearance of red raspberry plants of Gusar variety in variants with single fertilization and double mixed fertilization (on the 60th day of pre-growing): A - Control; B - Revitalize liquid marka Ya; C - Revitalize liquid marka U.

When double treatment was given at the pre-growing stage, mixed fertilization with Revitalize liquid marka Ya was the most effective in terms of effect on mean shoot length 8.8 ± 0.06 cm vs. 6.7 ± 0, 17 cm marka U, on mean total leaf area 94.7 ± 0.03 cm^2 vs. 82.5 ± 0.06 cm^2 marka U, on mean total root length 260.6 ± 0.06 cm vs. 202.3 ± 0.03 cm, on mean root length 12.8 ± 0.04 cm vs. 11.9 ± 0.07 cm.

4 Conclusions

1. At the stage of *ex vitro* adaptation of raspberry plants of Gusar variety it is recommended to apply exogenous treatment with Revitalize liquid marka Ya for a single treatment.
2. At the stage of pre-growth of *ex vitro* adapted raspberry plants of Gusar variety it is recommended to apply fertilization for single treatment, and for double treatment to apply exogenous treatment together with fertilization Revitalize liquid marka Ya.
3. Research in this area should be continued in the future.

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