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Full Length Research Paper

Optimization of somatic embryogenesis induction in Iranian melon (*Cucumis melo* cv. Khatooni)

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In this study, regeneration of *Cucumis melo* cv. Khatooni: one specific Iranian melon- via somatic embryogenesis was investigated. Three different types of explant (cotyledon, hypocotyl and cotyledonary petiole) from six days old seedlings were used. Growth regulator treatments were two levels of 6-benzyl aminopurine (0 and 0.1 mg/l) and four levels of 2,4-dichlorophenoxyacetic acid (0, 2.5, 5 and 7.5 mg/l). After seven weeks, cotyledonary petiole showed the highest potential in somatic embryo induction and the combination of 0.1 mg/l BA and 5 mg/l 2,4-D had significant effect on somatic embryogenesis of Khatooni melon.

Key words: Melon, somatic embryogenesis, regeneration, cotyledon, hypocotyl, cotyledonary petiole.

INTRODUCTION

Melon is one of the most important crops in Iran and there is a unique potential for its production due to the rich germplasm and diverse climatic conditions. Melons are reported to be indigenous to Africa (Kerje and Grum, 2000; Robinson and Decker-walters, 1999) however, secondary centers of its diversity exist in Iran, Afghanistan, India and China (Robinson and Decker-walters, 1999). Iran is the third largest melon producing country after China and Turkey (FAO, 2010) and "Khatooni" which belongs to a unique Iranian cultivar-group (Lotfi and Kashi, 1999) is the most popular commercial cultivar and has the highest area under cultivation (Salehi et al., 2010). However, this cultivar is subject to different stresses specially fusarial wilt. Therefore, we chose this cultivar in this study. This cultivar has large rugby-shaped fruits with striped and netted skin surface, very crispy and favorable white flesh (Figure 1), matures early (70 to 80 days) and has a good shelf life (Lotfi and Kashi, 1999).

Conventional breeding method of melon is difficult because of interspecific and intergeneric incompatibilities (Guis et al., 2000; Deakin et al., 1971; Niemirowiczszczytt and Kubicki, 1979), and genetic engineering could be effective and rapid for producing interesting new varieties (Rhimi et al., 2006). Application of this method requires reliable procedures for plant regeneration via *in vitro* culture (Debeaujon and Branchard, 1993). Somatic embryogenesis in melon has been developed before organogenesis and the first regenerated plant achieved by this method was reported by Blackmon et al. (1981). After that, several efforts have been made for plant regeneration of different genotypes of melon via somatic embryogenesis by different growth regulators, explants and culture conditions (Nakagawa et al., 2001; Kintzios et al., 2002; Rhimi et al., 2007; Shah et al., 2008; Nunez-Palenius et al., 2008; Comlekcioglu et al., 2009). There are limitation on the somatic embryogenesis in melon such as genotype, growth regulator, explant and culture media (Nunez-Palenius et al., 2008).

The goal of this study was to establish a simple and efficient regeneration protocol via somatic embryogenesis in this cultivar. Achievement of these data is believed to provide key information in genetic transformation and introduction of new economical traits such as fusarial wilt

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Abbreviations: BA, 6-Benzyl aminopurine; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **MS,** Murashige and Skoog basal medium

Figure 1. Fruits of *C. melo* L. (cv. Khatooni).

resistance.

MATERIALS AND METHODS

Explant preparation

Mature seeds of melon (*Cucumis melo* cv. Khatooni) were used as explant sources for the induction of somatic embryogenesis. After removing the seed coats, they were dipped in 70% ethanol for 2 min and after washing them with sterilized distilled water, they were put in 1.5% commercial bleach solution containing two drops of Tween 20 as wetting agent for 20 min and shacked slowly. At last, the seeds were rinsed with sterilized distilled water for four times. After drying the seeds, they were cultivated on MS basal medium (Murashige and Skoog, 1962) including vitamins, sucrose (1%), 6-benzyl aminopurine (BA) (0.5 mg/l) and agar (8%). After six days, seedlings were used as explant sources. Explants included hypocotyls, cotyledons and cotyledonary petioles. Hypocotyls were cut into 4 to 5 mm segments and transferred to medium culture. Proximal parts of 4 to 5 mm segments of cotyledons were transferred abaxial side down onto the induction medium and distal segments were discarded. For the preparation of cotyledonary petiole explants, distal parts of cotyledons were discarded and then each seedlings were stood on hypocotyl stub and hypocotyl were cut in a way that cotyledon was with half of the hypocotyl. The apical meristem was removed using a binocular and proximal parts of cotyledon with about 1 to 2 mm of hypocotyl and were cultured on the media abaxially.

Medium and culture condition

MS basal medium with 30 g/l sucrose solidified by 8 g/l agar were supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) (0, 2.5, 5 and 7.5 mg/l) and BA (0 and 0.1 mg/l). The pH of the medium was adjusted to 5.8 before autoclaving at 100 Kpa, 121°C for 15 min. Explants were incubated on the medium and kept in the dark at 25 ± 1°C. After four weeks, cultures were transferred to light condition (50 µmol/s/m) with a 16-h photoperiod. Explants that passed different stages to attain embryo were determined and cotyledonary embryos were transferred to hormone-free media to complete their procedure to become a perfect plant.

Histology and microscopical analysis

For histology, all explants were fixed in formalin/acetic acid/alcohol (FAA), dehydrated in tertiary butyl alcohol, embedded in paraplast and stained with iron haemotoxylin (Sass, 1958). Sections were cut by microtome and were observed under the Olympus electron microscope.

Experimental design and statistical analysis

Amount of callus, the number of explants that were able to form somatic embryogenesis and the average number of embryos per regenerating explant were determined after seven weeks. The obtained data after normal test were analyzed on factorial based on complete randomize design (CRD) with three replications. The factors included four levels of 2,4-D (0, 2.5, 5 and 7.5 mg/l), two levels of BA (0 and 0.1 mg/l) and three explants (cotyledon, hypocotyl and cotyledonary petiole). Data were compared with least-significant-difference (LSD) on the 0.5 probability level and analyzed using the SAS software.

RESULTS AND DISCUSSION

After 4 weeks, non-compact and friable callus were exhibited on the explants. At their surface, globular structures were observed which was the primary stage of embryogenesis. In some other explants, compact callus were exhibited, and they did not have embryogenic structures.

After transferring the explants to hormone-free medium, different stages of somatic embryos development (globular, heart and torpedo shape) were observed.

Moreover, some of them had attained the cotyledonary stage, exhibiting different shapes and sizes of a normal cotyledon. It has been reported that normal somatic embryos of melon have long and thin hypocotyls, in contrast to abnormal embryos, which have short hypocotyls (Kageyama et al., 1991). Nunez-Palenius et al. (2008) believe that the embryogenic pathway has two

Table 1. Variance analysis (MS) of callus amount, embryo induction and embryo number in each explant in response to explants type and different hormone concentration.

Source	df	Callus amount MS	Embryo induction (%) MS	Embryo number in each explant MS
Explant	2	2.310**	19.038**	0.295**
BA	1	1.717**	83.755**	1.050**
Explant *BA	2	0.029	7.229	0.098*
2,4-D	3	31.286**	39.410**	0.572**
Explant *2,4-D	6	0.276**	3.966	0.038
BA *2,4-D	3	0.233**	18.047**	0.140**
Explant *BA *2,4-D	6	0.068	1.181	0.023
Error	48	0.038	2.980	0.028

*P<0.05; **p<0.01.

Table 2. Effect of explant types on callus amount, embryo induction and embryo number in each explant in Khatooni melon.

Explant	Callus amount	Embryo induction (%)	Embryo number in each explant
Cotyledon	1.84[b]	13[a]	1.46[b]
Hypocotyl	1.53[c]	5[b]	0.71[c]
Cotyledonary petiole	2.15[a]	21[a]	2.58[a]

Values followed by different letter within the column were significantly different at 1% level.

stages of culturing explants on induction medium containing auxin and transferring the induced explants to hormone-free media for developing embryos.

Callus induction, embryo's growth and development of plantlet were not the same in the different explants and hormone concentrations. Among the three types of explant used in this study, cotyledons and especially cotyledonary petioles had the best potential for embryogenesis (Table 2). This was probably because of the regenerative meristem in the junction point of the cotyledon and hypocotyl. Although there are several documents available on using cotyledon and hypocotyl explants in melon somatic embryogenesis, there are few report on the application of cotyledonary petiole explants. Rhimi et al. (2006) used cotyledon, hypocotyl and zygotic embryo as explants for melon somatic embryogenesis induction. In their study, the calluses which were formed from cotyledon and zygotic embryo had higher performance in embryogenesis, while hypocotyl did not show any significant result. However, zygotic embryo was introduced as the suitable explants for maximum production of perfect plant via somatic embryogenesis and 51.5 and 44.5% plantlets from Mazoun and Beji cultivars produced root from zygotic embryo explants, respectively. Tabei et al. (1991) reported that the cotyledon of mature seed is the best explants for somatic embryogenesis in Earl's Favorite Harukei No.3 cultivar. They used four explants including cotyledon of mature seed, hypocotyl, petiole and leaf. Homma et al. (1991) used different types of explants for somatic embryo production in melon and obtained the best result from the explants which included radical, hypocotyl and proximal part of the cotyledon. Debeaujon and Branchard (1993) published a review article on somatic embryogenesis in cucurbitaceae. Although they reported that somatic embryogenesis was induced from different explants such as protoplast, they introduced seed-derived parts, especially cotyledon and hypocotyl as the best explants.

In our study, application of 2,4-D and BA and their interaction affected the callus induction (Table 1). 0.1 mg/l BA and 7.5 mg/l 2,4-D had significant effect on the amount of callus (Table 3). Auxin is known as the most important plant growth regulator for somatic embryogenesis. Embryogenic callus induction in melon has been widely reported using different types of auxin (Oridate et al., 1992; Gray et al., 1993; Guis et al., 1997; Nakagawa et al., 2001). 2,4-D is the most widely used auxin to induce somatic embryogenesis in melon explants (Oridate and Oosawa, 1986; Debeaujon and Branchard, 1993). In addition, NAA and IAA have also been effective on melon embryogenesis (Tabei et al., 1991).

2,4-D and BA and their combination had significant effects on embryogenesis too (Table 1) and the concentration of 5 and 7.5 mg/l 2,4-D with 0.1 mg/l BA produced the most promising result (Table 3). With respect to the number of embryos, 5 mg/l of 2,4-D and 0.1 mg/l BA was more effective (Table 3). Similar result were achieved by Homma et al. (1991) who reported that combination of 2-4 mg/l 2,4-D with 0.1 mg/l BA had the

Table 3. Effect of BA and 2,4-D concentration on callus amount, embryo induction and embryo number in each explant in Khatooni melon.

BA (mg/l)	2,4-D (mg/l)	Callus amount	Embryo induction (%)	Embryo number in each explant
0	0	0^{f}	0^{c}	0^{c}
	2.5	1.68^{e}	3^{c}	0.33^{c}
	5	2.27^{c}	14^{b}	1.66^{b}
	7.5	2.79^{b}	3^{c}	0.22^{c}
0.1	0	0^{f}	0^{c}	0^{c}
	2.5	1.97^{d}	14^{b}	2.22^{b}
	5	2.79^{b}	47^{a}	5.55^{a}
	7.5	3.21^{a}	25^{a}	2.66^{b}

Values followed by different letter within the column were significantly different at 1% level.

best performance. Although Gray et al. (1993) reported application of 5 mg/l 2,4-D and 0.1 mg/l BA for somatic embryogenesis in “Male Sterile A 147” genotype of melon, they achieved the best result from 2,4-D and TDZ. In another investigation by Stipp et al. (2001) on yellow queen and yellow king cultivars, the best result was achieved from 5 mg/l 2,4-D and 0.1 mg/l TDZ. They could not obtain any somatic embryo from the different combinations of BA and 2,4-D, and only observed non compact whitish callus. Rhimi et al. (2006) reported that the best result for somatic embryogenesis in Maazon and Beji cultivars were 0.25 mg/l 2,4-D and 0.5 mg/l BA. In Beji cultivar, in addition to the earlier mentioned concentrations, 0.1 mg/l 2,4-D and 0.25 mg/l BA were also effective. Akasaka et al. (2004) introduced 2 mg/l 2,4-D and 0.1 mg/l BA as the best concentration of growth regulators for somatic embryo induction in Vedrantais and Earl's Favorit Fuyua cultivars.

In this study, we demonstrated a novelty that cotyledonary petioles are more efficient and rapid than other commonly used explants for *in vitro* regeneration of melon, and combination of 5 mg/l 2,4-D and 1 mg/l BA was the best concentration for embryogenesis in Khatooni” cultivar. Several embryos were achieved from “non-compact friable callus and most of them were normal and had similar shapes with those reported previously on other melon cultivars (Rhimi et al., 2006; Kageyama et al., 1990). To our knowledge, this is the first report of this kind on the embryogenesis of “Khatooni” melon. Therefore, it can be said that the interaction of the genotype, explant types and the concentration and type of the plant growth regulators play a major role in melon embryogenesis.

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REFERENCES

Akasaka-Kennedy Y, Tomita KO, Ezura H (2004). Efficient plant regeneration and Agrobacterium-mediated transformation via somatic embryogenesis in melon (*Cucumis melo* L.). Plant Sci,. 166: 763-769.

Blackmon WJ, Reynolds BD, Postek CE (1981). Production of somatic embryos from callused cantaloupe hypocotyl explants. HortScience, 16: 451-451.

Comlekcioglu N, Mendi YY, Eldogan S, Unek C (2009). Effects of different combinations and concentrations of growth regulators and photoperiod on somatic embryogenesis of *Cucumis melo* var. *flexuosus*. Afr. J. Biotechnol., 8(22): 6228–6232.

Deakin JR, Bohn GW, Whitaker TW (1971). Interspecific hybridization in *Cucumis*. Econ. Bot., 25: 195-211.

Debeaujon I, Branchard M (1992). Induction of somatic embryogenesis and caulogenesis from cotyledon and leaf protoplast derived colonies of melon (*Cucumis melo* L). Plant Cell Rep., 12: 37-40.

Debeaujon I, Branchard M (1993). Somatic embryogenesis in Cucurbitaceae. Plant Cell Tiss. Org. Cult., 34: 91-100.

Food and Agriculture Organization (FAO). 2010. FAOSTAT Data. http://www.fao.org/biotech/stat.asp.

Gray DJ, Mccolley DW, Compton ME (1993). High-frequency somatic embryogenesis from quiescent seed cotyledons of *Cucumis melo* cultivars. J. Am. Soc. Hort. Sci., 118: 425-432.

Guis M, BenAmor M, Latche A, Pech JC, Roustan JP (2000). A reliable system for the transformation of cantaloupe Charentais melon (*Cucumis melo* L. var. *cantalupensis*) leading to a majority of diploid regenerants. Sci. Hortic-Amsterdam, 84: 91-99.

Guis M, Latche A, Pech JC, Roustan JP (1997). An efficient method for production of diploid cantaloupe Charentais melon (*Cucumis melo* L. var. *cantalupensis*) by somatic embryogenesis. Sci. Hortic-Amsterdam, 69: 199-206.

Homma Y, Sugiyama K, Oosawa K (1991). Improvement in production and regeneration of somatic embryos from mature seed of melon (*Cucumis melo* L.) on solid media. Jpn. J. Breeding. 41: 543-551.

Kageyama K, Yabe K, Miyajima S (1991). Somatic embryogenesis in suspension culture of mature seed of *Cucumis melo* L. Jpn. J. Breed., 41: 273–278.

Kerje T, Grum M (2000). The origin of melon, *Cucumis melo*: a review of the literature. In: The 7th EUCARPIA Meeting on Cucurbit Genetics & Breeding, pp. 37-44.

Kintzios S, Sereti E, Bluchos P, Drossopoulos JB (2002). Growth regulator pretreatment improves somatic embryogenesis from leaves of squash (*Cucurbita pepo* L.) and melon (*Cucumis melo* L.) Plant Cell Rep., 21: 1–8.

Lotfi M, Kashi A (1999). The Iranian melon as a new cultivar-group. Taxonomy of Cultivated Plants: Third International symposium, Royal

Botanic Gardens, Kew, pp. 447-449.
Nakagawa H, Saijyo T, Yamauchi N, Shigyo M, Kako S, Ito A (2001). Effects of sugars and abscisic acid on somatic embryogenesis from melon (*Cucumis melo* L.) expanded cotyledon. Sci. Hortic.-Amsterdam, 90: 85-92.
Niemirowiczszczytt K, Kubicki B (1979). Cross fertilization between cultivated species of genera *Cucumis* L. and *Cucurbita* L. Genet. Polonica, 20: 117-124.
Nunez-Palenius H, Gomez-Lim M, Ochoa-Alejo N (2008). Melon Fruits: Genetic Diversity, Physiology, and Biotechnology Features. Crit. Rev. Biotechnol., 28: 13-55.
Oridate T, Oosawa K (1986). Somatic embryogenesis and plant regeneration from suspension callus culture in melon (*Cucumis melo* L.). Jpn. J. Breed,. 36: 424-428.
Oridate T, Atsumi H, Ito S, Araki H (1992). Genetic differences in somatic embryogenesis from seeds in melon (*Cucumis melo* L.). Plant Cell Tiss. Org. Cult., 29: 27-30.
Rhimi A, Ben Fadhel, N, Boussaid M (2006). Plant regeneration via somatic embryogenesis from in vitro tissue culture in two Tunisian *Cucumis melo* cultivars Maazoun and Beji. Plant Cell Tiss. Org. Cult., 84: 239-243.
Rhimi A, Michel H, Mohamed B (2007). Agrobacterium mediated transformation of Tunisian *Cucumis melo* cv. Maazoun. Afr. J. Biotechnol., 6(18): 2162-2165.
Robinson RW, Decker-Walters DS (1999). *Cucurbits*. CAB International, Wallingford, NY.
Salehi R, Kashi A, Lee JM, Babalar M, Delshad M, Lee SG, Huh YC (2010). Leaf Gas Exchanges and Mineral Ion Composition in Xylem Sap of Iranian Melon Affected by Rootstocks and Training Methods. HortScience, 45(5): 766-770.
Sass JE (1958). Botanical microtechnique. Third edition. Ames, Iowa: Iowa State University Press, pp.73-74.
Shah P, Singh NK, Khare N, Rathore M, Anandhan S, Aref M, Singh RK, Das SC, Ahmed Z, Kumar N (2008). Agrobacterium mediated genetic transformation of summer squash (*Cucurbiata pepo* L. cv. Australian green) with cbf-1 using a two vector system. Plant Cell Tiss. Org. Cult., 95: 363-371.
Stipp LCL, Mendes BMJ, Piedade SMDS, Rodriguez APM (2001). *In vitro* morphogenesis of *Cucumis melo* var. inodorus. Plant Cell Tiss. Org. Cult., 65: 81-89.
Tabei Y, Kanno T, Nishio T (1991). Regulation of organogenesis and somatic embryogenesis by auxin in melon. *Cucumis melo* L. Plant Cell Rep., 10: 225-229.