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In vitro propagation of *Vitis:* The effects of organic substances on shoot multiplication

by

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Multiplication végétative de la vigne *in vitro:* Effets de substances organiques sur la multiplication de pousses herbacées

R é s u m é : Les effets de substances organiques sur la multiplication de pousses herbacées ont été étudiés avec des boutures terminales de l'hybride *Vitis* Remaily Seedless, obtenues *in vitro* et repiquées. Les sels minéraux utilisés étaient ceux de MURASHIGE et SKOOG (1962).

La cytokinine, benzylaminopurine (BAP), a une concentration optimale pour la multiplication de 5 µM. 60 % des pousses ont alors au moins 3 noeuds (1,5 cm de long), une taille considérée adéquate pour leur micropropagation.

L'addition du sulfate d'adénine en concentrations de 2,5 à 20 × 10^{-4} M déprime la multiplication des pousses, quand BAP est employée à sa concentration optimale.

La thiamine (2 et 4 µM) et l'inositol (50, 100 et 500 µM) ont été testés ensemble. Le nombre de pousses produites décroît quand la concentration de l'inositol est augmentée, la production étant maximum avec 4 µM de thiamine. Le meilleur taux de multiplication a été obtenue avec 4 µM de thiamine et 50 µM d'inositol. Le nombre de pousses, par pousse repiquée, est alors semblable à celui obtenu avec notre milieu nutritif standard qui contient en plus de l'acide nicotinique et de la pyridoxine. Cependant, le nombre de pousses ayant au moins 3 noeuds est double sur ce dernier.

Les acides aminés, aspartate, asparagine, glutamate, glutamine et tyrosine en concentrations de 2,5 à 40 × 10^{-4} M n'ont pas affecté la multiplication.

Dans les conditions d'incubation utilisées, une combinaison adéquate de constituants organiques du milieu de culture pour la multiplication de pousses herbacées de la vigne a été définie. Elle contient 3 µM de thiamine · HCl, 55,5 µM de myo-inositol, 8 µM d'acide nicotinique, 5 µM de pyridoxine · HCl et 5 µM de benzylaminopurine. Il est possible que l'addition d'aspartate à 4 µM puisse être bénéfique. Nous avons trouvé que définir des concentrations optimales pour les vitamines est important pour la multiplication des pousses. La cytokinine a été effective dans une marge très étroite. Cela peut signifier que sa concentration optimale varie selon le génotype.

K e y w o r d s : tissue culture, cytokinin, vitamin, amino acid.

Introduction

A potentially important use for tissue culture is rapid vegetative micropropagation. The feasibility of grapevine micropropagation was demonstrated in previous work where grapevines were obtained from 0.5—1 mm apices (apical dome plus 2—4 leaf primordia) (CHÉE and POOL 1982, 1983). We recognize four principal *in vitro* steps in micropropagation of grapevines: 1) establishment of the material in culture; 2) first shoot production; 3) shoot multiplication from subcultured shoots; and 4) rooting of the shoots produced. For rapid micropropagation, the shoot multiplication phase is most important. The goals are attainment of a high number of shoots of adequate size (3—4 nodes, 1.5 cm) and quality in a short time.

The organic constituents of the culture medium can be manipulated in order to reach these goals. Budburst and shoot elongation of unrooted cuttings of *Vitis labrus-*

cana BAILEY Concord cultured *in vitro* increased in response to benzylaminopurine (BAP) (POOL *et al.* 1974). BAP and naphthaleneacetic acid (NAA) at 10^{-7}—10^{-4} M were used in combination to establish 0.5—1 mm apices of the *Vitis* hybrid Rougeon in culture. Then BAP at 5 µM and NAA at 0.5 µM were used to produce shoots from the rosetted apices (CHÉE 1980). Further shoot multiplication was from subcultured shoots using BAP (10^{-7}—10^{-5} M) combined with NAA (0—5×10^{-7} M) (CHÉE and POOL 1982). Cultures established from axillary buds of *V. vinifera* 'Sylvaner Riesling' had greater shoot proliferation with 2×10^{-5} M than 10^{-5} M BAP but shoot elongation was slightly inhibited at the higher cytokinin concentration (JONA and WEBB 1978). ALDWINCKLE and BUTURAC (1980) used the same method with 12 *Vitis* genotypes to establish dual cultures of *Vitis* and obligate pathogens. Proliferation of adventitious buds from fragmented shoot apices of *V. vinifera* Cabernet Sauvignon and Sultana was optimal with 10 µM BAP (BARLASS and SKENE 1980). GOUSSARD (1981) has reported on the effects of BAP and zeatin riboside concentrations on shoot production while establishing 0.75—1 mm shoot apices of *V. vinifera* Chenin blanc in culture.

Adenine and various amino acids were reported to stimulate shoot production (BUTENKO 1968; MURASHIGE 1974). The effect of adenine may have been due to cytokinin properties. KLIEWER (1967) and ERIS (1980) studied the amino acid content of grapevines. Their research shows that the main free amino acids are: aspartate, glutamine, arginine, aminobutyrate, asparagine, glutamate, proline, alanine, leucine and serine. The amounts and ratios varied during the season. Arginine, glutamine and aspartate were most prominent during the March growth flush (ERIS 1980). Arginine was the main storage form of nitrogen (KLIEWER 1967). Amino acids are a readily available source of nitrogen and could improve growth and development and therefore shoot yield in micropropagation.

For our previous work we included thiamine, myo-inositol, nicotinic acid and pyridoxine in the media (CHÉE and POOL 1982). Thiamine has been reported essential and inositol clearly beneficial in nutrient media for growth of tissues in culture, but the need for other vitamins is less certain (GAUTHERET 1959; BUTENKO 1968; MURASHIGE 1974).

Most media used in tissue culture were developed to ensure adequate callus growth of a specific species. The same media were then used for other purposes or applied to other species with no changes made except for modifications in the growth regulators. No medium has been designed specifically with micropropagation of grapevines in mind.

Here we report on the effects of several organic substances on shoot multiplication from subcultured shoots of Remaily Seedless, a new grape variety released by the New York Agricultural Experiment Station.

Materials and methods

Plant material

The *Vitis* hybrid Remaily Seedless was established in culture and shoots were produced and continuously multiplied as described elsewhere (CHÉE 1982). This supply of shoots provided the explants used in the experiments. The explants were 3—4 node shoots (1.5 cm) cut 1—2 mm below the oldest node. They were placed horizontally and slightly pressed into the medium with the cut end submerged. The medium (30 ml) was contained in presterilized 25 × 100 mm plastic petri dishes.

Media

Culture media contained the inorganic nutrients of MURASHIGE and SKOOG (1962), 3 % sucrose, and 0.8 % 'Bacto-Agar' (DIFCO Laboratories). Before autoclaving, pH was ajusted to 5.7—5.8. Organic substances varied with the experiment and are given in Table 1. In the experiment testing the effects of thiamine and inositol, the control contains our standard formula of 3 μM thiamine · HCl, 55.5 μM myo-inositol, 8 μM nicotinic acid and 5 μM pyridoxine · HCl.

Incubation conditions

Illumination was by a 1 : 1 mixture of 'Gro-Lux' (F40GRO) and 'Cool White' (F40CW) fluorescent tubes (Lifeline series, manufactured by Sylvania). The tubes were 14 cm apart and 30 cm above the cultures. The total energy measured at the explant level was 1900 μW cm^{-2}. Daylength was 10 h. Temperature varied from 21 °C in dark to 27 °C in light.

Experimental design

The experimental designs were completely randomized with subsampling. Each treatment consisted of 6 plates containing 3 explants each. The treatment designs were

Table 1

Molar concentrations of the organic substances used in the experiments

Concentrations des substances organiques utilisées dans les experiences

Organic substance	Experiment			
	Effects of benzylamino-purine	Effects of thiamine and inositol	Effects of adenine sulfate	Effects of amino acids
Benzylaminopurine (× 10^{-6} M)	0 0.1 1 2.5 5 10 20 40 80	5	5	5
Adenine sulfate (× 10^{-4} M)	0	0	0 2.5 5 20	0
Thiamine · HCl (× 10^{-6} M)	3	2 4	3	3
myo-Inositol (× 10^{-6} M)	55.5	50 100 500	55.5	55.5
Nicotinic acid (× 10^{-6} M)	8	0	8	8
Pyridoxine · HCl (× 10^{-6} M)	5	0	5	5
Arginine (× 10^{-4} M)	0	0	0	0 2.5 5 10 20
Asparagine (× 10^{-4} M)	0	0	0	0 2.5 5 10 20 40
Aspartate (× 10^{-4} M)	0	0	0	0 2.5 5 10 20 40
Glutamine (× 10^{-4} M)	0	0	0	0 2.5 5 10 20
Glutamate (× 10^{-4} M)	0	0	0	0 2.5 5 10 20
Tyrosine (× 10^{-4} M)	0	0	0	0 2.5 5

Table 2

Analysis of variance · Effects of benzylaminopurine concentration on shoot production from *Vitis* Remaily Seedless shoot explants of 3—4 nodes (1.5 cm) subcultured *in vitro* for 6—7 weeks

Analyse des variances · Effets des concentrations de benzylaminopurine sur la production de pousses herbacées, à partir de boutures apicales de *Vitis* Remaily Seedless ayant 3—4 noeuds (1,5 cm) et repiquées *in vitro* · Résultats après 6—7 semaines

		Shoots/explant			% Shoots of ≧ 3 nodes			Nodes/shoot			Total nodes/explant		
Source of variation	df[1])	SS	% SS	F	SS	% SS	F	SS	% SS	F	SS	% SS	F
Among plates	51	8 822	80		106 517	67		439	79		1 001 534	79	
Among treatments	8	6 909	63	**	75 122	47	**	340	61	**	77 917	61	**
Linear	1	626	6	**	14 294	9	**	91	16	**	10 756	9	**
Quadratic	1	133	1	NS	1 918	1	NS	12	2	*	14	0	NS
Cubic	1	959	9	**	8 000	5	**	24	4	**	7 213	6	**
Deviations	5	5 191	47	**	50 909	32	**	214	38	**	59 934	47	**
Among plates within treatments	43 (2)	1 913	17		31 395	20		99	18		22 617	18	
Among explants within plates	103 (5)	2 347	21		54 610	34		129	23		28 131	22	
Among explants	154	11 167	102		161 127	101		568	102		128 664	101	

[1]) Missing values in parentheses.
* = Significant at P = 0.05, ** = significant at P = 0.01, NS = not significant.

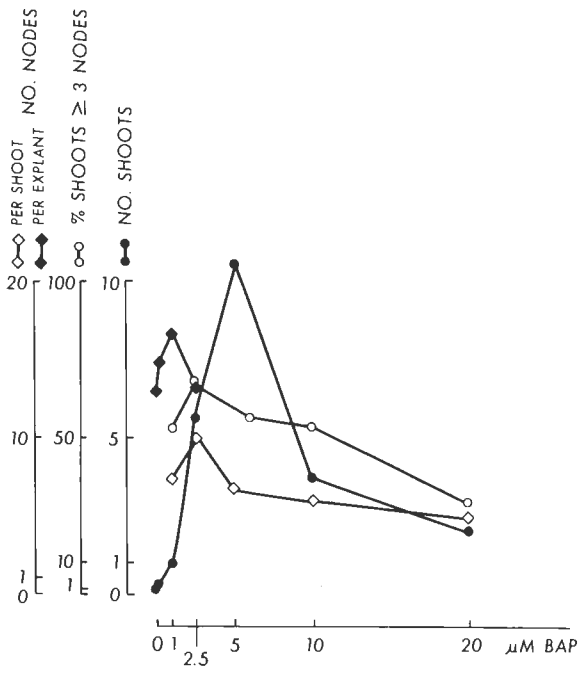
structured, our interest being to study the trends resulting from increasing concentrations of the organic substance under study. When investigating the combined action of thiamine · HCl and myo-inositol, a 2 × 3 factorial design was used. Statistical analysis was by components of variance method. Treatment means were compared using Duncan's Multiple Range Test (DMRT) when appropriate with a 0.05 level of significance.

The variates recorded after 6—7 weeks in culture were: number of shoots produced per explant (SH/EXP) and number of nodes on these shoots. From these observations we calculated per explant: number of shoots of at least 3 nodes and their percentage (%SH3/EXP), average number of nodes on the shoots produced (NOD/SH), and total number of nodes as a measure of vegetative vigor (TNOD/EXP). The number of expanded internodes (visibly elongated) on the explant itself was also recorded in the experiment on the effects of benzylaminopurine.

Results

Effects of benzylaminopurine (BAP) concentration on shoot multiplication and explant growth

For each variate the contribution of the cubic trend to the total sum of squares was significant (Table 2). The effects of BAP are presented in the figure.



Effects of benzylaminopurine (BAP) concentration on 3—4 node (1,5 cm) *Vitis* Remaily Seedless shoots (explants), subcultured *in vitro* for 6—7 weeks.

Effets des concentrations de benzylaminopurine (BAP) sur des pousses terminales de 3—4 noeuds (1,5 cm) de *Vitis* Remaily Seedless (explants), repiquées *in vitro* pour 6—7 semaines.

Analysis of variance showed significant deviations from the cubic trends. This indicates that a cubic curve does not best fit the data. However, for our purpose a cubic trend adequately explains the data. For instance when the cubic curve was fitted for SH/EXP on the domain BAP = 0—20 μM, the $r^2 = 0.90$ indicates a good fit. Therefore, the deviation from the cubic trend lies outside of the domain (for 40 and 80 μM). Similar curves could be fitted to the other variates.

Shoot production occurred from 1 to 40 μM BAP. Shoot proliferation increased with increased BAP level reaching a maximum at 5 μM. Higher concentrations were detrimental to shoot multiplication. Concentrations of 40 and 80 μM produced undifferentiated growth and curled leaves. The size of the shoots declined with increasing BAP levels as did the percentage of shoots of at least 3 nodes.

Shoots first developed from most axillary buds of the explant, and then from the basal axillary buds of the new shoots. This basitonic growth resulted in dense clumps of shoots. After 6 weeks in culture the bases of all the shoots were fused and had lost identity in the tissue of the original explant. The explants were identified with difficulty as a deformed stem of hard tissue. Callus, in contact with the medium, and root growth occurred when the shoots were numerous. Usually the shoots developed in the axil of a leaf or leaflike structure. However, as the cluster of shoots grew denser this was difficult to observe and formation of adventitious shoots cannot be ruled out.

The effect of BAP on the number of internodes expanded on the explant was also analyzed. Analysis of variance demonstrated a significant contribution to the total sum of squares for the quadratic trend. The effect of BAP on explants is shown in the figure. When BAP concentration was increased from 0 to 2.5 μM the number of expanded internodes increased, reached a maximum at 1 μM and decreased. At 1 μM BAP all the internodes present at excision (3—4 visible nodes plus 2—4 in the apical bud) had expanded indicating that there was no *de novo* node production. Without BAP the explants discolored over time and became brown after 6 weeks. With BAP the explants remained green and fresh looking.

In conclusion, varying BAP from 0 to 80 μM defined optimal concentrations for shoot multiplication (5 μM), for production of larger shoots (2.5 μM) and for a maximum of expanded internodes on the explant (1 μM).

Table 3

Effects of adenine sulfate concentration on shoot production from *Vitis* Remaily Seedless shoot explants of 3—4 nodes (1.5 cm) subcultured *in vitro* for 6—7 weeks

Effets des concentrations de sulfate d'adénine sur la production de pousses herbacées, à partir de boutures apicales de *Vitis* Remaily Seedless ayant 3—4 noeuds (1,5 cm) et repiquées *in vitro* · Résultats après 6—7 semaines

Adenine sulfate ($\times 10^{-4}$ M)	Shoots/ explant	% Shoots of ≥ 3 nodes	Nodes/ shoot	Total nodes/ explant
0	21.2 a	58 a	3.41 ab	71 a
2.5	4.7 b	64 a	3.66 a	16 b
5	4.4 b	57 a	2.91 b	16 b
20	0.0 b	0 b	0.00 c	0 b
Trend	Quadratic	Quadratic	Linear	Quadratic
Coeff. of variation	0.61	0.22	0.19	0.56

Mean separation within columns by Duncan's Multiple Range Test ($p = 0.05$).

Table 4

Analysis of variance · Effects of thiamine and myo-inositol concentrations on shoot production from *Vitis* Remaily Seedless shoot explants of 3—4 nodes (1.5 cm) subcultured *in vitro* for 6—7 weeks

Analyse des variances · Effets des concentrations de thiamine et myo-inositol sur la production de pousses herbacées, à partir de boutures apicales de *Vitis* Remaily Seedless ayant 3—4 noeuds (1,5 cm) et repiquées *in vitro* · Résultats après 6—7 semaines

		Shoots/explant			% Shoots of ≧ 3 nodes			Nodes/shoot			Total nodes/explant		
Source of variation	df[1])	SS	% SS	F	SS	% SS	F	SS	% SS	F	SS	% SS	F
Thiamine	1	1 779	9	*	968	2	NS	10	6	NS	5 602	8	NS
Inositol	2	3 664	18	*	1 031	2	NS	2	1	NS	11 566	16	*
Thiamine × inositol	1	113	1	NS	123	0	NS	2.4	1	NS	171	0	NS
Among plates within treatments	24 (1)	9 264	49		25 493	47		79.2	46		33 809	49	
Among explants within plates	52 (8)	5 470	29		26 454	49		82	47		21 143	31	
Among explants	80	20 070	106		54 298	101		176	101		71 687	104	

[1]) Missing values in parentheses.
* = Significant at P = 0.05, NS = Not significant.

Effects of adenine sulfate concentration on shoot multiplication

The analyses of variance for the variates SH/EXP, %SH3/EXP, NOD/SH and TNOD/EXP showed that the factor adenine sulfate significantly contributed to the total sum of squares for all variates. Quadratic trends were significant for SH/EXP, %SH3/EXP and TNOD/EXP (Table 3). For the variate NOD/SH the trend was linear.

Adenine sulfate reduced SH/EXP. %SH3/EXP was unchanged by the addition of adenine sulfate except for the treatment where no shoots were produced. NOD/SH was reduced by the higher concentrations. TNOD/EXP was reduced when adenine sulfate was included in the medium.

Effects of thiamine and myo-inositol concentrations on shoot multiplication

The analyses of variance for the effects on the variates are summarized in Table 4. For SH/EXP the contributions of thiamine and inositol to the total sum of squares were significant. For TNOD/EXP only the effect of inositol was significant. There were no effects of thiamine and inositol on %SH3/EXP and NOD/SH. The interaction thiamine-inositol was not significant for any of the variates.

The means for the treatments are given in Table 5. SH/EXP increased with higher thiamine levels and decreased with higher inositol levels. TNOD/EXP decreased with increasing inositol concentration as a result of diminishing shoot multiplication with no change in their size.

The different treatments were compared to our standard vitamins and inositol formula (control) which contains thiamine · HCl and myo-inositol but also nicotinic acid and pyridoxine · HCl (concentrations under Materials and methods). To obtain the correct standard error a new analysis of variance was performed for each of the variates. The number of treatments being those of the 2 × 3 factorial plus the standard. The means were compared with a DMRT in Table 5.

Table 5

Effects of thiamine and myo-inositol concentrations on shoot production from *Vitis* Remaily Seedless shoot explants of 3—4 nodes (1.5 cm) subcultured *in vitro* for 6—7 weeks

Effets des concentrations de thiamine et myo-inositol sur la production de pousses herbacées, à partir de boutures apicales de *Vitis* Remaily Seedless ayant 3—4 noeuds (1,5 cm) et repiquées *in vitro* · Résultats après 6—7 semaines

Myo-inositol (µM)	Shoots/explant Thiamine (µM)		% Shoots of ≥ 3 nodes	Nodes/ shoot	Total nodes/ explant
	2	4			
50	14 ab	27 a	23 b	1.7 b	42 b
100	5 b	13 ab	31 b	2.0 b	20 c
500	NA	5 b	28 b	1.9 b	13 c
Average effects	—	—	28	1.9	—
Control	23 a		56 a	3.5 a	64 a

Separation of means within variates by Duncan's Multiple Range Test (P = 0.05).
Control contains: 3 µM thiamine · HCl, 55.5 µM myo-inositol, 8 µM nicotinic acid, 5 µM pyridoxine · HCl.

Table 6

Effects of amino acids on shoot production from *Vitis* Remaily Seedless shoot explants of 3—4 nodes (1.5 cm) subcultured *in vitro* for 6—7 weeks

Effets de quelques acides aminés sur la production de pousses herbacées, à partir de boutures apicales de *Vitis* Remaily Seedless ayant 3—4 noeuds (1,5 cm) et repiquées *in vitro* · Résultats après 6—7 semaines

Substance	Concentrations ($\times 10^{-4}$ M) and observations	Shoots/ explant	% Shoots of ≧ 3 nodes	Nodes/ shoot	Nodes/ explant
Arginine	Average	20	52	3.0	58
	Coeff. of variation	0.60	0.22	0.15	0.52
Aspartate	0	21	58	3.4	71
	2.5	23	47	2.7	57
	5	25	45	2.8	64
	10	30	49	3.0	73
	20	31	42	2.6	67
	40	34	45	2.8	85
	Trend	NS	NS	NS	NS
	Coeff. of variation	0.65	0.27	0.24	0.46
Asparagine	Average	22	51	3.1	59
	Coeff. of variation	0.47	0.27	0.23	0.40
Glutamate	0	21	58	3.4	71 a
	2.5	14	48	2.7	37 b
	5	16	51	3.0	44 b
	10	13	49	2.7	38 b
	20	11	60	3.4	35 b
	Trend	Linear	Quadratic	Quadratic	Cubic
	Coeff. of variation	0.38	0.16	0.16	0.36
Glutamine	0	21 a	58	3.4	71 a
	2.5	10 b	48	2.7	27 b
	5	8 b	60	3.5	26 b
	10	10 b	53	3.3	31 b
	20	4 b	64	3.3	17 b
	Trend	Cubic	NS	NS	Cubic
	Coeff. of variation	0.51	0.21	0.20	0.43
Tyrosine	0	21 a	58 a	3.4 a	71 a
	2.5	13 a	44 b	2.7 b	31 b
	5	15 a	45 b	2.7 b	38 b
	Coeff. of variation	0.47	0.22	0.17	0.42

Mean separation within columns and substances by Duncan's Multiple Range Test (P = 0.05).

To conclude, the greatest shoot production was on the medium combining 4 µM thiamine and 50 µM inositol and on the control. All others produced significantly fewer shoots. However, the control produced double the percentage of shoots of adequate size for micropropagation (3 or more nodes).

Effects of amino acid concentration on shoot multiplication

Arginine. — The analysis of variance showed no difference among the treatments and no significant trend with increasing arginine concentration. Therefore, the average values for the variates were reported in Table 6. The addition of arginine had no effect on shoot multiplication.

Aspartate. — No significant differences among treatments and no significant trend with increasing aspartate concentration were shown. However, an examination of the data indicated a steady increase in SH/EXP with increasing aspartate concentration coupled with a high coefficient of variation (Table 6). Because of the large standard deviation the possible trend was not detected. To conclude, there was no statistically significant effect of aspartate on shoot multiplication.

Asparagine. — There were no significant differences among treatments or significant trends with increasing concentration of asparagine. The average values for each variate are given in Table 6. Asparagine had no effect on shoot multiplication.

Glutamate. — A significant linear trend for SH/EXP, significant quadratic trends for %SH3/EXP and NOD/SH, and significant differences for TNOD/EXP were shown.

The data in Table 6 reflect those trends. SH/EXP was decreased by adding glutamate. %SH3/EXP decreased with increased glutamate concentration then increased to the level of the control medium. A similar quadratic trend reflected the effects of glutamate on NOD/SH. TNOD/EXP was lower in the treatments with glutamate in consequence of the decrease in shoot production.

Glutamine. — There were significant differences among treatments for the variates SH/EXP and TNOD/EXP and no significant differences for %SH3/EXP and NOD/SH.

The means are reported in Table 6. Comparisons of the means using DMRT show that less shoots were produced per explant on media containing glutamine; glutamine addition depressed shoot multiplication.

Tyrosine. — The means of the treatments were compared for each variate with a DMRT in Table 6. There was no effect of tyrosine on SH/EXP or %SH3/EXP, however NOD/SH and consequently TNOD/EXP were reduced. Adding tyrosine to the medium did not improve shoot multiplication.

Discussion

Effects of benzylaminopurine (BAP)

The effect of BAP in the lower range was more directly on the quality and growth of the explant. The effect of cytokinins on retarding senescence observed is well documented (Osborn 1962). That there was no *de novo* node production confirms our findings reported elsewhere (Chée 1982). The number of expanded internodes decreased when BAP was increased above 1 µM and active shoot proliferation occurred. Internode expansion was a consequence of general growth stimulus by the cytokinin and a correct phytohormone balance rather than a direct effect of BAP concentration as for shoot multiplication.

The effect of BAP in shoot multiplication was on the number of shoots produced per explant and on their size measured in number of nodes. The narrow peak for maximum shoot production might indicate that BAP will need to be adjusted for other cultivars or incubation conditions. In regard to micropropagation, 5 μM BAP was the optimum concentration with 66 % of the 21 shoots produced having at least 3 nodes, a size considered adequate for rooting or repeated subculturing.

The number of shoots produced was not strictly related to the number of axillary buds of the explant that could be induced to grow. Shoots first developed from axillary buds and then from the basal buds of the new shoots. Shoot proliferation resulted from a continuous action of the cytokinin during culture. The basitonic growth would indicate that the effect of cytokinin was more directly on the buds in contact with the media.

Effects of adenine sulfate

The addition of adenine to a medium containing an optimum concentration of BAP depressed shoot production. A similar decrease in shoot production was observed on media without adenine when increasing the BAP concentration above the optimal level.

From our knowledge about the biosynthetic pathway of the purine bases (BONNER and VARNER 1976; LEHNINGER 1976), it appears that plants do not produce free adenine. Instead, the purine ring is built step by step on activated ribose-5-phosphate. Therefore, the addition of adenine would not facilitate production of purine nucleotides. However, free purines from the degradation of adenylic and guanylic acids may be used to produce cytokinins. The side chain essential for activity can be attached to the N_6 position of adenine using isopentenyl phosphate to produce the natural cytokinin isopentenyl adenine. Consequently, the addition of adenine to a medium with an optimum BAP concentration might result in a supraoptimal level of cytokinin in the explant and result in depressed shoot multiplication.

Effects of thiamine and inositol

All combinations of thiamine and inositol tried were adequate for explant growth and shoot production. The thiamine-inositol combination with best shoot proliferation was similar to the one of our control. Then the number of shoots produced were not found different. However, 56 % of the shoots produced on the control had at least 3 nodes (adequate for micropropagation) as opposed to only 28 % for any of the thiamine-inositol treatments. The control also contained nicotinic acid and pyridoxine.

Two important findings were made. The first and obvious is that while only thiamine and inositol were necessary for shoot production on grapevines, nicotinic acid and pyridoxine should not be omitted in a program for micropropagation. The second is that the vitamins included in a medium must be adjusted to the species and to the type of *in vitro* culture of concern and not simply taken from the literature. The vitamins used by other authors for grapevine shoot production were borrowed from media designed for other species and purposes (BARLASS and SKENE 1978; JONA and WEBB 1978; GOUSSARD 1981; RAJASEKARAN and MULLINS 1981).

Effects of amino acids

The amino acids, with the possible exception of aspartate, either had no effect or were inhibitory to shoot proliferation. The use of amino acids in the culture of plant tissue is discussed by GAUTHERET (1959). In general, they are not beneficial to tissue

proliferation when added singly but very effective when added in combination. Such amino acid mixtures were defined for different species.

In the case of aspartate, an improvement of growth could be expected. Aspartate is the precursor of asparagine which provides nitrogen to a range of pathways. Aspartate is important for nitrogen transport in woody plant and grapevines in particular (KLIEWER 1967; ERIS 1980). The assimilation of N via the glutamine synthase and glutamate synthetase pathway requires reducing energy provided by NADPH via ferredoxin of the photosynthetic apparatus in leaves. In tissue culture this energy might be limited due to low light intensities. If so, a readily available pool of aspartate that provides nitrogen in a reduced form would benefit growth.

Summary

The effects of organic substances on shoot multiplication from subcultured shoots of the *Vitis* hybrid Remaily Seedless were investigated. Culture media contained the inorganic nutrients of MURASHIGE and SKOOG (1962).

Benzylaminopurine (BAP) was most effective in promoting shoot multiplication at 5 μM. 60 % of the shoots had at least 3 nodes (1.5 cm total length), a size considered adequate for micropropagation.

The addition of adenine sulfate from 2.5 to 20 × 10^{-4} M depressed shoot multiplication when BAP was optimal.

Combinations of thiamine (2 and 4 μM) and inositol (50; 100; 500 μM) were tested. Shoot multiplication decreased with increasing inositol concentration and was higher with 4 μM of thiamine. Best multiplication was with 4 μM thiamine and 50 μM inositol and gave similar number of shoots per explant as obtained on our standard medium which contains in addition nicotinic acid and pyridoxine. However, twice as many 3-node shoots were produced on the standard medium.

The amino acids arginine, aspartate, asparagine, glutamate, glutamine and tyrosine at 2.5—40 × 10^{-4} M had no effect on shoot multiplication.

With the culture conditions used in this research, an adequate set of organic constituents for shoot multiplication of grapevines is defined as 3 μM thiamine · HCl, 55.5 μM myo-inositol, 8 μM nicotinic acid, 5 μM pyridoxine · HCl and 5 μM benzylaminopurine. The addition of 4 μM of aspartate may be beneficial. It was found that optimization of vitamin concentration is important in shoot multiplication. The restricted optimum cytokinin concentration for best shoot production suggests that its concentration may have to be adjusted for each cultivar.

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