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Research Note

An optimized medium for clonal micropropagation of grapevine

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A b b r e v i a t i o n s : indole-3-acetic acid = IAA, β-indole-butyric acid = IBA, naphthalene-acetic acid = NAA, 6-benzyl-aminopurine = BA, MURASHIGE & SKOOG (1962) = MS.

Full-strength or diluted medium of MS is used for micropropagation of different species though originally it was developed for tobacco callus culture. Using a mathematical experimental design, we conducted research with an intention to develop an adequate medium for clonal micropropagation of grapevine. Proliferation of axillary buds in liquid medium containing BA, rooting of shoots and obtention of cuttings from *in vitro*-grown plants were carried out according to the methods of HARRIS and STEVENSON (1982). The experiments were based on 4 grape genotypes: the rootstock Kober 5 BB and 3 cvs released by the Institute for Vine and Wine "Magarach": Podarok Magaracha (interspecific cross), Zhemchug Magaracha and Sverkhrannii bessemyannyi Magaracha (intraspecific crosses of *V. vinifera* genotypes).

To develop optimum media, we used homogeneous explants: nodes with removed leaves for proliferation of axillary buds and one-node-cuttings, *in vitro*-grown plants for the development of plants. The mathematical design of our experiment was based on the method of random balance (HARTMANN *et al.* 1977). First of all, we determined factors influencing the process and the degree of influence of these factors and their interrelationships. Then we conducted full polyfactorial experiments involving the factors influencing the process. Algorithms of multiple curvilinear step-by-step regression were applied and steps towards the optimization of the process were calculated using the steep ascent method of Box-Wilson modified for the case of nonlinear models. The amount of replications in the variants of the experiment was 10 and the level of significance was 0.05.

In our experiments we applied full-strength, 1/2 and 1/4 concentrations of each of macro-elements, ferrum chelate and micro-elements of the basic MS medium as well as myo-inositol, thiamine, pyroxidine, nicotinic acid, sodium hummate and sucrose in different combinations using the matrix of random numbers.

We conducted a full polyfactorial experiment (3 factors x 3 concentrations) to determine the affect of vitamins (thiamine, pyridoxine and nicotinic acid) on proliferation of axillary buds in liquid medium. The vitamins tested were applied at 0, 0.5 and 5.0 mg/l. The medium utilized was essentially MS supplemented with 0.5 mg/l BA. Based on a model system with homogeneous initial explants (single bud cuttings without leaves obtained from *in vitro*-grown plants), we found out that it was necessary to supplement the medium with 5 mg/l nicotinic acid and 5.5 mg/l pyridoxine to achieve the highest proliferation of axillary buds in the 4 genotypes tested. For Rannii Magaracha cultivar, optimum concentrations were 20 mg/l nicotinic acid and 30 mg/l pyridoxine. Good elongation of shoots in the genotypes tested was promoted by 5.5 mg/l thiamine and 5.5 mg/l pyridoxine.

We also studied the effects of $MgSO_4 \cdot 7\ H_2O$ (x_1) and $CaCl_2$ (x_2) on the proliferation of axillary buds in the genotypes tested at the 0.05 level of significance. The regression equation to be applied in this case is as follows: $y = 3.8 + 11.5\ x_1 + 7.9\ x_2$ and the determination coefficient is 0.9. Tab. 1 indicates an experimental design towards the optimization of the process and the results obtained. The optimum composition of medium 2 is as follows: 370 mg/l $MgSO_4 \cdot 7\ H_2O$ and 650 mg/l $CaCl_2$. Medium 1 contains mineral elements of MS supplemented with 170 mg/l $NaH_2PO_4 \cdot H_2O$. The composition of liquid medium we developed for the proliferation of axillary buds differs from MS in its contents (mg/l) of $CaCl_2$ (650), $NaH_2PO_4 \cdot H_2O$ (170), pyridoxine (5.5), nicotinic acid (5), para-amino benzoic acid (5), and BA (0.5). The results we obtained are due to the fact that the cytokinin effect is dependent on the Ca^{2+} supply of plants (ELLIOT 1983). TAKANO *et al.* (1988) reported on the effect of K^+ and Ca^{2+} on the proliferation of lettuce shoots.

T a b l e 1

Experimental design for the development of an optimum medium for the proliferation of axillary buds (47 d of culture); SL = shoot length (cm)

Nutrients (mg/l)		Kober 5BB		Podarok Magaracha		Zhemchug Magaracha		Sverkhrannii bessem. Mag.	
$MgSO_4 \cdot 7\ H_2O$	$CaCl_2$	SL	number of buds	SL	number of buds	SL	number of buds	SL	number of buds
370	331	1.6	8 ± 4	1.0	8 ± 6	1.8	5 ± 2	2.4	6 ± 4
370	650	3.4	16 ± 4	2.7	19 ± 2	2.8	12 ± 2	3.6	13 ± 3
566	910	4.4	12 ± 2	1.3	7 ± 4	1.2	9 ± 1	1.7	5 ± 1
652	1017	5.0	14 ± 5	1.2	7 ± 4	1.8	6 ± 4	2.8	6 ± 3

The genotypes tested differed in their responses to an increase in concentrations of $CaCl_2$ and $MgSO_4$ (Tab. 1). Kober 5BB displayed an increase in shoot length, number of buds practically unchanged on medium 4 compared to other genotypes tested.

After a long-term culture of shoot aggregations developed from axillary buds on the medium used for their proliferation except for thiamine (5.5 mg/l) used instead of nicotinic acid, shoots were formed which were transferred to an agar-solidified medium of the same composition containing 0.5-0.2 mg/l BA. Elongated shoots were cut into nodes with one leaf and established for rooting in a solid or liquid medium (on bridges of filtering paper in glass tubes 22 mm in diameter and 200 mm long which contained 7 ml of liquid medium).

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Shoot length, leaf quality, number, length and quality of roots served as criteria for optimized development of plants *in vitro*. Since increased concentrations of certain substances are beneficial for intense shoot development yet inhibit root development and *vice versa* (GALZY 1969), we used the total quality criterion which allowed to take into account all characters of the plant. Each character (x_i) varies to a larger or smaller extent depending on the medium composition. That is why to determine the total quality criterion, it is necessary to reduce variation of characters to certain limits (z_i), depending on their importance for the development and adaptability of plants. The total quality criterion (y) and variation of characters (z_i) were calculated according to the following equations:

$$a = \frac{x_{max} - M \cdot x_{min}}{M - 1} \qquad z_{1,2,3,\ldots,n} = \frac{x_i - a}{x_{max} - a}$$

$$y = z_1 \cdot z_2 \cdot z_3 \ldots z_n;$$

where x_{max}, x_{min} are extreme values of a character, x_i - value being estimated of a character, M is a random number depending on the usefulness of a character, e.g. if M=10, z_i varies over the range of 0.1 - 1; if M=2, z_i varies over the range of 0.5 - 1.

We studied the effect of the interaction between $MgSO_4 \cdot 7\ H_2O$ (x_1) and KH_2PO_4 (x_2) on total quality criterion of the development of the 4 genotypes tested at the 0.05 level of significance and the following regression equation was established: $y = 1.81 - 0.28\ x_1 \cdot x_2$, the determination coefficient is 0.54. The lower determination coefficient of the total quality criterion is due to the fact that, for each genotype, optimum concentrations of $MgSO_4$ and KH_2PO_4 exist. Within the genus *Vitis*, there are genetic differences of plants in the ability of uptake K^+, Ca^{2+} and Mg^{2+} (SCIENZA *et al.* 1986).

The medium we developed contains 308 mg/l NH_4NO_3, 922 mg/l KNO_3, 597 mg/l $MgSO_4 \cdot 7\ H_2O$, 122 mg/l KH_2PO_4, 331 mg/l $CaCl_2$, half strength 1/2 MS ferrum chelate, half-strength 1/2 MS microelements, 20 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.2 mg/l pyridoxine, 0.1 mg/l thiamine, 0.1 mg/l IAA, 10.0 g/l sucrose, 7.0 g/l agar for the solid medium. After 6 weeks of culture on that medium, a better development of the 4 genotypes was observed compared to the control (half-strength MS mineral substances for the solid medium and 1/4 MS mineral elements for the liquid medium (Tab. 2)).

The most reliable differences in shoot and root length were observed on the control medium and on those we developed in cvs Podarok Magaracha and Zhemchug Magaracha. As far as amount of roots is concerned, the genotypes showed slight differences depending on the medium composition. A better development of plants was observed in the liquid media compared to the solid ones.

NOVAK and JUVOVA (1983) reported, that the effect of auxins on the rooting of shoots depended on the mineral composition of nutrient media. With the half-strength MS, 0.1 mg/l IBA was the optimum concentration and with the full-strength MS NAA provided a better effect compared to IBA. With media containing mineral components which do not benefit rooting, stronger auxins or higher concentration of the above growth regulators should be applied. We adjusted optimum concentrations of medium components for 0.1 mg/l IAA. Prior to the transfer of plants to a greenhouse, they had been grown on medium containing 1 mg/l ferulic acid without IAA.

Table 2

Development of four grape genotypes on the control and proposed (Prop.) solid (S) and liquid (L) media

Genotype	Medium composition	Shoot length (cm)	Number of roots	Root length (cm)
Kober 5BB	1/2 MS - S	3.2 ± 1.1	3.3 ± 1.0	3.8 ± 1.7
	Prop. - S	4.4 ± 0.5	3.8 ± 1.0	4.6 ± 0.5
	1/4 MS - L	6.3 ± 0.8	1.9 ± 0.2	4.2 ± 1.2
	Prop. - L	8.5 ± 1.6	2.1 ± 0.6	7.6 ± 2.0
Podarok Magaracha	1/2 MS - S	2.7 ± 1.2	3.1 ± 1.0	3.7 ± 1.6
	Prop. - S	4.4 ± 0.4	3.2 ± 0.5	7.4 ± 0.7
	1/4 MS - L	5.2 ± 1.2	2.9 ± 0.8	6.7 ± 1.7
	Prop. - L	9.6 ± 0.7	2.1 ± 0.3	11.1 ± 1.7
Zhemchug Magaracha	1/2 MS - S	3.2 ± 0.6	8.2 ± 2.2	2.7 ± 1.1
	Prop. - S	4.5 ± 0.6	7.0 ± 1.3	5.8 ± 0.9
	1/4 MS - L	4.8 ± 0.7	4.5 ± 0.7	4.1 ± 0.6
	Prop. - L	8.0 ± 0.6	3.3 ± 0.5	7.1 ± 0.9
Sverkhrannii bessemyannyi Magaracha	1/2 MS - S	5.1 ± 0.9	5.1 ± 1.3	4.8 ± 1.0
	Prop. - S	6.3 ± 1.1	4.7 ± 1.1	6.2 ± 1.0
	1/4 MS - L	7.0 ± 1.6	3.8 ± 0.3	3.9 ± 1.1
	Prop. - L	10.1 ± 1.5	3.1 ± 0.3	5.6 ± 1.4

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