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Direct shoot organogenesis on shoot apex from seedling explants of *Capsicum annuum* L.☆

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Abstract

In vitro direct multiple shoot formation from seedling explants of Indian high pungent varieties of *Capsicum annuum* cv. Arka Abhir (AA) and Arka Lohit (AL) was successfully obtained. We were able to induce regeneration potency in these varieties by inverting the explant. Aseptically grown seedling explants with decapitated roots, apical meristem and cotyledonary leaves were inoculated in an inverted position in bud induction medium comprising of Murashige and Skoog's basal medium supplemented with 2-(*N*-morpholine) ethanesulphonic acid (MES) buffer along with 26.63 μM benzyl adenine (BA), 2.28 μM indole-3-acetic acid (IAA) and 10 μM silver nitrate. Profuse shoot bud induction with 20–25 shoot buds per explant was obtained. Supplementation of phloroglucinol in the bud induction medium resulted in 17 and 18% enhancement in bud induction response in Arka Abhir and Arka Lohit variety, respectively on the inverted hypocotyls. Auxin transport inhibitor triiodo benzoic acid (TIBA) in the bud induction medium resulted in induction of buds in a shorter period of 40–45 days when compared to bud induction (BI) medium which takes 55–65 days for bud induction. These buds were transferred to MS medium containing 2.8 μM gibberellic acid and 10 μM silver nitrate resulting in elongation of shoot buds. Transfer of shoots to MS basal medium induced rooting to produce plantlets. This protocol can be efficiently used for mass propagation and presumably also for regeneration of genetically transformed *C. annuum* tissues.

Keywords: Adventitious shoot buds; *Capsicum annuum*; Direct organogenesis; Hypocotyl explants; Polarity

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1. Introduction

Capsicum annuum L. is an important horticultural crop belonging to the family Solanaceae. Even though other Solanaceae members easily undergo morphogenesis, *Capsicum* sp. were found to be highly recalcitrant. Few common observations in *Capsicum* regeneration include formation of profuse leafy structure instead of shoot buds, or shoot buds that do not elongate (see, e.g. Hyde and Phillips, 1996), induction of somatic embryos but fail to develop a shoot (Steinitz et al., 2003). Application of cell and molecular biology techniques for genetic improvement of this crop has been limited because of the difficulties in plant regeneration (Liu et al., 1990). Though various reports on organogenesis and embryogenesis in *C. annuum* varieties are available (reviewed by Ochoa-Alejo and Ramirez-Malagon, 2001), none of them provided promising response in *C. annuum* var. Arka Abhir (AA) and Arka Lohit (AL), the two commercially important varieties in India. This may be attributed to intra varietal differences in regeneration from various explants (Jacobs and Stephens, 1990) and difficulties in elongation of shoot buds and somatic embryos reported in *C. annuum* (Phillips and Hubstenberger, 1985; Binzel et al., 1996; Steinitz et al., 2003).

The work reported here mainly addresses the regulatory role of polarity of the explant, exogenously administered auxin transport inhibitor tri-iodo benzoic acid (TIBA), phloroglucinol (PG) along with benzyl adenine (BA) and indole-3-acetic acid (IAA) on direct adventitious shoot formation in recalcitrant genotypes of *C. annuum* cv. Arka Abhir and Arka Lohit. Studies were also carried out on the role of light in elongation of shoot buds in presence of exogenously fed GA_3 and $AgNO_3$.

2. Materials and methods

2.1. Explant preparation

Arka Abhir, a low pungent variety and Arka Lohit, a high pungent variety of *C. annuum* were obtained from Indian Institute of Horticultural Research (IIHR), Bangalore, India. Seeds were thoroughly washed in running tap water, subsequently surface sterilized with 0.2% $HgCl_2$ (Hi-media India) for 3 min, washed copiously with sterile distilled water. The seeds were germinated in vitro and 15-day-old seedlings show well-developed roots, two cotyledonary leaves and an apical meristem. The apical meristem (1–2 mm), cotyledonary leaves and root portion were excised using a sharp scalpel and these entire hypocotyls of 5–7 cm length were used as explants. The explants were inoculated immediately in order to prevent the drying of cut edges of the explant.

2.2. Culture medium

Various media used are given in Tables 1–3. Basically, MS media (Murashige and Skoog, 1962) with adjuvant and growth regulators were prepared. Bud induction (BI) medium comprised MS salts, vitamins, 1.95 g/l MES (Sigma USA), 17.74–44.38 μM BA (Sigma USA), 1.44–4.57 μM IAA (Sigma USA) and 10 μM $AgNO_3$. Media pH was