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The effect of different hormone concentrations and dark pretreatment on adventitious shoot regeneration in snake melon (*Cucumis melo* var. *flexousus*)

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Abstract

Explants consisting of the proximal part of cotyledons from 7 day-old seedlings, germinated under dark and light conditions, were cultured on MS media containing different concentrations of BA (0, 0.5, 1.0, 2.0 mg/l) and IAA (0, 0.25, 0.5, 1.0 mg/l).

Germination ratio of the seeds under both light and dark conditions was found as 66.8 %, but shoot formation ratio obtained from the seeds germinated under dark condition was higher than the seeds germinated under light condition.

The highest frequency of adventitious shoot regeneration (42.8 %) was obtained on MS medium with 1.0 mg/l BA+ 0.25 mg/l IAA from the seeds germinated under dark condition. 1.0 mg/l BA+ 0.5 mg/l IAA (34.8 %), 1.0 mg/l BA(32.8 %), 2.0 mg/l BA+ 0.25 mg/l IAA (12.8 %) and 2.0 mg/l BA+ 0.5 mg/l IAA (7.1 %) resulted in a lower shoot regeneration frequency.

In the case of the seeds germinated under light conditions, the maximum frequency of adventitious shoot regeneration from cotyledon explants (2.8 %) was achieved on MS medium with 1.0mg/l BA+ 0.25 mg/l IAA. Hormonal supplements of 2.0 mg/l BA (1.8 %) and 2.0 mg/l BA+0.5 mg/l IAA (1.4 %) resulted in a much lower shoot regeneration frequency.

Keywords: Snake melon, organogenesis, cotyledon explants, adventitious shoots

Introduction

The origin of snake melon is known as South-Eastern Anatolia, Azerbaijan, Iraq, Palestine and Central Asia [15]. There are many species in Anatolia, the fruit (?) appearing as round, green, hairy or furrowed [2]. The amount of annual snake melon production and area sown in Turkey are not completely known, because it is grown mostly as a special crop in home gardens and market gardens.

Although optimization of regeneration was obtained in commercial *Cucurbitaceae* species, melon (*Cucumis melo* L.) [10, 9] watermelon (*Citrullus lanatus Thunb. Matsum. and Nakai)* [5], summer squash (*Cucurbita pepo* L.) [1], winter squash (*Cucurbita maxima* Duch.) [16], cucumber (*Cucumis sativus* L.) [7] and bottle gourd (*Lagenaria siceraria* Standl.) [12], there is no study on regeneration of snake melon. There are also some transformation studies in some Cucurbits species like melon [10], watermelon [4], cucumber [20, 18], bottle gourd [13].

C. melo var. flexousus is a good target for a genetic breeding program because snake melon cultivars are mostly wild genotypes and accessions from different regions like Asia [22]. According to the research of Salgam and Yazgan it is found out that early sowing increases yield but not the fruit length [23]. To acquire better fruit characteristics breeding for

disease resistance can be an important factor, because powdery mildew is one of the major problems in the snake melon crop. For the purpose of getting rid of this problem, some researches have been conducted for breeding resistant varieties [17]. In this respect, some modern techniques were used and the quality of the crop was raised. Thus tissue culture is necessary for upgrading snake melon and other cucurbit crops [21].

The optimization of a regeneration protocol is essential for understanding the regeneration process and for future genetic engineering studies. The aim of this study was to establish an effective regeneration system by using different hormone concentration and dark pretreatment in snake melon.

Material and Methods

Snake melon 46 KSU (*Cucumis melo* var. *flexousus*) seeds obtained from Kahramanmaras were sterilized with a diluted commercial bleach solution (5 %) for 15 minutes and rinsed with sterile distilled water. After removing the seed coats, the seeds were washed in ethanol (70 %) for 5 minutes, rinsed with distilled water and sterilized for 20 minutes with 1.25 % sodium hypochlorite plus 2 drops of Tween-20, followed by washing three times with sterile distilled water. Sterilized seeds were germinated in tubes on MS [19] medium supplemented with MS vitamins, 3 % (w/v) sucrose and 0.75 % (w/v) agar. The pH was adjusted to 5.7 prior to autoclaving. Cultures were incubated at 25±1°C with 75 µmol $m^{-2}s^{-1}$ cool white fluorescent light during 7 days.

Cotyledon explants were excised from *in vitro* grown seedlings after 7 days since germination. Each cotyledon was cultured with abaxial side down on regeneration medium variants, consisting of MS basal medium supplemented with different concentrations of BA (0, 0.5, 1.0, 2.0 mg/l) and IAA (0, 0.25, 0.5, 1.0 mg/l). Proximal parts of cotyledon explants from 7-day-old seedlings cultured on MS medium were evaluated for their regenerative capacity. Shoot regeneration ratios were scored after 4 weeks of culture on the regeneration medium.

Statistical analysis

An ANOVA using Duncan's multiple range test was used to compare the means of all treatments by JMP 7.0 statistic program (SAS Institute Inc.).

Results and Discussions

In this study, the proximal parts of the cotyledons were used as explants in regeneration studies such as were used by various authors in the other *Cucurbitaceae* species: melon [11], watermelon [5], winter squash [16], cucumber [18, 24] summer squash [1] and bottle gourd [12, 13], some of them mentioning that the proximal part of the cotyledons showed a higher frequency of adventitious shoot regeneration when compared with the distal part [16, 18].

Germination ratio of the seeds under both light and dark conditions was found as 66.8 % (Table 1), but shoot formation ratio obtained from the seeds germinated under dark condition was higher than that of the seeds germinated under light condition.

Table 1. Germination Rates of Snake Melon Seeds

Light condition	Germination (%)
Dark	66.8 (103/154)
Light	66.8 (137/205)

The highest frequency of adventitious shoot regeneration (42.8 %) was obtained on MS medium with 1.0 mg/l BA+ 0.25 mg/l IAA from the seeds germinated under dark condition. 2.0 mg/l BA+ 0.5 mg/l IAA (34.8 %), 1.0 mg/l BA(32.8 %), 2.0 mg/l BA+ 0.25 mg/l IAA (12.8 %) and 2.0 mg/l BA+ 0.5 mg/l IAA (7.1 %) resulted in a much lower shoot regeneration frequency (Table 2).

Table 2. Effect of Plant Growth Regulators on Adventitious Shoot Regeneration

PGR (mgl^{-1})		Shoot Regeneration Frequencies (%)	
BA	IAA	Germination in Dark	Germination in Light
0.0	0.00	0.0 f	0.0 d
0.0	0.25	0.0 f	0.0 d
0.0	0.50	0.0 f	0.0 d
1.0	0.00	32.8 c	0.0 d
1.0	0.25	42.8 a	0.0 d
1.0	0.50	34.8 b	0.0 d
2.0	0.00	0.0 f	1.8 b
2.0	0.25	12.8 d	2.8 a
2.0	0.50	7.1 e	1.4 c
LSD		1,13***	0,3***

The exact mechanism of how dark pretreatments stimulate subsequent light-dependent organogenesis was not completely understood but it is likely that incubation of plant tissues in darkness preserves light sensitive endogenous plant growth regulators and other compounds [8, 14, 6, 25]. The beneficial effect of a dark pretreatment before preparing cotyledon explants for subsequent organ regeneration was also reported in watermelon [6]. It has been reported that the induction of somatic embryogenesis in sugarcane was dependent on the type of explants, supplemented hormones and also light and dark conditions (26).

The maximum frequency of adventitious shoot regeneration (2.8%) was achieved on MS medium with 1.0 mg/l BA+ 0.25 mg/l IAA in cotyledon explants from the seeds germinated under light condition; 2.0 mg/l BA (1.8 %) and 2.0 mg/l BA+ 0.5 mg/l IAA (1.4 %) resulted in a much lower shoot regeneration frequency (Table 2). Similar observations were reported in watermelon, where the induction of multiple shoots in cotyledon explants varied with concentration and the type of plant growth regulators: BA alone induced direct shoot differentiation, while 2iP and IAA exhibited synergism with BA for this morphogenic response (27).

Conclusions

In this paper, the effects of dark and light pretreatment and different hormone concentrations (BA-IAA) on adventitious shoot regeneration in snakemelon were searched. Dark pretreatment was found more effective than light one. It was determined that the media containing 1 mg/l BA with 0.25 mg/l IAA showed the best regeneration capacity in snakemelon cotyledon explants.

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