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INFLUENCE OF GENOTYPE AND EXPLANT SOURCE ON THE *IN VITRO* REGENERATION ABILITY OF DIFFERENT MELON VARIETIES

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Nine genotypes of melon (*Cucumis melo* L.) were selected for the investigation of regeneration. Most of the tested varieties showed regeneration ability on medium containing 0.5 mg l^{-1} or 1 mg l^{-1} BA, but following the appearance of shoot buds, only six varieties produced leafy shoots. The effect of combinations of BA with different auxins (IAA, NAA, 2,4-D) and ABA in the culture medium on shoot regeneration was tested on cotyledon explants of 'Hógolyó' and 'Hale's Best'. To establish optimal conditions for the adventitious shoot induction six types of seedling-derived explants were prepared from seedlings of four different ages. The best results for shoot forming capacity were achieved with cotyledons followed by decapitated seedlings and hypocotyls derived from 4-day-old seedlings. Cotyledon segments of 'Hógolyó' and 'Hale's Best' were also cultivated on media with different concentrations of IAA and BA supplemented with 0.26 mg l^{-1} ABA. The highest number of well-formed plantlets was counted for 'Hógolyó' on the medium supplemented with 0.9 mg l^{-1} BA + 0.6 mg l^{-1} IAA + 0.26 mg l^{-1} ABA. This is the first report on the *in vitro* regeneration of 'Hógolyó' from decapitated seedling and hypocotyl explants and of 'Javított Zentai', 'Muskotály', 'Hógolyó', 'Tétényi csereshéjú' and 'Magyar Kincs' from cotyledon explants.

Keywords: Melon – *in vitro* – regeneration – cotyledon – decapitated seedlings

INTRODUCTION

Melon (*Cucumis melo* L.) is an important vegetable crop that is cultivated all over the world. In Hungary melon has been a popular vegetable for centuries. Breeders have made great efforts to satisfy the varying needs of consumers and producers. The development of genetic transformation for melon offers the potential of introducing valuable traits into this crop, e.g. disease resistance, high sugar content and high protein content, to improve its productivity and quality beyond the limits of conventional breeding. The regeneration of melon has been reported from cotyledonary

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explants [20, 22], hypocotyl explants [13, 20], leaves [14] and root explants [15]. Genetic transformation of melon has also been reported by several authors [5, 6]. Most of the reported studies have been conducted with *Cucumis melo* L. var. *cantalupensis* or var. *reticulatus*, with fewer data available for var. *inodorus* [17]. The influence of the genotype on the regeneration of *C. melo* L. was investigated on cotyledon explants by Molina and Nuez [19]. As for other plants, melon genotypes were found to differ substantially in their sensitivity to plant growth regulators and culture conditions in the regeneration process.

The purpose of this study was to screen the regeneration ability of melon varieties, which have good quality, bold flavour, distinct aroma, and high sugar content. These varieties included old genotypes and landraces [26]. Unfortunately, they have low shelf-life and most of them are susceptible to viral diseases, so genetic modification could clearly be advantageous. The aim of these experiments was to establish a reliable regeneration system as a basis for transformation studies.

MATERIAL AND METHODS

Plant material

Mature seeds of *C. melo* var. *inodorus* (‘Hógolyó’), var. *cantalupensis* (‘Javított Zentai’, ‘Ezüstananász’, ‘Muskotály’, ‘Topáz’) and var. *reticulatus* (‘Tétényi csereshéjú’, ‘Magyar Kincs’, ‘Fortuna’) [25] were used as explant sources. For comparison ‘Hale’s Best’, a cultivar widely used in regeneration experiments [6, 22] was also studied. All the cultivars were provided by the Hungarian Central Agricultural Office, except ‘Hógolyó’ (Vegetable Crops Research Institute) and ‘Hale’s Best’ (GeneBank of Corvinus Unversity, Budapest).

Following manual removal of the seed coats, the seeds were surface-sterilized for 15 minutes in 15% Clorox and washed three times with sterile distilled water. The seeds were sown onto MS [21] medium. For germination they were kept at 32 °C in darkness for two days followed by two days in a growth chamber at 25 °C, with a 16 h/8 h light/dark photoperiod provided by cool-white fluorescent lamps.

Explant preparation

Explants were prepared following germination from 2-, 4-, 8- or 14-day-old seedlings. After removing the tip and bottom part the cotyledons of all the varieties were halved transversely to give 2 explants per cotyledon. Eight explants were placed in each Petri dish with the abaxial side down (40 explants per experiment). Hypocotyls were excised from the seedlings and the remained segments were cut into three equal parts (upper, middle and basal part), and vertically inserted (1–2 mm) into the culture media. Decapitation was performed by cutting below the cotyledonary node to remove the cotyledons and apical bud while the roots remained intact in the culture

vessel (200 ml glass vessels, diameter 5 cm, 20 explants per experiment in both cases). Primary leaves, measuring about 0.5–0.9 cm, were excised from the 14-day-old seedlings and cultured intact on regeneration medium.

Culture media and conditions

Cotyledon segments were cultivated on MS medium supplemented with different combinations of growth regulators. In the first series of experiments, cotyledons of all the varieties were cultured on medium containing 0.5 mg l^{-1} or 1 mg l^{-1} 6-benzylaminopurine (BA). From the responding varieties a second series of experiments was conducted on two varieties selected for investigation of the effect of a possible interaction between different auxins (indole-3-acetic acid (IAA), 1-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) 1 mg l^{-1} of each) with BA (0.5 mg l^{-1}) on the efficiency of shoot induction. Abscisic acid (0.26 mg l^{-1} ABA) was also added to one medium containing 0.5 mg l^{-1} BA + 1 mg l^{-1} IAA.

In order to investigate the organogenic capacity of explants of different age and source, a comparative experiment was performed. The effect of the explant type on bud initiation was studied by culturing 'Hógolyó' and 'Hale's Best' cotyledon explants, hypocotyl explants, decapitated seedlings and primary leaves. The explants were cultured on MS medium containing 0.5 mg l^{-1} BA + 1 mg l^{-1} IAA + 0.26 mg l^{-1} ABA, except for the decapitated seedlings which remained on growth regulator-free MS medium.

The effect of seedling age on shoot initiation was studied on explants of all types taken from 2-day-old (*just germinated*), 4-day-old (*half-expanded cotyledons*), 8-day-old (*fully-expanded cotyledons*) and 14-day-old (*open cotyledons with first leaves*) seedlings of both varieties.

To find the most suitable medium for shoot induction, cotyledon segments of 'Hógolyó' and 'Hale's Best' were also cultivated on media with different concentrations of IAA (0, 0.6, 1.2 mg l^{-1}) and BA (0, 0.6, 0.9, 1.2 mg l^{-1}) supplemented with ABA (0.26 mg l^{-1}).

All the media were supplemented with 3% sucrose, MS vitamins and 2.5 g/l Phytagel (Duchefa) and the pH was adjusted to 5.6–5.8 before autoclaving (120 °C, 20 min). The explants were cultured in Petri dishes or in 200 ml glass vessels (diameter 5 cm). After 2–3 weeks of culture the regenerating areas of the explants were excised and transferred to fresh regeneration medium. After 7 weeks of culture the explants were scored under stereomicroscope for the number of explants forming shoot buds or leafy shoots.

Root induction and plant acclimatization

For root induction, excised shoots which had at least 3–4 fully opened leaves were transferred to regulator-free MS medium until they had a well-developed root system. The plantlets were acclimatized in soil/vermiculite mixture (1 : 1) and incubated at

25 ± 1 °C in a growth chamber for 2 weeks at 100% relative humidity and for another 2 weeks with gradually decreasing humidity. After these steps they were transferred to a greenhouse.

Data analysis

Three independent experiments were conducted for data analysis. Each treatment consisted of 40 cotyledon explants, 20 hypocotyls, 20 decapitated seedlings and 20 first leaves. For the growth regulator experiment eight cotyledon explants of the 'Hógolyó' and 'Hale's Best' were used. Data were collected after 7 weeks of culture. The number of regenerated shoots was calculated as the ratio of explant forming shoots and the total number of explants. The percentage of cotyledons that formed shoots and the number of shoots per regenerated cotyledon were also calculated. The data were evaluated by analysis of variance (ANOVA) and means were compared using Duncan's multiple range test ($p = 0.05$) to determine significant differences.

RESULTS

Comparison of genotypes

In the first series of experiments cotyledons of all the varieties were cultured on media containing 0.5 mg l^{-1} or 1 mg l^{-1} BA. On medium containing 1 mg l^{-1} BA shoot initials appeared on cotyledon segments of all the varieties within 2–3 weeks. However, complete development of shoots only took place in the case of 'Javított Zentai', 'Hógolyó', 'Magyar Kincs' and 'Hale's Best' while the other varieties tended to form callus or remained in the stage of leafy bunches without elongation of the shoot axis. On medium containing 0.5 mg l^{-1} BA shoot initials appeared on the explants of 'Javított Zentai', 'Muskotály', 'Hógolyó', 'Tétényi csereshéjú' and 'Hale's Best' after 2–3 weeks and developed into well-formed leafy shoots, while in 'Topáz' only shoot buds were formed. On the other three varieties only callus formation was observed. Among the regenerants, those originating from medium with 0.5 mg l^{-1} BA seemed to be more vigorous and the loss caused by hyperhydricity was less severe than in the case of 1 mg l^{-1} BA. For evaluation of the final results only leafy shoots showing the normal phenotype were counted. The data are given in Table 1.

From the responding varieties, a second series of experiments was conducted. The varieties 'Hógolyó' and 'Hale's Best' were selected to investigate the effect of a possible interaction between BA and the different auxins. The highest number of plantlets having three or four leaves was observed on explants of both varieties on medium containing 0.5 mg l^{-1} BA + 1 mg l^{-1} IAA + 0.26 mg l^{-1} ABA. The lack of ABA in the regeneration medium slightly decreased the number of shoots for both varieties. A significant difference was only found for 'Hale's Best'. Leafy shoots of 'Hógolyó'

Table 1
Shoot regeneration frequency on MS medium containing two different concentrations of BA (1 mg l^{-1}, 0.5 mg l^{-1})

Name of tested varieties	No. of shoots with 3–4 leaves per explant	
	1 mg l^{-1} BA	0.5 mg l^{-1} BA
Ezüstananász	0±0[a]*	0±0[a]
Javított Zentai	0.22±0.039[b]	0.23±0.021[b]
Muskotály	0±0[a]*	0.1±031[a]
Topáz	0±0[a]*	0±0[a]*
Hógolyó	0.65±0.047[d]	0.53±0.046[c]
Fortuna	0±0[a]*	0±0[a]
Magyar Kincs	0.12±0.021[b]	0±0a
Tétényi csereshéjú	0±0[a]*	0.1±0.027[a]
Hale's Best	0.34±0.033[c]	0.46±0.026[c]

* Only shoot initials appeared on the explants.

Each value represents the mean±SD of 40 explants in three repeated experiments.

Numbers followed by the same letter (a, b, c or d) are not significantly different at the 0.05 level according to Duncan's multiple range test.

were also formed on medium containing 0.5 mg l^{-1} BA with 1 mg l^{-1} NAA, but on explants of 'Hale's Best' only yellowish callus was formed with green nodules. On the medium containing 0.5 mg l^{-1} BA and 1 mg l^{-1} 2,4-D the explants expanded and became green, but none of the varieties showed shoot regeneration and only white, translucent callus formation occurred. The results are summarized in Table 2.

Table 2
Shoot regeneration frequency on cotyledon explants of Hógolyó and Hale's Best on media with different growth regulators

Growth regulators (mg l^{-1})					No. of shoots per explant	
BA	IAA	NAA	2,4-D	ABA	Hógolyó	Hale's Best
0.5	0	0	1	0	0±0[a]	0±0[a]
0.5	0	1	0	0	0.46±0.031[b]	0±0[a]*
0.5	1	0	0	0	0.89±0.041[c]	1.06±0.042[b]
0.5	1	0	0	0.26	0.97±0.039[c]	1.23±0.046[c]

* Only shoot initials appeared on the explants.

Each value represents the mean ± SD of 40 explants in three repeated experiments.

Numbers having the same letter (a, b, or c) are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table 3
Shoot regeneration frequency from explants of different source and age

Age of explants (day)	Hógolyó (No. of shoots per explant)			Hale's Best (No. of shoots per explant)		
	Cotyledon	Decapitated seedling	Hypocotyl (upper part)	Cotyledon	Decapitated seedling	Hypocotyl (upper part)
2	0.00 ± 0.00^{aA}	0.07 ± 0.03^{aB}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.12 ± 0.03^{aB}	$0.07\pm0.03a^{bB}$
4	0.89 ± 0.06^{cC}	0.32 ± 0.08^{bB}	0.13 ± 0.08^{bA}	1.03 ± 0.14^{cB}	0.45 ± 0.10^{cA}	0.33 ± 0.08^{cA}
8	0.27 ± 0.05^{bB}	0.20 ± 0.10^{bB}	0.00 ± 0.00^{aA}	0.32 ± 0.05^{bB}	0.28 ± 0.08^{bB}	0.13 ± 0.06^{bA}
14	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}

Each value represents the mean ± SD of 20 to 40 explants in three repeated experiments.

Numbers having the same letter (a, b, or c) are not significantly different at the 0.05 level according to Duncan's multiple range test. Small letters indicate the significant differences between the explant ages vertically and capital letters indicate the differences between explant sources horizontally.

Effect of age and explant type

In the case of 2-day-old seedlings of 'Hógolyó', only decapitated seedlings produced shoots. These regenerants often appeared 1–2 weeks later than those originating from 4-day-old seedlings. On 2-day-old cotyledon segments groups of shoot initials were formed and sometimes small leaves appeared but plant development was not completed. The other 2-day-old explant types showed no regeneration response at all, only producing white callus. The most efficient regeneration was obtained on 4-day-old seedlings. For shoot forming capacity the best results were achieved with cotyledons followed by decapitated seedlings and hypocotyls (upper part). On the middle part of the hypocotyl only shoot initials appeared, while only callus formation was observed on the basal part. All the 8-day-old seedling explants produced fewer shoots than the 4-day-old ones (cotyledons, decapitated seedlings). Shoot initials appeared on the upper part of the 8-day-old hypocotyls while white callus was formed on the basal and middle part of the hypocotyl. The results are shown in Table 3.

In the case of 2-day-old seedlings of 'Hale's Best', decapitated seedlings and the upper part of the hypocotyls produced shoots. Greenish callus formation was observed on the 2-day-old cotyledons, while white callus appeared on the middle and basal part of hypocotyl. The best results were given by 4-day-old seedlings, as observed for 'Hógolyó', except that on the middle part of the hypocotyl greenish callus was observed. Regenerated plantlets appeared on the cotyledons, decapitated seedlings, and upper part of hypocotyl from 8-day-old seedlings. On the middle part of the hypocotyl greenish callus was observed. The results are shown in Table 3. The explants of 14-day-old seedlings, including the first leaves, showed no response for either variety.

Comparing the explant sources, cotyledon segments showed significantly higher regeneration capacity than the other explant types (Table 3). The results indicate that the age of the seedlings and the source of explant had a great effect on the regeneration ability (number of shoots).

Growth regulator combination of 'Hógolyó'

After 2 weeks on regeneration medium shoot buds were formed on most of the cotyledon explants (Figure 1A, B, C). Some exhibited small clusters of shoot initials and after 3–4 weeks started to form callus. Others formed leaf primordia, but no shoots developed from them. On media supplemented with BA in the range of 0.6–1.2 mg l^{-1} combined with 0.6 mg l^{-1} IAA, the initiation of white callus was observed, besides shoot induction. Some of the regenerated shoots were malformed on media containing 1.2 mg l^{-1} IAA or 1.2 mg l^{-1} BA, so these were not counted in the final results. The highest number of well-formed plantlets (ready to transfer to rooting media) was counted on medium supplemented with 0.9 mg l^{-1} BA+ 0.6 mgl^{-1} IAA+0.26 mg l^{-1} ABA. On this medium 95.83% of the explants from 'Hógolyó' responded and 1.64 plants were regenerated per explant. The number of explants

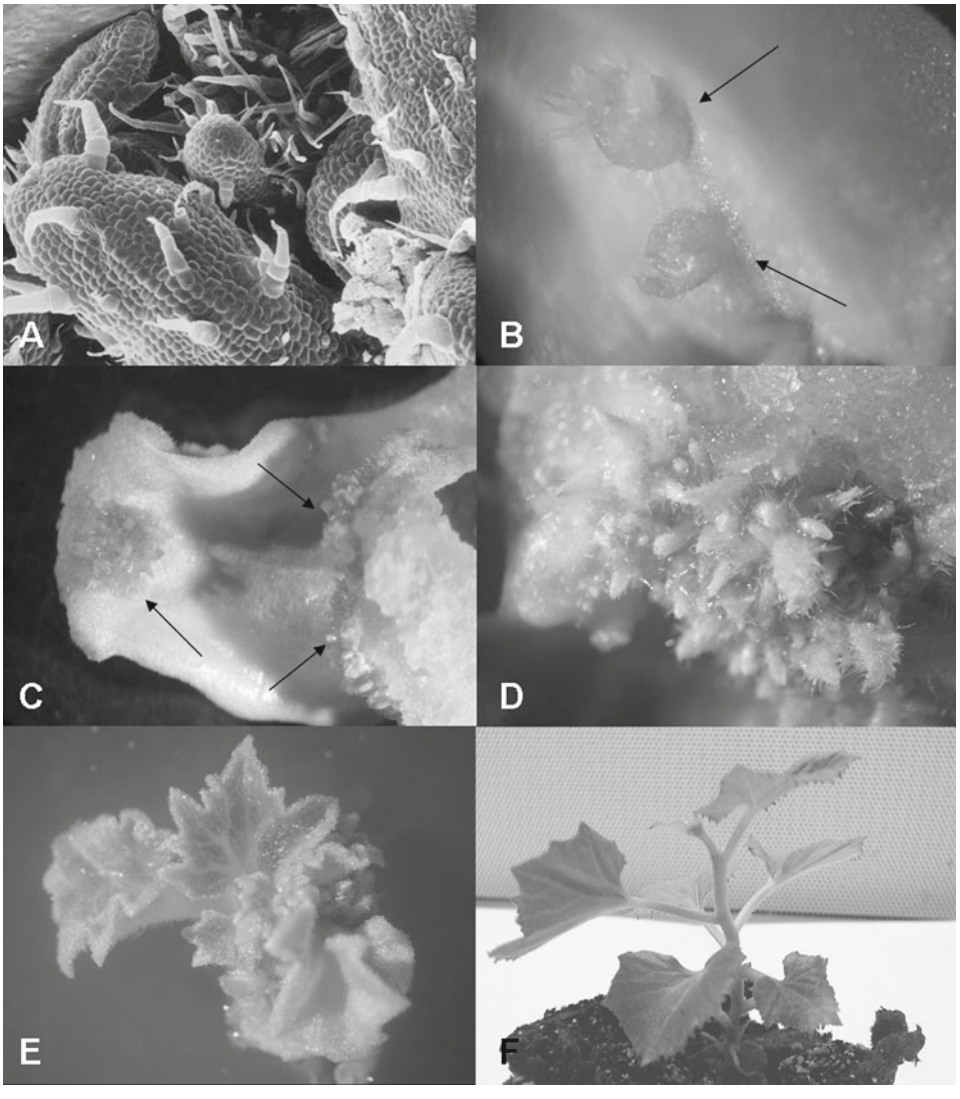

Fig. 1. Regeneration steps for variety Hógolyó. A – Scanning electron microscope photograph of shoot buds and shoot initials (Magnification: ×250,000) after 7 days on regeneration medium. B – Stereomicrograph of single shoot buds on the surface of cotyledon explant. The black arrows indicate the shoot buds. C – Stereomicrograph of cotyledon segment with callus and shoot bud formation on both sides of the cut surface after 14 days on regeneration medium. Regeneration occurs along the points indicated by the black arrows. D – Stereomicrograph of shoot initiation (multiple shoot cluster) after 14 days on regeneration medium. E – Leafy shoot after six weeks on regeneration medium. F – Rooted plant in soil/vermiculate mixture

Table 4
Shoot regeneration frequency from cotyledons

Growth regulators (mg l^{-1})			Hógolyó		Hale's Best	
BA	IAA	ABA	Explants forming shoots (%)	No. of shoots per regenerated explant	Explants forming shoots (%)	No. of shoots per regenerated explant
0	0	0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.6	0	0.26	54.17±5.89^{ab}	1.13±0.05^{a}	45.83±7.80^{a}	1.07±0.05^{a}
0.9	0	0.26	54.17±5.89^{ab}	1.38±0.16^{ab}	33.33±7.80^{a}	1.17±0.12^{ab}
1.2	0	0.26	29.17±2.95^{a}	1.11±0.08^{a}	37.50±8.84^{a}	1.07±0.05^{a}
0	0.6	0.26	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.6	0.6	0.26	83.33±7.80^{bc}	1.43±0.11^{ab}	87.50±5.10^{c}	1.52±0.12^{b}
0.9	0.6	0.26	95.83±2.95^{c}	1.64±0.09^{b}	83.33±2.95^{bc}	1.31±0.11^{ab}
1.2	0.6	0.26	75.00±5.10^{bc}	1.15±0.06^{a}	79.17±5.89^{bc}	1.14±0.10^{ab}
0	1.2	0.26	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.6	1.2	0.26	29.17±7.80^{a}	1.25±0.10^{ab}	79.17±5.89^{bc}	1.37±0.03^{ab}
0.9	1.2	0.26	66.67±2.95^{bc}	1.23±0.10^{ab}	58.33±7.80^{abc}	1.30±0.05^{ab}
1.2	1.2	0.26	58.33±17.92ab	1.18±0.08^{a}	54.17±7.80^{ab}	1.28±0.14^{ab}

Each value represents the mean ± SD of 8 explants in three repeated experiments.

Numbers having the same letter (a, b, or c) are not significantly different at the 0.05 level according to Duncan's multiple range test.

forming shoots was significantly higher than at any other concentration, as proved by Duncan's multiple range test. In the absence of BA, shoot regeneration was not observed. After two weeks all the explants became yellow and died on the regeneration medium. Increasing the concentration of BA resulted in a higher number of shoots and the best results were achieved on medium containing 0.9 mg l^{-1} BA but a further increase in the concentration decreased the number of shoots. The results are shown in Table 4.

Growth regulator combination of 'Hale's Best'

After 1 or 2 weeks on regeneration medium callus formation was observed on most of the explants on the cut surface of the cotyledon. In the third week the cotyledons enlarged and shoot initials appeared on the callus. The highest number of well-formed plantlets was counted on medium supplemented with 0.6 mg l^{-1} BA and 0.6 mg l^{-1} IAA. On this medium 1.52 plants were regenerated per explant. At higher concentrations of BA the formation of shoots decreased. In the absence of BA no shoot regeneration was observed. Increasing the concentration of IAA caused an increase in the number of shoots per explant. The results showed that the highest number of shoots

appeared on medium containing 0.6 mg l^{-1} IAA. Increasing the level of IAA decreased the number of developing shoots. Duncan's multiple range test showed that the medium supplemented with 0.6 mg l^{-1} IAA was significantly better than medium without IAA. The results are shown in Table 4.

Rooting of shoots

The shoots obtained from explants with 3 or 4 fully opened leaves were excised for *in vitro* rooting. On regulator-free MS medium 70% of the regenerated shoots developed 3–5 adventitious roots after 10–14 days of culture. The plants were successfully acclimatized in environment-controlled growth chamber (Figure 1D, E, F); they showed normal appearance and gave flowers in the greenhouse.

DISCUSSION

Comparison of genotypes and growth regulators

The regeneration ability of a number of melon cultivars was tested. Considerable differences were observed. Of the nine varieties, 'Hale's Best', 'Hógolyó' and 'Javított Zentai' showed better regeneration efficiency than 'Magyar Kincs', 'Muskotály' and 'Tétényi csereshéjú'. On explants of 'Fortuna', 'Ezüstananász' and 'Topáz' only the initiation of buds or callus was observed (Table 1). The genotype effect was also observed by Ficcadenti and Rotino [8], who examined eleven genotypes belonging to both *reticulatus* and *inodorus* types. Regeneration was achieved from 4- to 5-day-old cotyledons of all the genotypes in the presence of BA alone but the morphogenic response was affected by the genetic background. Debeaujon and Branchard [4] stated that genetic and environmental factors significantly determined the ability of melon plants to give an *in vitro* morphogenic response. Oridate et al. [24] also noted a significant genotype effect in somatic embryogenesis from melon seeds and concluded that the differences existed between cultivars rather than between varieties. Molina and Nuez [19] suggested that genetic variability rather than physiological differences was responsible for the different plant regeneration capacities from leaf explants of a seed population of melon. Kintzios and Taravira [16] concluded that only six of fourteen commercial cultivars responded positively to the shoot induction treatment, indicating that regeneration ability was genotype-dependent. Galperin et al. [11] screened thirty genotypes and found only one line (BU-21) with high regeneration capacity, making it suitable for transformation. Regeneration ability proved to be heritable in crossing experiments [12]. Nunez-Palenius et al. [23] also concluded that melon *in vitro* response is under genetic control; however, plant hormones have a paramount importance in the process. The present results confirm these findings: the varieties 'Hógolyó' and 'Hale's Best' gave a positive response in most of the hormone combinations in contrast to three other genotypes where no regeneration response

occurred at all. The regeneration ability of 'Hale's Best' was also confirmed by other studies [22, 28].

The comparison of varieties and media confirmed that BA alone was able to induce regeneration in most of the varieties (Table 1). A similar effect of BA was reported by Kathal et al. [14], Gaba et al. [9] and Ficcadenti and Rotino [8]. The present report confirmed the statement of Liborio-Stipp et al. [17] that cotyledon explants cultivated in absence of BA showed no organic response. They also observed that many of the multiple shoot buds became leaf primordia and very few real shoot buds were found when examined on histological sections. The lack of shoot apical meristems was previously described by Gaba et al. [10], and this phenomenon was also observed in some of the varieties, where multiple shoot initiation never led to leafy shoots, though leaves appeared (Fig. 1D).

The varieties responded differently to the auxins used in media containing BA. In general it can be stated that the efficiency of regeneration was decreased slightly by NAA and drastically by 2,4-D compared to IAA. Among the applied auxins, IAA applied together with BA greatly increased the number of regenerated shoots (Table 2). By contrast Niedz et al. [22] stated that auxin may not be required for organogenesis from cotyledons of melon. Moreno et al. [20] also observed that the presence of auxin was not essential for regeneration. Nevertheless both these authors used regeneration media containing combinations of cytokinin (BA or kinetin) and auxin (IAA). Tabei et al. [27] found that 1 mg l^{-1} IAA promoted shoot induction and that cytokinins influenced the regulation of morphogenesis. The present results underline the positive effect of IAA adding to BA on shoot induction and regeneration.

Ficcadenti and Rotino [8] stated that the addition of ABA and BA together increased the number of plantlets per explant. Niedz et al. [22] demonstrated that ABA in a concentration of 0.26 mg l^{-1} significantly increased the number of shoots when applied together with 1.1 mg l^{-1} BA and 0.88 mg l^{-1} IAA. A similar effect was found for 'Hógolyó' and 'Hale's Best' in the present work. The addition of 1 mg l^{-1} IAA and 0.26 mg l^{-1} ABA to the medium containing 0.5 mg l^{-1} BA increased the number of regenerated shoots though this was only significant for 'Hale's Best' (Table 2). However, the quality of the shoots was clearly better when adding ABA.

A separate experiment was conducted to optimize the hormone concentrations. At a standard level of ABA, the ratio of IAA and BA was changed. The combination of higher BA (0.9 mg l^{-1}) and lower IAA (0.6 mg l^{-1}) levels was found to give the best result in the case of 'Hógolyó' (Table 4). For 'Hale's Best' a lower concentration of BA (0.6 mg l^{-1}) was proved to be suitable. On growth regulator-free medium or medium supplemented only with IAA plus ABA, no shoot regeneration was observed ('Hógolyó', 'Hale's Best'). This confirms that the presence of a cytokinin, in the present case the BA, is crucial for the regeneration process (Table 4).

Effect of source and age of explant

Besides the medium, differences in the source and age of explants also influenced the rate of regeneration. Four-day-old cotyledon explants were found to be the most appropriate explant source for regenerating leafy shoots of the varieties 'Hógolyó' and 'Hale's Best'. In a study on the variety 'Hale's Best' [22] 4-day-old and 7-day-old explants resulted in a significantly higher percentage of regeneration than 18-day-old cotyledons but the difference between the 4- and 7-day-old cotyledons was not significant. Curuk et al. [2] examined Turkish melon varieties and stated that the regeneration rate of 4- and 5-day-old cotyledon explants was significantly higher than that of 6-day-old cotyledon explants, in contradiction to the results of Niedz et al. [22]. The present study confirmed the results of Curuk et al. [2], as significant differences were found in the reactions of 2-, 4-, 7- and 14-day-old cotyledon explants of 'Hógolyó' and 'Hale's Best'. As Tabei et al. [27] reported, the endogenous level of phytohormones may change during the growth of cotyledons in melon. Different ages of explants may correspond to different endogenous growth regulator levels; therefore, the age of the explants would have a critical impact on successful regeneration. Kathal et al. [13] successfully regenerated plants on 7-day-old hypocotyl explants of the variety Pusa Sharbati with 1 mg l^{-1} IAA + 0.5 mg l^{-1} kinetin. In the study of Curuk et al. [3] hypocotyl explants, attached to a fragment of cotyledon, gave more diploid explants than cotyledon explants. Although Moreno et al. [20] failed to regenerate shoots from hypocotyls, they were able to induce somatic embryos from this explant type. In agreement with the results of Curuk et al. [3] and Molina and Nuez [18] hypocotyls were found to be suitable explants for regeneration in the present work. However, the number of shoots obtained per explant was not as high as in the cited work or in the present experiments on cotyledons.

This is the first report on successful plant regeneration from decapitated melon seedlings. The shoot regeneration process was successful for both 'Hógolyó' and 'Hale's Best' but was less efficient than from cotyledons. The procedure was previously carried out by Fári et al. [7] on Solanaceous plants, but recently Amutha et al. [1] also induced adventitious shoot regeneration of greenhouse-grown squash (*Cucurbita pepo* L.) by a different method leaving a single cotyledon after removing the apical meristem.

In conclusion, regeneration from cotyledon explants appears to be an efficient method for obtaining leafy regenerants but finding the right age of seedlings is critical for successful regeneration. In the future alternative pathways, such as the use of decapitated seedlings or hypocotyl explants, should also be considered. Optimum regeneration was obtained with different auxin: cytokinin ratios for each variety. This may be due to differences in the endogenous hormone level of different genotypes and in diverse developmental stages and physiological states for individual plants. One of the varieties tested ('Hógolyó') proved to be a suitable candidate for transformation studies.

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