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Salicylic acid induced plantlet regeneration in pepper (*Capsicum fruitescens*) Calli

Ajay Sharma*, P.N. Mathur, G. Rajamani and A. Joshi
Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture
Maharana Pratap University of Agriculture and Technology, Udaipur - 313001(Rajasthan) India

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Cotyledonary explants from 4 week-old aseptic seedlings of *Capsicum fruitescens* cv. California Wonder were cultured on MS medium supplemented with sucrose (5%), agar (0.8%) and 2,4-D (1 mg l^{-1}) pH 5.7. One month old callus was subcultured on the same medium devoid of 2,4-D but with salicylic acid (0.5 to 2.0 mM) to study the role of Salicylic acid in triggering defense responses. However, the best response for salicylic acid induced plantlet regeneration was obtained at 0.5 mM concentration. Kinetin (0.5 to 5.0 mg l^{-1}) or Gibberellic Acid (each 0.5 to 5.0 mg l^{-1}) failed to do so. The salicylic acid induced cytodifferentiation and plantlet regeneration in pepper may function through the response regulator mediated two component signaling system.

Key words : Salicylic acid, Regeneration, Pepper.

INTRODUCTION

SALICYLIC acid plays a critical signaling role in the activation of plant defense responses against pathogens[1-7]. It is also an endogenous trigger of thermogenesis and increases the expression of an alternative oxidase linked to this generation of heat[8-9]. While studying its role in triggering defense responses in pepper cell culture, a novel response were observed. It triggered organogenesis and plant regeneration. The possible implications of this observation are discussed in this paper.

MATERIALS AND METHODS

Seeds of *Capsicum fruitescens* cv. California wonder were surface sterilized with sodium hypochlorite (1%) for two minutes, washed with sterile distilled water and placed on MS (Murashige and Skoog)[10] medium (pH, 5.7) supplemented with sucrose (3%) and agar (0.8%) and allowed to germinate under 16h photoperiod at 25°C. Cotyledonary explants from four week old seedlings were excised in approximately 1 cm. segments and cultivated in 100 ml conical flasks containing MS medium supplemented with sucrose (5%), potato dextrose agar (8%) and 2,4-D (1 mg l^{-1})[11].

A portion of the callus was subcultured after 20 days on the same medium (Figure-A). Small portions of this callus after one month were transferred on the same medium devoid of 2,4-D but supplemented with salicylic acid (0.5, 1.0 and 2.0 mg l^{-1}). It was observed that control flasks (Figure-B) showed no regeneration while the salicylic acid containing medium supported plantlet regeneration. The best response was obtained with salicylic acid at concentration of 1.0 mM (Figure-C).

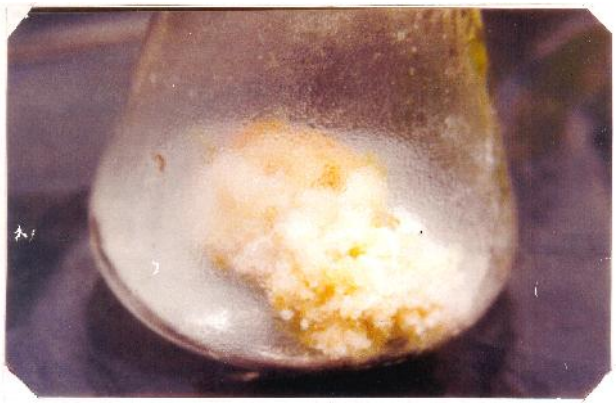
RESULTS AND DISCUSSION

Cellular differentiation and organogenesis are poorly understood. The classical reports of Skoog and Miller (1957)[12] revealed that undifferentiated callus cultures will form into roots or shoots depending upon the relative amount of cytokinins and auxin in the medium; the ratio rather than the absolute amount of these hormones is critical. A balanced ratio keeps the cells in an undifferentiated state, while high cytokinin to auxin ratios promote shoot, their low ratios promote root development.

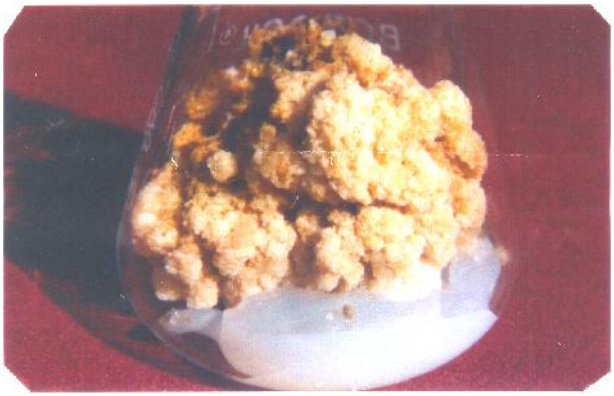
Cytokinins and auxins are not the only molecular triggers for cellular differentiation. Amino acids have been shown to be specific stimulators of somatic embryogenesis and differentiation. Rapid accumulation of polyamines occurs concomitantly with the initiation of cell division and the inhibition of polyamine biosynthesis induces differentiation.

Aminoethoxyvinyl glycine and silver nitrate, inhibitors of ethylene biosynthetic pathway cause high frequency regeneration from cultured explants, of *Zea mays*[20], *Brassica campestris*[2l] and *B. juncea*.[22] Recently Huang *et al*.[23] have shown that high levels of 1-amino-cyclopropane-1 carboxylic acid (ACC) and polyamines might be molecular prerequisites of differentiation during the induction of embryogenesis. Conversion of ACC to 1-

Fig. : Salicylic acid induced plantlet regeneration in pepper (*Capsicum fruitesence*) calli



A. Initiating Callus (20 days)



B. Callus growth on medium devold of 2,4-D and SA

* Author for Correspondence

C. Regenerants on medium containing SA (1.0 mM)

(malonylamino)-cyclopropane-l-carboxylic acid (MACC) and the maintenance of relatively high level of polyamines, especially spermidine appear to be important for further development of embryos.

Phytosulfokines, a novel class of polypeptide hormones[24] have also been shown to promote organogenesis in roots, buds and embryos besides their primary role in cell proliferation.[25]

The triggering of organogenesis and plantlet regeneration by salicylic acid is being reported for the first time. The diverse molecular triggers for differentiation can possibly be explained in light of the recent two component response regulator mediated signaling system[26].

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