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Influence of nutrient media on the physiological parameters of grape microclones

Natalia Zelenianska*
Doctor of Agricultural Sciences, Senior Researcher
National Scientific Centre "V.Ye. Tairov Institute of Viticulture and Winemaking" of NAAS
65496, 27 40th Anniversary of the Victory Str., Tairove vill., Ukraine
https://orcid.org/ 0000-0002-9303-8686
Iryna Ishchenko
Candidate of Agricultural Sciences, Associate Professor
Odessa State Agrarian University
65012, 99 Kanatna Str., Odessa, Ukraine
https://orcid.org/0000-0003-0255-4843
Mykhailo Samofalov
Postgraduate Student
National Scientific Centre "V.Ye. Tairov Institute of Viticulture and Winemaking" of NAAS
65496, 27 40th Anniversary of the Victory Str., Tairove vill., Ukraine
https://orcid.org/0000-0002-8785-0651

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Abstract. Successful rooting of grape microclones in uncontrolled environmental conditions (*in vivo*) depends on the level of resistance, which is formed at the stage of their passage and growth *in vitro*. An important role in this case is played by indicators of the water regime of the vegetative mass. The purpose of this study was to get acquainted with the results of determining the water-holding capacity, the intensity of transpiration of grape microclones *in vitro* and to establish the share of their influence on the adaptation potential *in vivo*. Biotechnological, laboratory, vegetation, and mathematical and statistical research methods were used in this study. The obtained results showed that to optimize the physiological processes in the tissues of the leaves and shoots of grape microclones, to increase their viability under *in vivo* conditions, it is advisable to cultivate them *in vitro* on structured nutrient media (MS + agroperlite, MS + vermiculite, MS + agroperlite + vermiculite) with the content of phytohormones IAA – 0.2 mg/l, and 6-BAP – 0.3 mg/l. Structured nutrient media contributed to an increase in water retention capacity and a decrease in the intensity of transpiration of tissues of leaves and shoots of microclones of both technical and rootstock varieties. During 60 minutes of research, from 0.006 g to 0.034 g of water evaporated from microclones of technical varieties, from 0.003 g to 0.053 g from microclones of

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*Corresponding author

rootstock varieties, respectively. Transpiration intensity (after 10 min) decreased by 1.7-1.8 times. On the control Murashige and Skoog nutrient medium, plants evaporated a larger amount of water during the corresponding period of time: from 0.006 g to 0.079 g (technical varieties) and from 0.008 g to 0.086 g (rootstock varieties); the intensity of transpiration was higher. After cultivation of grape microclones on structured nutrient media, they were characterized by a higher content of dry matter in the tissues of leaves and shoots (14.6-15.0%) and better survival rates *in vivo* (76.3-98.5%, at 58.5-65.2% in control). The reliability of the results obtained is confirmed by the results of multivariate analysis of variance. The obtained results expand the understanding of dynamic changes in the indicators of the water regime of the vegetative mass of grape microclones *in vitro*, their influence on plant survival under *in vivo* conditions

Keywords: *in vitro*; water retention capacity; transpiration intensity; survival rate; phytohormones; biologically active preparations; mineral substrates

INTRODUCTION

Today, the method of tissue and organ culture *in vitro* is widely used in agricultural practice for accelerated reproduction of valuable genotypes. This method is based on the activation of organogenesis from various types of initial explants, on artificial nutrient media, under sterile conditions. This process of plant reproduction consists of several stages: the introduction of initial explants into *in vitro* culture; propagation of shoots in culture *in vitro*; obtaining plants with roots and their preliminary adaptation to open soil conditions; transfer of plants to *in vivo* conditions (Riabovol & Riabovol, 2019). The final stage in this process is the adaptation of test tube plants to non-sterile and uncontrolled environmental conditions. It was established that at this stage the largest number of microclones dies. This is because *in vitro* culture plants are in conditions that differ from natural ones in terms of physico-chemical parameters: water, light, temperature regimes, gas composition of air in culture containers, nutrient medium consistency, etc. Cultivation of plants *in vitro* in closed culture containers also requires compliance with the sterility regime, a certain gas environment, and ensures constant maintenance of the relative humidity of the air inside the containers. All this in a complex leads to a change in the course of many morphophysiological processes and functional changes of plants *in vitro*: they are characterized by an underdeveloped waxy cuticle of leaves, a damaged stomatal apparatus, weak photosynthetic activity, vitrification, a weak vascular connection between the root and the shoot, an unbranched root system and underdeveloped root hairs (Gritsak & Drobyk, 2020). Therefore, for the successful acclimatization of microclonal plants to *ex vitro* conditions, on the one hand, it is necessary to ensure a number of optimal physical factors (suitable substrate, air humidity, ventilation, acid-alkaline balance (pH), etc.), on the other hand, the plants must be in such a physiological biochemical state to gradually adapt to new, uncontrolled environmental conditions, i.e., it is necessary to optimize the conditions of plant growth and development at the stage of *in vitro* cultivation.

One of the determining factors that affects the physiological-biochemical, anatomical-morphological state of microclonal plants, specifically, general watering, water-holding capacity, transpiration intensity, the content of leaf pigments, dry matter in shoots and leaves is the composition and quality of the nutrient medium.

Studies by many authors have established that plants can react differently to the composition of the nutrient medium *in vitro*. Therefore, it must be selected considering the specific and varietal specifics. This was manifested in changes in anatomical indicators (Martins, 2018), growth and development indicators (Coelho *et al*., 2021), reproduction coefficient (Vujovic *et al*., 2020), the course of physiological and biochemical processes (Manocari *et al*., 2023). And this is understandable because the nutrient medium provides plants with the necessary macro- and microelements, vitamins, phytohormones and removal of metabolic products. Therefore, the issue of determining the influence of the nutrient environment on the course of physiological processes of grape microclones *in vitro*, their regulation to increase the adaptation potential is relevant and timely.

The purpose of this study was to determine the individual physiological indicators of grape microclones of rootstock and technical varieties under the conditions of cultivation on different nutrient media *in vitro*; to establish their influence on the viability of microclones *in vivo*.

To fulfil the purpose, the following tasks were set: to determine indicators of water-holding capacity, transpiration intensity, dry matter content in the tissues of leaves and shoots of grape microclones *in vitro* on different types of nutrient media and with different content of phytohormones; to determine the viability of grape microclones *in vivo*, after cultivation on different types of nutrient media; to determine the share of influence of factors – grape variety, content of phytohormones in the Murashige and Skoog nutrient medium (MS) and its structured base on the physiological indicators of grape microclones *in vitro*; to establish the dependence of the viability of grape microclones *in vivo* on the physiological state of plants *in vitro*.

LITERATURE REVIEW

Few scientific works have investigated the physiological and biochemical state of plants *in vitro* and *in vivo*. And all of them are considered mainly from the standpoint of modelling, impact of abiotic stress and screening of resistant genotypes. The most common abiotic stresses that affect plant growth and productivity are drought and salinity. They cause enormous economic losses as a result of reduced agricultural productivity. In such conditions, plants face a violation of osmotic potential, nutrient transport, and a decrease in photosynthesis. Conventional breeding methods do not always lead to the desired result in the selection and evaluation of stress-resistant plant forms and species. However, thanks to the application of *in vitro* methods of tissue and organ culture, in a short period of time and with a small amount of plant material, it is possible to investigate the mechanism and carry out screening of resistant forms and varieties of plants.

Martínez-Santos *et al.* (2021) evaluated the physiological and biochemical status of *Vanilla planifolia* Jacks. ex Andrews under conditions of *in vitro* water stress induced by polyethylene glycol (PEG). Microclonal plants of *Vanilla planifolia* Jacks. were cultivated on the semi-solid Murashige and Skoog nutrient medium (MS) with the addition of PEG-6000 (0%, 1%, 2% and 3% w/v). After 60 days, the height of the plants was determined, in the tissues of leaves and shoots – the content of dry matter, chlorophyll, soluble proteins, proline, glycine, stomatal index and the number of open stomata per unit area of the epidermis. The obtained results showed that with an increase in the concentration of PEG-6000 in the nutrient medium, plant height, the content of chlorophyll, soluble proteins, and the number of open stomata decreased, while the content of dry matter and amino acids, on the contrary, increased.

Hernández-Pérez *et al.* (2021) using similar concentrations of PEG-6000 in the MS nutritional medium based on the determination of biometric growth indicators, the content of dry matter in tissues, total protein, proline, glycine-betaine, performed *in vitro* screening of *Saccharum spp*. Hybrids, varieties Mex 69-290, Mex 79-431, CP 72-2086 and MOTZ Mex 92-207 resistant to water stress. Based on the results obtained, the water stress-tolerant Mex 69-290 variety was established. As the microclonal plants of this variety met the biometric parameters of development with an increase in the concentration of PEG in the nutrient medium, an increase in dry matter and amino acids was observed in the tissues.

Gao *et al.* (2020) investigated the effect of osmotic stress on tissue hyperhydration of microclones of *Dendrobium officinale* L. The latter were cultured on a solid MS nutrient medium supplemented with plant growth regulators, various concentrations of sucrose and agar. The level of hyperhydration was assessed by water regime indicators, relative electrical conductivity, and enzyme activity. The results indicated that high concentrations of sucrose, agar, PEG-6000 in the nutrient medium considerably increased the total water content, free water content, relative electrical conductivity, peroxidase activity, and reduced the content of bound water, proline, soluble protein, and sugars.

According to Khalid *et al.* (2021), who investigated changes in the physiological and biochemical state and biometric growth indicators of *Camelina sativa* L. under model drought MS+PEG-6000 (1-7%), it was shown that with an increase in the concentration of PEG to 2.0%, the ability of seeds to germinate, the content of water, chlorophylls, and carotenoids in the leaves increased significantly, and the processes of cotyledons unfolding and the appearance of true leaves accelerated. Subsequently, increasing the concentration of PEG led to a decrease in these indicators compared to the control. This is evidence that *Camelina sativa* L. can tolerate moderate water stress without any adverse impact on growth and physiological and biochemical parameters.

Bareera *et al.* (2019) conducted *in vitro* screening of drought-resistant varieties of *Brassica napus* L. For this, callus cultures of varieties B-56, B-18, ZMR-4, ZM-21, KM-256, ZMR-10, Punjab Sarsoon, Cyclon, Rainbow and UAF-11 were obtained on nutrient media with PEG-6000, the viability of which were evaluated by the content of dry matter, proline, glycine, betaine, total content of soluble sugar. The authors showed that when exposed to low concentrations of PEG-6000 in the tissues of callus cultures, all the above indicators increased. The exception was the mass index of wet callus, which, on the contrary, decreased.

Kovalikova *et al.* (2020) investigated the reaction of microclonal plants of five varieties of *Malus domestica* L. ("Malinové holovouské", "Fragrance", "Rubinstep", "Idared", "Car Alexander"), five varieties of *Prunus avium* L. ("Regina", "Napolenova", "Kaštánka", "Sunburst", "P-HL-C") to osmotic stress simulated by increased concentrations of PEG-6000 in the medium. Stress, similar to drought, negatively affected the total water content, chlorophyll content, and leaf area of both plant species. Overall results indicate a wide range of water deficit tolerance among *Malus domestica* L. and *Prunus avium* L. cultures *in vitro*.

In separate scientific works, the salt tolerance of plants *in vitro* was evaluated based on physiological-biochemical, biometric indicators of microclonal plants. Thus, Putnik-Delić *et al.* (2019) investigated the effect of different concentrations of NaCl (0.2, 0.6, and 1.2 g/l) in the MS nutrient medium on the intensity of transpiration of microclones of *Brassica napus* L. of the Slavica cultivar. They proved that the intensity of transpiration decreased with increasing NaCl concentration in the nutrient medium. Al-Khateeb *et al.* (2020) investigated the effect of different NaCl salt content (from 0 to 300 mM)

in the composition of the nutrient medium on photosynthesis, transpiration intensity and stomatal conductance of *Phoenix dactylifera* L. *in vitro*. The results showed that an increase in the salt concentration in the nutrient medium reduced the intensity of these processes.

Cioć *et al.* (2019) investigated the effect of red and blue spectrum light (in a ratio of 7:3), different concentrations of 6-benzyladenine (BA) (1, 2.5, and 5 μM) in growing microclones of *Gerbera jamesonii* Bolus. They proved that an increase in the concentration of BA contributed to an increase in the height of plants, the number of leaf blades, and a modification of lighting – the area of leaf blades. However, the authors noted that an increase in the concentration of BA was accompanied by a decrease in the content of dry substances in the vegetative mass, an increase in the intensity of lighting – an increase in the content of leaf pigments. Ki Young Choi *et al.* (2022) determined the effect of the intensity of white LED light on the growth of microclones of *Malus domestica* (M-9). Microclones were grown for 30 days under the influence of five white light-emitting diodes (LEDs) of different intensities: 100-500 (L1), 250-500 (L2), 500-500 (L3), 250-250 (L4) and 100-100 (L5). The authors stated that the number of leaves, stem diameter, wet mass of shoots, roots, and dry mass of shoots under white LED light 500-500 (L3) were significantly greater than those cultivated under other light intensities. Furthermore, a positive correlation was established between stomatal conductivity and transpiration rate. These results indicate that the light intensity of PPFD 500-500 was favourable for microclone growth *in vitro*.

Ergasheva *et al.* (2022) indicate the need to determine the physiological and biochemical state of microclonal plants when transferring them to uncontrolled environmental conditions. This will determine their survival rate and the yield of standard seedlings. Thus, scientists of Gulistan State University (Uzbekistan) determined the general watering of leaves and the intensity of transpiration of *Punica granatum* L. in the pre-adaptation period *in vitro* and when transplanting plants into non-sterile soil of local varieties – "Qora qayim", "Qizil anor", "Oq dona (Tuyatish)", "Achchiq dona". As a result, they proved that when microclonal plants were transferred to uncontrolled conditions, a high intensity of transpiration was observed, a substantial decrease in the water content in the leaves, i.e., a water deficit was observed. Therefore, the author recommends increasing the level of humidity to 90-95% at the stage of *in vitro* pre-adaptation, and then gradually reducing it to 50%, to ensure optimal mechanisms of plant adaptation to *in vivo* conditions.

The University of Bonn (Germany) investigated the influence of different growing conditions of *Populus canescens* L. – conditions *in vitro*, *ex vitro*, climate chamber, greenhouse on the development of the leaf apparatus, the composition of cuticular wax and transpiration of detached leaves. Studies have shown that when plants are transferred *in vitro* to other conditions, they quickly dehydrate, regardless of the amount of cuticular wax (Grünhofer *et al.*, 2021).

Thus, a brief review of the literature shows that there are very few scientific studies providing the results of research on the determination of the main physiological indicators of plants in the culture of tissues and organs *in vitro*, depending on the modification of the mineral and phytohormonal basis of the MS nutrient medium. And for grapes, such studies were generally not conducted.

MATERIALS AND METHODS

The study was conducted in the Department of Grape Seeding, Propagation and Biotechnology of the National Scientific Centre "V.E. Tairov Institute of Viticulture and Winemaking" (NSC V.E. Tairov IVW) during 2018-2022.

The object of research was microclones of grapes – Yarylo, Zagrei (technical varieties), Dobrynia, Harant (rootstock varieties). Physical parameters of cultivation: air temperature – 24-25°C, air humidity 60-70%, photoperiod – 16 hours, lighting 2500-3000 lux. All works were carried out under sterile conditions of laminar flow and culture boxes equipped with ultraviolet irradiators.

To cultivate grape microclones in the culture of tissues and organs *in vitro*, a modified Murashige and Skoog nutrient medium (MS) was used. The modification of the environment consisted in changing the amount of phytohormones (indolylacetic acid (IAA), 6-benzylaminopurine (6-BAP), adding biologically active preparations (Radifarm, Klonex gel) and mineral substrates (agroperlite, vermiculite) (Table 1).

Table 1. *Research scheme*

Experiment variants	Composition of the nutrient medium			
	basis	phytohormone content, mg/l		additional components
		IAA	6-BAP	
Control 1	MS	0.3	0.2	-
Control 2	MS	0.6	0.5	-
Variant 1	MS	0.3	0.2	Radifarm (2.5 ml/l)

Table 1, Continued

Experiment variants	Composition of the nutrient medium			
	basis	phytohormone content, mg/l		additional components
		IAA	6-BAP	
Variant 2	MS	0.6	0.5	Radifarm (2.5 ml/l)
Variant 3	MS	0.3	0.2	Clonex gel
Variant 4	MS	0.6	0.5	Clonex gel
Variant 5	MS	0.3	0.2	agroperlite
Variant 6	MS	0.6	0.5	agroperlite
Variant 7	MS	0.3	0.2	vermiculite
Variant 8	MS	0.6	0.5	vermiculite
Variant 9	MS	0.3	0.2	agroperlite + vermiculite (1:1)
Variant 10	MS	0.6	0.5	agroperlite + vermiculite (1:1)

Source: *compiled by the authors*

The MS medium was prepared as prescribed, after which other components were added, except for Clonex gel. It was applied by immersing the basal part of micropropagules before planting on a nutrient medium. Agar-agar was used for gelation of modified nutrient media. In nutrient media for the first-fourth variants of the experiment, it was used in the amount of 7.0 g/l, for the fifth–tenth variants of the experiment – 6 g/l.

Nutrient media were autoclaved under a pressure of 1 atm. for 15 min. After autoclaving and solidification of modified nutrient media with mineral substrates, two-layer (structured) nutrient media were formed in glass culture containers. The nutrient medium: mineral substrates ratio was 1.0:0.5. The *Radifarm preparation* is an extract of plant origin, which contains amino acids, steroids, glycosides, polysaccharides, betaine, vitamins, and trace elements. It promotes rapid rooting of plants, uniform growth, development of vegetative mass, root system, reduces the stress caused by transplanting plants.

The *Clonex gel preparation* is a complex of substances: indolylbutyric acid, vitamins, hormones, trace elements, nutrients necessary for the development and growth of the root system of plants. *Mineral substrates agroperlite, vermiculite* are minerals of the hydromica group, the use of which helps increase the aeration properties of nutrient substrates (nutrient media).

After 90 days of cultivation, the following were determined in the vegetative mass of grape microclones: water-holding capacity (%), transpiration intensity (g×(m^2×h)), dry matter content (%) (Sherer, 2011); plant viability was determined 30 days after planting in the greenhouse.

RESULTS AND DISCUSSION

In cells and tissues, there are two forms of water – free and bound. Free water is characterized by sufficient mobility, is a solvent that ensures the course of all physiological and biochemical reactions. The action of the stress factor, first of all, leads to evaporation and reduction in the cells of this particular form of water. Bound water is divided into osmotically bound and colloidal bound, it is located either in the middle of the colloidal system or on the surface of the colloids, between them.

The ability of plants to retain a certain part of water due to osmotic forces and increased hydrophilicity of biocoloids is a universal reaction of the body to the deterioration of environmental conditions. Therefore, under any stressor, a decrease in water loss occurs due to an increase in the water retention capacity of tissues.

Water-holding capacity is an indicator that characterizes the loss of water by the vegetative organs of plants over a certain period of time. In the technology of *in vitro* reproduction of grapes, it is important from the standpoint of transferring plants to uncontrolled conditions *in vivo* and their survival.

Analysis of the dynamics of water loss by leaves and shoots of grape microclones *in vitro* (pre-adaptation stage) of rootstock and technical varieties cultivated on modified nutrient media with agroperlite, vermiculite and their mixture (fifth-tenth variants) showed that they decreased in relation to the control values (control 1, control 2).

Thus, grape microclones of rootstock varieties lost 0.003 g after 5 min, 0.008 g after 10 min, 0.011 g after 15 min, 0.022 g after 20 min, 0.032 g after 30 min and 0.053 g after 60 min of water, which is 50.0% (after 5 min), 36.3% (after 10 min), 33.3% (after 15 min), 26.6% (after 20 min), 32.0% (after 30 min) and at 37.2% (after 60 min) less than control values (Fig. 1).

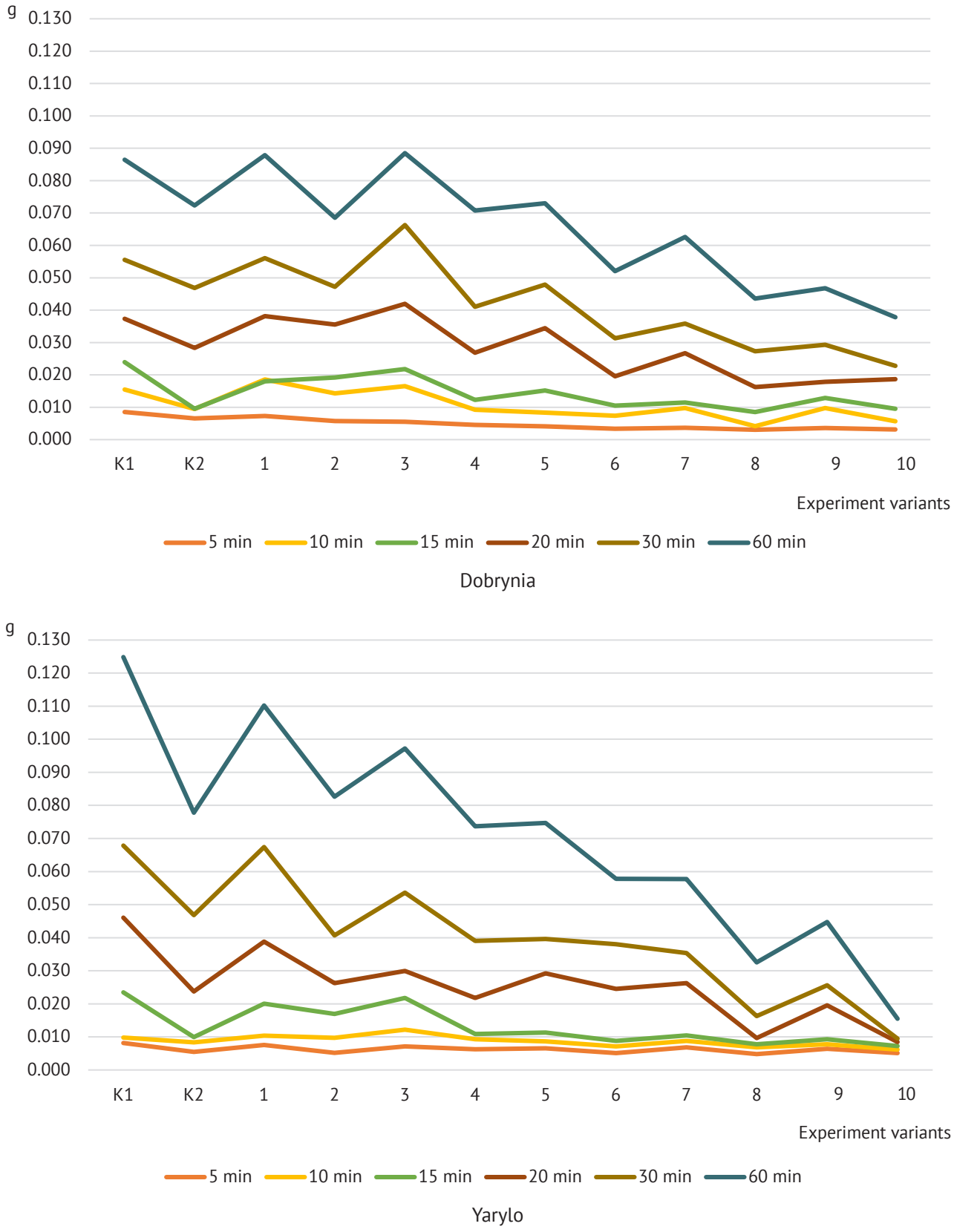

Figure 1. *Dynamics of water loss by grape microclones of rootstock and technical varieties on different types of nutrient media (average for 2018-2022)*

Source: *compiled by the authors*

Microclones of technical varieties lost 0.006 g, 0.007 g, 0.009 g, 0.014 g, 0.020 g, 0.034 g of water after the indicated time intervals, which was also less than the control values by 16.6%, 22.2%, 46.6%, 44.0%, 56.8%, 58.2%.

In grape microclones on nutrient mediums with Clonex gel and Radifarm (first-fourth variants), the water loss rate was at the control level. On average, plants of these variants lost from 0.007 g to 0.082 g of water (rootstock varieties) and from 0.006 g to 0.088 g of water (technical varieties) after 5-60 minutes.

Therefore, the results of the determinations indicate that the water-holding capacity of the tissues of grape microclones on structured nutrient media was the greatest, which indicates a potentially greater stability of these microclones in changing conditions. Furthermore, the indicator of water-holding capacity was generally higher in the experimental and control variants, where the content of phytohormones in the composition of the MS was equal to 0.2 mg/l 6-BAP, 0.3 mg/l IAA (first, third, fifth, seventh, ninth variants).

After analysing the experimental material, multiple analysis of variance was performed. The main influencing factors were the grape variety (factor 1), the content of phytohormones in the MS nutrient medium (0.2 mg/l 6-BAP, 0.3 mg/l IAA) (factor 2) and the structured base of the MS (addition of BAP or mineral substrates) (factor 3). According to the results obtained, the revealed differences in the results of the experiment are reliable, since the factual values of the Fischer criterion (for all the main factors of influence) at the significance level of P – 0.05 were greater than their tabular values (Table 1-2).

***Table 2**. Results of dispersion analysis on the effect of the type of nutrient medium on the water-retaining ability of grape microclones*

Factors of influence	$F_{fact.}/F_{theor.}$ Share of influence of factors, %				
	Water evaporation				
	5 min	10 min	20 min	30 min	60 min
Factor 1	314.23/2.69 20.14	591.66/2.69 24.72	523.34/2.69 27.11	476.35/2.69 31.84	439.83/2.69 26.22
Factor 2	887.63/3.94 18.96	960.05/3.94 13.37	887.02/3.94 17.58	681.06/3.94 15.17	769.04/3.94 15.28
Factor 3	356.00/2.30 38.03	557.59/2.30 38.83	278.83/2.30 31.63	338.37/2.30 37.70	465.00/2.30 46.20
Factor 1 × Factor 2	30.40/2.69 1.94	130.24/2.69 5.44	20.00/2.69 1.18	19.13/2.69 1.27	24.53/2.69 1.46
Factor 1 × Factor 3	14.76/1.77 4.73	52.37/1.77 10.94	32.35/1.77 9.61	17.46/1.77 5.83	20.62/1.77 6.14
Factor 2 × Factor 3	98.05/2.30 10.47	34.64/2.30 2.41	42.77/2,30 4.23	14.42/2.30 1.60	8.87/2.30 0.88
Factor 1 × Factor 2 × Factor 3	11.35/1.77 3.63	14.04/1.77 2.93	22.59/1.77 6.71	13.19/1.77 4.40	6.33/1.77 1.88
Error	2.10	1.36	1.95	2.59	1.94

***Source**: compiled by the authors*

This method of research helped establish the share of the influence of each factor on the water-holding capacity of the vegetative mass of grape microclones from their total population. Thus, Factors 3 (structured basis of MS) and 1 (grape variety) had the most significant share of influence. Depending on the variants, they were within 31.63-46.20% (factor 3) and 20.14-31.84% (factor 1) of the total 100% of the population. The influence of Factor 2 (the content of phytohormones in the nutritional medium of MS) slightly decreased and was within 13.37-18.96%, the interaction of these factors was within 1.18-10.94%. The share of unaccounted factors was small and amounted to 1.36-2.59%.

Transpiration is the process of evaporation of water from the surface of plants over a certain period of time. The amount of transpiration depends on many factors, including temperature, lighting, water supply, etc. Determining the intensity of transpiration of grape micrroclones *in vitro* showed that it depended on the structured basis of the nutrient medium and the content of phytohormones in it. It was the largest in control plants (C1) and after the use of biologically active preparations (first, second, third variants) (Fig. 2).

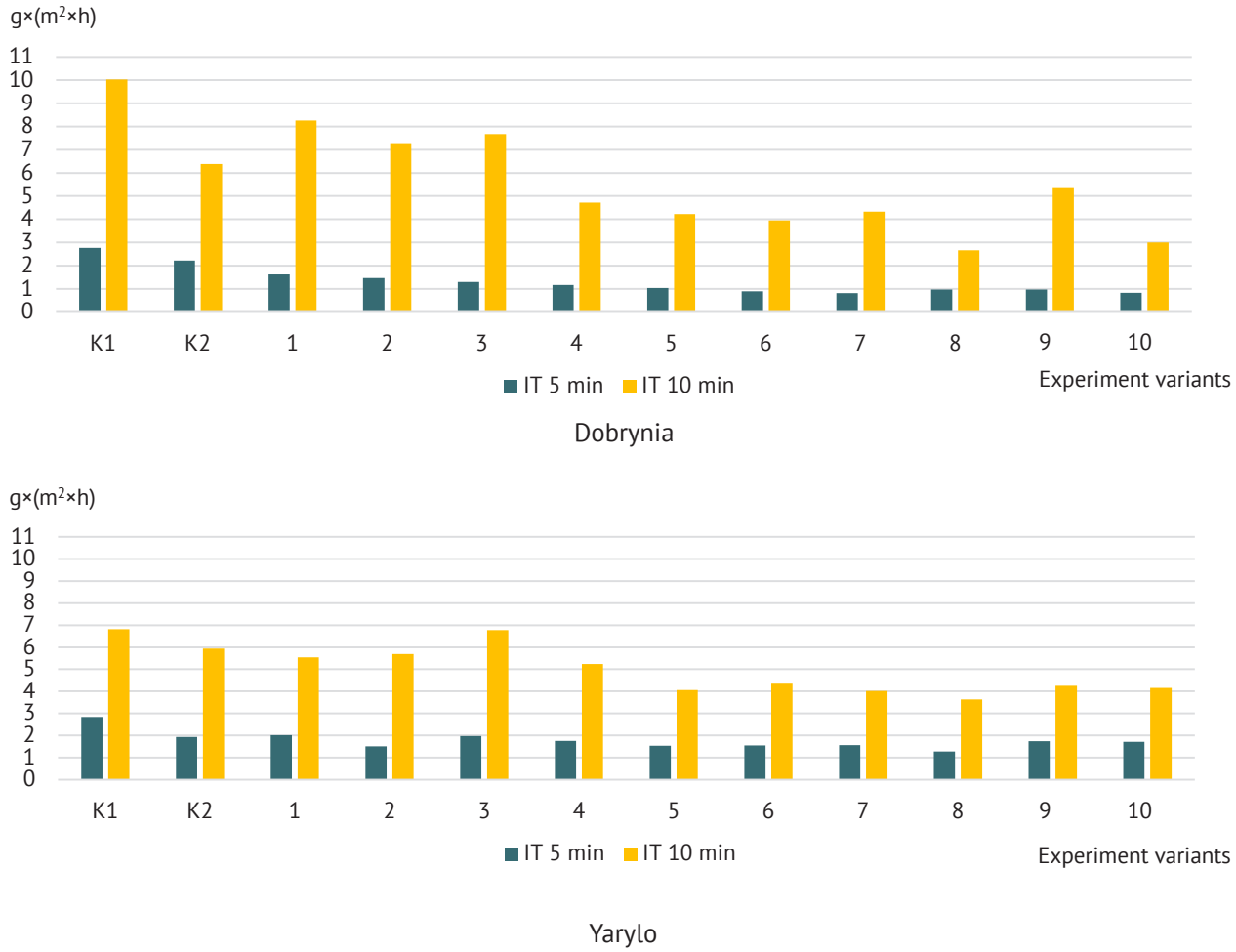

Figure 2. *The intensity of transpiration of grape microclones of grafted and technical varieties on different types of nutrient media (average for 2018-2022)*

Source: *compiled by the authors*

Thus, for microclones of grape rootstocks, the intensity of transpiration in the first variant (MS + Radifarm + IAA 0.3 mg/l; 6-BAP 0.2 mg/l) was the highest among the experimental variants and was equal to 1.6 g×(m²×h) after 5 min, 8.3 g×(m²×h.) after 10 min. Compared to the control values, a decrease of 1.1% (after 5 min) and 1.6% (after 10 min) was noted. In the second variant (MS + Radifarm + IAA 0.6 mg/l; 6-BAP 0.5 mg/l) the intensity of transpiration was equal to 1.5 g×(m²×h) after 5 min, 7.3 g×(m²×h) – after 10 min, which was more than the control (C2) by 1.3%, 2.7%. In the third variant, where Clonex gel was used (IAA 0.3 mg/l; 6-BAP 0.2 mg/l), the transpiration intensity was less than the control values by 1.5%, 2.7%. For grape microclones of technical varieties, according to the indicated experimental options, a similar pattern was noted, although the level of transpiration intensity was lower, and was within 5.5-6.8 g×(m²×h), while for microclones of rootstock varieties – within 7.3-8.3 g×(m²×h).

After cultivation of grape microclones on structured nutrient media (the fifth-tenth variants), the intensity of transpiration of the vegetative mass was lower, compared to both the first-fourth variants (MS+BAP) and the controls (C1, C2). In microclones of rootstock varieties, this difference with controls was equal to 1.5 (after 5 min) – 3.4 (after 10 min) g×(m²×h), with variants where BAP was applied – 0.4 (after 5 min) – 1.9 (after 10 min) g×(m²×h), in microclones of technical varieties – 0.8-2.5 g×(m²×h) and 0.3-1.3 g×(m²×h) according to variants.

The results of the variance analysis indiciated that the difference between the experimental and control variants was reliable only after determining the intensity of transpiration of the vegetative mass of plants after 10 min ($F_{act.}$>$F_{theor.}$); after determining this indicator, after 5 minutes, the value of $F_{fact.}$ was smaller than $F_{theor.}$ Data were also obtained that helped determine the proportion of influence of each factor on the intensity of transpiration. The substantial influence of Factor 3 (structured basis of MS (adding BAP or mineral substrates)) was proved – 52.7% and Factor 1 (grape variety) – 21.5%; the influence of Factor 2 (the content of phytohormones in the MS nutritional medium), albeit substantial, was estimated at 5.4%.

During preparation and transfer of microclonal grape plants from *in vitro* conditions to *in vivo* conditions, the tissue structure of leaves and shoots plays an important role. It is usually evaluated by dry matter content or total hydration. The determination of the mass of wet and dry growth of grape microclones followed by the determination of the content of dry substances showed that most of them were synthesized in the leaves and shoots of plants that were cultivated on structured nutrient media (Fig. 3).

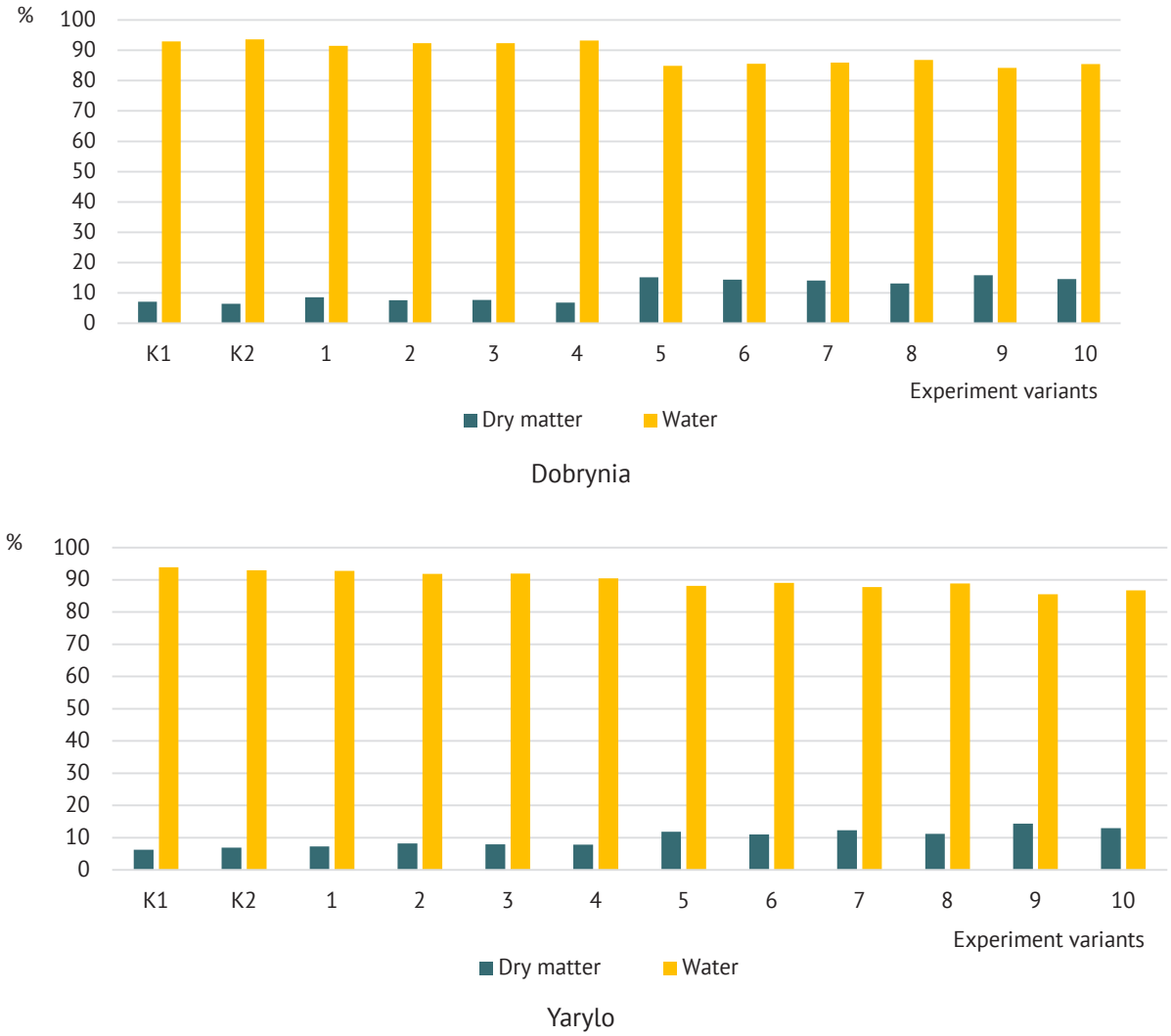

Figure 3. *The content of dry matter and water in the tissues of leaves and shoots of grape microclones of grafted and technical varieties on different types of nutrient media (average for 2018-2022)*

Source: *compiled by the authors*

Thus, in the indicated versions of the experiment, the dry matter content was equal to 12.0-15.2% (rootstock varieties), 11.0-14.9% (technical varieties). On nutrient media with the use of biologically active preparations Radifarm and Clonex gel, this indicator was equal to, on average, 6.8-8.7% (rootstock varieties) and 7.3-9.1% (technical varieties). In plants of control variants, the dry matter content in the tissues of the vegetative mass was 6.7-7.5% and 6.6-7.8%, respectively, in rootstock and technical grape varieties. Thus, in microclones of the first and fourth variants, the dry matter content was 0.8-1.2% higher than the control values, and in microclones of the fifth and tenth variants – by 5.6-7.8%.

The analysis of the results of the multiple variance analysis indicated that the identified differences in the results of the experiment are reliable ($F_{fact.} > F_{theor.}$) at the significance level P – 0.05. The factor structured MS basis had the greatest influence on the accumulation of dry matter in the vegetative mass of grape microclones during *in vitro* cultivation – 90.5%. Other main factors of influence (grape variety, hormonal basis of MS), albeit significant, were estimated at 2.2%.

Engraftment of grape microclones in vivo. The results of the study proved that the largest number of such plants were after cultivation of grape microclones *in vitro* on nutrient media with mineral substrates, but mainly on

those where the content of phytohormones in the composition of the MS was equal to 0.3 mg/l IAA, 0.2 mg/l BAP (fifth, seventh, ninth, and tenth variants). The viability of microclones of grape rootstock varieties was at 85.5% (Dobrynya) – 92.5% (Garant), microclones of technical varieties – at 82.4% (Zagrei) and 85.5% (Yarylo) (Fig. 4).

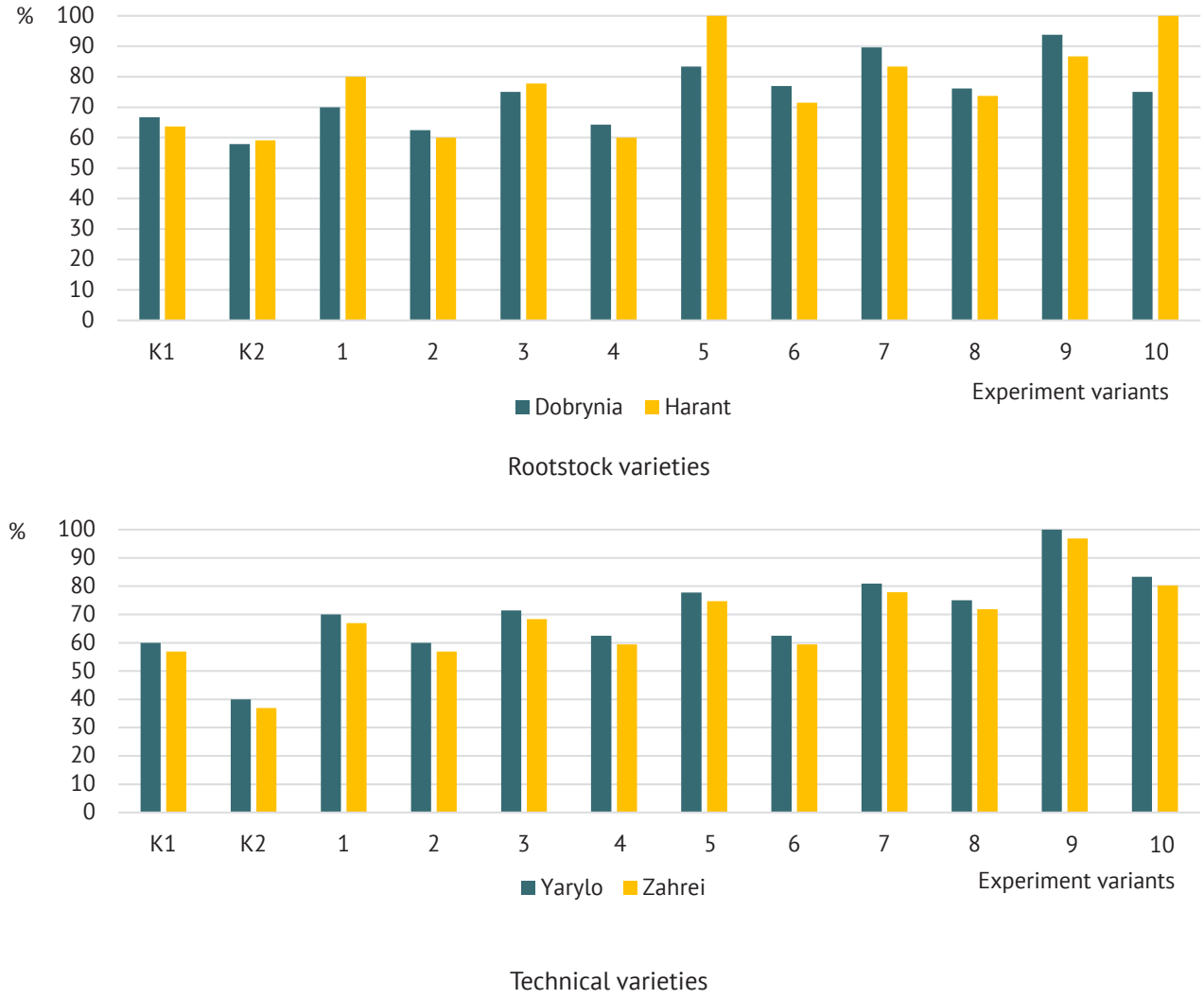

Figure 4. *Viability of grape microclones of grafted and technical varieties in vivo (average for 2018-2022)*
Source: *compiled by the authors*

Compared to Control 1, the difference was between 12.0% and 40.0%. After the use of biologically active preparations, the survival rate of grape microclones was at the level of control values. In similar variants, but with a higher content of phytohormones – 0.6 mg/l IAA, 0.5 mg/l BAP (second, fourth, sixth, eighth variants), the survival rate of microclones decreased and was at the level of Control 2.

A multiple correlation-regression analysis was conducted to establish the dependence of the viability of grape microclones *in vivo* on their physiological state formed *in vitro* (water regime, transpiration intensity, dry matter content). The results indicated that the multiple correlation coefficient between the independent variables water-holding capacity, transpiration intensity, dry matter content and the dependent variable microclonal survival *in vivo* was equal to 0.82, which indicates a high multiple correlation, according to the Chaddock scale. According to the coefficient of multiple determination R^2, the viability of grape microclones depended on the indicated independent variables by 70.0%. Determination of the standardized regression coefficient β helped compare the relative contribution of each independent variable to the prediction of the dependent variable. As evidenced by the received predictors, dry matter content and water-holding capacity are statistically significant and important – β(dry matter) = 1.04, β(water-holding capacity) = 0.36.

For *in vitro* plants to successfully take root in non-sterile conditions, they must be ready to overcome the stresses to which they are exposed during the adaptation process. According to the data of the authors

(Bag, 2019; Leite, 2021), to increase the percentage of survival of plants *in vivo*, it is necessary to start preparing them for new conditions *in vitro*, including a decrease in air humidity. In this regard, the quality and physical properties of the nutrient medium are of paramount importance. Research by many authors has established that different agricultural plants, their varieties, and forms react and manifest themselves differently in *in vitro* culture on different types of nutrient media, and therefore, the quantitative and qualitative composition of the nutrient medium should be selected considering varietal specificity. For the reproduction of grapes with the use of *in vitro* tissue and organ culture, modified nutrient media based on Murashige and Skoog medium are used. Its composition includes macro- and microsalts, calcium chloride, iron chelate, vitamins, indoleacetic acid, 6-benzylaminopurine, sucrose, agar (Zelenianska, 2022). According to the results of the research in this paper, for better growth and development of grape microclones of rootstock and technical varieties, the course of physiological processes in the tissues of leaves and shoots, optimal is the Murashige and Skoog nutrient medium with a minimum content of phytohormones in the composition: 0.3 mg/l IAA, 0.2 mg/l BAP. Analogous results were obtained (Sota, 2019; Okello, 2021) on the culture of *Aspilia africana* (Pers.) C.D. Adams and *Rosmarinus officinalis* L.).

At the moment of transfer of *in vitro* plants to *in vivo* conditions, they are exposed to a strong influence of water stress. As a result, this leads to dehydration of tissues, destruction of membranes. Plants are especially sensitive to dehydration immediately after their removal from culture containers, which is associated with non-functioning stomata, reduced water absorption, and high transpiration. The main water loss occurs in the first 10-14 days due to non-functional stomata. Therefore, it is important that before transferring the plants to *in vivo* conditions, they have functioning leaves. It is known that stomatal closure occurs at an air humidity of 65%, but at such humidity, rapid wilting and death of plants occur. Therefore, (Grytsak, 2020; Hannachi, 2021) recommend initially maintaining (*in vivo*) humidity within 99-95%, then reducing it to 50-60%. But without expensive temperature and humidity control systems, this is very difficult to achieve (Chen, 2021).

To obtain functional leaves from microclonal plants, it is necessary to achieve a balance of water exchange (optimize water exchange and transpiration) even *in vitro*. This can be done by reducing the temperature at the base of the culture containers, applying a thin layer of lanolin paste, vegetable oil, paraffin, polyethylene glycol, on a nutrient medium. However, experimental plants under such influences were characterized by weak growth and development. Based on this information, the authors suggested that adding agroperlite and/or vermiculite to the MS nutrient medium (creating structured nutrient media) would also help reduce humidity in the culture tanks and promote additional aeration of the nutrient medium. The obtained experimental results on indicators of the water regime, including the intensity of transpiration, confirmed our assumption. It was established that on structured nutrient media (MS + agroperlite, MS + vermiculite, MS + agroperlite+vermiculite) grape microclones were characterized (compared to the control, MS by prescription) by higher water-holding capacity and lower intensity of transpiration of leaf and shoot tissues. It is known that the greater the water retention capacity of plant leaves, the better they will tolerate the adverse impact of abiotic environmental factors (Gupta, 2020). In our case, the greater the water-holding capacity of grape microclones, the better they will take root in uncontrolled conditions. The obtained results proved that on structured nutrient media, which the authors of this paper determined to be the most effective (MS + agroperlite, MS + vermiculite, MS + agroperlite+vermiculite), the tissues of leaves and shoots of grape microclones had a higher water-holding capacity, a lower intensity of transpiration, and their viability *in vivo* was at 76.3-98.5%. For comparison; in the control, the viability of grape microclones *in vivo* was at 58.5-65.2%. This is explained by the fact that on the indicated nutrient media, grape microclones, already *in vitro*, used water more economically. Such savings are associated with structuring, adding mineral substrates to the nutrient medium. This, on the one hand, improved the aeration of the substrate, on the other hand – modelled more natural cultivation conditions. Comparable results were obtained (Hoang, 2020) upon cultivating *Eutrema japonicum*, on a nutrient medium with agroperlite.

Before planting grape microclones *in vivo*, the authors also determined the content of dry matter in leaves and shoots. According to our results, their largest number was synthesized on structured nutrient media. According to the data (Grytsak, 2020), it was established that at the maximum values of the indicators of wet and dry mass growth, plants form new leaves much faster *in vivo*.

As a result of the conducted multiple dispersion and correlation-regression analysis, the authors noted a prominent dependence of the formation of indicators of the water regime of grape leaves and shoots, the content of dry substances on the composition of the nutrient medium, and a high correlation between the indicators of the water regime of microclones and their viability *in vivo*.

CONCLUSIONS

To increase the adaptation potential of grape microclones *in vitro*, it is advisable to cultivate them on structured nutrient media with mineral substrates – MS (IAA 0.3 mg/l+6-BAP 0.2 mg/l) with the addition of agroperlite or vermiculite (MS+agroperlite, MS+vermiculite, MS+agroperlite+vermiculite).

On structured nutrient media, grape microclones were characterized (compared to the control) by higher water-holding capacity and lower transpiration intensity of leaf and shoot tissues. Drying the plants and determining the amount of moisture that evaporated after short intervals of time (after 5, 10, 15, 20, 30, and 60 min) showed that the water-holding capacity increased (on average for the variants) by 0.5-8.1% (technical varieties) and by 0.6-3.0% (rootstock varieties).

On the contrary, the intensity of transpiration (after 10 min) of grape microclones decreased, which was typical for both technical and rootstock varieties. Compared to the control, this indicator decreased by 1.8 times in plants on MS + agroperlite, MS + vermiculite nutrient media and by 1.6 times in plants on MS + agroperlite + vermiculite nutrient media. In the tissues of leaves and shoots of microclones of grapes, a larger amount of dry substances was synthesized. Compared to the control, microclones of technical varieties accumulated 6.0% (MS + agroperlite), 5.9% (MS + vermiculite), 7.4% (MS + agroperlite + vermiculite) more dry matter; in microclones of rootstock varieties – by 6.9%, 6.6%, and 8.1%, respectively.

The largest number of plants that took root *in vivo* and were further characterized by active growth and development was after *in vitro* cultivation on structured nutrient media. Compared to the control, this indicator increased by 19.8-26.5% in the MS + agroperlite variants, by 21.0-21.3% in the MS+vermiculite variants, and by 25.1-40.0% in the MS + agroperlite + vermiculite variants.

The reliability of the obtained results was confirmed by the results of multiple variance and correlation-regression analysis. It has been proved that factors such as the structured basis of MS (27-52%) and grape variety (20-30%) had the greatest influence on indicators of water-holding capacity, intensity of transpiration of the vegetative mass of grape microclones *in vitro*, only factor of the structured base of MS substantially influenced the indicator of dry matter content (90%). A positive correlative dependence of the viability of grape microclones *in vivo* on the physiological state formed *in vitro* (R=0.82) was established.

The perspective of further research is to determine the anatomical structure of the stomatal apparatus of the lower epidermis of leaves of microclones of grafted and technical varieties of grapes under the conditions of cultivation *in vitro* and *in vivo*, establishing its influence on the water regime and adaptation potential of plants.

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CONFLICT OF INTEREST

None.

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Вплив поживних середовищ на фізіологічні показники мікроклонів винограду

Наталя Миколаївна Зеленянська
Доктор сільськогосподарських наук, старший науковий співробітник
Національний науковий центр «Інститут виноградарства і виноробства імені В. Є. Таїрова» НААН
65496, вул. 40-річчя Перемоги, 27, смт. Таїрове, Україна
https://orcid.org/ 0000-0002-9303-8686

Ірина Олександрівна Іщенко
Кандидат сільськогосподарських наук, доцент
Одеський державний аграрний університет
65012, вул. Канатна, 99, м. Одеса, Україна
https://orcid.org/0000-0003-0255-4843

Михайло Олександрович Самофалов
Аспірант
Національний науковий центр «Інститут виноградарства і виноробства імені В. Є. Таїрова» НААН
65496, вул. 40-річчя Перемоги, 27, смт. Таїрове, Україна
https://orcid.org/0000-0002-8785-0651

Анотація. Успішне приживання мікроклонів винограду в неконтрольованих умовах довкілля (*in vivo*) залежить від рівня стійкості, який формується ще на етапі їх пасажування і росту *in vitro*. Важливу роль при цьому відіграють показники водного режиму вегетативної маси. Метою роботи було ознайомлення з результатами визначення водозатримуючої здатності, інтенсивності транспірації мікроклонів винограду *in vitro* та встановлення частки їх впливу на адаптаційний потенціал *in vivo*. У роботі використовували біотехнологічні, лабораторні, вегетаційні та математично-статистичні методи досліджень. Отримані результати показали, що для оптимізації фізіологічних процесів у тканинах листків та пагонів мікроклонів винограду, підвищення їх приживлюваності в умовах *in vivo* доцільним є їх культивування *in vitro* на структурованих поживних середовищах (МС + агроперліт, МС + вермікуліт, МС + агроперліт + вермікуліт) із вмістом фітогормонів ІОК – 0,2 мг/л, і 6-БАП – 0,3 мг/л. Структуровані поживні середовища сприяли підвищенню водозатримуючої здатності та зниженню інтенсивності транспірації тканин листків і пагонів мікроклонів як технічних, так і підщепних сортів. Протягом 60 хв. досліджень у мікроклонів технічних сортів випаровувалось від 0,006 г до 0,034 г води, у мікроклонів підщепних сортів – відповідно від 0,003 г до 0,053 г. Інтенсивність транспірації (через 10 хв.) зменшувалась в 1,7-1,8 рази. На контрольному поживному середовищі Мурасіге-Скуга за відповідний проміжок часу рослини випаровували більшу кількість води: від 0,006 г до 0,079 г (технічні сорти) та від 0,008 г до 0,086 г (підщепні сорти); інтенсивність транспірації була вищою. Після культивування мікроклонів винограду на структурованих поживних середовищах вони характеризувалися більшим вмістом сухих речовин у тканинах листків і пагонів (14,6-15,0 %) та кращими показниками приживлюваності в умовах *in vivo* (76,3-98,5 %, при 58,5-65,2 % у контролі). Достовірність отриманих результатів підтверджено результатами багатофакторного дисперсійного аналізу. Отримані результати розширюють уявлення про динамічні зміни показників водного режиму вегетативної маси мікроклонів винограду *in vitro,* їх вплив на приживлюваність рослин в умовах *in vivo*

Ключові слова: *in vitro*; водозатримуюча здатність; інтенсивність транспірації; приживлюваність; фітогормони; біологічно активні препарати; мінеральні субстрати