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Tissue culture and plant regeneration of watermelon (*Citrullus vulgaris* Schrad. cv. Melitopolski)

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Abstract

Plants were regenerated from cotyledon and hypocotyl explants of watermelon (Citrullus vulgaris). The explants were cultured on a Murashige and Skoog's basal nutrient medium supplemented with auxin, cytokinin and auxin-cytokinin combinations. Green healthy nodular and compact callus was obtained in medium containing naphthalene acetic acid and benzylaminopurine. Shoot differentiation and root differentiation from the cotyledon and hypocotyl after callus formation in different media containing benzylaminopurine or naphthalene acetic acid, respectively. Shoot formation required benzylaminopurine. Kinetin proved ineffective in inducing shoot buds or shoots. Root differentiation occurred in a medium containing naphthalene acetic acid or indole acetic acid. There was a greater proliferation of roots on medium supplemented with naphthalene acetic acid. The regenerated shoots developed roots when transferred to medium containing naphthalene acetic acid and complete plantlets could be transferred to soil for further growth.

Abbreviations:

BAP - 6 Benzylaminopurine
NAA - α-Naphthalene acetic acid
MS - Murashige and Skoog's medium
IAA - Indole acetic acid
KN - Kinetin

Introduction

Since the early demonstration of cellular totipotency and differentiation in vitro, plant tissue culture techniques have been widely used for the clonal multiplication of plants (Murashige 1974). To date, many economically important plants have been successfully propagated through this technique (George and Sherrington 1984). Watermelon (Citrullus vulgaris), a member of the Cucurbitaceae is an important food crop, and some genera of this family have already been reported to regenerate (Jelaska 1974; Malepszy and Nadolska-Orczy 1983; Moreno et al. 1985; Kathal et al. 1986; Trulson and Shahin 1986).

Being an economically important crop the application of genetic engineering in watermelon cultivation is of special value to obtain improved or desirable traits like disease resistance. This technology is now available for many plants (Shahin et al. 1986 and Trulson et al. 1986). We are interested in using genetic engineering techniques to introduce a cloned gene of δ-endotoxin from Bacillus thuringiensis (Evstratova et al. 1988) into watermelon genome. Transformations of plants can be expected to provide a new possibility for increasing genetic variability in crops. However, before undertaking this approach, it is important to know that somatic cells of this species are able to regenerate in such a way as to give rise to whole plants and the conditions required for such regeneration. The present investigation outlines the comparative morphogenetic responses of various explants of aseptically grown watermelon seedling cultured in different media combinations.

Material and methods

Seeds of watermelon (Citrullus vulgaris Schrad. cv. Melitopolski) were obtained from the National Research Institute of Irrigated Cultivation of Vegetable and Melon, Astrakhan, USSR. The seeds were aseptically germinated in flasks containing Murashige and Skoog's basal nutrient medium (Murashige and Skoog 1962). To assure uniform and rapid germination of seeds flasks were kept for 48 – 72 h at 28°C in the dark. Germinated seeds (radicle length approximately 5-6 mm) were then transferred to the culture room under 16 h photoperiod. After 7-8 days when the seedlings were green but the cotyledons partially unfolded they were used. The explant tissue was prepared by aseptically removing the seedling and cutting discs 5 mm in diameter from the cotyledon using a cork borer or cutting of 0.5-1.0 cm long sections of the hypocotyl. The explants were cultured horizontally on MS medium. The medium was supplemented according to experimental requirements using different hormones such as indole acetic acid, naphthalene acetic acid, kinetin and benzyl aminopurine (Table-1).

We have used MS medium supplemented with 3% sucrose, 100 mg/l inositol and gelled with 0.8% agar as the basal medium. The pH of the medium was adjusted to 5.7 prior to autoclaving. All cultures were incubated

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at 25±2°C with a 16 h protoperiod. The comparative morphogenetic responses of the explants to auxins, cytokinins and auxin-cytokinin combinations were scored after 30 days.

Results and Discussion

The culturing of explants of aseptically grown watermelon seedling evoked a clear-cut morphogenetic response. The presence of BAP in the nutrient medium alone promoted the regeneration of shoots from the cotyledonary and hypocotyl explants. Out of the various concentrations of BAP, 4.5 μM was highly effective for the regeneration of shoots from these explants followed by callus production (Fig.1 A,B). The percentage of shoot formation was very low from the hypocotyl explants. Kinetin was ineffective in inducing shoot bud or shoot formation, in - stead compact green calluses were produced on hypocotyl and cotyledonary explants. Some recent reports also indicate the formation of shoot buds or shoots when explants of Santalum (Bapat and Rao 1984), Ficus (Jaiswal and Narayan 1985; sugarbeet (Enomotos and Ohyama 1985) and mulberry (Ho-Rak et al. 1985) are cultured on BAP supplemented MS medium. Similarly, the presence of BAP in the growth medium has been shown to promote the development of shoot buds and shoots in Brassica explants cultured in vitro (Kartha et al. 1974). Gunay and Rao (1978) further reported a sporadic formation of shoots with zeatin and none with kinetin supplemented MS medium.

Callus formation was observed on all the explants that were cultured in media containing auxins. Apart from callus proliferation, the presence of NAA in the nutrient medium promoted the formation of roots on hypocotyl and cotyledon at the concentration of 0.5 μM and 1.0 μM, respectively (Fig.1-C,D). Root differentiation was also observed on hypocotyl and cotyledon explants that were cultured on IAA supplemented nutrient medium. The promotion of root differentiation in vitro in Brassica (Kartha et al. 1974), Petunia (Rao et al. 1973), Capsicum (Ganay and Rao 1978), Ficus (Jaiswal and Naryan 1985) and Abelmoschus (Mangat and Roy 1986) explants by NAA or IAA has been reported. A characteristic difference, however, between the two auxins was that on IAA medium, the roots were short and poorly developed while on NAA supplemented MS medium the roots were thick and long.

On transfer to the MS medium supplemented with 0.5 M NAA, the shoots (regenerated from all the explants) developed roots within two weeks and the complete plantlets could be transferred to pots for further growth. Prolonged culture in the same medium (shoot regeneration and root regeneration medium) resulted in callusing at the base.

A strong interaction of auxin and cytokinin was observed when the explants were cultured on MS medium supplemented with NAA (0.5 or 1.0 μM) and BAP (4.5 μM) which resulted in healthy nodular and compact cal - lus. It failed to form shoots or roots on the same medium. These results indicate that

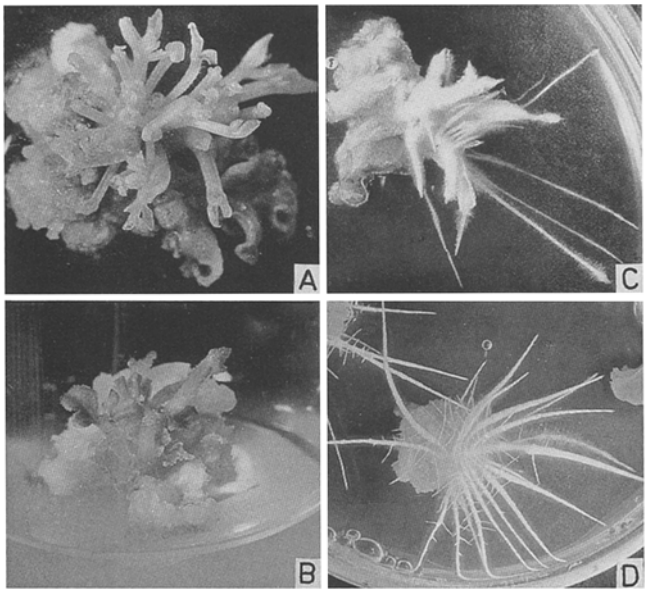

Fig. 1 A-D. Responses of various explants of watermelon (Citrullus vulgaris) to different growth substances and plant regeneration.
A. Regeneration of shoots from a cotyledon explant.
B. Regeneration of shoots from a hypocotyl explant.
C. Regeneration of roots from a cotyledon explant.
D. Regeneration of roots from a hypocotyl explant.

Table-I. Comparative morphogenetic responses of cotyledon and hypocotyl explants of aseptically grown watermelon (Citrullus vulgaris) seedlings cultured in MS medium supplemented with various hormones. Data were scored after 30-days. The explants used were of similar size and physiological age. NG-no growth, C-callus, R-root, Sh-shoot or shoot bud, C+R-callus and root, C+Sh-callus and shoot, MS-basal medium.

Medium	Cotyledon	Hypocotyl
MS + NAA 0.25 M	C	C
MS + NAA 0.50 M	C	C+R
MS + NAA 1.00 M	C+R	C
MS + IAA 0.25 M	C	C+R
MS + IAA 0.50 M	C	C+R
MS + IAA 1.00 M	C+R	C
MS + BAP 2.5 M	NG	NG
MS + BAP 3.0 M	C	C
MS + BAP 3.5 M	C	C
MS + BAP 4.0 M	C	C
MS + BAP 4.5 M	C+Sh	C+Sh*
MS + KN 3.0 M	NG	NG
MS + KN 3.5 M	C	C
MS + KN 4.0 M	C	C
MS + KN 4.5 M	C	C
MS + KN 5.0 M	C	C
MS + NAA 0.5 M + BAP 4.5 M	C	C

*The hypocotyl explants regenerate poorly on this medium

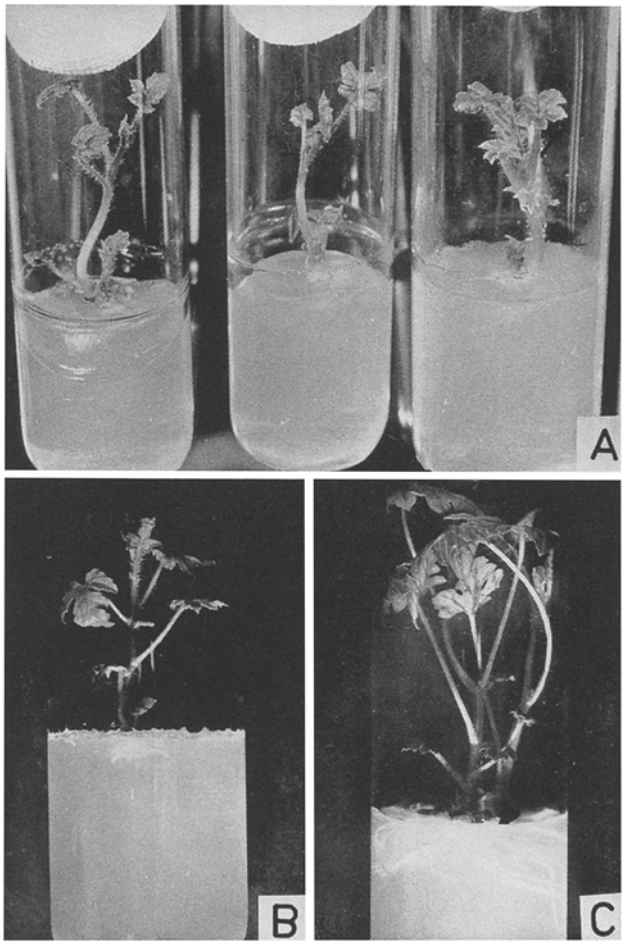

Fig.2 A-C. Regenerated plantlets exhibiting root growth.

A. Regenerated shoots are transferred to MS medium containing 0.5 M NAA.

B. Regenerated shoot shows the initiation of roots after 10-days of transfer.

C. Regenerated shoot shows the root growth after 20-days of transfer.

NAA counteracts the effect of BAP. A similar antagonistic effect of cytokinin to auxin was obtained in callus cultures of Aegle (Arya et al. 1981) and Ficus (Jaiswal and Naryan 1985).

Experiments described here on regeneration of whole plants from the explants indicate that seedling age conditions during the culture are as important as the correct hormonal balance for successful regeneration. Thus our experiments demonstrate the development into complete plants of the plantlets obtained from cultured explants of watermelon. This protocol allows the regeneration of watermelon plants within two months and the young watermelon plants can be transferred to soil for further growth.

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