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AN EFFICIENT AND RELIABLE PROTOCOL FOR SINGLE STEP *IN VITRO* REGENERATION OF *TRACHYSPERMUM AMMI*

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An efficient and reliable protocol for *in vitro* regeneration of *Trachyspermum ammi* was successfully accomplished. Complete plantlet formation was achieved on Murashige and Skoog's medium supplemented with either IAA (3.0 mg/l) or NAA (3.0 mg/l), singly within 3-4 weeks, thus eliminating the need of different hormonal combinations and concentrations for obtaining shoots and roots one after the other.

Keywords: *In vitro* regeneration; *Trachyspermum ammi.*

Introduction

Trachyspermum ammi commonly known as Bishop's weed or Ajowan is an annual herb, belonging to family Apiaceae. Seeds yield 2.5-4% essential oil which is valued considerably in medicines, on account of the presence of thymol therein. The fruits are considered antispasmodic, stimulant and carinative. The distilled dry fruits of Ajowan contain 15-17% proteins and 25-32% fats, thus making an excellent feed for cattle. In the present investigation, a successful attempt has been made to develop a quick *in vitro* regeneration system achieved in single step in Ajowan.

Materials and Method

Seed of *Trachyspermum ammi* procured from Agriculture Research Station, Durgapura were germinated on paper bridges with distilled water and then the shoot tips were excised from 2 week old seedlings. The explant thus constituted of two contyledons, the meristermatic cotyledonary node region and a part of hypocotly measuring 3-5mm in length. Murashige and Skoog's (MS) basal medium[1] was used for all experiments. Hormones used were auxins (IAA, IBA, NAA, & 2,4-D) and cytokinins (BAP & KN) singly or in combinations. For all the culture experiments, 100ml 'Borosil' Erlenmeyer flasks were used. The medium thus contained in the culture vessels was autoclaved at 1.06 kg/cm^2 pressure for 15-20 minutes.

All the manipulations were done aseptically under a laminar air flow bench (MH-104, Horizontal type). Presterilization was done by UV light for 40 minutes. All cultures after inoculation were kept in a growth chamber fitted with fluorescent tubes and incandescent bulbs, so as to provide continuous diffused illumination (3000-4000 Lux). Temperature was maintained at 26±2°C and relative humidity at 50-55%. The experiments were confirmed by repeatition of the same and simultaneously maintaining at least 25 replicates of each.

Result and Discussion

Shoot tips when implanted vertically on MS medium supplemented with various auxins (2, 4-D, IAA, NAA, & IBA) and Cytokinins (BAP & KN) singly or in various combinations gave valid results. Noteworthy observation was the response of shoot-tips cultured on 3.0mg/l of IBA, NAA and IAA incorporated singly in the MS medium.

After one week of inoculation of the shoot apices, an increase in the length of the shoots was observed with the emergence of new shoots from the meristematic cells present at the apex of the explant. The sensitivity of shoot apices to various hormones is due to the activity of meristematic cells, which are

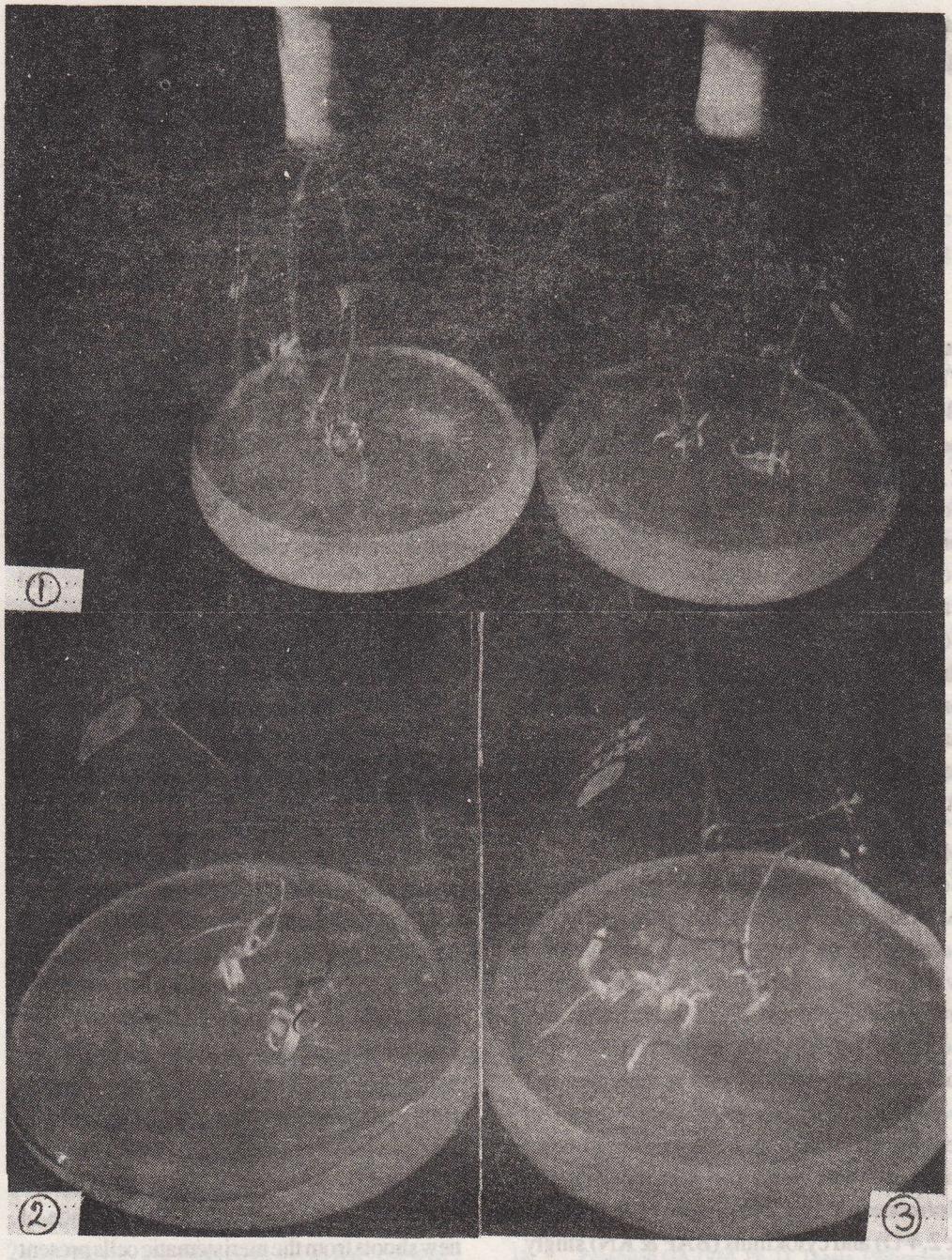

Fig. 1 Complete plantlet regeneration of *Trachyspermum ammi* via shoot tip culture on MS + IAA (3.0 mg/l).

Fig. 2 Plantlet regeneration of *T. ammi* via shoot tip on MS + NAA (3.0 mg/l).

Fig. 3 Plantlet regeneration of *T. ammi* via shoot tip on MS + IBA (3.0 mg/l).

Fig. 4 Hardening of *in vitro* regenerated plantlets.

actively dividing and homogeous composition. In general, these cells yield calli of higher regeneration ability than the nature and highly differentiated cells[2,3].

The shoots obtained grew vigorously by the second week, meanwhile, roots also started developing simultaneously at the cut end, which was embeded in the medium. Thus, the full length of the plants with well developed root and shoot system was observed by culturing the shoot tips on the medium incorporated with 3-0 mg/l of NAA, IAA or IBA (Fig. 1,2 & 3). Faster growth was seen in the medium containing 3.0 mg/l of IBA. The plants were ready within a period of three weeks. Auxins are usually required for shoot growth. Similarly, they proved to be highly effective for replacing out all the three developmental stages of micropropagation, as described by Murashige[4] viz. explant establishment, multiplication of the propagule and rooting and hardening for planting into soil just with a single step. Similar kind of effects of auxins (IAA) have also been reported by Dave[5] in *Cuminum cyminum.* Herrera *et al.*[6] however, faced problems of callus formation at the base of the shoot-tip explant of *Digitalis* which was overcome by lowering the concentration of cytokinins, whereas, no such complexities were encountered while culturing the shoot-tip explant of *Trachyspermum.* Thinmann[7] attributed the inhibitory effect were observed during the present studies while using auxins (IAA, NAA and IBA). The role of IAA for rooting has also been reported by several workers[8-11].

Out of all the developed plants some were taken for soil survival experiments, while others were allowed to grow *in vitro* on the same hormones. The plants *in vitro,* after maturation attained flowering within a time peiod of 45-55 days, thus proving the efficiency of tissue culture technique.

Under the soil survival experiments, the platlets were first transferred to soil, mixed with vermiculite (Fig. 4). After the hardening period of two weeks, they were planted in the soil alone where they survived well. The results presented are singificant, as successful regeneration was obtained only in a single step.

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