УДК 634.836:531.19

OPTIMIZATION OF NUTRIENT MEDIUM COMPOSITION BY THE MATHEMATICAL DESIGN OF EXPERIMENT FOR SHOOT TIP DEVELOPMENT IN FOUR GRAPEVINE GENOTYPES

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Nutrient media for shoot development from shoot tips 0.5-0.8 mm with several leaf primordia in four grapevine genotypes were optimized by means of a mathematical design of experiment. Over the range of component concentrations $CaCl_2$ had considerable the highest effect on shoot tip development compared to other components of the media. Grapevine genotypes were different in their needs for concentrations of macro-elements to optimize the process of their growth and development. Shoot development from shoot tips in each grapevine genotype as a function of macro-element concentrations in media must be described by an individual regression equation. The proposed method of result evaluation and the mathematical design of experiment may be used in physiological and agricultural research for optimization of processes affected by numerous factors. The optimized media for shoot tip development may by used for sanitation of grape plants from viruses in meristem cultures

Keywords: IN VITRO, MICROPROPAGATION, MINERAL ELEMENT, MERISTEM CULTURE, STORAGE, VITIS.

UDC 634.836:531.19

ОПТИМИЗАЦИЯ СОСТАВА ПИТАТЕЛЬНОЙ СРЕДЫ МАТЕМАТИЧЕСКИМ ПЛАНИРОВАНИЕМ ЭКСПЕРИМЕНТА ДЛЯ РАЗВИТИЯ ВЕРХУШЕК ПОБЕГОВ IN VITRO У ЧЕТЫРЕХ ГЕНОТИПОВ ВИНОГРАДА

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Питательная среда для развития побегов *in vitro* из верхушек побегов размером 0,5-0,8 мм с несколькими листовыми зачатками (примордиями) у четырех генотипов винограда была оптимизирована с применением математического планирования эксперимента. Среди исследуемых концентраций компонентов питательной среды $CaCl_2$ оказывал наиболее сильный эффект на развитие верхушек побегов по сравнению с другими компонентами среды. Генотипы винограда различались в их потребности в концентрациях макроэлементов для оптимизации процессов их роста и развития. Развитие побегов из верхушек побегов у каждого виноградного генотипа является функцией концентраций макроэлементов в среде и может быть описано (представлено) индивидуальным для каждого генотипа уравнением регрессии. Предлагаемый метод оценки результатов и математическое планирование эксперимента может быть использовано в физиологии и сельском хозяйстве для оптимизации процессов, зависящих от многих факторов. Оптимизированные среды для развития верхушек побегов могут быть использованы для оздоровления виноградных растений от вирусов в меристемных культурах *in vitro*

Ключевые слова: В ПРОБИРКЕ, МИКРОРАСПРОСТРАНЕНИЕ, МИНЕРАЛЬНЫЙ ЭЛЕМЕНТ, КУЛЬТУРА МЕРИСТЕМЫ, ХРАНЕНИЕ, ВИНОГРАД

Abbreviations

BA = N6-benzyladenine;

IAA = indole-3-acetic acid;

MS = Murashige and Skoog (1962) medium.

Introduction

Virus elimination in grapevine may be achieved by heat therapy and shoot tip culture (Gribaudo et al., 1994; Regner et al., 1995; Anaclerio et al., 1999). Nepoviruses may easily be eradicated by heat therapy at 36°C for four weeks and by shoot tip culture while closteroviruses is much more difficult to achieve, and the size of shoot tip explants is of crucial importance (Regner et al., 1995). Grapevine plants were obtained from shoot tip explants containing one or two leaf primordia and not more than 0.3 mm long (Bini, 1976; Blaich, 1984; Duran-Vila et al., 1988; Maekawa et al., 1993; Regner et al., 1995).

Genotypes of the genus Vitis differ in their ability to uptake macro-element ions from the soil and their optimum concentrations in shoots and roots which are needed for plant growth (Scienza et al., 1986). Various concentrations of $MgSO_4$ and KH_2PO_4 in liquid and solid media were optimum for plant growth of four grapevine genotypes (Slenco et al., 2001). For development of shoots from shoot tip explants of different grapevine genotypes, media containing N6-benzyladenine (BA) and different concentrations of macro-elements were used (Novak and Juvova, 1983; Harris and Stevenson, 1982; Gribaudo et al., 1994; Maekawa et al., 1993).

This report attempted to optimize component concentrations levels of the nutrient medium using a mathematical design of experiments in order to improve shoot tip survival rate and to increase shoot growth from the explants. Optimized media for shoot tip development may be used for sanitation of grapevine plants from viruses in meristem cultures.

Materials and methods

Plant material

The experiments were based on four grapevine genotypes: the root stock *Riparia* x *Rupestris 'Kober 5 BB'* and cultivars released by the Institute for Vine and Wine 'Magarach': 'Podarok Magaracha' (interspecific cross of a *Vitis vinifera L.* and a Franco-American hybrid), 'Zhemchug Magaracha' and seedless 'Sverkhrannii bessemyannyi' (intraspecific crosses of *Vitis vinifera L.*).

Shoot tip culture and conditions

Green shoots collected from a mature vine released from dormancy or from field-grown plants in the beginning of the vegetation period were used as a source of explants. Shoots, about 1.0-1.5 cm long, were surface sterilized in 70% (v/v) ethanol for 20 s and then in 1% sodium hypochlorite with 0.1%. Tween 20 for 15 min, and then rinsed four times in sterile water. Shoot tips with several leaf primordia (0.5-0.8 mm long) were excised under a stereomicroscope and cultured on different medium versions solidified with 7 g l^{-1} Difco agar. The medium versions were supplemented with 0.5 mg l^{-1} BA, and the pH was adjusted to 5.6-5.8 with NaOH prier to autoclaving at 103 kPa for 25 min. The compositions of the media used in experiments are indicated in 'Results and discussion'. All shoot tip cultures were maintained in culture tubes with 22 mm in diameter containing 5 ml of culture medium under a photon flux density at the culture surface of 55 □mol m^{-2} s^{-1} provided by cool white fluorescent tubes with a 16-h photoperiod at 27^{o}C.

Plant growth and development

To obtain rooted plants, shoots with well-developed leaves grown from shoot tips were divided into single buds with one leaf and established on solid medium for rooting. The thick basal portion of the arms was not used for rooting. Vitrified shoots and leaves sometimes developed on medium with BA. In such cases, only shoots with two or three leaves were established on the

rooting medium. The rooting medium composition was described by Zlenko et al. (1995) and Slenko et al. (2001) containing: 308 mg l^{-1} NH_4NO_3, 922 mg l^{-1} KNO_3, 597 mg l^{-1} $MgSO_4$ $7H_2O$, 122 mg l^{-1} KH_2PO_4, 331 mg l^{-1} $CaCl_2$ (MS), 1/2 Fe-EDTA and 1/2 micro-elements MS, 20 mg l^{-1} myo-inositol, 0.1 mg l^{-1} thiamine (MS), 0.5 mg l^{-1} nicotinic acid (MS), 0.2 mg l^{-1} pyridoxine, 0.2 mg l^{-1} indole-3-acetic acid (IAA), 10 g l^{-1} sucrose and 7 g l^{-1} Difco agar. Subsequent *in vitro* propagation of plants was by dividing them into single-bud explants with one leaf and establishing the latter on the same rooting medium (Slenko et al., 2001) except for the presence of 0.1 mg l^{-1} IAA.

Mathematical design of experiment and statistical analysis

Evaluation of results and calculation of the overall quality criterion.

The development of shoot tips after 50 days was evaluated using the following criteria: survival rates (%), shoot length (cm) and leaf development by means of a 1-5 point numerical scale. The trait ‘leaf development’ enters as the subjective (visual) evaluation of cytokhinin-induced vitrification and leaf lobation scored in points: 1 – very high vitrification and lobation or no leaf development at all, 2 – high vitrification and lobation, 3-4 – medium vitrification and lobation, and 5 – no lobation with the typical leaf shape of each cultivar.

Survival rates of shoot tips may be high but shoot tips may fail to develop into shoots with leaves. Therefore an overall quality criterion was needed calculated as follows (Zlenko et al., 1995). Since each trait (variables: $Ix_{1,2,...\ i}$; $IIx_{1,2,...\ i}$ .... $nx_{1,2,...\ i}$) varies to a larger or smaller extent depending on the medium composition, variation of the traits must be reduced to certain limits which reflected the usefulness of a trait, i.e. a more useful trait provided a wider scale of variation and the scale of variation of a less useful trait is narrower. Variation of traits (variables: $It_{1,2,...\ i}$; $IIt_{1,2,...\ i}$ .... $nt_{1,2,...\ i}$) on different

medium versions within limits determined by us and the overall quality criterion (o.q.c.) were calculated using the following equations:

$$Ia = -\frac{Ix_{max} - M \cdot Ix_{min}}{M - 1} \quad (1);$$

$$It_{1,2...i} = -\frac{Ix_i - Ia}{x_{max} - Ia} \quad (2);$$

$$o.q.c. = It \cdot IIt \ldots \cdot \ldots nt \quad (3)$$

where Ix_{max}; IIx_{max} ... . nx_{max} and Ix_{min}; IIx_{min} ... . nx_{min} are extreme values of traits; x_i is the value being estimated of a trait; M is a random number determined depending on the usefulness of a trait, e.g. if M = 10, t_i varies over a range of 0.1-1.0 and if M=2, t_i varies over a range of 0.5-1.0. In this paper M = 10 for the traits of all processes.

Mathematical design of experiment. To calculate component concentrations in medium enabling optimum shoot development from shoot tips, a mathematical design basing on the random balance method was used (Hartmann et al., 1977). Since it is practically impossible to do a full polyfactorial experiment if too many factors are involved, each at three or two levels (such factors were component levels in numerous medium versions used in the experiment). Algorithms of multiple curvilinear stepwise regression were applied. The influence of the factors was evaluated based on the significance of the members of regression equations describing processes. Stepwise optimization of each process was calculated using the steepest ascent method of Box-Wilson. Component concentrations in media were used to increase the overall quality criteria of shoot development from shoot tips in four grapevine genotypes, therefore, optimum medium for each process was determined. This mathematical design used in the experiment was described in the previous study (Zlenko et al., 1995).

Statistical analysis. Each medium version for each cultivar was in 15 replications. Confidence limits of average values of the traits were calculated

with significant at P<0,05. Deviations (variation coefficient *V* with significant at P<0,05) of shoot tip development from average values were not more than 23% for survival rates (%), 31% for the shoot lengths (cm), 34% for leaf development and 29% for the overall quality criteria (conventional units).

Results and discussion

Calculation of regression equations describing shoot tip development in four grapevine genotypes and individually in rootstock 'Kober 5BB'

Shoot growth in grapevine is affected by various nitrogen-containing salts presented in the nutrient medium and their concentrations (Villegas et al., 1992). Therefore, not only nitrogen sources of the MS formulation (NH_4NO_3 and KNO_3) but also some other substances [$(NH_4)_2SO_4$ and NH_4Cl], KCl and K_2SO_4 as a source of the K^+ ion were used in the media (Table I).

Table I. - Meaning of factors expressed as natural and coded variables. Experiment focused on optimizing medium for shoot tip development in four grapevine genotypes: 'Kober 5 BB', 'Podarok Magaracha', 'Zhemchug Magaracha' and 'Sverkhrannii bessemyannyi'.

Factors (variables)		Highest level		Intermediate level		Lowest level	
		In natural units, mg l^{-1}	In the coded form	In natural units, mg l^{-1}	In the coded form	In natural units, mg l^{-1}	In the coded form
(x_1)	NH_4NO_3	1650	+1	825	0	0	-1
(x_2)	$(NH_4)_2SO_4$	1360	+1	680	0	0	-1
(x_3)	NH_4Cl	1112	+1	556	0	0	-1
(x_4)	KNO_3	1900	+1	950	0	0	-1
(x_5)	KCl	1412	+1	706	0	0	-1
(x_6)	K_2SO_4	1637	+1	818	0	0	-1
(x_7)	$CaCl_2$	662	+2	331	0	166	-1
(x_8)	$MgSO_4$ $7H_2O$	370	+1	-	-	185	-1
(x_9)	KH_2PO_4	170	+1	-	-	85	-1
(x_{10})	NaH_2PO_4 H_2O	170	+1	-	-	0	-1
(x_{11})	Fe-EDTA	MS	+1	-	-	1/2 MS	-1
(x_{12})	micro-elements	MS	+1	-	-	1/2 MS	-1
(x_{13})	myo-inositol	100	+1	-	-	10	-1

(x_{14})	thiamine	5	+4	1	0	0	-1
(x_{15})	pyridoxine	5	+4	1	0	0	-1
(x_{16})	nicotinic acid	5	+4	1	0	0	-1
(x_{17})	para-aminobenzoic acid	5	+1	-	-	0	-1
(x_{18})	sucrose	40 g l^{-1}	+1	-	-	20 g l^{-1}	-1

These substances were applied at levels leading to the content of nitrogen in the form of ammonium or nitrate and the content of the K^+ ion equal to those in the macro-elements of the full- and the half-strength MS medium. High cytokinin levels induced development of malformed shoots with subsequent poor rooting (Slenko et al., 2001), so a low level of BA(0.5 mg l^{-1}) in the experiments were needed. To determine factor (component) levels and factor interactions influencing the process, the random balance method was used. The experiment included 18 factors, each at three or two levels and expressed in natural units (mg l^{-1} or g l^{-1}) and in the coded form (Table I). Design of the experiment using the random balance method is shown in Table II, with variables in the coded form. Green shoots collected from a nature vine released from dormancy were used as a source of shoot tip explants. Taking into consideration characteristics of shoot tip development (survival rates, shoot length and leaf development scored in points) after 50 days in culture on each of 20 medium versions, the overall quality criterion (see ‘Material and methods’) for each of the four genotypes and the average value of the overall quality criteria were calculated (Table II).

Shoot tip culture on the medium version 20 containing MS mineral elements did not improve shoot development in any of the four genotypes (Table II). In this respect, root stock ‘Kober 5 BB’ performed the best on the medium version 8, cvs. ‘Podarok Magaracha’ and ‘Zhemchug Magaracha’ did so on the medium version 6, and the version 11 was the most beneficial medium for cv. ‘Sverkhrannii bessemyannyi’. The highest value (0.58) of the average overall

quality criterion for the four genotypes was achieved on the medium version 6 (Table II).

Table II. - Design of experiment using variables in the coded form (Table I) and results pertaining to shoot tip development in four grapevine genotypes (expressed as units of the overall quality criterion). The variation coefficient (V) for each medium version of each genotype is not more than 29% ($P < 0.05$)

Medium versions	Meaning of factors as variables in the coded form																		Shoot tip development after 50 days of culture (expressed in units of the overall quality criterion)				
	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}	x_{13}	x_{14}	x_{15}	x_{16}	x_{17}	x_{18}	'Kober 5 BB'	'Podarok Magaracha'	'Zhemchug Magaracha'	'Sverkhrannii bessemyannyi'	Average value for the four genotypes
1.	+1	-1	+1	-1	+1	0	0	+1	-1	+1	-1	+1	-1	0	0	-1	-1	+1	0.02	0.06	0.03	0.11	0.05
2.	-1	0	0	+1	-1	-1	+2	-1	-1	-1	+1	+1	-1	-1	-1	-1	+1	+1	0.08	0.03	0.11	0.19	0.10
3.	+1	-1	-1	0	0	+1	-1	+1	-1	-1	+1	+1	-1	0	-1	-1	-1	-1	0.04	0.07	0.01	0.01	0.03
4.	-1	0	0	+1	+1	-1	+2	-1	+1	+1	-1	-1	+1	0	0	-1	+1	-1	0.57	0.32	0.25	0.46	0.40
5.	+1	-1	0	0	+1	+1	-1	-1	-1	-1	+1	+1	+1	+4	0	+4	+1	+1	0.01	0.01	0.03	0.02	0.02
6.	0	0	-1	0	-1	-1	+2	-1	+1	-1	-1	-1	-1	0	0	-1	+1	-1	0.01	1.00	1.00	0.31	0.58
7.	0	+1	0	0	+1	-1	0	-1	-1	-1	-1	+1	-1	+4	-1	0	+1	+1	0.04	0.12	0.11	0.47	0.19
8.	-1	0	-1	+1	0	+1	+2	-1	-1	+1	+1	+1	-1	0	+4	+4	-1	+1	1.00	0.08	0.09	0.18	0.33
9.	+1	0	+1	0	-1	0	-1	-1	+1	+1	-1	-1	-1	-1	0	-1	+1	-1	0.01	0.01	0.40	0.33	0.19
10.	+1	-1	+1	-1	+1	+1	+2	+1	-1	+1	-1	-1	+1	+4	+4	0	-1	-1	0.01	0.13	0.08	0.19	0.10
11.	0	0	0	-1	0	0	0	+1	+1	-1	+1	-1	+1	0	-1	+4	+1	+1	0.02	0.05	0.08	1.00	0.29
12.	-1	+1	-1	-1	0	+1	-1	-1	+1	+1	+1	-1	-1	-1	0	0	-1	-1	0.01	0.01	0.03	0.09	0.04
13.	0	-1	0	+1	+1	0	-1	+1	-1	-1	-1	-1	+1	+4	-1	0	+1	-1	0.02	0.16	0.23	0.04	0.11
14.	0	+1	+1	+1	0	0	0	+1	+1	-1	-1	-1	+1	+4	-1	0	-1	-1	0.01	0.08	0.03	0.07	0.05
15.	0	+1	-1	+1	-1	-1	-1	-1	-1	-1	+1	+1	-1	-1	-1	+4	-1	-1	0.01	0.04	0.02	0.25	0.08
16.	0	+1	0	0	0	0	+2	+1	+1	+1	+1	+1	+1	+4	+4	-1	-1	+1	0.04	0.10	0.03	0.25	0.10
17.	-1	+1	+1	-1	-1	+1	+2	+1	-1	+1	+1	-1	+1	+4	+4	0	+1	-1	0.01	0.01	0.02	0.08	0.03
18.	-1	-1	+1	-1	+1	-1	0	+1	-1	+1	-1	+1	+1	-1	+4	+4	-1	+1	0.02	0.01	0.03	0.04	0.02
19.	-1	+1	-1	-1	-1	+1	0	-1	+1	-1	-1	-1	-1	-1	+4	+4	-1	+1	0.01	0.01	0.04	0.03	0.02
20.	+1	-1	-1	+1	-1	-1	0	+1	+1	+1	+1	+1	+1	0	+4	+4	+1	+1	0.05	0.38	0.03	0.03	0.12

Based on the average values of the overall quality criteria, regression equations for shoot tip development as functions of concentrations of substances in medium versions (Tables I and II) were calculated for the four genotypes (y_1) and individually for the root stock 'Kober 5 BB' (y_2):

$$y_1 = 0.151 - 0.177x_7 \cdot x_8 + 0.143x_7 - 0.037x_7 \cdot x_8 \cdot x_{14} + 0.085x_1 - 0.056x_1 \cdot x_8 - - 0.042x_8 \quad (4)$$

$$y_2 = 0.156 + 0.077x_4 \cdot x_7 + 0.175x_7 \cdot x_{10} - 0.161x_7 \cdot x_8 - 0.052x_7 \cdot x_9 - 0.072x_8 + + 0.062x_{10} + 0.048x_9 - 0.030x_4 \cdot x_8 \quad (5)$$

with the following characteristics of the equations (Table III): the level of significance of y_1 is 0.001, the determination coefficient is 0.694, the standard

error is 0.097; the level of significance of y_2 is 0.000, the determination coefficient is 0.977, the standard error is 0.065. The factor x_7 ($CaCl_2$) provided the most significant effect on both equations since the first members of the equations are of the highest significance. They also are characterized with the highest determination coefficients, i.e. make the largest contribution to the description of the process by means of the equation (Table III).

Table III. - Improvement of statistical characteristics of regression equations (4 and 5) during sequential selection of equation members describing overall quality criteria of shoot tip development as a function of concentrations of substances in medium versions (Tables I and II).

Equation members	Coefficient of regression	Level of significance	Coefficient of determination	Standard error
Equation (4) for calculating average values of the overall quality criteria for the four genotypes (y_1):				
$x_7 \cdot x_8$	-0.177	0.009	0.324	0.127
x_7	0.143	0.006	0.423	0.121
$x_7 \cdot x_8 \cdot x_{14}$	-0.037	0.004	0.479	0.118
x_1	0.085	0.001	0.572	0.107
$x_1 \cdot x_8$	-0.056	0.001	0.631	0.103
x_8	-0.042	0.001	0.694	0.097
The free member=0.151				
Equation (5) for calculating the overall quality criteria separately for the root stock ‘Kober 5 BB’ (y_2):				
$x_4 \cdot x_7$	0.077	0.001	0.460	0.245
$x_7 \cdot x_{10}$	0.175	0.000	0.773	0.163
$x_7 \cdot x_8$	-0.161	0.000	0.859	0.133
$x_7 \cdot x_9$	-0.052	0.000	0.905	0.112
x_8	-0.072	0.000	0.927	0.102
x_{10}	0.062	0.000	0.963	0.075
x_9	0.048	0.000	0.973	0.068
$x_4 \cdot x_8$	-0.030	0.000	0.977	0.065
The free member=0.156				

Stepwise optimization of media for shoot tip development based on regression equations

Table IV shows stepwise optimization of macro-element concentrations in media for shoot tip development based on the regression equations 4 and 5.

Table IV. - Macro-element concentrations in media optimized based on regression equations with a view to improve shoot tip development in the four grapevine genotypes and individually in the root stock 'Kober 5 BB'. In addition to the macro-elements indicated in this Table, each medium version contained (Slenko et al., 2001): Fe-EDTA and micro-elements MS medium, 100 mg l^{-1} myo-inositol, 10 mg l^{-1} thiamine, 5 mg l^{-1} nicotinic acid; 0.2 mg l^{-1} pyridoxine, 10 mg l^{-1} glycine, 5 mg l^{-1} para-aminobenzoic acid, 0.5 mg l^{-1} BA, 30 g l^{-1} sucrose and 7 g l^{-1} Difco agar.

Macro-elements (variables in equations)		Concentrations of macro-elements (mg l^{-1}) in media for shoot tip development									
		MSM	Using the regression equation (4) for the four genotypes (based on the macro-element concentrations in medium version 6 from Table II)					Using the regression equation (5) for the stock 'Kober 5 BB' (based on the macro-element concentrations in medium version 8 from Table II)				
			1cv4	2cv4	3cv4	4cv4	5cv4	1Kob	2Kob	3Kob	4Kob	5Kob
(x_1)	NH_4NO_3	1650	833	1200	1520	1950	2153	-	-	-	-	-
(x_2)	$(NH_4)_2SO_4$	-	680	680	680	680	680	680	680	680	680	680
(x_3)	NH_4Cl	-	-	-	-	-	-	-	-	-	-	-
(x_4)	KNO_3	1900	950	950	950	950	950	1950	2090	2233	2337	2480
(x_5)	KCl	-	-	-	-	-	-	706	706	706	706	706
(x_6)	K_2SO_4	-	-	-	-	-	-	1637	1637	1637	1637	1637
(x_7)	$CaCl_2$	331	675	740	800	890	944	680	734	793	835	900
(x_8)	$MgSO_4\,7H_2O$	370	183	165	140	90	67	173	142	112	93	64
(x_9)	KH_2PO_4	170	170	170	170	170	170	85	84	83	83	81
(x_{10})	$NaH_2PO_4\,H_2O$	170	-	-	-	-	-	180	208	235	252	278

The optimization began (Table IV) by determining the optimum medium version for each of the process: macro-element concentrations in the medium version 6 (Table II) for shoot tip development of the four genotypes and macro-element concentrations in the medium version 8 (Table II) individually for the root stock 'Kober 5 BB'. Green shoots collected from field-grown plants were used as a source of shoot tip explants. Table V shows shoot tip development

after 50 days in culture on the media (Table IV) of the different optimization designs for each of the processes.

Table V. - Shoot tip development in four grapevine genotypes after 50 days in culture on media (Table IV) containing macro-element concentrations calculated by using the regression equations (4) and (5). Deviations of shoot tip development (*V*) from average values ($P < 0.05$) were not more than 31% for the shoot length and 34% for leaf development.

Designations of media	Shoot tip development in four grapevine genotypes after 50 d in culture							
	'Kober 5 BB'		'Podarok Magaracha'		'Zhemchug Magaracha'		'Sverkhrannii bessemyannyi'	
	Shoot length, cm	Leaf development, scored in points 1.0-5.0	Shoot length, cm	Leaf development, scored in points 1.0-5.0	Shoot length, cm	Leaf developmen, scored in points l.0-5.0	Shoot length, cm	Leaf development, scored in points l.0-5.0
Control MMS*	0.2	1.8±0.6	1.9±0.5	1.8±0.6	0.4±0.1	0.3±0.1	1.3±0.4	1.0±0.3
Control 11TableII**	0.1	1.7±0.5	0.6±0.2	1.6±0.5	0.5±0.2	1.8±0.5	2.3±0.7	4.4±0.6
Based on the regression equation (4) for shoot tip development in four grapevine genotypes								
Control 6TableII**	0.1	1.0	2.7±0.8	4.0±1.1	1.2±0.4	4.1±0.6	0,5±0,1	2.0±0.6
1cv4	0.1	1.0	3.1±0.9	4.2±1.3	1.8±0.6	4.2±0.5	0.7±0.2	1.9±0.6
2cv4	0.1	1.0	3.5±1.0	4.4±1.4	2.4±0.8	4.5±0.5	0.6±0.2	1.6±0.5
3cv4	0.1	1.0	3.8±1.0	3.9±1.2	1.9±0.6	3.3±0.8	0.7±0.2	1.3±0.4
4cv4	0.1	1.0	3.3±0.9	3.4±1.1	1.4±0.5	2.1±0.6	0.8±0.2	0.6±0.2
5cv4	0.1	1.0	2.8±0.8	2.8±0.9	1.0±0.3	1.4±0.4	0.6±0.2	0.8±0.2
Based on the regression equation (5) for shoot tip development in the root stock Kober 5 BB								
Control 8TableII**	1.9±0.6	4.0±0.5	0.9±0.3 callus	1.2±0.4	0.5±0.2	2.4±0.7	0.4±0.1	1.8±0.6
1Kob	2.4±0.7	4.3±0.4	0.6±0.2 callus	1.8±0.6	0.6±0.2	2.0±0.6	0.5±0.2	1.3±0.4
2Kob	3.2±1.0	4.5±0.4	0.8±0.2 callus	1.7±0.5	0.6±0.2	1.8±0.6	0.6±0.2	1.0±0.3
3Kob	3.0±0.8	3.9±0.8	0.7±0.2 callus	1.9±0.6	0.7±0.2	1.9±0.6	0.6±0.2	1.2±0.3
4Kob	1.4±0.3	2.0±0.7	0.8±0.2 callus	2.0±0.6	0.5±0.2	1.5±0.5	0.4±0.1	1.3±0.4
5Kob	1.1±0.3	1.5±0.5	1.1±0.3 callus	2.1±0.6	0.4±0.1	1.5±0.4	0.5±0.1	1.5±0.5

* Contains MS mineral elements supplemented with 170 mg l^{-1} NaH_2PO_4 H_2O. The remaining additives indicated in the Table IV.

** Macro-element concentrations in medium versions indicated in Table II used as controls to check the optimization efficiency. The remaining additives indicated in the Table IV.

The design of an experiment for four grapevine genotypes (equation 4) led

to encouraging results, though they were not reliable enough (macro-element concentrations of the medium version 6 from Table II, entering as control) for shoot tip development in cvs. ‘Podarok Magaracha’ (optimized medium version 3cv4, Table V, Fig. 1) and ‘Zhemchug Magaracha’ (optimized medium version 2cv4, Table V). On the contrary, the results were disappointing for root stock ‘Kober 5 BB’ and cv. ‘Sverkhrannii bessemyannyi’ due to the design of experiment by means of equation (4) began by using macro-element concentrations of the medium version 6 from Table II which was optimum for cvs. ‘Podarok Magaracha’ and ‘Zhemchug Magaracha’ but not so either for root stock ‘Kober 5 BB’ (for which macro-element concentrations of the medium version 8 from Table II, designated as 8 Table II in Table V, was optimum) or for cv. ‘Sverkhrannii bessemyannyi’ (for which macro-element content of the medium version 11 from Table II, designated as 11 Table II in Table V, was optimum). In root stock ‘Kober 5 BB’ (Table V) shoot tips performed the best on the medium version 2Kob achieved by stepwise optimization using the regression equation (5) particularly for this genotype. This medium version 2Kob contained 208 mg l^{-1} NaH_2PO_4 H_2O (Table IV). Macro-elements at 3/4 levels of the MS formulation medium, supplemented with 170 mg l^{-1} NaH_2PO_4 H_2O, were optimum for grapevine shoot tip development (Harris and Stevenson, 1982).

Fig. 1. Development of several shoots after 50 days of shoot tip culture in cv. ‘Podarok Magaracha’ on the medium version 3cv4 (Tables IV and V) (the explant removed from the culture tube, top view).

The addition of this substance stabilizes the pH of the medium during explant culture (Viskot and Bezdek, 1984). Callus was formed from shoot tips of cv. ‘Podarok Magaracha’ on media optimized for shoot development in the root stock ‘Kober 5 BB’ (Table V). Reliable differences were established in shoot tip development on MMS medium and on optimized medium versions for root stock ‘Kober 5 BB’ (2Kob, Table V), cv. ‘Podarok Magaracha’ (version 3cv4, Table V), cv. ‘Zhemchug Magaracha’ (version 2cv4, Table V) and cv. ‘Sverkhrannii bessemyannyi’ (macro-element content of the medium version 11 from Table II, designated as version 11 Table `II in Table V).

Within the genus *Vitis*, plants have genetically determined differences in the uptake of K^{+}, Ca^{2+} and Mg^{2+} (Scienza et al., 1986). Grapevine genotypes differed in their needs for nitrogen levels of the soil for their growth: some

genotypes were capable to grow well on the soils with low nitrogen levels (Murthy and Iygengar, 1997). Different root stock genotypes affected the levels of mineral substances (N, K and Fe) in the scion (Bavaresco, 2001) and induced different responses of the scion to soil nitrogen fertilization (Keller et al., 2001). Various levels of mineral substances found in different organs of the plant depending on the genotype and the levels of mineral substances in the soil or in the water culture may enter as an indirect proof of the fact that the grapevine genotypes used in our experiments needed different levels of mineral substances in their optimum media. The design of the experiment focused on medium optimization for one grapevine genotype did improve shoot tip development in the specific genotype while poorly-developed shoot tips were observed on the same medium in the other genotypes. Thus, macro-element content of the medium version 11 (Tables II and V) applied for cv. 'Sverkhrannii bessemyannyi' enabled the growth of high-quality shoots and leaves while extremely poor shoot tip development was observed on that medium version in the remaining three genotypes. Our report in this paper and other results concerned with shoot tip culture and whole plant culture (Slenko et al., 2001) led to the conclusion that optimized mineral salts levels for a certain grapevine genotype may not be so for other genotypes, though, of course, some of a great diversity of grapevine cultivars and root stocks may be expected to grow well on this or that optimized medium.

Experiments focused on medium optimization for shoot tip culture by means of the mathematical design (Tables I-V) are time-consuming and required large numbers of explants. It can be seen from the two experiments (Tables II and V) that the four grapevine genotypes have different optimum concentration of macro-elements. Table VI shows media that proved to be best efficient in the two experiments. Optimization of the medium composition may be achieved sooner and with good though not universally the best results by culturing shoot tips of any other genotypes on seven media presented in Table VI to determine

better medium version for each genotype. In some genotypes, shoot tip culture developed callus, the levels of thiamine, nicotinic acid and glycine presented in the media recommended should be reduced to 2 mg l^{-1}.

Table VI. - Compositions of nutrient media recommended for shoot tip development in various grapevine genotypes. In addition to the macro-elements indicated in the Table, each medium version contained other substances, indicated in Table IV.

Macro-elements	Concentrations of macro-elements in different medium versions, mg l^{-1}						
	Medium versions from Tables I and II				Medium versions from Tables IV and V		
	4	6	8	11	2cv4	3cv4	2Kob
1. NH_4NO_3	-	825	-	825	1200	1520	-
2. $(NH_4)_2SO_4$	680	680	680	680	680	680	680
3. NH_4Cl	556	-	-	556	-	-	-
4. KNO_3	1900	950	1900	-	950	950	2090
5. KCl	1412	-	706	706	-	-	706
6. K_2SO_4	-	-	1637	818	-	-	1637
7. $CaCl_2$	662	662	662	331	740	800	734
8. $MgSO_4 7H_2O$	185	185	185	370	165	140	142
9. KH_2PO_4	170	170	85	170	170	170	84
10. $NaH_2PO_4 H_2O$	170	-	170	-	-		208

Conclusions

Our findings lead us to the following conclusions:

1. Over the range of component levels in media (Tables I and II), macro-elements had a higher effect on grapevine shoot tip development compare to Fe-EDTA, micro-elements, vitamins and sucrose. In regression equations 4 and 5 describing shoot tip development in the four genotypes (y_1) and individually in the root stock ‘Kober 5 BB’ (y_2) as a functions of macro-element concentrations in media, the members including the variable x_7 (factor $CaCl_2$), based on the levels 166 mg l^{-1} (1/2 MS), 331 mg l^{-1} (MS) and 662 mg l^{-1} (2 MS), were the most significant and had the largest determination coefficients (Table III).

2. Regression equation (5) describing shoot tip development in one genotype (‘Kober 5 BB’) as a function of macro-element concentrations in media has a larger determination coefficient (0.977) relative to regression

equation (4) for the four genotypes as a whole (determination coefficient is 0.694) since different medium versions were optimum for different genotypes (Tables II and V). Stepwise optimization of the mineral composition of medium for grapevine shoot tip development should begin based on the optimum medium version for each genotype (Tables II and IV).

3. Different concentrations of macro-mineral elements were optimum for shoot tip development in the four grapevine genotypes, irrespective of their specific origin (*Vitis vinifera* or interspecific hybrids, Table V).

The regression equations describing the pattern of mineral elements in optimized media for shoot tip cultures of different genotypes reflect their considerable heterogeneity as concerns best efficient concentrations of macro-elements in media. The proposed assessment of results by reducing the number of parameters characterizing a process to the overall quality criterion (equations 1-3) and the mathematical design of experiment may be used in physiological and agricultural research for optimization of processes affected by numerous factors. The optimized media (Table IV) may be used for growing of virus-free shoots in meristem cultures of different grapevine genotypes.

Acknowledgements

The authors wish to honour the memory of their teacher, Professor P.Ya. Golodriga, former director of the Institute for Vine and Wine ‘Magarach’, and also wish to thank Dr. O. Adibekov for elaborating the mathematical design of their experiments.

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Résumé

OPTIMISATION DE LA COMPOSITION DU MILIEU NUTRITIF À L'AIDE DU DESIGN MATHÉMATIQUE DE L'EXPÉRIMENTATION SUR LE DÉVELOPPEMENT DES APEX DES POUSSES DE QUATRE GÉNOTYPES DE VIGNE

A l'aide de la méthode du design mathématique de l'expérimentation, nous avons procédé au choix des milieux nutritifs optimaux pour le développement des pousses de quatre génotypes de vigne dans la culture *in vitro* à partir des apex des pousses ayant la dimension de 0.5–0.8 mm, et qui se composaient de méristèmes avec des rudiments de feuilles. L'équation de regression, décrivant la dépendance du développement des apex des pousses d'un génotype – porte-greffe 'Kober 5 BB' – des concentrations des substances dans le milieu, reflétait le processus de façon plus adéquate (coefficient de détermination 0.977) par rapport à l'équation générale de regression pour les quatre génotypes (coefficient de détermination 0.694), puisque les concentration différentes des substances dans les milieux étaient optimales pour les génotypes différents. Vu le caractère très hétérozygote des génotypes de la vigne, suivant la concentration des substances dans le milieux le développement des apex des pousses de chacun d'eux peut être décrit par une équation de regression propre à chaque génotype. Dans les limites des concentrations des substances étudiées, les macroéléments (surtout le $CaCl_2$) exerçaient, par rapport à d'autres composants des milieux, l'influence la plus importante sur le développement des apex des pousses. Les milieux optimaux pour les génotypes différents se distinguaient par les concentrations des macroéléments, indépendamment de leur origine (*Vitis vinifera* ou hybrides interspécifiques). La méthode proposée de l'évaluation des résultats par la compilation d'un grand nombre de paramètres caractérisant un processus en un seul paramètre, ainsi que la méthode de la planification mathématique de l'expérimentation, peuvent être utilisées au cours des recherches dans les domaines de physiologie et d'agriculture pour optimiser les processus qui dépendent de plusieurs facteurs. Les milieux optimisés, servant pour le développement des apex des pousses, peuvent être employés pour la culture des pousses sans virus dans des cultures de méristèmes de différents génotypes de vigne.

Mots clés**:** CULTURE DU MÉRISTÈME, CONSERVATION, ÉLÉMENT MINÉRAL, *IN VITRO*, MULTIPLICATION, *VITIS*.
09.01.2010