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# The Effects of Different Combinations and Varying Concentrations of Growth Regulators on the Regeneration of Selected Turkish Cultivars of Melon

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## 1. Introduction

Cucurbits are an economically important family of plants. The majority of the vegetable production in Turkey, for example, derives from the species belonging to the family *Cucurbitaceae*. Despite the importance of cucurbits among vegetable crops worldwide, the development of genomic tools in these species has been rather limited. Although melon production has been improved by conventional plant breeding methods, output is still insufficient. One useful technique in overcoming such problems in melon is functional genomics' studies, and the other one is abiotic stress resistance and improved fruit quality has been gene transfer via *Agrobacterium tumefaciens* mediated transformation. The availability of an optimized plant regeneration system is crucial for genetic transformation techniques as well as obtaining an entire plant. Although Hasanbey and Cinikiz in Turkey, for example, are important commercial melon cultivars used in the breeding programs and molecular biology of fruit ripening and genetic mapping of melons, there is no study to date on the regeneration of these cultivars. The objectives of the present study are thus to develop and optimize an efficient *in vitro* regeneration protocol for *Cucumis melo* L. and investigate the effects of different genotypes and growth regulators on the *in vitro* regeneration of melon. In this paper, we discuss the following topics: general information on the family *Cucurbitaceae*, the importance of melon production both in Turkey and in the world; lastly, the efficiency of *in vitro* culture techniques on melon propagation are presented with data relevant to our laboratory research. We assume that statement of the research findings presented here lead to for further studies on *in vitro* propagation of melon.

### 1.1. The family cucurbitaceae

The family *Cucurbitaceae* consists of cucumbers and melon as two major commercial vegetable crops and two minor crops, the West Indian Gherkin and the Kiwano, respectively. They are cultivated, economically useful crops [1]. According to the infrageneric classification, the genus *Cucumis* is divided into two subgenera based on the different base chromosome numbers of *Cucumis*; *Cucumis* subgen. *Cucumis* and *Cucumis* subgen. *Melo*. Whereas *Cucumis* subgen. *Cucumis* has  $x=7$  chromosome numbers, *Cucumis* subgen. *Melo* has  $x=12$  [2]. *Cucumis melo* is the type of the genus *Melo*. As a cucurbit crop, *melon* (*Cucumis melo*) has 552 synonyms and can be divided into three types: cantaloupe melons, musk melons and winter melons [1,3]. Melon is a valuable human food source cultivated in arid and semiarid regions of the world [4]. The family *Cucurbitaceae* is hypothesized to be consisted of the species' open-pollination. Due to this open pollination within melon varieties, new melon species have emerged; hence *in vitro* propagation enables *in vitro* conservation of different melon genotypes carrying a variety of desired traits [5].

### 1.2. The importance of melon production in the world

Melon (*Cucumis melo* L.  $2n=2x=24$ ) is a diploid species with various phenotypic characters due to its adaptation under diverse agroecological conditions from the Mediterranean to Eastern Asia (Figure1).

Melons are grown in both temperate and tropical regions. Due to various morphological variations in its fruit characteristics such as size, colour, shape, taste and texture, melons are hence described an extensive diverse group. In addition, *Cucumis melo* L. can be divided into three groups or types: *Cantaloupensis* (Cantaloup or Musk melon) group, *Inodorous group* (Winter melon or Casaba) and *Reticulatus* (Ananas) group. Genotypes of these three groups can be crossbred [5, 6].

Although Africa is the origin of the melon, the diversification center for this fruit encompasses all Asian countries from Turkey to Japan. China, Turkey, Iran, and USA produce 57% of the melon annual production in the world [3-6].



**Figure 1.** General view of flower and fruit of melon (adapted from [7])

Melon fruits are the valuable food sources with robust vitamin and mineral composition (Table 1) as well as economic values [8].

Composition	<i>Inodorous Melon Groups</i>
<b>Overall Compositions</b>	
Water (g)	91.85
Minerals (mg)	218.41
Proteins (g)	1.11
Total Lipid (Fat) (g)	0.10
Carbohydrate (g)	6.58
Fibre, total dietary (g)	0.9
Sugars, total (g)	5.69
<b>Vitamins</b>	
Vitamin K (µg)	2.5
Vitamin C (mg)	21.8
Thiamin (mg)	0.015
Riboflavin (mg)	0.031
Niacin (mg)	0.232
Folate (mcg)	8
Vitamin B6 (mg)	0.163
Vitamin E (mg)	0.05

**Table 1.** Nutritional compositions of the media used for micropropagation of Hasanbey and Cinikiz melons (value per 100 g of edible portion) \*From USDA Nutrient Database, July 2012 [12]

Melons provide several nutrients involving protein (0,6-1,2%/100 g) vitamin E,, vitamin C, and Vitamin K for human metabolic reactions in daily dietary [9, 10]. Melon fruits are used in production of deserts, such as jam, ice cream, yogurt as well as soup (from the juice), pickling and cosmetics [11].

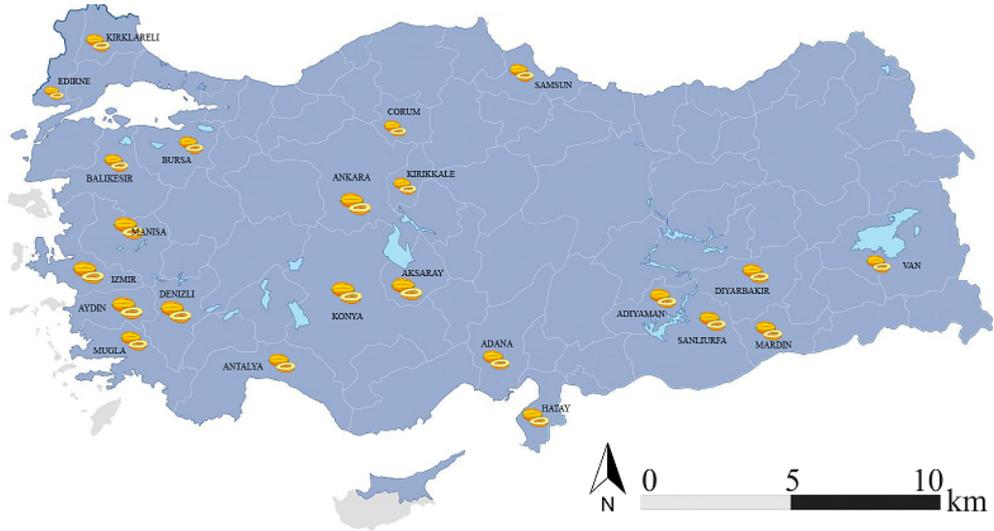
A characteristic skin color and aroma for melons are the primary traits sought by melon breeders. In addition, the development and ripening of melon fruits are very complicated due to the many biochemical and physiological changes comprising cell wall degradation, alteration in pigment biosynthesis, aromatic compounds, and increasing of sugar content. Therefore, performing the ex-situ regeneration of melon is very important for the research focused on improving the agronomic traits of melon *in vitro* conditions.

### 1.3. The importance of melon production in turkey

Turkey is an important country for cultivation of the economically important plant family *Cucurbitaceae* [13, 14]. Although the nation is not a primary center of melon diversification, it is the second largest producer after China in the world [15]. 38% of vegetable production in Turkey are *Cucurbitaceae* species which are watermelon, melon, cucumber, squash, and pumpkin. Of the 26,7 million tons of melon produced worldwide, 1,749 million tons of

production, emerges from Turkey [16, 17] Central Anatolia is the main melon production region in Turkey. Ankara, Balıkesir, Diyarbakir, Konya and Manisa are the provinces of highest melon production in Turkey [18]. The main production states are shown in Figure 2 [5,18].

In Turkey, the most popular cultivars are Yuva, Kirkagac, Kislik Sari Kuscular, Hidir, Cumra, Cinikiz and Hasanbey and melons are cultivated on landraces of less than 5 ha in size. Due to climatic conditions, melon harvesting in Turkey can change based on cultivation regions, but generally it is done from June to September [18].



**Figure 2.** Melon production regions in Turkey

#### 1.4. The importance of *in vitro* propagation of melon varieties

*In vitro* propagation provides a great number of clonal plants in a short time. This technique is based on the theory that a new plantlet can be derived from the use of any plant parts (leaf, shoot, root, etc.) on a suitable initial medium [19, 20]. The nutritional composition of a culture medium for optimal growth of a plant tissue is based on plant genotype [20]. Thus, the method established to manipulate plant tissues and cells is not only essential for *in vitro* propagation of valuable plants but also required to regenerate transgenic plants [1, 19]. Due to the fact that most commercial melon varieties have been subjected to viral pathogens, defects in fruit quality and absent yield have resulted in major economic losses [8]. For *in vitro* conservation of melon, *in vitro* propagation among melon varieties, has been implemented to obtain clonally propagated genotypes of melon [21]. In addition, due to the small-sized genome, high polymorphism, and short generation time of the melon, genetic transformation could be possible in the melon [22, 23]. There are several reports of the genetic transformation of melon with a variety of marker genes, as well as genes for viral resistance, abiotic stress resistance,

and fruit quality attributes [13, 24-34] Also some reports suggest that transformation in melon is strictly limited by genotype [26, 28]. This event makes this crop a good target for transformation protocols [35]. It has been shown that genetic transformation on melon via *Agrobacterium tumefaciens* is limited to a few varieties. Since the difficulty of that melon regeneration is well known [36, 37, 38] the findings of our study on melon regeneration thus hope to constitute a source for further studies on profitable melon breeding.

### 1.5. Two important turkish melon cultivars: Hasanbey and Cinikiz

The local melon genotypes are the primary production resources of melon production in Turkey [14]. Hasanbey (Figure 3) and Cinikiz melon (Figure 4) varieties are the domestic farmgate *inodorous* melon crops. 85% of melon production in Turkey consists of *Inodorous* (Hasanbey, Cinikiz, Kirkagac, Kuscular, Hirsiz Calmaz and Yuva) and 15% occurs in *Cantaloupe* (*Macdimum*, Galia, Polidor and Falez) and *Reticulatus* (Ananas, Topatan, Barada). Moreover, In Turkey Flexuosus melon cultivars and Dudaim type of melons are grown in small quantities [8]. *Cantaloupe* type melons are commonly cultivated in the Mediterranean region of Turkey, whereas the *Inodorous* melons are grown mainly in the Central Anatolia, Aegean and Southeastern Anatolia regions of Turkey. *Inodorous* group has the largest number of cultivars in Turkey [39].



**Figure 3.** Hasanbey melon fruits(adapted from [40])

Hasanbey melon is commonly grown in Western Anatolia in Turkey. This variety is round and dark green with a long shelf life. The Hasanbey melon cultivar is harvested from August to September due to its late ripening period [4]. Cinikiz melons are grown in the Central Anatolia in Turkey. This melon group has the highest ascorbic acid, sweetness and sugar content. The immature fruits of the Cinikiz melon have a light green skin color with dark green spots; mature fruits, a yellow colored skin [16, 18].



**Figure 4.** Cinikiz melon fruits (adapted from [41])

Individual plants of the Hasanbey or Cinikiz melon genotypes under *in vitro* conditions develop organs of similar size due to the elimination of environmental conditions and are, genetically controlled. Because of the interspecific and intergeneric incompatibility barriers in melons, some conventional methods such as hybridization and line fixing for improvement of melon cultivars are quite limited as well as expensive [42, 43]. We assume that the outcomes from the present study may provide good sources for further transformation studies on the genotypes of Hasanbey and Cinikiz melon groups [10, 44].

In the light of these facts, we hypothesize that an efficient regeneration method of economically important Turkish Cultivars, Cinikiz and Hasanbey, allows comparison of two different melon cultivars in regard to regeneration ability under an identical artificial medium. In the present study, two Turkish melon varieties were tested for *in vitro* regeneration ability cultured on Murashige and Skoog (MS) media containing different combinations and varying concentrations of growth regulators.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Seed sources

Mature seeds of *Cucumis melo* L. cv. Hasanbey and Cinikiz were used as explants sources. The seeds of Hasanbey and Cinikiz melon varieties were obtained from Laboratory for Plant Biotechnology in Horticulture Department, Agriculture Faculty, Cukurova University, Adana, Turkey.

### 2.1.2. Media and culture conditions

The achievement of *in vitro* tissue culture hinges on the composition of the medium, growth regulators, plant genotypes, explant sources, growth and culture conditions. Culture media used in this study are based on MS salts [20].

After surface sterilization, for the initiation of seed cultures, 10 seeds were placed into 100x15mm petri dishes containing basal MS basal medium with MS vitamins, 3% (w/v) sucrose and 0.75% (w/v) agar for three days. The pH level of the medium was adjusted to 5.7 prior to adding gelling agents. The media were sterilized by autoclaving at 121°C for 20 minutes.

Cultures were incubated in a growth room at 25±2°C at dark for three days. *In vitro* grown cotyledon pieces of mature seeds were transferred into regeneration medium containing different concentrations of IAA (0.0, 2.5, 5.0 mg L<sup>-1</sup>), Kin (0.0, 2.5, 5.0 mg L<sup>-1</sup>) and NAA (0.0, 0.5 mg L<sup>-1</sup>) for organogenesis. Cotyledon explants were incubated at 25±2°C under 16 h photoperiod provided by cool white fluorescent lamps.

## 2.2. Methods

### 2.2.1. Surface sterilization of seeds

Melon seed coats were removed and the seeds were dipped into 70% ethanol for ten minutes and kept in 20% sodium hypochlorite with 2 drops of Tween-20 per 100 ml solution with occasional shaking for 10 minutes. The seeds were then rinsed three consecutive times with sterile distilled water and blotted dry in a laminar flow cabinet. After straining the water, the seeds were placed directly on the culture medium under sterile conditions.

### 2.2.2. Seed germination

For seed germination, 10 seeds in each of the 100x15mm petri dishes containing the culture medium containing MS salts [20], MS vitamins and 3% (w/v) sucrose, gelling agent were placed.

Cultures were maintained on a hormone free MS medium for five days in a growth chamber at 25±1°C in darkness.

### 2.2.3. Plant regeneration treatments

*In vitro* plant regeneration is based on the balance of cytokinin and auxin and the quality of the explant sources during plant development. To evaluate the efficiency of various growth regulators, cotyledon explants were excised from *in vitro* grown seedlings after seed germination and then placed with the abaxial side onto the surface of a solidified regeneration medium variants, consisting of MS basal medium supplemented with growth regulators, auxins (NAA and IAA) and cytokinins (Kinetin) in different combinations (see Table 2 for concentrations of growth regulators).

The explants obtained from the part proximal to the apex of the seedling were taken to induction medium. All media were sealed with parafilm and maintained in a growth room at

25±2°C under dark conditions. Every combination of growth regulators was used in the medium for each melon genotype. The experiment was set as a total of 22 treatments for both of the melon genotypes; each treatment was carried out in triplicates containing ten explants in each culture medium.

Concentrations of growth regulators (mg L <sup>-1</sup> ) in the culture media used for propagation		
NAA	IAA	Kinetin
0	0	0
0	2,5	0
0	2,5	2,5
0	2,5	5,0
0	5,0	0
0	5,0	2,5
0	5,0	5,0
0,5	0	0
0,5	0	2,5
0,5	0	5,0
0,5	5,0	5,0

**Table 2.** MS media supplemented with different growth regulators for plant regeneration

The regeneration ability of each genotype was then scored weekly for a period of 6 weeks. The data on seed germination and plant regeneration were collected and regenerated plants less than 1 mm in length were not taken into consideration.

### 3. Results and discussion

#### 3.1. Seed germination

The germination ability of the melon seeds can be affected by both internal and external factors. Since seed size is considered important for better germination [45-47], seeds in similar size were selected for both genotypes. Germination ratio of the Cinikiz melon seeds was found higher (85%) than that of the Hasanbey seeds (78%) under the same culture conditions.

### 3.2. *In vitro* plant regeneration

In the study presented here, cotyledons from *in vitro* grown seedlings were explant sources. In all experiments, basic MS medium containing MS salts, MS vitamins and sucrose ( $30 \text{ g L}^{-1}$ ) was used.

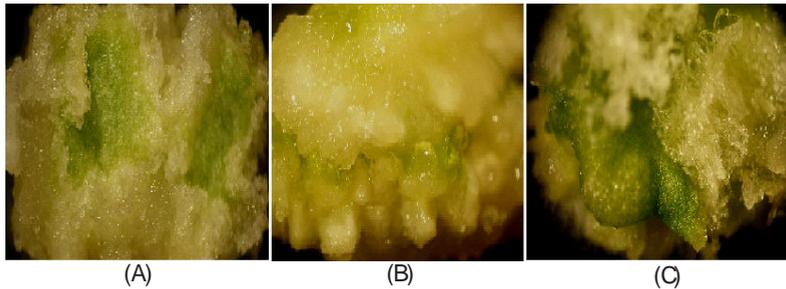
Different concentrations and combinations of IAA ( $0,0, 2,5, 5,0 \text{ mg L}^{-1}$ ), Kin ( $0,0, 2,5, 5,0 \text{ mg L}^{-1}$ ) and NAA ( $0,0, 0,5 \text{ mg L}^{-1}$ ) were investigated to optimize regeneration of two commercially important Turkish melon varieties: Hasanbey and Cinikiz.

There were significant differences between two melon varieties based on the growth regulator concentrations. According to our findings, comparison of the genotypes showed that the Cinikiz melon cultivar has better regeneration ability than did the Hasanbey melon. In addition, the maximum shoot regeneration was achieved on MS medium supplemented with  $2,5 \text{ mg L}^{-1}$  IAA and  $2,5 \text{ mg L}^{-1}$  Kin was determined the best regeneration medium for both cultivars. Moreover, NAA was found to be the best growth regulator in the induction of callus of both melon varieties. NAA alone induced direct callus formation, while Kin exhibited synergism with IAA for induction bud formation for both two varieties.

To date propagation of *Cucumis melo* has been reported by different research groups [34, 36, 48, 49, 50, 51]. The main differences between the present study and the earlier ones are the growth regulators used and melon cultivars selected. We used Hasanbey and Cinikiz melon cultivars as explants; while the others used 'Amarillo oro' [48, 51], 'Accent', 'Galia', 'Presto', and 'Viva' [36], 'Revigal' and 'Kirkagac' [34, 50], 'Topmark' [49, 50]. After three weeks on a MS medium free from plant growth regulator, approximately 80% of cotyledon explants gave rise to friable callus (Figure 5). Calli were found from the cut surface of cotyledon explants within 5 days. In a previous study, however, direct shoot formation was obtained from cotyledon explants cultured on MS medium supplemented with  $1,0 \text{ mg L}^{-1}$  BA [36].

In our study, the media containing  $0,5 \text{ mg L}^{-1}$  NAA showed that supplying NAA in the medium increased the callus formation, although the IAA and Kin concentration ( $5 \text{ mg L}^{-1}$ ) was the identical to that of previous study [36]. The addition of NAA alone in our experiment stimulated formation of the callus. The best callus formation ratios were observed from the media containing  $0,5 \text{ mg L}^{-1}$  NAA,  $5 \text{ mg L}^{-1}$  IAA and  $5 \text{ mg L}^{-1}$  Kin for Cinikiz and Hasanbey melon cultivars 100% and 87%, respectively. The medium containing  $0,5 \text{ mg L}^{-1}$  NAA,  $5 \text{ mg L}^{-1}$  IAA and  $5 \text{ mg L}^{-1}$  Kin stimulated callus growth for Hasanbey melon cultivar. On the contrary, the frequency of callus formation for Cinikiz melon genotype due to NAA concentration was significant. In all medium containing  $0,5 \text{ mg L}^{-1}$  NAA, high callus formation (100%) from Cinikiz melons' cotyledons was obtained (Table 3).

The calli were white to yellowish; their surface showed structures such as shoot formation and the calli were rarely regenerative. Our result agrees with the reported callus formation from the plants regenerated *in vitro* [50].



**Figure 5.** A) Callus formation from cotyledon explants on the MS hormone free medium, (B) Callus formation from 'Cinikiz' cotyledon explants and (C) 'Hasanbey' cotyledon explants on the MS medium supplemented with 0,5 mg L<sup>-1</sup> NAA, 5 mg L<sup>-1</sup> IAA and 5 mg L<sup>-1</sup> Kin.

In an earlier study on regeneration of melon, shoot buds were obtained at high rates in cotyledon explants [48] and well-developed shoots were observed from calli growing MS media containing 1,5 mg L<sup>-1</sup> IAA and 6,0 mg L<sup>-1</sup> Kin.

According to previous study, [48] Kin was essential for shoot formation. *Cucumis melo* L. cv. In our study, Cinikiz gave better shoot production than Hasanbey cultivar. The explants produce fragile and large callus; within 15 days direct shoot organogenesis had occurred. The frequencies of bud formation were increased by the combination of 2,5 mg L<sup>-1</sup> IAA and 2,5 mg L<sup>-1</sup> Kin compared to 2,5 mg L<sup>-1</sup> Kin and 0,5 mg L<sup>-1</sup> NAA. On the other hand, the bud formation of the Hasanbey melon genotype was higher than the Cinikiz melon genotype on the medium including solely 2,5 mg L<sup>-1</sup> IAA. When the concentration of IAA were increased, the frequency of bud formation for Hasanbey melon variety decreased. A high frequency of induction of bud for Cinikiz melon genotype occurred on the medium supplemented with same concentration of IAA and Kin, but when the concentrations of IAA and NAA were increased with the same level, bud formation for Hasanbey melon was not observed. It was found that IAA and Kin combination at the same concentration (2,5 mg L<sup>-1</sup>) increased the regeneration ratio. We were able to induce bud formation by culture of the explants on MS medium with 0,5 mg L<sup>-1</sup> NAA for both melon cultivars. This result is similar to those of early studies on melon regeneration [49, 50] where it was known that the medium containing NAA stimulated callus growth.

Of the growth regulators tested, NAA was the most effective at inducing callus creation from the cotyledons of both two melon genotypes presented here (Table 3).

The media used in the study presented containing IAA alone or in combination with Kin never trigger callus creation. This result is similar to previous studies [43, 48, 49].

Development in regeneration from the melon Hasanbey cotyledon explants on the medium added 2,5 mg L<sup>-1</sup> IAA and 2,5 mg L<sup>-1</sup> Kin was found to be slower than with melon Cinikiz.

After 4 weeks in the culture, there was little further development on the explants of Hasanbey melon cultivar compared to that of Cinikiz.

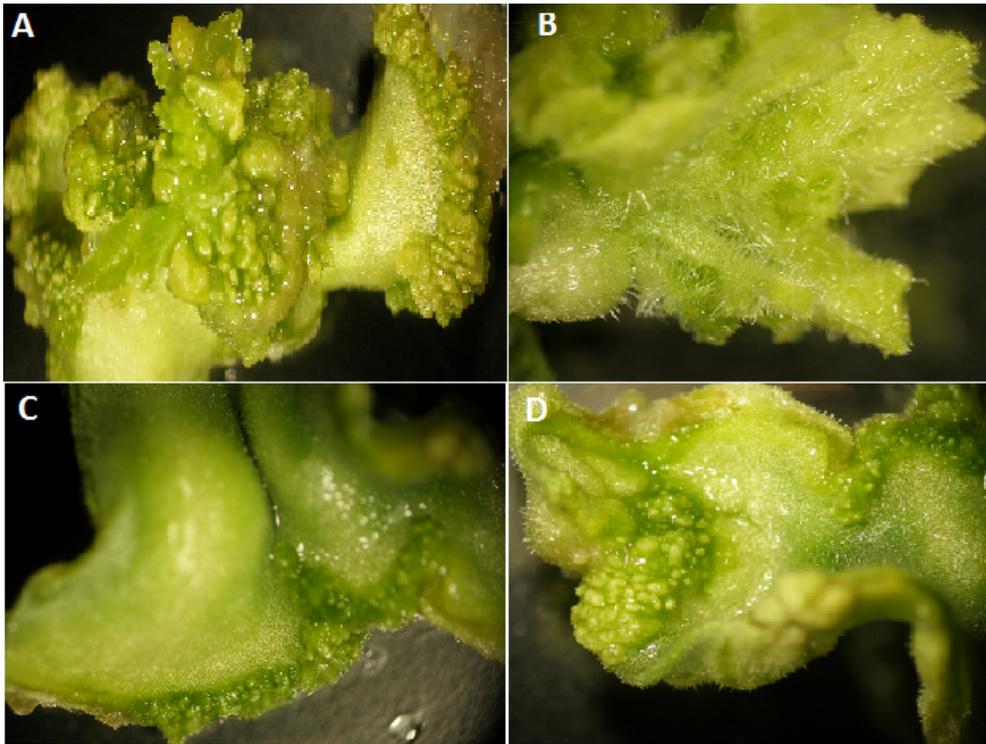
Treatments (mg L <sup>-1</sup> )			Variables						
			Callus creation (%)		Bud formation(%)		Shoot regeneration ratio (%)		
			Melon genotypes		Melon genotypes		Melon genotypes		
			Hasanbey	Cinikiz	Hasanbey	Cinikiz	Hasanbey	Cinikiz	
IAA	NAA	Kin							
0	0	0	0	0	10	12	0	0	
2,5	0	0	0	0	62	50	0	0	
2,5	0	2,5	0	0	50	78	50	75	
2,5	0	5,0	0	0	37	62	25	12	
5,0	0	0	0	0	50	75	0	5	
5,0	0	2,5	0	0	50	50	0	0	
5,0	0	5,0	0	0	0	62	0	0	
0	0,5	0	50	100	25	25	20	15	
0	0,5	2,5	50	100	0	12	0	2	
0	0,5	5,0	75	100	0	50	12	25	
5,0	0,5	5,0	87	100	25	12	0	0	

**Table 3.** Effect of IAA, NAA and Kin concentration on callus creation, bud formation and shoot regeneration percentages of Hasanbey and Cinikiz melon varieties' cotyledons on the medium containing different concentrations of IAA (0,0, 2,5, 5,0 mg L<sup>-1</sup>), Kin (0,0, 2,5, 5,0 mg L<sup>-1</sup>) and NAA (0,0, 0,5 mg L<sup>-1</sup>)

The best results for shoot regeneration were obtained from MS medium supplemented with 2,5 mg L<sup>-1</sup> IAA and 2,5 mg L<sup>-1</sup> Kin for Cinikiz and Hasanbey melon genotypes 75% and 50%, respectively. The results of the regeneration tests are summarized in Table 3.

The regeneration efficiency of Hasanbey and Cinikiz melon using cotyledon explants was evaluated in terms of regenerated plants. Cotyledon organogenesis was induced during incubation and bud formations located along the cut basal edge of the explant were visible on 8 day-old cultures of both of the two melon varieties. On the other hand, the first shoots formed 12 day-old explants of Cinikiz melon. According our results, the Cinikiz melon cultivar gave better results than did the Hasanbey cultivar (Figure 6).

Furthermore, the most important difference was observed in the number of bud induced between the two melons. In the Cinikiz melon young tubercles often clustered in the point of regeneration and shoot meristems developed into leaves(Figure 6-A). In the Hasanbey melon, the first regenerated shoots were observed in 15 day-old cotyledon explants and regenerated shoots were originated from the epidermal layer of the explants (Figure 6-B). These results as those of the previous studies show the clear cut effect of the genotypes on regeneration.

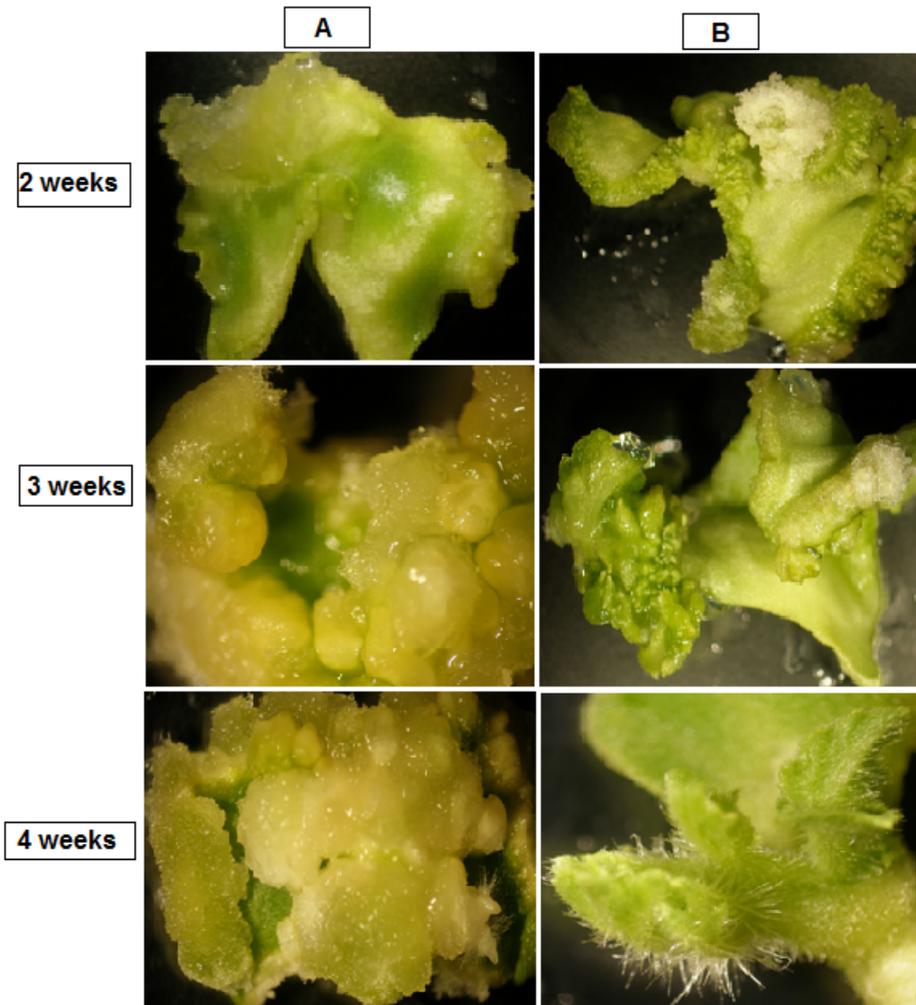


**Figure 6.** A, B) Shoot formation of Cinikiz melon cultivar on the MS medium added with 5,0 mg L<sup>-1</sup> IAA and 2,5 mg L<sup>-1</sup> Kin and (C, D) shoot formation of Hasanbey melon cultivar on the MS medium added 2,5 mg L<sup>-1</sup> IAA alone

For the Hasanbey genotype, explants which were cultured on the MS medium free from plant growth regulators, showed callus formation with different coloration and appearance after 2 weeks. Most of them were white to yellowish and friable. A mass of small cells initiated the regeneration of the shoot meristems form directly on explants *in vitro*. This result is similar to previous studies [43, 50].

After 3 weeks on the medium, explants developed into buds and about a month on culturing, root formation was observed. As they continued to grow, they became yellow and did not develop into shoots. (Figure 7-A). In addition, some demonstrated necrosis. Shoot formation (50%) for Hasanbey melon genotype was obtained on a MS medium consisting of 2,5 mg L<sup>-1</sup> IAA and 2,5 mg L<sup>-1</sup> Kin after 2 weeks. Explants after 3 weeks of culture formed shoot structures, continued to grow, bud formation was not observed at this stage (Figure 7-B).

As a result, it was observed that the melon possessed the ability to regenerate by means of direct organogenesis from cotyledon explants. After 4 weeks in culture the explants enlarged.

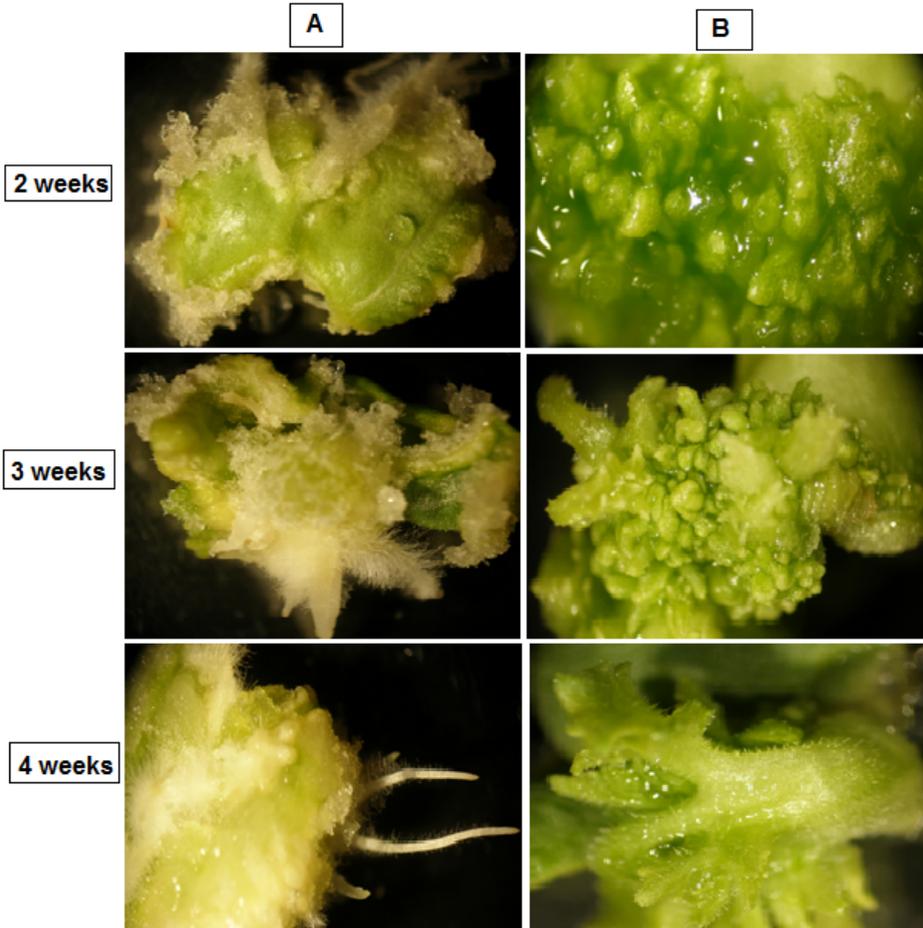


**Figure 7.** Hasanbey genotypes at different stages of their development. A: Control groups which developed on free plant growth MS medium, B: Explants which developed on MS medium supplemented with 2,5 mg L<sup>-1</sup> IAA and 2,5 mg L<sup>-1</sup> Kin

Control groups of Cinikiz melon cultivar developed on the MS medium free from plant growth regulators showed developing callus and roots (Figure 8-A). On the media containing 2,5 mg L<sup>-1</sup> IAA and 2,5 mg L<sup>-1</sup> Kin, the highest percentage of shoot regenerants from Cinikiz melon cultivar after 2 weeks was obtained (Figure 8-B).

In contrast by involving the control groups of Cinikiz melon cultivar on the MS medium free from plant growth regulators, shoot formation was observed. After 2 weeks, some young

protuberances clustered and developed; after 4 weeks, finger-like structures were observed (Figure 8-B). After 4 weeks, no difference could be determined between protuberances that became leaves from Cinikiz samples.



**Figure 8.** Cinikiz genotypes at different stages of their development. A: Control groups which developed on free plant growth MS medium, B: Explants which developed on MS medium added with 2,5 mg L<sup>-1</sup> IAA and 2,5 mg L<sup>-1</sup> Kin

#### 4. Conclusion

Our *in vitro* propagation results from these two melon varieties, Hasanbey and Cinikiz, were selected as research material for the study presented here due to their importance for the

Turkish agricultural production. Due to open pollination, melon varieties can be more or less stable against environmental factors from one generation to the next. In addition, as can be seen from the results presented here, Hasanbey and Cinikiz melon genotypes can be *in vitro* propagated.

On the other hand, further studies are needed to analyze other Turkish melon varieties and identify the optimum regeneration medium for each genotype. In addition, if the response of the two melon cultivars selected in this study is observed from the point of view of breeder significance, experimental fields should be conducted in future studies. Understanding the role of growth regulators in the development of selected melon cultivars has highly facilitated melon production under controlled environments. Moreover, the resulting information can be also used for research on melon developmental physiology, an intensive continuation of *in vitro* propagation studies is essential, as well.

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