constitutes a new record of the fungus on small cardamom.

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SHOOT TIP CULTURE OF PEPPER FOR MICROPROPAGATION

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LITERATURE on pepper (*Capsicum*) tissue culture has recently been reviewed[1]. Agrawal and Chandra[2] reported differentiation of multiple shoot buds and plantlets from cultured embryos of *Capsicum annuum* L. var. *mathania*. Regeneration of shoots has been reported from cotyledon[3], hypocotyl[3,4] explants and from protoplast derived callus tissue[5] of *C. annuum*. In a more recent report, regeneration from different organ explants of several genotypes of *C. annuum* has been described[6]. The present report describes the micropropagation of *Capsicum* through shoot tip cultures.

Seeds of *Capsicum annuum* var. *mathania* and an intervarietal hybrid 'Bharat' of *C. annuum* L. were obtained from the Agriculture Research Station, Durgapura and Indo-American Hybrid Seeds, Bangalore respectively. Seeds were soaked in tapwater for 24 h before surface sterilizing with 0.1% $HgCl_2$ solution for about 5 min and washed thoroughly with sterile water. They were germinated in culture tubes fitted with filter paper bridges and containing sterilized tapwater. Shoot tips (3–4 mm long) with 2–4 leaf primordia were excised from 15-day-old seedlings and cultured on MS[7] medium supplemented with kinetin (Kn), 6-benzylamino purine (BAP) alone or in combination with 3-indoleacetic acid (IAA), 3-indolebutyric acid (IBA) or naphthaleneacetic acid (NAA). The pH of the medium was adjusted to 5.8 before autoclaving. The cultures were maintained in diffused continuous light from fluorescent tubes and incandescent bulbs at 26±2°C.

Table 1 shows the morphogenetic responses of shoot tip explants of *C. annuum* var. *mathania* on MS medium supplemented with BAP, Kn, IAA,

Table 1 *Morphogenetic responses of shoot tip explants of Capsicum annuum var. mathania on MS medium with BAP, Kn, IAA, IBA or NAA added singly or in combinations*

	BAP		Kinetin	
	3	5	3	5
MS + mg/l	S**(60)	S***(80)	S*(80)	S**(60)
IAA	1R*(60)	S**(60)	S***(80)	S**(60)	S**(60)
IBA	1R**(60)	S**(60)	S**(60)	S**(80)	S**(60)
NAA	1 S* R**(100)	S* R**(100)	S* R**(100)	S* R**(100)	S* R**(80)
IAA or IBA	3R**(80)	S* R*(80)	S* R*(80)	S* R*(80)	S* R*(80)
IAA or IBA	5R*(100)	S* R*(70)	S* R*(90)	S* R*(80)	S* R*(70)

R, roots; S, shoots/shoot buds; *1–2; **3–6; ***7–12; Figures in parentheses are per cent cultures responded.

IBA or NAA added singly or in different combinations. On a medium supplemented with 1 mg/l of BAP, a green and compact callus was induced from the cut surface of explants and the apex elongated to form a shoot in three weeks. The concentration of BAP when raised to 5 mg l, the basal end of the explant callused and 7–12 shoot buds were formed after 2 weeks (figure 1A). With 5 mg/l of Kn, less number of buds differentiated as compared to BAP (5 mg l) (table 1). IAA and IBA evoked rhizogenesis only. NAA (1–5 mg/l) evoked root formation and elongation of the shoot apex. On media with BAP or Kn (1 mg l) in combination with IAA or IBA (1 mg l), callus formation and rooting were observed. On media with higher levels (3–5 mg/l) of BAP or Kn in combination with low levels of IAA or IBA (1 mg/l), a large number of shoot buds regenerated together with callus formation. Rooting also occurred with BAP (5 mg/l) and higher concentration of IAA or IBA (5 mg/l) (figure 1B). Shoot buds differentiated on any medium could be multiplied by subculturing them on a medium with

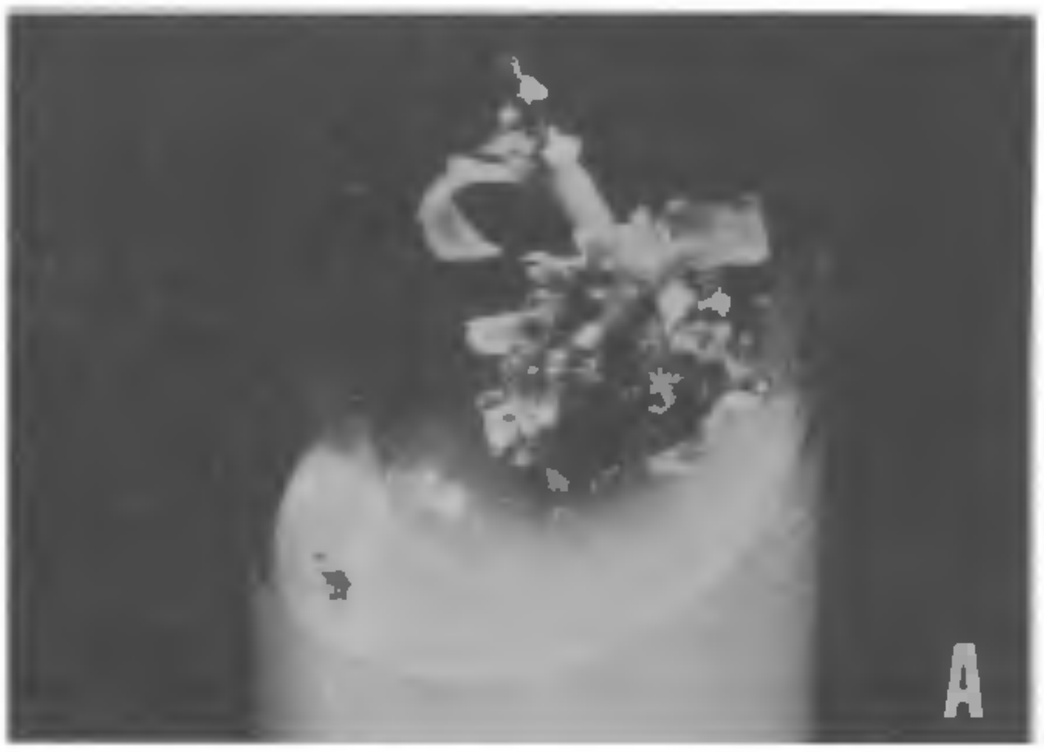

Figure 1A, B. A. Formation of vigorous shoots and a large number of shoot buds on MS medium with BAP (5 mg/l), and **B.** Formation of shoots, intervening callus and roots on MS medium with BAP (5 mg/l) + IBA (5 mg/l).

Figure 2. Plantlets derived from shoot tip explants. *In vitro* differentiated shoots were rooted on MS medium with IBA (0.1 mg/l).

BAP (5 mg/l). Thus, on this medium from an initial number of 2–3 buds placed together, a large number of shoot buds were obtained in 2 weeks and the number continued to increase for 3–4 weeks. This process of subculturing was repeated to get a continued supply of shoots. The shoots thus multiplied were isolated and placed on 0.1 mg/l of IBA or NAA containing medium to obtain complete plantlets. Rooting was induced on this medium along with the elongation of the axis of shoots and complete plantlets developed in 2–3 weeks (figure 2). Phillips and Hubestenberger[6] reported the necessity of an auxin with a cytokinin for rooting.

Shoot buds were induced from the shoot tip cultures in hybrid Bharat on media containing BAP or Kn (2–5 mg/l) alone or in combination with IAA or IBA (3–5 mg/l).

In this work, no loss of morphogenetic potential occurred if the callus together with some shoot buds (2–3) was regularly subcultured. Callus subcultured in this way could differentiate many more adventitious shoots and the process was repeatable in all the subcultures for 2 years. Shoot tip explants responded by forming many shoot buds, single shoots (elongation of parent shoot), a shoot with roots, only roots or callus depending upon the type, concentration and combination of growth regulators used. Shoot buds were induced in the presence

of high levels of BAP or Kn alone or in combination with IAA or IBA. They were formed *de novo* and not due to the release of axillary buds as reported in many plants[8]. Elongation of parent shoot tip was noted in the presence of low levels of BAP alone or in auxin-cytokinin combinations. In no instance the elongation of parent shoot was observed in a hormone-free medium.

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A COMPARATIVE STUDY OF *IN VITRO* SHOOT REGENERATION FROM COTYLEDON AND ROOT EXPLANTS OF FOUR VARIETIES OF *BRASSICA OLERACEA* L.

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INTERSPECIFIC variation for *in vitro* shoot differentiation in the genus *Brassica* has been reported earlier[1–3]. Several workers[1–3] have observed intraspecific variation for shoot differentiation in *Brassica oleracea*. Lazzeri and Dunwell[2], and Murata and Orton[3] used root and hypocotyl segments, respectively, as the explants for the comparison of regeneration potentiality of the various varieties, and commented that cotyledons showed very poor and sporadic response. Dietert *et al*[1] studied regeneration in callus cultures, the frequency of which was very poor. Moreover, the observations of these three workers with regard to intravarietal variation are at variance. In the present study, the intraspecific variation for regeneration in *B. oleracea* was examined using cotyledons and root segments as the explants. Interestingly, we found cotyledons to be more amenable to regeneration than root segments.

Seeds of 4 French varieties of *B. oleracea* (*botrytis*, *capitata*, *gemmifera*, *italica*) were bought from a nursery in Paris and brought to India. The seeds were surface-sterilized by treating them in 0.2% mercuric chloride solution for 10 min, washed repeatedly in sterile distilled water and planted on MS medium[4]. Cotyledons (lamina + petiole) and root segments (10 mm long) middle segment, were excised from 8-day-old aseptic seedlings and cultured on seven nutrient media (table 1). The pH of the medium was adjusted to 5.8 after adding all the constituents. The medium was then dispensed into culture tubes (15 ml medium/tube) and, after plugging with non-absorbent cotton, autoclaved at 1.06 kg/cm^2 for 15 min. All cultures were stored at 27 ± 3°C in light (15 μE m^{-2} S^{-1} irradiance provided by Mysore fluorescent lamps TL 40 W/54 cool day light). The percentage of cultures showing shoot differentiation after 4 weeks of culture was recorded.

The results are presented in table 1. In general, cotyledons showed consistently higher regeneration than root segments. This is contrary to the results of Lazzeri and Dunwell[5]. We observed that in the cotyledon cultures differentiation of shoot buds occurred invariably at the cut end of the petiole (figure 1A). A detailed study by Sharma and Bhojwani[6] had shown that excised or intact lamina of cotyledons of *B. juncea* lack the potentiality to differentiate shoot buds. These results are in agreement with the findings of poor and sporadic regeneration in the cultures of laminar discs[5] and cotyledon segments[6] reported earlier.

Brassica oleracea represents one of the most polymorphic species among Brassicas. Lazzeri and Dunwell[2] compared the regeneration potentialities of three varieties of *B. oleracea* and noted *capitata* to be the most responsive to regeneration and *gemmifera* to be the least and *italica* was intermediate. However, on the medium used for a comparative study *capitata* showed only 3% regeneration. Murata and Orton[3] studied shoot differentiation in the hypocotyl cultures of six varieties of *B. oleracea* (*acephala*, *botrytis*, *capitata*, *gemmifera*, *gongylodes* and *italica*). Of these *acephala* cultures showed