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HIGH-FREQUENCY SHOOT REGENERATION FROM COTYLEDON EXPLANTS OF WATERMELON CV. SUGAR BABY

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Summary

A protocol for *in vitro* shoot regeneration from cotyledon explants of *Citrullus lanatus* (Thunb.) Matsum. & Nakai cv. Sugar Baby is described. The cotyledons excised from 7-d-old aseptic seedlings showed the highest percentage of shoots on Murashige and Skoog (MS) + N^6-benzyladenine (BA; 3.0 μM) + N^6-[2-isopentenyl] adenine (2iP; 3.0 μM) and MS + BA (3.0 μM) + indole-3-acetic acid (IAA; 3.0 μM). Whereas the latter medium induced shoot regeneration after the callusing of the explant, the former stimulated direct shoot formation. The regenerated shoots were rooted and the resulting plants were established in earthen pots with 55% success.

Key words: Citrullus lanatus; Sugar Baby; cotyledon; regeneration.

Introduction

Watermelon is an economically important crop, and its fruits are rich in carbohydrates, vitamins and minerals (Anonymous, 1992). It is susceptible to a number of fungal, bacterial and viral diseases (Kim et al., 1998; Compton and Gray, 1999) requiring annual field rotation, frequent chemical sprays, and disease-resistant cultivars (Compton and Gray, 1999). Therefore, a major objective of watermelon breeding has been to develop cultivars with disease resistance by the application of recombinant DNA technology (Dong and Jia, 1991; Chen et al., 1998; Xiao et al., 1999). In addition, the production of polyploids through somaclonal variation in tissue culture could be exploited to produce new parental breeding lines which are useful in developing new seedless watermelon cultivars (Compton and Gray, 1999).

Tissue culture can be used to produce disease-resistant (Tabei, 1997; Xiao et al., 1999), fertile, non-chimeric tetraploid plants for use in triploid hybrid seed production. Cross-fertilization of putative tetraploids with diploid pollinators and the production of triploid seed has confirmed the efficacy of this approach (Compton et al., 1996a). Triploid plants produced seedless fruits that were superior or equal to fruits produced by currently available triploid hybrids (Compton et al., 1994a, 1996b).

In vitro regeneration of plants from cotyledons of mature seeds and young seedlings has received considerable attention in the recent past. This, most likely, is because of their easy accessibility, quick response and high ability for shoot organogenesis, somatic embryogenesis, protoplast culture (Tabei, 1997) and transformation studies (Compton et al., 1994b; Choi et al., 1994; Tabei, 1997). Also it would reduce the time required for commercial seed increase of new tetraploid parental lines.

There are a number of reports on adventitious shoot regeneration from excised cotyledons of watermelon (Blackmon and Reynolds, 1982; Srivastava et al., 1989; Adelberg et al., 1990; Compton and Gray, 1991, 1993, 1994; Dong and Jia, 1991; Compton et al., 1994a, 1996a, 1996b; Compton, 1997; Jaworski and Compton 1997; Hao and Wang, 1998). These show differences in genotype, optimum growth regulator concentrations and combinations, age of the explants, conditions of rooting and acclimatization. The objective of the present work was to study improvements in adventitious shoot regeneration of watermelon.

Materials and Methods

For regeneration studies, explants were derived from aseptically germinated seedlings. Seeds were decoated and surface-sterilized in 0.1% (w/v) $HgCl_2$ solution for 45 s. After several washings in sterile distilled water, seeds were soaked in it for 2 h. The seeds were then implanted in culture tubes (25 × 150 mm) containing 20 ml of Basal Medium (BM) comprising inorganic and organic nutrients of MS (Murashige and Skoog, 1962), 3.0% (w/v) sucrose, 100 mg l^{-1} *myo*-inositol (Sigma Chemical Co., St. Louis, MO, USA) and 0.8% agar (Bacteriological grade; Qualigens, India). The pH of the medium was adjusted to 5.8 prior to autoclaving for 15 min at 1.05 kg cm^{-2} at 121°C. The cultures were maintained at 25 ± 2°C under 16 h photoperiod. Each experiment was repeated three times with 24 cotyledons per treatment in each experiment.

Excised entire cotyledons from 7-d-old aseptic seedlings were cultured on MS medium supplemented with N^6-benzyladenine (BA), alone or in combination with another cytokinin N^6-[2-isopentenyl] adenine (2iP), and auxins indole-3-acetic acid (IAA) and α-naphthaleneacetic acid (NAA). The cotyledons were planted in such a way that their basal half was embedded in the medium. The morphogenic responses of cotyledons to 21 treatments are summarized in Table 1.

Results

BA alone induced adventitious shoot bud differentiation from cotyledon explants even at the lowest concentration. However, there

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TABLE 1

EFFECT OF CYTOKININ BA EITHER ALONE OR IN COMBINATION WITH ANOTHER CYTOKININ 2iP AND AUXINS IAA AND NAA AT DIFFERENT CONCENTRATIONS ON CALLUS AND SHOOT DIFFERENTIATION FROM 7-d-OLD COTYLEDON EXPLANT[a]

Treatments[b]	Per cent cultures showing callus	Per cent cultures with adventitious shoots		Number of shoots per explant
		Directly	Via callus	
Control (MS Basal Medium)	0	0	0	–
(a)				
BA 1.0 μ*M*	0	20.8 ± 0.3	–	15.3 ± 0.6
BA 3.0 μ*M*	0	24.9 ± 0.3	–	20.5 ± 1.4
BA 5.0 μ*M*	16.6 ± 0.2	29.2 ± 0.1	–	20.5 ± 1.6
BA 10.0 μ*M*	0	33.2 ± 0.1	–	25.0 ± 0.6
(b)				
BA 1.0 μ*M* + 2iP 1.0 μ*M*	33.2 ± 0.2	37.4 ± 0.1	–	24.0 ± 1.3
BA 3.0 μ*M* + 2iP 3.0 μ*M*	0	65.2 ± 0.2	–	35.5 ± 1.6
BA 5.0 μ*M* + 2iP 5.0 μ*M*	0	49.9 ± 0.0	–	12.2 ± 1.1
BA 10.0 μ*M* + 2iP 10.0 μ*M*	0	24.9 ± 0.3	–	12.0 ± 1.0
(c)				
BA 1.0 μ*M* + IAA 1.0 μ*M*	0	62.4 ± 0.0	–	20.4 ± 1.4
BA 3.0 μ*M* + IAA 3.0 μ*M*	100.0 ± 0.3	–	66.5 ± 0.2	32.5 ± 0.8
BA 5.0 μ*M* + IAA 5.0 μ*M*	100.0 ± 0.3	–	58.2 ± 0.3	25.0 ± 0.6
BA 10.0 μ*M* + IAA 10.0 μ*M*	66.4 ± 0.3	–	54.0 ± 0.3	20.0 ± 0.6
BA 10.0 μ*M* + IAA 1.0 μ*M*	0	35.5 ± 0.1	–	16.0 ± 0.3
BA 5.0 μ*M* + IAA 1.0 μ*M*	0	12.0 ± 0.1	–	23.0 ± 1.0
(d)				
BA 1.0 μ*M* + NAA 1.0 μ*M*	24.9 ± 0.3	41.6 ± 0.2	–	10.0 ± 0.8
BA 3.0 μ*M* + NAA 3.0 μ*M*	83.1 ± 0.2	–	33.2 ± 0.0	5.5 ± 0.7
BA 5.0 μ*M* + NAA 5.0 μ*M*	83.2 ± 0.1	–	24.9 ± 0.1	3.0 ± 0.6
BA10.0 μ*M* + NAA10.0 μ*M*	66.6 ± 0.3	0	0	–
BA10.0 μ*M* + NAA 1.0 μ*M*	0	24.9 ± 0.3	–	25.8 ± 1.4
BA 5.0 μ*M* + NAA 1.0 μ*M*	0	8.3 ± 0.1	–	2.0 ± 1.0

[a] Each value represents the mean ± SE of three replicate experiments with 24 cotyledons per treatment in each experiment.
[b] MS medium was used with all the growth regulators.

was a slight increase in the percentage of cultures responding with increase in the concentration of the cytokinin. With BA (10.0 μ*M*) >33% of explants formed 25 shoots per explant (Table 1a). 2iP acted synergistically with BA. Maximum regeneration occurred on a medium containing 3.0 μ*M* each of BA and 2iP (Fig. 1b). On this medium 65.2% of cultures exhibited regeneration with 35 shoots per explant (Table 1b).

In response to a combination of BA and IAA (3.0 μ*M* each), 66.5% of cultures exhibited regeneration with as many as 32 shoots per explant (Fig. 1c). However, the differentiation of shoots was preceded by callusing of the explants. At a lower concentration, IAA (1.0 μ*M*) induced direct shoot bud differentiation with a frequency comparable to BA (3.0 μ*M*) + 2iP (3.0 μ*M*). However, the intensity of regeneration was high with the latter treatment. These observations clearly indicate that the presence of IAA above 1.0 μ*M* promotes callusing, subsequently leading to shoot formation (Table 1c). Almost similar results were obtained with BA + NAA in which the presence of NAA above 1.0 μ*M* induced callusing and indirect shoot proliferation (Table 1d).

On induction medium the shoots remained stunted and with the passage of time showed formation of callus followed by differentiation of thick and vitrified shoots. Therefore, the explants bearing shoots were transferred *en masse* to medium containing only BA (1.0 μ*M*). On this medium shoots were healthy and well developed and attained a height of 5–6 cm after 20 d (Fig. 1d).

For the initiation of roots, 3.0 cm long shoots were individually transferred to MS medium supplemented with NAA (0.5 μ*M*). In this treatment 91.5% of shoots produced roots directly at the base within 6 d, with an average of six roots per explant (Fig. 1e). After another 6 d, plantlets were transferred to earthen pots containing a mixture of garden soil and sand (1:1). Over 55% of the plantlets survived transplantation to pots. The plants resumed growth, and their leaves turned dark green in 1 wk. In another week the plants attained a height of 7–8 cm (Fig. 1f).

Discussion

The induction of multiple shoots in cotyledon explants varied with concentration and the type of plant growth regulators. BA alone induced direct shoot differentiation. 2iP and IAA exhibited synergism with BA for this morphogenic response (Table 1).

Blackmon and Reynolds (1982) reported adventitious shoot formation from excised cotyledons of watermelon in response to 10.0 mg l^{-1} (49.21 μ*M*) 2iP + 0.1 mg l^{-1} (0.49 μ*M*) β-naphthoxyacetic acid (NOA). Dong and Jia (1991) also observed adventitious shoot differentiation from cotyledons of watermelon on a medium containing 5–7 mg l^{-1} (22.19–31.6 μ*M*) BA + 0–3 mg l^{-1} (0–17.13 μ*M*) IAA. These reports showed that the high-frequency shoot regeneration in watermelon required a high concentration of cytokinin. Compton and Gray (1991, 1993) also made similar observations on all the cultivars of watermelon tested. However, according to Tabei et al. (1993) presence of high concentrations of 10–20 mg l^{-1} (57.08–114.16 μ*M*) IAA was also essential for shoot formation in watermelon.

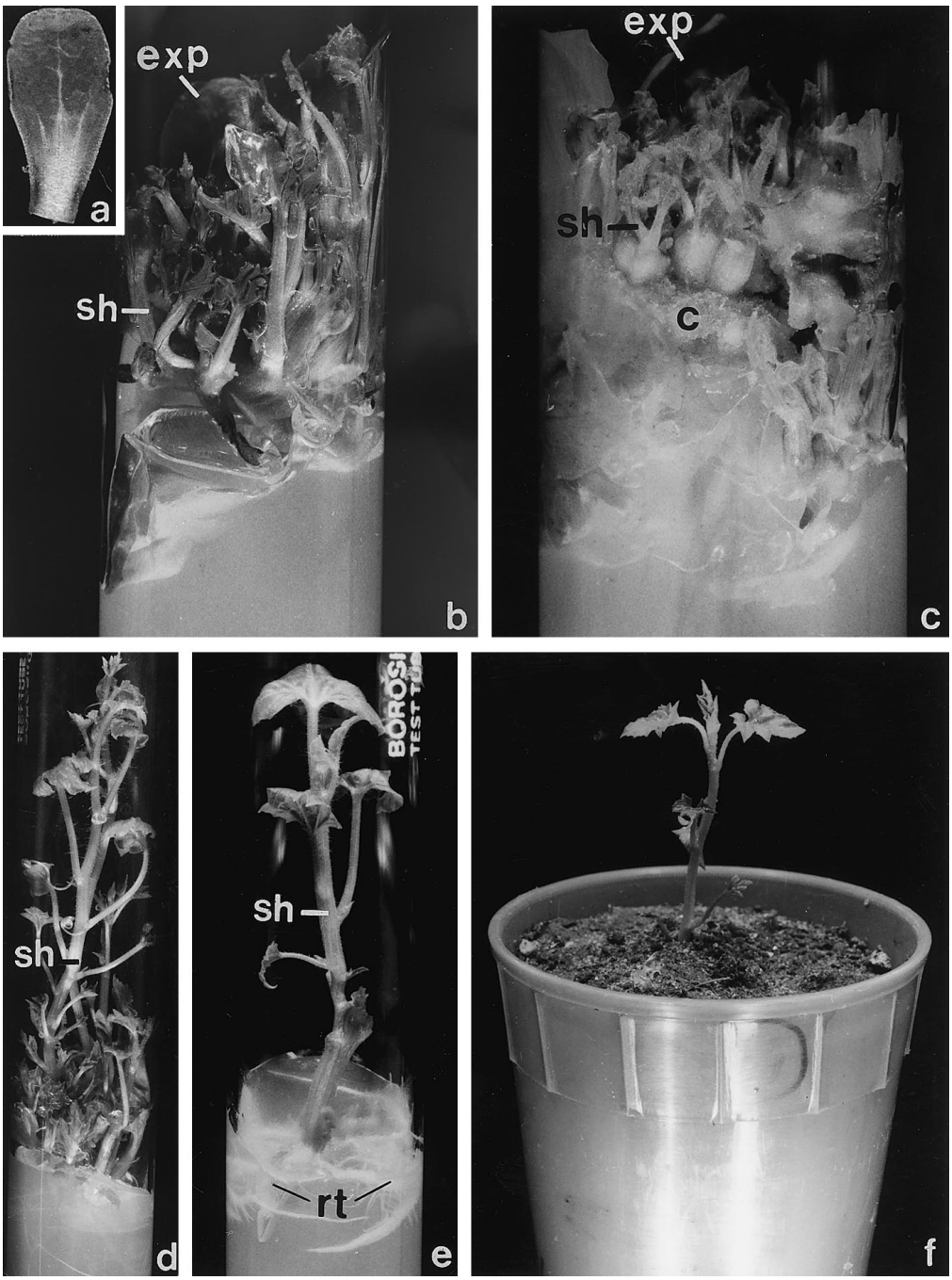

FIG. 1. a, Inset shows excised cotyledon from 7-d-old seedling, used as the explant. b, A 24-d-old culture on BM+3.0 μ*M* BA+3.0 μ*M* 2iP showing direct shoot regeneration from the cut end of the explant (×1.6). c, A 24-d-old culture on BM+3.0 μ*M* BA+3.0 μ*M* IAA, showing indirect shoot regeneration from the callus developed at the cut end (×1.7). d, Twenty-d-old culture on 1.0 μ*M* BA showing elongation (×0.8). e, Excised shoot on BM+0.5 μ*M* NAA showing well-developed roots at the end of 11 d (×0.8). f, A tiny plant 14 d after transfer to garden soil and sand (1:1) (×3.0). c, callus; exp, explant; sh, shoot; rt, root.

Our observations suggest that for shoot bud differentiation from cotyledons of watermelon, a combination of 3.0 μ*M* each of BA and 2iP is better than BA alone or BA + IAA or BA + NAA. Moreover, with BA (3.0 μ*M*) + 2iP (3.0 μ*M*) shoot differentiation occurred directly whereas with BA (3.0 μ*M*) + IAA (3.0 μ*M*) it was preceded by callusing. These results are consistent with those of Kathal et al. (1988) who noted that a combination of BA+2iP at equimolar concentration was best for shoot bud differentiation in leaf cultures of muskmelon. We have observed that in watermelon shoots regenerated directly from the cotyledons on BA+2iP-supplemented medium within 2 wk while it took 3 wk in BA+IAA-supplemented medium.

Whereas callus-mediated plant regeneration, associated with large-scale genetic variability, may offer somaclonal variant selection for use in agriculture, direct regeneration of shoots from BA+2iP-supplemented medium is recommended for clonal propagation.

Another important finding in the present work is that multiple shoots regenerated from explants must be transferred to elongation medium, as prolonged culture on shoot induction medium not only stimulates callus formation but also produces abnormal shoots. The same pattern of events is reported for muskmelon (Dirks and Van Buggenum, 1989).

To increase the percentage of successfully acclimatized plantlets, a well-developed root system and two or three leaves (at least 10.0 mm long lamina) (Ohki et al., 1991) and, preferably, high light intensity are needed (Kozai and Iwanami, 1988). In the present study, general growth was satisfactory in garden soil and sand mixture, and about 55% of the tissue-culture raised plants survived transplantation.

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