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Effects of E20 and Ms Based Media on *In vitro* Induction of Axillary Buds and Shoot Development from Haploid *Cucumis melo* Microcuttings

Ugur Bal, [1]Nebahat Sari and [1]Hale Yilmaz
Department of Horticulture, Faculty of Agriculture Trakya University Tekirdag, Turkey
[1]Department of Horticulture, Faculty of Agriculture, Cukurova University, Adana, Turkey

Abstract: The culture medium E20A was used as part of the dihaploidization technique in the genetic improvement of *Cucumis melo*. During long term maintenance of haploid stock plants via subculture of microcuttings, weak shoot development and death of plantlets were observed. Therefore in search of a suitable medium to induce vigorous development of the long term maintained plantlets, the present experiment was conducted. The basic media E20A and MS were used with or without only one concentration of IAA, i.e., 0.01 mg l^{-1}, in combination with one of the three concentrations of BAP, i.e., 0.225 mg l^{-1}, 1.125 mg l^{-1} and 2.250 mg l^{-1}. Plantlet length, number of nodiums, callus length and diameter, rates of root and shoot initiation were recorded weekly during culture. It was determined that while single shooting and up-to-the-standard plantlets were obtained using E20A medium containing 0.01 mg l^{-1} IAA (medium one), multi shooting plantlets with shorter internodiums were obtained from the E20A medium only with 0.225 mg l^{-1} BAP (medium three). The most desirable shoot development was obtained from the media without BAP but containing 0.01 mg l^{-1} IAA. Integration of BAP into the media resulted in callus development which in turn restricted shoot development. It was concluded that low salt media is more suitable for *in vitro* induction of axillary buds and shoot development in melon.

Keywords: Melon, *in vitro* haploid plant, IAA, BAP, high salt medium

Introduction

Haploidy is not a new subject but is a rapidly expanding field of research since the discovery in 1964 by Guha and Maheshwari of the possibility of developing haploid androgenetic plants from microspores of *Datura innoxia* (Guha and Maheshwari, 1964). Later, gynogenetic and androgenetic haploid plants were produced in many species. Until the 1980s, chromosome elimination following distant hybridization and anther culture or ovule-ovarium culture techniques were used in haploid production, but better results were obtained in some species including melon, via parthenogenetic haploid induction techniques, using incomplete pollen, i.e., using pollen irradiated or treated with chemicals, in the pollination. (Roger and Ellis, 1966; Montelongo-Escobedo and Rowe, 1969; Sauton and Dumas de Vaulx, 1987; Sauton, 1989; Sari *et al*., 1992; Sari *et al*., 1994).

Production of haploid melon plants (*Cucumis melo* L. var. *cantaloupensis*) was first reported by Sauton (1987) using irradiated pollen in the pollinations. Also, production of haploid plants of *C.melo* var. *inodorus* and *C.melo* var *reticulatus*, characteristically of larger fruit size, was first reported by Sari *et al.* (1992) using the same technique. Chromosome doubling techniques for the haploids produced were also established (Sauton, 1987) and more advanced techniques were developed to increase the number of doubled plants (Sari *et al*., 1999).

The culture medium E20A, developed by Sauton (1987), is used in the cloning and multiplication of haploid plants. The medium is also used successfully in the culture of both haploid embryos following rescue and microcuttings and in the induction of doubled shoot development following chromosome doubling treatments as well as *in vitro* rooting of the plantlets.

For multiplication purposes several microcuttings each carrying one axillary bud at the leaf axis, are obtained from melon plants developing *in vitro*. The microcuttings are then transferred to fresh E20A medium and expected to develop shoots from their axillary buds giving rise to new shoots which later develop into plantlets carrying many leaves and axillary buds. While the above is the case for initial stages of culture, in cases where microcloning lasts as long as 6-12 months even if plants are subcultured to fresh E20A medium monthly in the form of microcuttings with or without shoots, a gradual decrease in the vigor of the plants is observed and therefore microcuttings obtained from these become recalcitrant to shoot induction and development from the axillary buds when subcultured further. The unfavorable vigor decrease appears in the form of yellowing of the plantlets and

leaves as well as weak shoot development. Furthermore, in some cases microcuttings do not respond at all and die. Because of the conditions, research plants *in vitro* are maintained in long periods, the aforementioned problems seem inevitable.

De Fossard *et al.* (1974) divided tissue culture media into low, medium and high salt groups according to concentrations of the molecules available in a medium. The E20A medium is composed of molecules mainly of low to medium concentrations making E20A a low-medium salt medium. It was hypothesised therefore, that the use of a high salt medium, i.e. MS (Murashige and Skoog, 1962), together with high concentrations of a cytokinin (BAP in our experiment) may restore vigour and induce and maintain healthy shoot development.

It was furthermore determined in previous studies using melon for microclonning (Spetsidis *et al.*, 1996) that BAP integrated into the media increased the number of shoots developed. Such an addition, therefore, might induce multiple shooting conferring rapid multiplication of the desired material.

The present report describes efforts to overcome the above mentioned problems by testing MS (Murashige and Skoog, 1962) as a high salt medium, which is used extensively in tissue culture studies, against E20A (Sauton, 1987), i.e. a long established low-medium salt medium in melon.

Materials and Methods

The present research was carried out in Biotechnology Laboratories of the Horticulture Department of the Faculty of Agriculture, Cukurova University, Turkey, between 1997-1998. Haploid plants, developed through dihaploidization technique as part of a melon breeding program of the department, were used.

The basic media used were E20A (Sauton, 1987) and MS (Murashige and Skoog, 1962) (Table 1) and growth regulators tested, either singly or in combination, were a single concentration of IAA, i.e. 0.01 mg l^{-1} (Sauton, 1987) and three different concentrations of BAP, i.e. 0.225 mg l^{-1}, 1.125 mg l^{-1} and 2.250 mg l^{-1}, (Adelberg *et al.*, 1993; 1994) (Table 2). Aproximately one year old haploid microcuttings, subcultured earlier several times, were used in the experiment and 20 microcuttings, five cuttings for four replications, were inoculated on each of the media The total number of test tubes used for the experiment was 280, i.e. a combination of 14 different media and 20 microcuttings each. The experiment was set according to completely randomised plots design and the mean values were compared by Tukey test.

Duration between the inoculation of a micro cutting carrying a single axillary bud and a developed plant with many leaves and therefore many axillary buds, length of the shoots and other data obtained during four consecutive weekly observations were as follows.

Table 1: Ingredients of E20A and MS media (mg l^{-1})

Ingredients	E20A	MS
Macronutrients (mg l^{-1})		
KNO_3	1075.0	1900.00
NH_4NO_3	619.0	1650.00
$MgSO_4.7H_2O$	206.0	370.00
$CaCl_2.H_2O$	156.5	440.00
KH_2PO_4	71.0	170.00
$Ca(NO_3).4H_2O$	25.0	-
$NaH_2PO_4.4H_2O$	19.0	-
$(NH_4)_2SO_4$	17.0	-
KCl	3.5	-
Micronutrients (mg l^{-1})		
$MnSO_4.H_2O$	11.065	22.300
$ZnSO_4.7H_2O$	1.812	8.600
H_3BO_3	1.575	6.200
KI	0.345	0.830
$Na_2MoO_4.2H_2O$	0.094	0.250
$CuSO_4.5H_2O$	0.008	0.025
$CoCl_2.6H_2O$	0.008	0.025
Na_2EDTA	37.300	37.300
$FeSO_4.7H_2O$	27.800	27.800
Organic compounds (mg l^{-1})		
Meso-inositol	50.300	100.000
Pyrodoxine-HCl	5.500	0.500
Nicotinic acid	0.700	0.500
Thiamine-HCl	0.600	0.100
Ca pantothenate	0.500	-
Biotine	0.005	-
Glycine	0.100	-
Others:		
Sucrose (g/l)	20	20.00
Agar (g/l)	8	8.00
pH	5.9	5.90

Plant length (mm): Plantlet length from the surface of the medium up to the tip of the shoot was measured by a ruler and recorded in millimeters.

Number of nodiums: Number of nodiums were counted and recorded.

Callus diameter and length (mm): The calli developed were measured for length and width and the data were recorded in millimeters.

Rooting of plantlets: Degree of root development was evaluated visually according to a scale of 1-3, in which 1) no roots, 2) slightly rooted and 3) well rooted.

Degree of developing shoots: Calculated by dividing the number of developing shoots with the total number of microcuttings inoculated.

Sister plant production: The microcuttings initially inoculated and the new shoots developed from these initial microcuttings were counted together (ti) and the

Table 2: The concentrations of growth regulators

Medium No.	Basic medium with growth regulators	Medium no.	Basic medium with growth regulators
1	E20A+IAA 0.01 mg l^{-1} (Control)	8	MS+IAA 0.01 mg l^{-1}
2	E20A+BAP 0.225 mg l^{-1}+IAA 0.01 mg l^{-1}	9	MS+BAP 0.225 mg l^{-1}+IAA 0.01 mg l^{-1}
3	E20A+BAP 0.225 mg l^{-1}	10	MS+BAP 0.225 mg l^{-1}
4	E20A+BAP 0.125 mg l^{-1}+IAA 0.01 mg l^{-1}	11	MS+BAP 1.250 mg l^{-1}+IAA 0.01 mg l^{-1}
5	E20A+BAP 0.125 mg l^{-1}	12	MS+BAP 1.250 mg l^{-1}
6	E20A+BAP 2.250 mg l^{-1}+IAA 0.01 mg l^{-1}	13	MS+BAP 2.250 mg l^{-1}+IAA 0.01 mg l^{-1}
7	E20A+BAP 2.250 mg l^{-1}	14	MS+BAP 2.250 mg l^{-1}

degree of sister plant production was determined according to the formula below:

Degree of sister plant production= $\Sigma t_i * n_i / n$

t_i = number of sister plants (rooted) on the day of observation

n_i = number of sister plants in the same test tube

n = total number of test tubes

Results and Discussion

Differences in plant lengths were not recorded at the first week's observation because rooting had just started and consequently axillary growth had not occured, maintaining the inoculant's original length. In the second week, statistically significant differences at a 1% level on the effects of the media tested were observed. The longest plants, considering the mean value of the replicates, were from the control medium (E20A + 0.01 mg l^{-1}) which was followed by the medium number 2, the E20A in combination with 0.01 mg l^{-1} IAA and 0.225 mg l^{-1} BAP. The medium number 11, i.e. MS + 0.01 mg l^{-1} IAA and 1.125 mg l^{-1} BAP induced excessive callus growth resulting in short plantlets, i.e., an average of 0.48 mm, which almost disappeared in the calli. The medium number 1, on the third observation, enhanced plant length about 95% in two weeks, which was followed by the medium number 2 (13.2 mm) with a 68% length increase. Also, the medium number 8 (MS + 0.01 mg l^{-1} IAA) induced 79% length increase since the first observation. Plant length increase on the medium number 6 (E20A + 0.01 mg l^{-1} IAA + 2.225 mg l^{-1} BAP) was 25% in comparison to the first observation. Apart from these four media, callus developed in excessive amounts on the rest of the media resulting in remaining plant lengths to be short. As was the case in the second and third observations, the first medium on the fourth observation appeared to be the medium enhancing the most plant growth with 23.70 mm. Also at the end of a four week growing period of the 20 microcuttings inoculated to the medium number one, while 60% remained under 30 mm, 25% was between 30 to 50 mm and 15% was longer than 50 mm, showing that the average plant length was short and was the result of microcuttings which either developed very little or not at all. It was also striking, at the fourth observation that the initial average plant length on the medium number one, i.e. E20A + 0.01 mg l^{-1} IAA, was increased by 196%. The medium number one was followed by the medium number eight which enhanced growth by an average of 81% compared to the first observation. Significant differences, in the last observation, were not encountered amongst the rest of the media and the remaining 10 media followin the medium eight was evaluated in the same group (Table 3).

In the first observation for the number of nodiums, significant differences were not observed among the 14 media. In the second week's observation, however, the medium 3 (E20A + 0.225 mg l^{-1} BAP) induced the most number of nodiums with 3.8 nodiums per plant. While the medium 1 ranked second with 2.25, the medium 8 ranked third with 2.15 nodiums per plant. Also increases in the number of nodiums, of 24%, 18%, 18% and 18% were observed from the media number 2, 6, 7 and 5, respectively. Also, in the second observation the number of nodiums remained the same for medium four, whereas the number decreased for the rest of the media tested, which was due to the fact that the callus proliferating from the base of the explant took over the basal parts of the explant. At the third observation, plants on the third medium, continued developing with very short internodes, which was in line with our previous observations that is average 3.45 nodiums per plant falling in the same statistical group and this was followed by the medium one and eight with 2.9 and 2.65 nodiums per plant, respectively. Also on the medium 11, nodiums to be counted were not found since the callus proliferated took over the whole plant. As seen from the Table 4, the medium number 3 and 8, from the second week onwards, displayed the most number of nodiums, which were followed by the media 10, 5, 9 and 2.

In the fourth observation, the number of nodiums remained between 0.05 and 3.50 on all 14 media due to the adverse effects of excessive callus proliferation. In tissue culture studies, the number of nodiums alone is not descriptive enough, hence the length of internodiums should be taken into consideration as well. When taken a closer look at the medium number 3 (Table 3) which produced plants as long as 9.6 mm and when divided by the number of nodiums that is 2.65 (Table 4), average

Table 3: Plant length obtained from the media (mm)

Medium No.	1st observation 12.07.1998	2nd observation 19.07.1998	3rd observation 26.07.1998	4th observation 02.08.1998
1	8.00	11.70 a	15.65 a	23.70 a
2	7.85	10.95 a	13.20 ab	5.10 b
3	6.65	9.75 ab	4.80 bcd	9.60 b
4	7.35	6.95 abc	4.80 bcd	3.15 b
5	7.10	7.75 abc	6.20 a-d	5.25 b
6	6.30	5.55 abc	7.90 a-d	3.80 b
7	7.15	6.70 abc	3.45 cd	3.35 b
8	6.55	8.70 ab	11.75 abc	11.85 ab
9	5.90	5.00 abc	4.90 bcd	5.30 b
10	6.75	7.85 abc	6.90 a-d	9.00 b
11	6.15	0.48 c	0.00 d	0.00 b
12	5.20	2.35 bc	1.00 d	0.15 b
13	4.65	2.60 bc	0.90 d	0.65 b
14	8.35	5.20 abc	1.70 d	4.15 b
D	(%5) NS	(%1) 7.94	(%1) 9.56	(%1) 12.06

Table 4: Results for the number of nodiums on all the media

Medium No.	1st observation 12.07.1998	2nd observation 19.07.1998	3rd observation 26.07.1998	4th observation 02.08.1998
1	1.80	2.65ab	2.90ab	3.50a
2	1.85	2.30abc	2.55a-d	1.25bcd
3	1.80	3.80a	3.45a	2.65ab
4	1.70	1.70bcd	1.83a-d	0.65cd
5	1.65	1.95bc	2.30a-d	1.40bcd
6	1.70	2.00bc	1.85a-d	1.05bcd
7	2.25	2.00bc	0.80de	0.70cd
8	1.45	2.25abc	2.65abc	2.63ab
9	1.35	1.40bcd	1.55b-e	1.30bcd
10	1.40	1.75bc	2.20a-d	1.90abc
11	1.78	0.15d	0.00e	0.10d
12	1.45	0.90cd	1.00cde	0.05d
13	1.60	0.95cd	1.20b-e	0.20d
14	2.2	1.55bcd	1.70a-e	0.85cd
D	(%5) NS	(1%) 1.59	(1%) 1.79	(1%) 1.63

Table 5: Callus diameter (mm) developed by the media

Medium No.	1st observation	2nd observation	3rd observation	4th observation
1	2.95a	1.05bc	1.05c	1.35f
2	4.15a	12.18a	17.25a	17.85ab
3	2.15ab	7.65abc	10.25ab	13.10ce
4	1.15ab	9.25a	15.15	17.80ab
5	2.15ab	8.15abc	12.20	14.70a-e
6	1.75ab	10.30a	11.70	17.55abc
7	0.85b	9.65a	8.55	16.55a-d
8	0.65b	0.90c	1.05	1.70f
9	2.90ab	8.55abc	9.80	12.95de
10	2.95ab	8.90ab	10.80	11.40e
11	1.15ab	9.30a	12.55	17.90a
12	0.70b	11.65a	15.35	17.25a-d
13	1.15ab	10.05a	10.95	13.40b-e
14	1.45ab	11.45a	12.80	16.00a-d
D	(%5) 3.22	(%1) 7.89	(%1) 7.68	(%1) 4.49

Table 6: Callus length obtained from the media (mm)

Medium No.	1st observation 12.07.1998	2nd observation 19.07.1998	3rd observation 26.07.1998	4th observation 02.08.1998
1	1.70ab	1.40cd	1.05b	0.79d
2	2.60a	10.70a	10.70a	11.15abc
3	1.65ab	4.15bcd	6.15ab	7.90c
4	1.10ab	6.20abc	9.70a	11.95abc
5	2.50ab	6.30abc	8.40a	11.10abc
6	1.75ab	7.90ab	11.70a	13.95a
7	1.10ab	7.35ab	9.30a	13.05ab
8	0.50b	0.85d	0.90b	0.40d
9	2.40ab	6.75ab	7.35a	9.00bc
10	1.95ab	6.05abc	7.35a	7.50c
11	1.70ab	7.80ab	9.25a	14.20a
12	0.50b	9.30a	11.15a	12.60ab
13	1.20ab	6.40abc	8.40a	10.60abc
14	1.20ab	8.10ab	9.40a	11.85abc
D	(5%)2.02	(1%) 5.12	(1%) 5.61	(1%) 4.64

Table 7: Results of root induction by the media

Medium No.	1st observation 12.07.1998	2nd observation 19.07.1998	3rd observation 26.07.1998	4th observation 02.08.1998	Average
1	1.15	1.70	1.65	1.60	1.52
2	1.00	1.00	1.00	1.00	1.00
3	1.00	1.00	1.00	1.00	1.00
4	1.00	1.00	1.00	1.00	1.00
5	1.00	1.00	1.00	1.00	1.00
6	1.00	1.00	1.00	1.00	1.00
7	1.00	1.00	1.00	1.00	1.00
8	1.15	1.35	1.65	1.65	1.45
9	1.00	1.00	1.05	1.05	0.77
10	1.00	1.00	1.00	1.00	1.00
11	1.00	1.00	1.00	1.00	1.00
12	1.00	1.00	1.00	1.00	1.00
13	1.00	1.00	1.00	1.00	1.00
14	1.00	1.00	1.00	1.00	1.00

Table 8: Arithmethic means of the number of sister plants obtained

Medium No.	1st observation 12.07.1998	2nd observation 19.07.1998	3rd observation 26.07.1998	4th observation 02.08.1998
1	1.00	1.00	1.05	1.25
2	1.00	1.00	1.85	2.35
3	1.00	2.00	5.15	6.00
4	1.00	1.40	1.65	1.25
5	1.00	1.50	2.35	2.80
6	1.00	1.50	2.00	2.05
7	1.00	1.55	1.55	1.60
8	1.00	1.00	1.10	1.10
9	1.00	1.20	1.50	1.60
10	1.00	3.85	5.00	4.85
11	1.00	1.00	1.00	1.00
12	1.00	1.25	1.35	1.00
13	1.00	1.30	1.40	1.00
14	1.00	1.25	1.35	1.00

Table 9: Results of shoots development on the media

	1st observation 12.07.1998		2nd observation 19.07.1998		3rd observation 26.07.1998		4th observation 02.08.1998	
Medium No.	No. of developing shoots	%	No. of developing shoots	%	No. of developing shoots	%	No. of developing shoots	%
1	11	55	19	95	19	95	19	95
2	18	90	18	90	19	95	20	100
3	17	85	20	100	20	100	20	100
4	8	40	10	50	10	50	10	50
5	10	50	11	55	13	65	14	70
6	8	40	8	40	16	80	16	80
7	11	55	11	55	20	100	20	100
8	7	35	11	55	14	70	16	80
9	11	55	13	65	15	75	15	75
10	8	40	12	60	16	80	17	85
11	10	50	10	50	20	100	20	100
12	4	20	5	25	5	25	5	25
13	4	20	7	35	7	35	8	40
14	13	65	13	65	14	70	15	75

length of the internodiums can be calculated as 3.62 mm. In fact, on the medium number 1, the number of nodiums was 3.50 and the plant length was 23.70, when the two were divided an internodium length of 6.77 mm was obtained, which is a suitable internodium length for use in micropropagation. In the third medium, despite numerous nodiums, the plants themselves were short and so we can therefore conlude that plant development in this medium is unsatisfactory.

In Fig. 1, the most satisfactory plant development, that is on the medium number 1, is shown and in Fig. 2 plants with short internodiums and sister plant formation obseved on the medium number 3, after one month of culture, are shown. The effects of the growth regulators, integrated into the E20A media, i.e. the media 1-7 and the MS media, i.e., the media 8-14, on the plant development are shown in Fig. 3 and 4, respectively.

The presence and size of the calli developed as a result of existing balance of auxins and cytokinins were determined and measured from the outside of the test tubes and the results are presented in the Tables 5 and 6. At the first observation date, from the initial introduction of the microcuttings, the differences and resemblances among the 14 media, with regards to callus length, were

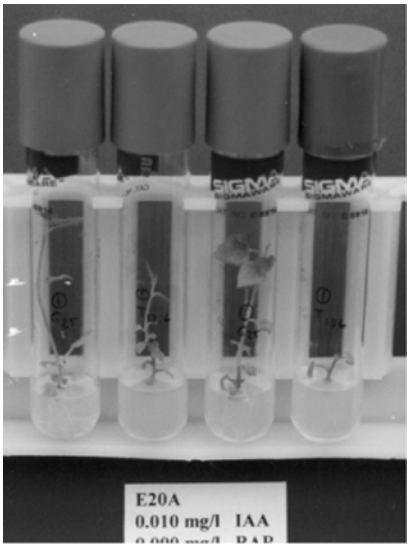

Fig. 1: Plant development on the medium number one (E20A+IAA 0.01 mg l^{-1})

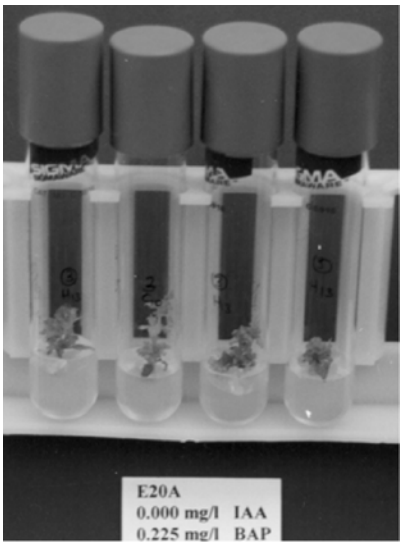

Fig. 2: Plant development on the medium number three

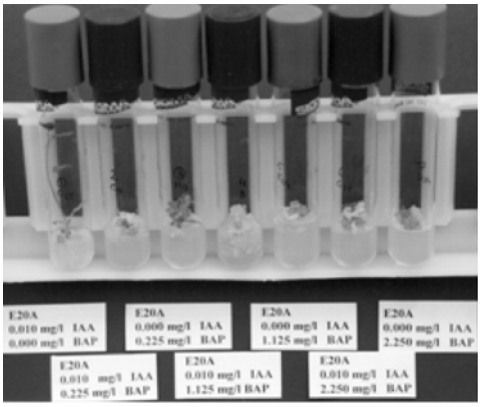

Fig. 3: Plant development on the E20A medium containing the growth regulator

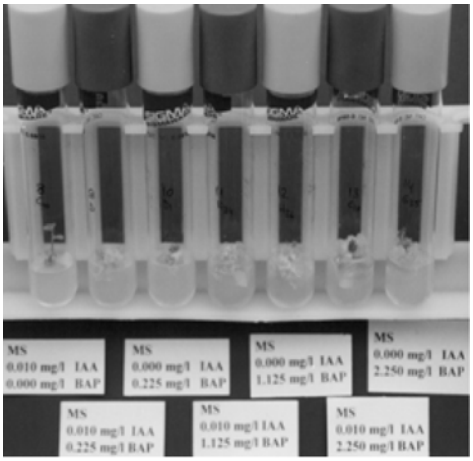

Fig. 4: Plant development on the MS medium containging the growth regulator

determined. While the medium 2 (E20A+0.01 mg l^{-1} IAA + 0.225 mg l^{-1} BAP) developed the largest callus with 4.15 mm length, the media number 7, 8 and 12 resulted in the smallest calli. Even though induced calli varied in size, the rest of the media,were statistically in the same group. In the second week's observations, while the callus in medium number two maintained the largest size, the calli developing in the media number 4, 6, 7, 11, 12, 13 and 14 fell in the same statistical group inducing large size calli. A decrease of 81% was observed in the size of the plants from the medium 1 was a result of root initiation from the plants. In the third week's observations while the E20A and MS containing only one dose of IAA, e.g., media number 1 and 8, respectively, resulted in the smallest callus size which was followed by the medium 7 and the medium 2 developed the largest size callus (Table 5). The difference among the rest of the media was determined to be statistically insignificant. In the last observation the media 1 and 8 were in the same statistical group, with an average callus size of 1.35 and 1.70 mm, respectively, which was followed by the medium 10 with 11.40, as was previously determined and the medium number 11, resulted in the largest callus, that is 13 times of the callus developed in the medium 1.

It was determined on the E20A media that the use of BAP alone resulted in smaller calli in comparison to the use of BAP in combination with IAA, however a similar case was not determined for the use of MS medium the growth regulators. In the first observation, the callus length determined in relation to the callus diameter, were in line with each other, except for the medium 7 (Table 6). While

the medium 2 resulted in the highest length callus, the media 8 and 12 developed the smallest calli and the rest of the media remained in the same statistical group.

At the week following the first observation, the media 2 and 12 maintained their undesirable characteristic of long callus induction, with a length of 10.70 mm and 9.30 mm, respectively, both falling in the same statistical group. The medium number 1, as was the case in the callus diameter, inhibited callus length as the root development progressed. The smallest callus length was measured on the medium 8 as 0.85 mm. Differences among the media appeared distinctively in the third observation. Since the root initiation and development progressed, the medium 1 with 1.05 mm callus length ranked behind the medium 8 with 0.90 mm and the medium 3 with 6.15 mm callus length appeared in the middle among the results obtained. Since the other 11 media developed long callus, they fell statistically into the same group, the lengths varying between 7.35 and 11.70 mm. In the last observation the smallest calli were determined from the media 1 and 8, which was the case in the earlier observations. The longest callus length was obtained from the media 11 and 6, with 14.20 mm and 13.95 mm, respectively (Table 6).

Haploid microcuttings *in vitro* displayed similar rooting characteristics on both media 1 (E20A+0.01 mg l^{-1} IAA) and 8 (MS+0.01 mg l^{-1} IAA) carrying IAA solely. Following two weeks of culture on medium 9, i.e., MS with 0.01 mg l^{-1} IAA and 0.225 mg l^{-1} BAP root development was limited. In the other combinations, however, rooting was not observed (Table 7).

The effect of the media on the sister plant production is presented in the Table 8. As seen in the table, the auxin, integrated into medium 1, which induced apical growth, blocked axillar shoot development. Shoots growing under apical dominance, i.e., as a single plantlet induced from just one microcutting, are dissected into several microcuttings each carrying one leaf therefore one axillar bud and transferred onto the media, i.e. the usual method of multiplication. In our experiment, however, the cytokinin, i.e., BAP, added to the original multiplication medium containing IAA, affected the auxin cytokinin balance and resulted in the initiation of axillar shooting, that is sister plant development.

In the observations, the number of new shoots including the original cutting and related arithmetic mean was determined. Since the microcuttings were just transferred and only one week as a period for development is insufficient for sister plant development, axillary shoot development was not observed in the first data recording date. In the second observation, cuttings on the media 1, 2, 8 and 11 did not initiate roots and kept their initial state that was recorded in the first observation. On the medium 10, however, 3.85 times more number of plants, compared to the previous week, were observed. Since 2 shoots per plant was obtained on the medium three, it followed the medium 10. On the other hand, it was determined that the quality of newly developed shoots was much more desirable on the medium 3, in that the sister plants appeared on the medium 10 were shorter, that is average 9 mm. In summation, axillar shoots developed were very little and not suitable for propagation by microcuttings. Whereas internodiums of the sister plants developed on the medium 3, were longer and at a suitable state to obtain microcuttings.

In the third week observation, the number of sister plants developed on the medium ten was 5 and ranked second statistically. It must be mentioned, however, that the problems encountered in this medium in the second observation continued in the third. The medium 3 ranked first by increasing the number of shoots 2.5 times resulting in 5.15 shoots, which was followed by medium 5 with 2.35 shoots. The number of shoots developed in the rest of the media varied between 1.05 and 1.85, being the results for the media 1 and 2, respectively.

On the fourth observation, the medium 3 maintained its characteristic as an inducer of the most number of sister plants in just one container and medium 3 was followed by the medium 10 with 4.85 sister plants. While the media 2, 5 and 6 displayed increases parallel to their state in the third observation, excess callus developed on the media 10 and 14 and the shoots counted on the third observation were hardly distinguishable from the callus developing in this observation. Hence a decrease was observed on the average number of sister plants (Table 8).

The number of developing shoots, in addition, was recorded weekly and percentages of developing shoots were calculated. The highest shooting percentage was observed from the medium 2 with 90% which was followed by the media 3 and 14 with 85 and 65%, respectively. The shooting percentages for the rest of the media were 55%, for the media one, seven and nine and 20% for the media 12 and 13 (Table 9).

In the second week, following initial transfer of the microcuttings, differences in the shooting percentages appeared on the media tested. A shooting of 100% was determined on the medium 3 which was followed by the medium 1 with 95%. Between the first and second week a 72% increase was observed in the shooting of medium 2. The medium 2 followed medium 1, the least shooting percentage was 25% on the medium 12 followed by medium 13 with 35%.

In the third week observations, media 7 and 10, by approximately 100% increase compared to the second week, displayed 100% shooting. While the shooting

percentage observed on medium 1 stabilized at the 95%, medium two displayed an increase from 90 to 95%. The shooting percentage obtained from the medium 6, i.e. 80%, became statistically significant in the third week by displaying 100% increase. The maximum shooting occurred on medium 12 was 25% which was the same as the one recorded in the previous observation.

The shooting rates determined from media 2, 3, 7 and 11 reached a 100% four weeks after the initial transfer of the microcuttings to the media tested, which was followed by medium 1 displaying 95% shooting rate. At the end of the experiment the least shooting was from medium 12 which displayed the same characteristics throughout the culture period. The medium 8 drew attention in the last week's observation with regards to increase in the shooting as 14%.

It was determined that the most satisfactory development was obtained from the E20A medium, a low-medium salt medium. It may be therefore said melon shoots develop satisfactorily on low to medium salt media together with growth regulators in low concentrations. It appears, therefore, that further experiments with regards to use of a basic medium should test low salt media. On the other hand half strength MS may take priority with the growth regulator IAA at 0.01 mg l^{-1} and BAP at 0.225 mg l^{-1} levels either alone or in combination, since a comparison might be useful with the data already available here for the full strength MS.

The effects of the growth regulators were of two types. The first is the induction of single shooting plantlets carrying optimal number of nodiums on the control medium, i.e. E20A with 0.01 mg l^{-1} IAA and the second is the induction of growth with multiple shoots (3-6), many nodiums and a shrubby appearance when BAP at a concentration of 2.25 mg l^{-1} is added to the E20A. Although the callus developing on the latter medium was undesirable, Spetsidis *et al.* (1996) working on the same plant determined that starting from the second week the plants could be isolated from the callus and when subcultured to fresh media, several shoots could be obtained.

In conclusion, it was determined that the media that could be satisfactorily used in healthy maintenance of the microcuttings and *in vitro* cloning of the plantlets, which earlier had been subcultured several times and therefore had been maintained in vitro a considerably long time, are E20A with 0.01 mg l^{-1} IAA (medium one) and E20A with 0.225 mg l^{-1} BAP (medium 3). While the development observed on the E20A medium with 0.225 mg l^{-1} BAP was not fully suitable for the propagation by microcuttings because of multishooting characteristic, i.e. 5-6 shoots per plant, it was still interesting since because several plants could be obtained following one time subculture. Alternatively this system does not seem to be useful for the melon plants at its current state because of excess callus and short internodes induced. It seems more prudent to suggest further studies, using preferably lower concentrations of the BAP, to search for media which are capable of inducing multiple shoots, i.e., microcuttings carrying several sister plants and shoots which can have internodiums long enough to be used in the propagation by microcuttings. Apart from this when inducing plant development with 5-6 shoots within 2-3 weeks on the medium with 0.225 mg l^{-1} BAP, useful plantlets for propagation by microcuttings could be obtained following the removal of the shoots from the callus and transferring to fresh media on the second week. If this procedure is a probably successful protocol used during the time required for repeated subculturing to multiply the haploids, required time for subculturing may be shortened. In addition to the use of BAP, integration of the GA_3 into the media, which induces shoot elongation, should be considered (Gamborg and Philips, 1995).

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