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ONE STEP REGENERATION VIA SHOOT TIP CULTURE IN CUMIN

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Shoot apices of the two weeks old seedlings of *Cuminum cyminum* L. (cumin), grown *in vitro*, were excised and inoculated on MS (Murashige & Skoog's) medium incorporated with IAA (8.0 mg/l). The shoot apices grew vigorously with fine branching. Rooting was induced right at the cut end of the shoot tip, which was embedded in the medium. Thus, complete plantlets were developed within four weeks time period in just one step eliminating the requisite for a separate rooting medium. However, shoot multiplication could not be achieved on the same medium.

Keywords : *Cuminum cyminum; in vitro.*

Introduction

Cuminum cyminum, which belongs to Family Apiaceae, is a well known spice, and besides, it has significant medicinal value. Complete regeneration obtained through callus subculturing has been reported earlier.[1] Shoot tip culture is preferred over meristem culture when viral elimination is not part of an objective because the explant is easier to dissect with higher survival and growth rates. Recent reports highlighting the use of the technique are there, for virus elimination[2,3] as well as clonal propagation[4] and micropropagation[5,6].

Present investigation lays an excellent model system to eliminate excess labour in micropropagation of plant species obtaining complete plantlet formation in a single step.

Materials and Methods

Cumin seeds (var.UC-19) were surface sterilized with 0.2% mercuric chloride and then washed 4 to 5 times with sterile distilled water. The seeds were inoculated on paper bridges. From the two weeks old seedlings, shoot apices were excised and inoculated vertically on Murashige and Skoog's Medium[7], congealed with 0.8% agar with addition of 3% sucrose, and finally incorporated with IAA (1.0-10.0 mg/l).

The pH of the medium was adjusted to 5.8, prior to autoclaving. All the manipulations were done aseptically in a horizontal type Lainar Flow. The cultures were incubated at 26±2^{0}C under fluorescent lights. The regenerated plantlets were transferred to sterile pots containing mixture of soil and vermiculite (2:1).

Results and Discussion

The results are summarised in Table 1. The shoot tips inoculated on MS-medium supplemented with lower concentrations of IAA (1.0-5.0 mg/l) showed very slow increase in length of the shoots without any root formation or sometimes feeble hair-like roots appeared which were not appropriate for survival of plant. However, IAA (8.0 mg/l) led to vigorous shoot development (Fig.1). Roots were induced

by the second week of inoculation and developed profusely proportionate to the shoot system (Fig.2). IAA (10.0 mg/l), when incorporated to MS-medium, showed very slow shoot length with 1-2 roots developed underneath. Complete platlets developed by IAA (8.0 mg/l) incorporation were able to survive in soil, making the micropropagation successful.

Usually, micropropagation is achieved in three steps i.e. establishment of the explant, multiplication of the propagule and then rooting and hardening for planting into soil[8]. Herrera *etal*[9] gave the first report on *Digitalis thapsi* describing a technique eliminating the requisite of a separate medium for rooting.However, they faced problem of callus formation at the base which was overcome by lowering the concentration of cytokinins. There was no such problem in cumin.

According to Skoog and Miller[10] *de novo* root formation depends on a low cytokinin to a high auxin ratio. Thus, when cytokinins are used for shoot multiplicition in stage II, it is usually avoided in stage III, where auxins are used for the induction of roots. Present investigation reflects upon the regeneration achieved in cumin in a single step in response to IAA, with no requisite of exogenous cytokinin. The result is important showing suitability of the shoot tip as an explant with its sufficient endogenous levels of cytokinin, and IAA as an effective hormone for root induction as well as shoot development.

Table 1. Shoot tip culture response in *Cuminum cyminum.*

Hormone	Concentration (mg/l)	Response		
		Shoot buds	Shoot length	Rooting
IAA	1.0	S^{+}	$L^{\#}$	R^{*}
	3.0	S^{+}	$L^{\#}$	R^{*}
	5.0	S^{++}	$L^{\#\#}$	R^{**}
	8.0	S^{++}	$L^{\#\#\#}$	R^{***}
	10.0	S^{+}	$L^{\#\#}$	R^{**}

S^{+} = 1-2 shoot buds; S^{++} = 3-5 shoot buds; L^{*} = 1-2 cm length; L^{**} = 3-5 cm length; L^{***} = 6-9 cm length; R^{*} = Meagre rooting; R^{***} = Profuse rooting.

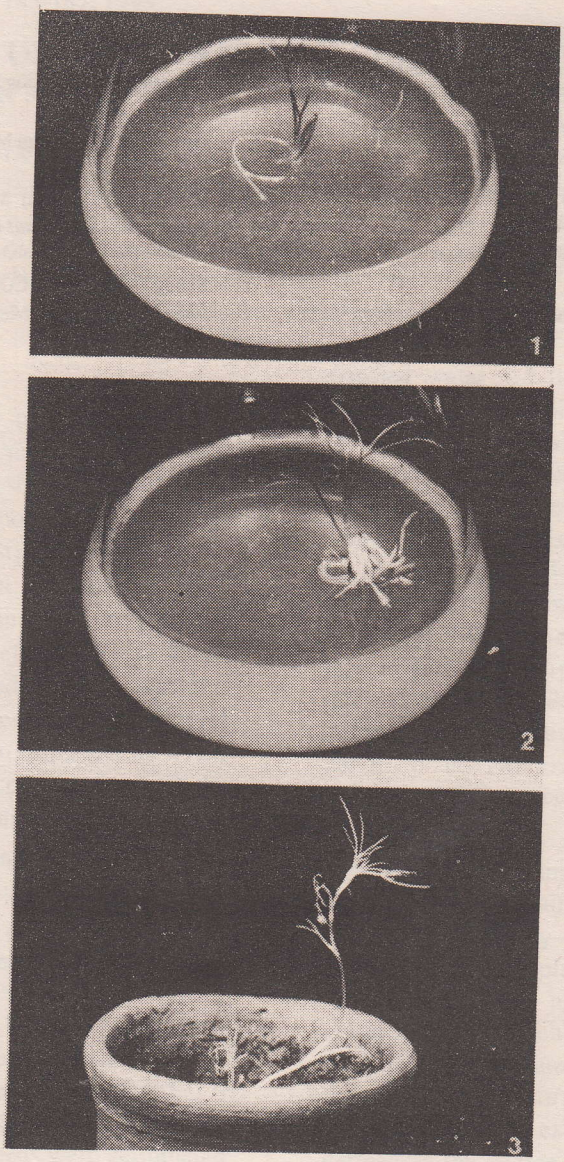

Fig. 1-3. Response of shoot-tip explant of *Cuminum cyminum* on MS-medium incorporated with 8.0mg/l of IAA.
1 Establishment of shoot-tip on the medium with increase in shoot length after the first week;
2 Rooting appeared beneath the explant embedded in the medium;
3 By the fourth week, the complete plantlet was formed which was able to survive in soil.

Reference :

1. Jha TB, Roy SC and Mitra GC 1983, *Plant Cell Tissue & organ culture* **2** 11
2. Ohkoshi K 1991, *Food Technol Center* **126** 7
3. Wang PJ and HU CY 1982, *Am. Potato J.* **59** 33
4. Whitehead HCM and Giles KL 1977, *New Zealand J. For. Sci.* **7** 40
5. Conchou O Nichterlein K and Vomel A 1992, *Planta Media* **58** 73
6. Mathew MK and Hariharan M 1990, *Annals of Botany* **65** 277
7. Murashige T and Skoog F 1962, *Physiol. Plant.* **15** 473
8. Hiraoka N and Oyangi M 1988, *Plant Cell Rep.* **7** 39
9. Herrera MT, Cacho M, C orchete MP and Tarrago JF 1990, *Plant Cell Tissue and Organ Culture* **22** 179
10. Skoog F and Miller CO 1957, *Sym. Soc. Exp. Biol.* **11** 188