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Adventitious Shoot Organogenesis and Plant Regeneration from Cotyledons of Diploid Diyarbakır Watermelon (*Citrullus lanatus* cv. "Sürme")

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Abstract: Adventitious shoots were obtained from the diploid Diyarbakır watermelon cultivar "Sürme" by culturing cotyledons on shoot regeneration medium for 3 weeks. The effects of two cytokinins, benzyl amino purine (BA) and kinetine (Kin), on shoot organogenesis were examined. The number of shoots per explant were approximately more than 50% higher when 0.5 mg/l BA was used compared to the most effective kinetin concentration (1 mg/l). Plants were obtained by transferring 1 to 2 cm shoots to MS medium supplemented with 1-4 mg/l naphthalene acetic acid. Most of the regenerated plants (more than 50%) were successfully transplanted to the field. This demonstrates that in vitro propagation can be used to produce high quality diploid Diyarbakır watermelon for use in breeding lines.

Key Words: Regeneration, Tissue culture, Watermelon

Diploid Diyarbakır Karpuzu (*Citrullus lanatus* cv. "Sürme") Kotiledonlarından Adventif Sürgün Organogenezisi ve Bitki Rejenerasyonu

Özet: Diploid Diyarbakır karpuzunun (*Citrullus lanatus* cv. "Sürme") kotiledonlarından 3 haftalık kültür sonucunda adventif sürgünler oluşturuldu. Sürgün organojenezisi üzerine iki tip sitokininin; benzil aminopürin (BA) ve kinetin (Kin) farklı konsantrasyonlarının etkileri araştırıldı. Eksplant başına oluşan sürgün sayısı bakımından en iyi sonuç, 0.5 mg/l BA içeren besi ortamından elde edilirken bu oran aynı zamanda kinetinin en iyi sonuç veren konsantrasyonundan (1 mg/l) yaklaşık %50 daha fazla olduğu tespit edilmiştir. Elde edilen sürgünlerin (1-2 cm) in vitro ortamda köklenmesi; Naftalen Asetik Asit'le (NAA) desteklenen MS besi ortamına aktarılmasıyla elde edilmiştir. Rejenere edilen bitkilerin %50'den fazlası başarılı bir şekilde toprağa aktarılmıştır. Sonuç olarak, yüksek kaliteli diploid Diyarbakır karpuzunun in vitro yöntemle çoğaltılabileceği ve geliştirilen bu yöntemin, karpuz çeşitlerinin ıslahında kullanılabileceği kanısındayız.

Anahtar Sözcükler: Doku kültürü, Karpuz, Rejenerasyon

Introduction

Potyviruses cause serious disease problems in commercial cucurbit planting across Turkey and in other production areas around the world. Resistance to viruses such as zucchini yellow mosaic virus exists in wild germplasm (1). However, the introduction of this germplasm into commercial watermelon cultivars would result in a loss of certain favourable characteristics and require years of selective breeding to obtain acceptable resistant cultivars.

Adventitious shoot regeneration from the cotyledons of diploid watermelon has promising applications in the areas of genetic transformation (2) and the regeneration of tetraploid somaclonal variants which can be used as

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parental lines to breed seedless watermelon (3). However, an efficient in vitro plant regeneration system must be in place before such techniques can be employed.

Watermelon plants have been obtained through micropropagation of shoot tips (4,6), somatic embryogenesis from cotyledons of immature embryos (7), and adventitious shoot regeneration from cotyledon pieces (4,8,9). Adventitious shoot regeneration systems have been demonstrated as useful for obtaining genetically transformed plants (10,11), but reports of shoot regeneration from watermelon cotyledons are conflicting and may not be suitable for many commercial cultivars. Therefore, the objective of this study was to develop an efficient adventitious shoot regeneration protocol for diploid Diyarbakır watermelon. The protocol presented here for the induction of adventitious shoot organogenesis from the cotyledons of seedlings germinated in vitro represents the first stage of a study of the pathways of in vitro tissue cultures of the Diyarbakır watermelon.

Materials and Methods

Seeds of diploid watermelon cultivar "Sürme", which is the most common local cultivar in the south-east of Turkey, were surface disinfected for 20 min in 3% sodium hypochlorite (NaOCl), rinsed five times with sterile distilled water and soaked for 2 h in sterile distilled water. Embryos were extracted by removing the seed coat then germinated in Magenta GA_7 vessels (Magenta, Corp., Chicago) containing 50 ml of germination medium Murashige and Skoog (MS; M0404) salts (12) plus (per litre) 30 g/l sucrose (S 5391), 50 mg/l ascorbic acid, and 7 g/l agar (A 1296). The pH of all media was adjusted to 5.7 before the addition of agar and autoclaving.

Unless otherwise stated, explants consisted of cotyledons from 5-day-old seedlings excised 2 to 3 mm above the point of attachment to the stem hypocotyl. The cotyledon margins (1 mm) were removed and explants cultured abaxial side down on autoclaved medium containing test levels of growth regulators for 3 weeks under a 16 h photoperiod (30 mmol m^{-2} s^{-1} from cool-white fluorescent lamps) at 25 ºC ± 2. Plant growth regulators were prepared fresh at the time of media preparation. Explants were subcultured to fresh medium of the same composition at 3-week intervals. The effect of BA and Kin on adventitious shoot organogenesis was examined using cotyledons from "Sürme" seedlings. Explants were obtained from the cotyledons of seedlings germinated in vitro for 5 days. "Sürme" cotyledons were incubated on medium supplemented with either BA or Kin, each at 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg/l for 3 weeks.

After 3 weeks on shoot elongation medium, shoots longer than 1 cm were excised and transferred to Magenta GA_7 vessels that contained 50 ml of rooting medium (as above) supplemented with naphthalene acetic acid (NAA) at 1.0, 2.0 and 4.0 mg/l for 3 weeks. After 3 weeks on rooting medium, the plantlets were transplanted to cell packs filled with autoclaved medium and covered with a glass or clear plastic lid, and grown under the same conditions as the tissue was cultured. After 7 days, the plants were acclimatised to ambient humidity levels by gradually removing the lid over 2 to 3 days and moved to the greenhouse or growth room.

A minimum of three plates were cultured per treatment with four or five explants per plate. Data recorded at 3 weeks included the number of shoots per explant and the length of newly developed shoots. To detect significant differences among treatment levels, data from recorded experiments were subjected to analysis of variance. Student's *t*-test was adjusted to the P = 0.05 probability level to separate mean differences when significant treatment effects were detected.

Results and Discussion

Effect of cytokinin type and concentration

The mean number of shoots per explant and mean shoot length were highest when the medium was supplemented with BA (0.5 and 1.0 mg/l) compared to all kinetin concentrations tested (Table 1). Shoot regeneration rates observed in this study are similar to those of Compton and Gray (7) who stated that the shoot regeneration of watermelon cotyledons was best on MS medium with BA (1 or 2 mg/l). Many of the shoots that originated from explants cultured on medium with more than 4 mg/l BA or Kin were abnormal (thick and stunted) and/or vitrified. Shoots originating from explants incubated on medium with BA were also highly elongated and easiest to handle; Compton and Gray (7) observed similar results for diploid, triploid and tetraploid watermelon.

Table 1. Effect of different cytokinins (BA and Kin) and concentrations (0.5-16 mg/l) on the number of shoots and shoot length of cultured explants from seedlings of "Sürme" after 21 days on MS media containing cytokinins.

PGRs	Concentration (mg/l)	Mean number of shoots per explant[1]	Mean shoot length (cm)[2]
Control		1.00 ± 0.00a	1.50 ± 0.00a
BA	0.5	5.22 ± 0.54b	2.58 ± 0.37b
	1.0	4.33 ± 0.59bc	3.00 ± 0.40b
	2.0	3.37 ± 0.91c	1.33 ± 0.16a
	4.0	1.42 ± 0.20d	1.16 ± 0.16a
Kin	0.5	2.80 ± 0.37b	1.66 ± 0.44a
	1.0	3.66 ± 0.33b	2.25 ± 0.32a
	2.0	3.00 ± 0.53b	1.83 ± 0.16a
	4.0	1.71 ± 0.28a	1.50 ± 0.00a

[1] The number of explants for each treatment ranged from 10 to 15.

[2] Data recorded on day 21 presents an average of 12 to 15 explants. Different lowercase letters in a row above following any two mean values indicate that these two means are not significantly different at P = 0.05 level of significance according to Student's t-test.

Description of cultures and regenerated plants

Green meristematic protrusions, which resembled young shoot apices, were observed at the base of enlarged cotyledons 7 to 10 days after initiating explants on MS medium with BA and Kin (Figure 1). Meristems were also observed in medium without plant grower regulators (PGRs); however, shoots that grew from explants incubated on medium with 0.5 or 1.0 mg/l BA or kin were of higher quality. Adventitious shoots were macroscopically visible 21 days after cotyledon explant were placed on medium with BA (Figure 2). Transferring explant with shoots regardless of BA or Kin concentration to medium without BA encouraged shoot elongation. Hyperhydricity was common among shoot-tip cultures that were maintained in the medium containing greater than 1.0 mg/l BA. These results are similar to previous reports for watermelon that employed high levels of thidiazuron (TDZ) (7).

Rooting

After 3 weeks on medium with Kin (1-2 mg/l) some shoots also rooted in vitro. Elongated shoots (1-3 cm) could be removed and rooted in vitro. Rooted plants were acclimatised in cell packs and grown in the laboratory at ambient humidity levels for 2 weeks before transfer to the growth room. The frequency of shoots per rooting explant was significantly influenced by the concentration of NAA (Table 2).

The ability of shoots to root or plants to survive acclimatisation was dependent on the concentration of MS medium . Medium containing 1 and 2 mg/l NAA gave 70% and 60% of rooted explants respectively. A previous study with watermelon shoots from tissue culture demonstrated that only shoots langer than 1.6 cm were capable of efficient rhizogenesis and acclimatisation (90-100%) (7). Chaturvedi and Bhatnaga (13) also reported

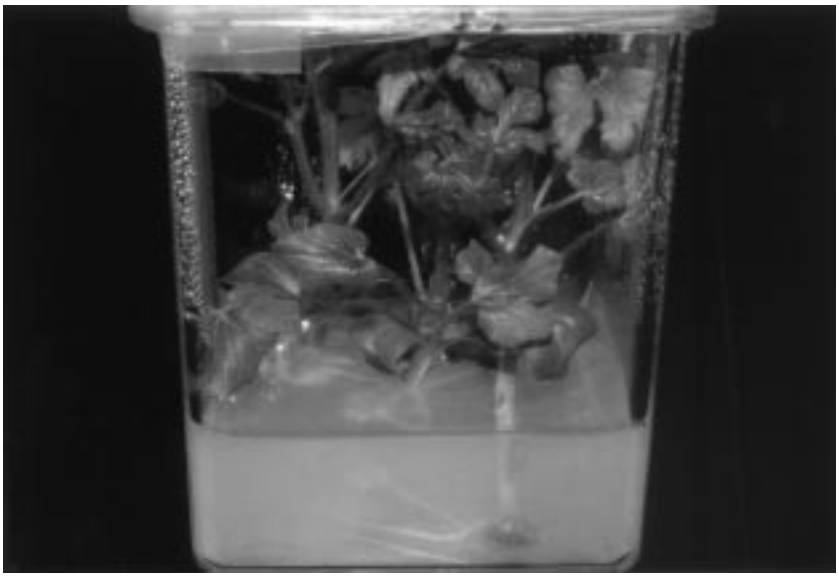

Figure 1. Green meristematic protrusions were observed on the base of enlarged cotyledons 15 days after culture initiation.

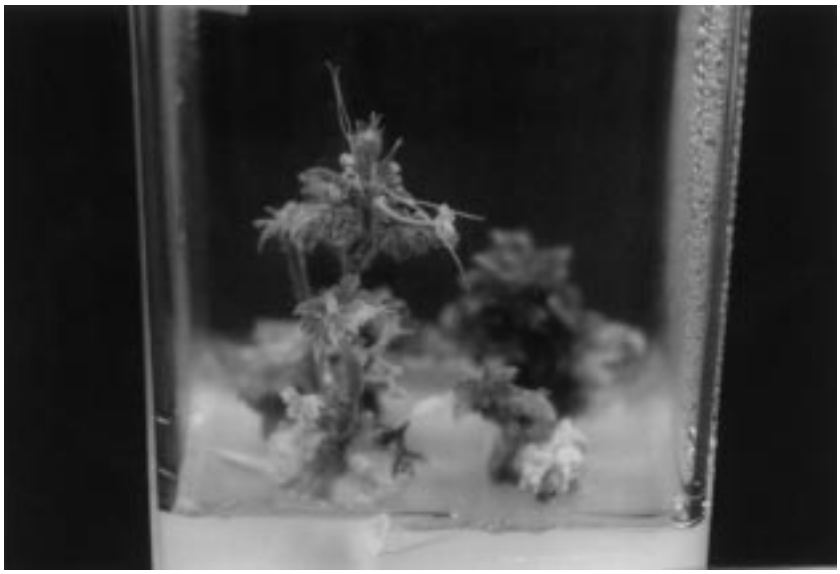

Figure 2. Explants with shoots proliferated on MS medium containing 0.5 mg/l BA 3 weeks after culture initiation.

that regenerated shoots and a resulting plant were established in earthen pots with 55% success. Other factors that affect rooting rates include the seed source and year the seed was collected, water source, the genotype, the amount of time in culture, and different technicians. Studies on these factors now are underway.

Conclusion

This study demonstrates that adventitious shoot regeneration from seedling cotyledons of diploid Diyarbakır watermelons can be used as a means of increasing the number of diploid unique individuals. The system could also be a useful tool to regenerate tetraploids for seedless watermelon production. Diploid tissue could also be subjected to an amenable genetic transformation protocol and the resulting genetically transformed diploid and tetraploid plants could then be used as parents for the development of genetically superior seedless watermelon cultivars.

The primary reason why this study is restricted to only one cultivar is that to date and studies only started a year ago. Consequently, efforts are now underway to expand these studies to all Diyarbakır watermelon cultivars. The establishment of a sound protocol for the production of new desirable cultivars of Diyarbakır watermelons is the first step towards a breeding line for the improvement of stocks.

Table 2. Effects of NAA on the frequency of root induction on the regenerated shoots of "Sürme".

NAA (mg/l)	Frequency of rooted shoots (%)[1]	Frequency of transplanted seedlings (%)[2]
Control	10	100
1.0	70	90
2.0	60	90
4.0	30	80

[1] Data recorded on the day 21 of culture.

[2] The number of explants for each treatment ranged from three to 10.

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