YYÜ TAR BİL DERG
(YYU J AGR SCI)
2013, 23(2): 164–171

Geliş Tarihi(Received):29.03.2013
KabulTarihi (Accepted):14.04.2013

Araştırma Makalesi/*Research Article (Original Paper)*

Callus Proliferation and Shoot Regeneration From Different Explant Types in Ornamental Gourd (*Cucurbita pepo* var. *ovifera*)

Burcu TUNCER

Department of Horticulture, Faculty of Agriculture, Yuzuncu Yil University, 65100 Van/Turkey
e-posta: brctuncer@gmail.com

Abstract: The effect of 4 different types of explants (proximal part, distal part, whole cotyledon, and hypocotyl) and 36 different hormone combinations on callus proliferation and shoot regeneration were evaluated in ornamental gourd (*Cucurbita pepo* var. *ovifera*). The explants were taken from 8-day-old seedling cultured on Murashige and Skoog (MS) media containing different doses of 2,4-dichlorophenoxyacetic acid (2,4-D) (2.0, 2.5, and 3.0 mg l^{-1}) for callus induction. Generated calli were cultured in combinations of 6-benzylaminopurine (BAP) (0, 0.5, 1.0 and 2.0 mg l^{-1}) with different doses of α-naphthaleneacetic acid (NAA) (0, 0.1 and 0.5 mg l^{-1}), indole-3-acetic acid (IAA) (0, 0.1 and 0.5 mg l^{-1}) and kinetin (0, 1.0 and 2.0 mg l^{-1}) in MS media. The maximum callus induction ratio (%) in all types of explants was obtained from MS media supplemented with 2.0 mg l^{-1} 2,4-D. BAP (1.0 and 2.0 mg l^{-1}) combinations with NAA (0.1 and 0.5 mg l^{-1}) were more successful in hypocotyl and proximal types of explants in terms of callus proliferation (%). The highest mean number of shoots per explant were obtained from 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} kinetin in hypocotyl (1.8) and proximal explants (2.6).

Key words: Callus, *Cucurbita pepo* var. *ovifera*, Organogenesis, Regeneration.

Süs Kabağında (*Cucurbita pepo* var. *ovifera*) Farklı Eksplant Tiplerinde Kallus Çoğalması ve Sürgün Rejenerasyonu

Özet: Süs kabağında (*Cucurbita pepo* var. *ovifera*), 4 farklı eksplant tipi (proksimal, distal, tüm kotiledon ve hipokotil) ve 36 farklı hormon kombinasyonunun kallus çoğalması ve sürgün rejenerasyonu üzerine etkisi araştırılmıştır. Kallus uyartımı için 8 günlük fidelerden alınan eksplantlar, Murashige and Skoog (MS) ortamında farklı dozlarda 2,4-D (2.0, 2.5 ve 3.0 mg l^{-1}) içeren ortamda kültüre alınmıştır. Oluşan kalluslar, MS ortamında BAP'ın (0, 0.5, 1.0, ve 2.0 mg l^{-1}) kombinasyonları ile birlikte NAA (0, 0.1 ve 0.5 mg l^{-1}), IAA (0, 0.1, 0.5 mg l^{-1}) ve kinetinin (0, 1.0 ve 2.0 mg l^{-1}) farklı dozlarında kültüre alınmıştır. Maximum kallus uyartımı (%) tüm eksplant tiplerinde MS+2.0 mg l^{-1}2,4-D ortamından elde edilmiştir. Kallus çoğalması (%) yönünden hipokotil ve proksimal eksplant tiplerinde BAP'ın (1.0 ve 2.0 mg l^{-1}) NAA (0.1 ve 0.5 mg l^{-1}) ile kombinasyonları daha başarılı bulunmuştur. Eksplant başına en yüksek sürgün sayısı hipokotil (1.8) ve proksimal eksplantlarında (2.6) 0.5 mg l^{-1} BAP+1.0 mg l^{-1} kinetin içeren ortamdan elde edilmiştir.

Anahtar kelimeler: *Cucurbita pepo* var. *ovifera*, Kallus, Organogenesis, Rejenerasyon.

Introduction

Ornamental gourds, affiliated to *Cucurbitaceae* family, are in general classified under *Cucurbita*, *Lageneria* and *Luffa* species. Smaller ornamental gourds, classified under *Cucurbita* species, and also known as pear bicolor ornamental gourd, are included within *Cucurbita pepo* var. *ovifera* botanical class. While stems and leaves of this species are prickly, and flowers thereof are in yellow color, its fruits are smooth, and in small pear shape, bottom half is in green, and top half is in yellow color. It is known that *C. pepo* ssp. *ovifera* var. *ovifera* originated in the Eastern USA (Decker 1988).

Plant biotechnology appears to be a viable option for the improvement of the *Cucurbita* species by means of plant tissue culture and genetic transformation. The establishment of an efficient *in vitro* plant regeneration system suitable for genetic transformation is the key step in this approach. Plant tissue

culture technique among vegetative proliferation techniques may successfully be applied among many gourd species of the *Cucurbitaceae* family, for instance in bottle gourd (*Lagenaria siceraria*) (Han et al. 2004; Saha et al. 2007; Shyamali and Hattori 2007; Haque et al. 2008; Yalçın-Mendi et al. 2009), figleaf gourd (*Cucurbita ficifolia*) (Kim et al. 2010), ash gourd (*Benincase hispida* L.) (Thomas and Sreejesh 2004), summer squash (*Cucurbita pepo* L.) (Ananthakrishnan *et al.* 2003; Pal *et al.* 2007), winter squash (*Cucurbita maxima* Duch.) (Lee et al. 2003), pumpkin (*Cucurbita moschata* Duch.) (Zhang et al. 2008), and thereby new plants may be generated by way of maintaining *in vitro* shoot regeneration.

The aim of this study was to investigate the effect of plant growth regulators on callus proliferation and adventitious shoot regeneration from different explant types of ornamental gourd. This is the first report on *in vitro* regeneration of *Cucurbita pepo* var. *ovifera.*

Materials and Methods

Explant preparation: Seeds of ornamental gourd (*Cucurbita pepo* var. *ovifera)* were obtained from Yalova province, Turkey. Seeds were disinfected with 10% sodium hypochlorite solution plus Tween-20 (2 drops per 100 ml of solution) for 10 min., then rinsed with bidistillated water 3 times. Sterilized seeds were placed on germination medium containing MS media (Murashige and Skoog 1962) supplemented with 3% sucrose and 0.7% agar in jars (3 seeds per jar). The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 20 min. The seedlings were exposed to 25±2° C, 16 h day^{-1} luminous photoperiodicity and 2000 lux intensity light. Cotyledon (proximal part, distal part and whole cotyledon) and hypocotyl explants were excised from 8-day-old seedlings.

Callus induction: Collected cotyledons (proximal part, distal part, and whole cotyledon) and hypocotyls from germinating seeds (8 days old) were cut into pieces. For callus induction, different doses of 2,4-D (2.0, 2.5 and 3.0 mg l^{-1}) were added to MS basic nutrient medium. All cultures were maintained at 25 ± 2 °C in the dark. After 4 weeks of culture, well-developed calli were recorded.

Callus proliferation and adventitious shoot induction: For adventitious shoot induction, calli were cultured in MS medium supplemented with different concentrations of BAP (0, 0.5, 1.0 and 2.0 mg l^{-1}) in combination with NAA (0, 0.1 and 0.5 mg l^{-1}), IAA (0, 0.1 and 0.5 mg l^{-1}) and kinetin (0, 1.0 and 2.0 mg l^{-1}). Cultures were kept at 25 ± 1°C under a 16/8-h (day $night^{-1}$) photoperiod with a light intensity of 2000 lux. After 4-5 weeks of culture, percentage of callus proliferation and nature of callus were determined.

Shoot elongation: Primary shoot primodium and calli were transferred to shoot elongation medium (0.1 mg l^{-1} GA_3 (gibberellic acid) + 1.0 mg l^{-1} BAP) containing MS nutrient medium. After 8 – 9 weeks of culture, plant regeneration efficiency was considered as number of shoots per explant.

Statistical analyses: In all experiments a minimum of three repetitions were cultured. Each single treatment consisted of five explants per jar. The obtained data were analyzed using factorial variance analysis. Angle and square root transformation were applied on the data prior to variance analysis. The data were analyzed statistically using SPSS ver. 13. and mean values were compared at the $p< 0.05$ level of significance using Duncan's multiple range test.

Results and Discussion

Effect of 2,4-D on callus induction: In the study, where various types of explants and 2,4-D (2.0, 2.5 and 3.0 mg l^{-1}) doses were tried, the highest callus induction (%) was obtained in all types of explants from MS medium, containing 2.0 mg l^{-1}2,4-D (Figure 1). While the highest callus induction was obtained from MS medium including 2.0 mg l^{-1} 2,4-D, where cotyledon (100%) and proximal part (97.9%) explants were cultured, they were followed by 2.5 mg l^{-1} 2,4-D whole cotyledon (95.8%) and 2.0 mg l^{-1} 2,4-D hypocotyl (91.6%) explants. Lower callus induction was observed from the distal parts of cotyledons in all of the media (Figure 1).

2,4-D is a synthetic auxin, widely used *in vitro* callus induction in numerous plant species. In a study conducted on 2 summer squash types, while the highest callus induction (86%) was achieved from the medium including MS+2.5 mg l^{-1} 2,4-D, where hypocotyl explants were cultured (Pal et al. 2007), the

same was achieved from cotyledon explants, and from MS medium including 4 µM 2,4-D in terms of ash gourd (Thomas and Sreejesh 2004).

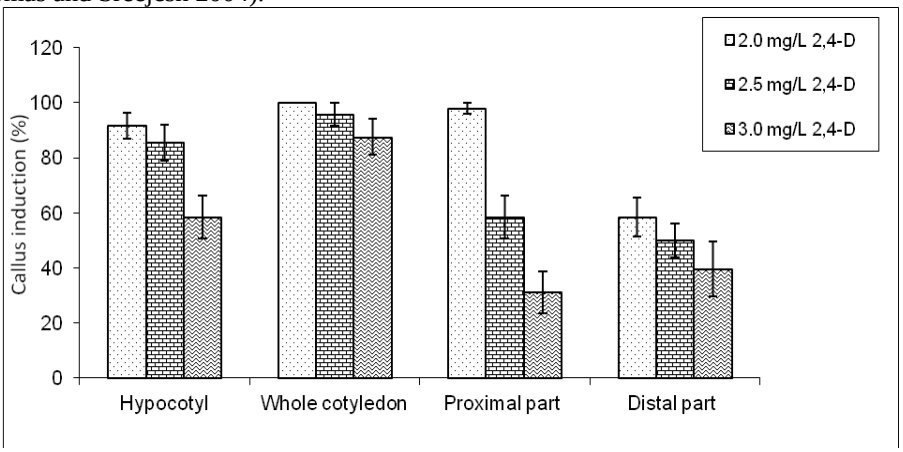

Figure 1. Effect of different concentrations of 2,4-D on callus induction in ornamental gourd. Data were recorded 4 weeks after culture. Vertical lines indicate standard error.

Callus proliferation and adventitious shoot induction: In order to stimulate the adventitious shoot formation, calli were cultured in MS medium including 36 different hormone combinations. Despite callus proliferation (Figure 2e-f-g-h), primary shoot primodium (Figure 2d) and different structures (Figure 2a-b-c) were found in the cultures 4-5 weeks later, no shoot elongation could have been achieved in any one of the medium and among any one of the types of explants. Callus proliferation (%) and nature of callus having occurred in different medium composition were given in Table 1.

While the highest values in terms of callus proliferation were obtained among hypocotyl explants by 100% from media no.8 (1 mg l^{-1} BAP+0.1 mg l^{-1} NAA), media no.12 (2 mg l^{-1} BAP+0.5 mg l^{-1} NAA), and media no.15 (0.5 mg l^{-1} IAA), among whole cotyledon explants by 93.7% from media no.3 (0.5 mg l^{-1} NAA), among proximal explants by 100% from media no.8 (1 mg l^{-1} BAP+0.1 mg l^{-1} NAA), media no.9 (1 mg/l BAP+0.5 mg l^{-1} NAA), and media no.11 (2 mg l^{-1} BAP+0.1 mg l^{-1} NAA), and among distal explants by 93.7% from media no.11 (2 mg l^{-1} BAP+0.1 mg l^{-1} NAA), and media no.12 (2 mg l^{-1} BAP+0.5 mg l^{-1} NAA) (Table 1). It has been found out that, calli having been obtained from hypocotyl and proximal explants were mostly in greenish compact callus (GC), and those from whole cotyledon explants were mostly in yellowish-brown compact (YBC) callus. Successful outcomes were achieved in terms of callus proliferation (%) generally from BAP (1-2 mg l^{-1}) and NAA (0.1-0.5 mg l^{-1}) combinations among hypocotyl and proximal explants (Table 1). Thiruvengadam et al. (2010) found that the combination of 7.7 µM NAA with 2.2 µM Thiadiazuron (TDZ) produced greenish compact callus from leaf explants in *Momordica charantia* L.

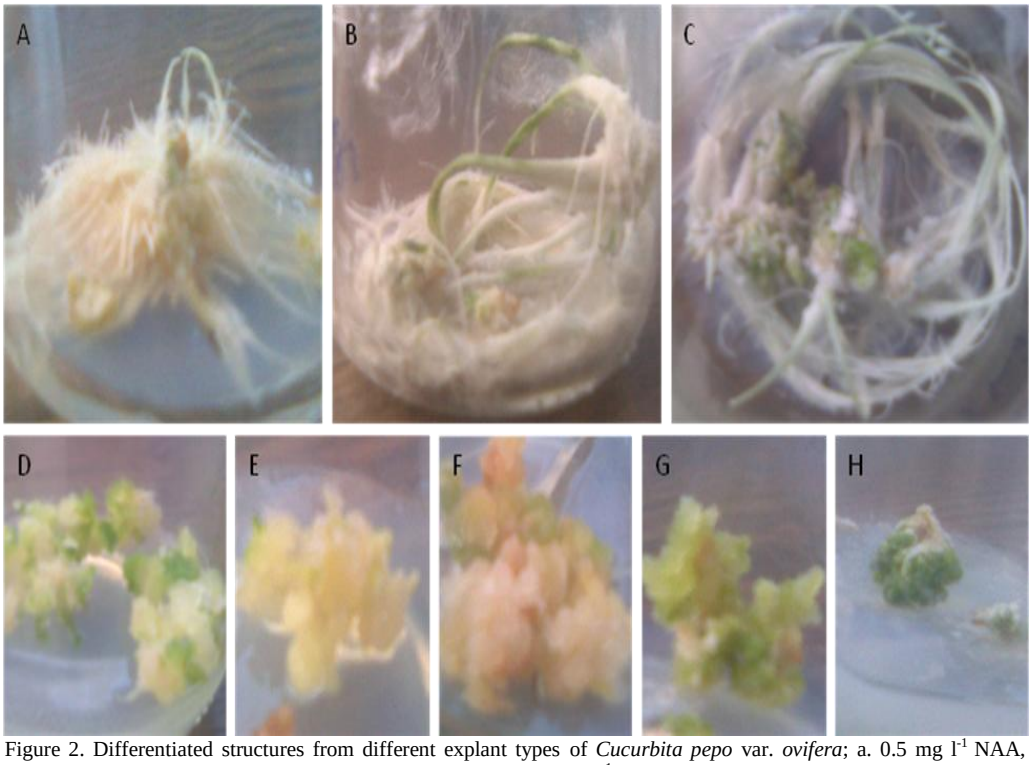

Figure 2. Differentiated structures from different explant types of *Cucurbita pepo* var. *ovifera*; a. 0.5 mg l^{-1} NAA, explant type: proximal part, b. 0.5 mg/l BAP + 0.1 mg l^{-1} NAA, explant type: proximal part, c. 2.0 mg/l BAP + 0.5 mg l^{-1} NAA, explant type: hypocotyl, d. Initiation of adventitious shoots from yellowish friable callus (2 mg l^{-1} BAP + 0.1 mg l^{-1} IAA, explant type: hypocotyl), e. Yellowish-brown friable callus (2 mg l^{-1} BAP, explant type: proximal part), f. Yellowish-brown compact callus (1 mg/l Kinetin, explant type: distal part), g. Yellowish-green friable callus (1 mg l^{-1} BAP + 0.5 mg l^{-1} NAA, explant type: proximal part), h. Greenish compact callus (1 mg l^{-1} BAP + 0.5 mg l^{-1} IAA, explant type: hypocotyl).

Shoot elongation: While callus proliferation was achieved in adventitious shoot induction medium, shoot elongation could not have been achieved in any one of the medium, and among any type of explants (Table 2).

While highest shoot regeneration was achieved among proximal explants via 2.6 shoot/explants from media no.29 (0.5 mg l^{-1} BAP + 1.0 mg l^{-1} kinetin), and via 1.6 shoot/explants from media no.30 (0.5 mg l^{-1} BAP+2 mg l^{-1} kinetin), and among hypocotyl explants via 1.8 shoot/explants from media no.29 (0.5 mg l^{-1} BAP+1 mg l^{-1} kinetin)(Table 2). Adventitious shoot regeneration obtained from calli was seen in Figure 3.

In this study, cultivation of hypocotyl and proximal explants of *Cucurbita pepo* var. *ovifera*, in MS medium including 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} kinetin, increased the number of shoots obtained per each of the explants. Combined use of different doses of BA and kinetin creates a synergistic effect on *in vitro* shoot induction (Shyamali and Hattori 2007; Saha *et al.* 2007). It was reported that, highest shoot regeneration was obtained from MS + 2 mg l^{-1} BA + 1 mg l^{-1} kinetin medium, in which cotyledon explants are cultured in the studies conducted on bottle gourds (*Lagenaria siceraria*) (Shyamali and Hattori 2007; Saha et al. 2007). It was reported in numerous literature that, success rate *in vitro* shoot induction varies, depending on the species, medium combinations, and on the types of explants. For instance, the highest shoot regeneration was achieved in *Cucurbita moschata* Duch., where cotyledon explants were cultured, from the medium including MS + 0.5 mg l^{-1} BA (63.7%) (Zhang et al. 2008), in *Cucurbita maxima* Duch., from the medium including MS + 1 mg l^{-1} BA (Lee et al. 2003). In a study conducted in *Cucurbita pepo* L., hypocotyl explants and MS + 0.5 mg l^{-1} thidiazuron medium were found to be successful in terms of shoot regeneration (85%) (Pal et al. 2007). On the other hand, it was reported that, while highest shoot regeneration was achieved among figleaf gourd from MS medium including 1.0 mg l^{-1} zeatin + 0.1 mg l^{-1} IAA, where cotyledon explants were cultured (Kim et al. 2010), in *Benincisa hispida* L., highest shoot regeneration (95%) among calli cultured from cotyledon explants was achieved from 4 µM BAP+0.2 µM NAA combination (Thomas and Sreejesh 2004).

Table 1. Effects of plant hormones on callus proliferation (%) from cotyledon (distal part, proximal part, whole cotyledon) and hypocotyl explants of ornamental gourd (*Cucurbita pepo* var. *ovifera)*

Media	**Percentage of callus proliferation and nature of callus**							
(mg/l)	**hypocotyl**	**NC**	**whole cotyledon**	**NC**	**proximal**	**NC**	**distal**	**NC**
BAP+NAA								
1	31.2±18.7$^{A\ def}$	GC	18.7±18.7$^{A\ def}$	YBC	37.5±23.9$^{A\ defgh}$	YBF	0.0±0.0$^{A\ g}$	-
2	50.0±10.2$^{A\ bcdef}$	GC	50.0±28.8$^{A\ abcdef}$	YBC	43.7±21.3$^{A\ cdefg}$	GC	43.7±18.7$^{A\ cde}$	GC
3	50.0±10.2$^{B\ bcdef}$	GC	93.7±6.2$^{A\ a}$	YF	62.5±16.1$^{B\ abcde}$	YBF	0.0±0.0$^{C\ g}$	-
4	0.0±0.0$^{A\ f}$	-	50.0±22.8$^{A\ abcdef}$	GC	31.2±6.2$^{A\ defgh}$	YBC	31.2±11.9$^{A\ def}$	YBC
5	75.0±25.0$^{A\ abcd}$	GC	75.0±17.6$^{A\ abc}$	GC	62.5±21.6$^{A\ abcde}$	GC	12.5±12.5$^{A\ fg}$	YBC
6	37.5±21.6$^{AB\ cdef}$	YBC	68.7±23.6$^{A\ abcd}$	GC	43.7±11.9$^{AB\ cdefg}$	GC	0.0±0.0$^{B\ g}$	-
7	6.2±6.2$^{A\ fg}$	YF	31.2±18.7$^{A\ cdef}$	GC	37.5±12.5$^{A\ defgh}$	GC	25.0±14.4$^{A\ efg}$	YBC
8	100.0±0.0$^{A\ a}$	GC	56.2±21.3$^{B\ abcde}$	GC	100.0±0.0$^{A\ a}$	GC	0.0±0.0C g	-
9	75.0±25.0$^{A\ abcd}$	GC	50.0±28.8$^{A\ abcdef}$	GC	100.0±0.0$^{A\ a}$	GC	75.0±10.2$^{A\ ab}$	GC
10	56.2±21.3$^{A\ abcde}$	GC	62.5±16.1$^{A\ abcde}$	YBC	37.5±12.5$^{A\ defgh}$	YBF	56.2±18.7$^{A\ bcd}$	YBC
11	43.7±25.7$^{A\ bcdef}$	GC	43.7±25.7$^{A\ abcdef}$	GC	100.0±0.0$^{A\ a}$	GC	93.7±6.2$^{A\ a}$	GC
12	100.0±0.0$^{A\ a}$	GC	75.0±14.4$^{A\ abc}$	YBC	93.7±6.2$^{A\ ab}$	GC	93.7±6.2$^{A\ a}$	GC
BAP+IAA								
13	0.0±0.0$^{A\ f}$	-	0.0±0.0$^{A\ f}$	-	56.2±11.9$^{A\ bcdef}$	YBF	0.0±0.0$^{B\ g}$	-
14	50.0±1.4$^{B\ bcdef}$	YF	0.0±0.0$^{C\ f}$	-	0.0±0.0$^{C\ h}$	-	87.5±7.2$^{A\ a}$	YBF
15	100.0±0.0$^{A\ a}$	GC	25.0±10.2$^{B\ cdef}$	YBC	18.7±11.9$^{B\ fgh}$	YBC	0.0±0.0$^{B\ g}$	-
16	43.7±25.7$^{AB\ bcdef}$	GC	18.7±11.9$^{B\ def}$	YBF	6.2±6.2$^{B\ gh}$	YF	75.0±10.2$^{A\ ab}$	YF
17	25.0±17.6$^{A\ def}$	GC	0.0±0.0$^{B\ f}$	-	12.5±7.2$^{B\ gh}$	GC	68.7±11.9$^{A\ abc}$	YBF
18	56.2±21.3$^{AB\ abcde}$	YBF	31.2±11.9$^{BC\ cdef}$	YBC	0.0±0.0C ^{h}	-	87.5±7.2$^{A\ a}$	YBF
19	18.7±11.9$^{A\ ef}$	YGC	37.5±12.5$^{A\ abcdef}$	YBC	25.0±14.4$^{A\ efgh}$	YBF	12.5±7.2$^{A\ fg}$	YBC
20	50.0±17.6$^{A\ bcdef}$	GC	56.2±11.9$^{A\ abcde}$	GC	6.2±6.2$^{B\ gh}$	YBC	6.2±6.2$^{B\ fg}$	YF
21	75.0±10.2$^{A\ abcd}$	GC	0.0±0.0$^{B\ f}$	-	56.2±25.7$^{A\ bcdef}$	GC	0.0±0.0$^{B\ g}$	-
22	0.0±0.0$^{C\ g}$	-	31.2±11.9$^{B\ cdef}$	YF	68.7±11.9$^{A\ abcd}$	YBC	0.0±0.0$^{C\ g}$	-
23	81.2±11.9$^{A\ abc}$	GC	37.5±12.5$^{B\ bcdef}$	YBC	0.0±0.0$^{C\ h}$	-	0.0±0.0$^{C\ g}$	-
24	12.5±12.5$^{A\ ef}$	YBF	0.0±0.0$^{A\ f}$	-	0.0±0.0$^{A\ h}$	-	0.0±0.0$^{A\ g}$	-
BAP+Kinetin								
25	43.7±15.7$^{AB\ bcdef}$	GC	31.2±15.7$^{AB\ cdef}$	YBF	56.2±6.2$^{A\ bcdef}$	GC	6.2±6.2$^{B\ fg}$	YBC
26	25.0±14.4$^{AB\ def}$	YF	37.5±7.2$^{A\ bcdef}$	YBF	0.0±0.0$^{B\ h}$	-	43.7±6.2$^{A\ cde}$	YBC
27	0.0±0.0$^{C\ f}$	-	0.0±0.0$^{C\ f}$	-	43.7±21.3$^{B\ cdefg}$	YBF	87.5±12.5$^{A\ a}$	YBC
28	0.0±0.0$^{B\ b}$	-	68.7±6.2$^{A\ abcd}$	YF	6.2±6.2$^{B\ gh}$	YBF	25.0±14.4$^{B\ efg}$	YBC
29	56.2±6.2$^{B\ abcde}$	GC	0.0±0.0$^{C\ f}$	-	93.7±6.2$^{A\ ab}$	GC	0.0±0.0$^{C\ g}$	-
30	56.2±6.2$^{B\ abcde}$	GC	43.7±6.2$^{B\ abcdef}$	YBF	81.2±6.2$^{A\ abc}$	GC	6.2±6.2$^{C\ fg}$	YBC
31	68.7±23.6$^{A\ abcd}$	GC	12.5±12.5$^{B\ b}$	YBF	31.2±6.2$^{B\ defgh}$	GC	18.7±11.9$^{B\ efg}$	YBC
32	87.5±7.2$^{A\ ab}$	GC	75.0±17.6$^{A\ abc}$	YBC	0.0±0.0$^{B\ h}$	-	0.0±0.0$^{B\ b}$	-
33	50.0±10.2$^{B\ bcdef}$	GC	12.5±7.2$^{C\ ef}$	YBC	0.0±0.0$^{C\ h}$	-	87.5±7.2$^{A\ a}$	YBF
34	43.7±11.9$^{A\ bcdef}$	YBF	50.0±0.0$^{A\ abcdef}$	YBC	18.7±6.2$^{B\ fgh}$	YBC	0.0±0.0$^{B\ g}$	-
35	0.0±0.0$^{B\ f}$	-	62.5±21.6$^{A\ abcde}$	YBC	87.5±7.2$^{A\ ab}$	GC	0.0±0.0$^{B\ g}$	-
36	75.0±10.2$^{A\ abcd}$	YBF	87.5±7.2$^{A\ ab}$	YBC	43.7±25.7$^{A\ cedfg}$	YBC	0.0±0.0$^{B\ g}$	-

Different small letters in a same column show significant differences among mean media ($p<0.05$)
Different capital letter in a same line show significant differences among the mean explant ($p<0.05$)
NC: Nature of callus, YF: Yellowish friable, YBF: Yellowish-brown friable, YBC: Yellowish-brown compact, YGC: Yellowish-green compact, GC: Green compact

Table 2. Combination effect of plant growth regulators on adventitious shoot regeneration from cotyledon (distal part, proximal part, whole cotyledon) and hypocotyl explants in ornamental gourd (*Cucurbita pepo* var. *ovifera)*

Media no	Media (mg/l)	Number of shoots per explant			
		hypocotyl	whole cotyledon	proximal	distal
	BAP+NAA				
1	0 + 0	$0.0\pm0.0^{A\ d}$	$0.6\pm0.4^{A\ ab}$	$0.8\pm0.4^{A\ cdef}$	$0.0\pm0.0^{A\ c}$
2	0 + 0.1	$0.0\pm0.0^{B\ d}$	$0.5\pm0.3^{AB\ ab}$	$1.4\pm0.5^{A\ bc}$	$0.8\pm0.5^{AB\ a}$
3	0 + 0.5	$0.0\pm0.0^{A\ d}$	$0.0\pm0.0^{A\ b}$	$0.4\pm0.3^{A\ ef}$	$0.0\pm0.0^{A\ c}$
4	0.5 + 0	$0.0\pm0.0^{A\ d}$	$0.0\pm0.0^{A\ b}$	$0.0\pm0.0^{A\ f}$	$0.0\pm0.0^{A\ c}$
5	0.5 + 0.1	$0.0\pm0.0^{A\ d}$	$0.4\pm0.3^{A\ ab}$	$0.0\pm0.0^{A\ f}$	$0.1\pm0.1^{A\ c}$
6	0.5 + 0.5	$0.0\pm0.0^{A\ d}$	$0.0\pm0.0^{A\ b}$	$0.0\pm0.0^{A\ f}$	$0.0\pm0.0^{A\ c}$
7	1 + 0	$0.2\pm0.1^{A\ d}$	$0.1\pm0.1^{A\ b}$	$0.3\pm0.2^{A\ ef}$	$0.4\pm0.2^{A\ abc}$
8	1 + 0.1	$0.0\pm0.0^{A\ d}$	$0.0\pm0.0^{A\ b}$	$0.0\pm0.0^{A\ f}$	$0.0\pm0.0^{A\ c}$
9	1 + 0.5	$0.5\pm0.3^{A\ d}$	$0.4\pm0.3^{A\ ab}$	$0.06\pm0.06^{A\ f}$	$0.1\pm0.1^{A\ c}$
10	2 + 0	$0.0\pm0.0^{A\ d}$	$0.4\pm0.3^{A\ ab}$	$0.3\pm0.1^{A\ ef}$	$0.4\pm0.2^{A\ abc}$
11	2 + 0.1	$0.0\pm0.0^{B\ d}$	$0.0\pm0.0^{B\ b}$	$0.6\pm0.3^{A\ def}$	$0.0\pm0.0^{B\ c}$
12	2 + 0.5	$0.0\pm0.0^{A\ d}$	$0.0\pm0.0^{A\ b}$	$0.0\pm0.0^{A\ f}$	$0.0\pm0.0^{A\ c}$
	BAP+IAA				
13	0 + 0	$0.0\pm0.0^{B\ d}$	$0.0\pm0.0^{B\ b}$	$0.5\pm0.3^{A\ ef}$	$0.0\pm0.0^{B\ c}$
14	0 + 0.1	$0.0\pm0.0^{B\ d}$	$0.0\pm0.0^{B\ b}$	0.0 ± 0.0^{B}	$0.5\pm0.2^{A\ abc}$
15	0 + 0.5	$1.3\pm0.0^{A\ ab}$	$0.2\pm0.1^{B\ ab}$	0.0 ± 0.0^{B}	$0.0\pm0.0^{B\ c}$
16	0.5 + 0	$0.0\pm0.0^{B\ d}$	$0.0\pm0.0^{B\ b}$	$0.2\pm0.2^{AB\ ef}$	$0.4\pm0.1^{A\ abc}$
17	0.5 + 0.1	$0.0\pm0.0^{B\ d}$	$0.0\pm0.0^{B\ b}$	$0.0\pm0.0^{B\ f}$	$0.4\pm0.1^{A\ abc}$
18	0.5 + 0.5	$0.6\pm0.3^{A\ d}$	$0.2\pm0.2^{B\ ab}$	$0.0\pm0.0^{B\ f}$	$0.6\pm0.2^{A\ ab}$
19	1 + 0	$0.2\pm0.1^{A\ d}$	$0.0\pm0.0^{A\ b}$	$0.2\pm0.1^{A\ ef}$	$0.0\pm0.0^{A\ c}$
20	1 + 0.1	$0.3\pm0.1^{B\ d}$	$0.9\pm0.5^{A\ a}$	$0.0\pm0.0^{B\ f}$	$0.0\pm0.0^{B\ c}$
21	1 + 0.5	$0.0\pm0.0^{B\ d}$	$0.0\pm0.0^{B\ b}$	$1.0\pm0.4^{A\ bcde}$	$0.0\pm0.0^{B\ c}$
22	2 + 0	$0.0\pm0.0^{B\ d}$	$0.0\pm0.0^{B\ b}$	$0.6\pm0.3^{A\ cdef}$	$0.0\pm0.0^{B\ c}$
23	2 + 0.1	$1.2\pm0.4^{A\ ab}$	$0.0\pm0.0^{B\ b}$	$0.0\pm0.0^{B\ f}$	$0.0\pm0.0^{B\ c}$
24	2 + 0.5	$0.1\pm0.1^{A\ d}$	$0.0\pm0.0^{A\ b}$	$0.0\pm0.0^{A\ f}$	$0.0\pm0.0^{A\ c}$
	BAP+Kin				
25	0 + 0	$0.0\pm0.0^{B\ d}$	$0.0\pm0.0^{B\ b}$	$1.0\pm0.4^{A\ bcde}$	$0.06\pm0.06^{B\ c}$
26	0 + 1.0	$0.0\pm0.0^{A\ d}$	$0.0\pm0.0^{A\ b}$	$0.0\pm0.0^{A\ f}$	$0.3\pm0.2^{A\ bc}$
27	0 + 2.0	$0.0\pm0.0^{B\ d}$	$0.0\pm0.0^{B\ b}$	$0.9\pm0.4^{A\ bcde}$	$0.0\pm0.0^{B\ c}$
28	0.5 + 0	$0.0\pm0.0^{B\ d}$	$0.6\pm0.4^{A\ ab}$	$0.0\pm0.0^{B\ f}$	$0.06\pm0.06^{B\ c}$
29	0.5 + 1.0	$1.8\pm0.6^{A\ a}$	$0.0\pm0.0^{A\ b}$	$2.6\pm0.5^{A\ a}$	$0.0\pm0.0^{A\ c}$
30	0.5 + 2.0	$1.2\pm0.4^{A\ bc}$	$0.6\pm0.3^{A\ ab}$	$1.6\pm0.3^{A\ b}$	$0.0\pm0.0^{B\ c}$
31	1 + 0	$0.0\pm0.0^{B\ d}$	$0.3\pm0.2^{AB\ ab}$	$0.0\pm0.0^{B\ f}$	$0.4\pm0.3^{A\ abc}$
32	1 + 1.0	$0.0\pm0.0^{B\ d}$	$0.6\pm0.3^{A\ ab}$	$0.0\pm0.0^{B\ f}$	$0.0\pm0.0^{B\ c}$
33	1 + 2.0	$0.2\pm0.1^{B\ d}$	$0.2\pm0.2^{B\ ab}$	$0.0\pm0.0^{B\ f}$	$0.8\pm0.3^{A\ a}$
34	2 + 0	$0.7\pm0.4^{A\ cd}$	$0.0\pm0.0^{B\ b}$	$0.4\pm0.2^{AB\ ef}$	$0.0\pm0.0^{B\ c}$
35	2 + 1.0	$0.0\pm0.0^{B\ d}$	$0.9\pm0.5^{A\ a}$	$1.3\pm0.4^{A\ bcd}$	$0.0\pm0.0^{B\ c}$
36	2 + 2.0	$0.4\pm0.3^{A\ d}$	$0.0\pm0.0^{A\ b}$	$0.1\pm0.09^{B\ f}$	$0.0\pm0.0^{B\ c}$

Different small letters in a same column show significant differences among mean media ($p<0.05$)
Different capital letter in a same line show significant differences among the mean explant ($p<0.05$)
That was why, calli having been obtained from adventitious shoot induction medium were taken to shoot elongation medium (0.1 mg l^{-1} GA_3 + 1.0 mg l^{-1} BAP).

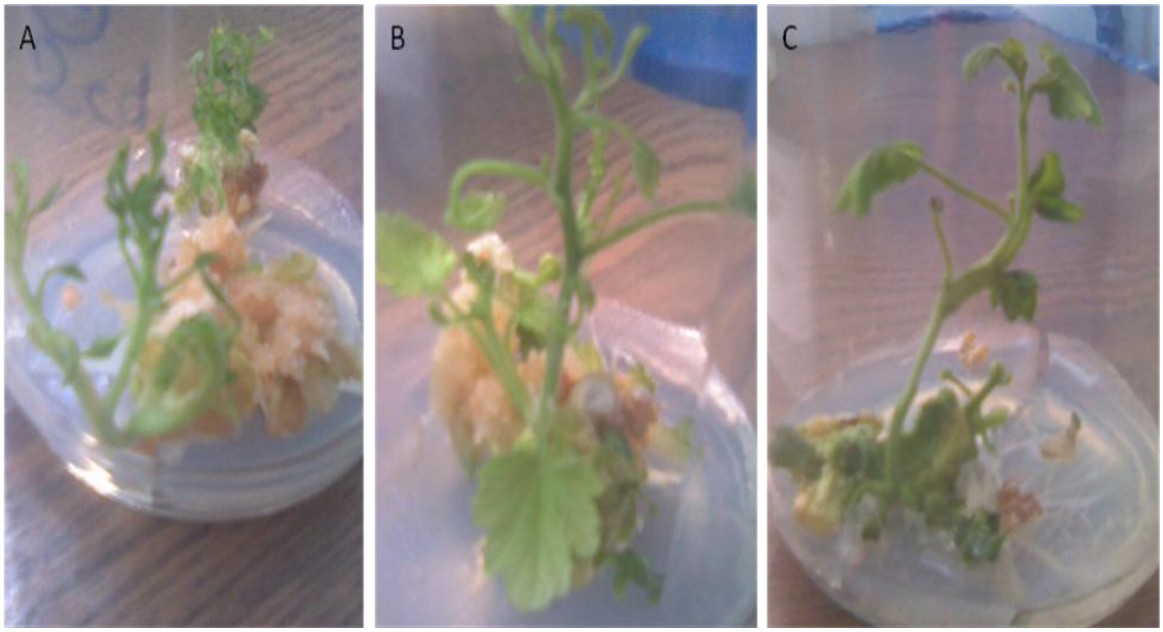

Figure 3. Regeneration of adventitious shoots from different explant types callus of *Cucurbita pepo* var. *ovifera* a. (0.5 mg l^{-1} BAP + 1.0 mg l^{-1} kinetin, explant type: hypocotyl), b. (0.5 mg l^{-1} BAP + 1.0 mg l^{-1} kinetin, explant type: proximal part), c. (0.5 mg l^{-1} BAP + 2.0 mg l^{-1} kinetin, explant type: proximal part)

Conclusion

In conclusion, in this study, where the effect of different types of explants and nutrient medium on regeneration was investigated, plant regeneration protocol was developed for *C.pepo* var. *ovifera*. In the study, where numerous media were tried, no problem was encountered in terms of callus proliferation; however limited number of shoots could have been obtained from the calli. That was why, calli having been cultured in adventitious shoot induction medium were taken to shoot elongation medium (0.1 mg l^{-1} GA_3 + 1.0 mg l^{-1} BAP) so as to achieve shoot elongation. The highest shoot regeneration from the calli taken to elongation medium has been obtained from MS medium of hypocotyl and proximal explants, including 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} kinetin. Due to the lower rate of success in shoot elongation, it is considered that further studies need to be conducted on this species regarding, combined and/or separate effect of different doses of BAP and GA_3 on *in vitro* shoot regeneration.

Acknowledgement: The author would like to thank Dr. Arzu Cig for her assistance during labarotory work. Thanks also to Associate Prof. Dr. Sıddık Keskin for performing the statistical analysis.

References

Ananthakrishnan G, Xia X, Elman C, Singer S, Paris H. S, Gal-On A, Gaba V (2003). Shoot production in squash (*Cucurbita pepo*) by in vitro organogenesis. Plant Cell Rep. 21: 739–746.

Decker DS (1988). Origin(s), Evolution, and Systematics of *Cucurbita pepo* (*Cucurbitaceae*). Economic Botany. 42 (1): 4-15.

Han JS, Oh DG, Mok IG, Park HG, Kim CK (2004). Efficient plant regeneration from cotyledon explants of bottle gourd (*Lagenaria siceraria* Standl.). Plant Cell Rep. 23:291–296.

Haque ME, Sarkar MAR., Mahmud MA, Rezwana D, Sikdar B (2008). *In vitro* propagation of pumpkin and ash gourd through nodal segments. J. Bio-Sci. 16: 67-71.

Kim KM, Kim CK, Han JS (2010). In vitro regeneration from cotyledon explants in figleaf gourd (*Cucurbita ficifolia* Bouche´), a rootstock for *Cucurbitaceae*. Plant Biotechnol Rep. 4: 101–107.

Lee YK, Chung WI, Ezura H (2003). Efficient plant regeneration via organogenesis in winter squash (*Cucurbita maxima* Duch.). Plant Science. 164: 413-418.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15: 473–497.

Pal SP, Alam I, Anisuzzaman M, Sarker KK, Sharmin SA, Alam MF (2007). Indirect Organogenesis in Summer Squash (*Cucurbita pepo* L.). Turk J Agric. For. 31: 63-70.

Saha S, Mori H, Hattori K (2007). Synergistic effect of kinetin and benzyl adenine plays a vital role in high frequency regeneration from cotyledon explants of bottle guard (*Lagenaria siceraria*) in relation to ethylene production. Breeding Science. 57: 197-202.

Shymali S, Hattori K (2007). Effect of polyamines and silver nitrate on the high frequency regeneration from cotyledon explants of bottle gourd (*Lagenaria siceraria*; sp. asiatica). Pakistan Journal of Biological Sciences. 10(8): 1288-1293.

Thiruvengadam M, Rekha KT, Yang CH, Jayabalan N, Chung IM (2010). High-frequency shoot regeneration from leaf explants through organogenesis in bitter melon (*Momordica charantia* L.). Plant Biotechnol Rep. 4: 321–328.

Thomas TD, Sreejesh KR (2004). Callus induction and plant regeneration from cotyledonary explants of ash gourd (*Benincasa hispida* L.). Scientia Horticulturae. 100: 359–367.

Yalçın Mendi Y, İpek M, Buzkan N, Aka Kacar Y, Curuk S (2009). Regeneration and histological analysis of regeneration in bottle gourd (*Lagenaria siceraria* (Molina) Stand.). Turk J Agric. For. 33: 165-172.

Zhang Y, J Zhou, Wu T, Cao J (2008). Shoot regeneration and the relationship between organogenic capacity and endogenous hormonal contents in pumpkin. Plant Cell Tiss Organ Cult. 93: 323–331.