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IN VITRO PROPAGATION OF BITTER GOURD (*Momordica charantia* L.)

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Abstract

Bitter gourd (Momordica charantia L.) belongs to the family Cucurbiteceae. It is widely consumed as a vegetable and especially as a folk medicine in Asia. This review outlines the work done on the tissue culture of Momordica charantia L.. Commonly known as karela or bitter melon and contains bright red seeds due to high lycopene, a pigment that can be used as an artificial food colorant. Bitter gourd protein has been reported to have HIV inhibitor properties. The fruits of bitter gourd contain nutritionally useful essential minerals and amino acids. It has hypoglycemic activity which reduces the blood glucose, antitumor activity and antispermatogenic and androgenic activities. It is a common food item of the tropics and is used for the treatment of cancer, diabetes, AIDS and many ailments. It has also include alkaloids, insulin like peptides, and a mixture of steroidal sapogenins known as charantin. Bitter gourd is tolerant to a range of limiting factors of the environments and can be grown in tropical and subtropical climates. Improvement of this crop and development of new varieties are obviously necessary which could be done through the applications of modern techniques of biotechnology. Plant tissue culture is one of the biotechnological technique to culture plant cells or tissues under controlled aseptic conditions on artificial medium and is used to some degree in the improvement of almost every major agronomic, vegetable and fibre crop species. It has value in basic research like cell biology, genetic transformation studies and biochemistry for the production of medicinally valuable secondary metabolites. Some limited efforts have been made for the improvement of this crop using biotechnological techniques like: type of explants (auxin, cytokinin), media composition, growth conditions, genotypes.

***Key words**: Bitter gourd, Momordica charantia L., propagation, tissue culture.*

INTRODUCTION

Bitter melon (*Momordica charantia* L.) is an annual, climbing, medicinal vine with small yellow flowers. It has cucumber-shaped fruits, 10-20 cm in size. The fruit has a warty structure and turns from green to orange-red color as it becomes ripe. Its seeds are 7-10 mm in length, flat and coarse. Its fruits and leaves are rich in iron, calcium, phosphorus and vitamin B. Its homeland is East India, and it is also grown in tropical regions of Caribbean, Africa and South America and in the Mediterranean countries including Turkey and Italy (Thiruvengadam et al., 2006). In Turkey, it is generally cultivated in Yalova, Bursa, in the Gazipaşa county of Antalya and the Silifke county of Mersin.

It is used in the treatment of stomach ulcer, eczema and other skin diseases, in viral diseases such as HIV and bacterial diseases. It has the ability to prevent tumor growth due to its lutein and lycopene content. It is also regarded as an insulin plant because of its ability to regulate the control of blood sugar (Simina et al., 2016).

Plant tissue cultures are used for plant breeding, commercial production and basic biological researches (Agarwal M., 2015). Callus and suspension cultures are extremely useful methods to produce herbal products with economic importance in terms of raw materials, especially to the pharmaceutical industry for the production of secondary metabolites. Auxines are growth regulators that are frequently used in callus formation from explants.

The auxin group hormones are divided into two goups, “natural auxins” and “synthetic auxins”. Natural auxines are indole-3-acetic acid (IAA), 4-chloro-indole acetic acid (4-CPA) and phenyl acetic acid (PAA). Synthetic auxins are naphthalene-acetic acid (NAA), β-naphthoxyasetic acid (BNOA), indole-3-butyric acid (IBA), 3-chlorophenoxypropionamide (3-CPA), 2,4-dichlorophenoxyacetic acid (2,4-D),

2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP) etc. (George et. al., 2008). 2,4-D is the most widely used auxin derivative for this purpose. Although *in vitro* propagation studies in plants (Al Munser et. al., 2007; Ananya et al., 2009; Nikam et. al., 2009, Malek et. al., 2010; Safdar et. al., 2013) have already been studied in bitter melon. Other techniques such as: *in vitro* callus regeneration, somatic embryogenesis, organogenesis and regeneration studies in bitter melon have not yet been conducted in Turkey. This research is the first study in this regard. In this study, *in vitro* and *in vivo* germination capacities of two genotypes of *Momordica charantia* L. and the effects and *in vitro* propagation of 2,4-D at different doses for callus formation on leaf and stem explants isolated from these conditions were investigated.

MATERIALS AND METHODS

The bitter melon seeds used in the study were obtained from local growers from Gazipaşa and Silifke in Turkey. Sodium hypochlorite (NaOCl) (commercial name ACE) was used for surface sterilization of seeds. The seeds were soaked for 15 minutes in solution at 20% NaOCl for *in vitro* cultivation and for 3 minutes at 10% NaOCl for *in vivo* cultivation. The stem and leaf explants were rinsed 3 times for 5 minutes each and the sterilization was completed. The seeds were then inoculated in MS (Murashige and Skoog, 1962) basal medium, each magenta vessel containing 5 seeds and 5 explants. Each application was carried out in 3 repetitions. Leaf and stem explants were cut to 1 cm size for using. As callus-forming medium, MS medium was supplemented with 2 mg/l, 4 mg/l, 6 mg/l and 8 mg/l 2,4-D. Also, 30 g/l sucrose and 5 g/l agar were added to the media. The pH of the medium was adjusted to 5.7 and sterilized at 121°C for 20 minutes under 1.5 atmosphere pressure autoclave conditions. The cultures were kept in the climate chamber at 25ºC for 16 hours of light and 8 hours of darkness. Lighting was provided with white fluorescent lamps under 2000 lux light.

RESULTS AND DISCUSSIONS

In *in vitro* and *in vivo* germination studies conducted with the Gazipaşa and Silifke bitter melon genotypes, the seeds, following the sterilization, were cultured in MS nutrient media and pots (Figure 1) in climate chambers. The first germination time was at 8 days after the start of culturing in both media. Germination development was recorded on the 8th, 15th, 25th and 35th days.

Figure 1. The Gazipaşa and Silifke bitter melon genotypes growing in the pot in the climate (*in vivo*) room

The effect of different germination times on the germination rates (%) of the seeds of Gazipaşa and Silifke genotypes were analyzed using analysis of variance, germination rates (%) at different times were given for *in vitro* (Table 1) and *in vivo* (Table 2).

Table 1. Germination rates at different times under *in vitro* conditions (%)

Germination Time (days)	Germination ratio (%)	
	Gazipaşa	Silifke
8	17.0[c]	12.0[b]
15	17.0[c]	16.0[a]
25	22.0[b]	16.0[a]
35	28.0[a]	16.0[a]

Letters show different groups at 0.01 level.

As seen in Table 1, germination ratios ranged from 12 to 28% under *in vitro* conditions. Germination ratios were higher in the Gazipaşa genotype than those in the Silifke genotype in all time periods observed under *in vitro* conditions. The germination rates and duration in the germination medium increased in both

genotypes. This increase was more evident in the Gazipaşa genotype.

Under *in vivo* conditions, germination rates varied between 20 and 50% in the pots (Table 2). Germination rates under *in vitro* conditions were higher in the Gazipaşa genotype than those in the Silifke genotype in all time periods observed under *in vitro* conditions.

Table 2. Germination rates at different times under *in vivo* conditions (%)

Germination Time (days)	Germination ratio (%)	
	Gazipaşa	Silifke
8	40.0^{c}	20.0^{c}
15	47.0^{b}	23.0^{b}
25	47.0^{b}	27.0^{a}
35	50.0^{a}	27.0^{a}

Letters show different groups at 0.01 level.

In callus culture studies conducted with the Gazipaşa and Silifke bitter melon genotypes, leaf and stem explants were isolated from 9-10 day old plantlets growing *in vitro* and *in vivo* media, and cultured in nutrient media containing 2,4-D at different concentrations (2 mg/l, 4 mg/l, 6 mg/l and 8 mg/l) (Figure 2). Analysis of variance was performed with the data obtained at the end of four weeks and the results of callus formation rates are given in Table 3 *(in vitro)* and Table 4 (*in vivo).*

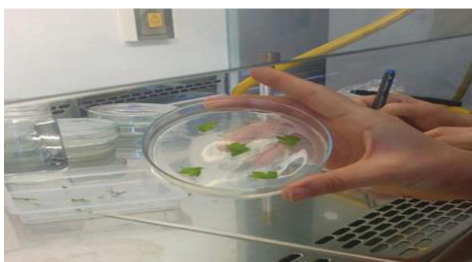

Figure 2. Cultivation of leaf explants of Gazipaşa bitter melon genotypes in MS + 2,4-D media

Table 3. Callus formation rate (%) after four weeks in leaf explants isolated from *in vitro* conditions

2.4-D (mg/l)	Callus Formation Rate (%)	
	Gazipaşa	Silifke
Control (MS0)	0.00^{c}	0.00^{c}
2.00	100.00^{a}	100.00^{a}
4.00	0.00^{c}	93.00^{b}
6.00	100.00^{a}	100.00^{a}
8.00	66.00^{b}	100.00^{a}

Letters show different groups at 0.01 level.

As shown in Table 3, the callus formation rate of leaves in the Gazipaşa and Silifke genotypes developed under *in vitro* conditions ranged from 0% to 100%. The highest callus formation rate in the Gazipasa genotype was observed in MS(1962) nutrient media containing 2% and 4 mg/l 2,4-D at 100%. The highest callus formation rate in the Silifke genotype was also obtained from MS medium containing 2, 6 and 8 mg/l 2,4-D with 100%.

Table 4. Callus formation rate (%) after four weeks in stem explants isolated from *in vitro* conditions

2.4-D (mg/l)	Callus Formation Rate (%)	
	Gazipaşa	Silifke
Control (MS0)	0.00^{c}	73.00^{b}
2.00	0.00^{c}	47.00^{c}
4.00	33.00^{b}	47.00^{c}
6.00	73.00^{a}	87.00^{a}
8.00	0.00^{c}	67.00^{b}

Letters show different groups at 0.01 level.

As shown in Table 4, the callus formation rate of stems in the Gazipaşa and Silifke genotypes developed under *in vitro* conditions ranged from 0% to 100%. In both of the Gazipaşa and Silifke genotypes, the highest callus formation rate was obtained on MS medium containing 6 mg/l 2,4-D with 73% and 87%, respectively.

One of the somatic embryos developed from the callus of the Silifke genotype in the control group [MS0] nutrient medium from the stem explant formed plantlets and this plantlet were adapted to the external conditions in the climate chamber (Şekil 3.). In both genotypes, callus formation occurred at the maximum level from medium containing 6 mg/l 2,4-D and a decrease in callus formation was observed in the Silifke genotype at 8 mg/l 2,4-D dose.

Callus formation was not observed in the Gazipaşa genotype on MS0 and MS medium containing 8 mg/l 2,4-D.

As shown in Table 5, the callus formation rate of leaves in the Gazipaşa and Silifke genotypes developed under *in vivo* conditions ranged from 0% to 87%.

Figure 3. Adapting the plantlet obtained from the stem explants of the Silifke genotypes through indirect somatic embryogenesis from the callus formed in the MS0 nutrient medium to the external conditions in the pot

The maximum callus formation rate was observed in the Silifke genotype with 87%. In the same genotype, there was a continuous decrease in callus formation after a dose of 2 mg/l 2,4-D, and no callus formation was observed in the medium containing 8 mg/l 2,4-D. In the Gazipaşa genotype, callus formation was observed only in the MS medium containing 2 mg/l 2,4-D.

Table 5. Callus formation rate (%) after four weeks in leaf explants isolated from *in vivo* conditions.

2.4-D (mg/l)	Callus Formation Rate (%)	
	Gazipaşa	Silifke
Control (MS0)	0.00^b	0.00^d
2.00	53.00^a	87.00^a
4.00	0.00^b	40.00^b
6.00	0.00^b	7.00^c
8.00	0.00^b	0.00^d

Letters show different groups at 0.01 level.

As shown in Table 6, the callus formation rate of stems in the Gazipaşa and Silifke genotypes developed under *in vivo* conditions ranged from 0% to 73%.

Table 6. Callus formation rate (%) after four weeks in stem explants isolated from *in vivo* conditions

2.4-D (mg/l)	Callus Formation Rate (%)	
	Gazipaşa	Silifke
Control (MS0)	20.00^c	67.00^b
2.00	47.00^a	60.00^c
4.00	40.00^b	73.00^a
6.00	40.00^b	60.00^c
8.00	0.00^d	73.00^a

Letters show different groups at 0.01 level.

The highest callus formation rate in the Gazipasa genotype was observed in MS food media containing 2% and 4 mg/l 2,4-D with 73%. In the Gazipaşa genotype, the highest callus formation occurred with 47% in the medium containing 2 mg/l 2,4-D, and callus formation started to decrease after this concentration. Callus formation was not observed on the medium containing 8 mg/l 2,4-D.

CONCLUSIONS

The difference in the germination rates of the Gazipaşa and Silifke bitter melon genotypes under *in vitro* and *in vivo* conditions may be due to seed shell hardness. The seeds of the Gazipaşa genotype are small, wrinkled and soft, while the seeds of the Silifke genotype are large, flat, and the shells are harder than those of the Gazipaşa bitter melon seed. The height of the plantlets growing under *in vivo* conditions in the pot were observed to be higher than that of the Silifke genotype. The germination rates of Gazipaşa and Silifke bitter melon seeds were high in both species under *in vivo* conditions. Also, under both conditions, the seeds of the Gazipaşa genotype showed higher germination.

In terms of callus formation rates, the media in which the explant was cultivated, 2,4-D concentrations, genotypes and explants were compared and a number of differences were observed in the obtained data. The highest callus formation was 100% in the explants obtained from *in vitro* media, whereas this rate was detected as 87% in the explants from *in vivo* media. The most effective 2,4-D concentration in explants taken from *in vitro* media was determined to be 6 mg/l in both genotypes and both used explants (leaf and stem). At the same time 2 mg/l 2,4-D in leaf explants caused maximum callus formation. The most effective concentration of auxin was found to be 2 mg/l in both types of leaf explants under in vivo conditions. The most effective concentration of auxin in the stem explant was 2 mg/l in the Gazipaşa genotype,4 and 8 mg/l 2,4-D in the Silifke genotype, resulting in maximum callus formation. Also, it was observed that the calli of the Gazipaşa

genotype were more easily dispersed than those of the Silifke genotype. In both *in vitro* and *in vivo* media, callus formation was found to be 73% and 67% in Silifke genotype, respectively, whereas it was 20% in Gazipaşa genotype under *in vivo* conditions.

The callus formation in control group was not detected at leaf explants for both culture conditions. Callus formation was observed higher in the leaf explant than that in the stem explant in both media from which the explant was taken.

However, some plantlets were obtained by indirect somatic embryogenesis from callus develoed from the stem explants of genotype Silifke and not from the leaf explants.

Although this is the case, one plantlet was obtained from callus formed from the stem explants of the Silifke genotype through indirect somatic embryogenesis and not from the leaf explants. The obtained plantlet was taken from *in vitro* conditions to the pots and transferred to external conditions and adaptation was maintained.

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