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Regeneration of plants from leaf explants of *Cucumis melo* cv. Pusa Sharbati

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ABSTRACT

Leaves of three different sizes excised from 14, 21, 28 and 35-day-old seedlings of Cucumis melo were cultured on a MS medium supplemented with a range and combination of growth regulators. Maximum shoot differentiation from the leaf explants occurred in the combined presence of BAP and 2iP at equimolar concentration of 1 μM. Regeneration potential of leaves declined with increasing size of the leaves and the age of the donor seedlings. For elongation the shoots were transferred to MS+BAP [1 μM]. Such shoots were rooted with 75% frequency on MS + IAA [0.5 μM]. The plants have been established in pots.

ABBREVIATIONS

BM [Basal Medium], MS [Murashige and Skoog], BAP [6-Benzyl amino-purine], 2iP [6- γ, γ –dimethylallylamino purine], IAA [Indole-3-acetic acid].

INTRODUCTION

The usefulness of cell culture techniques is dependent on in vitro regeneration of plants. In Cucumis melo [Muskmelon] regeneration of plants has been reported from excised cotyledons [Halder and Gadgil, 1982; Moreno et al., 1985; Bouabdhallah and Branchard, 1986; Trulson and Shahin, 1986; Oridate and Oosawa, 1986; Orts et al., 1987] and from hypocotyl segments [Blackman et al., 1981; Kathal et al., 1986]. There are only two reports of plant regeneration from leaf explant and leaf protoplast of Cucumis sativus [Malepszy and Nadolska, 1983; Orczyk and Malepszy, 1985]. Moreno et al. [1984] raised callus from leaf protoplasts of Cucumis melo which failed to differentiate. This paper describes plant regeneration from cultured leaf explants of C. melo cv. Pusa Sharbati as influenced by growth regulators, the age of the donor seedling and the size of the leaf explants.

MATERIAL AND METHODS

Seeds of C. melo cv. Pusa Sharbati were sterilized in 0.2% $HgCl_2$ for 5 minutes and then soaked in sterile distilled water for 24 h. The seeds were resterilized by soaking in 0.2% $HgCl_2$ for 5 min., decoated and transferred to culture tubes [25 x 150 mm] containing 20 ml of Basal Medium [BM]. The BM contained inorganic and organic nutrients of MS [Murashige and Skoog, 1962], 3% sucrose and 0.8% Agar [Hi Media Laboratories Pvt. Ltd.]. It was variously supplement with BAP [0.5 - 10 μM], 2iP [1 μM - 10 μM] and IAA [1 μM]. The pH of the medium was adjusted to 5.8 prior to autoclaving at 1.06 Kg cm^{-2}.

Leaves [lamina with small petiole of three different sizes [0.3 - 0.5, 0.6 - 0.9 and 1.0 - 1.2 cm; Fig. 1A] were excised from aseptic seedings of different ages [14, 21, 28 and 35 days]. While excising the leaves, the pre-existing axillary bud was carefully excluded. The whole excised leaves were cultured on the BM supplemented with BAP, either alone or in combination with 2iP and IAA, separately. The cultures were incubated under 15 watt m^{-2} intensity of continuous light at 25±2°C. Weekly observations were made but final data was collected after 40 days.

For transplantation, 8-10 cm long plantlets, 7 days after root initiation were transferred to plastic cups [7 cm in height] containing garden soil and sand [1:3] and irrigated with tap water. The plants were covered with polythene bags. After a week small holes were punched in the plastic and 4 days later the bags were removed.

RESULTS AND DISCUSSION

1. Regeneration of Shoots

On BM all the leaves showed slight overall expansion and callusing at the cut end of the petiole. Occasionally the leaves larger than 0.6 cm also formed roots at the cut end. All hormonal treatments induced shoot bud differentiation with varied frequencies [Fig. 2]. Individually, BAP induced maximum regeneration [91%] at 5 μM [Fig. 2e]. Further increase in the concentrations of the cytokinins did not improve the regeneration frequency. Substitution of 2iP [10 μM] [Fig. 2g] for the BAP resulted in better shoot elongation but it caused reduction in the percentage [58%]

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of cultures showing regeneration and number of shoot buds per culture. Combined presence of BAP and 2iP at an equimolar concentration of 1 μM promoted shoot buds differentiation in the leaf explants of all the three sizes [Fig.1B, 2h]. When

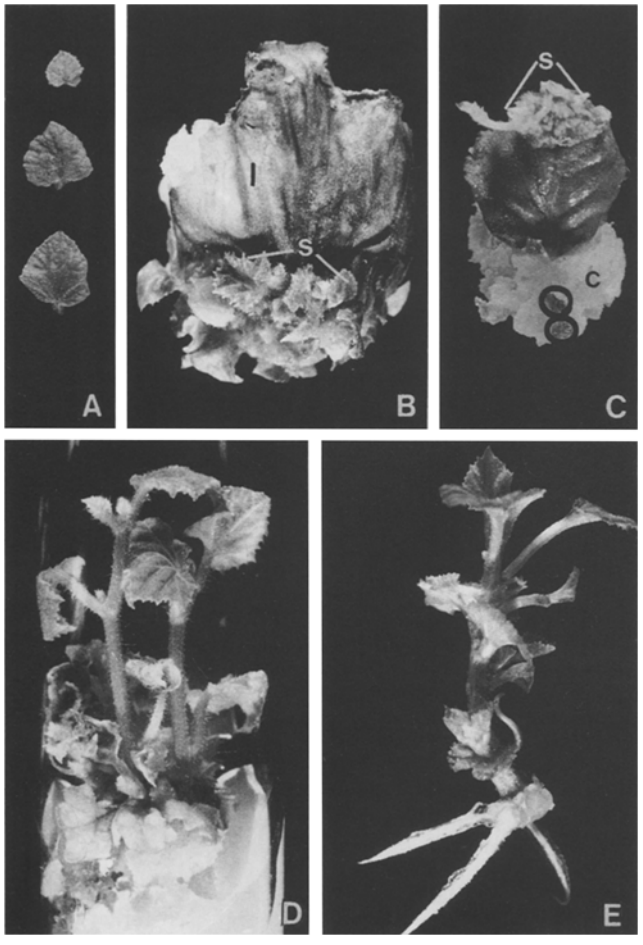

Fig. 1. [A-E] Regeneration of plants from cultured leaves of Cucumis melo cv. Pusa Sharbati [c-callus, l-lamina, s-shoot]. Fig. B-E showing culture of youngest leaf [0.3 cm]. A: Explants of different sizes [0.3, 0.6 and 1.0 cm] excised from aseptic seedlings. B: A 20-day-old culture of leaf on BM + BAP [1 μM] + 2iP [1 μM] showing several adventitious shoots differentiating directly from the junction of the lamina and the petiole. C: A 25-day-old culture of leaf on the same medium as in B, showing differentiation of shoots at the leaf tip and the initiation of friable callus from the cut end of the petiole. Also note shoot buds [encircled] arising from the callus. D: Culture of adventitious shoots excised from leaf explants cultured on BM + BAP [1 μM] + 2iP [1 μM] and transferred to BM + BAP [1 μM]. Note the elongation of the shoots 25 days after culture. E: An adventitious shoot rooted on BM+IAA [0.5 μM]; culture period 15 days.

both the cytokinins were used at a concentration of 3 μM, regeneration in the cultures of youngest leaf [100%] was as good as with lower concentration of the two cytokinins. However, the older leaves showed a drastic decline in the regeneration frequencies at the elevated concentrations of the cytokinins [Fig. 2i]. The addition of IAA [1 μM] in conjunction with BAP [3 μM] not only caused a decline in the number of cultures [66%] showing regeneration but also induced rooting [Fig. 2j]. The roots differentiated either directly or after slight callusing from the cut end of the petiole. However, the roots did not form a continuous axis with regenerated shoots.

On BM + BAP [1 μM] + 2iP [1 μM] which proved most responsive medium for shoot regeneration, the expansion of leaf was observed after two days of culture. Shoot buds and callus were initiated almost concurrently after 7 days. To begin with callus was friable and creamish in colour, which later turned compact. Shoot buds arose directly at the junction of lamina and petiole and some times from the lamina tip [Fig.1C]. Although histological studies have not been made so far the dissections of cultured leaves revealed that some shoot buds also differentiated from the callused petiole [Fig. 1C]. Initially the shoot buds formed by the callus were glassy and dark green and few in number. However, after 35 days, the compact callus gave rise to shoot buds, which developed into 0.5-2 cm long shoots.

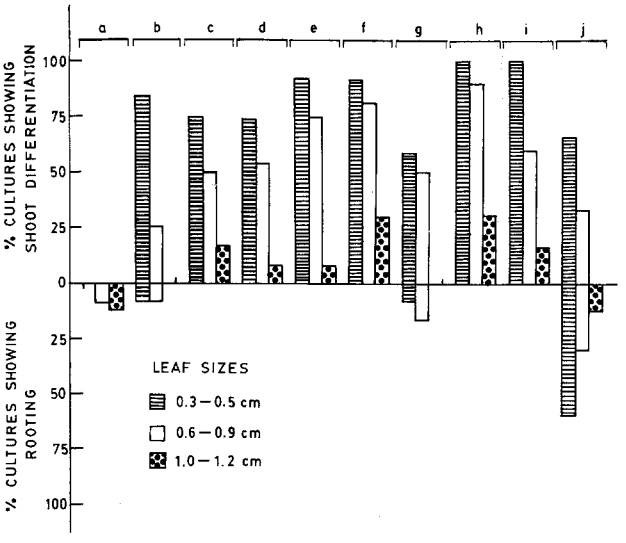

Fig. 2. Effect of auxin [IAA] and cytokinins [BAP & 2iP] on shoot and root differentiation from cultured leaves (a) BM; (b) BM + BAP 0.5 μM; (c) BM + BAP 1 μM; (d) BM + BAP 3 μM; (e) BM + BAP 5 μM; (f) BM + BAP 10 μM; (g) BM + 2iP 10 μM; (h) BM + BAP 1 μM + 2iP 1 μM; (i) BM + BAP 3 μM + 2iP 3 μM; (j) BM + BAP 3 μM + IAA 1 μM.

(a) Effect of the age of the seedling and leaf size

The age of the donor seedlings and size of the leaves showed inverse relationship with the percentage of cultures showing shoot regeneration. In all the hormonal combinations tested, the percentage of cultures showing shoot bud differentiation declined with increase in the size of the leaf [Fig.2] and age of the seedling [Fig.3].

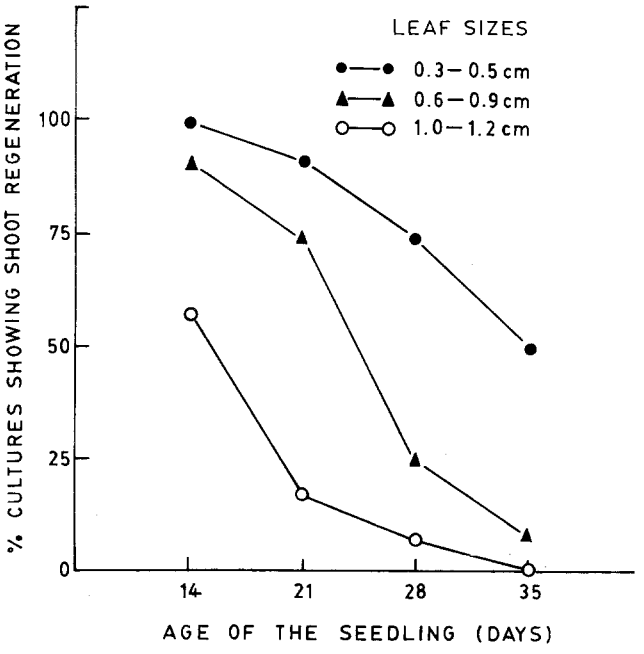

Fig. 3. Shoot regeneration in leaf cultures as affected by the age of the donor seedling and the size of the leaf.

(b) **Elongation and multiplication of shoots**

On the original medium, the regenerated shoots generally remained stunted. To promote shoot elongation the regenerants were excised and transferred to BM + BAP [1 μM]. On this medium the shoots multiplied well and showed good elongation upto 10 cm [Fig. 1D].

2. **Rooting of Regenerated Shoots**

The shoots longer than 2 cm were excised from the elongation medium and transferred to BM + IAA [0.5 μM]. On this medium 75% of the shoots developed 2-4 adventitious roots after 10-15 days of culture [Fig. 1E] which later developed laterals.

Sixty percent plants survived transplantation to pots. The plants resumed growth after one week of transplantation and attained a length of 15-18cm within three weeks. During this period 3-5 new leaves had developed.

It is concluded that the age and size of leaf explants determine their regeneration capacity.

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