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INDIRECT CLONAL MICROPROPAGATION OF TWO YEMENI LOCAL GRAPE CULTIVARS FROM SHOOT APICAL MERISTEMS *IN VITRO*

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ABSTRACT

In the present study, trials were done in order to micropropagate two ocal and popular Yemeni grape Cultivars (*Vitis vinifera L. cv. Asemi and cv. Razeki*) by obtaining multiple adventitious shoot bud proliferation *in vitro* from shoot apical meristems using MS basal salts culture media containing different benzyl adenine and 2,4 D concentrations. The experimental part of the study was carried out during 2002/2003 in the laboratories of the faculty of agriculture, Sana'a University. Results of the present study have revealed that multiple adventitious shoot bud formation was possible from the two selected cultivars under the effect of 1 or 2 mg/1. benzyl adenine plus 1 or 2 mg/1. of 2,4D within 4 to 6 weeks of incubation in the dark at room temperature. It was then possible to obtain regenerants from the adventitious shoot buds in culture tubes containing hormone free MS media. After acclimatization, the obtained regenerants were successfully transferred to soil. Results of analysis have shown that the obtained regenerants were identical to their corresponding controls morphologically but differed in their leaf chlorophyll contents.

INTRODUCTION

The propagation of grapevine by classical vegetative methods may be accompanied by more than 60 different types of infectious agents which in turn negatively affect both qualitative and quantitative characteristics of the yield.

Shoot tips and apical meristems of grapevine are being used now extensively for clonal micropropagation (Barlass *et.al.;* 1978), production of pathogen free plantlets (Barlass *et.al.',* 1982, Savino *et.al.;* 1990 and Goussard *et.al.;* 1991), prolonged preservation of germplasm (Ganeshan *et.al.;* 1992), transformation (Scorza *et.al.;* 1995), different genetic manipulations (Botallico *et.al.;* 1996), as well as crop improvement programs (Gray *et.al.;* 1997).

Cytokinins, alone or combined with auxins are being used for regeneration of whole plantlets from different explants of grapevine. The development of adventitious shoots from leaf explants of *Vitis vinifera* L. cvs. Cabemet, Sanvignon. French Colombard, Thompson Seedless and White reislingwas obtained by (Stamp *et.al.;* 1990 a) using either Murashige and Skoog (MS) or Nitsch and Nitsch basal salts supplemented with different benzyl adenine concentrations. The obtained shoots were easily elongated, rooted and were identical when compared to their corresponding controls. In another study ,Stamp *et.al* (1990 b) observed that shoot organogenesis from

leaf explants of *Vitis vinifera L. cv.* French Colombard and Thompson seedless was improved when explants were incubated on cytokinin containing and agar solidified MS basal salts. Dore-Swamy and Sahijram (1991) obtained fully developed plantlets from bud explants of *Vitis vinifera L.* cv. Dog Ridge using woody plant medium supplemented with cytokinins. Gray and Benton (1991) stated that multiple shoot bud proliferation was possible to obtain from *Vitis rotundifolia* shoot apical meristems under the effect of thiadiazuron (TDZ). With *vitis vinifera L. cv.* Thompson seedless, Gowda and Muralikrishna (1994) were able to obtain a profuse multiple shoot initiation from axillary bud explants using MS basal salts containing 0.1 mg/1. benzyl adenine and 0.1 mg/L. kinetin in the beginning and then kinetin alone was enough to induce the same response.

It has been repeatedly observed that the response of plants to different *in vitro* culturing techniques is largely dependant on both cultivar and genotype (Robacker 1991, Sudarsono and Goldy 1991,Xu *et al.*,1995).

Tapia and Read (1995) after their studies on the effect ofthiadiazuron on different genotypes of *Vitis vinifera L.* came to the conclusion that multiple shoot proliferation was much more pronounced when cytokinin was used in relatively higher concentrations (0.5 and 1.0 mg/L.). Further more, the same study have shown that different genotypes differ with respect to their response to cytokinin treatment. Similar results were obtained by Xu *et al.* (1995) illustrated that both embryogenesis and somatic embryo germination from different *Vitis rotundifolia* explants *in vitro* is a genotype dependant process. Even more, Meyerson *et al.* (1995) after their extensive studies on Vitis spp. came to the conclusion that significant differences exist when shoot micropropagation from nodal explants and shoot apices are compared.

The purpose of the present study is to micropropagate *Vitis vinifera L. cvs.* Razeki and Asemi which are two important local and popular Yemeni vinegrape cultivars which have been propagated by the classical vegetative propagation methods.

MATERIALS AND METHODS

Explants of *Vitis vinifera L. cvs.* Asemi and Razeki were collected from Khowlan vineyards at the southern east of Sana's city, Yemen Republic, and the experimental part of the study was carried out during 2002/2003 in the laboratories of the faculty of agriculture, Sana'a University. Shoot tips were cleaned by washing with tap water containing 2% detergent. The shoot tips were then sterilized under aseptic conditions by immersion in 1% Clorox with occasional shaking. Explants were then washed three times with sterile distilled water and dried over sterile filter paper in petri dishes. The apical meristems (2 to 4 mm.) were then separated carefully using a sterile scalpel. Explants were then divided into many groups and incubated on hormone free and hormone containing Murashige and Skoog (1962) basal salts containing 30gm sucrose and 10 gm agar per liter. Benzyl adenine and 2,4 D in different concentrations (0.1-3.0 mg/1.) were added to the culture media in order to

induce callogenesis and/or adventitious bud proliferation. Incubation was carried out at room temperature and 16 hours per day photoperiod. After adventitious shoot buds have induced they were carefully separated and allowed to grow on hormone free MS culture media. The obtained regenerants were compared to their controls with respect to their morphological characteristics, chlorophyll contents. Chlorophyll contents were determined according to the method ofVernon and Seely (1960).

RESULTS AND DISCUSSION

Descriptive analysis of the results of the present study, as illustrated in table (1), have shown that the hormonal composition of the culture media influenced the type of morphogenic response. Callus is being much faster when benzyl adenine was used in a concentration of 2 mg/1. regardless of the auxin concentration used. It was generally observed that the increase of auxin concentration resulted in direct and indirect root morphogenesis from stem apical meristems if benzyl adenine concentration in the culture media was not high (0.1 and 0.5 mg/1.). It is also possible to say that 1 or 2 mg/1. benzyl adenine plus 1 or 2 mg/1. auxin resulted in inducing callus formation and then the callus formation was followed by multiple adventitious bud formation without any need to change the hormonal composition of the culture media. No big differences were observed between the two cultivars under test. When apical meristems of both cultivars were incubated on culture media containing 3 mg/1. benzyl adenine plus 0.1 mg/1. 2, 4 D direct shoot morphogenesis was obtained from Asmi and not Razeki cultivar after 4 and 6 months of incubation.The induction of adventitious shoot buds formation that was obtained in the present study may confirm and agree with the results obtained by other authors with other Vitis species or cultivars e.g. (Stamp *et.al,* 1990 a, Dore-Swamy and Sahijram, 1991, Gray and Benton, l991, Gowda and Muralikrishna ,1994). It should be remembered that the differences in the type and concentration of the cytokinin required to induce shoot organogenesis among different species and different cultivars of the same species may be due to the differences in the physiological status of the explant or may be gene governed. The callus formation observed under the effect of different hormonal combinations may confirm the work of other authors e.g. (Gowda and Muralikrishna, 1994) who obtained round, compact, amorphous and friable calli from leaf discs of *Vitis vinifera* and *Vitis labrusca* using the same hormones that were used in this study.

The shoot buds that had developed from different hormonal treatments were then allowed to grow on hormone free MS culture media. After they have elongated to a suitable length in petri dishes, they were separately transferred to culture tubes. Each tube contained 30 ml of hormone free MS culture media. At the same time shoot apical meristems were also allowed to grow on induced adventitious shoot buds. Analysis of morphological characteristics of the cultivars (Asemi)the same conditions for comparison with the ones regenerated in vitro from hormone regenerants (table 2) and (Razeki) regenerants (table 3) after 3, 6 and 9 weeks of growth

have shown that the regenerants obtained after hormonal treatments were almost identical to their corresponding controls on the morphological levels. For the first 3 weeks of growth *in vitro,* root growth was not observed. Analysis of chlorophyll contents of leaves of both cultivars showed that chlorophyll a, b and total chlorophylls were increased significantly and highly significantly in comparison to their corresponding controls.
This may be explained on the basis that plantlets obtained from induced adventitious shoot buds were treated with high cytokinin concentrations.

Table (1) Descriptive analysis of the effect of BA and 2, 4 D on *V. vinifera* L. cv Asemi and Razeki after 2, 4 and 6 weeks of incubation *in vitro*

BA mg/ 1.	Growth (weeks)	2,4 D mg/1.									
		0.1		0.5		1.0		2.0		3.0	
		Asemi	Razeki	Asemi	Razeki	Asemi	Razeki	Asemi	Razeki	Asemi	Razeki
0.1	2	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	R	C	R	C	-	-
	6	C	C	C&R	C	R	R	C&R	R	R	C
0.5	2	-	-	-	-	-	-	-	-	-	-
	4	-	C	-	C	R	R	R	-	R	-
	6	C	C	C	C	C&R	C&R	C&R	C	R	R
1.0	2	-	-	-	-	-	-	-	-	-	-
	4	C	C	C	C	C	C	C	C	C	C
	6	C	C	C	C	C&S	C&S	C&R	C&R	C&R	C&R
2.0	2	-	-	C	C	C	C	C	C	C	C
	4	C	-	C	C	C&S	C	C&S	C&S	C&R	C
	6	C	C	C	C	C&S	C&S	C	C	C&R	C&R
3.0	2	-	-	-	-	-	-	-	-	-	-
	4	S	S	C	C	C	C	C&R	C	R	C
	6	S	C	C	C	C	C	C&R	C&R	R	R

(-) No change, (R) root morphogenesis, (S) shoots morphogenesis, (C) callus formation

Table (2): Morphological characteristics and leaf chlorophyll contents (mg/g. fresh weight) *of in vitro*regenerated Vitis vinifera cv. Asemi plantlets

Growth period	3 (weeks)		6 (weeks)		9 (weeks)	
Sample Criteria	C	R	C	R	C	R
Shoot length (cm)	1.4 ± 0.1	1.5 ± 0.1 N.S	2.4 ± 0.0	2.5 ± 0.1 N.S	3.5 ±0.1	3.6 ±0.1 N.S
No of leaves	1.6 ±0.0	1.7 ± 0.1 N.S	2.8 ± 0.1	2.9 ± 0.I N.S	3.8± 0.0	3.8 ± 0.0 N.S
Shoot fresh weight (mg) .	97 ± 4.0	98 ± 3.0 N.S	198 ± 6.0	193 ± 5.0 N.S	280 ± 7.0	293 ± 8.0 N.S
Shoot dry weight (mg)	10 ±-3.0	10 ±2.0 N.S	20 ±4.0	19 ±3.0 N.S	27 ±5.0	28 ±3.0 N.S
Root length (cm)	-	-	1.1± 0.2	1.1± 0.1 N.S	1.91 ±0.3	1.88 ±0.4 N.S
No; of rootlets	-	-	2.0 ± 0.0	2.0 ± 0.0 N.S	4.1 ± 0.1	4.0 ±0.0' N.S
Root fresh weight (mg)	-	-	28 ± 3.0	27 ± 2.0 * N.S	45 ± 4.0	46 ± 4.0 N.S
Root dry weight (mg)	-	-	4 ±1.0	3 ± 1.0 N.S	6± 1.0	6 ±1.0 N.S
Chlorophyll (a)	-	-	-	-	1.32 ±0.05	1.51± 0.03 +S
Chlorophyll (b)	-	-	-	-	0.43 ± 0.03	0.59±0.03 +H.S
Total chlorophyll	-	-	-	-	1.75 ±0.02	2.11±0.03 +H.S

Each value is a mean of 3 determinations ± standard error. (N.S) non significant, (S) significant, (H.S) highly significant, (C) Regenerants obtained from direct growth of apical meristems, (R) Regenerants obtained from induced adventitious shoot buds.

Table (3): Morphological characteristics and leaf chlorophyll contents (mg/g. fresh weight) *of in vitro* regenerated *Vitis vinifera* cv. Razeki plantlets.

Growth period	3 (weeks)		6 (weeks)		9 (weeks)	
Sample Criteria	C	R	C	R	C	R
Shoot length (cm)	1.5 ±0.1	1.5 ±0.1 N.S	2.6 ±0.2	2.5 ±0.2 N.S	3.8 ±0.2	3.7 ±0.1 N.S
No of leaves	1.6 ±0.0	1.6 ±0.0 N.S	3.0 ±0.0	3.0 ±0.0 N.S	4.1 ±0.1	4.2 ±0.1 N.S
Shoot fresh weight (mg)	111±5.0	117 ±4.0 N.S	211 ±7.0	205± 7.0 N.S	314 ±8.0	312 ±5.0 N.S
Shoot dry weight (mg)	14 ±6.0	19 ±4.0 N.S	27 ±5.0	25 ±5.0 N.S	38 ±6.0	35 ±4.0 N.S
Root length (cm)	-	-	1.2 ±0.1	1.3 ±0.1 N.S	200 ± 0.2	204 ±3.0" N.S
No; of rootlets	-	-	2.0 ±0.0	2.0 ±0.0 N.S	4.2 ±0.0	3.8 ±0.1 N.S
Root fresh weight (mg)	-	-	25 ±2.0	24 ± 2.0 N.S	43 ± 4.0	42 ± 3.0 N.S
Root dry weight (mg)	-	-	4 ±1.0	4 ±0.1 N.S	7 ±0.1	6±0.1 N.S
Chlorophyll (a)	-	-	-	-	1.38 ±0.04	1.55± 0.03 +S
Chlorophyll (b)	-	-	-	-	0.41 ± 0.02	0.69 ± 0.03 +H.S
Total chlorophyll	-	-	-	-	1.79± 0.02	2.24 ±0.02 +H.S

Each value is a mean of 3 determinations ± standard error. (N.S) non significant, (S) significant, (H.S) highly significant, (C) Regenerants obtained from direct growth of apical meristems, (R) Regenerants obtained from induced adventitious shoot buds.

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الإكثار الدقيق لإثنين من أصناف العنب اليمنية من القمم الميرستيمية للمجموع الخضري في الزجاج

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لقد تم إجراء التجارب في هذه الدراسة بمعامل كلية الزراعة ، جامعة صنعاء ، الجمهورية اليمنية في الأعوام ٢٠٠٢/٢٠٠٣ بغرض إكثار اثنين من أصناف الأعناب اليمنية الشائعة والتي عادة ما يتم إكثارها بالطرق التقليدية وهما الصنف عاصمي والصنف رزاقي . لقد تم فصل وتعقيم المناطق الإنشائية لقمم المجاميع الخضرية وزراعتها علي أملاح وسط موراشسج وسكوج محتويا علي تركيزات متدرجة من كل من البنزيل أدينين والـ ٢، ٤د . لقد أثبتت التجارب إمكانية الحصول علي كالوس النبات وكذلك نمو متضاعف للبراعم الخضرية العرضية في خلال ٤-٦ أسابيع باستخدام تركيز ١ أو ٢ ميلليجرام / اللتر من أي من الهرمونات المستخدمة في التجربة مضافا إلى الآخر . لقد أمكن الحصول علي بادرات كاملة من تلك البراعم في أنابيب تحتوي علي نفس الوسط الغذائي ولكن خاليا من الهرمونات كما أمكن أقلمة تلك البادرات ونقلها الى التربة . بمتابعة مظاهر النمو المورفولوجية وجد أن النباتات المستخلفة في الزجاج من البراعم العرضية لا تختلف عن نباتات المجموعة الضابطة والتي نمت مباشرة من القمم الميرستيمية للمجاميع الخضرية لكلا الصنفين بينما كان المحتوي الكلوروفيللي للنباتات الناتجة من البراعم العرضية زائداً بصورة معنوية عن نباتات المجموعة الضابطة.