Original Article

In vitro Regeneration of Persian melon (*Cucumis melo*) cv. Khatooni through Direct Organogenesis

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ABSTRACT

Melon breeding programs can highly benefit biotechnological tools. However, a breeding program associated with biotechnological tools depends upon the development of an efficient *in vitro* plant regeneration system. Organogenesis is used for new breeding purposes in biotechnology programs. The aim of current study was to examine the various growth regulations and explants on direct organogenesis of Persian melon cultivar 'Khatooni' using induction of regeneration in cotyledon leaf explants. For this purpose, leaf cotyledons *in vitro* explant (three-, five-, seven-, 10-, and 12-day-old seedlings) were cultured in Murashige and Skoog Medium (MS) supplement with 0, 0.5, and 1 mg.l^{-1} 6-benzylaminopurine (BAP). Adventitious buds were identified by the development of the shoot meristem tip and the beginning of the leaf. The results showed that *in vitro* organogenesis in melon was the highest multiplication rate (5.3) and shoot regeneration (63.7%) when cotyledon leaves from seven-day-old seedlings were cultured on MS medium supplement with 1 mg.l^{-1} BAP. Regenerated plantlets were transferred to MS medium supplement 0.1 mg.l^{-1} BAP and 1 mg.l^{-1} Gibelleric acid (GA_3) to elongate shoots. Then, by transferring to MS medium supplement with 1 mg.l-1 Indole butyric acid (IBA), regenerated plantlets with developed roots were obtained. According to the results of this research, we can start propagating and producing *in vitro* melon plants faster and healthier. Using this method in gene transfer program can increase the production efficiency of transgenic plants.

1. Introduction

Melon (*Cucumis melo* L.) is one of the most important crops from the Cucurbitaceae family. Iran ranks third in the world after China and Turkey (with a production of about 2 million tons and 100,000 hectares of arable land) [1]. For breeding and production of plant products with new genetic characteristics, through biotechnology, reliable methods are needed for *in vitro* regeneration [2].

Numerous reports have been described for the regeneration of melon cultivars, which are mainly in two ways: Organogenesis and somatic embryogenesis. Organ regeneration is either direct regeneration without callus growth between explant or indirect regeneration including callus growth before shoot production [3]. Most reports of regeneration of melon cultivars are related to direct

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organogenesis. Direct regeneration is relatively easier and does not have problems such as the long culture period, the problems we face in indirect organogenesis and somatic embryogenesis, and also less somaclonal diversity and ploidy abnormalities; however, perhaps the main drawback of this method is low regeneration and more sensitivity and hardiness in gene transfer processes [4,5,6,7,8].

Many factors in melon *in vitro* regeneration are involved; there are usually many differences between different genotypes of melon, in addition to the type and age of explants as well as the culture medium and growth regulator in regeneration potential. They also have a great effect on melon regeneration efficiency during organogenesis; in melon, cotyledon leaf explants usually had the highest shoot Multiplication rate with direct organogenesis [9, 10, 11]. In general, the amount of cytokinin to auxin more than one is used to induce shoot formation, although the presence of the cytokinin alone is sufficient. Among cytokinins, BAP is the most widely used in shoot induction. Shoot elongation requires the induction of cytokinin subject to the addition of gibberellin, if rooting occurs in a hormone-free medium or at low auxin levels [12, 13, 14, 15].

The effect of genotype, culture medium, and age of explants on shoot regeneration from cotyledon leaf explants of Turkish melon was studied, and the highest multiplication rate was observed in concentrations of 1.13, 0.88, and 0.26 $mg.l^{-1}$, respectively, BAP, IAA, and ABA in 4-day-old explant (90%) [12]. Also, by examining 3-day explants of 7 Costarican cultivars, the highest direct shoot regeneration with a level of 1 $mg.l^{-1}$BAP, on 70% of the total genotypes was shown [13].

2. Materials and Methods

Initially, peeled mature melon seeds of Khatooni cultivar were disinfected in ethanol 70% for 30 seconds and then re-immersed in sodium hypochlorite solution 1% for 20 minutes. The seeds were completely rinsed with distilled water 3 times. To ensure uniform germination and production of stronger cotyledon leaves, seeds were transferred to half MS medium supplement with 0.5 $mg.l^{-1}$BAP.

After that, using 2-5 mm cotyledon leaf explant along with the part under the cotyledon determined to be more regenerative, three, five, seven, 10 and 12-day old seedlings were divided into two parts and cultured on MS medium supplement with 0, 0.5 and 1 $mg.l^{-1}$ BAP. Then, the effect of seedling age and BAP concentration on shoot organogenesis was evaluated. Four weeks after initial planting, multiplication rate, i.e., number of shoots produced from each explant, and regeneration percentage, i.e., number the explants produced by the Adventive shoot, were measured vs. the total cultured explants.

Experiments were used in 5 replications with 20 explants based on a completely randomized design (CRD) in each treatment. Adventive shoots produced with part of the mother tissue of explants were transferred to elongate in MS medium supplement with 0.1 $mg.l^{-1}$ BAP with 1 $mg.l^{-1}$ GA_3 Adventive shoots. After several subcultures, the elongated shoots were separated from each other and transferred to MS rooting medium supplement with 1 mg.l-1of IBA. All MS media contained 30 $g.l^{-1}$ sucrose and 7 $g.l^{-1}$ agar and the pH was adjusted to 5.6-5.8. The explant was placed to 8-16 hours of the light cycle and 25 °C. After 4 Weeks, seedlings with developed roots were obtained.

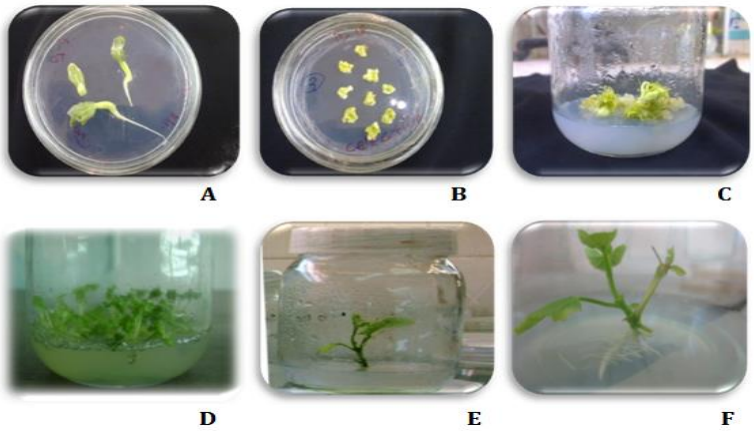

Figure 1. Stages of direct organogenesis of Persian melon cv. Khatooni; A) Seedling growth in a germination medium, B) Preparation of cotyledon leaf explants, C) Production of adventive shoot buds in induction medium, D) Elongation of the shoot in elongation medium, E) Transfer to rooting medium and F) Seedling rooting

For this purpose, this experiment aimed to propagate Persian melon cv. Khatooni through direct regeneration of cotyledon leaf explants by examining seedling age and BAP concentration in culture medium for gene transfer purposes in melon.

3. Results

The results showed that at seedling age, BAP concentration and the interaction of Both factors were significantly different (Table 1). In the experiment, the multiplication rate of 2.0 shoots per explant and the percentage of shoot regeneration from cotyledon explants were 33.5% (Tables 2 and 3). Cotyledon leaves were isolated from seedlings with 7-day seedling age in MS medium supplement with 1 mg.l^{-1}BAP, compared with other explants with the best Multiplication rate (5.3) and percentage of shoot regeneration (63.7%) (Tables 2 and 3).

Table 1. The ANOVA result for characteristics studied *in vitro* organogenesis of Melon cv. Khatooni.

S.O.V	DF	Mean Squares	
		Shoot Regeneration (%)	Multiplication rate
Seedling age	4	42.02 **	3.4 **
BAP Consentration	3	53.29*	6.23*
Seedling age × BAP Consentration	12	30.14*	2.05*
C.V. (%)	-	18.54	5.81

**, *, ns: Significant at 1% and 5% of probability levels, and non-significant, respectively.

Table 2. The effect of Seedling age and BAP Concentration on Shoot Regeneration (%) *in vitro* organogenesis of Melon cv. Khatooni

BAP Concentration	Seedling age					Mean
	3	5	7	10	12	
0	18.7^{cd}	32.8^{bc}	40.5^{b}	15.4^{cd}	12.9^{e}	24.1^{C}
0.5	35.1^{bc}	38.1^{bc}	44.0^{b}	23.0^{c}	18.6^{cd}	31.7^{B}
1	51.3^{ab}	55.1^{ab}	63.7^{a}	28.7^{c}	25.6^{c}	44.9^{A}
Mean	35.0^{B}	42.0^{AB}	49.4^{A}	22.3^{C}	19.0^{C}	33.5

The mean followed by the same letter (capital letters: simple effects and small letters: interaction effects) is not significantly different based on Duncan's multiple ranges.

Table 3. The effect of Seedling age and BAP Concentration on Multiplication rate *in vitro* organogenesis of Melon cv. Khatooni

BAP Concentration	Seedling age					Mean
	3	5	7	10	12	
0	0.5^{de}	1.1^{d}	1.1^{d}	0.1^{e}	0.1^{e}	
0.5	1.2^{d}	3.2^{c}	3.5^{c}	1.7^{d}	0.4^{de}	0.6^{C}
1	2.7^{cd}	4.1^{bc}	5.3^{a}	3.2^{c}	1.5^{d}	2.1^{B}
Mean	1.5^{B}	2.8^{A}	3.3^{A}	1.7^{B}	0.7^{C}	3.4^{A}

4. Discussion

One of the most important factors is involved in the success of *in vitro* plant regeneration to create optimal conditions. Factors that affect the *in vitro* regeneration of melon include genotype, type and age of explants, type of hormonal concentrations and culture medium, and others. *in vitro* regeneration of melon is strongly influenced by genotype so that sometimes the effect of other factors is negligible [15].

In our research, cotyledon leaves with 7-day-old seedlings cultured in MS medium supplement with 1 $mg.l^{-1}$BAP had a higher efficiency indirect regeneration (about 64%). There is no report on direct organogenesis of Khatouni cultivar but similar results have been reported on other melon cultivars. In different melon cultivars, high shoot regeneration from 7-day-old cotyledon leaf explants were obtained on MS medium supplement with 1 $mg.l^{-1}$BAP. It has been reported that the application of 1.5 mg L-1 BAP plus 250 mg L-1 cefotaxim and 1 mg L-1 BAP with 1000 mg L-1 cefotaxim could form the most efficient media for plant regeneration. On the other hand, more regenerants were obtained per explant on medium supplemented with combination of 0.5 mgL-1 BAP and 0.5 mg L -1 IAA (88%), compared with the use of 1.0 mg L-1 BAP (75%) alone (10). In the 'Amarillo' cultivar, the highest shoot production and organogenesis index were obtained from 7-day-old cotyledon leaf explants in MS medium supplement with BAP [18]. From 'Flexus' 7-day-old cotyledon leaf explants, only 42% of new shoots were obtained during direct regeneration; however, in this report, MS medium was in addition to 1 $mg.l^{-1}$BAP containing 0.25 $mg.l^{-1}$IAA [19]. Also, the positive effect of IAA adding to BAP on shoot induction and regeneration in melon was underlined [20]. However, the effect of explant age is highly dependent on genotype and different reports of best seedling age for direct regeneration of cotyledon and even subspecies explants of different melon cultivars have been reported, but in general, it is used for most plants of the cucurbit family from BAP for direct regeneration.

Shoot formation can be induced directly from cotyledons, leaves, or sub-cotyledon explants that have greater polarity for shoot formation, which is likely to be related to the accumulation of growth factors such as auxin [3]. The age of cotyledons is a vital factor; younger cotyledons usually have better efficiency for regeneration as well as a reduction in polyploidy due to somaclonal diversity. Appropriate choice of the explant significantly influences the morphogenic ability. In melons, younger, smaller leaves and very young cotyledons were found to be highly responsive (5). Polyploidy abnormalities have a high relative correlation with explant source and callus formation, so the use of dormant cotyledons of mature seeds and methods other than indirect organogenesis to avoid polyploidy has reduced the ability to produce regenerative plants (3).

5. Conclusion

The direct organogenesis of melon is influenced by genotype, type, and amount of phytohormone, type of explant and seedling age, etc. Considering the relatively higher efficiency of seven-day explants in MS medium supplement with 1 $mg.l^{-1}$BAP, the use of this protocol can be satisfactory.

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All authors have to declare their conflict of interest.

Consent for publications

All authors have to write this sentence that they read and approved the final manuscript for publication.

Availability of data and material

The authors have to declare that they embedded all data in the manuscript.

Authors' contributions

All authors should write their part in designing the idea, doing, analyzing and writing the article.

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The authors have to declare that they do not use human or animals in their research but if so, they have to write name of the ethics committee that has approved the study of enter the code of research ethics (provided by the institution or university where the research was conducted) in the article.

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