

Full Length Research Paper

Effects of different combinations and concentrations of growth regulators and photoperiod on somatic embryogenesis of *Cucumis melo* var. *flexuosus*

Nuray Comlekcioglu^{1*}, Yesim Yalcin Mendi², Selay Eldogan² and Ceren Unek²

¹Department of Horticulture, Faculty of Agriculture, University of Harran, Sanliurfa, Turkey.

²Laboratory for Biotechnology, Department of Horticulture, Faculty of Agriculture, University of Cukurova, Adana, Turkey.

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The present study describes the effects of medium, explant type and photoperiod on somatic embryogenesis of snake melon (*Cucumis melo* var. *flexuosus*). Two different types of explant (cotyledon and hypocotyls) obtained from 3 days old snake melon seedlings and culture conditions were investigated for somatic embryogenesis. Explants were cultured on Murashige and Skoog (1962) (MS) medium supplemented with 23 different combinations of auxins (2,4-dichlorophenoxyacetic acid, 2,4-D; α -naphthalene acetic acid, NAA) and cytokinin (6-benzylaminopurine, BAP; N6-[2-isopentyl]adenine, 2iP) either alone or in combination with each other. Embryogenic calluses were obtained from cotyledon explant on MS media supplemented with NAA and 2,4-D alone and combinations with cytokinin. Our findings showed that NAA and 2,4-D did not act synergistically with BAP or 2iP. Callus formation was the same whether BAP or 2iP was added into the media or not. The highest embryo formation was achieved from cotyledon explant which was 20.00 ± 7.94 somatic embryos per petri dish on medium supplemented with 4 mg/l 2,4-D and 0.5 mg/l BAP. The cotyledon and hypocotyls explants cultured on the media with 2 mg/l 2,4-D with 0.5 mg/l BAP showed embryogenic callus formation and number of somatic embryos were 17.33 ± 5.51 and 16.33 ± 3.06 per petri dish, respectively. Auxin was found critical for formation of somatic embryo and at the same time, the two tested cytokinins at any concentration acted synergistically with auxin.

Key words: *Cucumis melo* var. *flexuosus*, somatic embryo, cotyledon, hypocotyl.

INTRODUCTION

Snake melon (*Cucumis melo* var. *flexuosus*) is a traditionally cultivated vegetable crop grown during summer in tropical and subtropical conditions. It is commonly harvested while fruit is still green and consumed as a vegetable whether fresh, cooked or pickled in some Asiatic and African countries (Besirli and Yanmaz, 1999).

*Corresponding author. E-mail: nuray@harran.edu.tr, nuraycom01@hotmail.com. Tel: +90 414 344 00 72. Fax: +90 414 344 00 73.

Abbreviations: BAP, 6-Benzylaminopurine; 2,4-D, 2,4-dichlorophenoxyacetic acid; NAA, α -naphthalene acetic acid; 2iP, N6-[2-isopentyl]adenine; SE, somatic embryogenesis or somatic embryos; MS, Murashige and Skoog basal medium.

Somatic embryogenesis is a significant method for mass propagation, plant regeneration for successful transformation and to develop genetically uniform plants (Etienne et al., 2002; Thomas and Sreejesh, 2004; Thakare et al., 2008; Gatica-Arias et al., 2008; Lee et al., 2003; Martin, 2004). It is also used when evaluating growth, differentiation, de-differentiation and development of plant cells (Ikeda-Iwai et al., 2003; Martin, 2004). Somatic embryogenesis is also important to obtain artificial seed (Büyükalaca and Mavituna, 1995; Vicient and Martinez, 1998; Nakagawa et al., 2001; Maruyama et al., 2003; Grabowska et al., 2009) clonal propagation by somatic embryo culture and germplasm conservation through somatic embryogenesis (Maruyama et al., 2003).

Several protocols have been established for plant regeneration of *cucurbits* via somatic embryogenesis

from different explant type (Chee, 1990; Choi et al., 1994; Nakagawa et al., 2001; Chaturvedi and Bhatnagar, 2001; Lee et al., 2003; Thomas and Sreejesh, 2004; Park et al., 2005; Pal et al., 2007; Selvaraj et al., 2007; Cho et al., 2008; Shah et al., 2008) nevertheless, there are no reports on somatic embryogenesis of *Cucumis melo* var. *flexuosus*.

Somatic embryogenesis can be limited by various factors such as genotype, explant type, culture media composition, growth regulators, and photoperiod (Gonzalez et al., 2001; Corredoira et al., 2003).

The objectives of the present study were to develop and optimize an efficient *in vitro* regeneration protocol by somatic embryogenesis for *Cucumis melo* var. *flexuosus* and to investigate effects of different explant type, growth regulators and photoperiod on embryogenic callus formation and somatic embryo.

MATERIALS AND METHODS

Two explant types (hypocotyls and cotyledon) of *Cucumis melo* var. *flexuosus* using different concentrations and combinations of auxin (2,4-D - NAA) and cytokinin (BAP - 2 iP) were studied to obtain somatic embryo. Regeneration media were MS medium supplemented with different combinations of:

2,4-D (0, 2, 4 mg/l) and BAP (0.5, 0.1 mg/l),
NAA (2, 4 mg/l) and BAP (0.1, 0.5 mg/l),
2,4-D (0, 2, 4 mg/l) and 2ip (0.1, 0.5 mg/l)
MS medium without hormones (control).

All media were supplemented with 30 g.l⁻¹ sucrose, 7 g l⁻¹ Bacto agar and the pH was adjusted to 5.7 before autoclaving (121 °C, 15 min).

Pilled seeds were surface-sterilized by soaking in 70% (v/v) ethanol for 1-2 min, 10 min in 1% sodium hypochlorite containing 1% tween 20, rinsed three times in sterile water and germinated on hormone free MS (Murashige and Skoog, 1962) medium. The seeds were incubated at 25 °C under 8/16 h dark/light photoperiod. Three days-old cotyledons and hypocotyls were used as explants in this experiment. The cotyledons and hypocotyls were cut into two pieces and explants were cultured abaxial side down on the media. Six explants were cultured in each petri dish for primary callus induction. Each replicate contained five petri dishes (each experiment contained at least 30 explants). Cultures were incubated in a growth room at 25±2 °C in darkness for four weeks. The primary callus was then divided and subcultured on either hormone-free MS medium or supplemented with different combination of growth regulators, at 25±2 °C under 8/16 h dark/light photoperiod or complete darkness. Three petri dishes were used for each combination. Observations and counting of somatic embryos were made after seven weeks, and standard error of the mean was calculated. The data were subjected to standard analysis of variance for a randomized complete block design using TARIST (Acikgoz et al., 2004) statistical software. Duncan's multiple-range test was used for means separation.

RESULTS AND DISCUSSION

Callus induction

Different type of calluses developed from explants 4 to 6

weeks after culturing. This depends on the concentration and type of growth regulators in the medium. Callus formation was affected by the type of explants, concentration, combination and type of growth regulators in the medium. No callus formation was detected from both explant types on hormone free MS, both on the medium containing only 0.1 mg/l BAP at complete darkness or photoperiod conditions and on the medium containing only 2iP (0.1 and 0.5 mg/l) or only BAP (0.5 mg/l) at photoperiod. Small amount of callus developed at complete darkness from the medium with 2iP (0.1 and 0.5 mg/l). BAP or 2iP alone induced friable soft- spongy and yellowish or creamy-white callus (Plate 1, a and b) which turned to brown color and died or failed to regenerate to direct roots (Plate 1c and d). Green compact and nodular callus (Plate 1e and f), which developed into direct root proliferation or embryogenic structures, were obtained from culture media with NAA and 2,4-D alone and combinations with BAP or 2iP.

Maximum amounts of callus were obtained from media containing NAA and 2,4-D alone. The callus texture and amount developed on medium supplemented with NAA or 2,4-D in combination with BAP or 2iP were similar to those from NAA and 2,4-D alone. Our findings showed that NAA and 2,4-D did not act synergistically with BAP or 2iP. Ali et al. (2007) reported similar findings. Adding BAP or 2iP into the media did not show enough callus formation.

The callus developed on the medium high concentration 2,4-D or NAA was the most suitable for induction of somatic embryogenesis. Similar results were obtained by Gonzales et al. (2001), who reported that callus induction differ between genotypes and culture media with respect to the percentage of calli formed, their morphology, frequency and type of embryogenic callus. These could be manipulated by changing the composition of the medium. It was reported that NAA was necessary for callus formation and even at low concentration (0.05 mg/l) it was effective on callus formation (Ozden et al., 2008).

Somatic embryo formation

Somatic embryo (SE) formation was affected by the growth regulators and photoperiod respectively. Hormone free MS medium induced no somatic embryogenic response at all in both explant types. SE was obtained only from explants cultured at photoperiodic condition (Plate 2). No SE was observed from the culture at complete darkness. Similar to our results, Ali et al. (2007) reported that direct somatic embryos occurred on leaf explant incubated at 16 h light and 8 h dark conditions in sugarcane (*Saccharum officinarum*). The cultures placed under complete darkness did not produce somatic embryos. Hoshino and Cuello (2006) reported similar findings that light quality and intensity is one of the critical

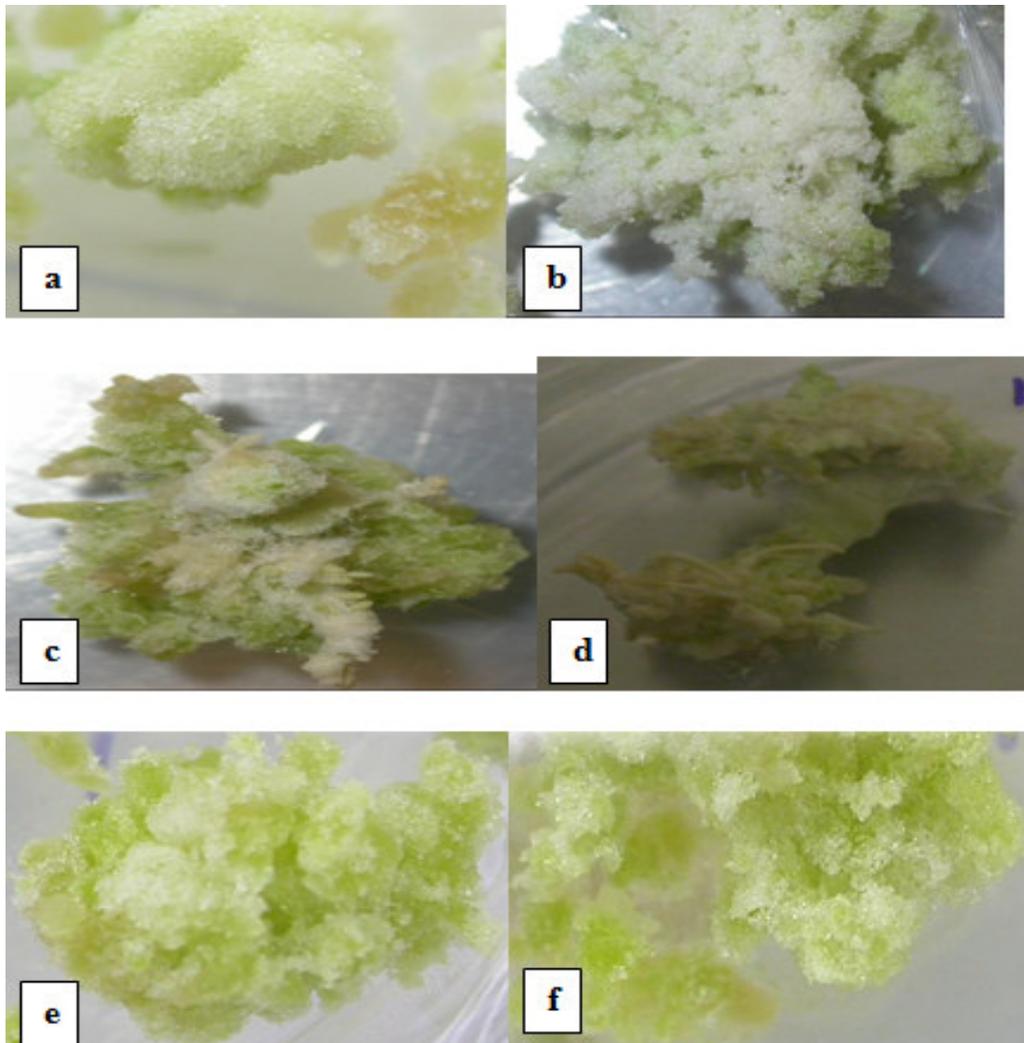


Plate 1. Different type of callus derived from cotyledon explants 4 to 6 weeks after culturing. **a** and **b**: Friable soft- spongy and yellowish or creamy-white callus on MS medium containing BAP. **c** and **d**: Regeneration of direct roots on medium containing BAP. **e** and **f**: green compact and nodular callus from media contained NAA.



Plate 2. Somatic embryos obtained from explant that cultured at photoperiodic condition, from media contained 4 mg/l 2,4-D and 0.5 mg/l BAP.

environmental factors that have effect on somatic embryos induction. Angoshtari et al. (2009) found that, light stimulated somatic embryo formation and maturation in *Brassica napus*. It has been reported that somatic embryo responses differed between genotypes. Somatic embryos were obtained at either a photoperiodic or completely dark condition in grapevine (Tangolar et al., 2008) and at dark in sweetpotato (Triqui et al., 2008). High frequency of somatic embryogenesis was observed on MS medium when 2,4-D and BAP was added. The best embryogenic response was obtained on the medium containing 4 mg/l of 2,4-D combined with 0.5 mg/l BAP from cotyledon explant (20.00 ± 7.94 SE per petri dish). SE numbers obtained from cotyledon and hypocotyls explants were 17.33 ± 5.51 and 16.33 ± 3.06 per petri dish, respectively, on the medium that 2 mg/l of 2,4-D and 0.5 mg/l BAP were added. Auxin was found critical for

Table 1. Somatic embryos (SE) from hypocotyl and cotyledons explants cultured in different growth regulators combination.

2,4-D (mg/l)	NAA (mg/l)	BAP (mg/l)	2iP (mg/l)	Hypocotyl (Mean number of SE Petri ⁻¹)	Cotyledon (Mean number of SE Petri ⁻¹)	Mean of media
-	-	-	-	0.00	0.00	0.00 l
2.0	-	0.0	-	1.67±1.15	4.67±3.51	3.17 i
2.0	-	0.1	-	8.67±5.51	2.00±2.00	5.34 e
2.0	-	0.5	-	16.33±3.06	17.33±5.51	16.83 a
2.0	-	-	0.0	0.00	0.00	0.00 l
2.0	-	-	0.1	1.67±1.15	5.00±3.61	3.33 h
2.0	-	-	0.5	1.33±1.15	2.00±1.00	2.00 k
4.0	-	0.0	-	1.67±1.53	1.67±1.15	2.00 k
4.0	-	0.1	-	2.00±1.00	8.67±2.08	5.34 e
4.0	-	0.5	-	6.33±3.21	20.00±7.94	13.17 b
4.0	-	-	0.0	0.00	0.00	0.00 l
4.0	-	-	0.1	0.00	0.00	0.00 l
4.0	-	-	0.5	5.67±3.79	6.67±3.99	6.17 d
-	2.0	0.0	-	1.33±0.58	2.00±2.00	2.00 k
-	2.0	0.1	-	4.33±3.51	3.00±2.00	3.67 g
-	2.0	0.5	-	9.67±2.53	6.33±5.13	8.00 c
-	4.0	0.0	-	1.00±1.00	2.33±2.08	2.50 j
-	4.0	0.1	-	2.33±2.08	3.33±4.16	4.25 f
-	4.0	0.5	-	3.33±2.08	4.00±3.61	3.67 g
-	-	0.1	-	0.00	0.00	0.00 l
-	-	0.5	-	0.00	0.00	0.00 l
-	-	-	0.1	0.00	0.00	0.00 l
-	-	-	0.5	0.00	0.00	0.00 l

Means having the same letter in columns are not significantly different by Duncan's multiple range test ($P < 0.01$).

induction of somatic embryogenesis and at the same time the two tested cytokinins (BAP and 2iP) at any concentrations acted synergistically with auxin. Increasing concentration of cytokinin resulted to higher SE formation. Somatic embryo formation increased when the medium contained 2,4-D and supplemented with high level of BAP. Few SE was observed on medium containing only auxin (2,4-D or NAA). However, when cytokinin was added, the number of SE increased. Number of SE on the medium of 0.5 mg/l BAP was higher than that obtained with 0.1 mg/l BAP (Table 1). In the present study it was seen that plant growth regulators and auxin-cytokinin balance were effective on somatic embryo formation. Cytokinins had significant effects on somatic embryogenesis and high concentration (0.5 mg l⁻¹) was more effective. Similar results were obtained by Singh and Chaturvedi (2009) who reported that the effects of cytokinins on somatic embryogenesis were significant and mandatory for the differentiation of somatic embryos. Triqui et al., (2008) reported that somatic embryogenesis varies with the genotype and interaction between the combination of growth regulators and the genotype was

significant. Corredoira et al. (2003) found that the explants incubated on the medium including 0.1 mg/l BA+ 0.1 mg/l NAA showed high percentage of somatic embryo.

In conclusion, the present study showed that, callus induction and SE were strongly affected by plant growth regulators and culture conditions. The medium containing both auxin and cytokinin at any concentration was capable of inducing the formation of SE but differences were observed based on the type and concentration of plant growth regulators and light conditions.

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