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THE DEVELOPMENT AND THE MICROCLONAL MULTIPLICATION OF *LYCIUM BARBARUM* L. (WOLFBERRY)

164.01 – BOTANY

Abstract of the doctoral thesis in biological sciences

The thesis was developed in the Embryology and Biotechnology Laboratory of the "Alexandru Ciubotaru" National Botanical Garden (Institute)

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The defence will take place at ***November 5, 2020***, at ***15^{00}*** o'clock, at the meeting of the Committee for public defense of the doctoral thesis, the Polyvalent Hall of the Dimitrie Cantemir State University, 3/2 Academiei Street, Chisinau.

The doctoral thesis and the abstract can be found at the "A. Lupan" Central Scientific Library (Institute) and on the official website of the ANACEC (www.cnaa.md) and on the SUDC website: http://edu.asm.md.

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PURPOSE AND OBJECTIVES OF RESEARCH

The topicality and the importance of the research. The favourable climatic conditions and soil fertility, in the Republic of Moldova, offer farmers the opportunity to cultivate various high-value crops. The quality of autochthonous fruits, vegetables and berries have always been appreciated both in our country and abroad. In recent years, there has been a tendency to plant bacciferous crops, but also to promote the consumption of berries [2, 27]. The diversity of bacciferous crops can be enriched by introducing new varieties of fruit shrubs, one of which is wolfberry (goji) – a functional food high in antioxidants, vitamins and important minerals [1, 25]. Until now, although intensively studied in terms of nutrition value and health benefits of its fruits, this species has been insufficiently studied in terms of morphobiological and structural development of the plants. The conventional methods of propagating this crop (productive cultivars) are not sufficiently effective [28], so, there is a need to introduce new, modern methods that would facilitate and improve this process. The increase of the efficiency of thr production of propagating material will be possible by achieving the objectives related to the propagation of the studied species by tissue culture, obtaining a large number of healthy, genetically homogeneous, high quality specimens, in a relatively short time [3, 9].

Lycium barbarum is a relatively new species in our country and the peculiarities of its cultivation have not yet been sufficiently studied. The cultivation of this species is opportune because the fresh fruits of this shrub would be a valuable addition to the modern diet, which is poor in foods that provide significant health benefits. In the Republic of Moldova, wolfberry is grown by 26 farmers, and the area of land planted with these shrubs increases annually [29]. The commercial growth of bacciferous crops in the Republic of Moldova is at an early stage of development, and the organizations ”Moldavian Berries”, ”High-performance agriculture in the Republic of Moldova” and ”Improving productivity in the cultivation of fruit shrubs and strawberries” have contributed to the development this sub-sector of agriculture [16].

The increased production of berries in the Republic of Moldova has generated a high demand for propagating material, the quality of which also influences plant productivity. For the development and support of fruit shrub nurseries, which sell high quality seedlings, the propagation of plants using various modern methods, especially tissue culture, offers certain advantages such as the production of healthy, disease-resistant plants, which grow faster than those obtained by conventional propagation methods.The correlative micropropagation, phenological, biochemical and anatomical research presented in this paper can serve as a methodological support in the development of agriculture, food, pharmaceutical and cosmetic industries based on autochthonous raw materials.

The **purpose** of the research has been the development of the technology of *in vitro* multiplication of *Lycium barbarum* L., the comparative biomorphological study and the biochemical evaluation of the spontaneous species and the cultivars grown under the pedoclimatic conditions of the Republic of Moldova.

The following **objectives** have been set in accordance with the goal of the research:

- The identification of wolfberry cultivars and the selection of the appropriate explants for initiating *in vitro* culture;
- The selection of growth media suitable for each stage of microcloning;
- The analysis of the developmental biology of the obtained plants, under *in vitro*, *ex vitro* and experimental field conditions;
- The anatomical study and the comparative biochemical evaluation of the content of flavonosides, tannins and ascorbic acid in *Lycium barbarum* L. plants from the spontaneous flora and in cultivated wolfberry varieties;
- The development of a micropropagation technology and description of the protocol that would allow the production of large numbers of wolfberry plants in a short amount of time and the expansion of the gene pool of fruit shrubs within the "Al. Ciubotaru " National Botanical Garden (Institute) (NBGI), (initiation of the collection).

Scientific novelty and originality. For the first time in the Republic of Moldova, wolfberry plants have been propagated through efficient micropropagation techniques and methods for the production of robust, uncontaminated and uniform propagating material. The tissue culture techniques of organogenesis and callus induction in promising wolfberry varieties have been optimized. A complex biological study (morphological, anatomical, biochemical) was carried out on wolfberry cultivars in comparison with the plants of the same species grown in the wild, under the pedoclimatic conditions of the Republic of Moldova.

The theoretical significance. The biotechnological research resulted in new scientific data on microcloning and micropropagation of this species, based on the principle of cell totipotency in tissue culture for the regeneration of plants *in vitro*. Besides, this study has been focused on the biological features of plant growth and development based on phenological, biometric, anatomical and biochemical aspects, under the pedoclimatic conditions of the Republic of Moldova, data that would contribute to a proper training of farmers on the use of drought-resistant and high-quality cultivars.

The applicative value of the paper. As a result of scientific research, the protocol for obtaining healthy and homogeneous planting material of wolfberry cultivars in the Republic of

Moldova has been developed and described in detail. The research results are used in the Embryology and Biotechnology Laboratory of NBGI. The obtained planting material served as a source to initiate the creation of the collection of 5 goji cultivars in NBGI: 'Amber Sweet', 'Erma', 'Ning Xia N1', 'New Big' and 'Licurici' – a cultivar bred by the scientific researchers of the Biotechnology and Embryology Laboratory of GBNI (it is currently tested by the State Commission for Crop Variety Testing of the Republic of Moldova). The wolfberry plants propagated by tissue culture can serve as planting material for the establishment of modern plantations on large areas in the Republic of Moldova. The results of the study can be included in the teaching process at the specialties: Botany, Ecology, Pharmacy and educational institutions with agricultural profile.

The implementation of scientific results. Based on the scientific research carried out by us, the methods of propagation of the species *Lycium barbarum* L. by tissue culture were implemented in the Biotechnology and Embryology Laboratory. The results obtained from phenological and biochemical studies will enrich the spectrum of the berry production sector in the Republic of Moldova. At the same time, they represent scientific-didactic material for the courses: *Applied Botany*, *Pharmaceutical Botany*, *Biological Chemistry* and in educational institutions with biological and agricultural profile and can be implemented in projects with private beneficiaries, farmers and amateurs interested in cultivating wolfberry varieties.

Publications on the topic of the thesis. The obtained results are reflected in 21 scientific papers: 3 articles in reviewed national journals (of which 2 without co-authors), 9 articles in national collections of scientific articles (of which 1 published abroad), 7 papers presented at national and international scientific events, 1 application for plant variety patent.

KEY WORDS

Lycium barbarum L., tissue culture, microcloning/micropropagation, callus induction, rhizogenesis, acclimatization, wolfberry cultivars, phenology, anatomy, biochemistry.

RESEARCH METHODOLOGY

The scientific study and the research methodology, which sum up an impressive number of methods and techniques, selected in this study allowed achieving the objectives set by us. The comprehensive study on the propagation of *Lycium barbarum* L. by tissue culture and the growth and development of plants *in vitro* and *ex vitro* was performed by applying a large number of methods and techniques that made it possible to achieve our objectives. The investigations on the biological processes of plant growth and development, under the climatic conditions of the Republic of Moldova, were carried out using morphological, anatomical and biochemical methods.

The presented research was carried out within the institutional project 15.817.02.26A (2015-2019) “Scientific substantiation regarding the elaboration technologies of *in vitro* multiplication of valuable species of economic interest for the Republic of Moldova”, with the continuation of investigations in the project within the State Program 2020-2023 (20.80009.7007.19) "The introduction and elaboration of conventional and microclonal technologies of cultivation of new woody plant species".

SYNTHESIS OF THE CHAPTERS

The Introduction addresses the topicality and the importance of the problem, the purpose and the objectives of the thesis, the scientific novelty of the achieved results, the theoretical importance and the applicative value of the paper, the approval of the results and the summary of the compartments of the thesis.

1. CURRENTLY AVAILABLE DATA ON THE DEVELOPMENT AND *IN VITRO* MULTIPLICATION OF THE SPECIES *LYCIUM BARBARUM* L.

This chapter contains an analysis of the latest data from the literature on the value of the species in terms of biochemistry and taxonomy, as well as the origin, the natural range and the cultivation of *Lycium barbarum* L. The technologies of plant tissue culture used worldwide, particularly those used to propagate fruit shrubs, are also described here. In this chapter, special attention was paid to the study on the requirements of this crop to pedoclimatic factors and its suitability to the natural environment of our country (requirements in terms of soil, temperature, rainfall, light and ecological characteristics), which will contribute to the proper training of horticulturists, regarding the adaptation of *Lycium barbarum* L. to the agro-pedo-climatic conditions of the Republic of Moldova.

2. THE STUDY OBJECT AND RESEARCH METHODS

The experimental activities carried out in order to develop an efficient micropropagation technology for *Lycium barbarum* L. and the assessment of the morphological and biochemical peculiarities of the spontaneous species and cultivated varieties were performed in the Embryology and Biotechnology Laboratory of the "Al. Ciubotaru" National Botanical Garden (Institute). The investigations by biochemical methods were performed in the Laboratories of Pharmacognosy and Pharmaceutical Botany of the "Nicolae Testemitanu" State University of Medicine and Pharmacy and Plant Biochemistry Laboratory of the Institute of Genetics, Physiology and Plant Protection, in 2016-2019.

Study material. The wolfberry cultivars 'Ning Xia N1', 'Erma', 'New Big', 'Amber Sweet', ‘Licurici’ and the species *Lycium barbarum* L. from the spontaneous flora of the Republic Moldova served as biological material.

Research methods. The research on multiplication by tissue culture included procedures of sterilization of the laboratory glassware, culture media and plant material, the preparation and testing of culture media, the maintenance of the culture of *Lycium barbarum* L. *in vitro* and the transfer of plantlets from *in vitro* conditions into *ex vitro*. The analysis of the adaptation of wolfberry cultivars to the pedoclimatic conditions of R. Moldova was carried out by phenological / biometric examinations regarding the growth of plants, namely: *plant height, number of branches per plant, length of branches per plant and number of leaves per plant*, and by the phytochemical and anatomical study of the cultivars 'Ning Xia N1' ,' Erma' and ‘Licurici’ in comparison with the leaves and fruits of plants from the spontaneous flora [4, 10, 14].

The statistical processing and interpretation of the data was performed by statistical tests (ANOVA, *Bonferroni*, *Duncan* test) that allowed the presentation and the clear interpretation of the statistical results of data processing.

3. MICROPROPAGATION AND *IN VITRO* DEVELOPMENT OF WOLFBERRY CULTIVARS

The study on micropropagation techniques of horticultural plants explores an avant-garde field of horticulture and agriculture that is essential for the production of planting material with high biological value that meets important criteria. The main advantages are the very high multiplication rate, the genetic uniformity, the quality of the propagating material and the higher chance that the new plants will be free of pathogens. In this sense, the aim was to develop an optimized *in vitro* multiplication technology based on simple, accessible, efficient methods, currently widely used in the field of micropropagation, including the production of mini-cuttings *in vitro* [13].

The first objective for obtaining a higher efficiency in the production, by micropropagation, of planting material of the wolfberry cultivars ’Ning Xia N1’, ’Erma’, ’New Big’, ’Amber Sweet’ and ’Licurici’ was to establish basic media and various combinations of growth regulators for the stages of initiation, multiplication and rhizogenesis *in vitro*, using basic nutrient media with the hormonal balance of cytokinins, auxins and less gibberellins. Different media have been considered for the induction of morphogenesis and callogenesis of wolfberry cultivars, the fruits of which have nutritional value for consumers and are of economic importance for farmers and for the country.

3.1. The initiation and the stabilization of *in vitro* cultures of wolfberry

The phase of tissue culture initiation and stabilization involves the sterilization of the biological material, the isolation of explants and their inoculation on an initiation medium. The biological material was taken from mother plants grown in the collection of the "Al. Ciubotaru " National Botanical Garden (Institute).

The choice of the explant. The explants were taken from young plants. In order to obtain vigorous plantlets by tissue culture, an essential condition is the good choice of the explant, which is characterized by a biochemical balance, depending on the age of the mother-plant, its physiological stage, the organ from which it was taken, as well as the structure and the size of the explant [3]. The following explants were tested: apical meristems with leaf primordia, apical meristems, shoot fragments, leaf blade fragments and root fragments.

The asepsis of the biological material. The efficiency of the sterilization of the biological material was achieved by testing four sterilizing agents: sodium hypochlorite, calcium hypochlorite, ethyl alcohol and mercuric chloride. Mercuric chloride proved to be the most efficient, with a plantlet viability rate of 85 %. Mercury chloride (0.01 %) was used as an aseptic reagent for 7 minutes for apical meristems and apical meristems with leaf primordia, followed by 5 rinses with autoclaved water and 3 % hydrogen peroxide solution (H_2O_2), for the researched cultivars. At the same time, the other sterilizing agents proved to be unsuitable, as fungal and bacterial infections occurred.

The culture medium. Two variants of the nutrient media were tested: MS (Murashige-Skoog) and B5 (Gamborg) supplemented with the BAP growth regulator in concentration of 0.1 mg/l and 0.2 mg/l: V1 - *MS modified, without growth regulators;* V2 - *B5 modified, without growth regulators;*V3 - *MS+BAP (0.1 mg/l);*V4 - *MS+BAP (0.2 mg/l;*V5 - *B5+BAP (0.1 mg/l);*V6 – *B5 BAP (0.2 mg/l)* (Figure 3.1).

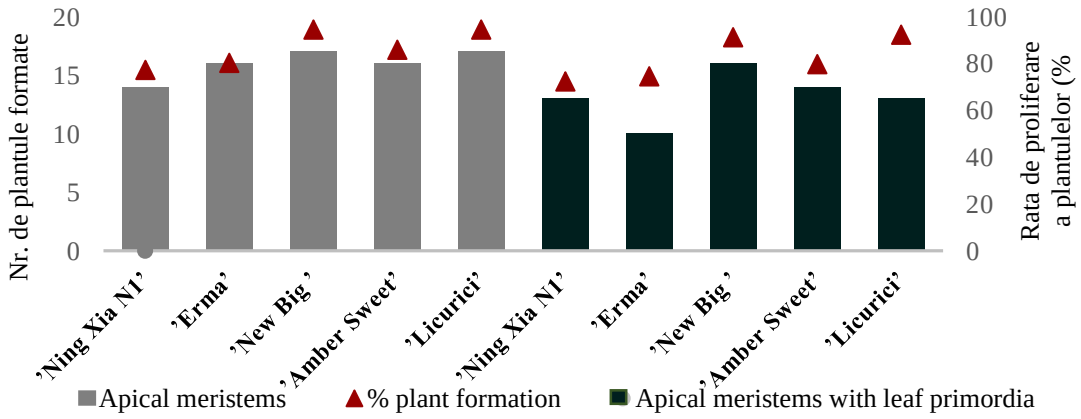

Fig. 3.1. The influence of the V4 medium on the *in vitro* initiation process

Note: V4 - Murashige-Skoog supplemented with BAP cytokine 0.2 mg/l

Due to the obvious vitrification phenomena and the appearance of the purple colour on the the leaves and stems of the plantlets, in the MS and B5 media, inositol concentrations were modified to 50 mg/l. Thus, in the 'Ning Xia N1' cultivar, the average initiation rate calculated at the end of a 30-day-period indicated a number of 14 plantlets initiated from apical meristems and 13 plantlets – from meristems with leaf primordia. In the 'New Big' and 'Licurici' cultivars, in the same culture medium, a maximum number of 17 initiated plantlets was obtained.

Concluding on the above results, we can see that there is a close correlation between the culture medium and the contribution of growth regulators in the process of initiation of *in vitro* culture, influenced to a lesser extent by the donor organs. Thus, depending on the purpose set, the following media were used: media for microcloning (proliferation of shoots) and micropropagation of plantlets; media for the rhizogenesis of plantlets (Table 3.1).

Table 3.1. Combinations and concentrations of growth regulators used to induce microcloning, to stimulate elongation and rhizogenesis in goji cultivars

MS 100%	**Variants of media**												
	Microcloning							**Stimulation of the elongation and rhizogenesis of shoots**					
Hormones (mg/l)	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**
Cytokinins, mg/l:	0,5	0,7	-	-	0,2	0,2	0,2	-	-	-	-	-	
BAP			0,5	0,7	-	-	-	-	-	-	-	-	-
KIN													
Auxins, mg/l:	-	-	-	-	0,2	-	-	-		0,2	0,5	-	-
AIB													
AIA	-	-	-	-	-	0,2	-	0,2	0,5	-	-	-	-
ANA	-	-	-	-	-	-	0,2	-	-	-	-	0,2	0,5
Giberillins,mg/l:	-	-	-	-	0,1	0,1	0,1	-	-	-	-	-	-
GA_3													

Note: **MS**- Murashige & Skoog medium, (1962); **BAP**- benzyl aminopurine; Kin- kinetin; **NAA**- α-naphthaleneacetic acid; **IBA**- indole-3-butyric acid; **IAA** – indole-3-acetic acid; **GA_3**- gibberellic acid

3.2 The influence of cytokinins on the multiplication rate of wolfberry cultivars

The commercial multiplication of a large number of plant species by tissue culture techniques is one of the successful applications of this technology, practiced in many laboratories and appreciated on the global market as an extremely profitable industry. The influence of the composition of the culture medium on the multiplication rate is a particularly important stage in the *in vitro* technological flow [7]. The multiplication phase is carried out on several subcultures with a duration of 3-4 weeks. Thus, the following main aspect is dedicated to increasing the multiplication rate and maintaining the genetic stability of the biological material.

The influence of cytokinins on the *in vitro* multiplication rate. They serve to maintain cell viability, supporting the survival capacity of inoculated explants, promoting the formation and

growth of shoots. Therefore, the amount of cytokinins in the medium was increased for the development of lateral, adventitious buds and the rapid proliferation of shoots from them. To test the response to the presence of cytokinins in the culture medium, BAP and KIN phytoregulators were used, which according to the literature [35] are of interest due to their extremely effective action on the growth and development of wolfberry explants in tissue culture. Thus, four variants of nutrient media with different concentrations (0,5 mg/l; 0,7 mg/l) of each cytokinin were tested. The results regarding the influence of the basic medium on the number of shoots obtained after 20 and 35 days: in this respect, it was relevant the difference from 2 shoots to 8 shoots formed per explant, initially calculated between the experimental variants defined by the MS medium, with concentrations of (0,5 mg/l; 0,7 mg/l) of the BAP and KIN cytokinins in all the studied cultivars. Higher vigor of the shoots was noticed in the experimental variants characterized by the nutrient medium supplemented with BAP cytokinin of (0,5 mg/l) (Figure 3.2).

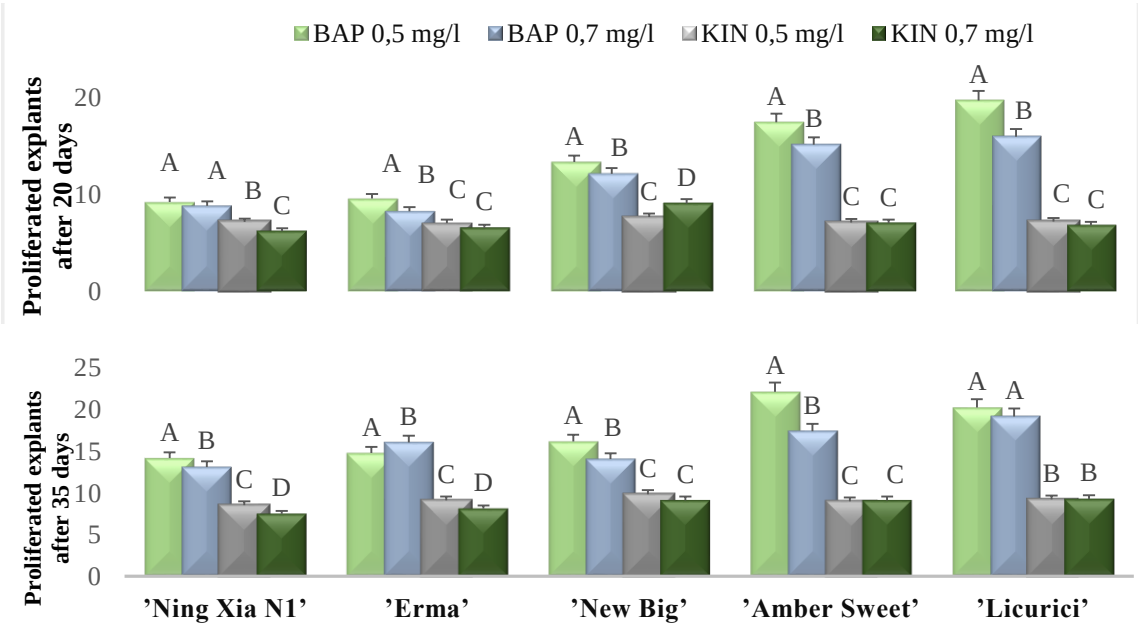

Fig. 3.2. Proliferation and microcloning of wolfberry shoots on the basic nutrient medium MS with different concentrations of BAP and KIN cytokinins (mg/l).

Note: the statistical analysis performed by ANOVA (the bars represent the standard deviation); A, B, C: the interpretation of the significance of differences, using the Duncan test, ($p < 0.05$) between varieties.

The average number of shoots formed per initial explant varies from (20.17) in the cultivar 'Licurici' to (22.06) in the cultivar 'Amber Sweet'. Although in the varieties 'Ning Xia N1', 'Erma' and 'New Big', the number of shoots did not differ significantly (14,11; 14,72 and 16,11 respectively); these differences are statistically significant ($p<0.05$). The height of the plantlets, after 20 days, was bigger in 'Amber Sweet' (4,09 cm), with significant differences as compared with the other cultivars. 'Amber Sweet', 'New Big' and 'Licurici' had the tallest plantlets (4,26; 4,04 and 4,01 cm respectively; $p<0.05$), as compared with the cultivars 'Erma' and 'Ning Xia N1',

which were statistically differentiated with shorter plantlets, respectively, the height of the stem reached 3,25 and 3,17cm after 35 days (Figure 3.3). As a result of the investigations carried out, it was found that, as the concentration of BAP cytokinin decreased to 0,5mg/l, the length of the shoots increased and with the increase of BAP concentration to 0,7mg/l, the height of the shoots decreased insignificantly, the same rule was noticed while using KIN cytokinin of the same concentrations.

In conclusion, adding BAP cytokinin in the MS medium at low concentrations, significantly influences the formation of the shoot, but inhibits the elongation of the root [8].

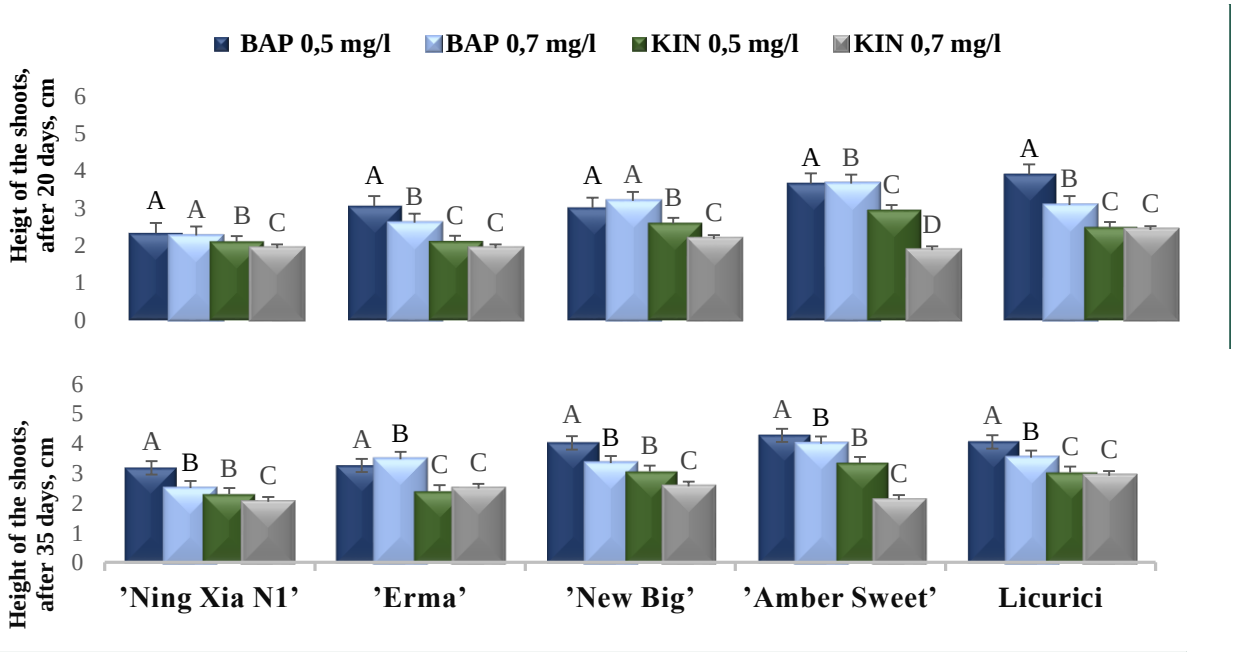

Fig. 3.3. The height (cm) of the shoots on media supplemented with BAP and KIN cytokinins

Note: statistical analysis performed by ANOVA, (bars represent the standard deviation; A, B, C, D: the interpretation of the significance of differences, using the Duncan test, $p < 0.05$) between cultivars.

3.3. The influence of the combination and concentration of growth regulators on the multiplication rate and the process of growth of wolfberry tissue cultures

The synergistic combination of auxin, cytokinin and gibberellin (GA_3) promoted the organogenesis processes in explants cultured *in vitro* by influencing the physiological processes [18]. Combinations of three types of growth regulators: cytokinins, auxins and gibberellins (BAP+IBA+GA_3, BAP+IAA+ GA_3, BAP+NAA+GA_3), each being supplemented with various concentrations of 0,3 + 0,2 + 0,1mg/l, gave optimal results in terms of the multiplication rate.

The highest results were obtained on the M6 medium with the hormonal balance composed of 0,2 BAP mg/l + 0,2 NAA mg/l + 0,1 GA_3 mg/l, which had a significant effect ($p<0.05$, according to the Duncan test) on the micropropagation rate, as compared with the other phytohormone combinations. The average number of proliferated shoots per initial explant was raised to (4,56

and 4,94) in the cultivars ’Ning Xia N1’ and ’Erma’, the highest proliferation of shoots was observed in the cultivars ’New Big’ of (6,94), followed by ’Amber Sweet’ (5,06) and ’Licurici’ (5,22). The combination of growth regulators on the M5 medium influenced the low share of shoot proliferation in all the studied cultivars, generating on average 2-4 shoots, with a length of 1,68-2,3 cm (Figure 3.4).

The concentration of the hormonal balance of the M7 medium revealed a lower share as compared with the M5 and M6 mediums. Thus, the proliferation of shoots showed statistically significant differences ($p<0.05$), the cultivar ’Licurici’ produced an average of 5,22 shoots, as compared with the varieties ’Amber Sweet’, ’New Big’, ’Erma’ and ’Ning Xia N1’ (2,78; 2,61; 2; 1,61 shoots). The minimum length was found in the ’Ning Xia N1’ cultivar – of 2cm per explant and the ’Amber Sweet’ and ’New Big’ cultivars – with a length of 2,52cm and 2,78cm. The cultivar ’Licurici ’ has developed a maximum shoot growth of 3,61cm.

Generalizing the data obtained in the study, we mention that by the experiments performed using three types of regulators from the group of cytokinins, auxins and gibberellins, a hormonal balance was created in a ratio of 2:2:1, significant for $p <0.05$ (according to the Duncan test), using meristems from apical and lateral buds as initial explants.

We can assert that the results obtained by us are similar to those reported by other researchers, who suggested for the micropropagation of wolfberry the same combinations of growth regulators [22].

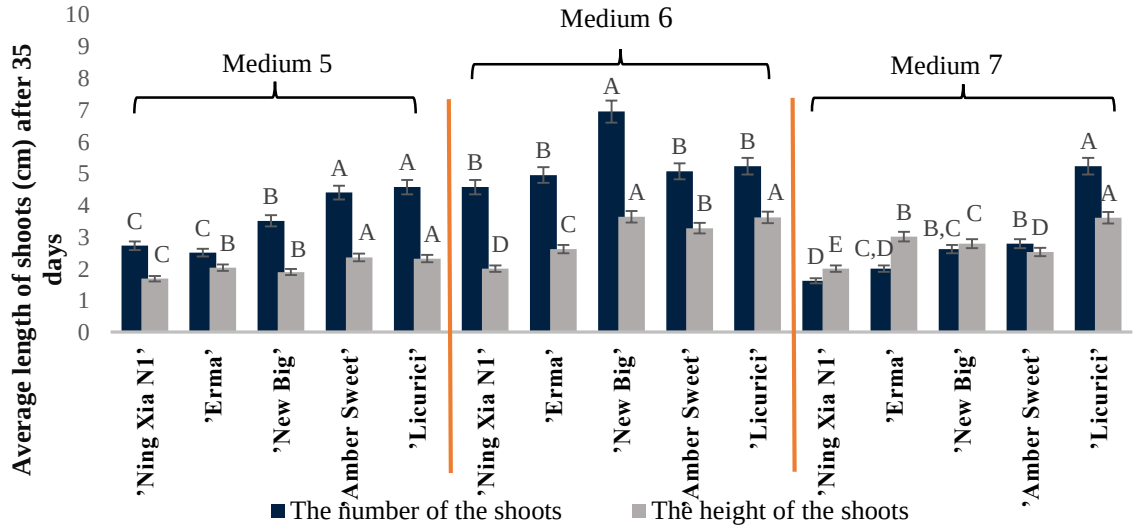

Fig. 3.4. The influence of growth hormone combination and concentration on *in vitro* growth and proliferation of wolfberry cultivars

Note: **Medium 5** - BAP (0.2 mg/l) +IBA (0.2 (mg/l) + GA_3 0.1 (mg/l); **Medium 6** - BAP (0.2 mg/l)+NAA(0.2 mg/l) + GA_3 (0.1 mg/l); **Medium 7** - BAP (0.2 mg/l) +IAA (0.2 mg/l) + GA_3 (0.1 mg/l).
statistical analysis performed by ANOVA, (bars represent the standard deviation; A, B, C, D: interpretation of the significance of differences, using the Duncan test, $p <0.05$) between cultivars.

3.4. The influence of auxins on the process of growth and rhizogenesis in wolfberry cultivars

Auxins, cytokinins and gibberellins predominated in the microcloning stage in low concentrations, and in the rhizogenesis stage, it is recommended to reinstall the apical dominance at the level of *in vitro*-generated stems and rooting them by supplementing the basic medium with auxins [12]. Assuming that the culture medium used for the multiplication of shoots derived from meristems can significantly influence the process of *in vitro* rhizogenesis, experiments aimed at determining the rooting capacity of plantlets were performed according to the multiplication medium.

Induction of the rhizogenesis process. For the induction of the root system, the basic media MS 100% and MS 50%, liquid and with agar, supplemented with low concentrations of growth regulators from the auxin group IBA, IAA and NAA, of 0,2 mg/l and 0,5 mg/l were tested.

The obtained results showed a positive correlation between the mineral composition of the basic medium MS 100% used for the multiplication of explants and the rooting capacity of shoots of the cultivars: ’Ning Xia N1’, ’Erma’, ’New Big’, ’Amber Sweet’and ’Licurici’, with a rooting rate between 40,3-100 % [31]. The analysis of the presented data on the influence of auxin concentrations in the culture medium on the rooting rate has shown that the medium M12 significantly surpasses the others, with over 95 % for all wolfberry cultivars (Figure 3.5).

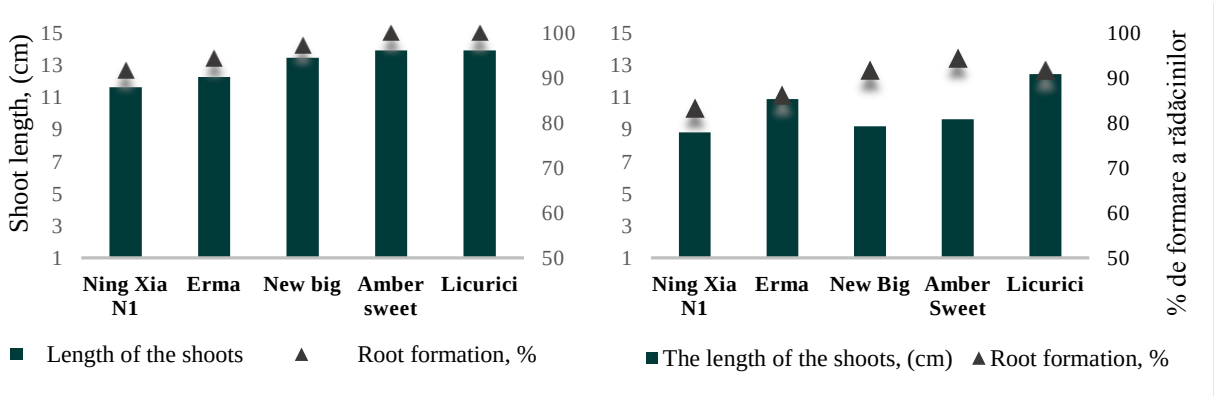

Fig. 3.5. The rooting capacity of wolfberry cultivars in the medium MS 100%, NAA of 0,2 mg/l (left) and 0,5 mg/l (right)

In the case of plantlets regenerated in the media M10 and M11, the increase of the IAA concentration from 0,2 to 0,5 mg/l determined a rooting rate of 46% and 73,3%, respectively. In the medium M12, the initiation of the roots was observed within 14 days after the *in vitro* transfer, and in 20-25 days, there was a considerable growth of the plantlet, which could already be used as material to take cuttings for further multiplication, and the lower part of the plantlet was transferred to *ex vitro*. The medium supplemented with IBA with the same concentration of 0,2mg/l showed rooting rates between 40,4% and 74,6% [30].

The nutrient medium MS 50% supplemented with NAA auxin of 0,2 mg/l, was used for conservation, over a certain period. The rooting of the researched cultivars, after 30 days, exceeded only 16%, and an increase in the rooting process, of over 90%, was recorded after 60 days. Another method of conservation is micropropagation by micro-cutting culture. Once the shoot reached a length of 10,0-12,0 cm, cuttings were taken from it and transferred to another nutrient medium for further micropropagation.

3.5. *In vitro* organogenesis in wolfberry cultivars (callus culture)

Most cultivated plants, especially those that are propagated vegetatively, are frequently infected by viruses and pathogenic microorganisms. The diseases of plants lead to a decrease in their productivity and quality. In addition to the use of meristem culture, another method of plant healing, widely applied to many crops, is clonal multiplication by using callus culture. Other horticultural species have also been propagated by callus culture: *Actinidia arguta, Rubus idaeus, Rubus fruticosus, Fragaria sp.* etc [20, 26, 34].

Seven nutrient media with different concentrations were tested: control (0 mg/l); cytokinin: BAP (0, 1 mg/l; 0,3 mg/l) and auxin: 2,4D (0,3 mg/l; 0,5 mg/l; 1,0 mg/l). Callus induction and the regeneration of shoots from callus in the wolfberry cultivars ’Ning Xia N1’, ’Erma’, ’New Big’, ’Amber Sweet’ and ’Licurici’ was initiated from petiole fragments (5-7 mm long) and leaf fragments (3-5 mm in diameter), harvested from plantlets regenerated *in vitro*.

The process of organogenesis in wolfberry. The callus developed from leaf blade discs and petiole segments on the experimental media M_1-M_7, in different combinations and concentrations of growth regulators in culture media, induced cell proliferation, characterized initially by the deformation of somatic explants, which varied in shape, color and consistency. A friable, morphogenic callus was well developed approximately 40-45 days after the *in vitro* inoculation of somatic explants. Because our goal was not only to obtain the callus itself, but its proliferation, we found that it best responded to the callusogenesis of petiole fragments, on M_6 medium with the addition of 2,4D (1,0 mg/l) in combination with BAP (0,1 mg/l), with 100% results, and – from the leaf blade disc – an average percentage of 55,7% was obtained for all wolfberry cultivars.

The explants from petiole fragments cultivated on the M_6 medium, became corrugated and enlarged after 14-20 days from inoculation, thus forming a rosette of 6-8 mini-shoots each. Thus, callus proliferation is different, depending on the cultivar analyzed. The best proliferation was observed in the case of the cultivar ’New Big’, with a maximum number of shoots of 8,22, followed by the cultivars ’Erma’, ’Licurici’ and ’Amber Sweet’, with an average number (7,17; 7,50; 7,94) of proliferated shoots, and minimum values were recorded in the cultivar ’Ning Xia

N1' – 6,83 shoots. Plantlet generation from leaf blade in the cultivar 'Ning Xia N1' resulted in an average number of 1,89 shoots, 'Licurici ' – 3,28 shoots and 'Amber Sweet' – an average number of 3,39 shoots, but, in the cultivars 'Erma' and 'New Big' there was zero shoot development.

The plantlets obtained from callus mass, on the M_6 medium, were healthy, robust, vigorous, they were not contaminated in the *in vitro* culture after subsequent steps, nor while being transferred to *ex vitro* [19]. The regenerated plants had normal morphological features like the mother-plant.

3.6. The acclimatization of propagating material obtained by *in vitro* culture

Ex vitro acclimatization is the final stage of micropropagation. In this phase, the seedlings are gradually transferred from the artificial environment, to the usual conditions in the greenhouse (*ex vitro*) or in the field, close to the natural conditions. The acclimatization of wolfberry seedlings was done by classical methods, in two stages, for 30-40 days. *Stage I:* V1 – substrate consisting of lawn soil, peat, perlite, sand, leaf soil, in proportions of 1:0,5:0,25:0,25:1; V2 – substrate consisting of lawn soil, commercial peat, perlite, sand and leaf soil at the ratio of 1:1:0,5:0,5:0,25 and V3 – substrate consisting of commercial peat, lawn soil and sand at the ratio of 1:1:0,25. The commercial peat used must have neutral pH. The phenotypic observations and the analysis of the recorded results showed that the most effective substrate proved to be V2. *Stage II* - wolfberry plantlets, which reached 5-7 cm in length and formed 5-7 leaves, were transferred to a substrate of lawn soil, peat, sand, at 1:0,25:0,25 ratio, in containers of 500 ml, covered with a cloth, placed so that the sunlight penetrates from only one direction.

After the acclimatization process, the plants were transferred to the experimental field (the collection of fruit shrubs) and we made observations on the process of their adaptation to the pedoclimatic conditions (described in Chapter 4).

4. COMPARATIVE MORPHO-ANATOMICAL AND BIOCHEMICAL STUDY ON THE SPECIES *LYCIUM BARBARUM* L.

Little research has been done until now on the biological peculiarities of growth and the phytochemical features of the species *Lycium barbarum* L., including the identification of the diversity and the amounts of biologically active substances, therefore it would be helpful to conduct a more detailed research. The aim of our research has been the individual adaptation of wolfberry varieties to the pedoclimateric conditions of the Republic of Moldova, such as: morphological, anatomical, physiological and biochemical changes. The increase in the economic value of the species is due to its multiple nutritional and therapeutic properties [24].

4. 1. Phenological traits of shrubs of the species *Lycium barbarum* L.

The phenological phases of *Lycium barbarum* L. were recorded based on the observations made since the plants came out of dormancy in the first year (March 2017) and the second year of their cultivation (April 2018).

The three cultivars had a relatively similar dynamics of the phenological phases, but the cultivar 'Licurici' entered the growing season later in 2017, but in 2018 it surpassed it. During the flowering stage, all three cultivars continued to produce flowers until the end of November, which indicates that in the area of the Republic of Moldova, the flowering and fruiting stages are longer than it was mentioned in the literature for other geographical areas [23].

4.2. Biometric features of the vegetative organs of *Lycium barbarum* L. shrubs

The biological characteristics of *Lycium barbarum* L. shrubs in 2016-2018 were analyzed based on four parameters such as: plant height, number of shoots, length of shoots and number of leaves on the cultivated plants.

The dynamics of plant growth in height. In terms of this feature, there were significant differences between taxa, depending on the analyzed period. At planting (18.11.2016), the average height of potted plants fluctuated between 40 and 50 cm.

After 12 months from planting (November 2017), the height of 1-year-old plants varied on average from 56,6 cm in the cultivar 'Ning Xia N1', 75.67 cm - 'Erma' to 93,87 cm - 'Licurici'. Although the plants did not differ significantly in height, these differences were statistically significant ($p<0.05$).

After 24 months from planting (November 2018), the comparative analysis of the average height of the 2-year-old shrubs was carried out. The cultivar 'Licurici' with 195,53 cm and 'Erma' – 184,33 cm were the tallest and showed statistically insignificant differences in plant growth, except for the variety 'Ning Xia N1' – 173,47 cm.

The plants allowed to grow without any pruning, in order to study their natural development, had several stems: between 1 and 5. The wolfberry shrubs had on average a single stem. The growth rate of the plants in the experimental group revealed the fact that, during 2016-2018, the *Lycium* shrubs had an average growth of 0,36 – 0,55 m/year.

The dynamics of branch development on the plants. The growth of *Lycium barbarum* L. branches in the first year of development was studied on each plant. The most representative stages in this study were: May, June-August and November.

After 12 months from planting (November 2017), the number of branches differed significantly between cultivars, thus, the plants of the cultivar 'Ning Xia N1' had on average 18.47 branches, less than the cultivars ’Erma’ and ’Licurici ’ (22,73 and 34,73, respectively).

After 18 months from planting (May 2018), the shrubs of the cultivar ’Licurici’ had more branches (34,73), with significant differences in comparison with the varieties ’Erma’ and ’Ning Xia N1’ (22,73 – 22,60).

The length of branches. The length and number of branches are important indicators in the formation of plant mass and in the dynamics of plant growth.

After 12 months from planting (November 2017), as for the indicator – *the average length of the shoots,* significant differences between cultivars were noticed. The differences turned out to be of a minimum value of 17,67 cm - ’Ning Xia N1’, 21,0 - ’Erma’ and a maximum of 23,13 cm - ’Licurici’.

After 18 months from planting (May 2018), the length of the shoots showed statistically significant differences ($p<0.05$) in the plants of the cultivar ’Ning Xia N1’ with a minimum value of 4,99 cm, as compared with the plants of the cultivars ’Licurici’ and ’Erma’ with maximum values of 7,67 and 6,83 cm, respectively, with statistically insignificant differences.

Thus, it was found that, in 2018, the average length of the shoots, as compared with the same period of the previous year (May 2017), had lower indices. This was true for all the studied cultivars. Analyzing the number of shoots in these two periods (May 2017 and 2018), we can assume that the low indices of the average length of shoots in 2018, were due to the fact that, in that year, the number of new shoots was much higher than in the previous one.

Leaf formation. The shape of the leaves varied considerably between the studied cultivars. The total number of leaves per plant, in the first year after planting, was determined by measurements made between May and June 2017. The maximum value was 195,47 leaves in ’Licurici’, with statistically significant differences as compared with the other investigated cultivars, but the minimum values, of 161,53 and 171,01 leaves, respectively, were recorded in the cultivars ’Ning Xia N1’ and ’Erma’. The number of leaves per plant increased significantly in the second year after planting in comparison with the first. Initially, the cultivar ’Licurici’ had a much higher average number of leaves on its plants than the ’Ning Xia N 1’ and ’Erma ’. However, in the second year, statistically significant higher values were recorded in the cultivars ‘Licurici’ and ‘Erma’ as compared with the cultivar ’Ning Xia N1’.

Phenological observations revealed that most of the plants of these three taxa of *Lycium barbarum* L. did not reach maturity until 2018. The plants of the cultivars ’Erma’ and ’Ning Xia N1’ had a more modest growth rate than those of ’Licurici’ in May and July-August 2017, but it

increased towards the end of that year. ’Erma’ and ’Ning Xia N1’ maintained this impressive growth rate in 2018 as well.

4.3. Adaptive anatomical features of leaves (in greenhouse and outdoors) in the species and cultivars of *Lycium barbarum* L.

In the present study, a comparative anatomical research on the species *Lycium barbarum* L. from the spontaneous flora and its cultivated varieties was conducted in order to establish structural adaptive characteristics of their drought resistance under the pedoclimatic conditions of the Republic of Moldova. The anatomical description of the leaves of the studied wolfberry taxa highlighted structural indices that play a major role in identifying these taxa.

Thus, in the leaves of *Lycium barbarum* L., the adaxial epidermis consisted of epidermal cells of polygonal shapes, covered with a slightly thicker cuticle, as compared with the abaxial epidermis. The epidermal cells, located above the mesophyll, had a thicker cuticle than the epidermal cells on the leaf veins. Another characteristic was the lining of the vascular bundles (consisting of idioblastic cells with brown or black content).

The cultivar ’Ning Xia N1’ contained anisocytic stomata on both epidermises, surrounded by 3 subsidiary cells. Epidermal cells were isodiametric. There were crystals with calcium oxalate grouped as druses.

In the cultivar ’Erma’, anomocytic stomata were present on both epidermises, but numerically, there were more on the lower one. The mesophyll of the leaf was differentiated dorso-ventrally, the palisade tissue consisted of 2 rows of slightly elongated cells, well arranged under the upper epidermis, the lower epidermis had round or oval cells.

The cultivar ’Licurici’ – oval-shaped epidermal cells with chloroplasts, in a single layer. The calcium oxalate druses were dispersed and arranged into the sheath, the palisade tissue cells were arranged in two rows, with elongated cells, with little intercellular space. From a histo-anatomical point of view, all the studied taxa were characterized by great uniformity and absence of hairs (trichomes). The epidermises were similar, consisted of a single layer and were strongly cutinized, with elongated cells, with thickened outer walls.

Comparative anatomy of the leaves of some studied taxa (greenhouse and field conditions). The study on the structural peculiarities of the leaves in the cultivars and the species *Lycium barbarum* L., which differ by origin, is of interest for the full understanding of the biological properties of the taxa, which are of great horticultural value. Although the analyzed cultivars where characterized by different thickness of the leaf blade, the analysis of the correlation between the mesophyll and the thickness of the leaf blade among cultivars showed

that the highest values of 0,85-0,89 were recorded in the taxa ’Erma’, ’New Big’, ’Amber Sweet’ and ’Licurici’ (Table 4.1).

Table 4.1. Anatomical characteristics of the leaves of the species *Lycium barbarum*, spontaneous and cultivated (under greenhouse and field conditions)

Species, cultivar	Anatomical characteristics						
	Thickness of the leaf blade (µm)	Thickness of the epidermises (µm)		Thickness of the mesophyll (µm)	Thickness ratio index		
		Adaxial	Abaxial		Mesophyll /leaf blade	Adaxial/ abaxial epidermis	Epidermis /leaf blade
***Lycium barbarum* L.** (SFF.)	135,00±0,50	20,30±0,62	7,23±0,33	107.0±0,62	0,79	2,8	4.90
Ning Xia N1 (G)	117,27±1,21	14,53±0,35	9,80±0,63	85,0±0,76	0,72	1.48	4.80
Ning Xia N1 (F)	126,17±4,07	15,90±0,66	12,25±0,41	123,40±1,52	0,74	1,29	4.48
Erma (G)	126,00±0,49	18,30±0,64	11,00±0,26	97,20±2,27	0,73	1,66	4,3
Erma (F)	143,20±0,85	19.60±0,62	14,70±0,54	155,50±1,29	0,89	1,33	4,17
Licurici (G)	187,40±0,74	27,30±0,99	18,95±0,85	162,30±0,30	0,86	1,44	4,05
Licurici (F)	195,00±0,82	35,60±1,17	27,7±0,84	167,0±2,02	0,85	1,28	3,08

Note: n – deviation; ***SFF*** – spontaneous flora, field; ***G*** – greenhouse; ***F*** – field.

The spontaneous species and the cultivar ’Ning Xia N1’ record values between 0,79 and 0,74. For the cultivars grown in the greenhouse, minimum values were achieved by ’Ning Xia N1’ – 0,72 and maximum - by ’New Big’, ’Amber Sweet’ and ’Licurici’ – 0,87.

The results of the research on the quantitative and qualitative anatomical structure (described in detail) and the comparative anatomical study on the leaves of the researched biotypes, focused on a complex of anatomical indicators, showed that the above-mentioned cultivars had a structural adaptive character in response to the action of environmental conditions. This fact was indicated by the adaptation of external structures such as: thick cuticle of external-internal type, the size and the degree of packing of epidermal and internal cells: the degree of development of the mesophyll, the presence and the distribution of calcium oxalate druses and the degree of development of the mechanical tissue.

4.4. The biochemical study of leaves and fruits of wild and cultivated *Lycium barbarum* L. plants

Bioactive compounds act like antioxidants and have ability to capture radicals in biological processes, that is why they posess phytotherapeutic effects. Tannins and flavonoids have demonstrated antibacterial, antiviral, antioxidant and anti-inflammatory effects. Also, the main

feature of an antioxidant, which can be enzymatic, polyphenolic, vitamin or pigment, is the ability to neutralize free radicals and reactive oxygen species [15].

4.4.1. The qualitative analysis of flavonoids and tannins

The description of the phytochemical *screening* of the specific chemical reactions in the analyzed samples, denotes that the degree of expression is more pronounced in the extracts from the leaves than from the fruits, which demonstrates the presence of a different flavonoid spectrum. Thus, various flavonoid compounds are present in the organs of the wild and cultivated wolfberry plants (plants obtained by tissue culture).

The analysis of the results of the application of analytical staining and sedimentation reactions demonstrates the presence of mostly condensed tannins (staining in blackish-green with the solution of iron ammonium alum, and greenish-yellow – with solution of acetic acid and lead acetate). The effects of the analytical reactions were much more pronounced in the leaves than in the fruits, especially in the extracts of the products harvested from the spontaneous flora.

4.4.2. The quantitative determination of biologically active compounds

The total flavonoid content. The spectrophotometric analysis of plant extracts resulted in quantitative data on the total flavonoid content determined in grams per 100 g of plant products expressed as rutoside (PV) (Figure 4.1). The obtained results show that the flavonoid content prevailed in the leaves of wild plants (50,33 mg/g PV). The leaves of cultivated plants had high values of 56,33 mg/g PV in the cultivar ’Licurici’, followed by ’Ning Xia N1’ and ’Erma’ with a content of 56,00 mg/g PV.

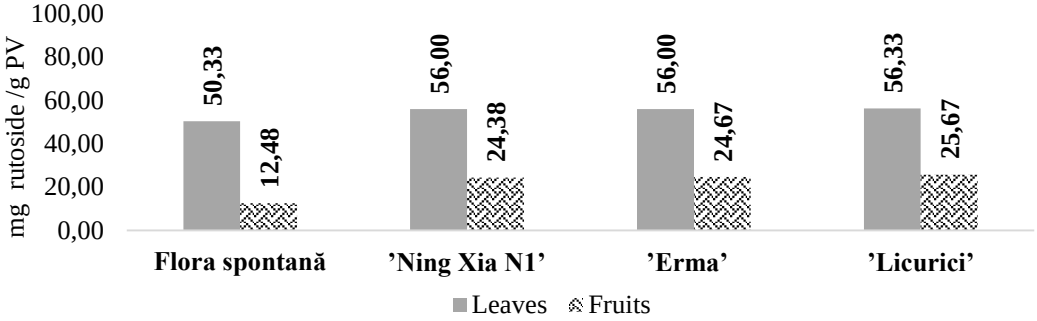

Fig 4.1. The total flavonoid content in leaves and fruits of wild and cultivated wolfberry

The fruits of spontaneous plants showed minimal values (12,48 mg/g PV), much lower than the cultivated ones. Thus, the cultivars ‘Licurici’ (25,67 mg/g PV) and ’Erma’ (24,67 mg/g PV) contained the highest amounts, and ’Ning Xia N1’ (24,38 mg/g) – the lowest.

The total tannin content. The determination of the tannin content was performed by applying the following methods: titrimetric (*permanganometry, permanganometry associated with*

the sedimentation of tannins with gelatin) and spectrophotometric (wavelength 275 nm, recalculation to the standard tannin solution). The results of determining the tannin content in the vegetal products (leaves and fruits) from the spontaneous and cultivated flora are summarized in Figure 4.2.

The permanganometric method + gelatin sedimentation - revealed maximum values for the leaves from the spontaneous flora with 5,84 %, followed by the cultivars ’Erma’, ’Licurici’ - with 3,96 % and ’Ning Xia N1’ with 3,90 %. As for the content of tannins in fruits, the percentage values ranged between 2,34 % - spontaneous flora and 1,94 % - cultivated varieties.

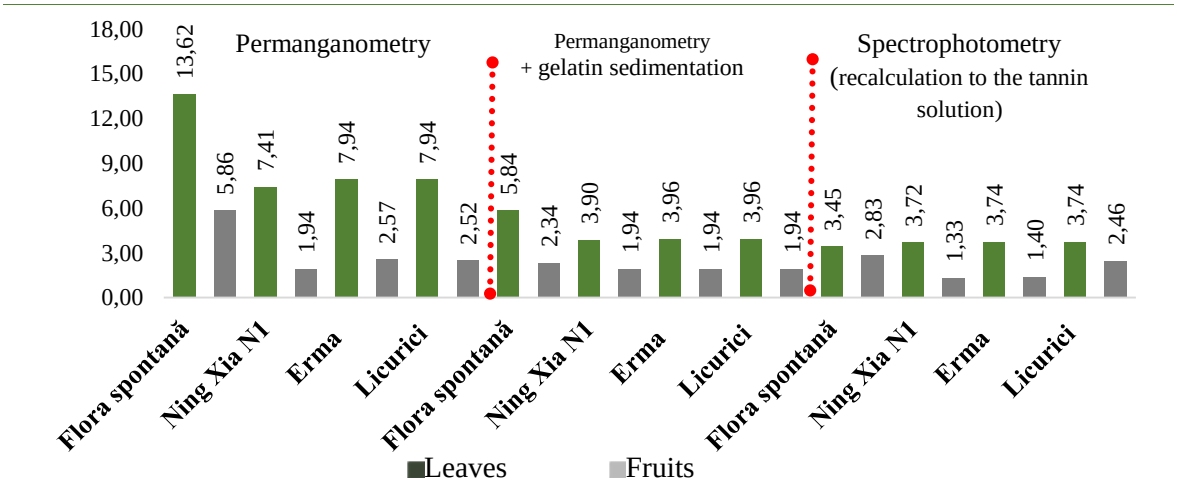

Fig. 4.2. The percentage of tannins determined titrimetrically and spectrophotometrically in plant products of spontaneous and cultivated *Lycium barbarum*

The results of the research showed that the leaves from the spontaneous flora and the cultivated varieties were characterized by a maximum content of tannins, determined permanganometrically. The most optimal method of measuring the content of tannins was *permanganometry associated with tannin sedimentation* by applying the reaction with gelatin because of its higher accuracy. The content of flavonoids and tannins in the analyzed plant products varies, but all the studied cultivars are of interest for the food industry and the pharmaceutical industry.

4.4.3. The analysis of biochemical indicators in fruits of wild and cultivated *Lycium barbarum* L. plants

The evaluation of taxa in terms of fruit quality was an important element in completing their description; quality is an important feature for fresh as well as processed fruits. Analyzing the content of ascorbic acid in fruits, we found that it varied depending on their origin, biosynthesis and its minimal accumulation was in the fruits harvested from cultivated plants: ’Ning Xia N1’ (0,107 %), ’Erma’ (0,111 %), ’Licurici’ (0,114 %) and the maximal accumulation – in those from

the spontaneous flora (0,118 %). Our results showed that the amount of ascorbic acid in goji berries varied from 0,107 to 0,118 % (Table 4.2).

Table 4.2. The analysis of biochemical indicators in fruits of wild and cultivated *Lycium barbarum* L.

Indices	Spontaneous Flora	Ning Xia N1	Erma	Licurici
Average fruit weight, g	0,689	0,801	0,848	0,852
Total sugar, %	4,548	7,158	6,454	8,514
Total titratable acidity, %	0,405	0,381	0,301	0,335
Vitamin C %	0,118	0,107	0,107	0,111

As for the sugar content, it fell within the normal limits of the species *Lycium barbarum* L. (the normal limits of the total sugar content of goji berries are 3,45-8,90 %) [11]. The highest value of the total sugar content was found in the cultivar 'Licurici' – 8,514 %, followed by 'Ning Xia N1' – 7,158 %, 'Erma' – 6,545 % and the wild plants of this species – 4,548 %. The titratable acidity values fell within the absolute limits, the content of citric acid in goji berries was between 0,58 % and 0,89 % [21]. A maximum value of the acid content was found in the fruits of wild plants (0,405 %), but lower values – in the cultivars: 'Ning Xia N1', 'Licurici' and 'Erma', of 0,381 %, 0,335 % and 0,301 %, respectively.

Thus, the amount of sugar determined in the cultivated goji berries is small, so they can serve as a dietary product, an excellent addition to the daily diet that will improve well-being and promote a healthy lifestyle. Some studies have shown that the highest sugar content was observed in the fruits collected at the end of the growing season (full ripening stage), from shrubs grown in full sun and well-drained soil. Goji berries are a valuable raw material for the production of foods, medicines, cosmetics and other products.

GENERAL CONCLUSIONS AND PRACTICAL RECOMMENDATIONS

General conclusions

The multiplication of the species *Lycium barbarum* L. by tissue culture, the comparative morphological assessment, the biochemical evaluation of the phytochemical compounds of the spontaneous species and several cultivars, under the pedoclimatic conditions of the Republic of Moldova, were performed for the first time in our country.

1. The The technology was developed and the in vitro multiplication protocol of goji varieties was described: 'Amber Sweet', 'New Big', 'Erma', 'Fireflies' and 'Ning Xia N1' for the production of quality planting material - robust, uncontaminated, uniform with a high morphogenetic potential for regeneration, initiated from apical meristems and apical meristems with leaf primordia, inoculated on MS100% medium with the addition of BAP - 0.2 mg/l [26, 30].

2. The research on the microcloning of wolfberry cultivars revealed an increased proliferation rate, exceeding on average 22,06 shoots per explant in the cultivar ’Amber Sweet’, followed by ’Licurici’ with 20,17, ’New Big’ with 16,11, ’Erma’ with 14,72 and ’Ning Xia N1’ with 14.11 shoots, on nutrient medium MS 100 %, supplemented with BAP cytokine (0,5 mg/l). The optimal hormonal balance in the process of multiplication by tissue culture proved to be: BAP (0,2 mg/l) + NAA (0,2 mg/l) + GA_3 (0,1 mg/l), which determined reasonable proliferation rates and relatively well developed shoots in the studied cultivars: ’Ning Xia N1’ (4,56), ’Erma’ (4,94), ’New Big’ (6,94), followed by ’Amber Sweet’ (5,06) and ’Licurici’ (5,22) [6].

3. The rhizogenesis of the micro-shoots was stimulated by supplementing the basic nutrient medium MS 100% with IBA, IAA and NAA auxins in concentration of 0,2 mg/l, as well as on the nutrient medium MS 50 %. The highest rooting rate, of about 90 % in all wolfberry cultivars, was observed on the medium supplemented with NAA in a concentration of 0,2 mg/l. The MS 50 % medium supplemented with the same concentration of NAA auxin proved cost-effective, optimal and efficient in order to preserve the wolfberry material for a period of 60 days. The wolfberry cultivars were acclimatized with a viability rate of over 95 %, and the most suitable season for this process is summer [17].

4. The process of callogenesis was induced in MS 100 % medium supplemented with 2,4D growth regulators of 1 mg/l and BAP 0,1 mg/l and the organogenesis of plantlets from callus was induced on MS 100 % medium supplemented with NAA (0,2 mg/l). The dynamics of callus induction in wolfberry cultivars for both types of explants (leaf and petiole fragments) was determined by the culture medium, the type of hormones and explant [32].

5. The microscopic study comparing the anatomical structure of the leaf epidermises in the studied plants (from spontaneous and cultivated flora) showed a great uniformity, but highlighted the structural-anatomical adaptive characters to unfavorable conditions (drought, low temperatures and insolation) such as: thicker leaf blade, the presence of stomata with an increased frequency on the lower epidermis and the location of calcium oxalate druses dispersed in the mesophyll and arranged in the sheath [33].

6. The pedoclimatic conditions of the Republic of Moldova are favorable for the growth and the production of fruits of wolfberry cultivars and led to an abundant growth during the research

years 2017-2019. The branching capacity, which determines the biological productivity of wolfberry plants, includes the length of the annual growth of fruit shoots influenced by the peculiarities of the cultivar, the climatic conditions and the quality of the planting material, the resistance to diseases and the age of plants.

7. The results of qualitative and quantitative biochemical research on some classes of chemical compounds show that the leaves and fruits of *Lycium barbarum* L. can serve as important sources of flavonoids (56.33mg /g in wild plants and 25,67 mg /g in cultivated plants), tannins (5,84 % in wild plants and 2,34 % in cultivated ones) and vitamin C (0,118 % in wild fruits and 0,111 % in cultivated fruits). The amount of sugar in the fruits of cultivated plants varies between 6,54 and 8,51 %, and their acidity is 0,30-0,38 %. Thus, the fruits have a high content of valuable nutrients and a low sugar content, which provides a high quality with low calorie intake and an optimal balance of nutrients [5].

Practical recommendations

1. The data obtained from the study are recommended for obtaining valuable uncontaminated and homogeneous planting material of goji varieties for the national economy through the technology of *in vitro* multiplication of plants of the species *L. barbarum* L.

2. In order to obtain the quality material, it is recommended to take the apical meristem and apical meristem explants with leaf primordia

3. The varieties ’Licurici’, ’Erma’, ’Ning Xia N1’, ’New Big’ and ’Amber Sweet’ are recommended for founding industrial plantations as a food source and as a decorative shrub for landscaping, in the pedoclimatic conditions of the of the Republic of Moldova.

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1. **TABĂRA M.**, Structura anatomică a laminei frunzei speciei spontane *Lycium barbarum* L. şi a soiurilor, Akademos – Revistă de ştiinţă, inovare, cultură şi artă, Nr. 1 (56), 2020, p. 15 – 20. ISSN 1857-0461.
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4. **TABĂRA M**., CIORCHINĂ N., TROFIM M., CUTCOVSCHI MUŞTUC A., Influenţa regulatorilor de creştere asupra procesului de multiplicare la specia *Lycium barbarum* L. Conferinţa ştiinţifico-practică „Instruire prin cercetare pentru o societate prosperă” consacrată jubileului „90 de ani ai Facultăţii Biologie şi chimie”, Chişinău 2020 p. 221-228. ISBN 978-9975-76-307-3.
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9. ЧОРКИНЭ Н.Г., **ТАБАРА М.А.**,. КУТКОВСКИ-МУШТУК А.И, ТРОФИМ М.И., ЛОЗИНСКИ М.Н. *Lycium barbarum* L. (гожи) в культуре *in vitro*, сорт Эрма. Conference: Annual Meeting Society of Plant Physiologists of Russia Mechanisms of resistance of plants and microorganisms to unfavorable environmental Book of Proceedings (in two parts) of the All-Russian Scientific Conference with International Participation and Schools of Young Scientists (Irkutsk, July 10–15, 2018**.** p. 1423-1427. ISBN 978-5-94797-324-2.

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- Biotehnologii avansate – realizări şi perspesctive, al IV-lea Simpozion naţional cu participare internaţională, 3-4 octombrie 2016, Chişinău.

ANNOTATION

Tabăra Maria, "Development and microclonal multiplication of *Lycium barbarum* L. (wolfberry)", PhD thesis in biological sciences, Chisinau, 2020.

Structure of the thesis. The thesis includes introduction, four chapters, general conclusions and practical recommendations, bibliography from 321 sources, total volume of 156 pages, 18 tables, 39 figures and 10 annexes. The obtained results are published in 21 scientific papers.

Keywords: *Lycium barbarum* L., *in vitro* culture, microclonation/micropropagation, callusogenesis, rhizogenesis, acclimatization, goji varieties, phenology, anatomy, biochemistry.

Field of investigation: 164.01 – Botany

The purpose of the research is to develop *in vitro* multiplication technology of *Lycium barbarum* L. and comparative morphological and biochemical evaluation of biochemical compounds of the spontaneous species and varieties grown under the pedoclimatic conditions of the Republic of Moldova.

Objectives of the thesis: *identification of goji varieties and highlighting the appropriate explants for initiating *in vitro* cultures *selection of nutrients suitable for each stage of microcloning *analysis of plant development biology obtained, *in vitro, ex vitro* and experimental conditions; *anatomical study and comparative biochemical evaluation of the content of flavonosides, tannins, and ascorbic acid in cultivated goji plants and spontaneous flora; *elaboration of micropropagation technologies and description of the protocol for obtaining a high multiplication coefficient for goji varieties and extension of the genus of fruit shrubs within GBNI (initiation of the collection).

Scientific novelty and originality. For the first time in the Republic of Moldova, goji plants have been propagated through efficient micropropagation techniques and methods for the production of robust, uncontaminated and uniform propagating material. This complex study is the first of its kind in our country, its importance being given by the need for biological characterization of shrubs in the experimental group, making observations and biometric measurements on: plant height, length and number of shoots, number of leaves on the rosette and on the plant, and the comparative highlighting of the morpho-anatomical and biochemical peculiarities with adaptive characters of the plant organs in the climatic conditions of the country of the cultivated plants and the native spontaneous flora.

The most important solved scientific problem in the thesis consist in the *elaboration* of the *in vitro* multiplication technology of the species *Lycium barbarum* L. and the obtaining of the uncontaminated, uniform and homogeneous quality propagating material for goji varieties in the climatic conditions of the Republic of Moldova. The planting material obtained through *in vitro* culture will *contribute* to the establishment of modern plantations in the Republic of Moldova and the organization in GBNI of the collection of fruit bushes.

The theoretical significance. The obtained results allow us to mention that, by using *in vitro* multiplication, it confirms the increase of the quantity of planting material and the improvement of its quality. Thus, this study highlighted the elucidation of biological features of plant growth and development based on phenological, biometric, anatomical and biochemical aspects in climatic conditions in the Republic of Moldova, data that would contribute to proper training of farmers on the use of drought-resistant and high-quality varieties.

The applicative value of the paper. The scheme of the technology for obtaining healthy and homogeneous planting material for goji varieties for the Republic of Moldova was elaborated. The research results are used in the Embryology and Biotechnology Laboratory of NBGI. The planting material obtained served as a source to initiate the creation of the collection of 5 goji varieties from GBNI: 'Amber Sweet', 'Erma', 'Ning Xia N1', 'New Big' and 'Licurici' (variety improved by scientific researchers of the Biotechnology and Embryology Laboratory of NBGI, is currently on the CSTSP test commission). In vitro multiplied goji plants can serve as planting material for the establishment of modern goji plantations in large areas of the Republic of Moldova.

Implementation of scientific results. Based on the scientific research carried out, the *in vitro* multiplication methods of the *Lycium barbarum* species were implemented in the Laboratory of Embryology and Biotechnology. The results obtained from phenological and biochemical investigations will enrich the spectrum of the existing berry sector in the Republic of Moldova. At the same time, they represent scientific didactic material for the specialties: Botany, Ecology, Pharmacy and in educational institutions with botanical and agricultural profile, as well as contracts with private beneficiaries, farmers and amateurs interested in goji varieties.

THE DEVELOPMENT AND THE MICROCLONAL MULTIPLICATION OF *LYCIUM BARBARUM* L. (WOLFBERRY)

164.01 BOTANY

Abstract of the doctoral thesis in biological sciences

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