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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

BIOTECHNOLOGICAL ASPECTS OF PRODUCTION OF CLONAL ROOTSTOCKS OF FRUIT CROPS AND GRAPES VARIETIES IN *IN VITRO* CULTURE, INDOOR AND OUTDOOR GROUNDS

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The dissertation work was performed in the biotechnological laboratories of the Research Institute of Fruit and Tea Growing of the Ministry of Agriculture of Azerbaijan and the Republican Unitary Enterprise "Institute for Fruit Growing" of the National Academy of Sciences of Belarus

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GENERAL DESCRIPTION OF WORK

Actuality of a problem and the degree of its study. Today, one of the urgent tasks of the horticultural industry in Azerbaijan is the lack of healthy high-quality planting material of domestic production. In 2019, 2748 thousand seedlings of fruit crops and 156120 seedlings of grapes were produced in the republic, which is not enough to establish new and replace existing orchards and vineyards. The main limiting factor in increasing the production of two-component seedlings of fruit crops is the lack of technology for propagation of healthy clonal rootstocks, since seed stocks do not meet modern requirements for planting material: they are carriers of systemic diseases, including viral ones; they do not allow planting with high planting density and normalized height.

A promising method that really makes it possible to solve this problem is the tissue culture method (*in vitro* culture), which makes it possible to obtain in a short time the most adapted to local soil and climatic conditions, characterized by homogeneity, healthy rootstocks. In many developed countries, *in vitro* micropropagation technology is an integral part of the nursery system.

In Azerbaijan, the industrial practice of micropropagation of fruit plants and grapes is practically absent or exists at an early stage of development. The reasons for the lack of application of a rather promising method in production are not new: high requirements for initial investment, lack of professional personnel in this industry, poor development or lack of technologies for obtaining planting material for many crops and varieties adapted for cultivation in Azerbaijan.

Aim and research objectives. The aim of the study was to develop a technology for *in vitro* propagation of clonal rootstocks of stone fruit crops, pears and local grape varieties, as well as to determine the adaptive potential of healthy rootstocks and seedlings in open ground in Azerbaijan.

Research objectives: - assessment of the characteristics of the proliferation of clonal rootstocks at the stages of introduction into *in vitro* culture, micropropagation, rooting and adaptation;

- development of an improved technology for *in vitro* propagation of clonal rootstocks of stone fruit crops and pear;

- development of an improved technology for in vitro propagation of local grape varieties;

- assessment of the adaptive potential of *in vitro* obtained clonal rootstocks in the open field and the possibility of growing them using intensive technology;

- determination of the compatibility of rootstocks grown in vitro with economically significant varieties of stone fruit crops and determination of the seasonal growth and development of seedlings.

Research methods: The process of introduction into *in vitro* culture, proliferation, rhizogenesis and initial adaptation was carried out according to the methods[1,2] of micropropagation of plants of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine and RUE "Institute for Fruit Growing" of the National Academy of Sciences of Belarus with innovations concerning the sterilization scheme for explants, the composition of nutrient media and cultivation conditions.

The first field of the fruit nursery was laid according to a new technology[3] for Azerbaijan, a two-line system for growing rootstocks on a black opaque mulching film. The agrobiological study of the rootstocks of fruit crops in the open field was carried out according to the methodology[4] of the Kuban State Agrarian University.

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[2] Размножение плодовых растений в культуре in vitro / Н.В.Кухарчик, М.С.Кастрицкая, С.Э.Семенас [и др.]. - Минск: Беларусская навука, - 2016. - 208 с.

[3] Курбанов, И.С., Сулейманова, С.Д., Гаджиев, А.А. Изучение роста и развития подвоев косточковых культур полученных методом in vitro // - Баку: Аграрная наука Азербайджана, - 2016. №5, - с. 32-34.

[4] Дорошенко, Т.Н. Биоэкология и питомниководство плодовых культур: учеб.-метод.пособие / Т.Н.Дорошенко, Л.Г.Рязанова, А.В.Рындин; - Краснодар: Кубанский ГАУ, - 2015. – 62 с.

The objects of the study were clonal rootstocks of stone fruit and pome fruit crops (GF 677, Myrobalan 29C, MaxMa 14, Garnem 15, OHF 87), grape varieties of national selection (Madrasa and Bayan Shirey).

The main provisions of the work submitted for the defense:

- the main factors that determine the high efficiency of *in vitro* culture initiation, micropropagation and rhizogenesis of clonal rootstocks of stone fruit crops (GF 677, Myrobalan 29C, MaxMa 14, Garnem 15), pears (OHF 87), local grape varieties (Madrasa and Bayan Shirey) were as follows: causes of explant contamination and the use of antibiotics for its elimination, nutrient media for all stages of *in vitro* cultivation, genotypes of rootstocks and grape varieties;
- the technology of *in vitro* propagation of clonal rootstocks of stone fruit crops and pears, developed for the first time for the conditions of Azerbaijan;
- for the first time *in vitro* sterile cultures of local grape varieties (Madrasa and Bayan Shirey) were obtained;
- a two-line system for growing clonal rootstocks of stone fruit crops obtained by *in vitro* culture on a mulching film, which ensures good adaptation (95%) in the open field, uniform growth of the rootstocks and the achievement of the sizes required for budding;
- seasonal growth and development of seedlings received by *in vitro* technology, which makes it possible to obtain (under the conditions of Azerbaijan) standard seedlings of almonds, cherries, plums, nectarines, etc.

Scientific novelty of the research. For the first time in Azerbaijan, the whole cycle of biotechnological works for *in vitro* propagation of clonal rootstocks of stone fruit crops (GF 677, Myrabolan 29C, MaxMa 14, Garnem 15), pears (OHF 87), grape varieties of national selection (Madrasa and Bayan Shirey) was developed. The factors of contamination of the explants during initiation of *in vitro* culture were determined, the optimal nutrient media, types of explants were specified, and sterile cultures of 7 genotypes were obtained. For cultivated forms of rootstocks and grape varieties, the efficiency of micropropagation was established depending on the nutrient media used and passages. For the first time

for conditions of Azerbaijan, a technology has been developed for *in vitro* reproduction of clonal rootstocks of stone fruit and pears, and the main parameters of adaptation of *in vitro* plants in greenhouses, protective nets and open ground have been determined.

New data have been obtained on the seasonal growth and development of clonal rootstocks propagated in vitro and tree seedlings of stone fruit crops grown using biotechnology when applying a two-line growing system on mulching film in open ground, which is new for Azerbaijan.

The theoretical and practical significance of the study. The results obtained are valuable contribution to the development of fruit growing and viticulture in Azerbaijan. The technology developed for *in vitro* propagation of clonal rootstocks of stone fruit crops and pears includes a scheme for the production of healthy planting material of clonal rootstocks *in vitro*; accelerated reproduction (technique of *in vitro* culture initiation, nutrient media, micropropagation, rooting, adaptation). The technology developed has no analogues in the Republic of Azerbaijan, its use is ecologically safe and allows to obtain a healthy planting material of high quality clonal rootstocks. Data on the growth and development of clonal rootstocks and seedlings of stone fruit crops when using a new for Azerbaijan two-line planting system on mulching film in the open field, make it possible to regularly obtain standard seedlings of almonds, cherries, plums, nectarines, etc. in Azerbaijan.

Approbation and implementation of the results of dissertation research. The main results of the dissertation were published in 14 papers, including 6 publications included in the list of the Higher Attestation Commission under the President of the Republic of Azerbaijan, 2 in the edition included in the RSCI system and the list of the Higher Attestation Commission of the Russian Federation, 1 in the edition included in the Index Copernicus system Int., 4 - in the materials of conferences (International scientific-practical conference "Problems of ensuring food security of the independent Azerbaijan state and increasing the competitiveness of the agricultural sector" (Baku, 2018), Republican scientific-practical conference "Academician Jalal Aliyev and genetic resources of biological

diversity" (Ganja, 2018), Conference of young scientists and students "Innovations in Biology and Agriculture to Solve Global Challenges" (Baku, 2018), International scientific and practical conference (Novosibirsk, RF, 2019) "Experimental and theoretical research in modern science"). In addition, the Scientific Council of the Research Institute of Fruit and Tea Growing of the Ministry Agriculture approved and recommended for publication (protocol No. 15, 13.12.2019) "Technology for reproduction of healthy planting material of clonal rootstocks using biotechnological methods (microclonal propagation)" developed by the author and "Methodological instructions for conducting laboratory works on micropropagation of fruit and berry crops in vitro "(published).

The results of the dissertation research were introduced in the nurseries of Azerting LLC and Zerdabi ETB LLC.

The name of the organization where the dissertation work was carried out. Research Institute of Fruit Growing and Tea Growing of the Ministry of Agriculture of Azerbaijan, RUE “Institute for Fruit Growing” of the National Academy of Sciences of Belarus.

Structure and volume of work. The thesis consists of an introduction, 7 chapters, conclusions, recommendations for the practical use of the results, a list of references and applications. The work includes 30 tables and 31 figures. The total volume of the thesis consists of 200 typewritten pages. The general text part of the thesis (without tables, figures, bibliography and annexes) consists of 117 typewritten pages and 210,133 characters.

Personal contribution of the applicant. The dissertation work includes the author's research carried out in the period 2016-2019. The applicant identified and formulated the goal and objectives of the research, personally and with his participation, the selection of research objects, the laying of the experimental site, the processing of research results, the rationale and formulation of the provisions put forward for defense were carried out. The applicant personally participated in laboratory and field work, processing of the obtained experimental material, generalization and analysis of research results, and their introduction into production. The applicant personally

prepared publications on the main provisions of the dissertation work.

THE MAIN CONTENT OF THE WORK

The introduction substantiates the relevance of the topic of the dissertation work, formulates the goals, objectives of the research and the main provisions of the work submitted for defense, shows the scientific novelty and practical value of the work.

The first chapter describes the state of the issue under study at the present stage. The advantages, methods and stages of plant propagation *in vitro*, the main conditions for obtaining high-quality regenerant plants on artificial nutrient media are described in detail.

Analysis of literature data shows that at present one of the promising directions for obtaining high-quality planting material of fruit plants is the combination of *in vitro* culture and modern nursery technologies. However, there is no consensus among the authors about the sterilization scheme for explants, the combination and concentration of macro-, microelements and growth regulators in the nutrient medium, and the methods of adaptation even for the same culture.

The second chapter describes the conditions, objects and methods of research. Laboratory work was carried out in the biotechnological laboratories of the Research Institute of Fruit and Tea Growing of the Ministry of Agriculture of Azerbaijan and the Republican Unitary Enterprise "Institute for Fruit Growing" of the National Academy of Sciences of Belarus. Research in indoor and outdoor covered ground conditions was carried out in the greenhouse complex and on the 1st and 2nd fields of the fruit nursery of the Research Institute of Fruit and Tea Growing of the Ministry of Agriculture of Azerbaijan.

The research was carried out in four consecutive stages:

• the first stage – sterilization, initiation of *in vitro* culture, *propagation* and rooting of plants in *in vitro* culture;

• the second stage - adaptation of regenerated plants to non-sterile environmental conditions in a greenhouse and under a shade net;

• third stage - planting of obtained rootstocks on the first field of the nursery;

• fourth stage - budding, obtaining of healthy planting material.

The objects of the study were clonal rootstocks of stone fruit and pome fruit crops GF 677, Myrobalan 29C, MaxMa 14, Garnem 15, OHF 87 and grape varieties of folk selection (Madrasa and Bayan Shirey).

At the stages initiation of in vitro culture, propagation and rooting, the optimal composition of nutrient media for each individual rootstock and variety was selected by modifying the culture media of Murashige-Skoog[5] (MS), Driver-Kuniyuki[6] (DKW) and Gamborg[7].

Evaluating the effectiveness of the microclonal reproduction technology, we took into account: the viability and the level of regeneration of explants (the ratio of the number of viable and regenerated explants to the number of planted ones); the multiplication factor (the ratio of the obtained microshoots to the number of initial ones); the number of rooted microplants; number of roots; the percentage of rooting, etc.

The conditions for the cultivation of microplants in vitro: illumination 2.5–3.0 thousand lux, temperature +21…+25°C, photoperiod 16/8 hours. Duration of subcultivation: 20-21 days.

The adaptation of regenerated rootstock plants obtained in *in vitro* culture was carried out in 4 stages: in a laboratory, in a greenhouse, under a shady net and in an open field.

The first field of the fruit nursery was laid out according to a new technology for Azerbaijan - a two-line system for growing rootstocks on a black opaque mulch film.

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[5] Murashige, T., Skoog, F. A revised medium for rapid growth and bioassays with tobaceo tissue cultures // - Rockville: Physiologia Plantarum, - 1962. 15(3), - p. 473- 497.

[6] Driver J.A., Kuniyuki A.H. In vitro propagation of Paradox Walnut root stock // - Alexandria: HortScience, - 1984. 18(4), - p. 506-509.

[7] Gamborg, O.L. The effect of amino acids ammonium of the growth of plant cells in suspens culture // Journal of Plant Physiology, - 1975. №45, - p. 372-375.

In **the third chapter,** the features of initiation, propagation and rooting stage of *in vitro* culture of rootstocks of fruit crops were studied. Factors affecting the efficiency of *in vitro* culture initiation are the sterilization scheme and the composition of the culture medium (which must be selected for each individual genotype) were considered.

The effectiveness of sterilization is determined by the presence of both external pathogens (which are easy enough to remove with sterilizing agents), and infection inside plant tissues, to eliminate which, most often, various antibiotics are used.

The influence of traditional antibiotics and antibiotics of a new generation on the level of contamination of explants on a nutrient medium, as well as on the growth and development of plants, was studied, the possibility of recovering of explants infected during the passage from bacterial or fungal pathogens was considered.

According to the results of the study, it is recommended to use the antibiotic nystatin at concentrations of 100 and 200 mg/l, tetracycline at a concentration of 100 mg/l, ceftriaxone at concentrations of 10 and 200 mg/l. Among the new generation antibiotics, a high proportion of regenerated explants in all variants is shown when using the antibiotic biotaxime at a concentration of 500 mg/l.

The optimal nutrient media for initiation of *in vitro* culture of clonal rootstocks and their propagation have been determined. For initiation of *in vitro* culture, 7 modified nutrient media were used. In the course of the study, a higher survival rate and intensive development of explants were observed for the rootstock MaxMa 14 on DKW + 0.6 mg/l BAP + 0.01 mg/l IBA + 2.0 mg/l thiamine + 1.0 mg/l nicotinic acid + 2.0 mg/l glycine + 100.0 mg/l inositol (variant VI) - 85.2% (control - 74.3%); for rootstock Myrobalan 29C on MS medium + 0.6 mg/l BAP + 0.01 mg/l NAA + 0.1 mg/l thiamine + 0.5 mg/l nicotinic acid + 2.0 mg/l glycine + 0.5 mg/l pyridoxine + 100.0 mg/l inositol (variant V) - 92.5% (control 75%); for rootstocks GF 677, Garnem 15, OHF 87 on MS medium + 0.6 mg/l BAP + 0.01 mg/l NAA + 2.0 mg/l thiamine + 1.0 mg/l nicotinic acid + 2.0 mg/l glycine + 100.0 mg/l inositol (variant IV) - 95.0 - 93.3 - 91.6%, respectively (control - 75.0 - 83.3 - 74.0%, respectively). Thus,

explants on the nutrient media modified by us were characterized by the highest survival rate and development, which made it possible to increase the efficiency of the initiation stage by 10 - 12%.

At the second stage (micropropagation), the features of the proliferation of explants at the first passages of micropropagation were determined depending on the hormonal composition of the nutritive Murashige-Skoog (MS) medium. Eight different combinations and concentrations of growth hormones have been studied.

It was found that the breeding coefficient of rootstocks, depending on the hormonal composition of medium, varied slightly, but significantly differed depending on the genotypes of rootstocks. The maximum multiplication factor was noted for the stock Myrobalan 29C - 5.39 on a nutrient medium containing 1 mg/l BAP + 0.2 mg/l NAA. The minimum intensity of reproduction was observed in the stock Garnem 15 (1.49) on a medium containing 1.0 mg/l BAP + 0.02 mg/l NAA. On average, the following multiplication factors are shown for rootstocks: Myrobalan 29C (4.13 to 5.39), MaxMa 14 (4.21 to 4.83), GF 677 (4.0 to 4.76), Garnem 15 (1.49 to 1.87).

Considering that the studied combinations and concentrations of hormones did not give the desired result, namely a high multiplication factor and intensive morphological development of microcuttings, it was decided to continue the study and conduct a comparative assessment of these media for rootstocks with our modified media, which showed the highest results at the initiation stage of *in vitro* culture.

As can be seen from Table 1, the media that showed a high result at initiation stage made it possible to obtain a relatively high result at the stage of micropropagation.

The maximum indices of the multiplication factor of rootstock microcuttings for variants of nutrient media I - IV were 5.82 - 6.27.

Thus, the developed composition of nutrient media significantly increased the multiplication factor of microcuttings of fruit crop rootstocks at the stage of micropropagation.

Table 1

Multiplication factor of rootstocks on modified nutrient media

Nutrient media		Multiplication factor				
		MaxMa 14	Myroba-lan 29C	GF677	Garnem 15	OHF87
I	MS+1.0 mg/l BAP+0.02 mg/l NAA	4.21±0.33	4.57±0.60	4.76±0.63	1.49±0.15	4.35±0.62
II	MS+0.6 mg/l BAP+0.01 mg/l NAA+ 2.0 mg/l thiamine + 1.0 mg/l nicotinic acid + 2.0 mg/l glycine + 100.0 mg/l inositol	4.75±0,45	6.02±0,25	**5.82±0,23**	**6.03±0,27**	**6.27±0,32**
III	MS+0.6 mg/l BAP+0.01 mg/l NAA+0.1 mg/l thiamine+0.5 mg/l nicotinic acid + 2.0 mg/l glycine + 0.5 mg/l pyridoxine + 100.0 mg/l inositol	4.32±0,33	**6.17±0,52**	4.20±0,42	5.32±0,24	5.32±0,23
IV	DKW + 0.6 mg/l BAP + 0.01 mg/l IBA+ 2.0 mg/l thiamine + 1.0 mg/l nicotinic acid + 2.0 mg/l glycine + 100.0 mg/l inositol	**6.02±0,42**	5.99±0,48	4.60±0,50	5.0±0,30	5.0±0,45

It was found that *in vitro* reproduction rate of rootstocks changes not only depending on the type of nutrient medium, genotype, but also on the duration of *in vitro* passage. On the example of the rootstock OHF 87, it was shown that when using the modified MS medium during 6 passages, the multiplication factor ranged from 2.5 to 6.22.

The number of microcuttings suitable for rhizogenesis also varied from 52.8% at the fifth passage to 69.9% at the fourth passage. On average, the number of microcuttings suitable for rhizogenesis was 60.5%.

It is noted that the addition of activated carbon at a concentration of 2-3 g/l to the nutrient medium at the passage preceding the rooting stage increases the number of microcuttings suitable for rhizogenesis up to 90-93%.

Optimized nutrient medium MS for micropropagation containing 2 g/l of activated carbon and modified liquid nutrient medium MS with a content of 3 g/l of activated carbon made it possible to stimulate the development and growth of microcuttings, obtain regenerants with a well-developed leaf apparatus, suitable for further passage and transplantation into nutrient medium for rhizogenesis.

The activation of microcutting growth upon the addition of a liquid nutrient medium MS with 3 g/l of activated carbon occurred much faster. Already after 7-10 days, the stem length of 2.2-2.6 cm (average) was noted for microplants, depending on the rootstock, which once again proves the positive effect of this medium on the elongation of stems of microplants.

At the rooting stage, the influence of Gamborg medium on the rooting rate of microcuttings of stone fruit rootstocks was studied. It was determined that the composition of the Gamborg medium for the Myrobalan 29C and GF 677 microcuttings did not fit at all, completely suppressed rhizogenesis, the plants stopped developing and died after a while. The best rooting result (100%) on this medium was shown by MaxMa 14 micro plants. But root formation on this medium was very long and reached 35-40 days, whereas usually the rooting stage takes about 20-25 days.

Further, a study was carried out to select the composition of the nutrient medium for rooting. The influence of the following nutrient media and conditions of 10-day etiolation in variants II-V on the development of microcuttings was studied:

Variant I (control). Nutrient medium Murashige and Skoog (MS) with the addition of growth regulators 0.1 mg/l BAP and 0.5 mg/l IBA;

Variant II. Nutrient medium Murashige and Skoog (MS) with the addition of growth regulators 0.1 mg/l BAP and 0.5 mg / l IBA;

Variant III. Nutrient medium Murashige and Skoog (MS) with the addition of growth regulators 0.5 mg/l IBA and 0.1 mg/l NAA,

Variant IV. Nutrient medium Murashige and Skoog (MS) with the addition of growth regulators 1.0 mg/l IBA and 0.1 mg/l NAA;

Variant V. Nutrient medium Driver and Kuniyuki (DKW) with the addition of growth regulators 1.0 mg/l IBA and 0.01 mg/l NAA.

It was found that for root formation in microplants of MaxMa14 rootstock, the most suitable medium was DKW medium with 1.0 mg/l IBA and 0.01 mg/l NAA (V variant). On this medium, the proportion of rooted microplants was 82.0%. Optimal rooting of rootstock Myrobalan 29C microplants (80.0%), Garnem 15 (88.0%), GF 677 (80%) and OHF 87 (80%) was observed on MS medium using 1.0 mg/l IBA and 0.1 mg/l NAA (IV option). Such root system development is maximal for the studied clonal rootstocks and should provide a high level of adaptation of microplants in non-sterile conditions, on substrates that differ sharply in physical and chemical parameters from nutrient media.

It was also found that root formation on fruit crop rootstocks of Myrobalan 29C, MaxMa 14, Garnem 15, GF 677 and OHF 87 occurs better under conditions of 10 days etiolation followed by cultivation under normal illumination of 2.5-3 kLx and a photoperiod of 16/8 hours.

In addition, no significant change in the efficiency of *in vitro* rooting of fruit crop rootstocks, depending on the passage, was observed.

The fourth chapter is devoted to initiation of *in vitro* culture of local Azerbaijani grape varieties, which is due to the need to start work on the recovery of grapes from systemic diseases.

For the first time, research was begun on the initiation of *in vitro* culture of local Azerbaijani grape varieties Madrasa and Bayan Shirey. For these varieties, a high efficiency of *in vitro* culture initiation on MS medium supplemented with 1.1 mg/l BAP was shown. For Madrasa variety, the lateral buds were characterized by the maximum viability (70.0%), the efficiency of using the meristem and apical bud is slightly lower (66.7%). For the Bayan Shirey variety, the maximum number of regenerated explants was observed for lateral bud (100.0%), for the meristems and apical buds - 66.7%.

At micropropagation stage, it was found that excessive use of growth regulators, in this case BAP, leads to callus formation, which has a negative effect on subsequent root formation.

The aim of the study at rooting stage was to select the most suitable combination and concentration of growth regulators to obtain completely developed plants of grape varieties of folk selection (Madrasa and Bayan Shirey) (Table 2).

When evaluating the results on the stage of rooting of microplants of Madrasa and Bayan Shirey varieties on nutrient media using 9 different hormonal combinations and concentrations, the following were taken into account: the number of shoots (pcs), the length of shoots (cm), the number of leaves on the shoots (pcs), the percentage of rooting (percentage of completely developed plant,%), the number of roots (pcs) and their length (cm).

Table 2

Development of micro plants of varieties Madrasa and Bayan Shirey at the stage of rooting

Variants		Number of shoots, pcs		Shoot length, cm		Number of leaves, pcs	
		Madrasa	Bayan Shirey	Madrasa	Bayan Shirey	Madrasa	Bayan Shirey
1	0.5 mg/l IBA (control)	1.1	1.3	1.38	3.02	2.12	4.4
2	1.0 mg/l IBA	1.0	1.0	**2.46**	**3.25**	**4.1**	**5.13**
3	2.0 mg/l IBA	1.23	1.33	1.68	2.67	1.75	4.16
4	0.5 mg/l IBA + 0.5 mg/l BAP	**2.11**	1.90	1.86	1.71	2.31	3.3
5	1.0 mg/l IBA + 0.5 mg/l BAP	1.12	**2.11**	1.74	1.9	1.70	3.45
6	2.0 mg/l IBA + 0.5 mg/l BAP	1.24	1.89	1.25	2.07	3.22	3.8
7	0.5 mg/l IBA + 0.5 мг/л 2iP	1.6	1.43	1.93	2.04	3.82	3.45
8	1.0 mg/l IBA + 0.5 mg/l 2iP	1.12	1.15	2.09	1.71	2.67	2.8
9	2.0 mg/l IBA + 0.5 mg/l 2iP	1.33	1.1	2.06	1.2	2.58	2.70

As can be seen from Table 2, the largest number of axillary shoots was obtained for Madrasa variety (2.11 pcs) in 4th variant MS + 0.5 mg/l IBA + 0.5 mg/l BAP, and for the Bayan Shirey variety (2.11

pcs) in 5th variant MS + 1.0 mg/l IBA+ 0.5 mg/l BAP. It should be noted that, in general, at the stage of rooting, the shoot formation of Madrasa microplants was lower than that of Bayan Shirey.

In terms of shoot length and activity of leaf formation, Madrasa microplants were also inferior to Bayan Shirey microplants.

Also, during the study, it was revealed that nutrient medium which inhibits the formation of shoots of both varieties, has a positive effect on shoot elongation. So, for the varieties Madrasa and Bayan Shirey in the 2nd variant on MS medium with the addition of 1.0 mg/l IBA was received the minimum number of shoots (1.0 pcs) and the maximum shoot length - 2.46 and 3.25 cm, respectively (Table 2).

Evaluating the root formation of both varieties (Table 3), it was found that the highest percentage of rooting for Madrasa variety was obtained in a medium containing 2.0 mg/l IBA (84%), and for Bayan Shirey variety on a medium containing 1.0 mg/l IBA (82%).

The maximum longest root was recorded in the 1st variant for Bayan Shirey variety (3.9 cm) and in the 2nd variant for Madrasa variety (3.47 cm) with a content of 0.5 mg/l and 1.0 mg/l IBA, respectively.

In terms of quantity, a larger number of roots both for Madrasa variety (7.6 pieces) and for Bayan Shirey variety (6.83 pieces) were formed on MS nutrient medium + 2.0 mg/l IBA. The maximum average length of roots formed *in vitro* for microplants of Madrasa variety (3.34 cm) was recorded on MS medium with a content of 1.0 mg/l IBA, and for micro plants of Bayan Shirey variety (2.33 cm) on MS + 1.0 mg/l IBA + 0.5 mg/l BAP.

Analyzing results of the study, the medium containing 0.5 mg/l IBA + 0.5 mg/l BAP and the medium containing 0.5 mg/l IBA + 0.5 mg/l 2ip completely inhibited the process of root formation in microplants of Bayan Shirey (Table 3).

In general, the optimal results for *in vitro* rooting of both grape varieties were obtained on MS medium containing 1.0 mg/l IBA, except for number of roots, the maximum number of which (Table 3) was obtained on medium containing 2.0 mg/l IBA. This medium (MS + 1.0 mg/l IBA) made it possible to obtain plants with good

morphometric parameters, shoots reached 3 cm or more, each microplant had 3–5 internodes and a well-developed leaf system.

Table 3
Root formation in microplants of grapes of Madrasa and Bayan Shirey varieties at the stage of rooting

	Variants	Rooting,%		Maxim. long root, cm		Average root length, cm		Average number of roots, pcs	
		Madrasa	Bayan Shirey	Madrasa	Bayan Shirey	Madrasa	Bayan Shirey	Madrasa	Bayan Shirey
1	0.5 mg/l IBA (control)	10,0	80,0	0,5	**3,9**	0,34	1,93	0,2	3,7
2	1.0 mg/l IBA	83,0	**82,0**	**3,47**	2,96	**3,34**	2,29	2,6	5,2
3	2.0 mg/l IBA	**84,0**	80,0	0,47	2,67	0,27	1,63	**7,6**	**6,83**
4	0.5 mg/l IBA + 0.5 mg/l BAP	10,0	0	0,21	0	0,17	0	0,44	0
5	1.0 mg/l IBA + 0.5 mg/l BAP	18,0	65,0	0,08	2,84	0,08	**2,33**	0,10	0,89
6	2.0 mg/l IBA + 0.5 mg/l BAP	49,0	62,0	1,64	2,4	1,24	1,71	2,78	1,33
7	0.5 mg/l IBA + 0.5 мг/л 2iP	25,0	0	0,84	0	0,61	0	0,60	0
8	1.0 mg/l IBA + 0.5 mg/l 2iP	32,0	30,0	2,09	0,31	1,40	0,23	1,0	0,4
9	2.0 mg/l IBA + 0.5 mg/l 2iP	60,0	70,0	2,36	2,23	0,24	1,31	0,33	3,8

The plant roots in *in vitro* culture were light, callus was absent at the base, the number of adventitious roots per plant was 3–5 pieces. These characteristics of microplants made it possible to use them for adaptation.

Fifth chapter. The transfer of plants from *in vitro* conditions to *ex vitro* conditions is the final and most stressful stage for tube plants, which determines the success of all work. In our studies, the adaptation of regenerated plants to ex-vitro conditions took place in four stages: 1) adaptation in laboratory conditions; 2) adaptation to greenhouse conditions; 3) adaptation under the shadow grid; 4) adaptation in open ground conditions.

Biometric measurements of morphological parameters of regenerant plants (the number of adapted microplants, %; stem

length, cm; root length, cm) were carried out after using a coconut substrate.

In general, the coconut substrate had a positive effect on the morphological development of microplants. The substrate contributed to intensification of growth processes of aboveground and root parts of plants. Good activity of root system on this substrate was also noted: the formation of large number of lateral and adventitious roots and elongation of the root system, which in turn contributed to the good development of aboveground part of the plant.

The highest percentage of adapted plants was shown by the stock Garnem 15 - 86.4%. The rootstock GF 677 demonstrated good stem formation 3.8 cm and the longest roots 3.54 cm.

This tendency was also noted after 45 days of adaptation, when the established plants were transferred to greenhouse (2nd stage of adaptation) and transferred into pots or cassettes in non-sterile peat substrate (a mixture of peat and agroperlite in a ratio of 5:1).

In a greenhouse, regenerant plants were kept at a temperature of +20...+23°C, periodically watered and enriched with fertilizers. When the plants reached a height of 30-35 cm, demonstrated good development of stems and leaf apparatus, they were taken out under the shade net.

Taking out of regenerated plants from the greenhouse under the shade net (3rd stage of adaptation) was carried out not earlier than the end of April - beginning of May. The adaptation under the shade net is an intermediate adaptation between the adaptation in the closed (greenhouse) and open ground (field) conditions. It is necessary in order to protect plants from direct rays of the sun for some time and adaptation to environmental conditions took place without losses. The adaptation process of plants under the shade net takes at least 10 days.

Then the plants were planted on the first field of the nursery, which was established according to the technology of two-line growing of rootstocks developed at the Research Institute of Fruit and Tea Growing of the Ministry of Agriculture.

The **sixth chapter** provides new data on the seasonal growth and development of *in vitro* cultured clonal rootstocks and biotechnologically grown stone fruit trees seedlings using a two-line open field mulching system.

The two-line growing system on mulch film ensures good adaptation in the open field of clonal rootstocks of stone fruit crops grown *in vitro*. The number of rootstocks adapted in the open field 3 months after planting was 95%.

As a result of studying the dynamics of growth and development of rootstocks in the first field of the nursery, it was found that plants begin to branch out in May, the formation of new shoots and their active growth is completed by the beginning of September. By the middle of the growing season, the plants of the rootstock GF 677 exceeded the requirements of the standard (30-45 cm) in height, and depending on the type and quality of the planting material, this indicator ranged from 80 to 120 cm. For MaxMa 14 and Myrobalan 29C rootstock plants, this parameter was 62- 127 cm and 60-110 cm, respectively.

By the time of budding, the diameter of rootstock stem at the grafting site averaged 8.03 mm for GF 677, 8.8 mm for Garnem 15, 8.6 mm for MaxMa 14, and 9.1 mm for Myrobalan 29C. The survival rate of budding was 92%.

As a result of field studies, it was established that there were no visual symptoms of incompatibility between scions and rootstocks and a high percentage of the survival rate of budding.

The tallest were the seedlings (Table 4) grown on GF 677 rootstock - up to 214.6 cm. Hereafter the highness of seedlings was on Garnem 15 (up to 176.2 cm), Myrobalan 29C (up to 149.03 cm), MaxMa14 (up to 148.5 cm).

An important parameter characterizing the quality of seedlings is the thickness of the rootstock stem at the grafting site and its ratio with the thickness of grafted component. The following variety-rootstock combinations were characterized by minimum difference in the diameter of scion and rootstock at the grafting site: CF 677 + Ferraduel; CF 677 + Ferragnes; Garnem 15 + Guara. The maximum difference in the diameter of scion and rootstock at the grafting site

was observed for all varieties on MaxMa 14 rootstock and for Black Diamond variety on Myrobalan 29C rootstock (Table 4).

Table 4

Parameters of growth rates of seedlings on rootstocks GF 677, Garnem 15, MaxMa 14 and Myrobalan 29C (September, 2019)

Rootstock	Scion (variety)	1 year old seedling (budding 2018)		
		stem diameter, mm (average)		stem height, cm (average)
		under budding	over budding	
CF 677	Guara (almond)	20.6	16.8	199.3
	Ferraduel (almond)	13.1	11.9	**214.6**
	Ferragnes (almond)	13.1	11.8	194.2
Garnem 15	Guayox 35 (peach)	15.8	13.1	154.1
	Guayox 30 (peach)	16.1	13.2	162.2
	Guara (almond)	13.6	12.2	176.2
MaxMa 14	Regina (sweet cherry)	18.5	13.9	139.5
	Ziraat 0900(sweet cherry)	19.6	15.32	148.5
	Yellow Drogan (sweet cherry)	18.8	14.1	139.1
Myrobalan 29C	Black Diamond (plum)	18.0	13.6	149.03
	Black Amber (plum)	17.4	14.0	124.2

Seventh chapter. An economic assessment of the application of the developed technology, namely the nutrient media modified by us, was carried out, taking as a basis the results of a study on rooting of rootstocks of fruit crops *in vitro*.

The results of the study give reason to conclude that the more effective the composition of nutrient medium, the lower the cost of plant, the higher the profitability of production and, accordingly, the profit. The developed modified nutrient media turned out to be not only vegetatively productive, but also economically effective.

CONCLUSION

1. The main parameters established determine the high efficiency of *in vitro* culture initiation, micropropagation and rhizogenesis of clonal rootstocks of fruit crops. The optimal

nutrient media for *in vitro* propagation of clonal rootstocks, providing high efficiency of initiation (85.2-95%), high rates of propagation (5.82-6.27) and high percentage of rooting (80-88%) have been identified [6-8; 10-12].

2. For the first time the *in vitro* culture of grape varieties of folk selection (Madrasa and Bayan Shirey) was established and showed a high efficiency of initiation (66.7-100.0%) and rhizogenesis (82-84%) [3-4; 9].

3. For the first time for the conditions of Azerbaijan, a technology has been developed for *in vitro* reproduction of clonal rootstocks of stone fruit crops and pears, which makes it possible to obtain healthy planting material of high quality all year round [6; 8; Annex 5].

4. The two-line growing system on mulch film ensures good adaptation in the open field of clonal rootstocks of stone fruit crops grown *in vitro* (95%) [1].

5. As a result of field studies, it was found that there are no anomalies in the rootstocks obtained in *in vitro* culture. There are no visual symptoms of incompatibility between scions and *in vitro* rootstocks. High percentage of bud survival (92%) was observed; standard seedlings of 11 variety-rootstock combinations were obtained.

RECOMMENDATIONS FOR PRACTICAL USE OF RESULTS

1. It is recommended to use the developed technology of *in vitro* propagation of clonal rootstocks of stone fruit crops and pears in modern nursery stock farms equipped with biotechnology laboratories.

2. It is recommended to use in nursery farms of the country a two-line system of growing rootstocks of stone fruit crops on mulch film.

3. It is recommended to use the obtained data in the educational process of higher educational institutions of agricultural and biological profile.

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